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# Development of a multi-residue analytical methodology based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters

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**Abstract**

This paper describes development, optimization and validation of a method for the simultaneous determination of 29 multi-class pharmaceuticals using off line solid phase extraction (SPE) followed by liquid chromatography–triple quadrupole mass spectrometry (LC–MS–MS). Target compounds include analgesics and non-steroidal anti-inflammatories (NSAIDs), lipid regulators, psychiatric drugs, anti-histaminics, anti-ulcer agent, antibiotics and β-blockers. Recoveries obtained were generally higher than 60% for both surface and wastewaters, with exception of several compounds that yielded lower, but still acceptable recoveries: ranitidine (50%), sotalol (50%), famotidine (50%) and mevastatin (34%). The overall variability of the method was below 15%, for all compounds and all tested matrices. Method detection limits (MDL) varied between 1 and 30 ng/L and from 3 to 160 ng/L for surface and wastewaters, respectively. The precision of the method, calculated as relative standard deviation (R.S.D.), ranged from 0.2 to 6% and from 1 to 11% for inter and intra-day analysis, respectively. A detailed study of matrix effects was performed in order to evaluate the suitability of different calibration approaches (matrix-matched external calibration, internal calibration, extract dilution) to reduce analyte suppression or enhancement during instrumental analysis. The main advantages and drawbacks of each approach are demonstrated, justifying the selection of internal standard calibration as the most suitable approach for our study. The developed analytical method was successfully applied to the analysis of pharmaceutical residues in WWTP influents and effluents, as well as in river water. For both, river and wastewaters, the most ubiquitous compounds belonged to the group of anti-inflammatories and analgesics, antibiotics, the lipid regulators being acetaminophen, trimethoprim, ibuprofen, ketoprofen, atenolol, propranolol, mevastatin, carbamazepine and ranitidine the most frequently detected compounds.


*Keywords:* Liquid chromatography–tandem mass spectrometry; Pharmaceuticals; Surface and wastewater analysis; Multi-residue analytical method; Ion suppression

## 1. Introduction

During the last three decades the impact of chemical pollution has focused almost exclusively on the conventional "priority" pollutants. However, the growing use of pharmaceuticals worldwide, classified as the so-called emerging contaminants, has become a new environmental problem, which has awakened great concern among scientists in the last few years. Even though they are found in very low concentrations, there is still a lack of knowledge about long-term risks that that the presence of a large variety of drugs may pose for non-target organisms as well as for human health.

Wastewater treatment plants (WWTPs) are major contributors of pharmaceuticals in the environment. Due to their high consumption, pharmaceuticals along with their metabolites are continuously introduced to sewage waters, mainly through excreta, disposal of unused or expired drugs or directly from pharmaceutical discharges [1–3]. Recently, it has been reported that the elimination of some pharmaceutical compounds during wastewater treatment processes is rather low and as a result, they are found in surface, ground and drinking waters [4–7]. For this reason, Pharmaceuticals may be able to cause the same exposure potential as persistent pollutants, since their high transformation and removal rates can be compensated by their continuous input into the environment.

Consequently, there is a growing need to develop reliable analytical methods, which enable their rapid, sensitive and selective

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determination in environmental samples, at trace levels. Several analytical methodologies are already available in the literature for the determination of pharmaceutical compounds in surface and wastewaters, and numerous papers reported occurrence of specific pharmaceuticals at levels ranging from ng/L to μg/L [8,15]. Nevertheless, the vast majority of them are focused on specific therapeutic classes, paying special attention to antibiotics which are believed to be the ones of biggest concern among all pharmaceuticals due to their potential for antibiotic resistance [1,16,19]. However, multi-residue analytical methodologies, including multiple-class pharmaceuticals, are becoming the required tools to provide reliable and wider knowledge about their occurrence, as well as for the monitoring of their removal, partition and ultimate fate in the environment [9,20–24]. 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 not obtaining the best performance for each one of the compounds. However, developing a multi-group method is rewarding as it can be applied in routine analysis, providing a large amount of data.

In the literature reported, pre-concentration and isolation of target analytes from water samples is mainly based on off-line solid phase extraction (SPE). However, the majority of multi-group methods generally include more than one extraction step, using two or more different sorbent materials or different elution solvents, fractionating target compounds in groups according to their physico-chemical properties and pharmaceutical classes, which results in an overall time-consuming sample preparation [9]. Instrumental analysis is performed by gas and liquid chromatography–mass spectrometry (GC–MS) [25–29], (LC–MS) [10,24,30] and LC–MS–MS [2,11,15,20,31] being the latter the method of choice due to its versatility, specificity and selectivity, enabling the detection of target compounds in the low ng/L range. However, matrix effects are one of the major drawbacks of LC–MS–MS, especially when working in electrospray ionization mode (ESI). Matrix effect may result in the suppression or, less frequently, the enhancement of analyte signals, leading sometimes to erroneous results [32–34]. Therefore, an exhaustive study to evaluate matrix effects should be included in the method validation, in order to ensure the reliability of the results obtained.

In light of these concerns, the aim of this work was the development of a sensitive multi-residue analytical method, based on off line SPE followed by LC–ESI–MS/MS (QqQ) for the simultaneous analysis of an extended list of 29 pharmaceuticals in both surface and wastewaters. Within this list different therapeutic classes are incorporated. Target compounds were selected due to their occurrence and ubiquity in the aquatic environment [35,36], according to the information found in the literature reported, as

Table 1
Target analytes and their physico-chemical properties

| Therapeutic groups | Compounds | Log $K_{ow}$ | p$K_a$ | MW | $P_v$ (mmHg) | Molecular formula |
|---|---|---|---|---|---|---|
| Analgesic and antiinflammatories | Ketoprofen | 3.12 | 4.45 | 254.29 | 3.72E−7 | $C_{16}H_{14}O_3$ |
| | Naproxen | 3.18 | 4.15 | 230.27 | 1.892E−6 | $C_{14}H_{14}O_3$ |
| | Ibuprofen | 3.97 | 4.91 | 206.23 | 1.162E−11 | $C_{13}H_{18}O_2$ |
| | Indomethacine | 4.27 | 4.5 | 357.8 | 9.89E−11 | $C_{19}H_{16}ClNO_2$ |
| | Diclofenac | 4.51 | 4.14 | 296.16 | 6.14E−8 | $C_{14}H_{10}Cl_2NO_2$ |
| | Mefenamic acid | 5.12 | 4.2 | 241.3 | 4.636E−7 | $C_{15}H_{15}NO_2$ |
| | Acetaminophen | 0.46 | 9.38 | 151.17 | 7E−6 | $C_8H_9NO_2$ |
| | Propyphenazone | 1.94 | nd | 230.31 | 5.2E−6 | $C_{14}H_{18}N_2O$ |
| Lipid regulators and cholesterol lowering statin drugs | Clofibric acid | nd | nd | 214.5 | nd | $C_{10}H_{11}O_3Cl$ |
| | Gemfibrozil | 4.77 | nd | 250.34 | nd | $C_{15}H_{22}O_3$ |
| | Bezafibrate | 4.25 | nd | 361.82 | nd | $C_{19}H_{20}ClNO_4$ |
| | Pravastatin | 3.1 | nd | 446.00 | nd | $C_{23}H_{36}O_7$ |
| | Mevastatin | 3.95 | nd | 390.51 | 2.19E−12 | $C_{25}H_{38}O_5$ |
| Psychiatric drugs | Carbamazepine | 2.47 | 7 | 236.27 | 1.84E−7 | $C_{15}H_{12}N_2O$ |
| | Fluoxetine | 3.82 | 8.7 | 309.33 | nd | $C_{17}H_{18}F_3NO$ |
| | Paroxetine | 3.95 | nd | 329.37 | nd | $C_{19}H_{20}FNO_3$ |
| Anti-ulcer agent | Lansoprazole | 2.58 | 8.73 | 369.36 | nd | $C_{16}H_{14}F_3N_3O_2S$ |
| Histamine $H_1$ and $H_2$ receptor antagonists | Loratadine | 5.20 | nd | 382.89 | 1.56E−9 | $C_{22}H_{23}ClN_2O_2$ |
| | Famotidine | −0.64 | nd | 337.43 | 6.02E−11 | $C_8H_{15}N_7O_2S_3$ |
| | Ranitidine | 0.27 | nd | 314.41 | 1.2E−7 | $C_{13}H_{22}N_4O_3S$ |
| Antibiotics | Erythromycin | 3.06 | 8.8 | 733.93 | 2.28E−27 | $C_{37}H_{67}NO_{13}$ |
| | Azythromycin | 4.02 | 8.74 | 748.99 | 3.9E−27 | $C_{38}H_{72}N_2O_{12}$ |
| | Sulfamethoxazole | 0.89 | 6.0 | 253.28 | 6.93E−8 | $C_{10}H_{11}N_3O_3S$ |
| | Trimethoprim | 0.91 | 7.12 | 290.32 | 9.88E−9 | $C_{14}H_{18}N_4O_3$ |
| | Ofloxacin | nd | nd | 361.37 | nd | $C_{18}H_{20}FN_3O_4$ |
| β-Blockers | Atenolol | 0.16 | 9.6 | 266.34 | 2.924E−10 | $C_{14}H_{22}N_2O_3$ |
| | Sotalol | 0.24 | nd | 272.37 | 5.3E−9 | $C_{12}H_{20}N_2O_3S$ |
| | Metoprolol | 1.88 | 9.68 | 267.37 | 2.88E−7 | $C_{15}H_{25}NO_3$ |
| | Propranolol | nd | nd | 259.80 | nd | $C_{16}H_{21}NO_2$ |

