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Determination of drugs in surface water and wastewater samples by liquid chromatography–mass spectrometry: methods and preliminary results including toxicity studies with *Vibrio fischeri*

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Abstract

In the present work a combined analytical method involving toxicity and liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) was developed for the determination of pharmaceutical compounds in water samples. The drugs investigated were the analgesics: ibuprofen, ketoprofen, naproxen, and diclofenac, the decomposition product of the acetyl salicylic acid: salicylic acid and one lipid lowering agent, gemfibrozil. The selected compounds are acidic substances, very polar and all of them are analgesic compounds that can be purchased without medical prescription. The developed protocol consisted, first of all, on the use Microtox® and ToxAlert®100 toxicity tests with *Vibrio fischeri* for the different pharmaceutical drugs. The 50% effective concentration (EC_{50}) values and the toxicity units (TU) were determined for every compound using both systems. Sample enrichment of water samples was achieved by solid-phase extraction procedure (SPE), using the Merck LiChrolut® EN cartridges followed by LC–ESI-MS. Average recoveries loading 1 l of samples with pH=2 varied from 69 to 91% and the detection limits in the range of 15–56 ng/l. The developed method was applied to real samples from wastewater and surface-river waters of Catalonia (north-east of Spain). One batch of samples was analyzed in parallel also by High Resolution Gas Chromatography coupled with Mass Spectrometry (HRGC–MS) and the results have been compared with the LC–ESI-MS method developed in this work.

Keywords: *Vibrio fischeri*; Water analysis; Toxicity; Drugs; Ibuprofen; Ketoprofen; Naproxen; Diclofenac; Salicylic acid; Gemfibrozil

1. Introduction

The growing use of pharmaceutical products is becoming a new environmental problem. For several years the use of pharmaceutical products are growing, the U.S. pharmaceutical industry for example, has been growing twice as fast as the rest of the U.S. economy, and it shows no signs of slowing down. This fact is fueled by several factors as the baby-boomer population in the U.S. and Europe into older age, a life phase in which people consume four times the healthcare resources of the general population. In Germany for instance, up to 100 t of individual drugs are prescribed every year [1]. However this amount

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underestimates the total usage of all pharmaceutical compounds, many of which can be purchased without prescription, as happens with a wide number of anti-inflammatory, lipid regulator and analgesic drugs. Due to this high distribution, relative concentrations in function of the usage, half life and metabolism, these compounds and their metabolites are found in sewage.

High concentration of pharmaceutical products reach wastewater treatment plants (WWTP), via human urinary or fecal excretion and from pharmaceutical manufacturing discharges. WWTP influent constituents have to face a complex mixture of various organic and inorganic substances and, detailed information on potential wastewater composition are often scarce. Pharmaceutical compounds are not totally eliminated in the WWTP and due to this fact variable concentrations of pharmaceutical drugs can reach surface and groundwater samples.

During the last few years, the interest to assess the presence of pharmaceutical compounds in the environment has been growing (see Refs. [2] or [3,4]). Anti-inflammatory drugs, analgesic products and their metabolites were detected in wastewater [5–7], surface water [8,9] and groundwater [3].

However, in any environmental study, there is the need to assess the toxic effects of new chemicals found, like pharmaceuticals. In the present work, the toxic effect of Ketoprofen, Naproxen, Ibuprofen, Diclofenac–Na, Gemfibrozil and Salicylic acid were evaluated by two methods based on the bioluminescence inhibition of *Vibrio fischerii*: ToxAlert®100 developed by Merck and Microtox® by Azur Environmental. These toxicity tests offer an ease of operation, reproducibility, sensitivity, low cost and standardization [10–12].

The analytical method developed involves a solid-phase extraction (SPE) step, followed by LC–ESI-MS. Up to now, most of the analytical methods reported in the literature for pharmaceutical residue analysis were based on GC–MS [7,8,13,14] which often requires derivatization, only few studies have been carried out using LC–MS [15–17].

Isolation is a critical step for extracting pharmaceutical compounds from environmental water samples. Generally, polymeric sorbents have a higher adsorption capacity than C_{18} sorbent [18] for polar analytes. In this work the isolation was achieved using LiChrolut® EN (Lic EN) extraction cartridges from Merck which is composed of an ethylvinylbenzene–divinylbenzene-copolymer. On the other hand, Oasis® HLB from Waters, which is based on a macroporous copolymer made from two monomers, the divinylbenzene and the *N*-vinylpyrrolidone was also used.

The combined methodology involving the toxicity study and the chemical analysis by SPE–LC–ESI-MS was applied to surface water and wastewater samples in Catalonia (north east of Spain).

The specific objectives of the present work were:

- To assess the toxic potential to *V. fischeri* of ketoprofen, naproxen, ibuprofen, diclofenac–Na, gemfibrozil and salicylic acid.
- To develop an analytical method based on SPE–LC–ESI-MS for the determination of these drugs in natural water samples.
- To apply a combined methodology involving toxicity evaluation by *V. fischeri* and chemical analysis based on SPE–LC–ESI-MS for the trace determination of pharmaceutical compounds in surface and waste water samples.

