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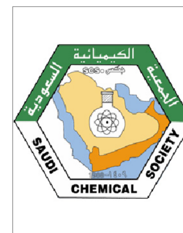

SPECIAL ISSUE: ENVIRONMENTAL CHEMISTRY

Fluorescence detection and identification of eight sulphonamides using capillary electrophoresis on released excipients in lake water

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KEYWORDS

Capillary electrophoresis;
Sulphonamides;
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Abstract A simple and sensitive capillary electrophoresis method with fluorescence detection was developed for the determination of sulphanilamide, sulphamerazine, sulphacetamide and sulphanilic acid, sulphathiazole, Sulphisomidine, sulphadoxine and sulphadiazine in lake water. The sulphonamides were extracted from lake water, derivatized with fluorescamine and determination of sulphonamide was achieved using 20 mM borate buffer of pH 9.5 at an applied voltage of 25 kV. Detection was performed using UG-11 excitation filter of 405 nm and 495 nm emission filters. A fast, simple and sensitive method with limit of detection in the range 0.89–1.43 n mol L^{-1} for all the four sulphonamides with good recoveries 80–110% is seen. Inter-day and intra-day validation of the separation method shows fairly good results. The detection and quantification limits for this newly developed method are too low to determine drug residues in lake water.

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1. Introduction

Sulphonamide cluster of medicine are N-derivatives of 4-amino-benzene-sulphonamide a diverse category of synthetic antibiotics. There in depth use in veterinary diseases for therapeutic growth and promotion results their presence as residue in animal tissues and lake water (Coly et al., 2005; Babić et al., 2006; Wang et al., 2007). Sulphonamides contaminants within the surroundings occur thanks to disposal of expired or unused drug by human and animal excretion (Long et al., 1990). As a result there's a risk of medicine remaining in edible animal merchandise like milk, meat, fish and egg (Lu et al., 2007; Huang et al., 2007; Fang et al., 2006; Posyniak et al. 2005). Hence, there's a requirement of quick correct and sensitive analytical strategies for observance sulphonamides residues in lake water and aquatic animals.

A number of the most important issues regarding this continual input to the surroundings embody potential persistent effects to non-target organisms (Fent et al., 2006). As their in depth usage, sizeable attention has been paid to the poten-

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tial human health risk thanks to their cancer efficiency and potential antibiotic resistance (Christian et al., 2003; Hirsch et al., 1999). To know the prevalence, fate and effects of those compounds within the surroundings remains happening (Richardson and Ternes, 2005). To know the behaviour of Sulphonamide within the surroundings, reliable and sensitive measure strategies should be established by measuring capillary electrophoresis process.

Sulphonamides have oftentimes been detected in n mol L^{-1} to μ mol L^{-1} concentration target lake water and lake water fishes (Sun et al., 2009; Montiu et al., 2007). Varied chromatographically strategies are developed for the analysis of sulphonamides among them are high-performance liquid chromatography (HPLC) with UV detector, (Lin and Huang, 2008) amperometry, (Preechaworapun et al., 2006) or mass qualitative analysis detection, (Msagati and Nindi, 2004; Heinig and Henion, 1999) and Gas chromatography (GC) for sensitive and selective determination of antibacterial drug, (Chiavarino et al., 1998). CE has been established to possess inherent blessings over HPLC and GC owing to its high sensitivity, high potency, short analysis time, low sample demand and low solvent consumption with minimum waste generation.

