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Keyword:	Multi-pumping, Sulfonamides, eco-friendly method, Water

An eco-friendly method for analysis of sulfonamides in water samples using a multi-pumping system

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ABSTRACT

A new methodology for determination of sulfonamides (sulfaquinoxaline, sulfathiazole, and sulfadimethoxine) in water samples was developed by coupling an automated multi-pumping flow system (MPFS) with a liquid waveguide capillary cell (LWCC, pathlength 100 cm) and a spectrophotometric detector. The method is based on the reaction between sulfonamides and *p*-dimethylaminocinnamaldehyde (*p*-DAC) in the presence of sodium dodecylsulfate (SDS) in dilute acid medium (hydrochloric acid), with measurement of the reaction products at 565 nm. Experimental design methodology was used to optimize the analytical conditions. The linear range obtained was 10.0 to 130.0 μg L^{-1}, and detection (LOD) and quantification (LOQ) limits were 3.1 and 10.1 μg L^{-1}, respectively. The method was successfully applied to the analysis of sulfonamides in water samples. By coupling the multi-pumping flow system with the LWCC, the sensitivity was enhanced, reagent consumption was low, and waste generation was minimized. The results obtained with the MPFS method were confirmed by LC-MS.

Keywords: Multi-pumping, Sulfonamides, Eco-friendly method, Water.

1. INTRODUCTION

The presence of pharmaceuticals in aquatic environments has been reported in many scientific studies,[1-3] with antibiotics being amongst the most studied compounds.[3] Antibiotics can enter the aquatic environment in many ways: from the production of pharmaceutical chemicals, excretion of residues after usage, and inappropriate discarding of unused medicines.[2] In addition, antibiotics are used in large quantities in livestock.[4] The increase in levels of antibiotics in soil, surface water, and groundwater poses a serious threat to human health, since compounds such as sulfonamides can persist in the environment and their high mobility means that they can be transferred to water.[3,5] Concentrations of antibiotics exceeding 1 mg L^{-1} have been detected in treated industrial effluents and in the receiving water bodies.[3]

Increasing awareness of the potentially harmful consequences of the presence of antibiotics in the aquatic environment has led to considerable growth in analytical methodologies for their determination in environmental matrices,[6] as well as in treated water.[7-11]

Sulfonamide antibiotics are widely used for therapeutic and prophylactic purposes in both human and veterinary medicine, and sometimes as growth promoter additives in animal feed.[12] The detection, in water wells, of sulfonamide antimicrobials approved strictly for use in veterinary medicine provides evidence of groundwater contamination from an animal waste source.[13] The presence of sulfonamide and other antibiotic residues in the aquatic environment can be toxic to the ecosystem and lead to effects in humans.[14-17]

The activity and behavior of sulfonamides depends on the types of substituents present on the aromatic rings.[18] The resulting differences enable the use of sulfonamides and their derivatives, alone or in combination with other compounds, in various situations as antibacterial, antitumor, antiviral, and antithyroid agents.[19-21]

The literature describes several classical methodologies for the determination of sulfonamides in aqueous matrices, including capillary electrophoresis,[22] high performance liquid chromatography-tandem mass spectrometry,[23] thin layer chromatography,[24] and immunoassay.[13,25] Most of these

methodologies necessitate laborious sample cleanup steps, consume large amounts of (organic) reagents, or are time-consuming.

Therefore, there is a need for rapid and sensitive methods that comply with the principles of Green Chemistry,[26,27] to help to ensure that water is safe for human consumption. The great advantage of the development of new green methodologies is the low production of toxic residues and minimal use of reagents. One of the main aims of green analytical chemistry is to minimize production of pollutant compounds, hence protecting humans and the wider environment.[28]

Multi-pumping flow systems (MPFS) are innovative analytical techniques that enable the use of smaller injection volumes and consequently lower generation of wastes.[29,30] These systems employ solenoid micropumps that can act as both commutators and propulsion devices to deliver microliter volumes of solutions. Reagent addition in multi-pumping flow systems is inherently intermittent, resulting in the ability to minimize reagent consumption and waste generation.[27]

