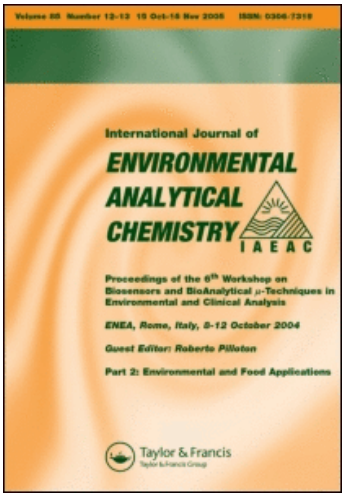


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# Pilot survey of chemical contaminants from industrial and human activities in river waters of Spain

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Over the past decades there has been significant progress in the treatment of sewage and industrial wastewaters in order to minimise discharges of urban effluents with lots of contaminants. Nowadays, the status of contamination of bodies of water continues to be a key task for further environmental management actions. This paper reports the presence of 88 contaminants belonging to several chemical groups (pharmaceuticals, personal care products, disinfectants and pesticides), in river waters of the region of Madrid, one of the more densely populated areas of Spain. Three different monitoring campaigns were performed over a period of seven months. For quality assessment of river water analyses, an analytical protocol was developed employing a solid-phase extraction (SPE) method, followed by two methods based on liquid chromatography-mass spectrometry (two LC-MS systems with ion trap and time-of-flight analysers) in order to carried out the unequivocal detection and quantification of the target contaminants. The method detection limits achieved were in range 0.1–60 ng $L^{-1}$. Recovery values were higher to 70% for the most of the compounds and only two analytes (amoxicillin and tamoxifen) were not recovered with the SPE method applied. The results obtained during the monitoring campaign were classified in turn into two categories: contaminants in general called 'emergents', and priority substances or candidate pollutants from domestic and industrial activities. This classification allows us to evaluate the impact of both contributions, typically domestic and industrial, on the river waters. At the same time it enables us to get a first idea about the effectiveness of the urban wastewater treatment plants (WWTPs) that release the effluents to those rivers in eliminating or removing contaminants. The concentration ranges detected were between 110 and 9942 ng $L^{-1}$ for emerging contaminants and 1 and 652 ng $L^{-1}$ for priority or candidate pollutants.

**Keywords:** river waters; priority substances; emerging contaminants; pollution indicators; chemical contaminations; LC-MS/MS

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## 1. Introduction

Social and industrial development brings together new sources of water contamination. European rivers have been unwisely used as sewers of discharges of industrial and domestic wastes, affecting, in consequence, the biodiversity of thousands of kilometres of waterways, and polluting coastal and marine waters. From 1970, thanks to a range of EU environmental directives, the quality of river water across Europe has improved significantly. Now, in the Member States of the European Union, the chemical quality of surface waters is controlled under the Water FrameWork Directive (WFD). Within this framework, the first strategy adopted, in terms of chemical contamination, was the Decision 2455/2001/EC which established a list of 33 substances or groups of substances of priority concern due to their persistence, toxicity and widespread use and detection in rivers, lakes, transitional and coastal waters. The current list includes certain pesticides and PAHs, benzene, halogenated solvents, flame retardants, plasticers, surfactants and anti-fouling agents. Because of their high risk to animal and plant life in the aquatic environment and to human health, another eight chemicals have increased the number of dangerous substances to be controlled to 41. As part of this strategy, the list is intended to be reviewed and updated every four years for defining environmental quality standards (EQSs) that allow a sufficient level of protection of inland surface waters and other surface waters (transitional, coastal and territorial waters). A list of 28 'candidate' pollutants is actually under a review process for identification as possible 'priority substances' or as 'priority hazardous substances' [1]. Among them, seven are pharmaceuticals and six are personal care products.



Many other organic contaminants from anthropogenic origin, many times called 'emerging' contaminants, are the subject of concern among the scientific community because of their frequent detection in wastewater effluents [2,3] and the potential contamination of surface water (rivers, lakes and coastal waters) if the effluents are discharged directly into the water bodies [3–5]. Numerous publications have been dedicated to assessing the removal capacity of the wasterwater treatment plants (WWTPs) [6,7] as well as the potential contribution in the contamination of surface waters [8–10].

In Spain, the region of Madrid is one of the most densely populated regions in the European Union and is characterised by two main areas, a large metropolitan area, predominantly with industrial and service activities, and the far north of the region, which is a predominantly rural area, mostly uninhabited and with less services and productive development infrastructures.

The study was focused in the determination of two chemical groups, some priority substances and candidate pollutants (total of nine chemicals), and emerging contaminants (79 chemicals, mainly pharmaceuticals and personal care products). From the last group special consideration is paid to nicotine, caffeine and their metabolites. Nicotine, cotinine, caffeine and paraxanthine can represent an interesting indicator of anthropogenic contamination as a consequence of their widespread detection in the environment and their potential relationship with water contamination levels, giving an indication of the presence of other chemicals of human activities [11]. This idea is also supported by the fact of their removal at very high percentages from the efficient urban wastewater treatment plants [6,12]. This work intended (1) to provide an initial assessment of the chemical quality of the rivers of the region of Madrid and (2) to evaluate the capability of nicotine and caffeine as indicators of river water contamination from human activities.

## 2. Experimental

### 2.1 *Chemicals, standards and materials*

All the chemicals included in this study were purchased at analytical grade (purity >90%) from Sigma-Aldrich (Steinheim, Germany), except codeine and diazepan, which were obtained by dissolving a Codeisan tablet (30 mg of codeine) from Lab. Belmac (Madrid, Spain) and a valium tablet (10 mg of diazepan) from Lab. Andreu (Barcelona, Spain), respectively. The reference compounds, used as surrogate standard, $^{13}$C-phenacetin and $^{13}$C-caffeine, were purchased from Lab. Dr. Ehrenstorfer (Augsburg, Germany). Stock standard solutions of individual compounds were prepared at a concentration of 1–2 mg mL$^{-1}$ in methanol. Working solutions of individual compounds and mixtures were prepared at different concentration levels, by appropriate dilution of the stock solutions in methanol or methanol : water (10 : 90, v/v). All the standard solutions were stored at −20°C.

The solvents used including methanol and acetonitrile HPLC grade, were supplied by Merck (Darmstadt, Germany). Water used for LC-MS analysis was generated from a Direct-Q$^{TM}$ 5 Ultrapure Water Systems from Millipore (Bedford, MA, USA) with a specific resistance of 18.2 MΩ cm. Commercial cartridges packed with Oasis$^{TM}$ HLB (divinylbenzene/N-vinylpyrrolidone copolymer, 200 mg, 6 cc) were purchased from Waters (Mildford, MA, USA). Formic acid (purity, 98%) was obtained from Fluka (Buchs, Germany).



The analytes included in this study, were selected on the basis of previous experience [2,6,13] and due to their widespread use and detection in the surface waters [3,5,14]. Four 'priority substances' (atrazine, diuron, isoproturon and simazine), three substances subject to review for identification as possible 'priority substances' (carbamazepine, diclofenac and iopamidol) and two as possible 'priority hazardous substances' (bisphenol A and clotrimazole) because they pose a particular risk to animal and plant life in the aquatic environment and to human health, were also included in this study [1]. They comprise a group of 88 organic pollutants belonging to different compound categories: pharmaceuticals, pesticides, disinfectants, and some of their major metabolites. Among the pharmaceuticals, the following representatives of different therapeutical groups were selected. *Analgesics/anti-inflammatories* (acetaminophen, indomethacine, codeine, mefenamic acid, ketorolac, naproxen, ibuprofen, diclofenac, fenoprofen, ketoprofen, propyphenazone), *antibiotics* (metronidazole, sulfamethoxazole, trimethoprim, ciprofloxacin, cefotaxime, ofloxacin, erythromycin, amoxicillin, lincomycin, sulfadiazine, sulfathiazole, sulfapyridine, norfloxacin, tetracycline, sulfamethazine, azithromycin, clarithromycin), *lipid regulators* (fenofibrate, bezafibrate, gemfibrozil, pravastatin, mevastatin, simvastatin), *β-blockers* (atenolol, propranolol, sotalol, metoprolol, nadolol), *antidepressants* (fluoxetine, paroxetine, venlafaxine, citalopram hydrobromide, amitriptyline hydrochloride, clomipramine hydrochloride), *antiepileptic/psychiatrics* (carbamazepine, diazepam, primidone), *ulcer healings* (ranitidine, omeprazole, famotidine, lansoprazole, loratadine), *corticosteroides* (methylprednisolone), *diuretics* (furosemide, hydrochlorothiazide), *bronchodilatadors* (salbutamol, terbutaline), *contrast agents* (iopamidol, iopromide), *antineoplastic agents* (cyclophosphamide monohydrate, iofosfamide), *antiseptics* (clotrimazole), *selective estrogen receptor modulator (SERM)* (tamoxifen), *stimulants* (nicotine, caffeine) and *anaesthetics* (mepivacaine). A group of major *metabolites* such as carbamazepine 10,11-epoxide, 1,7-dimethylxanthine (paraxanthine), clofibric acid, fenofibric acid, salicylic acid, cotinine, the active product of the antipyretic drug dypirone, 4-methylaminoantipyrine (4-MAA) and

some of its main metabolites (*N*-acetyl-4-aminoantipiryne (4-AAA), *N*-formyl-4-aminoantipiryne (4-FAA), 4-dimethylaminoantipiryne (4-DAA), 4-amino-antipiryne (4-AA) and antipyrine) were also included. In addition, a group of six *pesticides* (atrazine, chlorpyriphos methyl, chlorfenvinphos, diuron, isoproturon and simazine), one *plasticiser* (bisphenol A), and two well-known *disinfectants* (biphenylol and chlorophene), completed the group of target compounds.