well as their high human consumption worldwide [37]. Target compounds, classified by groups according to their therapeutical effect and their physico-chemical properties are listed in Table 1. The developed method consists of a single extraction step for all target compounds, simplifying considerably sample preparation. Moreover, an extensive study to evaluate the suitability of several approaches to compensate matrix effects, including standard addition, internal standard calibration and dilution of sample extracts, has been performed and included in the method validation. Analyte identification and confirmation was performed in compliance with the EU regulations (EU Commission Decision 2002/657/EC [38]). The developed method was successfully applied to the analysis of pharmaceutical residues in WWTP as well as in river water samples.

## 2. Experimental

### 2.1. Chemicals and reagents

All pharmaceutical standards used were of high purity grade (>90%). Ibuprofen, naproxen, ketoprofen, diclofenac and gemfibrozil were kindly supplied by Jescuder (Rubí, Spain). Indomethacine, acetaminophen, mefenamic acid, clofibric acid, bezafibrate, mevastatin, azythromycin dihydrate, erythromycin hydrate, carbamazepine, fluoxetine hydrochloride, lansoprazole, loratadine, famotidine, ranitidine hydrochloride, sulfamethoxazole, trimethoprim, ofloxacin, atenolol, metoprolol, propranolol hydrochloride and sotalol hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Propyphenazone, pravastatin and paroxetine hydrochloride were from LGC Promochem (London, UK). Isotopically labelled compounds, used as internal standards, were $^{13}$C-phenacetin obtained from Sigma–Aldrich, mecoprop-$d_3$ from Dr. Ehrenstorfer (Augsburg, Germany), ibuprofen-$d_3$, atenolol-$d_7$ and carbamazepine-$d_{10}$ from CDN Isotopes (Quebec, Canada).

Individual stock standard solutions were prepared on a weight basis in methanol and stored at −2 °C. A mixture of all pharmaceutical standards was prepared by appropriate dilution of individual stock solutions. Further dilutions of this mixture were prepared in methanol–water (25:75, v/v) before each analytical run and were used as working standard solutions. Stock solutions of ofloxacin, pravastatin and sulfamethoxazole were renewed monthly before their use due to their limited stability. Stock solutions of internal standards were also prepared in methanol and were stared at −2 °C. A mixture of these standards, used for internal standard calibration, was also prepared by diluting the individual stack solutions in methanol.

The cartridges used for solid phase extraction were Oasis HLB (60 mg, 3 mL and 200 mg, 6 mL) from Waters Corporation (Milford, MA, USA). Other cartridges tested were Oasis MCX (150 mg, 6 mL) also from Waters Corp., $C_{18}$ (500 mg, 3 mL) and ENV+ (200 mg, 6 mL) from Isolute (Mid Glamorgen, UK), 1 μm glass fiber and 0.45 μm nylon membrane filters were purchased from Whatman (UK).

HPLC-grade methanol, acetonitrile and water (LiChrosolv) were supplied by Merck (Darmstadt, Germany). Hydrochloric acid 37%, $NH_4Ac$ and HAc were from Merck (Darmstadt, Germany). Nitrogen for drying 99.995% of purity was from Air Liquide (Spain).

### 2.2. Sample pre-treatment and solid phase extraction optimization

The method was optimized using ground water, WWTP influent and effluent. The latter were collected from a WWTP located in Rubi (Barcelona, Spain), which receives urban, domestic and industrial wastewaters. Amber glass bottles pre-rinsed with ultra-pure water were used for sample collection. Wastewaters were vacuum filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters (Teknokroma, Barcelona, Spain). Otherwise, well waters were only filtered with 0.7 and 0.45 μm filters. 500 mL of well water, 200 of effluent and 100 mL of influent wastewaters were measured. In order to optimise the extraction method, the performances of different SPE materials were compared, testing the following cartridges: polymeric Oasis HLB (60, 3 mL), Isolute ENV+ (200 mg, 3 mL), Octadecylsilica Isolute $C_{18}$ (500 mg, 3 mL) and finally, Strong cation Exchange Oasis MCX (150 mg, 6 mL). For this purpose, water samples were spiked, prior to the extraction, with appropriate concentrations of the standard mixture of target analytes. Once the sorbent yielding the highest recoveries was selected, an evaluation if any sample pH adjustment was required, prior to extraction, was performed. For this purpose, recoveries of target analytes from samples with pH adjusted with HCl 1 M to pH 2 were compared with the ones obtained without any pH adjustment (neutral pH).

For the preconcentration step, a Baker vacuum system (J.T. Baker, The Netherlands) was used. Firstly, SPE cartridges were conditioned with 5 mL of methanol followed by 5 mL of deionized water (HPLC grade) at neutral pH (or pH 2 in the tests where water samples were acidified), at a flow rate of 1 mL/min. After the conditioning step, water samples were percolated through the cartridges at a flow rate of 10 mL/min. Afterwards the cartridge was rinsed with 5 mL of HPLC-grade water and the cartridge was then dried under vacuum for 15–20 min, to remove excess of water. Elution was performed with 2 mL × 4 mL of methanol at 1 mL/min. The extract was evaporated under a gentle nitrogen stream and reconstituted with 1 mL of methanol–water (25:75, v/v). Finally, 10 μL of a 10 ng/uL standard mixture of the internal standards mecoprop-$d_3$, ibuprofen-$d_3$, for the analysis in negative ion (NI) mode and $^{13}$C-Phenacetin, atenolol-$d_7$ and carbamazepine-$d_{10}$, for the analysis in positive ion (PI) mode, were added in the extract for internal standard calibration and to compensate possible matrix effects.

### 2.3. LC–ESI–tandem MS analysis

LC analysis was performed using a Waters 2690 HPLC system (Milford, MA, USA) coupled to a Waters Micromass Quattro triple quadrupole mass spectrometer, equipped with a Z-spray ESI interface (Manchester, UK). Chromatographic separation was achieved with a Purospher Star RP-18 endcapped column (125 mm × 2.0 mm, particle size 5 μm) and a $C_{18}$ guard column, both supplied by Merck (Darmstadt, Germany). For the

analysis in NI mode, eluent A was methanol and eluent B was water at a flow rate of 0.2 mL/min. The elution gradient started with 20% of eluent A, increasing to 80% in 20 min, raising to 90% in a 4 min gradient and then, back to initial conditions within 3 min. The column was re-equilibrated for 15 min before another injection. The analysis in PI mode was performed using as eluent A a mixture of acetonitrile–methanol (2:1) and as eluent B a buffer consisting in $NH_4Ac$ 5 mM/HAc at pH 4.7, also at a flow rate of 0.2 mL/min. The elution gradient started with 15% of eluent A, keeping isocratic conditions for three minutes. Then, eluent A increased to 95% in 22 min and was held for 7 min. Finally, initial conditions were reached again in five minutes, with a re-equilibration time of 15 min in order to restore the column. The sample injection volume was set at 10 μL.

