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RESEARCH ARTICLE

Development of fast and robust multiresidual LC-MS/MS method for determination of pharmaceuticals in soils

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Abstract The aim of this study was to develop a simple extraction procedure and a multiresidual liquid chromatography-tandem mass spectrometry method for determination of a wide range of pharmaceuticals from various soil types. An extraction procedure for 91 pharmaceuticals from 13 soil types, followed by liquid chromatography-tandem mass spectrometry analysis, was optimized. The extraction efficiencies of three solvent mixtures for ultrasonic extraction were evaluated for 91 pharmaceuticals. The best results were obtained using acetonitrile/water (1/1 *v*/*v* with 0.1 % formic acid) followed by acetonitrile/2-propanol/water (3/3/4 *v*/*v*/*v* with 0.1 % formic acid) for extracting 63 pharmaceuticals. The method was validated at three fortification levels (10, 100, and 1000 ng/g) in all types of representative soils; recovery of 44 pharmaceuticals ranged between 55 and 135 % across all tested soils. The method was applied to analyze actual environmental samples of sediments, soils, and sludge, and 24 pharmaceuticals were found above limit of quantification with concentrations ranging between 0.83 ng/g (fexofenadine) and 223 ng/g (citalopram).

Keywords Extraction efficiency · Extraction method · Liquid chromatography-tandem mass spectrometry · Matrix effects · Sediments · Sludge · Validation

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Introduction

Pharmaceuticals can enter the environment by a number of pathways and can be further distributed to various environmental media (Grabicova et al. 2015; Golovko et al. 2014; Fedorova et al. 2014). One prominent pathway could be the use of wastewater sludge or waste water (even after treatment) for field fertilization and irrigation. In water environments, a large variety of these compounds and their metabolites have been detected (Jones et al. 2005; Miao et al. 2002), and soil could be an important source of water contamination (Halling-Sorensen 2001). Nevertheless, pharmaceuticals, albeit at trace levels in the effluents, can accumulate in soils if long-term irrigation occurs, and this may result in environmental problems such as contamination of the groundwater (Ternes et al. 2007).

Pharmaceuticals include a wide range of chemical classes. Their adsorption behaviors vary compound to compound and are difficult to predict because sorption is often controlled by interactions with specific functional groups or complicated pH-dependent speciation (Kodešová et al. 2015). Soil properties also affect pharmaceutical sorption, as shown in the recent work by (Kodešová et al. 2015; Kodešová et al. 2016). The presence and distribution of pharmaceuticals in the soil via land application are far from known because of a lack of appropriate methodologies.

Several analytical methods for determination of pharmaceuticals in various solid matrices have been described previously (Okuda et al. 2009; Salvia et al. 2012; Petrović et al. 2005; Moreno-González et al. 2015; Białk-Bielińska et al. 2016). Techniques such as pressurized liquid extraction (Jacobsen et al. 2004; Jelić et al. 2009; Barron et al. 2008), ultrasonic extraction (Xu et al. 2008), microwave-assisted extraction (Rice and Mitra 2007), or supercritical fluid extraction (Yamini et al. 2002) have been introduced for extraction of pharmaceuticals from solid environmental samples. Selection of the best solvent or solvent mixture is also necessary as it is influencing recovery of the target compounds from the solid matrices. However, most studies focused on extraction methods for a few groups of pharmaceuticals from sludge and sediments. These matrices have different compositions and behaviors than soils. Therefore, the development of a sensitive analytical method for determination of various classes of pharmaceuticals at trace levels is necessary.

Liquid chromatography combined with mass spectrometry (LC-MS) or with tandem mass spectrometry (LC-MS/MS) are popular techniques currently being used in pharmaceutical analyses. The latter allows detection of extremely low concentrations (ng/L or ng/g) of these compounds in various complex liquid or solid matrices (Lindberg et al. 2014; Fedorova et al. 2014).

In this context, the objective of this work was to develop a fast and simple multiresidual LC-MS/MS method for determination of a wide range of pharmaceuticals in various soil types. To this end, different solvent mixtures were tested. To our knowledge, a method for the determination of pharmaceuticals with evaluated performance in such a various soil types has never been proposed.

Materials and methods

Chemicals

Methanol, acetonitrile, and isopropanol (LiChrosolv® Hypergrade) were purchased from Merck (Darmstadt, Germany). Formic acid (LC/MS grade) to acidify the mobile phases was purchased from Labicom (Olomouc, Czech Republic). Ultra pure water was produced using an Aqua-MAX-Ultra System (Younglin, Kyounggi-do, Korea). All analytical standards were of high purity (mostly 98 %).

