LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY DETERMINATION OF SIX PHARMACEUTICALS IN VEGETAL BIOTA. UPTAKE STUDY IN *LAVANDULA DENTATA*

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ABSTRACT

A procedure based on microwave assisted extraction for the determination of 6 pharmaceuticals in samples of *Lavandula dentata*, *Salicornia ramosissima* and *Juncus sp*. by liquid chromatography-quadrupole time of flight mass spectrometry (LC-QTOF/MS) was optimized and validated. Best results were obtained using microwave assisted extraction of 1.0 g of homogeneous lyophilized samples and 5 mL of a mixture ACN:H_2O (1:1 v/v) as extracting solvent. Analytical recoveries ranged from 60 to 107 % with relative standard deviation (RSD) lower than 15 %. Limits of quantitation (LOQ) for the 6 pharmaceuticals flumequine (FLM), carbamazepine (CBZ), ciprofloxacin (CPR), enrofloxacin (ENR), diclofenac (DCL), and ibuprofen (IBU) were in the range 20.8-125 ng g^{-1}. The method was satisfactory applied for an uptake study in *Lavandula dentata* samples finding quantifying concentrations of FLM and CBZ in roots, leaf and stem.

Keywords: liquid chromatography quadrupole time-of-flight, pharmaceuticals, microwave extraction, lavandula dentata, Juncus, Salicornia

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1. **Introduction**

It is well known that the processes used in wastewater treatment plants (WWTPs) do not eliminate many substances including those namely as emerging pollutants that include, among others, pharmaceuticals products (Tauxe-Wuersch et al, 2005; Hijosa-Valesco et al., 2011; Al-Qaim et al., 2013; Al-Qaim et al., 2015). The use of treated wastewater to irrigate many arid and semi-arid areas has become a usual practice years ago. Additionally, since 1991 the European Union Regulations established that all its member states must treat urban wastewaters prior to their discharge into riverbanks, lakes and seas (EC council directive, 1991).

Surface and groundwater can contain variable amounts of active emerging pollutants even at points far from urban and industrial effluents. Thus pharmaceuticals have been found in all kind of waters and sediments around the world (Tiedekena et al. 2017), even in those from protected areas such as Natural Parks (Cesário et al. 2016, Hrabovsky et al. 2004, Carmona et al. 2014).

In the last decades of the 20th century, environmental interest has focused on some kinds of substances that until then had not been considered contaminants: the emerging pollutants. It is also well known that several emerging pollutants can be easily incorporated to the biota from the aquatic media. Thus, for example, some antibiotics have been found in sea fish samples from the southern of Spain (Fernandez-Torres et al. 2011), green algal species have been proposed for depuration of pharmaceutical pollutants in urban wastewater (Gentili et al. 2016) and diverse pharmaceuticals (antibiotics, non-steroidal anti-inflammatory drugs, lipid regulators, steroid estrogens, β-adrenoceptor blocker, antiepilectic drug, antifungal and antidepressant) have been found in 14 aquatic species from a Chinese lake (Zhengxin et al. 2017).

The plants can incorporate, through the roots essentially, compounds dissolved in the water that irrigates them. The incorporation of heavy metals to all kind of plants and the analytical strategies for their analysis have been widely studied for many years (Arasimowicz et al. 2013, Martínez-Alcalá et al. 2017). On the contrary, less attention has been paid to how pharmaceuticals incorporate to plants. However, these processes have been studied in detail in the case of vegetables intended for human consumption (Riemenschneider et al. 2017a, Riemenschneider et al. 2017 b). Thus, Boxall et al. (2006) and Marsoni et al. (2014), among others, showed that pharmaceuticals were accumulated

by plants when soil was irrigated at environmentally-realistic concentrations. Some authors have measured variable amounts of antibiotics (Bao et al. 2010, Hu et al. 2010, Yao et al. 2010) or antiepileptic drugs (Shernker et al. 2011) in vegetables and several pharmaceuticals of diverse nature have been measured in root vegetables (Malchi et al. 2014). Other studies have shown that organic compounds from the soil and water can be accumulated in different parts of the plants at different concentrations and rates (Al Nasir et al. 2008, Redshaw et al. 2008, Shernker et al. 2011).

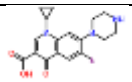
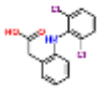
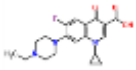
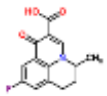
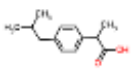
Additionally to uptake studies, the possible toxicity of certain pharmaceuticals for the plants has been also demonstrated. Thus, the toxicity of ENR in four agricultural crops was evaluated and it was found that water levels in the range 50-5000 μg L^{-1} produced toxic effects. Likewise, it was demonstrated that the plants metabolized enrofloxacin to ciprofloxacin, like in animals (Migliore et al. 2003). Kong et al. (2003) evaluated the uptake and phytotoxicity of oxytetracycline to alfalfa.

The analysis of organic compounds in vegetables and plants frequently include long and tedious extraction procedures followed by a clean-up step in order to eliminate interfering substances co-extracted with the interesting analytes. In general, the usual extraction procedures are ultrasounds assisted solid-liquid extraction or accelerated solvent extraction on dried or lyophilized samples. On the other hand, solid phase extraction (SPE) using different stationary phases is the most usual clean-up procedure. (Vaclaik et al. 2014, Emhofer et al. 2017).

In this work, our main objectives were (i) to develop and validate an analytical method based on high performance liquid chromatography coupled to quadrupole time of flight (UHPLC-Q-TOF) mass spectrometry detection to determine FLM, ENR, CPR, CBZ, DCL and IBU (see table 1 for physicochemical characteristics) in vegetal samples. Previous studies have reported the presence of these compounds, among others, in environmental samples (mainly water and sediments) due to a possible inefficient removal in wastewater treatment plants or even by direct discharge of untreated wastewaters (Gros et al. 2010, Camacho-Muñoz et al. 2010, Camacho-Muñoz et al. 2012, Verlicchi et al. 2012). Great interest show their presence in protected areas like Doñana National Park among others (Camacho-Muñoz et al. 2013, Carmona et al. 2014, Rivera-Jaimes et al. 2018) where their presence might suppose a potential risk for the surrounding ecosystem. The vegetal species selected for this study were *Lavandula*

dentata, Salicornia ramosissima and *Juncus sp*. These plants were selected for several reasons, they are much extended in the Mediterranean area ecosystems where several protected areas are located, they grow in different habitats which is important to evaluate the global risk on wild species, and lavandula was easily accessible to overcome an uptake study in the laboratory. Juncus sp. is an aquatic specie that usually grow in river banks and wetlands margings, Salicornia is a plant that grows in coastal salt marshes and inland salty habitats and Lavandula is a non-aquatic specie very extended through Mediterranean ecosystems. (ii) After validation, the optimized procedure was applied to overcome a study to evaluate the uptake of the selected drugs in plants irrigated with a mixture of them at three different levels of concentration for a period of one month. The analysis of the drugs was realized in leaves, stems and roots of the plant at different times in order to evaluate different behaviours in the ability of the plant to uptake the analytes through the time. Usually, these studies are carried out in short or long periods of irrigation, in this paper we have performed the uptake study of the selected drugs at several irrigation times in order to evaluate a possible change in the plant uptake behaviour throughout the time.