MS parameters for the analysis were the following: ESI source block and desolvation temperature: 150 and 350 °C, respectively; capillary voltage: 2.8 kV; argon collision gas $2.5 \times 10^{-3}$ mbar; cone nitrogen and desolvation gas flow: 43 and 636 L/h. After the selection of the precursor ions for each analyte, product ions were obtained with a combination of collision energies and cone voltages, parameters that were previously optimized. Instrument control, peak detection and integration were carried out using Masslynx NT software (version 3.4). For increased sensitivity and selectivity, data acquisition was performed working in multiple reaction monitoring mode (MRM). According to the performance characteristics defined in the EU Commission Decision 2002/657/EC for the confirmation and identification of Pharmaceuticals when using LC–tandem MS as the instrumental technique, a minimum of three identification points (IP) are required. According to this LC–MS–MS (QqQ) analysis using two MRM transitions is sufficient to confirm the identity of the compound. The MRM ratio, calculated as the relation between the abundances of both transitions and the LC retention time are criteria also used to confirm the presence of an analyte in the samples. Therefore, in this study transitions between a precursor ion and the two most abundant fragment ions were chosen for each analyte when working in MRM mode, earning four identification points, enough to accomplish the EU directive aforementioned. In the cases where compounds showed poor fragmentation only one transition could be moni-

Table 2
MS/MS parameters for the analysis of target analytes by MRM negative and positive ionization mode

| Target compounds | $R_t$ window (min) | $R_t$ (min) | Precursor ion | CV–CE | MRM1 | CV–CE | MRM2 | MRM ratio |
|---|---|---|---|---|---|---|---|---|
| Compounds analyzed by NI mode | | | | | | | | |
| Clofibric acid | 0–15.5 | 11.93 | 213 $[M-H]^-$ | 20–10 | 213 > 127 | 20–15 | 213 > 85 | 4.95 |
| Naproxen | | 13.89 | 229 $[M-H]^-$ | 26–10 | 229 > 169 | 26–20 | 229 > 185 | 1.40 |
| Mecoprop-$d_3$ | | 13.91 | 217 $[M-H]^-$ | 20–20 | 217 > 145 | – | – | – |
| Ketoprofen | | 14.71 | 253 $[M-H]^-$ | 30–10 | 253 > 209 | 30–15 | 253 > 197 | – |
| Bezafibrate | 15.5–30 | 16.02 | 360 $[M-H]^-$ | 20–20 | 360 > 274 | 20–20 | 360 > 154 | 5.79 |
| Ibuprofen-$d_3$ | | 17.99 | 208 $[M-H]^-$ | 25–10 | 208 > 164 | – | – | – |
| Ibuprofen | | 18.04 | 205 $[M-H]^-$ | 20–10 | 205 > 160 | – | – | |
| Diclofenac | | 18.11 | 240 $[M-H]^-$ | 20–20 | 294 > 250 | 20–15 | 294 > 214 | 5.75 |
| Mefenamic acid | | 19.00 | 240 $[M-H]^-$ | 20–20 | 240 > 196 | 20–30 | 240 > 180 | 16.17 |
| Indomethacine | | 20.03 | 356 $[M-H]^-$ | 20–20 | 356 > 297 | 20–20 | 356 > 312 | 3.47 |
| Gemfibrozil | | 21.19 | 249 $[M-H]^-$ | 20–30 | 249 > 121 | – | – | – |
| Compounds analyzed by PI mode | | | | | | | | |
| Atenolol-$d_7$ | 0–8 | 3.06 | 274 $[M+H]^+$ | 30–20 | 274 > 190 | – | – | – |
| Atenolol | | 3.09 | 267 $[M+H]^+$ | 30–20 | 267 > 190 | 20–30 | 267 > 145 | 1.05 |
| Ranitidine | | 3.49 | 315 $[M+H]^+$ | 20–20 | 315 > 176 | 20–30 | 315 > 130 | 1.66 |
| Famotidine | | 3.58 | 338 $[M+H]^+$ | 20–20 | 338 > 189 | 20–10 | 338 > 259 | 1.64 |
| Sotalol | | 3.82 | 273 $[M+H]^+$ | 20–10 | 273 > 255 | 20–20 | 273 > 213 | 1.22 |
| Acetaminophen | | 4.34 | 152 $[M+H]^+$ | 25–15 | 152 > 110 | 30–20 | 152 > 93 | 6.44 |
| Trimethoprim | 8–16 | 9.14 | 291 $[M+H]^+$ | 30–20 | 291 > 230 | 30–30 | 291 > 261 | 1.95 |
| Metoprolol | | 14.07 | 268 $[M+H]^+$ | 25–20 | 268 > 159 | 30–30 | 268 > 133 | 1.05 |
| Sulfamethoxazole | | 14.12 | 254 $[M+H]^+$ | 20–25 | 254 > 92 | 20–15 | 254 > 156 | 1.14 |
| $^{13}$C-Phenacetin | | 15.55 | 181 $[M+H]^+$ | 20–15 | 181 > 139 | – | – | – |
| Azythromycin | | 16.14 | 749 $[M+H]^+$ | 30–30 | 749 > 591 | 30–30 | 749 > 158 | 2.12 |
| Pravastatin | 16–25 | 17.14 | 447 $[M+Na]^+$ | 30–20 | 447 > 327 | – | – | – |
| Propranolol | | 17.34 | 260 $[M+H]^+$ | 25–20 | 260 > 183 | 25–20 | 260 > 116 | 1.78 |
| Ofloxacin | | 18.20 | 362 $[M+H]^+$ | 20–15 | 362 > 316 | 20–20 | 362 > 318 | 1.50 |
| Carbamazepine-$d_{10}$ | | 18.59 | 247 $[M+H]^+$ | 20–15 | 247 > 204 | – | – | – |
| Erythromycin | | 18.62 | 734 $[M+H]^+$ | 20–20 | 734 > 576 | 20–20 | 734 > 558 | 4.96 |
| Carbamazepine | | 18.71 | 237 $[M+H]^+$ | 20–20 | 237 > 194 | 25–20 | 237 > 192 | 4.09 |
| Propyphenazone | | 19.34 | 231 $[M+H]^+$ | 20–20 | 231 > 189 | 20–25 | 231 > 201 | 3.04 |
| Lansoprazole | | 20.00 | 370 $[M+H]^+$ | 20–15 | 370 > 252 | 20–20 | 37O205 | 2.88 |
| Paroxetine | | 20.44 | 330 $[M+H]^+$ | 30–20 | 330 > 192 | 30–30 | 330 > 123 | 4.51 |
| Fluoxetine | | 21.26 | 310 $[M+H]^+$ | 25–10 | 310 > 148 | 20–10 | 310 > 44 | 2.88 |
| Mevastatin | 25–32 | 27.91 | 391 $[M+H]^+$ | 20–15 | 391 > 185 | 20–30 | 391 > 159 | 1.18 |
| Loratadine | | 27.94 | 383 $[M+H]^+$ | 30–20 | 383 > 337 | 30–30 | 383 > 259 | 3.07 |

CV–CE: Cone volatge and collision energy.

tored. However, it only happened for three compounds, and their confirmation in the samples was performed matching their LC retention time with the ones obtained in the standards. Shifts in retention time were less than 3%, so the confirmation was considered accurate enough. For internal standards, only one transition was selected, as they are isotopically labelled compounds which are not likely to be found in environmental samples. In order to increase sensitivity, MRM transitions were classified in different elution time window. In almost each window one internal standard was included. MRM transitions, the optimum collision energies and cone voltages selected for each transition are indicated in Table 2. The first transition corresponds to the most abundant and was used for quantification and the second one for confirmation purposes.

### 2.4. Method validation

Extraction recoveries of target compounds were determined for ground water, WWTP effluent and influent using samples spiked at two concentration levels (50 ng/L and 1 μg/L for ground water, 100 ng/L and 1 μg/L for WWTP effluent and 1 and 10 μg/L for WWTP influent). For each matrix, recoveries were determined comparing the concentrations obtained, calculated by internal standard calibration, with the initial spiking levels. In each case, samples were analyzed in triplicate. As both surface and wastewaters spiked contained target compounds, blanks (no-spiked samples) were analysed in order to determine their concentrations, which were afterwards subtracted to the spiked waters. Matrix effects and the efficiency of internal standard calibration against standard calibration and sample extract dilutions were evaluated for each compound, and the results obtained are discussed in a specific section below.

The precision of the method was determined by repeated intra-day and inter-day analysis (five successive injection of a standard solution in 1 day and in five successive days, respectively), expressing it as the relative standard deviation (R.S.D.) of these replicate measurements. Method detection limits (MDL) and method quantification limits (MQL) were determined from spiked water samples, as the minimum detectable amount of analyte with a signal-to-noise ratio of 3 and 10, respectively. The instrumental detection limits (IDL) were estimated from the injection of a standard solution successively diluted until reaching a concentration level corresponding to a signal-to-noise ratio of 3.