2. Experimental

2.1. Chemical and reagents

Liquid-dried photo-bacteria reagents *Vibrio fischeri* NRRL B-111 77. NaCl was of analytical reagent grade and was from Parnreac (Barcelona, Spain). HPLC grade water, acetonitrile, acetone, and methanol were obtained from Merck (Darmstadt, Germany).

Hexane and phosphoric acid were also from Merck. High purity standards (99.5–100% pure) of all individual pharmaceuticals studied here were from Jescuder (Rubí, Spain).

LiChrolut®EN extraction cartridges from Merck and Oasis® HLB extraction cartridges from Waters Corporation (Milford, Massachusetts, USA). were used for solid-phase extraction.

BF_3–methanol 20% used for GC–MS derivatisation was purchased from Merck. Surrogate standards nitrobenzene-d_5, 2-fluorobiphenyl and 4-terphenyl-

d_{14} and internal standards anthracene-d_{10} and decachlorobiphenyl were from Supelco (Bellefonte, PA, USA).

2.2. Sample collection

Samples from four rivers of Catalonia: Llobregat, Cardener, Riera de Rubí and the Anoia and the treated effluent emitted to the rivers from two wastewater treatment plants (WWTP) of Terrassa and Manresa were analyzed. These WWTP consist of a primary settlement followed by a biological treatment and these plants receive both urban and industrial wastewater.

The pilot survey study took place during July, August and November 2000 with monthly sampling. Samples were collected in Pyrex borosilicate glass containers. Each bottle was rinsed with tap water and high-purity water prior to the sample addition. The samples were split in two sub-samples for the toxicity and chemical analysis. The preservation for the toxicity test sub-samples was accomplished by storing the bottles at 4°C immediately after sampling and the test was performed during the 24 h after the sampling. The sub-samples corresponding to the chemical analysis were stored at 4°C immediately after sampling and were acidified, filtered and extracted by SPE during the 24 h after sampling. The extracts were preserved at −15°C prior to the LC–MS analysis.

2.3. Cytotoxicity methodologies

Luminescent bacteria such as *V. fischeri*, naturally emit light. The enzyme involved is bacterial luciferase which catalyses the following reaction:

$$FMNH_2 + O_2 + R{-}CO{-}H \rightarrow FMN + R{-}COOH + H_2O + \textbf{LIGHT}$$

The reaction sequence involves incorporation of a molecule of oxygen into reduced flavin mononucleotide (FMN), and subsequent reaction with the aldehyde to form the $FMN{\cdot}H_2O$ complex, which breaks down with emission of light.

Bioluminescence is directly proportional to the metabolic status, of the cell. Toxic substances will cause changes to the metabolic cellular state, these changes are rapidly reflected in a bioluminescence decrease.

In the present work, the determination of the toxicity of selected pharmaceutical substances was performed measuring the bioluminescence inhibition of standard substance solutions by ToxAlert®100 from Merck, and Microtox®.The toxicity of real samples were determined by ToxAlert®100. The osmolality of all standards and samples was adjusted to 2% NaCl for the optimal reagents performance.

The percent of inhibition (%I) was determined by comparing the response given by a saline control solution to that corresponding to the sample (or diluted sample) or standard (or diluted standard). Therefore, the bioluminescence inhibition is $\%I = [1-(\text{dilution light}/\text{control light})]\times 100$. Measuring the inhibition values in a wide number of solutions produced for a standard substance, using those systems, it is possible to fit an inhibition curve and to the 50% effective concentration of this substance (EC_{50}). Together with the EC_{50} values for a more convenient graphical expression and interpretation of the toxicity data, the toxicity values of standard substances, can be converted in Toxicity Units, according to the formula of Sprague and Ramsay (1965).

$$TU = (EC_{50})^{-1} \times 100$$

This expression is the dilution factor which must be applied to the effluent so as obtain a 50% effect, and is directly proportional to the toxicity.

2.4. Sample preparation

An off-line solid-phase extraction (SPE) was used for the clean-up and pre-concentration of the samples. All the experiments were performed using an Automated Sample Preparation with Extraction Columns system (ASPEC XL) fitted with an external 306 LC pump for the dispensing of the samples through the SPE cartridges and with a model 817 switching valve for the selection of the samples, all from Gilson (Villiers-le-Bel, France). Disposable SPE cartridge columns packed with 200 mg of

LiChrolut® EN (Lic EN) sorbent, from Merck, were used.

The recovery studies were performed using groundwater samples (pH 8, 75 mg/l nitrate, 387 mg/l sulfate, 254 mg/Ca, 88 mg/l Mg and conductivity 2020 μS/cm). Groundwater was filtered through 0.7 μm glass microfiber filters from Scharlau (Barcelona, Spain) to remove suspended matter and acidified to pH 2 with sulfuric acid and spiked to final concentrations of 0.1, 1 and 10 μg/l of each pharmaceutical.

