Journal of Chromatography A, 1258 (2012) 1–15

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Optimization of matrix solid-phase dispersion for liquid chromatography tandem mass spectrometry analysis of 12 pharmaceuticals in sediments

Dragana Mutavdžić Pavlović*, Tea Pinušić, Martina Periša, Sandra Babić

Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 17 May 2012
Received in revised form 6 August 2012
Accepted 7 August 2012
Available online 16 August 2012

Keywords:
Pharmaceuticals
Matrix solid-phase dispersion
Liquid chromatography–tandem mass spectrometry
Sediment

ABSTRACT

The matrix solid-phase dispersion (MSPD) technique accompanied with LC–MS/MS detection for the purpose of determination of 12 pharmaceuticals (sulfaguanidine, sulfadiazine, sulfamethazine, sulfamethoxazole, trimethoprim, roxithromycin, praziquantel, febantel, enrofloxacin, ciprofloxacin, norfloxacin and procaine) applied to sediment samples has been described in this paper. Different parameters, such as the type of solid phase, the elution solvent and its volume have been investigated. The analytes were successfully extracted by C18 as an MSPD sorbent with 5 mL of acetonitrile:5% of oxalic acid = 6:4 (v/v) as an elution solvent. The proposed method provides a linear response over the concentration range of 0.0005–100 μg/g, depending on pharmaceuticals with correlation coefficients above 0.9928 in all cases except for trimethoprim (0.9889). Also, the method has revealed low limits of detection (0.125–500 ng/g), good precision (intra and inter-day), a relative standard deviation below 15% and recoveries above 80%, except for roxithromycin, febantel and enrofloxacin. The method has been successfully applied to analysis of different sediment samples.

1. Introduction

Pharmaceuticals represent a large and diverse group of compounds designed to prevent, cure and treat diseases and improve health. They are utilized in significant quantities throughout the world. For example, in the European Union (EU), around 3000 different pharmaceutically active compounds have been approved for use in human medicine. Although the effects of pharmaceuticals have already been identified as an emerging problem in environmental chemistry [1], the potential environmental impacts of their production and use are slightly understood and have recently become a topic of research interest [2]. Residues of pharmaceutical compounds end up in the environment due to common practices of their usage which facilitates improvement of the state of health of both humans and animals. The concentration of pharmaceuticals in the environment, their evolution with time and their possible effects depend not only on the quantity of manufactured drugs and the dosage frequency but also on their amount when being discharged from wastewater treatment plants (WWTPs) [3]. In contrast to the aquatic environment, the occurrence and the fate of pharmaceuticals in solid matrices, such as soil and sediment have not been thoroughly studied yet. Animal origin pharmaceuticals, including aquaculture-derived compounds, contribute significantly to the occurrence of pharmaceuticals in solid matrices due to their patterns of application.

The fate and degradation pathway of pharmaceuticals released into the environment vary depending on the physicochemical properties of compounds. The mobility of compounds greatly depends on water solubility, the octanol–water partitioning coefficient and the organic carbon contents of the sorbent. For example, tetracyclines show the highest sorption coefficient compared to other major antibiotics and sulfonamides which are usually relatively mobile. These trends help us predict where compounds can be found in the environment. In terms of the major antibiotic classes, sulfonamides are most commonly detected in groundwaters due to their high mobility. Several complex processes can be involved in the sorption mechanism of pharmaceuticals in solid matrices. This does not comprise only hydrophobicity but also cation exchange, cation bridging, surface complexation and hydrogen bonding, which all play important roles in retaining pharmaceuticals on a solid matrix [4]. The sorption and fixation of antibiotics is strongly governed by the property of numerous compounds to ionize depending on the pH of a medium. The log K_{ow} coefficients of ionizing compounds change considerably in a pH range around the pK_a. The adsorption coefficients K_d of sulfonamides increased when the soil pH decreased. This was related to the ionization of amphoteric sulfonamides [5].

Many analytical methods have been developed for determination of pharmaceuticals in environmental samples.

* Corresponding author. Tel.: +385 1 4597 204; fax: +385 1 4597 250.
E-mail addresses: dragana.mutavdzic@gmail.com, dmutavdz@fkit.hr (D. Mutavdžić Pavlović).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2012.08.025

Concerning pharmaceutical extraction procedures from soils and sediments, a number of alternative techniques exist along traditional extraction methods. The former include microwave assisted solvent extraction (MASE) [6], ultrasonic solvent extraction (USE) [7–9], microwave assisted micellar extraction (MAME) [3], accelerated solvent extraction (ASE) [7,10] and/or pressurized liquid extraction (PLE) [1,2,9,11]. These alternative procedures have shown clear advantages in terms of time and solvent consumption, though some of them require utilization of expensive equipment. Also, the above extraction techniques are followed by the clean-up step with solid-phase extraction (SPE). Matrix solid-phase dispersion (MSPD), a process allowing simultaneous extraction and clean-up of analytes from solid samples, can also be used as an alternative technique to classical extraction methods with significant reduction in solvent consumption and requires no particularly expensive instrumentation. MSPD has been mainly used for extraction of organic environmental pollutants from food and biological matrices [12], but to our knowledge it has not been applied to extraction of pharmaceuticals from sediment samples. The performance of MSPD is mainly affected by the column packing technique and the elution procedure. Particularly, analyzed samples (solid or semi-solid) are blended with a suitable adsorbent (e.g. C18) to form a homogenous packing material. After successful packing, the sample/adsorbent column is eluted by a stepwise solvent program similar to SPE [13].

Pharmaceuticals are usually determined by chromatographic methods, precisely GC or LC after the extraction and preconcentration step. LC is a more preferable chromatographic method since no time-consuming derivatization is needed because most pharmaceuticals are polar substances [1,14]. The majority of current analytical methods for separation and detection of pharmaceuticals refer to liquid chromatography coupled with mass spectrometry (LC–MS). LC with a single quadrupole MS analyzer offers good sensitivity, but when very complex matrices such as solid environmental samples are investigated, insufficient selectivity often impairs the unequivocal identification of analytes. Tandem MS guarantees superior performance in terms of sensitivity and selectivity in comparison with single quadrupole instruments. Therefore, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is the best choice when determining polar pharmaceuticals in environmental samples [15,16].

Simultaneous analysis of several groups of compounds with quite different physico-chemical characteristics generally requires a compromise in the selection of experimental conditions, which, in some cases, means failing to obtain the best performance for each compound. However, developing a multi-group method is rewarding as it can be applied in routine analysis, providing a large amount of data [17].

A novel, rapid, sensitive, an environmentally friendly and inexpensive multi-residue method has been proposed in this paper for the purpose of simultaneous extraction of 12 commonly used pharmaceuticals with a variety of structures and different physico-chemical properties (sulfaguanidine, sulfadiazine, sulfamethazine, sulfamethoxazole, trimethoprim, roxithromycin, febantel, praziquantel, enrofloxacin, norfloxacin, ciprofloxacin and procaine) from sediments. These compounds were selected on the basis of their vast production and consumption worldwide, especially in veterinary practices. The selected pharmaceuticals belong to different therapeutical classes: nine antibiotics, two anthelmintics and one anesthetic. The developed analytical method involves matrix solid-phase dispersion (MSPD) as a sample preparation technique and determination by LC–ESI-MS/MS. The advantage of the MSPD method over other reported applications thereof [12,18–27] reflects in its suitability for a wide range of compounds and matrices and for reduction of usage of organic solvents, which results in a decrease of the analysis costs and in safeguarding the integrity of the analyst and the environment. The performance of the method was evaluated through estimation of the linearity, sensitivity, repeatability and reproducibility. Finally, the method was successfully applied to the analyses of selected pharmaceuticals in different sediment samples.

2. Experimental

2.1. Reagents, standards, materials

The studied pharmaceuticals are as follows: praziquantel (PRAZ), febantel (FEBA), trimethoprim (TMP), norfloxacin (NOR), ciprofloxacin (CIPRO), enrofloxacin (ENRO), sulfaguanidine (SGUA), sulfadiazine (SDIAZ), sulfamethazine (SMET), sulfamethoxazole (SMETOX), procaine (PROC) and roxithromycin (ROXI). High purity (>99%) analytical standards of pharmaceuticals were obtained from Veterina Animal Health Ltd. (Kalinovica, Croatia) (PRAZ, FEBA, TMP, ENRO, SGUA, SDIAZ, SMET and PROC) or supplied by Sigma–Aldrich (NOR, CIPRO, SMETOX and ROXI). The studied pharmaceuticals and their physico-chemical properties are shown in Table 1.

A stock solution of a pharmaceutical mixture was prepared by dissolving accurate quantities of powdered standards in acetonitrile:water = 1:1 (v/v), and stored far away from light at 4 °C. The mass concentrations of each pharmaceutical in the stock solution were as follows: 10 μg/mL for PRAZ and FEBA; 50 μg/mL for ENRO, PROC, SMETOX, SMET and TMP; and 100 μg/mL for SGUA, SDIAZ, NOR, CIPRO and ROXI. Working standard solutions were prepared from this stock by serial dilution. All the used solvents belonged to the HPLC-grade and were supplied by Kemika (Zagreb, Croatia).

The polypropilene SPE empty reservoir (3 mL) and adequate 20 μm polyethylene frits were purchased from Agilent (Santa Clara, CA, USA).

MSPD sorbents Florisil PR and C18 ODS were purchased from Agilent (Santa Clara, CA, USA) with average particle size 45 μm and average pore size 60 Å.

2.2. Sampling and sample characterization

Several sediment samples were obtained from different Croatian regions, the Zadar County (sediment 1 and sediment 2), the Lika-Senj County (sediment 3) and the City of Zagreb (sediment 4). Once in the laboratory, samples were air-dried and grinded to pass through a sieve with 2-mm openings.

These sediment samples were characterized. The particle size analysis was set out by the Pippete method which is based on sedimentation of particles by their gravity [30]; organic matter (OM) content by the *Kochman* method which consists of organic matter oxidation using potassium permanganate and oxalic acid; sediment pH values in 1 M KCl with a sediment to solution ratio of 20 g:50 mL was determined with a pH meter (Mettler Toledo, USA); sediment electrical conductivity (EC) values in water with a sediment to water ratio of 20 g:50 mL was determined by an inoLab Cond 720 conductometer (Weilheim, Germany) and the content of calcium carbonate by volumetric measurements by calcimetry [30]. Their mechanical composition and basic chemical properties are presented in Table 2.

The sediment samples were different in texture (sandy, sandy loam and clay texture) with a different content of calcium carbonate, low level of organic matter (<5%) and pH values. Due to the above features of the sediments, it would be very interesting to investigate how their compositions influence the extraction efficiency of pharmaceuticals.