Capillary electrophoresis is one amongst the simplest instruments to find sulphonamides in tissue and in lake water. Capillary activity with UV detector has been used ordinarily for the determination of sulphonamides. But, determination of sulphonamides by capillary activity with fluorescence detector has been seldom used. lots of labour has been revealed exploitation UV detector, (Fuh and Chu, 2003; Lin et al., 1997; Hows et al., 1997), amperometric detection (Wang et al., 1999), applying the CZE mode (Ackermans et al., 1992; Lin et al., 1996; Berzas Nevado et al., 2001; Teshima et al., 2004) and micellar electrokinetic electrokinetic chromatography (MEKC) Lin et al., 1996; Dang, 1992; Reeves, 1999; Lamba et al., 2005 in numerous matrices like pharmaceutical compounds, biological fluids, or food of animal origin. The drawbacks of those strategies are that they need long analysis time and have low limit of detection (LOD). though exploitation MEKC, at high hydrogen ion concentration this was terribly high attributable to joule heating and therefore the bottom line separation was not attainable for few sulphonamides (Lamba et al., 2005; Stein et al., 1974), within the gift work, the relevancy of CE methodology for the analysis of residual sulphonamides in lake water has been evaluated involving presample derivatization with fluorescamine detection (Lara et al., 2009; Wang et al., 2012; Tong et al., 2013; Hruska and Franek, 2012).

Sulpha drug may be a variety of bactericide used since 1960. The drug is employed for treatment of urinary tract infection and respiratory infection. Despite its Unfavorable distribution in general body it's wide used throughout whole world. The exposure of this drug ends up in numerous major issues like cancer. A large range of studies show that patients World Health Organization consume antibacterial sulpha drugs unceasingly suffer from cancer as a result of its carcinogenicity. A number of these publications are as follow-up periods of up to 7 years, 9 years and 15 years. An oral dose of 1.0 g/kg sulpha drug was absorbed readily in mammals. Peak plasma concentration of approximately 1.0 mg/mL was achieved 1 h after administration. In human, high concentrations of antibacterial drug medicine were found in numerous organs like excretory organ, lung, liver, spleen and brain. The speed of elimination of the drug from these tissues paralleled that from blood.

Comparison of different method with present study.

Article topic	Methodology	Detector	Disadvantage/ advantage
Pressurized liquid extraction combined with capillary electrophoresis–mass spectrometry as an improved methodology for the determination of sulphonamide residues in meat • Guillermina Font, • Ana Juan-García, • Yolanda Picó	*r* between 0.996 and 0.997), precision (RSD < 14%) and recoveries (from 76% to 98%). The limits of detection and quantification (below 12.5 and 46.5 μg kg^{-1}	MS^2 experiments using an ion trap as analyser	Highly expensive, time consuming, low LOD
Use of capillary electrophoresis with laser-induced fluorescence detection to screen and liquid chromatography–tandem mass spectrometry to confirm sulphonamide residues: validation according to European Union 2002/657/EC	Six sulphonamide detection, Samples were prepared by homogenizing the tissue, with sodium hydroxide and acetonitrile. After evaporation, extracts were injected in the capillary electrophoresis	Tandem mass spectrometry confirmatory assay	Highly expensive, time consuming, heavy clean up procedure
Determination of sulphonamide residues in water samples by in-line solid-phase extraction-capillary electrophoresis	An analyte concentrator was constructed, based on the introduction of a small portion of a solid-phase extraction sorbent into the electrophoretic capillary to carry out an in-line concentration step, improving sensitivity. Five sulphonamides were detected	UV–vis	Analyte concentrator was constructed an additional instrument for routine use with filter
Present study	Eight sulphonamide pre column derivatization of sulphonamide with fluorescamine	Fluorescamine detector	6 min run time for result

2. Experimental

2.1. Chemicals and reagents

Sulphanilamide, sulphamerazine, sulphacetamide, sulphanilic acid, sulphathiazole, sulphamethoxazole, sulphaguanidine, sulphadiazine and sodium acetate were obtained from Sigma, St. Louis, MO, USA. Boric Acid was from Acros Organics, Springfield, NJ, USA. Methanol (HPLC grade), hydrochloric acid (analytical reagent grade), acetone (HPLC grade) were from Ranbaxy, Ropar, India. Glacial acetic acid and sodium hydroxide were obtained from E. Merck, Mumbai, India. Fluorescamine {4-phenylspiro[fura(3H),1-phthalan]-3,3′dione} was obtained from Fluka, Buchs, Switzerland, its 10 mM solution in acetone was prepared daily.