The recoveries were studied working in the neutral pH range using Oasis® HLB (Oasis) extraction cartridges with 300 mg, from Waters. Due to its chemical composition, the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone, the Oasis cartridges allow to work in the neutral pH range. Lichrolut EN cartridges were conditioned by passing 6 ml of hexane, 6 ml of acetone and 6 ml of HPLC water at pH=2 at a flow-rate of 1 ml/min. Afterwards, 1 l of filtered water sample was acidified to pH=2 and was loaded at a flow-rate of 10 ml/min. After pre-concentration, trapped compounds were de-sorbed using the following elution procedure: 1 ml of acetone, 2 ml of methanol and 2 ml of acetone. Finally, the extracts were evaporated to approximately 100 μl with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 300 μl. Finally, 20 μl were injected to the LC–MS system.

The conditions for Oasis extraction were as follows. SPE cartridges were conditioned with 6 ml of methanol followed by 6 ml of HPLC water at a flow-rate of 1 ml/min. One litre of filtered water sample was loaded at a flow-rate of 10 ml/min. The cartridges were then washed to reduce highly polar interferences by pulling 1 ml of 5% methanol in HPLC water through the cartridge. Elution of the analytes from the cartridge were carried out by first eluting with two 3 ml aliquots of acetonitrile:methanol 70:30 followed by two 2 ml aliquots of acetonitrile:methanol 70:30, and each solvent acidified to pH 3.7. The extracts were evaporated with a stream of nitrogen to approximately 100 μl. The sample is reconstituted with 500 μl of formate buffer. Finally, 20 μl were injected to the LC–MS system.

The samples corresponding to the November sampling campaign were selected to be also analyzed by HRGC–MS. These samples were extracted in parallel using a liquid–liquid extraction protocol according to Method 625, from the U.S. Environmental Protection Agency [19]. Acid compounds were derivatized using a treatment with BF_3–MeOH to their methyl esters previously to GC–MS analysis.

For the extraction protocol a surrogate standard mixture (nitrobenzene-d_5, 2-fluorobiphenyl and 4-terphenyl-$d_{1)}$ (10 μl) was added for 1 l of samples acidified with HCl until pH=2 and extracted twice with dichloromethane (150 ml and 100 ml) by stirring for 10 min. The organic extracts were combined and dried with anhydrous Na_2SO_4. Dichloromethane was removed under reduced pressure in a round bottom flask, until a volume of 0.5 ml. A solution of BF_3–methanol 20% (10 ml) was added to the extract and the mixture allowed to react overnight at room temperature, protected from light. A 10 ml aliquot of HPLC water was added and further extracted with *n*-hexane (3×10 ml). The combined extracts were concentrated under reduced pressure. The final concentrate was transferred to a 1 ml conical vial, washing the flask with isooctane, and dried under N_2 stream until a final volume of 100 μl for HRGC–MS. Internal standards mixture (anthracene-d_{10} and decachlorobiphenyl) (5 μl) were added to the extract.

2.5. LC–ESI-MS analysis

The HPLC system consisted of an HP 1100 autosampler with the volume injection set to 20 μl and HP 1090 A LC pump both from Hewlett-Packard (Palo Alto, CA, USA). Chromatographic separation was done using a reversed-phase C_{18} analytical column LiChrospher 100 RP-18 from Merck preceded by a guard column (4×4 mm, 5 μm) of the same packing material. Detection was carried out using a HP 1040 M diode array UV–Vis detector coupled in series with the LC–MSD HP 1100 mass selective detector, equipped with an atmospheric-pressure ionization source electrospray (ESI) interface. All extracts were analyzed using the ESI interface in negative ionization mode.

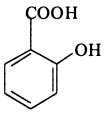
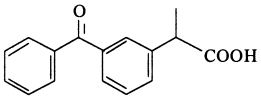
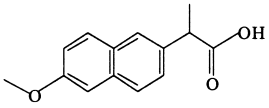
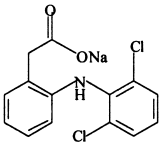
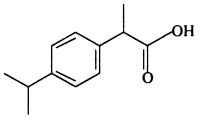
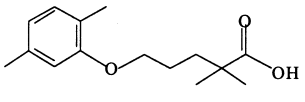
The extract samples were injected in the LC system using acetonitrile (A) and water acidified with formic acid to pH=2 (B), as a mobile phase. The

following solvent programming was used. Initial conditions 30% B and kept isocratic for 2 min, linearly increased to 40% B for 5 min, kept isocratic for 2 min followed by a linearly increased to 60% in 11 min and kept isocratic for 10 min. The flow-rate was 1 ml/min.