The use of a liquid waveguide capillary cell (LWCC) in combination with a multi-pumping flow system can provide the low detection limits required for trace analysis, which were previously difficult to achieve with molecular spectrophotometric methods.[31]

The aim of this work was to develop a simple, rapid, and environmentally friendly analytical method for the determination of sulfonamides in water samples, employing a multi-pumping flow system coupled with a liquid waveguide capillary cell and a spectrophotometric detector. In this study, three sulfonamides (sulfaquinoxaline (SQX), sulfathiazole (STZ), and sulfadimethoxine (SDX)) widely used in veterinary medicine were chosen as representative of the sulfonamide class. The MPFS method employed solenoid pumps to propel defined volumes of reagent and sample solutions to a confluence point, where the reaction between *p*-dimethylaminocinnamaldehyde (*p*-DAC) and sulfonamide, in an acidic micellar medium, produced a colored product that could be measured spectrophotometrically.

2. EXPERIMENTAL

2.1. Apparatus

The flow injection apparatus consisted of a multi-pumping system with different injection capacities (10 and 20 µL) and polytetrafluoroethylene (PTFE) tubes (0.8 mm i.d.) for propelling the fluids. Measurements of absorbance at 565 nm were carried out using optical fibers (600 µm diameter) connecting a liquid waveguide capillary cell (LWCC, World Precision Instruments, flow path of 100 cm, inner volume 250 µL), a halogen lamp light source (LS-1-LL, Ocean Optics), and a USB 4000 spectrophotometer detector (Ocean Optics). SpectraSuite data acquisition software (Ocean Optics) was employed for the absorbance measurements.

The multi-pumping flow system employed three Bio-Chem Valve micro-pumps for insertion and propulsion of the different solutions, as shown in Figure 1: P1 (10 µL), P2 (20 µL), and P3 (20 µL). The injections of the reagent and sample solutions were carried out in intercalation mode, followed by measurement of the absorbance. The main operating parameters of the multi-pumping system are given in Table 1.

Figure 1

Table 1

2.2. Reagents and solutions

All the reagents employed were analytical grade. Ultrapure water (18 MΩ cm, Milli-Q system, Millipore) was used to prepare the solutions.

An aqueous stock solution of 0.1 mol L^{-1} sodium dodecyl sulfate (SDS) (Sigma, St. Louis, USA) was prepared, and working solutions (5.0 x 10^{-3} mol L^{-1}) were prepared by dilution of the stock solution with deionized water.

A stock HCl solution (1.0 mol L^{-1}) was prepared by dilution of concentrated acid (Mallinckrodt, Xalostoc, Mexico) in deionized water, and was standardized using a volumetric procedure. Working solutions (1.85 x 10^{-2} mol L^{-1}) were prepared by appropriate dilution.

The chromogenic reagent (*p*-dimethylaminocinnamaldehyde, *p*-DAC) (Riedel-de Haën, Germany) stock solution (0.01%, w/v) was prepared in 1.85 x 10^{-2} mol L^{-1} HCl and kept refrigerated

for up to 1 week. Working solutions of *p*-DAC (0.0024%, w/v) were prepared by dilution of the stock solution.

Three sulfonamide standards (sulfaquinoxaline (SQX), sulfathiazole (STZ), and sulfadimethoxine (SDX)) were purchased from Sigma Aldrich, and an aqueous stock solution was prepared at a concentration of 100 mg L^{-1}. A further stock solution (5 mg L^{-1}) was prepared, and from this solution, working standards were prepared in the range 10.0-130.0 μg L^{-1} , in the presence of HCl (1.85 x 10^{-2} mol L^{-1}) and SDS (5.0 x 10^{-3} mol L^{-1}).

2.3. Water samples

Four water samples were collected from different rivers in Brazil, one water sample was collected from a private well in the municipality of Araraquara (São Paulo State), and one lake surface water sample was collected near an animal farm. The samples were filtered through 0.45 μm micropore membranes (Millex-HV, Millipore) and stored in polyethylene flasks under refrigeration at 4 ^{o}C. For recovery studies, portions of each water sample were spiked with stock solutions of the three sulfonamides (alone or in combination).