Most of the native standards were purchased from Sigma-Aldrich (St. Louis, MO, USA); cilazapril, clemastine, dicycloverine, felodipine, levomepromazine, meclozine, and orphenadrine were purchased from EDQM (Strasbourg, France); atracurium, bisoprolol, citalopram, sertaline, and venlafaxine were purchased from AK Scientific (Union City, USA); atorvastatin, donepezil, irbesartan, and rosuvastatin were purchased from CHEMOS GmbH (Regenstauf, Germany); oseltamivire was purchased from Roche (Germany); and sulfamethoxazole was obtained from Riedel-de Haen (Germany).

The internal standards (ISs) trimethoprim ($^{13}C_3$) and sulfamethoxazole ($^{13}C_6$) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA), while carbamazepine (D_{10}) and amitriptyline (D_6) were acquired from CDN Isotopes (Pointe-Claire, Quebec, Canada); atenolol (D_6) and metoprolol hydrochloride (D_7) were purchased from Alsachim (Strasbourg, France), and clarithromycin-N-methyl (D_3) was acquired from TRC (North York, Canada). A stock solution of each pharmaceutical was prepared in methanol at a concentration of 1 mg/mL. A spiking mixture of each was prepared by diluting the stock solution with methanol to a final concentration of 1 μg/mL. All stock and spiking solutions were stored at −20 °C.

Sampling, sample preparation, and extraction procedure

Thirteen types of soils, representative of the Czech Republic and central Europe, were selected for the experiments (see Supplemental Table SI 1). Each sample (about 20 kg) was collected from the field, dried, ground, and sieved through a 2-mm sieve. The basic chemical and physical properties were determined using methods published elsewhere (Kodešová et al. 2015).

Sediment samples were collected from fish pond bottoms, which consisted mainly of soil transported from surrounding fields, that were continuously exposed to effluent from a wastewater treatment plant (WWTP; from Vodňany, with 6500 connected inhabitants). Pond sediment samples were collected after pond harvesting as grab samples of the surface layer. Sediment samples were also taken at the influent and effluent of a biological fish pond known as Čežárka (length, 0.68 km) situated 500 m downstream from the WWTP and used as additional effluent treatment. An additional three samples were taken from a production pond known as Dřemliny (length, 7.3 km), which is the next pond in the system. Samples were taken from the WWTP sludge and four horizons (at depths of 0–35, 30–45, 45–60, and 60–60 cm) of a field fertilized with this sludge. They were analyzed to investigate possible transport of pharmaceuticals from the surface to lower soil horizons.

Fortified soil samples were prepared by weighing 2 g of each soil into 10-mL autosampler vials and dripping native pharmaceutical solutions in methanol onto the surface of each ones. Vials were then shaken and the solvent was allowed to evaporate. Final concentrations of the targeted compounds were 1000 ng/g dry weight (d.w.) for each soil. The ISs (10 ng/g) were added to all soils before the extraction procedure. Pharmaceuticals were extracted by following extraction procedures:

(1) P-1: extraction with acetonitrile and water (1/1 *v*/*v* with 0.1 % formic acid) followed by extraction with acetonitrile, 2-propanol, and water (3/3/4 *v*/*v*/*v* with 0.1 % formic acid)
(2) P-2: extraction with acetonitrile and water (1/1 *v*/*v* with 0.1 % formic acid)
(3) P-3: extraction with methanol and water (2/1 *v*/*v* with 0.1 % formic acid)

Four milliliters extraction solvent was added to the soil and ultrasonicated (DT 255, Bandelin electronic, Sonorex digitec, Berlin, Germany) for 15 min. The supernatant was filtered through a syringe filter (0.45 μm, regenerated cellulose, Labicom, Olomouc, Czech Republic) into 10-mL vials. The same step was repeated with the same solvent mixture volume (in procedures P-2 and P-3) or the same volume of different mixture (in procedure P-1). The soil extracts were stored in a freezer at −20 ° C until analysis. Each sample was prepared and analyzed in triplicate. All data were processed using the STATISTICA v.12 software for Windows (StatSoft, Czech Republic).

LC-MS/MS analysis

A triple stage quadrupole MS/MS TSQ Quantum Ultra mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela 1250 LC pump (Thermo Fisher Scientific) and an HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland) was used for analysis.

Two analytical columns, a Cogent Bidentate C18 column (50 mm × 2.1 mm i.d., 4 μm particle size from MicroSolv Technology Corporation Eatontown, NJ, USA) and a Hypersil Gold column (50 mm × 2.1 mm i.d, 3 μm particle size from Thermo Fisher Scientific, San Jose, CA, USA) were tested for chromatographic separation of target compounds. The former was chosen as the best suitable analytical column because it provided better separation and peak shapes. Method parameters are reported in Supplementary Table SI 2.