Operating parameters of MS-system were optimized in full scan mode (m/z values: 70–450) by flow injection analysis (FIA) of each target compound at 5 mg/l concentration. The optimal operating parameters of LC–ESI-MS in negative ion mode (NI) were set as follow: drying gas flow 13 l/min, drying gas temperature 300°C, nebulizer pressure 50 p.s.i., capillary voltage 3000 V and the fragmentation voltage 60 V. The main mass spectra ions and their relative intensities obtained under optimized conditions and three different fragmentation voltages are listed in Table 1.

The identification of target compounds was done in full scan mode by matching the retention time and mass spectrum with standards. Final quantification was performed in a selected ion monitoring mode (SIM) using external calibration. For the calibration curves, a series of injections of target compounds in

Table 1
Target compounds and their main m/z ions

Compound	Structure	m/z	Relative intensity		
			Fragmentation voltage (V) 60	Fragmentation voltage (V) 80	Fragmentation voltage (V) 100
Salicylic acid		137	100	100	80
		93	10	40	100
Ketoprofen		253	55	40	10
		209	10	90	85
		197	100	100	100
Naproxen		229	60	30	4
		185	100	85	38
		173	45	10	0
		170	20	100	100
		169	0	20	50
Diclofenac–Na		294	100	35	12
		250	60	100	100
		232	0	0	8
		214	0	0	6
Ibuprofen		205	100	85	10
		159	65	100	40
Gemfibrozil		249	100	100	15
		121	15	65	100

the range of 0.1 to 25 mg/l was used to obtain the equations. Calibration curves were generated using a linear regression analysis.

Fig. 1 shows an LC–MS chromatographic trace obtained when injecting 10 mg/l of standard mixture.

The recoveries of the method were determined by spiking experiment. As mentioned before, 1 l of filtered groundwater and acidified to pH 2 with sulfuric acid was spiked at 1 μg/l with each pharmaceutical, working in triplicates. The linearity of extraction methodology was checked by spiking experiments between 0.1 and 10 μg/l.

Five points calibration curves were constructed using a least-square linear regression analysis from the injection of standard solution of the mixture of all analytes at concentrations ranging from 0.5 to 25 μg/ml.

Limits of detection (LOD) were experimentally estimated from the injection of standard solution serially diluted until the signal-to-noise ratio for any single analyte reached a value of three.

The recoveries and limits of detection are reported in Table 2.

2.6. HRGC–MS analysis

The HRGC–MS analysis were performed on an

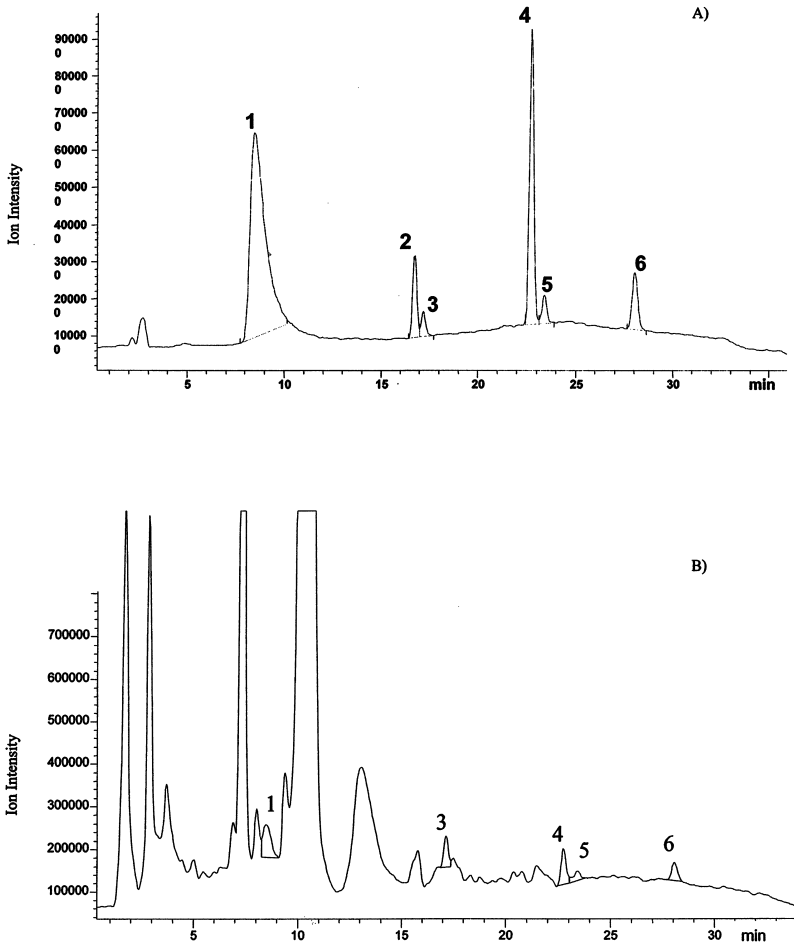


Fig. 1. (A) TIC chromatogram obtained by injecting a standard solution containing 10 mg/l of target compounds using ESI-MS detection in negative ion mode. The analytes are in order of elution: (1) salicylic acid, (2) ketoprofen, (3) naproxen, (4) diclofenac–Na, (5) ibuprofen and (6) gemfibrozil. (B) TIC chromatogram obtained by injecting a surface water sample corresponding to Riera Rubí. The chromatogram shows five of the six analyzed compounds: (1) salicylic acid, (3) naproxen, (4) diclophenac–Na, (5) ibuprofen and (6) gemfibrozil.