Table 1. Physicochemical properties of selected pharmaceutical chemicals.

Compound	Structure	Therapeutic class	log P[a]	pKa[a]	log Kow
Ciprofloxacin		Antibiotic	0.65	6.43	0.28[a]
Carbamazepine		Antiepileptic agent	2.67	13.9	2.45[b]
Diclofenac		Non steroidal antinflamatory	4.02	4.15	4.51[c]
Enrofloxacin		Antibiotic	1.88	6.43	0.70[a]
Flumequine		Antibiotic	2.41	5.70	2.60[a]
Ibuprofen		Non steroidal antinflamatory	3.72	4.41	3.97[c]

[a] Predicted values from database of Royal Society of Chemistry: http://www.chemspider.com.
[b] Dal Pozzo et al. 1989.
[c] Avdeef et al. 1997

The procedure substitutes the traditional solid phase extraction clean up by a dilution of the extract from a microwave assisted extraction to minimize the matrix effect obtaining good results in terms of recovery for the selected analytes. The simple procedure of

extraction allow the sample processing in a very short time as well as not loosing of information of the processed sample which would be compatible with the searching of metabolites of the selected compounds in future works.

2. Experimental

2.1 Chemicals and reagents

Solutions and dilutions were prepared using ultrapure water from a Milli-Q Plus (Millipore, Billerica, MA, USA) water purification system. Formic acid 98-100% Suprapur was supplied by Merck Darmstadt (Germany), acetonitrile and water LC-MS Ultra Chromasolv® were from Sigma-Aldrich (Madrid, Spain), sodium hydroxide in 2-propanol:water and Leucine Enkephalin were purchased from Waters (Barcelona, Spain).

2.2 Standards and stocks solutions

FLM, ENR, CPR, CBZ, DCL and IBU were purchased from Sigma–Aldrich (Madrid, Spain). The purity of all the standards was higher than 98% in all cases.

Methanolic stock solutions 100 µg mL^{-1} were prepared for all the pharmaceuticals except CPR that was prepared by diluting 2.5 mg in 5 mL ultrapure water to a final volume of 25 mL with methanol. Aqueous working solutions of the studied compounds were prepared daily by adequate dilutions from 100 µg mL^{-1} stock solutions.

2.3 Sample collection and preparation.

Blank samples of *Lavandula dentata, Salicornia ramosissima* and *Juncus sp* for optimization purposes were collected from Doñana´s Natural Park area. Samples for method development and validation were cleaned of remnants of soil with tap water first, subsequently with deionized water and finally dried with a paper towel. After that, the samples were lyophilized before being analysed by a freeze dry system (FreZone 2.5, Labconco, Mo, USA). Then they were homogenized using a grinder and kept at −80 ◦C until analysis.

To optimize the method, lyophilized samples were spiked with 5 mL aqueous solution, containing the analytes at the adequate concentration, added drop by drop using stir bar magnetic agitation to assure that the compounds spread throughout the sample and were in sufficient contact with the matrix. The spiked samples were left in contact for all night

at 4ºC and then freeze for 2 hours at -80ºC. The freeze samples were lyophilized again to eliminate the solvent and then submitted to the extraction procedure.

Samples for the uptake study were purchased in a local plant nursery and kept in our laboratory, under adequate light and water conditions for two weeks until the assay. Before extraction, plant leaves, stern and roots were washed with tap water to remove soil residues, then rinse with deionized water and dried with a paper towel. Then, each part of the plant was freeze at -80 ºC for 24 h and lyophilized. Dried samples were ground to powder and stored at -80°C until extraction.

2.4 Sample extraction

5 mL of a mixture ACN:H_2O (1:1 v/v) were added to 1.0 g of homogeneous lyophilized samples. Microwave energy was applied at 50 watts for 5 minutes to achieve the extraction. After that, the samples were centrifuged during 15 min at 6000 rpm and 1 mL of supernatant was taken and diluted 1:5 (v/v) with a mixture ACN:H_2O (1:1 v/v). Finally, the extracts were microfiltered through 0.22 µm PTFE filters (VWR, Spain) before injection into the UPLC system.

2.5 Liquid chromatography- mass spectrometry analysis

The analysis of the selected pharmaceuticals was performance in an Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) coupled to a Xevo G2S QTOF mass spectrometer (Waters, Micromass, Manchester, UK) with an orthogonal Z-spray lockspray and dual electrospray interface (ESI). Detection was achieved operating in positive and negative ionization modes. The LC separation was performed using a Zorbax Eclipse XDB-C18 analytical column (100 mm × 3.0 mm i.d., 3.5 µm particle size) preceded by a guard column Zorbax Eclipse XDB C-8 (12.5 mm × 2.1 mm i.d., 5 µm particle size) (Agilent Technologies Spain, Madrid, Spain). The column temperature was maintained at 25ºC. The mobile phase used for MS and MS/MS experiments consisted of aqueous formic acid solution 0.05% (solvent A) and acetonitrile (solvent B). Two different gradient elution programs at a flow rate of 0.5 mL min^{-1} were used for both positive and negative ionization modes. The elution in positive ionization mode starts at 90% A and 10% B increasing % B from 10% to 90 % in 8.0 min, returning then to initial conditions in one minute. Three minutes were waited between injections which allowed re-equilibration of the column to initial conditions. Negative ionization

183 elution programme starts at 70% A increasing the B percentage until 100% in 7.0 min
184 returning to initial conditions in one minute more. An equilibration time of 2 min was
185 needed before next injection. The injection volume was 5 µL in both cases.