A detailed description of MS/MS transitions and LC-MS/MS configuration and set up has been provided elsewhere (Grabic et al. 2012; Khan et al. 2012). The range of the analytes and the methods chosen were adopted from mentioned studies. The IS method was used for quantification of analytes. Information on the IS used is given in Table 1. The matrix effect was assessed for each compound, and corrections for ion suppression or enhancement were accomplished using matrix-matched standards for each soil type. Matrix-matched standards were prepared from soil extract by spiking with both IS and native compounds at 10 ng/mL and 100 ng/mL, respectively.

Table 1 Surrogate standard used, linearity (R^2), and limit of quantification (LOQ) ng/g, *RT* retention time (min) for 44 studied compounds

Compounds	Surrogate standard used	R^2	LOQ	RT
Analgesics/anti-inflammatories				
Diclofenac	Carbamazepine	1.000	1.0	9.09
Codeine	Trimethoprim	0.993	2.4	4.68
Tramadol	Trimethoprim	0.999	2.4	5.98
Anti-hypertensive (cardiovascular) drug				
Atenolol	Sulfamethoxazole	0.993	6.4	4.27
Metoprolol	Metoprolol	0.992	1.9	5.91
Bisoprolol	Metoprolol	0.990	1.1	6.73
Sotalol	Sulfamethoxazole	0.997	9.4	4.24
Valsartan	Carbamazepine	0.996	1.1	8.74
Antibiotics (ATB)				
Clarithromycin	Clarithromycin	0.994	2.5	8.58
Trimethoprim	Trimethoprim	0.998	3.2	5.19
Sulfamethoxazole	Sulfamethoxazole	1.000	2.0	6.52
Clindamycine	Clarithromycin	0.998	2.0	6.47
Sulfadiazine	Carbamazepine	0.999	1.7	4.8
Sulfathiazole	Carbamazepine	0.998	1.6	4.99
Sulfapyridine	Carbamazepine	1.000	1.5	5.05
Sulfamerazine	Carbamazepine	1.000	1.8	5.17
Sulfamethazine	Carbamazepine	0.999	1.5	5.46
Sulfamethizole	Carbamazepine	1.000	1.1	5.6
Sulfamethoxypyridazine	Carbamazepine	0.999	1.5	5.65
Sulfadimethoxine	Carbamazepine	1.000	1.7	7.26
Sulfaquinoxaline	Carbamazepine	1.000	1.4	7.29
Sulfaphenazole	Carbamazepine	1.000	1.4	7.38
Sulfasalazine	Carbamazepine	1.000	1.0	7.92
Oseltamivir	Trimethoprim	0.999	2.8	5.29
Antidepressants/psychiatric drugs				
Carbamazepine	Carbamazepine	0.992	2.1	8.02
Memantine	Trimethoprim	0.993	2.0	6.78
Naloxone	Sulfamethoxazole	0.993	1.7	4.63
Ropinirole	Metoprolol	0.998	1.2	5.54
Bupropion	Trimethoprim	0.999	1.2	6.81
Venlafaxine	Trimethoprim	1.000	2.2	6.84
Citalopram	Clarithromycin	0.999	2.2	7.97
Paroxetine	Clarithromycin	0.994	1.0	8.38
Amitriptyline	Amitriptyline	1.000	2.8	8.86
Mianserin	Clarithromycin	0.999	1.8	8
Lipid regulators				
Rosuvastatin	Carbamazepine	0.997	1.0	8.48
Bezafibrate	Carbamazepine	0.998	1.0	8.72
Antidiabetics				
Glibenclamide	Carbamazepine	0.996	1.0	9.08
Glimepiride	Carbamazepine	0.997	0.6	9.14
Antihistamine				
Fexofenadine	Carbamazepine	0.997	0.8	8.59

Table 1 (continued)

Compounds	Surrogate standard used	R^2	LOQ	RT
Clemastine	Clarithromycin	0.998	1.2	9.56
Muscle relaxant				
Atracurium	Trimethoprim	0.978	2.1	6.37
Urological				
Finasteride	Carbamazepine	1.000	0.8	9.18
Antifungal				
Fluconazole	Sulfamethoxazole	1.000	2.0	6.14
β2-adrenergic receptor agonist				
Terbutaline	Sulfamethoxazole	0.995	5.3	4.19

Results

Efficiency of extraction procedure

The extraction solvent is one of the most critical parameters influencing the final range of analytes and method performance. The recovery ratio achieved by each extraction procedure for each target pharmaceutical was evaluated at a fortification level of 1000 ng/g and was expressed as the ratio between the determined concentration and the nominal concentration. The complete data set is reported in Table SI 3, where the data shown is the average across the triplicate trials. Generally, parallels were below 30 % relative standard deviation (RSD), even at low recovery rates.

The recovery results achieved using various extraction solvents on 13 soil types for 91 pharmaceuticals are shown in Fig. 1. Included compounds were highly variable in their physicochemical properties, resulting in different sorption behaviors in sludge (Horsing et al. 2011). The most consistent results were achieved from the procedure P-1, where median recovery for individual soils ranged between 70 and 100 %. Extraction procedures P-2 and P-3 provided highly variable results across the range of pharmaceuticals, recovering from 3 % to more than 135 % (see Supplementary Table SI 3).