Table 2
Percent of recoveries and limits of detection obtained in select ion monitoring (SIM) mode

Compound	Lic EN pH 2			Oasis pH 7		
	Recovery	SD (%)	LOD (ng/l)	Recovery	SD (%)	LOD (ng/l)
Salicylic acid	76	13	15	<10	–	–
Ketoprofen	69	15	28	107.9	4.8	53
Naproxen	83	10	29	124.9	11.6	52
Diclofenac–Na	85	6	5	105.5	24.0	37
Ibuprofen	91	11	43	38.2	12.1	123
Gemfibrozil	70	6	56	109	2.54	72

integrated quadrupol GC6890 and MS HP-5973 from Hewlett-Packard (Waldbronn, Germany). Helium was used as carrier gas (at a constant flow of 0.7 ml/min) in a HP-5MS column (30 m×0.25 mm I.D., 0.25 μm film thickness). The program was from 90°C (held 2 min) to 150°C at 25°C/min with a second ramp to 200°C at 3°C/min and to 310°C (maintained for 10 min) at 8°C/min. Injector temperature was 280°C, and the injection mode was pulsed splitless for 90 s at 45 p.s.i. The scanning was 40–500 m/z at 1 scan/s. The data were processed with the Chemstation software that include the spectra libraries and Wiley 6th Edition (275 821 spectra).

The final quantification by HRGC–MS was carried out by an internal calibration using an isotope labeled standard (anthracene-d_{10}) and a calibration curve for every selected compound.

3. Results and discussion

3.1. Inhibition values for standard substances and toxicity units

Two bioluminescence inhibition assays, ToxAlert® 100 by Merck and Microtox® system have been used to establish the EC_{50} values, together with the TU of the selected drugs. Using ToxAlert®100 for every standard substance studied, a range of concentrations was prepared in saline solution (working in duplicate) and the %I were obtained. For each one a sigmoidal curve was fitted and the EC_{50} value was calculated (see Table 3). The concentration of each substance and EC_{50} are expressed in μg/ml. In order to compare the results the same procedure described above for some selected substances with Microtox® was used and the results are reported in Table 3. Fig. 2 shows the inhibition curves obtained using ToxAlert®100 for the pharmaceutical standard substances. Microtox® were carried out by using freeze-dried bacterial reagent, whereas the ToxAlert®100 were carried out with liquid-dried reagent. That could be the main cause of the differences obtained when applying these systems, as reported in Table 3.

Although, these compounds did not reveal a high toxic impact vs. *V. fischeri*, and they have toxic effects corresponding to the 50% affectivity ranging 10–50 mg/l, they act as toxic tracers. A few references can be found in the literature concerning the acute toxicity finding of non-steroidal anti-inflammatory drugs, but for instance, some work carried out studying the biological effects of Ibuprofen reveal that this analgesic exhibits high toxic effects over other organisms, like a high antimicrobial activity potential, against dermatophyte fungi [20], or the Ibuprofen activity as Gram-positive growth inhibitor [21].

3.2. SPE–LC–ESI-MS

Up to now, the chemical analysis for the com-

Table 3
Bioluminescence inhibition values (50% effective concentration, EC_{50} and toxicity units TUs), for six non-steroidal anti-inflammatory drugs using ToxAlert 100® and Microtox®

Compound	ToxAlert 100®		Microtox®	
	EC_{50} (μg/ml)	TUs	EC_{50} (μg/ml)	TUs
Salicylic acid	43.1	2.3	–	–
Ketoprofen	15.6	6.4	19.3	5.2
Naproxen	21.2	4.7	35.6	2.8
Diclofenac–Na	13.5	7.4	13.7	7.3
Ibuprofen	12.1	8.2	19.1	5.2
Gemfibrozil	18.8	5.3	31.5	3.2

