Talanta 80 (2009) 363–371

Contents lists available at ScienceDirect

Talanta

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

Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry

Aleksandra Jelić[a], Mira Petrović[a,b,*], Damià Barceló[a,c]

[a] Department of Environmental Chemistry, IDAEA-CSIC, c/Jordi Girona 18-26, 08034 Barcelona, Spain
[b] Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluis Companys 23, 80010 Barcelona, Spain
[c] Institut Català de Recerca de l'Aigua (ICRA), c/Pic de Peguera 15, 17003 Girona, Spain

ARTICLE INFO

Article history:
Received 6 April 2009
Received in revised form 26 June 2009
Accepted 30 June 2009
Available online 8 July 2009

Keywords:
Pharmaceuticals
Sewage sludge
Multi-residue method
Pressurized liquid extraction
Liquid chromatography–tandem mass spectrometry

ABSTRACT

A simple and sensitive method for simultaneous analysis of 43 pharmaceutical compounds in sewage sludge and sediment samples was developed and validated. The target compounds were extracted using pressurized liquid extraction (PLE) and then purified and pre-concentrated by solid phase extraction (SPE) using a hydrophilic–lipophilic balanced polymer. PLE extraction was performed on temperature of 100 °C, with methanol/water mixture (1/2, v/v) as extraction solvent. The quantitative analysis was performed by liquid chromatography tandem mass spectrometry using a hybrid triple quadrupole-linear ion trap mass spectrometer (LC–QqLIT-MS). Data acquisition was carried out in selected reaction monitoring (SRM) mode, monitoring two SRM transitions to ensure an accurate identification of target compounds in the samples. Additional identification and confirmation of target compounds were performed using the Information Dependent Acquisition (IDA) function. The method was validated through the estimation of the linearity, sensitivity, repeatability, reproducibility and matrix effects. The internal standard approach was used for quantification because it efficiently corrected matrix effects. Despite the strong matrix interferences, the recoveries were generally higher of 50% in both matrixes and the detection and quantification limits were very low. Beside the very good sensitivity provided by LC–QqLIT-MS, an important characteristic of the method is that all the target compounds can be simultaneously extracted, treated and analysed. Hence, it can be used for routine analysis of pharmaceuticals providing large amount of data. The method was applied for the analysis of pharmaceuticals in river sediment and wastewater sludge from three treatment plants with different treatment properties (i.e. capacity, secondary treatment, quality of influent waters). The analysis showed a widespread occurrence of pharmaceuticals in the sludge matrices.

1. Introduction

The pharmaceutical products have an important role in the treatment and prevention of disease in both humans and animals. They are designed either to be highly active and interact with receptors in humans and animals or to be toxic for many infectious organisms. Because of the nature they can also have unintended effects on animals and micro-organisms in the environment. Although the effects of the pharmaceuticals are investigated through safety and toxicology studies, the potential environmental impacts of their production and use are less understood and have recently become a topic of research interest [1].

The main point of collection and subsequent release of pharmaceuticals into the environment are wastewater treatment plants (WWTP), where they enter via domestic and hospital sewages or through industrial discharges. The studies of effluent waters and river sediment show that wastewater treatment achieves only partial removal of organic pollutants [2–4]. The analysis of effluent and receiving waters itself is not enough to understand comportment of pharmaceuticals during the whole wastewater treatment. Occurrence and distribution of pharmaceuticals in sewage sludge demands detailed investigations, especially because the digested sludge is disposed to landfills or used as agricultural fertilizer. This is another significant route of these micro-pollutants to the environment [5]. It is very difficult to presume the comportment of pharmaceuticals in wastewater treatment because the pharmaceutical products belonging to the same therapeutic groups do not

* Corresponding author at: Department of Environmental Chemistry, IDAEA-CSIC, c/Jordi Girona 18-26, 08034 Barcelona, Spain. Tel.: +34 934006172; fax: +34 932045904.
E-mail address: mpeqam@cid.csic.es (M. Petrović).

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.06.077

show the similar removal. This is caused by the fact that they have different chemical structure and thus they differ in issue. The available data just give very general overview. The existing analytical methods are mostly focused on specific therapeutic classes, paying special attention to the antibiotics due to their potential for antibiotic resistance [6]. But with the quantity and variety of pharmaceuticals and organic pollutants in general, the interactive and synergetic effects in environment are very possible. For this reason, new trends in analytical chemistry are focused on development of methods for simultaneous analysis of many various compounds. The qualitative and quantitative analysis is a good starting point for the further planning of wastewater treatment as well as the establishment of new regulations related to this subject.

Recent development of the advanced instruments and improved analytical methodologies made possible detection of pharmaceuticals in low levels in different environmental matrixes [5,7–11]. Several methodologies have been developed for determination of pharmaceuticals in solid environmental samples. In recent years, the target compounds have usually been extracted by liquid partitioning with ultrasonication (USE) [6,12–15], microwave assisted extraction (MAE) [16] or the more advanced pressurized liquid extraction (PLE). In order to minimize interferences with matrix components and to pre-concentrate target analytes, solid phase extraction (SPE) has been introduced in preparation procedure as a clean-up step. The combination of pressurized liquid extraction (PLE) with solid phase extraction (SPE) as clean-up step becomes mostly employed technique for preparation of solid samples for instrumental analysis. The PLE technique provides good recoveries, saves time and organic solvent, which makes this technique being preferred one for these kind of samples [5,17–21]. Recently, Radjenović et al. [7] used the PLE–SPE combination for the isolation of 31 pharmaceuticals from sludge samples proceeding from the conventional activated sludge treatment and pilot-scale membrane bioreactors. Barron et al. [5] used the same approach for sample preparation for analysis of 27 pharmaceuticals in soil and treated sludge. Also, Nieto et al. [17] and Göbel et al. [22] extracted the target compounds from sludge samples using the PLE–SPE. In all the mentioned methods a hydrophilic–lipophilic balance (HLB) reversed-phase sorbent was used as SPE packaging. This sorbent has been found suitable for multi-residue methods in neutral pH condition, with a proper selection of the eluent (solvent) [23–25].

The majority of current analytical methods for separation and detection of pharmaceuticals uses liquid chromatography–tandem mass spectrometry (LC–MS/MS) because of its versatility, specificity and selectivity [26]. Triple quadrupole (QqQ) [7,12,17,27] and ion trap MS (IT) [5,6,15] have been widely applied in quantitative target analysis. Recently, a hybrid instrument consisting of a quadrupole and a linear ion trap (QqLIT) have been applied for analysis of pharmaceuticals in waters, thus good results related to analysis of pharmaceuticals in sludge could be expected. This instrument allows powerful scan combinations which lead to rapid identification and confirmation of analytes [8,24,28,29].

The objective of the present study was to develop and validate a sensitive multi-residual method for determination of 43 pharmaceuticals in sludge and sediment. The target compounds belong to different therapeutic groups of pharmaceuticals. They are listed in Table 1 classified according to their therapeutic effects. The pharmaceuticals are extracted using PLE and purified by SPE (Oasis® HLB). High performance liquid chromatography (HPLC) coupled to a QqLIT-MS has been applied for separation and determination of the target pharmaceuticals. Quantitation was performed by the internal standard approach. The performance of the method was evaluated through the estimation of the linearity, sensitivity, repeatability, reproducibility and matrix effects.

The developed method offers simple and simultaneous extraction, treatment and analysis of 43 pharmaceutical compounds in sludge and sediment. Comparing to the previously published methods for determination of pharmaceuticals in environmental solid matrices this method exhibits significantly improved sensitivity due to the QqLIT capabilities. Finally, the method was successfully applied for analysis of pharmaceuticals in sludge from three WWTPs which proves that it can be used for routine analysis of pharmaceuticals providing a large amount of data.