### 2.2 *Area of study and sampling*

The river samples analysed in this study were collected from three different zones (north, centre and south) located in the centre of Spain (Madrid, Figure 1). This area is the most developed and densely populated of Spain. It is about 8050 $km^2$ in area and has a population of about 6 million. The streams run through several residential, industrial and agricultural areas. So, three areas of the region of Madrid were considerate as benchmarks to study the industrial/agricultural influence and population density in the pollution of rivers. Area A (north) presents a lower population density and with less services and productive development infrastructures (see Figure 1). In areas B and C (centre and south)



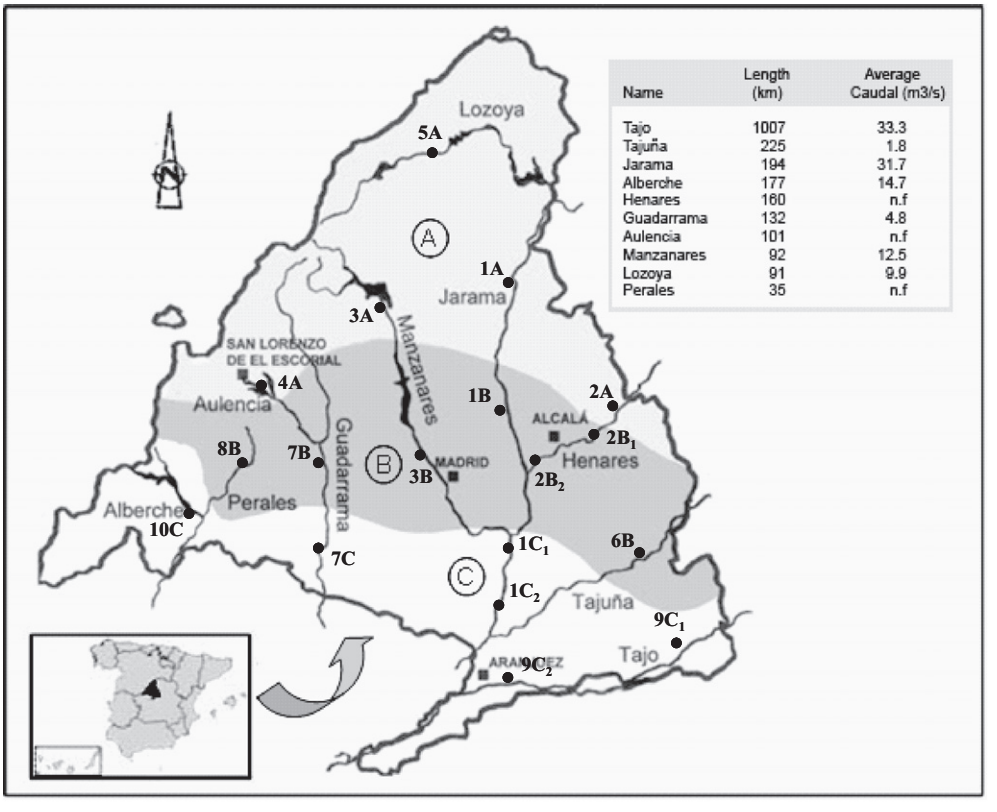


Figure 1. Map of the studied area and sampling points.*
*Area: A, B and C (north, centre and south, respectively). 1: Jarama; 2: Henares; 3: Manzanares; 4: Aulencia; 5: Lozoya; 6: Tajua; 7: Guadarrama; 8: Perales; 9: Tajo; 10: Alberche. $B_X$ and $C_X$: Different sampling points. n.f: not found.

there is a greater potential contribution to a possible contamination due to increased industrial activity and due to a higher population in both areas.

Ten rivers were subject of research in this study. A total of 51 water samples were analysed in three different monitoring campaigns performed in February, July and September 2008. Grab water samples (1L) were collected in clean amber glass bottles. Before sample collection, each bottle was properly pre-rinsed. The samples were sent in boxes packed with ice to the laboratory for posterior analysis. Upon reception, samples were filtered through a 0.7 μm glass fiber filter (Teknokroma, Barcelona, Spain), stored in the dark at 4°C until analysis and extracted within 48 h in all the cases.

### 2.3 *Sample preparation and extraction method*

Before extraction, samples were previously spiked with 0.1 μg of each surrogate standard, $^{13}$C-phenacetin and $^{13}$C-cafeine. A solid phase extraction (SPE) procedure was applied to the river samples using commercial Oasis$^{TM}$ HLB (divinylbenzene/N-vinylpyrrolidone copolymer) cartridges (200 mg, 6 cc) from Waters (Mildford, MA, USA). An automated sample processor ASPEC XL fitted with an 817 switching valve and an external 306 LC pump from Gilson (Villiers-le-Bel, France) was used for this purpose. HLB cartridges were conditioned at pH 8, according to the results obtained in previous experiments [2]. The procedure for carrying out the extraction of target compounds was the following. The Oasis HLB cartridges were preconditioned with 6 mL of MeOH and 5 mL of deionised water HPLC-grade (pH adjusted to 8 employing $NH_4OH$) at a flow rate of 3 mL min$^{-1}$. After the conditioning step, aliquots of 400 mL of sample (pH adjusted 8) were loaded into the cartridge. The samples were passed through the cartridges at a flow rate of 10 mL min$^{-1}$. After that, the cartridges were dried by nitrogen stream during approximately 15 min to remove excess of water and, finally the analytes retained, were eluted with $2 \times 4$ mL of MeOH at a flow of 3 mL min$^{-1}$. The extracts were evaporated until almost dryness using a Turbo-Vap from Zymark (Hopkinton, Massachusetts), with a water temperature at 35°C. Before analysis, the samples were reconstituted with 1 mL of AcN : water, 10 : 90 (v/v) and were then, filtered directly into an analysis vial using a 0.45 μm PTFE syringe filter (Millipore, USA).



### 2.4 *Liquid chromatography-QLIT-mass spectrometry analysis*

The method for the analysis of target compounds was developed with a 3200 QLIT MS/MS system (Applied Biosystem, Concord, Ontario, Canada) equipped with an electrospray ionisation source (ESI). The triple quadrupole/linear ion trap (QqQLIT) is a hybrid system in which the final cuadrupole can operate as conventional mass filter or as linear ion trap [2]. Chromatographic separation of the analytes was carried out using an HPLC system (Agilent Series 1100) equipped with a reversed phase C-18 analytical column of 250 mm length $\times$ 3.0 mm I.D and 5 μm particle size (ZORBAX SB, Agilent Technologies). The analyses were performed using a turbo ion spray source operating in both positive and negative modes.

For the analysis in positive mode, the compounds were separated using acetonitrile (mobile phase A) and HPLC-grade water with 0.1% formic acid (mobile phase B) at a flow rate of 0.2 mL min$^{-1}$. A linear gradient progressed from 10% of A (initial conditions) to 100% of A in 40 min, after which the mobile-phase composition was maintained at 100%

of A for 10 min. The re-equilibration time was 15 min. Compounds analysed in negative mode were separated using acetonitrile (mobile phase A) and HPLC-grade water (mobile phase B) at a flow rate of 0.3 mL $min^{-1}$. LC gradient started with 10% A and linearly was increased to 100% A, in 10 min, after which the mobile-phase composition was maintained at 100% A for 10 min. The re-equilibration time was 15 min. The volume of injection was of 20 μL in both modes.

The operation conditions for the analysis in positive ionisation mode were the following: ion spray voltage, 5000 V; curtain gas, 10 (arbitrary units); GS1 and GS2, 50 and 40 psi, respectively; probe temperature, 500°C. The GS1 parameter controls the nebuliser gas for the TurboIonSpray. It helps generate small droplets of sample flow and affects spray stability and sensitivity. The GS2 parameter controls the auxiliary, gas for the TurboIonSpray probe. It is used to help evaporate the spray droplets. GS2 works in conjunction with the temperature parameter. The parameters used for the analysis in negative ionisation mode were as follows: ion spray voltage, −3500 V; curtain gas, 10 (arbitrary units); GS1 and GS2, 50 psi; probe temperature, 500°C. Nitrogen served as nebuliser gas and collision gas in both modes. The mass spectrometer was calibrated manually with a solution of poly-propylene glycol which was introduced via a syringe pump to the interface, according to the manufacturer's instructions.



In order to obtain the maximum sensitivity for identification and detection of the target compounds, a careful optimisation of all MS parameters was performed for each analyte by flow injection analysis (FIA) in the spectrometer of 1 mg $L^{-1}$ solution of individual compounds in methanol. The parameters optimised were: declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP). The values of the parameters optimised and the transitions selected in the analytical method are shown in the Table 1. The MS operated in selected reaction monitoring mode (SMR) [2] with a resolution set to Low and Unit for Q1 and Q3, respectively. Table 1 also present the compounds analysed in both positive and negative modes. To obtain well defined and reproducible peaks a number of 10–15 points per peak was needed to achieve it. For this the dwell time values (time used for monitoring each ion transition) per each SRM transition were optimised, using an extract of river sample spiked at a concentration of 100 μg $L^{-1}$. For confirmation of analytes, the acquisition at least two SRM transitions for each compound together with retention time matching and the monitoring of the SMR ratio (which is the relationship between abundances of transitions selected for identification and quantification) were needed. The most intense SRM transition was selected for quantitation purposes (see Table 1). The data acquisition and processing was carried out using commercial software (Analyst, Applied Biosystems/MDS SCIEX).

An additional experiment was developed for the analysis in negative ionisation mode of the compounds, ibuprofen, iopamidol and iopromide, for which the second transition was not detected and therefore additional structural information was necessary for confirmatory purposes. For this case, the QTRAP system operated combining in the same run a SRM mode and two enhanced ion scan modes (enhanced product ion, EPI modes) [2]. Two EPI experiments were performed with Q1 set at Low resolution and the linear ion trap scanning from 80 to 800 amu at a rate of 4000 amu/s and a dynamic fill time, with a step size of 0.12 amu. The following parameters were used during the scans: DP:-20 V/CE:-10 eV and DP:-40 V/CE:-40 eV; CES (collision energy spread): 0 arbitrary units. The CES parameter controls the spread of the collision energies used when filling the LIT. It is used in conjunction with the Collision Energy (CE) parameter. CES applies only to EPI