The sediment samples which were free of pharmaceuticals (sediment 1) by means of preliminary analysis were used as a control blank and for optimization and validation of the method. Spiked

Table 1
Chemical structure and physico-chemical properties of studied pharmaceuticals.

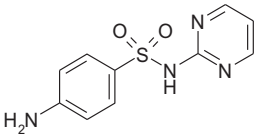
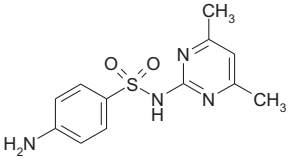
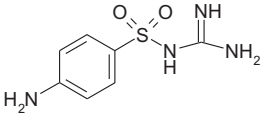
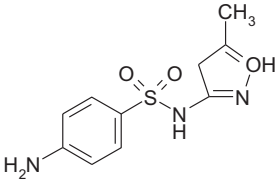
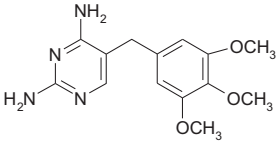
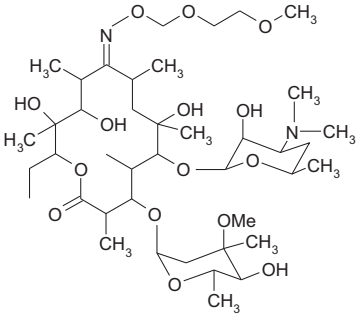
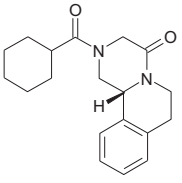
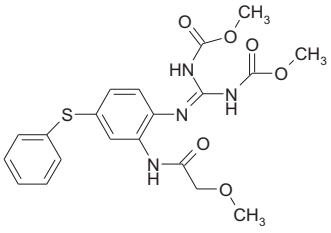
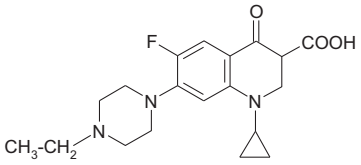
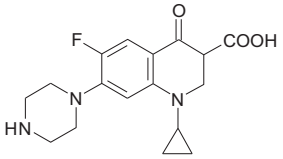
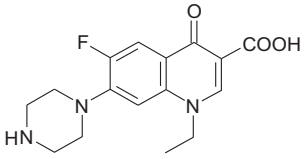
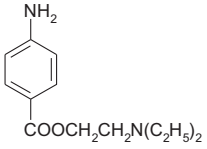
	Empirical formula	CAS no.	pK_a [28]	log K_{ow} [29]	log K_{oc} [29]
SDIAZ		68-35-9	2.10; 6.28	−0.09	1.87
SMET		57-68-1	2.28; 7.42	0.19	2.28
SGUA		57-67-0	1.55; 11.24	−1.22	1.70
SMETOX		723-46-6	1.83; 5.57	0.89	2.41
TMP		738-70-5	3.23; 6.76	0.91	2.86
ROXI		80214-83-1	9.17	2.75	3.98
PRAZ		55268-74-1	n.a.	2.42	3.55
FEBA		58306-30-2	n.a.	1.53	3.62

Table 1 (*Continued*)

	Empirical formula	CAS no.	pK_a [28]	log K_{ow} [29]	log K_{oc} [29]
ENRO		93106-60-6	5.86; 8.24	0.70	1.17
CIPRO		85721-33-1	6.68; 8.63	0.28	0.90
NOR		70458-96-7	6.22; 8.38	−1.03	1.27
PROC		59-46-1	2.24; 8.84	2.14	2.40

n.a., not available.

sediment samples were prepared by adding 2 mL of the standard solution of investigated analytes to 2 g of the sediment. The spiked sediments were allowed to air dry at the room temperature for 24 h.

2.3. Matrix solid-phase dispersion (MSPD)

C18 and Florisil sorbents were air-dried and cleaned before use. Both sorbent types were first washed three times with *n*-hexane and then three times with methanol. Actually, Florisil was activated at 160 °C before the washing. 50 mg of the sediment was placed in a glass mortar with 100 mg of the previously cleaned sorbent material. The materials were mixed in the glass mortar using a glass pestle to obtain a homogeneous material for the MSPD column. After the blending had been completed (after 2–3 min), the sample was packed into an empty column containing a polyethylene frit at the bottom. The second frit was placed on top of the sample by careful compression with a syringe plunger. The packed column was attached to a vacuum manifold (Visiprep™ 24, Supelco) coupled with a small vacuum pump while the flow was adjusted to 1 mL/min. The pharmaceuticals were then extracted using 5.0 mL of acetonitrile:5% of oxalic acid = 6:4 (v/v). All the extracts were evaporated to dryness using a rotary vacuum evaporator in a water bath at 40 °C, redissolved in 1.0 mL of acetonitrile:water = 1:1, and filtered through a 0.45 μm membrane filter before HPLC analysis. The extraction solvent and volume described above are based on the optimized procedure. During the optimization assays, a similar experimental procedure was followed using different pure solvents (methanol, ethanol, acetone, 1-prophanol, 2-prophanol, acetonitrile, dichloromethane and water (different pH values)) and also mixtures of methanol and acetonitrile with water (methanol:water = 6:4, methanol:water = 7:3, methanol:water = 8:2, acetonitrile:water = 6:4, methanol:water = 7:3 and acetonitrile:water = 8:2) and different acids (oxalic acid, acetic acid, hydrochloric acid, phosphoric acid and trifluoroacetic acid). Different elution volumes (5.0, 7.0, and 9.0 mL) and solid supports (C18 and Florisil) were also applied. Fulfilling the final optimized conditions, 50 mg (weighed accurately) of the sediment sample was first soaked in 50 μL of a saturated methanolic potassium hydroxide solution in a glass mortar and then 100 mg of Florisil and 0.5 g of anhydrous sodium sulphate were added. The mixture was thoroughly blended with the pestle to obtain complete disruption and dispersion of the sample on the solid support. The blending was followed (after 1 min) by packing the homogeneous mixture into a column containing a layer of 100 mg of Florisil at the bottom. These materials act as co-column or clean-up phases in the cartridge elution.

2.4. LC–ESI-tandem MS analysis

The LC analysis was performed using an Agilent Series 1200 HPLC system (Santa Clara, CA, USA) equipped with a Synergy Fusion C18 embedded column (150 mm × 2.0 mm, particle size 4 μm) and supplied by Phenomenex. The mobile phase used in the chromatographic separation consisted of a binary mixture of eluent A (0.1% formic acid in MilliQ water) and eluent B (0.1% formic acid in acetonitrile). A simultaneous mobile phase gradient program was

Table 2
Textural and chemical properties of the four tested sediments.

Sediment	Coarse sand (%)	Clay (%)	Silt (%)	Fine sand (%)	pH	EC (μS/cm)	OM (%)	$CaCO_3$ (%)
Sediment 1	15.40	0.35	0.15	84.10	7.52	124.0	2.91	8.34
Sediment 2	30.25	0.15	0.35	69.25	7.73	200.2	1.69	33.3
Sediment 3	0.65	0.25	0.45	98.65	3.97	20.0	2.79	3.17
Sediment 4	66.25	0.05	0.10	33.60	7.09	150.6	4.50	3.58

Table 3
LC–ESI-MS/MS conditions for the analysis of selected pharmaceuticals in MRM mode.

Compound	Retention time (min)	Precursor ion (*m*/*z*)	Quantification transition[a] (*m*/*z*)	Confirmation transition[a] (*m*/*z*)	Fragmentor voltage	CE[b] (eV)
SGUA	2.7	215	92	156	100	20
SMET	17.5	279	92	156	130	30
SDIAZ	14.5	251	156	92	110	20
SMETOX	19.7	254	92	156	120	20
TMP	14.3	291	230	123	135	30
ROXI	19.7	838	158	679	135	20
PRAZ	22.4	313	83	174	135	30
FEBA	23.5	447	383	415	110	30
ENRO	16.4	360	245	342	135	30
CIPRO	15.9	332	314	288	130	20
NOR	15.7	320	302	276	130	20
PROC	10.9	237	100	120	100	20

[a] Transition = precursor ion → product ion.
[b] Collision energy.

used: the elution started with a 2.5 min linear gradient from 100% A to 8% B, followed by a 3.5 min linear gradient to 10% B, a 5 min linear gradient to 30% B, a 4 min linear gradient to 60% B and finally a 3 min linear gradient to 95% B which was being maintained for 10 min and then a 0.1 min linear gradient back to 100% of A. After the gradient elution, the column was being equilibrated for 12 min before another injection. The flow rate amounted to 0.2 mL/min. An injection volume of 5 μL was applied in all analyses. The tandem MS analyses were carried out on an Agilent 6410 triple quadrupole mass spectrometer equipped with an ESI interface. The analyses were conducted in the positive ion (PI) mode regarding all the investigated analytes. The parameters for the analyses were as follows: drying gas temperature 350 °C; capillary voltage 4.0 kV; drying gas flow 11 L/min and nebulizer pressure 35 psi. The optimal collision energies, fragmentor voltages and transitions chosen for the multiple reaction monitoring (MRM) experiment are listed in Table 3.

The instrument control, data acquisition and evaluation were carried out with the MassHunter Agilent 2003–2007 Data Acquisition for Triple Quad B.01.04 (B84) software.

2.5. Validation of analytical procedure

Each compound was analysed by MRM, using the two highest characteristic precursor ion/product ion transitions. The positive identification criterion of the target analytes was founded on the LC retention time and the ratio of abundances of two specific precursor ion/product ion transitions.

The extraction recoveries of target compounds were identified using the sediment samples spiked with the analytes at three concentration levels. The recoveries were determined comparing the concentrations obtained with the initial spiking levels. In each experiment, the samples were analyzed in triplicate.

The linearity of the method was assessed using standard solutions and matrix-matched calibration and analyzing 10 concentration standards in triplicate (concentration ranges from 25 ng/L to 10 mg/L for FEBA and PRAZ, 125 ng/L to 50 mg/L for ENRO, TMP, SMET, SMETOX and PROC and from 250 ng/L to 100 mg/L for SGUA, SDIAZ, CIPRO, NOR and ROXI) to obtained concentration between 0.000025 and 100 μg/g in the final sediment samples, depending on pharmaceuticals. Calibration curves were constructed for each compound extracted from the sediment samples (spiked with calibration standards) by plotting the peak area versus the analyte concentration. Blanks were also prepared as a quality control tool, but were not included in the regression analysis. The results were analyzed by the linear regression method.