## 3. Results and discussion

### 3.1. Sample pre-treatment and solid phase extraction

The performance of different solid phase extraction materials was tested, including two polymeric sorbents (Lichrolut ENV+ and Oasis HLB), a mixed polymeric and cation exchange sorbent (Oasis MCX) and a non polar one ($C_{18}$). In Fig. 1 the performance of the materials tested is summarized. Only the results obtained for the most representative compounds of each therapeutic group in surface waters are illustrated. The analytes not shown, belonging to the same medicinal classes, as well as in other matrices (effluent and influent wastewaters) followed a similar pattern. As it can be observed, quite good recoveries were obtained for acidic compounds when using Oasis MCX, whereas basic and neutral compounds, except carbamazepine, were poorly recovered. This could be attributed to the fact that Oasis MCX is a mixed reversed phase-cation exchange cartridge which, at low pH values, it can efficiently extract acidic, basic and neutral compounds, since the cation exchanger binds the basic compounds and the reversed phase can retain both acidic and neutral ones. As our experiment was performed at neutral pH and analytes were subsequently eluted with pure methanol, only acidic and neutral compounds were recovered. In order to extract efficiently basic analytes, samples should have been adjusted at low pH values and eluted afterwards with methanol–ammonia.

Lichrolut ENV+ was only effective for few compounds. Generally, this cartridge is recommended for the extraction of polar

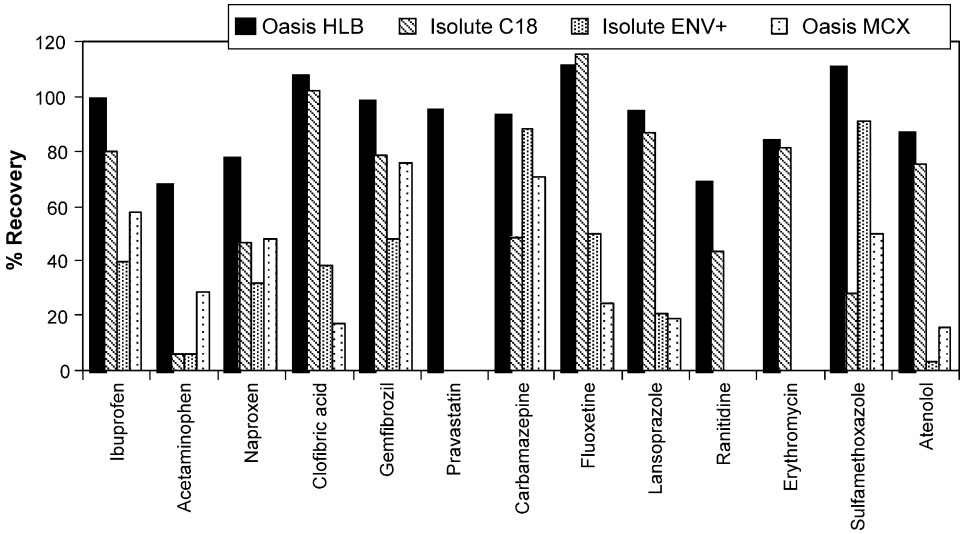


Fig. 1. Recoveries obtained for the extraction of selected Pharmaceuticals in 500 mL of river water, spiked at 1 μg/L without sample pH adjustment using different SPE materials.

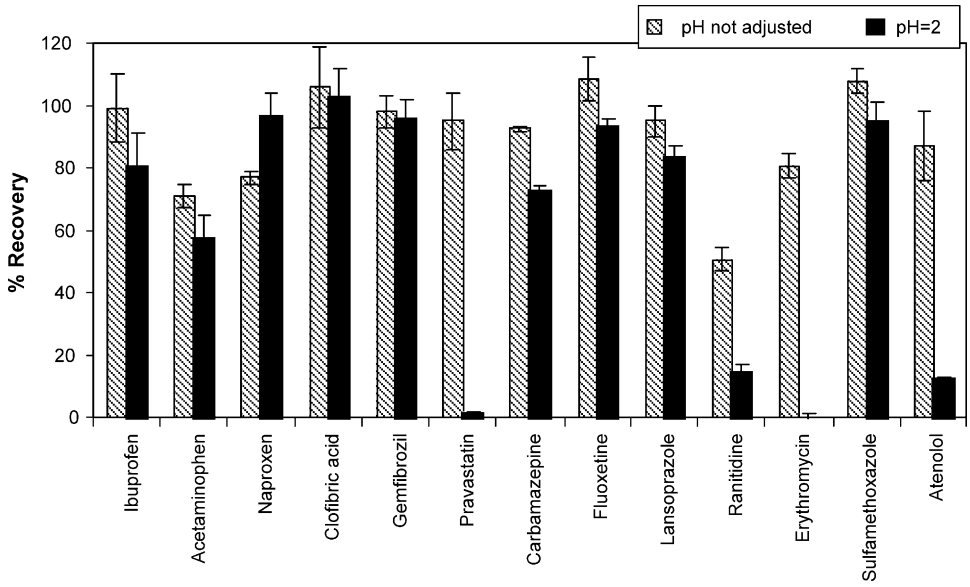


Fig. 2. Influence of pH adjustment, (pH 2 and no pH adjustment), on the recoveries obtained using Oasis HLB SPE cartridges (matrix: river water and spiking level 1 μg/L).

Table 3
Recoveries obtained for target analytes in surface and wastewaters at the two spiking levels tested for each type of water

| Therapeutic group | Compound | Recoveries at high spiking level (1 and 10 μg/L for river, effluent and influent wastewater) (R.S.D.%) | | | Recoveries at low spiking level (50,100 ng/L 1 μg/L for river, effluent and influent wastewaters) (R.S.D.%) | | |
|---|---|---|---|---|---|---|---|
| | | Surface waters | WWTP effluent | WWTP influent | Surface waters | WWTP effluent | WWTP influent |
| Analgesics and anti-inflammatories | Ketoprofen | 51 (8) | 61 (2) | 89 (7) | 121 (9) | 53 (12) | 52 (4) |
| | Naproxen | 77 (2) | 51 (1) | 60 (1) | 73 (6) | 81 (9) | 34 (5) |
| | Ibuprofen | 99 (11) | 90 (8) | 63 (9) | 70 (12) | 87 (7) | 111 (9) |
| | Indomethacine | 64 (2) | 60 (4) | 79 (2) | 50 (12) | 50 (13) | 51 (5) |
| | Diclofenac | 102 (3) | 78 (2) | 80 (2) | 81 (12) | 60 (3) | 89 (4) |
| | Mefenamic acid | nd | nd | 90 (3) | 93 (5) | 65 (4) | nd |
| | Acetaminophen | 71 (5) | 45 (1) | 48 (3) | 60 (5) | 50 (3) | 47 (1) |
| | Propyphenazone | nd | nd | 71 (4) | 60 (8) | 60 (15) | nd |
| Lipid regulators and cholesterol lowering statin drugs | Clofibric acid | 106 (13) | 32 (4) | 62 (11) | 79 (5) | 30 (12) | 56 (4) |
| | Gemfibrozil | 98 (5) | 61 (2) | 88 (3) | 80 (4) | 71 (11) | 80 (9) |
| | Bezafibrate | 78 (15) | 88 (13) | 82 (4) | 62 (2) | 107 (3) | 79 (5) |
| | Pravastatin | 95 (10) | 93 (4) | 85 (6) | 80 (8) | 70 (5) | 113 (8) |
| | Mevastatin | 60 (9) | 34 (3) | 60 (11) | 60 (5) | 50 (11) | 34 (2) |
| Psychiatric drugs | Carbamazepine | 93 (1) | 97 (5) | 98 (3) | 67 (6) | 93 (12) | 105 (10) |
| | Fluoxetine | 105 (6) | 60 (2) | 108 (4) | 74 (12) | 74 (2) | 67 (12) |
| | Paroxetine | 95 (5) | 65 (3) | 96 (1) | 110 (12) | 76 (12) | 84 (4) |
| Antiulcer agent | Lansoprazole | 75 (13) | 86 (4) | 77 (5) | 80 (7) | 75 (4) | 87 (5) |
| Histamine $H_1$ and $H_2$ receptor antagonists | Loratadine | 72 (9) | 86 (12) | 115 (3) | 76 (8) | 93 (12) | 91 (3) |
| | Famotidine | 50 (5) | 61 (4) | 50 (4) | 40 (7) | 104 (5) | 50 (3) |
| | Ranitidine | 51 (8) | 109 (12) | 88 (4) | 94 (6) | 86 (11) | 38 (1) |
| Antibiotics | Erythromycin | 81 (5) | 116 (4) | 48 (3) | 70 (4) | 50 (13) | 98 (9) |
| | Azythromycin | 104 (7) | 93 (3) | 40 (5) | 30 (7) | 30 (15) | 63 (2) |
| | Sulfamethoxazole | 108 (4) | 50 (5) | 80 (4) | 90 (6) | 50 (3) | 101 (3) |
| | Trimethoprim | 96 (9) | 88 (7) | 111 (1) | 93 (6) | 83 (15) | 106 (2) |
| | Ofloxacin | 93 (3) | 106 (2) | 95 (4) | 80 (5) | 75 (3) | 106 (3) |
| β-Blockers | Atenolol | 87 (12) | 92 (5) | 82 (5) | 96 (6) | 96 (5) | 97 (2) |
| | Sotalol | 50 (6) | 43 (3) | 111 (5) | 115 (11) | 71 (7) | 56 (5) |
| | Metoprolol | 60 (8) | 103 (5) | 104 (4) | 103 (5) | 114 (6) | 86 (12) |
| | Propranolol | 102 (3) | 85 (4) | 55 (1) | 81 (11) | 44 (4) | 70 (6) |

