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THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Sciences de l'Eau

École doctorale GAIA - Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau

Unité de recherche HydroSciences Montpellier

Uptake of organic micropollutants by vegetable crops irrigated by reclaimed wastewater: Analytical developments to conduct field studies

Présentée par Rayana MANASFI

Le 24 novembre 2020

Sous la direction de Serge CHIRON

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Abstract

The use of wastewater in agricultural sector for irrigation and fertilization purposes is not a recent practice. However, until the past couple decades this practice has been of increasing interest regarding the global safety, particularly human health. Besides to the useful nutrients and organic matter that wastewater introduces to the agricultural lands, pharmaceutical active compounds (PhACs) and other organic micropollutants are also co-introduced leading to the presence of undesired chemicals in agroecosystems, most remarkably in crops intended for human consumption. Incredible effort has been made in last couple decades to analyze such contaminants in environmental samples, yet there is still a lack of robust analytical methods for the extraction and quantification of a large number of PhACs and other wastewater pollutants, and a lack of knowledge regarding their fate and behavior in the entire agroecosystem including soil and crops.

In this context, the first objective of this thesis was to develop robust and sensitive analytical methodologies specific to soil and crops matrices (e.g., root and leaf) to analyze a large number of wastewater organic contaminants mainly PhACs and their metabolites. QuEChERS-based methods followed by detection and quantification on high-resolution mass spectrometry were successfully developed for soil, lettuce/leek root and lettuce/leek leaves. Good recoveries were obtained for the 48 studied compounds in all matrices (in general between 80 and 120 % for most of the compounds). Detection and quantification of compounds was performed on a high-resolution mass spectrometry on quadrupole-time of flight (Q-TOF) coupled to liquid chromatography provided from SCIEX technology. Comparing the two recently developed acquisition modes high-resolution multiple reaction monitoring (MRM^{HR}) and Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH) gave similar results, though MRM^{HR} gave more consistent results for most of the compounds. Finally, satisfying detection and quantification limits were obtained for all 3 matrices, for instance lettuce leaves matrix in MRM^{HR} acquisition gave detection limits ranging from 0.01 to 0.12 ng/g and quantification limits ranging from 0.04 to 0.38 ng/g dry weight (d.w).

Later, the following objective was to apply these methodologies on real agricultural field irrigated with local treated domestic wastewater. The second objective allowed us to understand the distribution of selected contaminants in soil and crops and calculate their bioconcentration factors, to discriminate their degradation in soil and crops, and finally to

assess the potential risk for human consumption of wastewater irrigated crops. Two years of successive growth cycles of lettuce and leek under greenhouse field conditions were successfully implemented. Different kinds of water were used for irrigation mainly domestic treated wastewater, following real drip irrigation conditions. Little accumulation in soil and crops was found, which was explained by the intensive degradation of contaminants in soil and metabolism in crops. A few metabolites were detected in lettuce leaves, particularly carbamazepine-epoxide which was produced by the lettuce metabolism. Finally, this study revealed a *de minimis* human health risk related to the consumption of raw vegetable irrigated with treated wastewater.

The last objective was to study the fate of selected contaminants introduced in soil by wastewater irrigation, when they are exposed to the ubiquitous filamentous soil fungi *Trichoderma*. Implemented experiments in liquid medium revealed for the first time an important ability of *Trichoderma* species, namely *T. harzanium* and *T. asperellum* to degrade two fluoroquinolones antibiotics (ciprofloxacin and ofloxacin), and one fungicide (climbazole). Equally important, new metabolites of ofloxacin and climbazole were tentatively identified in this study for the first time.

Key words: pharmaceuticals, fate in agroecosystems, biodegradation, plant uptake, QuEChERS, high resolution mass spectrometry.

Résumé

L'utilisation des eaux usées dans l'agriculture à des fins d'irrigation et de fertilisation n'est pas une pratique récente. Cependant, au cours de ces deux dernières décennies, cette pratique a suscité un intérêt croissant au regard des risques potentiels qu'elle représente pour la santé humaine. Outre les nutriments utiles et la matière organique que ces eaux introduisent dans les terres agricoles, des produits pharmaceutiques (PhACs) et d'autres micropolluants organiques sont également co-introduits, conduisant à la présence de composés chimiques indésirables dans les agroécosystèmes, plus remarquablement dans les cultures destinées à la consommation humaine. Des efforts incroyables ont été faits au cours des deux dernières décennies pour analyser ces contaminants dans des échantillons environnementaux, mais il y a encore un manque de méthodes analytiques robustes pour l'extraction et la quantification des multiples micropolluants organiques présents dans des matrices complexes comme les sols et les végétaux, et un manque de connaissances concernant leur devenir dans les agroécosystèmes.

Dans ce contexte, le premier objectif de cette thèse est de développer des méthodologies analytiques robustes pour analyser de nombreux micropolluants organiques présents dans les eaux usées, particulièrement les PhACs et leurs métabolites dans le sol et les racines/feuilles de laitue et poireau. Ces méthodes sont basées sur QuEChERS suivies d'une détection par LC-HRMS-QTOF. Des bons taux de récupérations ont été obtenus pour les 48 composés étudiés dans toutes les matrices (en général entre 80 et 120% pour la plupart des composés). La comparaison des deux modes d'acquisition récemment développés, high-resolution multiple reaction monitoring et Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra ont donné des résultats similaires, bien que la MRM^{HR} ait donné des résultats plus cohérents pour la plupart des composés. Enfin, des limites de détection et de quantification satisfaisantes ont été obtenues pour les 3 matrices (par exemple, 0,01 à 0,12 ng/g et 0,04 à 0,38 ng/g poids sec ont été obtenus par les feuilles de laitue, respectivement). L'objectif suivant consiste à appliquer ces méthodologies analytiques pour suivre le comportement et devenir de certains micropolluants sur un champ agricole irrigué avec des eaux usées domestiques traitées. Le deuxième objectif nous a donc permis de comprendre leur distribution dans le sol et les cultures en calculant des facteurs de bioconcentration, en discriminant leur dégradation dans le sol à celle dans les plantes, ceci afin d'évaluer le risque potentiel pour la consommation humaine de végétaux irrigués avec des eaux usées traitées.

Deux années successives de culture de laitue et poireau sous serre ont été mises en œuvre, et irrigués goutte à goutte avec différents types d'eau, principalement les eaux usées domestiques traitées. Une faible accumulation dans le sol et les végétaux a été constatée, ce qui s'explique par leur dégradation intensive dans le sol et la plante. Quelques métabolites ont été détectés dans les feuilles de laitue, en particulier la carbamazépine-époxyde issue du métabolisme de la carbamazépine par la plante. Enfin, cette étude a révélé un risque minimum pour la santé humaine lié à la consommation de légumes crus irrigués avec des eaux usées traitées.

Le dernier objectif est d'étudier le devenir de certains contaminants introduits dans le sol par l'irrigation des eaux usées, lorsqu'ils sont exposés aux champignons filamenteux du genre *Trichoderma* omniprésents dans le sol. Des expériences mises en œuvre en milieu liquide ont révélé pour la première fois une capacité importante des espèces de *Trichoderma* (*T. harzanium* et *T. asperellum*) à dégrader deux antibiotiques (ciprofloxacine et ofloxacine) et un fongicide (climazole). Tout aussi important, de nouveaux métabolites de l'ofloxacine et du climazole ont été identifiés pour la première fois.

Mots clés: produits pharmaceutiques, devenir dans les agroécosystèmes, biodégradation, assimilation par les plantes, QuEChERS, spectrométrie de masse haute résolution

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Publications and international communications

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- Development of methods for the determination of PhACs in soil/earthworm/crop system irrigated with reclaimed water. Rayana Manasfi, Francesc Labad, Nicola Montemurro. Book chapter, 2020.
- Soil Sorption and Degradation Studies of Pharmaceutical compounds present in Recycled Wastewaters based on Enantiomeric Fractionation. Monica Brienza, Belinda Huerta Buitrago, Rayana Manasfi, Serge Chiron. Book chapter, 2020.
- Nitric oxide reactivity accounts for N-nitroso-ciprofloxacin formation under nitrate-reducing conditions. Monica Brienza, Rayana Manasfi, Andrés Sauvêtre, Serge Chiron. *Water Research* 185 (2020) 116293.
- Effect of the pharmaceuticals diclofenac and lamotrigine on stress responses and stress gene expression in lettuce (*Lactuca sativa*) at environmentally relevant concentrations. Yvonne Bigott, Soumitra Paul Chowdhury, Sandra Pérez, Nicola Montemurro, Rayana Manasfi, Peter Schröder. *Journal of Hazardous Materials* 20 (2020) 31870-7.
- Relevance of N-nitrosation reactions for secondary amines in nitrate-rich wastewater under UV-C treatment. Monica Brienza, Rayana Manasfi, Serge Chiron. *Water Research* 162 (2019) 22-29.
- Impact of repeated irrigation of lettuce with treated municipal wastewater on pharmaceutical plant uptake and degradation under field cultivation conditions. Rayana Manasfi, Monica Brienza, Nassim Aït-Mouheb, Nicola Montemurro, Sandra Perez, Serge Chiron. *Science of the Total Environment*, (2020) 142742.
- Comparison of MRMHR and SWATH acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce leaves using a modified QuEChERS method. Nicola Montemurro, Anastasia Orfanoti, Rayana Manasfi, Nikolaos S. Thomaidis, Sandra Pérez. *Journal of Chromatography A*, 1631 (2020) 461566.

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- Evaluation of different modified QuEChERS-based methods for the extraction of 48 wastewater-derived organic contaminants from soil and lettuce root using high resolution LC-QTOF with MRMHR and SWATH acquisition modes. Rayana Manasfi, Nicola Montemurro, Serge Chiron, Sandra Perez. Paper, Analytical Biochemistry, 2020.

Orale communications

- 15 – 16 October 2020. Oral presentation: “Wastewater-borne organic micropollutants accumulation in soil and lettuce leaves using QuEChERS extraction methods and LC-QTOF-MS: a field study”. 16th Annual Workshop On Emerging High-Resolution Mass Spectrometry (HRMS) And LC-MS/MS Applications In Environmental Analysis And Food Safety. Online.
- 28 - 29 November 2019. Oral presentation: “Pharmaceutical plant uptake and soil accumulation in crops irrigated with treated wastewater: A field study”. 2nd Internal Conference on Risk Assessment of Pharmaceuticals in the Environment. Barcelona, Spain.
- 02 - 04 October 2019. Oral presentation: “The fate of water borne contaminants in wastewater treatment and reuse chain”. International REUSE MUSE Workshop: Agricultural Water Reuse - how to address health and environmental challenges? Montpellier, France.
- 16 - 20 June 2019. Oral presentation: “Biodegradation and fate of PPCPs by fungi *Trichoderma* species and identification of degradation products”. 17th International Conference on Chemistry and Environment, ICCE 2019. Thessaloniki, Greece.

Poster communications

- 18 – 19 December 2018. Poster presentation: “Biodegradation of emerging contaminants by *Trichoderma* strains”. Globaqua Final conference: Water river management under water scarcity and multiple stressors. Barcelona, Spain.

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List of abbreviations

AHFS. American Society of Health System Pharmacists

ASE. Assisted Solvent Extraction

ATC. Anatomical Therapeutic Chemical

CNHIM. Hospital National Center of Drugs Information

d.w. dry weight

EphMRA. European Pharmaceutical Marketing Research Association

HRMS. High Resolution Mass Spectrometry

Kd. Soil-water partition coefficient

Kow. Distribution coefficient of octanol-water

MEA. Microwave-Assisted Extraction

mg/g, mg/Kg. Milligram per gram, milligram per kilogram

ng/g, ng/Kg. Nanogram per gram, nanogram per kilogram

µg/g, µg/Kg. Microgram per gram, microgram per kilogram

OWC. Organic Water Contaminants

PhACs. Pharmaceutically active compounds

QuEChERS. Quick, Easy, Cheap, Effective, Rugged and Safe

STP. Sewage Treatment Plant

TPs. Transformation Products

USE. Ultrasound Solvent Extraction

WHO. World Health Organization

WWTP. Wastewater treatment plant

Chapter 1:

Introduction and aims

Pharmaceutically active compounds (PhACs) are chemicals used in everyday life for their biological activity, as they contribute to our health and high standards of living. Thus, it is expected that PhACs consumption will increase in the future. However, since the past two decades, it has been recognized that PhACs themselves are presenting a new type of environmental pollution and potential human and environmental health risks. Thanks to analytical field development, and its ability to detect these compounds at very low concentration (ng/L to µg/L) in the complex environmental matrices, PhACs have been found present in the environment at trace levels (Kümmerer, 2010; Patel et al., 2019; Petrie et al., 2015). These compounds end up in the environment by several pathways including proper and improper use, in addition their load into the environment is expected to increase in the coming years (Kümmerer, 2010).

It is reported that 30 to 70 % of ingested PhACs are excreted unmetabolized via urine and feces (Liu et al., 2017; Qin et al., 2016), hence their introduction into domestic waste systems. Consequently, domestic wastes are transported to wastewater treatment plants (WWTPs) for treatment and disinfection processes. Lately, it has been largely reported that WWTPs are not able, or in other words not designed to remove PhACs from wastewater (Tran et al., 2018). Thus, the resulted wastewater effluents are constantly contaminated with PhACs. Taking into account the worldwide problem of water scarcity and shortage, wastewater effluents are considered as important alternative of natural surface and ground water. Furthermore, it was incorporated in many practices as a fundamental requirement for water resource management, mainly in agricultural sector which accounts about 70% of freshwater consumption. However, to date there is a lack of legislation on the minimum requirements for water quality and treatment. Besides treated wastewater is rich of nutrient and organic matter that can reduce the need of mineral fertilizers application. All in all, the reuse of treated wastewater in agriculture lands contributes to the promotion of the circular economy by recovering nutrients and saving money.

In these circumstances, crops irrigation with treated wastewater constitutes one main introduction pathway of PhACs to agroecosystems (Li, 2014). PhACs residues and their transformation products (TPs) in the agroecosystems have been a topic for several years now (Carter et al., 2019; Du and Liu, 2012; Fatta-Kassinos et al., 2011), they can present a potential risk not only to soil and groundwater underneath but also to crops and their consumers. Once PhACs are introduced to the agroecosystem, they can undergo numerous fate and behavior

processes in agricultural soil, besides they are also taken up into the growing irrigated crops, hence they enter the food chain leading to undesired exposure of humans and animals to these bioactive substances. Thereupon, there are growing concerns about (1) the safety of irrigation with treated wastewater as it contains PhACs and other contaminants and their metabolites, (2) human health following the consumption of wastewater irrigated crops. Finally, studies on the fate of PhACs in real agroecosystems are very scarce or limited to studies under controlled or laboratory conditions, this refers mainly to the lack of appropriate, reliable and robust analytical methods for their determination in plant tissues and soil.

Given what have been said, this PhD research has been framed in the following form:

Chapter 1 describes the literature current knowledge regarding PhACs introduction occurrence and fate in agroecosystems.

Chapter 2 is written as a book chapter “Development of Methods for the Determination of PhACs in Soil/ Earthworm/Crop System Irrigated with Reclaimed Water”. It describes all the analytical procedures published so far for the extraction and analysis of PhACs and their related compounds from plant tissues and soil irrigated with treated wastewater.

Chapter 3 is written as a scientific article “Comparison of MRMHR and SWATH acquisition modes for the quantitation of 48 wastewater-borne pollutants (PhACs and metabolites) in lettuce leaves using a modified QuEChERS method”. It describes a high-resolution mass spectrometry approach for developing quantification methods of 48 different wastewater-borne contaminants in lettuce leaves by the means of QuEChERS method. Finally, the article also reported the application of the developed method to lettuce crops grown under controlled conditions and irrigated with fresh local domestic wastewater effluents.

Chapter 4 is written as a scientific article “Evaluation of different QuEChERS-based methods for the extraction of 48 wastewater-derived organic contaminants from soil and lettuce root using high resolution LC-QTOF with MRMHR and SWATH acquisition modes”. It describes the optimization and development of a common high-resolution analytical method for soil

and lettuce root. The method comprises 48 wastewater-borne contaminants (PhACs and metabolites), and it follows modified simple steps of QuEChERS method. Finally, the article also reported the application of the developed method to soil and lettuce roots irrigated with local domestic wastewater effluents.

Chapter 5 is written as a scientific article “Impact of long-term irrigation with municipal reclaimed wastewater on the uptake and degradation of organic contaminants in lettuce and leek”. It describes the fate/behavior of 14 selected PhACs and other wastewater-borne contaminants (PhACs and metabolites) in soil and vegetables under 2 years realistic field conditions. It also describes the ability of growing vegetables to uptake contaminants from irrigation water, and the related potential risks to human consumption.

Chapter 6 is written as a scientific article “Biodegradation of fluoroquinolone antibiotics and the climbazole fungicide by *Trichoderma* species”. It describes the ability of the filamentous fungi *Trichoderma* to eliminate two antibiotics (ciprofloxacin and ofloxacin) and one personal care products (climbazole). The generated TPs were tentatively identified, as well as kinetics study was done for parent compounds and their metabolites.

Finally, conclusions and perspectives of this work have been described at the end of the thesis.

1. Pharmaceuticals

1.1. Classification

Since the industrial revolution we are depending on the use of anthropogenic chemicals in our everyday life more than ever. Besides to the classical organic pollutants such as industrial chemicals, heavy metals, pesticides, fertilizers, detergents, surfactants, plasticizers and many other, a new group of organic pollutants (emerging pollutants including pharmaceutically active compounds, PhACs) have reached the focus of interest. Thanks to the technical development in the field of environmental analysis during the last couple decades, the occurrence of these pollutants in the environment is recognized, and has become a priority concern. In 2002, United States Geological Survey (USGS) released the first nationwide study of the occurrence of pharmaceuticals, hormones and other organic wastewater contaminants (OWCs) in US streams (Kolpin et al., 2002). They have found 82 out of 95 selected OWCs in 80% of the streams sampled in 30 states during 1999 and 2000. However, the number of studies reporting the occurrence of pharmaceuticals and other OWCs in the environment is in continuous increase ever since (aus der Beek et al., 2016; Caliman and Gavrilescu, 2009; Christian et al., 2003; Gunther et al., 2010; Kümmerer, 2010, 2008; Patel et al., 2019; Petrie et al., 2015). In spite of the fact that until now pharmaceuticals are found at low concentrations in the environment (aus der Beek et al., 2016), there are some concerns that continuous exposure to pharmaceuticals may increase human and ecosystem health risk (Du and Liu, 2012).

As definition, pharmaceuticals or PhACs are a broad range of substances with wide spectrum of therapeutic classes. They are used for prevention, diagnosis or treatment of a human or animal disease, and for restoring, correcting, or modifying organic functions (Daughton and Ternes, 1999). They are classified according to their chemical name, galenic form, therapeutic class, etc. Among the classification methodologies there are: the “American Society of Health-System” (AHFS), the “European Pharmaceutical Marketing Research Association” (EphMRA), and the “Anatomical Therapeutic Chemical” (ATC). The latest is established by the World Health Organisation (WHO) (Abou-Atmé et al., 2006; Tollier et al., 2005). The “Hospital National Center of Drugs Information” (CNHIM) has given the ATC classification official status in France by publishing a French translation in 1996 (BOMAS, 1996). ATC classification consists

of 14 principal groups and five levels of hierarchy, in level 1 PhACs are divided according to the organ on which they act and depending on their pharmacological and therapeutic properties, this level comprises the 14 principal groups (Table 1), later on the other 4 levels have been introduced as subdivisions of level 1 (Table 2) (Tollier et al., 2005).

Table 1. The 14 principal groups of level 1 in ATC classification

Code	Wording
A	digestive tract and metabolism
B	blood and hematopoietic organs
C	cardiovascular system
D	dermatological drugs
G	genitourinary system and sex hormones
H	systemic hormones, sex hormones excluded
J	general anti-infectives for systemic use
L	antineoplastics and immunomodulators
M	muscle and skeleton
N	nervous system
P	pest control, insecticides
R	respiratory system
S	sensory organs
V	diverse

Table 2. The 4 levels established by ATC classification

Level 1	according to the organ on which PhACs act and depending on their pharmacological and therapeutic properties
Level 2 & 3	define therapeutic and/or pharmacological subgroups
Level 4	defines chemical and/or pharmacological and/or therapeutic subgroup
Level 5	defines the chemical active substance

2. Entry of pharmaceuticals to agroecosystem

PhACs can enter the environment via different routes (Figure 1), which have been extensively documented. However, the reuse of treated wastewater (effluent) for crops irrigation is a key pathway, besides to the application of sludge and animal manure and slurry as natural fertilizers onto agricultural fields (Gros et al., 2019; Li, 2014).

Among the most frequently occurring PhACs classes in treated wastewater and biosolids are antibiotics, non-steroidal anti-inflammatories, and anti-convulsants, with concentrations between ng/L to low $\mu\text{g/L}$ in treated wastewater (Kim et al., 2007; Sui et al., 2011; Vanderford and Snyder, 2006) and $\mu\text{g/kg}$ to low mg/kg (dry weight) in biosolids (Clarke and Smith, 2011; McClellan and Halden, 2010).

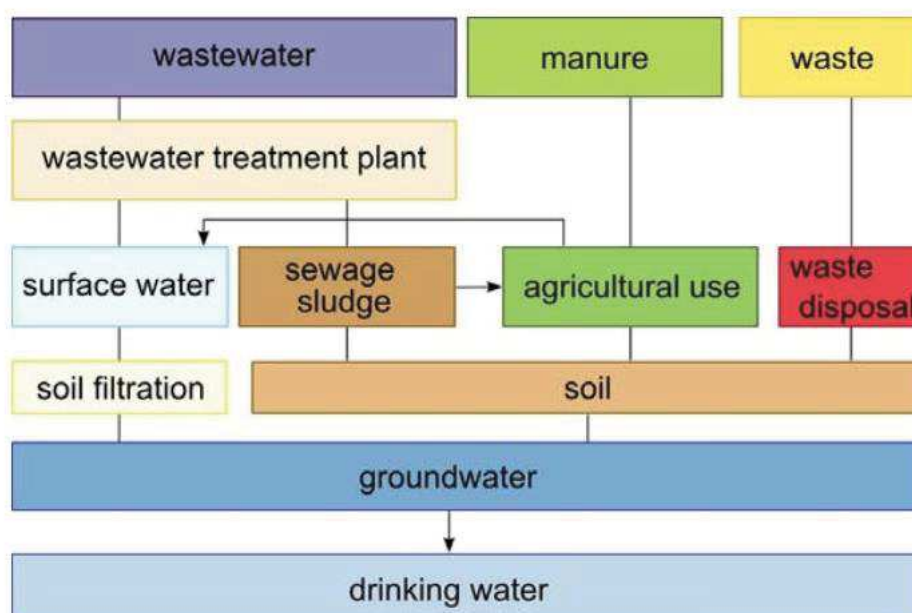


Figure 1. Sources and fate of PhACs in the environment (Bartha, 2012)

2.1. Reuse of treated wastewater (TWW)

In order for a substance to develop its intended effect in the human body, the intact molecule should reach the diseased cell before any degradation occurs by the biochemical processes of the body. Therefore, pharmaceuticals are designed for stability. Moreover, they are designed to not bioaccumulate and instead be rapidly eliminated from the treated body.

Thus, this has 2 consequences: (1) PhACs are excreted via urine and feces not metabolized and thus reach the domestic wastewater, (2) the stability of PhACs hinders their degradation by wastewater treatment plants (WWTPs). Several pharmaceuticals have shown to be partially removed while others not at all. An instance for this, PhACs as carbamazepine, diclofenac, propranolol and clofibrac acid have shown remediation efficiencies less than 10% (Jones et al., 2005; Petrović et al., 2005; Stackelberg et al., 2004; Ternes et al., 2002). It is believed that domestic wastewater (human activity) is the main source of the occurrence of PhACs in the environment (Roig, 2010).

30 to 70 % of ingested PhACs are excreted via urine and feces (Liu et al., 2017; Qin et al., 2016). Once PhACs are released to the domestic waste system, they will typically be transported to a WWTP where wastewater will be treated through several processes. Conventional WWTPs are not designed to remove PhACs, thus the final effluent is likely to contain PhACs at concentration in the $\mu\text{g/L}$ range (Patel et al., 2019). However, effluents have lately been used for fields irrigation, a practice sometimes described as “recycled wastewater irrigation” or “reclaimed wastewater irrigation” (Kretschmer et al., 2006). 70 % of all water use worldwide, including river and groundwater rivers, goes for irrigation, while 20 %, and 10 % goes for industry and residences, respectively (Kretschmer et al., 2006). Water supply is one of today’s challenges in a world of growing population and industrialization. Thus if the world is facing water shortage, it is also facing a food shortage. Which made of this practice an attractive option to satisfy the demands for irrigation (Hamilton et al., 2007). Many countries have adopted this practice to cope with their water shortage, including United States, Australia, Singapore, South Africa, Japan, China, Mexico and New Zealand (Levine and Asano, 2004). Moreover, in many low-income countries, even untreated wastewater is used for irrigation (Kinney et al., 2006). With the continuous drought, climate change and population growth, the future use of treated wastewater is set to increase (Hamilton et al., 2007), which will result in greater loads of PhACs reaching the agroecosystem.

2.2. Fertilization

Sewage sludge. Sewage sludge is applied to agricultural fields as fertilizers for several reasons. It is a cheap way of disposal for sewage treatment plants (STPs), in addition it is an important source of nitrogen, phosphorus, organic matter and other nutrients all of which can enhance

soil physical properties and crop yield (Chitdeshwari and Savithri, 2007; Joshua et al., 1998; Webber et al., 1996). In European countries, an average of 37 % of sewage sludge is applied on agriculture soils, equivalent to 2.39×10^6 tons (dry weight) per year (Chang et al., 2002). However, land application of sewage sludge on the other side does have its drawbacks, it is at the same time a source of many contamination such as heavy metals, pathogens and organic compounds (Rogers, 1996). Therefore, the use of sewage sludge as fertilizer is a potential introduction pathway of PhACs to the agroecosystem. Recently, several studies have detected PhACs in sewage sludge effluents (Ivanová et al., 2018; Kinney et al., 2008; Metcalfe et al., 2003; Nieto et al., 2010). Carbamazepine is one of the most frequently detected pharmaceuticals in sewage sludge in few to hundred $\mu\text{g}/\text{Kg}$ concentration range (Díaz-Cruz et al., 2009). Kinney and coworkers have detected carbamazepine in all the nine sewage sludge samples studied (Kinney et al., 2006). Also, sulfonamides antibiotics are frequently detected in sewage sludge, and recently their metabolites have also been detected in sludge samples at concentrations $<9.81 \text{ ng}/\text{g}$ (García Galán et al., 2012).

Biosolids. Biosolid refer to solid, semisolid and liquid residues generated from municipal WWTPs. They are applied to cropland as a source of nutrient and organic matter to improve crop yield, besides it constitutes a major pathway of nutrient recycling. On the other hand, the application of biosolids to agriculture is economically beneficial, for farmers because of reduced synthetic nutrient costs, and for municipalities because of reduced disposal costs of the generated solid sewage waste. However, since they are originally obtained from WWTPs, they have been found to be contaminated with variety of PhACs (Clarke and Smith, 2011; Clarke and Cummins, 2015).

Animal slurries and manure. Residual PhACs in medicated animal manure and slurries contribute as the major sources of PhACs introduction in agricultural soils via fertilization (Carvalho and Santos, 2016; Kumirska et al., 2015; Massé et al., 2014). PhACs and veterinary pharmaceuticals are used regularly in the livestock industry and animal production for treatment and disease prevention, to improve feed efficiency and as growth promoter. Antimicrobials, steroid hormones and beta agonists are the mainly used PhACs in animal agriculture. However, up to 90% of PhACs administered to animals are excreted in manures

unmetabolized (Joy et al., 2013). PhACs concentration in manure have been reported to range from trace level to hundreds of mg/Kg (Sassman and Lee, 2005; Soni et al., 2015).

3. Fate of pharmaceuticals in agroecosystem

The fate of PhACs in the agroecosystems depends heavily on their physico-chemical properties as well as on the properties of the receiving soil matrices. Several reviews have highlighted the current knowledge on the fate of PhACs after their introduction into the agroecosystem and soil (Du and Liu, 2012; Li, 2014; O'Bannon, 2020; Qin et al., 2015; Wu et al., 2015). Once PhACs reach the soil, they can undergo several processes such as biotic/abiotic degradation, sorption onto soil, persistence and accumulate, leaching, and they can be uptaken by the growing crops or soil living organisms. Sorption and biodegradation are the major processes for PhACs removal from agricultural soil, especially for those resistant to abiotic degradation such as photodegradation and hydrolysis (Estevez et al., 2014; Fang et al., 2012).

3.1. Degradation in soil

3.1.1. Biotic degradation

Bacteria and fungi are the two groups of organisms that are able to degrade PhACs in soil. Biodegradation has been reported to play a major role in the fate of PhACs in soil (Xu et al., 2009b; Yu et al., 2013). For example, after 45 days of incubation, clofibric acid and diclofenac showed higher degradation rate in non-sterile soil (88 - 100%) than in sterile soil (33 - 43%), indicating the significant role of microorganisms in degrading PhACs in soil (Xu et al., 2009b). However, biodegradation is affected by several factors including PhACs chemical structure and physicochemical properties, and soil characteristics. For instance, 4-n-Nonylphenol showed higher degradability than 4-tert-Octylphenol due to its straight side chain (Ying and Kookana, 2005). Besides weakly acidic PhACs such as diclofenac, ibuprofen, bezafibrate, gemfibrozil and naproxen exhibited more rapid degradation than neutral PhACs (Grossberger et al., 2014). Soil with higher organic matter content reduce the bioavailability of PhACs due to sorption processes, hence inhibit their biodegradation (Das et al., 2004; Xu et al., 2009b). Moreover, it was also reported that biodegradation was generally inhibited when biosolids are used for soil amendment in agriculture, thus prolonging their persistence in soil (Li et al., 2014, 2013; Monteiro and Boxall, 2009). This was explained by the addition of organic matter

from biosolids, which lead to higher sorption of PhACs hence decreasing their bioavailability. Besides, biosolids may serve as readily available nutrient and carbon source over PhACs for microorganism, which may contribute to the decrease of PhACs degradation in soil.

3.1.2. Abiotic degradation

Abiotic processes such as photodegradation, hydrolysis and mineralization, may also contribute significant degradation to PhACs in soil with low moisture level and suppressed microbial activity. However, only few studies to date reported the transformation of PhACs via abiotic processes in soil.

Photodegradation is the direct or indirect chemical transformation of a compound by a light-induced mechanism (Boreen et al., 2003). It represents an important process in environmental fate, Thiele-Bruhn and Peters observed an a significant photodegradation of various tetracyclines in soil surfaces (Thiele-Bruhn and Peters, 2007). For the hydrolysis processes of PhACs in field soil. Recently, Jin and colleagues have revealed an important hydrolysis decomposition of chloramphenicol antibiotics in field soil, especially in soils with low moisture content and suppressed microbial activity (Jin et al., 2019). So far little information is available regarding mineralization process of PhACs in soil, because of the limited availability of ^{14}C -labeling compounds. ^{14}C -naproxen and ^{14}C -diclofenac were found to be 50 to 80% mineralized to $^{14}\text{CO}_2$ in different soils (Dodgen et al., 2014; Topp et al., 2008) while only 1.2% of ^{14}C -carbamazepine and 17% of ^{14}C -acetaminophen were mineralized (Li et al., 2014, 2013).

However it is clear that PhACs can be degraded in soil, their continuous input ensures that some of them are persistent.

3.2. Sorption on soil

Soil and soil components sorption is the main physiochemical process that prevents PhACs from leaching to some extent, as well as it is an important contribution to PhACs bioavailability (Carter et al., 2016; Drillia et al., 2005; Wang and Wang, 2015). Sorption is related to many factors, such as PhACs properties, soil characteristics including pH and organic matter content, as well as external factors such as temperature (Wang and Wang,

2015; Xu et al., 2020). As definition, sorption is the partitioning of the compound between the water and solid phase (Schwarzenbach et al., 2003). It can be characterized by a soil-water partition coefficient (K_d), which is the ratio of the concentration in the solid phase to the concentration in the solution phase at equilibrium. Hence higher K_d values allow higher sorption, such as bisphenol A, whilst smaller K_d values, such as ibuprofen, allow less PhACs sorption and higher leaching possibility into groundwater or nearby streams (Chefetz et al., 2008; Xu et al., 2009a).

Physicochemical properties specific to the PhAC including the octanol-water partition (K_{ow}), the degree of ionization, the charge of the molecule, the hydrophobicity, and the pKa value, are important in determining the sorption of the chemical to soil. For example, depending on the pKa values of the PhAC and soil pH, PhAC can be non-ionic, anionic, cationic or zwitterionic (Schaffer and Licha, 2015). Briefly, non-ionic PhACs are sorbed to soil organic matter by hydrophobic partitioning via weak van der Waals and electron donor-acceptor interactions. Ionic compounds are sorbed to soil by electrostatic interactions such as cation exchange, cation bridging and complexation. Cationic PhACs are sorbed on the negatively charged clay soil content, while anionic PhACs are sorbed on the positive charged soil surface (Call et al., 2019). Regarding K_{ow} , it indicates the tendency of a chemical to partition between the organic and the aqueous phases. Therefore, high K_{ow} indicates high hydrophobicity and lower water solubility, hence the compounds should sorb to a greater extent to soil generating high K_d values. An instance for this, triclosan have shown an extremely high K_d (127 L/Kg) and $\log K_{ow}$ (4.8) (Aranami and Readman, 2007; Barron et al., 2009). However, this positive correlation between K_{ow} and K_d cannot be generalized, as other sorption processes might occur. For example, tetracyclines have low K_{ow} , yet they bind to soil via complexation with soil's metal ions such as Ca^{2+} and Mg^{2+} (Avisar et al., 2010; Bui and Choi, 2010).

Soil composition is very complex, and can be divided to 3 categories: (1) poor sorption capacity (scree and sandy soil), (2) medium sorption capacity (soil containing iron oxide), and (3) strong sorption capacity (soil containing humic acids and clay). Ciprofloxacin antibiotic showed K_d values range from 726.8 to 1277873 L/Kg across 13 different soil types in Brazil (Leal et al., 2013). In general, higher clay and organic matter content and lower sand content result in higher sorption capacity contributed to the intense exchange capacity of clay, thus a decrease in the mobility of PhACs (Borgman and Chefetz, 2013; Tang et al., 2018). Humic acids are negatively charged at environmental pH (Christl et al., 2016), hence PhACs with positively

charged moieties, such as tetracyclines and zwitterions, can interact with (Trellu et al., 2016). In another example, clofibric acid have showed higher sorption capacity (K_d of 200.73 ± 59.93 L/Kg in a silt clay soil, while a much lower sorption capacity (k_d of 3.36 ± 1.55 L/Kg) was observed in silt loam soil (Xu et al., 2009b).

It is worthy to mention that PhACs sorption to soil, depending on soil and PhACs properties, occurs via several mechanisms, such as electrostatic interactions (cation exchange, electrostatic attraction and electrostatic repulsion), cationic bridging, surface complexation, π - π interaction and cation- π bonding, hydrogen bonding, hydrophobic interaction and van der Waals attractions (Xu et al., 2020).

3.3. Persistence and accumulation in soil

The chemical properties of PhACs, such as high water solubility, high polarity and low volatility are largely responsible for their persistence and thus their accumulation in terrestrial environments (Kümmerer, 2008). As it is mentioned above, PhACs need to be stable to resist metabolism in the human body to ensure an adequate pharmacological effect, and to have a useful shelf life (Richman and Castensson, 2008). In addition, PhACS persistence in soil depends on their photostability, binding and sorption ability, biodegradation rate and leaching potential (Díaz-Cruz et al., 2003).

Some PhACs incorporated soil through irrigation and fertilization showed to be persistent and accumulative, which results in an increasingly residual concentrations ranged from few μg up to g/Kg (Thiele-Bruhn, 2003). Grossberger and colleagues reported that neutral PhACs were more persistent in soil irrigated with treated wastewater, while weakly acidic PhACs, namely ibuprofen, gemfibrozil, diclofenac, naproxen and bezafibrate, showed more rapid degradation explained by the susceptibility of their carboxylic groups to microbial transformation (Grossberger et al., 2014). However, the persistence of the same PhACs is specific to soil properties as much as to the PhACs properties, and thus can vary across soil types and the surrounding environment. For instance, it was reported that tetracycline could persist in soil for over 1 year (Zuccato et al., 2000). Later in 2002, Hamscher and colleagues reported a moderate degradation of tetracyclines that occurs in 180 days, as it was detected at concentrations up to 0.3 g/Kg in farmland soil fertilized with manure (Hamscher et al., 2002).

However, it is pointed out that the routes of entry of PhACs into the environment, through irrigation with wastewater or application of fertilizers, may also have an important role on their persistence behavior. For instance, Albero and colleagues reported a dissipation half-life ($t_{1/2}$) of antibiotics in the range of 8 to 92 days, except for fluoroquinolones was higher than 90 days. When soil was amended with composted manure, $t_{1/2}$ values have increased between 6 and 53% (Albero et al., 2018). Persistence of these antibiotics were in the same range in other study, although they were very variable because of other factors that affect compounds dissipation and persistence (Riaz et al., 2018). Finally, Yang and colleagues reported that the dissipation, expressed as $t_{1/2}$, of sulfonamide could be from 18.6 to 213 days for sulfonamides, 1 to 2 months for tetracyclines, and 7 to 8 months for enrofloxacin (Yang et al., 2018).

3.4. Leaching

Mobility and leaching of PhACs are important processes involved in their fate in agroecosystems. Leaching into deeper soil or into groundwater depend on their sorption capacity onto top soil. However, several studies have reported the leaching ability of some PhACs. For instance, Paz and colleagues reported the leaching ability of lamotrigine, carbamazepine and its metabolites (10,11-epoxycarbamazepine and 10,11-dihydro-10,11-trans-dihydroxycarbamazepine) in soil with lesser organic matter content (Paz et al., 2016). Similar results were obtained with Leal and colleagues for sulfonamides, whereas fluoroquinolones showed high sorption even low organic matter content soil (Leal et al., 2013). Furthermore, several studies have reported the ability of highly mobile and persistent PhACs (such as gemfibrozil, ibuprofen, estriol, carbamazepine and sulfamethoxazole) to be transported to surface water through field runoff, or leach to ground water (Avisar et al., 2010; Bondarenko et al., 2012; Kodešová et al., 2015; Qin et al., 2015; Tolls, 2001) thus affecting aquatic organisms and entering human drinking water posing a potential risk to human health.

3.5. Uptake by growing crops

High level accumulation of PhACs in edible crops may raise potential human health risk through food chain. Several studies have reported the ability of growing crops to uptake PhACs introduced by wastewater irrigation or by application of contaminated natural fertilizers such as sewage sludge, biosolids, animal manure and slurries (Carvalho et al., 2014; Picó et al., 2019; Wu et al., 2011, 2011). Generally, the uptake rate depends on many factors including PhACs properties, soil properties, plants species and environmental factors (Chuang et al., 2019). Besides, sorption and degradation processes in soil can affect the bioavailability of PhACs to be uptaken by crops.

Briefly, crops grown in soil with lower organic matter and clay content are at greater risk for uptake and accumulation of PhACs (Goldstein et al., 2014). Regarding PhACs properties, most PhACs are ionizable, either acids or bases, which may undergo dissociation in water or soil according to their pH. Besides, they can go from extremely hydrophilic (such as sulfamethoxazole, $\log K_{ow} = 0.89$) to highly hydrophobic (such as fenofibrate; $\log K_{ow} = 5.19$). Malchi and colleagues reported that non-ionic PhACs such as, carbamazepine, caffeine and lamotrigine, were detected at significantly higher concentrations than ionic PhACs such as metoprolol, bezafibrate, clofibrac acid, diclofenac, gemfibrozil, ibuprofen, ketoprofen, naproxen, sulfamethoxazole, and sildenafil in root vegetables (carrot and potato) irrigated with treated wastewater in field scale (Malchi et al., 2014). In other study, basic PhACs (positively charged) were found at comparable levels with neutral PhACs, while acidic PhACs (negatively charged) existed at significantly lower levels (Wu et al., 2014). Previously, Wu and colleagues have reported that the hydrophobicity of neutral PhACs plays also an important role for their uptake via roots, as a positive linear relationship was observed between the root uptake and the PhACs hydrophobicity, suggesting that hydrophobicity is a primary factor affecting the uptake of neutral compounds by roots (Wu et al., 2013). However this model cannot be applied to ionic PhACs, with which additional mechanisms such as electrical attraction and repulsion and ion trap may affect the uptake by roots. In general, roots are negatively charged thus anions (acidic PhACs) are poorly taken up by plants due to repulsion to the negatively charged anion (González García et al., 2019). While for the positively charged basic PhACs, Wu and colleagues reported 3 possible processes that may lead to plant uptake: (1) electrical attraction of the cation due to the negative charge of roots. (2) accumulation into the vacuole by ion trap; and (3) partitioning on to the root solids (Wu et al., 2013).

4. Occurrence of pharmaceuticals in agroecosystem

Daughton and Ternes, and Heberer (Daughton and Ternes, 1999; Heberer, 2002) have reviewed the occurrence of prescribed and nonprescribed PhACs in the environment. These two well-known reviews present evidences that significant quantities of PhACs are potentially discharged to the environment. After all, PhACs are designed to be stable for many reasons, for instance, stability makes them suitable for longer storage and steady against enzymes and acidic pH to allow stomach passage. These properties make such compounds at the same time mobile and persistent in the environment and may thus have risk on human and environmental health (Du and Liu, 2012; Park et al., 2016), as well as ecotoxicological effects on non-target organisms (Biel-Maeso et al., 2017).

4.1. Soil

A range of pharmaceuticals has been detected in agricultural soil with concentrations of anti-inflammatory drugs, antimicrobial agents and anticoagulants being reported up to $\mu\text{g}/\text{kg}$ levels (Borgman and Chefetz, 2013; Ho et al., 2014; Kinney et al., 2006; Qin et al., 2015; Walters et al., 2010). Other studies reported their presence in agricultural soils at trace levels ranging from ng/Kg up to g/Kg (Fang et al., 2012; Karnjanapiboonwong et al., 2011; Kinney et al., 2006). The most common pharmaceuticals found in soil are the antibiotics (trimethoprim, sulfadiazine and triclosan), analgesics (ibuprofen and diclofenac) and antiepileptic (carbamazepine), with carbamazepine the most frequently detected among the studies (Biel-Maeso et al., 2017; Chen et al., 2011; Gibson et al., 2010; Ho et al., 2012a, 2012b; Kinney et al., 2008). Additionally, carbamazepine is known to be resistant to degradation thus it has the accumulation potential over time, which verify its frequent occurrence in soil (Gibson et al., 2010). However, until today, little is known about the concentration of PhACs in soil, because of the lack of appropriate instrumentation and methods able to measure their occurrence at low concentrations in such complex matrix as soil. Kinney and colleagues (Kinney et al., 2006) have detected pharmaceuticals at concentration ranging from 0.02 to 15 $\mu\text{g}/\text{kg}$ dry weight in soil irrigated with treated wastewater in Colorado city. Additionally, a number of PhACs in their study, (such as acetaminophen, caffeine, carbamazepine, erythromycin, sulfamethazole, 1, 7-dimethylxanthine and dehydronifedipine) appeared to persist for months after

irrigation. Also Biel-Maeso and colleagues (Biel-Maeso et al., 2018) have reported the detection of PhACs, at lower concentration range, in surface soil irrigated with treated wastewater, 17 out of 45 of their target PhACs were detected, the total concentration detected were in the range of 2 and 15 ng/g.

4.2. Crops

The introduction of PhACs to crops via plant uptake by the application of treated wastewater and natural fertilizers (such as sewage sludge, biosolids, animal manure and slurries) have received a major public concern lately. Contamination of food by these chemicals may pose potential risks to humans. To date many laboratory scales studies or greenhouse settings have reported the occurrence of PhACs in crops via plant uptake from soil receiving treated wastewater irrigation or contaminated fertilizers amendment. Conversely, little information is available at realistic field conditions. Carbamazepine was commonly detected in various plants after irrigation with treated wastewater (Goldstein et al., 2014; Shenker et al., 2011; Wu et al., 2010) and application of biosolids as fertilizer in greenhouse experiments (Holling et al., 2012; Wu et al., 2012, 2010). While sulfamethoxazole and trimethoprim were reported to have limited uptake in plants grown in biosolids amended soil (Holling et al., 2012). Which could be explained by the higher bioavailability of carbamazepine. Bhalsod and colleagues reported that concentrations in fresh lettuce shoots varied from $0.05 \pm 0.04 \mu\text{g}/\text{kg}$ for sulfadiazine to $345 \pm 139 \mu\text{g}/\text{kg}$ for carbamazepine, in greenhouse under overhead and soil-surface irrigations using PhACs contaminated water (Bhalsod et al., 2018).

Regarding field experiments, few studies have been reported to date, thus we are going to report most of them. Gottschall and colleagues did not detected any PhACs in the grain of wheat grown for about 1 year following a high single application of municipal biosolids (Gottschall et al., 2012). However, Sabourin and colleagues reported the detection of atenolol, cocaine, ciprofloxacin, metformin, minocycline, norfloxacin, DEET, naproxen, glyburide, sulfamerazine, penicillin G, triamterene, and trimethoprim, with concentrations ranging from 0.02 to 14 ng/g (dry weight), in tomato, carrot, potato and sweet corn from field soils treated with municipal biosolids (Sabourin et al., 2012). Calderon and colleagues reported the occurrence of hydrocinnamic acid, salicylic acid, caffeine and ibuprofen, in apple tree leaves and alfalfa irrigated with reclaimed wastewater, with concentrations of 0.016–

16.9 ng/g (wet weight) (Calderón-Preciado et al., 2011). Jones-Lepp and colleagues have reported the detection of the industrial flavoring agent N,N'-dimethylphenethylamine at 48-180 ng/g (dry weight) in their 4 studied crops irrigated with the effluent of a local WWTP, while the two antibiotics, azithromycin and clarithromycin, were found only in Bermuda grass roots at concentrations of 90 and 135 ng/g (dry weight), respectively (Jones-Lepp et al., 2010). Wu and colleagues detected 8 PhACs and personal care products (including caffeine, meprobamate, primidone, DEET, carbamazepine, dilantin, naproxen, and triclosan) in 8 vegetables with 64% detection frequency, at concentration range from 0.01 to 3.87 ng/g (dry weight) (Wu et al., 2014). Riemenschneider and colleagues reported the detection of 12 PhACs and micropollutant and 6 carbamazepine metabolites in 10 vegetable species (among them carrot, lettuce, potato and zucchini) at concentrations ranging from 1.7 to 216 ng/g (dry weight) (Riemenschneider et al., 2016). Martínez-Piernas and colleague revealed the presence of 17 OMCs in tomato leaves (0.04 - 32 ng/g), and 8 in the fruits (0.01 - 1.1 ng/g); 5 of them not reported before in real field samples (Martínez-Piernas et al., 2019). Higher concentration levels were detected when untreated wastewater was applied for irrigation. Pan and colleagues have studied the uptake of 5 antibiotics by 5 edible crops irrigated with largely untreated domestic wastewater or fishpond water. Their results revealed that norfloxacin was consistently detected at the highest concentrations (4.6 - 23.6 µg/kg) in crop tissues, followed by chloramphenicol (2.6–22.4 µg/kg) and tetracycline (4.0 - 10.1 µg/kg), while sulfamethazine and erythromycin were not detected in most of the vegetable crops (Pan et al., 2014).

Based on the research findings reported to date, and referring mainly to field studies, PhACs detected in crops grown in natural fertilizers amended or treated wastewater irrigated soil were in the ng/g concentration range, which is much smaller than the exposure in a single medical dose (typically in the 20 – 200 mg range). A field study showed that carbamazepine and caffeine would require an adult to consume hundreds of kilograms of treated wastewater irrigated sweet potatoes and carrots daily to reach the threshold of toxicological concern (TTC) level (Malchi et al., 2014). Matamoros and colleagues predicted that human consumption of vegetable crops irrigated with PhACs contaminated water would cause an exposure of 500 ng/day of each compound, a level well below the therapeutic dose for individual pharmaceuticals (Matamoros et al., 2012). On the whole, the concentrations of PhACs in the edible crops represent a *de minimis* risk to human health. However, it may be

still overearly to draw a concrete conclusion, as the field studies are too limited and they lack comprehensive assessment of the human health risk associated with PhACs exposure through edible crops. Finally, it is worthy to mention that the maximum residue value for PhACs in plant-based products has not been established yet (JECFA, 2006).

5. Aims of the thesis

It is crucial to understand how PhACs behave in agroecosystem after their introduction via treated wastewater irrigation, including their distribution between soil and crops, their fate in soil and crops, and finally their degradation rate when exposed to soil organisms namely the ubiquitous filamentous *Trichoderma* soil fungi (Figure 2). However, such comprehensive study needs robust and sensitive analytical methodologies specific to the agroecosystem samples.

The aim of this PhD research was therefore to explore the behavior of PhACs and other wastewater contaminants, introduced to a realistic field by irrigation with domestic wastewater effluents. The specific objectives were to:

- 1) Recapitulate all analytical methodologies reported to date for the extraction and analysis of PhACs and related compounds in soil and plants (Chapter 2).
- 2) Optimize and develop analytical methodologies suitable for high-resolution mass spectrometry detection and quantification for a large number of wastewater-borne contaminants mainly PhACs and their TPs, frequently detected in WWTPs effluents (including analgesics and anti-inflammatories, anti- hypertensives, antifungal agents, lipid regulators, psychiatric drugs and stimulants, β - blockers, antibiotics, antimycotics, and sweeteners) by the means of modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method in agroecosystem samples including soil, lettuce roots and lettuce leaves. In fact, the application of QuEChERS method for soil and lettuce root matrices is not very common, yet according to our knowledge, this was the first time a study was dedicated for the optimization and development of an analytical method specific to lettuce root matrix. Besides, there is a lack of analytical

methods for detecting such large number and interfamily wastewater-borne pollutants in crops (Chapter 3 and 4).

- 3) Investigate the fate and behavior of 14 selected wastewater contaminants including PhACs and metabolites in realistic agroecosystem conditions, by the application of the previously developed methods. Thus, to assess the human health risks related to wastewater irrigated fresh vegetables. It should be noted that very few studies at field scale have been reported, even fewer have followed PhACs in the agroecosystem allowing for bioconcentration factor calculations. Hence an important knowledge gap regarding their fate in soil and crops, and realistic human health risk assessment (Chapter 5).
- 4) Investigate the fate of two fluoroquinolone antibiotics (ciprofloxacin and ofloxacin) and one personal care product (climbazole) when exposed to the ubiquitous filamentous *Trichoderma* soil fungi in liquid medium. To our knowledge, it is the first time that the ability of *Trichoderma* species in degrading PhACs has been investigated (Chapter 6).

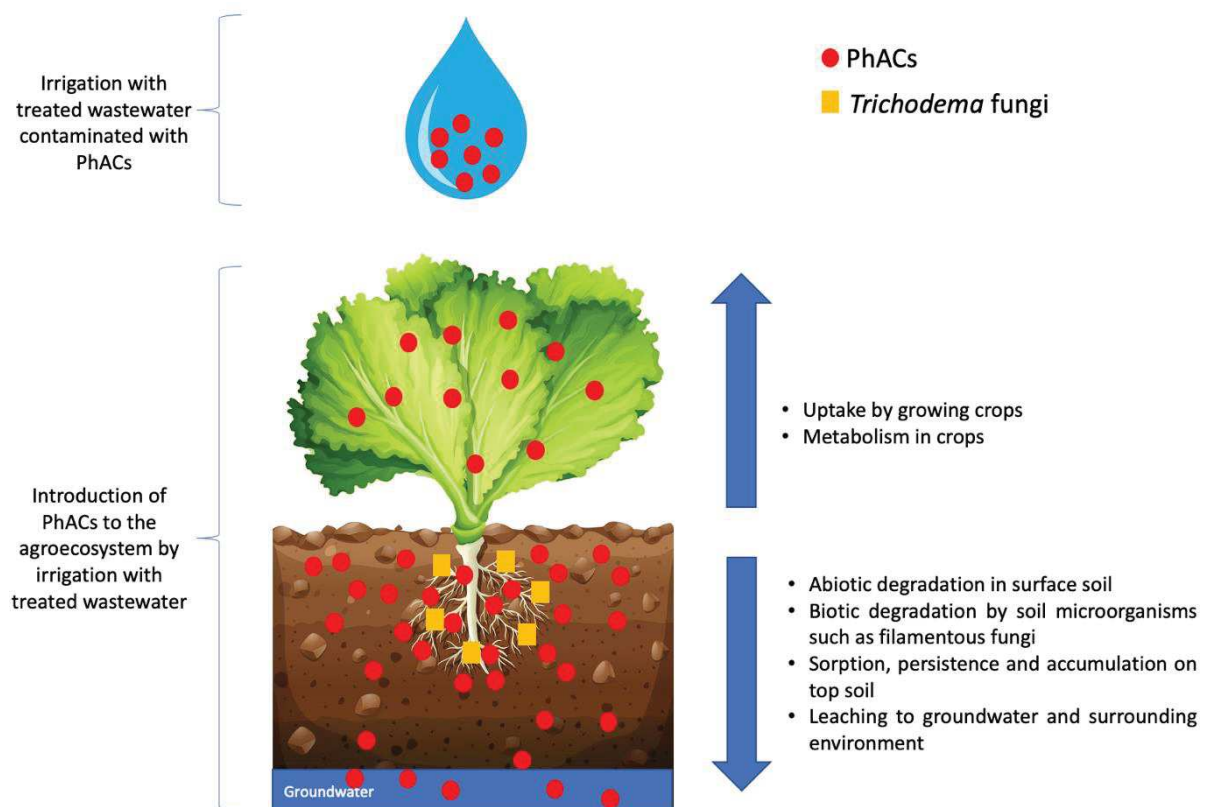


Figure 2. A conceptual model of the fate of PhACs in agroecosystem emitted by treated wastewater irrigation

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Chapter 2:

Sample pretreatment, extraction, cleanup and analysis of pharmaceuticals: plants and soil

1. Development of Methods for the Determination of PhACs in Soil/Earthworm/Crop System Irrigated with Reclaimed Water

1.1. Context

Treated wastewater is an incredible alternative of natural water, especially for agricultural sector. Moreover, wastewater is an important source of nutrient and organic matter, which can lead to an increase in crops productions. Thus irrigation of crops with treated wastewater constitutes one important solution to cope with water shortages. However, the main drawback is that this practice can lead to the introduction of PhACs into agricultural land and water since WWTPs are not designed for their elimination during wastewater treatment. Hence growing crops can uptake these chemicals from soil and water, leading to their accumulation at trace level in fresh vegetables for human consumption. In fact, the potential contamination of water, soil and crops is the main disadvantage of wastewater reuse in agriculture, as well as the following risk that PhACs pose to environmental and human health. However, there is a general lack of knowledge on the effects of PhACs on the environment, which is explained in part by the difficulty of extracting and quantifying these compounds at trace level from complex matrices such as soil and vegetables. Besides to the high prices of the analytical instruments needed either for their extraction or for their detection.

1.2. Objective and main results

The objective of this chapter book is to discuss all the analytical procedures (including microwave assisted extraction (MAE), ultrasound solvent extraction (USE), pressurized liquid extraction (PLE) and QuEChERS) used for the extraction of PhACs and their related compounds from plants tissues and soil irrigated with treated wastewater. As well as it describes all that is known to date about the cleaning techniques of the extracts, and separation and detection techniques of PhACs by liquid or gas chromatography. From this built review, it is clear that today several successful extraction methods have been developed for the extraction, detection and quantification of PhACs from soil and vegetables. However, in some cases these methods are time consuming, expensive, and require expert analysts. Yet, several studies have been published with a significant effort to overcome these obstacles by developing fast,

cheap and environmentally friendly methods. It is therefore necessary to carry on the research on developing even simpler, robust, time saving and green analytical methods.

Development of Methods for the Determination of PhACs in Soil/ Earthworm/Crop System Irrigated with Reclaimed Water



Rayana Manasfi, Francesc Labad, and Nicola Montemurro

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- 7 Analysis of Pharmaceuticals in Earthworms
 - 7.1 Sampling, Sample Preparation, and Extraction
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Abstract Pharmaceuticals have been becoming a major concern of environmental pollution since the beginning of the century. The ways in which these contaminants are introduced into the environment are very different, but almost always associated with wastewater. In fact, current wastewater treatment plants are not designed for the removal of pharmaceutical products. Indeed, the problem of water scarcity has played an important role in the introduction of pharmaceutical products into the environment, particularly in the agricultural sector. Because of the drought, more and more countries are resorting to the use of treated wastewater to irrigate vegetables for human consumption. Consequently, the reuse of wastewater in agriculture constitutes a continuous introduction of these molecules into the soil.

The effects of this practice are not entirely clear. However, the probability that these compounds can enter the food chain directly is high. In fact, through radical absorption, plants could uptake pharmaceuticals from soil and water, leading to the accumulation of drugs in the tissues.

The development of analytical methods of solid matrices such as soil or plant tissues requires substantial work due to the great complexity of the matrices and the differences between the physico-chemical properties of analytes of interest. Several multi-class methods have recently been developed to determine a large number of pharmaceutical products in soil or plants using different extraction techniques.

This chapter addresses to list all the analytical procedures published so far used for the extraction and analysis of pharmaceutical products from plant tissues and from the soil irrigated with treated wastewater.

Keywords ASE, Crop uptake, Pharmaceuticals, QuEChERS, Soil contamination, USE

1 Introduction

Treated wastewater is an incredible resource to cope with the increasing demand of water to meet the agricultural sector, undermined by the continued lack of water and frequent water shortages. In fact, the use of wastewater in irrigation as well as stabilizing the nutrient content in the soil can lead to an increase in the production of crops in arid areas due to the constant input of organic and mineral components into the soil through the wastewater. Despite the high content of nutrients, especially nitrogen immediately available for plant growth [1], irrigation of crops with recycled water can lead to the spread of pharmaceutical active compounds (PhACs) in agricultural land and waters. These substances are part of the products of daily use

and are not completely removed from the activated sludge treatment plants. Hence, they are a source of concern due to the harmful effects that these substances can cause in a variety of aquatic and terrestrial organisms due to their continuous entry into the environment.

The main drawback arising from the application of wastewater effluents is that vegetables could uptake PhACs from soil and water, leading to their accumulation at trace level in fresh products for human consumption [2]. In fact, the main disadvantage of wastewater reuse in agriculture is the potential contamination of soil, crops, and water sources and the inherent risk of harmful effects that contamination poses to exposed organisms. However, the PhAC concentrations in crops and vegetables should be much lower than the dosage for an effective therapy.

Every day, due to the constant development of the pharmaceutical industry, new compounds are approved and marketed. However, there is a general lack of knowledge of the effects that these substances cause on the environment. This is explained in part due to the reduced number of studies or the intrinsic difficulty of extracting and isolating and quantifying organic compounds at trace levels in soil and plants. Moreover, the analytical techniques used for this type of pollutants are relatively expensive.

Although several analytical methods have been developed to extract PhACs from water and soil samples, one of the greatest efforts of recent years is to try to develop robust analytical methods for the analysis of PhACs in plant tissues. Given the small number of these publications, it is evident how this issue represents a difficult challenge to overcome. Furthermore, the use of an inappropriate or low sensitive method with relatively high limits of quantification could contribute to give unreliable results as a consequence of the low concentrations with which these compounds are present in water or in the soil.

Due to the complexity of the sample matrix, the analysis of environmental samples involves several difficulties. The analyses of solid or semi-solid matrices such as soil, sludge, or sediment or of biological samples (animal, vegetable, or plasma tissues) are more complex than liquid samples, which usually require fewer pretreatment phases, due to their liquid form. In the case of wastewater, for example, an initial filtration is sufficient to remove the particulates followed by a solid-phase extraction using cartridges with different absorbent resins. The latest technological advances also allow for the analysis of surface or wastewater by direct injection avoiding all the problems related to the handling of the sample [3, 4].

Sample preparation has simply the purpose of transferring the analytes in a measurable form [5]. The first step for the success of an analytical method is sampling. In fact, the determination of pollutants in soil or plants initially requires specific sampling techniques that take into account the heterogeneity of the soil or plant matrix [6].

Furthermore, the sample preparation for the analysis of pharmaceutical residues is a very critical aspect since the analytes of interest are intimately bonded to the components of the matrix to be studied. Indeed, prior to the analysis, specialized extraction techniques are needed that can effectively isolate the analytes without any component of the matrix potentially interfering with the detection of the analyte. The

goal is to develop the most selective extraction method, optimizing the extraction conditions to exploit the chemical differences between analytes and matrix components. In the event that part of the matrix is co-extracted with the analytes, it is often advisable to carry out a further step consisting of techniques for cleaning the matrix from interfering co-extractives with the compounds of interest.

In fact, to obtain a selective method, it is necessary to consider in particular the removal of the matrix components such as organic matter, lipids, waxes, sugars, or pigments. The removal of these co-extracts must be controlled to minimize any adverse effects that could affect the detection of the compounds of interest. Therefore, several post-extraction cleaning procedures have been developed for the removal of the co-extracted matrix components.

Another often overlooked difficulty is the optimization of the extraction conditions of a new analytical method; it is necessary to use a relevant matrix containing the analytes of interest. For persistent organic contaminants or for pesticides, certified reference materials (CRM) are commercially available. However, as far as PhACs are concerned, these materials are not available, and the development must be based on internally produced materials. In most cases, producing internally contaminant-free material means growing vegetables until fully harvested (60–90 days in the case of a lettuce). This may lead to a delay in the development of the analytical method which is not always feasible. Very often then, we resort to the use of vegetables from organic farming [7].

This reference material thus obtained is then doped with known concentrations of the compounds of interest in order to study the recovery tests and thus guarantee the ability to perform a quantitative analysis of the compounds with the developed method.

In an attempt to obtain a rapid and efficient extraction of the analytes from solid matrices, different extraction techniques such as microwave-assisted extraction (MAE), ultrasound solvent extraction (USE), pressurized liquid extraction (PLE), or the most recent QuEChERS method have recently been the subject of in-depth study, due to good efficiency and reliability. In fact, these are the extraction techniques currently most used for the determination of PhACs in soil or plants. They are preferred to traditional extraction methods such as Soxhlet, as they guarantee greater contact between the solvent and the soil particles, with consequent greater coverage of the analytes and a lower consumption of organic solvents. In fact, the Soxhlet method involves very long extraction times with the consumption of large quantities of organic solvents.

The analysis of PhACs is commonly performed using liquid or gas chromatography techniques, although liquid chromatography is preferred as it is more suitable for the analysis of polar compounds.

The purpose of the chapter is to discuss all the analytical procedures used for the extraction and analysis of pharmaceutical products and their related compounds from plant tissues and from the soil irrigated with treated wastewater. This chapter will describe all that is known about the analytical procedures published so far. In the coming section, we will discuss the most popular previously reported methods for pharmaceuticals extractions (USE, PLE, MAE, QuEChERS) from soil and plant

tissues. The different cleanup techniques of the extracts will also be addressed, while the last section will be dedicated to separation and detection techniques. Given the lack of analytical methods in the literature, the determination of drugs in earthworms will be only partially treated at the end of the chapter.

2 Most Common Extraction Techniques

2.1 Assisted Solvent Extraction (ASE)

Assisted solvent extraction (ASE) is also known as pressurized liquid extraction, (PLE), pressurized fluid extraction (PFE), enhanced solvent extraction (ESE), or high-pressure solvent extraction (HPSE). ASE and PLE are the most popular names.

It is considered as an advanced, reasonably uncomplicated extraction technique, time saver, and easy to learn. Briefly, the samples are mixed with a proper cell matrix and filled in the cell. Then the cell is placed in a carousel; the latter rotates automatically in a way to put the cell in the oven chamber. After preheating the oven chamber, the cell is filled with the extraction solvent and kept for an optimized static time, under the desired temperature and pressure. Finally, a solvent containing the extracted analytes is obtained and collected in a vial, while the cell is then washed and purged with a nitrogen flow [8]. All of those steps constitute one cycle and can be repeated several times. The number of cycles is also optimized during method development.

It is usually employed at temperature above the boiling point of the used extraction solvent, along with high pressure to keep the solvent in liquid state during the extraction process. Further, solvent's viscosity decreases as the temperature increases, which enhances its ability to wet the matrix and solubilize the target analytes [9]. Therefore, besides its rapid extraction process, ASE has the advantage of less solvent consumption, less toxicity, more environmentally friendly, and possibility automation. All of these criteria have made ASE popular in pharmaceutical extraction field. It has been successfully applied to a wide range of analytes and pharmaceuticals since polar and non-polar solvents, or a mixture of solvents, may be used [10], though many parameters need to be optimized in order to obtain the highest analyte recoveries, such as solvent selection, temperature, pressure, extraction time, and other parameters such as extraction mode, extraction time, number of cycles, cell matrix, and flush volume.

2.2 Ultrasound Solvent Extraction (USE)

Ultrasound solvent extraction is based on cavitation effect generated by ultrasound radiations in a water bath. Usually solid samples and the extractant are mixed in a centrifuge tube and placed into tube rack in the ultrasonic water bath. The ultrasound

radiations generate a great number of tiny bubbles in liquid media (the extraction solvent added to the sample in the centrifuge tube) and mechanical erosion of solids (soil and crops samples); thus, particles rupture [11]. However, USE does not always reach high efficiency as efficiencies reached by other methods. Additionally, ultrasonic irradiation enhances the release of matrix components simultaneously with the analytes, hence an important matrix effect.