Table 1. Values of the parameters optimised with the developed method by LC-QLIT-MS/MS.*

| Compound | tr(min) | Precursor Ion (*m*/*z*) | DP | SRM 1 | CE1 | SRM 2 | CE2 | SRM 3 | CE3 | [SRM2]/ [SRM1] (%RSD) |
|---|---|---|---|---|---|---|---|---|---|---|
| Positive | | | | | | | | | | |
| Nicotine | 5.1 | 163.1 | 40 | 117.2 | 34 | 130.1 | 27 | 84.1 | 27 | 0.8 (6) |
| Cotinine | 5.8 | 177.0 | 45 | 80.0 | 36 | 98.0 | 26 | – | – | 0.3 (6) |
| Salbutamol | 6.8 | 240.3 | 44 | 148.2 | 26 | 222.2 | 14 | 166.2 | 16 | 0.5 (5) |
| Atenolol | 6.8 | 267.4 | 30 | 145.2 | 35 | 190.2 | 20 | 116.1 | 25 | 0.8 (4) |
| Famotidine | 6.8 | 338.0 | 25 | 189.3 | 24 | 259.4 | 15 | 70.7 | 40 | 0.9 (20) |
| Terbutaline | 6.9 | 226.3 | 47 | 152.2 | 20 | 107.1 | 40 | 125.2 | 32 | 0.3 (8) |
| Amoxicillin | 7.0 | 366.2 | 32 | 114.2 | 27 | 208.0 | 15 | 349.1 | 10 | 0.5 (5) |
| Ranitidine | 7.5 | 315.3 | 38 | 176.2 | 21 | 130.1 | 30 | 224.2 | 20 | 0.7 (2) |
| Sotalol | 7.9 | 273.3 | 45 | 133.2 | 37 | 213.2 | 22 | 255.2 | 14 | 0.8 (1) |
| 4-MAA | 9.1 | 218.2 | 35 | 56.1 | 30 | 97.2 | 16 | 159.2 | 17 | 0.4 (4) |
| 4-DAA | 10.0 | 232.2 | 48 | 113.2 | 17 | 111.2 | 21 | 98.2 | 25 | 0.4 (11) |
| 4-AA | 10.3 | 204.2 | 45 | 56.2 | 30 | 159.2 | 16 | 94.2 | 28 | 0.2 (9) |
| 1,7-dimethylxanthine | 10.4 | 181.2 | 50 | 124.2 | 25 | 69.2 | 43 | 96.1 | 32 | 0.1 (12) |
| Acetaminophen | 10.9 | 152.2 | 40 | 110.1 | 20 | 64.8 | 45 | 93.0 | 35 | 0.2 (1) |
| Metronidazole | 12.0 | 172.1 | 35 | 128.1 | 20 | 82.1 | 30 | 111.1 | 30 | 0.6 (4) |
| Codeine | 12.1 | 300.3 | 35 | 165.2 | 50 | 199.2 | 35 | 153.2 | 55 | 1.0 (12) |
| Lincomycin | 14.3 | 407.1 | 50 | 126.3 | 45 | 359.3 | 23 | – | – | 0.05 (10) |
| Sulfadiazine | 16.6 | 251.2 | 40 | 92.1 | 35 | 156.3 | 17 | 108.1 | 30 | 1.0 (7) |
| Nadolol | 16.8 | 310.2 | 45 | 254.4 | 20 | 236.1 | 25 | 201.2 | 30 | 0.4 (1) |
| Caffeine | 17.0 | 195.1 | 40 | 138.2 | 25 | 110.2 | 30 | 123.1 | 40 | 0.3 (12) |
| 4-AAA | 17.2 | 246.2 | 46 | 83.1 | 40 | 228.1 | 18 | 104.2 | 28 | 1.0 (13) |
| 4-FAA | 17.4 | 232.2 | 45 | 214.2 | 18 | 104.1 | 28 | 83.1 | 28 | 0.9 (3) |
| Sulfathiazole | 17.6 | 256.2 | 45 | 156.0 | 18 | 92.2 | 34 | 108.4 | 33 | 0.7 (1) |
| Trimethoprim | 17.7 | 291.3 | 45 | 230.2 | 28 | 123.2 | 30 | 261.2 | 30 | 0.9 (14) |
| Sulfapyridine | 18.1 | 250.1 | 47 | 156.1 | 21 | 108.3 | 34 | 184.3 | 20 | 0.5 (6) |
| Norfloxacin | 18.2 | 320.0 | 50 | 276.4 | 23 | 233.3 | 30 | 302.3 | 18 | 1.0 (19) |
| Ofloxacin | 18.3 | 362.3 | 47 | 261.3 | 33 | 318.3 | 25 | – | – | 0.9 (15) |
| Ciprofloxacin | 18.6 | 332.3 | 50 | 231.2 | 48 | 314.3 | 25 | 245.1 | 32 | 0.8 (14) |
| Cefotaxime | 19.0 | 456.1 | 40 | 324.1 | 15 | 396.1 | 10 | 241.3 | 20 | 0.7 (9) |
| Mepivacaine | 19.2 | 247.4 | 28 | 98.1 | 23 | 70.1 | 53 | – | – | 0.3 (8) |

(*Continued*)

Table 1. Continued.

| Compound | tr(min) | Precursor Ion (*m*/*z*) | DP | SRM 1 | CE1 | SRM 2 | CE2 | SRM 3 | CE3 | [SRM2]/ [SRM1] (%RSD) |
|---|---|---|---|---|---|---|---|---|---|---|
| Tetracycline | 19.4 | 445.3 | 40 | 154.2 | 37 | 410.2 | 28 | 337.1 | 40 | 1.0 (5) |
| Metoprolol | 20.6 | 268.2 | 30 | 116.2 | 25 | 159.2 | 28 | 133.2 | 30 | 0.6 (5) |
| Primidone | 20.8 | 219.2 | 35 | 91.1 | 35 | 162.3 | 16 | 119.2 | 20 | 0.6 (9) |
| Sulfamethazine | 20.9 | 279.0 | 42 | 186.2 | 20 | 124.1 | 30 | 156.1 | 26 | 0.9 (18) |
| Azithromycin | 21.0 | 749.6 | 65 | 83.1 | 84 | 158.2 | 51 | 591.4 | 42 | 0.6 (3) |
| Antipyrine | 21.2 | 189.2 | 48 | 77.1 | 51 | 104.1 | 32 | 106.2 | 40 | 0.5 (5) |
| Omeprazole | 22.2 | 346.3 | 35 | 198.2 | 15 | 151.2 | 25 | 136.2 | 35 | 0.4 (16) |
| Venlafaxine | 23.0 | 278.4 | 50 | 58.1 | 45 | 260.4 | 15 | 121.3 | 38 | 0.2 (4) |
| Propanolol | 24.4 | 260.0 | 35 | 116.2 | 23 | 183.2 | 23 | 155.2 | 30 | 0.7 (2) |
| Ifosfamide | 24.5 | 261.1 | 60 | 91.9 | 33 | 154.3 | 29 | 182.2 | 21 | 0.6 (6) |
| Sulfamethoxazole | 24.8 | 254.2 | 47 | 108.2 | 30 | 156.1 | 21 | 147.2 | 22 | 0.8 (2) |
| Cyclophosphamide monohydrate | 24.9 | 261.1 | 55 | 140.1 | 29 | 120.1 | 31 | 233.2 | 16 | 0.3 (6) |
| Erythromycin | 25.1 | 734.6 | 58 | 158.3 | 40 | 576.5 | 28 | 316.1 | 25 | 0.02 (5) |
| Carbamazepine 10.11-epoxide | 25.2 | 253.2 | 75 | 180.2 | 40 | 236.2 | 11 | 210.2 | 20 | 0.6 (2) |
| Lansoprazole | 25.7 | 370.0 | 45 | 252.2 | 15 | 119.2 | 27 | 205.4 | 25 | 0.7 (19) |
| Citalopram hydrobromide | 25.9 | 325.3 | 42 | 109.1 | 30 | 262.1 | 25 | 234.1 | 35 | 0.3 (1) |
| Paroxetine | 26.9 | 330.3 | 70 | 192.2 | 25 | 151.2 | 30 | 123.2 | 30 | 0.4 (8) |
| Clarithromycin | 28.1 | 748.4 | 45 | 158.4 | 35 | 590.4 | 18 | 558.4 | 25 | 0.01 (10) |
| Amitriptyline hydrochloride | 28.5 | 278.4 | 30 | 233.1 | 22 | 91.2 | 35 | 117.0 | 30 | 0.8 (1) |
| Carbamazepine | 28.6 | 237.2 | 50 | 194.3 | 25 | 192.1 | 28 | – | – | 0.4 (10) |
| Fluoxetine | 28.8 | 310.3 | 30 | 44.2 | 25 | 148.2 | 10 | – | – | 0.2 (5) |
| Simazine | 28.9 | 202.2 | 45 | 124.2 | 23 | 132.0 | 26 | 104.1 | 34 | 1.0 (11) |
| Ketorolac | 30.0 | 256.3 | 70 | 105.1 | 25 | 178.1 | 34 | – | – | 0.01 (15) |
| Clomipramine hydrochloride | 30.1 | 315.2 | 40 | 58.1 | 60 | 86.1 | 27 | 242.1 | 27 | 0.9 (5) |
| Clotrimazole | 30.4 | 344.9 | 30 | 277.3 | 15 | 165.0 | 43 | 241.2 | 40 | 0.9 (2) |
| Propyphenazone | 30.9 | 231.3 | 55 | 189.2 | 22 | 201.2 | 30 | – | – | 0.4 (2) |
| Methylprednisolone | 31.1 | 475.3 | 40 | 457.2 | 9 | 321.1 | 17 | 339.3 | 16 | 0.9 (9) |
| Loratadine | 31.4 | 383.1 | 50 | 337.3 | 29 | 267.2 | 40 | 259.4 | 40 | 0.6 (8) |
| Isoproturon | 32.3 | 207.3 | 45 | 72.1 | 35 | 134.2 | 29 | 165.1 | 19 | 0.1 (17) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Atrazine | 32.5 | 216.1 | 45 | 174.1 | 24 | 104.1 | 40 | 132.1 | 31 | 0.4 (7) |
| Ketoprofen | 33.0 | 255.2 | 47 | 209.2 | 16 | 105.1 | 33 | 177.2 | 22 | 0.8 (14) |
| Naproxen | 33.3 | 231.2 | 35 | 185.3 | 20 | 170.1 | 32 | – | – | 0.3 (1) |
| Diazepan | 35.4 | 285.2 | 50 | 154.2 | 35 | 193.2 | 40 | 222.3 | 35 | 1.0 (2) |
| Biphenylol | 35.6 | 171.2 | 40 | 153.2 | 23 | 152.0 | 37 | 127.2 | 45 | 0.6 (20) |
| Indomethacine | 37.4 | 358.2 | 50 | 139.1 | 25 | 174.3 | 15 | – | – | 0.1 (8) |
| Fenofibric acid | 37.6 | 319.1 | 65 | 233.1 | 22 | 139.1 | 42 | 121.0 | 40 | 0.4 (8) |
| Tamoxifen | 38.1 | 372.2 | 50 | 72.1 | 42 | 129.0 | 35 | 91.1 | 35 | 0.05 (7) |
| Mefenamic acid | 40.4 | 242.2 | 36 | 180.2 | 53 | 224.2 | 34 | 209.2 | 20 | 0.5 (10) |
| Chlorfenvinphos | 40.5 | 359.1 | 55 | 155.2 | 15 | 127.1 | 21 | 205.1 | 24 | 0.9 (1) |
| Mevastatin | 41.2 | 391.3 | 55 | 229.3 | 20 | 185.2 | 25 | 159.3 | 30 | 0.7 (9) |
| Chlorpyriphos methyl | 43.4 | 322.1 | 43 | 125.1 | 21 | 290.1 | 19 | 109.1 | 30 | 0.7 (1) |
| Simvastatin | 45.1 | 419.1 | 45 | 199.1 | 15 | 285.3 | 15 | 243.1 | 15 | 0.6 (3) |
| Fenofibrate | 46.5 | 361.2 | 60 | 139.1 | 35 | 233.1 | 25 | 121.1 | 40 | 1.0 (7) |
| Negative | | | | | | | | | | |
| Salicylic acid | 3.1 | 137.1 | 28 | 93.0 | 25 | 65.0 | 45 | – | – | 0.1 (6) |
| Iopamidol | 3.7 | 776.0 | 40 | 126.9 | 40 | – | – | – | – | – |
| Iopromide | 4.2 | 790.0 | 45 | 126.9 | 40 | – | – | – | – | – |
| Furosemide | 11.1 | 329.1 | 35 | 205.1 | 30 | 285.1 | 20 | 126.2 | 45 | 0.8 (6) |
| Clofibric acid | 11.3 | 213.0 | 30 | 127.1 | 25 | 85.0 | 17 | – | – | 0.2 (3) |
| Pravastatin | 11.6 | 423.1 | 43 | 100.8 | 47 | 321.1 | 22 | 143.0 | 32 | 0.6 (10) |
| Benzafibrate | 11.8 | 360.1 | 30 | 274.2 | 30 | 154.1 | 40 | 85.0 | 35 | 0.6 (8) |
| Hydrochlorothiazide | 12.0 | 296.0 | 56 | 205.0 | 29 | 269.1 | 25 | 126.0 | 41 | 0.9 (4) |
| Bisphenol A | 14.0 | 227.1 | 60 | 133.0 | 35 | 211.2 | 40 | 92.8 | 40 | 0.5 (11) |
| Diclofenac | 14.1 | 294.0 | 25 | 250.0 | 15 | 214.1 | 28 | – | – | 0.1 (2) |
| Fenoprofen | 14.5 | 241.1 | 17 | 197.1 | 13 | 93.1 | 48 | – | – | 0.6 (7) |
| Diuron | 14.7 | 231.1 | 50 | 186.1 | 23 | 149.8 | 33 | 122.1 | 46 | 0.2 (1) |
| Ibuprofen | 15.6 | 205.1 | 30 | 161.2 | 10 | – | – | – | – | – |
| Chlorophene | 16.0 | 217.0 | 61 | 181.2 | 27 | 153.0 | 30 | 101.0 | 43 | 0.7 (8) |
| Gemfibrozil | 16.5 | 249.2 | 35 | 121.0 | 25 | 127.1 | 15 | – | – | 0.1 (3) |