2.4. Experimental design

The significant parameters were evaluated by means of experimental design methodology. Selected variables were optimized by multivariate analysis, with application of a 2^{7-3} fractional factorial design, where 2 means the minimum (-1) and maximum (+1) levels for generation of the experiments and 7 means the number of variables considered in the study. To reduce the number of experiments, without interfering in the final statistical analysis, it was decided to use a 2^{7-3} fractional factorial design, resulting in 16 experiments.

The selected variables were the concentrations of *p*-DAC (0.0011 and 0.0024%, w/v), SDS (0.005 and 0.010 mol L^{-1}), and HCl (0.0185 and 0.0550 mol L^{-1}), the pulse numbers of the sample and reagent solutions (binary experiment with 5 and 15 pulses), the pulse number of the carrier solution (10 and 60 pulses), the pulse intervals for the sample and reagent solutions (0.4 and 0.8 s), and the pulse

interval for the carrier solution (0.4 and 0.8 s). The multivariate analyses were carried out using the Minitab 16 and Statistica 8.0 software packages.

2.5. Study of interferences

An analysis of interferences was carried out considering many of the major species commonly present in water samples (Fe^{3+}, Na^{+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Mn^{2+}, NO_3^-, NO_2^-, CH_3COO^-, SO_4^{2-}, PO_4^{3-}, and Cl^-). The study of interferences for cations was performed using nitrate as a common anion, and for anions, the study was performed using sodium as a common cation. In addition, the presence of glyphosate or pharmaceuticals such as bromopride, metoclopramide hydrochloride, aceclofenac, mefenamic acid, and furosemide was also tested. Solutions containing 100 μg L^{-1} of sulfonamide and each potential interferent at concentrations 1 and 10 times greater than that of the analyte were evaluated under the same conditions.

2.6. Analytical curves

Under optimized conditions, sulfonamide standard solutions (10, 15, 25, 40, 55, 70, 85, 100, and 130 μg L^{-1}) were injected together with the reagent, in triplicate, using the multi-pumping system. Formation of the colored product was monitored at 565 nm.

2.7. Reference method

LC-MS analysis was performed using a high performance liquid chromatography system (Model 1200, Agilent Technologies) coupled to a QTRAP 3200 linear ion trap quadrupole mass spectrometer (AB SCIEX). The solvents used were H_2O:methanol:acetic acid (89:10:1) (phase A) and methanol (phase B), in gradient elution.[32]

The MS system employed positive mode electrospray ionization. A standard solution of each analyte was used at a concentration of 500 μg L^{-1} in aqueous solution, and optimization was carried out by direct infusion at 10 μL min^{-1}, using an automatic syringe. The analytes were detected by Multiple Reaction Monitoring (MRM) analysis. The optimized parameters are shown in Table 2.

Table 2

3. RESULTS AND DISCUSSION

In an acidic micellar medium, *p*-DAC reacts with sulfonamides containing primary aromatic amine groups to produce a colored compound (Schiff base) that can be measured at 565 nm. When the sulfonamide is protonated by HCl in a micellar medium, a reaction occurs between the amino group of the sulfonamide and the carbonyl group of the reagent. The effect of surfactant micelles on the condensation of aldehydes (such as *p*-DAC) with amines has been the subject of a number of publications.[33-36]

In this work, the MPFS system employed solenoid micropumps for insertion and propulsion of the microliter volumes of solutions. By using the multi-pumping flow system, the reagent consumption was low and waste generation was minimized. In addition, two strategies were used to increase the sensitivity of the MPFS method. Use of the micellar medium (SDS) caused a significant increase in the absorbance values. In the absence of SDS, the reaction was very slow. An additional strategy to increase the analytical sensitivity of the spectrophotometric measurements was the use of a liquid waveguide capillary cell (LWCC), which, in combination with the presence of SDS micelles, significantly enhanced the sensitivity.