The matrix effects were studied by the evaluation of signal suppression or enhancement for each pharmaceutical. Several approaches for the evaluation of matrix effects were described and, among them, Matuszewski et al. [31] proposed a procedure which leads to a quantitative information. Residual compounds still present after sample preparation can interfere with the MS ionization process leading to the well-known signal suppression or enhancement situations. The influence of endogenous compounds on LC–MS (i.e. matrix effect, ME) is evaluated by the ratio of peak areas from a matrix sample fortified after the sample preparation and a neat standard [31,32]. The matrix effect (ME) were calculated as follows:

$$\mathrm{ME}\,(\%) = \frac{\text{Area of pharm. after extraction spike}}{\text{Area of pharm. in standard solution}} \times 100$$

The precision of the method was calculated by repeated ($n = 3$) intra-day and inter-day analysis of the spiked sediment at the concentration level in the middle of the linearity range. The precision of the method was expressed as the relative standard deviation (RSD) of replicate measurements. The limits of detection (LOD) and limits of quantification (LOQ) were derived from the spiked sediment samples and expressed as a concentration producing an S/N ratio of 3 and 10, respectively.

The recoveries of the analytes from the spiked sediment samples were evaluated by the ratio of the response (peak area) obtained from the measurements of extracts to the response of a corresponding standard solution.

3. Results and discussion

3.1. Optimization of the MSPD method

In an attempt to extract the 12 studied compounds simultaneously, it was important to consider their physicochemical

Table 4
Sorption data for some investigated pharmaceuticals in soils and sediment samples [5].

Compound	Matrix; texture/pH/OM%	K_d (L kg^{-1})	K_{oc} (L kg^{-1})
SDIAZ	Silt loam/7.0/1.6	2.0	124
	Clay-loam/6.2/3.1	2.5	81
SMET	Sand/5.2/0.9	1.2	174
	Loamy sand/5.6/2.3	3.1	125
	Sandy loam/6.3/1.2	2.0	208
	Clay silt/6.9/1.1	1.0	82
ENRO	Clay/4.9/1.63	3037	186,340
	Loam/5.3/0.73	5612	768,740
	Loamy sand/6.0/1.23	1230	99,980
	Loam/7.5/1.58	260	16,510
	Loamy sand/5.3/0.70	496	70,910

OM, organic matter.

properties which include the degree of binding of investigated compounds to sediment samples.

The pharmaceuticals of different structural classes vary considerably according to their molecular structures and physicochemical properties (Table 1). Some substances are hydrophobic or nonpolar whereas others are completely water soluble or dissociate at pH values typical for soils. Thus, distribution coefficients (K_d) for the adsorption of antibiotics to soil materials and aquatic sediments vary, for example, for sulfonamides from 0.6 to 4.9 and for fluoroquinolones from 310 to 6310. However, the sorption of pharmaceuticals to soil minerals is weaker than to the soil organic matter. Although the adsorption of various sulfonamides was stronger to clay than to the sand size fraction of a soil, the opposite was true for the increase of the partition coefficients at the desorption step. The sorption parameters for some of the investigated pharmaceuticals are shown in Table 4.

Sorption of antibiotics is particularly influenced by the soil pH, the soil organic matter and soil minerals. Correspondingly, the strong adsorption of fluoroquinolones to soils, especially to clay minerals was accompanied by expansion of the spacing of montmorillonite. The main mechanism for fluoroquinolones is adsorption of anionic antibiotics via cation bridging to clay minerals. Thereby, the deprotonated carboxylic group of fluoroquinolone-carboxylic acids is fixed to the clay minerals while the sorption of the decarboxylated derivative is much smaller [5].

The extraction of pharmaceuticals from sediment samples was carried out by MSPD. The preliminary experiments were carried out to optimize the main parameters affecting MSPD, such as the type of sorbent and the solvent polarity. In this procedure, it is vital to select a suitable adsorbent because the chosen adsorbent is not only used as an adsorption separation material but also as a blending solid support to disrupt and disperse the sample [13]. Florisil and C18 with different extraction solvents were initially tested to obtain an optimal extraction sorbent. For that purpose, several elution solvents, such as methanol, ethanol, acetonitrile, 1-prophanol, 2-prophanol, dichloromethane, acetone and water were used for pharmaceutical extraction from the sediment samples. The extraction recoveries were almost the same for all pharmaceuticals, but chromatogram of extracts which obtained with the C18 sorbent shown better peak shape with less unknown compounds than Florisil.

Beside the sorbent selection, the nature of the elution solvent also matters since target analytes should be efficiently desorbed while the remaining matrix components should be retained in the column [13,33]. In this context, the elution profile is also an important factor in the MSPD procedure because it also plays two roles, the first one refers to separation wherein the profile appears as a general mobile phase and the second one relates to the dissolution/extraction of target compounds [13].

Therefore, methanol, acetonitrile and water were selected as an extraction solvent in the second set of the experiments after the selection of C18 as an optimal sorbent. The former selection was based on the first set of experiments. In fact, these three solvents showed more potential for extracting selected pharmaceuticals than the other solvents and because of that, different acetonitrile–water and methanol–water mixtures (6:4; 7:3; 8:2) were used. The results of these experiments were presented in Fig. 1.

From the presented results, it is obvious that the extraction results for all the investigated pharmaceuticals were almost the same for both organic solvents. The largest extraction differences referred to febantel. The extraction recoveries ranged from 55 to 71% for methanol–water mixtures, and from 67 to 97% for acetonitrile–water mixtures, bearing in mind that the score of 97% was achieved with acetonitrile:water = 6:4 (v/v). Roxithromycin was extracted with very low efficiencies (2.5–42.5%) and on the other hand, fluoroquinolones (ciprofloxacin, norfloxacin and enrofloxacin) were not extracted from the sediment samples at all with respect to the above extraction mixtures. The extraction efficiencies for all other pharmaceuticals (sulfonamides, trimethoprim, procaine and praziquantel) amounted to or exceeded 90%. The lower values obtained for roxithromycin can be ascribed to its partial binding to the lipophilic matrix. This possibility is supported by relatively high K_{ow} values of roxithromycin which is the highest value between all selected pharmaceuticals. Due to its high liposolubility, this pharmaceutical could be absorbed and retained by sediment particles [25]. The fact that procaine and praziquantel also have relatively high K_{ow} values ($\log K_{ow} > 2$) differences between the obtained extraction results compared to those of roxithromycin can be probably attributed to the feature of roxithromycin being a big molecule which is hardly douching and move through the sediment. The reason for very bad fluoroquinolones results is probably the strong interaction between the fluoroquinolones and the sediment, which makes them difficult to extract. Additional features, such as the interactions of quinolones with silica structures or their ability to form stable complexes with Al(III), Fe(III) and other metal ions must be considered. Apart from these features, quinolones with a different type of substituents have rather different physical properties. The carboxylic group makes the compounds acidic. However, 7-piperazinyl quinolones also contain basic amine substituents. In an attempt to extract quinolones with different acid-base properties, Turiel et al. [34] have evaluated different mixtures of various organic solvents with several acids and bases to find a suitable solution for extraction of quinolones [35].

For that reason and based on the previous results in another set of experiments, several 5% acid solutions such as oxalic acid, acetic acid, hydrochloric acid, phosphoric acid and trifluoroacetic acid were used in combination with acetonitrile. Acetonitrile:5% acid = 6:4 (v/v) was selected as the ratio for experiments with acids. The results of these experiments are shown in Fig. 2.

The result of the tested acetonitrile:5% acid mixture indicates that it is clear that the most advantageous results can be linked to the mixture of oxalic and acetic acid. Phosphoric acid provides for very high extraction recoveries (>100%), especially for CIPRO and NOR. The higher peak areas can be explained by the matrix effect enhancing the chromatographic response to pharmaceuticals (especially fluoroquinolones) but the real reason for that is not known. One of the potential reasons is probably hidden in the fact that phosphoric acid shows the tendency to form different complexes with sediment ingredients or, possibly, with pharmaceuticals. These results were the reason for investigation of different percentages (1, 2 and 10%) of the above acids in a mixture with acetonitrile. The results are shown in Figs. 3 and 4.

In case different percentages of acetic acid are used, extraction recoveries increase with the increase of the acetic acid percentage for almost all pharmaceuticals. The only exception is connected with 5% of the acidic acid.

On the other hand, the increase of oxalic acid percentage has shown a different influence on pharmaceuticals extraction recoveries. The extraction recoveries were increased for almost all pharmaceuticals. In few cases, the pharmaceutical extraction recovery did not exceed 2% (e.g. SMET, PRAZ, and ENRO) and 5% (e.g. SGUA), respectively. Regarding some pharmaceuticals, the increase of the oxalic acid percentage showed a negative influence (e.g. FEBA and PROC) and as far as SMETOX is concerned, there was no detected influence.

The most important thing in the last set of experiments is that the obtained extraction results for fluoroquinolones were satisfactory considering the fact that they seemed to be a big problem during the whole extraction procedure. Experiments with a mixture of natrium hydroxide and methanol were also done but the

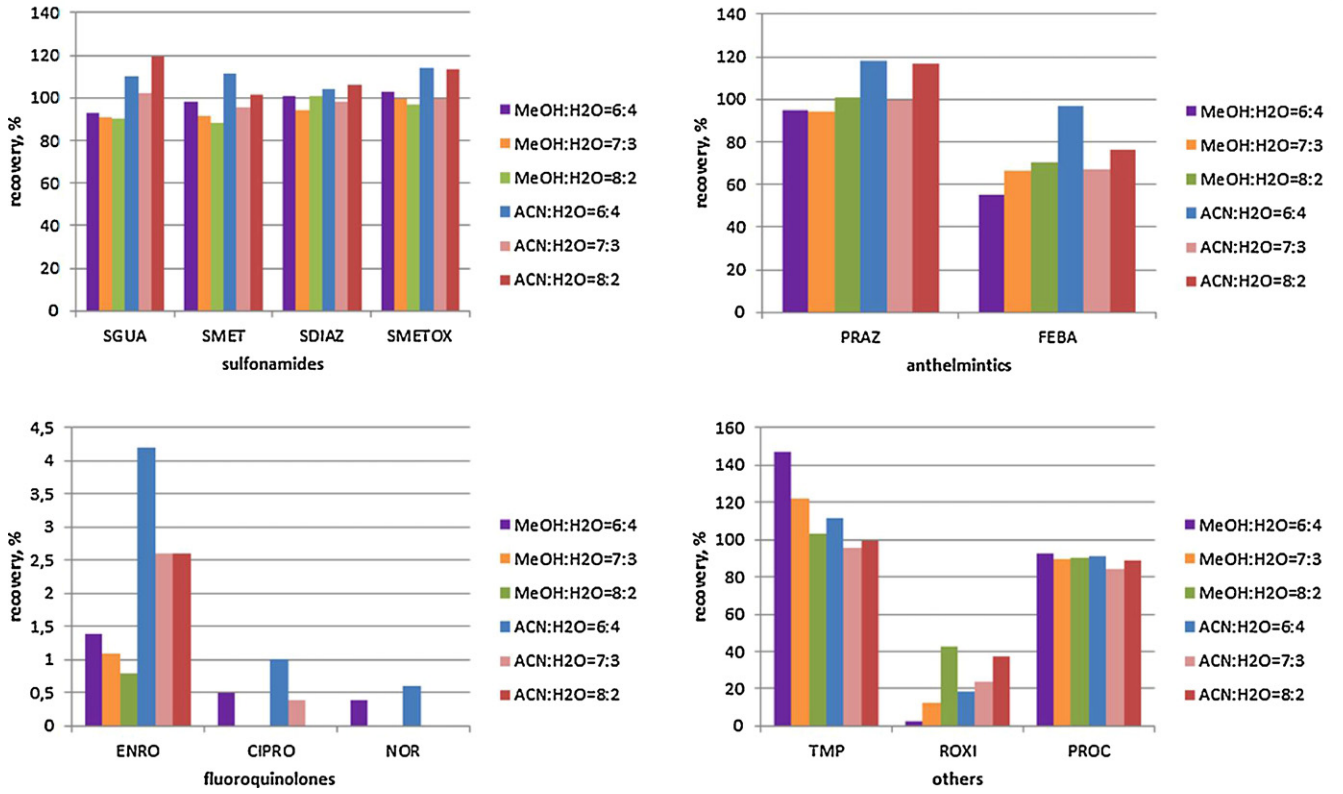


Fig. 1. Comparison of MSPD recoveries obtained using different extraction solvent mixture and C18 sorbent ($n = 3$).

