Occurrence and analysis of selected pharmaceutical compounds in soil from Spanish agricultural fields

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Abstract This work describes the analysis of 15 pharmaceutical compounds, belonging to different therapeutic classes (anti-inflammatory/analgesics, lipid regulators, antiepileptics, β-blockers and antidepressants) and with diverse physical–chemical properties, in Spanish soils with different farmland uses. The studied compounds were extracted from soil by ultrasound-assisted extraction (UAE) and determined, after derivatization, by gas chromatography with mass spectrometric detection (GC–MS). The limits of detection (LODs) ranged from 0.14 ng g^{-1} (naproxen) to 0.65 ng g^{-1} (amitriptyline). At least two compounds where detected in all samples, being ibuprofen, salicylic acid, and paracetamol, the most frequently detected compounds. The highest levels found in soil were 47 ng g^{-1} for allopurinol and 37 ng g^{-1} for salicylic acid. The influence of the type of crop and the sampling area on the levels of pharmaceuticals in soil, as well as their relationship with soil physical–chemical properties, was studied. The frequent and widespread detection of some of these compounds in agricultural soils show a diffuse contamination, although the low levels found do not pose a risk to the environment or the human health.

Keywords Pharmaceuticals · UAE · Soil · Agricultural fields · Environment levels · GC–MS · Spain

Introduction

At present, approximately 3,000 different pharmaceutical ingredients are used in the European Union (Termes and Joss 2006). Pharmaceutical compounds are administered in large quantities to humans and animals, and have been detected in diverse environmental matrices (Antonic and Heath 2007, Pintado-Herrera et al. 2013, Quintana et al. 2007). After consumption, these drugs are released into the environment mainly through discharge of wastewater treatment plants (WWTPs) or from biosolids applied to land (Al Aukidy et al. 2012). Septic system effluents discharge and disposal of sewage sludge are other potential sources that can introduce pharmaceuticals into the environment (Kinney et al. 2006). The presence of pharmaceutical chemicals in the environment has long been recognized as a concern because it is likely that some of these compounds may pose a risk to flora and fauna (Oetken et al. 2005, Pomati et al. 2004, Pounds et al. 2008, Stuart et al. 2012). Moreover, residual pharmaceuticals have the potential to be taken up by edible plants and then enter the food chain (Shenker et al. 2011, Winker et al. 2010). Most of the studies carried out for the determination of pharmaceuticals in the environment have focused on the aquatic compartment, as a consequence of the polar nature of the majority of these compounds and since the main route to enter the environment is through the discharge of wastewater effluents into surface water. Although most pharmaceutical drugs are designed to be hydrosoluble and biodegradable, many compounds have high log Kow (Table 1) and therefore, present a high affinity to sludge or soil. The presence of pharmaceuticals in soil may be explained by the spreading of sludge or manure to fertilize agricultural soils together with the use of contaminated water for irrigation. Residues of pharmaceutical compounds, usually found at low levels, may cause negative effects due to their continuous introduction into the environment (Xu et al. 2009a).

Table 1 Physicochemical properties of the target compounds

Compound	Therapeutic class	Log Kow	Pk_a	Water solubility, mg mL^{-1} at 25 °C
Clofibric acid	Lipid regulator	2.6	3.35	0.583
Ibuprofen	NSAID	3.97	4.47	0.022
Salicylic acid	NSAID	1.2	3.5	2
Allopurinol	Antigout	2.9	9.3	0.48
Paracetamol	NSAID	0.5	9.4	0.014
Gemfibrozil	Lipid regulator	4.7	4.4	0.01
Fenoprofen	NSAID	3.9	4.21	15
Amitriptyline	Antidepressant	4.92	9.76	0.01
Metoprolol	β-blocker	1.9	9.5	157
Naproxen	NSAID	3.2	4.8	0.016
Mefenamic acid	NSAID	5.1	4.2	0.02
Ketoprofen	NSAID	3.1	4.45	0.05
Carbamazepine	Antiepileptic	2.45	13.9	0.017
Diclofenac	NSAID	4.5	4.1	0.02
Fenofibrate	Lipid regulator	4.8	−4.9	0.25

NSAID nonsteroidal anti-inflammatory drugs

Among the pharmaceutical compounds, anti-inflammatory/analgesics, lipid regulators, antiepileptics, β-blockers and antidepressants are often consumed (Fent et al. 2006). Analgesics are consumed in large quantities and have been frequently found in water (Kosjek et al. 2005, Vazquez-Roig et al. 2011) and sediments (Antonic and Heath 2007). Other pharmaceuticals, such as lipid regulators and β-blockers, have been also found in sediments due to its elevated lipophilicity with log Kow in the range 3–5 (Perez-Carrera et al. 2010). A number of researchers have detected pharmaceutical compounds in environmental samples throughout North America and Europe (Al Aukidy et al. 2012, Antonic and Heath 2007, Metcalfe et al. 2003, Verenitch et al. 2006), generally originated from discharge of treated wastewater effluents, use of reclaimed water for irrigation, or soil application of biosolids. Although they report levels of pharmaceuticals in soils located in different environmental areas, information regarding the concentration of these compounds in agricultural soils growing different crops is scarce in the available scientific literature, and only residues of a small number of pharmaceuticals have been reported (Braganca et al. 2012, Walker et al. 2012).

The analysis of trace quantities of pharmaceuticals requires powerful and selective techniques, such as, high performance liquid chromatography (Vazquez-Roig et al. 2010) or gas chromatography (GC) (Bisceglia et al. 2010, Weigel et al. 2004) coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS). The presence of polar functional groups with active hydrogens requires the use of a derivatization procedure to reduce their polarity and enhance their volatility before GC analysis. Ultrasound-assisted extraction (UAE) is a technique frequently applied to the extraction of various contaminants from soil (Sanchez-Brunete et al. 2010). Nevertheless, no method has been found on its application to the simultaneous analysis of acid, neutral, and basic pharmaceuticals in soil. The short extraction time and low solvent consumption of UAE, besides its robustness and easy handling, are some of the advantages of this extraction technique.