3.1. Optimization of variables

3.1.1. Factorial design

The factorial design matrix is presented in Table 3, considering the different combinations of the factors and levels. For each variable, a lower (-1) and an upper (+1) level were selected, based on the preliminary experiments. This resulted in sixteen experiments being carried out (in triplicate) for analysis of the significance and influence of the variables. In all the experiments, the concentrations of the sulfonamides were kept constant at 100 μg L^{-1}.

Table 3

A 2^{7-3} factorial design was carried out and the resulting graph (Fig. 2) was used to visualize the estimated effects of the main factors, in terms of their magnitude and importance. In Figure 2, a statistically significant influence is indicated when the bar for a factor crosses the line corresponding to a significance level of p = 0.05. It can be clearly seen that the reagent/sample pulses was the factor that had the greatest influence, with the best results obtained when this factor was adjusted to a high level (+1). The variables with no significant effects (considering a 5% significance level) were the *p*-DAC, HCl, and SDS concentrations, the pulse number of the carrier solution, the pulse intervals of the sample and reagent solutions, and the pulse interval of the carrier solution.

Figure 2

The optimized values (in parentheses) of the variables were: *p*-DAC concentration (0.0024% w/v), SDS concentration (0.005 mol L^{-1}), HCl concentration (0.0185 mol L^{-1}), pulse number of the sample and reagent solutions (15, with the binary analysis system), pulse number of the carrier solution (60), pulse interval of the sample and reagent solutions (0.4 s), and pulse interval of the carrier solution (0.4 s).

3.2. Analytical features of the MPFS method

The analytical parameters considered were: LOD (limit of detection), LOQ (limit of quantification), precision, accuracy, and linear range. Repeatability was evaluated using intra- and inter-day results for a 100 μg L^{-1} standard solution, and the results obtained (% RSD) were 1.70 and 3.80%, respectively. These repeatability results are acceptable and show that the method can be satisfactorily applied for the analysis of sulfonamides in water samples.

Under the optimized experimental conditions, linear analytical curves were constructed using concentrations of the sulfonamides in the range 10-130 μg L^{-1}. The analytical curves and their parameters are described in Table 4.

Table 4

The LOD and LOQ values were determined according to IUPAC recommendations,[37] using the expressions 3x(s/b) and 10x(s/b), respectively, where s is the standard deviation of measurements of the blanks (n = 10) and b is the slope of the linear range. The calculated LOD and LOQ values were 3.1 and 10.1 μg L^{-1}, respectively.

3.3. Interferences

The study of interferences was carried out considering species commonly present in water samples. A difference of >5% in the absorbance signal was assumed to indicate the existence of interference.[38] No interference in the MPFS method was observed for excess levels of the ionic species, glyphosate, and some of the pharmaceuticals tested (see Section *2.5*). At room temperature (25 °C), these compounds do not react with *p*-DAC under the conditions employed in the MPFS method.

3.4. Sample analysis and recovery

The efficiency of the MPFS method was evaluated by application of the technique in the determination of sulfonamides in water samples. The results showed that only one unspiked sample presented a positive result for sulfonamides. The quantification results obtained using the MPFS and comparative methods for analysis of unspiked water samples and samples spiked with a mixture of sulfonamides are summarized in Table 5.

Table 5

Recovery analyses were performed in order to evaluate the accuracy of the technique and detect possible interferences from the matrix. Water samples were spiked with different concentrations (25, 55, 100, and 130 μg L^{-1}) of the three sulfonamides (individually or in mixtures), and each analysis was carried out in triplicate. In order to confirm the results obtained by the MPFS method, the samples containing the sulfonamides were also analyzed using the LC-MS reference method.[32] Figure 3 shows representative chromatograms obtained for water samples spiked with 55 μg L^{-1} of sulfaquinoxaline, sulfathiazole, and sulfadimethoxine, respectively. Table 6 shows the recovery results for the MPFS and comparative LC-MS methods, indicating good accuracy and an absence of matrix effects. Good agreement between the two methods was found by application of the Student's paired t-test, with values below the tabulated value indicating that there was no significant difference between the two methods. The technique developed here therefore represents a valuable tool that could be used for the fast screening of sulfonamides.