186 2.6 Mass detection conditions

187 The nebulisation gas (nitrogen, supplied by a high purity nitrogen generator Nitrogen
188 Zefiro 35 LC-MS, Cinel-gas, Italy) was set at 600 L/h and the cone gas (nitrogen) was set
189 to 30 L h^{-1}, desolvation temperature was set to 400ºC and source temperature to 100ºC
190 for positive polarity and 120ºC for negative polarity. MS and MS/MS experiments were
191 operated in both (negative and positive) ionization modes. The resolution of TOF mass
192 spectrometer was approximately 30.000 at full width half maximum at m/z 556 and
193 22.000 at m/z 554. The capillary voltage was set to 1.5 kV for positive ionization and -
194 2.00 kV for negative, and sample cone voltage at 40 V. For MS spectra data being
195 collected in continuum between *m/z* 50 and 1000 in a scan time of 1.0 seconds. For
196 MS/MS experiments the following conditions were used: cone voltage 40, collision
197 energy (see table 2), collision gas was argon (99.995%, Praxair). The precursor ion was
198 selected using the quadrupole analyser, fragmented in the collision cell and subsequently
199 product ion spectra was recorded in the TOF analyser from *m/z* 50 to 800. All the obtained
200 spectra were lock mass corrected. An independent reference spray (lockSpray) to ensure
201 accuracy and reproducibility was used for all analyses. Leucine Enkephalin (200 pg μL^{-1}
202 in ACN/H_2O 1:1 v/v at 0.1% HCOOH) was used as lock mass (*m/z* $[M+H]^+$ 556.268 and
203 *m/z* $[M-H]^-$ 554.2615) at a flow rate of 10 µL min^{-1}. The lockSpray frequency was set at
204 30 s, meaning that every 30 s the flow from the lockSpray needle was introduced into the
205 mass spectrometer for 0.3 s, thus giving the software the possibility to perform on going
206 correction of the exact mass of the analyte. The lockSpray capillary was set at 2.54 KV.
207 Data were averaged over 3 spectra min^{-1}. Xevo G2S Q-TOF mass spectrometer was
208 calibrated in mass range of *m/z* 50-1200 with a mixture of NaOH 0,1M and formic acid
209 of 10% in ACN:H_2O (80:20 *v/v*) daily. Data were acquired and processed by MassLynx
210 v4.1 software.

211 2.7 Method Validation

212 The proposed method was validated checking the quality parameters of the analytical
213 method. We have mainly considered the items related to the estimation of the well-known
214 performance characteristics parameters. The following criteria were considered: linearity,

sensitivity (limit of detection and quantitation), matrix effect, precision and recovery (trueness).

Linearity was evaluated by matrix-matched calibration curves by spiking blank extracts at ten concentration levels in the range 0.5 to 500 ng mL^{-1}.

Instrumental detection and quantitation limits (ILOD, ILOQ) and method detection and quantitation limits (LOD, LOQ) were calculated as the minimum concentration of analyte giving peaks whose signal to noise ratios are 3 and 10, respectively, for each vegetal matrix in spiked samples (n=3).

To evaluate the precision of the method, the repeatability (in one single day) and intermediate precision (in different days) were studied. Precision study was performed using the prediction of actual concentrations from the validation standards selected for the analytical assay and calculating a RSD.

Matrix effect was evaluated by comparing the slopes of the matrix-matched calibration for each part (leaf, stem and root) of the plant for the three selected plants, and a calibration prepared in solvent (external calibration). A matrix effect percentage (ME%) was calculated by ME (%) = [(Sm/Se)-1]x100 where Sm is the slope of the matrix-matched calibration and Se the slope of the external calibration in solvent, considering that 0% indicates no matrix effect, and values above indicates enhancement effect and values below indicates ion suppression.

The trueness was evaluated by means of recovery assays on spiked samples (see section 2.3 for details). The recoveries were calculated as percentage of extracted compound at low and high level of concentration for each kind of matrix and compound. Spiked samples at 25 (125 for IBU) and 500 $ng \cdot g^{-1}$ final concentration (in triplicate) were submitted to the extraction procedure.

2.8 Uptake study design

Lavandula dentata plants were purchased in a local plant nursery in order to ensure the homogeneity of the sample as much as possible and then they were subjected to an uptake study of a mixture of the six drugs for 30 days. Before beginning the experiment, the plants were left to achieve acclimatization to the new light and temperature conditions for

two weeks and those that were not healthy or seemed to be affected by the change of environment were discarded.

Eight plants of 30-40 cm length were used for the experiment at three concentration levels: 10 $ng \cdot mL^{-1}$ (low level of exposition); 700 $ng \cdot mL^{-1}$ (medium level of exposition) and 10 $\mu g \cdot mL^{-1}$ mL (high level of exposition). Two plants per level were irrigated with 20 mL of solution, containing all the analytes in the concentration described, every 2 or 3 days for one month by pouring the solution directly onto the soil. The low volume of water used assured that all of the solution containing the analytes remained in the soil until the next water addition. Additionally, two plants were selected as blanks (controls) and they were irrigated with tap water following the same irrigation pattern as uptake study plants.

Cuts of leaves and stems were made every 7 days in order to evaluate the accumulation of the compounds through time. Once the assay was finished, roots were also collected.

3. Results and discussion

3.1 UPLC-QTOF/MS method development

Initially, mass spectra for each target compound individually was analyzed by direct infusion of 0.25 $\mu g \cdot mL^{-1}$ aqueous standard solutions into the mass detector, looking for the optimum conditions for all compounds that produce less fragmentation. Several ionization conditions were studied including the use of formic acid, ammonium formiate, acetonitrile and methanol as modifiers to evaluate the enhancement or suppression effects on the ionization produced by their presence. Both negative and positive ionization modes were evaluated finding that DLC was also detected in both modes. Additionally, adducts formation ($[M+H]^+$; $[M+Na^+]$ and $[M+NH_4^+]$) was also evaluated finding that for all compounds under the optimum conditions the $[M+H]^+$ and $[M-H]^-$ were the most abundant ions. Desolvation and interface temperatures were high to produce efficient ionization due to the high percentage of water in the mobile phase. Table 2 shows the retention times as well as the elemental compositions for all the analysed compounds.

Full MS acquisition mode was used for quantification purposes using the extracted ion chromatograms of $[M+H]^+$ and $[M-H]^-$. MS/MS experiments for confirmation purposes were realized for each compound evaluating several collision energies in the range 6-20

275 eV. Table 2 shows the optimum collision energies for each compound as well as the
276 elemental composition of all the fragment ions obtained in MS/MS experiment.

Table 2. Retention times, full MS and MS/MS fragments ions elemental composition and collision energy for each analyte.

