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Trace analysis of sulfonylurea herbicides in water by on-line continuous flow liquid membrane extraction–C_{18} precolumn liquid chromatography with ultraviolet absorbance detection

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Abstract

An on-line system that consists of continuous-flow liquid membrane extraction (CFLME), C_{18} precolumn, and liquid chromatography with UV detection was applied to trace analysis of sulfonylurea herbicides in water. During preconcentration by CFLME, five target compounds, including metsulfuron methyl, bensulfuron methyl, tribenuron methyl, sulfometuron methyl, and ethametsulfuron, were enriched in 960 μl of 0.5 mol l^{-1} Na_2CO_3–$NaHCO_3$ (pH 10.8) buffer used as acceptor. This acceptor was on-line neutralized and transported to the C_{18} precolumn where the analytes were absorbed and focused. Then the focused analytes were injected onto a C_{18} analytical column for separation and detection at 240 nm. The proposed method was applied to determine sulfonylurea herbicides in water, river, and reservoir water with detection limits of 10–50 ng l^{-1} when enriching a 120-ml sample. Throughput is typically one sample per hour.
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Keywords: Trace analysis; Water analysis; Continuous-flow liquid membrane extraction; Sulfonylureas; Pesticides

1. Introduction

Sulfonylureas are widely used herbicides that have characters of high selectivity, very low dosage rates (4–20 g active ingredient per hectare) and acute mammalian toxicity (LD_{50}>4000 mg kg^{-1}) [1]. Because of the low dose used and chemical instability, sulfonylureas are present at very low concentrations in environmental waters, which presents a challenge for their determination in recipient waters.

Although many methods such as gas chromatography [2], supercritical fluid chromatography [3], capillary electrophoresis [4] and immunoassay [5] have been proposed for analysis of sulfonylureas in various matrices, liquid chromatography (LC) [1,4,6–10] is the most commonly used one because of the polarity and thermal instability of sulfonylureas. LC–mass spectrometry (MS) methods [1,4,7–10], which have the advantages of high sensitivity and higher degree of selectivity, have been used increasingly during the last few years. However, the LC–MS instrument is very expensive and unavailable for most environmental laboratories. Therefore, developing inexpensive LC–UV methods

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with sufficient selectivity and sensitivity for routine analysis of sulfonylureas is attractive.

Due to the low level present, clean-up and enrichment before analysis are necessary and become a crucial step for the determination of sulfonylureas in environmental samples. A number of methods have been reported for the enrichment of these compounds, and solid-phase extraction (SPE) is the most widely used one [1,4,7–9].

Supported liquid membrane (SLM) extraction is an alternative trace-enrichment technique with high degree of sample clean-up. SLM has been coupled on-line with LC for the determination of sulfonylureas [6]. However, it suffers from relatively low enrichment rate.

In our previous work, continuous-flow liquid membrane extraction (CFLME) was developed for trace enrichment of sulfonylureas [11,12] with high enrichment rate. To obtain the same detection limits for the two studied sulfonylureas (50–100 ng l^{-1}), the enrichment sample volume and time were 20 ml and 10 min for CFLME [12], and 250 ml and 5 h for SLM [6], respectively.

In this present study, CFLME and a C_{18} precolumn were combined for high sensitivity and selective sample enrichment, and further coupled on-line with LC–UV for the trace determination of sulfonylureas in water.

2. Experimental

2.1. Apparatus and materials

Fig. 1 shows the schematic diagram of the CFLME–C_{18}-LC system. P1 and P2 are the two peristaltic pumps, and V2 is the six-port valve of the FIA 5020 Analyzer (Tecator, Sweden). P3 is a WZ-50G microinfusion pump (The Medical Instrument Factory of Zhejiang University, China), and P4 is a MiniPump (Laboratory Date Control, Division of Milton Roy Company). V1 is the laboratory-made six-port valve, and V3 is a 7725 injector (Rheodyne, USA) whose sample loop was replaced by a C_{18} precolumn. The mixing coils (MC1, 30 cm×0.5 mm I.D; MC2, 60 cm×0.5 mm I.D), extraction coil (EC, 60 cm×0.5 mm I.D) and T-shaped three-way connectors were all made of polytetrafluoroethylene (PTFE).