2. Experimental part

2.1. Chemicals

All the pharmaceutical standards for target compounds were of high purity grade (>90%). Ibuprofen, neproxen, ketoprofen, diclofenac and gemfibrozil were supplied by Jescuder (Rubí, Spain). Acetaminophen, indometacin, mefenamic acid, phenazone, bezifibrate, mevastatin, fenofibrate, Pravastatin (as sodium salt), carbamazepine, famotidine, ranitidine (as hydrochloride), cimetidine (as hydrochloride), erythromycin (as hydrate), azithromycin (as dehydrate), roxitromycin, clarithromycin, josamycin, tylosin A, sulfamethazine, trimethoprim, chloramphenicol, atenolol, sotalol, metoprolol (as tartrate), timolol, pindolol, nadolol, salbutamol, clenbuterol (as hydrochloride), enalapril (as maleate), glibenclamide, furosemide, hydrochlorothiazide and metronidazole were purchased from Sigma–Aldrich (Steinheim, Germany). Standard atorvastatin (as calcium salt) was provided by LGC Promochem (London, UK), while diazepam, lorazepam and butalbital were from Cerilliant (Texas, USA).

Isotopically labelled compounds, used as internal standards, were sulfathiazole-d_4 from Toronto Research Chemicals, diazepam-d_5 and phenobarbital-d_3 from Cerilliant (Texas, USA), atenolol-d_7, carbamazepine-d_{10}, ibuprofen-d_3, clotrimazole-d_5, enalapril-d_5, hydrochlorothiazide-d_2, glyburide-d_3, albuterol-d_3, cimetidine-d_3, ethyl clofibrate-d_4, antipyrine-d_3, acetaminophen-d_4, diclofenac-d_4 from CDN Isotopes (Quebec, Canada), mecoprop-d_3 from Dr. Ehrenstorfer (Augsburg, Germany) and ^{13}C-erythromycin and ^{13}C-phenacetin from Sigma–Aldrich (Steinheim, Germany).

The solvents, HPLC grade methanol, acetonitrile, water (Lichrosolv) and formic acid 98% were provided by Merck (Darmstadt, Germany). Nitrogen used for drying from Air Liquide (Spain) was of 99.995% purity.

The cartridges used for solid phase extraction were Oasis® HLB (200 mg, 6 ml) from Waters Corporation (Milford, MA, USA). The syringe filters of 0.45 μm pore size were purchased from Pall Corp (USA).

The individual standard solutions as well as isotopically labelled internal standard solutions were prepared on a weight basis in methanol. Furosemide and butalbital were obtained as solutions in acetonitrile, while lorazepam and diazepam were dissolved in methanol, at a concentration of 1 mg/ml. The solutions were stored at −20 °C. Fresh stock solutions of antibiotics were prepared monthly due to their limited stability while stock solutions for the rest of substances was renewed every three months. A mixture of all pharmaceuticals was prepared by appropriate dilution of individual stock solutions in methanol–water (25:75, v/v) and it was renewed before each analytical run. A separate mixture of isotopically labelled internal standards, used for internal standard quantification, was prepared in methanol and further diluted in methanol–water (25:75, v/v) mixture.

2.2. Sample pretreatment

River sediment and sludge samples, provided from three wastewater treatment plants (WWTP), were used for development

Table 1
Target compounds and the corresponding isotopically labelled compounds. SRM transitions and MS/MS parameters for the analysis of the target compounds.

Therapeutic groups	Compounds	CAS number	Internal standards	Precursor ion (m/z)	SRM1	DP-CE-CXP	SRM2	DP-CE-CXP	SRM ratio (SRM1/SRM2)
Analgesics/anti-inflammatories	Ketoprofen	22071-15-4	Mecoprop-d_3	253 $[M-H]^-$	209	40-12-11	197	40-6-9	15.6
	Naproxen	22204-53-1	Ibuprofen-d_3	229 $[M-H]^-$	185	35-10-13	169	35-38-9	2.1
	Ibuprofen	15687-27-1	Ibuprofen-d_3	205 $[M-H]^-$	161	45-10-7	–	–	–
	Indomethacine	53-86-1	Ibuprofen-d_3	356 $[M-H]^-$	312	50-12-3	297	50-24-17	4.7
	Diclofenac	15307-86-5	Diclofenac-d_4	294 $[M-H]^-$	250	40-16-1	214	40-30-15	17.6
	Mefenamic acid	61-68-7	Ibuprofen-d_3	240 $[M-H]^-$	196	45-20-5	180	45-38-35	19.1
	Acetaminophen	103-90-2	Acetaminofen-d_3	150 $[M-H]^-$	107	55-22-7	–	–	–
Phenazone type drugs	Phenazone	60-80-0	Phenazone-d_3	189 $[M+H]^+$	56	76-40-4	147	76-33-4	1.9
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	41859-67-0	Ethyl clofibrate-d_4	360 $[M-H]^-$	274	70-26-1	154	70-38-5	2.9
	Fenofibrate	49562-28-9	Ethyl clofibrate-d_4	361 $[M+H]^+$	139	76-43-10	–	–	–
	Gemfibrozil	25812-30-0	Ibuprofen-d_3	249 $[M-H]^-$	121	85-20-7	127	85-14-5	21.7
	Mevastatin	73573-88-3	Carbamazepine-d_{10}	391 $[M+H]^+$	185	56-19-16	159	56-39-14	1.6
	Pravastatin	81093-37-0	Carbamazepine-d_{10}	447 $[M+H]^+$	327	81-29-10	–	–	–
	Atorvastatin	134523-00-5	Carbamazepine-d_{10}	559 $[M+H]^+$	440	71-27-20	250	71-63-4	2.0
Psychiatric drugs	Diazepam	439-14-5	Diazepam-d_5	285 $[M+H]^+$	193	91-45-8	154	91-50-15	1.9
	Lorazepam	846-49-1	Diazepam-d_5	323 $[M+H]^+$	174	66-45-18	229	66-45-8	1.2
	Carbamazepine	298-46-4	Carbamazepine-d_{10}	237 $[M+H]^+$	194	76-29-19	–	–	–
Histamine H2 receptor antagonists	Ranitidine	66357-35-5	Cimetidine-d_3	315 $[M+H]^+$	176	56-25-14	130	56-39-6	2.0
	Famotidine	76824-35-6	Cimetidine-d_3	338 $[M+H]^+$	189	56-27-4	259	56-20-8	1.6
	Cimetidine	51481-61-9	Cimetidine-d_3	253 $[M+H]^+$	95	46-30-8	159	46-23-12	1.5
Macrolide antibiotics	Erythromycin	114-07-8	Erythromycin ^{13}C	734 $[M+H]^+$	158	71-41-8	576	71-35-8	3.8
	Roxithromycin	80214-83-1	Erythromycin ^{13}C	838 $[M+H]^+$	158	56-49-14	679	56-31-8	4.2
	Clarithromycin	81103-11-9	Erythromycin ^{13}C	748 $[M+H]^+$	591	61-35-12	158	61-40-12	12.6
	Josamycin	16846-24-5	Erythromycin ^{13}C	828 $[M+H]^+$	174	101-45-14	600	101-37-18	6.6
	Tylosin A	1401-69-0	Erythromycin ^{13}C	916 $[M+H]^+$	174	86-63-14	773	86-41-10	14.2
Sulfonamid antibiotics	Sulfamethazine	57-68-1	Sulfathiazole-d_4	279 $[M+H]^+$	186	71-25-0	124	71-33-10	1.0
Other antibiotics	Trimethoprim	738-70-5	Carbamazepine-d_{10}	291 $[M+H]^+$	230	76-33-0	261	76-31-20	1.2
	Chloramphenicol	56-75-7	Ibuprofen-d_3	323 $[M-H]^-$	152	75-22-13	194	75-18-27	3.5
	Metronidazole	443-48-1	Clotrimazole-d_5	172 $[M+H]^+$	172	61-21-8	82	61-37-6	1.8
ß-blockers	Atenolol	29122-68-7	Atenolol-d_7	267 $[M+H]^+$	145	60-35-8	190	60-35-14	3.6
	Sotalol	3930-20-9	Atenolol-d_7	273 $[M+H]^+$	213	60-25-6	255	60-25-6	1.1
	Metoprolol	37350-58-6	Atenolol-d_7	268 $[M+H]^+$	121	60-35-10	133	60-35-8	1.0
	Timolol	26839-75-8	Atenolol-d_7	317 $[M+H]^+$	261	60-30-20	244	60-30-6	1.2
	Nadolol	42200-33-9	Atenolol-d_7	310 $[M+H]^+$	254	46-30-2	201	46-35-4	1.4
	Pindolol	13523-86-9	Atenolol-d_7	249 $[M+H]^+$	116	60-30-8	98	60-30-14	7.0
ß-agonists	Clenbuterol	37148-27-9	Albuterol-d_3	277 $[M+H]^+$	203	61-23-14	132	61-33-10	2.2
	Salbutamol	18559-94-9	Albuterol-d_3	240 $[M+H]^+$	148	61-25-12	166	61-20-12	1.7
Barbiturates	Butalbital	77-26-9	Phenobarbital-d_5	223 $[M-H]^-$	180	60-16-9	85	60-18-5	3.8
Antihypertensive	Nifuroxazide	965-52-6	Phenacetine ^{13}C	276 $[M+H]^+$	121	81-25-10	65	81-73-4	7.3
	Enalapril	75847-73-3	Enalapril-d_5	377 $[M+H]^+$	234	91-29-12	303	91-35-6	16.8
Diuretic	Hydrochlorothiazide	58-93-5	Hydrochlorthiazide-d_2	296 $[M-H]^-$	78	90-28-17	–	–	–
	Furosemide	54-31-9	Ibuprofen-d_3	329 $[M-H]^-$	205	65-22-19	285	65-32-11	1.2
Antidiabetic	Glibenclamide	10238-21-8	Glibenclamide-d_3	494 $[M+H]^+$	369	81-23-6	169	81-55-12	1.8