Compound	Elemental Composition	Mr	Precursor ion	extracted m/z (precursor)	RT (min)	Collision Energy (eV)	m/z Frag. Ions	Fragment ions
CPR	C17H18FN3O3	331.1332	$[M+H]^+$	332.1407	2.7	15	288.1501	C16H19FN3O
							314.1303	C17H17FN3O2
							245.1084	C14H14FN2O
							268.1430	C11H17FN6O
ENR	C19H22FN3O4	359.1645	$[M+H]^+$	360.1723	2.9	15	316.1728	C18H23FN3O
							342.1618	C19H21FN3O2
							245.1090	C14H14FN2O
CBZ	C15H12N2O	236.0950	$[M+H]^+$	237.1024	5.0	20	194.0964	C14H12N
FLM	C14H12FNO3	261.0801	$[M+H]^+$	262.0875	5.4	20	244.0776	C14H12FNO2
							202.0305	C11H5FNO2
							220.0410	C11H7FNO3
DCL+	C14H13Cl2NO2	295.0167	$[M+H]^+$	296.0240	7.2	20	214.0418	C10H6N4O2
							250.0191	C13H12Cl2N
							278.0135	C14H12Cl2NO
IBU	C13H18O2	206.1306	$[M-H]^-$	205.1230	5.3	6	161.1336	C11H18
DCL-	C14H13C12NO2	295.0167	$[M-H]^-$	294.0081	5.1	20	214.0418	C10H6N4O2
							215.0504	C14H13ClNO
							250.0191	C13H12Cl2N
							278.0142	C14H12Cl2NO

3.2 Extraction procedure optimization

The microwave and ultrasound assisted extraction was assayed based on previous experience developed by our group (Fernandez-Torres et al.) with complex matrixes for pharmaceutical analysis. Several conditions were tested using ultrasound assisted extraction and microwave assisted extraction. Recoveries in the range 20-100 % were obtained using ultrasound extraction with water as extracting agent for 5 min. Higher times of extraction produces degradations and undesirable matrix effects through the determination. On the other hand, best results were obtained when microwave assisted extraction was applied. Several powers (25-100 W), extracting solvents (methanol, water, acetonitrile, mixtures MeOH:H_2O and ACN:H_2O both at several ratios), times of extraction (1-20 min) as well as the final reconstituting volume (0.5-5 mL) were properly evaluated. Best extraction recoveries for all matrices were obtained when a mixture ACN:H_2O (1:1 v/v) was employed at 50 watts of microwave power for 5 minutes. The optimum amount of sample was also evaluated under these conditions, 0.5g, 1.0 g and 2.5 g of lyophilized samples were spiked with all the analytes, finding no great differences in terms of recovery (higher than 40% for all matrices) when this was under 1.0 grams. However, low recoveries, around 40 or 50%, were obtained for IBU and CPR which might be due to a matrix effect over ionization process. In order to improve the low recoveries obtained for such analytes, two different final treatments for the extract were studied avoiding the use of clean up procedures such as solid phase extraction which lengthen the processing time and also may produce loss of information in terms of metabolites and degradation products for further future studies. In order to improve the low recoveries obtained for these analytes, a final dilution as well as an evaporation of the extract were evaluated (clean up procedures such as solid phase extraction were avoided because they lengthen processing time and may lead to loss of information in terms of metabolites and degradation compounds for further studies). Previous dilution of the extract before injection was tested by means of several solvents and mixtures (methanol, water, acetonitrile and mixtures of them at several ratios). Results obtained showed that a dilution 1:5 of the extract with a mixture ACN:H_2O (1:1 v/v) reduced the matrix effects considerably leading to improve the recoveries to 65-98%. An evaporation step followed by subsequent reconstitution of the extract was assayed using methanol, water, formic acid, acetonitrile and several mixtures of them at different ratios to final volumes of 1 mL and 2.5 mL. Lower recoveries were obtained than dilution 1:5 with a mixture ACN:H_2O (1:1 v/v) probably due to ionization suppression on the detection step because of an undesirable matrix effect. Therefore, the evaporation and reconstitution steps were discard for the proposed procedure.

3.3 Method Validation

The method validation was realized according to the procedure described in section 2.7 for all the matrices selected in this study. Linearity was evaluated by matrix-matched calibration curves obtained by spiking blank extracts at ten concentration levels in the range 0.5 to 500 $ng \cdot mL^{-1}$. In general, linearity achieved was good for all the analytes measured with regression coefficients higher than 0.99 in all cases. Instrumental detection and quantitation limits (ILOD, ILOQ) and method detection and quantitation limits (LOD, LOQ) were calculated as the minimum concentration of analyte giving peaks whose signal to noise ratios are 3 and 10, respectively, for each vegetal matrix. All were determined in spiked samples (n=3) and table 3 shows the obtained results for each matrix and compound analysed.

Table 3. Instrumental and method limits of detection and quantitation.

			FLM	CBZ	CPR	ENR	DCL+	IBU	DCL-
ILOD ($ng \cdot mL^{-1}$)			0.31	0.25	0.42	0.44	0.56	1.51	1.43
ILOQ ($ng \cdot mL^{-1}$)			1.03	0.83	1.40	1.47	1.87	5.03	4.77
LOD ($ng \cdot g^{-1}$)	*Salicorni*	leaf and Stem	5.72	6.25	5.25	6.41	4.26	20.8	15.3
		Root	5.54	6.16	4.21	5.92	2.16	18.3	13.2
	Juncus	Stem	4.85	5.48	5.31	7.92	8.47	30.2	22.1
		Root	4.64	4.83	4.95	6.54	6.32	10.9	14.8
	Lavandula	leaf	3.78	4.62	5.32	9.22	7.21	25.2	15.7
		Stem	7.75	6.25	10.5	11.0	14.0	37.7	35.7
		Root	5.22	5.45	6.41	5.38	7.35	32.5	15.7
LOQ ($ng \cdot g^{-1}$)	*Salicorni*	leaf and Stem	19.1	20.8	17.5	21.4	14.2	69.3	51.0
		Root	18.5	20.5	14.0	19.7	7.22	61.0	44.1
	Juncus	Stem	16.2	18.3	17.7	26.4	28.2	100.7	73.7
		Root	15.5	16.1	16.5	21.8	21.1	36.3	49.3
	Lavandula	leaf	12.6	15.4	17.7	30.7	24.0	84.0	52.3
		Stem	25.8	20.7	35.0	36.7	46.7	125	119
		Root	17.4	18.2	21.4	17.9	24.5	108	52.3

The relative standard deviations for 6 replicate determinations of 10 ng·mL^{-1} of the six analytes studied were between 2.5 and 4.3 % (intraday repeatability). The interday repeatability (n=3, 6 days) were between 5.1 and 7.2 %.