*DP: declustering potencial (V); CE: collision energy (eV); EP: entrance potencial. 5 V; CXP: collision cell exit potencial. 2.5 V; SRM 1: quantitation; SRM 2–3: confirmation.

and MS/MS/MS scans. The spectrums generated by an extract of river sample spiked at a concentration of 1000 $\mu g\,L^{-1}$ and 100 $\mu g\,L^{-1}$, acquired in EPI mode, were stored in a mass spectral library, which enables further confirmation of organic compounds in real positive samples. In this case, confirmation criteria applied to the target compounds in the samples were: presence of the characteristic SRM transition at the correct retention time, and the correct relative ions abundance. However, at low concentrations (approx. 20 $\mu g\,L^{-1}$), it was not possible to carry out the confirmation of the compounds iopromide and iopamidol with any of the modes previously described (SRM and EPI modes), because the structural information achieved was not sufficient since with the conditions employed, it was only obtained one ion for each compound, being necessary for get an adequate confirmation to use a Time-of-Flight (TOF) as complementary tool.

### 2.5 *Liquid chromatography-time-of-flight-mass spectrometry*

A liquid chromatography-electrospray-ionisation-time-of-flight mass spectrometry (LC-ESI-TOF MS) system, in negative ionisation mode, was used to confirm two compounds in the samples, iopromide and iopamidol. The analytes were separated using a HPLC system (consisting of vacuum degasser, autosampler and binary pump from Agilent Series 1100, Agilent Technologies) equipped with a reversed-phase C8 analytical column of 4.6 × 150 mm, 5 μm particle size (ECLIPSE XDB, Agilent Technologies). Compounds analysed in this mode were separated using acetonitrile as mobile phase A and 0.05% ammonium formate in HPLC-grade water as mobile phase B at a flow rate of 0.4 $mL\,min^{-1}$. LC gradient started with 20% A and linearly was increased to 100% A, in 8 min, after which the mobile-phase composition was maintained at 100% A for 5 min. The re-equilibration time was 10 min. The volume of injection was of 30 μL. The HPLC system was connected to a time-of-flight mass spectrometer (MSD-TOF, Agilent Technologies, Santa Clara, CA) equipped with an electrospray interface operating under the following conditions: capillary, 4000 V; nebuliser, 40 psi; drying gas, 9 $L\,min^{-1}$; gas temperature, 325°; skimmer voltage, 60 V; octapole dc1, −42.5 V; octapole rf, 250 V; fragmentor 190 V. Spectra were acquired over the $m/z$ 50–1000 range at a scan rate of 1 s/spectrum. A second orthogonal sprayer with a reference solution was used as a continuous calibration in negative ion using the following reference masses: 119.036320 and 966.000725 $m/z$ (resolution: 11500 (500 at 966.000725 $m/z$). The full mass spectra data recorded were processed with Agilent MassHunter MSD TOF software. Based on the accurate mass obtained, all possible elemental compositions for ion fragments with a maximum deviation of 3–4 ppm from the measured mass were calculated.

### 2.6 *Validation study*

All the validation studies were performed by using river extracts. Because of the difficulty of obtaining blanks, the samples were previously analysed and the presence of the target compounds considered. To minimise matrix effects, due to the presence of matrix interferences, matrix-matched calibration curves were used for quantitative determinations. The linearity in the response was studied by using matrix-matched calibration solutions prepared by spiking river extracts at seven concentration levels, ranging from the quantitation limit of each analyte to 0.5 $mg\,L^{-1}$ in the final extract. Each point was obtained as the average of three injections. Integrated peak area data of the selected

quantification SRM transitions (SRM1; see Table 1) were used to construct the curves. The recovery studies ($n = 3$) were carried out, as has been described in Section 2.3 above, by spiking river samples at the concentration level of $0.25\,\mu g\,L^{-1}$. Precision of the analytical response, determined as relative standard deviation (RSD), was obtained from repeated injection ($n = 5$) of a spiked extract at $100\,\mu g\,L^{-1}$ during the same day (repeatability) and on different days (reproducibility). The method detection limits (MDLs) and method quantification limits (MQLs) were determined experimentally from the injection of spiked river samples, calculated using the minimum concentration of analyte providing signal-to-noise ratios of 3 (for the SRM2 transition) and 10 (for the SRM1 transition) respectively, and considering the preconcentration provided by the SPE method applied. They were estimated from the spiked extracted ion chromatograms at the lowest analyte concentration assayed. Confirmation criteria applied to the target compounds in the water samples were as follows: presence of two characteristic SRM transitions at the correct retention time and the correct SRM ratio, relative ion intensity or accurate mass, depending of the system or mode of analysis employed.

## 3. Results and discussion

### 3.1 *Analysis of river water samples*



The extraction efficiency was investigated for a total of 88 compounds using only one method. As has been detailed in Section 2.3 above, the recovery studies were performed by triplicate, giving relative standard deviation ranging from 2 to 18%. Recoveries of $^{13}$C-Phenacetin and $^{13}$C-Caffeine (use as surrogate standards) were 78% and 77%, respectively, and they allow us to verify an adequate performance of the extraction method and the elution step. The recovery values for the target analytes were higher than 50% in the majority of the cases (74 compounds). From them recoveries higher than 70% was achieved for 65 of these compounds. The extraction efficiency was lower than 50% in 12 cases and only 2 analytes were not recovered: amoxicillin and tamoxifen. Among the target compounds of this survey monitoring, the extraction method was satisfactory for most of the priority substances and 'candidate' pollutants selected with recovery values higher than 80%, except for diuron (63%), clotrimazole (34%) and iopamidol (20%). These values can be considered acceptable, taking into consideration the wide range of polarities involved and the good reproducibility obtained. For a positive identification of the analytes in the water river samples by LC-QLIT-MS/MS system, the following criteria were taken into account for each compound: monitoring of at least two SRM transitions, retention time and SRM ratio, verifying which these analytical responses were within a margin of ±2% and ±20%, respectively, in comparison with the response obtained for the standards. Table 1 presents both SRM1, SMR2 transitions used for identification and quantification, retention time and SRM ratios values. Relative standard deviation of SRM ratio obtained from the matrix-matched calibration curves do not surpass, in any case, a RDS value of 20%. In three target compounds (ibuprofen, iopamidol and iopromide), the second transition was not detected at low concentration. The confirmation criteria applied to those compounds in the samples were: presence of the characteristic SRM transition and the correct relative ions abundance by EPI scans (see Section 2: Experimental, above). In addition, the presence of the compounds (iopamidol and iopromide) in river samples was confirmed by LC-TOF-MS analysis, where the criterion was based on the determination of the accurate masses at the correct retention time of the target compounds. The results of