Figure 3

Table 6

4. CONCLUSIONS

A simple and rapid method was developed for the determination of sulfonamides in water samples, using a multi-pumping flow system coupled to a liquid waveguide capillary cell for increased sensitivity. Most of the existing methods for such measurements require an initial sample cleanup step, resulting in slow analyses. An important advantage of the new technique is that it is possible to determine sulfonamides in water without the need for cleanup. Good accuracy (recoveries of 78.4 to 109.2% for sample A and 81.6 to 108.7% for sample B) and an absence of matrix effects were found

for the developed procedure, as well as high sensitivity and speed, showing that it can be used as a useful tool for the fast screening of sulfonamides at low concentrations in water samples. Additional advantageous features of the MPFS method include automated analysis, no use of organic solvents, low reagent consumption, and minimal waste generation (about 1.85 mL per analysis). This MPFS system therefore constitutes a valuable addition to green analytical methodologies.

ACKNOWLEDGEMENTS

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398 **FIGURE CAPTIONS**

399

400 Fig. 1 Schematic illustration of the multipumping flow system. RC: reaction coil (80 cm); X:
401 confluence point; P1: *p*-DAC (0.0024%, w/v) in HCl (0.0185 mol L^{-1}); P2: sample/standard solutions
402 in HCl (0.0185 mol L^{-1}) and SDS (0.005 mol L^{-1}); P3: SDS (0.005 mol L^{-1}) and HCl (0.0185 mol L^{-1});
403 D: detector (LWCC pathlength = 100 cm); W: waste.

404

405 Fig. 2 Estimated effects graph, showing the influence of the different system variables.

406

407 Fig. 3 Representative chromatograms for real spiked water samples. A: spiked sample with 55 μg L^{-1}
408 of sulfaquinoxaline; B: spiked sample with 55 μg L^{-1} of sulfathiazole; C: spiked sample with 55 μg L^{-1}
409 of sulfadimethoxine.

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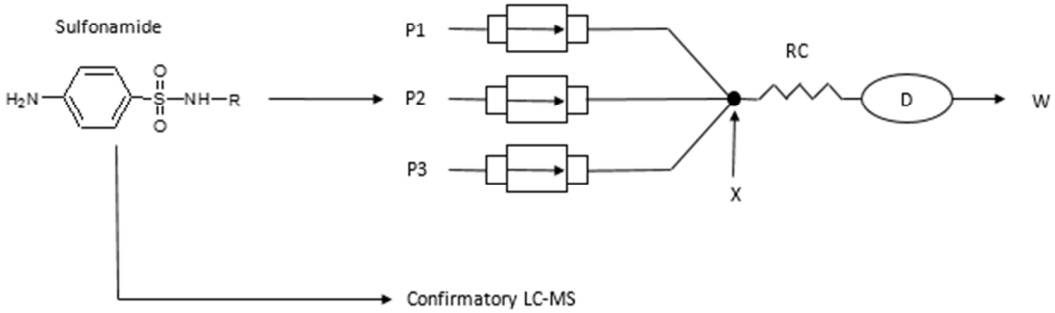
Graphical Abstract



An eco-friendly method using multi-pumping flow system was developed in this work for the determination of sulfonamides in water.

446

447 **FIGURES**

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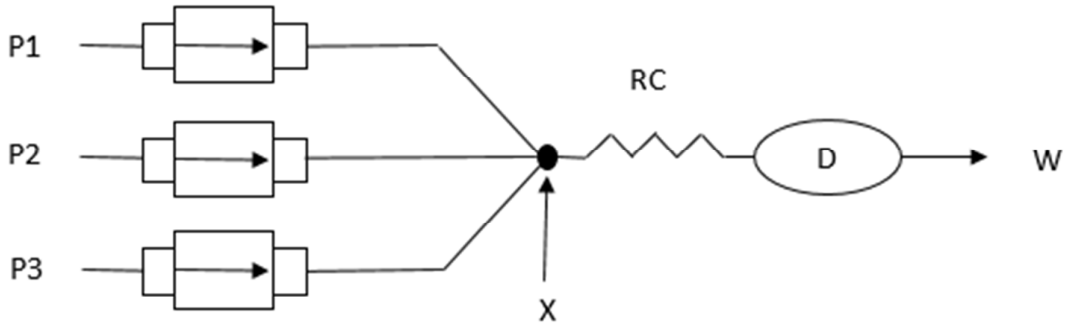

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452 D: detector (LWCC pathlength = 100 cm); W: waste.