Stock solutions of sulphonamides, 10^{-2} M, were ready by dissolving correct quantity of the sulphonamides in 3 mL of 3 M HCl and created up to 10 mL with distilled deionized water. All operating standards were ready by diluting glorious aliquots of the stock resolution with milli pore water. The solutions were unbroken in dark at concerning 4 °C and located to be stable for a minimum of 1 month. Acetate buffer of 15 mM, pH 4 was used to conduct the derivatization reaction. The buffer used for the separation of sulphonamides was 20 mM borate buffer at pH 9.5.

Sulphanilamide, sulphamerazine, sulphacetamide, sulphanilic acid, sulphathiazole, sulphamethoxazole, sulphaguanidine, sulphadiazine and Na acetate were obtained from alphabetic character, St. Louis, MO, USA. Chemical element acid was from Acros Organics, Springfield, NJ, USA. Wood spirit (HPLC grade), acid (analytical chemical agent grade), dissolving agent (HPLC grade) were from Ranbaxy, Ropar, India. Glacial carboxylic acid and hydrated oxide were obtained from E. Merck, Mumbai, India. Fluorescamine was obtained from Fluka, Buchs, Schweiz, its 10 mM solution in dissolving agent was prepared daily.

2.2. Instrumentation and separation conditions

A Prince-C 255 instrument with programmable injector and high voltage source (Prince Technologies, The Netherlands) was used as instrumental analysis. Separation was carried out at 25 kV applied voltage. AN ARGOS 250 B instrument (Flux Instruments, Switzerland) equipped with a 75 W Xenon-mercury lamp was used as light detector. Hydrodynamically sample introduction by applying pressure of 40 mbar for 6 s. The excitation light was filtered through a Schott glass UG-11 filter and an emission wave length of 495 nm and excitation filter of 405 nm was applied for the limited light. For data processing, DAX 7.1 data acquisition and analysis software (Prince Technologies, The Netherlands) was used for analysis. Fused silica capillary 50.3 cm × 75 μm internal diameter was used as separation column. A detection window was created by burning off the coating at 35.2 cm from capillary inlet.

2.3. Sample analysis

The method was analysed with spiked lake water samples. Water samples were collected from a lake in Bhavnagar (city), Gujarat, India, and unbroken at 20 °C during a white goods once not in use. The samples were filtered at temperature through a 0.45 μm nylon membrane filter and subjected to derivatization and analysed by Capillary activity.

2.4. Derivatization procedure

A 100 μL portion of binary compound sample containing 10^{-5} mol L^{-1} of sulphonamides, and sulphanilic acid, 860 μL of acetate buffer of pH 4 and 40 μL of 40 mM fluorescamine solution were mixed throughly. The mixture was allowed to for 5 min at close temperature for complete derivatization then injected into capillary.

3. Results and discussion

3.1. Derivatization reaction conditions

3.1.1. Impact of pH

All the sulphonamides at 1 mol L^{-1} were optimally derivatized over the pH 3–10 (Fig. 1). At higher pH, as a result of probable

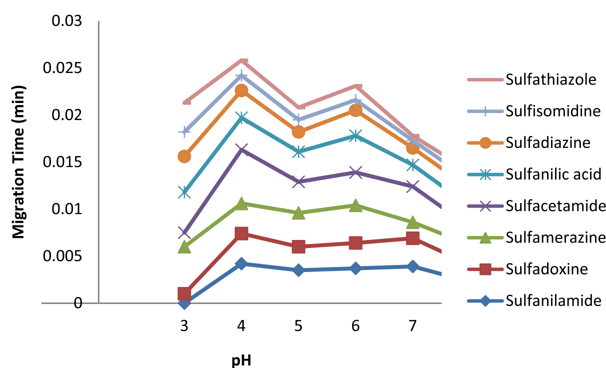

Figure 1 Effect of pH on derivatized Sulphonamides with fluorescamine. Background electrolyte is borate buffer of 9 pH. Applied voltage: 25 kV. Line diagram of different sulpha drugs are 1, sulphanilamide; 2, sulphamerazine; 3, sulphacetamide; 4, sulphanilic acid 5, sulphathiazole; 6, sulphamethoxazole; 7, sulphaguanidine; 8, sulphadiazine.