and validation of the method. The sludge samples are products of aerobic (WWTP Tudela) and anaerobic digestion (WWTP Arazuri and WWTP Terrassa) of sludge.

The sediment was collected from the middle course of river Ebro (Spain) in 2004. The samples from WWTP Tudela (Sludge I) were collected in October 2007 and July 2008. This plant serves 37,300 inhabitants, with a total capacity of 110,000 equivalent inhabitants. Sludge processing is based on thermophilic aerobic digestion in bacterial beds with approximately 9 days of retention time. The samples from WWTP Arazuri (Pamplona) (Sludge II) were collected in October 2007 and from WWTP Terrassa in April 2007. The WWTP Arazuri serves around 356,000 inhabitants, with a total capacity of 722,000 equivalent inhabitants in 2007. The sludge treatment involves anaerobic digestion with retention time of 19 days. The WWTP Terrassa has a total treatment capacity of 277,000 equivalent inhabitants. The sludge is treated by anaerobic digestion with the solid retention time of approx. 10 days.

All the solid samples were freeze-dried (LioAlfa 6, Telstar) at −40 °C and with 0.044 bar vacuum and stored at −20 °C until the analysis.

2.3. Extraction and clean-up

The samples of sludge and sediment were extracted by PLE using ASE 300 accelerated solvent extractor (Dionex, Sunnyvale, CA) equipped with 11 ml stainless extraction cells. Aliquots of freeze-dried and grinded sludge and sediment (1 g) were mixed in the extraction cells with Hydromatrix. This dispersing agent is used

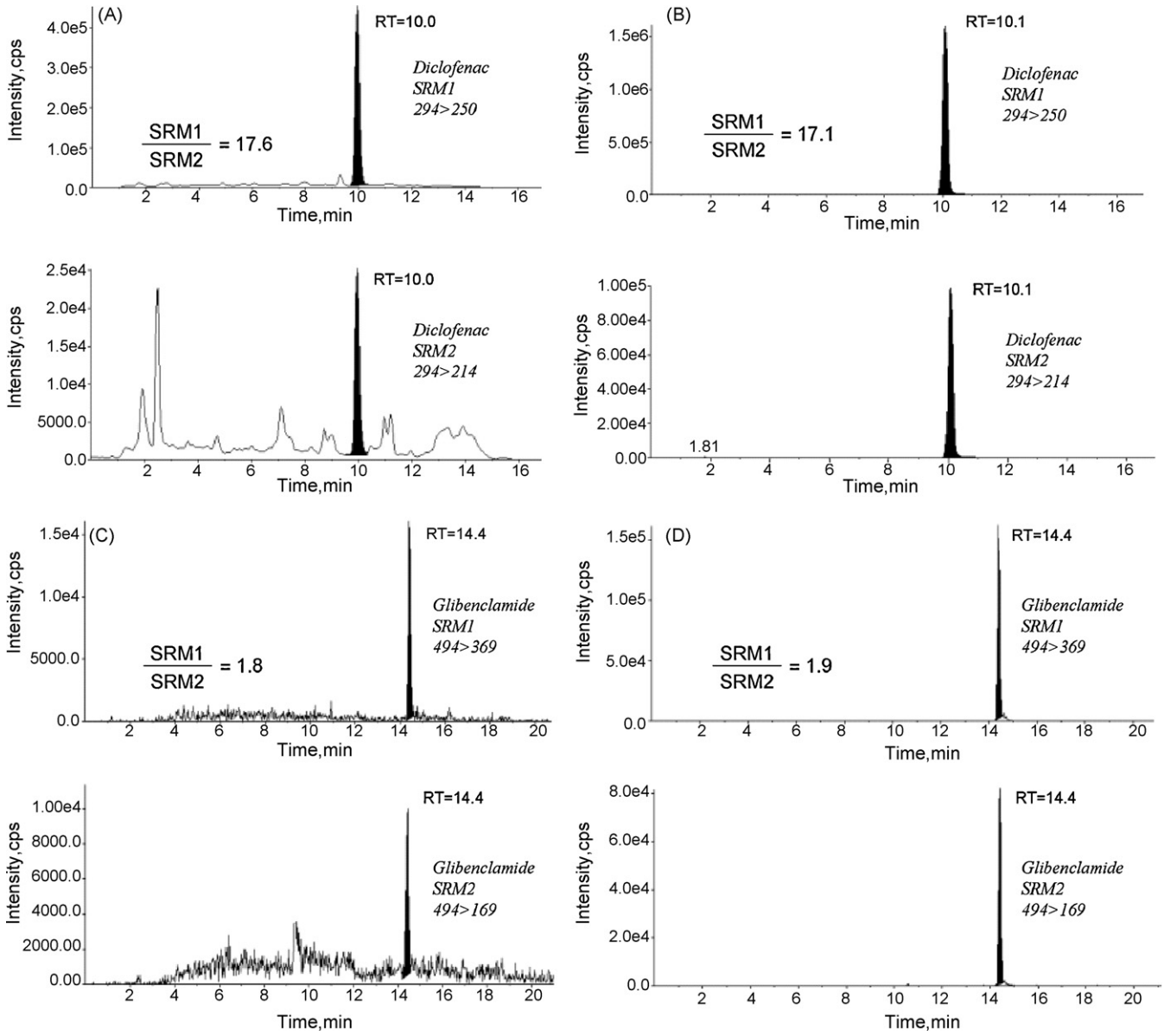

Fig. 1. Chromatograms of the two SRM transitions of diclofenac and glibenclamide in Sludge II (A and C, respectively) and in standard solution (B and D).

to prevent aggregation of sample particles and reduce interstitial volume in the cells [30]. Optimization of extraction parameters included selection of solvent and variation of temperature, time and number of extraction cycles. The extraction method was established with the following parameters: methanol/water, 1/2 (v/v) as extraction solvent, temperature of 100 °C, a preheating period of 5 min, 3 static cycles, each lasting 5 min, total flush volume of 100% of cell with 60 s of nitrogen purge.