The aims of this study were to develop a rapid and robust analytical method, based on UAE and gas chromatography with mass spectrometric detection (GC–MS), for the simultaneous determination in soil of 15 widely used pharmaceutical compounds, belonging to different therapeutic classes with a wide range of physical–chemical properties, and to evaluate their presence in soil from agricultural fields located in three Spanish regions (Valencia, Murcia and Segovia). In addition, the influence of the type of crop and the sampling area on the levels of pharmaceuticals in soil, as well as their relationship with soil physical-chemical properties, was also studied.

Materials and methods

Soil sampling and area description

A number of 31samples were collected from several Spanish areas: 8 samples from rice fields, 6 from citrus orchards, 5 from horticultural fields (potatoes and cabbage), and 12 from cereal farmland (wheat and barley). Samples were taken from surface soil (0–10 cm), where the contamination is generally higher (Chen et al. 2011), using a manual sampling drill, collected in glass jars (100–250 g) and stored in a portable refrigerator during transport to the laboratory. Samples were air dried overnight at room temperature, ground to pass through a 2 mm screen and stored frozen −20 °C until analyzed.

For recovery assays, an alfisol soil (Soil Survey Staff. 2006) was collected from the plough layer (0–10 cm) of an experimental plot located in the region of Madrid (Spain), sieved (2 mm), and stored frozen (−20 °C) until being used. The characteristics of this control soil sample were as follows: pH 7.69, total organic matter content (OM) 0.97 %, sand 44.34 %, silt 37.44 % and clay 18.22 %.

Valencia is a known area of rice and citrus production in Spain. All the rice fields samples were taken from the Natural Park of "L'Albufera" (Valencia) at two sampling areas: one with irrigation water from either Júcar River or the Albufera Lake and the other with water coming from Pinedo WWTP. Citrus and vegetables fields in this area, irrigated with water from Turia River, were also sampled. In Murcia, soil samples were taken from citrus, vegetables, and cereal (barley and wheat). Fields were irrigated with groundwater from the aquifer "Campo de Cartagena" that is around 150 m deep and impermeable. Soil from barley and wheat fields in Segovia

was sampled as it is a representative area of dry-land crops that is irrigated using groundwater from a more superficial and permeable aquifer ("Los Arenales").

Soil physical–chemical properties

Soil samples were air-dried overnight at room temperature, and standard soil analyses were carried out. Three soil granulometric fractions (sand, silt, and clay) were determined for each sample following the pipette method. Soil pH was measured in a 1:5 (soil/distilled water) extract, shaken for 5 min and left standing for 2 h. OM was analyzed by the Walkley–Black method. USDA soil taxonomy classification was used (Soil Survey Staff. 2006). All the physical–chemical properties of the soils sampled and the soil taxonomy classification of Valencia, Murcia, and Segovia are described in Table 2.

Reagents

Acetonitrile (ACN) and ethyl acetate (EtAc), residue analysis grade, and ammonium hydroxide (NH_4OH)≥32 % were purchased from Scharlab (Barcelona, Spain). Anhydrous sodium sulfate was obtained from Aldrich (Steinheim, Germany), heated for 24 h at 180 °C and, after cooling, stored in a dark vessel in a desiccator before use. All the pharmaceutical chemicals (purity ≥98.8 %), the derivatization agent *N*-(*tert*-butyldimethylsilyl)-*N*-methyl-trifluoroacetamide (MTBSTFA, purity ≥95 %) with 1 % *tert*-butyldimethylchlorosilane (TBDMCS) and 96 % formic acid were purchased from Aldrich (Steinheim, Germany).

Separate stock solutions of individual compounds made up at 50 μg mL^{-1} were prepared in ACN and stored at −18 °C. A working mixture solution containing each compound at 1 μg mL^{-1} was prepared weekly by dilution of the stock solution in ACN. Table 1 reports the pharmaceutics selected in this study, showing their therapeutic class, and their physical–chemical properties.

Instruments

Extraction

An ultrasonic water bath (Raypa, Barcelona, Spain) was used in the extraction step. The generator of this ultrasonic water bath has an output of 150 W and a frequency of 35 kHz. A vacuum manifold (Supelco, Visiprep, Madrid, Spain) was employed for collecting the extracts that were then evaporated to dryness using an evaporator (Genevac EZ-2 NET Interlab, S.A.L., Spain).

Gas chromatography and mass spectrometry

All measurements were performed by GC–MS with an Agilent 6890 gas chromatograph (Waldbronn, Germany) equipped with an automatic injector, Model HP 7683, and an inert mass spectrometric detector (MSD), Model HP 5973 N, equipped with an inert ion source. A fused silica capillary column ZB-5MS, 5 % phenyl polysiloxane as non-polar stationary phase (30 m×0.25 mm i.d. and 0.25 μm film thickness), from Phenomenex (Torrance, CA), was used for the analysis. Operating conditions were as follows: injector port temperature 285 °C; helium (purity 99.995 %) as carrier gas at a flow rate of 1.2 mL min^{-1} and pulsed splitless mode (pulsed pressure 45 psi=310 kPa for 1.5 min) with the splitless injector purge valve activated 1.5 min after injection of 2 μL volume into a liner with deactivated glass wool. The column temperature was maintained at 80 °C for 0.5 min, then programmed at 20 °C min^{-1} to 285 °C and held for 2 min. The total analysis time was 12.75 min, and the equilibration time was 3 min.

Mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV, an ion source temperature of 230 °C, and a quadrupole temperature of 150 °C. The electron multiplier (EM) voltage was operated with a gain of 3 and a solvent delay of 7.5 min. Table 3 lists the compounds along with their retention times and selected ions. The target and qualifier abundances were determined by

Table 2 Soil sample properties and USDA classification

Area	Crop	USDA	*n*[a]	pH (1:5)	% Sand	% Clay	% OM
Segovia	Cereal	Alfisol	1–4, 9	6.2–9.1	21–97	1–55	0.2–4.3
	Cereal	Inceptisol	5, 7	5.5–7.8	55–72	16–31	0.7–1.2
	Cereal	Psamments	6, 8	7.6–8.2	53–63	11–18	1.1–2.6
Murcia	Citric	Xerosols	10–12	7.8–8.3	23–57	21–30	1.6–2.1
	Vegetable	Xerosols	13–14	8.2–8.3	34–56	20–21	1.5–1.6
	Cereal	Xerosols	15–17	8.1–8.2	34–43	11–38	0.9–1.5
Valencia	Vegetable	Fluvents	18–20	8.2–9	30–40	32–45	3.2–3.9
	Citric	Fluvents	21–23	7.9–8.4	36–39	37–39	2.9–3.4
	Rice	Fluvaquents	24–31	8–8.4	17–36	37–50	3.3–4.7

[a] *n*: sample number

Table 3 Retention times (t_R, min) and selected ions (m/z) of the compounds studied

Name	t_R	T^a	$Q1^b$	$Q2^b$
Clofibric acid-TBDMS	7.93	143	271	185
Ibuprofen-TBDMS	8.13	263	264	117
Salicylic acid-TBDMS	8.66	309	310	195
Allopurinol-diTBDMS	9.16	307	308	193
Paracetamol-diTBDMS	9.33	322	248	323
Gemfibrozil-TBDMS	9.76	243	179	307
Fenoprofen-TBDMS	9.98	299	197	206
Amitriptyline	10.07	58	202	277
Metoprolol-TBDMS	10.30	72	324	223
Naproxen-TBDMS	10.40	287	185	288
Mefenamic acid-TBDMS	10.96	298	224	355
Ketoprofen-TBDMS	10.98	311	295	105
Carbamazepine-TBDMS	11.26	193	194	293
Diclofenac-TBDMS	11.47	352	354	214
Fenofibrate	11.57	121	273	139

[a] *T*: target ion

[b] *Q1* and *Q2*: qualifier ions

injection of standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from 45 to 450 m z^{-1}. The compounds were confirmed by their retention times and the identification of target and qualifier ions. Retention times must be within ±0.2 min of the expected time and qualifier-to-target ratios within a 20 % range for positive confirmation.

Sample preparation

Two Whatman No. 1 paper circles of 2 cm diameter (Whatman, Maidstone, UK) were placed at the end of a 20 mL glass column (Normax, Portugal) and anhydrous sodium sulfate (3 g) was added as a layer over the paper filter then, sieved soil (5±0.001 g) was placed in the column. For the recovery studies, soil samples were previously fortified with a mixture of the different pharmaceuticals to reach final concentrations of 20–10—5 ng g^{-1}. Samples were left 60 min before extraction to reach equilibrium. ACN containing 2 % of NH_4OH (8 mL) was added to the column that was placed for 15 min in an ultrasonic water bath at room temperature. The water level in the bath was adjusted to equal the extraction solvent level inside the columns, which were supported upright in a tube rack and closed with one-way stopcocks. After extraction, the columns were placed on the multiport vacuum manifold where the solvent was collected in graduated tubes, samples were washed with 1 mL of additional basic solvent (ACN containing 2 % of NH4OH), and extracts were evaporated to dryness. The soil samples were extracted a second time in the ultrasound bath (15 min) with 8 mL of ACN containing 2 % of formic acid, and samples were then washed with 1 mL of acidic solvent. The extracts were collected in the graduated tubes used in the first extraction step, evaporated to dryness, and reconstituted to 1 mL with ACN. An aliquot (100 μL) of the extract was transferred into a 2 mL reaction vial, followed by the addition of 50 μL of MTBSTFA:TBDMCS (99:1, v/v). The vials were closed and the mixture left to react for 1 h at 70 °C before the GC–MS analysis. Chromatographic standards were prepared spiking blank soil extracts.

Quality assurance and quality control

The quality assurance and quality control criteria used for this method included analyses of laboratory blanks (solvent blanks) and laboratory control samples. One laboratory blank was run with each set of samples to check for contamination from the preparative steps and to demonstrate laboratory background levels. Using the proposed procedure, background levels of laboratory blanks were below the limits of detection (LODs). Control soil samples were used in the recovery assay. A recovery in the range of expected levels was carried out in each set of samples to confirm that results were in the accepted recovery range. Solvent was injected every four samples and after each sample containing potentially high levels of the target contaminants to avoid possible cross-contamination. To overcome memory effects, the inlet was flushed every day, prior to the analysis of samples, by heating at 300 °C for 30 min. Repeatability and reproducibility were determined at two concentrations, namely 5 and 10 ng g^{-1}. Repeatability was evaluated by performing extraction and injection of both solutions ten times on the same day. To determine reproducibility, extraction, and injection were performed three times per day on six different days for each level.

Statistical analyses

A standard statistical analysis (mean, median, and standard deviation) was carried out to determine the levels of pharmaceutical compounds in soil. Significant differences between means (crops and agricultural areas) were assessed through nonparametric ANOVA and Kruskal–Wallis tests (α=0.01). To study the relationship between soil properties and levels of pharmaceutical compounds found in soil, canonical correlation analyses (CCorA) were used, where the aim was to maximize the covariance between two sets of variables and to minimize their respective variance. All the statistical analyses were carried out using the XLSTAT (Addinsoft Version 2012.2.02).

Results and discussion

Sample preparation

Acetonitrile and EtAc were evaluated as extraction solvents in the optimization of the extraction of the studied pharmaceutical compounds from soil. Although there were only slight differences in recovery yields with both solvents, the GC–MS chromatograms showed less interferences when ACN was used as the extraction solvent (data not shown). Moreover, ACN is the solvent recommended to carry out the derivatization process; hence, it was selected as extraction solvent. Nevertheless, when UAE was initially performed using ACN, extraction yields >70 % were only achieved for three compounds (paracetamol, carbamazepine, and fenofibrate) (Fig. 1). An increase of the polarity of the solvent was considered, and ACN with 10 % of methanol was assayed, but no significant improvement was observed with this modification. In order to enhance the recovery of acidic compounds, such as clofibric, salicylic, and mefenamic acids, ACN containing 2 % of formic acid was tested. These acidic compounds showed a clear improvement (Fig. 1), but the recoveries were still very low for compounds like allopurinol, amitriptyline, and metoprolol. For this reason, another extraction step with ACN containing 2 % NH_4OH (Li et al. 2013) was implemented in the UAE procedure before the extraction with acidic ACN. As shown in Fig. 1, recoveries with this two-step UAE were close to 100 % for all compounds except for allopurinol, which presented recoveries around 50 %, but with good reproducibility. A purification step of sample extracts was not necessary, and clean chromatograms without interferences from other components were obtained with this UAE method.