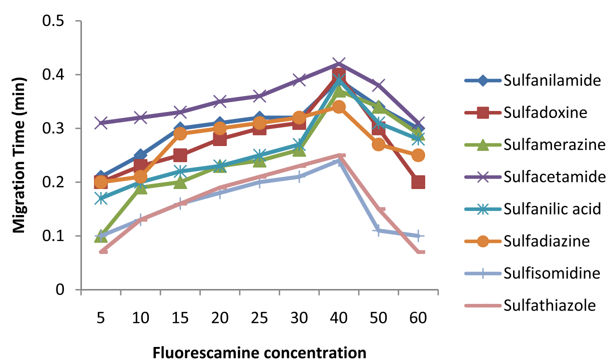

Figure 2 Effect of fluorescamine on sulphonamide of different concentrations. Background electrolyte is borate buffer of 9 pH. Applied voltage: 25 kV. Line drawing of sulphonamides drugs are1, sulphanilamide; 2, sulphamerazine; 3, sulphacetamide; 4, sulphanilic acid 5, sulphathiazole; 6, sulphamethoxazole; 7, sulphaguanidine; 8, sulphadiazine.

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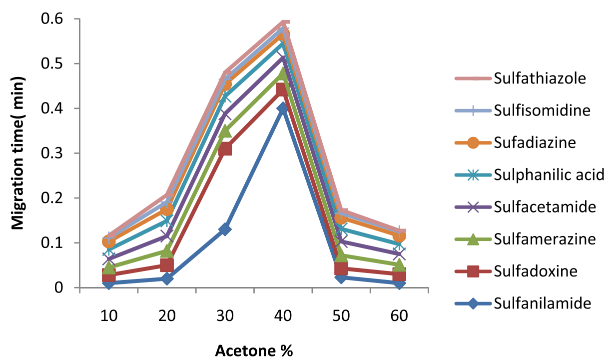

Figure 3 Effect of acetone percentage on the reaction performance. Background electrolyte is borate buffer of 9 pH. Applied voltage: 25 kV. Peaks: 1, sulphanilamide; 2, sulphamerazine; 3, sulphacetamide; 4, sulphanilic acid 5, sulphathiazole; 6, sulphamethoxazole; 7, sulphaguanidine; 8, sulphadiazine.

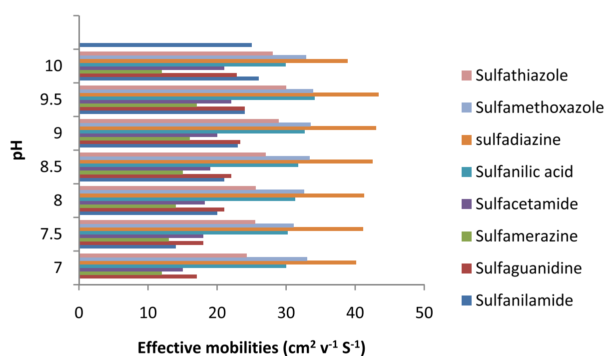

Figure 4 Effective mobility of sulphonamides obtained between pH 7.0 and 10. Background electrolyte is borate buffer of 9 pH. Applied voltage: 25 kV. Sulpha drugs are 1, sulphanilamide; 2, sulphamerazine; 3, sulphacetamide; 4, sulphanilic acid 5, sulphathiazole; 6, sulphamethoxazole; 7, sulphaguanidine; 8, sulphadiazine.

chemical reaction decrease in peak space of fluorescamine. The amino group of sulphonamides was protonated, and did not bear reaction with fluorescamine as a result of low pH vary, (Reeves, 1999). Therefore, 15 mM acetate buffer of pH 4 was used for derivation reaction, because it offers the utmost yield.