As it has been previously published by other authors (Hu et al.2014), matrix effects on ESI is a very frequent undesirable effect, thus, a matrix matched calibration is usually followed. In this work, the matrix effect of all the studied compounds was evaluated following the procedure described in section 2.7 and results are shown in figure 1. Values lower than 20% were considered as low matrix effects, values in the range 20-40% were consider as medium matrix effect, values in the range 40-60 % were consider as high matrix effect and values over 60% were consider as very high matrix effect. As it can be seen in figure 1, higher matrix effects are shown for analytes ionized in negative mode for most matrices. ENR and CPR are the compounds with a higher matrix effect on most parts of the plants; this fact is in agreement with other published papers in vegetal matrices. Additionally, a very high matrix effect was observed by IBU in Juncus sp. FLM was the compound that presented the lower matrix effect for all matrices being about 30 % for *Salicornia ramosissima* and below 10 % for the rest of matrices, so matrix-matched calibration was used as for results obtained.

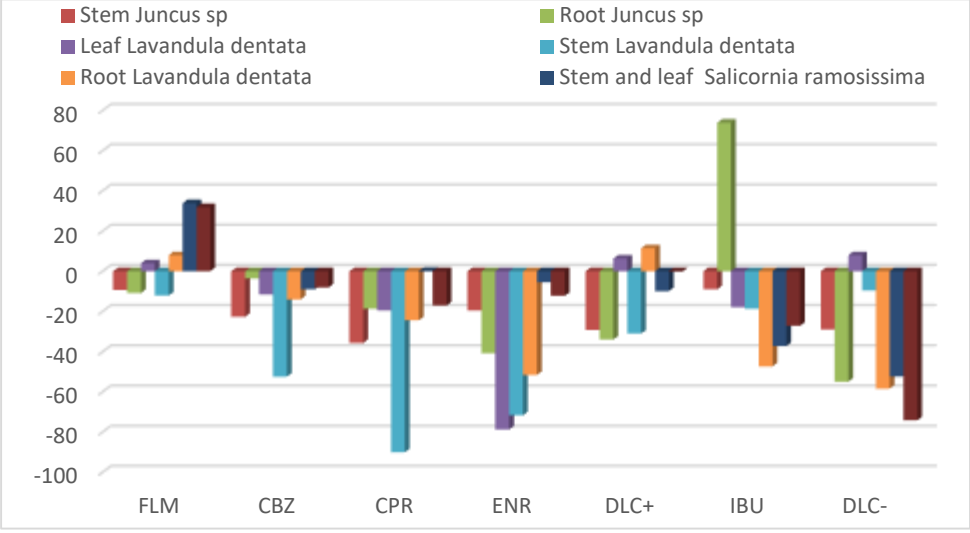


Figure 1. Matrix effect by ion suppression or enhancement for each compound and matrix analyzed.

The recoveries were calculated as percentage of extracted compound at a low and high level of concentration for each kind of matrix and compound. Spiked samples (in triplicate) to obtain final

concentrations of 50 and 500 $ng \cdot g^{-1}$ for positive ionization compounds and 150 and 500 $ng \cdot g^{-1}$ were submitted to the extraction procedure described above. Table 4 depicts the results, and as it can be seen, the overall recoveries ranges from 60 to 107 %. Higher recoveries were obtained for CBZ in all the matrices evaluated which is in accordance with results obtained by other authors previously for vegetal matrices (Marsoni et al. 2014, Riemenschneider C et al. 2017b). In general, recoveries for ENR and CPR were 70% approximately. This results are quite similar to those reported by Hu et al. 2014 in vegetal matrices. IBU showed 70% recovery for most matrices and DCL showed recoveries ranged between 80-90 % as average. Recoveries reported by Montemurro et al. (2017) in lettuce for IBU and DCL (in the order 90-120%) were higher compared to our data. FLM was the analyte that showed more dependence with the matrix, thus results for stem are in all cases nearly 90-100% but low recoveries around 60% were obtained with root of salicornia and lavandula. The results were corrected according to the obtained recoveries percentages. Figure 2 shows a typical chromatogram of Juncus stem extract spiked at 50 (150 for IBU) $ng \cdot g^{-1}$ of each compound and figure 3 the corresponding full mass spectra obtained at the retention time of each analyte.

Table 4. Recoveries (average % ±SD, n=3) for each matrix at low and high spiked concentration level.

		FLM		CBZ		CPR		ENR		DCL		IBU	
		50 ng·g^{-1}	500 ng·g^{-1}	50 ng·g^{-1}	500 ng·g^{-1}	50 ng·g^{-1}	500 ng·g^{-1}	50 ng·g^{-1}	500 ng·g^{-1}	50 ng·g^{-1}	500 ng·g^{-1}	150 ng·g^{-1}	500 ng·g^{-1}
Salicor.	*Stem*	100 ± 15	105 ± 10	101 ± 7.6	90.3 ± 5.7	75.7 ± 2.8	69.2 ± 5.6	62.5 ± 1.5	71.2 ± 3.6	95.6 ± 3.4	99.8 ± 2.4	75.2 ± 19	69.8 ± 7.6
	Root	67.1 ± 6.4	87.1 ± 10	66.5 ± 0.6	67.1 ± 6.5	75.7 ± 0.2	73.2 ± 6.5	74.1 ± 0.2	75.6 ± 6.9	61.5 ± 3.8	89.2 ± 7.6	82.7 ± 1.1	75.4 ± 4.6
Juncus	*Stem*	89.6 ± 8.4	77.3 ± 9.0	99.5 ± 1.3	89.9 ± 9.4	72.1 ± 0.5	65.3 ± 7.9	72.1 ± 0.1	75.0 ± 5.0	101 ± 13	95.1 ± 5.1	69.3 ± 9.4	74.1 ± 7.7
	Root	97.7 ± 5.5	87.6 ± 5.9	100 ± 8.7	97.7 ± 3.4	71.2 ± 3.6	81.6 ± 7.3	74.5 ± 0.6	78.5 ± 6.4	78.1 ± 1.6	88.8 ± 2.9	65.7 ± 1.1	70.0 ± 6.6
Lavandula	Leaf	100 ± 8.9	90.1 ± 11	88.4 ± 5.4	90.6 ± 9.9	70.4 ± 2.2	77.0 ± 8.4	71.2 ± 5.2	71.3 ± 4.3	100 ± 6.8	89.7 ± 9.6	81.9 ± 5.1	67.9 ± 2.3
	Stem	87.8 ± 7.8	67.8 ± 12	77.5 ± 0.2	77.8 ± 7.2	78.2 ± 2.3	82.3 ± 4.9	78.3 ± 8.4	77.9 ± 2.3	68.9 ± 3.4	96.3 ± 6.9	70.5 ± 1.5	70.7 ± 4.8
	Root	60.1 ± 2.2	70.3 ± 5.2	107 ± 8.9	80.1 ± 6.2	72.5 ± 0.3	66.8 ± 2.6	66.9 ± 0.8	74.1 ± 9.3	72.1 ± 1.1	92.8 ± 12	79.4 ± 5.6	73.2 ± 0.9