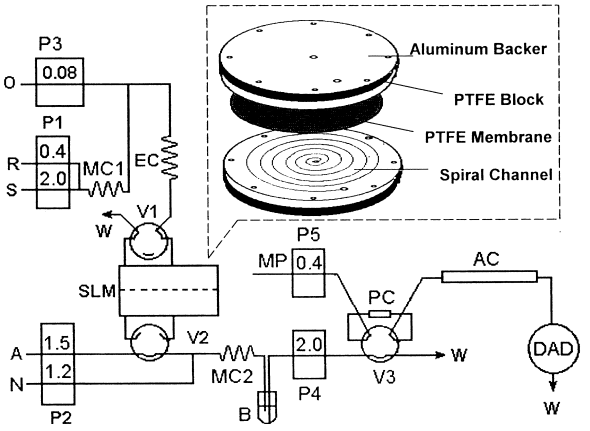


Fig. 1. Schematic diagram of the experimental set-up. The flow-rates of each solution are shown as ml min^{-1} in the symbols of pumps. See text for more details. P1, P2, P3, P4, P5, pumps; V1, V2, V3, valves; MC1, MC2, mixing coils; EC, extraction coil; SLM, supported liquid membrane device; B, 5-ml test tube; PC, C_{18} precolumn; AC, analytical column; DAD, detector; S, sample; R, 0.5 *M* H_2SO_4; O, organic solvent (dichloromethane); A, acceptor (0.5 mol l^{-1} Na_2CO_3–$NaHCO_3$ buffer, pH 10.8); N, neutralization reagent (0.75 mol l^{-1} H_2SO_4); MP, mobile phase; W, waste.

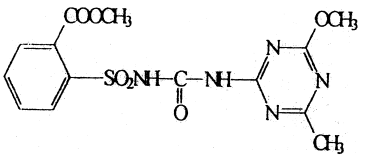
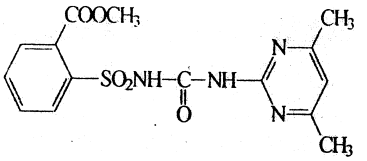
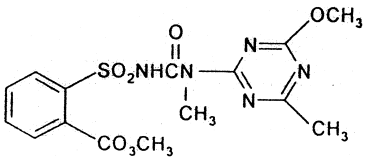
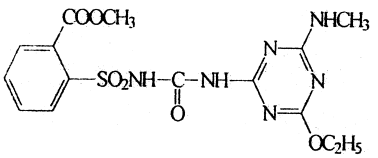
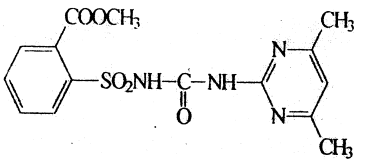
The custom-made SLM device shown in Fig. 1 is similar to that described elsewhere [6], but the acceptor and the donor channel grooves were each 0.3 mm deep, 2.0 mm wide and 160 cm long with a volume of 960 μl. Fluoropore FG PTFE membrane (average pore size 0.2 μm, porosity 0.7; Millipore, Bedford, MA, USA) was used to support the organic liquid.

The precolumn used in the CFLME and column-switching procedures was a C_{18} column (16 mm× 4.0 mm I.D, Three Dimension Chromatography, Tianjin, China).

2.2. Reagents and chemicals

The five sulfonylureas (Table 1) were obtained from Tianjin Pesticides Factory (Tianjin, China). Individual stock solutions were prepared by dissolving 10 mg of each standard in 100 ml of HPLC-grade methanol, respectively. Working solutions were

Table 1
The molecular structure and some physiochemical parameters of the five studied sulfonylurea herbicides

Name	Structure	Water solubility (mg l^{-1})	pK_a
Metsulfuron methyl (MSM, 98.5%)		270 (pH 4.59) 9500 (pH 6.11)	3.3
Sulfometuron methyl (SMM, 74.5%)		8 (25 °C, pH 5) 300 (25 °C, pH 10)	5.7
Tribenuron methyl (TBM, 90.5%)		28 (pH 4) 50 (pH 5) 280 (pH 6)	5.0
Ethametsulfuron (EMS, 90.5%)		50 (pH 6)	3.1±0.5
Bensulfuron methyl (BSM, 97.7%)		2.9	5.2

obtained daily by appropriate dilution of the stock solutions with water. Standard stock solutions were stored at 4 °C. All other chemicals were from Beijing Chemicals and were at least analytical grade. Deionized water was used throughout.

2.3. CFLME procedure

The CFLME set-up is shown in Fig. 1. Dichloromethane was used as liquid membrane. The CFLME procedure is at first conducted as described else-

where [11,12]. After enrichment for, typically 30 or 60 min, P2 was activated to introduce the analytes that had been enriched in the acceptor channel of the SLM device, and a stream of neutralization reagent (N, 0.75 *M* H_2SO_4) to the mixing coil (MC2). The acceptor was acidified to about pH 2.5 in MC2 and delivered into a 5-ml test tube (B) where the produced CO_2 bubbles were eliminated. At the same time, P4 was activated to transport the mixture in the test tube onto the C_{18} precolumn (PC) where the analytes were focused. Next, 3 ml of 0.01 *M* H_2SO_4 were added into the test tube twice to completely transport the analytes onto the precolumn and at the same time wash away the salt in the precolumn. Finally, P4 was stopped and V3 switched to the injection position to transport the analytes from the precolumn to the analytical column.

2.4. Column-switching procedure

The system set-up was the same as that of Fig. 1 except that the CFLME device was not used. Samples were transported directly onto the C_{18} precolumn and then injected onto the analytical LC column.

2.5. LC analysis

LC separations were performed on an LC-VP (Shimadzu, Japan) instrument that consisted of an SCL-10Avp system controller, an LC-10ATvp pump (P5), and an SPD-M10Avp diode array UV detector (DAD) set at 240 nm. Data acquisition and processing were accomplished with a Class-VP Workstation (Shimadzu). The analytical column was a 150 mm× 6.0 mm I.D. C_{18} column (Shim-pack CLC-ODS, 5 μm particles). The mobile phase was filtrated with 0.45 μm micropore membrane.