Nd: not calculated.

organic compounds at low pH values, but it can also retain neutral drugs at pH 7, such as carbamazepine and macrolide antibiotics, through hydrophobic interactions.

$C_{18}$ provided good results for the majority of the compounds. However, comparing to Oasis HLB, the latter was much more efficient, yielding high recoveries for all target compounds. This sorbent, with the combination of the hydrophilic–lipophilic polymer, can extract acidic, neutral and basic analytes at a wide range of pHs, including neutral pH.

In the next step, the effect of prior acidification of the sample is evaluated. Fig. 2, shows the recoveries obtained using Oasis HLB with and without pH adjustment. Basic and neutral pharmaceuticals yielded significantly higher recoveries without acidifying the sample. However, for acidic compounds results were quite similar, except for clofibric acid, ketoprofen, naproxen and mevastatin. As the aim of this work was to extract all target analytes in one single step and recoveries obtained were good without sample acidification, this protocol was selected as the optimum one for further experiments. Recoveries (mean of three replicates ± R.S.D.) are given in Table 3 and discussed in the method validation section.

### 3.2. LC–ESI–tandem MS analysis

To optimize the chromatographic separation, a serial of preliminary experiments were performed, testing different mobile phases consisting of methanol, acetonitrile or mixture of acetonitrile and methanol as an organic phase and water with different mobile phase additives, such as ammonium acetate and acetic acid at various concentrations. The optimal separation of 22 compounds detected in PI mode was achieved using 5 mM aqueous $NH_4Ac$/HAc (pH 4.8) and a mixture of acetonitrile/methanol (2:1, v/v). For the 10 compounds detected in NI mode, the best separation was obtained using methanol–water. Representative chromatograms of a 1 μg/mL standard mixture of the compounds analyzed in NI and PI mode and the internal standards fitted in each retention time window are illustrated in Fig. 3.

The optimization of MS parameters (cone voltage and collision energy) was performed by flow injection analysis (FIA) for each compound. The analgesics and anti-inflammatories ibuprofen, diclofenac, mefenamic acid, ketoprofen, naproxen, indomethacine and the lipid regulators gemfibrozil, bezafibrate and clofibric acid were more sensitive in NI mode, whereas the analysis of the rest of compounds was carried out in PI mode.

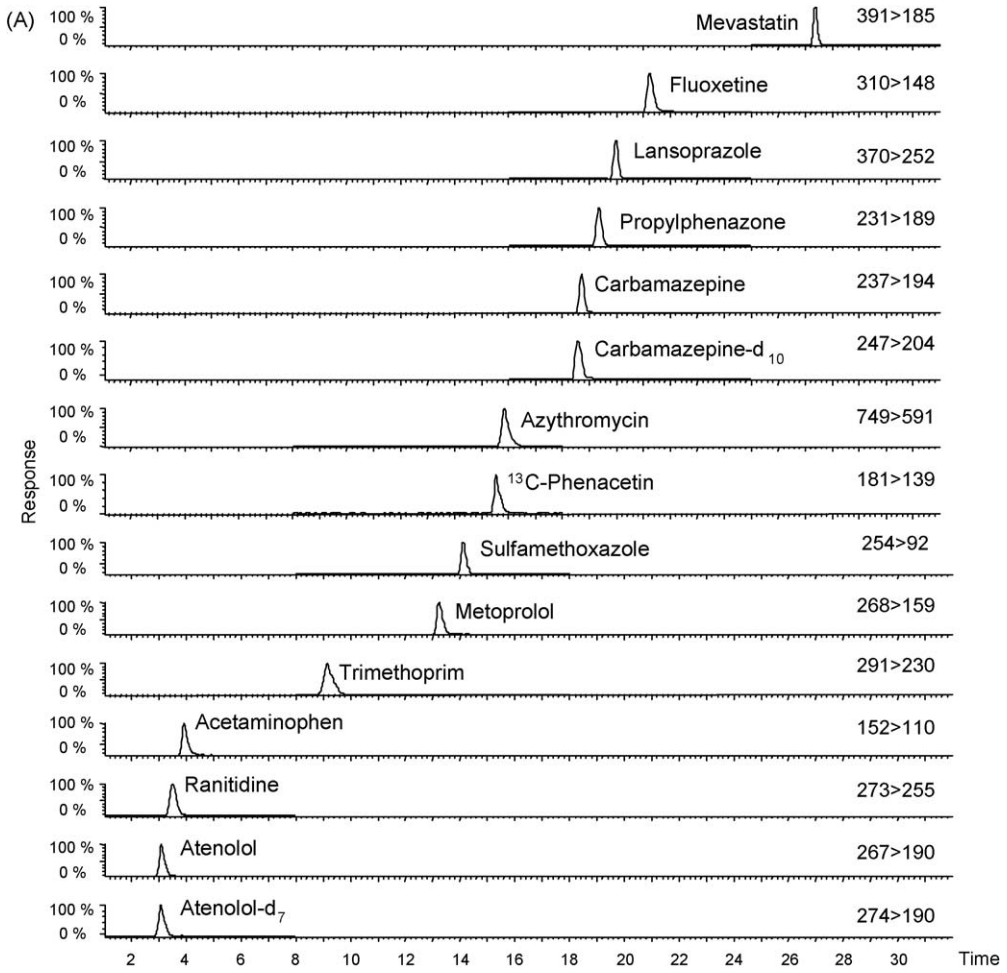


Fig. 3. Example of a MRM chromatogram of a standard mixture at 1 ng/μL for target compounds analyzed by (A) positive ionization mode and (B) negative ionization mode.