3.4 Uptake study in *Lavandula dentata*.

Lavandula dentata plants were submitted to the uptake study described in section 2.8. The plants that were irrigated with 700 $ng \cdot mL^{-1}$ and 10 $\mu g \cdot mL^{-1}$ solutions, showed a sick appearance "nearly dead" after 21 days exposition, whereas the rest of the plants remained alive for all the assay. The plants were submitted to the procedure described in the above sections and finally analyzed (a triplicate of analysis was performed at each cut) in the UPLC-QTOF/MS instrument. The results are detailed in table 5. Only FLM and CBZ were detected in the plants. This fact, previously reported by other authors, is due to chemicals uptake depends on great extent on the hydrophobic nature (log P) and ionization state of the molecules which highly depends on the pKa and the pH of the soil (Marsoni et al 2014, Wu et al. 2013). Thus, in our study, drugs with different octanol-water partition coefficients and pKa values were selected. FLM and CBZ were quantified in all samples nearly at all concentration levels, finding, as expected, higher concentrations in the plants at high irrigation concentration. As it can be seen, data show a clear trend of accumulation in root samples for both drugs, reaching very high concentrations in the case of high level exposure concentration for CBZ. However in leaf samples, it can be seen how initially the plant accumulates the highest concentration of drug and later these values gradually decrease until almost disappears, this results do not agree with those previously reported by Tanoue et al. (2012) and Wu et al. (2014) who found higher concentrations for CBZ in leaves than in roots. This could be due to the damage suffered by the plant throughout the exposure, which would cause the leaf to stop absorbing and what it is observed is the degradation of the drug until the plant ends up dying. The same behavior was followed by FLM. Nevertheless, a different behavior was observed in stem samples, FLU concentration remained stable until 21 days and then a considerable decrease in concentration was observed probably due to the damage suffered by the plant. A quite similar behavior was followed by CBZ at low concentration level of irrigation but a surprising increase in concentration was observed at high concentration levels of exposure which would disagree with the above suggested damage in the plant. At medium exposure level, both plants showed the same anomalous behavior, thus a high accumulation was suffered by both plants at 21 days and then decrease, however concentrations at 7 and 14 days were very low compared with the rest of results obtained by the other samples which would suggest a problem that could alter the absorption of CBZ by the plant at the beginning of the assay.

CPR, ENR were not found in any sample, however they were expected to be found as their absorption have been previously reported by Lillenberg et al. 2010 and they low values for log P and log *K*ow. This could be due to the plant physiology itself or compound degradation in the soil.

DCL and IBU were not identified in any samples probably due to their low pKa value the were mainly present as anions which implies low absorption by the roots (Marsoni et al.2014). This results agree with some published previous papers (Tanoue et al. 2012, Montemurro et al. 2017), however Emhofer at al. (2017) found unaltered DCL in cress roots cultivated under hydroponic conditions after seven days irrigation, this discrepancy might be attributed to the low potential for uptake of these pharmaceuticals (see table 1, log P and log *K*ow show high values) due to its low lipophicity or even due to the experimental conditions (Marsoni et al. 2014).

From the data depicted in Table 5 some other conclusions can be drawn. As it can be seen in the minigraphs included in the table, the accumulation trends in leaves for CBZ and FLM do not depend on the irrigated concentration, showing quite the same behavior for the three concentrations studied. Initially a strong adsorption occurs that subsequently decreases with time as already mentioned above. However, this behavior is different in stem samples that show different trends at the three concentrations studied. Thus, CBZ as of FLM at low and medium concentration, show a slight or constant accumulation during the first three weeks and a decrease is observed in the concentration in the last week. However, at high concentrations, the accumulation of FLM occurs more rapidly in the first or second week and subsequently a decrease is observed. In contrast, CBZ exhibits a completely opposite behavior to that of FLM at this concentration level. To the best of our knowledge, the founded concentration in stem dependence on the concentration level of irrigation have not been previously reported.

Additionally, as can be seen, concentration average values obtained remain in the same order between the two plants used for each assay which means that plants suffers the same accumulation when the uptake study is performed under quite similar conditions.

400 Table 5. Results obtained in *Lavandula dentata* after uptake assay.

Matrix	level	Cuts	Found compounds concentrations							
			Flumequine[a] P1		Flumequine[a] P2		Carbamazepine[a] P1		Carbamazepine[a] P2	
			Average[b]	SD	Average[b]	SD	Average[b]	SD	Average[b]	SD
Leaf	10 ng mL^{-1}	7 days	111	23	220	14	141	22	145	18
		14 days	58	0.2	70	8	69	10	132	8
		21 days	<MLOQ		20	4	36	8	83	12
		30 days	<MLOQ		<MLOQ		24	3	21	10
	700 ng mL^{-1}	7 days	1554	68	1326	49	1148	68	1754	24
		14 days	449	20	363	23	312	57	413	11
		21 days	187	6	420	28	223	22	676	18
		30 days	206	19	346	22	163	53	353	2
	10 µg mL^{-1}	7 days	1156	67	2076	43	2498	38	1390	17
		14 days	631	68	468	82	1672	37	545	31
		21 days	242	34	361	35	245	13	368	61
		30 days	150	20	62	13	150	27	207	4
Stem	10 ng mL^{-1}	7 days	172	14	209	37	109	25	84	39
		14 days	151	3	184	15	101	5	156	25
		21 days	646	19	79	20	129	8	48	18
		30 days	335	25	57	9	54	15	49	4
	700 ng mL^{-1}	7 days	502	21	559	4	102	6	167	16
		14 days	666	5	565	14	101	9	103	14
		21 days	417	14	561	8	647	11	717	33
		30 days	160	1	331	12	102	12	103	28
	10 µg mL^{-1}	7 days	764	17	804	47	464	9	633	7
		14 days	1020	45	686	16	3837	37	1862	57
		21 days	875	14	490	35	3074	20	2318	37
		30 days	494	27	308	14	15635	272	4630	113
Root	10 ng mL^{-1}		127	4	92	3	832	9	443	43
	700 ng mL^{-1}		195	7	241	9	1354	32	722	72
	10 µg mL^{-1}		1904	16	1924	16	15035	110	14076	41

[a]concentration expressed in ng g^{-1}
[b]average concentration (n=3)

4. **Conclusions**

The developed analytical method allows the efficient extraction of the 6 selected pharmaceuticals from different parts of the selected plants in five minutes by means of microwave energy with no additional clean-up step. Regarding the extraction procedure, several procedures, solvents and treatments were evaluated looking for the best results in terms of recoveries and sensitivity, obtaining results for quantification limits in the order of $ng \cdot g^{-1}$, with good accuracy and precision in the order of the recommended values for pharmaceutical determinations when 5 mL of a mixture ACN:H_2O (1:1 v/v) was used as extracting solvent.