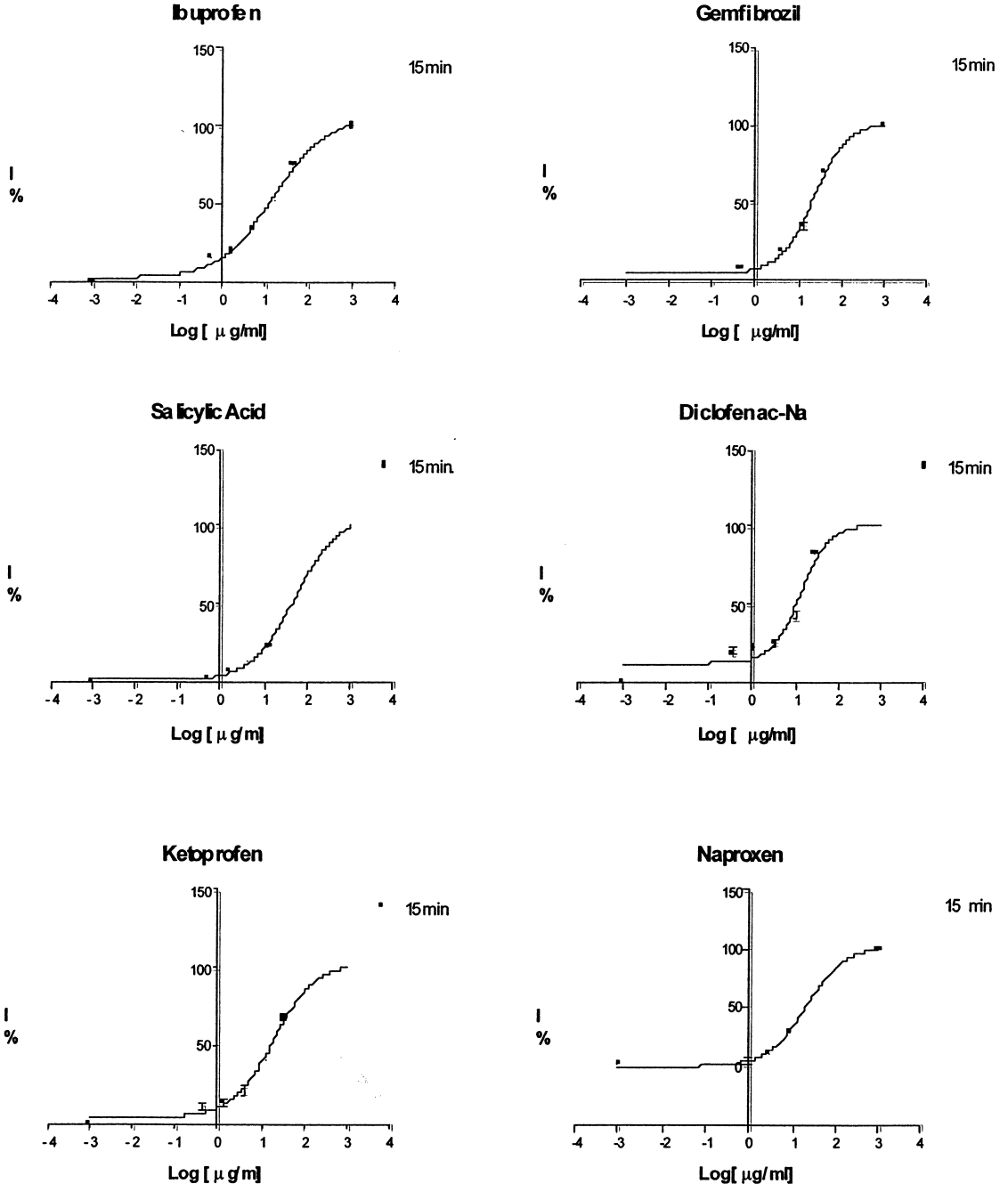


Fig. 2. Toxicity bioluminescence inhibition curves obtained for the six pharmaceutical compounds using ToxAlert®100.

pounds studied here is generally carried out using GC–MS determinations [22]. The main limitation of this technique is that a prior derivatization step is needed.

The isolation for the six selected compounds was achieved in the present work using LiChrolut® EN when the extraction is carried out working at pH 2. When using Oasis® HLB and working at the neutral

pH the extraction is only achieved for the five pharmaceuticals: Ketoprofen, Naproxen, Diclofenac–Na, Ibuprofen and Gemfibrozil (see Table 2). In this range of pH the Salicylic acid (principal metabolite of the acetylsalicylic acid) cannot be extracted. The other compounds will be extracted because their molecular hydrophobic zones can interact with the sorbent. Ibuprofen is the second compound, after acetylsalicylic acid, with less hydrophobic zones and that could explain the low recovery obtained with Oasis at neutral pH. To avoid the problems observed with Oasis the real samples were extracted after acidification to pH 2 and the extraction cartridges used were LiChrolut® EN.

The LC–ESI-MS response strongly depends on the optimization of interface parameters. A series of flow injection mode sequences were used for optimization. Eluents were acetonitrile and water acidified to pH 2 with formic acid. Successive direct injections of each target compound at 5 mg/l were done. The detection parameter values were optimized evaluating not only the sensitivity but also signal-to-noise ratio and fragmentation of each analyte in the scan mode (m/z 70–450). Nebulizer pressure is dependent on column flow. For a flow-rate of 1 ml/min nebulizer pressures varying from 45 to 60 p.s.i. were evaluated. The optimum was 50 p.s.i. The drying gas flow was evaluated in the range of 11–13 l/min and the optimum for most compounds was at 13 l/min. The optimum drying gas temperature was 300°C, at the optimum capillary voltage of 3000 V.