Procedure P-1 was selected for further validation because of its consistent recoveries for most pharmaceuticals groups. However, we found that some compounds were ineffectively recovered from all matrices and others showed unacceptably high variability in recoveries from various matrices. It has been shown that there is no correlation between lipophilicity (log D) and sorption in sludge for various pharmaceuticals (Horsing et al. 2011). Sorption in soil is additionally complicated by the highly variable content of inorganic matter, which affects, in particular, the interaction between ionized pharmaceuticals and the charged surfaces found on minerals (Kodešová et al. 2015). Both mentioned facts result leads to understandably poor performance for lipophilic compounds (e.g., miconazole, fluphenazine, and meclozine), which have high affinities to sludge (Horsing et al. 2011). Some compounds that have low sorption to sludge were also ineffectively recovered (orphenadrine and hydroxyzine). Since we aimed to keep the method as simple as possible, we decided not to improve extraction efficiency by increasing solvent volume or adding another step with a less polar solvent. Instead, the array of target pharmaceuticals was reduced to 63 compounds, and we aimed for a target recovery between 55 and 135 % in all soils.

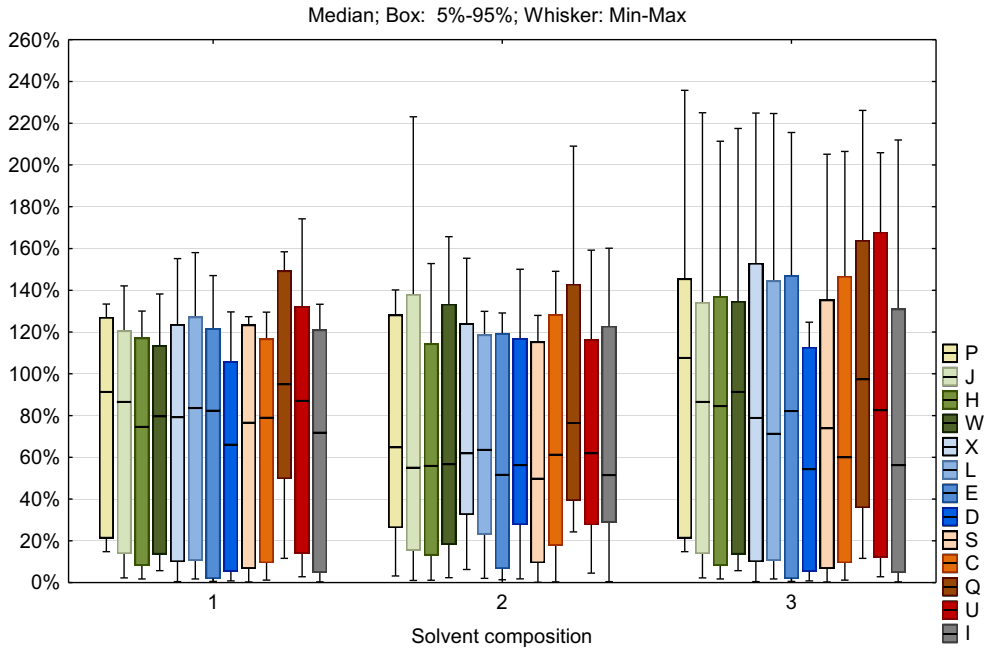

Fig. 1 Recoveries of 91 pharmaceuticals obtained using different extraction solvents for 13 soil types (from P to I soils, detailed soil properties are shown in Supplementary materials Table SI 1). (*1*) P-1: extraction with acetonitrile and water (1/1 *v/v* with 0.1 % formic acid) followed by extraction with acetonitrile, 2-propanol, and water (3/3/4 *v/v/v* with 0.1 % formic acid). (*2*) P-2: extraction with acetonitrile and water (1/1 *v/v* with 0.1 % formic acid). (*3*) P-3: extraction with methanol and water (2/1 *v/v* with 0.1 % formic acid)