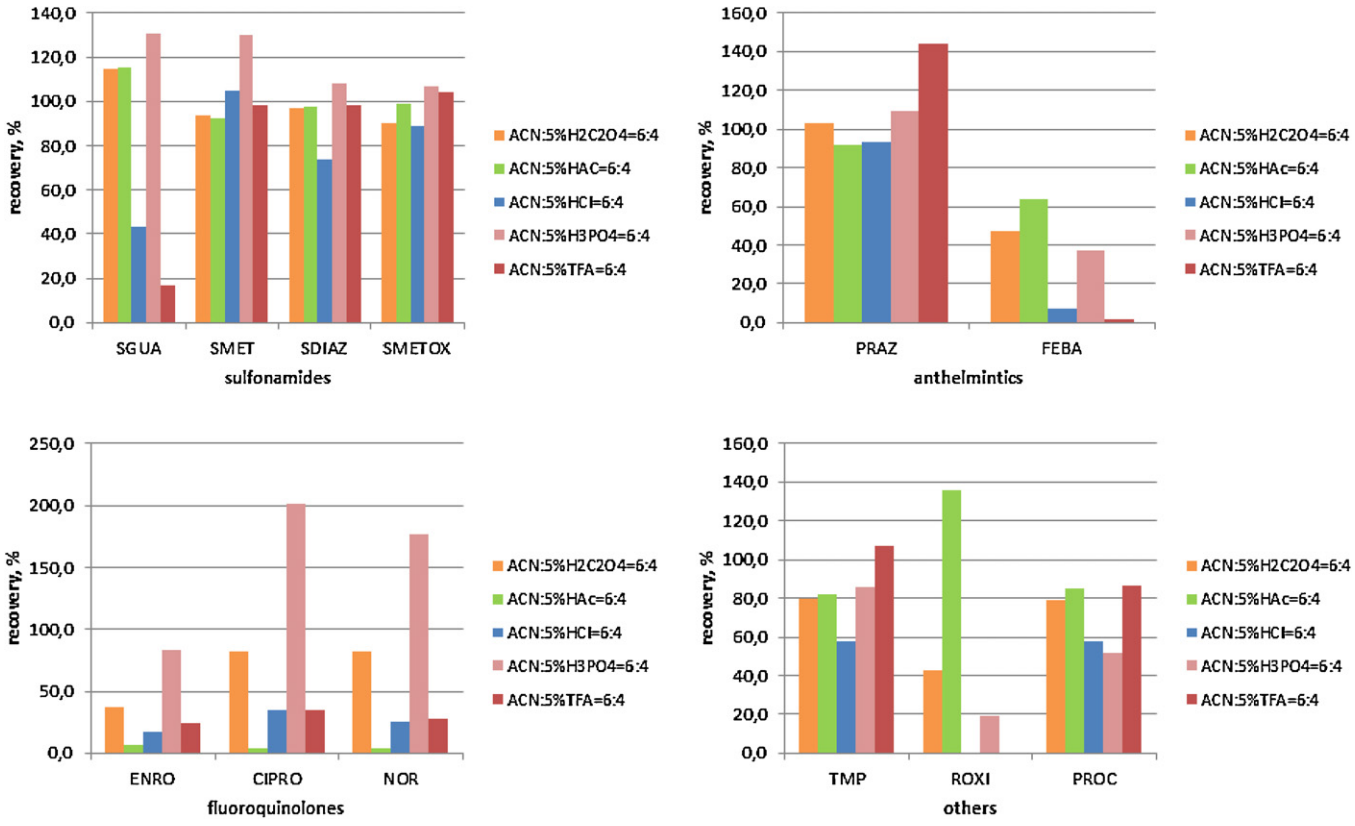


Fig. 2. Comparison of MSPD recoveries obtained using different acetonitrile:5% acid mixtures and C18 sorbent ($n = 3$).

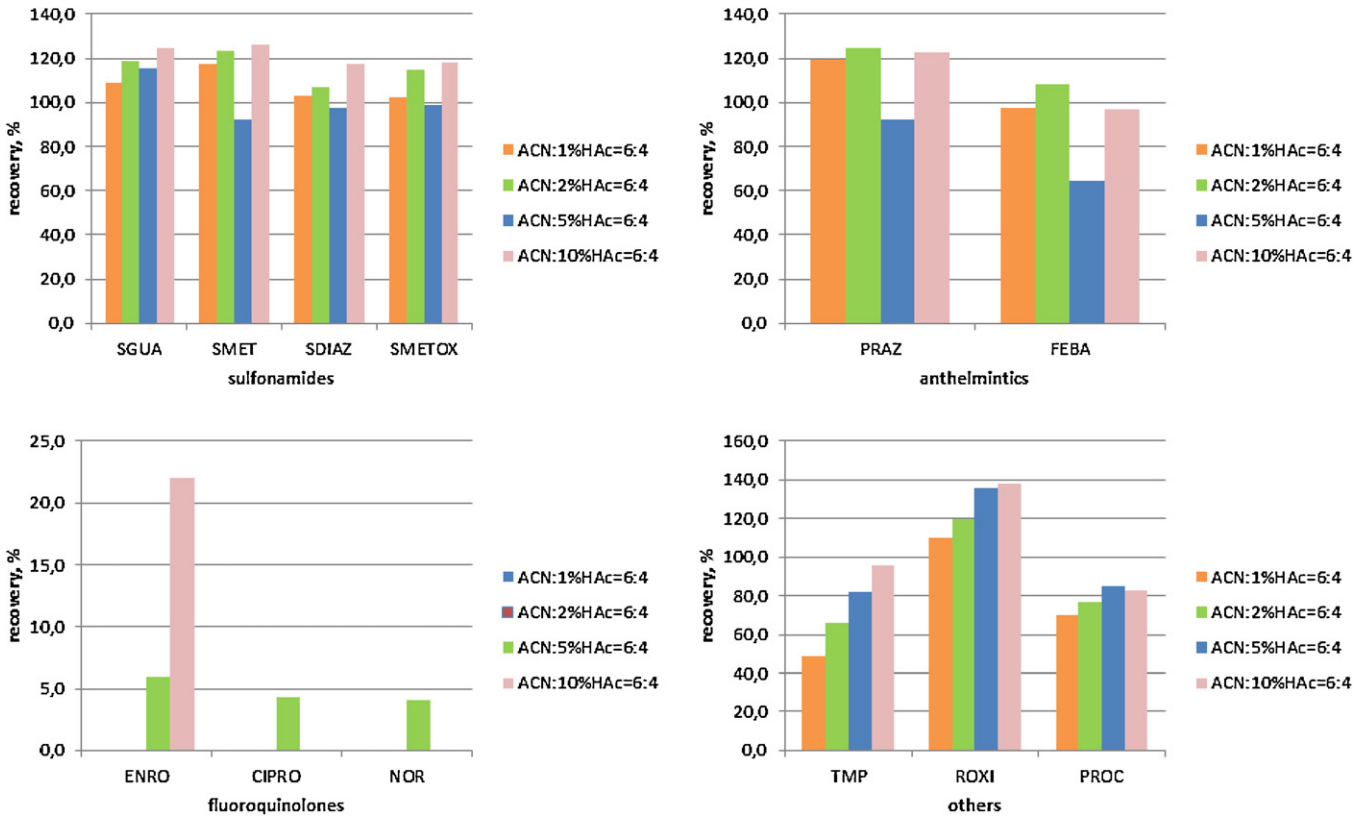


Fig. 3. Comparison of MSPD recoveries obtained using different % of acetic acid in mixture with acetonitrile and C18 sorbent ($n = 3$).