3.1.2. Effect of fluorescamine

To study the effect of fluorescamine concentration on derivatization of sulphonamides, 05–40 mM concentration of fluorescamine reagent reacted with 1 μ mol L^{-1} fixed amount of sulphonamides. It was found that 20 mM fluorescamine was sufficient to achieve the maximum peak areas (Fig. 2). It was also observed that on using higher concentration of derivatizing agent the reaction mixture became turbid and the peak areas decreased due to fluorescence quenching by the hydrolysis product of fluorescamine (Stein et al., 1974).

3.1.3. Effect of acetone concentration

Acetone was observed to slow down the reaction of sulphonamide with fluorescamine (Lamba et al., 2005; Stein et al., 1974), from several min to several hours. Effect of acetone concentration on the fluorescence response of derivatives was investigated as shown in Fig. 3. There was a marginal increase in fluorescence intensity in the medium containing acetone around 40%.

3.2. Separation conditions

3.2.1. Effect of pH

The separation of derivatized sulphonamides at pH 7–10 was studied at 25 kV applied voltage in CE system. As indicated in Fig. 4, the optimum separation pH was determined to be 9.5. A 20 mM borate buffer of pH 9.5 was used for separation of all eight sulphonamides (Fig. 5).

3.2.2. Effect of injection volume

Increasing injection volume gave better limit of detection but it seriously affected the resolution. Thus, a compromise was needed between the limit of detection and peaks resolution

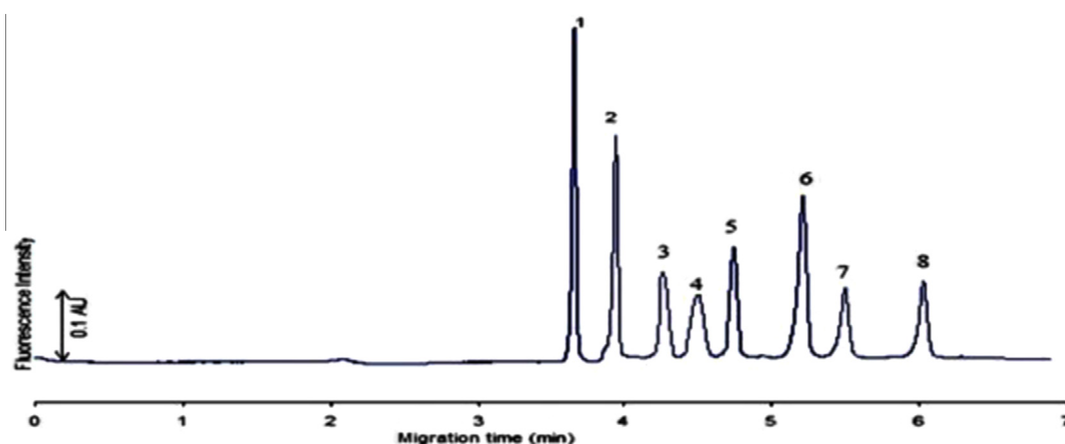

Figure 5 Separation by capillary zone electrophoresis of fluorescamine derivatives of sulphonamides. Sample concentration: 10^{-6} mol L^{-1}. Peaks: electro-osmotic flow; sulphanilamide; 2, sulphamerazine; 3, sulphacetamide; 4, sulphanilic acid 5, sulphathiazole; 6, sulphamethoxazole; 7, sulphaguanidine; 8, sulphadiazine.

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Table 1 Calibration curves and detection limits.

Sulphonamide	SD* ($y = mx$ [a] $+ c$)	r^2	LOQ[e]	LOD[d]
Sulphanilamide	0.41	0.9919	2.94	0.97
Sulphamerazine	1.65	0.9910	6.25	2.06
Sulphacetamide	3.31	0.9973	10.41	3.43
Sulphanilic acid	2.09	0.9901	5.72	1.89
Sulphathiazole	1.61	0.9384	7.16	2.36
Sulphamethoxazole	1.30	0.9875	5.06	1.67
Sulphaguanidine	0.64	0.9743	2.04	0.67
Sulphadiazine.	1.56	0.9548	7.14	2.36

Calibration graph constructed over ten concentration levels; results are the averages of three replicate analyses.
y = peak area.
[a] x = concentration, mol L^{-1}.
[b] Standard deviation in intercept.
[c] Standard deviation in slope.
[d] LOD = limit of detection ($S/N = 3$), nmol L^{-1}.
[e] LOQ = limit of quantitation ($S/N = 10$) nmol L^{-1}.
* SD (Standard Deviation) = SD[b](a**) + SD[c](b***).
** a = intercept.
*** b = slope.