Gas chromatographic determination

Table 3 lists the compounds with their retention times and their target and qualifier ions. The GC method was optimized, seeking to reduce time and increase the sensibility in the determination of our target analytes. Different inlet temperatures, 325, 300, 275, and 250 °C, were evaluated, and 275 °C was selected because it provided the best results in terms of abundance and peak shape. The oven was programmed to start at 60, 70, 80 or 90 °C and reach 285 °C at 20 °C min^{-1}, and an initial oven temperature of 80 °C was selected because it provided high peak abundances while reducing the analysis time from 17.5 to 12.25 min.

Prior to the GC–MS determination, the studied analytes need to be derivatized. The derivation agent MTBSTFA with TBDMCS as catalyst was selected, as it was used in various works where pharmaceutical compounds are derivatized

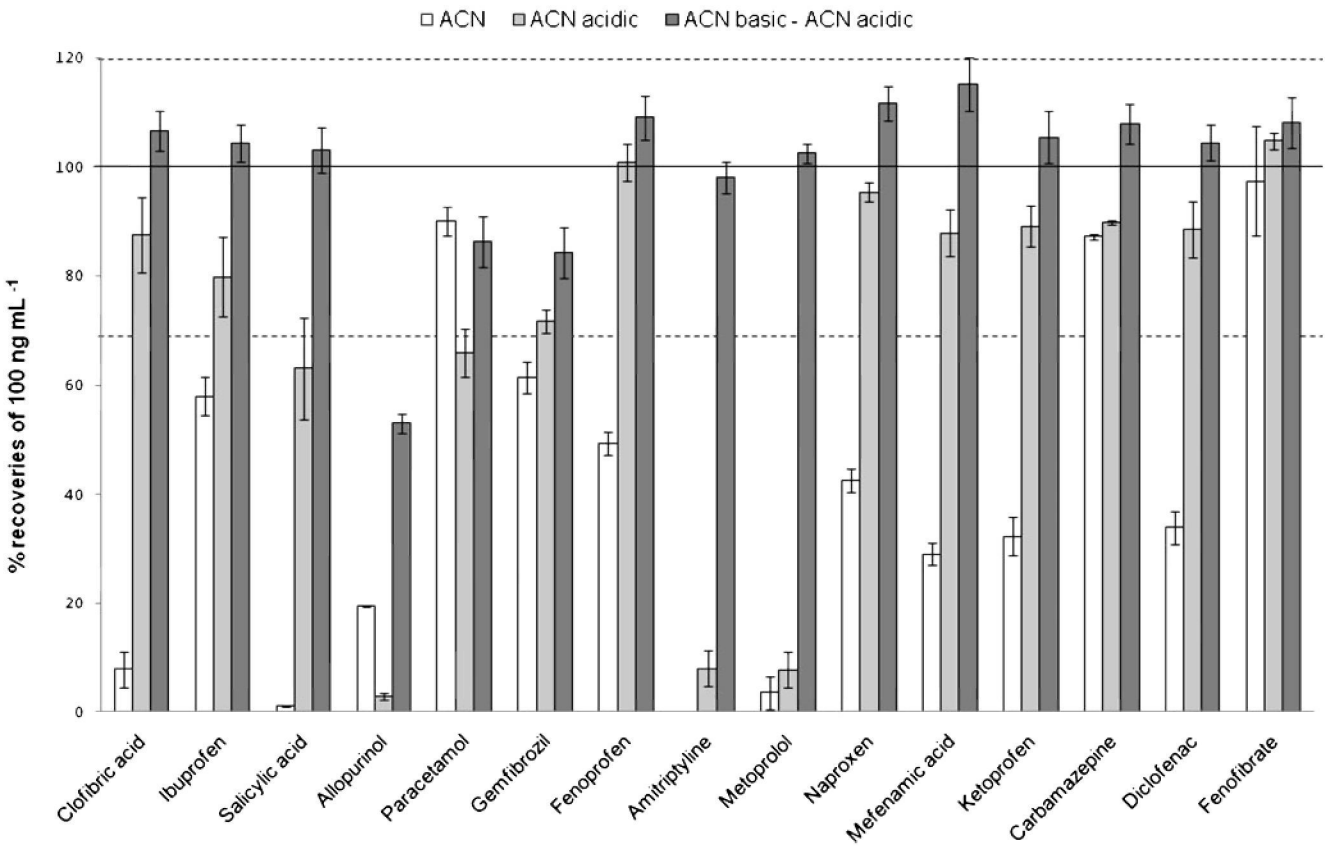

Fig. 1 Comparison of the extraction efficiency of different solvents in samples spiked at 20 ng g^{-1}, n=4