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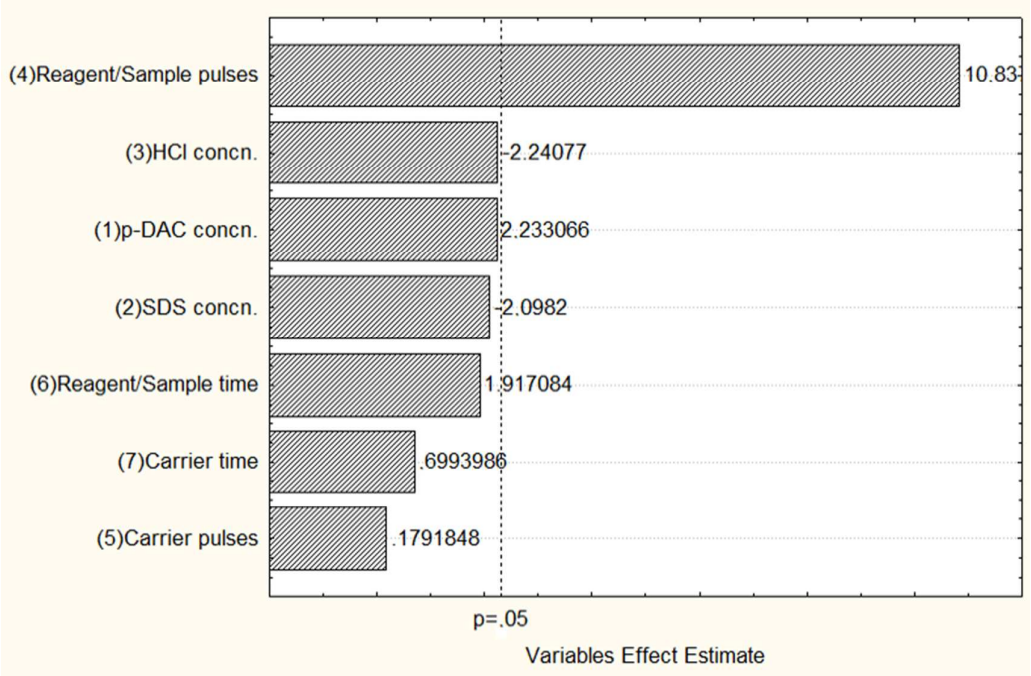

Fig. 2 Estimated effects graph, showing the influence of the different system variables.