Table 2 Precision of analytical method.

Sulphonamides	RSD (%) of corrected peak area	
	Intra-day	Inter-day
Sulphanilamide	1.05	1.56
Sulphamerazine	2.30	2.49
Sulphacetamide	1.27	4.82
Sulphanilic acid	2.35	4.93
Sulphathiazole	3.84	1.17
Sulphamethoxazole	1.02	1.45
Sulphaguanidine	2.45	1.29
Sulphadiazine	1.03	1.67

All results are the averages of three replicate analysis.

while optimizing the sample volume injection. Hydrodynamic injection of 40 nl of sample at 40 mbar for 6 s was found to be the best compromise between the two parameters.

3.2.3. Features of merit

The linearity range for eight sulphonamides derivatives was verified by determining different concentrations of each sulphonamide when a rectilinear calibration graph was obtained over the range 100-1000 nmol L^{-1} of sulphonamides (Table 1). The inter-day and intra-day repeatability data of corrected peak areas are given in Table 2. Inter-day repeatability was measured over a period of 5 days. The average RSD obtained was ⩽1.913% for intra-day and ⩽2.42% for inter-day which recommended that the method can be used in routine process.

The limit of detection ($S/N = 3$) for all the eight sulphonamide was found to be between 0.67 and 3.43 nmol L^{-1}, and the limit of quantification ($S/N = 10$) was found in the range 2.04–10.41 nmol L^{-1} (Table 1).

3.2.3.1. Precision of analytical method. Determination of Precision of the analytical method is by comparing the effective mobilities of sulphonamide standards. The method was effectively performed for validation study.

3.2.4. Recovery studies

The recovery of sulphonamides by the present method was determined by comparing the peak areas of analyte in spiked water samples with that of the standards containing the same amount of sulphonamides. For recovery experiments in lake water sample, three replicate analyses of samples spiked at 5 and 10 µmol L^{-1} were done. The average recovery ranged to 80–110% with the RSD in range 0.99–4.19% (Table 3). Fig. 6 shows the chromatograms obtained for spiked and unspiked lake water samples.

3.3. Applicability of method

Because sulphonamides are soluble in water and having chelating effect with soil, and have potential to enter the ground water quickly. Sulphonamides can be quantitated easily from any water source using a variety of extraction methods, but the goal of present method is to screen out large numbers of sulphonamide to find out potential carcinogenic markers of sulpha group. Clean up procedure is small enough to get good chromatographic peak in both unspiked and spiked condition. The method can be used as routine process for determination of level of sulpha drugs in aquatic organisms and drinking water.

4. Conclusions

Sulphonamides square measure found as terribly persistent residues at trace within the effluents of lake water. These resi-

Table 3 Recovery of sulphonamides in spiked lake water samples.

Sulphonamide	Recovery (%)			
	5 µmol L^{-1}		10 µmol L^{-1}	
	Average ± SD	RSD (%)	Average ± SD	RSD (%)
Sulphanilamide	95.98 ± 2.20	1.29	102.80 ± 3.76	3.66
Sulphamerazine	97.52 ± 0.46	0.99	101.91 ± 1.21	1.42
Sulphacetamide	101.33 ± 2.11	4.19	97.16 ± 2.13	1.98
Sulphanilic acid	93.86 ± 0.44	1.47	91.23 ± 0.88	1.90
Sulphathiazole	92.14 ± 4.32	8.91	105.61 ± 4.91	3.29
Sulphamethoxazole	101.08 ± 1.32	2.31	91.87 ± 1.75	1.02
Sulphaguanidine	88.12 ± 1.29	3.69	86.69 ± 2.60	1.71
Sulphadiazine.	110.73 ± 3.82	2.30	91.42 ± 0.92	1.43

All results are the averages of three replicate analyses.