Table 2. Analytical performance of the proposed LC-QTRAP-MS/MS method.*

| Compound | Recovery (RSD%, $n=3$) | IDL (pg injected) | MDL ($ng\,L^{-1}$) | MQL ($ng\,L^{-1}$) | Lineality ($r^2$) |
|---|---|---|---|---|---|
| Positive | | | | | |
| Nicotine | 76 (9) | 6 | 1 | 4 | 0.976 |
| Cotinine | 100 (8) | 3 | 0.5 | 2 | 0.995 |
| Salbutamol | 95 (4) | 1 | 0.2 | 1 | 0.975 |
| Atenolol | 89 (7) | 2 | 0.3 | 1 | 0.982 |
| Famotidine | 72 (17) | 7 | 2 | 5 | 0.993 |
| Terbutaline | 82 (10) | 1 | 1 | 2 | 0.994 |
| Amoxicillin | – (–) | 231 | 29 | 97 | 0.999 |
| Ranitidine | 76 (9) | 1 | 0.5 | 2 | 0.983 |
| Sotalol | 89 (11) | 3 | 0.5 | 2 | 0.999 |
| 4-MAA | 13 (6) | 2 | 2 | 6 | 0.992 |
| 4-DAA | 73 (2) | 1 | 0.3 | 1 | 0.981 |
| 4-AA | 53 (9) | 4 | 0.4 | 1 | 0.987 |
| 1,7-dimethylxanthine | 44 (9) | 6 | 0.3 | 1 | 0.987 |
| Acetaminophen | 42 (13) | 11 | 0.5 | 2 | 0.990 |
| Metronidazole | 51 (12) | 9 | 1 | 3 | 0.999 |
| Codeine | 75 (12) | 20 | 3 | 11 | 0.992 |
| Lincomycin | 75 (18) | 2 | 2 | 8 | 0.992 |
| Sulfadiazine | 22 (15) | 8 | 2 | 7 | 0.996 |
| Nadolol | 86 (18) | 1 | 0.4 | 1 | 0.998 |
| Caffeine | 82 (16) | 6 | 1 | 4 | 0.995 |
| 4-AAA | 93 (10) | 1 | 0.5 | 2 | 0.989 |
| 4-FAA | 94 (14) | 4 | 2 | 5 | 0.990 |
| Sulfathiazole | 73 (6) | 3 | 2 | 6 | 0.995 |
| Trimethoprim | 83 (6) | 1 | 1 | 2 | 0.991 |
| Sulfapyridine | 94 (15) | 7 | 1 | 4 | 0.986 |
| Norfloxacin | 52 (13) | 3 | 3 | 10 | 1.000 |
| Ofloxacin | 88 (3) | 5 | 1 | 3 | 0.993 |
| Ciprofloxacin | 50 (3) | 6 | 2 | 6 | 0.996 |
| Cefotaxime | 24 (7) | 207 | 60 | 200 | 0.982 |
| Mepivacaine | 83 (3) | 1 | 0.1 | 0.3 | 0.984 |
| Tetracycline | 25 (9) | 18 | 7 | 23 | 0.998 |
| Metoprolol | 83 (5) | 5 | 0.4 | 1 | 0.998 |
| Primidone | 88 (7) | 3 | 2 | 5 | 0.994 |
| Sulfamethazine | 98 (2) | 2 | 0.4 | 1 | 0.995 |
| Azithromycin | 73 (11) | 8 | 4 | 15 | 0.988 |
| Antipyrine | 91 (2) | 2 | 0.5 | 2 | 0.997 |
| Omeprazole | 88 (7) | 2 | 0.2 | 1 | 0.986 |
| Venlafaxine | 79 (7) | 1 | 0.4 | 1 | 0.995 |
| Propanolol | 95 (12) | 1 | 0.3 | 1 | 0.999 |
| Ifosfamide | 81 (8) | 5 | 0.4 | 1 | 0.996 |
| Sulfamethoxazole | 65 (3) | 9 | 0.4 | 1 | 0.996 |
| Cyclophosphamide monohydrate | 84 (2) | 2 | 1 | 4 | 0.996 |

(*Continued*)

the validation studies of the analytical method are summarised in Table 2. The analytical method developed shown a satisfactory performance in terms of precision determined by inter and intra-day studies (with RSD values for lower than 20% in all the cases), and sensitivity (with lower MDLs values to $10\,ng\,L^{-1}$ for the most of compounds except amoxicillin, cafotaxime, ketorolac, biphenylol, tamoxifen, iopamidol and iopromide).

Table 2. Continued.

| Compound | Recovery (RSD%, $n=3$) | IDL (pg injected) | MDL ($ng\,L^{-1}$) | MQL ($ng\,L^{-1}$) | Lineality ($r^2$) |
|---|---|---|---|---|---|
| Erythromycin | 76 (6) | 2 | 3 | 10 | 0.997 |
| Carbamazepine 10,11-epoxide | 93 (6) | 4 | 0.2 | 1 | 0.990 |
| Lansoprazole | 91 (2) | 25 | 2 | 7 | 0.998 |
| Citalopram hydrobromide | 86 (7) | 1 | 0.4 | 1 | 0.992 |
| Paroxetine | 55 (7) | 2 | 0.5 | 2 | 1.000 |
| Clarithromycin | 86 (4) | 7 | 7 | 25 | 0.991 |
| Amitriptyline hydrochloride | 74 (6) | 1 | 0.3 | 1 | 0.993 |
| Carbamazepine | 91 (1) | 1 | 0.3 | 1 | 0.994 |
| Fluoxetine | 35 (5) | 1 | 0.5 | 2 | 0.997 |
| Simazine | 81 (4) | 2 | 0.4 | 1 | 0.998 |
| Ketorolac | 95 (4) | 4 | 16 | 55 | 0.998 |
| Clomipramine hydrochloride | 51 (6) | 1 | 0.5 | 2 | 0.994 |
| Clotrimazole | 34 (11) | 22 | 4 | 12 | 0.994 |
| Propyphenazone | 79 (12) | 2 | 0.2 | 1 | 0.998 |
| Methylprednisolone | 79 (18) | 43 | 8 | 27 | 0.990 |
| Loratadine | 76 (14) | 2 | 0.3 | 1 | 0.983 |
| Isoproturon | 87 (3) | 1 | 0.1 | 0.3 | 0.997 |
| Atrazine | 80 (4) | 1 | 0.2 | 1 | 0.987 |
| Ketoprofen | 83 (12) | 1 | 0.5 | 2 | 0.988 |
| Naproxen | 99 (7) | 18 | 6 | 20 | 0.997 |
| Diazepan | 90 (4) | 2 | 0.3 | 1 | 0.996 |
| Biphenylol | 56 (13) | 303 | 51 | 170 | 0.995 |
| Indomethacine | 91 (16) | 3 | 1 | 2 | 0.996 |
| Fenofibric acid | 100 (14) | 2 | 0.5 | 2 | 0.990 |
| Tamoxifen | – (–) | 4 | 11 | 39 | 0.998 |
| Mefenamic acid | 81 (6) | 4 | 1 | 2 | 0.998 |
| Chlorfenvinphos | 87 (4) | 4 | 0.4 | 1 | 0.991 |
| Mevastatin | 93 (6) | 7 | 1 | 3 | 0.997 |
| Chlorpyriphos methyl | 25 (10) | 21 | 3 | 10 | 0.994 |
| Simvastatin | 76 (18) | 7 | 2 | 8 | 0.999 |
| Fenofibrate | 28 (12) | 5 | 2 | 6 | 0.997 |
| Negative | | | | | |
| Salicylic acid | 10 (8) | 3 | 0.3 | 1 | 0.999 |
| Iopamidol | 20 (5) | 45 | 15 | 51 | 0.952 |
| Iopromide | 98 (14) | 32 | 11 | 30 | 0.999 |
| Furosemide | 80 (11) | 1 | 0.5 | 2 | 0.988 |
| Clofibric acid | 92 (11) | 1 | 1 | 3 | 0.991 |
| Pravastatin | 120 (5) | 9 | 2 | 6 | 0.989 |
| Benzafibrate | 70 (9) | 2 | 0.1 | 0.2 | 0.999 |
| Hydrochlorothiazide | 83 (9) | 2 | 2 | 6 | 0.981 |
| Bisphenol A | 105 (17) | 241 | 7 | 25 | 0.976 |
| Diclofenac | 112 (12) | 3 | 0.4 | 1 | 0.998 |
| Fenoprofen | 93 (10) | 9 | 1 | 3 | 0.995 |
| Diuron | 63 (16) | 5 | 5 | 17 | 0.942 |
| Ibuprofen | 71 (8) | 19 | 2 | 7 | 0.981 |
| Chlorophene | 71 (16) | 13 | 2 | 8 | 0.982 |
| Gemfibrozil | 78 (10) | 3 | 2 | 7 | 0.976 |

*IDL: instrumental detection limit; MDL: method detection limit; MQL: method quantification limit.

MQLs values ranged from 1 to 10 ng $L^{-1}$ for 72 compounds. The analytical method show also a good performance in terms of linearity (with correlation coefficients ($r^2$) $\geq$ 0.98 for the most of compounds), obtained by using matrix-matched calibration curves.

### 3.2 *Concentration levels of priority substances and 'candidate' pollutants*

Table 3 shows the concentrations measured of the target compounds for this survey monitoring of rivers of the region of Madrid. The survey study was carried out during three monitoring campaigns, where water samples were collected from rivers of different areas of the region of Madrid (north, centre and south). The most important hydrological collector in the region is the Tajo basin, that is nourished by the Jarama, the Manzanares, the Alberche and the Guadarrama rivers. Other major rivers in the region of Madrid are the rivers Lozoya, Guadalix and Henares. The results of the Table 3 are presented in function of the north area (zone A), centre (zone B) and south (zone C), and for each zone, the sampling points are indicated by numbers. As it has been indicated in Section 2.2 above, the three zones are different in terms of population and activities developed. Zone A (north) is characterised by a lower population density and with less services and productive development infrastructures. Zone B and C (centre and south) are characterised by an important demographic weight and where there is a growing industrial activity and service activities. The data shown in Table 3 correspond to the range of concentrations observed in the river waters during the three monitoring campaigns carried out over a period of seven months.



From the priority substances and 'candidate' pollutants analysed, atrazine, diclofenac and carbamazepine were detected in zone A, and in the zones B–C, diuron, simazine, iopamidol, and bisphenol A were also detected. Isoproturon and clotrimazol were not detected in any of the water samples collected. As can be seen in Table 3, the measured concentrations of the priority substances (atrazine, simazine, isoproturon and diuron) did not surpass the maximum allowable concentration (MAC) established as environmental quality standards (EQS) for rivers under the WFD. The survey monitoring was carried out over a period of seven months and taking into account the average concentration during this period, the measured concentration neither surpasses the annual average (AA) set as EQS. The level of concentration detected for the priority substances was at low ng $L^{-1}$ while the EQS are at low μg $L^{-1}$. The 'candidate' pollutants diclofenac and carbamazepine were detected at higher frequency and higher levels of concentration in the zones B and C than in zone A. Up to now, 'candidate' pollutants are not subject to EQS, but the available toxicological information for representative aquatic species can be benchmarks for environmental risk assessment. Diclofenac was measured at concentrations ranging from 1 to 529 ng $L^{-1}$, so at these levels of concentrations, some aquatic organisms do not show acute toxic effects; however, long-term effects or chronic effects could not be discarded [15,16]. Bisphenol A seems to be a ubiquitous contaminant but it was found at the limit of detection (7 ng $L^{-1}$) of our methods in most of sampling points.