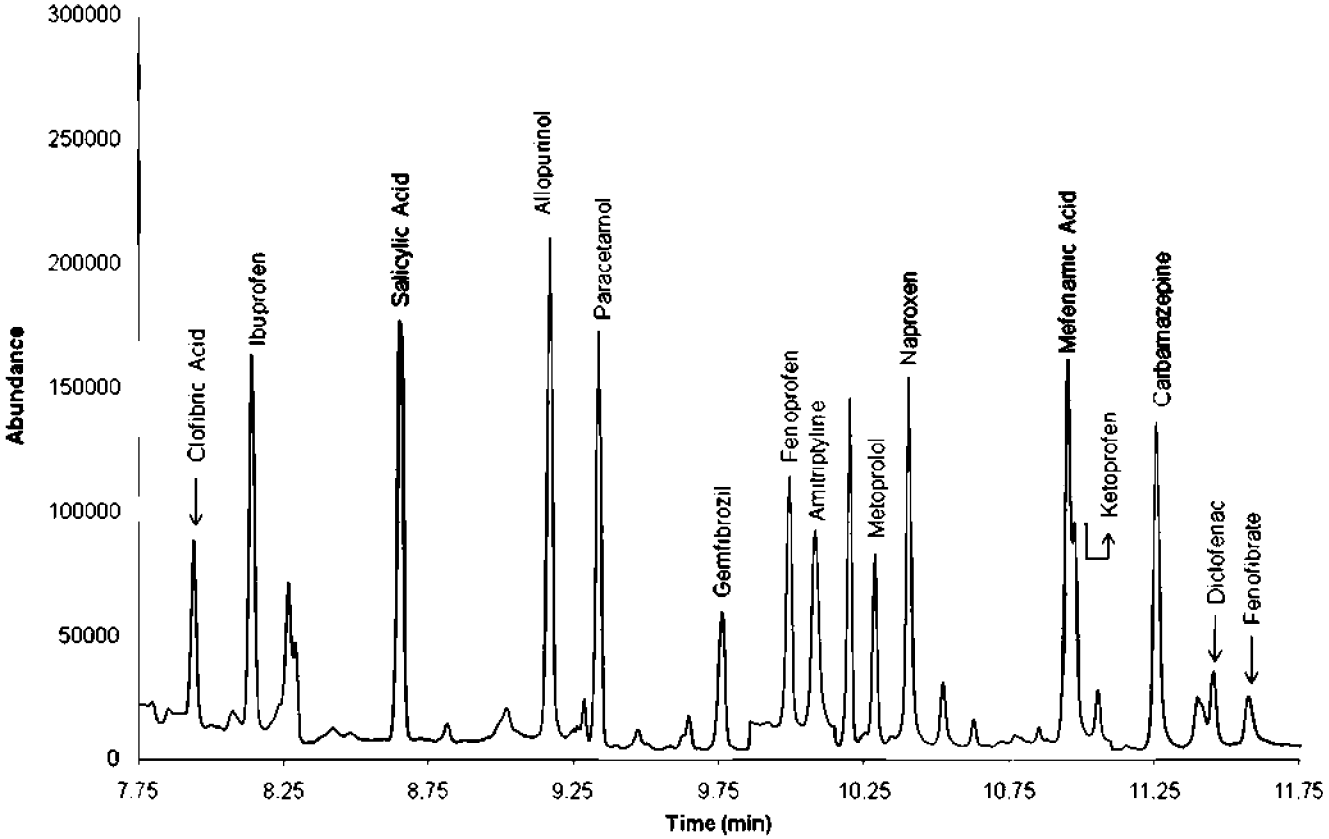

Fig. 2 A GC–MS chromatogram of a blank soil extract spiked at 10 ng g^{-1}

before the GC determination (Rice and Mitra 2007). After testing different oven temperatures and reaction times, the derivatization at 70 °C during 1 h provided the highest reaction yields. Figure 2 presents a chromatogram of a blank soil extract spiked at 10 ng g^{-1} obtained in the conditions indicated above.

Method validation

After optimization, the developed method was evaluated in terms of linearity, accuracy, precision, reproducibility, repeatability, and LODs before it was used to determine pharmaceutical residues in soil.

The linearity of the method was evaluated injecting seven blank soil extracts spiked at concentration ranging from 0.2 to 20 ng g^{-1} for all the studied compounds. Each calibration solution was injected four times. A good linearity was obtained with correlation coefficients equal or higher than 0.99 for all the compounds studied.

The accuracy of the method was evaluated performing the recovery of target analytes from soil samples spiked with standard solutions at three concentration levels 20, 10, and 5 ng g^{-1}. Good recoveries, ranging from 80 to 115 %, were obtained for all the compounds except for allopurinol, which were near 50 % with good reproducibility (Table 4). In this work, higher extraction yields were achieved for fenofibrate and diclofenac in comparison to those obtained in soil by using pressurized liquid extraction (Vazquez-Roig et al.

Table 4 Recoveries (%) with their relative standard deviations (RSD %, n=8) and limit of detection and quantification (LOD, LOQ, ng g^{-1}) (n=10) of the studied contaminants

Compound	Recovery±RSD (%)			LOD	LOQ
	20 ng g^{-1}	10 ng g^{-1}	5 ng g^{-1}		
Clofibric acid	106.6±3.7	112.8±1.7	104.5±6.4	0.14	0.38
Ibuprofen	104.4±3.4	102.8±3.6	97.8±3.4	0.07	0.21
Salicylic acid	103±4.2	93.8±4.1	80.3±3.3	0.14	0.38
Allopurinol	53.0±1.7	40.8±2.2	50.0±5.8	0.07	0.21
Paracetamol	86.2±4.7	97.4±7.2	92.0±4.4	0.07	0.24
Gemfibrozil	84.2±4.6	108.5±1.6	92.8±2.9	0.04	0.16
Fenoprofen	109.0±4.0	102.6±3.0	100.8±4.7	0.09	0.26
Amitriptyline	98.0±2.9	105.1±2.1	101.8±3.1	0.24	0.65
Metoprolol	102.5±1.8	87.3±2.5	96.3±2.4	0.18	0.55
Naproxen	111.6±3.2	101.5±1.7	98.5±4.9	0.04	0.14
Mefenamic acid	115.1±4.8	107.5±3.3	94.3±3.6	0.16	0.42
Ketoprofen	105.4±4.7	99.2±1.6	95.6±4.5	0.14	0.38
Carbamazepine	107.8±3.7	92.8±3.9	99.7±4.7	0.16	0.44
Diclofenac	104.4±3.3	97.5±3.1	106.6±3.2	0.16	0.48
Fenofibrate	108.1±4.6	94.3±4.5	105.8±3.9	0.17	0.53