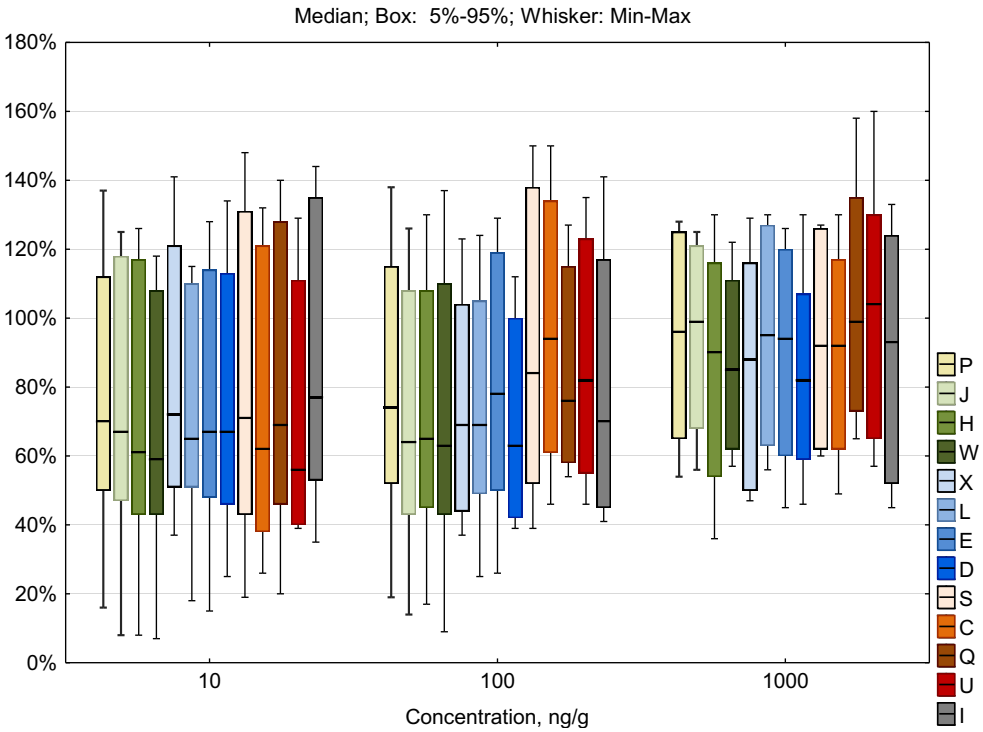

Fig. 2 Recoveries of 63 pharmaceuticals obtained using P-1 from 13 soil types (from P to I soils, detailed soil properties are shown in Supplementary materials Table SI 1) at three concentration levels: 10, 100, and 1000 ng/g

As sorption (and consequently extraction) is a competitive process, it can have different effects at high vs. low concentration levels. Subsequently, we evaluated procedure P-1 for extraction of 63 pharmaceuticals at three fortification levels (10, 100, and 1000 ng/g d.w.) in all 13 soils. The complete data set is given in Supplementary Table SI 4 and illustrated in Fig. 2. There is an apparent trend in both median and minimum recoveries for all soils. Lower minimal recoveries were found at the 10 ng/g fortification level compared to the other two. This trend, which can be explained by saturation of sorption capacity in highly fortified soils, is clear for a few pharmaceuticals, such as atorvastatin, rosuvastatin, and furosemide. Consistent, but relatively low, recoveries (between 40 and 55 %) were found for a group of pharmaceuticals (sertraline, irbesartan, felodipine, and sulfamoxole) at both the lower fortification levels. Some compounds were difficult to extract from only two of the tested soils, U and C. The reasons for this cannot be exactly defined, and finally they are irrelevant from analyst's point of view.

Using the same criterion for recovery as mentioned above, and omitting some pharmaceuticals that displayed border performance (all lipid regulators, sulfaphenazole, sulphamethazine, and finasteride); we were able to include 44 of the 63

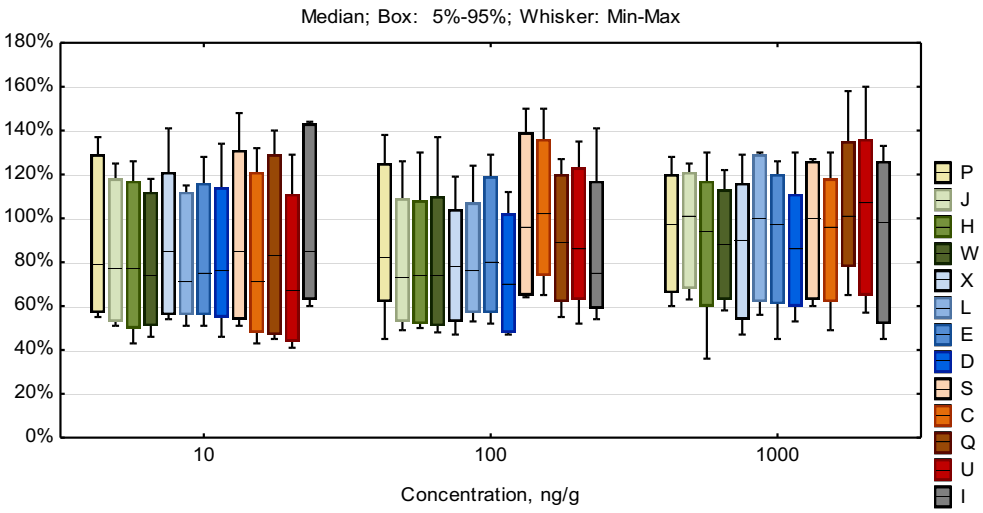

Fig. 3 Recoveries of 44 pharmaceuticals obtained using P-1 from 13 soil types (from P to I soils, detailed soil properties are shown in Supplementary materials Table SI 1) at three concentration levels: 10, 100, and 1000 ng/g