The extract obtained in PLE (~22 ml) was diluted in 500 ml of HPLC water (methanol < 5%), and processed by SPE. Oasis HLB cartridges (200 mg, 6 ml) from Waters Corporation (Milford, MA) were used for clean-up. The cartridges were conditioned with 5 ml of methanol followed by 5 ml of HPLC water at neutral pH. Then the dilution of ASE extract was percolated through the cartridges using a Baker vacuum system (J.T. Baker, The Netherlands). Finally, the compounds were eluted with 8 ml of methanol at a 1 ml min^{-1} flow and then the SPE extracts were evaporated under a nitrogen stream and reconstituted with 1 ml of methanol–water mixture (25:75, v/v). Prior to the LC–MS/MS analysis, the samples were passed through 0.45 μm filters and fortified with a standard mixture of the internal standards to the concentration of 20 ng ml^{-1}.

2.4. LC–ESI-(QqLIT)-MS^2 analysis

LC analysis was performed using Symbiosis™ Pico (SP104.002, Spark, Holland), equipped with an autosampler and connected in series with a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved with a Purospher Star RP-18 endcapped column (125 mm × 2.0 mm, particle size 5 μm) preceded by a C_{18} guard column (4 × 4, 5 μm), both supplied by Merck (Darmstadt, Germany).

Knowing that an aqueous, purified and pre-concentrated extract of a solid environmental sample is quite similar to a water sample, already established protocol by Gros et al. [8] was used for quantitative analysis of water samples.

The elution gradients were adapted for the LC by Symbiosis™ Pico. For the analysis in negative ionization mode, solvent A was a mixture of acetonitrile–methanol (1:1, v/v) and solvent B was HPLC water. The elution started with 20% of eluent A, increasing to 80% in 15 min, raising to 90% in 2 min and then back to initial conditions within 3 min. The column was re-equilibrated for 10 min before another injection. The analysis in positive ionization mode was performed using acetonitrile as solvent A and HPLC water with 0.1% formic acid as solvent B. The elution started with 5% of eluent A, increasing to 95% in 20 min, raising to 100% in the following 2 min and then back to initial conditions within 5 min. The re-equilibration time was 10 min. The sample injection volume was set at 20 μl and the flow on 0.2 ml/min in both modes.

Data acquisition was performed in selective reaction monitoring (SRM) mode. For each compound two SRM transitions between the precursor ion and two most abundant fragment ions were monitored, as illustrated in Fig. 1. Only one transition was monitored for the isotopically labelled standards since they are normally not present in environmental samples. In total, 70 transitions in positive ionization mode (corresponding to 30 compounds and 12 internal standards) and 29 transitions in negative ionization mode (13 compounds and 7 internal standards) were monitored. In order to obtain additional confirmation, especially for compounds showing poor fragmentation, an Information Dependent Acquisition (IDA) experiment was performed, with SRM as the survey scan and an enhanced product ion scan (EPI), at three different collision energies, as dependent scan. The obtain spectra were compared with library data based on EPI spectra at the three collision energies used. This allows broad accomplishment of the requirements set by the EU regulations (EU Commission Decision 2002/657/EC) [31] related to identification and confirmation of pharmaceuticals in LC–tandem MS analysis.

2.5. Method validation

The performance of the method was evaluated through estimation of the linearity, sensitivity, repeatability, reproducibility and matrix effects of the method.

Quantification, based on peak areas, was performed by internal standard calibration. The internal standards used for quantification of the compounds were following: sulfathiazole-d_4, diazepam-d_5, phenobarbital-d_3, atenolol-d_7, carbamazepine-d_{10}, ibuprofen-d_3, clotrimazole-d_5, enalapril-d_5, hydrochlorothiazide-d_2, glyburide-d_3, albuterol-d_3, cimetidine-d_3, ethyl clofibrate-d_4, antipyrine-d_3, acetaminophen-d_4, diclofenac-d_4, mecoprop-d_3, ^{13}C-erythromycin and ^{13}C-phenacetin. Seven-point calibration curves (0.5–100 ppb) were generated using linear regression analysis. The linearity was qualified by linear correlation coefficient, r^2.

To determine the recoveries, sediment and sludge samples were spiked in triplicate with a standard mixture of analytes in methanol/water, 25/75 (v/v) to 50 ng g^{-1} concentration. The spiked samples were stirred vigorously in order to enable better contact of analytes with the matrix. After 24-h equilibration, these samples together with the correspondent blank samples were extracted and treated by the previously described protocol. The internal standards for correcting matrix effects were added to the final sample extract. The recoveries were determined in triplicate comparing the obtained concentrations, after subtraction of concentrations found in blank samples, with the initial spiking level.

Method detection limits (MDL) and method quantification limits (MQL) were determined as the minimum detectable amount of analyte with a signal-to-noise of 3 and 10, respectively.

Intra-day and inter-day precision were determined from five repeated injections 50 ng g^{-1} standards during the same day (repeatability) and in five successive days (reproducibility). These two parameters were expressed as relative standard deviation of result (RSD, %).

Matrix effect was evaluated. In order to express it as percentage of suppression or enhancement, Eq. (1) was applied. The peak-areas from the analysis of spiked sludge and sediment extracts ($\text{area}_{\text{matrix}}$) reduced by the peak areas corresponding to the native analytes present in the sample ($\text{area}_{\text{blank}}$), were compared with the peak areas from spiked solvent at the same concentration ($\text{area}_{\text{solvent}}$). The spiked concentration was 25 ng g^{-1} for all the solid samples.

$$\text{signal suppression } (\%) = 100 - \left(\frac{(\text{area}_{\text{matrix}} - \text{area}_{\text{blank}}) \times 100}{\text{area}_{\text{solvent}}}\right) \quad (1)$$

The efficiency of internal standard and standard addition calibration were evaluated comparing the relations of calibration curves made in pure solvent with those prepared in matrix extracts.

3. Results and discussion

3.1. PLE conditions

The combination of solvent, temperature, flush volume, number and time of extraction cycles were investigated in order to obtain optimum extraction conditions for analysis of 42 pharmaceutical compounds. This method is one step forward in development of multi-residual analytical methods in our group [7,8]. Thus the starting point for PLE extraction were conditions reported by Radjenović et al. [7], adopted for the extraction of extended list of compounds.

The influence of the parameters was investigated simultaneously. The experiments were organized combining different solvent mixtures with different temperatures in 30 extractions, and then the other parameters were examined (time and number of cycles). Extraction pressure was set to 1500 psi for all PLE experiments, because it is not considered as a critical experimental parameter and it has negligible impact on analytical recovery [30].