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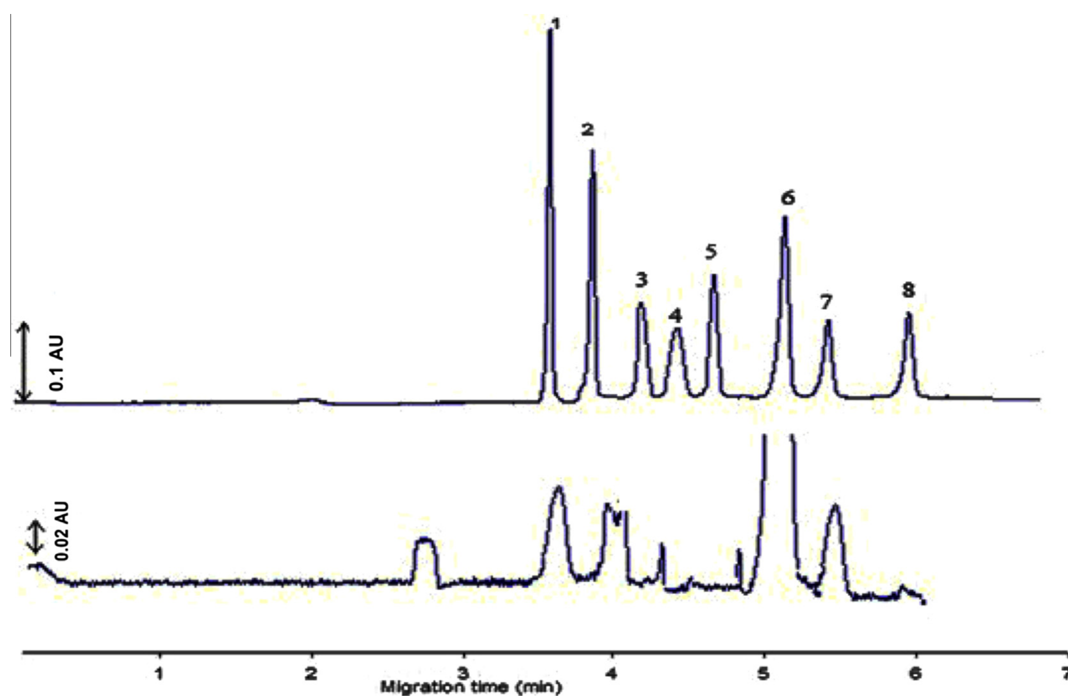

Figure 6 The electropherogram of lake water samples, (A) spiked with sulphonamides at 10 μmol L^{-1}. and (B) unspiked sample. Electro-osmotic flow; other peaks designation and operating conditions of 1, sulphanilamide; 2, sulphamerazine; 3, sulphacetamide; 4, sulphanilic acid 5, sulphathiazole; 6, sulphamethoxazole; 7, sulphaguanidine; 8, sulphadiazine.

dues square measure discharged into the surface waters where they are conjointly detected at concentrations up to the mg/l level in samples collected from many lakes, and rivers. Many of the sulpha drug excipients were known as markers for sewage contamination in surface and ground waters because of their persistence and since they are not significantly assimilated in sewerage sludges or aquatic organisms. An alternative strategies for groundwater measures employed in beverage production, designated compounds will leach from the contaminated watercourses into the groundwater aquifers. Reckoning on the methodologies used for drinking water purification and excipients free may additionally seem at trace-level concentrations at the low ng/l level in water also in tissues of organism that ends up in carcinogenic effect. Underneath the optimized conditions, separation of eight sulphonamides by CE was complete in 6 min. The detection and quantification limit at low nmol L^{-1} level was adequate to verify residues of those medicines in lake water.

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