Table 2 Recoveries and recovery uncertaninties of 44 pharmaceuticals from four soil samples at fortification level 100 ng/g dry weight

Compound	100 ng/g dry weight							
	Soil P		Soil I		Soil C		Soil U	
	Recovery	$n = 10$ RSD	Recovery	$n = 10$ RSD	Recovery	$n = 10$ RSD	Recovery	$n = 10$ RSD
Analgesics/anti-inflammatories								
Diclofenac	63 %	7 %	60 %	8 %	53 %	14 %	54 %	9 %
Codeine	89 %	5 %	74 %	8 %	74 %	11 %	65 %	9 %
Tramadol	112 %	7 %	86 %	6 %	84 %	10 %	72 %	8 %
Anti-hypertensive (cardiovascular) drug								
Atenolol	117 %	17 %	82 %	8 %	71 %	13 %	77 %	14 %
Metoprolol	110 %	8 %	97 %	16 %	97 %	16 %	100 %	11 %
Bisoprolol	105 %	8 %	94 %	16 %	90 %	15 %	93 %	11 %
Sotalol	95 %	13 %	86 %	12 %	74 %	14 %	85 %	9 %
Valsartan	72 %	9 %	63 %	10 %	61 %	11 %	54 %	9 %
Antibiotics (ATB)								
Clarithromycin	122 %	20 %	126 %	22 %	112 %	14 %	113 %	18 %
Trimethoprim	114 %	7 %	103 %	9 %	98 %	6 %	92 %	9 %
Sulfamethoxazole	113 %	9 %	101 %	8 %	101 %	7 %	102 %	7 %
Clindamycine	105 %	22 %	114 %	21 %	103 %	10 %	102 %	19 %
Sulfadiazine	82 %	6 %	73 %	7 %	74 %	5 %	73 %	5 %
Sulfathiazole	86 %	7 %	77 %	7 %	75 %	6 %	73 %	6 %
Sulfapyridine	74 %	6 %	66 %	8 %	65 %	6 %	65 %	5 %
Sulfamerazine	78 %	7 %	71 %	6 %	74 %	7 %	66 %	6 %
Sulfamethazine	67 %	6 %	65 %	8 %	64 %	5 %	62 %	5 %
Sulfamethizole	68 %	7 %	66 %	7 %	65 %	5 %	62 %	6 %
Sulfamethoxypyridazine	84 %	5 %	76 %	7 %	82 %	4 %	77 %	6 %
Sulfadimethoxine	95 %	7 %	91 %	7 %	90 %	5 %	86 %	5 %
Sulfaquinoxaline	80 %	7 %	81 %	8 %	76 %	4 %	74 %	6 %
Sulfaphenazole	66 %	8 %	66 %	5 %	60 %	6 %	60 %	5 %
Sulfasalazine	78 %	13 %	47 %	19 %	67 %	11 %	49 %	11 %
Oseltamivir	99 %	7 %	77 %	9 %	78 %	7 %	44 %	9 %
Antidepressants/psychiatric drugs								
Carbamazepine	105 %	5 %	96 %	4 %	91 %	12 %	88 %	8 %
Memantine	58 %	6 %	43 %	8 %	42 %	12 %	34 %	7 %
Naloxone	97 %	16 %	65 %	13 %	78 %	17 %	96 %	8 %
Ropinirole	75 %	13 %	70 %	16 %	70 %	17 %	78 %	8 %
Bupropion	105 %	8 %	94 %	10 %	80 %	13 %	81 %	8 %
Venlafaxine	90 %	7 %	58 %	8 %	65 %	12 %	54 %	8 %
Citalopram	96 %	25 %	102 %	21 %	99 %	16 %	103 %	18 %
Paroxetine	84 %	25 %	103 %	21 %	87 %	18 %	92 %	19 %
Amitriptyline	97 %	12 %	100 %	9 %	89 %	18 %	79 %	17 %
Mianserin	86 %	25 %	85 %	20 %	80 %	16 %	83 %	18 %
Lipid regulators								
Rosuvastatin	66 %	12 %	58 %	5 %	53 %	11 %	51 %	10 %
Bezafibrate	64 %	13 %	59 %	8 %	53 %	16 %	53 %	4 %
Antidiabetics								
Glibenclamide	68 %	7 %	64 %	8 %	59 %	12 %	59 %	9 %
Glimepiride	74 %	7 %	68 %	7 %	60 %	13 %	58 %	8 %

Table 2 (continued)