Solvent: The solvent must be able to solubilize the target analytes leaving the sample matrix integrate. Since the analysed pharmaceuticals vary in physicochemical properties, the choice of the solvent mixture was limited and it could not match all the compounds in their polarity. The neutral conditions were required. In this study the following extraction solvents tested were: methanol/water (1/1, 1/2, 1/3, 2/1, 3/1, v/v) and acetonitrile/water (1/1, 1/2, 1/3, 2/1, 3/1, v/v). The combination methanol/water, 1/2, (v/v) yielded relatively better recoveries than other ones. For enalapril, metronidazole, bezifibrate and chloramphenicol, higher percent of water (i.e. methanol/water, 1/3, v/v) gave higher recoveries. More methanol (i.e. methanol/water, 1/1, v/v) gave better results for gemfibrozil, sulfamethazine and erythromycin. In the group of experiments with mixtures of acetonitrile and water, the best ratio was 1/2 (v/v) as well, but still it gave lower recoveries than methanol/water combination.

Temperature: This is very important parameter in PLE extraction. Application of higher temperature in PLE decreases the viscosity of solvents, thus allowing its better penetration into the sample matrix. The increase of temperature decreases significantly the dielectric constant of the water so the organic solvents can be used in smaller amount or avoided. But too high temperature can lead to degradation of the compounds or loss in method selectivity due to more efficient extraction of interfering matrix components. In the described experiments, all the combinations of the solvents on three extraction temperatures were tested: 60, 80 and 100 °C. The temperature higher of 100 °C was not tested since thermal

Table 2
Method performance parameters: reproducibility (RSD% for $n=5$), repeatability (RSD% for $n=5$), recoveries (%) and method precision (RSD%), matrix effect (%), method detection (MDL, ng g^{-1}) and quantification limits (MQL, ng g^{-1}) obtained in sludge and sediment samples.

Therapeutic groups	Compounds	Repeat. (RSD%) ($n=5$)	Reprod. (RSD%) ($n=5$)	Sludge				Sediment			
				Recovery,% (RSD,%) ($n=3$)	Matrix effect (%)	MDL (ng g^{-1})	MQL (ng g^{-1})	Recovery, % (RSD, %) ($n=3$)	Matrix effect (%)	MDL (ng g^{-1})	MQL (ng g^{-1})
Analgesics/anti-inflammatories	Ketoprofen	2	2	98.4 (10)	14	0.56	1.86	105 (0.6)	35	0.93	3.11
	Naproxen	5	5	125 (4.6)	41	0.07	0.24	105 (5.2)	48	0.84	2.79
	Ibuprofen	1	2	118 (13)	38	0.10	0.32	129 (1.0)	45	0.12	0.40
	Indomethacine	2	3	107 (7.0)	87	0.22	0.73	81.4 (0.7)	68	0.15	0.49
	Diclofenac	3	2	81.4 (9.1)	79	0.94	3.13	101 (1.2)	54	0.03	0.09
	Mefenamic acid	8	7	69.3 (12)	68	0.07	0.24	95.2 (5.3)	58	0.22	0.74
	Acetaminophen	3	3	40.7 (8.8)	-24	0.07	0.24	60.3 (9.8)	9	0.22	0.74
Phenazone type drugs	Phenazone	6	6	194 (4.1)	76	1.12	3.72	169 (1.8)	73	0.34	1.15
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	7	13	107 (5.1)	47	0.01	0.05	95.1 (3.8)	56	0.02	0.06
	Fenofibrate	3	11	204 (9.0)	81	1.04	3.46	123 (7.4)	75	0.79	2.62
	Gemfibrozil	4	11	76.1 (13)	82	0.51	1.69	77.7 (4.9)	72	1.24	4.14
	Mevastatin	13	14	94.1 (1.0)	66	8.84	29.4	89.0 (1.5)	85	3.16	10.5
	Pravastatin	12	15	215 (5.4)	78	1.32	4.42	186 (6.9)	57	0.71	2.38
	Atorvastatin	6	5	72.9 (4.5)	97	0.99	3.31	34.6 (3.3)	98	0.72	2.41
Psychiatric drugs	Diazepam	1	4	96.8 (2.5)	93	1.83	6.10	107 (9.3)	77	0.23	0.77
	Lorazepam	2	10	70.2 (13)	79	5.75	19.2	125 (6.3)	71	3.20	10.7
	Carbamazepine	2	8	134 (3.5)	89	0.04	0.13	137 (1.7)	81	0.03	0.09
Histamine H_2 receptor antagonists	Ranitidine	1	9	106 (13)	99	0.03	0.10	126.2 (13)	89	0.02	0.06
	Famotidine	4	14	83.5 (14)	97	0.01	0.05	97.8 (8.1)	73	0.02	0.07
	Cimetidine	7	7	78.3 (14)	80	0.07	0.24	87.4 (7.2)	72	0.01	0.02
Macrolide antibiotics	Erythromycin	6	5	43.2 (1.2)	100	1.17	3.88	68.0 (12)	92	0.01	0.02
	Roxithromycin	5	6	146 (15)	97	6.75	22.5	149 (9.9)	89	0.04	0.13
	Clarithromycin	8	14	38.2 (9.1)	95	3.51	11.7	130 (11)	86	0.10	0.34
	Josamycin	9	8	42.1 (13)	94	0.08	0.27	206 (7.0)	88	0.64	2.12
	Tylosin A	7	5	142 (1.8)	93	0.81	2.69	157 (8.8)	84	0.04	0.14
Sulfonamide antibiotics	Sulfamethazine	6	9	40.3 (14)	74	0.14	0.48	45.7 (8.9)	74	0.32	1.06
Other antibiotics	Trimethoprim	10	11	93.1 (12)	96	0.14	0.47	97.2 (9.6)	83	0.25	0.83
	Chloramphenicol	10	14	76.0 (9.3)	65	0.03	0.09	72.9 (0.3)	45	0.10	0.32
	Metronidazole	9	11	80.5 (15)	62	0.55	1.83	41.7 (3.9)	69	0.38	1.26
ß-blockers	Atenolol	5	4	97.1 (11)	77	0.16	0.54	90.4 (14)	66	0.11	0.36
	Sotalol	3	5	105 (12)	84	0.06	0.20	105 (8.8)	74	0.06	0.20
	Metoprolol	4	8	195 (2.9)	98	0.30	1.00	144 (11)	84	0.21	0.71
	Timolol	9	7	169 (9.0)	98	0.08	0.25	109 (14)	85	0.05	0.17
	Nadolol	6	4	46.1 (8.7)	94	0.06	0.19	66.7 (8.7)	82	0.01	0.03
	Pindolol	4	5	53.0 (7.9)	99	0.42	1.41	77.2 (12)	90	0.03	0.10
ß-agonists	Clenbuterol	5	4	86.4 (14)	88	0.02	0.07	168 (3.3)	97	0.07	0.25
	Salbutamol	0	3	86.0 (4.8)	82	0.08	0.27	84.1 (11)	68	0.02	0.08
Barbiturates	Butalbital	8	7	44.5 (2.1)	69	0.39	1.31	33.2 (1.2)	77	0.05	0.16
Antihypertensives	Nifuroxazide	13	9	65.0 (6.4)	78	0.06	0.20	125 (8.8)	96	0.05	0.17
	Enalapril	6	14	100 (8.8)	96	0.10	0.32	97.7 (5.6)	81	0.01	0.03
Diuretics	Hydrochlorothiazide	9	8	42.3 (12)	73	0.08	0.27	71.0 (13)	65	0.06	0.20
	Furosemide	10	13	106 (3.3)	51	0.19	0.64	77.7 (3.3)	57	0.76	2.52
Antidiabetics	Glibenclamide	6	5	87.8 (11)	89	1.67	5.56	89.1 (9.4)	85	0.29	0.96

degradation can occur at higher temperatures [22]. The recoveries obtained on 60 °C were low for most of analysed compounds. Salbutamol, gemfibrozil, mefenamic acid and diazepam gave better recoveries at temperature of 80 °C. For the compounds that were recovered in small or exaggerated percent is difficult to conclude which temperature suits better (i.e. prevastatine, atorvastatine, macrolides).