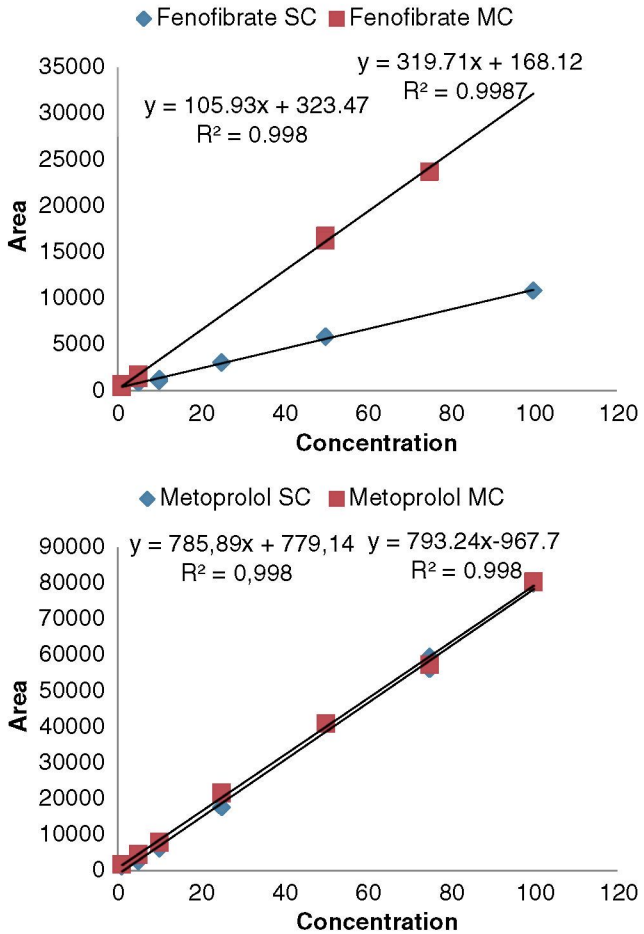

Fig. 3 Comparison of calibration curves of metoprolol and fenofibrate obtained by injection of standards in neat solvent (SC, ◆) and spiked soil extracts (MC, ■)

2011). Our results are similar to those reported by other authors that have applied microwave-assisted extraction for the determination of pharmaceuticals in soil (Azzouz and Ballesteros 2012).

The repeatability and reproducibility were determined at two concentrations, 5 and 10 ng g^{-1}. Repeatability values ≤ 8.0 %, expressed as relative standard deviation (RSD), were obtained for most of the compounds, indicating good precision. The highest reproducibility RSD value was 12 %, also indicating the robustness of the method.

Limits of detection and quantification (LOQ) of the developed method were determined using ten replicates of soil extracts, spiked at 0.2 ng g^{-1}. The equation to calculate the LOD was the following: $LOD = t_{99} \times S$, where t_{99} is the Students's value for a 99 % confidence level and $n-1°$ of freedom, and S is the standard deviation of the replicate analyses. The LOQ was calculated as ten times the standard deviation of the results of the replicate analysis used to determine LOD. Low limits were obtained due to the high selectivity and sensitivity of GC–MS, allowing the determination of these

Table 5 Concentration (n=31) of studied pharmaceutical compounds in soil

Compound	Range (ng g^{-1})	Mean±SD (ng g^{-1})	Detection rate (%)
Clofibric acid	n.d–0.7	0.7	3
Ibuprofen	n.d–1.5	0.5±0.4	81
Salicylic acid	1.4–37.1	4.4±6.2	100
Allopurinol	n.d–47	1.3±16.2	45
Paracetamol	n.d–0.5	0.4±0.1	71
Gemfibrozil	n.d–5.4	3.6±2.1	6
Fenoprofen	n.d–3.2	0.8±1.1	42
Amitriptyline	n.d	n.d	n.d
Metoprolol	n.d–n.q	n.q	3
Naproxen	n.d–5.9	0.7±2.2	19
Mefenamic acid	n.d–2.7	1.5±0.8	32
Ketoprofen	n.d	n.d	n.d
Carbamazepine	n.d–2.8	1.2±1.0	45
Diclofenac	n.d	n.d	n.d
Fenofibrate	n.d–7.8	7.0±1.0	6

n.d not detected, *n.q* not quantified

compounds at the trace levels found in soil. As shown in Table 4, LODs ranged from 0.04 to 0.24 ng g^{-1} and LOQs from 0.14 to 0.65 ng g^{-1}, being amitriptyline, metoprolol, and fenofibrate the compounds with the highest limits. The LODs and LOQs determined in this work are lower than those reported by other authors (Vazquez-Roig et al. 2011).

The matrix effect occurring in the GC analysis of some organic compounds has a negative impact on the accuracy of the results. There are several approaches to counteract matrix-induced effects, such as exhaustive cleanup-steps, the use of inert surfaces in the GC system, and different calibration methods (Sanchez-Brunete et al. 2008). Matrix effect was evaluated preparing two sets of calibration solutions, one set was solvent-based ranging from 1 to 100 ng mL^{-1}, and the other was prepared spiking blank soil extracts at the same concentrations. In both cases, a good linearity of the calibrations curves was obtained in the studied range. The slopes obtained by plotting seven concentration levels against peak area, following linear regression analysis, were compared. A significant increase of the chromatographic response was observed for ketoprofen, carbamazepine, diclofenac, and fenofibrate. Plots of calibration curves for metoprolol (without matrix effect) and fenofibrate (with a significant matrix effect) in neat solvent (SC) and matrix-matched (MC) soil extracts are shown in Fig. 3. Analysis of pharmaceuticals in SC produced for most compounds calibration curves with lower slopes when compared to MC standards; thus, quantification was carried out using MC standards.