For a maximum pharmaceuticals' recovery, several parameters should be optimized such as type of solvent, irradiation conditions (temperature and amplitude of sonication), sonication time, number of cycles, and sample amount. Generally, to obtain the highest extraction efficiency with the lowest matrix interferences, several extraction cycles are employed, each with a fresh and small solvent volume [12]. Thus, solvents from the different extraction cycles are combined and processed to the following step, usually clean-up step, unless it is skipped to analysis.

Several ultrasound devices are available, such as the water bath, probes, sonireactors, or microplate horn [13]. Yet, an ultrasonic water bath was always employed for soil and crop analysis, which is also known to be the cheapest and the most available [14–16]. An important aspect is that these types of devices are almost always available in every laboratory. Finally, USE is an environment-friendly technique, less solvent consumer, and energy and time saving.

2.3 Microwave-Assisted Extraction (MAE)

MAE is also called microwave-assisted solvent extraction (MASE). In this technique, microwave energy is used to directly heat the solvent in contact with the sample, thus achieving the partition of target analytes from the sample to the solvent and accelerating the speed of extraction. The microwave energy released is nonionizing radiation that causes molecular motion by migration of ions and rotation of dipoles [12]. The efficiency of MAE depends strongly on the nature of the solvent and the matrix [17]. Solvents used for MAE should be able to absorb microwaves. In other words, solvent should have dipole leading (polar). However, a combination of polar and non-polar solvents has extended MAE usage [10].

It is an automated green extraction technique offering many advantages such as less solvent consumption and short extraction times, even less than USE technique [17, 18]. Additionally, several samples can be extracted simultaneously, hence increasing the number of samples analyzed daily. It also offers protection for thermolabile compounds. Further, the equipment for MAE is relatively expensive, which probably is the main reason why a small number of studies are dealing with MAE. Finally, several factors should be optimized in order to obtain the best efficiency, such as solvent selection, temperature, and extraction time.

2.4 *QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe)*

QuEChERS (acronym of quick, easy, cheap, effective, rugged, safe) extraction technique, as its name tells, is known to be fast, easy, and cheap. It consists of two consecutive steps, the extraction/partitioning step usually using acetonitrile as extractant and salts for partitioning and a clean-up step using dispersive solid-phase extraction (d-SPE). Additionally, QuEChERS is one of the widely known green extraction methods. It requires small amount of low toxic, non-halogenated solvents and reagents, as well as laboratory equipment and no external energy supply. Furthermore, this method has also introduced the concept of d-SPE for clean-up purposes. Also, the low cost and short time allow the extraction of a reasonable number of samples. It was first introduced for the determination of pesticides in vegetables [19]; later it was successfully employed for other compounds (e.g., pharmaceuticals, hormones, chlorinated compounds, etc.) in different matrices (eggs, blood, earthworms, and environmental matrices) [20–24].

Nowadays, three main QuEChERS methods are officially known and widely used. Briefly, the original method (OR) [19] is the first method developed by Anastassiades and coworkers in 2003, who are known as the fathers of QuEChERS. They employed acetonitrile as extractant and $\text{MgSO}_4 + \text{NaCl}$ as partitioning salts. Later in 2007, Lehotay and coworkers [25] introduced a modified QuEChERS, in which they employed acidified acetonitrile with 1% acetic acid as extractant and the acetate-buffered salts ($\text{MgSO}_4 + \text{CH}_3\text{COONa}$) for partitioning; it is adopted as an American official method (AOAC). Finally, Anastassiades and coworkers modified their method in 2007 [26] to employ the citrate-buffered salts ($\text{MgSO}_4 + \text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O} + \text{Na}_2\text{C}_6\text{H}_6\text{O}_7 \cdot 1.5\text{H}_2\text{O}$) for partitioning, and it is adopted as a standard method of the European Committee for Standardization (CEN). Lastly, $\text{MgSO}_4 + \text{PSA}$ (primary secondary amine) were used as d-SPE salts in all three previously mentioned methods.

However, many authors have introduced own modifications according to their needs, including the extractant and d-SPE salts. Details concerning extraction and clean-up steps developed in the last couple decades are reported in Sects. 4 and 5 for soil and crops, respectively. At last, several QuEChERS commercial kit versions are now available and sold by many vendors, which played an important role in the usage expansion and wide application of this method, since salts are already precisely weighed and mixed, avoiding the extra effort and time loss for this step.

Thereupon, QuEChERS and modified QuEChERS methods have reached extraction yields for multiresidue analysis in the same order or even better than the three previously reported techniques, without the need of the sophisticated expensive equipment. However, to date and according to our literature survey, only 6 studies have reported multiresidual extraction methods from soil using QuEChERS technique, whereas more than 30 studies are reported for crop extraction.

2.5 Other Extraction Methods (Soxhlet and Solid-Liquid Extraction)

Soxhlet extraction is known for being a traditional extraction procedure. The sample is placed in a thimble-holder, and fresh extraction solvent from a distillation flask fills the sample. When solvent completely fills the thimble-holder, a siphon takes the solvent, with the extracted compounds, returning it back to the distillation flask [27]. This operation is repeated several times as extraction is completed according to the criteria of the study. The complexity, the high amounts of solvent needed and the great time of run required, has caused Soxhlet to be relegated to the background (at least in the pharmaceutical area), giving way to the latest techniques. Moreover, extraction methods such as USE have the ability to penetrate deep into the solid matrix, usually by means of the creation of cavities, which allows to obtain a good extraction performance that Soxhlet is not available to achieve [27].

For liquid matrices such as the analysis of surface water or wastewater, solid-liquid extraction (SLE) is the most effective and easiest to use technique. It does not need major pretreatments. Normally, prior filtration helps remove particulate matter from the samples. It is very effective and used to clean the matrix in combination with other extraction techniques. In order to extract the target compounds of the matrix, an organic solvent or water is mixed with the sample until equilibrium, and the liquid is removed from the mixture (extract). Commonly, heat is applied in order to enhance the extraction efficiency, as well as buffer compounds to have control of the pH. SLE has advantages such as simplicity and few amounts of solvent; however, this extraction procedure is little selective, and that is the main reason that modern extraction techniques have broader paths than SLE.

Only one report showed the capability of SLE to extract acetaminophen from *Brassica juncea* using 1 mL of HCl 0.1 M and nitrogen [28]. A comparative analysis of the most current techniques in terms of costs/speed of execution is reported in Table 1.

Table 1 Comparison between the extraction methods used in terms of time, instrumentation required, solvent volume (green chemistry), and total cost per sample

Extraction method	Time (minutes)	Specific instrumentation	Solvent volume (mL)	Cost
ASE	10–30	Yes	15–100	\$\$\$
USE	30–60	Yes	8–30	\$\$
MAE	5–20	Yes	5–10	\$\$\$
QuEChERS	20	No	10	\$
Traditional methods (Soxhlet)	360	Yes	220	\$\$\$ \$

3 Sample Preparation

Sample preparation concept is to convert a field matrix to a sample suitable for analysis. It is a crucial and important step especially for food and environmental matrices such as crops and soil, which are characterized by their complexity, besides the presence of pharmaceuticals in these matrices in trace amounts [29]. This step also allows to weaken the interactions established between pharmaceuticals and matrices, which is highly dependent on the physical and chemical properties of both pharmaceuticals and matrices. Further these interactions affect the applicability of different extraction methods, their factor conditions, as well as their efficiency and reproducibility.

Additionally, sample pretreatment is needed to assure its homogeneity and a good contact with the solvent during the extraction process. On the other hand, most of the analytical devices are unable to handle those matrices directly, which requires a pretreatment step before extraction for any matrix. In general, soil preparation includes three steps [12]: the first step is drying of sample, either by the use of the oven, by air-drying, or by lyophilization. However, sample exposure to elevated temperature may risk analyte degradation and alteration. Whenever lyophilization is employed, analytes are neither degraded nor evaporated, and the drying time is shorter. Generally, lyophilization is the most advantageous drying technique [30]. The second step consists in the homogenization and finely grounding of the soil samples generally using a mortar. The third and last step is soil sieving at 2 mm to remove coarse particles to increase sample homogeneity. Thereafter, soil samples are stored at -20°C or $+4^{\circ}\text{C}$ in the dark until analysis.

To perform ASE, from 0.5 to 20 g (Table 2) of soil are placed in a stainless-steel extraction cell (33 mL extraction cell volume is the most commonly used) and capped with filters at both ends. Soil sample is usually mixed with a cell matrix (dispersant) before loading in the cell, to enhance the extraction efficiency. Finally, the extraction cell is placed in the oven, and the system automatically starts setting the desired temperature and pressure. For MAE, 0.2–3 g (Table 4) were used, and no common step was found between the reported studies; they differ largely according to the MAE procedure employed. Finally, USE and QuEChERS are the easiest, where soil samples in both methods were introduced in centrifuge tubes. For USE 0.5–5 g (Table 3) of soil were used, the extraction solvent is therefore added, and the tube is placed in the sonication bath. While from 1 to 10 g (Table 5) of soil were used for QuEChERS, the extraction solvent and salts were added and the tube was immediately vortexed and hand shaken.

Plants are normally harvested when they have reached commercial size. When treating branched root plants, care should be taken, while their extraction from soil is done in order to preserve the root part completely. Once samples are recollected, they are handwashed and rinsed with tap water in order to remove residues such as the remaining soil and other interferences [7]. When plant samples are composed of a root part and a vegetative part or fruits, normally each part is treated differently due to its great difference between them [31]. Plant sample pretreatments also consist of

Table 2 ASE extraction and analysis conditions in soil

	ASE extraction conditions						Analysis					
	Soil mass (g)	Solvent	T (°C) P(psi or bar)	Static extraction time (min) × number of cycles	Flush volume (%)	Cell matrix (dispersant)	Clean-up	Recovery (%)	RSD (%)	LOD	Instrument	References
Pharmaceuticals Diclofenac, allopurinol, verapamil, carbamazepine, spironolactone, salbutamol, omeprazole, atenolol, bendroflumethiazide, diltiazem, acetylsalicylic acid, furosemide, prednisolone, cyproterone acetate, erythromycin, enalapril, metoprolol, paracetamol, citalopram, cimetidine, diazepam, zopiclone, oxazepam, amoxicillin, ramipril, terbuthaline, penicillin G, ibuprofen, morphine, gestodene, losartan potassium, amitriptyline besylate, simvastatin	20 g	Methanol/aqueous ammonia solution (0.1 mol L ⁻¹) (1:1, v:v)	80°C 1,500 psi	5 min × 5	50%	Otawa sand	Tandem MAX-HLB SPE cartridges	66–114%	1–14%	0.1–<2.5 ng/g	LC-MS/MS	[64]
Chlortetracycline, oxytetracycline, sulfadiazine, erythromycin, and tylosin (degradation products: TYL A, B, C, and D)	10 g	Methanol/0.2 M citric acid buffer (pH 4.7 adjusted with NaOH) (1:1, v:v)	Room T °C 1,500 psi	10 min × 1 and 3 min × 1	100%	Otawa sand	Tandem SAX-HLB SPE cartridges	50–100%	Not reported	0.6–5.6 g/kg	LC-MS/MS	[58]
Ciprofloxacin and norfloxacin	0.5 g	50 mM aqueous phosphoric acid (pH 2.0) and acetonitrile mixture (1:1, v/v)	100°C 100 bar	15 min × 6	150%	Quartz sand	MPC disk cartridge	75–92%	8–11%	0.05 mg/kg of dry matter	LC-FLD	[68]
Paracetamol, salicylic acid, propranolol hydrochloride, clofibric acid, ketoprofen, diclofenac sodium salt, bezafibrate, warfarin, flurbiprofen, indomethacin, ibuprofen sodium salt, meclofenamic acid sodium salt, gemfibrozil, atenolol, salbutamol, sulfamethoxazole, sulfamethazine sodium	2.7 g	MeOH:H ₂ O (50:50, v/v)	60°C 1,500 psi	5 min × 2	100%	Sea sand	HLB cartridges	>60% (for 20 analytes)	Not reported	<20 ng/g (for 20 analytes)	LC-MS/MS	[55]

salt, furosemide, pravastatin sodium salt, carbamazepine, nimesulide, (±)-metoprolol, (±)-tartrate salt, clonidine, trimethoprim, caffeine, S(±)-2-(6-methoxy-2-naphthyl)-propionic acid (naproxen), and igrasun (trilosan)	4 g	Milli-Q water at neutral pH	100°C 1,500 psi	5 min × 3	60%	Hydromarix	HLB cartridges	50–140%	Not reported	<0.01–0.83 ng/g	UPLC-MS/MS	[53]
Acetaminophen, diclofenac, fenoprofen, ibuprofen, indomethacin, ketoprofen, mefenamic acid, naproxen, phenazone, bezafibrate, clofibrate acid, gemfibrozil, pravastatin, caffeine, carbamazepine, famotidine, ramitidine, atenolol, metoprolol, nadolol, pindolol, propranolol, timolol, furosemide, hydrochlorothiazide, amphenicol, clindamycin, flumequine, lincomycin, metronidazole, novobiocin, ornidazole, sulfadiazine, sulfamethoxime, sulfaguanidine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxyipyridazine, sulfanilamide, sulfathiazole, sulfisoxazole, trimethoprim	3 g	50% phosphate buffer and 50% acetonitrile with pH = 3	70°C 1,500 psi	10 min × 1	60%	Diatomite	Tandem SAX-HLB SPE cartridges	60.2–97.6%	<10%	0.2–1.7 µg/kg	HPLC-MS/MS	[70]
Sulfapyridine, sulfamerazine, sulfadimethoxime, tetracycline, chlortetracycline, oxytetracycline, doxycycline, erythromycin, roxithromycin	3 g	50% methanol and 50% 0.2 M citric acid with	70°C 1,500 psi	10 min × 1	60%	Sea sand and diatomite	Tandem SAX-HLB SPE cartridges	67.3–97.4%	<9%	0.2–1.1 µg/kg	HPLC-MS/MS	[71]

(continued)

Table 2 (continued)

	ASE extraction conditions							Analysis				
	Soil mass (g)	Solvent	T (°C) P (psi or bar)	Static extraction time (min) × number of cycles	Flush volume (%)	Cell matrix (dispersant)	Clean-up	Recovery (%)	RSD (%)	LOD	Instrument	References
Pharmaceuticals oxytetracycline, doxycycline, erythromycin, roxithromycin		pH adjusted to 4.7 by NaOH										
Chlortetracycline and oxytetracycline, and their TPs: 4-epitetracycline, 4-epianhydroxytetracycline, 4-epichlortetracycline, epoxytetracycline, α-apo-oxytetracycline, and β-apo-oxytetracycline	5 g	Methanol/water (3:1, v/v) containing 25 mM EDTA and 0.6 M sodium chloride (pH 8.0)	Room T°C 10 MPa	20 min × 2	60%	Hydromatrix VR diatomaceous earth	Tandem SAX-HLB SPE cartridges	41%–110%	0.12–0.34%	0.08–0.3 mg/g	HPLC-MS	[59]
Methylparaben, methyl propylparaben, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 1,3,7-trimethylxanthine (caffeine), 1,7-dimethylxanthine, curbumazepine, 3-hydroxycarbamazepine, 10,11-dihydro-10-hydroxycarbamazepine, curbumazepine-10,11-epoxide, diclofenac, 4-hydroxydiclofenac, ibuprofen, 1-hydroxyibuprofen, 2-hydroxyibuprofen, carboxyibuprofen, sulfamethoxazole, and N4-acetylsulfamethoxazole	2 g	MeOH (0.5% v/v, formic acid)	50°C 1,500 psi	5 min × 2	60%	Diatomaceous earth	In-cell clean-up with C18 and PSA	60–103%	<11%	0.04–4.31 ng/g	HPLC-MS/MS	[69]
Sulfisomidine, sulfamerazine, sulfacetamide, sulfadoxine, sulfabenzamide,	5 g	MeOH-water (90:10, v/v)	100°C 1,500 psi	5 min × 3	100%	Hydromatrix	HLB cartridges	60–130%	<23%	0.01–4.19 ng/g	HPLC-MS/MS	[61]

succinylsulfathiazole, sulfamethoxazole, sulfadiazine, sulfadimethoxine, sulfamethizole, sulfamethoxazole, sulfamethoxypridazine, sulfapyridine, sulfathiazole, sulfisoxazole, sulfanitran and N4-acetylsulfamethazine, and the acetylated metabolites	10 g	Acetone/hexane/ acetic acid (50:50:2; v/v/v)	100°C 1,000 psi	5 min × 2	50%	Diatomaceous earth	HLB cartridges	54–118%	< 10%	0.25–2.5 ng/g	GC-MS	[54]
Clofibrate acid, ibuprofen, salicylic acid, 2,4-D, gemfibrozil, naproxen, ketoprofen, diclofenac, carbamazepine, 4-nonylphenols, triclosan, bisphenol A, di-n-butyl phthalate, butyl benzyl phthalate, bis-2-ethylhexylphthalate, estrone, 17-estradiol, 17-ethinyloestradiol	5 g	Methanol/water (1:1, v/v)	40°C 100 bar	5 min × 3	75%	Sea sand	HLB cartridges	63–80%	9–15%	LOQ: 0.5– 2.5 ng/g	LC-MS/MS	[72]
Sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, and sulfathiazole	4 g	0.1 M Tris (pH 8.8) and acetonitrile (85:15)	200°C 100 bar	5 min × 1		Diatomaceous earth	No clean-up	62–93%	< 10%	<15 g/kg	LC-MS/MS	[56]
Oleandomycin, erythromycin, tylosin, roxithromycin, framulin fumarate, monensin, ivermectin, salinomycin	30 g	1% (v/v) aqueous ammonia in methanol	80°C 140 bar	10 min × 2	70%;	Ottawa sand	Diol SPE cartridges	43–118%	19– 29%	0.2–1.6 mg/kg	LC-MS/MS	[73]
Acetaminophen, codeine, carbamazepine,	3 g	Water	90°C 500 psi	7 min × 3	100%	Sea sand	Tandem SAX-HLB	59–119%	< 16%	0.1–6.8 ng g ⁻¹	LC-MS/MS	[9]

(continued)

Table 2 (continued)

	ASE extraction conditions						Analysis				References	
	Soil mass (g)	Solvent	T (°C) P(psi or bar)	Static extraction time (min) × number of cycles	Flush volume (%)	Cell matrix (dispersant)	Clean-up	Recovery (%)	RSD (%)	LOD		Instrument
Pharmaceuticals ciprofloxacin, clofibrate acid, diazepam, diclofenac, fenofibrate, ibuprofen, metoprolol, norfloxacin, ofloxacin, oxytetracycline, sulfamethoxazole, tetracycline, propranolol, trimethoprim, and 4-epitetracycline hydrochloride, 4-epoxytetracycline							SPE cartridges					
Chlorotetracycline, doxycycline, oxytetracycline, and tetracycline antibiotics	5 g	5% (w/v) sodium acetate, 100 mM EDTA, in a 50:50 solution with methanol, adjusted to pH 8 with sodium hydroxide	60°C 104 bar	5 min × 2		Hydromatrix	Strata-X SPE cartridges	22–99%			LC-MS	[50]
17 α -Ethinylestradiol, 17 β -estradiol, 17 α -estradiol, sulfamethazine, sulfadimethoxine, sulfamer, sulfamethizole, sulfamerazine, sulfachloropyridazine, sulfadiazine, sulfamethoxazole, tetracycline, and oxytetracycline. Estrone and 4-epitetracycline. 17 β -Estriol and sulfathiazole. Conjugated estrogen metabolites, estrone-3-sulfate, estrone-3-glucuronide, 17 α -ethinylestradiol-3-glucuronide, 17 β -estradiol-3-sulfate, 17 α -estradiol-3-	5 g	Water/methanol/acetone (50/25/25, v/v/v) containing 25 mM EDTA, 2% ammonium hydroxide, and 0.6 M sodium chloride	Room T °C 100 bar	10 min × 2	60%	Hydromatrix	HLB SPE cartridges	21–105%	4–24%	0.01–0.1 ng/g	LC-MS/MS	[60]

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sulfate, 17 β -estradiol-17-sulfate, and 17 β -estradiol-3-gluconide. Anhydrotetracycline hydrochloride, anhydrochlorotetracycline hydrochloride, 4-epichlorotetracycline hydrochloride, and chlortetracycline hydrochloride	5 g	Deionized water (pH 7.0-6.8)	70°C 1,500 psi	10 min \times 1	100%	Sea sand	SAX and Strata-X cartridges	71-96%	8-15%	<1-3 $\mu\text{g kg}^{-1}$	LC-MS/MS	[52]
Chlortetracycline, doxycycline, oxytetracycline, and tetracycline Flubendazole, erythromycin, erythromycin (-H ₂ O), dicloxacillin, ciprofloxacin, sulfamethoxazole, oxytetracycline, carazolol, diclofenac, meclofenamic acid, carbamazepine, clofibrate acid, natamycin, eniconazole, ketoconazole, fluconazole, clotrimazole, miconazole, itraconazole, griseofulvin, voriconazole, thiabendazole, difenoconazole, hexaconazole, penconazole, propiconazole, paclitaxel, prochloraz, tebuconazole, bromuconazole, cyproconazole, epoxiconazole, fenbuconazole, fluquinconazole, flusilazole, flutriafol, metconazole, prothioconazole, terconazole, myclobutanil, triticonazole, carbendazim, metalaxyl	5 g	Acetone/citric acid 0.2 M (50:50) (pH adjustment at 4.5 with sodium hydroxide)	50°C 1,500 psi	5 min \times 2	50%	Diatomaceous earth	Strata-X SPE cartridges	>70%		$\leq 10 \mu\text{g kg}^{-1}$	UHPLC-MS	[74]

Table 3 USE extraction and analysis conditions in soil

	USE extraction conditions				Analysis				References	
	Soil mass (g)	Solvent	Time (min) × number of cycles	Temp. (°C)	Clean-up	Recovery (%)	RSD (%)	LOD		Instrument
Pharmaceuticals Tetracycline, chlortetracycline, oxytetracycline, trimethoprim, sulfadiazine, sulfadiazine, sulfadoxine, sulfathiazole, sulfamethoxazole, ciprofloxacin, enrofloxacin	3 g (for tetracyclines, sulfonamides, and trimethoprim) 1 g (for ciprofloxacin and enrofloxacin)	MeOH:EDTA-McIlvaine buffer pH = 6 (90:10)	20 min × 3		SPE-C18 cartridge (for tetracyclines, sulfonamides, and trimethoprim) n-Hexane (for ciprofloxacin and enrofloxacin)	61–105%			LC-MS/MS	[76]
4-Epioxytetracycline, 4-epitetracycline, acetylcysteine, allopurinol, alprazolam, atorvastatin, azithromycin, bezafibrate, bisphenol A, butylparaben, carbamazepine, chloramphenicol, clofibrac acid, ciprofloxacin, clarithromycin, codeine, diazepam, diclofenac, ethylparaben, etoricoxib, fenofibrate, flufenamic acid, gemfibrozil, ibuprofen, indomethacin, metformin, methylparaben, metoprolol, naproxen, norfloxacin, ofloxacin, oxytetracycline, paracetamol, propranolol, propylparaben, salicylic	1 g	Mix of methanol, distilled water, and of McIlvaine-EDTA buffer	30 min × 1		Strata-X 33 U polymeric reversed phase SPE cartridges	38–104%	<30%		UHPLC-MS/MS	[77]

acid, sulfamethoxazole, telmisartan, tetracycline, thiamphenicol, triclocarban, triclosan, trimethoprim, vildagliptin, warfarin, tetrahydrocannabinol, 11-nor-9-carboxy-9-tetrahydrocannabinol	1 g	Aqueous solution of MgNO ₃ 50% (w/v) containing 4% of ammonia	30 min × 1	Room temperature	No clean-up	> 82%	< 12%	0.04-0.08 µg g ⁻¹	HPLC-UV	[78]
Quinolones (ciprofloxacin, oxolinic acid, nalidixic acid and flumequine) and fluoroquinolones (norfloxacin, enrofloxacin, enoxacin, ciprofloxacin and danofloxacin)	2 g	Mixture of potassium phosphate buffer and acetonitrile (ACN) (1/1, v/v, pH 3.2) and 0.4 g Na ₂ EDTA	10 min × 3		Tandem SAX-HLB SPE cartridges	56-97%	1-20%	8.9 µg/kg	LC-MS/MS	[75]
Norfloxacin, ciprofloxacin, enrofloxacin, tetracycline, Chlortetracycline, oxytetracycline, sulfacetamide, sulfachloropyridazine, sulfamethoxime, sulfamerazine, sulfadimidine, sulfamethoxazole, sulfathiazole, sulfamethizole, sulfamethoxy-pyridazine, sulfapyridine and sulfisoxazole, ofloxacin	5 g	Mix of acetone and ethyl acetate	15 min × 4		C18 cartridge	63.8-110.7%			GC-MSD	[79]
Clofibric acid, ibuprofen, naproxen, ketoprofen, diclofenac, triclosan, 4-tert-octylphenol, 4-nonylphenol, bisphenol A, and estrone										

(continued)

Table 3 (continued)

	USE extraction conditions				Analysis				References	
	Soil mass (g)	Solvent	Time (min) × number of cycles	Temp. (°C)	Clean-up	Recovery (%)	RSD (%)	LOD		Instrument
Pharmaceuticals Acetylsalicylic acid, ibuprofen, paracetamol (acetaminophen), flurbiprofen, naproxen, diflunisal, ketoprofen; diclofenac sodium salt, valproic acid; primidone, nadolol, propranolol (hydrochloride), diethylstilbestrol, estrone, 17- β -estradiol, 17- α -ethinyloestradiol, estriol, amitriptyline, imipramine, and clomipramine in the form of hydrochlorides	5 g	Ethyl acetate/formic acid (50:1, v/v)	15 min × 3		Silica gel columns	> 80%	1.1–10.0%	0.3–1.7 ng g ⁻¹	GC-MS	[80]
4-tert-Octylphenol, benzo(a)pyrene, bisphenol A, carbamazepine, indole, p-cresol, phenanthrene, triclosan	2 g	80:20 IPA:H ₂ O	10 min × 2	Room temperature	HLB cartridges and Florisil cartridges	46.1–110%	4–48%	7.6–60.7 μ g/g	GC-MS	[81]
Difloxacin, sarafloxacin, enrofloxacin, ciprofloxacin, enoxacin, norfloxacin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, sulfaminoxaline, sulfaclozine, sulfamethoxydiazine,	5 g	ACN:Na ₂ EDTA-McIlvaine buffer (pH 4.0, 5:5, v/v) and 0.2 M NaOH	10 min × 2		HLB cartridge	> 60%	< 20%	0.01–2 μ g/kg	LC-MS/MS	[82]

sulfamonomethoxine, sulfadimidine, sulfamethoxazole, tylosin, roxithromycin, kiasamycin, erythromycin, tilimicosin, clindamycin, valnemulin, and tiamulin	0.5 g	ACN:MeOH (1:1, v/v) and 0.5% formic acid in ACN:MeOH (1:1, v/v)	20 min × 3		HLB cartridge	71–122%	< 10%	0.03–1 ng g ⁻¹	LC-MS/MS	[83]
Carbamazepine, diclofenac, cis-diltiazem, lamotrigine, methadone, midazolam, oxcarbazepine, sulfamethoxazole, trimethoprim, valsartan, cocaine, acridone, 4'-hydroxycyclofenac, and valsartan acid	4 g	MeOH:0.1 M EDTA:MeI/vaine buffer, 50:25:25	10 min × 3		SAX-HLB SPE cartridges	27–105%		18–40 µg kg ⁻¹	HPLC-UV	[84]
Clofibric acid, ibuprofen, salicylic acid, allopurinol, paracetamol, gemfibrozil, fenoprofen, amitriptyline, metoprolol, naproxen, melenamic acid, ketoprofen, carbamazepine, diclofenac, fenofibrate	5 g	ACN containing 2% of NH ₄ OH and ACN containing 2% of formic acid	15 min × 2	Room temperature	No clean-up	80–115%	8–12%	0.04–0.24 ng g ⁻¹	GC-MS	[85]
Doxycycline, erythromycin, progesterone, tylosin tartrate, amoxicillin, norfloxacin, sulfadiazine, trimethoprim, enrofloxacin, flumequine, tilimicosin	1 g	MeOH:ACN:0.1 M EDTA:MeI/vaine buffer (pH 4), 30:20:25:25	10 min × 3		HLB cartridges	63–121%	≤ 20%	0.5–3 µg/kg	LC-MS/MS	[86]

(continued)

Table 3 (continued)

	USE extraction conditions				Analysis				References	
	Soil mass (g)	Solvent	Time (min) × number of cycles	Temp. (°C)	Clean-up	Recovery (%)	RSD (%)	LOD		Instrument
Pharmaceuticals Acetylsalicylic acid, ibuprofen, paracetamol (acetaminophen), flurbiprofen, naproxen, diflunisal, ketoprofen, diclofenac sodium salt, valproic acid, primidone, nadolol, propranolol (hydrochloride), diethylstilbestrol, estrone, 17- β -estradiol, 17- α -ethinyloestradiol, estriol, amitriptyline, imipramine, and clomipramine in the form of hydrochlorides	5 g	Ethyl acetate/formic acid (50:1, v/v)	15 min × 3		Silica gel columns	52–112.6%	≤ 9.0%	0.3–1.7 ngg ⁻¹	GC-MS	[80]

Table 4 MAE extraction and analysis conditions in soil

	MAE extraction conditions				Clean-up	Analysis				References
	Soil mass (g)	Solvent (power in watt)	Time (min) × number of cycles	Temp (°C)		Recovery (%)	RSD (%)	LOD	Instrument	
Pharmaceuticals Acetylsalicylic acid, carbamazepine, chloramphenicol, clofibrac acid, diclofenac, florfenicol, flunixin, ibuprofen, ketoprofen, mefenamic acid, metoprolol, naproxen, niflumic acid, paracetamol, phenylbutazone, propranolol, pyrimethamine, trichlosan and thiamphenicol, 17 α -ethinyloestradiol, 17 β -estradiol, and estrone	1 g	3:2 methanol/water (500 W)	6 min × 1		HLB cartridges	91–101%	< 6%	0.8–5.1 ng/kg	GC-MS	[18]
Acetylsalicylic acid, ibuprofen, paracetamol, flurbiprofen, naproxen, diflunisal, ketoprofen, diclofenac sodium salt, diethylstilboestrol, estrone, 17 β -estradiol, 17 α -ethinyloestradiol, and estriol	5 g	Acetonitrile (400 W)	15 min × 1	115°C	HLB cartridges	> 50%		0.3–5.7 ng/g	GC-MS	[87]
Oxolinic acid and flumequine		1 M phosphoric acid buffer at pH 2 and dichloromethane	22 min × 1	90°C	1 M aqueous sodium hydroxide	79–94%	3–7%		LC-FLD	[88]
Norfloxacin and ciprofloxacin	0.2 g	Water (120 W)	5 min × 1		No clean-up	98%	5.21%		HPLC-photometric detector	[89]

(continued)

Table 4 (continued)

	MAE extraction conditions					Clean-up	Analysis				References
	Soil mass (g)	Solvent (power in watt)	Time (min) × number of cycles	Temp (°C)	Recovery (%)		RSD (%)	LOD	Instrument		
Pharmaceuticals Caffeine, 17-estradiol, ibuprofen, ketoprofen, musk ketone, naproxen, triclosan, and epicoprostanol	3 g	2:1 (v/v) methylene chloride/methanol	15 min × 3	115°C	89.6%	2.89%		GC-MS	[90]		
Sulfadiazine, sulfapyridine, sulfamerazine, sulfamer, sulfadimidine, sulfamethoxazole, sulfamonomethoxine, sulfaphenazole, sulfaminoxaline, and sulfamitran	2 g	Triton X-114 (1.5%, v/v) (800 W)			69.7–102.7%	< 7%	0.42–0.68 ngg ⁻¹	LC-UV	[91]		
Methylparaben, ethylparaben, propylparaben, butylparaben, isopropylparaben,	0.5–2 g	MeOH (350 W)	3 min × 1		92–102%	5.4–6.9%	0.5–4.5 ng kg ⁻¹	GC-MS	[92]		

isobutylparaben, benzylparaben, 2-phenylphenol, 4-phenylphenol, nonylphenol, 4-tert-octylphenol, bisphenol A, and triclosan	1 g	Aqueous 20% (w/v) Mg(NO ₃) ₂ ·6 H ₂ O and 2% (v/v) NH ₃ solution	20 min × 1	80°C	No clean-up	70–130%	1–6%	Few nanograms per gram	HPLC-FD	[93]
Norfloxacin and danofloxacin	3 g	Methanol	10 min × 1 (200 W)	120°C	No clean-up	70–110%	< 11%		HPLC-UV	[94]
1H-Benzotriazole, 5-methyl-1H-benzotriazole, benzothiazole, 2-hydroxybenzothiazole, 2-methylthiobenzothiazole, benzenesulfonamide, and toluenesulfonamide	1 g	ACN/water (90/10)	15 min × 1	120°C	SPE cartridge				UHPLC-MS/MS	[95]
Abamectin, doramectin, and ivermectin	2 g	Triton X-114 (5.0%, v/v)			No clean-up	81.2–93.7%	4.6–9.5%	3.2–5.7 ng g ⁻¹	HPLC-UV	[96]
Sulfadiazine, sulfamerazine, sulfamethoxydiazine, sulfamonomethoxine, sulfadimethoxine, sulfamethoxazole, and sulfaquinoxaline	2 g	ACN (320 W)			On line SPE	82.6–93.7%		1.4–4.8 ng g ⁻¹	LC-MS/MS	[97]
Sulfadiazine, sulfamerazine, and sulfamonomethoxine, and its two major metabolites, N-acetylsulfadiazine and 4-hydroxysulfadiazine	10 g	Acetonitrile/water 1:4 (v/v)	15 min × 1	150°C						[98]

(continued)

Table 4 (continued)

	MAE extraction conditions				Clean-up	Analysis				References
	Soil mass (g)	Solvent (power in watt)	Time (min) × number of cycles	Temp (°C)		Recovery (%)	RSD (%)	LOD	Instrument	
Pharmaceuticals Sulfaguanidine, sulfacetamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamethazine, sulfamerazine, sulfamethoxazole, and sulfadimethoxine	1 g	Methanol	45 min × 1	110°C			Sub- to low ng per g	LC-MS/MS	[99]	
Tetracycline, deoxytetracycline, oxytetracycline, chlortetracycline	1 g	Methanol (400 W)	20 min	60°C	μ-SPE device consisted of cop-per (II) isonicotinate	70.6–110.5%	<15.1% 0.1–6.3 ng/g	HPLC-UV	[100]	

Table 5 QuEChERS extraction and analysis conditions in soil

	QuEChERS extraction conditions			Clean-up salts	Analysis				References
	Soil mass (g)	Solvent	Extraction salts		Instrument	Recovery (%)	RSD (%)	LOD	
Pharmaceuticals									
Bentazone, atrazine, carbamazepine, phenytoin, and its metabolite	5 g	CH ₃ CN:H ₂ O 70:30, 5% CH ₃ COOH	Original salts: anhydrous MgSO ₄ and NaCl	d-SPE: C18 and anhydrous MgSO ₄	HPLC-UV	88–113%	≤14%	4–493 µg/kg	[102]
5-(p-hydroxyphenyl)-5-phenylhydantoin									
14 veterinary products, 11 hormonal steroids, 6 human contaminants (paracetamol, sulfamethoxazole, flvoxamine, carbamazepine, ibuprofen, bisphenol A)	5 g	Milli-Q water and ACN	AOAC salts	SAX and Strata-X SPE cartridge	LC-MS/MS	20–90%	≤30%		[106]
Ibuprofen and its metabolites (hydroxyibuprofen and carboxyibuprofen)	5 g	Acidified purified water (pH 2.5, hydrochloric acid) and for sonication acidified ACN (1% HCOOH)	EN salts: MgSO ₄ , NaCl, 1 g of sodium citrate dehydrate (NaCit), and 0.5 g of sodium citrate sesquihydrate (Na ₂ Cit)	No clean-up	LC-FLD	>80%	<5%		[101]
Ceftiofur, clopidol, florfenicol, monensin, salinomycin, sulfamethazine,	10 g	Distilled water and CAN acidified with acetic acid	Sodium acetate	C18	LC-MS/MS	60.25–120.3%	<13% except for		[103]

(continued)

Table 5 (continued)

	QuEChERS extraction conditions				Clean-up salts	Analysis				References
	Soil mass (g)	Solvent	Extraction salts			Instrument	Recovery (%)	RSD (%)	LOD	
Pharmaceuticals sulfathiazole, sulfamethoxazole, tiamulin, and tylosin							clopidol (25.2%)			
Sulfacetamide, sulfafisomidine, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamer, sulfamidine, sulfamethizole, sulfadoxine, sulfamethoxazole, sulfamoxole, sulfamonomethoxine, sulfisoxazole, sulfabenzamide, sulfaminoxaline, sulfadimethoxine, erythromycin, roxithromycin, clarithromycin	5 g	McIlvaine buffer	Na ₂ EDTA and NaCl		d-SPE: PSA, C18, and MgSO ₄	LC-MS/MS	61.4–118.9%	<20%		[105]
73 target compounds (pharmaceuticals and TPs)	1 g	Milli-Q H ₂ O and 1% acetic acid in ACN	Anhydrous MgSO ₄ and sodium acetate		Anhydrous MgSO ₄ and C18	LC-MS/MS	70–120% (this approach achieved to extract a total of 53 over 73 compounds)	≤20%		[104]

the removal of the water content and also a crushing process in order to obtain fine powder samples [32]. Although not all the studies have decided to eliminate the sample moisture, water content removal could provide more stabilization to the sample when stored [33, 34]. Besides, working with wet matrices makes it necessary to work with a higher amount of sample than working with dry samples. Commonly, freeze-drying process is performed ensuring a complete water removal. On the other hand, the crushing process is performed to ensure homogenization of the sample, but it also improves the subsequent extraction process. Depending on the extraction method performed and the use of dry or wet samples, the quantity of sample required varies. In general, 0.1 to 15 g of samples are used for analysis. Small quantities up to 5 g are used for dry samples [35], while larger amounts are required when wet samples are analyzed [36]. Freeze-drying samples, apart from preserving the stability of compounds of interest, allows to store in a small space originally voluminous samples. Lyophilization is the most common freeze-drying technique used [37] due to its rapidity but also the certainty of not wasting sample during the process [38].

As before-mentioned, sample quantity strongly depends on the extraction method used. Similar sample weights are used for the extraction of pharmaceutical products from plant tissues. From 0.1 to 8 g samples [39, 40] were used to perform ASE as its methodology is mainly focused on parameters such as solvent, temperature, and pressure, while sample amount plays a secondary role. In the case of USE, weights between 0.1 and 10 g sample were used [41–44]. However, compared to ASE, less quantity of sample is needed when USE was employed. Little amount of sample enhances the extraction efficiency due to the need to create cavities inside the matrix that are easily formatted when lower amount of sample is analyzed. On the other hand, ASE is not as sensitive to this factor owing to other parameters such as temperature and pressure which could help in the creation of these cavities inside the sample. Finally, as regards the QuEChERS method, several authors used weight ranges from 0.5 to 15 g [7, 36, 45, 46]. Looking at the original method developed by Anastassiades et al. [19], the established weight was 10 g of wet sample. This quantity was established on the basis of the amount of salts used by the QuEChERS methodology. It has to be noted that, depending on the nature of the analyzed sample (low-fat samples, high-fat samples, low water amount samples, etc.), different weights and hydration volumes should be applied in order to achieve a successful extraction performance. When 10 g of fruits between 25 and 80% of water content are analyzed, the amount of hydration water added varies depending on its water content. For example, for fruit with 60% water content, 4 mL of water should be added. On the other hand, 5 g of cereals and honey requires 10 mL of hydration water, while fruits with a water content above 80% do not need any amount of water hydration [47]. All these considerations are based on pesticide extraction though; extraction variations could be observed when PhACs are the target analytes.

4 Extraction Procedures for Pharmaceuticals from Soil

The fate of pharmaceuticals in soil depends strongly on their physico-chemical properties and soil texture; they might be sorbed on soil particles and its organic matter content, degraded by soil microbial community, volatilized, or leached to groundwater [48]. In order to better understand the fate of pharmaceuticals in soil, accurate and reliable analytical methods are needed as in to identify and quantify these molecules at low environmental concentrations ranging from a few $\mu\text{g}/\text{kg}$ up to g/kg [48].

Soil is a very complex matrix, and complex interactions between pharmaceuticals and soil particles and organic content are established. Besides, in solid environmental matrices, pharmaceuticals are present at very low concentrations with a large number of potentially interfering compounds. Due to this, analytical method development is a challenging task that requires adequate extraction and clean-up procedures. Extraction techniques should be selective and highly effective. For example, target analytes should be better recovered when co-extracts (matrix impurities) are fewer. Further, they should be fast, easy, and cheap and require minimal organic solvent use. A following clean-up step is essential in these methods since soil is very complex, and a co-extracted matrix contaminant is involuntarily co-extracted (such as humic and fulvic substances), especially for USE, PLE, and MAE, which they employ energy to extract pharmaceuticals [49]. Moreover, this step helps in sustaining analytical devices and to improve limits of detection and quantification as pharmaceuticals are present at low concentrations in soil. Solid-phase extraction (SPE) is the most commonly used clean-up technique for the extraction techniques discussed in this section. Notwithstanding the foregoing, few studies have omitted this step since it allows the loss of the analytes along with the removal of matrix interferences. Despite everything, all of those modern analytical techniques include decreased sample amounts, less solvent consumption, time saving, high recoveries, good reproducibility, repeatability, and detection limits.

4.1 Assisted Solvent Extraction (ASE)

The selection of the extractants for trace residues from soil is crucial. One advantage of ASE is the possibility to choose a wide range of solvents. Several extraction solvents and buffer solutions have been employed to extract pharmaceuticals simultaneously from soil, with varying degrees of target compound recoveries [50, 51]. Acetonitrile (ACN), methanol (MeOH), hexane, and water are the most commonly used solvents for the soil and recognized as safe and environmentally friendly. Among these, the use of water as a solvent represents the most ecological extraction method and has already been successfully used with recoveries of over 50% [9, 52, 53]. Generally, a mixture of solvents of different polarity is more successful for the ASE application. This mixture allows to extract the majority of

the analytes covering the entire polarity range of the compounds, reducing the presence of other components of the matrix in the final extracts. An instance for this, Durán-Alvarez and coworkers [54] obtained improved recoveries when the mix of solvents acetone/hexane (1:1, modified with 2% acetic acid) was employed instead of single solvent, with which acetone is the polar solvent and hexane is the non-polar solvent. The addition of some buffers such as ammonia, citric acid, phosphoric acid, formic acid, sodium acetate, and ethylenediaminetetraacetic acid (EDTA) has also shown an improvement in recoveries. The commonly used mixtures are generally methanol/water or acetonitrile/water with some of the previously mentioned buffers. In some cases, those mixtures were also used unbuffered [54, 55].

The use of EDTA in the ASE method has not been recommended in most cases for several reasons. An example is the greater co-extraction of the matrix with consequent lower sensitivity and clogging of the ASE apparatus (cells and device tube) [56]. However, in some cases, its addition did not significantly improve analyte recoveries [52]. In contrast, EDTA has been successfully employed in the techniques discussed below (USE, MAE, and QuEChERS).

In addition to the selection of the solvent, several other parameters must be carefully considered in the development of the ASE method, such as temperature, pressure, extraction time and extraction mode, number of cycles, cell matrix, and flush volume. Those parameters are related and should be balanced to obtain acceptable recoveries for all target analytes. By far, temperature has proven to be the most important parameter. Theoretically, the high temperature helps to stop the strong interactions between pharmaceutical products and soil components; therefore, the higher the temperature, the higher the extraction yield [51, 57]. Furthermore, the temperature influences the physico-chemical properties of the solvents, therefore viscosity, density, and polarity. For example, the dielectric constant of pure water drops from 79 to 35 when the conditions are changed from room temperature and pressure to 200°C and 1.5 MPa, obtaining a water solvent similar to methanol at room temperature, with low density and polarity [57]. In other words, under high temperature conditions, the properties of the solvents are modified so as to reduce the viscosity and surface tension and greater diffusivity, which improves the wetting of the matrix and improves the mass transfer from the matrix to the solvent. All in all, the reduced interactions between analytes and matrix and the greater diffusion of the solvent in the high temperature matrix, in addition to the increasing solubility of the analytes, allow a faster mass transfer and a complete extraction process. On the other hand, in addition to all these strengths of the high temperature used in the ASE, some weaknesses are also presented, and it is worth mentioning them: (1) the co-extraction of other unwanted compounds from the matrix due to the bonds which break under high temperature conditions, thus converting the ASE into a less selective method; (2) the degradation of the thermolabile analytes at high temperature; (3) and, finally, the formation of toxic compounds due to chemical reactions that occur at high temperature. However, these weaknesses can be avoided by minimizing the extraction time and the number of cycles. Overall, the extraction temperature should be optimized to extract the analytes of interest with less matrix interference, loss of

selectivity, degradation, and toxic components. The temperature range used for ASE soil extraction ranges from 40 to 200°C, with 100°C being the most used temperature. A good temperature choice is 100°C as it exceeds the boiling point of most of the organic solvent and is low enough to avoid the degradation of the analytes, the excess of co-extracts, and the formation of toxic compounds. Since tetracyclines can undergo unwanted transformations at high temperature (they can be converted into their epi or anhydro form), some studies have performed their extraction at room temperature [58–60]. Tetracyclines and fluoroquinolones are extracted with lower temperatures from ambient to 100°C [58], whereas sulfonamides showed the necessity of higher temperature ranging from 50 to 200°C [61]. Many studies [62–64] have demonstrated that the extraction pressure has no influence on the recoveries and extraction efficiency. However, an elevated pressure is important to maintain the solvent in its liquid state. Additionally, it helps to wet the sample, resulting in an improved extraction efficiency [65]. Pressures from 500 to 1,500 psi were usually employed, with 1,500 psi the most common pressure.

The extraction time is the time in which the solvent and the matrix are in contact with the desired temperature and pressure and depends on the matrix and the target analyte characteristics [57]. It should be as short as possible but also adequate for mass transfer. Moreover, extraction time depends on the extraction mode (static or dynamic). Static mode was always chosen for pharmaceutical extraction from soil rather than the dynamic mode. It is worthy to mention that different instruments are designed for each mode. In the static mode, the solvent and the sample are maintained for a specific time at constant temperature and pressure, and the solvent is only replaced partly or completely when another cycle is employed [66]. If this time is longer than necessary, thermal degradation may occur for the extracted analytes resulting in a slower and less efficient extraction procedure. In addition, an equilibrium point of the analytes is established in the matrix and solvent in static mode, and beyond this point, the extraction efficiency will not increase. Hence, static extraction time should be optimized carefully. Most often, a static extraction time of 5 min (2×5 min cycles) or 10 min (1×10 min cycle) is employed.

The volume of the solvent can be introduced in several cycles or in one cycle. Fractioning the solvent in several cycles can improve extraction efficiency by avoiding the analyte equilibrium point to take place. However, it could also provide lower recoveries due to high co-extraction of matrix interferences [67]. Hence, a moderate number of cycles should be employed. Mainly 1–3 cycles are used.

The soil samples are generally mixed with a cellular matrix (dispersant) to prevent clogging of the cell and improve soil contact with the solvent. Among the different cellular matrices available, Ottawa sand [64], quartz sand [68], sea sand [55], diatomaceous earth [69], and Hydromatrix diatomaceous earth [59] are usually the dispersants commonly used with the soil. Lastly, flush volume is the least mentioned in studies, even though it is important to know the final volume of the extract. It is expressed as the percentage of the cell volume, and it ranges from 50 to 150% in all studies dealing with soil as matrix. This interval is sufficient to extract most of the analytes suitable for quantitative analysis, in a minimum volume of solvent, reducing the solvent consumption and concentrating the analytes as much as possible. Finally,

in a comparison with conventional methods such as Soxhlet, ASE is favored by time and by the reduction of the volume of solvent. However, compared to other more sustainable techniques such as MAE, USE, and QuEChERS, ASE may no longer be considered in terms of time or cost savings, but in some cases, it can provide better efficiency, reproducibility, and robustness. Therefore, ASE is a choice of interest for the development of analytical methods to determine pharmaceutical products from the soil (Table 2).

4.2 *Ultrasound Solvent Extraction (USE)*

Different extraction solvents were employed for pharmaceutical extraction from soil with ultrasonication, such as acidified aqueous solutions, EDTA solutions, and phosphate and McIlvaine buffers, mixed with organic solvent. Acetonitrile, methanol, and ethyl acetate are the main organic solvents employed with sonication methods (solvent selection depends on its viscosity). Na₂EDTA (disodium ethylenediaminetetraacetate) is a chelating agent and is used extensively to prevent pharmaceutical products such as tetracyclines and fluoroquinolones from forming chelated complexes with soil matrix (such as metal ions present in the soil). Sulfonamides are amphoteric and are best extracted in an acid medium, while tetracyclines are better extracted with a McIlvaine buffer. However, a combination of buffer and organic solvent has been used for the simultaneous extraction of multiple pharmaceutical residues. Hu and colleagues used potassium phosphate buffer with ACN (1/1, v/v, pH = 3.2) with 0.4 g of Na₂EDTA in order to obtain the best recoveries for fluoroquinolones, tetracyclines, and sulfonamides [75] (Table 3).

The sonication time and the number of cycles are important parameters and should be optimized so as to obtain the maximum recovery of the target analytes with a minimum matrix that interferes with the compounds (this can be explained by the distribution coefficients of pharmaceutical products rather than by the kinetics of desorption process). The sonication time ranged from 10 to 30 min, and the number of cycles ranged from 1 to 4 cycles at maximum. The temperature of the ultrasonic bath was not controlled in most studies. However, USE extraction was always performed at room temperature.

4.3 *Microwave-Assisted Extraction (MAE)*

In MAE, extraction parameters such as time, power, solvent (type and volume), pressure, temperature, number of cycles, and sample size are important parameters that require vigorous optimization to obtain the maximum recovery yield. The extraction solvents for MAE should be capable of absorbing microwaves, with permanent dipole leading (polar), although the combination of solvents with and

without dipole has extended the MAE application to a wide variety of analytes (polar and non-polar) (Table 4). Methanol, acetonitrile, and water are the most commonly used as a solvent for green extraction. The presence of water in the extraction solvent has been shown to increase extraction rates. This can be explained by the absorbance of the microwave energy by the water, resulting in efficient heating of the samples.

High pressure and temperature include the risk of degradation of pharmaceutical products and should be carefully optimized. The temperature is generally set between 60 and 120°C, while the extraction time is generally very short when applying MAE, since the solvent is heated directly with microwave energy and can last from 3 to 20 min.

4.4 QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe)

The selection of the extraction solvent is a crucial parameter. Acetonitrile (ACN) has been shown to provide the best extraction efficiency of a wide range of compounds, including pharmaceutical ones, with the lowest matrix interference. Furthermore, it was used by the QuEChERS fathers when the method was first introduced [19]. ACN was used as extraction solvent in all the studies dealing with soil extraction by QuEChERS technique, besides water or McIlvaine buffer as hydration solvent of the sample [101–106]. Hydration of the soil with water is important to weaken the interactions of the target analytes with the adsorption sites of the soil humic substance, promoting desorption and allowing the ACN to obtain better access to the soil pores. Likewise, the McIlvaine buffer is able to prevent the complexation of analytes with cations such as Mg^{2+} or Ca^{2+} . To the greatest extent, the hydration step allows the reconstitution of the dry samples with a high water percentage, for which the QuEChERS method was originally designed [107]. However, satisfactory results were obtained with water in almost all the abovementioned studies. Only Meng and coworkers employed McIlvaine buffer in their extraction procedure [105]. Besides, the acidification of the hydration solution and/or ACN with hydrochloric acid or acetic acid has shown to improve analyte recoveries [101–104].

Three QuEChERS salts are known which are used intensively: (1) the original non-buffered method ($MgSO_4 + NaCl$), (2) the AOAC method with acetate buffer ($MgSO_4 + CH_3COONa$), and (3) the EN method with citrate buffer ($MgSO_4 + C_6H_5Na_3O_7 \cdot 2H_2O + Na_2C_6H_6O_7 \cdot 1.5H_2O$). The AOAC and EN buffers have the ability to acidify the medium. The extraction pH is a very important parameter, and a small variation can affect extraction efficiency, especially for acidic and basic compounds [19]. In the EN method, the citrate buffer provides a pH of 5–5.5, while in the AOAC method, the acetate buffer provides a more acidic pH of 4.8 [108]. Higher recoveries and bigger number of compounds were extracted using AOAC method [103, 104, 106]. This is referred to the acidic conditions obtained by

citrate buffer, which improves the extraction of some pharmaceuticals such as sulfonamides, macrolides, and β -lactams. However, satisfactory recoveries were obtained using the original unbuffered method [102, 105] and the EN citrate-buffered method [101] as reported in Table 5.

5 Extraction Procedures for Pharmaceuticals from Plant Tissues

The irrigation with reclaimed wastewater represents a source of xenobiotics as PhACs that are not removed by conventional WWTPs [109–111]. Consequently, agricultural soils contain a wide range of pharmaceuticals that could be uptaken by plants through the roots [40, 112]. Vegetables have different types of more or less branched roots (hairy roots in lettuce, napiform roots of radish, or tuberous roots of potatoes). However, the type or shape of the root does not influence the radical absorption which is similar for all the roots [2]. The PhACs dissolved in water arrive in the rhizosphere, come into contact with the root through the epidermis, and could enter the vascular system with the absorption of the roots [2]. Depending on several PhACs, physico-chemical properties such as polarity, ionizable ease, or lipophilicity could affect the pass through the endodermis and then arriving to the vascular tissue which is responsible for transporting nutrients to the rest of the plant [113]. Therefore, the PhACs capable of entering the vascular tissue can be translocated from the root to the aerial tissues such as leaves and fruits. In fact, the main source of PhACs in the leaves is related to their translocation from the roots to the leaves of the most polar compounds that move with water [114]. Contrary, non-polar (high K_{ow}) PhACs are more susceptible to remain in the root compartment commonly in the cell vacuoles and then bioaccumulating in the root [2]. Moreover, when PhACs are inside the tissues, they could be metabolized, creating new molecules, usually with unknown fates and properties [115]. Therefore, in order to be able to detect and quantify the pharmaceutical products absorbed by plants at low environmental concentrations, accurate and reliable analysis methods are required to be applied to the different tissues of which the plants are made [116]. In fact, each fabric presents a separate challenge to overcome. Plants are characterized by containing large quantities of sugars, waxes, fatty acids, and chlorophyll, which complicates the analysis due to the difficulty of eliminating these substances. These components of the matrix, if not carefully removed, can interfere with instrumental analysis by acting as co-eluent of the target compounds [117, 118]. Taking this into account, the different extraction processes (QuEChERS, ASE, USE, MAE, etc.) are followed by a subsequent cleaning phase to obtain clean extracts for accurate detection and quantification. Ideally, a selective extraction technique allows the recovery of the compounds of interest, while the interferents remain without being extracted into the matrix [45, 119, 120]. Extraction methods that require the use of high temperatures such as ASE or MAE and in some cases due to the USE are more likely to obtain

extracts containing co-extracted elements of the matrix. Nevertheless, to overcome this problem, modern analytical techniques include strategies such as the reduction of the sample quantities, the lower solvent consumption, or the reduction of the extraction time, to favor the reduction of unwanted components of the matrix. The most used clean-up processes are SPE and d-SPE which allow to obtain fairly clear extracts. However, the skip of this step could improve the recoveries of some compounds that are retained by the SPE adsorbents. LC-MS is usually the instrumental analysis performed, thanks to its ability to achieve low limits of quantification and detection, good repeatability, and reproducibility [7, 121]. Indeed, LC-MS/MS have been the instrumental analysis with great confidence to confirm the analytes and its capability to reach very low limits of detection and quantification [122, 123]. All details for each extraction methodology for pharmaceuticals in plants are reported in Table 6.