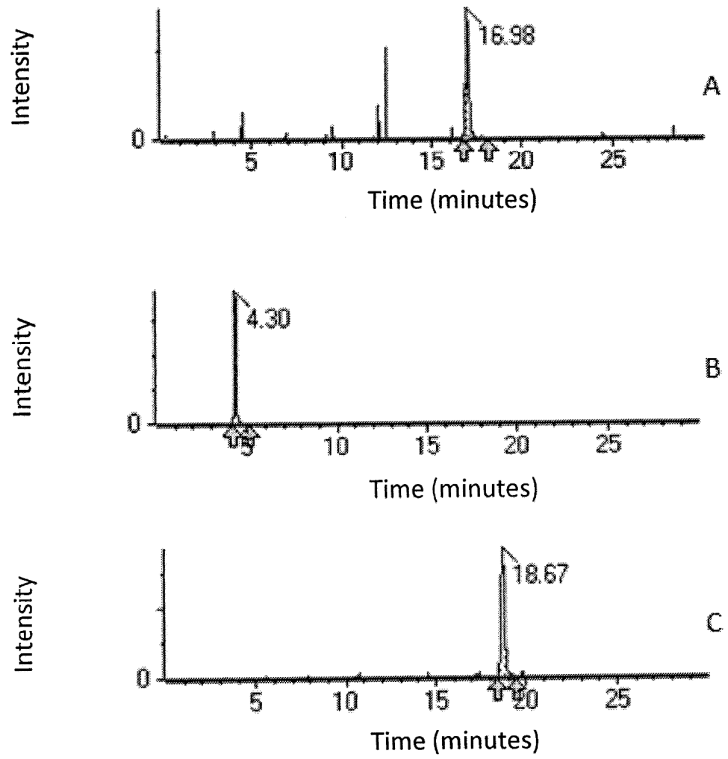

Fig. 3 Representative chromatograms for real spiked water samples. A: spiked sample with 55 μg L^{-1} of sulfaquinoxaline; B: spiked sample with 55 μg L^{-1} of sulfathiazole; C: spiked sample with 55 μg L^{-1} of sulfadimethoxine.

TABLES

Table 1. Multipumping system parameter values for the determination of sulfonamides.

Step	Pump volume delivered per stroke (µL)			Number of pulses	Time per pulse (s)	Action
	P1	P2	P3			
1	10	20	-	15	0.4	Binary sampling, introduction of sample and reagent solutions
2	-	-	20	70	0.4	Transport of the reaction mixture to the LWCC and signal recording

Table 2. Optimized parameters for the LC-MS method.

Analyte	Declustering potential (DP) (V)	Entrance potential (EP) (V)	Cell entrance potential (CEP) (V)	MRM[a] 1 *m/z* $[M+H]^+$	Collision energy (V)	MRM[a] 2 *m/z* $[M+H]^+$	Collision energy (V)	MRM[a] 3 *m/z* $[M+H]^+$	Collision energy (V)
STZ	21	9	14	256>156	17	256>92	35	256>108	27
SDX	31	9	18	311>156	27	311>92	45	311>108	39
SQX	36	8	16	301>156	19	301>92	43	301>65	69

[a] Multiple Reaction Monitoring.

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555 Table 3. Fractional factorial design matrix (2^{7-3}).

Experiments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sample/reagent pulse number [a]	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Carrier pulse number [b]	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Sample/reagent pulse interval [c] (s)	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Carrier pulse interval [d] (s)	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
[p-DAC] [e] (% w/v)	-	+	+	-	+	-	-	+	-	+	+	-	+	-	-	+
[HCl] [f] (mol L^{-1})	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	+
[SDS] [g] (mol L^{-1})	-	+	-	+	+	-	+	-	+	-	+	-	-	+	-	+

556 [a] -1 for 5 and +1 for 15. [d] -1 for 0.4 and +1 for 0.8. [g] -1 for 0.005 and +1 for 0.010.
557 [b] -1 for 15 and +1 for 60. [e] -1 for 0.0011 and +1 for 0.0024.
558 [c] -1 for 0.4 and +1 for 0.8. [f] -1 for 0.0185 and +1 for 0.0550.
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Table 4. Figures of merit of the MPFS method.

Compound	Linear range	R*	Linear equation	LOD (μg L^{-1})	LOQ (μg L^{-1})	Wavelength
SQX	10-130 μg L^{-1}	0.9994	$A=0.0491+0.0096C_{SQX}$	3.10	9.50	565 nm
SDX		0.9995	$A=0.0593+0.0067C_{SDX}$	3.50	10.1	
STZ		0.9997	$A=0.0430+0.0052C_{STZ}$	3.20	9.70	

*Correlation coefficient based on linear equation.

Table 5. Results obtained using the developed MPFS method and the confirmatory method in the analysis of unspiked water samples and samples spiked with a mixture of sulfonamides.