Extraction cycles: Optimization of the conditions included the screening of number and duration of static cycles. Each static cycle introduce fresh solvent which is very useful for the samples with complex matrix as sludge, while the longer time of a cycle can allow better diffusion of analytes into the extraction solvent. It is recommended to divide the extraction into more cycles [30]. The extracts of individual cycles of 5 min were collected as well as extracts from 2 and 3 cycles. Through the first and second fraction had almost all the compounds extracted completely, the third cycle was introduced to insure the complete extraction of diclofenac, indometacin, mefenamic acid, gemfibrozil, bezifibrate and lorati-

dine. With the three-cycle extraction, it is presumed that all the spiked and native analytes were removed. Finally, the PLE extraction was performed under the following conditions: 1 g of sample, temperature of 100 °C, as extraction solvent—methanol/water, 1/2 (v/v), a preheating period of 5 min, 3 static cycles, each lasting 5 min, total flush volume was 100% of cell with 60 s of nitrogen purge.

3.2. Method validation

The quantification was based on peak area. The shift of peaks in both transitions was noticed in the matrices comparing to the position in pure solvent. But, the retention times for all the analytes in the matrix varied less than 1%. In Fig. 1 are illustrated the chromatographic peaks of the two SRM transitions of diclofenac and glibenclamide in sludge and standard solution. The parts of the figure marked by A and C stand for the chromatograms of analytes in Sludge II, and B and D for chromatograms of standard solutions of diclofenac and glibenclamide, respectively. As illustrated, the retention times and SRM ratios concord well. Reproducibility and repeatability expressed as relative standard deviation, RSD%, were lower than 13% for intra-day and 15% for inter-day analysis, respectively (Table 2). Seven points calibration curves gave very good fits, $r^2 > 0.99$, over the established concentration range of 0.5–100 ng g^{-1} for all the compounds in all the matrices. The limits of detections in sludge and sediment were lower than 1 ng/g for the most compounds. The method provided lower sensitivity for phenazone, fenofibrate, prevastatine, mevastatine, diazepam, lorazepam, erythromycin, roxitromycin and

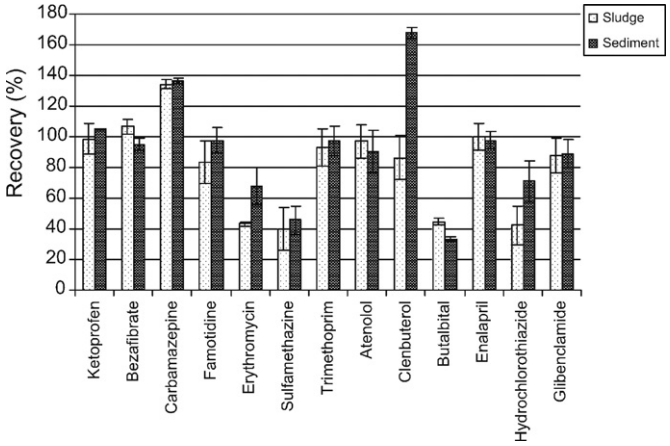

Fig. 2. Recoveries obtained for the representative compounds of each therapeutic group.

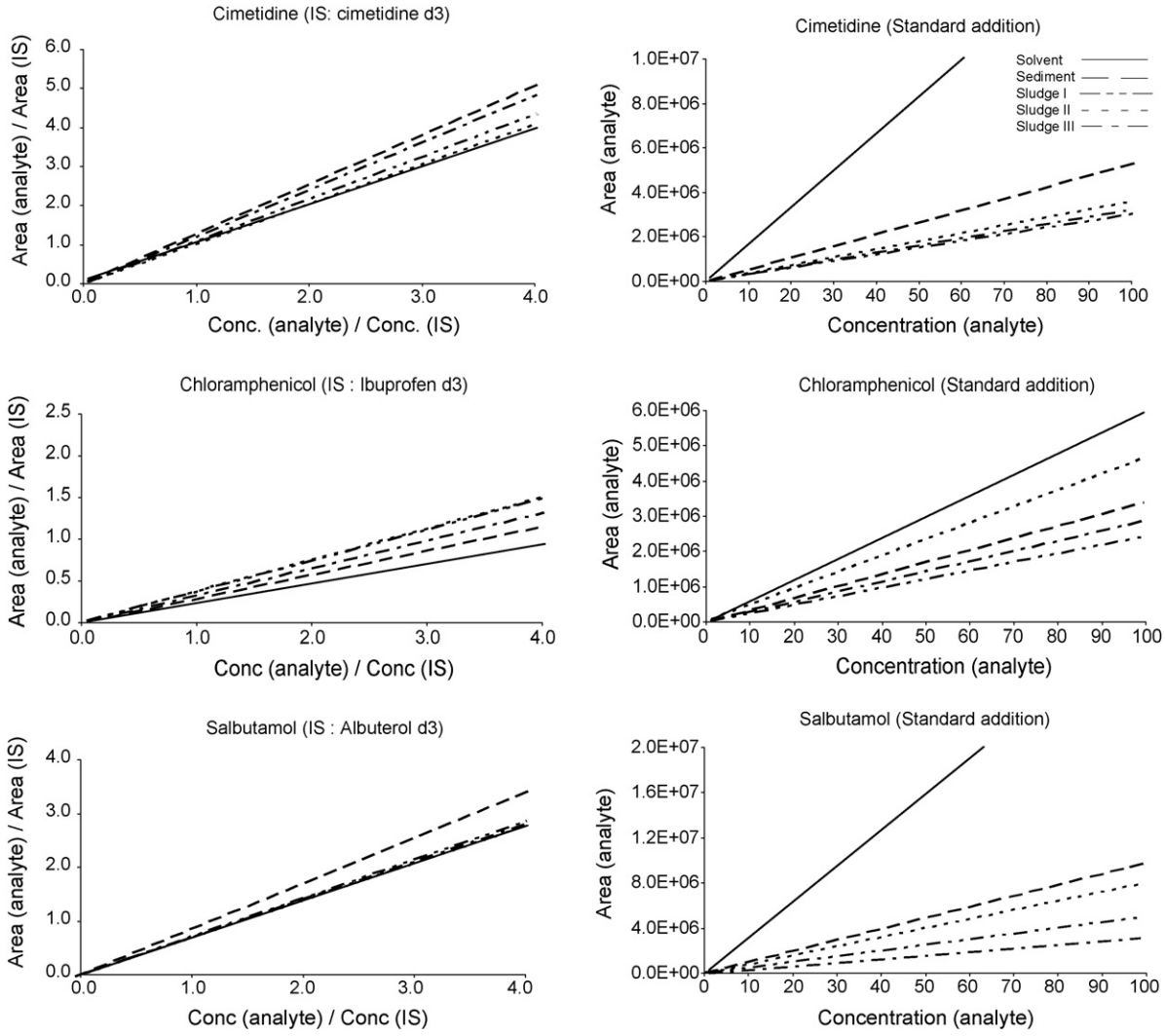

Fig. 3. Internal standard calibration curves (left) and standard addition calibration curves (right) of spiked solvent and extracts of sludge and sediment for cimetidine, salbutamol and chloramphenicol.

clarithromycin in sludge (1.12 < MDL < 6.75) and for mevastatine, gemfibrozil, lorazepam in sediment samples (1.24 < MDL < 3.20). Generally, the sensitivity was lower for sludge than for sediment samples, due to the more complex matrix. The values of MDL and MQL for sludge (Sludge I) and sediment are presented in Table 2. Compared to the method detection limits obtained for pharmaceuticals in solid matrices using some other instruments [5,7,12,13,32], with the LC–QqLIT-MS the improvement in sensitivity is significant.