### 3.3 *Concentration levels of emerging contaminants*

As shown in Table 3, there is a clear difference between the detection of emerging contaminants in the three zones studied. Zone A has a much lower load of emerging contaminants than zones B and C. That profiles respond with the general increase in

Table 3. Summary of concentration ranges ($ng\,L^{-1}$) obtained in the monitoring campaigns.*

| | Zone A | | | | | Zone B | | | | | | | Zone C | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound group | 1A | 2A | 3A | 4A | 5A | 1B | $2B_1$ | $2B_2$ | 3B | 6B | 7B | 8B | $1C_1$ | $1C_2$ | 7C | $9C_1$ | $9C_2$ | 10C |
| Priority S./Candidate P. (*) | | | | | | | | | | | | | | | | | | |
| Pesticides | | | | | | | | | | | | | | | | | | |
| Atrazine | – | 3–6 | – | – | D | D | 7–15 | 1–7 | 1–2 | 1–3 | D | D | 5–8 | 6–22 | D | D | 8–18 | – |
| Diuron | – | – | – | – | – | 17–34 | D | D | 18–35 | – | D | D | 31–47 | 14–28 | 17–59 | D | 17–21 | D |
| Isoproturon | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Simazine | – | – | – | – | – | D | D | D | 2–3 | D | 2–3 | D | 2–3 | 3–5 | 3–4 | D | 2–4 | 1–3 |
| Contrast agent | | | | | | | | | | | | | | | | | | |
| Iopamidol | – | – | – | – | – | – | – | – | D | – | – | – | D | D | – | – | D | – |
| Plasticiser | | | | | | | | | | | | | | | | | | |
| Bisphenol A | D | D | D | D | D | D | D | D | D | D | D | D | – | D | – | D | D | D |
| Analgesics/anti-inflammatories | | | | | | | | | | | | | | | | | | |
| Diclofenac | – | D | 1–29 | – | D | 36–202 | 38–150 | 4–165 | 31–462 | 1–12 | 2–129 | 2–108 | 55–529 | 20–405 | 91–285 | 1–17 | 4–192 | 4–10 |
| Antiepileptic | | | | | | | | | | | | | | | | | | |
| Carbamazepine | – | D | 1–55 | – | D | 47–202 | 9–13 | 3–31 | 45–137 | 1–8 | 45–56 | 2–76 | 41–129 | 46–112 | 51–78 | 1–5 | 29–73 | 2–36 |
| Antiseptic | | | | | | | | | | | | | | | | | | |
| Clotrimazole | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Emergents | | | | | | | | | | | | | | | | | | |
| Analgesics/anti-inflammatories | | | | | | | | | | | | | | | | | | |
| Acetaminophen | 3–7 | 3–27 | – | – | – | 8–11 | 10–18 | 4–15 | 13–22 | 3–38 | 3–7 | 3–21 | 8–33 | 7–35 | 5–25 | D | 4–25 | 3–7 |
| Indomethacine | – | – | – | – | – | 12–18 | – | 4–5 | 8–18 | – | 6–9 | 2–8 | 10–13 | 5–8 | 8–10 | – | 2–4 | – |
| Codeine | – | – | – | – | – | 1859–2533 | 201–898 | 534–1276 | 680–1440 | – | 265–832 | 100–368 | 873–3141 | 404–1707 | 427–735 | – | 173–978 | – |
| Mefenamic acid | – | – | – | – | – | 7–9 | D | 3–12 | 7–12 | – | D | D | 7–10 | 3–8 | 5–6 | – | 3–7 | – |
| Ketorolac | – | – | – | – | – | – | – | D | – | – | – | – | – | – | – | – | – | – |
| Naproxen | – | – | – | – | – | 224–395 | 43–303 | 21–258 | 109–137 | 23–51 | 100–379 | 21–221 | 42–338 | 46–444 | 61–216 | 25–44 | 40–164 | – |
| Ibuprofen | – | – | – | – | – | 296–642 | 165–1423 | 7–209 | 7–301 | 16–152 | 7–249 | 10–575 | 154–250 | 231–257 | 7–129 | – | 134–226 | – |
| Fenoprofen | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Ketoprofen | – | – | D | – | – | 52–74 | 2–9 | 3–91 | 48–81 | – | 2–5 | 3–5 | 18–72 | 5–24 | 9–27 | D | 2–5 | – |
| Propyphenazone | – | – | – | – | – | 7–8 | – | 2–3 | 13–22 | – | 9–14 | 4–19 | 12–18 | 9–13 | 7–15 | – | 5–12 | – |

(*Continued*)

Table 3. Continued.

| | Zone A | | | | | Zone B | | | | | | | Zone C | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound group | 1A | 2A | 3A | 4A | 5A | 1B | $2B_1$ | $2B_2$ | 3B | 6B | 7B | 8B | $1C_1$ | $1C_2$ | 7C | $9C_1$ | $9C_2$ | 10C |
| Antibiotics | | | | | | | | | | | | | | | | | | |
| Metronidazole | – | – | – | – | – | 10–32 | – | 3–15 | 9–20 | – | 5–12 | D | 8–22 | 4–15 | 6–18 | – | 3–12 | – |
| Sulfamethoxazole | – | D | D | – | – | 44–80 | 7–35 | 7–55 | 56–112 | 2–14 | 65–81 | 2–73 | 67–106 | 41–92 | 76–140 | D | 24–91 | 3–33 |
| Trimethoprim | – | – | D | – | – | 22–59 | 3–13 | 3–25 | 32–112 | D | 21–49 | 2–17 | 20–78 | 13–62 | 20–46 | D | 6–42 | – |
| Ciprofloxacin | – | – | – | – | – | 18–20 | – | 7–139 | 12–26 | – | – | – | – | – | – | – | – | – |
| Cefotaxime | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Ofloxacin | – | D | D | – | D | 29–402 | 14–98 | 51–296 | 13–34 | 3–15 | D | – | 14–169 | 7–104 | 5–12 | – | 5–67 | D |
| Erythromycin | – | – | D | – | – | 12–38 | 2–11 | 4–55 | 12–39 | 2–5 | 8–24 | 2–6 | 13–43 | 8–32 | 5–26 | – | 4–326 | – |
| Amoxicillin | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Lincomycin | – | – | – | – | – | 12–644 | – | – | – | – | – | – | D | D | – | – | D | – |
| Sulfadiazine | – | – | – | – | – | 7–34 | – | – | 7–15 | – | D | – | 7–12 | D | D | – | D | – |
| Sulfathiazole | – | – | – | – | – | D | – | – | D | – | D | D | D | D | D | – | D | – |
| Sulfapyridine | – | – | 4–19 | – | – | 51–77 | 5–12 | 21–24 | 73–166 | – | 48–81 | 4–78 | 51–118 | 34–102 | 52–94 | – | 12–79 | 4–7 |
| Norfloxacin | – | – | – | – | – | D | – | – | – | – | – | – | – | – | – | – | – | D |
| Tetracycline | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Sulfamethazine | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Azithromycin | – | – | – | – | – | – | – | 15–50 | – | D | – | – | D | – | – | – | 15–100 | – |
| Clarithromycin | – | – | – | – | – | 25–119 | D | 25–96 | 25–83 | – | 25–47 | – | 25–110 | 25–108 | 25–57 | – | 51–179 | – |
| Lipid regulators | | | | | | | | | | | | | | | | | | |
| Fenofibrate | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Bezafibrate | – | D | 1–36 | – | D | 18–118 | 2–21 | 3–70 | 18–97 | 1–4 | 8–84 | 3–12 | 32–155 | 15–113 | 13–109 | 1–6 | 8–155 | 1–4 |
| Gemfibrozil | – | D | 7–82 | – | D | 353–599 | 44–180 | 12–234 | 13–336 | 7–28 | 7–187 | 7–203 | 207–1164 | 203–529 | 186–1002 | 9–28 | 102–322 | 8–13 |
| Pravastatin | – | – | – | – | – | 14–72 | 7–10 | 7–60 | 12–26 | – | 7–38 | – | 17–65 | 11–55 | 7–10 | – | 7–34 | – |
| Mevastatin | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Simvastatin | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| $\beta$-blockers | | | | | | | | | | | | | | | | | | |
| Atenolol | – | D | 2–21 | – | 2–13 | 447–674 | 10–74 | 12–278 | 411–636 | 3–29 | 191–373 | 4–193 | 187–628 | 107–623 | 210–311 | 2–25 | 48–443 | 2–13 |
| Propranolol | – | – | – | – | – | 7–8 | 1–4 | 1–8 | 13–15 | – | 4–5 | – | 9–15 | 5–10 | 8–15 | – | 3–10 | – |
| Sotalol | – | – | – | – | – | 15–18 | D | 3–7 | 23–39 | – | 21–22 | D | 17–32 | 10–24 | 12–26 | – | 4–18 | – |
| Metoprolol | – | – | – | – | – | 13–20 | – | 3–7 | 10–18 | – | 6–9 | D | 14–17 | 6–14 | 8–9 | – | 4–12 | – |
| Nadolol | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Antidepressants | | | | | | | | | | | | | | | | | | |
| Fluoxetine | – | – | – | – | – | – | – | 3–5 | – | 2–4 | – | – | – | – | – | – | – | – |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Paroxetine | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Venlafaxine | – | – | 2–11 | – | – | 45–68 | 37–110 | 28–168 | 78–134 | 1–9 | 54–71 | 1–57 | 66–116 | 40–104 | 73–195 | 1–7 | 20–79 | D |
| Citalopram hydrobromide | – | – | – | – | – | 10–15 | 4–7 | 2–19 | 9–17 | – | D | – | 7–23 | 6–14 | 4–7 | – | 3–19 | – |
| Amitriptyline hydrochloride | – | – | – | – | – | 1–3 | – | – | – | – | – | – | 1–3 | 1–3 | – | – | 1–4 | – |
| Clomipramine hydrochloride | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Antiepileptic/psychiatrics | | | | | | | | | | | | | | | | | | |
| Diazepam | – | – | – | – | – | 2–4 | – | D | 5–9 | – | 1–3 | 1–21 | 6–7 | 4–9 | 4–13 | – | 2–4 | – |
| Primidone | – | – | 5–29 | – | – | 31–34 | 10–24 | 14–27 | 50–72 | – | 35–56 | 12–45 | 43–76 | 42–75 | 37–82 | – | 26–48 | 5–18 |
| Corticosteroides | | | | | | | | | | | | | | | | | | |
| Methylprednisolone | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Diuretics | | | | | | | | | | | | | | | | | | |
| Furosemide | – | – | – | – | – | 81–126 | 10–18 | 10–70 | 47–176 | 2–13 | 2–25 | 2–9 | 128–150 | 10–96 | 54–57 | 2–13 | 2–57 | – |
| Hydrochlorothiazide | – | D | 17–135 | – | D | 80–565 | 34–38 | 46–406 | 114–365 | 7-128 | 62–180 | 8–51 | 181–699 | 51–1002 | 368–788 | 7–50 | 35–857 | 7–149 |
| Ulcer healings | | | | | | | | | | | | | | | | | | |
| Ranitidine | – | – | – | – | – | 19–63 | 3–18 | 3–54 | 11–31 | – | 3–5 | – | 9–54 | 3–28 | 13–16 | – | 2–24 | – |
| Omeprazole | – | – | – | – | D | 2–222 | – | 38–66 | – | – | – | – | D | – | – | – | – | – |
| Famotidine | – | – | – | – | – | D | – | 9–100 | D | – | – | – | D | D | – | – | 4–217 | – |
| Lansoprazole | – | – | – | – | – | 4–36 | – | 4–96 | – | – | – | – | – | – | – | – | – | – |
| Loratadine | – | – | – | – | – | D | – | 2–10 | – | – | – | – | D | D | – | – | D | — |
| Bronchodilatadors | | | | | | | | | | | | | | | | | | |
| Salbutamol | – | – | – | – | – | 2–4 | – | – | 2–4 | – | 1–2 | – | 1–2 | 2–3 | 2–3 | – | – | – |
| Terbutaline | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Contrast agents | | | | | | | | | | | | | | | | | | |
| Iopromide | – | – | – | – | – | – | – | – | D | – | – | – | 28–58 | 28–36 | 28–32 | – | D | – |
| Serm | | | | | | | | | | | | | | | | | | |
| Tamoxifen | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Anesthetics | | | | | | | | | | | | | | | | | | |
| Mepivacaine | – | – | – | – | – | 5–8 | 1–2 | 1–4 | 7–14 | – | 2–3 | 1–3 | 9–15 | 6–10 | 3–5 | – | 3–10 | – |
| Antineoplastic agents | | | | | | | | | | | | | | | | | | |
| Cyclophosphamide monohydrate | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Iofosfamide | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