5.1 Assisted Solvent Extraction (ASE)

In order to perform a good extraction by means of ASE, the selection of an appropriate organic solvent as well as temperature is crucial. In fact, ASE provides the advantage to choose between several solvents allowing to extract compounds of different families in different matrices [124]. ASE uses temperature and pressure in its procedure, which means that modification on the solvent physico-chemical properties occurs facilitating the release of pharmaceuticals. As an example, solvent density decreases allowing a more extensive wet into the sample, whereas solvent boiling point increases due to the effect of the pressure [124]. However, high temperatures could deteriorate the PhACs, especially the thermolabile ones; then, a compromise between enhance on the extraction efficiency and the temperature is established. First, a dispersing agent (sand, hydromatrix, or Florisil) is mixed with the sample in order to avoid sample agglomerates, obtaining a greater surface and, consequently, facilitating the solvent interaction. Moreover, Florisil is the dispersant agent more used before the extraction starts, in order to improve the extraction efficiency through the adsorption of sugar and waxes with the subsequent retention [125, 126]. Samples are placed in a stainless-steel extraction cell and capped with filters at both ends to retain the sample into the cell but letting out the extraction solvent containing the extracted compounds [127]. Once the solvent is introduced into the system (different volumes could be employed), the extraction cell is placed in the oven, and the system starts setting the desired temperature and pressure. Another important parameter to consider consists of the time and cycles used, as the more time the solvent is in contact with the sample, the more extraction efficiency is performed. However, the co-extractive compounds are also released. Then, a compromise between time and extraction efficiency is created and should be optimized [124]. SPE was usually performed as the clean-up step after the extraction, removing interferences and obtaining cleaner extracts [74, 128]. For this reason, it is easier to adjust the pressure than the temperature of the ASE [128, 129]. Similar to

Table 6 Extraction methodologies from plants

Matrix and target molecules	Weight/volume of sample	Extraction solvent	Salts	Buffer	Clean-up	Analytical method	Reference
Honeys, honeybees, and pollens – pesticides, veterinary drugs, and a synergist	2 g dry weight	8 mL H ₂ O, 10 mL ACN, 3 mL hexane	4 g anhydrous MgSO ₄ , 1 g NaCl	1 g sodium citrate dehydrate, 500 mg disodium citrate sesquihydrate	15 mL solution for d-SPE (900 mg of anhydrous MgSO ₄ , 150 mg PSA bonded silica, 150 mg C18 bonded silica)	HPLC-MS/MS, GC-ToF	[146]
Milk and chicken feed – benzimidazoles and coccidiostats	10 g dry weight	10 mL deionized water, 10 mL MeCN	4 g of MgSO ₄ , 1 g NaCl	None	150 mg MgSO ₄ , 50 mg C18, 50 mg PSA	DART-HRMS	[147]
Animal feed – 13 sulfonamides	5 g dry weight	10 mL H ₂ O, 10 mL acetic acid 0.1% (v/v) in ACN; MeOH (75:25 v/v)	4 g anhydrous MgSO ₄	0.5 g sodium acetate	200 mg PSA	HPLC-MS/MS	[148]
Tomato fruit – lufenuron	15 g wet weight	15 mL ACN	6 g anhydrous MgSO ₄ , 1.5 g NaCl	None	100 mg PSA, 600 mg anhydrous MgSO ₄	HPLC	[36]
White radish, Chinese cabbage, cucumber, string bean, and green pepper – 26 veterinary antimicrobials	10 g wet weight	10 mL MeCN/MeOH (85:15, v/v)	4 g of anhydrous MgSO ₄ and 1 g of NaCl	1 g of H ₃ Cit·H ₂ O, 0.5 g of Na ₃ Cit·2H ₂ O	70 mg PSA	HPLC-MS/MS	[149]
Herbal dietary supplements – 96 pharmaceuticals, plant toxins, and other plant secondary metabolites	1 g dry weight	10 mL H ₂ O, 10 mL ACN, ACN (2% formic acid)	4 g anhydrous MgSO ₄ , 1 g NaCl	None	0.22 µm Teflon filter, d-SPE (100 mg C18, 300 mg MgSO ₄)	UHPLC-HRMS	[143]

(continued)

Table 6 (continued)

Matrix and target molecules	Weight/volume of sample	Extraction solvent	Salts	Buffer	Clean-up	Analytical method	Reference
Celery and lettuce – 11 pharmaceutical compounds	500 mg dry weight	7 mL ACN/MeOH/H ₂ O (74.1:0:28.6), (60.7:10.7:28.6), (53.6:17.9:28.6), (35.7:35.7:28.6)	2 g anhydrous Na ₂ SO ₄ , 0.5 g NaCl	150 mg L ⁻¹ Na ₂ EDTA	d-SPE (12.5 mg C18, 12.5 mg PSA, 225 mg Na ₂ SO ₄)	HPLC-MS/MS	[119]
Lettuce leaves – carbamazepine, flumequine, and thiabendazole	10 g wet weight	10 mL of ACN	6 g of MgSO ₄	1.5 g of NaOAc	d-SPE (125 mg PSA, 150 mg C18, 750 mg MgSO ₄), 0.45 µm PTFE filter	LC-QqLIT-MS/MS	[122]
Sedative functional foods (based on <i>Sativa miltiorrhiza</i> and <i>Schisandra chinensis</i>) – 11 blockers	2 g wet weight	5 mL of acetic acid/ACN/MeOH (0.1:3:7, v/v/v)	15 mL of acetic acid/ACN/MeOH (0.1:3:7, v/v/v) (second round)	None	d-SPE (50 mg PSA, 500 mg MgSO ₄)	UPLC-MS/MS	[150]
Baby food (some of vegetal origin) – sulfonamides	5 g dry weight 15 g wet weight	10 mL ultrapure water, 15 mL of 1% acetic acid in ACN 15 mL of 1% acetic acid in ACN	6 g MgSO ₄	1.5 g NaOAc	150 mg PSA, 150 mg C18, 900 mg MgSO ₄	UHPLC-HR/MS	[139]
Buckwheat and related products – atropine and scopolamine	5 g dry weight	10 mL H ₂ O, 10 mL ACN containing 1% (v/v) of formic acid	4 g of anhydrous Na ₂ SO ₄	1 g NH ₄ Ac	25 mg of PSA, 25 mg of GBC	UHPLC-MS/MS	[35]
Lettuce – 13 wastewater-derived contaminants	0.5 g dry weight	20 mL ACN-MeOH (1:1, v/v), MTBE-MeOH (1:1, v/v), hexane-	4 g MgSO ₄ , 1 g NaCl	1 g sodium citrate	0.2 µm PTFE filters, liquid-liquid extracted with hexane (hexane)	HPLC-MS	[7]

Lettuce, cabbage, and tomato – 28 organic micropollutants	0.5 g dry weight	acetone (1:1, v/v), MTBE:ACN (1:1, v/v), EtAc:MeOH (1:1, v/v), 0.5% formic acid in ACN:MeOH (1:1, v/v)	4 g anhydrous MgSO ₄ , 1 g NaCl	None	150 mg PSA, 900 mg MgSO ₄ , 0.2 µm syringe RC filter	LC-MS/MS	[45]
Plant food supplements – 26 pharmaceutical compounds	0.5 g or 5 mL	10 or 5 mL H ₂ O (0.1% formic acid), 10 mL ACN	4 g anhydrous MgSO ₄ , 1 g NaCl	None	0.22 µm PTFE filter	UHPLC-MS/MS	[140]
Radish roots and leaves – chlortetracycline, enrofloxacin, sulphathiazole	10 g wet weight	20 mL MeCN with 1% HAc	None	1 g SCTD, 500 mg SCDS, 100 mg EDTA-Na ₂	d-SPE (30 mg PSA, 30 mg C18)	LC-MS/MS	[141]
Lettuce, grapes, milk, chicken, pork, beef – 17 pesticides, 16 alkaloids, 18 drugs	1 g wet weight	1 mL H ₂ O (only in low water content samples), 1 mL ACN	400 mg MgSO ₄ , 100 mg NaCl	1 mL of pH 10 glycine buffer	d-SPE (75 mg, MgSO ₄ :PSA:C18: GCB 3:1:1 (w/w/w/w))	GC-MS, LC-MS/MS	[151]
Lettuce shoots and roots – 11 pharmaceutical compounds	0.25 or 0.5 g dry weight	2 mL H ₂ O, 5 mL ACN/MeOH (65:35, v/v)	2 g Na ₂ SO ₄ , 0.5 g NaCl	150 mg/L Na ₂ EDTA	12.5 mg C18, 12.5 mg PSA, 225 mg Na ₂ SO ₄	HPLC-MS/MS	[32]
Radish and enzyme extracts – 15 pharmaceuticals	0.5 g dry weight	2 mL H ₂ O, 5 mL ACN/MeOH (65:35, v/v)	2 g Na ₂ SO ₄ , 1 g NaCl	150 mg/L Na ₂ EDTA	250 mg Na ₂ SO ₄ , d-SPE (25 mg C18, 25 mg PSA), d-SPE (25 mg C18, 25 mg PSA)	LC-MS/MS	[46]

(continued)

Table 6 (continued)

Matrix and target molecules	Weight/volume of sample	Extraction solvent	Salts	Buffer	Clean-up	Analytical method	Reference
Lettuce, radish, and strawberry – 74 microcontaminants including pharmaceuticals, metabolites, and pesticides	10 g wet weight	10 mL of 1% acetic acid in MeCN and 20 μ L of the extraction quality control solution, 10 mL of MeCN	6 g of anhydrous MgSO ₄ 4 g of anhydrous MgSO ₄ , 1 g of NaCl	1.5 g of NaOAc 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogencitrate sesquihydrate	25 mg PSA, 10 mg GCB) d-SPE (750 mg of anhydrous MgSO ₄ and 125 mg of C18) d-SPE (750 mg of anhydrous MgSO ₄ , 125 mg of C18, and 125 mg of PSA)	HPLC-MS/MS	[121]
Cabbage, cucumber, cauliflower, leek, and other commonly consumed vegetables – 49 antibiotics (17 sulfonamides, 16 quinolones, 6 macrolides, 5 β -lactams, 5 tetracyclines)	10 g wet weight	10 mL ACN (0.248% H ₃ PO ₄ in phosphate buffer and 0.1% of formic acid in formic acid buffer)	4 g MgSO ₄ , 1 g NaCl	McIlvaine buffer (0.049, or 0.19, or 0.46 g Na ₂ HPO ₄ , 0.5 g citrate) Phosphate buffer (0.5 g KH ₂ PO ₄) Formic acid buffer	d-SPE (10 mg PSA, 25 mg C18, 2.5 mg GCB) Other concentrations were tested with less recovery	UHPLC-MS/MS	[152]
14 different fruits and vegetables – penicillins G and V	1 g wet weight	2 mL of ACN	0.5 g of NaCl	40 mg of EDTA	0.45 μ m membrane filter	HPLC-QToF-MS/MS	[123]
<i>Lepidium sativum</i> – diclofenac, ketoprofen, naproxen, and mefenamic acid	1 g wet weight	3 mL ACN/0.1 M HCl (2/1, v/v) 4 mL ACN/0.1 M HCl (1/1, v/v)	0.8 g MgSO ₄ and 0.2 g NaCl 1.2 g MgSO ₄ , 0.3 g NaCl	None	None	HPLC-MS/MS	[153]
Leafy vegetables – 20 antibiotics	10 g wet weight	10 mL of ACN/MeOH (85:15, v/v)	4 g of anhydrous MgSO ₄ , 1 g of NaCl	1 g of citric acid monohydrate, 0.5 g of trisodium citrate dihydrate	C18 (30, 50, and 70 mg) and GCB (10, 30, and 50 mg)	UHPLC-MS/MS	[154]

Lettuce crops – 24 carbamazepine transformation products	10 g wet weight	10 mL of MeCN at 1% of acetic acid	6 g of MgSO ₄	1.5 g of NaOAc	d-SPE (125 mg PSA, 125 mg C18, 750 mg anhydrous MgSO ₄)	LC-QTOF-MS/MS	[155]
<i>Lepidium sativum</i> – atorvastatin, fluvastatin, simvastatin	1 g wet weight	4 mL ACN:H ₂ O (1:1, v/v)	1.2 g MgSO ₄ , 0.3 g NaCl	None	None	HPLC DTIM QTOF-MS, HPLC QQQ/MS/MS	[156]
Soil and beet root – bisoprolol, metoprolol, sulfamethoxazole, trimethoprim, caffeine	10 g dry weight	10 mL ACN (1% acetic acid)	4 g anhydrous MgSO ₄ , 1 g NaCl	1 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate sesquihydrate	150 mg PSA, 900 MgSO ₄ mg, 150 mg C18	LC-MS/MS	[157]
Lettuce, maize, and radish – atenolol, carbamazepine, and triclosan	1 g dry weight	10 mL ACN	4 g MgSO ₄ + 1 g NaCl	Bond Elut QuEChERS Dispersive Universal Kit	None	LC-MS/MS QqQ	[158]
Chicory – diclofenac	2 g wet weight	4 mL ACN	2 g MgSO ₄ + 0.5 g NaCl	0.5 g sodium citrate tribasic dihydrate + 0.25 g sodium citrate dibasic sesquihydrate	150 mg MgSO ₄ + 25 mg PSA + 25 mg C18E	UPLC-MS/MS QTOF	[159]
Lettuce – 4-hydroxydiclofenac, 5-methyl-benzotriazole, acesulfame, acetaminophen, acridone, benzotriazole, bezafibrate,	1 g dry weight	10 mL CAN + 50 µL formic acid	4 g MgSO ₄ + 1 g NaCl	None	150 mg PSA, 900 MgSO ₄ mg, 150 mg C18	HPLC-MS/MS QTOF	[160]

(continued)

Table 6 (continued)

Matrix and target molecules	Weight/volume of sample	Extraction solvent	Salts	Buffer	Clean-up	Analytical method	Reference
bisphenol A, caffeine, carbamazepine, carbamazepine-10,11-epoxide, chloramphenicol, ciprofloxacin, citalopram, climbazole, clofibrac acid, diclofenac, diltiazem, fipronil, fipronil-desulfinyl, fipronil sulfone, fluconazole, furosemide, gemfibrozil, hydrochlorothiazide, ibuprofen, indomethacin, irbesartan, ketoprofen, lamotrigine, metoprolol, metronidazole, N-acetylsulfamethoxazole, oxcarbamazepine, propranolol, sucralose, sulfamethazine, sulfamethoxazole, triclosan, valsartan, valsartan acid, verapamil	500 mg dry weight	8 mL MeOH:HCl (95:5, v/v)	None (sonication method)	None	SPE clean-up (Oasis HLB 60 mg cartridge)	LC-MS/MS (ion trap mass spectrometer)	[112]
Cucumber, tomato, and lettuce – tetracycline, oxytetracycline, chlortetracycline, sulfamethazine, sulfamethoxazole, sulfadimethoxine							

Soil and radish leaf – chlordiazepoxide, clonazepam, diazepam, flurazepam, nordiazepam, oxazepam, temazepam, triazolam	1 g soil 1 g wet weight plant	Soil: 1 g sand +2*5 mL MeOH +1*5 mL acetone Plant: 1 g sand +3*5 mL ACN:H ₂ O (70:30, v/v) (15 mL total)	None (sonication method)	None	SPE clean-up (Oasis HLB 6 mL 200 mg)	LC-MS/MS	[31]
Radish and ryegrass – carbamazepine, diclofenac, fluoxetine, propranolol, sulfamethazine, triclosan	1 g wet weight of plant (leaf and root)	1 g sand +3*5 mL ACN:H ₂ O (70:30, v/v)	None (sonication method)	None	SPE clean-up (Oasis HLB 6 mL 200 mg)	LC-MS/MS (Thermo Finnigan TSQ Quantum Discovery Max)	[133]
Chinese white cabbage, water spinach, Chinese radish, corn, and rice – tetracycline, sulfamethazine, norfloxacin, erythromycin, chloramphenicol	1 g weight crop	30 mL acidified ACN:acetone (1:1, v/v, pH = 3)	None (ultrasonication)	None	SPE clean-up HLB cartridges	LC-MS/MS QqQ	[136]
Tomato – diclofenac, sulfamethoxazole, and trimethoprim	10 g wet plant	TMP and DCF: 10 mL MeOH + NaHCO ₃ (pH 6–7) SMX: 10 mL MeOH (pH 3–4)	None (sonication method)	None	None	UPLC-MS/MS QqQ	[44]
<i>Pisum sativum</i> L. var. <i>axiphium</i> – carbamazepine, atenolol, sulfamethoxazole, and metabolites	0,1 g dry weight	1 mL ACN:H ₂ O (1:1, v/v, 0,1% formic acid)	None (sonication method)	None	None	LC-MS/MS QqQ	[43]

(continued)

Table 6 (continued)

Matrix and target molecules	Weight/volume of sample	Extraction solvent	Salts	Buffer	Clean-up	Analytical method	Reference
Chickpea – metformin	0,1 g dry weight	2 mL dichloromethane	None (sonication method)	1,9 mL (ammonium acetate 450 mmol L ⁻¹ formic acid 50 mmol L ⁻¹)	SPE (Bond Elut-LMS cartridges 1 mL, capacity 25 mg)	LC-ToF-MS	[42]
Celery, lettuce, cabbage, spinach, carrot, cucumber, bell pepper, tomato – 16 PhACs and 3 PCP	0,2 g dry weight	20 mL tert-butyl ether	None (USE method)	None	SPE clean-up (HLB cartridge 150 mg)	UPLC-MS/MS	[137]
Lettuce, tomato, cauliflower, and broad bean seeds – sulfathiazole, sulfamethizole, sulfadiazine, sulfamethazine, sulfadimethoxine, sulfamethoxazole, ofloxacin, enrofloxacin, clindamycin, and trimethoprim	1 g wet weight	10 mL MeOH	None (USE method)	None	SPE clean-up (Strata-X cartridge, 100 mg/6 mL)	LC-MS/MS (Waters TQ detector)	[134]
Lettuce, spinach, arugula, and radish – carbamazepine, atenolol, sulfamethoxazole	0,1 g dry weight	4 mL ACN:H ₂ O (1:1, v/v, acidified 0,1% formic acid)	None (USE method)	None	None	LC-MS/MS QqQ	[41]
Alfalfa – diclofenac, sulfamethoxazole, trimethoprim	5 g wet weight	10 mL MeOH:H ₂ O (1:1, v/v)	None (USE method)	None	SPE clean-up (Oasis HLB)	UPLC-MS/MS TQD	[161]
Cucumber – 17 PPCs	0,2 g dry weight	20 mL tert-butyl ether	None (USE method)	None	SPE clean-up (Oasis HLB 150 mg)	UPLC-MS/MS QqQ	[37]
Lettuce – 19 PPCs	0,2 g dry weight	20 mL tert-butyl ether +20 mL ACN	None (USE method)	None	SPE clean-up (Oasis HLB)	UPLC-MS/MS	[120]

<i>Typha latifolia</i> and <i>Berula erecta</i> – iopamidol, iohexol, iomeprol, iopromide, propranolol, carbamazepine, naproxen, ibuprofen, diclofenac	0,2 wet weight	10 mL MeOH:acetone (95:5, v/v)	None (ultrasonic solvent extraction)	None	SPE clean-up (reversed phase Phenomenex Strata-X 200 mg/6 mL + normal phase Supelclean LC-Florisil 1 g/6 mL)	HPLC-DAD	[162]
<i>Typha angustifolia</i> , <i>Arundo donax</i> , and <i>Lemna minor</i> – 31 CECS	1 g wet weight	8 mL EtAc 3% NH ₄ OH + 2*5 mL ACN 4% formic acid	4 g Florisil +2 g MgSO ₄ (ultrasound-assisted)	None	Glass-column clean-up (1 g MgSO ₄ + 1 g C18)	GC-MS	[135]
Cress – sertraline, clomipramine, and trazodone	1 g wet weight	2 mL ACN	None (sonication + centrifugation)	None	None	LC-MS/MS	[163]
Pea – trimethoprim, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine, crotamiton, gliclazide, carbamazepine, losartan, cyclophosphamide, acetaminophen, ketoprofen, diclofenac, indomethacin	Wet weight	ACN – MeOH 0,5% formic acid	None (sonication + centrifugation)	None	SPE (Oasis HLB)	LC-MS/MS	[164]
Cucumber – carbamazepine, carbamazepine epoxide, 10,11-dihydroxycarbamazepine, 2-hydroxycarbamazepine, 3-hydroxycarbamazepine, lamotrigine	1 g wet plant	MeOH + 1 g Florisil	None (ASE extraction)	None	None	LC-MS/MS QqQ	[126]

(continued)

Table 6 (continued)

Matrix and target molecules	Weight/volume of sample	Extraction solvent	Salts	Buffer	Clean-up	Analytical method	Reference
Cabbage – salbutamol, trimethoprim, sulfamethoxazole, carbamazepine, sertraline, triclosan	8 g wet plant	50 mL ACN:H ₂ O (55:45, v/v)	None (pressurized liquid extraction, PLE)	None	None	LC-MS	[40]
Carrot, potato – bezafibrate, carbamazepine, diclofenac, gemfibrozil, ibuprofen, clofibrac acid, sulfapyridine, ketoprofen, sulfamethoxazole, 10,11-epoxycarbamazepine	1 g dry weight	MeOH + 1 g Florisil	None (ASE method)	None	None	LC-MS	[125]
Lettuce and carrot – tetracycline and amoxicillin	0,1 g dry weight	100% Milli-Q water (final extract 30 mL)	None (PLE method)	None	SPE clean-up (Oasis HLB cartridges hydrophilic-lipophilic balance, 200 mg/6 mL)	LC-MS/MS QTrap Sciex	[39]
Grass – oxytetracycline, sulfamethoxazole, ketoconazole	1 g wet weight	MeOH: citric acid (0,2 M, 50:50, v/v, pH = 4,5 with NaOH)	None (PLE method)	Na ₂ EDTA	SPE clean-up (Strata-X 200 mg/6 mL, reverse phase)	LC-MS Orbitrap Thermo Fisher	[165]
Soybean and wheat – ketoprofen, naproxen, diclofenac, ibuprofen	0,5 g dry weight	0,01 M NaOH	None (pressurized hot water extraction – ASE)	None	Hollow fiber liquid-phase microextraction clean-up	LC-MS	[131]

Wheat – sulfamethoxazole, trimethoprim, ofloxacin, carbamazepine	1 g dry weight	MeOH 70 mL	None (ASE method)	None	SPE clean-up (Oasis HLB)	LC-MS/MS	[129]
Cowpea, turnip, chard, collards, basil, lettuce, and cilantro – atenolol, ofloxacin, and diclofenac	– Cowpea, turnip: 3 g dry weight – Chard, collards: 1,5 g dry weight – Basil, lettuce cilantro: 1 g dry weight	70–75 mL methylene chloride:MeOH (75:25, v/v)	None (ASE method)	None	Turnip and Chard: SPE clean-up (Oasis HLB) 3 mL, 540 mg	LC-MS	[128]
Lettuce, spinach, carrot – azithromycin, roxithromycin, clarithromycin, clindamycin, methamphetamine, MDMA, pseudoephedrine, DMPEA	1 g dry weight	MTBE:MeOH (90:10, v/v) 5 mL final extract	None (ASE method)	None	TurboVap clean with hexane	LC-MS/MS ion trap	[130]
<i>Phragmites australis</i> – 81 micropollutants	0,5 g dry weight	25 mL MeOH:H ₂ O (25:75, v/v)	None (microwave-assisted extraction)	None	SPE clean-up (Oasis HLB)	UHPLC-MS/MS QqQ	[145]
Cereals (wheat, barley, oat, and rice) – 4 fluoroquinolones, 3 tetracyclines, 7 sulfonamides,	2 g wet weight	MAE: 6 mL ACN: MeOH:phosphoric acid 5% (7:1:2, v/v) USE: 7 mL ACN:	None (MAE, USE method)	None	d-SPE clean-up (250 mg PSA)	LC-MS/MS QqQ	[33]

(continued)

Table 6 (continued)

Matrix and target molecules	Weight/volume of sample	Extraction solvent	Salts	Buffer	Clean-up	Analytical method	Reference
4 macrolides, and lincomycin		MeOH:phosphoric acid 5% (7:1:2, v/v)					
Lettuce – lincomycin, roxithromycin, doxycycline hyclate, demeclocycline, sulfamethoxazole, oxytetracycline	2 g wet weight	10 mL (80% isopropyl alcohol, 20% 0,04 M citric acid solution)	None (freeze-thaw extraction)	None	SPE clean-up (200 mg Oasis HLB)	LC-MS/MS	[166]
<i>Brassica juncea</i> – acetaminophen	0,5 g wet weight (root and leaf)	1 mL HCl 0,1 M	None (solid-liquid extraction)	None	SPE method, Phenomenex Strata-X 300 mg	LC-MS	[28]
Cucumber, eggplant, long bean, and wheat – sulfamonomethoxime, sulfathiazole, acetylsulfamethoxazole, sulfisoxazole, sulfachlorpyridazine, sulfamethazine, sulfamerazine, trimethoprim, chloramphenicol, ibuprofen, sulfamethoxazole, and triclosan	5 g wet grain crop 2 g wet fruit and vegetables	220 mL ACN	None (Soxhlet extraction)	None	None	LC-MS/MS QqQ	[34]

soil, MeOH, ACN, and water are the most used solvents for the extraction procedure. Different mixtures have been reported in order to achieve the optimum polarity conditions to extract the targeted compounds and avoiding interferences from the matrix plant [40, 74, 130]. Additionally, buffer mixtures such as citric acid and NaOH are included providing the properly pH into the mixture and enhancing the extraction efficiency. The most used extraction solvent, MeOH, provided good results in carrot and potato, coming to detect ten PhACs in a single analysis [40] but also in wheat [129] and in cucumber [126]. Using water as extractant solvent helped Azanu et al. to extract tetracycline and amoxicillin from lettuce and carrot [39]. On the other hand, Cortés et al. managed to extract four pharmaceuticals from soybean and wheat using buffered water (0.01 M NaOH) [131]. It is worth to mention that the greenest method consists of using water as a unique extraction solvent.

Up to 15 different plant matrices were studied under ASE methodology in only 9 reports (Table 6), indicating the versatility of ASE. Moreover, lettuce and carrot were the matrices most studied (three reports for each matrix). Reports showed the capability to develop one method for the detection of PhACs in several matrices [39, 130].

5.2 *Ultrasound Solvent Extraction (USE)*

The USE methodology is certainly the most widely performed extraction method in the environmental field. The base of the USE is centered in the formation of small cavities inside the sample by means of ultrasound, for a subsequent penetration of the solvent causing a greater contact surface between the sample and the extraction solvent, enhancing PhACs extraction [132]. A further comminution of the samples could occur due to the increase in collisions between the particles due to the vibration of ultrasound and to the disturbances created for cavitation [132]. Dispersant agents such as sand are commonly employed to help the formation of cavities into the samples by impact of sand particles into the sample [133]. USE is known to be an easy extraction method but also for the use of low quantities of solvent. However, the heat that is usually generated by particle collisions on the one hand could facilitate the release of some classes of target compounds; in the case of PhACs, it could lead to negative effects due to a possible thermal degradation. The sonication time and the number of cycles are parameters that must be optimized to ensure good extraction performance. Normally, the sonication time varied from 10 to 30 min, and 1 to 4 cycles are performed [112]. The organic solvents most used in the extraction of PhACs from plants are mainly the same as previously seen as MeOH, ACN, and water, used individually or in different mixtures [41, 134]. In particular, the ACN/water mixture has been used successfully for the extraction of pharmaceutical residues in radish, ryegrass, *Pisum sativum*, lettuce, spinach, and rocket with satisfactory results [31, 41, 43, 133]. Buffered mixtures containing NaHCO₃, formic acid, or NH₄OH were also used providing an adequate pH for a better extraction

performance especially for the acidic compounds which are more sensitive to the pH [44, 112, 135].

Up to 16 studies have been reported to employ USE in order to extract PhACs from plant matrices (Table 6). The versatility and applicability of USE have been widely demonstrated on about 20 different vegetable matrices with good results. Lettuce, radish, and tomato were the most studied matrices [31, 41, 44, 112, 120, 133, 134, 136, 137].

The most interesting compounds studied were antibiotics, benzodiazepines, and nonsteroidal anti-inflammatory drugs (NSAIDs), such as carbamazepine, diazepam, sulfamethoxazole, sulfamethazine, trimethoprim, etc.

5.3 *QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe)*

The QuEChERS method is the most used extraction technique for pharmaceutical residues. In fact, more than 25 studies using QuEChERS for drugs in plant tissues are reported in Table 6. The extraction of QuEChERS mainly depends on the selection of three phases: the solvent, the salts required, and the cleaning phase. Many of the reported studies are based on more or less important modifications of the original procedure developed by Anastassiades et al. for the determination of pesticides in food [19]. In the original method, ACN was the solvent selected for its ability to extract non-polar and relatively polar compounds and has the ability to leave lipophilic substances without being extracted [138]. In addition, ACN mixes easily with water allowing the penetration of the organic solvent into the matrix [138]. Reports have shown that ACN is by far the most widely used solvent, sometimes buffered with acidifying compounds to improve extraction efficiency. Different solvents such as H₂O or MeOH have also been used less frequently but also providing good extraction recoveries. The original method requires a sample weight of 10 g of fresh material with a water content greater than 80%. Sometimes, for dry, dehydrated, or fat-rich samples, water is added up to the minimum quantity required for the extraction [35, 139, 140]. Acidification of the organic solution, majorly with acetic acid or formic acid, has shown to improve the recoveries of compounds susceptible to pH [121, 141]. The main difference with the other extraction methods consists in the use of salts which force the analytes to pass from the aqueous phase to the organic phase, known as the salting out process [142]. Taking as a reference the original method, MgSO₄ with NaCl were the salts employed [19]. MgSO₄ is used for several reasons. In fact, it helps in the separation between phases (ACN/water) by saturation and is also used for its drying capacity. Finally, the drying process involves an exothermic process with consequent release of heat which leads to a better extraction of the non-polar compounds [138]. On the other hand, the main purpose of NaCl consists of the reduction of polar interferences and co-extractives coming from the plant [138]. Three main types of salts are used in

plants, the original non-buffered salts ($\text{MgSO}_4 + \text{NaCl}$), the EN-buffered salts consisting of the original salts and the addition of citrate buffers ($\text{MgSO}_4 + \text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O} + \text{Na}_2\text{C}_6\text{H}_6\text{O}_7 \cdot 1.5\text{H}_2\text{O}$), and the AOAC method with acetate buffer ($\text{MgSO}_4 + \text{CH}_3\text{COONa}$). The pH is extremely important in this extraction procedure. The EN and AOAC extraction salt kits consist of buffered salts which result in a pH between 5 and 5.5 for the EN method and a $\text{pH} < 5$ for the AOAC. In contrast, the final pH of the original method mainly depends on the nature of the initial matrix [142]. However, current studies suggest that the original salt kit is the most used salt mix to extract pharmaceutical residues from plant tissues, with excellent results [45, 140, 143]. On the other hand, AOAC salts and EN salts were widely used providing more stability to the target compounds [7, 121]. Aside from the extraction phase, Anastassiades and colleagues also developed a new clean-up methodology to remove any interferences based on a solid-phase extraction in the dispersive phase (d-SPE) [19]. Further considerations are shown in the clean-up section. The matrices studied so far are mainly vegetables such as lettuce, radish, and cabbage (Table 6). In particular, plants consisting of a branched root are the most studied samples, but fruits such as cucumber, tomato, and strawberry are also being studied. QuEChERS has become the most used extraction method with exponential growth in recent years, and everything seems to indicate that thanks to its versatility and its possible future automation, this growth seems even more pronounced in the near future.

5.4 Other Extraction Methods (MAE, Soxhlet, Solid-Liquid Extraction)

The microwave-assisted extraction method (MAE) is a technique widely used in soils but has not had the same diffusion with plants, at least for now. The MAE consists in the use of a correctly selected organic solvent for the extraction of target drugs which is heated by microwaves, combining sonication and temperature properties [144]. This technique is widely used for the analysis of metals and heavy metals in plant material. However, it is also used in the field of PhACs, although the quantity of solvent is reduced and the obligation to operate at high temperatures could adversely affect the extraction of the target compounds. The organic solvents used are the same as previously described for other methodologies. Basically, ACN, MeOH, and water, including their mixtures, are the solvents selected for vegetables. Only two studies with plants have been reported, one with *Phragmites australis* and the other with cereals [33, 145].

Recent works have demonstrated the ability of the classic Soxhlet extraction method to detect pharmaceutical products in plants and to detect 12 pharmaceutical products in different vegetables [34]. However, Soxhlet extraction is not usually used because, due to the high working temperatures required by Soxhlet and also the long exposure times, this method could alter the properties of the target compounds,

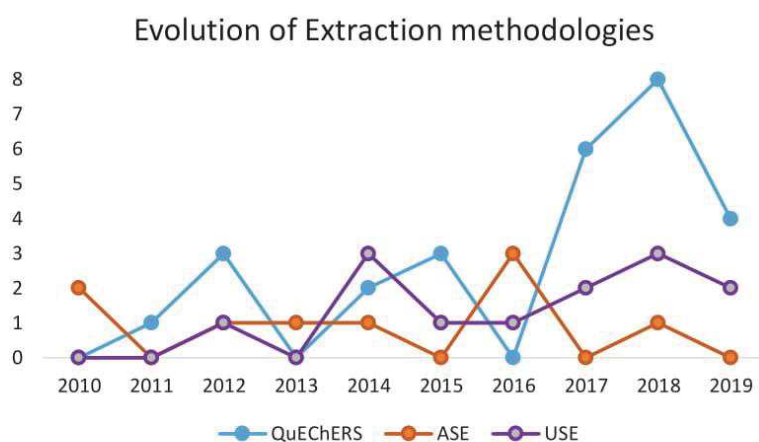


Fig. 1 Comparison of the use of the three main extraction methods (USE, ASE, and QuEChERS) for pharmaceutical residues in plant tissues reported in the last 10 years

in particular thermolabile ones. In addition, a high amount of solvent is required, making the procedure more expensive and polluting than modern methods.

Finally, solid-liquid extraction (SLE) is a simple but effective extraction method used when a liquid matrix contains the target compounds. It is a technique widely used for water analysis. Although the new methodologies (USE, ASE, QuEChERS, etc.) are able to provide more selectivity and also great results, SLE can be used in combination with these techniques as an extract clean-up phase. Despite this, Bartha et al. managed to analyze the paracetamol from the root and leaves of *Brassica juncea* [28].

Figure 1 shows the evolution of the three main extraction techniques for plants over the past 10 years. The use of these techniques between 2010 and 2016 was quite similar, but since 2017 QuEChERS has been the main methodology followed by the authors to extract PhACs from plant tissues, thus demonstrating its versatility in this area.

6 Clean-Up Procedures

Environmental and food samples have very complex matrices, and part of its constituents are involuntarily co-extracted during the usual extraction procedures. Furthermore, although the high temperature used in ASE and MAE and the strong sonication waves used in USE are very effective in the extraction of the compounds of interest, as a counterpart, they facilitate the migration of matrix interference in the final extracts [56]. Consequently, as a main effect, these elements can mask the detection of analytes during chromatography. Therefore, cleaning the extracts is a crucial step. A large diversity of sorbents is today available and successfully

employed for extract clean-up from matrices interfering components. These include, for example, SPEs in the reverse phase (HLB, C8, C18, etc.), normal phase SPEs (alumina, diol, Florisil, silica, etc.), and the ion exchange mode SAX (strong anion exchange) or MCX (mixed-mode cation exchange). The various sorbents mentioned are used in the form of discs, columns, dispersive phase or cartridges. However, the latest format is the most widely used. In this section, we will discuss the commonly used sorbents for soil and crop extract clean-up, as well as the newly introduced sorbents.

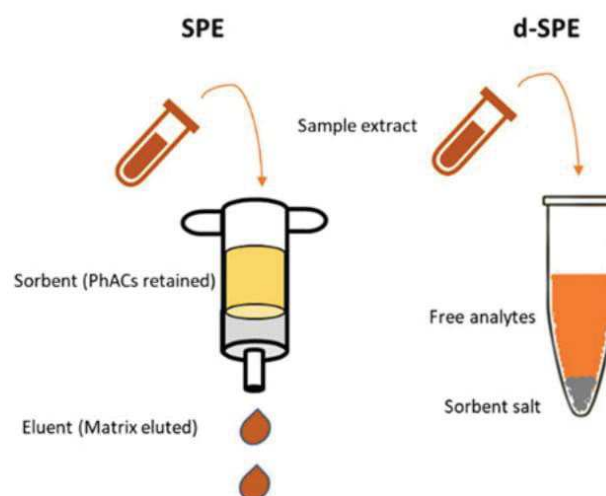
6.1 Commonly Used Sorbents

Solid-phase extraction (SPE) in cartridge format is one of the most clean-up techniques used over the last years. The great selectivity of SPE in retaining the targeted compounds and simultaneously eluting the matrix interferences, or vice versa, has made this technique gain wide popularity. Moreover, they allow for a large volume of extract to be purified and concentrated at the same time. As previously mentioned, a large diversity of sorbents is today available; however, for soil and crop extracts, SPEs using hydrophilic and lipophilic balance (HLB) sorbent cartridges solely [72, 82] or in combination with the strong anion exchange sorbent (SAX) [70, 75], which is more expensive, are the purification techniques predominantly employed. HLB cartridges are able to bind non-polar and polar pharmaceuticals at the same time if they are in their neutral form in the extract, whereas, when HLB is employed in tandem with SAX on top of it, SAX cartridges are able to retain matrix interferences such as the negatively charged humic and fulvic acids present in soil extract or the waxes, fatty acids, chlorophyll, and pigments present in plant extracts, while pharmaceuticals are retained on HLB cartridges, resulting in further purified extracts [58].

Those two dominant SPE sorbents are in general used packed in cartridges, and they are mostly employed after a main extraction with ASE, MAE, and USE. However, Malvar and coworkers [69] have recently employed an in-cell clean-up with PLE (or ASE), also known as selective PLE (SPLE), where they added C18 and PSA to the extraction cell with the sample in dispersive form, which allowed to avoid the need of further treatment of sample extract. To our knowledge, this is the only study in which SPLE was employed.

Besides SPE cartridges, dispersive SPE (d-SPE) is nowadays very popular. The concept of d-SPE was first introduced by the fathers of QuEChERS [19], and from that time, it was always employed for cleaning up the extracts of diverse kinds of matrices obtained from QuEChERS extraction. SPE cartridges and d-SPE have similar purpose; the main difference between them remains on the usage mode. For the SPE cartridges, sample extract is loaded into the cartridges, whereas for the d-SPE, sorbents are in powder form (sorbent salts) and are added on the extract, where they absorb matrix interferences resulting in a free analyte extract (Fig. 2). By comparing both SPE techniques, d-SPE is easier to use and saves time.

Fig. 2 Employment differences between SPE and d-SPE



Since QuEChERS requires no external forces, less matrix components are extracted. However, most of the reported studies employed the easy and rapid clean-up step using d-SPE, in order to reduce matrix effect and improve limits of detection and quantification. C18, primary secondary amine (PSA), and magnesium sulfate (MgSO_4) are the most known and commonly used d-SPE sorbents for extract clean-up. MgSO_4 is used as dehydrating agent to remove water excess in organic solvents; C18 eliminates non-polar matrix interferences such as fats, lipids, and some minerals; and PSA is commonly used to retain polar organic acids such as fatty acids and pigments by strong hydrogen bonds. Since only a few studies (there are exactly six studies) employed QuEChERS for soil extraction, we will discuss them in details from here on. The C18 is nearly always used in all the reported studies, either solely or in combination with MgSO_4 and PSA. Lee and coworkers used C18 sorbent solely for extract clean-up, and satisfactory recoveries were obtained ranging between 60.2 and 120.3% [103]. The addition of magnesium sulfate and primary secondary amines was excluded in their study, because the first liberates energy that might influence on the stability of the tested compounds whereas the second have the ability to chelate/bind with compounds such as sulfonamides. De Carlo and coworkers [102] tested C18 + MgSO_4 and PSA + MgSO_4 separately, and the best results were obtained with C18 + MgSO_4 . The clean-up performed adding C18 + MgSO_4 allowed to recover all target analytes, whereas with PSA + MgSO_4 , only two out of the five target analytes were recovered. This can be explained by the adsorption of analytes on the PSA by strong hydrogen bonds established between the hydrogen-donor hydroxyl group of the studied analyte and the hydrogen-acceptor nitrogen of PSA, together with other weaker hydrogen-bonding interactions. Same observation was obtained with Martínez-Piernas and coworkers [104], where higher recoveries were obtained using the mixture C18 + MgSO_4 while the presence of PSA reduced the extraction efficiency. This is also referred to the ability of PSA to act as a chelating agent with acidic compounds such as clofibric acid, furosemide,

indomethacin, ketoprofen, ketorolac, mefenamic acid, and methylprednisolone. Finally, similar recovery yields were obtained by those two studies (88–113% [102] and 70–120% [104]). On the contrary, this PSA effect was not observed with Meng and coworkers [105] when it was added in a reasonable amount (25 mg), and better recoveries (61.4–118.9%) and matrix effect (–40 to 54%) were obtained using the combination 25 mg of PSA, 10 mg of C18, and 100 mg of MgSO₄. On the other hand, when the clean-up step was omitted, chromatograms with less quality were obtained, due to the presence of a noisy baseline and interfering peaks derived from co-extracted components [102]. However, no clean-up was employed by Bragança and coworkers (recoveries >80%) [101], and no significant matrix effect was observed in their results. Finally, Salvia and coworkers [106] tested the d-SPE using several sorbents: PSA, PSA + C18, Florisil, silica, aluminum oxide, and SAX. However, great matrix effect was always obtained (> 80%), whereas satisfactory results were obtained using SPE cartridges (SAX and Strata-X cartridges) which was adopted in their final method.

Finally, despite the advantages provided from the clean-up, some authors believed that SPE step causes loss of analytes, so they omitted the clean-up step in both soil and crop matrices [140, 153].

6.2 New Sorbents

Several sorbents have been introduced recently for a better clean-up step, among them we list: Z-Sep, Z-Sep+, Z-Sep/C18, ChloroFiltr, CarbonX, Cleanert NANO, Oasis PRiME, and finally EMR-Lipid.

Z-Sep and Z-Sep+ sorbents are based on zirconium dioxide (ZrO₂) and can replace the use of PSA and C18. They were used to clean up extracts with high amounts of fat for the analysis of pesticide residues [167, 168]. Z-Sep is recommended for the clean-up of samples with hydrophobic analytes. Z-Sep+ is used for samples containing greater than 15% fat, and finally Z-Sep/C18 is used for samples containing less than 15% fat [167, 169]. Z-Sep/C18 was used for pharmaceutical analysis from fish samples [170].

ChloroFiltr, CarbonX, and Cleanert NANO are used to remove co-extracted chlorophyll from plant matrices as for GCB (graphitized carbon black) [171–173]. ChloroFiltr is a polymeric-based sorbent; it was used in combination with MgSO₄ and PSA without scarifying the recovery of planar analytes [171, 172]. CarbonX in a non-friable form of GCB, and similar to GCB, it reduces the recoveries of planar analytes. It was effectively used in combination with MgSO₄, PSA, C18, and Z-Sep for the clean-up of pesticides and environmental contaminants in shrimps [37]. However, CarbonX retain pesticides less strongly than GCB, and it is easier to work with since it is available as filter-vial d-SPE [174] or SPE minicartridges [173, 175]. Cleanert NANO is used to remove colorant and fatty acids. It is composed of functionalized MWCNTs (multiwalled carbon nanotubes),

with deactivated surface to ensure the recovery of pesticides with benzene ring [138].

Oasis PRiME HLB (acronym for Process, Robustness, Improvements, Matrix effects, Ease of use) is the next-generation SPE device. It is a simpler, faster, and cleaner SPE cartridge. It is simple because it does not require any conditioning and equilibration steps; also no SPE expertise is required. Equally important, it can be employed by reversed phase “catch-and-release” SPE or “pass-through” SPE. The three-step catch-and-release protocol consists of loading of the pretreated sample, washing the cartridge, and finally eluting the caught analytes with the proper solvent suitable for target analytes, whereas in the two-step pass-through protocol, the sample is loaded and collected at same time, because in this case matrix interferences are retained while analytes are passing through. It is faster because it provides faster flows with less plugging. Finally, it provides cleaner extracts since it removes more than 95% of common matrix interferences, such as salts, proteins, and phospholipids, with the generic three-step protocol (load, wash, elute) and at least 90% more phospholipids than the generic protocol with Oasis HLB. It is effectively used for pesticide analysis in spices and fruits [176, 177].

Finally, EMR-Lipid sorbent is introduced by Agilent Technologies in 2015 for the removal of phospholipids and proteins [178]. Water should be added to the extract before EMR-Lipid clean-up, adding an additional step to the method. However, it does not function as solid d-SPE; conversely, it dissolves to saturation in the extracts; and the extraction mechanism is based on size exclusion and hydrophobic interactions. It was effectively used for the analysis of multiresidue pesticides and environmental contaminants in kale, salmon, avocado, and pork [179].

7 Analysis of Pharmaceuticals in Earthworms

Invertebrates, living in crop fields, play an important role in terms of accumulation of PhACs but also acting as soil purifiers [180]. Specifically, earthworms are recognized for their use as sentinel organisms belonging to the soil microfauna, in order to evaluate soil contamination by organic contaminants. In addition, they are the main soil-dwelling organism that has the ability to absorb pharmaceutical products from soils since earthworms represent the vast majority of the biomass of total soil life [180, 181].

Numerous studies have shown their ability to absorb pharmaceutical products from soil [182, 183] through consumption or direct contact with the soil, as well as other contaminants such as heavy metals [184, 185] and polychlorinated benzene [186]. Furthermore, unlike plants, earthworms move constantly in the soil favoring contact with large quantities of PhAC that remain in the soil [187].

Therefore, the analysis of worms could assess the global contamination of pollutants in a cultivated field, thanks to its bioaccumulation capacity. On the other hand, terrestrial worms are soil invertebrates widely used for the assessment of the ecotoxicological risk of soil contamination by pharmaceutical products [188]

and pesticides [189]. Therefore, they are a key organism in the terrestrial environment, and their presence reflects a healthy soil environment.

Furthermore, earthworms constitute 60–80% of the soil biomass [190], allowing them to be the preferred organisms for identifying the level of contamination in the soil and in the agro-ecosystem, therefore the contamination of the food chain. In addition, they maintain an integral position being the basis of the food chain. Moreover, wide ranges of PhACs are susceptible to be absorbed by earthworms. Lipophilic and hydrophilic compounds could enter into the earthworm system due to absorption on its skin and diffusion by means of the mucous skin membrane, respectively [183, 191, 192].

Furthermore, earthworms are incredible metabolic machines given the presence of numerous enzymatic processes that take place in their tissues. Recently, vermicomposting, a biotechnological composting process, has shown that some terrestrial worms are capable of transforming organic compounds into a less toxic final product [187].

The quantification of the pharmaceutical products taken and accumulated in earthworms is important not only for assessing the direct risk on earthworms themselves, but it is also the first step to estimate the transfer of contamination through the food chain to the best predators such as birds. For example, Spurgeon and colleagues studied the potential risk of secondary poisoning of metals transferred to earthworms' predators, such as birds, through food chain [193].

However, very few studies have been directed toward the extraction, detection, and quantification of pharmaceutical products in earthworms (Table 7), which may represent an important limitation for the assessment of environmental, ecotoxicological, and human health risk. Furthermore, these reported studies were addressed for risk assessment rather than for method development and validation for analytical purposes. Again, this type of complex biological matrix requires selective and clean extraction to be analyzed with precision. According to our literature survey, only two research groups have undertaken a study dedicated to the multiresidual extraction of pharmaceutical products in earthworm tissues [20, 181], although in the next paragraph we will discuss the different steps and conditions for the preparation, extraction, cleaning, and analysis of the samples reported in the literature.

7.1 Sampling, Sample Preparation, and Extraction

For earthworm sampling from soil field, the International Organization for Standardization (ISO) has standardized a protocol for soil invertebrate (earthworms) sampling (ISO, 2006). Briefly, the field should be distributed to several subplots, then two holes (dimension of one hole: 25 × 50 cm and 20 cm deep) are hand-sorted in each subplot, and earthworms are forced out by pouring in the holes diluted solution (0.5%) of formaldehyde. Collected earthworms are then transferred alive on a moist tissue to the laboratory for analysis. This protocol was followed by [192]. On the other hand, Kinney and coworkers [183] followed another sampling protocol,

Table 7 Extraction methodologies in earthworms

Pharmaceuticals	Extraction method	Sample mass	Extraction solvent	Clean-up	Recoveries (%)	RSD (%)	LOD	Instrument	References
Carbamazepine, diclofenac, fluoxetine, orlistat	USE		Methanol, ethyl acetate, acetonitrile/water (70:30 v/v), and acetonitrile	No clean-up	86.3–100.9%			Liquid scintillation counting (LSC)	[133]
Caffeine, carbamazepine, thiabendazole, trimethoprim, diphenhydramine, d-limonene, galaxolide (HHCB), tonalide (AHTN), acetophenone, indole, isoborneol, camphor, isoquinoline, menthol, 4-tert-octylphenol, para-nonylphenol-total, nonylphenol monoethoxy-total, nonylphenol, diethoxy-total, 4-cumylphenol, octylphenol, monoethoxy octylphenol, diethoxy, phenol, triclosan, 3-beta-coprostanol, cholesterol, beta-sitosterol, stigmastanol, naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]pyrene, 2-methylnaphthalene,	ASE	3–5 g	70:30 ACN/water		27–117%			HPLC-MS and GC-MS	[183]

Table 7 (continued)

Pharmaceuticals	Extraction method	Sample mass	Extraction solvent	Clean-up	Recoveries (%)	RSD (%)	LOD	Instrument	References
Fluconazole, climbazole, valsartan, valsartan acid, irbesartan, ciprofloxacin, citalopram, metronidazole, sulfamethazine, sulfamethoxazole, 4-nitro-sulfamethoxazole, N-acetyl-sulfamethoxazole, sulfamic acid, sulfamidamide, diclofenac, 4-hydroxydiclofenac, benzotriazole, 5-methylbenzotriazole, carbamazepine, carbamazepine-11,12-epoxide, oxcarbamazepine, metoprolol, diltiazem, propranolol, caffeine, verapamil, acridone, fenofibrate, sucralose, clarithromycin, lamotrigine, lamotrigine N2-oxide, N2-methyl-lamotrigine, 5-des-5-oxo-lamotrigine, acesulfame, hydrochlorothiazide, acetaminophen, ibuprofen, gemfibrozil, bisphenol A, chloramphenicol, furosemide, indomethacin, bezafibrate, clofibrac acid, fipromil, fipromil sulfone, fipromil-desulfinyl, and cocaine	QuEChERS	0,5 g	Acetonitrile	SPE (Oasis PRiME HLB)	70–99%	1–54%	0.01 ng g ⁻¹	LC-QToF-MS	[181]

which consists of removing a soil circle of 40 cm diameter and 25 cm of depth using a cleaned metal-blade spade, and then undamaged earthworms were extracted from the collected soil. It is an older protocol used by Salagovic and coworkers in 1996 [194] in order to assess the genotoxicity of polluted soil on earthworm.

Usually, the very first step before earthworm's tissues extraction is to let them empty their guts. For this depuration step, earthworms are left on wet filter paper for 24 h, and then they are washed with deionized water, dried with a towel, and frozen and/or lyophilized. Only one study reported the use of freeze-drying the earthworms [181]. After lyophilization, earthworms are crushed, homogenized, and stored at -20°C until analysis. Other studies used homogenized fresh earthworm tissue for the analysis [20, 183].

The weights of the samples vary according to the extraction method followed, since for QuEChERS weights of 0.25 and 0.5 g were employed, respectively [20, 181]; when ASE was performed, weights between 3 and 5 g were needed [183].

We have been able to identify at least four different methods applied to the analysis of drugs in earthworms (Table 7). Kinney et al. managed to detect 77 anthropogenic organic waste indicators means of the ASE methodology, although 20 were detected in real samples. Approximately, 50 mL of a 70:30 ACN/water solvent mixture were employed during five static cycles at a temperature of 130°C and 10,300 kPa. Recoveries from 27 to 117% were observed, but overall good recoveries and accuracy results were reported [183].

One of the first studies used a previous extraction method used for the soil but also applicable to earthworms. The method consists of an ultrasonic extraction for the determination of four pharmaceutical drugs (carbamazepine, diclofenac, fluoxetine, and orlistat). Different extraction solvents have been used depending on the compound. For carbamazepine, 20 mL of MeOH was used while for fluoxetine and orlistat 20 mL of ACN/water (7:3, v/v). Finally, 20 mL of ethyl acetate was used for diclofenac. After centrifugation, no cleaning process was performed to avoid reducing recovery results [182].

The use of the most versatile QuEChERS method has been reported by two different studies. In the most recent work by Montemurro et al., an analytical method was developed for the determination of over 50 pollutants present in earthworm wastewater using a rapid extraction method based on QuEChERS with an innovative cleaning step with SPE using Oasis PRiME HLB. The method shows good recovery results from 70 to 99% for most of the studied compounds. By applying this method, the authors managed to detect and quantify 19 PhACs in earthworms grown under controlled conditions, whereas 8 analytes were detected in earthworm samples collected from a cultivated field irrigated with treated wastewater [181].

Bergé and colleagues also used the QuEChERS method as an extraction method to detect 11 steroids, 14 veterinary antibiotics, and 6 human pharmaceutical products. In this case, the AOAC-buffered salt kit was used. For the hydration phase, an emulsion of 9 mL of water/hexane (67:33, v/v) was used, while acetonitrile was the extraction solvent. The cleaning procedure was performed using PSA and C18 (950 mg of MgSO_4 , 150 mg of PSA, and 150 mg of C18). Recoveries between 45.2 and 105% have been observed with good results of linearity and precision [20].

Finally, all the reports employed the analysis by means of LC-MS/MS. Concretely, Fourier transform (LC-FTMS), single quadrupole ion trap (QTrap), triple quadrupole (QqQ), and time of flight (LC-QToF-MS) mass spectrometers were employed for the detection of analytes of interest (Table 7).

8 Separation and Detection

It is widely known nowadays that pharmaceuticals are present in solid environmental and food matrices, such as soil and crops, at very low concentrations (few $\mu\text{g}/\text{kg}$ up to g/kg). Advances in chromatography techniques and mass spectrometry instruments have facilitated the separation and the detection of pharmaceuticals extracted from any kind of environmental and food matrices even at trace levels. Despite this, these samples are analytically very difficult to analyze because of their complexity and their numerous components that interfere with the detection of pharmaceutical products.

Liquid chromatography (LC) and gas chromatography (GC) are both used for pharmaceutical analysis, depending on their polarity and/or volatility. Chromatography techniques used for soil and crops are reported in Tables 2, 3, 4, 5, and 6. However, liquid chromatography (LC) is most widely used since most pharmaceutical products have high polarity and low volatility. Furthermore, whenever GC is used, the extracted pharmaceutical products need an additional derivatization procedure and/or the replacement with a GC-compatible organic solvent before their injection. BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) + TMS (trimethylsilyl) are the derivatization reagents commonly used [54, 80, 81, 85, 92]. Aside from the fact that most pharmaceutical products are polar and non-volatile, some are also thermolabile, such as tetracycline [31, 195], making the derivatization step an essential phase for their detection. In general, organic reactions, such as methylation, silylation, and acetylation, undergo the derivatization of the hydroxyl and carboxyl groups of pharmaceutical products. However, in this way, a further step is added to the analytical protocol, which can affect the efficiency of the method due to the loss of analytes, incomplete reactions, or the introduction of unwanted contaminants [195]. Therefore, LC has an advantage over GC since no derivatization step is necessary. However, it should be remembered that GC is convenient, suitable for routine analysis, and less subject to matrix effects [54, 196].

LC and GC are basically coupled to mass spectrometers (MS). In addition, several studies have used LC coupled with ultraviolet (UV), fluorescence (FLD), or diode array (DAD) detectors for the detection of a few numbers of pharmaceutical products [68, 78, 162]. However, lower detection and quantification limits were obtained compared to LC coupled to MS. Furthermore, the high-resolution mass spectrometry (HRMS) allows to perform a non-target analysis of pharmaceutical, as well as their environmental transformation products, or even the products of the metabolism without compromising the sensitivity of the analysis [196].

The availability of different columns played an important role in multiresidue separation in one single injection. However, the reversed phase (RP) C18 and C8 columns are dominantly used for pharmaceutical separation for LC [72, 197], while fused silica capillary and DB5 columns are commonly used for GC [80, 92].

For GC-MS, helium is always used as carrier gas, and 1–2 μL of samples are injected in split/splitless mode, while the column temperature is programmed from 50 to 300°C, EI ionization temperature is between 200 and 250°C, and finally the standard ionization energy of 70 eV is always used. The traditional ionization mode, electronic impact (EI), was always used as ionization source coupled with GC.

LC chromatography techniques are available as high-performance LC (HPLC) and ultrahigh-performance LC (UHPLC). The latter has a higher sensitivity with 2–3 orders of magnitude compared to HPLC, since it uses columns with particles of smaller dimensions ($<2 \mu\text{m}$), with consequent better chromatographic separation, better resolution, narrow peak shapes, and a reduced chromatographic run. However, UHPLC is used in only a few numbers of studies [53, 77, 95, 154, 159]. The composition of the mobile phase is an equally important factor for obtaining good ionization and separation efficiencies, reproducible retention times, and peak shapes [198]. Generally, the methanol/water or acetonitrile/water mixtures, at different pH values, are commonly used for the separation of pharmaceutical products under gradient elution. For better ionization and separation of pharmaceutical products, some modifiers are added to the mobile phases. Formic acid, acetic acid, ammonium acetate, and ammonium formate are the modifiers commonly used in the positive/negative ionization modes [58, 74].

When the mass spectrometer is coupled to LC, electrospray ionization (ESI) is always the most used ionization source for PhACs. It is a soft atmospheric ionization technique, easily coupled to LC. The analytes are dissolved in an organic solvent and introduced into the ionization chamber through a fine needle in the form of a spray. A high electrical potential is applied to the needle, resulting in the formation of charged droplets. The droplets are then vaporized by introducing neutral gas (generally nitrogen). Under these conditions, the charged droplets decrease in size as they move inside the source, the droplet of the solvent evaporates, and the charged analytes pass through the ionization chamber toward the analyzer. Therefore, ESI is the atmospheric pressure ionization technique mostly preferred and used since it is excellent with polar and non-polar compounds and for compounds with low thermal stability [199].

Advances and developments in mass spectrometry allow the detection of pharmaceutical by target analysis (using reference standards) with consequent quantification at the trace and ultra-trace levels or by providing the possibility to perform suspect or non-target screening. Additionally, the use of tandem MS/MS offers higher specificity, provided with MS^2 of compounds, thus reducing co-elution problems of matrix interferences. Therefore, it is preferred in the analysis of solid complex matrices. Mass spectrometers with single (MS) or multiple analyzers (MS/MS tandem) are both highly used. Single quadrupole (Q) [54], Orbitrap [74], and ion trap (IT) [59] are used as single MS, while triple quadrupole (QqQ) [58], quadrupole-time of flight (QTOF) [123, 156], and triple quadrupole-linear ion trap

(QTRAP or QLIT) [61] are used as tandem MS/MS for pharmaceutical analysis from soil and crops. However, MS/MS systems offer high sensitivity, selectivity, and precision and lower limits of detection and quantification, particularly in the analysis of complex solid matrices. In other words, hybrid mass spectrometers are built by combining two different analyzers in a single instrument and provide more information on the sample in shorter analysis times, therefore an easy differentiation between target analytes and interfering components of the matrix. Jacobsen and coworker [58] used QqQ for the analysis of chlortetracycline, oxytetracycline, sulfadiazine, erythromycin, and tylosin (TYL) and its degradation products, TYL A, B, C, and D, from soil, obtaining low detection and quantification limits (from 0.6 to 5.6 g/kg and from 25.7 to 73.9 g/kg, respectively). Martínez-Piernas and coworkers [155] used QTOF for the analysis of carbamazepine transformation products from lettuce obtaining detection limits less than 3 ng/g in dry weight samples.

In addition, IT analyzers have the ability to perform multiple compound fragmentation steps (MS^n) and to trap fragment ions resulting in full-scan spectra with high sensitivity. Barron and coworkers [55] used LC-ESI-ion trap-MS in single and tandem MS modes, and they obtained detection limits below 20 ng/g for 20 pharmaceuticals out of 27 from soil, reporting poorer sensitivity for paracetamol, salbutamol, caffeine, pravastatin, indomethacin, and clotrimazole. However, they assumed that this poor sensitivity might be due to their low % recovery. The combination of IT with quadrupole analyzers (QTRAP or QLIT) offers the robustness of a QqQ with the full scan and high sensitivity of IT. Low method detection limits were obtained with García-Galán and coworkers [61] using this instrument, ranging from 0.03 to 2.23 ng/g, for sulfonamide extraction from soil. However, for a better selectivity, QTOF and Orbitrap are the chosen instrument. They provide high-resolution and accurate masses for parent and fragment ions in full-scan spectra. Accordingly, they remove the interfering signals, making it easier to identify the non-target compounds in complex environmental and food matrices [155, 200]. Orbitrap was used by Chitescu and coworkers [74], for the extraction of oxytetracycline, sulfamethoxazole, and ketoconazole from grass, obtaining detection limits lower than 10 µg/kg.

The methodologies managed in full-scan mode or single ion monitoring (SIM) with a single quadrupole showed the need for a highly efficient cleaning phase. On the other hand, the use of multiple reaction monitoring (MRM) mode with MS/MS systems offers maximum selectivity with a reduced matrix effect. It allows identification by monitoring the target compound and usually the most abundant transitions (product ions, generally two) [197].

Finally, to deal with the evaluation of the matrix effects, which can cause an enhancement or reduction of the analyte response, most of the studies used isotopically labeled compounds. However, not all deuterated compounds associated with each analyte are available for purchase or are sometimes very expensive.

9 Conclusions and Future Perspectives

The reuse of urban wastewater has opened up new possibilities for the use and availability of water. Indeed, in order to reduce the human impact on the environment, wastewater can be used to irrigate agricultural fields in areas of the world affected by drought or where availability is normally limited. Furthermore, the reuse of wastewater certainly has a positive impact on the soil as a mean for the development of plants and on the farmers themselves because of the economic advantage they could obtain by using wastewater as a fertilizer and a source of water for crops.

However, it is known that wastewater treatment plants are unable to remove small organic compounds such as PhACs, which remain in the soil and can subsequently enter the plant system where they are absorbed by the roots. Once inside these plants, these compounds can be consumed by grazing animals or even by humans or remain in the environment. For this reason, the study of these compounds in soil and plants is at the center of interest of many scientists.

Determining the presence and concentration of contaminants in soil and plant tissues requires significant effort. Hence, from the research papers reviewed, it is clear that several successful analytical methods have been developed in the last decade to extract, detect, and quantify most of the pharmaceutical products commonly used in various matrices such as vegetable and soil. However, in most cases, these methods are time-consuming, are expensive, and require the use of specialized reagents and personnel. Various research teams have made significant efforts to overcome the obstacles associated with these methods, but in some cases further development is needed. It is therefore necessary to continue the research developing even simpler and more robust analytical techniques that are at the same time environmentally friendly.

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Chapter 3:

Optimization and validation of wastewater-borne
organic pollutants including pharmaceuticals
extraction from lettuce leaves

1. Comparison of MRMHR and SWATH acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce leaves using a modified QuEChERS method

1.1. Context

The development of a method that is able to screen a large number of PhACs is highly desirable, especially for the analysis of agroecosystem samples irrigated with treated wastewater. The presence of these chemicals in crops for human consumption is an important source of concern because of the potential risk that may pose for human health. However, there is a lack of analytical methods for detecting PhACs and other wastewater-borne organic contaminants in crops. Thus, an important effort was dedicated in the past decade to develop robust, rapid, fast, simple, sensitive and cheap analytical methods for the analysis of these chemicals in plant tissues.

1.2. Objective and main results

The objective of this article was to develop a robust analytical method, for the extraction of 48 PhACs and other wastewater-borne organic contaminants and their TPs frequently detected in wastewater (including analgesics and anti-inflammatories, anti-hypertensives, antifungal agents, lipid regulators, psychiatric drugs and stimulants, β -blockers, antibiotics, antimycotics, and sweeteners). A QuEChERS-based method followed by detection and quantification on a high-resolution mass spectrometry was developed to follow the selected compounds in lettuce leaves. The performance of the method was evaluated in terms of accuracy, repeatability, sensitivity, linearity and matrix effect. Furthermore, method performance was compared using two acquisition modes: high resolution multiple reaction monitoring (MRMHR) and Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH). Accordingly, the developed method was able to simultaneously extract and quantify the 48 selected compounds. Both acquisition modes provided similar recoveries between 80 and 120% in lettuce leaves, with few compounds of exception. However, MRMHR provided more consistent results for a greater number of compounds. Finally, the method was successfully applied to lettuce grown in controlled conditions and irrigated with treated

wastewater. 14 out of 48 selected compounds were detected with concentrations ranging from 2.9 ng/g (metoprolol) to 196.3 ng/g (citalopram).



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Comparison of high resolution mrm and sequential window acquisition of all theoretical fragment-ion acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce



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ABSTRACT

Screening of a large number of chemicals of emerging concern is highly desirable for the control of crops irrigated with reclaimed water since it is considered an alternative water source of great value. This study describes a high resolution mass spectrometry approach for developing methods for quantification in lettuce leaves of 48 different wastewater-borne pollutants (including analgesics and anti-inflammatories, anti-hypertensives, antifungal agents, lipid regulators, psychiatric drugs and stimulants, β -blockers, antibiotics, antimycotics, and sweeteners) frequently found in water resources. In this respect, a simple and fast QuEChERS-based method for the determination of contaminants in lettuce has been developed. During extraction, the use of formic acid was adopted to further improve the results of some problematic compounds (e.g., fenofibrate, furosemide, metronidazole, oxcarbazepine, sulfanilamide). High resolution multiple reaction monitoring (MRM^{HR}) and SWATH acquisition were compared in term of accuracy, repeatability, sensitivity, linearity and matrix effect. Both methods provided similar recoveries between 80 and 120% in lettuce leaves, although sulfanilamide, ciprofloxacin, and sulfamethazine presenting values of 26.8, 27.8, and 28.4% in MRM^{HR} and 25, 33.9, and 35% in SWATH, respectively. The effectiveness of a two-step cleanup on analyte recovery was also assessed and matrix effects were also taken into consideration during the method validation. The developed method allows the simultaneous quantitative analysis of 48 compounds (drug residues and metabolites) in lettuce leaves irrigated with treated wastewater for human consumption. Application of the present method to lettuce crops growth in controlled conditions showed the presence of 14 out 48 studied compounds with similar concentrations in both acquisition modes ranging from 3.3 and 1.3 ng g⁻¹ for climbazole (for MRM^{HR} and SWATH, respectively) to 33.2 and 17.7 ng g⁻¹ for sulfamethazine. Drug residues such as carbamazepine (6.0 and 8.5 ng g⁻¹), and its metabolite carbamazepine epoxide (18.1 and 16.5 ng g⁻¹), frequently found in wastewater effluents, were also detected.

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1. Introduction

In communities where water is a limited commodity, traditional water resources such as surface and ground water cannot meet their demands. Therefore, to address present and future water shortages, alternative water sources are considered. The use of reclaimed water is of a great interest as a response to the high

water demand in urban and rural areas, and, in fact this practice is already well established in agriculture which accounts for about 70% of freshwater consumption. However, reclaimed wastewater can contain salts, inorganic nitrogen and pathogens, heavy metals and organic contaminants such as pharmaceuticals which can present a potential risk not only to soil and the groundwater underneath but particularly to the crops. When pollutants are taken up into these plants during the growth phase but are not eliminated by the time of harvest, they enter the food chain ultimately leading to undesired exposure of humans and animals to inherently bioactive substances. Consequently, there is growing concern

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about the human health impact of crops irrigated with reused water.

Lettuce is one of the fresh crops most consumed raw around the world [1] and as a leafy vegetable has a very high ability to take up pharmaceuticals in its edible tissues [2]. However, few studies have evaluated the presence of wastewater-borne in lettuce because of the lack of suitable analytical methods [1]. The development of multi-analyte extraction methods for the determination of trace levels of wastewater-borne in lettuce is challenging for two major reasons: on the one hand, drugs differ widely in their structures and consequently in their physicochemical properties, and thus behave differently in extraction and clean-up processes. On the other hand, plant tissues are of complex composition containing numerous endogenous components, such as pigments, fat, cellulose and wax, which are prone to interfere with the sample extraction and subsequent measurement of the analytes, if not removed during sample treatment [3–7].

In recent years, several analytical methods have been developed to extract wastewater-borne pollutants from plant tissues using traditional approaches such as solid-liquid extraction [8,9], accelerated solvent extraction [7], and ultrasound extraction [1,5,10–13]. However, in order to assess the food quality and safety with respect to the presence of microcontaminants, a quick, selective, and sensitive analytical protocol is needed for its quantification in harvested vegetables. For an innovative, rapid, simple, robust and sensitive method only few publications proposed the use of a quick, easy, cheap, effective, rugged and safe (QuEChERS)-based method for the determination of pharmaceuticals in lettuce or other vegetable commodities [7,9,14–18]. Chuang et al. compared the performance of accelerated solvent extraction and QuEChERS for the suitability to extract eleven drugs spiked in lettuce from a local supermarket [7]. Both optimized methods provided satisfactory extraction recovery and precision to allow for quantification of the pharmaceuticals in vegetable tissues. Compared to the accelerated solvent extraction method, the QuEChERS method provided better performance for the determination of drugs in vegetables in terms of ease, speed, and solvent consumption [7]. In contrast, the comparison of solid-liquid extraction with QuEChERS for the analysis of 28 wastewater-borne contaminants and their potential metabolites in lettuce reported better performance parameters for the former method [9]. In a recent study with a broader range of analytes [14], covering as many as 74 micro-contaminants, some of which were not previously investigated, extraction with QuEChERS yielded to satisfactory results. Up to 84% of the compounds were recovered within a 70 to 120% range.

The detection of the analytes in the aforementioned studies was accomplished with compound-specific acquisition on triple-quadrupole mass spectrometers (QqQ-MS) operated in a targeted mode. Recently, the development of very fast data acquisition modes in high resolution-mass spectrometry (HR-MS) on quadrupole-time of flight (Q-TOF) instruments has enabled novel approaches offering rapid and reliable results for a large number of compounds in a target acquisition mode. The so-called high resolution multiple reaction monitoring (MRM^{HR}) is a robust targeted quantitation mode through two stages of mass selection, to provide high data richness and excellent specificity and sensitivity. First-, the quadrupole mass filter selects a given precursor ion, fragments it by collision-induced dissociation, and then the user chooses among the products ions one that provides the best combination of sensitivity and selectivity for quantification. As for all of the analytes, full HR-MS² spectra are recorded, their identities can be confirmed by checking them for the presence of additional fragment ions of diagnostic value. Conversely, some new Q-TOF hybrid systems have gained wide acceptance thanks to the Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH mode) providing high quality MS/MS data that can

be used for quantitation with fast acquisition speed and excellent mass accuracy [19,20] SWATH is a data-independent acquisition technique, separating into fixed or variable size m/z windows stepped across the entire m/z range of interest. In this way, fragment ions formed in a given window cause more easily associated to their precursor ion, resulting in high specific MS and MS/MS spectra [21]. Although the main applications are proteomics and metabolomics [22–24], SWATH acquisition generates comprehensive and high-quality MS/MS spectra comparable to “MRM-like” fragments that can be used to confirm unequivocally the detection of specific compounds after comparing the SWATH data with pre-assembled MS/MS spectral libraries [23,25,26].

Under this scenario, the main objective of the present study was to compare the performance of two high resolution mass spectrometry modes namely MRM^{HR} and SWATH using LC-QToF-MS for the determination of 48 wastewater-borne pollutants (including analgesics, antibiotics anti-inflammatories, antifungal agents anti-hypertensives, antimycotics, β -blockers, industrial pollutants, lipid regulators, psychiatric drugs and stimulants and sweeteners) in lettuce. Moreover, we also developed and validated an analytical method based on the QuEChERS extraction of lettuce leaves for the final determination of wastewater-borne pollutants of widespread use and commonly present in reclaimed water. The performance of 16 different modified QuEChERS procedures (with formic acid and PSA clean up step) to extract the selected analytes from this matrix were compared. After validation, the optimized analytical method was applied to the analysis of the selected compounds in lettuce plants grown in soil pots under controlled conditions and irrigated with treated wastewater for the whole crop cycle. Both MRM^{HR} and SWATH acquisition were achieved using a hybrid QTOF mass spectrometer

2. Materials and methods

2.1. Chemicals and reagents

Analytical reference standards (Acesulfame, acetaminophen, acridone, benzotriazole, 5-methyl-2H-benzotriazole, bezafibrate, bisphenol A, caffeine, carbamazepine, carbamazepine-10,11-epoxide, chloramphenicol, ciprofloxacin, citalopram, clarithromycin, climbazole, clofibrac acid, diclofenac, 4'-hydroxydiclofenac, diltiazem, fenofibrate, fipronil, fipronil desulfinyl, fipronil sulfone, fluconazole, furosemide, gemfibrozil, hydrochlorothiazide, ibuprofen, indomethacin, irbesartan, lamotrigine, lamotrigine N2-oxide, 5-desamino 5-oxo-2,5-dihydro lamotrigine, metoprolol, metronidazole, N2-methyl-lamotrigine, N-acetyl-sulfamethoxazole, 4-nitro-sulfamethoxazole, oxcarbazepine, propranolol, sucralose, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfanilic acid, valsartan, valsartan acid, and verapamil) were of high purity and were acquired from Sigma Aldrich (St. Louis, MO, U.S.).