In Table 2 are listed only the recovery results obtained for Sludge I and sediment, with the corresponding relative standard deviations. The RSD% was less than 15% for all the compounds in both matrices, which is considered as good method precision. The recoveries varied significantly depending on compound and matrix. For the pharmaceuticals in Sludge I only acetaminophen (40.7%), josamycin (42.1%), sulfamethazine (40.3%) and hydrochlorthiazide (42.3%) had recoveries <45%, wherein acetaminophen and hydrochlorthiazide were quantified with their deuterated standards. For the samples of sediment, recoveries were <50% for atorvastatin (34.6%), sulfamethazine (45.7%), metronidazole (41.7%) and butalbital (33.2%). In Fig. 2 are illustrated the results for some representative compounds for each therapeutic group, and the rest of the compounds followed a similar pattern. Some compounds yielded extremely high recoveries (»100%) in both matrices, like prevastatine, phenazone, fenofibrate and metoprolol. Elevated recoveries have already been reported in some studies about pharmaceuticals in sludge [5,7,32]. The properties of matrix itself and the complexity of the interaction with analytes, as well as possible errors in procedure could be the reason for the extreme (high or low) recoveries. Hence, it is difficult to give logical explanation and expect certain values of recoveries. If the accurate results are requested, the recoveries in the investigated matrix must be determined prior to quantification.

Matrix effect: The performance of HPLC–ESI-MS/MS analysis is strongly affected by the ionisable impurities coming from matrix (e.g. natural organic matter, salts, ion-pairing agents, non-target contaminants, etc.) that can interfere with the ionization processes. This may result in a signal suppression or enhancement leading to low sensitivity and inaccurate results. These effects are more extensive when the matrix is more complex as sludge matrix, for example. It is advisable to evaluate matrix effect as a part of validation of the method to ensure the reliability of results obtained. In this study, standard addition and internal standard addition experiments were performed to investigate and minimize matrix effect.

The calibration curves obtained from real sludge and sediment extracts with standard addition were compared with those in pure solvent (methanol/water, 25/75, v/v). All curves were linear over the concentration range of 0.5–100 ng g^{-1} with correlation factors $r^2 > 0.99$. For illustration, Fig. 3 (right) presents calibration curves of cimetidine, chloramphenicol and salbutamol in 4 real matrices (sediment and three samples of sludge) and one in solvent. Significant difference in slopes was observed proving the existence of matrix effects.

Fig. 3 (left) shows the calibration curves of the same compounds acquired by isotopically labeled compounds added into sludge and sediment extracts and in the pure solvent. The curves were linear over the concentration range of 0.5–100 ng g^{-1} with good correlation factors of $r^2 > 0.99$. Despite the fact that the matched isotopically labeled standards were not available for all the target compounds, the calibration curves appear to be overlapped well enough. This means that internal standards compensated the matrix effects considerably; therefore the internal standard addition was used for quantification in this study. Although the selected isotopically labeled standards seem to be appropriate for the target compounds (Fig. 3), cannot be expected that they give very accurate results. More precise results could be achieved using the surrogate standards of the target compounds. It is expected that the surrogate standards compensate for any error that can occur during the sample preparation. But since the surrogate standards were not available for all the analysed compounds and the internal standard quantification gave repeatable results, we decided to use only internal standard approach. The application of only one approach allowed more comparable and reproducible results. The lack of fully compatible surrogate/internal standards is considered as the main limitation of one multi-residual method for pharmaceuticals in environmental samples [33].

Matrix effect was quantified comparing the areas of compounds in spiked matrix samples with the areas obtained in spiked solvent (methanol/water, 25/75, v/v). The effect was expressed by percentage of signal suppression/enhancement and the results are summarized in Table 2. Knowing that the nature of matrix effect is pretty varying, the percentage is just a relative indicator of the degree of suppression and enhancement. The percentage varies from 14 to 100% in sludge and from 9 to 98% for sediment samples. The impact of matrix interferences was different for each compound and except acetoaminophen, all the compounds were subjected to ion suppression. Strong MS signal suppression effects were observed for most of the compounds.

3.3. Application of the method

The developed method was applied for determination of pharmaceuticals in sediment from middle course of river Ebro and in the sewage sludge from WWTPs Tudela, Pamplona and Terrassa. Target pharmaceuticals were not detected in sediment samples. Of

Table 3
Average concentrations of target compounds detected in sludge-samples from WWTP Tudela (Sludge I), Pamplona (Sludge II) and Terrassa (Sludge III).

Compound	Concentration (ng g^{-1}, d.w.)		
	Sludge I	Sludge II	Sludge III
Ketoprofen	BLD[a]	18.9 ± 1.42	21.1 ± 1.3
Naproxen	BLD	5.9 ± 0.7	4.27 ± 0.1
Ibuprofen	43.2 ± 5.5	117 ± 5.9	91.5 ± 3.8
Indomethacine	BLD	2.5 ± 0.3	2.9 ± 0.1
Diclofenac	27.5 ± 0.5	69.1 ± 7.6	74.9 ± 4.1
Mefenamic acid	26.2 ± 3.2	19.3 ± 2.3	14.3 ± 2.2
Acetaminophen	103 ± 9.0	77.8 ± 10.3	42.1 ± 6.4
Phenazone	3.2 ± 0.1	16.0 ± 1.4	BLD
Bezafibrate	2.9 ± 0.2	7.2 ± 0.6	18.7 ± 0.7
Fenofibrate	3.3 ± 0.3	BLD	17.1 ± 3.7
Gemfibrozil	14.3 ± 1.9	33.9 ± 4.61	31.8 ± 3.8
Atorvastatin	42.1 ± 2.8	65.0 ± 3.21	21.4 ± 2.7
Diazepam	3.20 ± 0.1	8.5 ± 0.2	4.6 ± 0.4
Carbamazepine	10.1 ± 0.1	11.0 ± 1.60	12.7 ± 1.4
Ranitidine	0.2 ± 0.02	2.3 ± 0.3	BLD
Famotidine	2.14 ± 0.3	12.4 ± 1.3	14.7 ± 2.3
Cimetidine	0.5 ± 0.06	2.5 ± 0.2	6.1 ± 0.5
Roxithromycin	BLQ	BLQ[b]	BLQ
Clarithromycin	BLD	47.0 ± 2.9	27.0 ± 2.1
Josamycin	BLD	4.8 ± 0.5	47.8 ± 4.8
Sulfamethazine	BLD	BLQ	1.1 ± 0.4
Trimethoprim	BLD	9.2 ± 0.8	11.2 ± 1.2
Chloramphenicol	BLD	1.2 ± 0.3	BLD
Metronidazole	BLD	10.6 ± 0.6	BLD
Atenolol	10.8 ± 1.1	8.8 ± 0.7	3.96 ± 0.4
Sotalol	1.7 ± 0.2	BLD	BLD
Nadolol	0.8 ± 0.1	3.3 ± 0.5	2.70 ± 0.3
Pindolol	13.6 ± 1.1	23.1 ± 1.9	BLD
Clenbuterol	BLD	40.2 ± 2.5	BLD
Salbutamol	BLD	3.8 ± 0.6	BLD
Nifuroxazide	BLD	0.5 ± 0.05	BLD
Hydrochlorothiazide	29.0 ± 3.6	126 ± 8.5	30.5 ± 1.9
Furosemide	10.1 ± 0.3	16.8 ± 1.4	11.7 ± 0.4
Glibenclamide	7.7 ± 0.6	15.8 ± 1.7	42.4 ± 1.5

BLD and BLQ determined for each matrix individually.
[a] BLD—below limits of detection.
[b] BLQ—below limits of quantification.