Table 3. Continued.

| Compound group | Zone A | | | | | Zone B | | | | | | | Zone C | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1A | 2A | 3A | 4A | 5A | 1B | $2B_1$ | $2B_2$ | 3B | 6B | 7B | 8B | $1C_1$ | $1C_2$ | 7C | $9C_1$ | $9C_2$ | 10C |
| Pesticides | | | | | | | | | | | | | | | | | | |
| Chlorpyriphos methyl | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Chlorfenvinphos | – | – | – | – | – | 2–16 | 2–5 | – | – | – | 2–24 | – | D | D | 2–20 | – | – | – |
| Disinfectants | | | | | | | | | | | | | | | | | | |
| Biphenylol | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Chlorophene | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Stimulants | | | | | | | | | | | | | | | | | | |
| Nicotine | 91–148 | 46–168 | 34–114 | 36–187 | 40–150 | 16–38 | 20–162 | 34–614 | 23–55 | 35–94 | 11–28 | 30–129 | 23–41 | 23–41 | 20–43 | 23–53 | 18–142 | 37–67 |
| Caffeine | 23–123 | 55–191 | 42–166 | 41–145 | 23–186 | 470–1131 | 81–480 | 66–280 | 206–864 | 75–261 | 105–209 | 66–576 | 76–403 | 107–382 | 93–147 | 46–131 | 100–262 | 48–122 |
| Metabolites | | | | | | | | | | | | | | | | | | |
| Cotinine | 10–16 | 4–24 | 9–22 | 5–17 | 7–28 | 44–190 | 18–57 | 36–87 | 15–53 | 22–69 | 43–64 | 26–103 | 47–159 | 36–77 | 14–75 | 17–37 | 25–93 | 26–58 |
| 1,7-dimethylxanthine | 8–23 | 7–44 | 13–44 | 5–16 | 12–21 | 63–349 | 55–67 | 35–51 | 53–134 | 47–91 | 55–78 | 49–293 | 44–243 | 53–83 | 52–99 | 34–75 | 43–68 | 32–69 |
| Carbamazepine 10,11-epoxide | – | – | 2–12 | – | – | 9–12 | – | 6–7 | 10–25 | – | 7–11 | 6–13 | 8–14 | 7–16 | 8–17 | – | 6–12 | 2–9 |
| Clofibric acid | – | – | – | – | – | 4–69 | – | 3–20 | 3–10 | – | – | – | 7–39 | 4–26 | D | – | – | – |
| Fenofibric acid | – | – | – | – | – | 15–34 | 2–6 | 7–38 | 20–32 | – | – | – | 2–37 | 2–7 | 2–6 | – | – | – |
| Salicylic acid | 10–108 | 8–119 | 19–34 | 4–70 | 16–37 | 13–53 | 5–30 | 6–33 | 7–32 | 37–109 | 9–24 | 3–11 | 10–917 | 12–35 | 4–45 | 6–53 | 5–25 | 5–20 |
| 4-Maa | – | – | – | – | – | 10–28 | D | D | 8–28 | – | – | – | 15–20 | 6–22 | 7–22 | – | 6–10 | – |
| 4-Aaa | D | 2–116 | 54–257 | – | 3–63 | 842–1322 | 253–466 | 124–1169 | 724–1135 | 9–153 | 745–1054 | 5–715 | 943–1572 | 518–1361 | 811–1255 | 22–98 | 410–1207 | 67–85 |
| 4-Faa | – | 23–52 | 28–112 | – | 23–35 | 623–791 | 139–280 | 74–552 | 671–883 | 8–11 | 552–620 | 14–747 | 526–877 | 359–876 | 684–1043 | 18–40 | 292–697 | 35–56 |
| 4-Daa | – | – | – | – | – | – | – | – | – | – | – | – | D | D | – | – | – | – |
| 4-Aa | – | – | – | – | – | 4–13 | 2–7 | 2–8 | 7–35 | – | 2–4 | – | 2–19 | 2–20 | 8–14 | – | 2–10 | – |
| Antipyrine | – | – | – | – | – | 10–18 | D | 3–10 | 19–67 | D | 14–24 | 2–28 | 17–35 | 15–35 | 18–44 | D | 16–36 | – |
| Total load of ($ng\,L^{-1}$) | | | | | | | | | | | | | | | | | | |
| Priority S./Candidate P. (*) | – | 3–6 | 2–84 | – | – | 116–432 | 62–170 | 8–203 | 112–619 | 2–22 | 50–187 | 2–184 | 138–652 | 119–550 | 171–421 | 1–22 | 73–296 | 5–47 |
| Emergents | 116–442 | 185–677 | 254–989 | 157–432 | 111–469 | 6633–9942 | 1194–4783 | 597–7032 | 5080–6726 | 110–1175 | 2597–4882 | 296–4679 | 4122–8485 | 2818–8085 | 3506–5723 | 128–423 | 1877–6360 | 266–578 |

*Zone: A, B and C (north, centre and south, respectively). 1: Jarama; 2: Henares; 3: Manzanares; 4: Aulencia; 5: Lozoya; 6: Tajuña; 7: Guadarrama; 8: Perales; 9: Tajo; 10: Alberche. $B_X$ and $C_X$: Different sampling points. (*) Priority Substances and Candidate Pollutants. D: detected (<MQL).

concentration when moving downstream related to the increase in population density and to discharges from WWTPs. The compounds detected in zone A were: acetaminophen, ketoprofen, sulfamethoxazole, trimethoprim, ofloxacin, erytromicyn, sulfapyridine, bezafibrate, gemfibrocil, atenolol, venlafaxine, primidone, hydrochlorothiazide, omeprazole, and the metabolites: carbamazepine 10,11-epoxide, salicylic acid, 4-AAA and 4-FAA. It represents a total of 18 contaminants from the 75 analysed. However, most of them (56) were measured in most of the sampling points of zones B and C that can be explained in a similar way as above. The compounds which were not measured in zones B and C were: fenoprofen, cefotaxime, amoxicillim, tetracycline, sulfamethazine, fenofibrate, mevastatin, simvastatin, nadolol, paroxetine, clomipramine hydrochloride, methylprednisolone, terbutaline, cyclophosphamide monohydrate, iofosfamide, tamoxifen, chlorpyriphos methyl, biphenylol and chlorophene. Ketorolac was only detected in one sample. Among the emerging contaminants detected, some compounds of the therapeutical groups of analgesic/anti-inflamatories (codeine, ibuprofen), lipid regulators (gemfibrocil) and diuretics (hydrochlorothiazide) were detected at higher concentrations than the rest of compounds surpassing concentrations of $1\,\mu g\,L^{-1}$. Similar values have been reported for ibuprofen in surface waters from United Kingdom [17] and for hydrochlorothiazide in effluents from Italy [18]. This is probably due to the amounts of these compounds consumed in the world, in particular, ibuprofen is the third active compound more consumed in Spain during 2007 [19]. Other pharmaceuticals, such as naproxen and atenolol, were detected at maximum concentrations of approx. 400 and $600\,ng\,L^{-1}$, respectively. The frequency of those detections was very similar in the three samples analysed. The metabolites of dypirone, 4-AAA and 4-FAA, were also detected at concentrations over $1\,\mu g\,L^{-1}$ and at a maximum concentration of approx. $800\,ng\,L^{-1}$, respectively. This fact confirms that the monitoring of metabolites and transformation products is necessary in these studies [13]. On the other hand, codeine was detected at a highest range of concentration ($100–2533\,ng\,L^{-1}$) and the metabolites, salicylic acid, 4-AAA and 4-FAA, were the most often detected compounds (>80%) in the river waters during the monitoring campaign. Figure 2 shows the results of the total load of contaminants measured for each category (priority substances/'candidate' pollutants and emerging contaminants), during the three monitoring campaigns and for the different zones of monitoring. The data is presented as the sum of concentrations (cumulative levels) for each category. In view of the results shown in Table 3, the load of emerging contaminants in the rivers waters of zones B and C is clearly much higher than in zone A which is less densely populated (see Figure 2). Furthermore, considering intra zone B and C data, it is observed higher values in concentration and number of contaminants in rivers 1, 2, 3 and 7 with respect to 6, 8, 9 and 10. It can be explained as a consequence of the higher population of those river areas called Jarama, Manzanares, Henares and Tajo. It could draw a relationship between an increased water contamination in function of the density of population and the use of pharmaceuticals. Regarding the detection of pesticides in the three zones, there is no apparent difference, for the pesticides analysed among agricultural zone (zone A) and industrial zones (B and C).