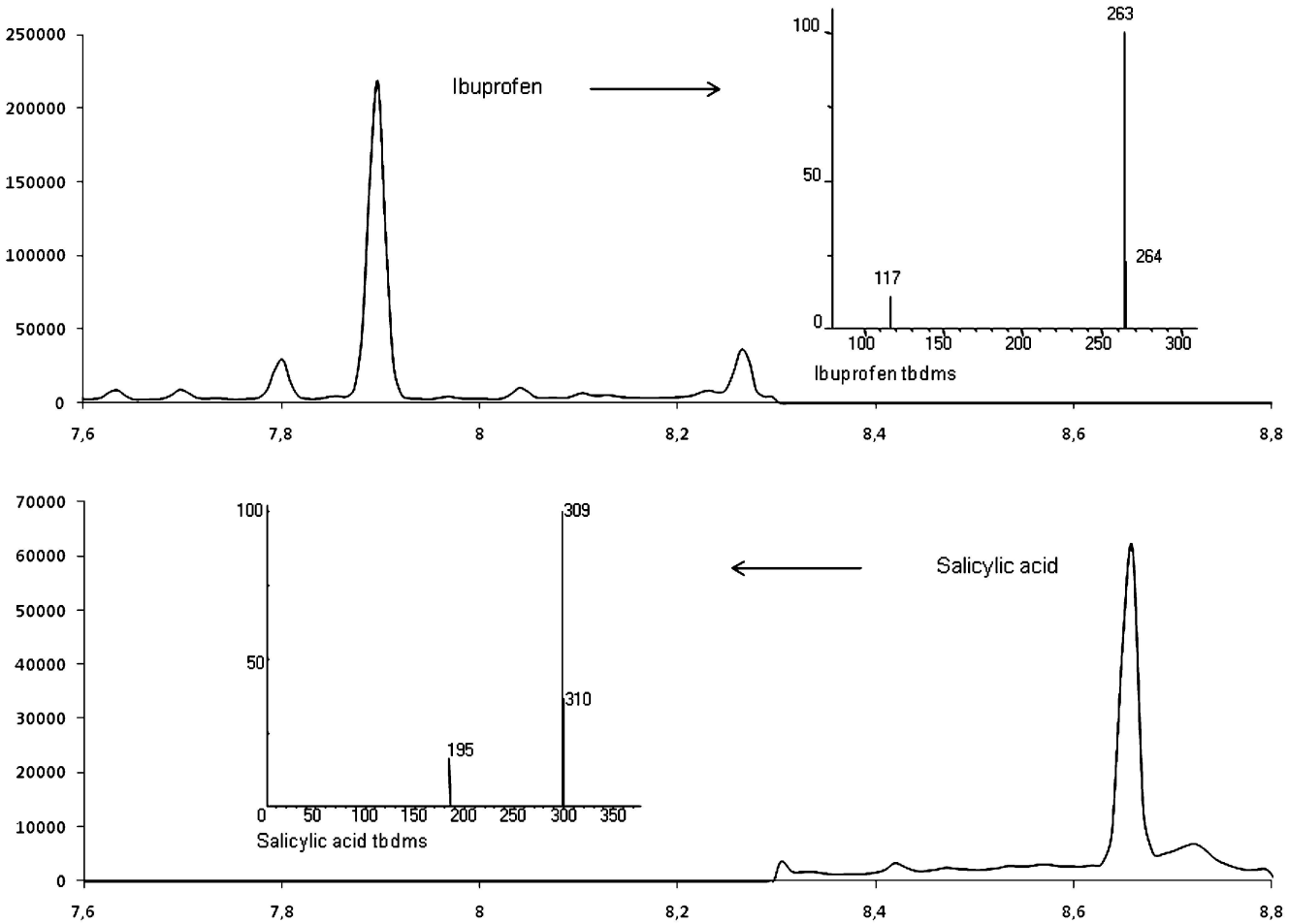

Fig. 4 Ion chromatograms of a rice soil extract containing ibuprofen (0.7 ng g^{-1}) and salicylic acid (6.8 ng g^{-1}) with the main ions of their mass spectra

Analysis of real samples

The proposed method was applied to analyze pharmaceutical drug residues in soil samples collected from agricultural fields. Table 5 summarizes the range of concentrations found together with the detection frequencies for each compound. Three out of the 15 pharmaceuticals studied (amitriptyline, ketoprofen, and diclofenac) were not detected in any of the samples, and one compound (metoprolol) was detected but not quantified. This fact could be explained by their low consumption and also because some of them have a low soil adsorption (Lin and Gan 2011, Xu et al. 2009a, Xu et al. 2009b). The rest of the pharmaceuticals studied were found at concentration levels up to 47 ng g^{-1}. Figure 4 shows a representative GC–MS chromatogram of a soil sample containing ibuprofen (0.7 ng g^{-1}) and salicylic acid (6.8 ng g^{-1}). As shown in Table 5, salicylic acid was found in all samples, followed by ibuprofen (81 %) and paracetamol (71 %), which can be explained by the high consumption of these compounds by the population. The concentration of ibuprofen in all samples is relatively small (<1.5 ng g^{-1}), due to its low soil adsorption and high biodegradability (Lin and Gan 2011, Winker et al. 2010). In comparison with other studies on pharmaceuticals in soil, the levels found for the majority of compounds were similar to those reported in our study. In soil samples from a Spanish marsh area, (Vazquez-Roig et al. 2011) found some of the compounds studied in our work but with a low detection frequency and concentration of fenofibrate and ibuprofen. In soil samples from the USA (Xu et al. 2009b), clofibric acid, ibuprofen, and naproxen were detected at slightly higher levels. Salicylic acid was reported at higher concentrations in samples from China (Chen et al. 2011) and in UK, similar levels of carbamazepine where detected in soil (Walker et al. 2012).