The method has been applied for the identification and quantification of the analytes in samples of *Lavandula dentata* after irrigation the soil of the plant with a mixture of six drugs. The uptake assay developed lasted 30 days. From this assay can be stated that FLM and CBZ were the compounds more accumulated in leaf and stem of the plant, showing similar trends at low concentration levels of exposure. Contrary to what it was initially expected at high and medium level of exposure the plants showed a higher accumulation at 21 days and then a high decrease at 30 days, behavior that could be related to the damage of the plant. However additional studies should be done to assure this supposition. As it was expected the concentrations of the compounds in root increased with the higher concentration of the mixture used in the assay.

On the other hand, CPR, ENR, DCL and IBU were not identified in the samples as unaltered form of the drug.

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References

Al Nasir, F., Batarseh, M.I. 2008, Agricultural reuse of reclaimed water and uptake of organic compounds: Pilot study at Mutah University water treatment plant, Jordan. Chemosphere 72, 1203-1214.

Al-Qaim, F. F., Abdullah, M. P., Othman, M. R., Mussa, Z. H., Zakaria, Z., Latip, J., Afiq, W. M. 2015. Investigation of the environmental transport of human pharmaceuticals to surface water: A case study of persistence of pharmaceuticals in effluent of sewage treatment plants and hospitals in Malaysia. J. Braz. Chem. Soc. 26(6), 1124-1135.

Al-Qaim, F. F., Abdullah, P., Othman, M. R., Latip, J., & Afiq, W. M. 2013. Development of analytical method for detection of some pharmaceuticals in surface water. Trop. J. Pharm. Res. 12(4), 609-616.

Arasimowicz M., Wisniowska-Kielian B., Niemiec M. 2013, Efficiency of antioxidative system in spinach plants growing in soil contaminated with nickel. Ecol. Chem. Eng. A. 20(9), 987-997.

Avdeef A., Box K.J., Comer J.E.A., Hibbert C., Tam K.Y. 1998. pH-Metric logP 10. Determination of Liposomal Membrane-Water Partition Coefficients of Ionizable Drugs. Pharm. Res. 15, 209-215.

Bao Y.P., Li Y.W., Mo C.H., Yao Y., Tai Y.P., Wu X.L., Zhang Y. 2010, Determination of six sulfonamide antibiotics in vegetables by solid phase extraction and high performance liquid chromatography. Environ. Chem. 29(3), 513–518.

Boxall, A.B.A., Johnson, P., Smith, E.J., Sinclair, C.J., Stutt, E., Levy, L. 2006. Uptake of veterinary medicines from soils into plants. J. Agric. Food Chem. 54(6), 2288-2297.

Camacho-Muñoz, D., Martín, J., Santos, J. L., Aparicio, I., Alonso, E. 2012. Effectiveness of conventional and lowcost wastewater treatments in the removal of pharmaceutically active compounds. Water Air Soil Pollut., 223, 2611–2621.

Camacho-Muñoz, D., Martín, J., Santos, J. L., Aparicio, I., Alonso, E. 2013. Distribution and risk assessment of pharmaceutical compounds in river sediments from Doñana Park (Spain). Water Air Soil Pollut.. 224, 1665-1680.

Camacho-Muñoz, M. D., Santos, J. L., Aparicio, I., Alonso, E. 2010. Presence of pharmaceutically active compounds in Doñana Park (Spain) main watersheds. J. Hazard. Mater., 177, 1159–1162.

Carmona E., Andreu V., Picó Y. 2014. Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water. Sci. total Environ. 484, 53-63.

Cesário R., Monteiro C.E., Noguira M., O'Driscoll J.N., Caetano M., Hintelmann H., Mota A.M., Canário J. 2016. Mercury and methylmercury dynamics in sediments on a protected area of Tagus estuary (Portugal). Water Air Soil Pollut. 227, 475-XX.

Dal Pozzo A., Donzelli G., Rodriquez L., Tajana L. 1989. "In vitro" model for the evaluation of drug distribution and plasma protein-binding relationships. Inter. J. Pharm. 50, 97-101.

EC COUNCIL DIRECTIVE of 21 May 1991 concerning urban wastewater treatment (91/271/EEC). Official Journal of the European Communities. 30/5/91. No L 135/41.

Emhofer L., Himmelsbach M., Buchberger W., Klampfl C.W. 2017, High-performance liquid chromatography-mass spectrometry analysis of the parent drugs and their metabolites in extracts from cress (*Lepidium sativum*) grown hydroponically in water containing four non-steroidal anti-inflammatory drugs. J. Chrom. A 1491, 137-144.

Fernández-Torres R., Bello-López M.A., Olias-Consentino M., Callejon-Mochon M., Ramos-Payan M. 2011. Enzymatic-microwave assisted extraction and high-performance liquid chromatography–mass spectrometry for the determination of selected veterinary antibiotics in fish and mussel samples. J of Pharm. Biomed. Anal. 54, 1146–1156.

Gentili, F.G.; Fick, J. 2016. Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants. J. Appl. Phycol. 29(1), 255-262.

Gros, M., Petrovic, M., Ginebreda, A., & Barceló, D. 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Intern., 36, 15–26;

Hijosa-Valesco M., Fink G., Schlüsener P., Sidrach-Cardona R., Martín-Villacorta J., Temes T., Bécares E. 2011. Removal of antibiotics from urban wastewater by constructed wetland optimization. Chemosphere. 83, 713-719.

Hrabovsky V., Dzendzelyuk O.,Katerynchuk I., Furgala Y. 2004. Monitoring of radionuclides contamination of soils in Shatsk National Natural Park (Volyn Region, Ukraine) during 1994-2001. J. Environ. Radioac. 72, 25-33.