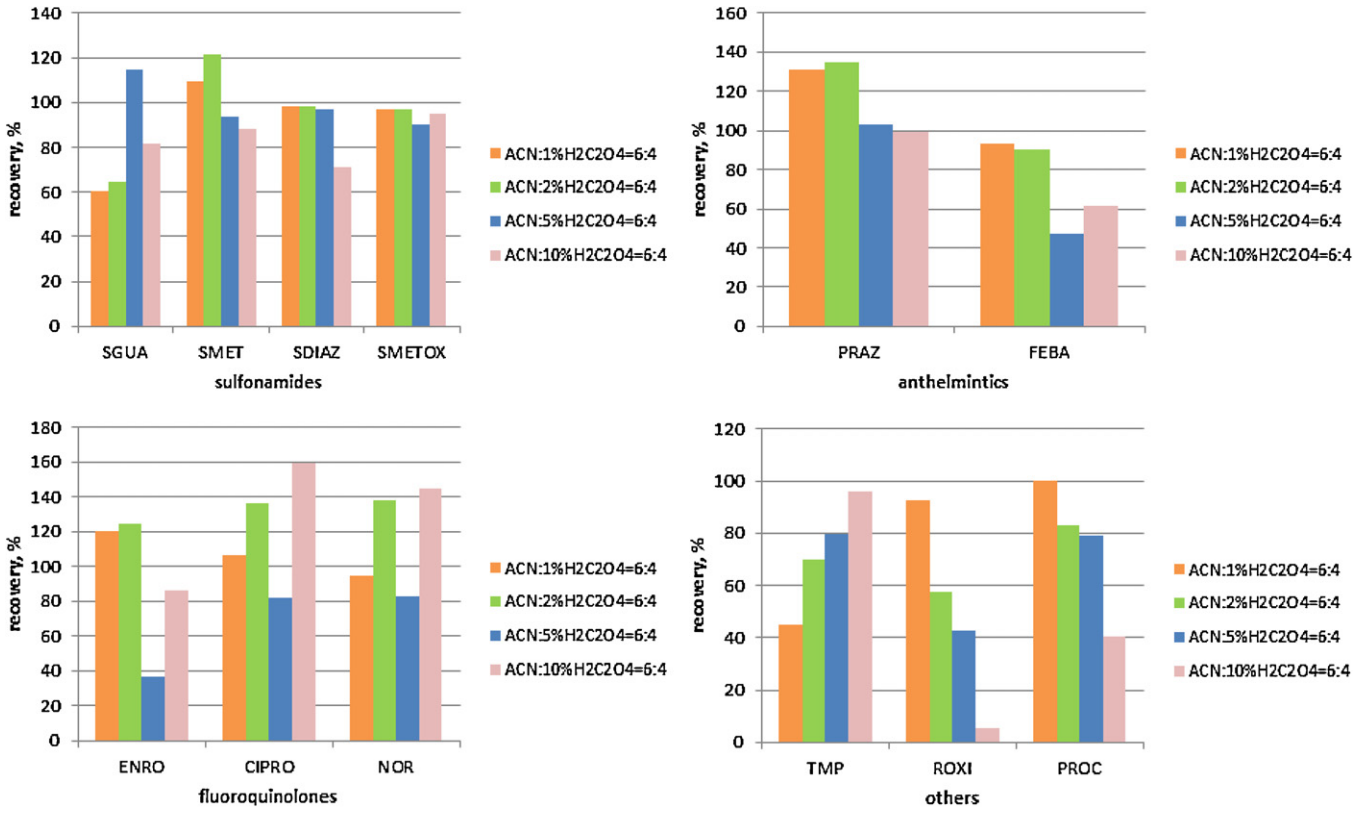


Fig. 4. Comparison of MSPD recoveries obtained using different % of oxalic acid in mixture with acetonitrile and C18 sorbent ($n = 3$).

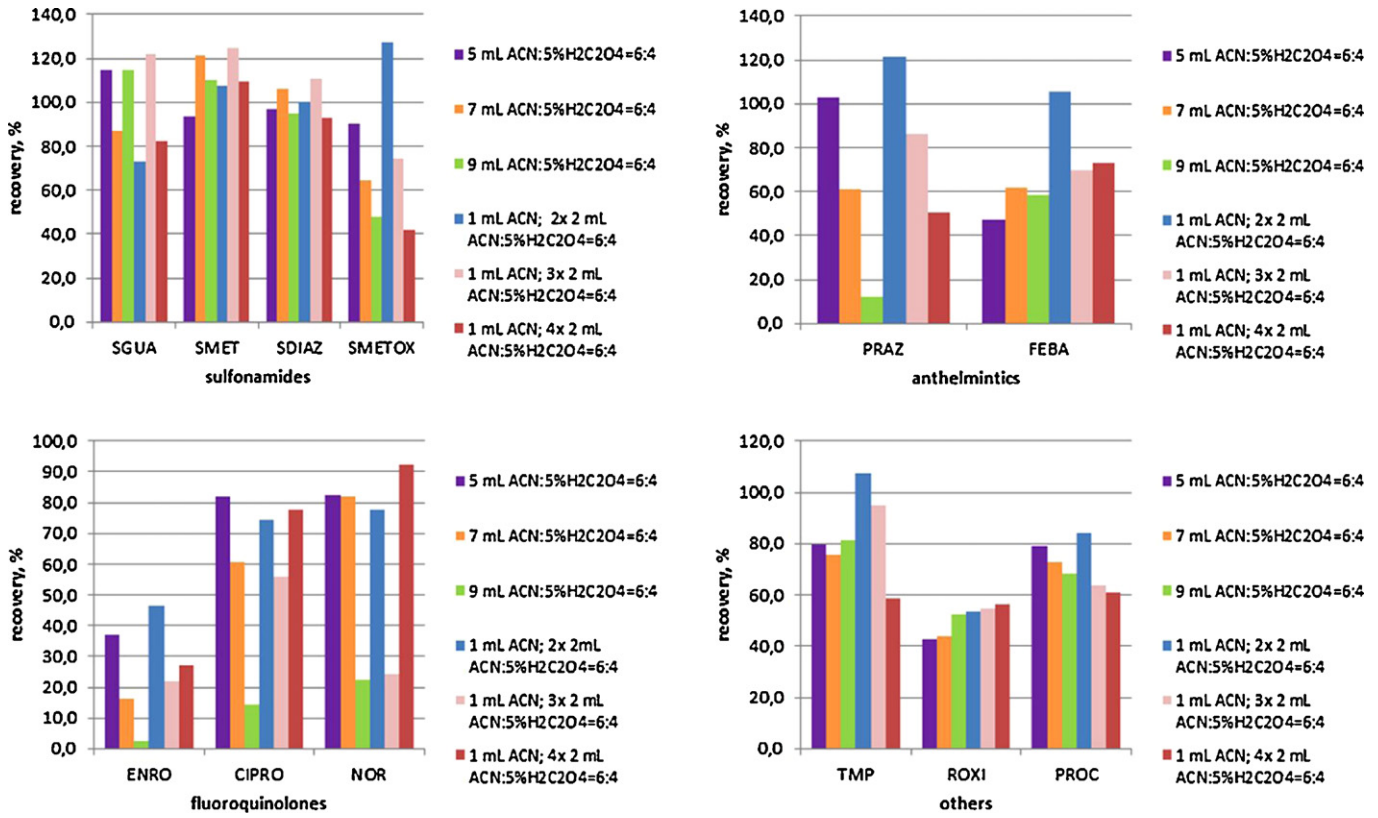


Fig. 5. Comparison of MSPD recoveries obtained using different extraction solvent, their combinations and different volume on C18 sorbent ($n = 3$).

results were not so good for all of the 12 pharmaceuticals and the matrix effect was greater than in other extraction experiments. Therefore, the mixture of acetonitrile and 5% of oxalic acid in the 6:4 (v/v) ratio was selected as extraction solvents for the investigated mixture of pharmaceuticals in further experiments.

With respect to the selected extraction solvent, the obtained extraction recovery results were not so good for all pharmaceuticals, especially for roxithromycin and febantel. What can be said for roxithromycin and febantel based on the previous experiments is that roxithromycin show affinity to pure methanol while febantel shows affinity to pure acetonitrile. A pretty good extraction efficiency of investigated pharmaceuticals using these pure organic solvents was achieved. Taking account of this fact, the following set of experiments for extraction of selected pharmaceuticals from sediments included combinations of the aforementioned pure organic solvents with a selected extraction solvent were used. Accordingly, the first series allowed methanol or acetonitrile to pass through the MSPD column followed by acetonitrile:5% of oxalic acid = 6:4 (v/v) while in the second series, the order was reverse. To sum up, these experiments have disclosed that the best extraction efficiency can be obtained with 1 mL of acetonitrile followed 2× 2 mL of acetonitrile:5% of oxalic acid = 6:4 (v/v). The aforementioned combination of solvents facilitates an increase of the extraction efficiency of almost all pharmaceuticals, especially for roxithromycin and febantel. Still, the advantage of this extraction solvent in comparison with acetonitrile:5% of oxalic acid = 6:4, was not so high when selecting it for an optimal extraction solvent. For that reason both extraction solvents were used in the following sets of experiments.

Whereas the selectivity of the MSPD procedure depends on an applied sorbent/solvent combination, the aforementioned method can be improved and this is what was tried. But on the other hand, the intentional ionization or suppression of analytes ionization and matrix components can greatly affect the nature of interactions of specific target analytes with the matrix and eluting solvent. Accordingly, this should be considered as a variable for attaining reproducible and efficient extractions [33]. In some publications

Table 5
Linearity range, correlation coefficient, limits of detection and quantification of the MSPD–LC–ESI-MS/MS method.

Compound	Linearity range (μg/g)	Linearity[a] equation	r^2	LOD (μg/g)	LOQ (μg/g)
SGUA	5–100	$A = 60.724\gamma + 1732.6$	0.9941	0.5	5
SMET	0.0125–50	$A = 869.52\gamma + 1267.1$	0.9979	0.0025	0.0125
SDIAZ	0.025–100	$A = 364.79\gamma + 1182.4$	0.9973	0.005	0.025
SMETOX	2.5–50	$A = 179.57\gamma - 171.41$	0.9971	0.25	2.5
TMP	0.0025–50	$A = 1105.7\gamma + 2259$	0.9889	0.00125	0.0025
ROXI	5–100	$A = 52.37\gamma + 150.82$	0.9986	0.5	5
PRAZ	0.5–10	$A = 294.88\gamma + 223.86$	0.9978	0.05	0.5
FEBA	0.0005–10	$A = 68.902\gamma + 564.61$	0.9928	0.00025	0.0005
ENRO	0.00125–50	$A = 247.41\gamma + 211.18$	0.9982	0.000125	0.00125
CIPRO	0.0025–100	$A = 477.67\gamma + 1885.6$	0.9981	0.00025	0.0025
NOR	0.0025–100	$A = 560.07\gamma + 2365.5$	0.9977	0.00025	0.0025
PROC	0.0125–50	$A = 907.3\gamma + 603.67$	0.9993	0.0025	0.0125

[a] Calculated as peak areas versus concentration.

[20,25,33], authors have added acids, bases, salts, chelating or dechelating agents, etc. to co-blending with the sample and solid support because these additives could affect the elution sequence or retention of the target analyte. The real reason for that is modification of the chemistry of the sample, which could increase the extraction efficiency of some compounds.

Therefore, this aspect of experiments was also checked. Methanolic saturated potassium hydroxide was added to the sediment in the dispersion stage. The same was done with anhydrous sodium sulphate using C18 as a solid support to examine the aforementioned effect on the extraction efficiency. Besides, many MSPD procedures also employ the use of co-columns to obtain a further degree of fractionation and sample clean-up [36]. For that purpose, Florisil was used in another experiment as a co-column material packed into the bottom of the same column as the MSPD material (sediment and C18). This way, the authors were curious how these two aspects (modifying the nature of the sample and co-column) exercise effect on the extraction efficiency of selected compounds. Nevertheless, these experiments have provided unsatisfactory recoveries.

The next step in the MSPD method development was optimization of the extraction solvent volume. Experiments with different volumes of selected extraction solvents (acetonitrile follow acetonitrile:5% of oxalic acid = 6:4 and acetonitrile:5% of oxalic acid = 6:4) were put under the spotlight since it was difficult to decide which extraction solvent is the best choice for extraction of 12 pharmaceuticals from sediment samples. For that purpose, 5, 7 and 9 mL of the total volume were tested. Results are shown in Fig. 5.