Isotope-labelled compounds (acetaminophen-d4, acesulfame-d4, benzotriazole-d4, bezafibrate-d4, bisphenol A-d8, caffeine-13C3, carbamazepine-d10, ciprofloxacin-d8, citalopram-d6, climbazole-d4, diclofenac-13C6, fenofibrate-d6, fluconazole-13C3, furosemide-d5, gemfibrozil-d6, hydrochlorothiazide-d2, ibuprofen-d3, indomethacin-d4, irbesartan-d6, lamotrigine-13C3, metoprolol-d7, metronidazole-d4, naproxen-d3, sucralose-d6, sulfamethazine-d4, sulfamethoxazole-d4, valsartan acid-d4, valsartan-d3) were purchased from Cerilliant (Sigma Aldrich, St. Louis, MO, U.S.), Alschim (Illkirch-Graffenstaden, France), Santa Cruz Biotechnology (Dallas, TX, U.S.), or Toronto Research Chemicals (Toronto, ON, Canada).

CAS numbers, molecular formulas, molecular weight, and other relevant properties of all target compounds are reported in Table A.1 (Supplementary Material, SM).

For standards and samples preparation, LC-MS grade acetonitrile ($\geq 99.9\%$), methanol ($\geq 99.9\%$), ethyl acetate ($\geq 99.9\%$), dimethyl sulfoxide ($\geq 99.9\%$), and HPLC water were purchased from Merck (Darmstadt, Germany). Formic acid ($\geq 96\%$, ACS reagent), ammonium acetate ($\text{NH}_4\text{CH}_3\text{CO}_2$), and ammonium formate (NH_4HCO_2) were supplied by Sigma-Aldrich while ammonium fluoride was bought from Fisher Chemical (Fisher Scientific SL, Madrid, Spain). For high purity mobile phase solutions, acetonitrile and water (Optima™ LCMS Grade) were purchased from Fisher Chemical (Fisher Scientific SL, Madrid, Spain).

QuEChERS extraction salts and dispersive solid phase extraction (dSPE) were obtained from BEKOlut GmbH & Co. KG (Hauptstuhl, Germany). The Original non-buffered kit was composed by 4 g MgSO_4 and 1 g NaCl, while the buffered European EN 15,662 kit was constituted by 4 g MgSO_4 ; 1 g NaCl; 1 g sodium citrate; 0.5 g disodium citrate sesquihydrate. The dSPE clean-up mixture was made of 150 mg PSA (primary secondary amine), 150 mg of C18-bonded silica, and 900 mg MgSO_4 .

2.2. Preparation of standard solutions

Stock solutions ($1000 \mu\text{g mL}^{-1}$) of individual pharmaceuticals standards were prepared in either acetonitrile, methanol, dimethylsulfoxide, or HPLC water depending on the solubility of each compound and stored in the dark at -20°C . Working mixtures of pharmaceuticals and the isotopically labeled compounds ($2 \mu\text{g mL}^{-1}$), used for spiking the lettuce blank samples during the method development, in the validation studies, and for calibration purposes were prepared by diluting an appropriate volumes of the stock solutions in methanol.

2.3. LC-MS/MS analysis

Samples were analyzed on a SCIEX X500R QTOF system (Sciex, Redwood City, CA, U.S.) equipped with Turbo V™ Electrospray Ionization (ESI) source. Depending of the analytes, they were detected in negative or positive polarity mode. The total chromatographic run time for each injection was 12 min for positive or negative acquisition and the separation of the analytes was achieved on a Hibar HR Purospher STAR RP- C18 column ($100 \text{ mm} \times 2.1 \text{ mm}$ i.d., $2\text{-}\mu\text{m}$ particle size, Merck, Darmstadt, Germany), maintained at 40°C . The fast elution was carried out using as mobile phases consisting of aqueous mobile phase (A), either 5 mM ammonium acetate + 0.1% formic acid (positive ion mode) or 2 mM ammonium fluoride (negative mode), and (B) acetonitrile. The flow rate was 0.5 mL min^{-1} , the injection volume was $10 \mu\text{L}$, and the autosampler temperature was 8°C . The elution gradient is reported in Table A.5.

Any possible drift in the mass accuracy of the SCIEX Q-TOF-MS was automatically corrected during batch acquisition by infusing a reserpine solution ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_9$, m/z 609.28066) for positive mode, and a cluster of trifluoroacetic acid ($[(\text{CF}_3\text{COONa})^5 + \text{CF}_3\text{COO}]^-$, m/z 792.85963) for negative mode. The instrument provided a typical resolving power (FWHM) of 31,000 to 44,000 at m/z 132.9049 and 829.5395, respectively with a mass error of 0.2 ppm. Calibration was performed before or after a control vial in the batch sequence making use of the Calibrant Delivery System (CDS).

All HR-MS data were acquired using either MRM^{HR} or SWATH modes. Quantitation was performed in the MRM^{HR} fragment scanning mode which provides the noise in the chromatogram to the minimum due to the selection of specific ions at specific collision energies (CE), decluttering potentials (DP), and fragmentation voltages (V). The SWATH acquisition in turn, lacked the selectivity of MRM^{HR} but the MS data set could be used for retrospective analysis.

Both modes consisted of a single TOF-MS experiment over a range from m/z 100 to 950 with an accumulation time (AT) of 120 ms; DP and CE were set to 80 V and 10 V and -80 V and -10 V, for positive and negative, respectively). The source conditions were as follows: source temperature and nitrogen gas flows (Atomizing gas, GS1 and Auxiliary gas, GS2) were set to 550°C , 55, and 55 psi, respectively. Ion Spray Voltage was set to 5500 V (-4500 V for negative); Collision gas (CAD) was set to 7, while Curtain gas was set to 30 psi. The MRM^{HR} experiments were acquired in fragment scanning mode. The Guided MRM^{HR} tool from SCIEX was used for the optimization of transitions. The selected ionization mode, the optimized CEs and Vs for each compound have been reported in electronic supplementary material (Table A.2), while the AT for each TOF MS/MS experiment was fixed at 12 ms. The SWATH acquisition consisted of 10 MS/MS experiments with variable Q1 window widths (m/z 100 to 950, 40 ms AT) using a CE of 35 V with ± 15 V spread. The variable Q1 windows were generated using the SCIEX SWATH variable window calculator (Ver. 1.1). The MS survey scan obtained for lettuce extract spiked with all the compounds was run in the window calculator to generate the variable window widths, for positive and negative acquisition. The outcomes are reported in Figure A.1.

Qualitative analysis was performed using SCIEX OS™ Software version 1.6 (Sciex, Redwood City, CA, USA). Two ions were used for each compound, the most abundant product ion for the quantification and the precursor ion for the confirmation (Table A.2, ESM). Only the accurate mass of molecular ion obtained from the TOF-MS experiment was used for the isotopically labeled compounds. For SWATH acquisition, high confidence identification was based on unique fragment ions and their ion ratios as well as HR-MS/MS library searching using high resolution spectral libraries supplied by SCIEX.

2.4. Sample preparation

Lettuce (*Lactuca sativa* L. "Maravilla de Verano-Canasta") was selected as the matrix of this study for its fast growth, its high cultivation and consumption worldwide, its ability to grow easily in greenhouse conditions, and its extensive root system that can facilitate the uptake of organic contaminants from soil. Furthermore, it is usually consumed without being cooked and its vegetative part consists of green leaves, making the sample preparation easier [1]. To produce sufficient of contaminant-free matrix for method optimization and validation, several lettuce seedlings at the four leaf stage were grown for 60 days using organic potting soil purchased from a local garden store (Barcelona, Spain) [27]. At the harvest, lettuce plants were carefully hand washed with tap water and then rinsed with purified water. The heads then, were separated from the roots and blotted dry with a paper tissue and stored at -20°C for at least 48 h. The lettuce leaves were freeze-dried, using a LyoAlfa 6 system (Telstar Technologies, Terrassa, Spain) and ground to a fine powder with a knife mill with a stainless steel grinding chamber (Grindomix GM 200, Retsch GmbH, Haan, Germany) and stored at -20°C until extraction.

2.5. Extraction and clean up

The recovery studies were performed using a modified QuEChERS approach which was optimized by evaluating different extraction and clean-up conditions. The Original non-buffered (OR) and the European EN 15,662 method (EN) QuEChERS extraction salts kits were compared. To assess the influence of acidification (formic acid) in the extraction efficiency, different concentrations were added to the extraction solvent (0.5 and 1%). To avoid the risk of base-catalyzed degradation following the use of PSA, acidification was also evaluated after the cleaning phase by adding 0.05% formic

Table 1
Different procedures of modified QuEChERS performed, including all variants tested during the study.

	Type of salt		Hydration			Formic acid		Cleanup	
	ORIGIN AL	EN 15662	HPLC water	Ammoniu m acetate	Ammoniu m formiate	0.5 %	1 %	PSA-C18	PSA-C18 0.05 % Formic acid
Protocol 1									
Protocol 2									
Protocol 3									
Protocol 4									
Protocol 5									
Protocol 6									
Protocol 7									
Protocol 8									
Protocol 9									
Protocol 10									
Protocol 11									
Protocol 12									
Protocol 13									
Protocol 14									
Protocol 15									
Protocol 16									

acid [28]. The efficiency of removal of undesirable co-eluent by the use of dSPE PSA-C18 clean-up was also tested. Finally, the alternative use of ammonium acetate and ammonium formate solutions instead of water during hydration step was also evaluated to improve the recoveries of challenging compounds. Protocols of the different extraction procedures are reported in Table 1 and described in details in the electronic supplementary material (Table A.6 and Table A.7) and discussed in Results section.

The streamlined procedure provided below which was adopted for extraction and clean-up in the final method. Briefly, 1 g freeze-dried blank lettuce was placed in 50-mL disposable polypropylene centrifuge tube and 9 mL HPLC water, (90% hydration). The tubes were vortexed for 2 min at 2500 rpm using a BenchMixer XLQ QuEChERS Vortexer (Benchmark Scientific, Sayreville NJ, US). After a 1-hour hydration phase, the sample was spiked with 50 μ L of standard solution containing all target compounds (2 μ g mL⁻¹ in methanol) to achieve a final concentration in the lettuce of 100 ng g⁻¹ dry weight (d.w.), corresponding to 10 ng g⁻¹ of fresh weight (f.w.) after hydration step. The tube was vortexed again and the sample was allowed to stand for 30 min. Then 10 mL acetonitrile and 50 μ L of concentrated formic acid were added followed by a vortex. The OR extraction salts were added directly into the tube and the mixture was instantly shaken in order to prevent the formation of crystalline agglomerates owing to MgSO₄ hydration. The sample was vortexed again and centrifuged (4000 rpm, 10 min, 4 °C). The resulting supernatant (organic phase) was transferred into a glass tube and left overnight at -20 °C, enabling the precipitation of fatty acids and waxes, co-extracted from the lettuce leaves. The

following day, the d-SPE clean-up took place. While avoiding to resuspend the material deposited on the bottom, 6 mL of the organic phase were transferred into the PSA tube (150 mg PSA, 150 mg C18, 900 mg MgSO₄) and the mixture was shaken for 1 min manually, vortexed for 2 min, and centrifuged at 4000 rpm for 5 min, 4 °C. Then, 1 mL of the supernatant was evaporated until dryness under a gentle stream of nitrogen at room temperature and then reconstituted with 1 mL of water/10% methanol solution and injected for LC-MS/MS analysis.

2.6. Clean-up efficiency

To determine the amount of co-extracts removed in the matrix through the 2-step cleanup, a gravimetric measurement was conducted according to [29]. Nine 50-mL Falcon tubes were prepared weighting 1 g of blank lettuce matrix in each tube. Three samples were extracted using only the OR QuEChERS kit according to optimized protocol. Then, 5 mL of the organic supernatant layer (5 g equivalent) was transferred into three previously weighted test tubes. Three more samples were extracted in the same way but they were left overnight at -20 °C, to enable the precipitation of fatty acids or waxes. The following day, taking care not to pick up the material deposited on the bottom, 5 mL of the organic phase (5 g equivalent) were transferred into 3 other pre-weighed glass test tubes. 5 mL (5 g equivalent) of each extract after freezing out and clean-up (150 mg PSA, 150 mg C18, 900 mg MgSO₄) were transferred to pre-weighed test tubes. All nine test tubes were then evaporated until total dryness under a gentle stream of nitrogen at

room temperature until constant weight. The difference in weight was recorded to estimate the amount of matrix co-extracts in the initial and final extracts. The results are reported in Figure A.2.

2.7. Method performance

The analytical method was validated for specificity, accuracy, intraday precision, linearity, limits of detection (MDLs) and quantification (MQLs), and matrix effect (ME) using spiked lettuce samples.

To ensure the quality of the results, the specificity of the method was evaluated by analyzing untreated lettuce samples. The absence of signal above signal-to-noise ratio (S/N) of 3 at the retention time of the analytes of interest eliminates a false positive by contamination in the extraction process. The accuracy was determined with spiking the matrix at concentrations of 2, 5, 10, 50, and 200 ng g⁻¹ f.w. Relative recoveries (R%) were calculated by comparing the peak areas obtained in samples spiked before the extraction ($n = 3$) and after the extraction ($n = 3$) at five concentration levels, according to Eq. (1):

$$R\% = 100 \times (\text{Area spiked pre extraction}) / (\text{Area spiked post extraction}) \quad (1)$$

The precision of the assay expressed by repeatability (intraday) was calculated as relative standard deviation (RSD%) obtained from the relative recoveries of the recovery study for each concentration level. Values were considered Acceptable when recoveries were ranged between 70 and 120% and RSDs $\leq 20\%$. Both MRM^{HR} and SWATH acquisition were compared in term of accuracy and precision. Mean values of accuracy and precision for MRM^{HR} and SWATH are reported in Table 2.

In order to assess the ME extracts of untreated lettuce samples ($n = 3$ replicates) were spiked with the mix of the target compounds at the same concentration levels as used in the recovery study (2, 5, 10, 50, and 200 ng g⁻¹ f.w.), before LC-MS/MS analysis. The peak areas produced by these samples were compared with those obtained the solvent (water/10% methanol), spiked at equivalent concentrations. ME (%), were expressed according to the following Eq. (2):

$$ME (\%) = 100 \times [(Area in spiked extract / Area in spiked solvent) - 1] \quad (2)$$

Hereinafter, ME values of $\pm 40\%$ were considered acceptable, whereas ME values outside this range indicated significant matrix effect.

To compensate for ME and to evaluate the linearity of the method, a matrix-matched calibration curve approach was employed. An 11-point calibration curve was prepared by spiking blank lettuce extracts with proper amounts of standard solution are a ranged 0.05 to 300 ng mL⁻¹, corresponding to 0.5 and 3000 ng g⁻¹ f.w. in lettuce leaves. Each concentration was injected three times on the same day and the calibration curve was constructed by plotting the ratio of the analyte signal to its surrogate standard signal against the analyte concentration. Calibration curve was constructed by linear weighted least-squares regression (1/x as weighting factor). For the majority of the compounds, at least 8 calibration points were considered. Linearity was evaluated by calculating the coefficient of determination (r^2) for each analyte. The acceptance criterion was that the coefficient of correlation ≥ 0.99 . Surrogate standards used in each case are shown in Table A.2.

Method detection limit (MDL) was defined as the lowest concentration of an analyte that could be distinguished of the matrix signal with a S/N greater than 3. Method quantification limit (MQL) was defined as the lowest concentration of a given compound giving a response that could be quantified, with a S/N greater than 10

and a RSD $\leq 20\%$. MDL and MQL were estimated from the matrix-matched calibration curves based on the following equations according to [30]:

$$MDL = 3 \times S_b / \text{slope} \quad (3)$$

$$MQL = 10 \times S_b / \text{slope} \quad (4)$$

where S_b is the standard deviation of the intercept.

3. Results and discussion

Aiming to optimize the extraction of target compounds contaminants from lettuce leaves, the Original QuEChERS protocol developed by Anastassiades et al. for pesticide residues in food commodities [28] was only marginally modified. For the extraction of wastewater-derived compounds, the traditional liquid-liquid partition formed by the addition of anhydrous MgSO₄ and NaCl now involves the addition of acetonitrile containing 0.5% formic acid. Moreover, a two-step clean-up consisting of a preliminary freezing step followed by the conventional dSPE was performed. The acquisition of MRM^{HR} and SWATH were compared and, therefore, the optimal conditions were selected. Finally, some parameters were studied that influence the performance and efficiency of the extraction including the matrix effect.

3.1. Method performance: optimization of LC-MS/MS conditions

In the present study, 3 replicates, each at 5 spiking levels (2, 5, 10, 50, and 200 ng g⁻¹ f.w.), in lettuce leaves were prepared using the selected QuEChERS protocol and analyzed by LC-QToF-MS. The best chromatographic separation of molecules of interest was performed with a Merck Hibar HR Purospher STAR RP-C18 column. Separation of all studied target analytes was successfully performed, and this column was chosen for further analysis. To achieve the optimal separation and high sensitivity for MS detection gradient elution based on mobile phase consisting of acetonitrile and water (5 mM ammonium acetate + 0.1% formic acid) for the positive electrospray ionization and acetonitrile and water (2 mM ammonium fluoride) for the negative ionization, at a flow rate of 0.5 mL min⁻¹. For development and comparison experiments, only the MRM^{HR} approach was used by acquiring data in fragment scanning mode. The Guided MRM^{HR} tool from SCIEX was used for the optimization of high resolution transitions. High resolution MRM parameters including ionization mode, CEs and Vs were carefully studied for each compound to provide the best possible sensitivity. Both electrospray ionization ESI+ and ESI- modes were tested. According to SANTE European Commission guideline for pesticides (SANTE/11,813) [31], two ions with mass accuracy equal or mass difference lower than 5 ppm are necessary for confirming a positive finding for the identification in HR-QToF-MS analysis. In the present study, each compound was confirmed by comparing the signal of two high resolution ions, the most abundant product ion with the best signal intensity for the quantitation while the precursor ion for the confirmation. Results of the optimized mass spectrometric conditions in MRM^{HR} for each compound are shown in Table A.2. In contrast, for SWATH acquisition a fixed value of DP of 80 V and -80 V, for positive and negative, respectively with a CE of 35 V with a collision energy spread of ± 15 V was employed since they are essential for comparison with the high resolution spectral libraries supplied by SCIEX. In order to estimate its sensitivity, the validation of targeted compounds was also performed acquiring data with SWATH acquisition and afterwards both modalities were compared. For an accurate quantitation, the MRM^{HR} fragment scanning mode provided high selectivity and sensitivity of product ion transitions, decreasing the noise

Table 2
Method performance parameters for target analytes in MRM^{HR} and SWATH acquisition including Retention time (RT), Linearity range, Coefficient of determination (r^2), Accuracy (%), Precision (RSD,%), and Method detection limits (MDL) and Method quantitation limits (MQL).

Analyte	RT (min.)	MRM ^{HR} Linearity		SWATH Linearity		MRM ^{HR} Intraday performance		SWATH Intraday performance		MRM ^{HR}		SWATH	
		Range (ng g ⁻¹)	r^2	Range (ng g ⁻¹)	r^2	Accuracy ^a (%)	Precision ^b (RSD,%)	Accuracy ^a (%)	Precision ^b (RSD,%)	MDL ^c (ng g ⁻¹)	MQL ^d (ng g ⁻¹)	MDL ^c (ng g ⁻¹)	MQL ^d (ng g ⁻¹)
4'-Hydroxydiclofenac	7.54	2.5 - 2000	0.9921	5 - 3000	0.9935	83.3	17.5	78.9	13.7	0.09	0.26	0.05	0.15
4-Nitro-sulfamethoxazole	6.86	2.5 - 2000	0.991	1 - 500	0.9958	92.6	10.8	90.9	5.4	0.06	0.18	0.06	0.18
5-Desamino 5-Oxo-2,5-dihydro Lamotrigine	4.64	5 - 2000	0.984	2.5 - 1000	0.997	80.8	19.0	80.1	6.9	0.08	0.25	0.04	0.12
5-Methyl-2H-benzotriazole	4.41	2.5 - 2000	0.9927	2.5 - 2000	0.9972	100.	14.5	89.6	13.0	0.05	0.16	0.04	0.12
Acesulfame	1.18	2.5 - 3000	0.9873	2.5 - 1000	0.9955	65.6	9.0	72.4	7.6	0.12	0.38	0.08	0.25
Acetaminophen	2.01	5 - 3000	0.9954	5 - 2000	0.9985	84.6	22.3	85.8	7.0	0.04	0.13	0.67	2.04
Acridone	5.82	0.5 - 1000	0.9886	5 - 500	0.987	96.5	11.4	93.6	2.9	0.02	0.05	0.08	0.25
Benzotriazole	3.23	0.5 - 3000	0.9953	0.5 - 1000	0.993	91.1	10.7	95.6	16.4	0.06	0.18	0.18	0.54
Bezafibrate	5.38	1 - 1000	0.9866	1 - 500	0.9946	90.8	4.9	91.6	6.5	0.03	0.09	0.05	0.15
Bisphenol A	7.39	1 - 3000	0.9904	2.5 - 2000	0.9914	90.9	6.1	89.8	3.4	0.08	0.26	0.05	0.16
Caffeine	2.82	1 - 3000	0.9956	10 - 2000	0.9954	87.6	9.9	90.4	5.4	0.02	0.07	0.10	0.29
Carbamazepine	6.48	5 - 1000	0.9988	0.5 - 500	0.9958	99.3	4.1	96.8	1.9	0.06	0.18	0.02	0.06
Carbamazepine-10,11-epoxide	5.32	0.5 - 2000	0.9947	1 - 500	0.9995	97.2	10.2	97.8	4.9	0.04	0.11	0.02	0.06
Chloramphenicol	5.18	0.5 - 1000	0.9938	0.5 - 500	0.9913	90.4	9.4	94.8	3.2	0.02	0.06	0.02	0.06
Ciprofloxacin	3.54	2.5 - 2000	0.9914	2.5 - 2000	0.9797	27.8	9.2	33.9	12.4	0.01	0.04	0.07	0.22
Citalopram	6.22	1 - 1000	0.9935	1 - 1000	0.9818	89.1	7.2	90.2	4.4	0.07	0.21	0.68	2.05
Clarithromycin	7.22	2.5 - 2000	0.9963	2.5 - 1000	0.982	88.8	4.1	90.6	3.2	0.01	0.04	0.04	0.12
Climbazole	8.39	1 - 1000	0.9917	1 - 500	0.9746	86.7	10.4	85.8	8.8	0.05	0.14	0.08	0.25
Clofibrate acid	4.07	2.5 - 3000	0.9911	1 - 1000	0.9951	90.3	9.5	89.0	4.8	0.02	0.05	0.06	0.18
Diclofenac	6.95	5 - 2000	0.9917	2.5 - 1000	0.9894	87.0	14.6	90.6	14.5	0.05	0.17	0.08	0.23
Diltiazem	6.37	2.5 - 2000	0.9945	2.5 - 1000	0.9917	93.9	4.7	94.7	4.1	0.04	0.11	0.04	0.11
Fenofibrate	9.89	1 - 1000	0.9956	2.5 - 1000	0.9946	83.7	18.4	90.4	16.0	0.06	0.20	1.63	4.93
Fipronil	8.92	0.5 - 100	0.9988	0.5 - 100	0.9706	95.7	6.4	95.1	5.8	0.03	0.08	0.04	0.14
Fipronil desulfinyl	9.05	2.5 - 100	0.9669	0.5 - 50	0.9542	94.9	4.1	94.6	4.3	0.02	0.05	0.02	0.07
Fipronil sulfone	9.22	1 - 100	0.9888	0.5 - 50	0.9258	93.3	6.1	93.1	7.7	0.01	0.04	0.02	0.05
Fluconazole	4.02	2.5 - 3000	0.9957	2.5 - 2000	0.9947	90.3	5.6	92.3	3.6	0.02	0.07	0.03	0.10
Furosemide	4.42	1 - 3000	0.999	5 - 1000	0.9974	64.1	7.9	68.9	5.3	0.11	0.32	0.16	0.50
Gemfibrozil	8.78	1 - 3000	0.9937	2.5 - 500	0.9699	87.6	11.2	96.0	20.3	0.08	0.26	0.08	0.23
Hydrochlorothiazide	2.77	2.5 - 1000	0.9867	2.5 - 500	0.9864	87.5	9.1	99.5	11.8	0.06	0.18	0.44	1.33
Ibuprofen	7.68	10 - 2000	0.9628	0.5 - 100 ^e	0.9944 ^e	106.0	16.6	101.7	24.3	0.07	0.22	0.14 ^e	0.42 ^e
Indomethacin	7.24	2.5 - 2000	0.9888	5 - 2000	0.9858	92.6	8.3	105.6 ^e	12.7 ^e	0.10	0.31	0.12	0.37
Irbesartan	6.49	1 - 1000	0.9902	1 - 500	0.9865	79.3	16.5	81.0	20.8	0.03	0.08	0.03	0.09
Lamotrigine	4.02	2.5 - 1000	0.9926	2.5 - 500	0.9912	65.5	4.5	66.7	7.8	0.05	0.14	0.01	0.04
Lamotrigine N2-oxide	3.91	5 - 1000	0.9835	2.5 - 1000	0.999	81.3	9.3	79.9	5.9	0.03	0.09	0.05	0.14
Metoprolol	4.17	2.5 - 2000	0.9989	0.5 - 1000	0.9986	86.2	5.3	87.6	6.9	0.09	0.27	0.10	0.29
Metronidazole	2.16	5 - 2000	0.9953	1 - 1000	0.9913	86.6	14.0	92.3	10.2	0.10	0.32	1.74	5.28
N2-Methyl-Lamotrigine	4.62	2.5 - 1000	0.9945	5 - 500	0.9472	87.1	5.1	87.3	7.9	0.03	0.09	0.09	0.28
N-Acetyl-sulfamethoxazole	3.72	1 - 1000	0.9945	2.5 - 1000	0.9945	86.5	4.8	89.1	7.2	0.04	0.12	0.11	0.35
Oxcarbazepine	5.6	5 - 2000	0.9905	5 - 1000	0.9968	55.5	6.6	62.2	4.5	0.04	0.11	0.07	0.22
Propranolol	5.64	2.5 - 2000	0.9951	1 - 1000	0.9984	92.9	7.1	103.1	8.9	0.08	0.24	0.04	0.13
Sucralose	3.35	5 - 3000	0.9809	10 - 3000	0.9718	78.9	7.7	77.2	5.4	0.10	0.30	0.17	0.52
Sulfamethazine	3.62	2.5 - 2000	0.9918	2.5 - 3000	0.9993	28.4	7.5	35.0	7.2	0.07	0.22	0.05	0.16
Sulfamethoxazole	4.16	1 - 2000	0.9928	1 - 1000	0.9957	40.5	8.3	46.3	7.7	0.04	0.11	0.07	0.20
Sulfanilamide	1.28	5 - 3000	0.9951	2.5 - 2000	0.9987	26.8	9.4	25.0	9.9	0.04	0.13	0.04	0.11
Sulfanilic acid	0.47	5 - 3000	0.9946	10 - 2000	0.9789	60.9	10.3	52.7	9.9	0.08	0.25	0.25	0.76
Valsartan	5.37	2.5 - 2000	0.9952	5 - 2000	0.9962	81.1	17.2	73.5	18.7	0.05	0.15	0.09	0.27
Valsartan acid	2.54	5 - 2000	0.9928	2.5 - 1000	0.9991	40.7	14.5	44.0	14.3	0.03	0.08	0.06	0.17
Verapamil	7.1	1 - 1000	0.9933	0.5 - 500	0.9888	92.8	7.9	94.6	4.9	0.03	0.09	0.02	0.06

^a Accuracy was expressed as mean of relative recoveries calculated from the five studied levels.

^b Precision was calculated as average relative standard deviation (RSD%) obtained from the relative recoveries at each concentration level.

^c MDLs were estimated from the matrix-matched calibration curves using linear regression analysis.

^d MQLs were estimated from the matrix-matched calibration curves using linear regression analysis.

^e For ibuprofen in SWATH acquisition, linearity range, r^2 , LOD and LOQ were calculated using 5 calibration curve points and the molecular ion.

* Indomethacin was recovered only at 5 and 200 ng g⁻¹ in SWATH acquisition.

in the chromatogram to the minimum due to the use of specific DPs and CEs. On the other hand, the information acquired through the SWATH mode can always be useful at a later time for querying the data to identify new unidentified metabolites through a retrospective analysis. In fact, SWATH mode combines in the same run high quality HR-MS and HR-MS/MS data that can be used for quantitation or for a retrospective analysis. To enhance the selectivity

[25], the SWATH Variable Window Calculator was used to optimize the Q1 isolation window pattern for the matrix of interest, in this case lettuce, to achieve the right balance of compound coverage and specificity. Ten sequential Q1 variable windows were generated by injecting a lettuce matrix sample fortified with all target compounds in full-scan. The obtained MS survey scan contains the list of the m/z values of all precursors and the intensities from all

the peaks detected in the spectrum within the same retention time window of the chromatographic gradient. Variable windows were generated by computing the number of precursor ions and taking into account their intensities as a weighting factor. The generated windows based on the precursor ion distribution within the retention time of the LC gradient for both positive and negative SWATH acquisition were reported in supplementary material (Figure A.1).

Instrumental and method performances were assessed by considering accuracy, intra-day precision linearity, and limits of detection and quantitation. Data can be found in Table 2. Both MRM^{HR} and SWATH acquisition were compared in term of recoveries and precision. In term of accuracy, most of the compounds exhibited similar relative recoveries between 80 and 120% for both methods. However, not all compounds were adequately recovered. In fact, few compounds showed poor recoveries such as sulfanilamide, ciprofloxacin, and sulfamethazine presenting values of 26.8, 27.8, and 28.4% in MRM^{HR} and 25, 33.9, and 35% in SWATH, respectively. Moreover, indomethacin was recovered only at 50 and 200 ng g⁻¹ in SWATH acquisition (Table A.3). The failure to detect this compound at lower levels was likely caused by a coeluting spectral interference which was not sufficiently resolved from the fragment ion of indomethacin.

The intra-day precision expressed by repeatability was calculated as relative standard deviation (RSD%) obtained from the relative recoveries studied for each concentration level. Most of compounds are very precise with a deviation less than 10%, below the recommended 20% for both acquisition methods. Only one compound was >20% (acetaminophen, 22.3%) for MRM^{HR}, while in SWATH just three compounds presented an inaccuracy higher than 20% (Gemfibrozil 20.3%, irbesartan 20.8% and ibuprofen 24.4%). Individual values of relative recoveries and precision for each concentration for MRM^{HR} and SWATH are reported in Table A.3. Recovery values are in line with values reported elsewhere in similar methods [14,32]. Interestingly, although the recoveries are quite similar between the two acquisition modes, some differences are observable in terms of linearity and correlation coefficient. The linearity of the method was assessed using the matrix-matched calibration approach with a calibration curve constructed between 0.05 and 300 µg L⁻¹ (equivalent to 0.5–3000 ng g⁻¹ f.w. of lettuce leaves) taking into account at least eight calibration points. Calibration curves were constructed using linear weighted least-squares regression (1/x as weighting factor) by plotting the ratio of the analyte peak area to that of its corresponding IS. In MRM^{HR}, the chromatographic response was linear up to 2000 ng g⁻¹ for most compounds covering several order of magnitude with correlation coefficients (r²) above 0.99 for most compounds (Table 2). Only ibuprofen and fipronil desulfinil presented r² < 0.98, with values of 0.9628 and 0.9669, respectively. Despite the reliable results of MRM^{HR}, SWATH acquisition provided an overall shorter linearity response for all compounds with values up to 500 ng g⁻¹ for most compounds. This may be due to the detector which in this case must manage a multitude of ions at the same time and for this reason it is saturated. Furthermore, due to a reduction in orders of magnitude, r² was also affected. More in details, r² ranged from 0.9699 to 0.9995 for all compounds except for fipronil sulfone, N2-Methyl-Lamotrigine, and fipronil desulfinyl presenting r² of 0.9258, 0.9472, and 0.9542, respectively. In addition, linearity and r² for ibuprofen could not be calculated, given that this compound generally requires a quite large accumulation time that was possible only working in MRM^{HR}. Although the estimated number of cycles between MRM and SWATH is quite similar, 1167 and 1184, for MRM^{HR} and SWATH respectively, the accumulation time varies substantially between the two methods. In fact, while in MRM^{HR} each analyte has an accumulation time of 12 ms, in SWATH the entire Q1 window has a unique AT shared among all the analytes present in that window, corresponding to 40 ms. This AT

is insufficient for some less sensitive compounds such as, for example, ibuprofen. Furthermore, comparing with a recent study of Kadokami and coworkers [33], it is evident that the main factor that could have influenced the measurement of ibuprofen (or other weak acids such as indomethacin) is precisely the accumulation time. In fact, although our method has an MS-MS scanning range in which ibuprofen is contained smaller than that reported in [33] (165.8 - 214.8 Da vs 149.5 - 213.7 Da), the AT of this latter interval is 70 ms, sufficient for the determination of ibuprofen.

Regarding sensitivity, the Method Detection Limits (MDLs) and Method Quantification Limits (MQLs) were estimated from the matrix-matched calibration curves using linear regression analysis. As the Table 2 shows, both methods reported overlapping limits between them for the majority of compounds. For MRM^{HR}, MDLs and MQLs ranged from 0.01 to 0.12 ng g⁻¹ and 0.04 to 0.38 ng g⁻¹, respectively. As for SWATH, although most of the compounds presented acceptable MDLs and MQLs ranging between 0.01 to 0.16 and 0.04 to 0.50 ng g⁻¹, respectively, eight compounds (sucralose, benzotriazole, sulfanilic acid, hydrochlorothiazide, acetaminophen, citalopram, fenofibrate, and metronidazole) provided higher values (MDLs and MQLs ranging between 0.17 to 1.74 and 0.52 to 5.28 ng g⁻¹, respectively). Only for ibuprofen in SWATH acquisition, linearity range, r², LOD and LOQ were calculated using 5 calibration curve points and the molecular ion.

3.2. Evaluation of matrix effect

Lettuce leaves are a very complex matrix, and its extraction by aqueous organic solvent mixtures often leads to the presence of co-extracted matrix components in the final extracts [1,34–36]. Although it is necessary to extract a wide range of analytes as efficiently as possible, these co-extractive components of the matrix are not desired and may alter the ionization efficiency of the analytes in the ionization source influencing the signal intensity due to competition for available charged ions [37]. In fact, co-extractives can dramatically influence analysis performance causing suppression or improvement of the analyte response by decreasing or increasing the instrumental response factors of the target analytes, compared to those observed in the solvent [34,35]. In the quantitative analysis, ME can negatively influence an accurate quantification especially according to the type of sample and the properties of the analyte [38]. The mean ME of the five spiking levels for all compounds comparing both acquisition modes (MRM^{HR} and SWATH) are reported in Fig. 1 whereas they are well detailed in Table A.4.

To reduce the number of co-extracts and decrease the effects of the matrix, the use of a cleaning phase during sample preparation is often indispensable. [1,39–41]. As a result, besides recoveries, the impact of matrix effect (ME) was also assessed to evaluate the effect on the response of the analytes. The extent of matrix effects was measured by comparing the response in blank lettuce extracts and in solvent spiked at the same concentration levels used for recovery study (2, 5, 10, 50, 200 ng g⁻¹), before LC-MS/MS analysis. The extent of the effects due to the components of the matrix classified according to the average ME% for each compound is shown in Fig. 1 and is well detailed for each level in Table A.4. Both MRM^{HR} and SWATH acquisition have given similar effects matrix between them regardless of the instrumental conditions used. In fact, as reported in Fig. 1, the patterns observed are quite overlapping with respect to the matrix, indicating low effect (from -40 to 40%) for the 50% of the investigated compounds. ME values outside the acceptable range of ±40% denote strong signal suppression or enhancement with a consequent impact on method performance. For instance, the most susceptible are compounds with acidic groups (i.e. valsartan, fenofibrate, gemfibrozil, and sulfanilic acid) resulted the compounds more af-

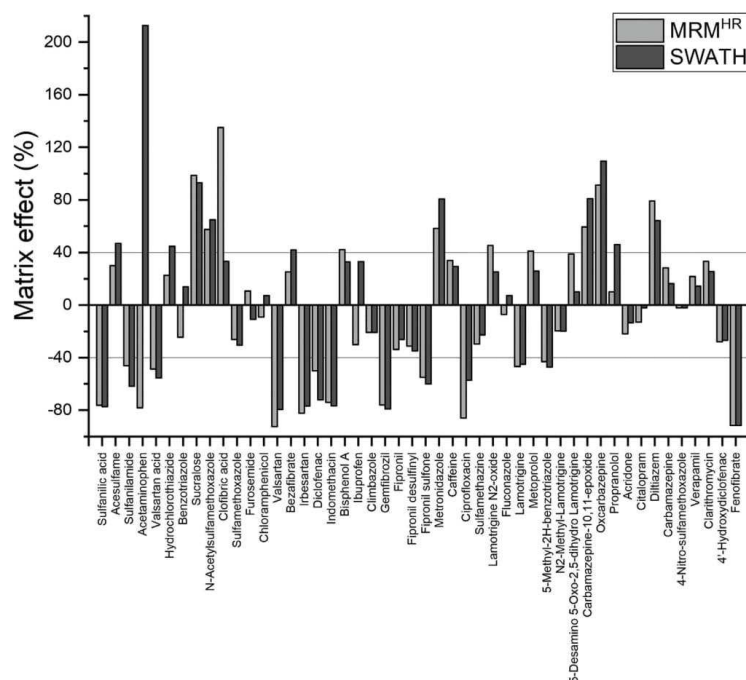


Fig. 1. Comparison of Matrix effect (%) for target compounds in different acquisition modes (MRM^{HR} and SWATH) at five different spiking levels. Bars refer to the mean values of the five concentrations. Compounds are ordered by retention time and ionization mode. From sulfanilic acid to fipronil sulfone for negative ionization, and from metronidazole to fenofibrate for positive ionization.

ected by the ion suppression close to -90% for both MRM^{HR} and SWATH. In contrast, sucralose and clofibrac acid showed substantial enhancement for MRM^{HR} (98.6 and 135%, respectively) while oxcarbazepine and acetaminophen for SWATH (109.4 and 212.6%, respectively). Curiously, acetaminophen shows an opposite performance depending on acquisition method used (-78.3% for MRM^{HR} and 212.6% for SWATH). Since co-eluted substances of the matrix may reduce the ion intensity of the target compounds, the use of a matrix-matched calibration curve combined with the internal standard approach should solve this problem, improving the accuracy of the quantification and reducing the signal suppression/enhancement of the analyte.

3.3. Optimization of the sample extraction procedure: comparison of different methods

Freeze drying process prolong the stability of compounds of interest in commodities with a high amount of water without causing a sensible reduction in their amount [42]. For this reason, we decide to use freeze-dried lettuce with a low residual water for the development of the present method. However, the original QuEChERS method was designed for fresh samples of 10 g with more than 80% water [28]. To keep the salting out as similar as possible to the original method with the use of 10 g of fresh plant tissue and due to the normal water content of a lettuce greater than 90%, we have chosen to use 1 g of freeze-dried lettuce leaves and fix the hydration volume to 9 ml of water (ammonium acetate or ammonium formate).

The effect of salt and buffer addition, on the partitioning of the compounds was preliminary evaluated comparing two commercially available kits (OR and EN). In this first screening, a dispersive SPE clean-up using PSA/C18 and/or the addition of 1% of formic acid were also evaluated (Table A.6). The OR kit resulted more effective allowing the recovery of the 62% of the compounds ($R > 60\%$) compared to EN (only 55%) (Figure A.3). The addition of formic acid prior to the cleanup step seems to slightly reduce the overall recoveries. For example, benzotriazole, caffeine, carbamazepine-10,11-epoxide, and furosemide resulted significantly influenced by the use of the acid (Table A.6). Since the pK_a of these compounds is > 7.5 , in an acidic pH, it would already be positive charged and the extraction in the organic solvent is less efficient [43]. Also the clean-up step tends to reduce the recovery of some analytes. In particular, the PSA, being a weak anion exchanger, could affects the recovery of more polar organic compounds due to hydrophilic interactions. [32]. The combined use of 1% HCOOH and PSA/C18 leads to a slightly increase in recoveries especially in the case of OR protocol (67%). The EN buffer seems to mitigate this effect (55%). In this first evaluation OR 1% HCOOH PSA/C18 appeared the best choice.

In follow-up experiments, the efficiency of the selected QuEChERS method (OR) was assessed by comparing the addition of a proper amount of HCOOH in combination of PSA and alternative hydration solvents. In this second phase, three new compounds, of which the analytical standards were not available at the beginning, were added (5-desamino 5-oxo-2,5-dihydro Lamotrigine, lamotrigine N2-oxide, and N2-methyl-lamotrigine). Suitable modifications of the OR method were compared in Fig. 2. The Original QuECh-

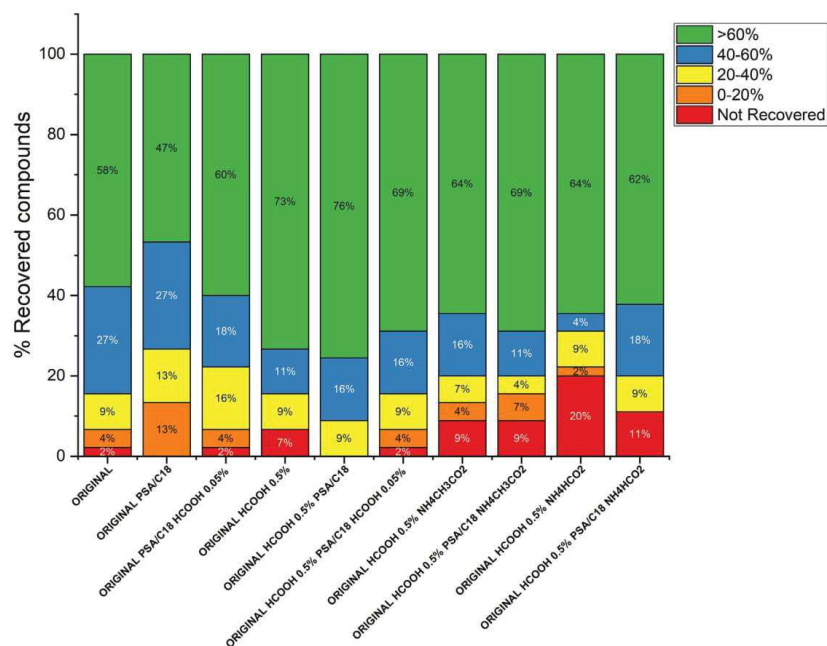


Fig. 2. Comparison of recoveries (%) of target analytes in lettuce spiked at 10 ng g^{-1} using the Original QuEChERS extraction salts kit and different modifications of the Standard method including the hydration of the sample, and/or the clean-up step.

ERS and Original PSA/C18 were compared against the addition of $50 \mu\text{L}$ formic acid (0.5%). Due to the prolonged contact with PSA [28], the pH of the extracts could increase compromising the stability of some pharmaceuticals with acidic groups (e.g. fenofibrate, ibuprofen, indomethacin) and reduce the overall recoveries. Hence, the possible degradation of such basic compounds was also tested by an immediate acidification of the extracts after the PSA/C18 step by adding $10 \mu\text{L}$ of a 5% formic acid solution in acetonitrile per mL (corresponding to a final concentration of 0.05% HCOOH). This step was tested with or without the initial acidification (Fig. 2). The addition of acid after the cleanup step seems to adversely affect some compounds such as ciprofloxacin, indomethacin, and valsartan (Table S7, ESM). The addition of ammonium formate or ammonium acetate were also tested to enhance the extraction efficiency. Both solutions were prepared by adding 200 mg in 9 mL of pure water with a final pH of 6.6 and 6.7, respectively. These ammonium salts, when added during the hydration of the sample, are supposed to compete with the target compounds improving the absorption of interfering substances on the sorbent. As a result, the pure water replacement should induce phase separation and extraction due to a lower adsorption of the target analytes to the matrix [44]. However, the addition of ammonium salts during the hydration showed that no relevant improvement was observed when we used ammonium acetate. About 9% of compounds were not recovered in presence of ammonium acetate, whether or not PSA is employed (Fig. 2). On the other hand, when we used ammonium formate a significant decrease of the average recoveries was indeed observed. In fact, about 20% of the compounds have not been recovered at all when OR is used without PSA. This percentage is reduced to 11% in the presence of PSA, however insufficient to justify its use. Also in this case, the most susceptible compounds are ciprofloxacin and some drugs with acidic groups (fenofibrate,

ibuprofen, indomethacin) (Table A.7). Finally, the addition of $50 \mu\text{L}$ formic acid (0.5%) to the Original QuEChERS following by a PSA/C18 clean-up step seems to provide significant benefits to justify the change of our initial addition of 1% formic acid. The use of 0.5% HCOOH led to improvement in recoveries enabling the highest recoveries of all compounds. In this way, this combination was more effective given that all the compounds were effectively recovered reporting values above 20%. In particular, 76% of the compounds presented values higher than 60%. (Fig. 2 and Table A.7). Only 4 compounds presented values below 40% (ciprofloxacin 21.8%, sulfamethazine 20.5%, sulfamethoxazole 23.9%, and sulphanilamide 38.7%). Based on the results of the experiments described in Fig. 2, Original HCOOH 0.5% PSA/C18 was selected for the further validation study.

3.4. Effect of 2-step clean-up on co-extractives

The amounts of undesirable co-extractives from lettuce leaves samples were determined by weighting after evaporation of the extracts to dryness in pre-weighted test tubes, according to optimized protocol. In general, the use of acetonitrile in the partitioning step minimized only the fat co-extractives [19,28]. The dSPE clean-up with PSA was not initially developed to remove chlorophyll and sterols from vegetable extracts [45]. In fact, the PSA can only retain fatty acids and other polar compounds in the matrix due to the presence of the primary and secondary amine moieties. Furthermore, the reverse phase absorbent C18 is able to effectively remove starch and sugar from samples by trapping them [46]. A dispersive SPE formed of a combination of PSA and GCB (Graphitized Carbon Black) is generally used to remove chlorophyll from samples with a high content of pigments [28]. To prevent the compounds of interest from being absorbed by the GCB, we

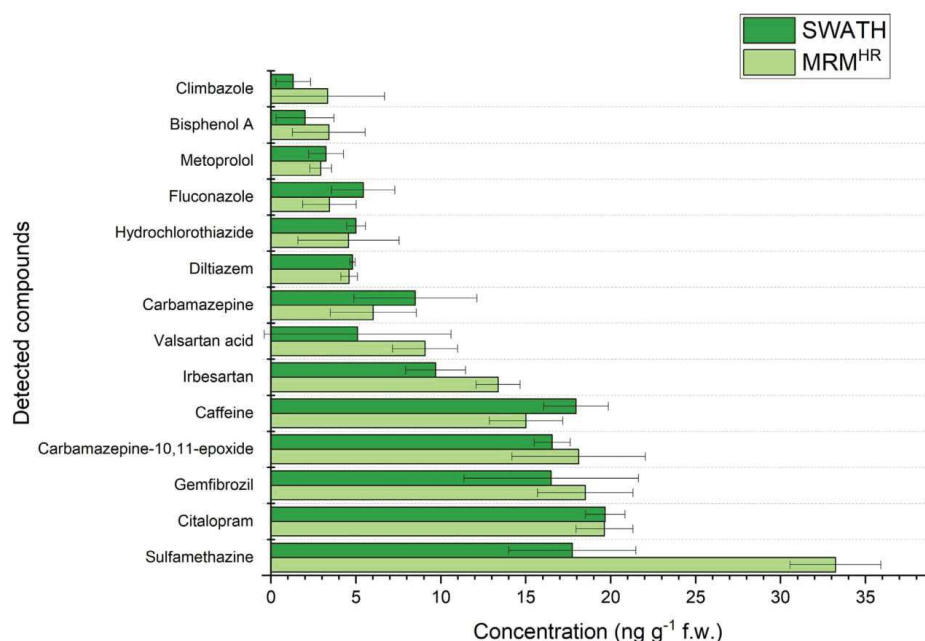


Fig. 3. Presence of target compounds in lettuce samples irrigated with treated wastewater effluent by comparing MRM^{HR} and SWATH acquisitions.

opted for an additional simple and inexpensive cleaning procedure which consists in leaving the extracts overnight at $-20\text{ }^{\circ}\text{C}$ (freezing-out) before the following clean-up step with dispersive PSA. This approach that does not require extra solvents such as hexane [1], which is generally used to promote the precipitation of fats at the low temperature [19], and can be used to remove co-extractives in bulk from aqueous solvents or other relatively polar solvents [47]. Figure A.2.A shows the amount of co-extractives in the acetonitrile extracts using only OR protocol, the effect of temperature, and the reduction of co-extractives in presence of the 2-step clean-up. The gravimetric results demonstrated the reduction of the amount of co-extractives in acetonitrile extracts by almost 60% by weight using the freezing-out step. In addition, after the dispersive-SPE clean-up, another large part of co-extractives was removed from extracts. The freezing procedure together with the dispersive clean-up led to a rather substantial reduction of co-extractives (-83.5%) corresponding to a residual amount of 5 mg in the final extract. Although it has not been evaluated, the addition of formic acid seems to favor the precipitation of chlorophyll during freezing-out, as shown in Figure A.2.B. Another important advantage of the freezing-out is that part of the residual water that MgSO_4 was unable to remove during the salting out, is deposited on the bottom of the tube, frozen together with the co-extractives.

3.5. Application to real samples

In order to evaluate the presence of pharmaceuticals in lettuce leaves, the validated method was applied to lettuce plants growth in controlled condition (greenhouse experiment). During the study, 12 lettuce seedlings (*Lactuca sativa* L.) cv. Maravilla de Verano-Canasta at the approximately four-leaf stage were transplanted in 12 plastic pots (22 cm diameter) filled with 3 kg of pristine soil collected from the Parc Agrari of El Prat de Llobregat (Barcelona,

Spain). For the first five days after transplant, all plants were irrigated with tap water. Afterwards, eight plants were irrigated with 100 mL of treated wastewater effluent provided by the wastewater treatment plant EDAR of El Prat de Llobregat (Barcelona, Spain) each two days for the entire growing period. The remaining four pots were used as controls and were irrigated with 100 mL of tap water each two days. After 60 days, all plants were harvested, washed with deionized water to remove any soil residues, and gently blot dried with paper towel. Then, the samples were extracted according to the optimized procedure.

Quantification of the target analytes in plant samples was achieved in MRM^{HR} and SWATH acquisition modes using the internal standard method. Each analyte was quantified by using its corresponding deuterated standard. An isotopically labelled compounds with similar retention time or from the same group was used only for the quantification or those compounds in which isotopically analogues compounds were not available (Table A.2). Only 14 out of 48 studied compounds were detected and were reported in Fig. 3. Most of detected compounds presented very similar levels of contamination. Six analytes presented concentrations close to 5 ng g^{-1} , such as climbazole (3.3 and 1.3 ng g^{-1} , for MRM^{HR} and SWATH, respectively), bisphenol A (3.4 and 2.0 ng g^{-1}), metoprolol (2.9 and 3.2 ng g^{-1}), fluconazole (3.4 and 5.4 ng g^{-1}), hydrochlorothiazide (4.5 and 5.0 ng g^{-1}), and diltiazem (4.6 and 4.8 ng g^{-1}). Carbamazepine is taken up below 10 ng g^{-1} (6.0 and 8.5 ng g^{-1} , for MRM^{HR} and SWATH, respectively). A comparison of carbamazepine obtained in the two modes, and SWATH MRM^{HR}, is shown in Figure A.4. Four compounds were detected in the leaves at concentrations below 20 ng g^{-1} , like caffeine (15.0 and 17.9 ng g^{-1}), carbamazepine epoxide (18.1 and 16.5 ng g^{-1}), gemfibrozil (18.5 and 16.5 ng g^{-1}), and citalopram (19.6 and 16.7 ng g^{-1}).

Some minor differences in concentrations were found for only three compounds comparing MRM and SWATH. Valsartan acid, irbesartan, and sulfamethazine presented higher values in MRM (9.0, 13.3, and 33.2 ng g⁻¹) compared to SWATH (5.1, 9.7, and 17.7 ng g⁻¹). These results are comparable with those previously reported by other authors working with the same matrix [5,7,9,48]. Irrigation with reclaimed water or contaminated water containing trace levels of pharmaceuticals could lead to uptake and the consequent accumulation of pharmaceuticals in green parts of lettuce crops, posing potential risks to human health.

4. Conclusions

Based on the results of this study, we concluded that the simple changes made to the classic QuEChERS method provided good possibilities to achieve our goals and simultaneously improve the overall recoveries for a large number of compounds without sacrificing the performance of a multi-residual method. Method performances were also studied by comparing two different acquisition techniques provided by the same instrument. Although the two techniques provide quite similar results in term of accuracy and limits of detection, specifically, in the SWATH acquisition, except ibuprofen and indomethacin which presented sensitivity and recovery problems, respectively, 46 compounds presented similar results to the MRMR^{HR} mode. Nonetheless, we will explore in a future study the full potential of SWATH mode not only to quantify small molecules but also to evaluate its capabilities in suspect screening and non-target analysis in a complex matrix like lettuce, taking full advantage of high resolution spectral libraries.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Nicola Montemurro: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. **Anastasia Orfanoti:** Formal analysis, Methodology, Software, Validation. **Rayana Manasfi:** Formal analysis, Methodology, Software, Validation. **Nikolaos S. Thomaidis:** Writing - review & editing. **Sandra Pérez:** Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2020.461566.

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Chapter 4:

Optimization and validation of wastewater-borne organic pollutants including pharmaceuticals extraction from lettuce root and soil

1. Evaluation of different QuEChERS-based methods for the extraction of 48 wastewater-derived organic contaminants from soil and lettuce root using high resolution LC-QTOF with MRMHR and SWATH acquisition modes

1.1. Context

Crops irrigation with treated wastewater is an important source to spread PhACs in the agroecosystem. Understanding the accumulation of these chemicals in soil and plant tissues such as roots, leaves and fruits is crucial for a better assessment of the risk to human health following the consumption of treated wastewater irrigated crops. Therefore, simple, fast, cheap yet reliable analytical methods are required depending on the matrix under study. However, agricultural matrices are very complex, and each matrix has very different components. Therefore, the availability of a common yet rigorous extraction method for multiple matrices is very beneficial.

1.2. Objective and main results

The objective of this article was to develop a common and robust extraction method for two matrices belonging to agroecosystem: the lettuce root and soil. QuEChERS-based method followed by detection and quantification on a high-resolution mass spectrometry was successfully developed for 48 PhACs and other wastewater-borne organic contaminants and their TPs frequently detected in wastewater (including analgesics and anti-inflammatories, anti-hypertensives, antifungal agents, lipid regulators, psychiatric drugs and stimulants, β -blockers, antibiotics, antimycotics, and sweeteners). Method performance was compared using two acquisition modes, high resolution multiple reaction monitoring (MRMHR) and Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH), in terms of recovery, linearity, intra-day precision, method detection limits (MDLs), method quantification limits (MQLs), and matrix effect (ME). Though similar results were obtained with both acquisition modes, MRMHR provided more consistent results for most of the compounds. Recoveries in MRMHR mode ranged from 63 to 121% and 45 to 104% for lettuce root and soil, respectively, for most of compounds. finally, the developed method was

successfully applied on real agricultural field samples, irrigated with domestic treated wastewater for 2 years under greenhouse conditions. Four and 13 compounds were detected in lettuce root and soil, respectively.

1 **Evaluation of different QuEChERS-based methods for the extraction of 48 wastewater-**
2 **derivated organic contaminants from soil and lettuce root using high resolution LC-**
3 **QTOF with MRM^{HR} and SWATH acquisition modes**

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25 **Abstract**

26 The reuse of treated wastewater in agriculture is an important route of introducing a large
27 number of organic contaminants into the agroecosystem. In this study, a modified QuEChERS-
28 based approach was developed for rapid, simple and simultaneous extraction of 48 organic
29 wastewater-derived contaminants from soil and lettuce root. Twenty two modified methods
30 were tested in order to obtain best and well-compromised recoveries for all target compounds
31 for soil and roots. Finally, a common method was chosen for both matrices consisting of a single
32 extraction step using EDTA-McIlvaine buffer and the unbuffered Original QuEChERS salts.
33 Method performance was accomplished by liquid chromatography-mass spectrometry on a
34 QToF-MS system using two different acquisition modes, the ultra-fast high-resolution multiple
35 reaction monitoring (MRM^{HR}) mode and the innovative Sequential Window Acquisition of All
36 Theoretical Fragment-Ion (SWATH) mode. Validation was evaluated in terms of recovery,
37 linearity, intra-day precision, method detection limits (MDLs), method quantification limits
38 (MQLs), and matrix effect (ME). Recoveries in MRM^{HR} mode ranged from 63 to 121% and 45 to
39 104% for lettuce root and soil, respectively, for most of compounds. The method was then
40 applied to follow the target compounds in soil and lettuce root, where the system lettuce-soil
41 was irrigated with treated wastewater under real greenhouse conditions. Four and 13
42 compounds were detected in lettuce root and soil, respectively.

43 **Keywords:** Pharmaceuticals, wastewater reuse, soil contamination, root uptake, modified
44 QuEChERS, LC-HRMS/MS.

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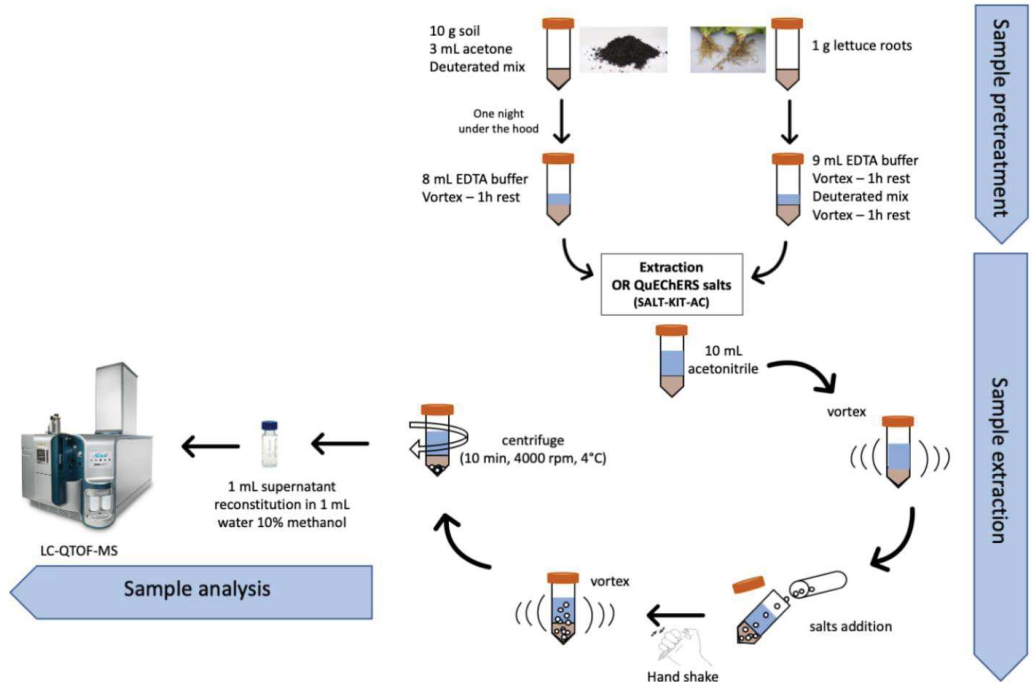
46 **Highlights**

- 47 • Development of a robust modified QuEChERS-based method for soil and lettuce roots
- 48 • Simultaneous extraction and analysis of 48 wastewater-borne organic pollutants
- 49 • Comparison of high resolution MRM and SWATH acquisition modes in LC-QTOF
- 50 • 5 to 14 compounds out of 48 were detected in real soil and lettuce samples

51

52

53 Graphical abstract



54

55

56 **Introduction**

57 Meeting the lack of water for agricultural purposes is a global problem today. To alleviate this
58 problem, countries in arid and semi-arid areas use treated wastewater (TWW) for crop irrigation
59 as an alternative to natural water resources. Furthermore, more than 50% of freshwater
60 consumption in southern Europe refers to the agricultural sector [1]. This practice constitutes
61 an important entry route for organic micropollutants such as pharmaceutical active compounds
62 into the soil and crops [2]–[4], as traditional wastewater treatment plants (WWTPs) are not
63 designed for such purposes, ensuring in some cases only partial removal of organic
64 micropollutants. Once these pollutants are spread on agricultural soil through the reuse of
65 wastewater, they can be accumulated in the soil and/or taken up by plants through the root
66 system [5]. Based on their physicochemical properties, some of them remain confined to the
67 rhizosphere. Others, on the other hand, have the potential to be absorbed by plant roots [6],
68 and be translocated to the aerial parts with consequent introduction of undesirable compounds
69 into the food chain and potential risks for human health, as well as for the health of the
70 ecosystem [7]–[13]. In fact, the roots constitute the main point of entry of contaminants through
71 the soil, regulating the translocation of pollutants to the leaves and fruits [7], [14]. Therefore,
72 pollutants that are unable to reach the leaves/fruit, either accumulate in the roots or remain
73 confined to the soil [6]. For example, Beltrán and colleagues found the highest concentration of
74 triclosan in the root part of corn [15].

75 Understanding the accumulation of pollutants in soil and plant tissues such as roots, leaves and
76 fruits is crucial for a better assessment of the risk to human health following irrigation of
77 vegetable crops with TWW. Furthermore, in order to respond to the scientific need for risk
78 assessment, simple, fast, cheap but reliable extraction methods are required depending on the
79 matrix under study. Environmental samples are known to exhibit numerous analytical
80 complexities. Each of these samples has very different constituents of the matrix. They are
81 involuntarily co-extracted during sample preparation, and their presence in the extracts can
82 mask the detection of the analytes of interest during chromatographic analysis [16]. For
83 example, the soil is rich in organic matter, humic and fulvic acids, while the leaves of vegetables,
84 especially lettuce, are rich in waxes, fatty acids and chlorophylls. These matrix components
85 establish different interactions with target analytes and influence their extraction yields.
86 Therefore, specific and rigorous extraction methods and cleanup procedures are required for
87 each of these matrices.

88 In addition to sample preparation and extraction, LC-MS / MS analysis as well as data analysis
89 are also time-consuming. So having a common extraction method for multiple matrices is very
90 beneficial. It provides a time-saving and less complications for the analyst who performs the
91 extraction which use fewer objects because the same equipment and laboratory materials will
92 be used for both matrices. In addition, the detection of these pollutants in environmental
93 matrices, such as water, soil, plants or biota, is challenging due to their presence at low
94 concentrations, and consequently to the low detection limits required for their analysis.

95 Several extraction methods have been reported for organic pollutants derived from wastewater
96 from water [17], soil [18], sediments [19], and crops [3]. In particular, in recent years, various
97 techniques have been introduced for the analysis of wastewater derived organic pollutants in
98 soil samples, such as pressurized liquid extraction (PLE) [20]–[22], microwave assisted extraction
99 (MAE) [23], [24], and ultrasound assisted extraction (UAE) [25]–[27], which allowed to obtain
100 high extraction recoveries. However, these techniques are time, materials and solvents
101 consuming, and require advanced instrumentation and analysts with high expertise to
102 manipulate them.

103 Generally, the roots of herbaceous plants and vegetables are intimately in contact with the soil,
104 placing themselves in the first 50 cm of depth. In particular, the lettuce has a root system with
105 a short tap-root from which depart numerous thin roots that remain superficial. This feature
106 means that separating the roots from the soil without damaging them is quite complicated.
107 Furthermore, lettuce roots have no commercial or food value, so even the possibility of
108 obtaining real field samples of roots exposed to organic contaminants is quite limited and
109 remains confined only to some greenhouse or small-scale studies. For this reason, according to
110 our literature survey, although lettuce is a model plant for studying the absorption of organic
111 contaminants, there is a lack of studies related to the development of specific extraction
112 methods for lettuce roots. Furthermore, although the QuEChERS method is known for its
113 simplicity and speed of execution, and despite the soil being a fairly studied matrix previously,
114 however, to our knowledge, only 5 studies have been conducted for the extraction of
115 wastewater-derived organic compounds from the soil using this method [28]–[32]. However,
116 none of the previously mentioned studies are able to extract 48 organic micropollutants
117 simultaneously, belonging to very different families such as, pharmaceuticals, fungicides, food
118 additives, etc.

119 Taking into account the limitations mentioned above, there is a clear need to develop a common
120 extraction method applicable at the same time for both matrices, the soil and the lettuce roots.

121 In this context, the aim of our study consisted in to develop and validate a fast and easy multi-
122 residue extraction method for lettuce root and soil, using a modified QuEChERS approach, in
123 order to facilitate the detection and quantification of 48 wastewater-borne organic pollutants
124 between the soil-root system for a better risk assessment for human health or the environment.
125 A different QuEChERS method for lettuce leaves was developed based on the different needs of
126 extraction yields and matrix cleanup. Further details about lettuce leaves extraction method are
127 available in our recent article [15].

128 Finally, method performance and validation was performed on a SCIEX X500R LC-QTOF-MS
129 hybrid system, comparing two different high resolution mass spectrometry modes: high
130 resolution multiple reaction monitoring (MRM^{HR}) and the Sequential Window Acquisition of All
131 Theoretical Fragment-Ion (SWATH) acquisition modes. The performance of 22 different
132 modified QuEChERS approaches was performed by varying the pH of the extraction solvent,
133 QuEChERS salts, water content and purification absorbents. After validation, the optimized
134 analytical methods were applied to the analysis of the selected compounds in real soil and
135 lettuce root samples irrigated with TWW under greenhouse conditions for 2 years.