To study the relation between soil properties (Table 2) and the concentration of the pharmaceutical compounds CCorA were applied. The CCorA results were plotted in Fig. 5 and proved to be highly significant. The first two factors (F1 and F2) in the multivariate analysis represented an 83 % of total variance and showed, as expected, that the concentration of pharmaceutical compounds are negatively affected by the sand percentage in soil. On the other hand, OM, pH, and clay content are positively related with carbamazepine, allopurinol, and paracetamol. Regarding carbamazepine and allopurinol,

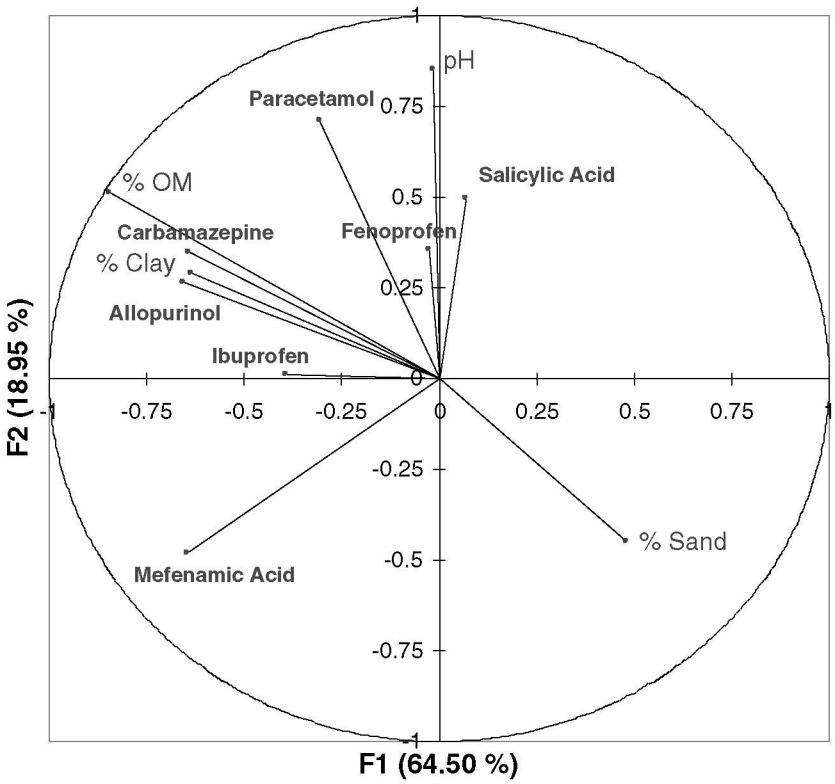

Fig. 5 Ordination diagram based on the CCorA of the soil parameters (% OM, pH, % sand and % clay) and levels of pharmaceutical compounds (ibuprofen, salicylic acid, allopurinol, paracetamol, fenoprofen, mefenamic acid. and carbamazepine)

they tend to group with a higher OM and clay content, which may favor the retention of these compounds in the soil matrix.

Table 6 shows the obtained data grouped into the area of sampling and type of crop. Soil samples from rice fields showed the highest levels of ibuprofen, allopurinol, mefenamic acid, and carbamazepine ($p<0.01$). Gemfibrozil and fenoprofen were detected in half of the rice fields, which were irrigated with wastewater treatment plant (SI—Table 1 of the Supplementary Information).

Regarding the area of sampling, Valencia showed the highest concentration of three compounds, paracetamol (although at low levels), allopurinol found (in 85.7 % of the Valencia's samples), and carbamazepine (a pharmaceutical persistent in the environment (Williams et al. 2006)), present in all the samples. Moreover, fenoprofen (42 % of the total samples) and mefenamic (32 % of the total samples) were found predominantly in Segovia, in cereal crops. This fact could be explained by the type of irrigation (Shenker et al. 2011), with surface water in the area of Valencia, water from a shallow aquifer in Segovia, and from a deeper and impermeable aquifer in Murcia.

Although the levels found of the compounds studied are relative low, the continuous release and chronic exposure to these substances make necessary monitoring their environmental levels to avoid adverse effects to aquatic life and the potential risk to human health (Stuart et al. 2012).

Table 6 Statistical summary (mean±standard deviation) of the levels of pharmaceutical compounds

	Crop				Agricultural areas		
	Cereal	Vegetables	Citrus	Rice	Murcia	Segovia	Valencia
Ibuprofen	0.0±0.1*	0.0±0.1*	0.1±0.2*	0.4±0.4**	0.1±0.1*	0.1±0.1*	0.1±0.3*
Salicylic acid	3.2±2.2*	2.8±1.8*	3.8±11.4*	4.8±2.5*	3.5±2.0*	3.8±2.3*	3.9±7.2*
Allopurinol	0.0±0.0*	0.0±3.5*	0.0±15.5*	0.1±0.7**	0.0±0.1*	0.0±0.1*	0.1±9.7**
Paracetamol	0.1±0.1*	0.1±0.1*	0.1±0.2*	0.1±0.1*	0.0±0.0*	0.0±0.1*	0.1±0.2**
Fenoprofen	0.0±0.7*	0.0±0.0*	0.0±1.0**	0.0±0.4*	0.0±0.0*	0.3±0.8**	0.0±0.7*
Mefenamic acid	0.0±0.5**	0.0±0.0*	0.0±0.0*	0.0±0.4**	0.0±0.0*	0.1±0.6**	0.0±0.3*
Carbamazepine	0.0±0.0*	0.0±0.3**	0.0±0.3**	1.4±1.0***	0.0±0.0*	0.0±0.0*	0.5±1.0**

*,**,*** Significant difference ($p<0.01$) between types of crop (cereal, vegetables, citrus, and rice) and agricultural areas (Murcia, Segovia and Valencia)

Conclusion

An analytical method has been developed for the simultaneous extraction and determination of 15 pharmaceutical compounds with diverse physical–chemical properties in different farmland soils. The developed method, based on an ultrasound assisted extraction procedure followed by GC–MS determination, is rapid, reliable, and accurate, allowing the determination of these compounds in soil at the low levels expected.

The application of this method to the analysis of soil samples from agricultural fields in several Spanish areas showed the presence of various pharmaceutical compounds although at low concentrations, highlighting the ubiquitous presence of the widely used anti-inflammatory drugs ibuprofen, salicylic acid and paracetamol in the three areas studied, as well as fenoprofen in Segovia and allopurinol and carbamazepine in Valencia.

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