Samples		MPFS method ($\mu g\ L^{-1}$)	LC-MS method ($\mu g\ L^{-1}$)
Sample 1	(us)	(-)	(-)
	(s)	(+) 60.0 ± 0.05	56.4 ± 0.11
Sample 2	(us)	(-)	(-)
	(s)	(+) 57.7 ± 0.04	51.2 ± 0.10
Sample 3	(us)	(-)	(-)
	(s)	(+) 59.4 ± 0.04	54.4 ± 0.09
Sample 4	(us)	(-)	(-)
	(s)	(+) 57.7 ± 0.05	56.3 ± 0.10
Sample 5	(us)	(-)	(-)
	(s)	(+) 57.7 ± 0.03	57.1 ± 0.06
Sample 6	(us)	(+) (<LOQ; >LOD)	< 3.70*
	(s)	(+) 58.4 ± 0.04	60.1 ± 0.10

(us) unspiked and (s) spiked samples (25 $\mu g\ L^{-1}$ SQX + 15 $\mu g\ L^{-1}$ STZ + 15 $\mu g\ L^{-1}$ SDX).
(-) undetected; (+) detected; * LOQ (LC-MS method) = 3.70.
Student's paired t-test, calculated value = 2.075 and tabulated value = 2.571 (95% confidence level).
Samples 1-4: river water; Sample 5: well water; Sample 6: lake surface water near an animal farm.

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623 Table 6. Recovery data for sulfonamides spiked in water samples.

Sample and Compound	Added value ($\mu g\ L^{-1}$)	MPFS method		LC-MS method	
		Found value ($\mu g\ L^{-1}$)	Recovery (%)	Found value ($\mu g\ L^{-1}$)	Recovery (%)
Sample A[a]					
SQX	25	24.6	98.4	21.3	85.2
	55	55.3	100.5	57.8	105.1
	100	97.0	97.0	93.7	93.7
	130	127.9	98.4	120.0	92.3
STZ	25	23.7	94.8	27.3	109.2
	55	46.4	84.4	49.1	89.3
	100	91.9	91.9	91.3	91.3
	130	118.5	90.8	118.7	91.3
SDX	25	23.4	91.2	19.6	78.4
	55	54.5	99.1	51.1	92.9
	100	95.7	95.7	98.3	98.3
	130	128.8	99.1	124.7	95.9
9 $\mu g\ L^{-1}$ SQX + 8 $\mu g\ L^{-1}$ STZ + 8 $\mu g\ L^{-1}$ SDX	25	22.9	91.6	25.1	100.4
25 $\mu g\ L^{-1}$ SQX + 15 $\mu g\ L^{-1}$ STZ + 15 $\mu g\ L^{-1}$ SDX	55	57.7	104.9	51.2	93.1
40 $\mu g\ L^{-1}$ SQX + 30 $\mu g\ L^{-1}$ STZ + 30 $\mu g\ L^{-1}$ SDX	100	103.8	103.8	103.2	103.2
50 $\mu g\ L^{-1}$ SQX + 40 $\mu g\ L^{-1}$ STZ + 40 $\mu g\ L^{-1}$ SDX	130	138.9	106.8	139.9	107.6
Sample B[b]					
SQX	25	21.3	85.2	20.4	81.6
	55	59.3	107.8	59.8	108.7
	100	91.3	91.3	92.0	92.0
	130	138.7	106.7	140.1	107.8
STZ	25	24.1	96.4	22.7	90.8
	55	55.8	101.5	51.5	93.6
	100	93.4	93.4	93.4	93.4
	130	129.5	99.6	128.7	99.0
SDX	25	21.7	86.8	21.0	84.0
	55	58.2	105.8	57.7	104.9
	100	98.1	98.1	99.3	99.3
	130	127.4	98.0	123.3	94.8
9 $\mu g\ L^{-1}$ SQX + 8 $\mu g\ L^{-1}$ STZ + 8 $\mu g\ L^{-1}$ SDX	25	24.9	99.6	23.3	93.2
25 $\mu g\ L^{-1}$ SQX + 15 $\mu g\ L^{-1}$ STZ + 15 $\mu g\ L^{-1}$ SDX	55	57.7	104.9	57.1	103.8
40 $\mu g\ L^{-1}$ SQX + 30 $\mu g\ L^{-1}$ STZ + 30 $\mu g\ L^{-1}$ SDX	100	98.0	98.0	94.1	94.1
50 $\mu g\ L^{-1}$ SQX + 40 $\mu g\ L^{-1}$ STZ + 40 $\mu g\ L^{-1}$ SDX	130	135.2	104.0	137.7	105.9

624 [a] River water sample [b] Well water sample

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