136 **1. Experimental section**

137 **1.1. Material and reagents**

138 Commercially available Original non-buffered QuEChERS salts (OR-a, containing 4 g MgSO₄ + 1 g
139 NaCl, and OR-b, containing 6 g MgSO₄ + 1.5 g NaCl), the citrate buffered European EN 15662
140 salts (EN containing 4 g MgSO₄ + 1 g NaCl + 0.5 g disodium citrate sesquihydrate) used for salting
141 out extraction process and dispersive solid phase extraction (dSPE) sorbents for the cleanup step
142 (900 mg MgSO₄ + 150 mg PSA + 150 mg C18e) were supplied from BEKOLut GmbH & Co. KG
143 (Hauptstuhl, Germany). Ammonium acetate (NH₄CH₃CO₂), ammonium formate (NH₄HCO₂),
144 sodium sulfate (Na₂SO₄) and sodium chloride (NaCl) were supplied from Sigma-Aldrich (St. Luis,
145 MO, U.S), while ammonium fluoride was obtained from Fisher Chemical (Fisher Scientific SL,
146 Madrid, Spain).

147 LC-MS grade acetonitrile (ACN) (≥ 99.9%), methanol (MeOH) (≥ 99.9%), ethyl acetate (EtAc) (≥
148 99.9%), dimethyl sulfoxide (DMSO) (≥ 99.9%), and HPLC water were purchased from Merck
149 (Darmstadt, Germany). Formic acid (≥ 96%, ACS reagent) and ammonium acetate were supplied
150 by Sigma-Aldrich while ammonium fluoride was bought from Fisher Chemical (Fisher Scientific
151 SL, Madrid, Spain). For high purity mobile phase solutions, ACN and water (Optima™ LCMS
152 Grade) were purchased from Fisher Chemical (Fisher Scientific SL, Madrid, Spain).

153 Di-sodium hydrogen phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), citric acid monohydrate
154 ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$) and anhydrous ethylenediamine tetraacetic acid (EDTA) ($\geq 99\%$) for the EDTA-
155 McIlvaine buffer (pH 4) preparation (Electronic Supplementary Material, ESM) were obtained
156 from Sigma-Aldrich (St. Luis, MO, U.S).

157 High purity ($>90\%$) reference standard of all target compounds (acesulfame, acetaminophen,
158 acridone, benzotriazole, 5-Methyl-2H-benzotriazole, bezafibrate, bisphenol A, caffeine,
159 carbamazepine, carbamazepine-10,11-epoxide, chloramphenicol, ciprofloxacin, citalopram,
160 clarithromycin, climbazole, clofibrac acid, diclofenac, 4-hydroxydiclofenac, diltiazem,
161 fenofibrate, fipronil, fipronil desulfinyl, fipronil sulfone, fluconazole, furosemide, gemfibrozil,
162 hydrochlorothiazide, ibuprofen, indomethacin, irbesartan, lamotrigine, lamotrigine N2-oxide, 5-
163 desamino-5-oxo-2,5-dihydro lamotrigine, methadone, metoprolol, metronidazole, N2-methyl-
164 lamotrigine, N4-acetyl-sulfamethoxazole, oxcarbazepine, propranolol, sucralose,
165 sulfamethazine, sulfamethoxazole, 4-nitro-sulfamethoxazole, sulfanilamide, sulfanilic acid,
166 valsartan, valsartan acid, verapamil) were purchased from Sigma-Aldrich (St. Luis, MO, U.S).
167 Analytical standards were individually weighted and dissolved in 100% ACN, 100% HPLC water,
168 100% MeOH or 100% DMSO, at a concentration of 5 or 10 mg mL^{-1} , according to compound
169 solubility, and stored in the dark at -20°C . The CAS numbers, molecular formulas, molecular
170 weight, and other relevant properties of all target compounds are reported in Table 1SM.

171 The corresponding isotope-labelled internal standards (IS) used as surrogates were obtained
172 from Cerilliant (Round Rock, TX, USA), Alsachim (Illkirch-Graffenstaden, France), Santa Cruz
173 Biotechnology (Dallas, TX, USA), or Toronto Research Chemicals (Toronto, ON, Canada) as
174 solutions at a concentration of 1 mg mL^{-1} or prepared in MeOH or DMSO at a final concentration
175 of 1 mg mL^{-1} .

176 Working solution mixtures used for analysis and calibration purposes were prepared separately
177 for all compounds and labeled standards by serial dilution in MeOH at concentration of 2 $\mu\text{g mL}^{-1}$
178 and stored in the dark at -20°C .

179 Deuterated compounds (hydrochlorothiazide- d_2 , ibuprofen- d_3 , acetaminophen- d_4 , bezafibrate-
180 d_4 , carbamazepine- d_{10} , fenofibrate- d_6 , indomethacin- d_4 , metoprolol- d_7 , valsartan- d_3 , naproxen-
181 d_3 , sulfamethazine- d_4 , sulfamethoxazole- d_4 , gemfibrozil- d_6 , valsartan acid- d_4 , furosemide- d_5 ,
182 sucralose- d_6 , benzotriazole- d_4 , metronidazole- d_4 , irbesartan- d_6 , bisphenol A- d_8 , ciprofloxacin- d_8 ,
183 climbazole- d_4 , acesulfame- d_4 , caffeine- $^{13}\text{C}_3$, fluconazole- $^{13}\text{C}_3$, citalopram- d_6 , diclofenac- $^{13}\text{C}_6$,
184 lamotrigine $^{13}\text{C}_3$) were obtained from Cerilliant (Round Rock, TX, USA), Alsachim (Illkirch-

185 Graffenstaden, France), Santa Cruz Biotechnology (Dallas, TX, USA), or Toronto Research
186 Chemicals (Toronto, ON, Canada).

187 **1.2. Sample pretreatment and extraction**

188 *Lettuce root sample.* Lettuce was selected as plant in this study for several reasons, in particular,
189 it is the most cultivated plant worldwide, it grows quickly in greenhouse and open fields, and it
190 has a complex roots' structure that may facilitate the uptake of organic contaminants from soil.
191 Organic lettuce (*Lactuca sativa* L., "Maravilla de verano-Canasta" sp.) were purchased from a
192 local supermarket in Barcelona, Spain. Lettuce roots were separated from the rest of the lettuce,
193 washed and freeze dried. Thereafter, dried roots were homogenized by a mortar and stored in
194 the dark at -40 °C until method optimization and validation.

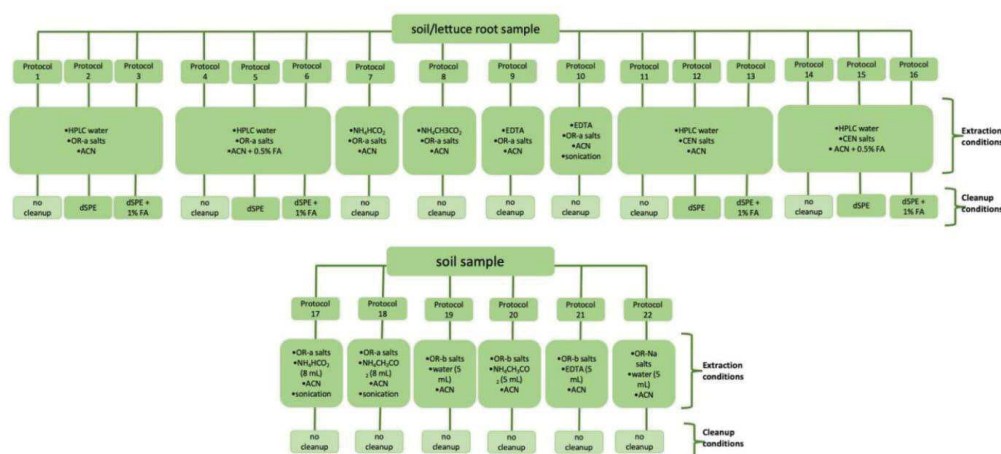
195 *Lettuce root pretreatment and spiking.* 1 g were weighted in 50 mL centrifuge tube, hydrated
196 with 9 mL EDTA-McIlvaine buffer, vortexed and left for one hour, thereafter roots were spiked
197 with a proper volume of the standard mix (excluding deuterated compounds for method
198 optimization), vortexed, and rested for another hour.

199 *Soil sample.* The contaminants-free soil used in this work was collected from L'Hospitalet de
200 Llobregat in Barcelona, Spain. It was homogenized by a mortar and sieved at 2 mm pore size to
201 remove coarse particles and increase its homogeneity. Thereafter, soil was left under the hood
202 for 2-3 days to assure its total dryness. Finally, it was stored in the dark at -40 °C until method
203 optimization and validation.

204 *Soil pretreatment and spiking.* 10 g of soil were weighted in a 50 mL centrifuge tube; 3 mL of
205 acetone were added followed by spiking with a proper volume of the standard mix (excluding
206 deuterated compounds for method optimization) to obtain the desired concentration (10 ng/g
207 for method optimization and 2, 5, 10, 50 and 200 ng/g for method validation). The tubes were
208 then vortexed and left under the hood at room temperature for one night, to allow solvent
209 evaporation and compounds interaction with soil matrix. Consequently, soil sample were
210 hydrated by adding 8 mL of EDTA-McIlvaine buffer, vortexed, and rested for one hour before
211 extraction step.

212 *Sample extraction.* Recoveries of all target compounds were optimized following the different
213 protocols reported in Fig. 1 (see Table 2SM for further extraction and cleanup details). A
214 common modified QuEChERS method, was finally selected for both matrices (Protocol 9), which
215 consisted of one single extraction step according to the following protocol: 10 mL of ACN were
216 added to the hydrated sample and vortexed. The original QuEChERS salt tube (OR-a, containing

217 4 g MgSO₄ + 1 g NaCl) was emptied in the mixture of ACN and sample, the obtained mixture was
 218 immediately hand shaken for one minute in order to prevent salts agglomeration then vortexed
 219 for another minute. Finally, the tube was centrifuged for 10 min at 4000 rpm and 4 °C. 1 mL of
 220 the obtained supernatant was evaporated under gentle nitrogen flow at room temperature until
 221 total dryness. Residues were collected with water/MeOH (90/10, v/v) for injection. In parallel,
 222 three more tubes for both matrices were treated the same way excluding the spiking of standard
 223 mix, in order to obtain a 'blank extract'.



224

225 *Figure 1. Extraction and cleanup condition of protocols 1 to 22 for lettuce root and soil matrices*
 226 *(protocols 17 to 22 are for soil matrix only)*

227 **1.3. LC-MS/MS analysis**

228 Target compounds recoveries were evaluated using the SCIEX ExionLC™ AD system with Turbo
 229 V™ source and Electrospray Ionization (ESI) operating in positive and negative mode, coupled
 230 with the SCIEX X500R QTOF system (Sciex, Redwood city, CA, U.S.). 10 µL of injection volume
 231 was run in the system with an auto-sampler temperature of 8 °C, for 12 min of injection time.
 232 Chromatographic separation was achieved on a Hibar® HR Purospher® STAR RP-C18 column (100
 233 mm x 2.1 mm i.d., 2 µm particle size, Merck, Darmstadt, Germany), with 40 °C of temperature
 234 in the column oven. Mobile phases used for the positive electrospray ionization mode (ESI+) and
 235 negative electrospray ionization mode (ESI-) were (A) 5 mM of ammonium acetate and 0.1% FA
 236 in water and (B) ACN, and (A) 2 mM ammonium fluoride in water and (B) ACN, respectively. The
 237 elution gradient both modes started with 5% of B for 20 s, then increasing to 40% in 6 min and
 238 to 96% in the following 4 min, held at 98% for 50 s, and finally returning to the initial conditions
 239 in the last min. The Guided MRM^{HR} tool from SCIEX was used for the optimization of high-

10

240 resolution fragment ions at specific ionization mode, DP, and CE for each compound, results are
241 shown in Table 3SM. The SWATH acquisition mode was performed with optimised variable Q1
242 windows (100 to 950 m/z, 40 ms AT) generated by an active Excell spreadsheet template known
243 as SWATH variable window calculator (Ver. 1.0) provided from SCIEX. More details about the
244 optimisation of both acquisition modes are presented in ESM.

245 High resolution data were acquired using two distinct workflows for target (high resolution
246 multiple reaction monitoring, MRM^{HR}) and non-target (SWATH) screening modes. Both modes
247 consisted of a single TOF-MS experiment over a range from m/z 100 to 950 with 120 ms of
248 Accumulation Time (AT) in MRM^{HR} and 40 ms in SWATH; fixed values of Declustering Potential
249 (DP) and Collision Energy (CE) were set to 80 V and 10 V and -80 V and -10 V, for positive and
250 negative mode of ionization, respectively.

251 Qualitative and quantitative analysis were performed using SCIEX OS™ Software version 1.6
252 (Sciex, Redwood City, CA, U.S.). Two high resolution ions were used for each compound, the
253 most abundant product ion for the quantification and the precursor ion for the confirmation
254 (Table 3SM). For isotopically label compounds only the accurate mass of molecular ion was used.
255 For SWATH acquisition, high confidence identification was based on unique fragment ions and
256 their ion ratios as well as HR-MS/MS library searching using high resolution spectral libraries
257 supplied by SCIEX. Five main confidence criteria were used for positive identification
258 determination, meeting the EU criteria of identification points, which were Mass Error,
259 Fragment Mass Error, RT Error, Isotope Ratio, and Library Score [33].

260 **1.4. MRM^{HR} vs. SWATH:**

261 MRM (Multiple Reaction Monitoring) acquisition mode is data dependent acquisition (DDA)
262 strategy, and it is the most common method for quantitation target analytes in complex matrix
263 providing high accuracy, sensitivity and selectivity. The advances in mass spectrometry enabled
264 the development of high-resolution multiple reaction monitoring (MRM^{HR}), allowing more
265 specific analysis of target compounds. this is important for the analysis and quantification of
266 poorly abundant and/or poorly ionizable analytes. Moreover, fragmentation parameters such
267 as Declustering Potential (DP) and Collision Energy (CE) are optimised for each target analyte in
268 order to obtain a product ion with the best signal intensity. However, the number of MRMs that
269 can be monitored in a single run is limited, which has pushed us to move our directions to a
270 more comprehensive quantitation technique, known as SWATH, which brings together data
271 independent acquisition and target data processing, all in one single run. SWATH® acquisition
272 method is data independent acquisition (DIA) strategy that allows to identify and quantify every

273 detectable compound in a sample in one single run, eliminating the risk of missing a relevant
274 analyte. Every detectable analyte in the sample is fragmented, giving a complete MS and MS/MS
275 picture of every detectable in the sample (MS/MS^{All}), without the need for repeated runs or re-
276 analysis.

277 In MRM^{HR}, ions are selected to make it through Q1 and the collision chamber. These ions are
278 fragmented in the collision chamber, and certain fragments are selected to make it through the
279 TOF. The selected fragments are chosen by the user and they must have the best combination
280 of selectivity and sensitivity. It should be noted that the obtained chromatograms provide high
281 selectivity and sensitivity with the minimum noise due to the usage of specific DP and CE. SWATH
282 uses successive Q1 windows across the entire m/z mass detection range, in which ions are
283 distributed along run time. Thereafter, ions in each window are transferred to the collision
284 chamber and broken into fragments MS/MS, each fragment is then traced back to the parent
285 ion through the powerful software function. This technique ensures that fragments of low-
286 content analytes are included.

287 **1.5. Method validation**

288 The performance of the final selected method (i.e. protocol 9) was validated according to the
289 following factors: recovery, linearity, intra-day precision, method detection limit (MDL), method
290 quantification limit (MQL), and matrix effect (ME). The performance of the method was also
291 compared using both MRM^{HR} and SWATH acquisition modes.

292 *Recovery.* Percentage relative recoveries (RR%) of five concentration levels (2, 5, 10, 50, 200
293 ng/g) were determined by the mean value of a triplicate for each concentration level. Obtained
294 mean area were compared to the mean area of a triplicate set of blank extract spiked with the
295 same concentration levels. Finally, RR% were calculated using the following equation:

$$296 \quad RR (\%) = 100 \times (\text{area of spiked sample} / \text{area of spiked blank extract})$$

297 *Linearity.* Method linearity was evaluated using a matrix matched calibration curve with
298 concentration range from 0.05 to 300 ng/mL, corresponding to (0.5 to 3000 ng/g for lettuce
299 root, and 0.05 to 300 ng/g for soil). calibration curves were constructed using linear regression
300 by plotting the ratio of the analyte signal to that of its corresponding deuterated compound
301 (Table 3SM). The last was added at a fixed concentration level (1000 µg/L) to all the calibration
302 curves points. Finally, linearity was evaluated by calculating the coefficient of determination (r^2)
303 for each analyte in each matrix, $r^2 \geq 0.99$ is the acceptance criterion.

304 *Intra-day precision.* Intra-day precision was evaluated by calculating the relative standard
305 deviation (RSD%) for the above mentioned five concentration levels. Each concentration was
306 evaluated in triplicate.

307 *Method detection and quantification limits.* MDL and MQL were determined as the
308 concentration level that gave a peak signal 3 times and 10 times the background noise from the
309 chromatogram, respectively. MDL and MQL were estimated from the matrix-matched
310 calibration curves using the following equations:

$$311 \quad \text{MDL} = 3 \times (\text{Sb} / \text{slope})$$

$$312 \quad \text{MQL} = 10 \times (\text{Sb} / \text{slope})$$

313 Where Sb is the standard deviation of the intercept.

314 *Matrix effect (ME %).* For matrix effect evaluation, a blank extract for both matrices were
315 prepared following the final selected method (Protocol 9), without spiking the standard mix
316 before extraction implementation. A pool of blank extract was prepared from a set of triplicate
317 samples. Aliquots of 1 mL were spiked at 2, 5, 10, 50 and 200 ng/g concentration level. Finally,
318 ME (%) was calculated using the following equation:

$$319 \quad \text{ME (\%)} = 100 \times ((\text{area of spiked blank extract} / \text{area of spiked MeOH}) - 1)$$

320 It should be noted that ME (%) higher than |40%| were considered as high impact on the
321 performance of the method.

322 **1.6. Application to real samples**

323 The developed method was applied to real soil and lettuce root samples, irrigated with TWW
324 under greenhouse conditions for 2 years. The greenhouse was located at Murviel-Lès-
325 Montpellier village (Hérault, France), where lettuces were grown in two tanks filled with soil
326 obtained from a local field. Baby lettuces (*Lactuca sativa variety Batavia*) were obtained from a
327 local organic market. Four lettuce were placed in each tank and cultivated for 6 weeks, the time
328 needed for a lettuce to get its maturity stage. Surface drip irrigation system was used, making
329 root uptake the only uptake pathway of organic contaminants. Further, it is the most efficient
330 and common irrigation system used in arid and semi-arid areas, as it complies with the existing
331 guidelines for a safe TWW reuse for irrigation. Lettuces were irrigated with two kinds of water,
332 (1) tap clear water used as reference and (2) fresh TWW obtained from Murviel-Lès-Montpellier
333 WWTP. After 6 weeks of cultivation, the whole lettuce was harvested, as well as its surrounding
334 soil with 15 cm of depth were sampled. Soil and lettuce samples were transported directly to

335 the laboratory, where lettuce roots were separated from leaves, and together soil samples were
336 treated as reported in section “1.3. Sample preparation and extraction”. Finally, since MRM^{HR}
337 was showing better reliability and cleaner chromatograms, it was used for the quantification of
338 organic contaminants in the obtained real samples. A corresponding deuterated standard was
339 used for the quantification of each analyte. However, whenever a corresponding deuterated
340 compound was lacking, it was replaced by another from the same family or with similar
341 retention time (Tables 3SM).

342 **2. Results and discussion**

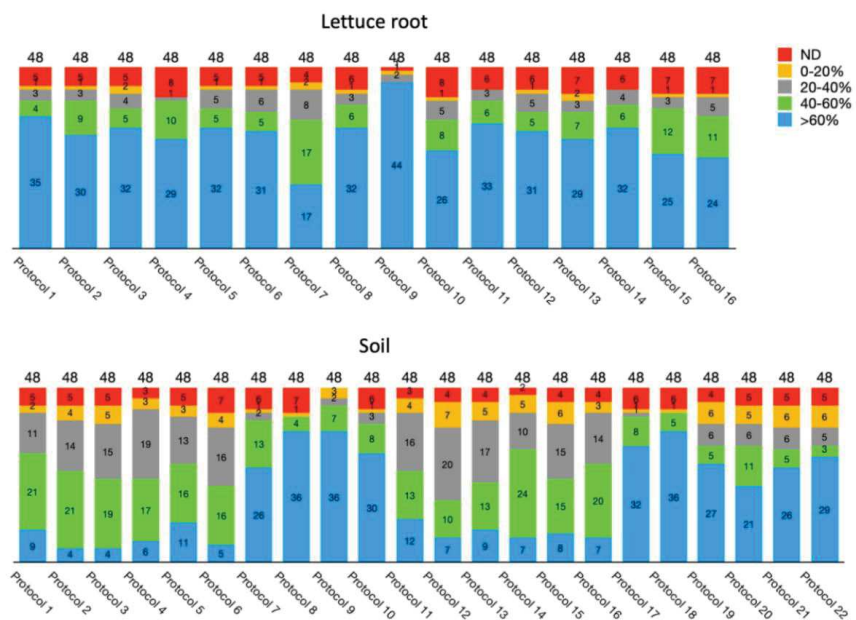
343 **2.1. Modified QuEChERS method development**

344 The selection of the investigated wastewater-derived organic compounds was based on their
345 occurrence in treated wastewater, and their previous detection in edible crops [34].
346 Additionally, they cover a wide range of polarity ($\log P = -2.16$ to $\log P = 5.31$), which made
347 method development more difficult. They include analgesics and anti-inflammatories, anti-
348 hypertensives, antifungal agents, lipid regulators, psychiatric drugs and stimulants, β -blockers,
349 antibiotics, and sweeteners.

350 For method development, all samples were injected in MRM^{HR} acquisition mode only. The 48
351 studied compounds exhibited different physico-chemical properties and characteristics. In order
352 to obtain well compromised recoveries for all of them, different extraction salts, pH conditions,
353 and an additional dispersive SPE clean-up step were tested. Details of all implemented protocols
354 are shown in Table 2SM for both matrices, lettuce root and soil. Each protocol for each matrix
355 was tested in triplicate. The obtained results and hereafter discussed are shown in details in Fig.
356 1 and Table 5SM to Table 12SM for lettuce root and soil.

357 Sample preparation for lettuce root and soil were different, yet same extraction protocols were
358 applied for both. Details concerning sample preparation are reported in the experimental
359 section. 9 mL of hydration solution was used for lettuce root based on the original QuEChERS
360 method developed by Anastassiades and co-workers [35], where they referred to the natural
361 hydration percentage (90%) of fruits and vegetables. On the other hand, 8 mL of hydration
362 solution was used for soil referring to the minimum hydration percentage applied in QuEChERS.
363 Hydration is considered as one of the most important steps in this method, with which water
364 molecules can interact and bend with the adsorption sites of the matrix promoting the
365 desorption of analytes. Recent studies showed the importance of this step, for instance, De Carlo
366 and co-workers [29] tried several extraction solvents for the extraction of bentazone, atrazine,
367 carbamazepine, phenytoin and its metabolite 5-(p-hydroxyphenyl)-, 5-phenylhydantoin from

368 soil using QuEChERS, their results showed that the addition of water to the extraction solvent
 369 significantly enhanced the recovery of the analytes except for atrazine. Consequently, different
 370 hydration methods were evaluated in combination with different QuEChERS extraction salts.



371

372 *Figure 2. Number of compounds with recoveries > 60%, 40-60%, 20-40%, 0-20% and ND (not*
 373 *detected) for lettuce root and soil matrices*

374 **2.1.1. HPLC water as hydrating solution and OR-a QuEChERS salts):**

375 In protocols 1 to 6 (Fig. 1, Tables 2SM), HPLC water as hydrating solution and original QuEChERS
 376 unbuffered salts (OR-a, containing 4 g MgSO₄ + 1 g NaCl) were used for both matrices. On the
 377 other hand, pure acetonitrile (protocols 1, 2 and 3) or acidified acetonitrile 0.5% formic acid
 378 (protocols 4, 5 and 6) were used as extraction solvent, 10 mL of volume were used referring to
 379 original QuEChERS method [35], and an additional dispersive SPE clean-up step (containing 900
 380 mg MgSO₄ + 150 mg PSA + 150 mg C18e) was tested (protocols 2, 3, 5 and 6). The clean-up step
 381 allows for matrix interferences removal, but it can also cause the loss of analytes not only during
 382 clean-up performance but also during storage due to change of pH conditions. Hence, this step
 383 is sometimes omitted (Protocols 1 and 4). In order to test the effect of this step on analytes'
 384 recoveries, we performed an additional test in which formic acid was added to the final extract
 385 obtained after dispersive SPE clean-up (protocols 3 and 6). Further details are discussed later in
 386 this section.

387 As illustrated in Fig. 1, in protocol 1, pure acetonitrile was used for the extraction, after salts
388 addition and centrifugation, 1 mL of the supernatant was evaporated, reconstituted with 1 mL
389 MeOH/water (10/90) and injected in the HPLC-MS/MS system. Whereas in Protocol 4, pure
390 acetonitrile was replaced with acidified acetonitrile (0.5% formic acid) to test the effect of acidic
391 medium on the extraction of our compounds of interest. No clean-up step was performed in
392 these two above mentioned protocols. In the case of lettuce root, results of protocols 1 and 4
393 showed similar overall recoveries: from 47.5 to 215.1%, and from 50.8 to 132.3% for protocol 1
394 and 4 respectively. However, 5 compounds (valsartan acid; ibuprofen; ciprofloxacin; fenofibrate;
395 indomethacin) were not extracted with protocol 1, whereas 8 compounds (ciprofloxacin (pka =
396 6.09); fenofibrate; furosemide (pka1 = 3.8 and pka2 = 7.5); indomethacin (pka = 4.5 and
397 sulfanilamide, pka = 10.58); sulfanilamide; propranolol; sulfamethazine; ibuprofen) were not
398 extracted with protocol 4 (Table 5SM). This can be explained by their pka values, with which
399 they are positively charged under acidic condition, thus decreasing their extraction efficiency.
400 However, in the case of soil (Table 6SM), a remarkable decrease of recoveries (data not shown)
401 was observed by adding formic acid (protocol 4), also the number of compounds higher than
402 60% recoveries has increased from 9 (protocol 1) to 6 (protocol 4) (Fig. 2). Consequently,
403 protocol 1 recorded better results than protocol 4.

404 For Protocols 2 and 5, same steps of Protocol 1 and 4 were repeated, respectively, followed by
405 a dispersive SPE clean-up step (containing 900 mg MgSO₄ + 150 mg PSA + 150 mg C18e), in order
406 to test the influence of the clean-up salts on analytes' recoveries and matrix interferences. PSA
407 (or primary secondary amines) is a polar adsorbent, and originally used to remove fatty acids
408 and other polar organic acids compounds, C18 is a nonpolar adsorbent and used to remove fats,
409 lipids, and some minerals, and finally MgSO₄ is a conventional desiccant and used to remove
410 water residues in organic solvents. As expected, recoveries of most of the compounds have
411 decreased in protocols 2 and 5 after the addition of dSPE cleanup for both matrices (data not
412 shown), further the number of compounds recording recoveries higher than 60% have increased
413 from 35 (protocol 1) to 30 (protocol 2) in the case of lettuce root; and from 9 (protocol 1) to 4
414 (protocol 2) in the case of soil (Fig. 2). The drop of recoveries after the application of a cleanup
415 step was also observed in previous studies [32]. As far, protocol 1 is still with best recoveries
416 record.

417 As already mentioned, PSA can remove acidity from the extracts which increases its pH, thus
418 increasing the risk of basic compounds degradation. In order to test the effects of the final
419 extract pH on the recoveries of the target compounds, protocols 3 and 6 were implemented as
420 a follow up for Protocols 2 and 5, respectively, in which 1 mL of the cleaned-up extract was 1%

421 acidified with 10 µL formic acid to preserve the acidity of the medium. However, no significant
422 difference was observed between protocols 2 and 3, and protocols 5 and 6 for both matrices.
423 Hence this step was neglected. Once again, protocol 1 is still with best recoveries record.

424 To sum up, results comparison of the 6 above mentioned protocols (Protocols 1 to 6 in Fig. 1
425 and Tables 2SM) showed that Protocol 1 exhibited the best results for both matrices, in which
426 most of the compounds were recovered above 40-60%, and the minimum number of not
427 extracted (ND, not detected) compounds was obtained (ibuprofen, valsartan acid ciprofloxacin,
428 fenofibrate and indomethacin in case of lettuce roots matrix; benzotriazole, indomethacin,
429 valsartan, acid 5-methyl-benzotriazole and fenofibrate in the case of soil matrix; see Tables 5SM
430 and 6SM for the list of compounds recording different recoveries for lettuce root and soil
431 respectively). Whereas, in Protocols 2 to 6, most of the compounds were extracted with
432 recoveries under 60% in the case of soil. It should be noted that recoveries obtained in protocols
433 1 to 6 for lettuce roots were much higher than the recoveries obtained for soil (data not shown).
434 In addition, the number of compounds recovered above 60% for lettuce roots were also higher
435 than the ones of soil, 29 to 35 compounds were recovered above 60% from roots while 4 to 11
436 compounds were recovered above 60% from soil in Protocols 1 to 6. This can be explained by
437 the high complexity of soil matrix, on which compounds are more retained. The usage of
438 acidified acetonitrile has lowered the recoveries of most of the compounds. However, this was
439 not the case in previous studies [28], [29], where recoveries of their target compounds were
440 significantly improved after acid addition. Moreover, the additional dispersive SPE clean-up has
441 in general lowered compounds recoveries (data not shown), due to the tendency of compounds
442 to be retained on salts. Finally, the addition of formic acid to the final extract after clean-up
443 showed no relevant difference (data not shown). The above-mentioned observation was
444 recorded in both matrices.

445 2.1.2. HPLC water as hydrating solution and CEN QuEChERS salts:

446 In order to test the influence of salts type used during extraction under the same above-
447 mentioned conditions, the same 6 protocols were repeated using the CEN QuEChERS citrate
448 buffered salts (Citate-kit-01 (4 g MgSO₄ + 1 g NaCl + 0.5 g disod. Citrate sesquihydr.)) (Protocols
449 11 to 16 in Fig. 1). Protocol 11 and Protocol 14, in which no cleanup was performed, gave the
450 best results for lettuce root and soil (Fig. 2). However, Protocol 1 was still showing similar or
451 even better results than Protocols 11 and 14. Therefore, protocol 1 employing pure acetonitrile
452 and original QuEChERS salts (OR-a, containing 4 g MgSO₄ + 1 g NaCl) was chosen for the rest of
453 the method optimization.

454 2.1.3. *Buffer solutions as hydrating solution and OR-a QuEChERS salts:*

455 So as to improve the analytes' recoveries obtained in Protocol 1, we replaced the HPLC water by
456 other solutions used by previous studies [36]–[38]. An instance for this, we used ammonium
457 formate in Protocol 7, ammonium acetate in Protocol 8, and EDTA-McIlvaine buffer in Protocol
458 9 (Fig. 1). Recoveries were remarkably improved with the use of these hydration solutions for
459 soil matrix (data not shown). This can be explained by a better partitioning of the analytes into
460 the organic phase (acetonitrile). As illustrated in Fig.2, Protocol 9 employing EDTA buffer
461 solution gave the best results for both matrices, recoveries and number of compounds above
462 60% recovery were even higher than Protocol 1. This can be explained by the ability of EDTA to
463 enhance analytes extraction by avoiding their complexation with cations as Mg^{2+} or Ca^{2+} .
464 However, one of our compounds of interest (ciprofloxacin) was not extracted from soil matrix,
465 besides it was poorly extracted from lettuce roots (13%). For further improvement of
466 ciprofloxacin recoveries obtained by Protocol 9, a 5 min sonication step was added right after
467 acetonitrile addition (Protocol 10 in Fig. 1). No significant enhancement was observed, so this
468 step was abandoned.

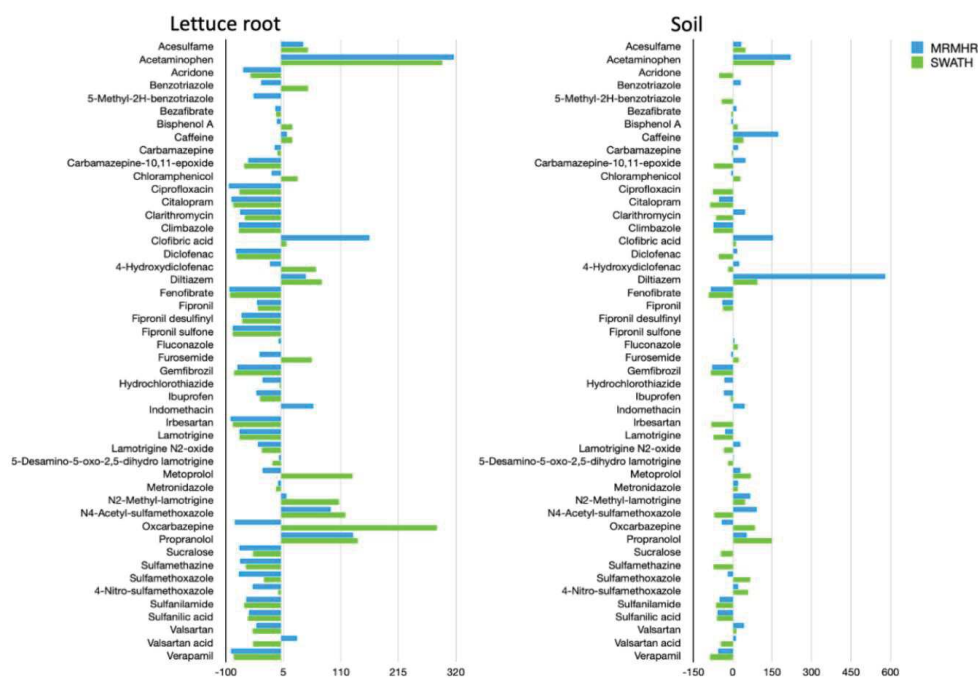
469 2.1.4. *Buffer solution as hydrating solution and 3 different OR QuEChERS salts (OR-a, OR-b
470 and OR-Na):*

471 6 more methods were performed for soil matrix only in order to extract ciprofloxacin (Protocols
472 17 to 22 in Fig. 1, Table 2SM). In protocols 17 and 18, we repeated protocols 7 and 8 respectively
473 with the addition of a 5 min sonication step after acetonitrile addition. In protocols 19, 20 and
474 21, we replaced OR-a salts (4 g $MgSO_4$ + 1 g NaCl) with OR-b (6 g $MgSO_4$ + 1.5 g NaCl), and we
475 reduced hydration volume from 8 mL to 5 mL of HPLC water (protocol 19), ammonium acetate
476 (protocol 20) and EDTA-McIlvaine buffer (protocol 21). The increase of salts mass and the
477 decrease of hydration solution volume was suggested to increase the partitioning of the analytes
478 in the organic solvent (acetonitrile). Finally, in order to test the hypothesis that ciprofloxacin is
479 attached on magnesium of QuEChERS salts, we prepared in the laboratory salts that contain
480 sodium instead of magnesium (6 g $NaSO_4$ and 1.5 g NaCl, protocol 22). Results are shown in Fig.
481 2. Protocols 17 to 22 failed to extract ciprofloxacin, moreover, obtained recoveries were lower
482 than the recoveries obtained with protocol 9.

483 Finally, based on the above reported discussion, Protocol 9 gave the best compromise and hence
484 was selected as extraction method for lettuce root and soil. Method performance validation was
485 hereafter conducted with protocol 9 for both matrices.

486 **2.2. Matrix effect (ME) evaluation**

487 Lettuce root and soil are complex matrices, and co-extracted matrix components are often
488 present in the final extract to be injected, thus decreasing or increasing the instrumental
489 response of the target analytes. In this work, the ME was evaluated in MRM^{HR} and SWATH
490 acquisition modes for the 5 concentration levels, detailed results are present in Table 7SM and
491 Table 8SM for lettuce root and soil, respectively. ME was calculated by comparing the response
492 in blank extracts and in solvent spiked at the same concentration levels (2, 5, 10, 50 and 200
493 ng/g). Fig. 3 shows the mean ME% in MRM^{HR} and SWATH, for lettuce root and soil. Similar ME%
494 were obtained by both acquisition modes MRM^{HR} and SWATH for both matrices. Moreover, 50%
495 of the compounds showed acceptable ME% (from -40% to +40%) in both matrices. However,
496 some compounds have shown an opposite performance between both acquisition modes such
497 as propranolol (-83.8% in MRM^{HR} and 285.5% in SWATH), furosemide (-39.1% in MRM^{HR} and
498 56.4% in SWATH), benzotriazole (-35.6% in MRM^{HR} and 49.2% in SWATH), N4-acetyl-
499 sulfamethoxazole (-32.7% in MRM^{HR} and 131.5% in SWATH), bisphenol A (-7.0% in MRM^{HR} and
500 20.5% in SWATH) and valsartan acid (29.3% in MRM^{HR} and -50.4% in SWATH) in the case of
501 lettuce root. However, it should be noted that the purification step was excluded in the final
502 selected protocol, hence higher matrix effects were expected. Acetaminophen showed the
503 highest ME% in both acquisition modes (316.5% in MRM^{HR} and 295.2% in SWATH) in the case of
504 lettuce, while in the case of soil diltiazem showed the highest ME% (579.7%) in MRM^{HR} only.
505 One way to overcome analytes response enhancement or reduction, is the use of a matrix
506 matched calibration curve spiked with deuterated standards. Indeed, analytes and internal
507 standards should have the same behavior during sample preparation, and same or similar
508 retention times during chromatographic separation. Hence, the use of internal standard method
509 is recommended and was further used for the quantification of real environmental samples.



510

511 *Figure 3. Mean of matrix effect (ME%) of five concentration levels for lettuce root and soil in*
 512 *MRMHR and SWATH acquisition modes*

513

514 **2.3. Method validation**

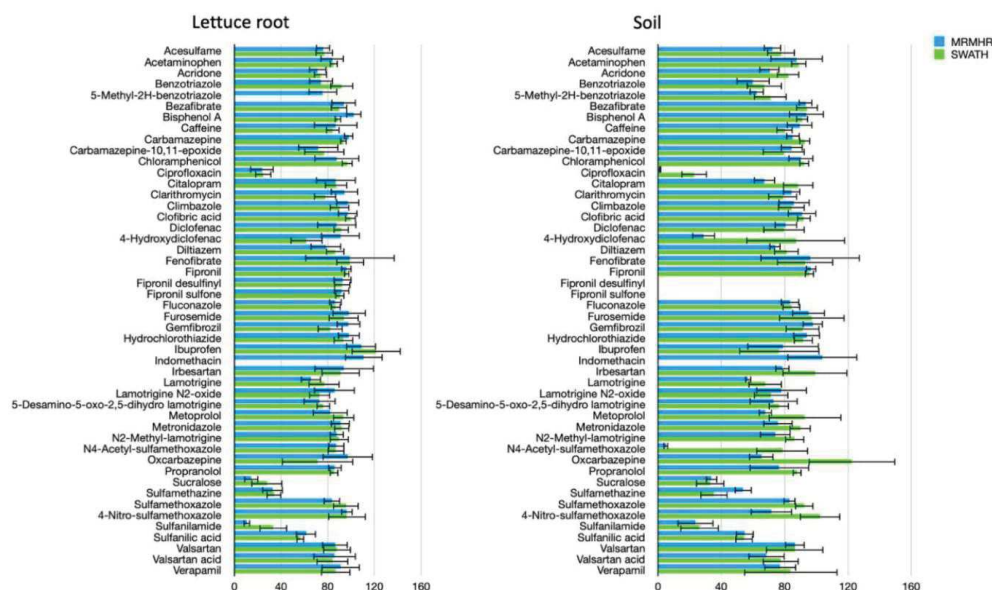
515 For method validation, MRM^{HR} and SWATH acquisition modes were used. The optimized method
 516 was validated for both matrices in terms of recovery, linearity, intra-day precision, method
 517 detection limit (MDL), and method quantification limit (MQL). Validation factors are summarized
 518 in Table 9SM to Table 12SM for lettuce root and soil.

519 *Recoveries.* Relative recoveries were obtained by the mean value of three replicates for each
 520 concentration level, in both acquisition modes (MRM^{HR} and SWATH). Results are summarized in
 521 Table 9SM and Table 10SM for lettuce root and soil, respectively. Fig. 4 shows the mean
 522 recoveries of lettuce root in MRM^{HR} and SWATH acquisition modes. Soil results are shown in Fig.
 523 10SM. The method exhibited good accuracy with relative recoveries between 80 and 120% for
 524 most of the studies compounds in both acquisition modes and for both matrices. However, some
 525 compounds were extracted with relatively low recoveries from both matrices (see Fig. “ for
 526 lettuce root and Fig. 10SM for soil) such as ciprofloxacin (23.6% in MRM^{HR} and 24.7% in SWATH
 527 from lettuce root; and 1.2% in MRM^{HR} and 22.8% is SWATH from soil), sulfamethazine (14.3% in

20

528 MRM^{HR} and 27.8% in SWATH from lettuce root; and 33.8% in MRM^{HR} and 33.0% in SWATH from
529 soil), sulfamethoxazole (32.7% in MRM^{HR} and 39% in SWATH from lettuce root; and 35.5% in
530 SWATH from soil), sulfanilamide (10.8% in MRM^{HR} and 33.3% in SWATH in lettuce root; and
531 23.7% in MRM^{HR} and 26.2% in SWATH), and finally oxcarbazepine that recorded 4.9% in MRM^{HR}
532 from soil. Since and according to our knowledge, this study is the first that reports the
533 development of an extraction method from lettuce root, thus comparison between results were
534 restricted to soil matrix. Besides, few data are available in the literature for the compounds
535 included in this study, dealing with soil matrix. Thereupon, comparable recoveries were
536 obtained in previous studies for the compounds in common [32], [39], though Salvia and
537 coworkers recorded higher recoveries for sulfonamide (50%) [32]. However, this could be
538 explained by the application of a clean-up step in their study.

539 *Intraday precision.* Intraday precision expressed by repeatability was calculated as relative
540 standard deviation (RSD%) obtained from the relative recoveries described above, at each
541 concentration level. Results are summarized in Table 9SM and Table 10SM for lettuce root and
542 soil, respectively. The bars in Fig. 4 shows the mean RSD% for the 5 concentration levels studied
543 for lettuce root and soil. Most of the compounds recorded high precision with RSD% below 20%
544 for both acquisition modes and both matrices. However, some exceptional compounds with
545 RSD% outside the acceptance criterion are always recorded, such as irbesartan (25.6% in
546 MRM^{HR}) and propranolol (30.6% in SWATH) for lettuce roots; and fenofibrate (31.4% in MRM^{HR})
547 and N₂-methyl-lamotrigine (22.8% in SWATH).



548

549 *Figure 4. Mean of relative recoveries (RR%) of five concentration levels for lettuce root and soil*
 550 *in MRMHR and SWATH acquisition modes. Error bars show the range of relative standard*
 551 *deviation (RSD%)*

552 *Linearity.* The linearity of the method was assessed using the matrix-matched calibration curve
 553 approach with a calibration curve constructed between 0.05 and 300 ng/mL, corresponding to
 554 (0.5 to 3000 ng/g dry weight for lettuce root, and 0.05 to 300 ng/g dry weight for soil), taking
 555 into account at least 8 points. Calibration curves were constructed using linear weighted least-
 556 squares regression by plotting the ratio of analyte's area to that of its corresponding deuterated
 557 compounds (internal standard). Results are summarized in Table 11SM and Table 12SM for
 558 lettuce root and soil, respectively. Analytes linearity was up to 1000, 2000 or 3000 ng/g in the
 559 case of lettuce and 100, 200 or 300 ng/g in the case of soil for most of the compounds with r^2
 560 higher than 0.99 in both acquisition modes. However, fipronil and its two studied transformation
 561 products namely fipronil sulfone and fipronil desulfinyl, showed shorter linearity, up to 100 ng/g
 562 in lettuce root and 10 ng/g in soil in MRM^{HR} and SWATH. On the other hand, all of the compounds
 563 exhibited $r^2 > 0.99$, except for irbesartan ($r^2 = 0.96436$ in MRM^{HR}) for lettuce root, while in the
 564 case of soil, caffeine recorded $r^2 = 0.98766$, and sulfanilic acid recorded $r^2 = 0.98147$ in MRM^{HR}
 565 and $r^2 = 0.97912$ in SWATH.

566 *MDL and MQL.* Sensitivity of the methods was asses through the calculation of MDL and MQL
 567 from the matrix-matched calibration curve using linear regression analysis. Results are
 568 summarized in Table 11SM and Table 12SM for lettuce root and soil, respectively. In general,

22

569 MDL and MQL of lettuce root were higher than soil in both acquisition modes. More in details,
 570 MDL ranged from 0.01 to 0.27 ng/g in MRM^{HR} and from 0.01 to 0.23 ng/g in SWATH in the case
 571 of lettuce root, while MDL ranged from 0.01 to 0.17 ng/g in MRM^{HR} and from 0.01 to XX ng/g in
 572 SWATH for soil. Fr MQL, it ranged from 0.1 to 0.92 ng/g in MRM^{HR} and from 0.15 to XX ng/g in
 573 SWATH for lettuce root, and from XX to XX ng in SWATH for soil.

574 2.4. Application to real samples

575 Four compounds were detected in lettuce root with concentrations ranging from 0.18 to 18.99
 576 ng/g, and 13 compounds were detected in soil with concentrations ranging from <LOQ to 14.78
 577 ng/g. Results are shown in Table 1. Organic micropollutants detected in roots were also detected
 578 in leaves part of the lettuce [40], except for clarithromycin which was detected only in roots.
 579 However, the highest concentrations were always recorded in leaves, then in roots followed by
 580 soil. In instance for this, carbamazepine recorded 6.0, 0.41 and 0.18 ng/g in leaves, roots and
 581 soil, respectively. However, valsartan acid is an exception, which recorded 0.18 ng/g in roots and
 582 1.54 ng/g in soil. It should be noted that degradation processes were out of scope of this study,
 583 besides to the leaching process that might take place in soil. Comparable results were obtained
 584 with Martinez-Piernas and coworkers [39], after analysis of agricultural soil irrigated with
 585 treated wastewater for a long period. Thereupon, the developed method proved its applicability
 586 to real environmental samples, moreover the irrigation of soil-lettuce system with TWW
 587 contaminated with trace levels of organic micropollutants may lead to their uptake and
 588 accumulation in the different parts of lettuce (leaves and roots), as well as to their accumulation
 589 in soil, posing potential risks to human and ecological health.

590 *Table 1. Quantification of wastewater-borne organic contaminants detected in soil and lettuce*
 591 *root samples irrigated with TWW under real greenhouse cultivation conditions (ND means not*
 592 *detected)*

Wastewater-borne organic contaminants	Soil (ng/g)	Lettuce root (ng/g)
Climbazole	0.30 ± 0.19	ND
Sulfamethoxazole	0.11 ± 0.22	ND
Citalopram	0.42 ± 0.19	ND
Valsartan	0.03 ± 0.06	ND
Valsartan acid	1.54 ± 1.11	0.18 ± 0.32
Irbesartan	0.58 ± 0.56	ND
Benzotriazole	1.66 ± 0.37	ND
Carbamazepine	0.18 ± 0.05	0.41 ± 0.28
Carbamazepine-11,12-epoxide	<LOQ	<LOQ
Clarithromycin	14.78 ± 3.34	18.99 ± 10.57
Acesulfame	0.84 ± 0.91	ND
Fipronil	0.03 ± 0.01	ND

Hydrochlorothiazide	0.21 ± 0.08	4.75 ± 8.22
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593

594 **3. Conclusions**

595 This work presents a new, robust, simple, yet common extraction method of 48 wastewater
596 borne organic micropollutants for soil and lettuce root, based on QuEChERS method. According
597 to our knowledge, it is the first time that a work was dedicated to optimize and validate a
598 method for lettuce root matrix. The developed method was validated in terms of recovery,
599 linearity, intra-day precision, method detection limit (MDL), method quantification limit (MQL)
600 and matrix effect (ME) in two different acquisition modes, MRM^{HR} (high resolution multiple
601 reaction monitoring) and SWATH^{HR} (Sequential Window Acquisition of All Theoretical Fragment-
602 ion), in order to compare their performance. Though similar results were obtained with both
603 acquisition modes, MRM^{HR} provided more consistent results for most of the compounds. This
604 method was successfully applied to real samples cultivated under greenhouse conditions, 4 and
605 13 pollutants were detected in lettuce root and soil, respectively.

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769 results.

Electronic Supplementary Material

Evaluation of different modified QuEChERS-based methods for the extraction of 48 wastewater-derived organic contaminants from soil and lettuce root using high resolution LC-QTOF with MRM^{HR} and SWATH acquisition modes

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Experimental section:

EDTA McIlvaine buffer preparation:

1.5 g of disodium hydrogen phosphate dehydrate, 1.3 g of citric acid monohydrate, and 0.372 g EDTA were dissolved in 100 mL HPLC water.

Table 1SM. physical and chemical properties of the target compounds.

Compound	CAS No.	Molecular formula	Molecular weight	Water solubility	Log Kow	pKa	Chemspider ID	Family group
Acesulfame	33665-90-6	C ₆ H ₅ NO ₄ S	163.15	9.10E+05	-1.33	3.0	33607	Artificial sweetener
Acetaminophen	103-90-2	C ₉ H ₉ NO ₂	151.16	3.03E+04	0.46	7.0	1906	analgesic
Acridone	578-95-0	C ₁₃ H ₉ NO	195.22	1.31E+02	1.69	N/A	10188539	TP of carbamazepine
Benzotriazole	95-14-7	C ₆ H ₅ N ₃	119.12	1.10E+04	1.44	8.4	6950	Drug precursor
5-Methyl-2H-Benzotriazole	136-85-6	C ₇ H ₇ N ₃	133.15	3.07E+03	1.71	N/A	8381	Industrial product
Bezafibrate	41859-67-0	C ₁₉ H ₂₀ ClNO ₄	361.82	1.22E+00	4.25	3.8	35728	lipid regulators
Bisphenol A	80-05-7	C ₁₅ H ₁₆ O ₂	228.29	1.73E+02	3.32	9.6	6371	plasticizer
Caffeine	58-08-2	C ₈ H ₁₀ N ₄ O ₂	194.19	2.16E+04	-0.07	14.0	2424	stimulants
Carbamazepine	298-46-4	C ₁₅ H ₁₂ N ₂ O	236.27	1.77E+01	2.45	7.0	2457	psychiatric drug
Carbamazepine-10,11-epoxide	36507-30-9	C ₁₅ H ₁₂ N ₂ O ₂	252.27	2.77E+02	0.95	N/A	2458	Metabolite carbamazepine
Chloramphenicol	56-75-7	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₅	323.13	2.50E+03	1.14	5.5	5744	antibiotic
Ciprofloxacin	85721-33-1	C ₁₇ H ₁₈ FN ₃ O ₃	331.34	1.15E+04	0.28	6.1	2662	fluoroquinolones
Citalopram	59729-33-8	C ₂₀ H ₂₁ FN ₂ O	324.39	3.11E+01	3.74	9.8	2669	psychiatric drug and stimulant
Clarithromycin	81103-11-9	C ₃₈ H ₆₉ NO ₁₃	747.95	1.69E+00	3.16	9.0	10342604	macrolides
Climbazole	38083-17-9	C ₁₅ H ₁₇ ClN ₂ O ₂	292.76	8.28E+00	3.76	N/A	34752	antifungal agent
Clofibric acid	882-09-7	C ₁₀ H ₁₁ ClO ₃	214.65	5.83E+02	2.57	3.2	2695	lipid regulator
Diclofenac	15307-86-5	C ₁₄ H ₁₁ Cl ₂ NO ₂	296.15	4.52E+00	4.51	4.2	2925	Analgesic
4-Hydroxydiclofenac	64118-84-9	C ₁₄ H ₁₁ Cl ₂ NO ₃	312.15	1.74E+04	3.70	3.8	104192	Analgesic
Diltiazem	42399-41-7	C ₂₂ H ₂₈ N ₂ O ₄ S	414.52	1.23E+01	2.70	8.1	35850	anti-hypertensive
Fenofibrate	49562-28-9	C ₂₀ H ₂₁ ClO ₄	360.83	1.96E-01	5.19	N/A	3222	lipid regulator
Fipronil	120068-37-3	C ₁₂ H ₄ Cl ₃ F ₆ N ₄ OS	437.15	4.00E+00	1.90	7.0	3235	insecticide
Fipronil desulfinyl	205650-65-3	C ₁₂ H ₄ Cl ₃ F ₆ N ₄	389.08	4.86E-01	4.22	7.0	11542895	insecticide
Fipronil sulfone	120068-36-2	C ₁₂ H ₄ Cl ₃ F ₆ N ₄ O ₂ S	453.15	1.30E-01	4.42	7.0	2336427	insecticide
Fluconazole	86386-73-4	C ₁₃ H ₁₂ F ₂ N ₆ O	306.27	3.36E+02	0.25	1.8	3248	antifungal agent
Furosemide	54-31-9	C ₁₂ H ₁₁ ClN ₂ O ₅ S	330.74	7.31E+01	2.03	3.9	3322	diuretic
Gemfibrozil	25812-30-0	C ₁₅ H ₂₂ O ₃	250.33	4.96E+00	4.77	4.5	3345	lipid regulator
Hydrochlorothiazide	58-93-5	C ₇ H ₈ ClN ₃ O ₄ S ₂	297.74	7.22E+02	-0.07	7.9	3513	diuretics
Ibuprofen	15687-27-1	C ₁₃ H ₁₈ O ₂	206.28	2.10E+01	3.97	5.3	3544	analgesics
Indomethacine	53-86-1	C ₁₉ H ₁₆ ClNO ₄	357.79	9.37E-01	4.27	4.5	3584	antiinflammatory
Irbesartan	138402-11-6	C ₂₉ H ₂₈ N ₆ O	428.53	5.99E-02	5.31	4.1; 4.3	3618	anti-hypertensive
Lamotrigine	84057-84-1	C ₉ H ₇ Cl ₂ N ₅	256.09	3.13E+03	0.99	8.5	3741	psychiatric drug and stimulant
Lamotrigine N2-oxide	136565-76-9	C ₉ H ₇ Cl ₂ N ₅ O	272.09	N/A	N/A	N/A	29790380	Metabolite of lamotrigien
5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	252186-78-0	C ₉ H ₆ Cl ₂ N ₄ O	257.08	1.01E+02	2.73	N/A	13519082	Metabolite of lamotrigine
N2-Methyl-Lamotrigine	1152091-68-3	C ₁₀ H ₁₀ Cl ₂ N ₅	271.13	N/A	N/A	N/A	52083157	Metabolite lamotrigine
Metoprolol	51384-51-1	C ₁₅ H ₂₅ NO ₃	267.36	4.78E+03	2.88	9.7	138781	beta-blocker

Metrodinazole	443-48-1	C ₆ H ₆ N ₃ O ₃	171.15	7.89E+04	-0.6	7.0	62916	Antifungal agent
Oxcarbazepine	28721-07-5	C ₁₅ H ₁₂ N ₂ O ₂	252.27	2.03E+02	1.11	N/A	31608	psychiatric drugs and stimulants
Propranolol	525-66-6	C ₁₆ H ₂₁ NO ₂	259.34	2.28E+02	3.48	9.4	4777	beta-blockers
Sucralose	56038-13-2	C ₁₂ H ₁₉ Cl ₃ O ₈	397.63	2.28E+04	-1.00	7.0	64561	Artificial sweetener
Sulfamethazine	57-68-1	C ₁₂ H ₁₄ N ₄ O ₂ S	278.33	1.50E+03	0.89	2.7; 7.7	5136	sulfonamide antibiotics
Sulfamethoxazole	723-46-6	C ₁₀ H ₁₁ N ₃ O ₃ S	253.28	6.10E+02	0.89	1.6; 5.7	5138	sulfonamide antibiotics
4-Nitro-Sulfamethoxazole	29699-89-6	C ₁₀ H ₉ N ₃ O ₅ S	283.26	5.69E+02	1.22	N/A	626159	TP of sulfamethoxazole
N4-Acetylsulfamethoxazole	21312-10-7	C ₁₂ H ₁₃ N ₃ O ₄ S	295.31	1.22E+03	1.21	N/A	58771	Metabolite sulfamethoxazole
Sulfanilamide	63-74-1	C ₆ H ₈ N ₂ O ₂ S	172.21	7.50E+03	-0.62	6.1	5142	antibiotic
Sulfanilic acid	121-57-3	C ₆ H ₇ NO ₃ S	173.19	1.08E+04	-2.16	5.3	8166	antibiotic
Valsartan	137862-53-4	C ₂₄ H ₂₉ N ₅ O ₃	435.52	1.41E+00	3.65	3.9; 48	54833	anti-hypertensive
Valsartan acid	164265-78-5	C ₁₄ H ₁₀ N ₄ O ₂	266.25	N/A	N/A	5.0	N/A	TP of Valsarta
Verapamil	52-53-9	C ₂₇ H ₃₈ N ₂ O ₄	454.60	4.47E+00	3.79	8.9	2425	anti-hypertensive

Physicochemical properties, water solubility (in mg/L), polarity (log K_{ow}), and pka of selected compounds of study classified by their uses were estimated and obtained from the platforms Chemspider (www.chemspider.com) and PubChem (<https://pubchem.ncbi.nlm.nih.gov>) or estimated from log K_{ow}, by EPISuite platform, extracted from Chemspider database.

Table 25M. Details of the tested protocols for lettuce root and soil matrices (protocols 17 to 22 are for soil matrix only).

Protocol	HPLC water	Hydration solution*			1 minute vortex and 1h rest – addition of the standard mix and 1h rest – salts and extraction solvent addition	Extraction solvent		Sonication	Type of salts				Cleanup			Injection	
		NH ₄ HCO ₃ 0.35 mol L ⁻¹	NH ₄ C ₂ H ₃ O ₂ 0.28 mol L ⁻¹	EDTA buffer (pH 4)		ACN	ACN + 0.5% FA		OR-a	CEN	OR-b	OR-Na (homemade)	dSPE	dSPE + 1% FA	no cleanup		
Protocol 1	X									X						X	
Protocol 2	X									X						X	
Protocol 3	X									X						X	
Protocol 4	X									X						X	
Protocol 5	X									X						X	
Protocol 6	X									X						X	
Protocol 7		X								X						X	
Protocol 8			X							X						X	
Protocol 9										X						X	
Protocol 10								X	X							X	
Protocol 11	X									X						X	
Protocol 12	X									X						X	
Protocol 13	X									X						X	
Protocol 14	X									X						X	
Protocol 15	X									X						X	
Protocol 16	X									X						X	
Protocol 17		X (8 mL)						X	X							X	
Protocol 18			X (8 mL)	X				X	X							X	
Protocol 19	X (5 mL)										X					X	
Protocol 20			X (5 mL)								X					X	
Protocol 21											X					X	
Protocol 22	X (5 mL)											X				X	

*Hydration solution volume is 9 and 8 mL for lettuce root and soil respectively, unless it is indicated elsewhere.

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Table 35M. MRMHR optimized detection parameters in positive and negative ionization modes

Analyte	Chemical formula	Adduct/Charge	Precursor mass (m/z)	Fragment mass (m/z)	Declustering potential (V)	Collision energy (V)	Retention Time (min)	IS name
Acesulfame	C ₈ H ₉ NO ₅ S	[M-H] ⁻	161.9866	82.0297	-25	-15	1.18	Acesulfame-d4
Acetaminophen	C ₉ H ₉ NO ₂	[M-H] ⁻	150.0560	107.0379	-95	-20	2.01	Acetaminophen-d4
Acridone	C ₁₃ H ₉ NO	[M+H] ⁺	196.0757	167.0644	150	40	5.82	Carbamazepine-d10
Benzotriazole	C ₆ H ₅ N ₃	[M-H] ⁻	118.0411	50.0037	-95	-35	3.23	Benzotriazole-d4
5-Methyl-2H-Benzotriazole	C ₇ H ₇ N ₃	[M+H] ⁺	134.0713	77.0344	90	35	4.41	Benzotriazole-d4
Bezafibrate	C ₁₉ H ₂₀ ClNO ₄	[M-H] ⁻	360.1008	274.0648	-100	-20	5.38	Bezafibrate-d4
Bisphenol A	C ₁₅ H ₁₆ O ₂	[M-H] ⁻	227.1077	212.0845	-165	-20	7.39	Bisphenol A-d8
Caffeine	C ₈ H ₁₀ N ₄ O ₂	[M+H] ⁺	195.0877	138.0685	110	25	2.82	Caffeine- ¹³ C3
Carbamazepine	C ₁₅ H ₁₂ N ₂ O	[M+H] ⁺	237.1022	194.0981	115	25	6.42	Carbamazepine-d10
Carbamazepine-10,11-epoxide	C ₁₅ H ₁₂ N ₂ O ₂	[M+H] ⁺	253.0972	180.0736	60	55	5.32	Carbamazepine-d10
Chloramphenicol	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₃	[M-H] ⁻	321.0050	152.0352	-95	-20	5.18	Bisphenol A-d8
Ciprofloxacin	C ₁₇ H ₁₈ FN ₃ O ₃	[M+H] ⁺	332.1405	314.1292	155	35	3.54	Ciprofloxacin-d8
Citalopram	C ₂₀ H ₂₃ FN ₂ O	[M+H] ⁺	325.1711	109.0396	30	35	6.22	Citalopram-d6
Clarithromycin	C ₃₈ H ₆₀ NO ₁₃	[M+H] ⁺	748.4842	158.116	60	40	7.22	Ciprofloxacin-d8
Climbazole	C ₁₃ H ₁₇ ClN ₂ O ₂	[M-H] ⁻	291.0906	67.0304	-110	-20	8.39	Climbazole-d4
Clofibric acid	C ₁₈ H ₁₁ ClO ₃	[M-H] ⁻	213.0324	126.9961	-80	-20	4.07	Benzotriazole-d4
Diclofenac	C ₁₆ H ₁₁ Cl ₂ NO ₂	[M-H] ⁻	294.0094	250.0205	-65	-15	6.95	Diclofenac- ¹³ C6
4-Hydroxydiclofenac	C ₁₆ H ₁₃ Cl ₂ NO ₃	[M+H] ⁺	312.0189	230.0277	65	45	7.54	Diclofenac- ¹³ C6
Diltiazem	C ₂₁ H ₂₈ N ₂ O ₅ S	[M+H] ⁺	415.1686	178.0261	35	35	6.37	Carbamazepine-d10
Fenofibrate	C ₂₀ H ₃₁ ClO ₄	[M+H] ⁺	361.1201	139.0002	100	35	9.89	Fenofibrate-d6
Fipronil	C ₁₇ H ₄ Cl ₂ F ₆ N ₄ OS	[M-H] ⁻	434.9314	329.96	-25	-20	8.92	Climbazole-d4
Fipronil desulfinyl	C ₁₂ H ₈ Cl ₂ F ₆ N ₄	[M-H] ⁻	386.96444	350.9889	-100	-20	9.05	Climbazole-d4
Fipronil sulfone	C ₁₂ H ₈ Cl ₂ F ₆ N ₄ O ₂ S	[M-H] ⁻	450.9263	414.9508	-25	-20	9.22	Climbazole-d4
Fluconazole	C ₁₃ H ₁₂ F ₂ N ₆ O	[M+H] ⁺	307.1113	238.0834	105	20	4.02	Fluconazole- ¹³ C3
Furosemide	C ₁₂ H ₁₁ ClN ₂ O ₅ S	[M-H] ⁻	329.0004	285.0115	-110	-20	4.42	Furosemide-d5
Gemfibrozil	C ₁₅ H ₂₂ O ₃	[M-H] ⁻	249.1496	121.0671	-95	-20	8.78	Gemfibrozil-d6
Hydrochlorothiazide	C ₇ H ₈ ClN ₃ O ₄ S ₂	[M-H] ⁻	295.9572	268.9476	-140	-25	2.77	Hydrochlorothiazide-d2
Ibuprofen	C ₁₃ H ₁₈ O ₂	[M-H] ⁻	205.1234	161.1341	-25	-10	7.68	Ibuprofen-d3

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Indomethacine	C ₁₉ H ₁₄ ClNO ₄	[M-H] ⁻	356.0695	312.0798	-100	-10	7.24	Indomethacin-d4
Irbesartan	C ₂₅ H ₂₈ N ₆ O	[M-H] ⁻	427.2247	193.1347	-150	-35	6.49	Irbesartan-d6
Lamotrigine	C ₉ H ₇ Cl ₂ N ₃	[M+H] ⁺	256.0151	210.9719	145	35	4.02	Lamotrigine- ¹³ C3
Lamotrigine N2-oxide	C ₉ H ₇ Cl ₂ N ₃ O	[M+H] ⁺	272.0106	242.0051	120	25	3.91	Lamotrigine- ¹³ C3
5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	C ₉ H ₆ Cl ₂ N ₃ O	[M+H] ⁺	256.9991	228.9985	160	25	4.64	Lamotrigine- ¹³ C3
N2-Methyl-Lamotrigine	C ₁₀ H ₈ Cl ₂ N ₃	[M+H] ⁺	270.0308	57.039	160	40	4.62	Lamotrigine- ¹³ C3
Metoprolol	C ₁₅ H ₂₂ NO ₃	[M+H] ⁺	268.1907	116.1077	120	25	4.17	Metoprolol-d7
Metronidazole	C ₆ H ₉ N ₃ O ₃	[M+H] ⁺	172.0717	128.0429	85	15	2.16	Metronidazole-d4
Oxcarbazepine	C ₁₃ H ₁₂ N ₂ O ₃	[M+H] ⁺	253.0972	180.0726	115	40	5.6	Carbamazepine D10
Propranolol	C ₁₆ H ₂₁ NO ₂	[M+H] ⁺	260.1645	116.109	100	25	5.64	Metoprolol_D7
Sucralose	C ₁₂ H ₂₁ Cl ₃ O ₆	[M-H] ⁻	395.0073	359.0283	-130	-15	3.35	Sucralose d6
Sulfamethazine	C ₁₂ H ₁₄ N ₄ O ₂ S	[M+H] ⁺	279.091	124.0937	50	30	3.62	Sulfamethazine d4
Sulfamethoxazole	C ₁₀ H ₁₁ N ₃ O ₂ S	[M-H] ⁻	252.0448	156.0131	-35	-20	4.16	Sulfamethoxazole-d4
4-Nitro-Sulfamethoxazole	C ₁₀ H ₉ N ₃ O ₂ S	[M+H] ⁺	284.0336	189.0202	130	30	6.86	Sulfamethoxazole-d4
N4-Acetylsulfamethoxazole	C ₁₂ H ₁₃ N ₃ O ₄ S	[M-H] ⁻	294.0554	198.0233	-110	-20	3.72	Sulfamethoxazole-d4
Sulfanilamide	C ₈ H ₈ N ₂ O ₂ S	[M-H] ⁻	172.0069	79.9591	-110	-45	1.28	Sulfamethoxazole-d4
Sulfanilic acid	C ₆ H ₇ NO ₃ S	[M-H] ⁻	171.0229	78.9205	-100	-40	0.47	Sulfamethoxazole-d4
Valsartan	C ₂₄ H ₂₉ N ₅ O ₃	[M-H] ⁻	434.2197	179.0863	-135	-30	5.37	Valsartan-d3
Valsartan acid	C ₁₄ H ₁₉ N ₄ O ₂	[M+H] ⁺	267.0877	206.0519	110	20	2.54	Valsartan acid-d4
Verapamil	C ₂₇ H ₃₈ N ₂ O ₄	[M+H] ⁺	455.2904	165.0883	25	40	7.1	Carbamazepine-d10

LC-MS/MS analysis:

The MRMHR scanning mode was used for accurate quantification of product ion transitions. The Guided MRMHR tool from SCIEX was used for the optimization of high-resolution transitions, and fragment ions. The best signal intensity was selected for quantification while the precursor was used for confirmation. The optimized ionization mode, DP, and CE for each compound have been reported in Table 2SM and Table 3SM. The SWATH acquisition mode which combines in the same run data in full-scan, targeted independent MS/MS data and non-targeted independent MS/MS data acquisition, of a single TOF-MS experiment over a m/z range from 100 to 950 Da with an accumulation time of 120 ms (AT), followed by 10 MS/MS experiments with variable Q1 windows (100 to 950 m/z, 40 ms AT) using a Collision Energy (CE) of 35 V with a collision energy spread of ± 15 V, respectively for positive and negative mode of ionization. For generating SWATH Q1 variable windows, SCIEX provided an active Excel spreadsheet template known as SWATH variable window calculator (Ver. 1.0). The 10 sequential Q1 variable windows were created based on the precursor ion distribution within the retention time of the LC gradient. A sample containing all target compounds and lettuce roots or soil extracts was injected in full-scan to obtain MS survey scan. This scan contained the list of the m/z values of all precursors and the peak intensities obtained from the peaks detected in a spectrum merged by all the spectra within a same retention time window of the chromatographic gradient. Variable windows were generated by computing the number of precursor ions and taking into account their intensities as a weighting factor. The SWATH isolation window plot generated by this calculator based on the same MS survey scan including the variable windows were reported in Fig. 1SM and Fig. 2SM for lettuce root and soil, respectively. In order to evaluate the sensitivity of SWATH acquisition mode, the validation of targeted compounds was also performed acquiring data with SWATH acquisition and then both modalities were compared.