When varying the fragmentation voltage between 60 and 150 V, the best sensitivity is achieved in the low voltage range. When this voltage increases (see Table 1), the fragmentation for certain compounds, like salicylic acid and ibuprofen, does not increase but, then the sensitivity for all target analytes decrease significantly, especially for naproxen.

3.3. Environmental samples

The method involving SPE (using Lichrolut-EN and acidified samples) followed LC–ESI-MS was applied to the analysis of 12 surface water samples

Table 4
Toxicity results expressed as percent of bioluminescence inhibition and Lic-EN SPE–LC–MS results from surface water samples and effluents of two WWTP

Sample	Month	Toxicity %I	SPE–LC–MS (ng/l)					
			Salicylic acid	Ketoprofen	Naproxen	Diclofenac–Na	Ibuprofen	Gemfibrozil
Castellgalí	July	9.1	219	Bld	Bld	41	153	100
Llobregat	August	18	201	Bld	17	Bld	100	Bld
	November	<5	18	Bld	Bld	56	Bld	113
Castellgalí	July	11.4	280	Bld	Bld	120	Bld	236
Cardener	August	20	480	26	234	Bld	124	75
	November	<5	55	Bld	117	51	130	116
Martorell	July	12.5	449	Bld	Bld	610	1100	255
Anoia	August	28.5	961	17	Bld	31	2700	236
	November	6.3	80	Bld	120	147	468	220
Riera	July	36.8	1800	Bld	866	180	230	457
Rubí	August	38	8800	300	2000	Bld	1650	985
	November	14	1600	Bld	1500	484	1500	1550
Effluent WWTP	August	36	2000	23	271	60	1500	319
Manresa	November	20.6	570	348	1540	381	868	1200
Effluent WWTP Terrassa	August	55.8	13 000	871	Bld	Bld	85 000	–

Bld: Below limit of detection.

Table 5
Comparison of SPE–LC–MS and SPE–GC–MS data (mg/l) for the samples collected in November 2000 at the different sites

	Salicylic acid		Ibuprofen		Gemfibrozil		Naproxen		Ketoprofen		Diclofenac–Na	
	HRGC–MS	SPE–LC–MS	HRGC–MS	SPE–LC–MS	HRGC–MS	SPE–LC–MS	HRGC–MS	SPE–LC–MS	HRGC–MS	SPE–LC–MS	HRGC–MS	SPE–LC–MS
Castellgalí Llobregat	16	17	16	0	71	113	46	0	0	0	21	56
Castellgalí Cardener	28	55	50	130	72	116	99	117	0	0	30	51
L'Anoia Martorell	26	80	220	468	0	220	65	120	0	0	120	147
Riera de Rubí	2300	1600	1600	1500	0	1550	1450	1500	0	0	850	484
Effluent WWTP Manresa	900	570	680	868	1000	1200	1500	1540	370	348	411	381

and three WWTP effluents, the toxicity assessment of the samples using ToxAlert®100 also was determined. Fig. 1b shows an example of an LC–ESI-MS trace of a real sample.

Table 4 shows the toxicity inhibition values and the results of the chemical analysis. According to the EN ISO 11348-1,2 and 3 (draft from March 1997) standards, can be considered a toxic sample when the percent of inhibition is higher than the 20% for the 100% of the sample assayed. For this reason seven of the 15 analyzed samples are toxic.

The most toxic samples studied in this work are those corresponding to the effluents of WWTP, as was to be expected. Despite the complexity of the wastewaters discharged into the urban collecting systems, the performance of WWTP are good and most of the toxic polar organic substances are eliminated during the biological treatment. Even though some substances cannot be completely removed and some other substances are formed during the treatment process as happens with nonylphenol, according to the results obtained in previous works [23–25]. In accordance with these studies, most of the polar organic toxicants present in these WWTP effluents are linear alkyl benzenesulfonates (Cn-LAS), nonylphenol, polyethoxylated nonylphenol carboxylates and short chain nonylphenol ethoxylates, in conjunction with a mixture of pollutants and micro-pollutants, many of them unknown.

The whole toxicity potential of an effluent is proportional to all toxic compounds present on it and their synergistic effects in these concentrations.

Samples from the effluents of the WWTP exhibit a high influence to the acute toxicity due to the contribution of surfactants and their degradation products [25]. The results about the pharmaceutical concentrations in those samples were the highest for the present work, with levels of the different analytes varying from non-detected values up to 13 and 85 μg/l for Salicylic acid and Ibuprofen, respectively. Most of the samples analyzed were in the 20–1000 ng/l range. Although the pharmaceutical contribution to the whole toxicity is low, according to the acute toxicity studies carried out with pharmaceutical standard solutions, they can be considered as tracers of toxicity. The pharmaceuticals present may reflect the general toxicity of the complex mixture of organic and inorganic constituents that comprise

treated wastewater effluents and wastewater contribution to surface water.