The presented results have shown that an increase of the extraction solvent volume brings to a fall in the extraction efficiency for almost all selected drugs. 5 mL of the total volume of the extraction solvent was sufficient for complete the elution of pharmaceuticals from the MSPD column for both extraction solvents. This is why 5 mL of methanol was chosen for the elution solvent volume. In case acetonitrile followed by acetonitrile:5% of oxalic acid = 6:4 (v/v) were used as extraction solvents, the extraction efficiencies were a little bit more acceptable, particularly for febantel and then on the occasion when only acetonitrile:5% of oxalic acid = 6:4 was used. However, it is important to pay attention to the obtained chromatograms as well as to the simplicity of the procedure. Taking into considerations these conclusions, acetonitrile:5% of oxalic acid = 6:4 (v/v) was selected as the optimal extraction solvent for investigated pharmaceuticals from the sediment. With this extraction solvent, good recoveries and better peak shape of compounds were achieved. A representative chromatogram of spiked sediment sample is shown in Fig. 6.

3.2. Validation of the MSPD–LC–ESI-MS/MS method

Once the factors that affect the MSPD procedure had been optimized, the performance characteristics of the MSPD–LC–MS/MS method were established by validation of the method with spiked sediment samples. In terms of quantitative purpose linearity, the limits of detection (LOD) and quantification (LOQ), precision and recovery were evaluated.

The most intensive fragment ion from each precursor ion was selected as transition ion for detection and quantification. For this purpose, two criteria for positive identification were used: (a) the correlation of retention time with the standards (±2%) and (b) first selected precursor/product ion transition. Less intensive second transition was used for confirmation purposes.

3.2.1. Specificity and selectivity of the method

The specificity and selectivity of the method were established by the analysis of blank samples. The absence of any chromatographic peak in sediment extracts, at the same retention times as target pharmaceuticals, indicated that there were not matrix compounds that might give a false positive signal in these blank samples.

3.2.2. Linearity and matrix effects

The linearity in the concentration range was assessed for each pharmaceutical in sediment samples of 0.025–100,000 ng/g, depending on a pharmaceutical using five to ten standard mixtures. Calibration curves were prepared for each compound from the spiked sediment by plotting the peak area versus the analyte concentration. Blanks were also prepared as a quality control tool but were not included in regression analysis. The results were analyzed by the linear regression method. The coefficients of correlation exceeded 0.9928, except for trimethoprim (0.9889), thus confirming the linearity of the method (Table 5).

As described before, calibration was performed by the use of matrix-matched standards. The use of matrix-matched calibration standards was done to compensate for the matrix effect, i.e. signal suppression or enhancement of studied pharmaceuticals in matrix solution. It is well known that matrix effects are one of the main drawbacks of LC–MS/MS methods, making quantification in samples problematic in some cases [37]. The mechanism and the origin of the matrix effect is not fully understood, but it may originate from the competition between an analyte and the coeluting, undetected matrix components reacting with primary ions formed in

Table 6
Recoveries obtained for target analytes at three different spiking levels tested over the linearity range.

Compound	Concentration level (μg/g)	Recovery ± RSD (n = 3), %
SGUA	5	129.8 ± 1.9
	25	98.3 ± 6.4
	100	102.2 ± 7.4
SMET	2.5	136.7 ± 3.5
	12.5	99.3 ± 11.0
	50	102.5 ± 6.4
SDIAZ	5	137.1 ± 10.8
	25	119.5 ± 15.5
	100	108.7 ± 5.2
SMETOX	5	77.9 ± 6.4
	25	80.3 ± 11.9
	100	73.9 ± 0.5
TMP	0.25	114.6 ± 11.4
	6.25	93.6 ± 1.7
	25	110.5 ± 2.9
ROXI	5	40.3 ± 4.7
	25	47.0 ± 16.0
	100	37.0 ± 5.9
PRAZ	0.5	116.0 ± 2.6
	2.5	108.6 ± 0.0
	10	89.4 ± 4.5
FEBA	0.05	101.5 ± 9.3
	1.25	96.1 ± 8.8
	5	50.4 ± 2.3
ENRO	0.25	29.8 ± 18.5
	6.25	58.1 ± 10.9
	25	85.3 ± 3.7
CIPRO	0.5	73.4 ± 22.9
	12.5	100.6 ± 7.9
	50	79.9 ± 3.0
NOR	0.5	71.3 ± 28.7
	12.5	100.9 ± 4.6
	50	94.4 ± 5.4
PROC	2.5	101.3 ± 10.4
	12.5	101.5 ± 9.2
	50	107.2 ± 8.8

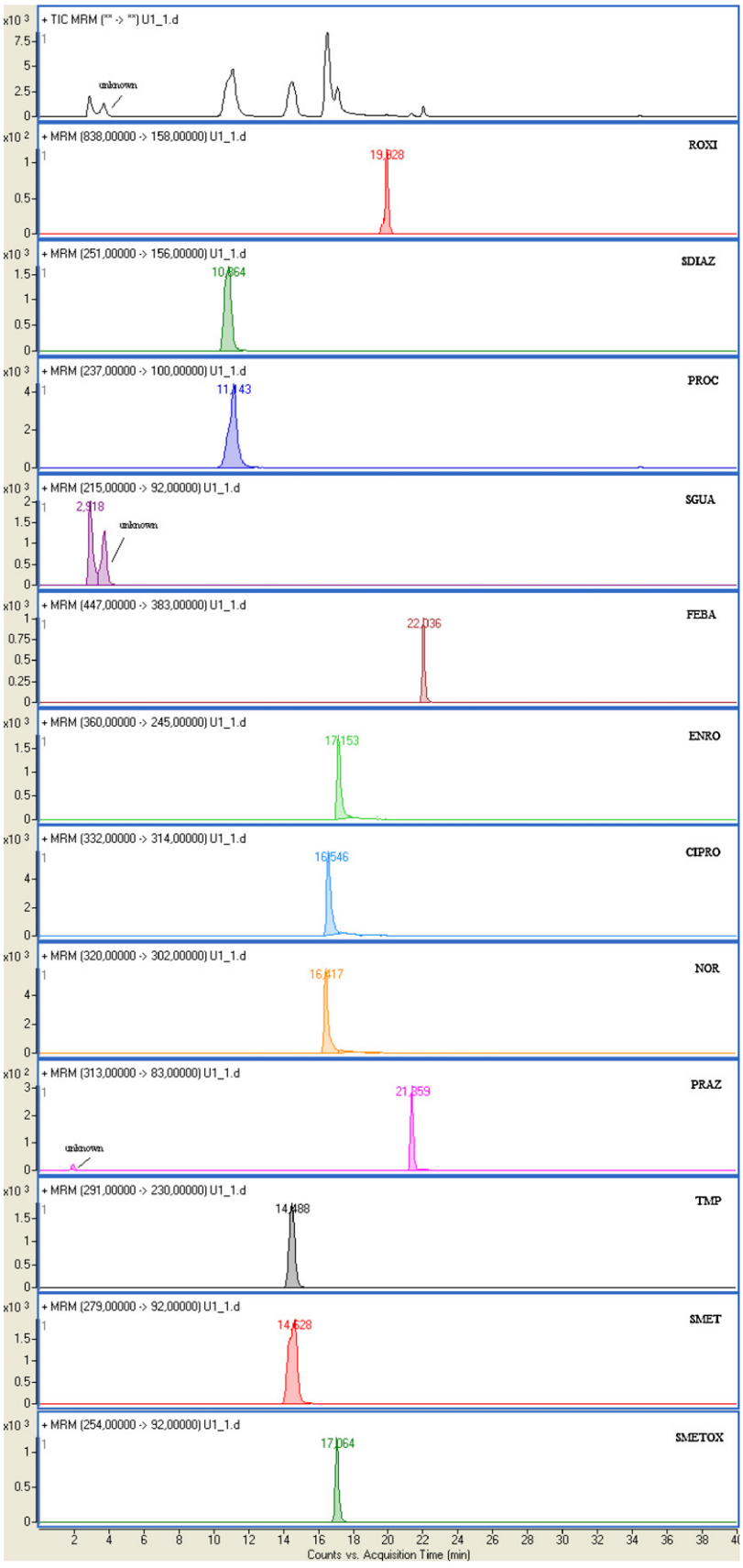


Fig. 6. Chromatogram of spiked sediment samples: total ion chromatogram and extracted MRM chromatograms for target analytes.

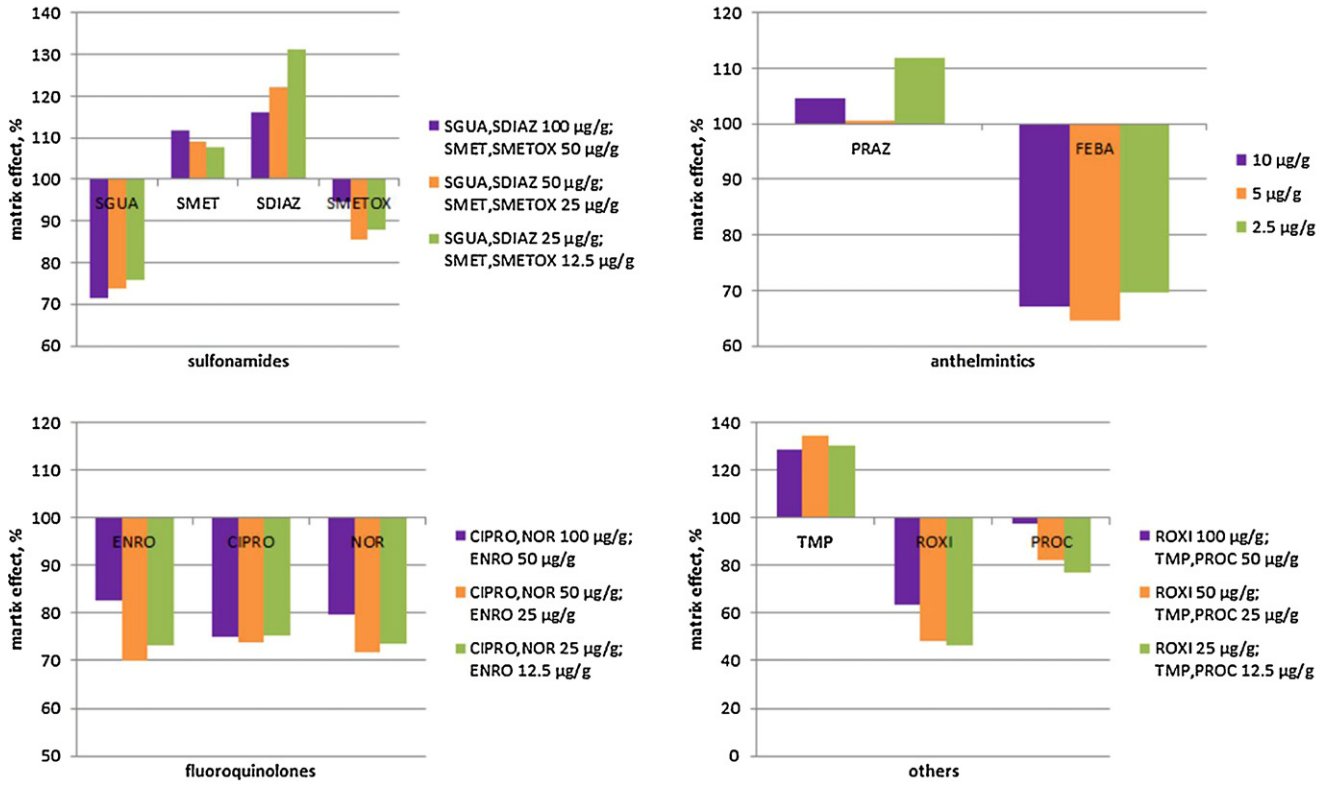


Fig. 7. Matrix effect at the three concentration levels.