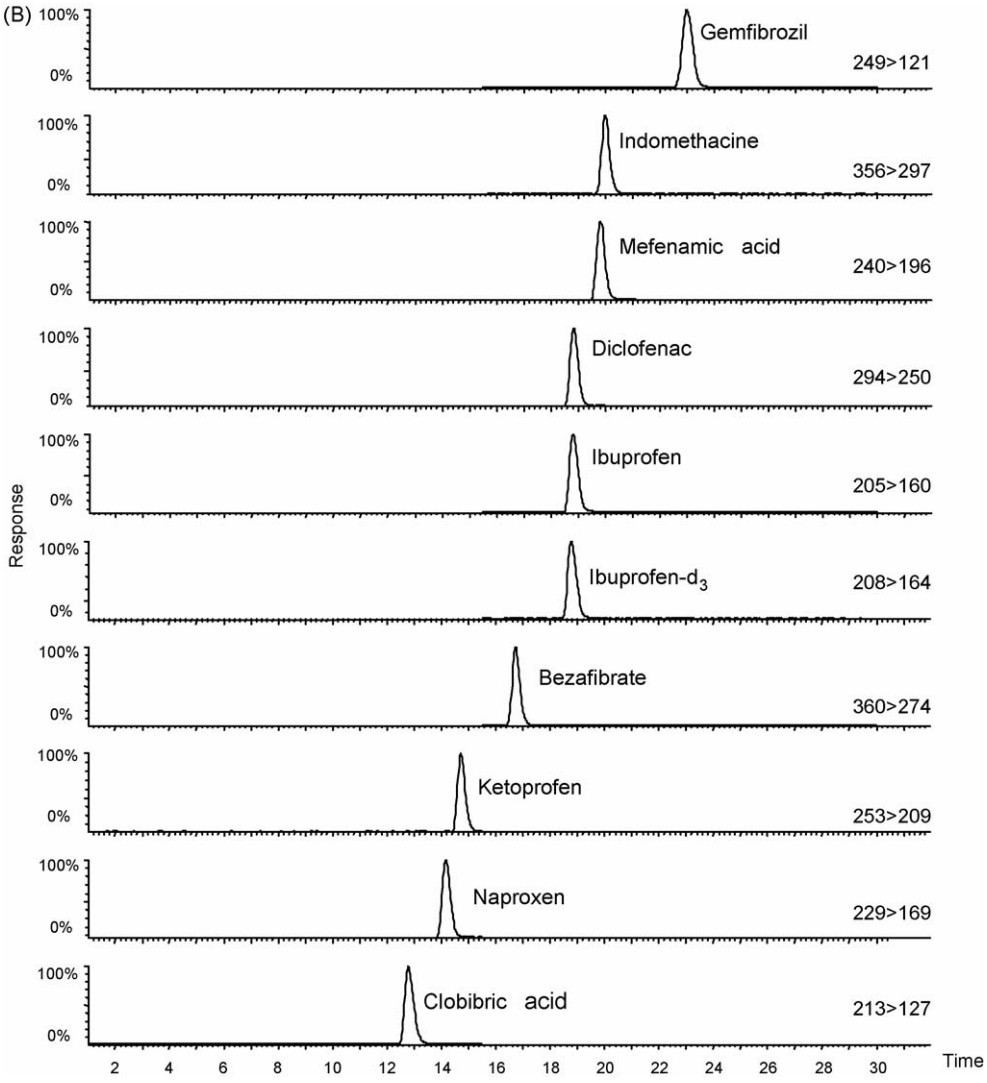


Fig. 3 (*Continued*).

In order to select precursor ions for each analyte, chromatograms were recorded in full scan mode. In all cases, $[M-H]^-$ for NI mode, and $[M+H]^+$ for PI mode were selected, with only exception being pravastatin which formed the sodium adduct. Once precursors were selected, product scans were recorded, testing different values of cone voltage and collision energies. Cone voltages were selected according to the sensitivity of precursor ions, whereas collision energies were chosen to give the maximum intensity of the fragment ions obtained. Product ions observed for each target compounds are indicated in Table 2 and were in good agreement with the ones reported in the literature [2,15,20].

### 3.3. Method validation

Sensitivity, linearity, recoveries, precision and the study of matrix effects were considered as the criteria for the validation of the analytical methodology developed. Validation data, determined for each matrix studied, are presented in Tables 3 and 4, Calibration curves were generated using linear regression analysis and over the established concentration range (0.01–1 μg/mL) gave good fits ($r^2 > 0.99$). Five-point calibration was performed daily, and the possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each 8–10 injections.

Recoveries achieved for all target compounds ranged from 60 to 102% and from 50 to 116% for surface and wastewaters, respectively. Only ranitidine, sotalol and famotidine, as well as mevastatin and clofibric acid, in the case of wastewater samples, showed lower recovery rates (50 and 35%, respectively). Nevertheless, as other performance data, such as repeatability and sensitivity, were good, the low recovery was not considered to be an obstacle for their reliable determination.

Detection limits obtained are reported in Table 4. MDL ranged from 1 to 30 ng/L and from 1 to 60 ng/L for surface and wastewaters, respectively. MQL were from 3 to 160 ng/L for both matrices. To ensure correct quantification, precision of the method was studied by analysing five replicates of a 100 μg/L standard. Results are indicated in Table 4 for each compound, showing a precision from 0.2 to 6% and

Table 4
Linearity (regression coefficient), instrumental detection limits (IDL), detection and quantification limits for the method (MDL, MQL), repeatability and reproducibility of the LC–MS/MS method without SPE

| Therapeutic group | Compounds | Linearity ($r^2$) | IDL (pg injected) | MDL (ng/L) | | | MQL (ng/L) | | | Repeatability % R.S.D. ($n=5$) | Reproducibility % R.S.D. ($n=5$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Surface waters | WWTP effluent | WWTP influent | Surface waters | WWTP effluent | WWTP influent | | |
| Analgesics and anti-inflammatories | Ketoprofen | 0.9985 | 60 | 30 | 21 | 28 | 70 | 73 | 95 | 1 | 6 |
| | Naproxen | 0.9924 | 30 | 7 | 9 | 9 | 20 | 32 | 32 | 1 | 5 |
| | Ibuprofen | 0.9989 | 60 | 8 | 12 | 12 | 42 | 20 | 20 | 3 | 9 |
| | Indomethacine | 0.9986 | 60 | 6 | 7 | 7 | 20 | 20 | 20 | 1 | 7 |
| | Diclofenac | 0.9997 | 42 | 2 | 10 | 10 | 5 | 30 | 30 | 1 | 3 |
| | Mefenamic acid | 0.9997 | 2 | 0.5 | 1 | 3 | 2 | 20 | 26 | 0.5 | 3 |
| | Acetamino phen | 0.9997 | 29 | 17 | 10 | 10 | 40 | 58 | 58 | 3 | 3 |
| | Propyphenazone | 0.9999 | 11 | 3 | 10 | 7 | 23 | 9 | 30 | 1 | 1 |
| Lipid regulators and cholesterol lowering statin drugs | Clofibric acid | 0.9996 | 6 | 1 | 2 | 2 | 3 | 6 | 6 | 1 | 5 |
| | Gemfibrozil | 0.9996 | 21 | 1 | 1 | 3 | 3 | 3 | 9 | 2 | 11 |
| | Bezafibrate | 0.9998 | 5 | 1 | 2 | 9 | 4 | 6 | 31 | 5 | 7 |
| | Pravastatin | 0.9994 | 200 | 47 | 60 | 60 | 160 | 160 | 160 | 1 | 1 |
| | Mevastatin | 0.9938 | 11 | 7 | 20 | 18 | 24 | 70 | 60 | 1 | 3 |
| Psychiatric drugs | Carbamazepine | 0.9999 | 6 | 2 | 10 | 18 | 10 | 40 | 61 | 4 | 6 |
| | Fluoxetine | 0.9959 | 39 | 20 | 20 | 35 | 66 | 70 | 100 | 2 | 2 |
| | Paroxetine | 0.998 | 18 | 8 | 7 | 6 | 20 | 26 | 22 | 3 | 5 |
| Antiulcer agent | Lansoprazole | 0.9999 | 62 | 5 | 8 | 14 | 16 | 20 | 47 | 4 | 5 |
| Histamine $H_1$ and $H_2$ receptor antagonists | Loratadine | 0.9965 | 18 | 2 | 3 | 4 | 7 | 10 | 11 | 0.2 | 4 |
| | Famotidine | 0.9974 | 12 | 5 | 10 | 12 | 18 | 20 | 26 | 1 | 4 |
| | Ranitidine | 0.9967 | 23 | 2 | 20 | 24 | 5 | 70 | 80 | 1 | 8 |
| Antibiotics | Erythromycin | 0.9946 | 50 | 4 | 6 | 6 | 14 | 20 | 20 | 2 | 6 |
| | Azythromycin | O.9989 | 27 | 1 | 3 | 7 | 3 | 10 | 23 | 1 | 1 |
| | Sulfamethoxazole | 0.9976 | 27 | 5 | 20 | 42 | 16 | 60 | 120 | 2 | 9 |
| | Trimethoprim | 0.9962 | 13 | 1 | 10 | 25 | 4 | 40 | 82 | 3 | 4 |
| | Ofloxacin | 0.999 | 52 | 16 | 43 | 43 | 56 | 110 | 110 | 1 | 5 |
| β-Blockers | Atenolol | 0.9954 | 10 | 9 | 10 | 42 | 30 | 40 | 141 | 1 | 6 |
| | Sotalol | 0.9975 | 10 | 18 | 60 | 29 | 60 | 100 | 97 | 4 | 9 |
| | Metoprolol | 0.9966 | 34 | 3 | 10 | 12 | 12 | 30 | 36 | 6 | 2 |
| | Propranolol | 0.9986 | 16 | 2 | 7 | 12 | 7 | 20 | 40 | 1 | 8 |

from 1 to 11% from intra- and inter-day analysis, respectively.