The samples corresponding to November were also analyzed by HRGC–MS, after a derivatization step with BF_3–MeOH. Although the techniques are different, Table 5 reveals a very good result correlation between both methods using both techniques, except for the case of gemfibrozil which derivatization was not completely achieved in some cases.

4. Conclusions

Solid-phase extraction with Lichrolut EN followed by LC–ESI-MS is an appropriate method for the trace determination of the most widely used non-steroidal anti-inflammatory drugs in water matrices. The new analytical methodology based on the SPE–ESI-MS and the commonly used GC–MS method are in good agreement. The LC–MS method offers an improvement versus GC–MS since the derivatization step is avoided. Toxicity data for selected compounds vs. standardized organism, *V. fischeri*, was presented. While pharmaceuticals are present, and their concentrations increase with observed toxicity, their contribution to the whole toxicity cannot be determined because these substances are acting as tracers of toxicity.

In general, we suggest that the combined methodology presented here can help to evaluate the environmental risk of pharmaceutical compounds in water samples and contributes to the achievement of a more complete picture of toxicity of wastewaters.

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References

[1] T.A. Ternes, Division of Environmental Chemistry Preprints of Extended Abstracts: Vol. 40 (2000) March.
[2] C.G. Daughton, T.A. Ternes, Environ. Health Perspectives 17 (1999) 907.
[3] B. Halling-Sorensen, S.N. Nielsen, P.F. Lanksky, F. Ingerslev, H.C. Holten-Lützhoft, S.E. Jorgensen, Chemosphere 36 (1997) 257.
[4] H.J. Stan, T. Heberer, Anal. Mag. 25 (1997) 20.
[5] H.-R. Buser, T. Poiger, M.D. Müller, Environ. Sci. Technol. 33 (1999) 2529.
[6] N. Huppert, M. Würtele, H.H. Hahn, Fres. J. Anal. Chem. 362 (1998) 529.
[7] T.A. Ternes, R. Hirsch, J. Mueller, K. Haberer, Fres. J. Anal. Chem. 362 (1998) 329.
[8] H.-R. Buser, M.D. Müller, Environ. Sci. Technol. 32 (1998) 188.
[9] H.-R. Buser, T. Poiger, M.D. Müller, Environ. Sci. Technol. 32 (1998) 3449.
[10] J.O. Lambertson, T.H. Dewitt, C. Swartz (Eds.), Sediment Toxicity Assessment, Lewis, Boca Raton, FL, USA, 1992, p. 183.
[11] M. Salizzato, V. Bertato, B. Pavoni, A. Volpi, P.-F. Ghirardini, Environ. Toxicol. Chem. 17 (1998) 655.
[12] M.W. Toussaint, T.R. Shedd, W.H. Van der Schailie, G.R. Leather, Environ. Toxicol. Chem. 14 (1995) 907.
[13] T. Heberer, H.-T. Stan, Int. J. Environ. Anal. Chem. 67 (1997) 113.
[14] S. Öllers, H.-P. Singer, P. Fássler, S.-R. Müller, J. Chromatogr. A 911 (2001) 225.
[15] R. Hirch, T. Ternes, K. Haberer, A. Mehlinch, F. Ballwanz, K.-L. Krantz, J. Chromatogr. A 815 (1998) 213.
[16] T.A. Ternes, R. Hirsch, Environ. Sci. Technol. 34 (2000) 2741.
[17] W. Ahrer, E. Scherwenk, W. Buchberger, J. Chromatogr. A 910 (2001) 69.
[18] R. Loos, R.L. Niessner, Chromatogr. A 822 (1998) 291.
[19] U.S. Environmental Protection Agency Method 625. Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act: Final Rule and Interim Final Rule and Proposed Rule. Federal Register Vol. 49 (209, pp. 153–174) October 26, 1984.
[20] A.K. Sanyal, D. Roy, B. Chowdhury, A.B. Banerjec, Lett. Appl. Microb. 17 (1993) 109.
[21] K.T. Elvers, S.J.L. Wright, Lett. Appl. Microb. 20 (1995) 82.
[22] M. Strumpf, T.A. Ternes, K. Haberer, P. Seel, W. Baumann, Vom Wasser 86 (1996) 291.
[23] M. Farré, M.-J. García, Ll. Tirapu, A. Ginebrada, D. Barcelò, Anal. Chim. Acta 427 (2001) 181.
[24] M. Farré, O. Pasini, M.-C. Alonso, M. Castillo, D. Barceló, Anal. Chim. Acta 426 (2001) 155.
[25] M. Castillo, E. Martínez, A. Ginebreda, Ll. Tirapu, D. Barceló, The Analyst 125 (2000) 1733.