The electrospray ionization source (ESI) conditions were optimized as follow. Ion Spray Voltage was set to 5500 V (-4500 V for negative); Source temperature and nitrogen gas flows (Atomizing gas, GS1 and Auxiliary gas, GS2) were set to 550° C, 55, and 55 psi, respectively. Curtain gas was set to 30 psi, while collision gas (CAD) was set to 7. Any drift in the mass accuracy of the SCIEX Q-TOF was automatically corrected and maintained throughout batch acquisition by infusion of Reserpine reference standard (C33H40N2O9, m/z 609.28066) for positive ionization, and a cluster of trifluoroacetic acid ([5(TFA-Na)+TFA]-, m/z 792.85963) for negative mode. Calibration was running every 5 samples during the batch acquisition making use of the Calibrant Delivery System (CDS).

XXX

Figure 1SM. Variable Q1 Window Widths for SWATH Acquisition in positive (A) and negative (B) ionization for lettuce root

XXX

Figure 2SM. Variable Q1 Window Widths for SWATH Acquisition in positive (A) and negative (B) ionization for soi

Results and discussion:

Table 4SM. List of compounds with recoveries > 60%, 40-60%, 20-40%, 0-20% and ND (not detected) for lettuce root matrix

	R > 60%	40 < R% < 60	20 < R% < 40	0 < R% < 20%	ND
Protocol 1	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-methyl-benzotriazole; acesulfame; acetaminophen; acridone; benzotriazole; bezafibrate; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; fluconazole; furosemide; gemfibrozil; hydrochlorothiazide; irbesartan; lamotrigine; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; propranolol; sucralose; sulfanilic acid; valsartan; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	lamotrigine N2-oxide; oxcarbazepine; bisphenol A; N2-methyl-lamotrigine	sulfamethazine; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; sulfamethoxazole	sulfanilamide	valsartan acid; ibuprofen; ciprofloxacin; fenofibrate; indomethacin
Protocol 2	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; acetaminophen; benzotriazole; bisphenol A; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; diltiazem; fluconazole; furosemide; gemfibrozil; hydrochlorothiazide; ibuprofen; lamotrigine N2-oxide; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	bezafibrate; oxcarbazepine; sulfanilic acid; irbesartan; sulfamethoxazole; acridone; caffeine; lamotrigine; diclofenac	sulfamethazine; 4-hydroxydiclofenac; clofibrac acid	sulfanilamide	valsartan acid; valsartan; ciprofloxacin; fenofibrate; indomethacin
Protocol 3	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acetaminophen; acridone; benzotriazole; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; diclofenac; diltiazem; fluconazole; gemfibrozil;	4-hydroxydiclofenac; bisphenol A; oxcarbazepine; furosemide; acesulfame	sulfamethazine; sulfamethoxazole; bezafibrate; clofibrac acid	valsartan; sulfanilamide	ciprofloxacin; fenofibrate; ibuprofen; indomethacin; valsartan acid

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Protocol 4	hydrochlorothiazide; irbesartan; lamotrigine N2-oxide; lamotrigine; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; sulfanilic acid; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	N2-methyl-lamotrigine; diclofenac; acridone; benzotriazole; irbesartan; metronidazole; sulfamethoxazole; lamotrigine N2-oxide; bisphenol A; acetaminophen	oxcarbazepine	none	ciprofloxacin; fenofibrate; furosemide; indomethacin; sulfanilamide; propranolol; sulfamethazine; ibuprofen
Protocol 5	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; acetaminophen; benzotriazole; bezafibrate; caffeine; carbamazepine; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; irbesartan; lamotrigine N2-oxide; lamotrigine; metoprolol; metronidazole; N2-methyl-lamotrigine; propranolol; sucralose; sulfanilic acid; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	bisphenol A; acridone; valsartan; carbamazepine-10,11-epoxide; 37. N4-acetyl-sulfamethoxazole	valsartan acid; sulfamethazine; oxcarbazepine; sulfamethoxazole; chloramphenicol	sulfanilamide	ciprofloxacin; fenofibrate; ibuprofen; furosemide; indomethacin
Protocol 6	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; acetaminophen; benzotriazole; caffeine; carbamazepine; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; irbesartan; lamotrigine N2-oxide; lamotrigine;	bisphenol A; acridone; carbamazepine-10,11-epoxide; 37. N4-acetyl-sulfamethoxazole; bezafibrate	valsartan acid; valsartan; sulfamethazine; oxcarbazepine; sulfamethoxazole; chloramphenicol	sulfanilamide	fenofibrate; furosemide; indomethacin; ciprofloxacin; ibuprofen

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	metoprolol ; metronidazole; N2-methyl-lamotrigine; propranolol; sucralose; sulfanilic acid; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl				
Protocol 7	37. N4-acetyl-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; irbesartan; sulfanilic acid; 4-nitro-sulfamethoxazole; chloramphenicol; sucralose; hydrochlorothiazide; 5-methyl-benzotriazole; carbamazepine; benzotriazole; gemfibrozil; fluconazole; furosemide; fipronil; fipronil sulfone; fipronil desulfinyl	4-hydroxydiclofenac; acesulfame; acetaminophen; bezafibrate; caffeine; carbamazepine-10,11-epoxide; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; lamotrigine; metoprolol; metronidazole; propranolol; valsartan; verapamil	sulfamethoxazole; oxcarbazepine; sulfamethazine; acridone; N2-methyl-lamotrigine; bisphenol A; caffeine; lamotrigine N2-oxide	valsartan acid; sulfanilamide	ciprofloxacin; fenofibrate; ibuprofen; indomethacin
Protocol 8	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acetaminophen; acridone; bezafibrate; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; irbesartan; lamotrigine; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; valsartan; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	bisphenol A; caffeine; acesulfame; sulfanilic acid; benzotriazole; lamotrigine N2-oxide	sulfamethazine; sulfamethoxazole; oxcarbazepine	sulfanilamide	ciprofloxacin; valsartan acid; fenofibrate; indomethacin; furosemide; ibuprofen
Protocol 9	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; acetaminophen; acridone; benzotriazole; bezafibrate; bisphenol A; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; fenofibrate; fluconazole; furosemide; gemfibrozil; hydrochlorothiazide; ibuprofen; indomethacin; irbesartan; lamotrigine N2-oxide; lamotrigine; metoprolol; metronidazole; 37. N4-acetyl-	none	ciprofloxacin; sulfamethoxazole	sulfamethazine	sulfanilamide

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	sulfamethoxazole; N2-methyl-lamotrigine; oxcarbazepine; propranolol; sucralose; sulfanilic acid; valsartan acid; valsartan; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl				
Protocol 10	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; acesulfame; acetaminophen; acridone; bezafibrate; bisphenol A; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; clofibrac acid; fluconazole; hydrochlorothiazide; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; oxcarbazepine; sucralose; sulfamethoxazole; valsartan acid; valsartan; fipronil; fipronil sulfone; fipronil desulfinyl	clarithromycin; N2-methyl-lamotrigine; 5-methyl-benzotriazole; diclofenac; benzotriazole; lamotrigine N2-oxide; gemfibrozil; climbazole	citalopram; irbesartan; diltiazem; propranolol; lamotrigine	verapamil	ciprofloxacin; sulfamethazine; ibuprofen; furosemide; indomethacin; sulfanilamide; sulfanilic acid; fenofibrate
Protocol 11	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-methyl-benzotriazole; acesulfame; acetaminophen; acridone; benzotriazole; bezafibrate; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; irbesartan; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; propranolol; sucralose; sulfanilic acid; valsartan acid; valsartan; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; lamotrigine; N2-methyl-lamotrigine; bisphenol A; caffeine	lamotrigine N2-oxide; sulfamethazine; oxcarbazepine	none	ciprofloxacin; fenofibrate; indomethacin; sulfanilamide; furosemide; ibuprofen
Protocol 12	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; acetaminophen; acridone; benzotriazole; bisphenol A; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; diltiazem; fluconazole; furosemide; gemfibrozil; hydrochlorothiazide; irbesartan; lamotrigine; metoprolol; metronidazole; 37. N4-	oxcarbazepine; clofibrac acid; sulfanilic acid; N2-methyl-lamotrigine; diclofenac	sulfamethazine; bezafibrate; sulfamethoxazole; 4-hydroxydiclofenac; lamotrigine N2-oxide	sulfanilamide	valsartan acid; fenofibrate; ibuprofen; indomethacin; valsartan; ciprofloxacin

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	acetyl-sulfamethoxazole; propranolol; sucralose; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl				
Protocol 13	4-nitro-sulfamethoxazole; 5-methyl-benzotriazole; acesulfame; acetaminophen; acridone; benzotriazole; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; diclofenac; diltiazem; fluconazole; hydrochlorothiazide; irbesartan; lamotrigine; metoprolol; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; sulfanilic acid; fipronil; fipronil sulfone; fipronil desulfinyl	clofibrac acid; oxcarbazepine; bisphenol A; sulfamethoxazole; lamotrigine N2-oxide; gemfibrozil; metronidazole	4-hydroxydiclofenac; bezafibrate; 5-desamino-5-oxo-2,5-dihydro-lamotrigine	sulfanilamide; sulfamethazine	valsartan acid; valsartan; ciprofloxacin; fenofibrate; ibuprofen; indomethacin; furosemide
Protocol 14	4-nitro-sulfamethoxazole; 4-nitro-sulfamethoxazole; 5-methyl-benzotriazole; acesulfame; acridone; bezafibrate; bisphenol A; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; clofibrac acid; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; irbesartan; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; valsartan acid; valsartan; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	sulfanilic acid; benzotriazole; caffeine; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; acetaminophen; lamotrigine	sulfamethazine; sulfamethoxazole; lamotrigine N2-oxide; oxcarbazepine	none	ciprofloxacin; fenofibrate; furosemide; indomethacin; sulfanilamide; ibuprofen
Protocol 15	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; benzotriazole; caffeine; carbamazepine; citalopram; clarithromycin; climbazole; diclofenac; diltiazem; fluconazole; hydrochlorothiazide; irbesartan; lamotrigine N2-oxide; metoprolol; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; sulfanilic acid; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	acetaminophen; bisphenol A; gemfibrozil; clofibrac acid; acridone; chloramphenicol; bezafibrate; metronidazole; 4-hydroxydiclofenac; lamotrigine; carbamazepine-10,11-epoxide; acesulfame	sulfamethazine; oxcarbazepine; sulfamethoxazole	sulfanilamide	valsartan acid; valsartan; ciprofloxacin; fenofibrate; ibuprofen; indomethacin; furosemide

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Protocol 16	5-methyl-benzotriazole; acesulfame; acetaminophen; acridone; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; diclofenac; diltiazem; fluconazole; hydrochlorothiazide; irbesartan; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	clofibrac acid; bisphenol A; sulfamethoxazole; sulfanilic acid; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; lamotrigine; 4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; gemfibrozil	benzotriazole; sulfamethazine; bezafibrate; oxcarbazepine; lamotrigine N2-oxide	sulfanilamide	valsartan acid; valsartan; fenofibrate; furosemide; indomethacin; ciprofloxacin; ibuprofen
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Table SSM. List of compounds with recoveries > 60%, 40-60%, 20-40%, 0-20% and ND (not detected) for soil matrix

	R > 60%	40 < R% < 60	20 < R% < 40	0 < R% < 20%	ND
Protocol 1	caffeine; valsartan; chloramphenicol; irbesartan; sucralose; climbazole; fipronil; fipronil sulfone; fipronil desulfinyl	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; acridone; benzotriazole; bisphenol A; carbamazepine-10,11-epoxide; ciprofloxacin; citalopram; diclofenac; diltiazem; fenofibrate; fluconazole; furosemide; gemfibrozil; hydrochlorothiazide; indomethacin; lamotrigine N2-oxide; metoprolol; 37. N4-acetyl-sulfamethoxazole; oxcarbazepine; propranolol; sulfamethoxazole; sulfanilamide; valsartan acid; verapamil	acetaminophen; 4-hydroxydiclofenac; ibuprofen; sulfamethazine; clarithromycin; N2-methyl-lamotrigine; sulfanilic acid; clofibrac acid; carbamazepine; metronidazole; bezafibrate	ciprofloxacin; oxcarbazepine	benzotriazole; indomethacin; valsartan acid; 5-methyl-benzotriazole; fenofibrate
Protocol 2	caffeine; fipronil; fipronil sulfone; fipronil desulfinyl	4-nitro-sulfamethoxazole; 5-methyl-benzotriazole; acesulfame; acridone; benzotriazole; bisphenol A; carbamazepine-10,11-epoxide; carbamazepine; clarithromycin; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; ibuprofen; irbesartan; lamotrigine N2-oxide; lamotrigine; metronidazole; oxcarbazepine; sulfamethazine; sulfamethoxazole; sulfanilic acid	sulfanilic acid; acetaminophen; clarithromycin; verapamil; citalopram; propranolol; metoprolol; 37. N4-acetyl-sulfamethoxazole; sucralose; N2-methyl-lamotrigine; chloramphenicol; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; climbazole; sulfanilamide	ciprofloxacin; 4-hydroxydiclofenac; bezafibrate; clofibrac acid	fenofibrate; indomethacin; valsartan acid; valsartan; furosemide
Protocol 3	caffeine; fipronil; fipronil sulfone; fipronil desulfinyl	4-nitro-sulfamethoxazole; acesulfame; acridone; benzotriazole; bisphenol A; carbamazepine-10,11-epoxide; carbamazepine; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; irbesartan; lamotrigine; metoprolol;	clofibrac acid; 5-methyl-benzotriazole; acetaminophen; clarithromycin; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; verapamil; propranolol; metronidazole; citalopram; climbazole; lamotrigine N2-oxide; N2-methyl-lamotrigine;	valsartan; ciprofloxacin; 4-hydroxydiclofenac; bezafibrate; furosemide	fenofibrate; ibuprofen; indomethacin; sucralose; valsartan acid

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		oxcarbazepine; sulfamethazine; sulfamethoxazole; sulfanilic acid	sulfanilamide; 37. N4-acetyl-sulfamethoxazole; chloramphenicol;		
Protocol 4	Verapamil; caffeine; climbazole; fipronil; fipronil sulfone; fipronil desulfinyl	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-methyl-benzotriazole; acridone; benzotriazole; bezafibrate; bisphenol A; carbamazepine; citalopram; diltiazem; fluconazole; gemfibrozil; ibuprofen; lamotrigine; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sulfanilic acid; valsartan;	sulfamethoxazole; lamotrigine N2-oxide; valsartan acid; acetaminophen; oxcarbazepine; clarithromycin; chloramphenicol; sucralose; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; carbamazepine-10,11-epoxide; diclofenac; hydrochlorothiazide; acesulfame; irbesartan; clofibrac acid	ciprofloxacin; sulfanilamide; sulfamethazine	fenofibrate; indomethacin; furosemide;
Protocol 5	bisphenol A; 5-methyl-benzotriazole; metronidazole; benzotriazole; metoprolol; ibuprofen; caffeine; sucralose; fipronil; fipronil sulfone; fipronil desulfinyl	37. N4-acetyl-sulfamethoxazole; acesulfame; sulfanilic acid; sulfamethazine; N2-methyl-lamotrigine; diltiazem; fluconazole; gemfibrozil; climbazole; carbamazepine-10,11-epoxide; hydrochlorothiazide; citalopram; propranolol; verapamil	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; acesulfame; acetaminophen; acridone; carbamazepine; chloramphenicol; clarithromycin; clofibrac acid; diclofenac; irbesartan; lamotrigine N2-oxide; lamotrigine; oxcarbazepine; sulfamethoxazole; sulfanilamide	ciprofloxacin; 4-hydroxydiclofenac; bezafibrate	furosemide; indomethacin; valsartan acid; valsartan; fenofibrate
Protocol 6	caffeine; sulfanilic acid; fipronil; fipronil sulfone; fipronil desulfinyl	propranolol; 4-nitro-sulfamethoxazole; sulfamethazine; benzotriazole; carbamazepine; acridone; oxcarbazepine; metoprolol; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; sulfamethoxazole; fluconazole; metronidazole; verapamil; carbamazepine-10,11-epoxide; 5-methyl-benzotriazole; bisphenol A	acesulfame; acetaminophen; chloramphenicol; citalopram; clarithromycin; climbazole; diclofenac; diltiazem; gemfibrozil; hydrochlorothiazide; irbesartan; lamotrigine N2-oxide; lamotrigine; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; sulfanilamide	ciprofloxacin; bezafibrate; 4-hydroxydiclofenac; clofibrac acid	fenofibrate; furosemide; ibuprofen; indomethacin; sucralose; valsartan acid; valsartan

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Protocol 7	acridone; diclofenac; valsartan; hydrochlorothiazide; bisphenol A; sulfamethoxazole; citalopram; acesulfame; 4-nitro-sulfamethoxazole; sucralose; diltiazem; 37. N4-acetyl-sulfamethoxazole; metronidazole; irbesartan; carbamazepine-10,11-epoxide; propranolol; caffeine; carbamazepine; chloramphenicol; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; gemfibrozil; ibuprofen; fluconazole; fipronil; fipronil sulfone; fipronil desulfinyl	lamotrigine N2-oxide; 4-hydroxydiclofenac; acetaminophen; bezafibrate; clarithromycin; N2-methyl-lamotrigine; sulfanilamide; clofibrac acid; sulfamethazine; climbazole; lamotrigine; verapamil; metoprolol	benzotriazole; 5-methyl-benzotriazole	oxcarbazepine	valsartan acid; fenofibrate; furosemide; indomethacin; sulfanilic acid; ciprofloxacin
Protocol 8	lamotrigine; N2-Methyl-Lamotrigine.; clarithromycin; acetaminophen; clofibrac acid; sulfanilamide; citalopram; benzotriazole; metronidazole; gemfibrozil; 4-nitro-sulfamethoxazole; sulfamethazine; irbesartan; acridone; acesulfame; diltiazem; ibuprofen; sucralose; propranolol; valsartan; sulfamethoxazole; hydrochlorothiazide; 37. N4-acetyl-sulfamethoxazole; carbamazepine; carbamazepine-10,11-epoxide; fluconazole; diclofenac; verapamil; metoprolol; chloramphenicol; climbazole; bisphenol A; caffeine; gemfibrozil; ibuprofen; fipronil; fipronil sulfone; fipronil desulfinyl	4-hydroxydiclofenac; lamotrigine N2-oxide; bezafibrate; 5-methyl-benzotriazole	none	oxcarbazepine	5-desamino-5-oxo-2,5-dihydro-lamotrigine; valsartan acid; fenofibrate; furosemide; indomethacin; sulfanilic acid; ciprofloxacin
Protocol 9	benzotriazole; diltiazem; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; sulfanilic acid; metronidazole; diclofenac; fluconazole; lamotrigine N2-oxide; metoprolol; irbesartan; ibuprofen; valsartan acid; 37. N4-acetyl-sulfamethoxazole; carbamazepine; clarithromycin; verapamil; carbamazepine-10,11-epoxide; 4-nitro-sulfamethoxazole; acesulfame; valsartan; indomethacin; sucralose; fenofibrate; caffeine; hydrochlorothiazide; climbazole;	sulfamethoxazole; lamotrigine; 5-methyl-benzotriazole; citalopram; N2-methyl-lamotrigine; acridone; propranolol	4-hydroxydiclofenac; sulfamethazine	ciprofloxacin; oxcarbazepine; sulfanilamide	none

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Protocol 10	chloramphenicol; clofibrac acid; furosemide; acetaminophen; bezafibrate; gemfibrozil; bisphenol A; fipronil; fipronil sulfone; fipronil desulfinyl		sulfamethoxazole; sulfanilamide; climbazole	oxcarbazepine	fenofibrate; chloramphenicol; furosemide; indomethacin; sulfanilic acid; ciprofloxacin;	
Protocol 11	4-hydroxydiclofenac; 4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; acetaminophen; acridone; benzotriazole; bezafibrate; bisphenol A; caffeine; carbamazepine-10,11-epoxide; carbamazepine; citalopram; clarithromycin; clofibrac acid; diclofenac; diltiazem; fluconazole; gemfibrozil; hydrochlorothiazide; ibuprofen; irbesartan; lamotrigine N2-oxide; lamotrigine; metoprolol; metronidazole; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; sulfamethazine; valsartan acid; valsartan; verapamil; fipronil; fipronil sulfone; fipronil desulfinyl	propranolol; diltiazem; fluconazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; irbesartan; acesulfame; verapamil; 4-nitro-sulfamethoxazole; bezafibrate; citalopram; diclofenac; hydrochlorothiazide; valsartan	5-methyl-benzotriazole; acridone; benzotriazole; carbamazepine-10,11-epoxide; clarithromycin; clofibrac acid; furosemide; lamotrigine; metoprolol; metronidazole; N2-methyl-lamotrigine; oxcarbazepine; sulfamethazine; sulfamethoxazole; sulfanilamide; valsartan acid	ciprofloxacin; sucralose; lamotrigine N2-oxide; acetaminophen	fenofibrate; ibuprofen; indomethacin	
Protocol 12	carbamazepine; sulfanilic acid; 4-hydroxydiclofenac; caffeine; 37. N4-acetyl-sulfamethoxazole; gemfibrozil; chloramphenicol; climbazole; bisphenol A; fipronil; fipronil sulfone; fipronil desulfinyl	ibuprofen; fenofibrate; metronidazole; bisphenol A; fipronil; fipronil sulfone; fipronil desulfinyl	sulfanilic acid; sulfamethazine; carbamazepine-10,11-epoxide; benzotriazole; acridone; chloramphenicol; gemfibrozil; oxcarbazepine; caffeine; fluconazole	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; acesulfame; carbamazepine; citalopram; clarithromycin; climbazole; diclofenac; diltiazem; furosemide;	ciprofloxacin; 4-hydroxydiclofenac; acetaminophen; bezafibrate; clofibrac acid; irbesartan; lamotrigine N2-oxide	

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			hydrochlorothiazide; indomethacin; lamotrigine; metoprolol; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; propranolol; sucralose; sulfamethoxazole; sulfanilamide; valsartan acid; valsartan; verapamil			
Protocol 13	sucralose; fenofibrate; ibuprofen; caffeine; sulfanilic acid; bisphenol A; fipronil; fipronil sulfone; fipronil desulfinyl	37. N4-acetyl-sulfamethoxazole; sulfamethazine; citalopram; verapamil; metoprolol; oxcarbazepine; acridone; chloramphenicol; benzotriazole; sulfamethoxazole; hydrochlorothiazide; fluconazole; carbamazepine	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; aceulfame; bezafibrate; carbamazepine-10,11-epoxide; clarithromycin; climbazole; diltiazem; gemfibrozil; irbesartan; lamotrigine N2-oxide; lamotrigine; metronidazole; N2-methyl-lamotrigine; propranolol; sulfanilamide	ciprofloxacin; 4-hydroxydiclofenac; diclofenac; acetaminophen; clofibrac acid	indomethacin; valsartan acid; valsartan; furosemide	
Protocol 14	citalopram; propranolol; climbazole; sulfanilic acid; fipronil; fipronil sulfone; fipronil desulfinyl	4-nitro-sulfamethoxazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 5-methyl-benzotriazole; aceulfame; acridone; bezafibrate; bisphenol A; caffeine; carbamazepine; chloramphenicol; clarithromycin; clofibrac acid; diltiazem; fluconazole; gemfibrozil; ibuprofen; lamotrigine; metoprolol; 37. N4-acetyl-sulfamethoxazole; N2-methyl-lamotrigine; sulfamethoxazole; valsartan acid; valsartan; verapamil	5-methyl-benzotriazole; acridone; benzotriazole; bisphenol A; carbamazepine-10,11-epoxide; carbamazepine; clarithromycin; diclofenac; fluconazole;	carbamazepine-10,11-epoxide; irbesartan; diclofenac; benzotriazole; metronidazole; oxcarbazepine; sulfamethazine; furosemide; sucralose; hydrochlorothiazide	ciprofloxacin; acetaminophen; 4-hydroxydiclofenac; lamotrigine N2-oxide; sulfanilamide	fenofibrate; indomethacin
Protocol 15	metoprolol; caffeine; gemfibrozil; sucralose; fenofibrate; fipronil; fipronil sulfone; fipronil desulfinyl	5-methyl-benzotriazole; acridone; benzotriazole; bisphenol A; carbamazepine-10,11-epoxide; carbamazepine; clarithromycin; diclofenac; fluconazole;	clofibrac acid; bezafibrate; sulfamethoxazole; chloramphenicol; aceulfame; climbazole; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; propranolol; irbesartan; citalopram; verapamil;	acetaminophen; ciprofloxacin; sulfanilamide; lamotrigine N2-oxide; 4-hydroxydiclofenac; sulfamethazine	indomethacin; valsartan acid; valsartan; furosemide	

		hydrochlorothiazide; ibuprofen; metronidazole; 37. N4-acetyl-sulfamethoxazole; oxcarbazepine; sulfanilic acid	lamotrigine; N2-methyl-lamotrigine; 4-nitro-sulfamethoxazole; diltiazem		
Protocol 16	fenofibrate; gemfibrozil; verapamil; sulfanilic acid; fipronil; fipronil sulfone; fipronil desulfinyl	4-nitro-sulfamethoxazole; 5-methyl-benzotriazole; acridone; benzotriazole; bezafibrate; bisphenol A; caffeine; carbamazepine-10,11-epoxide; carbamazepine; chloramphenicol; citalopram; clarithromycin; climbazole; diclofenac; diltiazem; hydrochlorothiazide; lamotrigine; metoprolol; 37. N4-acetyl-sulfamethoxazole; oxcarbazepine; propranolol;	bezafibrate; clofibrac acid; acetaminophen; lamotrigine N2-oxide; sulfamethazine; sulfamethoxazole; irbesartan; aceulfame; N2-methyl-lamotrigine; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; fluconazole; metronidazole; sucralose; furosemide	4-hydroxydiclofenac; ciprofloxacin; sulfanilamide	valsartan acid; valsartan; indomethacin; ibuprofen
Protocol 17	bezafibrate; sulfamethoxazole; diclofenac; lamotrigine; aceulfame; chloramphenicol; valsartan; ibuprofen; citalopram; verapamil; acridone; benzotriazole; irbesartan; N2-methyl-lamotrigine; carbamazepine; lamotrigine N2-oxide; 4-nitro-sulfamethoxazole; diltiazem; sucralose; fluconazole; 37. N4-acetyl-sulfamethoxazole; metoprolol; gemfibrozil; bisphenol A; climbazole; caffeine; carbamazepine-10,11-epoxide; hydrochlorothiazide; propranolol; chloramphenicol; fipronil; fipronil sulfone; fipronil desulfinyl	4-hydroxydiclofenac; acetaminophen; sulfanilamide; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; metronidazole; sulfamethazine; clofibrac acid; clarithromycin	5-methyl-benzotriazole	oxcarbazepine	valsartan acid; furosemide; fenofibrate; indomethacin; sulfanilic acid; ciprofloxacin
Protocol 18	sulfamethazine; 5-methyl-benzotriazole; benzotriazole; lamotrigine; sulfamethoxazole; aceulfame; clarithromycin; metronidazole; lamotrigine N2-oxide; 4-nitro-sulfamethoxazole; verapamil; citalopram; caffeine; propranolol; diltiazem; hydrochlorothiazide; bezafibrate; acridone; sucralose; N2-methyl-lamotrigine; climbazole; carbamazepine-10,11-epoxide;	sulfanilamide; 4-hydroxydiclofenac; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; acetaminophen; clofibrac acid	none	oxcarbazepine	fenofibrate; furosemide; valsartan acid; indomethacin; sulfanilic acid; ciprofloxacin

	37. N4-acetyl-sulfamethoxazole; metoprolol; diclofenac; fluconazole; bisphenol A; carbamazepine; valsartan; irbesartan; fipronil; fipronil sulfone; fipronil desulfinyl				
Protocol 19	clofibrac acid; lamotrigine; climbazole; irbesartan; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; metronidazole; fluconazole; 4-nitro-sulfamethoxazole; N2-methyl-lamotrigine; sucralose; acesulfame; diltiazem; metoprolol; acridone; citalopram; caffeine; carbamazepine; carbamazepine-10,11-epoxide; clarithromycin; verapamil; hydrochlorothiazide; diclofenac; 37. N4-acetyl-sulfamethoxazole; bisphenol A; fipronil; fipronil sulfone; fipronil desulfinyl	sulfamethazine; acetaminophen; valsartan; sulfamethoxazole; propranolol	5-methyl-benzotriazole; lamotrigine N2-oxide; fenofibrate; sulfanilamide; benzotriazole; 4-hydroxydiclofenac	oxcarbazepine; bezafibrate; indomethacin; chloramphenicol; ibuprofen; gemfibrozil	ciprofloxacin; furosemide; sulfanilic acid; valsartan acid;
Protocol 20	fluconazole; diltiazem; metoprolol; 4-nitro-sulfamethoxazole; diclofenac; hydrochlorothiazide; sucralose; propranolol; verapamil; 4-nitro-sulfamethoxazole; bisphenol A; carbamazepine; acridone; acesulfame; carbamazepine-10,11-epoxide; benzotriazole; metronidazole; fipronil; fipronil sulfone; fipronil desulfinyl	sulfamethoxazole; sulfanilamide; acetaminophen; sulfamethazine; clofibrac acid; citalopram; climbazole; lamotrigine; irbesartan; N2-methyl-lamotrigine; clarithromycin	lamotrigine N2-oxide; fenofibrate; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; 4-hydroxydiclofenac; 5-methyl-benzotriazole; valsartan	ibuprofen; bezafibrate; oxcarbazepine; gemfibrozil; chloramphenicol	ciprofloxacin; valsartan acid; furosemide; indomethacin; sulfanilic acid
Protocol 21	valsartan; benzotriazole; diltiazem; metronidazole; 4-nitro-sulfamethoxazole; irbesartan; carbamazepine-10,11-epoxide; valsartan acid; sucralose; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; citalopram; acridone; clarithromycin; fluconazole; carbamazepine; bisphenol A; clofibrac acid; hydrochlorothiazide; 37. N4-acetyl-sulfamethoxazole; diclofenac; caffeine; verapamil; acesulfame; fipronil; fipronil sulfone; fipronil desulfinyl	propranolol; acetaminophen; N2-methyl-lamotrigine; 5-methyl-benzotriazole; metoprolol	sulfamethazine; 4-hydroxydiclofenac; lamotrigine N2-oxide; lamotrigine; climbazole; sulfamethoxazole	bezafibrate; chloramphenicol; oxcarbazepine; gemfibrozil; ibuprofen; sulfanilamide;	ciprofloxacin; furosemide; sulfanilic acid; indomethacin; fenofibrate
Protocol 22	sulfamethazine; lamotrigine; citalopram; benzotriazole; 5-methyl-benzotriazole; propranolol; caffeine; climbazole; 4-nitro-	4-hydroxydiclofenac; valsartan; sulfanilamide; N2-methyl-	lamotrigine N2-oxide	indomethacin; bezafibrate; chloramphenicol;	fenofibrate; ciprofloxacin; valsartan acid; furosemide; sulfanilic acid

	sulfamethoxazole; 37. N4-acetyl-sulfamethoxazole; metronidazole; diltiazem; sucralose; acridone; fluconazole; acesulfame; 5-desamino-5-oxo-2,5-dihydro-lamotrigine; metoprolol; irbesartan; diclofenac; hydrochlorothiazide; carbamazepine-10,11-epoxide; carbamazepine; bisphenol A; verapamil; clarithromycin; fipronil; fipronil sulfone; fipronil desulfinyl	lamotrigine; acetaminophen; clofibrac acid; sulfamethoxazole		oxcarbazepine; gemfibrozil; ibuprofen	
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Table 6SM. Comparison of Matrix effect (%) for target analytes in MRMHR and SWATH acquisition modes in lettuce root matrix

Compound	ESI	MRM ^{HR} acquisition mode					SWATH acquisition mode				
		2 µg L ⁻¹	5 µg L ⁻¹	10 µg L ⁻¹	50 µg L ⁻¹	200 µg L ⁻¹	2 µg L ⁻¹	5 µg L ⁻¹	10 µg L ⁻¹	50 µg L ⁻¹	200 µg L ⁻¹
Acesulfame	-	115.1	34.9	20.9	30.7	5.5	97.1	60.2	54.1	27.2	7.8
Acetaminophen	-	500.9	341.8	292.8	265.7	181.2	548.4	334.9	243.1	214.0	135.4
Acridone	+	-68.3	-72.8	-71.7	-71.5	-57.1	-40.7	-64.8	-70.0	-53.8	-45.8
Benzotriazole	-/+	-52.8	-54.6	-34.0	-18.9	-17.6	153.0	74.1	42.6	-0.1	-23.3
5-Methyl-2H-Benzotriazole	+	-34.8	-64.7	-60.3	-48.0	-37.1					
Bezafibrate	-	-2.8	-22.5	-13.8	15.7	-28.8	-12.2	-19.0	-5.6	7.7	-18.1
Bisphenol A	-	-2.7	-15.9	2.5	-3.0	-16.0	51.0	20.6	18.9	17.6	-5.6
Caffeine	+	47.5	16.6	-1.4	1.4	-8.7	85.7	40.3	15.6	11.3	-9.4
Carbamazepine	+	-5.0	-13.9	-17.0	-9.3	-11.2	-2.0	-13.1	-7.5	-5.2	-4.2
Carbamazepine-10,11-epoxide	+	-45.1	-52.4	-30.6	-86.5	-82.8	-45.6	-66.5	-52.7	-87.7	-82.7
Chloramphenicol	-	-23.6	-15.1	-22.6	-5.5	-18.8	69.0	38.7	29.9	13.2	2.2
Ciprofloxacin	+	-96.0	-95.2	-96.5	-93.4	-92.1	-45.1	-85.6	-86.0	-80.4	-81.9
Citalopram	+	-89.6	-93.8	-89.5	-92.3	-86.0	-84.0	-90.3	-88.8	-88.9	-82.7
Clarithromycin	+	-72.1	-83.5	-65.2	-78.6	-73.8	-66.4	-77.3	-63.5	-65.2	-56.4
Climbazole	-	-65.5	-80.8	-77.0	-82.2	-77.8	-66.7	-78.8	-75.6	-80.5	-78.9
Clofibrac acid	-	189.2	196.9	139.7	182.6	102.9	13.0	-1.7	6.1	29.5	5.9
Diclofenac	+	-79.5	-79.5	-81.8	-84.6	-82.8	-78.4	-85.1	-83.3	-78.5	-73.9
4-Hydroxydiclofenac	+	-42.5	-45.0	-51.9	-58.4	-58.2	5.7	25.0	1.4	-1.9	-54.0
Diltiazem	+	146.5	-47.8	285.0	-83.6	-69.7	236.6	-18.5	290.4	-75.7	-60.5
Fenofibrate	+	-92.3	-96.0	-87.8	-96.5	-95.4	-91.1	-95.1	-94.9	-93.8	-89.4
Fipronil	-	-46.4	-41.3	-43.7	-38.5	-50.5	-33.3	-41.7	-43.5	-38.8	-51.2
Fipronil desulfinyl	-	-89.9	-92.5	-88.9	-85.0	-84.1	-88.2	-92.2	-89.4	-84.9	-84.3
Fipronil sulfone	-	-76.7	-76.8	-73.9	-62.4	-69.1	-71.2	-76.8	-74.0	-62.6	-70.2
Fluconazole	+	30.5	-21.2	-14.7	-6.7	-8.7	5.6	2.0	-4.8	2.0	-0.8
Furosemide	-	-26.9	-54.0	-44.0	-24.1	-46.8	272.9	53.7	10.6	-17.0	-38.1
Gemfibrozil	-	-75.4	-82.1	-81.3	-81.3	-75.2	-91.6	-84.3	-88.7	-80.8	-79.7
Hydrochlorothiazide	-	-27.2	-46.1	-31.8	-20.5	-38.6	35.9	-8.4	-9.2	-11.6	-15.8
Ibuprofen	-	-25.5	-54.5	-42.6	-47.3	-53.0	-39.2	-34.7	-59.1	-19.5	-37.8
Indomethacine	-	194.5	42.2	229.2	-82.1	-87.7					
Irbesartan	-/+	-89.5	-91.9	-88.9	-93.5	-93.2	-86.7	-91.6	-91.6	-89.9	-79.1
Lamotrigine	+	-79.8	-80.4	-76.4	-72.8	-67.5	-77.4	-77.5	-74.8	-75.1	-72.5
Lamotrigine N2-oxide	+	-63.3	-64.0	-57.3	-19.1	-3.6	-45.2	-46.5	-53.2	-10.6	-18.2

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5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	+	57.8	-26.3	-16.1	-14.2	-15.8	-23.5	-15.9	-10.2	-8.2	-15.9
N2-Methyl-Lamotrigine	+	-73.0	-77.9	-78.6	-75.1	-76.7	30.2	-44.7	-36.9	-54.8	-50.9
Metoprolol	+	-16.7	-1.9	16.3	-2.1	-18.8	-9.9	-9.3	-4.9	-2.2	-20.6
Metrodinazole	+	58.4	5.0	3.7	-4.5	-13.3	301.7	140.5	98.9	0.9	-9.9
Oxcarbazepine	+	84.3	150.1	66.8	142.8	11.4	133.4	190.8	113.8	131.0	22.8
Propranolol	+	-89.1	-89.0	-78.9	-80.3	-81.8	926.5	403.5	179.5	-17.4	-64.9
Sucralose	-	216.7	132.3	211.4	71.0	30.1	258.2	135.9	213.9	69.8	25.7
Sulfamethazine	+	-66.5	-80.8	-76.5	-78.2	-74.3	-24.4	-40.9	-54.5	-71.8	-60.7
Sulfamethoxazole	-/+	-76.6	-78.3	-74.6	-73.9	-67.6	-71.2	-69.8	-69.2	-60.9	-48.9
4-Nitro-Sulfamethoxazole	+	-26.7	-24.5	-27.2	-1.6	-19.5	96.6	68.2	63.0	68.1	24.7
N4-Acetylsulfamethoxazole	-/+	-30.8	-39.3	-40.1	-16.1	-37.4	258.3	145.0	102.6	123.4	28.0
Sulfanilamide	-	-67.6	-62.6	-67.8	-65.6	-51.7	-67.3	-74.7	-70.3	-66.1	-53.4
Sulfanilic acid	-	-59.4	-47.3	-46.9	-65.2	-69.3	-64.0	-51.5	-56.9	-63.6	-65.0
Valsartan	+/-	-21.3	-42.0	-39.8	-57.4	-62.7	-18.9	-54.1	-56.8	-59.7	-64.8
Valsartan acid	-/+	22.4	35.7	42.1	33.6	12.7	-38.3	-61.4	-61.7	-42.0	-48.7
Verapamil	+	-83.0	-94.2	-87.9	-94.9	-92.0	-79.0	-89.5	-88.1	-89.5	-83.1

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Table 7SM. Comparison of Matrix effect (%) for target analytes in MRMHR and SWATH acquisition modes in soil matrix

Compound	ESI	MRM ^{HR} acquisition mode					SWATH acquisition mode				
		2 µg L ⁻¹	5 µg L ⁻¹	10 µg L ⁻¹	50 µg L ⁻¹	200 µg L ⁻¹	2 µg L ⁻¹	5 µg L ⁻¹	10 µg L ⁻¹	50 µg L ⁻¹	200 µg L ⁻¹
Acesulfame	-	40.0	26.0	32.2	47.7	12.4	97.3	62.6	44.3	26.1	7.5
Acetaminophen	-	147.5	119.1	243.5	315.4	272.9	241.5	158.3	126.8	158.4	107.7
Acridone	+	27.3	-4.3	3.3	-1.7	-7.7	-42.1	-61.2	-64.3	-50.0	-43.3
Benzotriazole	+	85.4	43.8	18.5	6.0	-7.6	-25.7	15.9	55.5	4.3	-30.0
5-Methyl-2H-Benzotriazole	+/-	16.3	-3.4	1.4	3.7	-3.6	-30.1	-61.3	-51.7	-41.4	-29.5
Bezafibrate	-	13.3	38.3	-1.9	32.1	-10.8	-8.0	-16.7	-1.3	11.4	-17.3
Bisphenol A	-	1.4	13.0	-12.6	-9.3	-24.1	45.1	20.5	17.1	16.0	-8.4
Caffeine	+	463.8	234.6	155.3	12.3	-8.0	131.1	47.1	26.1	1.4	-5.2
Carbamazepine	+	45.7	18.7	33.5	-0.6	4.0	3.4	-11.1	-2.5	-5.0	-4.9
Carbamazepine-10,11-epoxide	+	66.7	39.2	127.0	9.2	-3.3	-62.0	-67.2	-59.5	-87.8	-80.8
Chloramphenicol	-	0.0	-12.5	-18.2	6.5	-9.1	67.1	30.5	23.6	13.1	2.5
Ciprofloxacin	+	69.2	9.4	-6.4	-34.3	-33.0	-34.5	-88.2	-89.2	-87.0	-84.2
Citalopram	+	-47.6	-65.6	-50.7	-61.1	-32.0	-81.5	-89.5	-88.3	-88.6	-81.2
Clarithromycin	+	50.1	14.8	114.3	38.5	14.7	-65.3	-76.2	-62.7	-64.8	-55.4
Climbazole	-	-64.7	-77.6	-75.6	-78.4	-74.7	-64.6	-77.8	-70.3	-80.6	-78.6
Clofibric acid	-	171.7	183.5	132.2	182.6	95.4	13.7	4.6	6.3	21.0	8.9
Diclofenac	+/-	11.8	16.6	33.2	21.2	-6.1	-30.1	-51.8	-54.9	-65.2	-66.8
4-Hydroxydiclofenac	+	-1.1	24.3	57.5	34.3	7.3	26.1	10.6	-24.1	-51.5	-56.5
Diltiazem	+	921.2	178.2	1819.8	-7.3	-13.2	267.2	-13.7	351.3	-74.4	-57.9
Fenofibrate	+	-84.6	-91.5	-83.0	-84.6	-76.6	-89.5	-94.8	-92.8	-93.5	-89.2
Fipronil	-	-33.2	-39.7	-44.8	-38.8	-48.7	-29.9	-37.2	-39.0	-38.5	-50.6
Fipronil desulfinyl	-										
Fipronil sulfone	-										
Fluconazole	+	22.3	-10.9	20.5	1.4	-8.5	55.5	8.4	6.0	17.4	0.1
Furosemide	-	66.2	-21.1	-30.5	-7.1	-34.4	128.5	39.9	-9.5	-13.7	-35.0
Gemfibrozil	-	-77.4	-81.5	-81.9	-75.6	-74.3	-85.5	-90.7	-86.2	-78.6	-79.1
Hydrochlorothiazide	-	-30.4	-33.4	-36.9	-23.2	-34.5	48.9	0.3	-2.7	-11.8	-15.5
Ibuprofen	-	-16.7	-52.5	-34.4	-17.7	-49.3	-16.8	50.9	-15.5	-25.2	-32.6
Indomethacin	-	151.6	3.2	232.3	-79.1	-85.3					
Irbesartan	+	5.1	-6.3	4.7	0.3	-2.2	-71.9	-87.6	-90.5	-87.8	-75.0
Lamotrigine	+	-25.4	-37.9	-28.7	-25.7	-31.7	-70.7	-78.8	-74.6	-74.8	-71.8
Lamotrigine N2-oxide	+	10.8	-0.7	39.5	48.2	38.3	-26.9	-55.3	-50.6	-17.1	-21.7
5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	+	18.1	22.8	8.7	-8.7	-16.8	-16.9	-21.8	-24.4	-12.5	-19.1
N2-Methyl-Lamotrigine	+	-13.0	-30.6	-4.5	-24.3	-29.5	271.2	108.3	18.7	-34.8	-37.7

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Metoprolol	+	44.9	20.0	32.1	10.2	-8.3	52.4	14.4	19.7	15.0	-11.7
Metrodinazole	+	181.4	57.9	93.0	3.4	-5.6	174.0	42.6	37.5	-13.4	-15.9
Oxcarbazepine	+	111.2	140.0	101.3	97.0	3.0	-57.5	-67.3	-58.9	-87.9	-80.7
Propranolol	+	-36.9	-40.3	-33.2	-47.1	-51.6	332.0	118.0	87.1	-47.1	-66.4
Sucralose	+/-	95.2	17.6	136.7	21.3	-10.8	238.5	155.0	237.2	75.2	31.2
Sulfamethazine	+	5.7	-12.1	2.2	2.0	-4.2	-8.0	-46.3	-55.3	-68.1	-55.6
Sulfamethoxazole	+/-	0.2	-11.4	11.0	9.2	-1.1	-79.6	-78.8	-77.6	-72.5	-61.1
4-Nitro-Sulfamethoxazole	+	48.2	14.1	28.6	7.1	4.1	79.5	61.1	55.5	68.0	27.9
N4-Acetylsulfamethoxazole	+	68.1	18.0	27.8	32.0	-4.9	107.0	76.1	72.0	60.6	25.3
Sulfanilamide	-	-12.7	-65.8	-58.9	-58.2	-51.2	-63.9	-69.7	-67.6	-65.9	-51.9
Sulfanilic acid	-	-56.4	-47.1	-50.9	-64.1	-69.3	-63.0	-48.5	-55.7	-65.8	-67.1
Valsartan	+	39.9	34.1	59.9	55.6	15.8	115.3	26.2	10.4	-39.6	-46.3
Valsartan acid	+	28.4	-2.5	26.5	7.5	-3.0	-31.2	-50.4	-54.9	-48.7	-47.5
Verapamil	+	-49.3	-69.2	-60.1	-60.2	-40.0	-83.8	-90.6	-88.5	-88.7	-82.8

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Table 8SM. Relative recoveries and intraday precision (RSD%) at 5 concentrations (2, 5, 10, 50, 200 µg L⁻¹) with MRM and SWATH acquisition modes for lettuce root matrix

compound	ESI	MRM acquisition mode										SWATH acquisition mode									
		2 µg L ⁻¹	RSD (%)	5 µg L ⁻¹	RSD (%)	10 µg L ⁻¹	RSD (%)	50 µg L ⁻¹	RSD (%)	200 µg L ⁻¹	RSD (%)	2 µg L ⁻¹	RSD (%)	5 µg L ⁻¹	RSD (%)	10 µg L ⁻¹	RSD (%)	50 µg L ⁻¹	RSD (%)	200 µg L ⁻¹	RSD (%)
Acesulfame	-	73.7	10.3	88.2	5.2	72.8	4.7	71.2	4.8	75.4	4.5	72.3	15.6	72.2	5.8	88.8	10.5	72.1	3.0	80.9	2.6
Acetaminophen	-	88.4	15.5	93.9	15.3	82.9	5.6	65.7	3.7	89.2	10.2	84.0	6.1	89.0	1.8	92.6	3.4	78.1	3.0	82.5	3.0
Acridone	+	69.0	5.1	74.3	0.7	65.5	10.8	66.8	5.6	81.6	14.8	67.2	7.4	78.2	5.4	72.5	2.9	73.3	7.3	78.2	5.5
Benzotriazole	-/+	73.6	9.3	99.9	20.0	72.5	9.4	58.9	3.9	67.2	8.3	114.6	10.9	128.7	9.6	77.1	9.1	74.6	4.2	65.9	15.3
5-Methyl-2H-Benzotriazole	+	54.1	11.1	111.0	17.9	80.9	12.0	63.7	11.9	69.7	8.0										
Bezafibrate	-	86.1	11.3	92.4	17.8	102.4	13.2	83.9	8.0	104.1	2.7	74.8	9.3	103.1	16.3	95.7	1.1	85.0	2.8	90.7	6.2
Bisphenol A	-	109.8	7.6	129.8	5.8	103.0	3.3	82.5	7.9	86.6	7.5	84.1	7.8	98.9	1.0	91.7	1.4	81.1	2.2	88.2	1.4
Caffeine	+	108.1	53.8	112.6	20.2	82.1	8.5	62.7	5.4	68.5	5.4	103.3	6.5	95.8	10.6	81.8	2.9	65.0	3.9	75.3	5.1
Carbamazepine	+	98.0	6.4	110.9	6.9	103.4	2.8	86.5	1.5	91.1	2.2	90.7	1.9	103.9	2.2	93.6	3.1	84.3	2.1	93.8	4.7
Carbamazepine-10,11-epoxide	+	69.6	10.4	62.4	9.4	81.2	17.5	69.4	23.8	77.7	25.3	69.3	22.8	82.2	10.3	87.3	15.4	66.6	16.9	80.2	19.9
Chloramphenicol	-	85.7	41.9	92.6	17.1	89.2	24.5	76.1	9.5	95.6	3.3	96.9	12.8	103.4	5.7	98.4	1.1	90.7	3.1	93.9	1.3
Ciprofloxacin	+	36.6	0.9	28.3	30.9	22.3	8.4	15.3	3.3	15.7	6.4	30.3	14.5	39.5	1.5	24.9	3.5	13.1	7.5	15.6	9.0
Citalopram	+	75.4	17.1	86.7	20.8	71.8	11.0	100.7	16.3	101.1	19.6	69.6	10.0	85.6	3.0	98.0	12.4	89.7	3.5	93.0	18.4
Clarithromycin	+	89.2	3.5	125.2	5.2	66.8	15.8	82.4	9.9	109.3	24.3	59.9	7.0	86.6	8.4	77.7	13.6	72.4	8.2	93.0	11.2
Climbazole	-	91.8	4.7	104.6	9.8	104.4	14.8	86.7	6.8	98.6	13.9	80.0	4.5	93.0	7.9	103.7	13.4	84.7	4.1	89.8	11.4
Clofibric acid	-	102.8	18.9	105.8	10.8	102.8	3.5	84.7	5.9	89.5	2.4	103.2	6.1	113.6	4.6	106.4	4.2	82.4	4.6	92.7	3.9
Diclofenac	+	73.5	35.2	81.3	15.5	102.9	3.2	92.8	20.8	88.8	9.0	75.3	4.0	98.7	14.5	98.7	4.0	87.4	7.1	97.7	2.9
4-Hydroxiclofenac	+	93.4	35.8	74.6	22.3	86.1	8.5	106.2	2.4	95.1	13.8	ND	ND	46.4	2.5	54.4	23.6	42.9	11.2	104.1	18.4
Diltiazem	+	47.1	3.8	94.5	10.4	75.7	16.4	85.9	14.4	89.3	20.2	71.7	3.1	90.0	7.2	93.9	10.2	84.8	3.8	91.9	15.1
Fenofibrate	+	63.6	34.4	136.3	25.5	63.1	34.7	110.9	39.6	121.4	56.6	81.5	0.7	100.1	9.7	117.7	19.1	96.3	7.6	100.9	23.7
Fipronil	-	91.7	4.2	106.4	3.1	102.9	4.7	86.5	6.4	93.7	3.7	93.1	0.6	106.3	1.6	101.7	4.1	86.8	2.8	94.2	3.0
Fipronil desulfinyl	-	79.9	5.3	108.9	3.4	102.1	15.2	67.8	2.0	105.5	11.9	80.0	1.3	107.3	3.8	107.8	16.2	68.3	4.3	99.5	9.9
Fipronil sulfone	-	94.1	6.6	102.5	8.0	102.2	10.6	66.8	5.1	94.8	2.9	86.8	3.8	105.8	2.2	98.1	7.5	67.4	3.5	94.2	3.4
Fluconazole	+	76.2	7.0	112.3	7.2	88.3	4.8	78.1	6.3	77.9	2.3	94.1	2.6	90.2	1.8	93.5	1.1	76.4	5.7	80.9	10.5
Furosemide	-	107.9	16.8	98.4	17.3	100.0	20.7	82.2	6.8	103.0	9.0	75.7	26.6	101.3	18.2	91.0	14.6	97.0	3.6	103.5	1.8
Gemfibrozil	-	86.4	16.4	107.9	9.5	93.4	9.7	99.1	8.8	102.6	6.5	74.7	14.2	47.6	7.1	106.1	14.4	87.8	16.5	95.4	1.7
Hydrochlorothiazide	-	100.3	6.1	109.7	26.2	96.0	4.5	88.2	3.6	96.1	7.0	94.4	16.1	101.7	9.6	94.5	7.2	84.6	5.9	91.8	3.8
Ibuprofen	-	111.3	25.5	136.0	6.9	107.8	21.4	90.8	6.9	98.1	3.9	77.7	5.6	172.7	60.7	177.9	22.1	87.4	11.4	91.8	6.2
Indomethacin	-	103.3	15.0	119.4	23.3	121.0	11.2	98.6	9.8	112.4	21.9										
Irbesartan	-/+	72.2	24.4	89.4	22.7	89.6	37.6	94.6	9.9	124.7	33.1	92.7	39.2	86.4	12.2	97.1	6.4	87.9	4.7	91.7	20.0
Lamotrigine	+	57.5	6.2	67.7	6.6	67.6	9.1	65.8	8.1	69.9	13.0	74.5	32.4	99.3	6.3	73.0	6.0	63.4	7.7	74.6	14.0
Lamotrigine N2-oxide	+	103.5	25.6	74.8	15.1	87.2	29.6	77.9	6.6	85.4	10.4	68.0	12.6	62.7	7.1	67.6	9.8	75.3	9.3	91.3	7.1

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5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	+	67.8	10.8	69.7	31.2	74.9	16.3	75.0	5.0	78.1	6.3	72.7	12.0	88.4	4.3	78.9	2.0	68.4	8.3	72.9	3.0
N2-Methyl-Lamotrigine	+	73.1	15.7	91.3	12.1	89.4	23.7	76.9	8.8	79.9	14.2	81.6	12.3	92.8	10.4	127.3	11.5	80.3	4.9	86.1	6.3
Metoprolol	+	122.9	21.2	94.9	7.2	75.6	6.0	84.5	2.0	76.8	5.1	111.4	11.0	91.2	7.5	93.7	8.8	79.3	3.8	84.6	2.6
Metrodiazole	+	95.1	11.3	90.7	7.8	96.7	4.7	70.6	3.6	86.1	2.7	101.3	15.8	99.6	11.7	86.8	4.4	81.0	7.3	80.0	1.4
Oxcarbazepine	+	84.8	4.3	94.7	13.9	96.7	7.1	79.0	2.1	81.8	6.8	95.7	15.8	94.3	10.8	80.0	2.7	80.7	3.4	84.1	3.3
Propranolol	+	92.4	38.4	160.8	8.9	73.9	25.1	75.5	9.8	84.1	25.7	101.0	43.9	55.3	24.3	58.2	36.6	55.8	38.3	86.0	9.7
Sucralose	-	84.9	14.5	96.5	6.3	90.7	3.3	75.1	1.1	83.1	3.9	88.2	7.7	92.0	5.1	90.9	1.3	73.8	2.9	82.3	2.2
Sulfamethazine	+	17.3	10.0	10.0	5.0	17.4	10.6	14.3	1.8	12.7	2.4	23.5	13.6	43.2	25.3	34.6	20.2	20.4	5.3	17.3	2.9
Sulfamethoxazole	-/+	31.1	14.3	41.0	14.9	30.6	3.2	30.2	6.3	30.8	4.9	30.8	12.0	37.4	10.4	38.1	2.3	30.3	2.1	32.8	4.1
4-Nitro-Sulfamethoxazole	+	78.5	8.0	90.4	5.6	87.2	9.4	77.4	7.7	83.8	4.3	110.5	36.2	97.2	2.0	99.9	9.1	82.7	3.4	88.1	4.1
N4-Acetylsulfamethoxazole	-/+	97.9	12.0	96.9	2.1	98.8	5.4	82.9	0.5	105.2	4.7	119.7	39.6	104.8	19.0	104.9	13.9	65.8	3.4	87.5	4.0
Sulfanilamide	-	ND	ND	ND	ND	ND	ND	11.1	3.3	10.5	2.3	75.1	17.4	40.3	15.4	28.9	24.3	10.1	1.9	12.4	1.1
Sulfanilic acid	-	106.3	4.3	83.3	5.6	66.8	2.4	39.8	24.1	11.4	6.6	99.4	4.0	78.2	5.4	63.4	1.6	33.1	3.3	9.1	1.9
Valsartan	+/-	74.3	14.1	94.0	16.6	82.1	3.8	97.7	4.1	83.7	16.4	90.3	7.8	95.5	25.4	100.2	8.4	78.8	0.1	74.7	17.6
Valsartan acid	-/+	105.9	23.4	90.2	13.3	91.9	27.1	68.6	4.4	73.4	23.4	90.8	14.0	101.9	17.8	95.1	9.2	64.5	4.8	68.6	21.1
Verapamil	+	40.8	11.2	88.8	3.9	63.0	19.8	111.3	24.3	152.0	22.2	65.8	2.9	72.2	12.2	94.4	15.0	102.8	9.3	98.8	23.9

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Table 9SM. Relative recoveries and intraday precision (RSD%) at 5 concentrations (2, 5, 10, 50, 200 µg L⁻¹) with MRM and SWATH acquisition modes for soil matrix

compound	ESI	MRM acquisition mode										SWATH acquisition mode										
		2 µg L ⁻¹	RSD (%)	5 µg L ⁻¹	RSD (%)	10 µg L ⁻¹	RSD (%)	50 µg L ⁻¹	RSD (%)	200 µg L ⁻¹	RSD (%)	2 µg L ⁻¹	RSD (%)	5 µg L ⁻¹	RSD (%)	10 µg L ⁻¹	RSD (%)	50 µg L ⁻¹	RSD (%)	200 µg L ⁻¹	RSD (%)	
Acesulfame	-	77.9	11.0	76.3	3.9	77.6	3.9	57.5	4.6	71.7	4.2	82.1	18.6	78.6	10.2	73.9	6.4	73.8	5.0	80.0	4.6	
Acetaminophen	-	113.2	52.6	73.5	14.9	99.9	7.1	75.4	5.0	75.4	3.5	92.1	8.1	95.9	1.7	95.0	9.4	78.2	2.3	83.0	3.3	
Acridone	+	68.9	5.7	83.0	12.7	55.1	2.5	63.1	6.5	81.9	4.9	93.2	13.2	86.0	8.8	75.5	5.9	73.9	3.9	82.5	3.8	
Benzotriazole	+	56.1	10.0	63.7	17.8	59.7	15.2	56.3	4.7	63.6	5.0	76.9	13.7	10.7	3.1	90.3	12.7	72.5	4.3	85.0	21.5	
5-Methyl-2H-Benzotriazole	+/-	68.4	7.2	66.2	3.5	52.6	5.6	54.8	4.7	70.0	1.4	50.7	7.1	95.2	7.1	77.4	16.1	64.5	6.0	66.5	15.4	
Bezafibrate	-	87.7	6.0	97.1	6.0	101.4	5.1	83.1	2.6	96.5	2.3	85.4	15.1	112.7	5.9	96.2	5.4	82.0	5.3	93.1	3.0	
Bisphenol A	-	95.0	27.2	97.7	2.1	104.0	9.1	82.5	3.6	89.0	13.1	87.8	7.1	100.5	3.5	96.6	6.2	82.2	0.8	87.3	0.7	
Caffeine	+	115.5	7.6	95.0	5.7	83.1	16.1	83.8	7.2	69.8	3.8	87.9	7.5	89.0	5.8	76.9	3.7	72.4	3.6	74.0	5.5	
Carbamazepine	+	76.8	7.3	90.8	4.7	72.1	6.3	93.3	2.1	92.9	1.2	92.6	1.8	98.0	4.8	94.5	5.4	85.0	4.1	92.8	1.2	
Carbamazepine-10,11-epoxide	+	76.9	11.1	93.7	5.6	75.3	1.8	84.0	6.5	91.8	9.5	85.5	1.2	65.0	11.5	94.7	14.2	64.8	17.9	86.9	21.1	
Chloramphenicol	-	90.8	4.6	97.3	14.9	91.2	11.3	81.6	6.3	90.6	2.4	88.4	2.5	97.8	6.8	96.8	2.8	87.0	3.5	91.7	0.5	
Ciprofloxacin	+	3.2	2.2	1.6	0.5	0.8	0.2	0.3	0.1	0.4	0.1	34.7	20.1	40.7	2.4	10.1	7.7	16.6	2.4	11.9	7.5	
Citalopram	+	65.7	15.5	75.2	3.7	52.8	7.4	74.1	5.5	68.3	2.0	73.5	7.3	84.5	3.9	103.3	17.3	88.5	4.9	92.3	14.4	
Clarithromycin	+	86.9	8.5	93.3	8.7	72.5	5.7	81.6	3.6	86.9	1.8	57.8	6.7	83.7	6.4	80.3	14.5	76.5	8.6	94.9	8.8	
Climbazole	-	79.3	14.7	85.2	8.1	88.0	12.0	90.0	4.0	87.2	10.5	68.8	5.4	82.0	7.9	89.8	8.0	91.1	9.6	89.2	12.2	
Clofibrac acid	-	90.0	21.9	106.2	8.7	93.1	4.3	78.6	7.3	86.0	2.9	82.9	1.5	102.8	2.1	99.0	5.3	86.9	7.4	89.2	5.1	
Diclofenac	+/-	87.7	8.3	79.8	2.5	65.4	5.4	81.3	12.6	89.3	6.5	56.1	12.1	54.7	16.2	102.0	16.5	93.4	11.9	91.4	8.9	
4-Hydroxydiclofenac	+	33.5	20.7	26.4	5.1	24.9	4.0	27.8	1.7	31.8	4.7	66.9	69.3	99.1	45.8	85.4	25.0	91.6	10.1	92.2	5.7	
Diltiazem	+	66.3	10.0	81.9	1.1	60.3	0.9	75.5	2.0	85.6	3.8	64.7	3.9	79.6	5.3	89.1	12.6	82.2	2.1	90.0	14.1	
Fenofibrate	+	129.1	75.6	99.0	20.5	80.5	49.2	91.3	5.7	80.3	6.1	79.3	25.8	97.7	15.4	91.1	18.4	91.6	14.1	104.9	15.0	
Fipronil	-	92.2	6.0	105.7	0.7	103.3	5.7	88.4	3.0	92.9	0.9	90.1	2.2	106.3	3.2	98.0	6.5	87.6	1.7	95.4	1.2	
Fipronil desulfinyl	-																					
Fipronil sulfone	-																					
Fluconazole	+	90.7	7.8	92.7	5.6	66.8	6.5	78.3	6.8	88.1	3.3	84.0	9.3	97.0	1.7	86.4	1.9	75.2	6.9	78.4	9.1	
Furosemide	-	94.2	18.0	105.5	12.0	99.5	12.3	79.2	4.8	96.8	4.6	112.6	61.6	97.4	14.7	98.4	18.2	77.8	7.2	99.7	2.0	
Gemfibrozil	-	104.8	8.5	100.5	9.7	102.6	11.9	85.1	0.3	96.2	1.5	79.1	6.3	114.4	23.2	84.0	16.0	88.2	4.0	90.2	3.8	
Hydrochlorothiazide	-	92.9	6.7	118.2	13.4	86.5	11.1	84.2	6.4	87.9	4.9	91.0	7.4	94.9	1.0	94.6	11.5	85.3	7.1	92.7	3.5	
Ibuprofen	-	68.4	39.7	102.5	62.7	68.7	4.7	65.5	2.2	89.6	3.7	93.5	53.0	53.4	18.0	62.9	36.7	84.8	14.4	88.7	3.9	
Indomethacin	-	140.7	18.1	110.6	21.8	79.6	48.0	92.7	6.5	94.3	16.1											
Irbesartan	+	72.2	3.3	79.0	5.5	68.7	7.3	83.2	2.6	88.8	3.9	99.7	38.2	101.0	24.8	111.0	18.4	90.6	4.9	93.0	16.0	
Lamotrigine	+	53.4	3.3	57.7	1.7	50.3	1.7	59.9	1.0	63.4	2.3	64.3	20.5	74.8	13.1	64.5	2.3	64.1	5.5	71.0	10.9	