### 3.4 *Concentration levels of caffeine, nicotine and their metabolites*

The utilisation of caffeine, nicotine and their metabolites as potential tracers or indicators of human impacts on surface water systems due to their ubiquity is still a subject for

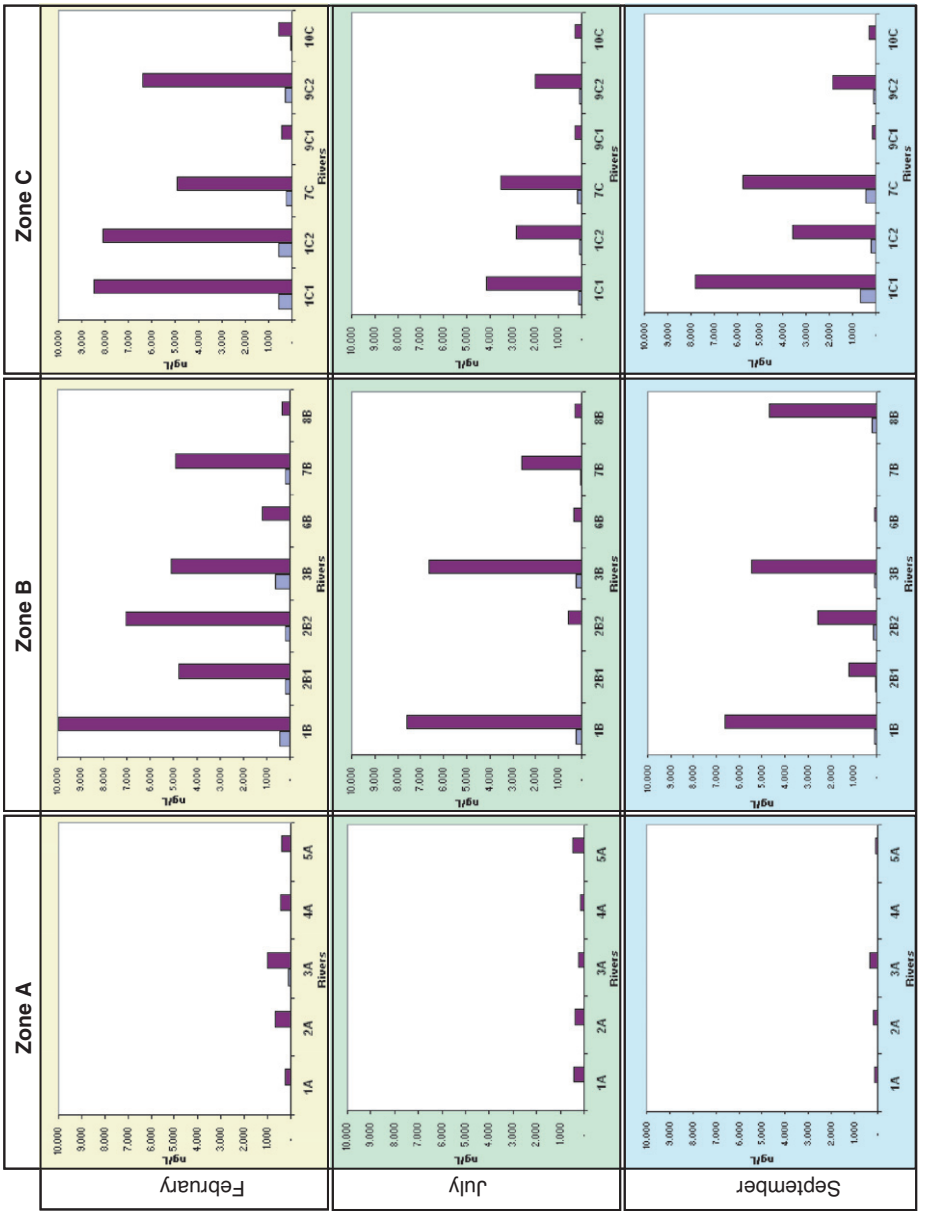


Figure 2. Graphs corresponding to the total load of contaminants measured for each category (priority substances "candidate" pollutants, emerging contaminants and pollution indicators), during the three monitoring campaigns and zones of study*.
*1: Jarama; 2: Henares; 3: Manzanares; 4: Aulencia; 5: Lozoya; 6: Tajua; 7: Guadarrama; 8: Perales; 9: Tajo; 10: Alberche. $B_X$ and $C_X$: Different sampling points. Sampling points: 4A/September; 2B1/July and 7B/September were not colletted.

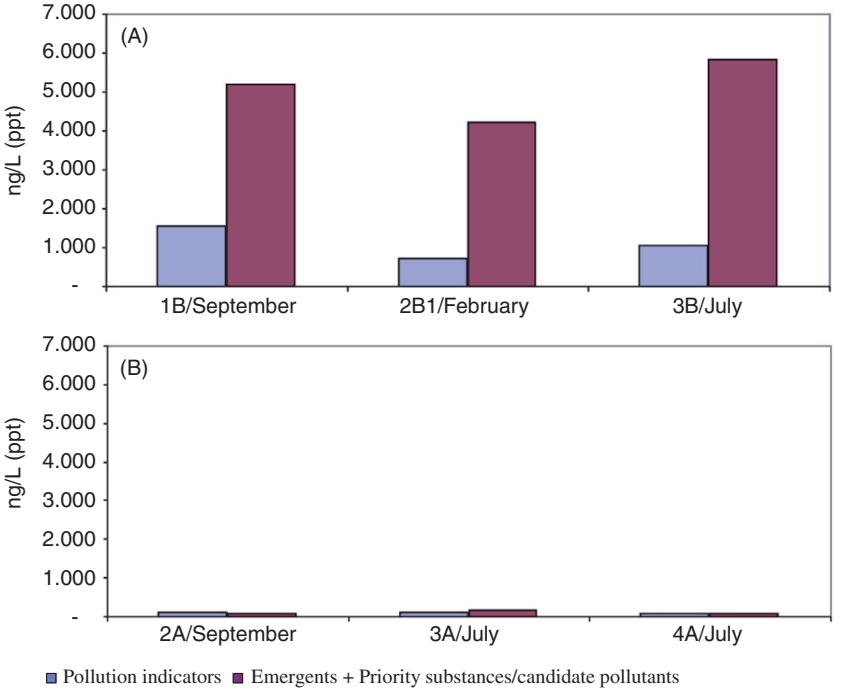


Figure 3. Concentration values of 'pollution indicators' (caffeine, nicotine and their metabolites) *vs.* the total load of contaminants (emergents + priority substances/candidate pollutants)*.
*Graph A: Sampling points (zone B) with total load of pollution indicators in excess of 600 $\mu g\,L^{-1}$. Graph B: Sampling points (zone A) with total load of pollution indicators of less than 200 $\mu g\,L^{-1}$. 1: Jarama; 2: Henares; 3: Manzanares; 4: Aulencia.



further research, mainly due to the need of a better understanding of how stable they are in the environment [11] and the need for data to know their background levels expected. Nicotine, caffeine and their metabolites have been previously reported as promising indicator candidates of water contamination [20,21]. A widespread detection of these chemicals in the environment may give weight to their potential relationship with water contamination due to anthropogenic sources. In this study, levels of caffeine, nicotine and metabolites have also been determined. As is reported in Table 3, caffeine, nicotine and their metabolites were detected in all water samples analysed, that is, in all sampling points and during the three monitoring campaigns. Caffeine was detected at loads higher than those found for nicotine, except for the sampling points 1A, 4A and $2B_2$. Although its metabolites (paraxanthine and cotinine) were also detected, their concentrations were lower than their parent compounds, except for cotinine in the points 1B, 7B, $1C_1$, $1C_2$ and 7C. The concentration values observed in zone A with concentrations typically around or below 200 $ng\,L^{-1}$ that could considered as a background level in our study. On the contrary, in zones B and C the values are typically in the range of 200–700 and 180–500 $ng\,L^{-1}$, respectively. Similar values have been obtained in other studies from various developed areas [22]. Figure 3 shows some examples of a more detailed look at the concentration values of caffeine, nicotine and their metabolites *versus* the total load contaminants from human activities. It can be noted, in general a relationship between

high load of contaminants when the considered indicators presented higher values of concentration. The results pointed out the interest in using such compounds as indicators of water contamination but obviously more data are necessary to confirm the efficacy of those indicators.

### 3.5 *Risk to the aquatic environment*

A preliminary screening of the risks to the aquatic compartment has been carried out, by comparing the predicted environmental concentrations (PEC) of the monitored substances to the predicted no effect concentration (PNEC), thus obtaining the so-called risk characterisation ratio [23]. Existing PNEC values for aquatic organisms have been obtained from Muñoz *et al*. [24] for 42 of the 88 substances monitored, and an average concentration for each substance has been calculated from data in Table 3. This preliminary screening shows that three substances, namely ciprofloxacin, ibuprofen and 4-AAA, exceed the PEC/PNEC value of 1, indicating a potential risk for aquatic organisms. It must be borne in mind that this simple screening has been performed considering average and not peak concentrations, therefore in particular locations PEC/PNEC values above 1 could be found for other substances. However, assessing the environmental risks of the 88 pharmaceuticals deserves a more elaborated study, which is beyond the scope of this paper.



## 4. Conclusions

This paper reports the first results obtained of a survey monitoring of chemical residues in river waters of the region of Madrid. As part of this study, an analytical protocol including SPE procedure and analysis by LC systems with ion trap and time-of-flight detectors has been developed for the detection of a total of 88 compounds. The results obtained during the monitoring campaign carried out over a period of seven months were classified in turn into three different categories: emerging contaminants, priority substances or candidate pollutants and pollution indicators. The concentration ranges detected were between 110 and 9942 ng $L^{-1}$ for emerging contaminants and 1 and 652 ng $L^{-1}$ for priority substances/candidate pollutants. Zones B (centre) and C (south) of the region of Madrid characterised by an important demographic weight also provide a higher load of contaminants in river water, and are higher than zone A (north) which is less densely populated. A preliminary screening of environmental risks suggests that some of the monitored contaminants might be exceeding aquatic risk thresholds. However, a more detailed study specifically focusing on this subject should be carried out in order to properly assess these risks. Caffeine, nicotine and their metabolites have shown as adequate indicators of contamination from human activities in the area studied.

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