### 3.3.1. Matrix effects

One significant drawback in ESI MS quantitative analysis is what is known as matrix effect. It occurs because the ESI source is highly susceptible to other components present in the matrix, which may result in a signal suppression or enhancement leading to erroneous results. There are several strategies to reduce matrix effect, e.g. selective extraction, effective sample clean-up after the extraction, or improvement of the chromatographic separation. Sometimes, these approaches are not the appropriate solutions because they could lead to analyte losses as well as long analysis times [39]. Other plausible and effective strategies, reported in the literature [30,33,40], consist in the use of suitable calibration approaches, such as external calibration using matrix-matched samples, standard addition or internal standard, as well as the dilution of sample extracts. Standard addition is a reliable method, but it is time-consuming. On the other hand, appropriate internal standard (structurally similar unlabeled compound or isotopically labelled standard) are not always commercially available or they are expensive. Therefore, in case of lack of matrix-matched materials and isotopically labelled standards, sample extract dilution is an effective alternative solution. In this work the efficiencies of these three strategies have been extensively studied for each analyte in WWTP effluent and influent samples, in order to select the best approach for quantitation purposes.

In order to evaluate the degree of ion suppression and in what extent the target compounds were sensitive to it calibration curves were prepared using WWTP effluent and influent extracts. Blanks (samples with no addition of the standards) were analyzed simultaneously in order to subtract the levels of target compounds present in the samples. The calibration curve obtained was compared to the one achieved for the same standards in methanol–water (25:75). When both curves are parallel and totally overlapped, compounds are not subjected to ion suppression. Fig. 4 shows an example of this approach, giving calibration curves for ketoprofen and ranitidine, analyzed by NI and PI mode, respectively. The curve above corresponds to the signals of the analyte in the solvent, whereas the second one represents the signals in a spiked influent extract. As it can be observed, curves obtained in a spiked influent extracts have lower slope, which indicates that compounds are susceptible to signal suppression. Signal suppression measured for compounds analyzed under NI conditions (analgesics and anti-inflammatories and some lipid regulators) ranged between 15 and 50% for effluent samples and from 40 to 60% for influent wastewaters, respectively. For the compounds analyzed under PI conditions, ion suppression ranged from 40 to 60% for effluent wastewaters, and over 60% for influent samples, reaching for some compounds values of 80% (ranitidine) and 90% (atenolol).

In order to evaluate the efficiency of the internal standards used in this work for the correction of these signal losses, internal calibration curves in methanol–water (25:75, v/v) and in spiked matrix extracts were compared. The overlapping of both curves means that signal losses experienced by the analytes are corrected by the internal standards. On the other hand, when internal standards are not able to compensate signal losses for a particular compound, curves are not parallel, showing a difference in slopes. Fig. 5 shows an example for clofibric acid, atenolol and carbamazepine. Internal standards used were mecoprop-$d_3$ (for clofibric acid), atenolol-$d_7$ and carbamazepine-$d_{10}$, respectively. It is clearly depicted that internal standards corrected in a great extent signal losses due to ion suppression. Similar results were observed for the rest of the compounds, except for diclofenac, indomethacine, mefenamic acid, azythromycin, metoprolol, erythromycin and ofloxacin, where this correction was not so successful. When signal losses are successfully corrected by internal standards, the difference of slopes between both curves is reduced to a value close to 1, as they are overlapped. Even though signal suppression was considerably reduced for these substances, a difference ranging from 27 to 60% was still observed, which means that the internal standard used does not successfully correct signal losses. These results justify the use of internal standards, since they reduce and efficiently compensate signal losses.

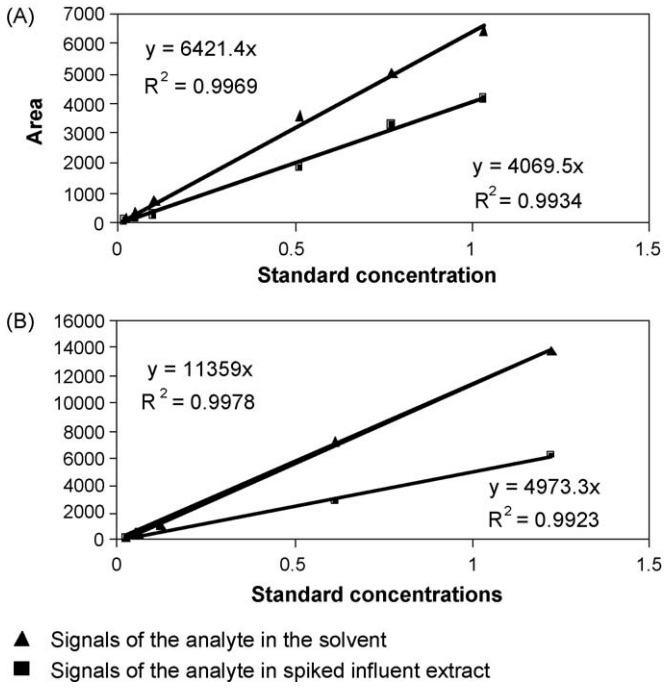


Fig. 4. Calibration curves in solvent vs. those prepared in spiked influent extracts for (A) ketoprofen and (B) ranitidine.

In addition, the efficiency of sample extract dilutions was studied. For this purpose, the signals obtained after sequential dilution of a WWTP effluent and influent extract, (1:2, 1:4 and 1:8), were compared with the ones obtained for the corresponding concentrations of the standards in solvent. It is considered that matrix effects have been corrected when both signals are equal. An example is illustrated in Fig. 6 for bezafibrate and atenolol. For effluent wastewaters, dilution 1:2 was enough to avoid the signal decrease for the compounds analyzed by both NI and PI mode, whereas for influent wastewaters, dilution 1:4 was required to solve this problem. However, for WWTP influents, dilution 1:4 could lead to a considerable decrease in sensitiv-

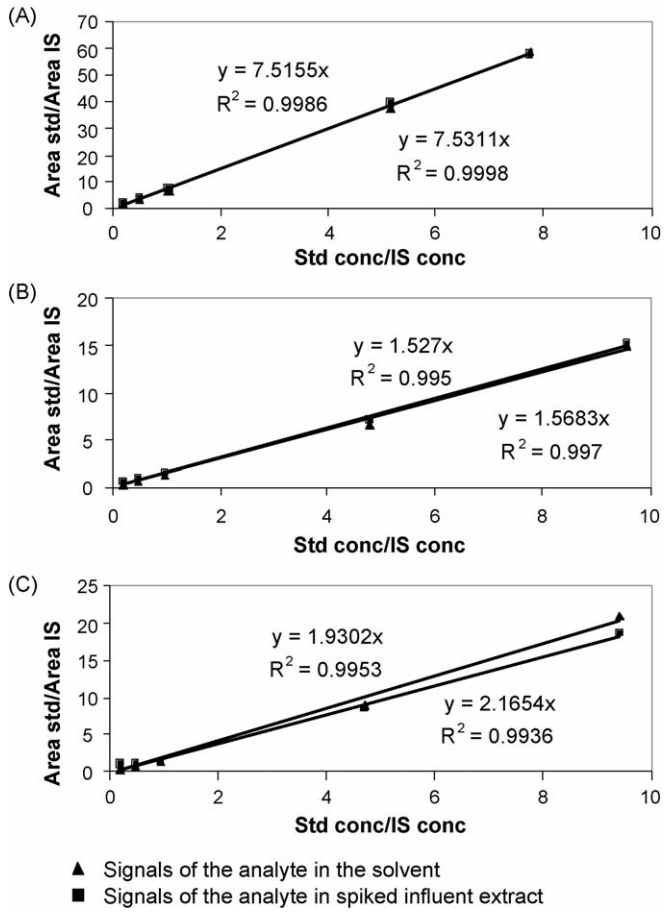


Fig. 5. Internal standard calibration curves in solvent and in spiked influent extracts for (A) clofibric acid (IS – mecoprop-$d_3$), (B) atenolol (IS – atenolol-$d_7$) and (C) carbamazepine (IS – carbamazepine-$d_{10}$).

ity, which is an important drawback that should be taken into account. According to these results, internal standard calibration is as efficient approach to assess the loss of signal intensity and therefore, can be used for quantitation purposes, with the advantage that it is simpler and not so time-consuming as matrix-matched calibration or standard addition.