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Lamotrigine N2-oxide	+	68.1	21.2	86.0	49.7	67.8	4.6	81.3	2.1	86.3	2.5	38.5	23.5	76.1	9.0	66.9	4.0	83.7	9.0	92.0	9.7
5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	+	61.4	30.7	85.2	17.0	61.9	6.1	78.1	16.4	78.4	5.7	77.4	18.9	85.5	4.8	75.5	5.8	68.7	0.3	74.4	1.7
N2-Methyl-Lamotrigine	+	58.7	3.8	82.4	6.6	53.2	4.1	73.2	2.2	71.4	3.8	116.1	67.2	80.8	11.3	98.5	22.2	77.0	5.0	91.3	8.3
Metoprolol	+	63.0	13.8	85.5	5.9	67.9	11.6	74.4	5.4	87.9	9.4	104.0	10.7	96.9	9.6	86.4	5.4	77.9	2.5	84.0	5.3
Metrolidinazole	+	53.8	12.6	89.4	13.0	65.2	12.7	76.9	3.9	84.6	6.2	92.3	12.5	101.7	9.1	84.4	3.3	69.9	3.9	82.8	1.0
Oxcarbazepine	+	4.5	2.6	4.1	1.7	6.6	2.1	4.0	0.4	5.2	0.8	80.2	12.9	66.9	10.7	94.8	14.9	65.6	18.1	84.9	24.6
Propranolol	+	73.5	14.3	68.4	11.0	56.2	7.6	62.1	3.9	66.3	2.5	195.0	29.7	163.1	55.9	79.7	19.5	85.5	13.6	88.7	18.9
Sucralose	+/-	77.8	25.0	69.7	42.4	79.9	19.7	70.3	2.0	84.8	5.1	95.6	3.9	97.2	4.5	90.6	1.7	75.1	3.1	80.6	1.4
Sulfamethazine	+	23.1	4.6	42.5	4.1	36.1	1.6	29.5	3.5	37.7	3.6	58.9	5.0	32.8	21.3	32.1	12.7	22.7	2.4	18.4	3.4
Sulfamethoxazole	+/-	54.6	7.9	63.4	6.8	45.8	6.7	49.0	2.0	56.2	3.5	34.6	24.8	38.7	10.2	35.8	0.5	34.6	3.0	32.8	4.2
4-Nitro-Sulfamethoxazole N4-	+	79.2	3.9	84.5	7.5	77.4	5.3	87.3	2.1	86.6	1.1	87.7	14.3	108.1	4.3	95.8	2.7	81.6	4.9	88.0	2.9
Acetylsulfamethoxazole	+	56.8	14.8	66.9	39.5	71.4	6.8	71.9	2.7	91.7	1.8	134.7	42.2	111.7	14.4	91.6	0.9	84.2	4.4	89.3	2.8
Sulfanilamide	-	48.7	21.9	27.8	16.3	16.7	8.8	11.6	6.2	13.8	3.6	60.3	37.3	32.5	10.4	18.9	7.4	8.7	4.4	10.8	1.4
Sulfanilic acid	-	85.0	6.0	79.9	8.3	64.0	5.5	35.0	7.4	9.8	1.5	95.5	12.3	73.7	2.2	58.9	4.5	33.9	5.8	9.8	2.6
Valsartan	+	98.4	8.2	88.9	6.7	79.2	4.2	79.9	2.5	86.2	9.4	131.0	30.8	77.3	27.8	64.0	7.0	84.3	8.6	74.8	16.9
Valsartan acid	+	43.3	23.2	74.6	12.9	70.4	7.9	71.6	8.1	82.4	5.5	77.5	18.9	79.7	4.3	86.8	8.3	75.6	4.7	67.8	20.3
Verapamil	+	78.3	25.3	84.1	2.0	72.6	12.9	76.5	2.6	73.8	6.5	44.6	50.8	79.7	7.1	99.6	51.3	95.4	12.0	99.5	26.8

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Table 10SM. Linearity, MDL and MQL with MRM and SWATH acquisition modes for lettuce root matrix

Compound	ESI	MRM acquisition mode			SWATH acquisition mode				
		Linearity (ng g ⁻¹)	r ²	MDL (ng g ⁻¹)	MQL (ng g ⁻¹)	Linearity (ng g ⁻¹)	r ²	MDL (ng g ⁻¹)	MQL (ng g ⁻¹)
Acesulfame	-	2.5 - 3000	0.99653	0.04	0.12	1 - 2000	0.99747	0.07	0.22
Acetaminophen	-	5 - 3000	0.99512	0.03	0.09	10 - 2000	0.99457	0.09	0.29
Acridone	+	2.5 - 3000	0.99604	0.05	0.16				
Benzotriazole	-/+	50 - 2000	0.99035	0.12	0.35				
5-Methyl-2H-Benzotriazole	+	10 - 2000	0.99848	0.11	0.32				
Bezafibrate	-	2.5 - 3000	0.99624	0.09	0.28	5 - 1000	0.99640	0.10	0.30
Bisphenol A	-	2.5 - 3000	0.99320	0.17	0.52	0.5 - 2000	0.99457	0.05	0.15
Caffeine	+	5 - 3000	0.99812	0.10	0.29				
Carbamazepine	+	2.5 - 1000	0.99763	0.04	0.11				
Carbamazepine-10,11-epoxide	+	2.5 - 2000	0.99968	0.15	0.45				
Chloramphenicol	-	1 - 2000	0.99211	0.09	0.27	2.5 - 2000	0.99534	0.07	0.20
Ciprofloxacin	+	50 - 2000	0.99347	0.14	0.41				
Citalopram	+	5 - 3000	0.99691	0.04	0.11				
Clarithromycin	+	2.5 - 3000	0.99605	0.09	0.27				
Climbazole	-	1 - 3000	0.99509	0.12	0.36				
Clofibrac acid	-	5 - 3000	0.99290	0.08	0.25	50 - 3000	0.99381	0.19	0.58
Diclofenac	+	50 - 2000	0.99405	0.14	0.42				
4-Hydroxydiclofenac	+	50 - 2000	0.99720	0.30	0.92				
Diltiazem	+	2.5 - 2000	0.99767	0.13	0.38				
Fenofibrate	+	10 - 2000	0.99964	0.05	0.14				
Fipronil	-	0.5 - 100	0.98953	0.02	0.06	0.5 - 100	0.99618	0.004	0.01
Fipronil desulfinyl	-	0.5 - 100	0.98276	0.01	0.03	0.5 - 100	0.99128	0.01	0.03
Fipronil sulfone	-	1 - 100	0.98235	0.01	0.04	0.5 - 100	0.98777	0.02	0.05
Fluconazole	+	2.5 - 3000	0.99368	0.03	0.10				
Furosemide	-	5 - 3000	0.98989	0.14	0.41	2.5 - 2000	0.99982	0.06	0.17
Gemfibrozil	-	5 - 1000	0.95746	0.16	0.48	1 - 3000	0.98928	0.05	0.15
Hydrochlorothiazide	-	2.5 - 100	0.99254	0.03	0.10	10 - 3000	0.99008	0.23	0.70
Ibuprofen	-	10 - 3000	0.99734	0.1	0.30	ND	ND	ND	ND
Indomethacin	-	2.5 - 3000	0.99330	0.10	0.30	50 - 3000	0.99128	0.06	0.18
Irbesartan	-/+	10 - 1000	0.96436	0.21	0.64				
Lamotrigine	+	1 - 2000	0.99874	0.01	0.04				
Lamotrigine N2-oxide	+	10 - 2000	0.99448	0.09	0.26				
5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	+	10 - 1000	0.99803	0.12	0.38				

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N2-Methyl-Lamotrigine	+	5 - 3000	0.99813	0.05	0.16				
Metoprolol	+	5 - 3000	0.99622	0.08	0.23				
Metrodinazole	+	5 - 3000	0.99621	0.03	0.09				
Oxcarbazepine	+	2.5 - 2000	0.99947	0.05	0.14				
Propranolol	+	2.5 - 2000	0.99784	0.06	0.19				
Sucralose	-	50 - 3000	0.96886	0.23	0.68	50 - 3000	0.99981	0.24	0.72
Sulfamethazine	+	50 - 2000	0.99910	0.12	0.37				
Sulfamethoxazole	-	50 - 3000	0.99376	0.08	0.23	50 - 3000	0.99859	0.27	0.82
4-Nitro-Sulfamethoxazole	+	10 - 2000	0.99874	0.14	0.43				
N4-Acetylsulfamethoxazole	-/+	1 - 3000	0.99754	0.07	0.20				
Sulfanilamide	-	50 - 2000	0.99908	0.09	0.27	10 - 2000	0.99136	0.07	0.20
Sulfanilic acid	-	2.5 - 3000	0.99680	0.16	0.48	500 - 3000	0.99985	0.77	2.33
Valsartan	+/-	50 - 3000	0.99364	0.27	0.83	50 - 3000	0.99353	0.19	0.58
Valsartan acid	-/+	2.5 - 3000	0.99860	0.12	0.37				
Verapamil	+	50 - 3000	0.99509	0.08	0.25				

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Table 11SM. Linearity, MDL and MQL with MRM and SWATH acquisition modes for soil matrix

Compound	ESI	MRM acquisition mode			SWATH acquisition mode				
		Linearity (ng g ⁻¹)	r ²	MDL (ng g ⁻¹)	MQL (ng g ⁻¹)	Linearity (ng g ⁻¹)	r ²	MDL (ng g ⁻¹)	MQL (ng g ⁻¹)
Acesulfame	-	0.1 - 300	0.99615	0.003	0.01	0.1 - 300	0.99804	0.002	0.01
Acetaminophen	-	0.25 - 100	0.99497	0.003	0.01	0.25 - 300	0.99732	0.01	0.02
Acridone	+	0.25 - 300	0.99849	0.11	0.33				
Benzotriazole	+	0.25 - 300	0.99406	0.14	0.44				
5-Methyl-2H-Benzotriazole	+/-	0.1 - 100	0.99546	0.03	0.10	0.25 - 300	0.99744	0.01	0.02
Bezafibrate	-	0.1 - 100	0.99619	0.01	0.02	0.05 - 200	0.99437	0.005	0.01
Bisphenol A	-	1 - 300	0.99057	0.02	0.06	0.05 - 100	0.99816	0.01	0.04
Caffeine	+	10 - 300	0.98766	0.11	0.34				
Carbamazepine	+	0.1 - 100	0.99614	0.01	0.03				
Carbamazepine-10,11-epoxide	+	0.1 - 300	0.99850	0.01	0.03				
Chloramphenicol	-	0.5 - 300	0.99112	0.02	0.05	0.05 - 50	0.99723	0.002	0.01
Ciprofloxacin	+	0.25 - 100	0.98827	0.01	0.03				
Citalopram	+	0.1 - 200	0.99406	0.07	0.21				
Clarithromycin	+	0.05 - 200	0.99262	0.17	0.53				
Climbazole	-	0.5 - 300	0.99718	0.01	0.03	0.5 - 100	0.99554	0.02	0.05
Clofibrac acid	-	0.5 - 300	0.99741	0.01	0.03	0.1 - 300	0.99829	0.01	0.03
Diclofenac	+/-	0.1 - 300	0.99840	0.05	0.16	5 - 300	0.99937	0.03	0.08
4-Hydroxydiclofenac	+	0.5 - 200	0.99595	0.02	0.06				
Diltiazem	+	0.05 - 100	0.99907	0.01	0.03				
Fenofibrate	+	0.25 - 200	0.99746	0.003	0.01				
Fipronil	-	0.05 - 10	0.99671	0.002	0.01	0.05 - 10	0.99034	0.002	0.01
Fipronil desulfinyl	-	0.05 - 10	0.99702	0.002	0.01	0.05 - 10	0.99487	0.001	0.003
Fipronil sulfone	-	0.05 - 10	0.99427	0.001	0.004	0.05 - 10	0.99198	0.002	0.005
Fluconazole	+	0.25 - 200	0.99659	0.01	0.03				
Furosemide	-	0.5 - 300	0.99531	0.01	0.04	1 - 300	0.99496	0.02	0.06
Gemfibrozil	-	0.05 - 100	0.99812	0.01	0.02	0.5 - 100	0.99461	0.01	0.02
Hydrochlorothiazide	-	0.1 - 100	0.99713	0.002	0.01	0.1 - 100	0.99339	0.01	0.02
Ibuprofen	-	0.25 - 300	0.99751	0.01	0.03				
Indomethacine	-	0.5 - 300	0.99753	0.01	0.02	5 - 300	0.99649	0.03	0.08
Irbesartan	+	0.25 - 100	0.99787	0.01	0.03				
Lamotrigine	+	0.05 - 100	0.99435	0.002	0.01				
Lamotrigine N2-oxide	+	0.25 - 200	0.99591	0.01	0.02				
5-Desamino 5-Oxo-2,5-dihydro-Lamotrigine	+	0.25 - 300	0.99878	0.13	0.40				

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N2-Methyl-Lamotrigine	+	0.05 - 200	0.99693	0.13	0.38				
Metoprolol	+	0.25 - 300	0.99875	0.003	0.01				
Metrodinazole	+	0.5 - 100	0.99397	0.11	0.33				
Oxcarbazepine	+	0.25 - 100	0.99346	0.01	0.02				
Propranolol	+	0.5 - 100	0.99745	0.02	0.06				
Sucralose	-	5 - 300	0.99976	0.01	0.05	5 - 300	0.99737	0.01	0.02
Sulfamethazine	+	0.25 - 200	0.99837	0.0001	0.0003				
Sulfamethoxazole	+/-	1 - 200	0.99351	0.01	0.03	1 - 300	0.99552	0.01	0.04
4-Nitro-Sulfamethoxazole	+	1 - 200	0.99715	0.02	0.07				
N4-Acetylsulfamethoxazole	+	5 - 300	0.99167	0.07	0.22				
Sulfanilamide	-	5 - 300	0.99135	0.01	0.03	1 - 300	0.99756	0.01	0.03
Sulfanilic acid	-	1 - 300	0.98147	0.005	0.01	0.25 - 300	0.97912	0.01	0.03
Valsartan	+	0.5 - 300	0.99717	0.01	0.03				
Valsartan acid	+	0.1 - 100	0.99690	0.04	0.12				
Verapamil	+	0.1 - 300	0.99814	0.01	0.03				

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Chapter 5:

Application of the previously developed methods
on realistic field agricultural samples

1. Impact of repeated irrigation of lettuce with treated municipal wastewater on pharmaceutical plant uptake and degradation under field cultivation conditions

1.1. Context

The ability of growing crops to uptake PhACs and wastewater-borne pollutants introduced by irrigation with treated wastewater have been largely reported to date. However, most of these studies are conducted at laboratory scales and under controlled conditions, hence a poor comprehensive assessment of the real risk posed by human consumption of treated wastewater irrigated crops. Besides, very limited data are available to date under real field conditions, and for a long term of wastewater irrigation practice. Since plant uptake is related to several factors including soil properties, physicochemical properties of contaminants and plant physiology, besides to contaminants fate/behavior in soil before they reach the plants, it is important today to fill the gaps of knowledge related to plant uptake under field scales, their fate/behavior in soil at long term, and their following risk to human consumption.

1.2. Objective and main results

The objective of this article was to follow the uptake of 14 selected PhACs and other wastewater-borne pollutants and their TPs by lettuce and leeks irrigated with different kind of water, including treated wastewater, under realistic agricultural growing and irrigation conditions over successive 2 years' crop cycles. Furthermore, we have reported the concentrations of the studied contaminants in soil and the edible part of the crops, allowing for bioconcentration factor calculations, and human health risk assessment. In this context, we have applied the analytical methods developed and previously reported in Chapter 3 and Chapter 4, to the obtained field agricultural samples. Results revealed a limited accumulation of these contaminants in soil and crops leaves, with concentration ranging from 1 to 30 ng/g in soil, and from 1 to 660 ng/g in leaves. This was explained by the intensive degradation of contaminants in soil and metabolism in plants. Carbamazepine-epoxide was detected at level 1 of confirmation in lettuce leaves at concentration 6.1 ± 0.7 ng/g, conversely it was not detected in soil, thus carbamazepine transformation was exclusively related to lettuce

metabolism. Furthermore, two metabolites of metoprolol were confirmed at level 2, another two and one for irbesartan were identified at level 4 of confirmation in lettuce leaves. Their estimated concentration suggests their presence at low concentrations, even lower than their parent compounds. On the whole, this study confirmed a *de minimis* human health risk related to the consumption of raw vegetable irrigated with treated wastewater.



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Impact of long-term irrigation with municipal reclaimed wastewater on the uptake and degradation of organic contaminants in lettuce and leek

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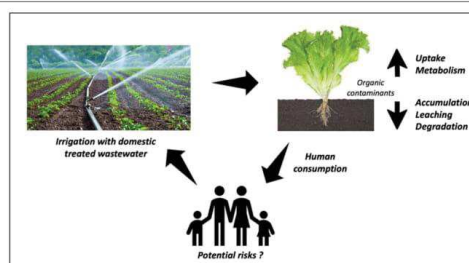
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HIGHLIGHTS

- Low accumulation of PPCPs in soil and in lettuce leave over successive crop cycles
- Clarithromycin was found a compound of concern for leaching and plant uptake in spiked water study.
- Enantiomer fractionation of climbazole was a good indicator of biodegradation in soil.
- SWATH® acquisition mode was reliable for TPs identification at low concentrations.
- *A de minimis* human health risks related to consumption of raw leafy green vegetable.

GRAPHICAL ABSTRACT



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ABSTRACT

A two years drip irrigation of lettuce and leek crops with treated municipal wastewater without and with spiking with fourteen wastewater relevant contaminants at 10 µg/L concentration level was conducted under greenhouse cultivation conditions to investigate their potential accumulation in soil and leaves and to assess human health related risks. Lettuce and leek crops were selected as a worse-case scenario since leafy green vegetable has a high potential for organic contaminants uptake. The results revealed limited accumulation of contaminants in soil and plant leaves, their concentration levels being in the range of 1–30 ng/g and 1–660 ng/g range in soil and leaves, respectively. This was likely related to abiotic and biotic transformation or simply binding processes in soil, which limited contaminants plant uptake. This assumption was underpinned by studies of the enantiomeric fractionation of chiral compounds (e.g. climbazole and metoprolol) in soil as pieces of evidence of biodegradation and by the identification of transformation products or metabolites in leaves by means of liquid chromatography - high resolution - mass spectrometry using a suspect screening workflow. The high bioconcentration factors were not limited to compounds with intermediate D_{ow} (100 to 1000) such as carbamazepine but also observed for hydrophilic compounds such as clarithromycin, hydrochlorothiazide and the food additives acesulfame and sucralose. This result assumed that accumulation was not only driven by passive processes (e.g. lipoidal diffusion through lipid bilayer cell membranes or Casparian strip) but might be supported by carrier-mediated transporters. As a whole, this study confirmed earlier reports on the *a de minimis* human

Abbreviations: TWW, treated wastewater; PPCPs, pharmaceuticals and personal care products; RWW, raw wastewater; TPs, transformation products; DMSO, dimethyl sulfoxide; TW, tap water; WWTP, wastewater treatment plant; OM, organic matter; STWW, spiked treated wastewater; BCF, bioconcentration factor; HQ, hazard quotient; EDI, estimated daily intake; ADI, acceptable daily intake; DOM, dissolved organic matter; LODs, limits of detection.

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health risk related to the consumption of raw leafy green vegetable irrigated with domestic TWW containing organic contaminants residues.

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1. Introduction

In arid and semi-arid agricultural area, in both developed and developing countries, irrigation of crops with treated wastewater (TWW) is already a common practice as a result of water scarcity due to climate change as well as to continuously growing population (Carter et al., 2019; Ait-Mouheeb et al., 2018). A large body of literature has demonstrated that crop plants can accumulate organic contaminants and more specifically pharmaceuticals and personal care products (PPCPs) (Fu et al., 2019). However, studies have been carried out by using different plant-growth methods (e.g., hydroponic cultivation, crops in pots under greenhouse, field cultivation conditions) with different sets of contaminants, different types of plants and different irrigation methods resulting to some inconsistency in conclusions. Limited data is actually available under real field growing conditions of crops using appropriate irrigation regimes (Goldstein et al., 2014; Riemenschneider et al., 2016; Wu et al., 2014; Christou et al., 2017; Picó et al., 2019), besides most of the studies did not focus on long term introduction of contaminants in soil-plant system through irrigation, neither their fate/behavior in soil which can affect their fate and uptake in plants at the same time. Therefore, it is crucial to conduct such experiments following the risk introduced by wastewater irrigation at long-term field conditions, taking in consideration contaminants fate in the whole soil-plant system. PPCPs concentration levels, which have been found in edible parts of food crops are in the ng/g to low µg/g range, hence revealing the potential for human exposure to PPCPs as a result of irrigation with TWW (Gonzalez Garcia et al., 2019). The European Commission has proposed in May 2018 new guidelines to facilitate water reuse in the EU for agricultural irrigation (EC European Commission, 2018) which are mainly focused on water physico-chemical constituents and pathogen indicators. However, the problem of organic contaminants has been mentioned in annexes, calling for more research in this field before including their levels as stricter requirements for water quality, if needed. Soil properties, water quality, the nature of the PPCPs and their physico-chemical properties as well as plant physiology (i.e. type of crops) have been considered major governing factors in determining plant uptake (Wu et al., 2015). Generally, plant uptake of neutrally charged PPCPs is greater than ionic compounds, because anionic PPCPs are repelled by cell membranes with negative electrical potential, and cationic species are attracted to the cell membranes thus limiting their movement into plants (Chuang et al., 2019). In addition, experimental results revealed that the potential for PPCPs uptake by crop plants decreased in the order of leafy green vegetables > root vegetables > cereals and fodder crops > fruit vegetables (Christou et al., 2019). Water flow is believed to be the primary carrier for uptake and transport of PPCPs in plants and transpiration may be a strong predictor for the accumulation of structurally diverse PPCPs in above-ground plant tissues (Nason et al., 2019). Others studies have shown the relevance of the PPCPs concentrations in soil pore water and consequently their bio-availability to plant uptake (Li et al., 2019). In contrast, the role of the behavior/fate of PPCPs in soil under field cultivation conditions has been poorly considered because research efforts have typically focused on short-term TWW irrigation scenarios, when plants were exposed for only short-time periods to PPCPs. The role of soil as a regulator and/or attenuator of the amount of PPCPs available for plant uptake due to sorption and degradation processes has probably been underestimated. One of the research need is therefore a better understanding of the behavior and fate of PPCPs in soil following chronic or long terms irrigation of TWW in agro-food systems to investigate the relevance of PPCPs exposure time on the plant uptake processes and on the subsequent

effects on PPCPs degradation. The reduction of PPCPs uptake by plants is generally related to sorption processes to soil, especially for those chemicals with strong hydrophobicity or positive charge but also to transformation processes such as photolysis at the soil-surface and biodegradation (Fu et al., 2019). Plant uptake might be therefore reduced, if attenuation processes outbalance the continuous input of chemicals. In addition, there is the potential for agricultural soil to develop accelerated biodegradation due to microorganisms adaptation processes, accounting for the non-accumulation of drugs such macrolide antibiotics over multiple crop cycles (Topp et al., 2016). Another research gap might be the role of active transport carriers in PPCPs plant uptake. Most current literature quantifying PPCPs accumulation in crop plants focuses on passive processes such as diffusion across membranes, ion trapping, and sorption. However, some results suggest that in some cases plant transporter proteins may be important for accumulation of certain PPCPs, as has been assumed for metformin (Eggen and Lillo, 2016) and amitriptyline (Nason et al., 2019). Understanding the biological aspects of plant accumulation of PPCPs is important not only in environmental risk assessment but also from a remediation perspective (e.g. removal in engineered treatment wetlands). Consequently, the major aim of this work was to contribute to fill these scientific gaps by investigating the behavior of selected organic contaminants (mainly PPCPs) in the wastewater-soil-plant system for the specific conditions of greenhouse-grown of lettuce and leek irrigated with different kinds of wastewater including raw municipal wastewater (RWW), lagoon-based secondary treated wastewater (TWVW) and spiked TWW with 14 pollutants under realistic agricultural scenarios over multiple crop cycles. Specific objectives were i) to investigate the impact of the fate of targeted PPCPs in soil on plant uptake ii) to understand the main driving processes of their uptake in plant, iii) to investigate the fate of selected PPCPs in lettuce leaves to evaluate implications of irrigation with TWW containing these PPCPs for food safety and human exposure via dietary intake.

2. Material and methods

2.1. Chemicals

Fourteen organic contaminants that were commonly detected in TWW were selected in this study including pharmaceuticals (ciprofloxacin (CIP), sulfamethoxazole (SMX), citalopram (CTP), diclofenac (DCF), valsartan (VAL), irbesartan (IRB), carbamazepine (CBZ), metoprolol (MTP), hydrochlorothiazide (HCT), clarithromycin (CLT)), food additives (acesulfame (ASF), sucralose (SUC)), the fungicide climbazole (CLB, human medicine) and the corrosion inhibitor 1H-benzotriazole (BNZ). The transformation products (TPs), valsartan acid (VAL-AC), oxcarbazepine (Ox-CBZ) and carbamazepine-10, 11-epoxide (CBZ-EPX) were also investigated as they were also frequently detected in investigated TWW. Reference standards for all compounds and their TPs were obtained from Sigma-Aldrich (S¹ Quentin-Fallavier, France) and were of high purity (>95%). Deuterated compounds including climbazole-d₄, valsartan-d₃, irbesartan-d₆, ciprofloxacin-d₈, citalopram-d₆, sulfamethoxazole-d₄, carbamazepine-d₁₀, benzotriazole-d₄, metoprolol-d₇ were used as internal standards. The above-mentioned standards were prepared individually in 100% acetonitrile, 100% HPLC water, 100% methanol or 100% DMSO according to compounds solubility and stored at -20 °C. Working solutions were prepared in serial dilution in methanol by mixing all compounds and stored at -20 °C. All LC-MS grade organic solvents and HPLC water were of purity higher than 99.9% and purchased from Merck (Darmstadt, Germany). Formic acid (≥96%, ACS reagent) and ammonium acetate

were supplied by Sigma-Aldrich while ammonium fluoride was bought from Fisher Chemical (Fisher Scientific SL, Madrid, Spain). For EDTA-McIlvaine buffer preparation, di-sodium hydrogen phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) was obtained from Merck, citric acid monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$) and ethylenediaminetetraacetic acid anhydrous (EDTA) ($\geq 99\%$) from Sigma-Aldrich.

EDTA-McIlvaine buffer preparation. 1.5 g of di-sodium hydrogen phosphate dihydrate, 1.3 g of citric acid monohydrate, and 0.372 g EDTA were dissolved in 100 mL HPLC water.

Two QuEChERS salts, BEKOLUT SALT-KIT-AC (4 g $\text{MgSO}_4 + 1$ g NaCl) known as original salt and QuEChERS Extract pouches (4 g $\text{MgSO}_4 + 1$ g NaCl + 0.5 g di-sodium citrate sesquihydrate) known as European salts (CEN) were used for compounds extraction from soil and leaf samples. The dispersive solid phase extraction salts BEKOLUTPSA-Kit-04 (900 mg $\text{MgSO}_4 + 150$ mg PSA + 150 mg C18e) were used for extracts clean-up. All the above-mentioned salts were obtained from BEKOLUT (Hauptstuhl, Germany) except for the CEN salts, which were obtained from Agilent Technologies.

2.2. Plant-growth and exposure conditions

The experiments were conducted in a greenhouse located at the Murviel-Lès-Montpellier village (Hérault, France, 43.605034° N, 3.757292° E) where lettuces and leeks were grown in large tanks (1 m² in surface and 60 cm soil depth) and irrigated with tap water (TW, 1 replicate, 4 lettuces), raw domestic wastewater (RWW) originating from the village (1 replicate, 4 lettuces), TWW (2 replicates, 8 lettuces) and TWW spiked with a mixture of fourteen organic contaminants at 10 µg/L concentration level, each (3 replicates, 6 lettuces), as shown in Fig. 1 Supplementing Material (SM). Those experiments were possible thanks to an administrative authorization from the Health Agency of the Occitanie region. The WWTP operated on the basis of stabilization ponds with three successive lagoons (13,680, 4784 and 2700 m³, respectively) and had a nominal capacity of 1500 Inhabitant Equivalent. The plant-growth method in greenhouse was selected to avoid water supply from rainfall but in contrast, this cultivation shaped/accelerated the evaporation rate due to hot conditions and therefore accelerated water, nutrient and contaminants plant uptake. Lettuces were cultivated during 3 successive cultivation cycles of 6 weeks in 2018 from June to December. Cultivation was then stopped during January and February 2019 and resumed for two additional cycles between March and June 2019. Leek crop was conducted during two successive cultivation campaigns of 16 weeks, one in 2018 and one in 2019. The cultivation schedule for lettuces and leeks is illustrated in Fig. 2SM. Under the greenhouse, the mean temperature and relative humidity during all cultivation cycles were 27.2 ± 7.2 °C and 54 ± 21.7%, respectively.

2.2.1. Soil

Sandy silty clay soil with 50.3% sand (19.5% very fine sand, 16.4% fine sand and 14.4% coarse sand), 25.9% silt and 24% clay (see Table 3SM for others parameters) was obtained from a local field to fill up the tanks. This texture is representative of alluvial soils near waterways where WWTPs are very often located and where TWW are often reused in irrigation, even though this kind of soil with a rather high proportion in clay and OM (2.7%) was known to restrict the uptake of organic contaminants in comparison to sandy soils. The collected soil was characterized by a regular cation exchange capacity of 11.1 milliequivalents/100 g soil and pH near neutrality (i.e. 7.6 in KCl). The soil was tested before starting the experiments and was found to be free of investigated organic contaminants.

2.2.2. Selected crops

Baby lettuces (*Lactuca sativa* variety Batavia) and leeks (*Allium porrum*) were obtained from a local organic market. Four lettuces and 16 leeks were placed in different tanks (see Fig. 1SM). It was known

that plant species have different patterns of contaminants uptake. Lettuce growing corresponded to a worse-case scenario because leafy green vegetable constitutes the crop plants with the highest ability to uptake and accumulate PPCPs in their edible tissue in comparison to root vegetables (e.g. carrot) or fruit-bearing vegetables (e.g. tomato) (Christou et al., 2019). Leek was selected due to a long growing period of 16 weeks (6 weeks for lettuce) potentially allowing for PPCPs accumulation or conversely longer time for dissipation processes to operate. Further, lettuce and leek are consumed raw and human health risk is likely greater than for cooked vegetable. No fertilization was practiced during the experiments, neither pesticides application. Leaves were collected at mature stage after removing roots. As under field experiments, growing conditions were less controllable and reproducible, some heterogeneity in results was expected. To limit this issue, three lettuces and leeks from each tank were mixed after freeze-drying and extracted separately so the analytical results were presented as a mean of two replicates.

2.2.3. Irrigation regime

Surface drip irrigation system with polyethylene irrigation pipe (16 mm i.d.) was chosen for this study, making root uptake the only relevant uptake pathway and avoiding soil runoff. In addition, drip irrigation system is the most efficient and a common irrigation practice in all arid and semi-arid regions, as it complies with the existing guidelines for a safe reuse of TWW for irrigation. RWW was selected as a worst-case scenario of irrigation water, as it can be used in some low-income countries. Non-compensate drippers working at a flow rate of 2 L/h with an inlet pressure of 1 bar were placed along the pipe. An inlet disk filter with a mesh opening size of 0.13 mm was installed to reduce the physical clogging of emitters. The irrigation was optimized to limit contaminants transport into soil and avoiding the occurrence of any leaching events. Consequently, plants were irrigated in short intervals that were every day in summer and every week in fall and spring. Irrigation flow was 0.5 L/2d in spring and fall and 1 L/2d in summer.

2.3. Experiments with spiked TWW (STWW)

Spiking took place manually, by routinely spiking a freshly obtained large volume of TWW with a mix solution of all studied 14 compounds. In real agricultural practices, TWW storage is actually unavoidable. This was the reason why a large volume (20 to 40 L) was spiked with selected contaminants in a tank. Such a large volume fitted to 8–15 d irrigation volume need according to the season. STWW was only used for lettuce irrigation.

2.4. Analytical procedures

Chemicals residues in soil as well as in leek and lettuce leaf samples were analyzed following the validated analytical methods described in Montemurro et al. (2020). Briefly, after harvest, leaf samples were directly transported to the analytical laboratory of Montpellier University where they were frozen, lyophilized, grounded to fine powder and stored at -40 °C until analysis. Rhizospheric soil samples and soil samples at the bottom of the tanks were collected at the end of some campaigns of the two years study. They were homogenized by a mortar and sieved at 2 mm, left under hood at room temperature for 2 or 3 d to ensure their total dryness and finally stored at -40 °C until analysis. 1 g of lyophilized lettuce and leek leaves and 10 g soil samples were extracted using QuEChERS extraction methods after spiking with a proper volume of deuterated mix. Soil, leek and lettuce leaves extracts were analyzed using the SCIEX ExionLC™ AD system coupled with the SCIEX X500R QTOF system (Sciex, Redwood city, CA, U.S.) with Turbo V™ source and Electrospray Ionization (ESI) operating in positive and negative mode. Target and non-target acquisition screening were used by employing the Multiple Reaction Monitoring (MRM^{HR}) acquisition mode and the SWATH® acquisition mode, respectively. Shifts in mass

accuracy were corrected by infusion of reserpine reference standard ($C_{33}H_{40}N_2O_9$, m/z 609.28066) for positive ionization, and a cluster of trifluoroacetic acid ($5(TFA-Na)^+ TFA^-$, m/z 792.85963) for negative mode. Calibrant Delivery System (CDS) was employed for mass calibration every 5 samples during the batch injection. Both acquisition modes were employed over a m/z range from 100 to 950 Da. MRM^{HR} acquisition was employed to quantify the target compounds, by following the precursor ion and one selected fragment ion chosen according to its highest intensity obtained on a specific collision energy (CE) and declustering potentials (DP). For MRM, MDLs and MQLs ranged from 0.01 to 0.12 ng/g and 0.04 to 0.38 ng/g, respectively. SWATH@ acquisition mode was employed to identify potential TPs that might be formed in lettuce leaves during the experiments, for which analytical standards were not available. SWATH@ acquisition mode allows to detect and fragment every compound in the sample in one single run, without the risk of missing a relevant analyte. Therefore, every compound in the sample has a complete MS and MS/MS spectrum, eliminating the need for re-analysis. More details on these analytical methods are made available in SM.

Chiral analysis of metoprolol and climbazole were carried out by LC-HRMS following the analytical procedures already published in Souchier et al. (2016) and Brienza and Chiron (2017), respectively. Briefly, enantiomers of metoprolol were separated using a ASTEC vancomycin-based analytical column (Chirobiotic V) using a reverse phase isocratic mode of elution with a mobile phase consisting of water +30 mM ammonium acetate/methanol, 10/90 (v/v). Enantiomers of CBZ were separated using a Phenomenex Lux Amylose-2 analytical column only using water/acetonitrile (35/65, v/v) as mobile phase in an isocratic mode of elution. LODs down to 10 ng/g were obtained with both analytical methods, which made them suitable for the analysis of metoprolol and climbazole in soil irrigated with STWW.

2.5. Calculation of BioConcentration Factor (BCF) and Hazard Quotient (HQ)

The BioConcentration Factor (BCF) of the studied compounds in lettuce was calculated in order to estimate the ability of lettuce crop to uptake organic contaminants from soil irrigated with contaminated water (Bianco et al., 2013). Thus, BCF was calculated for each compound detected in lettuce irrigated with the several types of water used in this experiment (TWW, RWW and STWW), as the ratio of the concentration in lettuce over the concentration in soil (dry weight):

$$BCF = \text{concentration in lettuce (ng/g)} / \text{concentration in soil (ng/g)}$$

The consumption of contaminated lettuce constitutes one pathway of human exposure. The HQ was calculated as the ratio of estimated daily intake (EDI) of PPCPs and the acceptable daily intake (ADI) which is the amount of contaminants that can be consumed daily over a person's lifespan without evocating an adverse effect. ADI values of the targeted pharmaceuticals were calculated by dividing the lowest therapeutic dose (mg/d) by a safety factor of 1000 and a body weight of 70 kg while EDI was calculated using the greatest concentration in lettuce leaves among the different lettuce crop seasons (worse case) following the equation proposed Prosser and Sibley (2015).

$$EDI = \frac{C_{\text{lettuce}} \times IR_{\text{veg}} \times \beta_{\text{g/cup}} \times \beta_{\text{dw/ww}}}{m}$$

C lettuce represents the concentration of contaminants in lettuce leaves (ng/d dry weight), IR veg represents 2.8 cup equivalents of lettuce per day, $\beta_{\text{g/cup}}$ represents the mass of a cup of fresh lettuce tissue, which is equal to 218.6 g of wet weight per cup (Prosser and Sibley, 2015), $\beta_{\text{dw/ww}}$ is the wet to dry weight conversion factor for lettuce which is equal to 0.052, and m is the mass of the consumer, which is considered 70 kg for adult (20–65 years of age) (USEPA, 1996).

3. Results and discussion

3.1. Occurrence and accumulation patterns of contaminants (mainly PPCPs) in soil and leaves

Only a few studies have reported the uptake and accumulation of organic contaminants by crop plants irrigated with TWW under realistic agricultural growing and irrigation conditions. Even fewer studies have reported their concentration values both in soil and in the edible part of the crop allowing for BCF calculation. In spite of poor controllable and reproducible conditions, field studies were implemented because they enabled an appropriate risk assessment of wastewater-borne contaminants to consumers as they integrated irrigation, soil and plant processes.

The average values of physico-chemical properties of the TWW are presented in Table 1SM and complied with irrigation water quality criteria as established by FAO's guidelines (Ayers and Westcot, 1985). This lagoon-based TWW was characterized by a high DOM content (>200 mg/L) which could prone interactions between DOM and ionizable PPCPs as well as by a high content of ammonium ions (>50 mg/L) which could lead to rhizosphere acidification due to excess cations plant uptake over anions uptake. This acidification process could result in loss of two pH units (Nason et al., 2018). This had strong implication for compound such as climbazole with a pKa of 7.5 because at pH 5.6, it would have been 100% ionized limiting its possible plant uptake while at pH 7.6 both neutral and cationic states of climbazole co-existed. Concentrations of investigated PPCPs were measured periodically in TWW. Highly variable levels were found and consequently, mean values are reported in Table 2SM. Irbesartan, benzotriazole and aceulfame were detected with relatively higher concentrations (>450 ng/L) than the others compounds, which were detected at concentration levels in the 5–200 ng/L range. Due to the low contaminants occurrence levels in small municipality effluents, it was decided to spike TWW with 14 compounds to avoid the risk of no detection in lettuce leaves. The spiking level was set to 10 $\mu\text{g/L}$ (each compound) which was a good compromise between the possibility to work at concentrations not so far from environmental concentrations and the potentiality to identify some TPs. The selection of the compounds was driven by the frequency of detection in TWW and to encompass a large range of different physico-chemical properties such as polarity (D_{ow}), charge, water solubility, sorption (k_d) and contrasting fate under photodegradation and aerobic/anaerobic biodegradation, expressed as half-lives values (see Table 1). For instance, X-ray contrast media, which have often been the most frequently detected compounds in domestic TWW, were excluded because their plant uptake has been found restricted under field growing conditions probably due to high molecular weight of 777–821 Da, slowing down their diffusion through cell membranes (Riemenschneider et al., 2016).

No detectable chemicals residues were found in soil samples prior to the experiments and under irrigation with tap water (results not shown). This latter experiment was carried out for a rough evaluation of the phytotoxicity of RWW, TWW and spiked TWW. Dry weigh of lettuces and leeks under different experimental irrigation conditions are reported in Table 4SM. Values were always higher than the dry weigh of lettuces grown with tap water, excluding potential phytotoxicity of RWW, TWW and spiked TWW, which could have impacted the lettuce uptake rates across the different cultivation cycles. Following the field irrigation experiments with spiked TWW, trace residues of 13 out of 14 compounds were detected in soil samples collected near the rhizosphere at the concentration levels in the 1–30 ng/g (d.w.) range. One exception was sucralose, which was never detected due to its high water solubility and lack of sorption onto soil ($k_d < 10$). The highest concentrations were for citalopram, climbazole, carbamazepine and clarithromycin. This result was rather well correlated with their rather high k_d values (542–1883, 123–200, 12–20 and 262–400 L/kg,

Table 1
Physico-chemical properties of investigated compounds and their reported half-lives in the literature under biodegradation and photolysis.

Compound	Mw (g/mol)	SW (mg/L)	pH 7	pKa	Kd (soil) (L/kg)	Log D _{ow} (pH = 7)	Molecule vol. (cm ³)	Charge pH 7.5	Half-life (d) Aerobic biodeg.	Half-life (d) Anaerobic biodeg.	Half-life photolysis
Ciprofloxacin	331	<30		6.1; 8.7	427–4844	0.28	227	Zwitterion	>100	n.f.	0.5 h
Sulfamethoxazole	253	610		1.6; 5.7	0.6–4.9	-1.05	173	Anion	4–10	3–7	3.7 h
Citalopram	324	6.1		9.78	542–1883	3.76	273	Cation	n.f.	n.f.	14–65 d
Diclofenac	296	17.8		4.15	1–18	1.66	207	Anion	0.4–5	>100	0.17 h
Valsartan	435.5	16.8		3.9; 4.7	n.f.	-1.20	359	Anion	0.5	n.f.	Stable
Irbesartan	428	0.35		4.1; 7.4	n.f.	4.4	328	Zwitterion	20–30	n.f.	Stable
Carbamazepine	236	153		-	12–20	2.7	187	Neutral	125–233	n.f.	34–42 d
Metoprolol	267	>10,000		9.7	20	-0.58	259	Cation	23	Stable	26–41 d
Hydrochlorothiazide	298	722		7.9; 9.2	11.9	-0.07	176	Neutral	35.8	n.f.	0.2–0.43 h
Clarithromycin	748	0.33		8.99	262–400	3.16	632	Cation	>100	n.f.	Stable
Climbazole	293	58		7.5	123–200	3.27	248	Neutral/cation	4.4–5.2	6.2	70 min
Benzotriazole	119	>10,000		8.2	0.1–0.8	1.17	88.3	Neutral	38–82	n.f.	42–54
Acesulfame	201	>10,000		2.0	0.1	-0.55	108	Anion	11–19	n.f.	Stable
Sucralose	398	>10,000		-	2.5	-0.47	235	Neutral	14–30	30–65	Stable

n.f: not found.

respectively) accounting for their retention in soil. Ciprofloxacin was detected in experiments with TWW but not in experiments with spiked TWW. Sorption on the TWW storage tank walls or fast ciprofloxacin photodegradation (half-life of 0.5 h has been reported for direct photolysis) during the storage event might account for this difference. Compounds concentrations profiles across lettuce cultivation cycles (in weeks) in soil and in lettuce leaves are reported in Fig. 1 for spiked TWW experiments. As a general trend, analysis of soil samples revealed no carryover of contaminants over multiple crop seasons. This lack of cumulative pattern in soil was in line with previous results (Dalkmann et al., 2012; Wu et al., 2014). Only 8 out of 14 compounds were consistently detected in lettuce leaves. The frequency of detection was 100% for hydrochlorothiazide, carbamazepine, metoprolol, clarithromycin, acesulfame, sucralose, citalopram and climbazole and 25% for benzotriazole, while diclofenac, ciprofloxacin, irbesartan and valsartan were never detected. All these compounds were usually detected in higher

concentrations in spring/summer than fall/winter, which confirmed a good correlation between crop transpiration and crop accumulation (Nason et al., 2018). Their distribution in the aerial organs of the plants was usually limited with concentration levels in the range of 1–660 ng/g (d.w.) which was consistent with those found in previous studies under field growing conditions (Fu et al., 2019). The highest concentration (i.e. 660 ng/g) was found for carbamazepine.

As far as RWW and TWW irrigation experiments were concerned, only 5 compounds were detected in soil and lettuce leaves including acesulfame, sucralose, hydrochlorothiazide, oxcarbazepine and carbamazepine as well as one human metabolite, carbamazepine-epoxide (see Fig. 2). In general, concentration levels in RWW and TWW experiments were much lower than those in spiked TWW experiments. One exception was acesulfame with concentrations in leaves exceeding those in spiked TWW experiments without any clear explanation for this experimental observation. However, this result meant that higher

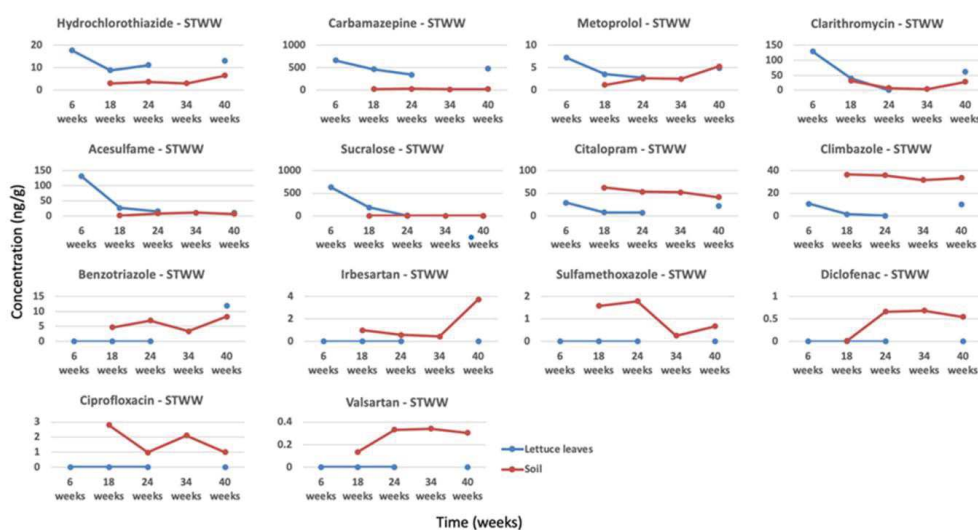


Fig. 1. Organic contaminants concentrations in rhizospheric soil and in lettuce leaves across successive crop cycles under spiked TWW irrigation at a 10 µg/L concentration level (each compound).

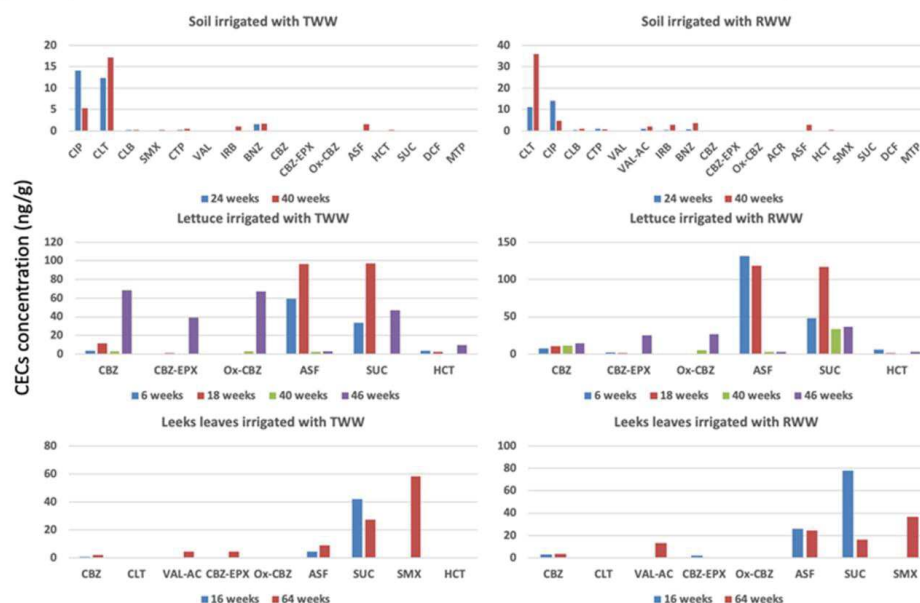


Fig. 2. Organic contaminants concentrations across successive crop cycles in rhizospheric soil and in lettuce and leek leaves samples irrigated with treated wastewater (TWW) and raw wastewater (RWW).

compound concentration in water did not necessarily mean higher concentration in leaves. In fact, many other uncontrollable processes including biotic and abiotic degradation, sorption, leaching, etc... were also taking place in soil and/or irrigation water, besides to the physico-chemical properties of CECs, soil and water, and the weather conditions, making the uptake level by crops unpredictable. Experiments were also carried out with leeks, another leafy vegetable with a longer growing period (16 weeks) than lettuce (6 weeks). Similar compounds than in lettuce experiments were detected in leek leaves including carbamazepine, carbamazepine-epoxide, sucralose and aceulfame (see Fig. 2). One exception was sulfamethoxazole which was detected at 35.5 ng/g with TWW and 58.6 ng/g with RWW at the end of the second growing crop cycle (i.e. 64 weeks). However, concentration levels were usually lower in leek than in lettuce leaves. Longer growing period did not imply higher contaminants accumulation probably because longer time for dissipation processes to operate was also allowed. The main and common feature of all those experiments was the lack of accumulation likely related to abiotic and biotic transformation processes both in soil and leaves (see Sections 3.2 and 3.3). The longer growing period could contribute to the low accumulation of CECs in leek leaves since uptaken CECs were available for metabolism for longer time before harvest. Sorption and degradation in soil likely minimized contaminants uptake by leek and lettuce.

Experimental results in spiked TWW experiments could also be interpreted according to contaminants physico-chemical properties and their persistence under photochemical and biodegradation processes. *Non-ionic compounds*: carbamazepine, hydrochlorothiazide and sucralose. Experiments confirmed that carbamazepine is highly uptaken by plants (Goldstein et al., 2014) even more than more hydrophilic compounds such as hydrochlorothiazide which was also detected in RWW and TWW experiments and previously in lettuce crop (0.18–0.49 ng/g) irrigated with municipal TWW at 0.18–0.49 ng/g concentration level (Martinez-Piernas et al., 2018). To the best of our

knowledge, this was the first time that sucralose uptake by lettuce was observed. Sucralose is structurally similar to sucrose with three chlorine atoms replacing three hydroxyl groups. The presence of multiple hydroxyl groups makes this compound very soluble in water and very hydrophilic, preventing its retention in soil. In spite of its extremely high stability in the environment (Soh et al., 2011), sucralose uptake was therefore not expected due to its low ability to partition into lipophilic cell structure. The uptake process appeared to be somewhat inconsistent across the successive lettuce growing campaigns and between the different types of water. But, when this latter occurred, the uptake rate was always high. One explanation could be that sucralose was uptaken by crops via water. Aquatic plants such as *Lemna* spp. have shown the capacity to assimilate carbon from sucralose (Amy-Sagers et al., 2017). Similarly to sucrose, sucralose may be a plant-accessible sugar, possibly accounting for high uptake values in our experiments. *Cationic compounds*: climbazole, metoprolol, citalopram and clarithromycin. As previously observed, the concentrations of cationic compounds in lettuce leaves were usually much lower than those of the non-ionic PPCPs. Citalopram and climbazole uptake was higher than metoprolol uptake because their larger accumulation in soil probably increased the available concentration for plant uptake resulting in higher accumulation in leaves. One exception was clarithromycin with a high uptake rate. Clarithromycin uptake was not expected due to its high molecular volume (632 cm³) which limited its uptake to upper ground leaves, and rather low D_{ow} (3.16 at pH 7) and k_d (in the 260–400 L/kg range) values and was only been reported once in Bermuda grass roots grown in biosolid amended soil (Jones-Lepp et al., 2010). However, clarithromycin was found to be tightly bound to DOM fraction with negatively charged functional groups (Sibley and Pedersen, 2008). Those interactions were largely reversible and reached a maximum at pH close to neutrality and might make clarithromycin more available for plant uptake by limiting strong sorption on negative mineral surfaces such as clays. *Anionic compounds*:

valsartan, diclofenac, sulfamethoxazole and acesulfame. Diclofenac was never detected in leaves probably because it underwent fast degradation under photochemistry during the TWW storage event and/or at the soil surface. Valsartan was also never detected in lettuce leaves. Valsartan biodegradation in activated sludge was reported to be very fast (<0.5 d, Helbling et al., 2010). Even though this value was reported in activated sludge experiments, similar behavior may be expected in soil irrigated with organic rich WW because it seems to be possible to read across from half-lives determined in highly efficient biotransformation experiments such as activated sludge to soil half-lives due to similarity in enzymatic transformations of organic contaminants (Fenner et al., 2020). With a pKa of 5.6, sulfamethoxazole may partly exist as non-ionic compound in the acidic rhizosphere facilitating plant uptake. However, sulfamethoxazole has been found to be trapped as an anionic specie in the cytosol (pH 7.2) and preferentially being translocated in the phloem rather than the xylem, ending in root (Chuang et al., 2019). Acesulfame was found highly up-taken in leaves similarly than in a previous field study (Riemenschneider et al., 2016), but mainly during the first growing campaign (131 ng/g, d.w.). This uptake rate steadily decreased during the following campaigns, being 26.2, 14.5 and 9.7 ng/g during the 2nd, 3rd and 4th campaign, respectively. *Swittierionic compounds*: ciprofloxacin and irbesartan were never detected in lettuce leaf. Ciprofloxacin was probably eliminated during the TWW storage stage due to fast photodegradation. For irbesartan, strong sorption to soil was anticipated ($\log D_{ow}$ 4.4) limiting its bioavailability.

The ability of lettuce plant to accumulate targeted contaminants from TWW irrigated soil in leaves was also assessed through the determination of BCF, which was established as the ratio of the concentration of each compound in leaves ng/g and in soil (ng/g). The linear regression between D_{ow} and BCF demonstrated a poor relationship, BCF being unusually high for polar compounds as it is shown by the red dots in Fig. 3a, where D_{ow} lower than 500 (polar compounds) were showing BCF relatively high (Fig. 3a). This result assumed that accumulation was not only driven by passive processes (e.g. lipoidal diffusion through lipid bilayer cell membranes or Casparian strip) but might be supported

by carrier-mediated transporters. In this respect, mammalian systems may provide guidance for identifying specific transporter proteins in plant systems. Human cellular uptake of hydrochlorothiazide is facilitated by diffusion through organic anion transporters (Hasannejad et al., 2004). Macrolide antibiotics and clarithromycin in particular possess several interesting features, among which are exceptionally high levels of accumulation and retention in cells and tissues. More and more data support the idea of the existence of an active transport system for macrolides in human cell (Bosnar et al., 2005). The active transport of metoprolol and citalopram have been also reported (Dobson and Kell, 2008). The contribution of transporter proteins might account for a higher BCF values for these compounds (3.3, 4.6, 12.9, 1.7 for hydrochlorothiazide, clarithromycin, metoprolol and citalopram, respectively) than that could be anticipated by their D_{ow} values (0.26, 50.1, 0.13 and 3326, respectively) and their positive charge (except for hydrochlorothiazide). This result means that potential high BCF values are not restricted to intermediate D_{ow} between 100 and 1000 such as the D_{ow} of carbamazepine. Similarly, amitriptyline, a cationic psychoactive drug, has known to be actively transported in human cell and its BCF of 0.35 in spinach was higher than that could be anticipated with its D_{ow} = 64,655 (Nason et al., 2019). All these findings supported the existence of proteins that transport xenobiotic organic cations in plants even though they have not been specifically identified yet. These organic cation transporters have been found in all organisms and have been implicated in the uptake of the cationic antidiabetic drug metformin in plant for instance (Eggen and Lillo, 2016). The public health risk assessment associated with the intake of targeted contaminants through the consumption of lettuce irrigated with spiked TWW, TWW and RWW was conducted through the hazard quotient (HQ) for adults (most of the targeted PPCPs are not prescribed to toddlers). Results are reported in Table 2. EDI were lower than ADI and HQ values were <0.4 for spiked TWW experiments. For TWW and RWW experiments only 4 compounds out of 14 were determined in lettuce restricting the potential toxicity additivity of mixtures of chemicals. A conservative approach for assessing the risk of a mixture of chemicals would be to sum the HQ values, which was always found <0.1. As lettuce was selected as a worse-case scenario since leafy vegetables has a high potential for PPCPs uptake (Christou et al., 2019), the daily consumption of lettuce was considered to not pose any health problem for lettuce grown in greenhouse conditions and soil irrigated with TWW during consecutive growing campaigns. This conclusion held true for leek.

3.2. Fate of contaminants in soil: chiral analysis of metoprolol and climbazole

In soil irrigated with TWW, several processes are in competition for organic contaminants removal including sorption and formation of non-extractable residues, phototransformation at the soil surface, leaching to deeper soil layers than the rhizosphere layer, plant uptake and biotransformation (Li et al., 2019). Their behavior and fate in soil is therefore very complex due to interconnected processes and it is often impossible to discriminate between abiotic and biotic processes. Enantiomeric fractionation of two chiral compounds, namely metoprolol and climbazole, was investigated during the successive lettuce growing seasons to highlight the significance of biodegradation processes over abiotic processes. Enantiomeric fractionation occurs when the biodegradation of one enantiomer of a chiral compound is favored over the other. Enantioselectivity reflects biological processes because abiotic enantioselective processes such as sorption on environmental mineral surfaces have been found negligible with respect to biodegradation-related enantioselectivity. Metoprolol and climbazole were selected because metoprolol specifically undergoes enantioselective biodegradation under aerobic conditions (Souchier et al., 2016) and climbazole under denitrifying/anaerobic conditions (Brienza and Chiron, 2017), thus covering different redox conditions in soil irrigated with TWW. The major

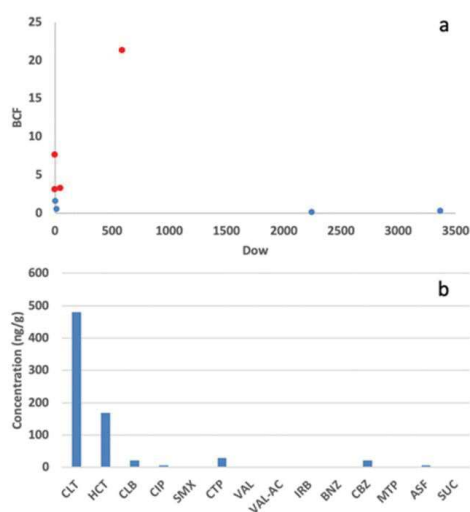


Fig. 3. a) Relationship between D_{ow} of targeted contaminants and their bioconcentration factors (BCF) and b) organic contaminants concentrations in a deep soil layer (50–60 cm) for leaching assessment.

Table 2
Estimated daily intake (EDI) of targeted PPCPs based on the highest concentration determined in lettuce leaf and associated Hazard Quotient (HQ) for adult.

Compound	Lettuce irrigated with STWW				Lettuce irrigated with TWW			Lettuce irrigated with RWW		
	Greatest conc. in leave (ng/g)	EDI (ng/kg/d)	ADI (ng/kg/d)	HQ	Greatest conc. in leave (ng/g)	EDI (ng/kg/d)	HQ	Greatest conc. in leave (ng/g)	EDI (ng/kg/day)	HQ
Ciprofloxacin	–	–	–	–	–	–	–	–	–	–
Sulfamethoxazole	–	–	–	–	–	–	–	–	–	–
Citalopram	29.1	43.3	285	0.15	–	–	–	–	–	–
Diclofenac	–	–	–	–	–	–	–	–	–	–
Valsartan	–	–	–	–	–	–	–	–	–	–
Irbesartan	–	–	–	–	–	–	–	–	–	–
Carbamazepine	660.0	982.8	2850	0.34	68.4	101.8	0.04	14.7	21.9	7×10^{-3}
Metoprolol	7.2	10.7	360	0.03	–	–	–	–	–	–
Hydrochlorothiazide	17.6	26.2	360	0.07	9.6	14.3	0.04	6.1	9.1	0.03
Clarithromycin	128.8	191.8	7140	0.03	–	–	–	–	–	–
Climbazole	10.7	15.9	–	–	–	–	–	–	–	–
Benzotriazole	11.8	17.6	–	–	–	–	–	–	–	–
Acetulfame	131.5	195.8	5×10^6	3×10^{-5}	148.7	221.4	4×10^{-5}	131.5	195.8	4×10^{-5}
Sucralose	632.0	941.2	15×10^6	6×10^{-5}	146.4	218.0	1×10^{-5}	116.8	173.9	1×10^{-5}

advantage of the enantiomeric fractionation approach over isotopic fractionation has been the relative simple analysis of enantiomers ratios in a routine way using chiral LC-HRMS. The chiral analysis of metoprolol and climbazole by chiral LC-HRMS which were previously developed for assessing the biodegradation rates of metoprolol (Suchier et al., 2016) and climbazole (Brienza and Chiron, 2017) in activated sludge were applied in this study to go deeper in the understanding PPCPs fate in soil. These methodologies were precise and reproducible with appropriate LODs of ng/g in solid matrices and were applied to a subset of field soil samples of spiked TWW experiments. As an illustrative example, Fig. 4a shows the enantiomer profile of metoprolol in a soil sample collected at the end of the last lettuce growing campaign. Enantiomers ratio showed an enantiomeric profile similar to that of metoprolol standard. This profile was constant across time and metoprolol remained racemic. This result very likely means that biodegradation did not operate because anoxic conditions already prevailed in the top layer soil (0–20 cm) due to the high content in DOM provided with TWW. No known TPs of metoprolol such as metoprolol acid, *o*-desmethylnmetoprolol or α -hydroxymetoprolol were detected in soil extracts (see Fig. 3SM). This constituted an additional piece of evidence for a lack of metoprolol biodegradation under

anaerobic conditions. Fig. 4b shows the chiral analysis of climbazole in soil at the end of the first lettuce growing campaign. In contrast to metoprolol, climbazole enantiomeric fractionation clearly took place along the growing campaigns, supporting that biodegradation was effective under denitrifying conditions. Identification of TPs of climbazole in soil extracts using C-18 LC-HRMS revealed the occurrence of climbazole-OH which confirmed the biotransformation of climbazole but also revealed the reductive dechlorination of climbazole (see Fig. 4c) due to photochemical processes (Castro et al., 2016). Consequently, surface drip irrigation did not completely prevent from climbazole photodegradation. After irrigation, upward movement of water to the soil surface where PPCPs phototransformation could occur has to be considered and phototransformation need to be taken into account when deriving microbial degradation rates from field studies as previously suggested (Buerge et al., 2019). It is well recognized that the anoxic/anaerobic biotransformation of organic micropollutants is energetically less favorable than their aerobic counterpart, which advocates for poor efficiency of biodegradation processes in soil irrigated with domestic TWW. However, certain aerobically recalcitrant contaminants are biologically degraded under anaerobic conditions and enzymatic reactions involved in their degradation

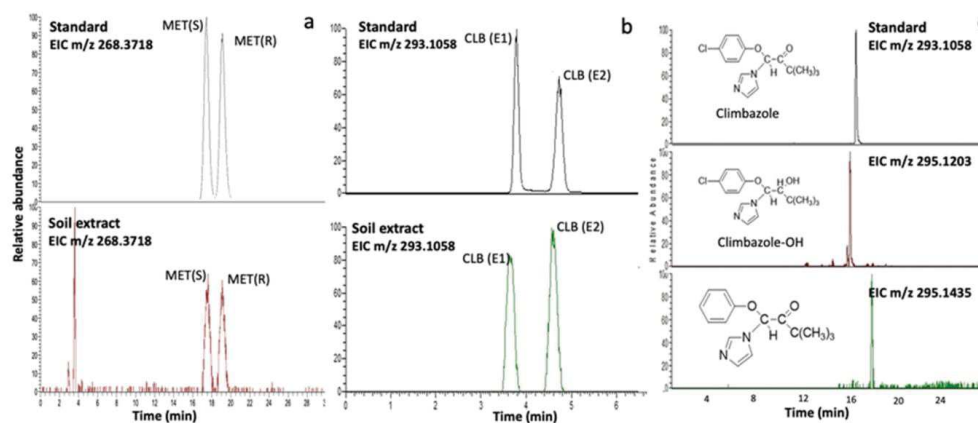


Fig. 4. Chiral LC-HRMS analysis of a) metoprolol (MET(S) and MET(R)) and b) climbazole (CLB(E1) and CLB(E2)) in soil irrigated with spiked treated wastewater. C) Extracted ion chromatogram of the identified transformation products of climbazole.

are becoming known (Ghattas et al., 2017). For instance, sulfamethoxazole (Wu et al., 2012) and irbesartan (Boix et al., 2016) are known to be quickly biotransformed under anaerobic conditions which probably contributed to their very low occurrence levels in soil. Finally, contaminants analysis were carried out in a soil layer (50–60 cm) deeper than the rhizosphere layer at the end the experiments to investigate their potential leaching. The results are reported in Fig. 3b. Clarithromycin and hydrochlorothiazide were detected at high concentrations (481.2 ± 67.3 and 169.6 ± 30.5 ng/g, respectively), while the others compounds were found at similar concentrations than those encountered near the rhizosphere. Many studies have reported the sorption of DOM to amorphous clay minerals (Sibley and Pedersen, 2008). Many clay surfaces in upper soil horizons irrigated with TWW were probably already coated with DOM and thus were not accessible for contaminants binding. The presence of high amount of free DOM in soil could give rise to interactions mainly through contaminant-DOM complexes. This kind of interactions was already demonstrated for clarithromycin (Sibley and

Pedersen, 2008) and might also apply for hydrochlorothiazide. They could facilitate the co-transport of DOM and contaminants downward through soil horizons with the flow of water. Sorption of PPCPs to DOM has appeared to be governed by their chemical structures and difficult to be predicted at the moment (Maoz and Chefetz, 2010) probably justifying further investigations.