Hu F., Bian K., Liu Y., Su Y., Zhou T., Song X., He L. 2014. Development of a modified Quick, Easy, CHeap, Effective, Rugged and Safe method for the determination of multi-class antimicrobials in vegetables by liquid chromatography tándem mass spectrometry. J. Chrom. A. 1368, 52-63.

Hu X.G., Zhou Q.X., Luo Y. 2010, Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ. Pollut. 158(9), 2992–2998.

Kong, W.D., Zhu, Y.G., Liang Y.C., Zhang J., Smith F.A., Yang M. 2007, Uptake of oxytetracycline and its phytotoxicity to alfalfa (medicago sativa L.). Environ. Pollut., 147(1), 187- 193.

Lillenberg M., Litvin S.V., Nei L., Roasto M., Sepp K. 2010. Enrofloxacin and Ciprofloxacin Uptake by Plants from Soil. Agron. Res. 8, 807–814.

Malchi T., Maor Y., Tadmor G., Shenker M., Chefetz B. 2014, Irrigation of Root Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and the Associated Human Health Risks. Environ Sci. Technol. 48(16), 9325-9333.

Marsoni M, De Mattia F, Labra M, Bruno A, Bracale M, Vannini C. 2014. Uptake and effects of a mixture of widely used therapeutic drugs in Eruca sativa L. and Zea mays L. plants. Ecotox. Environ. Saf. 108, 52-57.

Martínez-Alcalá I., Guillén-Navarro J.M., Fernández-López C. 2017, Pharmaceutical biological degradation, sorption and mass balance determination in a conventional

512 activated-sludge wastewater treatment plant from Murcia, Spain. Chem. Eng. J. 316, 332-
513 340.

514 Migliore L., Cozzolino S., Fiori M. 2003. Phytotoxicity and uptake of enrofloxacin in
515 crop plants. Chemosphere 52, 1233-1244.

516 Redshaw, C.H., Wootton, V.G., Rowland, S.J. 2008. Uptake of the pharmaceutical
517 Fluoxetine hydrochloride from growth medium by brassicaceae. Phytochem. 69(25),
518 2510-2516.

519 Riemenschneider C, Seiwert B, Goldstein M, Al-Raggad M, Salameh E, Chefetz B.
520 2017a. An LC-MS/MS method for the determination of 28 polar environmental
521 contaminants and metabolites in vegetables irrigated with treated municipal wastewater.
522 Anal. Meth. 9, 1273-1281.

523 Riemenschneider C, Seiwert B, Moeder M, Schwarz D, Reemtsma T. 2017b. Extensive
524 Transformation of the Pharmaceutical Carbamazepine Following Uptake into Intact
525 Tomato Plants. Environ. Sci. Tech. 51, 6100-6109.

526 Rivera-Jaimes J.A.,Postigo C., Melgoza-Alemán R.M., Aceña J., Barceló D., López de
527 Alda M. 2018. Study of pharmaceuticals in surface and wastewater from Cuernavaca,
528 Morelos, Mexico: Occurrence and environmental risk assessment. Sci. Total Environ.
529 613-614, 1623-1274.

530 Shernker, M., Harush, D., Ben-Ari, J., Chefetz, B. 2011. Uptake of carbamazepine by
531 cucumber plants- A case study related to irrigation with reclaimed wastewater.
532 Chemosphere 125, 963-988.

533 Tanoue R., Sato Y., Motoyama M., Nakagawa S., Shinohara R., Nomiyama K. 2012.
534 Plant Uptake of Pharmaceutical Chemicals Detected in Recycled Organic Manure and
535 Reclaimed Wastewater. J. Agric. Food Chem. 60, 10203−10211.

536 Tauxe-Wuersch A., De Alencastro L.F., Grandjean D., Tarredellas J. 2005. Occurrence
537 of several acidic drugs in sewage treatment plants in Switzerland and risk assessment.
538 Water Res. 39, 1761-1772.

539 Tiedekena E.J., Tahara A., McHughb B., Rowan N.J. 2017. Monitoring, sources,
540 receptors, and control measures for three European Union watch list substances of
541 emerging concern in receiving waters – A 20 year systematic review. Sci. Total Environ.
542 574, 1140–1163.

543 Vaclaik L., Krynitsky A.J., Rader J.I. 2014. Targeted analysis of multiple
544 pharmaceuticals, plant toxins and other secondary metabolites in herbal dietary
545 supplements by ultra-high performance liquid chromatography-quadrupole-orbital ion
546 trap mass spectrometry. Anal. Chim. Acta 810, 45-60.

547 Verlicchi, P., Al Aukidy, M., & Zambello, E. 2012. Occurrence of pharmaceutical
548 compounds in urban wastewater: removal, mass load and environmental risk after a
549 secondary treatment: a review. Sci. Total Environ. 429, 123–155.

550 Wu X., Conkle J.L., Ernst F., Gan J. 2014. Treated wastewater irrigation: Uptake of
551 pharmaceutical and personal care products by common vegetables under field conditions.
552 Environ Sci Technol. 48, 11286–93.

553 Wu X., Ernst F., Conkle J.L., Gan J. 2013. Comparative uptake and translocation of
554 pharmaceutical and personal care products (PPCPs) by common vegetables. Environ. Int.
555 60, 15-22.

556 Yao J.H., Niu D.K., Li Z.J., Liang Y.C., Zhang S.Q. 2010, Effects of antibiotics
557 oxytetracycline on soil enzyme activities and microbial microbial biomass in wheat
558 rhizosphere. Scientia Agricultura Sinica 43(4), 721–728.

559 Zhengxin X., Guanghua L., Zhenhua Y., Jianchao L., Peifang W., Yonghua W., 2017.
560 Bioaccumulation and trophic transfer of pharmaceuticals in food webs from a large
561 freshwater lake. Environ. Pollut. 222, 356-366.

562

Figure Captions

Figure 2. Total (TIC) and extracted ion chromatograms of a sample of Juncus stem spiked with all the analytes at 50 $ng \cdot g^{-1}$. a) Diclofenac-; b) Enrofloxacin+; c) Ciprofloxacin+; d) Flumequine+; e) Carbamazepine+; f) Diclofenac+; g) ibuprofen-; h) Total ion chromatogram for negative ionization; i) Total ion chromatogram for positive ionization.

Figure 3. Mass spectra of a sample of Juncus stem spiked with all the analytes at 50 $ng \cdot g^{-1}$(150 for IBU). a) Ibuprofen-b) Diclofenac-; c) Diclofenac+; d) Flumequine+; e) Carbamazepine+; f) Enrofloxacin+; g) Ciprofloxacin+.