the HPLC–MS/MS interface [31]. However, LC–MS is highly selective method in selected ion monitoring and in multiple reaction monitoring mode still the other compounds – although invisible in the LC–MS signal – may and very often do interfere. The suppression or enhancement of ionization by the co-eluting compounds occurs in the ESI source before any mass-spectrometric detection and it is thus in principle impossible to compensate it by mass spectrometry [38]. Matrix effect was quantified comparing the areas of compounds in spiked matrix samples after extraction with the areas obtained in standard solutions. Fig. 7 shows matrix effects for every tested pharmaceutical substance at three concentration levels within the linearity range.

Knowing that the nature of matrix effect is pretty varying, the percentage is just a relative indicator of the degree of suppression and enhancement. Most of the investigated pharmaceuticals displayed the suppression of the signal, but it was not a general pattern because the impact of matrix interferences was different for each compound. Sulfamethazine, sulfamethoxazole, praziquantel and procaine were not significantly affected by the matrix components, since the signal change was less than 20% in the whole investigated concentration range. All other compounds show some degree (<30%) of signal suppression or enhancement. Roxithromycin showed the highest signal suppression (from 63.5% to up 46.2%). This fact about roxithromycin explains his relatively low extraction efficiency (Fig. 5).

Evidently, the influence of the matrix was very variable. Namely, for one specific combination of pharmaceuticals and matrix, the matrix effect can vary from one set of measurements to other. This means that it is not possible to test matrix effect only once and considered to be constant. Therefore, for an accurate quantification, the use of matrix-matched standards is required [27]. However, the use of matrix-matched standards compensated quite well for the suppression effect achieving accurate quantification [1].

3.2.3. *Limits of detection and quantification, precision and recoveries*

The limits of detection (LOD) and quantification (LOQ) were experimentally estimated for each pharmaceutical using the signal to noise ratios from the mass chromatograms obtained in the SRM mode for the spiked sediment samples. The obtained detection and quantification limits are stated in Table 5. The LOD ranged from 0.000125 to 0.5 µg/g and the LOQ from 0.0005 to 5 µg/g.

To ensure correct quantification, precision of the method was studied by analysing three replicates of standard with concentration level in the middle of linearity range. Obtained results showing very good precision; the intra-day precision were up to 10% and inter-day precision up to 15%. The recoveries data were calculated by comparison of the extracted analyte amounts with appropriate working standard solutions. The untreated sediment samples were fortified at three different concentrations from the linearity range, depending on pharmaceuticals. Standard solutions were injected after every 10 samples to monitor changes in chromatographic conditions. Table 6 presents the recoveries of the 12 pharmaceuticals at three concentration levels for the sediment tested. Each recovery analysis was repeated 3 times and the recoveries of pharmaceuticals were expressed as average values of these three determinations.

In perfect conditions, the extraction recovery should not be sample concentration dependent. In other words, a useful method must not imply any significant difference in recovery over the expected concentration ranges of analyzed compounds. Still, it have been noticed that extraction efficiencies for few pharmaceuticals at the lowest concentration range were much higher than 100% or the RSD value was much higher than 10% (especially for sulfaguanidine, sulfadiazine, sulfamethazine and fluoroquinolones). These results could be explained by the matrix effect. However, it could

Table 7
Comparison of several methods for pharmaceuticals determination in solid environmental samples.

Compound	Matrix	Method	LOD	LOQ	Recovery	Detected levels	Reference
CIPRO	Sediments	PLE–SPE–LC–ESI-MS/MS	4.0 ng/g	na	55–62%	5.95 ng/g	[1]
NOR			5.2 ng/g	na	67–72%	na	
TMP			0.3 ng/g	na	93–97%	na	
SMETOX			0.3 ng/g	na	84–87%	na	
CIPRO	Soils	PLE–SPE–LC–ESI-MS/MS	4.1 ng/g	na	52–63%	na	[1]
NOR			4.7 ng/g	na	64–70%	na	
TMP			0.2 ng/g	na	91–105%	na	
SMETOX			0.6 ng/g	na	70–79%	na	
ROXY	Sediments	PLE–SPE–HPLC–QqLIT–MS/MS	na	na	na	0.42 ng/g	[2]
SDIAZ			na	na	na	5.49 ng/g	
SMETH			na	na	na	1.70 ng/g	
TMP			na	na	na	2.34 ng/g	
Other pharmaceuticals	Sediment	MAME–SPE–LC–UV–DAD	4–167 ng/g	12–556 ng/g	6–114%	0.1–2.3 μg/g	[3]
2 FQs	Sevage sludge	ASE–SPE–LC–FLD	na	450 μg/kg	82–94%	1400–2420 μg/kg	[4]
2 FQs	Soil	ASE–SPE–LC–FLD	na	180 μg/kg	75–92%	270–400 μg/kg	[4]
7 antibiotics	Manure	LLE–ESI-MS^2	na	100 μg/kg	47–89%	100–12,400 μg/kg	[4]
18 pharmaceuticals	Sediment	USE–APCI-MS^2 USE–ESI-MS^2	na	0.4–20 μg/kg	56–151%	na	[4]
8 antibiotics	Soil	PLE–SPE–ESI-MS^2	na	1–11 μg/kg	31–143%	1–57 μg/kg	[4]
SAs	Pig manure	–	na	na	na	≤3.5 mg/kg	[5]
MAs	Agricultural soils	–	na	na	na	13–67 μg/kg	[5]
FQs			na	na	na	6–52 μg/kg	
ROXY	Sediment	PLE–SPE–LC–QqLIT–MS/MS	0.04 ng/g	0.13 ng/g	149%	<LOQ	[11]
SMETH			0.32 ng/g	1.06 ng/g	45.7%	1.1 ng/g	
TMP			0.25 ng/g	0.83 ng/g	97.2%	11.2 ng/g	
ENRO	Soil	USE–HPLC–UV	0.04 μg/g	0.15 μg/g	na	Realistic environm. conc. levels = low μg/g range	[34]
NOR			0.06 μg/g	0.20 μg/g	na		
CIPRO			0.05 μg/g	0.18 μg/g	na		
FQs	Soil	USE–LC–UV	na	na	na	40–80 μg/kg	[35]
CIPRO, NOR	Soil	MAE–LC–FLD	na	95–98%	na	150 μg/kg	[35]
MAs, SAs	Sediment	USE	na	20 ng/g	na	na	[39]
MAs	Soils	PLE	na	1–1.4 μg/kg	na	na	[39]
CIPRO, NOR	Sludge	–	na	na	na	0.06 μg/g	[39]
ENRO	Soil	USE–HPLC(MIP)	0.30 μg/g	na	87.9%	na	[40]
NOR			0.35 μg/g	na	97.2%	na	
CIPRO			0.21 μg/g	na	93.4%	na	
ENRO		USE–SPE(MIP)–HPLC(MIP)	0.06 μg/g	na	85.3%	na	[40]
NOR			0.07 μg/g	na	75.2%	na	
CIPRO			0.05 μg/g	na	82.1%	na	
SMET	Manure	USE–SPE–LLE–LC–MS/MS	na	0.22 μg/kg	101%	20 mg/kg	[41]
SDIAZ			na	0.29 μg/kg	83%	na	
SMETOX			na	0.35 μg/kg	95%	na	
TMP			na	0.11 μg/kg	77%	na	
ENRO			na	1.5 μg/kg	na	2.8–8.3 mg/kg	
CIPRO			na	1.7 μg/kg	na		
SMET	Arable soil	USE–(LLE)–SPE–LC–MS/MS	na	1.0 μg/kg	69%	na	[41]
SDIAZ			na	1.1 μg/kg	89%	na	
SMETOX			na	1.5 μg/kg	74%	na	
TMP			na	0.49 μg/kg	61%	0.1 mg/kg	
ENRO			na	24 μg/kg	na	0.37 mg/kg	
CIPRO			na	25 μg/kg	na	0.45 mg/kg	
SAs	Agricultural soils	USE–SPE–HPLC–UV/FLD	na	na	na	≤40 mg/kg	[42]
ENRO			na	na	na	0.02–0.05 mg/kg	
SMETOX	Manure	USE–SPE–HPLC–UV/FLD	na	na	na	3.76 mg/kg	[42]
ENRO			na	na	na	2.80 mg/kg	
SMETOX	Soil	USE–SPE–LC–MS/MS	0.4 μg/kg	1.7 μg/kg	na	0.03–0.9 μg/kg	[43]
CIPRO			0.7 μg/kg	2.8 μg/kg	na	0.8–30.1 μg/kg	
SMETOX	Manure	MAE–SPE–LC–MS/MS	5 μg/kg	19 μg/kg	na	0.23–5.7 μg/kg	[43]
CIPRO			21 μg/kg	84 μg/kg	na	0.1–4.3 μg/kg	
SGUA	Sediment	MSPD–LC–ESI-MS/MS	0.5 μg/g	5 μg/g	115.0%		This work
SMET			2.5 ng/g	12.5 ng/g	93.4%		
SDIAZ			5 ng/g	25 ng/g	96.8%		
SMETOX			0.25 μg/g	2.5 μg/g	89.9%		
TMP			1.25 ng/g	2.5 ng/g	79.8%		
ROXI			0.5 μg/g	5 μg/g	42.6%		
PRAZ			0.05 μg/g	0.5 μg/g	102.9%		

Table 7 (*Continued*)

Compound	Matrix	Method	LOD	LOQ	Recovery	Detected levels	Reference
FEBA			0.25 ng/g	0.5 ng/g	47.0%		
ENRO			0.125 ng/g	1.25 ng/g	37.1%		
CIPRO			0.25 ng/g	2.5 ng/g	81.7%		
NOR			0.25 ng/g	2.5 ng/g	82.4%		
PROC			2.5 ng/g	12.5 ng/g	78.8%		

na, not available; FQs, fluoroquinolones; MAs, macrolides; SAs, sulfonamides.

be concluded that the extraction recoveries of investigated pharmaceuticals decrease disproportionally to their concentrations.