3.3. Fate of contaminants in leaves: Identification of metabolites and/or TPs

Till now, identification of metabolites in plants has been mainly carried out at high spiked concentrations (e.g. 1 mg/L) to elucidate metabolic pathways and under hydroponic conditions to discriminate between soil-generated TPs and plant-generated metabolites and because plants absorb higher levels of organic contaminants in hydroponic conditions than in soil experiments. For instance, such experiments were conducted to elucidate TPs of clarithromycin (Tian et al., 2019), carbamazepine (Martines-Piernas et al., 2019) and ofloxacin (Tadic et al., 2020) in lettuce crop. Metabolites identification are worthy to

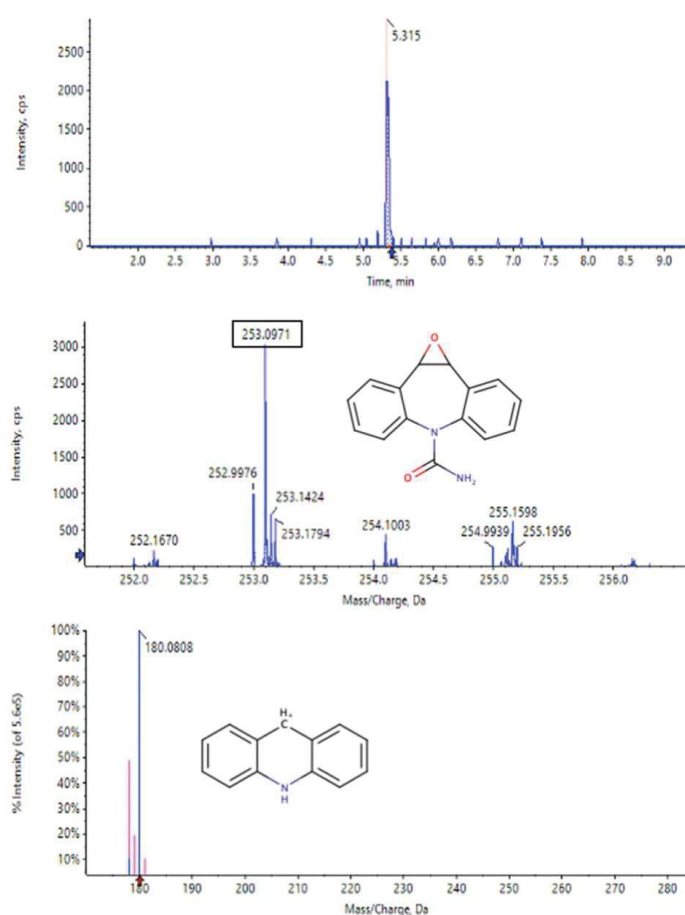
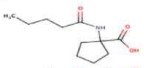
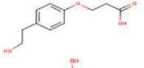
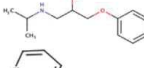
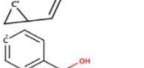
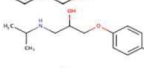
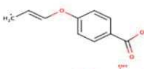
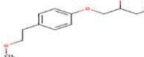



Fig. 5. Identification of carbamazepine-epoxide by LC-TOFMS in lettuce leaves at a concentration level of 6.1 ± 0.7 ng/g using the SWATH® acquisition mode. Upper plot: EIC m/z 253.0972; middle plot: MS spectrum; lower plot: MS/MS spectrum using m/z 253.0971 as precursor ion.

assess the consequences of long-term exposure to PPCPs because TPs are stored in cell walls and vacuoles and because some PPCPs can be transformed into more toxic products during plant metabolism. This is the case of carbamazepine, which is transformed into carbamazepine-epoxide (Sauvêtre et al., 2018). In this study, the objective was to tentatively identify previously reported metabolites or TPs of investigated compounds in order to assess their relevance under real growing conditions by using a suspect screening workflow based on a list of compounds and their metabolites/TPs with their respective exact m/z (see Table 6SM). In a first step, TPs with intensities lower than 1×10^4 cps, signal to noise ratios lower than 10, isotopic ratios higher than 10%, and mass accuracy errors higher than 5 ppm were eliminated. When possible, after preliminary identification, the potential metabolites/TPs were further confirmed by including the screening of known fragments ions and the MS/MS spectrum information was compared with that reported in previous literature reports. The level of confidence for the identification of detected TPs was classified according to Schymanski et al. (2014). This approach was first applied to clarithromycin and to carbamazepine for which metabolic pathways in lettuce were previously elucidated (Martines-Piernas et al., 2019; Tian et al., 2019). In case of carbamazepine, only carbamazepine-epoxide was detected and its structure was confirmed at a level 1 of confidence through matching with an authentic standard. Fig. 5 shows results on the identification of carbamazepine epoxide at a concentration level of 6.1 ± 0.7 ng/g in a lettuce leaves extract, demonstrating the reliability of the SWATH® acquisition mode for compound identification at a very a concentration level close to LODs in a complex matrix. Carbamazepine-epoxide was also detected in TWW (10.7 ± 1.3 ng/L) but not in soil. This amount found in leaves was therefore exclusively related to in-plant metabolism and not the combination of plant uptake and metabolism. This is reasonable since carbamazepine-epoxide has been known to undergo in soil hydrolysis leading to 10,11-dihydro-10-hydroxycarbamazepine and/or ring contraction leading to acridone derivatives (Li et al., 2013). Differently, valsartan acid, which was also identified with of level 1 of confidence due to the availability of an analytical standard

was found in TWW ($111.17 \pm$ ng/L) and in soil ($1.7 \pm$ ng/L) in accordance with its high environmental persistency but not in lettuce leave excluding valsartan acid plant uptake. None of the known metabolites of clarithromycin was detected in this study, even though clarithromycin was found to be extensively and quickly metabolized in lettuce (Tian et al., 2019) with a proportion of metabolites, which was estimated to account for more than 70% of the initial clarithromycin concentration. This finding might result from very low formation rates of clarithromycin metabolites, thus escaping from analytical determination. The suspect screening approach was then extended to known bio- and photo-TPs of ciprofloxacin, citalopram, diclofenac, valsartan, irbesartan, metoprolol, hydrochlorothiazide, climbazole and acesulfame (Table 6SM). Only TPs of metoprolol and irbesartan were detected. Metoprolol acid, a very common bio-TPs of metoprolol was never detected. In contrast, TP239 originating from the benzylic hydroxylation of metoprolol acid and TP253 resulting from further oxidation of TP239 into the corresponding carboxylic acid (see Fig. 4SM) were identified at level 2 of confidence (probable structure through matching with literature). These two TPs were previously observed in river-simulating flumes (Posselt et al., 2020) similarly to TP210 and TP226 but these two TPs were only identified at level 4 of confidence in our experiments (unequivocal molecular formula which can be unambiguously assigned with the spectral information). Finally TP213 resulting from the hydrolysis of irbesartan could be also identified at level 4 of confidence and will deserve further investigation (Fig. 5SM). Proposed structures and relative mass errors of the detected TPs and their fragments are presented in Table 3. Low mass error values (<5 ppm) were obtained comparing with their theoretical masses. Due to the lack of authentic standards, it was difficult to obtain accurate abundances of all these TPs. A semi-quantitative method was applied using the pseudo-molecular ion abundances and considering that the structure of metabolites/TPs were closely related to those of their parent compounds. Following this method, their concentrations were always estimated below the concentrations of their parent compounds. All these results demonstrated that PPCPs were likely highly metabolized in lettuce, precluding

Table 3
Proposed structures and mass error of the detected TPs of metoprolol and irbesartan in lettuce leaves.

TPs	Molecular formula	RT (min)	Observed m/z	Theoretical m/z	Polarity	Mass error (ppm) ^a	Proposed structure
Irbesartan sTP213	C ₁₁ H ₁₉ NO ₃	4.5	214.1438	214.1443	POS	-2.3349	
Metoprolol sTP210	C ₁₁ H ₁₄ O ₄	2.7	211.0965	211.09644	POS	0.2842	
Metoprolol sTP239	C ₁₃ H ₂₁ NO ₃	2.1	240.1593	240.15944	POS	-0.5829	
Metoprolol sTP239-fragment 91	C ₇ H ₇	-	91.0543	91.05453	POS	-2.5260	
Metoprolol sTP239-fragment 107	C ₇ H ₇ O	-	107.0493	107.04926	POS	0.3737	
Metoprolol sTP253	C ₁₃ H ₁₉ NO ₄	1.5	254.1386	254.1386	POS	0.0000	
Metoprolol sTP253-fragment 177	C ₁₀ H ₉ O ₃	-	177.0549	177.0545	POS	2.2592	
Metoprolol sTP226	C ₁₂ H ₁₈ O ₄	2.2	225.1129	225.1127	NEG	0.8884	

^a Mass error (ppm) = ((observed mass - theoretical mass) / (theoretical mass)) × 1,000,000.

the accumulation of parent compounds in leaves. The levels of metabolites/TPs concentrations in leaves were always low, limiting potential health risks.

4. Conclusions

Crops irrigation with TWW represents a recognized pathway for human exposure to organic contaminants with possible health implications. However, this study confirmed that the accumulation of 14 compounds in soil and in lettuce leaves irrigated with spiked TWW at 10 µg/L concentration level was very limited. These results relied on the implementation of real greenhouse cultivation conditions, the repeated application of spiked TWW over five successive lettuce crop cycles and a large number of compounds covering not only different physico-chemical properties but also showing contrasting behavior with respect to photo- and bio-transformation rates. This likely represented the originality of this study. Poor accumulation was also observed with non-spiked TWW and RWW and with another leafy vegetable, leek. The main reason for these low accumulation rates was an intensive degradation in soil and metabolic transformations in plant which were supported by the enantiomeric fractionation of climbazole in soil and the identification of suspected metabolites/TPs in lettuce leaves, respectively. Metabolites/TPs concentrations were always estimated below those of the parent compounds. Unexpected pharmaceuticals such as clarithromycin and hydrochlorothiazide where yet detected in lettuce leaves. This might be linked to active transport processes similarly to what has been observed in human cells. Information on pharmacokinetics and toxicokinetics of PPCPs in humans and animals should be more mined and used to anticipate the potential plant uptake of PPCPs as evenly suggested by Nason et al. (2019). The food additive sucralose was also encountered at high concentration in lettuce and leek leaves for the first time because sucralose may be a plant-accessible sugar similarly to sucrose. As a whole, this study confirmed *a de minimis* human health risk related to the consumption of raw green vegetable (e.g. lettuce) irrigated with TWW. More research is today needed to fully understand the environmental risks of TWW irrigation practices and specifically the impact of such practices on long-term soil quality.

CRediT authorship contribution statement

Rayana Manasfi: Analysis – writing – original draft, review & editing
 Monica Brienza: Experimental data interpretation
 Nassim Ait-Mouheb: Implementation of field experiments
 Nicola Montemurro: Analytical methods for organic contaminants
 Sandra Perez: Analytical methods for organic contaminants
 Serge Chiron: Conceptualization – writing, review & editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.142742>.

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Chapter 6:

Fate of two selected antibiotics and one personal care product by soil microorganisms

1. Biodegradation of fluoroquinolone antibiotics and the climbazole fungicide by *Trichoderma* species

1.1. Context

Trichoderma species belong to the most widespread filamentous fungi genus in soil. They are able to grow and survive under unfavorable conditions, colonize root and grow in symbiont relationship with plants. Therefore, *Trichoderma* have been used as natural fungicide in agriculture sector, regarding their ability to fight against pathogens and enhance crops productivity. Many studies have showed the ability of fungi, especially the White-Rot Fungi (WRF) such as *Trametes versicolor*, to degrade PhACs with good removal efficiencies. However, the use of filamentous fungi from *Trichoderma* genus has been poorly investigated for PhACs and other organic contaminants elimination. Therefore, it is interesting to investigate the ability of *Trichoderma* in degrading such contaminants. Finally, this research will enhance the possibility of treated wastewater reuse in irrigation without any additional treatment.

1.2. Objective and main results

The objective of this article was to study for the first time the removal kinetics of the filamentous fungi *Trichoderma*, namely *T. harzanium* and *T. asperellum* to degrade two fluoroquinolones antibiotics (ciprofloxacin and ofloxacin), and one fungicide (climbazole), as well as their transformation mechanisms and products in liquid medium. The obtained results revealed good removing efficiencies by both *Trichoderma* strains. However, contrasting results were obtained: *T. asperellum* removed 81% of ciprofloxacin, while *T. harzanium* removed 91% of climbazole. While ofloxacin was removed with degradation rate around 40% with both strains. Biosorption process played a limited role in their elimination. By the means of the liquid chromatography coupled to high resolution mass spectrometry, 3 TPs were identified for climbazole, 3 for ciprofloxacin and 4 for ofloxacin. Biotransformation included hydroxylation, oxidation/reduction, and N- and O-dealkylation reactions. Two metabolites from ofloxacin and one from climbazole were tentatively identified for the first time in this study. In brief, promising results have been obtained on the ability of *Trichoderma* species to

degrade PhACs and the fungicide. However further experiments at environmental concentrations and more realistic conditions such wastewater effluent or agricultural soil field are still needed, besides to toxicity assessment of the determined TPs.



Biodegradation of fluoroquinolone antibiotics and the climbazole fungicide by *Trichoderma* species

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Abstract

Filamentous fungi *Trichoderma* have been able to efficiently degrade fluoroquinolone antibiotics namely ciprofloxacin (CIP) and ofloxacin (OFL) as well as the fungicide climbazole (CLB) that are persistent in conventional activated sludge processes. All targeted compounds were biotransformed by whole cells of *Trichoderma* spp., exactly *T. harzianum* and *T. asperellum*, and biosorption played a limited role in their elimination. However, contrasting results were obtained with the two strains. *T. asperellum* was more efficient against CIP, with a 81% degradation rate in 13 days of incubation, while *T. harzianum* was more efficient against CLB, with a 91% degradation rate. While in the case of OFL, both strains showed same efficiency with degradation rate around 40%. Adding a cytochrome P450 enzyme inhibitor hardly resulted in the modification of degradation kinetics supporting the implication of extracellular enzymes in chemical biotransformation. Transformation products were identified by liquid chromatography–high resolution–mass spectrometry and transformation pathways were proposed. Biotransformation of selected compounds included hydroxylation, oxidation/reduction and N- and O-dealkylation reactions, similarly to those reported with white rot fungi. CIP underwent transformations at the piperazinyl ring through oxidation and conjugation reactions, while OFL mainly underwent hydroxylation processes and CLB carbonyl reduction into alcohol. Consequently, *Trichoderma* spp. likely possess a machinery of unspecific enzymes, which makes their application in removal of pharmaceutical and personal care products attractive.

Keywords *Trichoderma* · Biodegradation · Transformation products · Climbazole · Fluoroquinolone

Introduction

Soil and water pollution by contaminants of emerging concern (CECs) in the meantime is a worldwide problem. Among all CECs, pharmaceuticals and personal care products (PPCPs) are a large family of chemicals that can be bio-accumulated, persistent and toxic and may have a

negative effect on the environment and/or human health, even at trace level. Their main direct entrance to the environment is through municipal or industrial wastewater effluents (Chen et al. 2004; Couto et al. 2019; Kümmerer 2011) because they are not completely removed by biological wastewater treatment plants. The inefficiency of current activated sludge processes for PPCPs removal calls for innovative processes (Couto et al. 2019; Petrie et al. 2015; Phonsiri et al. 2019). Advanced oxidation processes have demonstrated high potential to remove PPCPs but the high energy consumption and the formation of toxic by-products can limit their implementation (Brienza et al. 2019; Fatta-Kassinos et al. 2011; Huang et al. 2018). In this context, bioremediation techniques using fungal strains for the removal of PPCPs have several advantages. They can be eco-friendly, feasible and cost-effective alternative treatments in comparison with other remediation techniques (Kumar et al. 2019; Olicón-Hernández et al. 2017). Many studies (Čvančarová et al. 2015; Jelic et al. 2012; Prieto et al. 2011) have been focused on the white rot

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fungi (WRF) such as *Trametes versicolor*. Good removal efficiencies accomplished by these WRF have been attributed to the action of a nonspecific enzymatic system that includes extracellular ligninolytic enzymes such as laccase, lignin peroxidase, manganese peroxidase and versatile peroxidase. The extracellular enzymatic system has not been found the only responsible for PPCPs degradation. In fact, the intracellular enzyme system also plays an important role thanks to the action of cytochrome P450 enzymes (CYP450) (Asgher et al. 2008; Čvančarová et al. 2015; Durán and Esposito 2000; Prieto et al. 2011; Tanaka et al. 1999). Extracellular ligninolytic enzymes are only produced by some organisms in contrast to CYP450 that are ubiquitous in all biological kingdoms. However, one limitation of the use of WRF in PPCPs remediation has been the difficulty for the inoculated fungus to successfully compete with the other microorganisms growing in bioreactors (Badia-Fabregat et al. 2017).

In contrast to WRF, the use of filamentous fungi from the genus *Trichoderma* has been poorly investigated for PPCP elimination from domestic wastewaters (Buchicchio et al. 2016) in spite of several appealing features: (1) Their prevalence in the environment is primarily due to their ability to rapidly grow and propagate in adverse environmental conditions, their ability to use different sources of carbon and nitrogen as well as their ability to effectively compete with bacteria through the synthesis of antibiotics. (2) They are able to accumulate heavy metals (Hoseinzadeh et al. 2017) and nutrients (Hultberg and Bodin 2017) and to grow in wastewater effluents to produce cellulase (Libardi et al. 2017). (3) There is a possibility for treated wastewater reuse in irrigation without any additional treatment as *Trichoderma* spp. are beneficial for plant growth.

The overall purpose of this study was to investigate the removal kinetics and mechanisms of targeted PPCP degradation by ubiquitous soil *Trichoderma* spp. (i.e., *Trichoderma asperellum* and *Trichoderma harzianum*) and to identify their biotransformation products (TPs) in liquid phase and under aerobic condition. Fluoroquinolone antibiotics (i.e., ciprofloxacin (CIP) and ofloxacin (OFL)) are widely used in both human and veterinary medicines as antimicrobial agents, hence their high detection (ng/L up to µg/L) in treated wastewater effluent (Kim and Aga 2007; Rabiet et al. 2006; Togola and Budzinski 2008). They were selected as probe compounds because their biotransformation have been widely investigated by WRF and others fungi species offering a starting point for comparison (Gros et al. 2014; Prieto et al. 2011; Wetzstein et al. 1999). Climbazole (CLB) is an antimycotic imidazole drug used as an active ingredient in antidandruff shampoo. It was selected on its ubiquitous occurrence in wastewater effluents (Richter et al. 2013), on its low biodegradability in activated sludge processes and due to its antimycotic properties.

Material and methods

Chemicals and fungi strains

Analytical standards of CIP, OFL, CLB, desethylene ciprofloxacin (hydrochloride), ciprofloxacin formamide and 4-chlorophenol were of high purity grade (>98%) and obtained from Sigma-Aldrich (St Quentin-Fallavier, France). Climbazole alcohol (CLB-OH) was synthesized as previously reported (Brienza and Chiron 2017). Ultra-pure water (UPW) was obtained using a Millipore system. LC-MS grade acetonitrile and HPLC water were purchased from Merck (Darmstadt, Germany). Fomic acid (≥96%, ACS reagent), ammonium fluoride, ammonium acetate, HPLC grade methanol (MeOH), 0.45 µm and 0.22 µm PTFE syringe filters, potato dextrose agar and malt extracts broth were supplied by Sigma-Aldrich. Oasis HLB (30 mg sorbent/1 cm³ and 200 mg sorbent/6 cm³) solid phase extraction (SPE) cartridges were from Waters Corporation (Milford, MA).

Stock solutions of CIP and OFL were prepared in water/MeOH (50/50, v/v) with 2% of hydrochloric acid (>90%) at a concentration of 400 mg/L, while CLB was prepared in MeOH at a concentration of 1000 mg/L. Serial dilutions were performed with UPW and stored at 5 °C, while stock solutions were stored at -20 °C. Standards were sterilized using PTFE 0.22-µm syringe filter before their usage in the experiments.

Trichoderma harzianum (TH, CBS 226.95) and *Trichoderma asperellum* (TA, CBS 433.97) were obtained from Westerdijk Fungal Biodiversity Institute (Utrecht, Netherlands) as pure strains. The two strains were cultivated separately in sterile petri dishes (9 cm of diameter) filled with potato dextrose agar at 25 °C. Fungi replications were routinely made every week by transferring one plug to a new potato dextrose agar petri dish. Potato dextrose agar was prepared by dissolving 39 g in 1 L of UPW and boiled until complete dissolution. Prepared solutions were sterilized in an autoclave for 15 min at 121 °C. Finally, 1 mL of 10% lactic acid sterilized using 0.22-µm PTFE syringe filter was added to suppress bacterial growth. In vivo degradation experiments were carried out using the mycelial formed from both *Trichoderma* strains.

Biodegradation experiments

Degradation experiments were carried out in sterile specimen cups, each containing 60 mL of low nutrient liquid medium made up by 0.25 g/L of peptic digest of animal tissue and 0.15 g/L of beef extract (pH 4.9), which was sterilized in an autoclave for 15 min at 121 °C. Each cup was inoculated with three mycelial plugs of 0.8 cm². The medium was spiked from a stock solution containing the selected compounds achieving a 200 µg/L initial concentration for each one. The spiking concentration was low enough to avoid any possible toxic

effect on fungi. Each compound was spiked separately with each of the two *Trichoderma* strains. The degradation of selected compounds was assessed by comparing their concentrations with the abiotic control cups during time experiments. Thereafter, cups were incubated at 25 °C in an orbital shaker at 135 rpm for 13 days. Experiments were carried out in the dark to avoid any compound photolysis. Samples (1 mL) from each cup were taken at specific time points (0, 2, 5, 7, 13 days), filtered at 0.45 µm and purified by SPE as described in the following section.

Besides the experimental treatment, abiotic controls (where the matrix contained target compounds exposed to the same experimental conditions but without fungi) were used to assess potential abiotic degradation. An additional experiment was carried out, where 5 mM of 1-aminobenzotriazole (ABT) was added as a CYP450 inhibitor in order to understand the role of intracellular enzymes in degradation processes.

To evaluate the role of the sorption processes in the CECs removal, two methods (i.e., ultrasonication and heat-killed fungi) were applied as reported by Buchicchio and co-authors (Buchicchio et al. 2016). Briefly, the remaining liquid medium at $t = 13$ days was passed through Oasis HLB cartridges (200 mg sorbent/6 cm³). For the ultrasonication method, the SPE sorbent was removed and sonicated in MeOH for 5 min. Extracts were subsequently centrifuged and the liquid phase recovered. When the heat-killed fungi approach was employed, the retained analytes were eluted with 2 × 5 mL MeOH, while fungal cells remained absorbed in the cartridges. Subsequently, SPE cartridges were autoclaved for 30 min at 121 °C. The sorbent phase was then washed with 2 × 5 mL MeOH. In both methods, eluates were concentrated until full dryness under a gentle nitrogen flow. Residues were collected with 1 mL of LC-MS mobile phase (ACN/water, 5/95%, v/v), filtered with 0.22-µm PTFE syringe filters and finally transferred to vials ready for injection.

Analytical methods

For the biodegradation analysis, an extraction and purification SPE method was implemented using Oasis HLB cartridges (30 mg sorbent/1 cm³). The conditioning step was performed with 1 mL MeOH followed by 1 mL UPW. A total of 1 mL of filtered sample was passed through the cartridge followed by 1 mL of UPW for washing. Cartridges were dried under vacuum until total dryness and finally eluted with 2 × 1 mL of MeOH. Eluates were totally dried under a gentle nitrogen flow at 55 °C, reconstituted with 1 mL of LC-MS mobile phase (ACN/water, 5/95%, v/v) and filtered with 0.22-µm PTFE syringe filters before injection. The employed SPE extraction provided high recoveries for all studied parent compounds (> 90%).

Kinetics studies and TPs identification were performed using a SCIEX ExionLC™ AD Quadrupole–Time of Flight mass spectrometer (Q-TOF, Redwood City, CA, USA). For TPs,

identification samples were injected at 7 days of incubation time, where the highest TP concentrations were often reached. A total of 5 µL of purified samples were injected with an auto-sampler temperature maintained at 8 °C. Separation was performed using a Hibar® HR Purospher® STAR RP-C₁₈ column (100 × 2.1 mm i.d., 2 µm particle size, Merck, Darmstadt, Germany), maintained at 40 °C in the column oven. A fast elution of 10 min was carried out using a binary linear gradient composed of 2 mM ammonium fluoride in water (A) and ACN (B) for negative electrospray ionization, and 5 mM ammonium acetate and 0.1% of formic acid in water (A) and ACN (B) for positive ionization mode, at a flow rate of 0.5 mL/min.

High resolution mass spectrometry data were acquired using the SWATH acquisition mode workflow consisting of a single TOF-MS experiment over a m/z range from 70 to 950 Da with an accumulation time (AT) of 130 ms, followed by 3 MS/MS experiments with variable Q1 windows (30 to 900 m/z , 50 ms AT) using a Collision Energy (CE) of 10, 20, 30 and 35 eV and a collision energy spread of ± 15 eV for the latest. The source conditions for the system were optimized as follows: Ion spray voltage was set to 5500 V; source temperature and nitrogen gas flows (atomizing gas, GS1 and auxiliary gas, GS2) were set to 550 °C and 60 psi, respectively. Curtain gas was set to 35 psi and Declustering Potential (DP) to 80 V. Any drift in the mass accuracy was automatically corrected and maintained throughout batch acquisition by infusion of reserpine reference standard (C₃₃H₄₀N₂O₉, m/z 609.28066) for positive ionization and a cluster of trifluoroacetic acid (5(TFA-Na) + TFA⁻, m/z 792.85963) for negative ionization. Calibration was running every 5 samples during the batch acquisition using the Calibrant Delivery System (CDS). All data were acquired and processed using SCIEX OS software version 1.5. A suspect screening workflow was used. A csv-database was made up of a list of possible TPs with their molecular formula, exact mass and structure. This list was generated from a literature search of TPs of OFL, CIP and CLB generated during photochemical experiments, other oxidative treatments and biodegradation/metabolism experiments and prioritizing some metabolic products estimated using the BiotransformationMassDefects software tool provided from Agilent (Agilent Technologies, Barcelona, Spain). High confidence identification of new TPs was based on accurate masses of pseudo-molecular ions and unique fragment ions interpretation.

Results and discussion

Biotransformation of CIP, OFL and CLB by *Trichoderma* species

Abiotic controls showed high compound stability during the incubation time (data not shown). Due to these results, the

concentration variations have been assigned to sorption on fungi and to biodegradation processes. Sorption processes including absorption (contaminants inside the biomass) and adsorption to the biomass surface were indirectly evaluated by setting up a control flask with thermal inactivated fungus or directly evaluated by compounds ultrasonic extraction. The sorption kinetics was not considered. Results showed that biosorption played a very limited role in the observed contaminant removal (< 10%) (data not shown). This lack of CECs biosorption is consistent with the results of previous experiments in which no sorption processes of carbamazepine, clarithromycin and tebuconazole on *Trichoderma* biomass occurred (Buchicchio et al. 2016; Obanda et al. 2008).

Concentrations of contaminants were measured at the beginning and at the end of the experiments, and the difference in concentrations is mainly attributed to biodegradation processes. The results are shown in Fig. 1 as eliminated percentage of each compound by both *Trichoderma* sp. (*T. harzianum* and *T. asperellum*), in presence and in absence of CYP450 inhibitor 1-aminobenzotriazole (ABT). Further explanations on the eliminated fraction by *Trichoderma* spp. are discussed in the rest of this section.

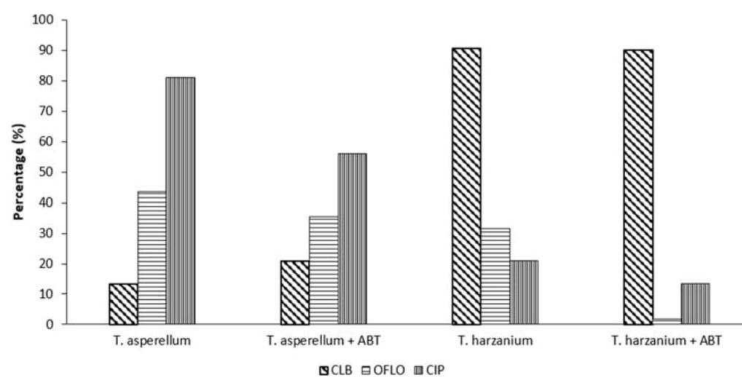
All targeted compounds were biotransformed by whole cells of *Trichoderma* spp. Biodegradation kinetics of parent compounds and transformation products (TPs) generation kinetics for CLB and CIP are shown in Figs. 2 and 3, respectively, in presence and in absence of ABT. The different experiments are all spiked at 200 µg/L as initial concentration. Recovery values for CLB, CIP and OFLO were determined to be 92 ± 8%, 94 ± 13% and 90 ± 20%, respectively. Complex extracts were obtained for these biological samples, and high matrix effects were observed accounting for the high coefficients of variation. OFL could not be properly quantified due to strong matrix effects in LC/MS neither its TPs due to the lack of analytical standards. OFL kinetic profiles are therefore reported in Supporting Material (SM, Fig. 1SM) on the basis of determination of peak area. Strong differences were

observed between both fungi strains. At the end of incubation time (13 days), *T. harzianum* achieved almost complete CLB removal (91%) (Fig. 2c) instead of only 32% (Fig. 3c) and 21% (Fig. 1SM) for OFL and CIP, respectively.

In contrast, *T. asperellum* was the most efficient strain towards the two fluoroquinolone antibiotics, with the highest biodegradability observed for CIP. After 13 days of incubation, 82% (Fig. 3a) and 44% (Fig. 1SM) of CIP and OFL were eliminated respectively, while only a slight biodegradation rate was observed for CLB (14%) (Fig. 2a). Biphasic kinetics including firstly a fast degradation phase and secondly a slower degradation phase might be related to nutrient privation after 2–3 days of incubation because biodegradation experiments were carried out in low nutrient liquid medium to prompt enzymes induction. The difference of the degradation rates observed between CIP and OFL may be justified by the difference in their molecular structures. However, this was not conclusive since both fluoroquinolones showed similar elimination rates when cultivated with the WRF *T. versicolor* and *P. ostreatus* (Čvančarová et al. 2015; Prieto et al. 2011). Higher elimination rates were observed for CIP when cultivated with *P. tigrinus*, while OFL underwent higher elimination rates when cultivated with *D. squalens* after 14 days of incubation (Čvančarová et al. 2015).

The contribution of internal CYP450 enzymes was evaluated by adding 5 mM of 1-aminobenzotriazole (ABT) in whole cultures. After 1 h, targeted compounds were added to fungal cultures. Degradation rates were 30% (Fig. 1SM) and 37% (Fig. 2d) less for OFL and CLB, respectively for *T. harzianum*, while no inhibition was observed for *T. asperellum* (deficit around 7%, (Fig. 1SM and Fig. 2b). On the contrary, CIP degradation was slightly affected when CYP450 inhibitor was added to *T. harzianum* (8% less, Fig. 3d), while it was 25% (Fig. 2b) less when added to *T. asperellum*. This result is in contrast with WRF for which the ABT addition led to significant inhibition of pharmaceuticals biodegradation (Prieto et al. 2011). However, it was

Fig. 1 Percentage degradation (%) of CLB, OFL and CIP by *T. asperellum* and *T. harzianum* in presence and in absence of 1-aminobenzotriazole (ABT) after 13 days of incubation



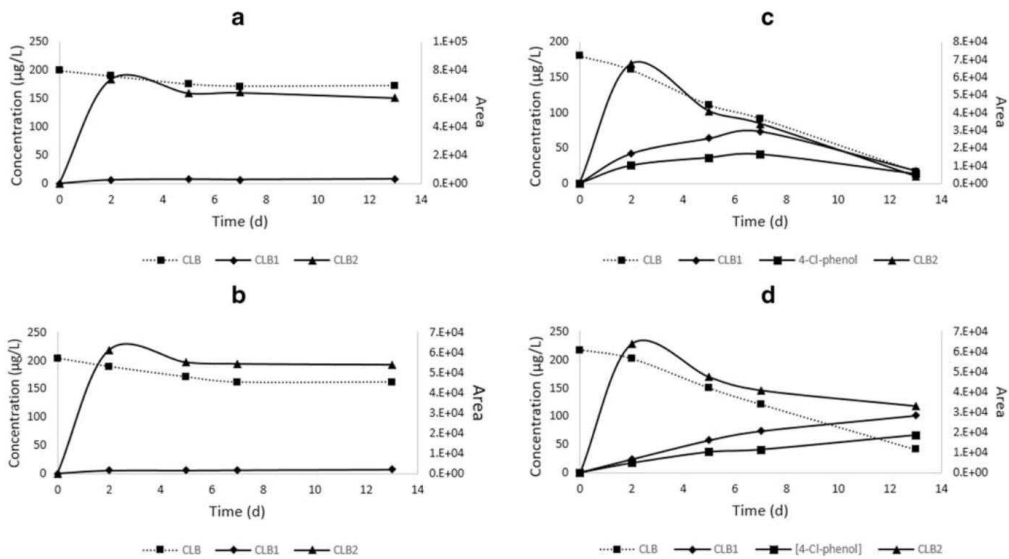


Fig. 2 Biodegradation and TPs formation kinetics of CLB by *T. asperellum* (a absence, b presence of 1-aminobenzotriazole (ABT)) and *T. harzanium* (c absence, d presence of 1-aminobenzotriazole (ABT)) after 13 days of incubation; CLB2 is plotted in function of area

shown that CYP450 are not the sole intracellular enzymes, which might be responsible for the non-steroidal anti-inflammatories (NSAIs) by *Trametes hirsuta* (Haroune et al. 2017). At

this stage, compounds uptake and intracellular transformations by others enzymes than CYP450 could not be ruled out. This assumption was reasonable because it is known that

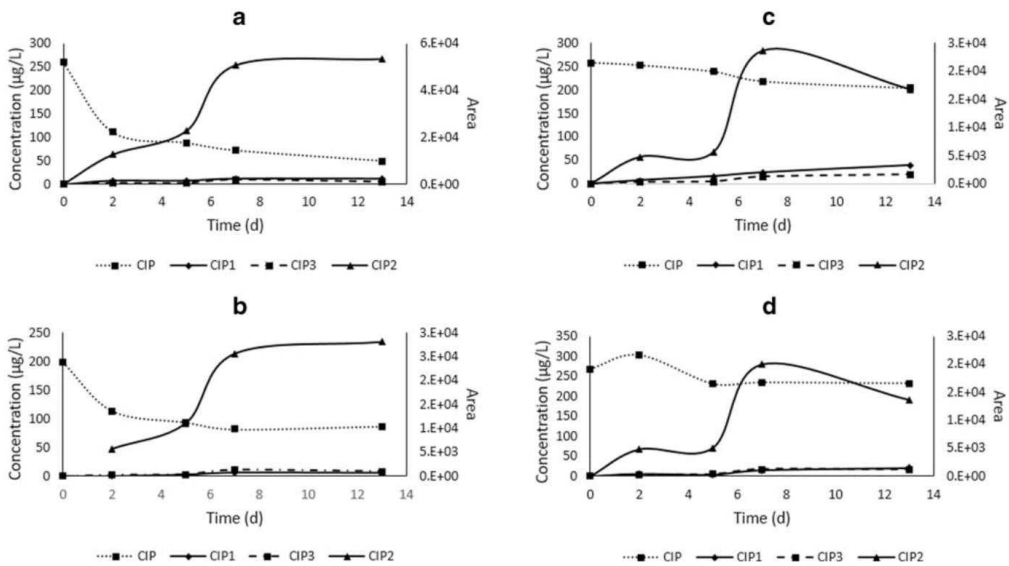


Fig. 3 Biodegradation and TPs formation kinetics of CIP by *T. asperellum* (a absence, b presence of 1-aminobenzotriazole (ABT)) and *T. harzanium* (c absence, d presence of 1-aminobenzotriazole (ABT)) after 13 days of incubation; CIP2 is plotted in function of area

T. asperellum and *T. harzanium* strains are able to secrete CYP450 enzymes (Chadha et al. 2018). TPs appeared to accumulate against time except for TPs of CLB for which bell shape profiles were observed implying a further degradation of these TPs after 7 days of incubation (Fig. 2a and c). In contrast, after ABT addition, TPs of CLB accumulated against time with a potential contribution of CYP450 in their further transformations (Fig. 2b and d). Most of CIP and OFL TPs were only slightly affected by ABT addition. Exceptions were CIP1 and OFL1 whose formation only started after 5 days and 7 days of incubation, respectively (Fig. 3b, d; Fig. 1SM). This might reflect a slower fluoroquinolone uptake by fungi in presence of ABT as previously reported for the ketoprofen analgesic (Marco-Urrea et al. 2010).

On the whole, these results indicated that both intracellular and extracellular enzymes were participating in CECs biodegradation. According to the results, it is reasonable to say that the contribution of intracellular enzymes contributed in a lesser extent than extracellular enzymes. This is somewhat different to the results obtained with WRF where it was shown that the contribution of intracellular and extracellular enzymes was equally important.

Identification of transformation products and transformation pathways

To the authors' knowledge, this work is the first one to report on the biodegradation of CLB by fungi species (i.e., *Trichoderma* spp.), while fluoroquinolones biodegradation has been previously investigated by different species including WRF (Čvančarová et al. 2015; Maia et al. 2014; Parshikov et al. 2001; Prieto et al. 2011; Wetzstein et al. 1999). Two different strategies were followed to identify TPs: (a) a suspect exact mass screening of pseudo-molecular ions $[M + H]^+$ or $[M - H]^-$ of plausible TPs identified in previous degradation studies and (b) suggested masses obtained from theoretical transformation reactions using the BiotransformationMassDefects software, where full-scan MS data of treated samples ($t > 0$) are compared with a sample at $t = 0$ h and controls to identify compound masses formed during biodegradation experiments. The only masses considered as TP candidates were the ones whose intensity changed over the time course of the biodegradation experiments and which were not present in the control experiments.

Following these analytical procedures, three, three and four TPs were identified for CLB, CIP and OFL, respectively. Extracted Ion Chromatograms showing identified TPs are included in SM (Fig. 2SM, Fig. 3SM, Fig. 4SM, Fig. 5SM and Fig. 6SM). Accurate mass measurements, MS/MS data of each TP together with their LC retention time (RT) and proposed chemical formula are reported in Table 1. MS/MS spectra of newly identified TPs are also reported in SM (Fig. 7SM, Fig. 8SM, Fig. 9SM, Fig. 10SM, Fig. 11SM and Fig. 12SM).

CIP transformation products Identical TPs (CIP1, CIP2 and CIP3) were identified with both *Trichoderma* strains, but if a mass balance was nearly achieved with *T. harzanium*, this was not true with *T. asperellum* (see Fig. 3), underlying the inability to detect all TPs in this latter case. CIP1 exhibited a protonated molecular ion at m/z 306.1252 and a fragment ion corresponding to water losses (m/z 288.1146) (Table 1). The structure of this TP was assigned to desethylene CIP and was confirmed by using an authentic standard. CIP1 was obtained by piperaziny ring cleavage. CIP2 and CIP3 (m/z 374.1516 and m/z 360.1359) were identified as N-acetyl CIP and formamide CIP, respectively; CIP2 was identified by the suspect mass screening approach, while CIP3 was confirmed by the use of a reference standard. Acetylation and formylation are common strategies of microorganisms to reduce fluoroquinolones toxicity and were previously reported under different biological treatment systems including biodegradation by WRF (Prieto et al. 2011). All these TPs were also detected with different others fungi (Čvančarová et al. 2015; Parshikov et al. 2001; Prieto et al. 2011; Wetzstein et al. 1999). The degradation of CIP from artificial contaminated soil was achieved thanks to the enzymatic activities of *Penicillium notatum*, *Aspergillus fumigatus*, *Penicillium frequentans* and *Penicillium expansum* (Zhang et al. 2012), while Parshikov and co-workers showed the ability of *Trichoderma viride* to produce conjugated CIP metabolites through chemical processes (Parshikov et al. 2001).

OFL transformation products Identical TPs (OFL1, OFL2, OFL3 and OFL4) were tentatively identified using both *Trichoderma* strains. A mass balance could not be established due to the lack of analytical standards for quantification purposes. However, on the basis of peak area, OFL2 was found to be the predominant TP. With a pseudo molecular ion at m/z 348.1350 and a fragment ion corresponding to water losses, OFL1 was identified as N-desmethyl-OFL as previously reported (Gros et al. 2014). OFL1 was also detected under biodegradation by *T. versicolor* (Čvančarová et al. 2015) and by mixed bacterial medium (Maia et al. 2014). OFL2 was detected by suspect screening approach and was characterized by an increase of an oxygen atom mass unit (m/z 378.1461) with respect to OFL likely due to hydroxylation processes. Due to non-specific fragmentations (H_2O and CO_2 losses), it was difficult to assign a definitive chemical structure to this TP with a high degree of certainty. In particular, it was not possible to discriminate between alkane hydroxylation and aromatic ring hydroxylation. The structure of OFL3 (m/z 318.1612) was tentatively assigned to decarboxylated OFL while with a loss of $2 \times H$ mass units with respect to OFL, OFL4 (m/z 364.1573) was likely generated after the reduction of the carbonyl function into a secondary alcohol. Čvančarová and co-authors (Čvančarová et al. 2015) reported a metabolite with m/z 364. However, its exact mass and its structural formula

Table 1 Summary of CIP, OFL and CLB identified TPs together with their parent compounds, retention time (RT), measured and theoretical $[M + H]^+$ or $[M - H]^-$ ions, MS/MS fragment ions and tentative structures

Compound	Ion	RT (min)	Trichoderma sp.	Relative mass error (ppm)	Observed m/z	Theoretical m/z	Formula	
CIP	$[M + H]^+$	2.6	<i>T. harzanium</i>	-1.50	332.1405	332.1410	$C_{17}H_{18}FN_3O_3$	
		2.6	<i>T. asperellum</i>	-2.10	332.1403			
CIP1	$[M + H]^+$	2.4	<i>T. harzanium</i>	-0.32	306.1252	306.1253	$C_{15}H_{16}FN_3O_3$	
				1.04	288.1146			288.1143
				-16.78	268.1036			268.1081
	$[M + H - H_2O - 20]^+$	2.4	<i>T. asperellum</i>	0.65	306.1255	288.1134		
				-3.12	288.1134			
				5.96	268.1097			
CIP2	$[M + H]^+$	3.8	<i>T. harzanium</i>	0.26	374.1516	374.1515	$C_{19}H_{20}FN_3O_4$	
				-0.28	356.1404			356.1405
	$[M + H - H_2O]^+$	3.8	<i>T. asperellum</i>	0.26	374.1516	356.1403		
				-0.56	356.1403			
CIP3	$[M + H]^+$	3.7	<i>T. harzanium</i>	0.00	360.1359	360.1359	$C_{18}H_{18}FN_3O_4$	
				2.63	342.1257			342.1248
				0.00	360.1359			
				-0.29	342.1247			
OFL	$[M + H]^+$	2.5	<i>T. harzanium</i>	-1.38	362.1510	362.1515	$C_{18}H_{20}FN_3O_4$	
				-1.93	362.1508			
OFL1	$[M + H]^+$	2.5	<i>T. harzanium</i>	-2.59	348.1350	348.1359	$C_{17}H_{18}FN_3O_4$	
				0.30	330.1249			330.1248
				0.29	348.1360			
				-4.54	330.1233			
OFL2	$[M + H]^+$	2.7	<i>T. harzanium</i>	-1.06	378.1461	378.1465	$C_{18}H_{20}FN_3O_5$	
				0.28	360.1355			360.1354
				0.30	334.1562			334.1561
	$[M + H - H_2O]^+$	2.7	<i>T. asperellum</i>	-0.53	378.1463	360.1351		
				-0.83	360.1351			
				-0.60	334.1559			
OFL3	$[M + H]^+$	2.5	<i>T. harzanium</i>	-1.57	318.1612	318.1617	$C_{17}H_{20}FN_3O_5$	
				0.00	261.1034			261.1034
				0.00	318.1617			
				8.43	261.1056			
OFL4	$[M + H]^+$	2.5	<i>T. harzanium</i>	3.1	364.1573	364.1672	$C_{18}H_{22}FN_3O_4$	
				-2.51	318.1604			318.1612
				2.5	364.1562			
				5.03	318.1628			
CLB	$[M + H]^+$	4.9	<i>T. harzanium</i>	3.07	293.1065	293.1056	$C_{15}H_{17}ClN_2O_2$	
				7.85	293.1079			
CLB1	$[M + H]^+$	4.3	<i>T. harzanium</i>	-4.07	295.1201	295.1213	$C_{15}H_{19}ClN_2O_2$	
				0.00	69.0443			69.0447
				16.26	295.1261			
				1.45	69.0448			
CLB2	$[M + H]^+$	3.9	<i>T. harzanium</i>	1.93	311.1168	311.1162	$C_{15}H_{19}ClN_2O_3$	
				27.52	69.0466			69.0447
				8.04	311.1187			
				26.07	69.0465			
4-chlorophenol	$[M - H]^-$	5.3	<i>T. harzanium</i>	3.15	126.9955	126.9951	C_6H_5ClO	
				-5.49	91.0184			91.0189

did not match to the one in our experiments. This led us to propose another structure for this TP. Due to the small amount of OFL2, OFL3 and OFL4 found in the sample extracts, the obtained mass spectra information did not permit a definitive structure elucidation of these TPs.

CLB transformation products CLB1 and CLB2 were detected with both *Trichoderma* strains, while an additional TP (i.e., 4-chlorophenol) was also detected with the *T. harzanium* strain in the negative mode of ionization as shown in Fig. 4. CLB1 exhibited a protonated molecular ion at m/z 295.1201

corresponding to an increase of $2 \times H$ mass unit with respect to CLB due to the reduction of the carbonyl group to a secondary alcohol to give CLB-OH. CLB1 was confirmed by the injection of an authentic standard and was detected under anaerobic activated sludge biodegradation conditions (Brienza and Chiron 2017) and under biodegradation by the freshwater algae *Scenedesmus obliquus* (Pan et al. 2018). CLB2 was characterized by a pseudo-molecular ion $[M + H]^+$ at m/z 311.1168 (Fig. 4), which corresponds to an increase of an oxygen mass unit probably related to the hydroxylation of CLB2. Due to the higher reactivity of the benzene ring in comparison with the tert-butyl alkyl group, hydroxylation is highly expected on the aromatic ring. 4-chlorophenol was likely obtained by ether cleavage of CLB and was detected for the first time.

On the basis of the identification of TPs by LC-HRMS, transformation pathways of CIP, OFL and CLB are proposed in Fig. 5. As observed, CIP and OFL did not share common transformation pathways. CIP transformation was mainly featured by oxidation of the piperazinyl ring as well as by conjugation reactions leading to acetyl-N-CIP and formyl-N-CIP. These transformations were previously reported with WRF and with filamentous fungi (Olicón-Hernández et al. 2017).

In contrast, OFL mainly underwent hydroxylation processes. This was due to the requirement of N-desmethylation of the piperazine ring before this latter been oxidized. Others minor pathways involved OFL decarboxylation reaction together with the carbonyl function reduction into secondary alcohol.

As far as CLB was concerned, the main transformation pathway involved the reduction of the carbonyl moiety. This

was also in compliance with the possible involvement of carbonyl reductases, which catalyze ketone reduction by direct hydride transfer from NAD(P)H cofactor. This reduction reaction was followed by a hydroxylation pathway. More interestingly was the detection of 4-chlorophenol as a major TP resulting from O-dealkylation reactions. These O-dealkylation reactions were related to fungal peroxygenase (Poraj-Kobielska et al. 2011) and was previously observed during the course of the biotransformation of clarithromycin by *T. asperellum* leading to the formation of descladonisyl-clarithromycin (Buchicchio et al. 2016).

As a whole, biotransformation of selected CECs included hydroxylation, oxidation/reduction and N- and O-dealkylation reactions, indicating that the oxidoreductase extracellular enzymes, such as laccase and peroxidase, were mainly responsible for CEC degradation processes. Laccase enzymes are known to perform soft oxidation reactions such as hydroxylation, methylation, demethylation and deacetylation (Barr and Aust 1994), while peroxidases are more potent enzymes able to perform harder oxidative reactions such as N- and O-dealkylation reactions. Consequently, *Trichoderma* spp. likely possess a machinery of unspecific enzymes, which may be similar to that of WRF and which could make them suitable for CEC residue elimination in wastewater or soil. The internal mechanism of detoxification mediated by CYP450 family has been pointed out as a main contributor for transforming different xenobiotics by fungi particularly by fungi characterized by a low secretion of lignin-modifying enzymes (e.g., ascomycota). However, this contribution was not so obvious in this study probably accounting for a slower degradation kinetics in comparison with studies with WRF.

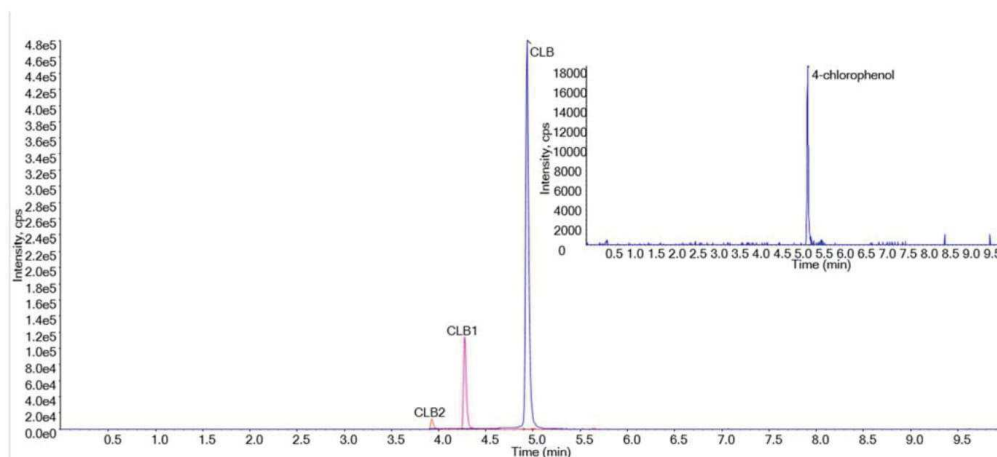


Fig. 4 Extracted Ion Chromatogram (XIC) corresponding to the analysis of TPs of climbazole after 7 days incubation in *T. harzanium* culture. Insert corresponds to analysis in negative ionization mode

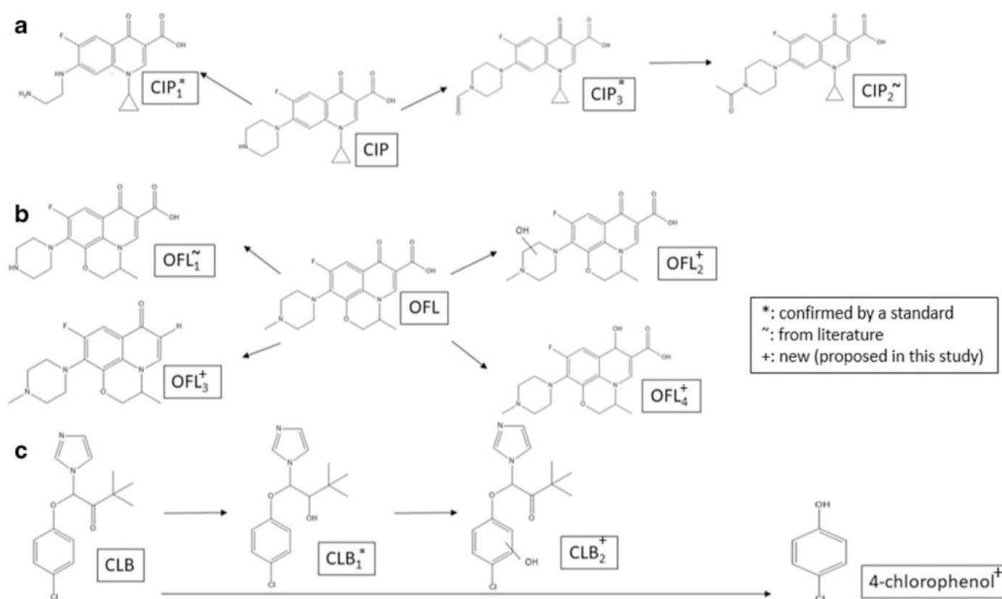


Fig. 5 Proposed transformation pathways of CIP (a), OFL (b) and CLB (c)

Conclusion

Filamentous fungi-mediated treatment of CECs is a promising and environmentally friendly technology. In this study, the fluoroquinolone antibiotics CIP and OFL and the CLB fungicide were efficiently removed by *Trichoderma* spp. in liquid medium. This study was one of the first ones reporting the capacity of these fungi to degrade CECs. The tentative identification of their transformation products was achieved, and biotransformation has turned out to be the predominant degradation process. The evidence of their degradability by *Trichoderma* spp. is of interest since CECs are poorly eliminated in conventional activated sludge treatments. Thanks to the high selectivity and mass accuracy provided by LC-HRMS, a significant number of unknown TPs were tentatively identified in experiments at Erlenmeyer scale. The toxicity tests of the generated TPs were not taken into consideration in this work because of the low experimental concentrations applied. Transformation mechanisms relied on a wide array of enzymatic reactions including conjugation, hydroxylation and oxidation/reduction reactions likely due to an unspecific extracellular enzyme system. This result is promising because the degradation of a wide range of pharmaceuticals can be contemplated. Even though *Trichoderma* spp. have been found to grow rapidly in adverse environmental conditions, more research is needed to evaluate the effectiveness of these fungi to remove pharmaceutical residues at environmental

concentrations in more realistic conditions such as in domestic wastewater effluents. Further experiments are also needed to optimize the capacity of *Trichoderma* spp. in degrading CECs and to assess the toxicity of the generated TPs.

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Conclusions and perspectives

The entry of PhACs to agroecosystems are very diverse, though crops irrigation with treated wastewater appeared to be the main pathway as a result of water shortages. In Europe, around 70 % of water consumption goes to the agricultural sector. Wastewater effluents irrigation, besides to natural fertilizers application such as sewage sludge, biosolids, and medicated animal manure and slurries are considered valuable resources rather than waste products in line with circular economy rules. However, the poor removal ability of PhACs from wastewater in conventional WWTPs lead to the continuous introduction of undesired compounds to agroecosystems through crops irrigation, and land fertilization.

Once PhACs attend agricultural soil they undergo diverse processes. For instance, PhACs can be sorbed onto soil particles, persist and accumulate in soil, degraded by biotic and abiotic processes, leach to ground water and runoff to surface water and surrounding environment, and most interestingly taken up by growing crops. PhACs deriving from treated wastewater or natural fertilizers enter crops through plant roots and may accumulate in various plant parts. Plant uptake depend on various parameters including PhACs physico-chemical properties, soil properties, plant physiology and environmental factors. To understand the fate of PhACs in the agroecosystem once they have been emitted from treated wastewater irrigation, sensitive analytical methodologies are required for their detection and quantification at trace levels as well as for the identification of their metabolites in the various agroecosystem matrices like soil and different plant parts. Up to the present, numerous analytical methods have been reported for the detection and quantification of PhACs and their metabolites in wastewater and surface water. On the contrary, the number of robust analytical methods for their determination in the agroecosystem matrices is still quite few.

In this context we have settled the first objective in this PhD research, coping with analytical methods optimization and development for detecting and quantifying a large number of PhACs and other wastewater contaminants and their metabolites in soil, lettuce/leek roots and lettuce/leek leaves. The vegetable selection in this PhD research refer to various reasons, most importantly they are commonly consumed raw worldwide, lettuce is known to has high ability to take up PhACs in its edible tissues, and leeks are slow growing crops allowing for likely higher PhACs accumulation. Generally, the roots of plants have no commercial or food value, yet it plays an important role for PhACs uptake to leaves and edible tissues. Moreover, PhACs that are not able to translocate from roots to shoots, reside in the root's part. Hence

the study of PhACs occurrence in root matrix highly contribute to conduct a comprehensive figure of PhACs fate in agroecosystems. To our knowledge, this is the first time a study was fully dedicated to develop an analytical method for detecting and quantifying PhACs in root matrix. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) based methods were successfully used for the simultaneous extraction of 48 contaminants from the desired matrices. Detection and quantification were accomplished on a high-resolution mass spectrometry coupled to liquid chromatography, using the recently developed high-resolution multiple reaction monitoring (MRM^{HR}) acquisition mode provided from the high-resolution mass spectrometry on quadrupole-time of flight (Q-TOF) instrument from SCIEX technology. Owing to the big differences between matrices components of agroecosystem, hence the various cleanup necessity for each matrix, we made our way to develop one method specific to lettuce leaves and another common method specific to lettuce root and soil. Methods performance was compared with a recently developed acquisition mode “SWATH, Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra” in terms of accuracy, repeatability, sensitivity, linearity and matrix effect. Satisfactory recoveries were obtained for most of the compounds in all 3 matrices (in general from 80 to 120%), with relatively low detection and quantification limits with lowest values of 0.01 ng/g and 0.04 ng/g d.w, respectively. Similar results were obtained from MRM^{HR} and SWATH acquisition modes, yet more consistent results for a greater number of compounds were achieved using MRM^{HR} acquisition. On the whole, QuEChERS proved to be as good or even better than other sophisticated, time consuming and expensive extraction methods (such as ASE, USE and MAE), not to forget that QuEChERS is the greenest between them. Few modifications in the original methods allowed for higher recoveries of a large number of compounds. Finally, SWATH proved to be as good as MRM^{HR}, and could be promising for non-target analysis in future work.

The presence of PhACs in edible crops is nowadays an important source of concern to human health. Despite the fact of their presence at quit low concentrations, the continuous exposure of PhACs may increase human and ecosystem health risk. Moreover, a large number of studies has demonstrated the ability of growing crops to uptake PhACs form contaminated irrigation water. However, most of those studies have been carried out at laboratory scale, under controlled conditions and for short time. Limited data are available as of now under

real field growing conditions, which give realistic appropriate risk assessment of human health related to wastewater irrigated crops consumption at long term. Under this scenario, our second objective of this PhD research was settled: by the means of the previously developed analytical methods, we aimed to follow the fate of 14 wastewater-borne contaminants including PhACs and their metabolites in realistic growth and irrigation conditions. A greenhouse is built in Murviel-Lès Montpellier wastewater treatment plant, lettuce and leek were grown in successive crop cycles for 2 years in soil collected from local agricultural field, and irrigated with different kind of water, mainly local domestic treated wastewater. We have also followed the distribution of contaminants in soil and edible parts of lettuce and leek, allowing for bioconcentration factor calculations and assessment of the risks related to human consumption. Results revealed limited accumulation of contaminants in soil and crops, explained mainly by the degradation processes taking place on surface soil. a few metabolites have been found in lettuce leaves with different confirmation levels, namely carbamazepine-epoxide detected at level 1 of confirmation thanks to the availability of analytical standard, two metabolites of metoprolol were confirmed at level 2, another two and one metabolite of irbesartan were confirmed at level 4. Finally, this study confirmed earlier reports on the a *de minimis* human health risk related to the consumption of raw leafy green vegetable irrigated with domestic TWW containing organic contaminants residues. However, further work is still needed for other kinds of crops and vegetables in order to withdraw concrete and wider conclusions on human health risks, especially for long term irrigation and chronic exposure.

Agricultural soil accommodates numerous microorganisms. The filamentous *Trichoderma* fungi is ubiquitous in all soil types, they are able to grow and live under severe conditions, they colonize plants roots and live in symbiont relationship with plants. many studies were focused on the ability of the White-Rot fungi, especially *Trametes versicolor*, to eliminate PhACs. However, and according to our knowledge, no studies up to now are reporting the ability of *Trichoderma* genus in removing PhACs. Granting the fact that they can be beneficial in removing the PhACs introduced to agriculture soil by wastewater irrigation. In this context, our third objective in this PhD research was to follow the fate of selected contaminants following their exposure to *Trichoderma* fungi, exactly *T. harzanium* and *T. asperellum*, in liquid medium for the first time. The selected contaminants were two fluoroquinolone

antibiotics (ciprofloxacin and ofloxacin) and one personal care product (climbazole). Good removal efficiencies were observed with both *Trichoderma*, yet contrasting results were observed between them after 13 days of incubation: *T. asperellum* removed 81% of ciprofloxacin, while *T. harzanium* removed 91% of climbazole. While ofloxacin was removed with degradation rate around 40% with both strains. Many TPs have been detected during the time course of the experiment, they were mainly formed by hydroxylation, oxidation/reduction, and N- and O-dealkylation reactions. Equally important, two metabolites from ofloxacin and one from climbazole were tentatively identified for the first time in this study. The analysis of *Trichoderma* biomass at the end the incubation time revealed that biosorption process played a very limited role in their elimination. On the whole, this study revealed the ability of *Trichoderma* to eliminate organic contaminants, however this should be taken to an environmental level and real agricultural soil in further studies. Additionally, more concern should be focused on the toxicity of the produced TPs.

Taking into account that today's solutions should be simple, fast and cheap, we mention few perspectives and future research and responsibilities:

1. Till the present there is no regulations about the minimum acceptable concentration of PhACs and wastewater-borne contaminates in edible crops. Regarding that water scarcity and shortages are kind of making the reuse of treated wastewater for crops irrigation an initial solution, it is therefore on the top list of emergencies to establish regulations on the acceptable concentrations of PhACs in treated wastewater destined for crops irrigation, and their occurrence in the edible crops destined for human consumption.
2. Therefore, more work should be dedicated for the development of green, fast and cheap analytical methodologies, for the detection and quantification of a large number of PhACs in environmental matrices, most importantly agroecosystems including soil and edible crops. In addition, these methodologies should provide high accuracy, reliability and robustness allowing for continuous monitoring of PhACs in the environment, particularly in crops intended for human consumption, and not to forget drinking water. In consequence this will be a key for promoting environmental public policies.

3. Many research studies have been done to improve wastewater treatment processes, recently there is a growing trend focusing on improving sustainability and reducing energy demand of wastewater treatment. Our findings regarding *Trichoderma* fungi ability to eliminate PhACs are very promising. The following work should take these fungi to another level, such as: (1) investigate its success and incorporate it in wastewater treatment processes, (2) investigate if *Trichoderma* is able to eliminate the introduced PhACs through irrigation in soil. Equally important, resulting TPs toxicity should be also taken in considerations.
4. Finally, in view of the incredible effort made by scientists during the last couple decades to follow the fate of PhACs in the environment including agroecosystems and to assess their potential risks to human health, at the same time efforts should also be focused to reduce or prevent their improper discharge to the environment. For instance, to conduct awareness campaigns and educate people on the potential risk of these compounds on the environment, coming back to their own health. Prevent their overuse and the improper disposal procedures of unused PhACs in the toilets, the drain and into the waste, and instead for example prepare specific disposal trash cans for each neighborhood or district.