43 analysed compounds, 34 were detected in sewage sludge samples from WWTPs. The average concentrations ($n = 5$) determined in those samples are summarized in Table 3. The recoveries and limits of detection and quantification were determined for each kind of sludge individually, prior to quantification. The concentrations below the limits of detection and quantification are not presented. In general, the pharmaceuticals were identified in concentrations of 0.1–120 ng/g. Ibuprofen, acetaminophen, diclofenac, atorvastatin and hydrochlorthiazide were detected in the highest concentrations in samples from all 3 WWTP. These compounds were present in average concentrations from 43.2 to 117 ng g^{-1}, 42.1 to 103 ng g^{-1}, 27.5 to 74.9 ng g^{-1}, 21.4 to 65 ng g^{-1} and 29.0 to 126 ng g^{-1}, respectively. Carbamazepine, mefenamic acid, gemfibrozil, furosemide and glibenclamide were frequently detected as well. The presence of these compounds was reported in recent works related to pharmaceuticals in sludge in similar concentrations [7,13,14]. WWTP Pamplona serves more inhabitants and has higher capacity of treatment than the other two, so the samples from this plant are more contaminated by analysed pharmaceutical residues.

4. Conclusion

The study involved development and validation of a method for simultaneous analysis of 43 pharmaceutical compounds in sludge and sediment. Differences in physicochemical properties of the compounds required neutral experimental conditions. The pharmaceuticals were isolated from solid samples using PLE followed by SPE clean-up step, and submitted to the analysis in LC–QqLIT-MS.

The influence of extraction parameters (i.e. solvent composition, temperature, and number and time of extraction cycles) on the extraction and overall recoveries were evaluated. Strong effects of signal suppression and extreme values of recoveries ($\gg$100%) were noticed as result of matrix interferences. The chosen isotopically labelled compounds compensated for the matrix effects well enough; therefore the internal standard approach was used for quantification. Linearity of the method was satisfactory ($r^2 < 0.99$) in the defined concentration range (0.5–100 ng g^{-1}). The instrument exhibited very good sensitivity with the MDLs and MQLs lower than 1 ng g^{-1} for most of the compounds. The instrumental precision was acceptable with RSDs < 15% for intra- and inter-day analysis and <1% for peak position shifts.

The applicability of the method for routine multi-residue analysis was demonstrated through the analysis of sludge samples from three WWTPs. The recoveries were higher of 50% for most of the compounds and the variance of values was observed depending on the origin of the sludge samples. The presence of 35 pharmaceuticals was confirmed, wherein ibuprofen, acetaminophen, diclofenac, atorvastatin and hydrochlorothiazide were detected in the highest concentrations.

Acknowledgements

The work was financially supported by the Spanish Ministry of Education and Science, project CEMAGUA (CGL2007-64551/HID), and project SOSTAQUA (led by Aguas de Barcelona and founded by CDTI in the framework of the Ingenio 2010 Programme under the CENIT call) and Spanish Ministry of the Environment and Rural and Marine Affairs through the project MMAMRM 010/PC08/3-04. A. Jelic gratefully acknowledges the JAE Program (Junta para la Ampliación de Estudios–JAE Predoc), co-financed by CSIC (Consejo Superior de Investigaciones Científicas) and European Social Funds, for a predoctoral grant. Waters (Milford, USA) is gratefully acknowledged for providing the SPE cartridges and Merck (Darmstadt, Germany) for providing the HPLC columns.

References

[1] A.B.A. Boxall, EMBO Rep. 5 (2004) 1110.
[2] A. Nikolaou, S. Meric, D. Fatta, Anal. Bioanal. Chem. 387 (2007) 1225.
[3] J.L. Zhou, Z.L. Zhang, E. Banks, D. Grover, J.Q. Jiang, J. Hazard. Mater. 166 (2009) 655.
[4] T.A. Ternes, TrAC Trends Anal. Chem. 20 (2001) 419.
[5] L. Barron, J. Tobin, B. Paull, J. Environ. Monit. 10 (2008) 353.
[6] R.H. Lindberg, P. Wennberg, M.I. Johansson, M. Tysklind, B.A.V. Andersson, Environ. Sci. Technol. 39 (2005) 3421.
[7] J. Radjenović, A. Jelicĭ, M. Petrović, D. Barcelo, Anal. Bioanal. Chem. 393 (2009) 1685.
[8] M. Gros, M. Petrović, D. Barceló, Anal. Chem. 81 (2009) 898.
[9] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, J. Chromatogr. A 1161 (2007) 132.
[10] D. Fatta, A. Achilleos, A. Nikolaou, S. Meriç, TrAC Trends Anal. Chem. 26 (2007) 515.
[11] M.J. Gómez, M. Petrović, A.R. Fernández-Alba, D. Barceló, J. Chromatogr. A 1114 (2006) 224.
[12] A.L. Spongberg, J.D. Witter, Environ. Sci. Technol. 397 (2008) 148.
[13] W. Chenxi, A.L. Spongberg, J.D. Witter, Chemosphere 73 (2008) 511.
[14] K. Kimura, H. Hara, Y. Watanabe, Environ. Sci. Technol. 41 (2007) 3708.
[15] A.K.W.B. Matthias Ferdig, J. Sep. Sci. 28 (2005) 1448.
[16] S. Morales, P. Canosa, I. Rodríguez, E. Rubí, R. Cela, J. Chromatogr. A 1082 (2005) 128.
[17] A. Nieto, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 30 (2007) 979.
[18] A. Göbel, A. Thomsen, C.S. McArdell, A.C. Alder, W. Giger, N. Theiß, D. Löffler, T.A. Ternes, J. Chromatogr. A 1085 (2005) 179.
[19] M.S. Díaz-Cruz, M.J. López de Alda, D. Barceló, J. Chromatogr. A 1130 (2006) 72.
[20] A.M. Jacobsen, B. Halling-Sørensen, F. Ingerslev, S. Honoré Hansen, J. Chromatogr. A 1038 (2004) 157.
[21] T.A. Ternes, M. Bonerz, N. Herrmann, D. Löffler, E. Keller, B.B. Lacida, A.C. Alder, J. Chromatogr. A 1067 (2005) 213.
[22] A. Göbel, A. Thomsen, C.S. McArdell, A.C. Alder, W. Giger, N. Theiβ, D. Lo□ffler, T.A. Ternes, J. Chromatogr. A 1085 (2005) 179.
[23] K.A. Krogh, E. Björklund, D. Loeffler, G. Fink, B. Halling-Sørensen, T.A. Ternes, J. Chromatogr. A 1211 (2008) 60.
[24] W.W. Buchberger, Anal. Chim. Acta 593 (2007) 129.
[25] R.A. Trenholm, B.J. Vanderford, J.C. Holady, D.J. Rexing, S.A. Snyder, Chemosphere 65 (2006) 1990.
[26] M. Petrović, M.D. Hernando, M.S. Díaz-Cruz, D. Barceló, J. Chromatogr. A 1067 (2005) 1.
[27] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958.
[28] M.J. Martinez Bueno, A. Aguera, M.J. Gomez, M.D. Hernando, J.F. Garcia-Reyes, A.R. Fernandez-Alba, Anal. Chem. 79 (2007) 9372.
[29] G. Hopfgartner, E. Varesio, V. Tschappat, C. Grivet, E. Bourgogne, L.A. Leuthold, J. Mass Spectrom. 39 (2004) 845.
[30] Technical note—DIONEX; http://www.dionex.com/en-us/webdocs/4736_TN208.pdf.
[31] Commission Decision (2002/657/EC) of 12 August 2002 Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities L221, Brussels, Belgium, pp. 8–36.
[32] A. Nieto, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 1174 (2007) 125.
[33] M. Gros, M. Petrović, D. Barceló, Talanta 70 (2006) 678.