Anyway, the extraction efficiency allows trace analysis in real sample. As listed in Table 7, some reported methods for the pharmaceuticals determination in few environmental solid samples were compared.

The proposed method in this work showed high sensitivity and wide linear range. Exception exists only in case of sulfaguanidine, sulfamethoxazole, roxithromycin and praziquantel. In case of mentioned pharmaceuticals developed method could be apply at points of pharmaceuticals entry to environment because these are the place which could expected their higher concentrations (low μg/g range).

3.3. Analytical applications

The described method was applied for determination of target pharmaceuticals in several spiked natural sediment samples collected throughout Croatian regions with different characteristics (Table 2). The unspiked samples (blanks) were previously analysed using the proposed method and no target compounds were detected.

The sediment samples were spiked with a stock solution of investigated pharmaceuticals and analysed by MSPD–LC–MS/MS. Fig. 8 suggests that all pharmaceuticals were determined in all tested samples with recoveries over 60%, except in the case of roxithromycin, febantel and enrofloxacin. The RSDs of all the recovery experiments did not reach 10%.

Fig. 8 indicates that it is obvious that there are composition differences between sediment samples, which are reflected on the extraction efficiency of investigated pharmaceuticals. As already mentioned, the sorption of pharmaceuticals is influenced by the soil pH, the soil organic matter and soil minerals.

The above results and sediment characteristics (Table 2) make evident that sediment 4 was poorer with clay but richer with different salts (concluded from the electrical conductivity) and organic matter, which resulted in somewhat lower extraction efficiencies of sulfonamides than of other sediment samples and better extraction efficiencies of enrofloxacin. This was the reason that the matrix effect was greater, particularly for ciprofloxacin and norfloxacin. On the other hand, sediment 3 had a totally opposite character. What has to be emphasized here is that this sediment was the most acidic sediment sample. This is probably the reason for the worst extraction efficiency of some pharmaceuticals (febantel and fluoroquinolones) in comparison with other investigated sediment samples.

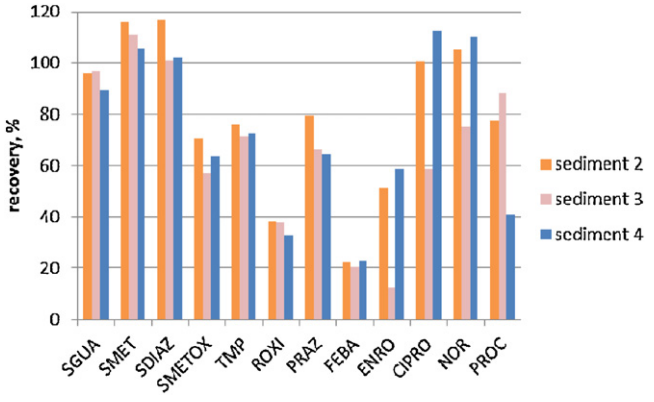


Fig. 8. Application of the optimized MSPD–LC–MS/MS procedure to different Croatia sediment samples.

Table 8
Application of MSPD procedure to a sediment samples containing a mixture of investigated pharmaceuticals.

	Added (μg/g)	Found (μg/g)
SGUA	25	20.9 ± 7.4
SMET	12.5	10.4 ± 1.7
SDIAZ	25	22.4 ± 2.4
SMETOX	12.5	12.4 ± 10.2
TMP	12.5	12.0 ± 5.6
ROXI	25	12.3 ± 13.4
PRAZ	2.5	2.6 ± 8.4
FEBA	2.5	2.2 ± 10.5
ENRO	12.5	11.4 ± 2.6
CIPRO	25	22.0 ± 4.2
NOR	25	25.4 ± 4.1
PROC	12.5	8.6 ± 12.8

To check application of developed MSPD–LC–ESI-MS/MS method to a real sediment samples, spiked sediment aged for 3 months are analysed. Result of this experiments shown in Table 8.

This experiment was performed due to impossibility to supply the sediment polluted with pharmaceuticals.

The obtained results demonstrate that the proposed method can be applied for determination of pharmaceuticals studied in different kinds of sediments and can guarantee satisfactory levels of accuracy and precision.

4. Conclusions

This work presents a new validated method for simultaneous determination of 12 pharmaceuticals with different structures and physico-chemical properties in sediment samples. The pharmaceuticals were isolated from sediment samples by using MSPD followed by LC–MS/MS. This ensures efficient recoveries (over 80%) for most investigated compounds and effective, expeditious removal of matrix interferents. The method is quite sensitive and precise. The usefulness of the proposed method for routine multiresidue analyses was demonstrated by applying it to different sediment samples.

This paper also proves that MSPD can be an attractive, affordable and effective alternative to existing extraction methods for organic contaminants from solid matrices. The major advantages of MSPD compared to other extraction methods such as PLE or Soxhlet reflect in its simple usage, low cost and, in some cases, reduced extraction time. Organic contaminants can be extracted more selectively and more quickly and can show similar or better recoveries with this method than with conventional extraction process.

References

[1] P. Vazquez-Roiga, R. Segarra, C. Blasco, V. Andreu, Y. Picó, J. Chromatogr. A 1217 (2010) 2471.
[2] B. Ferreira da Silva, A. Jelić, R. López-Serna, A.A. Mozeto, M. Petrović, D. Barceló, Chemosphere 85 (2011) 1331.
[3] R. Cueva-Mestanza, Z. Sosa-Ferrera, M.E. Torres-Padrón, J.J. Santana-Rodríguez, J. Chromatogr. B 863 (2008) 150.
[4] S.-C. Kim, K. Carlson, Trends Anal. Chem. 24 (2005) 635.
[5] S. Thiele-Bruhn, J. Plant Nutr. Soil Sci. (2003) 145.
[6] M. Varga, J. Dobor, A. Helenkár, L. Jurecska, J. Yao, G. Záray, Microchem. J. 95 (2010) 353.
[7] C.L. Chitescu, E. Oosterink, J. de Jong, A.A.M. (Linda) Stolker, Talanta 88 (2012) 653.
[8] J. Xua, L. Wu, W. Chen, A.C. Chang, J. Chromatogr. A 1202 (2008) 189.
[9] T. Okuda, N. Yamashita, H. Tanaka, H. Matsukawa, K. Tanabe, Environ. Int. 35 (2009) 815.
[10] J.C. Durán-Alvarez, E. Becerril-Bravo, V.S. Castro, B. Jiménez, R. Gibson, Talanta 78 (2009) 1159.
[11] A. Jelić, M. Petrović, D. Barceló, Talanta 80 (2009) 363.
[12] C. Sánchez-Brunete, E. Miguel, J.L. Tadeo, J. Chromatogr. A 1148 (2007) 219.
[13] H.B. Xiao, M. Krucker, K. Albert, X.M. Liang, J. Chromatogr. A 1032 (2004) 117.
[14] V.B. Reeves, J. Chromatogr. B 723 (1999) 127.
[15] M. Petrović, M.D. Hernando, M.S. Díaz-Cruz, D. Barceló, J. Chromatogr. A 1067 (2005) 1.
[16] A. Kot-Wasik, J. Dębska, J. Namieśnik, Trends Anal. Chem. 26 (2007) 557.
[17] S. Babić, D. Mutavdžić Pavlović, D. Ašperger, M. Periša, M. Zrnčić, A.J.M. Horvat, M. Kaštelan-Macan, Anal. Bioanal. Chem. 398 (2010) 1185.
[18] J.O. Fernandes, C. Soares, J. Chromatogr. A 1175 (2007) 1.
[19] A.C. Manhita, D.M. Teixeira, C.T. da Costa, J. Chromatogr. A 1129 (2006) 14.
[20] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, J. Chromatogr. A 1165 (2007) 32.
[21] J. Blesa, J.M. Soriano, J.C. Moltó, R. Marín, J. Mañes, J. Chromatogr. A 1011 (2003) 49.
[22] K. Kishida, N. Furusawa, J. Chromatogr. A 937 (2001) 49.
[23] Y. Picó, M. Fernández, M.J. Ruiz, G. Font, J. Biochem. Biophys. Methods 70 (2007) 117.
[24] S. Bogialli, A. Di Corcia, J. Biochem. Biophys. Methods 70 (2007) 163.
[25] M.G. Dantas Silva, A. Aquino, H. Silveira Dórea, S. Navickiene, Talanta 76 (2008) 680.
[26] J. Cai, Y. Gao, X. Zhu, Q. Su, Anal. Bioanal. Chem. 383 (2005) 869.
[27] M. Radišić, S. Grujić, T. Vasiljević, M. Laušević, Food Chem. 113 (2009) 712.
[28] S. Babić, A.J.M. Horvat, D. Mutavdžić Pavlović, M. Kaštelan-Macan, Trends Anal. Chem. 26 (2007) 1043.
[29] EPIweb 4.0, http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, February 2012.
[30] M. Pansu, J. Gautheyrou, Handbook of Soil Analysis—Mineralogical, Organic and Inorganic Methods, Springer, New York, 2006.
[31] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[32] I. Marchia, V. Viette, F. Badoud, M. Fathi, M. Saugy, S. Rudaz, J.-L. Veuthey, J. Chromatogr. A 1217 (2010) 4071.
[33] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, Anal. Chim. Acta 626 (2008) 155.
[34] E. Turiel, A. Martín-Esteban, J.L. Tadéo, Anal. Chim. Acta 562 (2006) 30.
[35] V. Andreu, C. Blasco, Y. Picó, Trends Anal. Chem. 26 (2007) 534.
[36] S.A. Barker, J. Chromatogr. A 885 (2000) 115.
[37] J.M. Marín, E. Gracia-Lor, J.V. Sancho, F.J. López, F. Hernández, J. Chromatogr. A 1216 (2009) 1410.
[38] A. Kruve, A. Künnapas, K. Herodes, I. Leito, J. Chromatogr. A 1187 (2008) 58.
[39] J. Beausse, Trends Anal. Chem. 23 (2004) 753.
[40] E. Turiel, A. Martín-Esteban, J.L. Tadeo, J. Chromatogr. A 1172 (2007) 97.
[41] E. Martínez-Carballo, C. González-Barreiro, S. Scharf, O. Gans, Environ. Pollut. 148 (2007) 570.
[42] A. Karcı, I. Akmehmet Balcıoğlu, Sci. Total Environ. 407 (2009) 4652.
[43] X. Hu, Q. Zhou, Y. Luo, Environ. Pollut. 158 (2010) 2992.