Only for the compounds that internal standards are not able to compensate ion suppression, the other strategies should be used for quantitation. In our study the approach selected was the dilution of sample extracts.

### 3.4. Analysis of real surface and wastewater samples

To demonstrate the applicability of the developed method, 10 river waters from the Ebro river basin in Spain, and samples from 5 WWTP (influent and effluent) in Croatia were analyzed. Results obtained are summarized in Table 5. Anti-inflammatories and analgesics, lipid regulators, β-blockers and some antibiotics are the major groups detected in WWTP and among them acetaminophen, ketoprofen, ibuprofen, diclofenac, mevastatin, atenolol, propranolol, sulfamethoxazole and trimetroprim were the most abundant, with concentrations in high ng/L or low μg/L levels. Maximum concentrations were detected for acetaminophen (paracetamol), with average concentration of 10 μg/L in WWTP influent and 2.1 μg/L in effluent and for antimicrobial trimethoprim (1.1 and 0.29 μg/L in WWTP influents and effluents, respectively). Other compounds frequently detected in WWTP samples were carbamazepine and ranitidine, with average concentrations of 400 ng/L for carbamezapine in both influent and effluent samples (no elimination) and 188 and 135 ng/L for ranitidine in influent and effluent, respectively. In general, levels detected in Croatian WWTP are similar to those previously reported for WWTP in Spain, Germany, Italy and USA [7,10,20,24,25]. In the Ebro

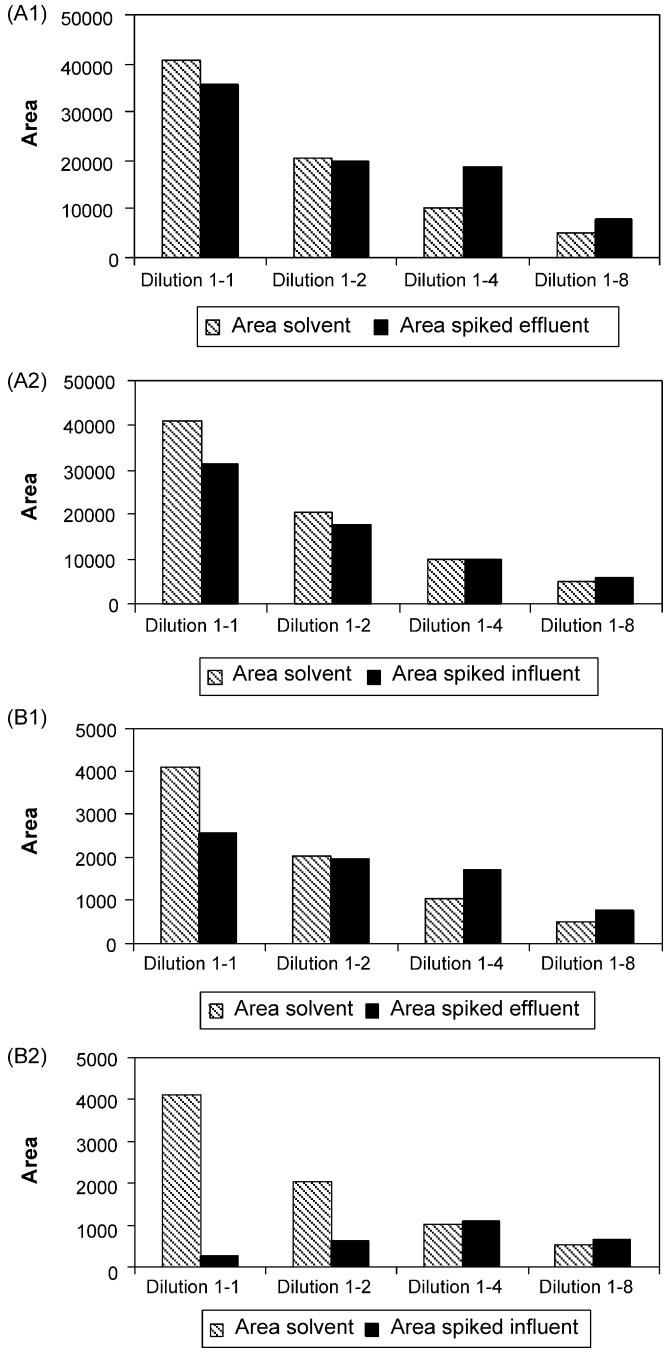


Fig. 6. Sample extract dilutions for (A1) bezafibrate in WWTP effluent and (A2) bezafibrate in WWTP influent; (B1) atenolol in WWTP effluent and (B2) atenolol in WWTP influent.

Table 5
Range of concentrations and average level (expressed in brackets) detected for target Pharmaceuticals in influent and effluent wastewaters from five Croatian urban WWTP and in 10 points from the Ebro river basin

| Therapeutic groups | Target compounds | Minimum–maximum concentrations (ng/L) | | |
|---|---|---|---|---|
| | | Surface waters | Effluent wastewaters | Influent wastewaters |
| Analgesics and anti-inflammatories | Ketoprofen | bld | 130–620 (318) | 160–970 (451) |
| | Naproxen | bld–50 (33) | bld–160 (108) | blq–190 (99) |
| | Ibuprofen | bld–150 (60) | 40–800 (266) | nd-900 (516) |
| | Indomethacine | bld–10 | bld | bld |
| | Diclofenac | bld–60 (29) | bld–390 (215) | 50–540 (250) |
| | Mefenamic acid | bld–3 (2) | bld–10 (7) | bld–5 (5) |
| | Acetaminophen | bld–250 (42) | blq–5990 (2102) | 130–26090 (10194) |
| | Propyphenazone | bld | bld | bld |
| Lipid regulators and cholesterol lowering statin drugs | Clofibric acid | 10–20 (11) | 20–30 (28) | bld–110 (72) |
| | Gemfibrozil | bld–60 (46) | bld–320 (120) | bld–360 (155) |
| | Bezafibrate | bld–10 (8) | bld–10 | bld–50 (23) |
| | Pravastatin | nd | nd | nd |
| | Mevastatin | bld | bld–800 (383) | bld–1170 |
| Psychiatric drugs | Carbamazepine | bld–110 (30) | bld–630 (410) | bld–950 (420) |
| | Fluoxetine | bld | bld | bld |
| | Paroxetine | bld | bld | bld |
| Antiulcer agent | Lansoprazole | bld | bld | bld |
| Histamine $H_1$ and $H_2$ receptor antagonists | Loratadine | bld–20 | bld | bld |
| | Famotidine | bld | bld | bld |
| | Ranitidine | bld–10 | bld–200 (135) | bld–290 (188) |
| Antibiotics | Erythromycin | bld–30 (17) | bld | bld |
| | Azythromycin | bld–20 (8) | 50–210 (96) | blq–300 (152) |
| | Sulfamethoxazole | bld–<blq | blq–820 (390) | bld–870 (590) |
| | Trimethoprim | bld–20 (11) | 70–310 (290) | bld–4220 (1172) |
| | Ofloxacin | bld | bld | bld |
| β-Blockers | Atenolol | bld–250 (72) | bld–1150 (400) | bld–740 (395) |
| | Sotalol | bld–70 | bld–210 (185) | 120–200 (167) |
| | Metoprolol | bld | bld | bld |
| | Propranolol | bld | 100–470 (290) | 80–290 (168) |

bld: below limit of detection; blq: below limit of quantification.

river water concentration of compounds positively identified rarely exceeded 100 ng/L, with average concentrations (for 10 samples collected along the river) in the low ng/L range.

## 4. Conclusions

The multi-residue analytical method developed, based on SPE–LC–MS/MS allowed the simultaneous extraction of 29 multiple-class Pharmaceuticals in a single extraction step, simplifying considerably sample preparation. Recoveries obtained for all target compounds, using Oasis HLB cartridges were higher than 60%, except for ranitidine, sotalol, famotidine (50%) and mevastatin (34%). The application of LC–MS/MS operating in the MRM mode, with two transitions (if available) monitored for each compound, provided good sensitivity and selectivity of detection according to the EU Commission Decision 2002/657/EC requiring a minimum of 3 IP for the confirmation of Pharmaceuticals in environmental samples. Only for ketoprofen, iburopfen, gemfibrozil and pravastatin the numer of IP required were not achieved due to their low fragmentation. The method yielded detection limits in the low ng/L range for both river and wastewater thus providing a reliable and robust tool that can be used for routine analysis of multi-class Pharmaceuticals in aqueous samples.

## Acknowledgements

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