Compound	100 ng/g dry weight							
	Soil P		Soil I		Soil C		Soil U	
	Recovery	$n = 10$ RSD	Recovery	$n = 10$ RSD	Recovery	$n = 10$ RSD	Recovery	$n = 10$ RSD
Antihistamine								
Fexofenadine	93 %	7 %	73 %	5 %	73 %	14 %	61 %	9 %
Clemastine	89 %	24 %	103 %	21 %	88 %	19 %	96 %	19 %
Muscle relaxant								
Atracurium	61 %	7 %	45 %	10 %	50 %	13 %	44 %	9 %
Urological								
Finasteride	64 %	15 %	62 %	11 %	54 %	16 %	55 %	5 %
Antifungal								
Fluconazole	99 %	9 %	86 %	6 %	82 %	14 %	86 %	8 %
β2-adrenergic receptor agonist								
Terbutaline	99 %	15 %	81 %	13 %	75 %	16 %	94 %	9 %

pharmaceuticals in the final method (Fig. 3). Overall, extraction procedure P-1 was simple, amply efficient for many compounds of interest.

Method validation

The method was simplified to a single LC-MS/MS run and further validated. A six-point calibration curve was prepared by spiking process blanks with target compounds at concentrations between 1 and 1000 ng/mL and ISs at 10 ng/mL. This range corresponds to concentrations in soil between 4 and 4000 ng/g. The calibration curves showed sufficient linearity in studied range with squares of residues $r^2 > 0.99$ for all compounds. The limit of quantification (LOQ) was derived from the response at the lowest calibration point with the relative response factor (RRF) not deviating more than 30 % from the average RRF. The peak area corresponding to this point, instead of the peak area found in the samples, was divided by a factor of four and put into a calculation template of validated samples. Thus, all factors influencing the final value (IS recovery, matrix effect, and other uncertainties of the method) were included. Averages of resultant LOQs together with linearity parameters are presented in Table 1. They ranged from 0.6 ng/g for glimepiride to 9.4 ng/g for sotalol and had a median value of 1.7 ng/g.

The matrix effect was evaluated as the difference between matrix-matched standards' RRF and average RRF obtained from the calibration curve. The matrix effect was less than 30 % for most pharmaceuticals, probably because of the relatively high dilution of the extracts (Supplementary Table SI 5). However, there were two groups of compounds that showed significant matrix effects. The larger, which contained paroxetine, bupropion, glibenclamide, glimepiride, fexofenadine, clemastine, and finasteride showed ionization enhancement independent of soil type. Early eluted pharmaceuticals (atenolol and terbutaline) belong to another group, where strong ionization suppression and significant differences between soil types were found (between −6 and −112 % for atenolol and between 2 and −84 % for terbutaline). This effect can be attributed to co-extracted ionic or polar interferences, because both affected analytes were eluted relatively close to the dead volume.

The accuracy and precision of the method for the selected 44 pharmaceuticals were evaluated in four soils (P, I, C, and Q) of variable properties at a fortification level 100 ng/g. Average recoveries and RSDs for ten parallel trials are reported in Table 2. According to requirements for analytical method performance at this concentration level (Commission Decision 2002), the recovery (trueness) of the method must fall within the range 50 to 120 %, and repeatability (RSD) should not exceed 23 %. The trueness of the method was acceptable for all pharmaceuticals in all soils except for atracurium in soils I and U. Repeatability of the analysis in all matrices and for all compounds varied between 4 and 25 %, which is also considered acceptable.

Application of the method

The developed method was applied for determination of pharmaceuticals in two possible contamination transfer scenarios.

Table 3 Average concentration of 44 target compounds detected in different sample types: fish pond Dřemliny (D_1, D_2, and D_3); biological fish pond Čežárka (*Čežárka _Eff* effluent samples, *Čežárka _Inf* influent samples); soil samples (loess) were taken from surface (S_1 0–35 cm) and subsurface horizons (from the depths of S_2 30–45 cm, S_3 45–60 cm, S_4 60–68 cm); sludge samples: SL_A, SL_B; *BLOQ* below the limit of quantification

Concentration ng/g dry weight

Compounds	D_1	D_2	D_3	SD_Eff	SD_Inf	S_1	S_2	S_3	S_4	SL_A	SL_B
Analgesics/anti-inflammatories											
Diclofenac	BLOQ	BLOQ	BLOQ	3.9	7.6	BLOQ	BLOQ	BLOQ	BLOQ	23	23
Codeine	BLOQ	BLOQ	BLOQ	BLOQ	4.5	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Tramadol	BLOQ	BLOQ	BLOQ	14	57	BLOQ	BLOQ	BLOQ	BLOQ	15	18
Anti-hypertensive (cardiovascular) drug											
Atenolol	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Metoprolol	4.8	2.2	3.1	83	56	BLOQ	BLOQ	BLOQ	BLOQ	65	67
Bisoprolol	BLOQ	BLOQ	BLOQ	16	13	BLOQ	BLOQ	BLOQ	BLOQ	3.9	3.6
Sotalol	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Valsartan	BLOQ	BLOQ	BLOQ	BLOQ	1.5	BLOQ	BLOQ	BLOQ	BLOQ	12	9.2
Antibiotics (ATB)											
Clarithromycin	BLOQ	BLOQ	BLOQ	15	11	BLOQ	BLOQ	BLOQ	BLOQ	71	59
Trimethoprim	BLOQ	BLOQ	BLOQ	6.9	10	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfamethoxazole	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Clindamycine	3.7	BLOQ	2.0	4.9	3.9	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfadiazine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfathiazole	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfapyridine	BLOQ	BLOQ	BLOQ	1.6	1.9	BLOQ	BLOQ	BLOQ	BLOQ	14	13
Sulfamerazine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfamethazine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfamethizole	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfamethoxypyridazine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfadimethoxine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfaquinoxaline	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Sulfaphenazole	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	1.9
Sulfasalazine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	1.1
Oseltamivir	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Antidepressants/psychiatric drugs											
Carbamazepine	BLOQ	BLOQ	BLOQ	7.1	11	BLOQ	BLOQ	BLOQ	BLOQ	65	62
Memantine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Naloxone	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	14	4.5
Ropinirole	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Bupropion	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Venlafaxine	BLOQ	BLOQ	BLOQ	49	37	BLOQ	BLOQ	BLOQ	BLOQ	42	50
Citalopram	3.5	BLOQ	BLOQ	100	98	3.3	4.6	BLOQ	BLOQ	199	223
Paroxetine	BLOQ	BLOQ	BLOQ	BLOQ	2.0	BLOQ	BLOQ	BLOQ	BLOQ	10	15
Amitriptyline	BLOQ	BLOQ	BLOQ	3.6	6.6	BLOQ	BLOQ	BLOQ	BLOQ	24	26
Mianserin	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	3.0	2.7
Lipid regulators											
Rosuvastatin	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	1.1	BLOQ
Bezafibrate	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	3.5	17
Antidiabetics											
Glibenclamide	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Glimepiride	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ

Table 3 (continued)

Concentration ng/g dry weight											
Compounds	D_1	D_2	D_3	SD_Eff	SD_Inf	S_1	S_2	S_3	S_4	SL_A	SL_B
Antihistamine											
Fexofenadine	0.83	BLOQ	BLOQ	7.8	12	BLOQ	BLOQ	BLOQ	BLOQ	31	32
Clemastine	BLOQ	BLOQ	BLOQ	BLOQ	1.8	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Muscle relaxant											
Atracurium	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	4.1
Urological											
Finasteride	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Antifungal											
Fluconazole	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
β2-adrenergic receptor agonist											
Terbutaline	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ

One scenario included sediments from fish pond bottoms, created from soil transported from surrounding fields continuously affected by the effluent from the WWTP, and another one included soil from an experimental field annually enriched with WWTP sludge. Twenty-four of 44 target compounds were found above the LOQ in at least one of the studied samples. The average concentrations ($n = 3$) determined in those samples are summarized in Table 3.

Concentrations of the pharmaceuticals ranged between 0.83 and 223 ng/g d.w. The samples, which can be assigned as sources of contamination (biological pond sediment and applied sludge), had significantly higher levels of studied compounds compared to the assumed targets of transport. Citalopram and venlafaxine prevailed in all sludge and biological pond samples over pharmaceuticals present at high concentrations in effluent (clarithromycin, carbamazepine, metoprolol, tramadol, and trimethoprim). There were relatively large differences between sludge and biological pond sediments. The absence of some compounds in sludge could be attributed to the age of the sludge. While the sediments were sampled just after water level lowering, the sludge stayed in the pond for an extended time before it was used as fertilizer. Only citalopram, metoprolol, and clindamycine were found in the production pond sediment samples at levels close to the LOQs. Citalopram only was found above the LOQ in the two upper soil horizons. This finding agrees with the predicted concentrations based only on the dilution factor derived from the ratio between citalopram found in sludge and that found in soil.

Conclusion

A simple and efficient extraction method, together with a sensitive LC − MS/MS method for the simultaneous determination of the concentrations of 44 pharmaceuticals in various soil samples, was developed. The range of recoveries obtained (55–135 %) implies that the method could be used for regular monitoring of various soil samples. By using this simple extraction method, recoveries were, for some pharmaceuticals, lower than 55 %, restricting its use to 44 pharmaceuticals out of 91 evaluated, in real soil matrices. Greater diversity in the soil results in the need for a more complex extraction procedure. Analytical complexities presented by the soil samples, their properties, and subsequent unknown matrix effects in analyses based on LC − MS/MS remain intractable issues. However, these gaps can be bridged by the simultaneous recoveries of target compounds to validate the analysis used during regular monitoring.

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