2.6. Extraction efficiency

Extraction efficiency, *E*, is a measure of the mass transfer rate through the membrane. It is defined as the percentage of analyte extracted from the donor solution to the acceptor solution and was calculated from the equation:

$$E = A_a/A_0$$

where A_a is the peak area of analyte in the acceptor after enrichment by CFLME, A_0 is the peak area of analyte in the sample before extraction. A_a was determined by the proposed procedure. A_0 was determined by direct focusing of the same volume and concentration of sample solution onto the C_{18} precolumn, and injecting the focused analyte onto the separation column.

3. Results and discussion

3.1. LC separation

MSM, EMS, SMM, TBM and BSM were baseline separated within 40 min using methanol–67 mmol l^{-1} KH_2PO_4–Na_2HPO_4 (pH 5.9) buffer (55:45, v/v) at a flow-rate of 0.4 ml min^{-1} as mobile phase. Though gradient elution would be much more suitable to get better separation of analytes in shorter time and probably a better separation of analytes and impurities, this procedure was not tried as no additional pump was available in this laboratory. On the other hand, the separation time, which is relatively long, is compatible with enrichment time.

3.2. Parameters for CFLME

Since the donor and acceptor channel of the SLM device were much shallower and longer (2×0.3× 1600 mm) than in our previous work [11], it is necessary to re-optimize CFLME-related parameters such as the extraction coil length and the flow-rate of dichloromethane.

Experiments showed that the extraction coil length has no significant influence on the peak area. Obviously this is because the donor and the acceptor channel of the present SLM device is long enough for analyte extraction into the organic phase to be completed. A 60-cm-long extraction coil was adopted in the following studies.

No significant influence on the peak area was observed when the flow-rate of dichloromethane was in the range of 3.0–7.0 ml h^{-1}. Though a low flow helps to reduce the consumption of dichloromethane, a flow-rate of 4.8 ml h^{-1} was adopted in this study as lower flows resulted in an unstable flow system

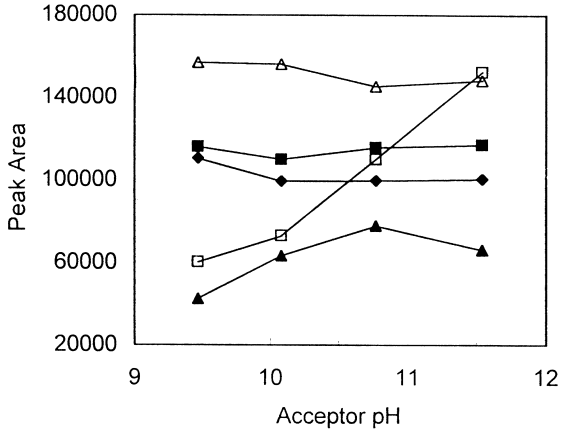


Fig. 2. Effect of acceptor pH on the peak area of sulfonylurea herbicides. Mixed standard solution containing 0.2 μg l^{-1} MSM, SMM and 0.4 μg l^{-1} TBM, EMS, BSM were determined with an enrichment time of 30 min and volume of 60 ml. The acceptor was a 0.5 mol l^{-1} Na_2CO_3–$NaHCO_3$ (pH 10.1) buffer. (♦), MSM; (■), SMM; (▲), TBM; (△), EMS; (□), BSM.

due to the relatively low pressure the microinfusion pump can provide.

Fig. 2 shows the influence of acceptor (0.5 mol l^{-1} Na_2CO_3–$NaHCO_3$ buffer) pH on the peak area when standard sulfonylurea solutions were enriched. As can be seen, while there is no significant difference for the other sulfonylureas the peak area of BSM increased significantly with increasing pH in the range 9.5–11.5. This probably is because the solubility of BSM at pH 7 is relatively low, the increase of acceptor pH will significantly increase its solubility and thus increase the extraction efficiency.

3.3. Matrix effect

In CFLME and SLM, interferants with the same acid–base characteristics as the analytes can be co-extracted into the acceptor, which will result in a decrease of the acceptor pH and thus the low recovery of analytes if the capacity of buffer is not large enough.

When 0.2 mol l^{-1} Na_2CO_3–$NaHCO_3$ buffer solution (pH 10.0) was used as acceptor, according to our previous work [12], recoveries for MSM and EMS in spiked tap water were satisfactory but their recoveries from river water were less than 30%. Therefore, in this present work, the concentration and pH of the Na_2CO_3–$NaHCO_3$ buffer were optimized in order to quantitatively recover all five sulfonylureas in water with different matrices.

Using 0.5 mol l^{-1} $NaCO_3$–$NaHCO_3$ buffer with different pH as acceptor and with an enrichment time of 30 min, the recoveries of the five sulfonylureas in tap and river water were investigated. Recoveries of the five compounds from both sample types were 76–119% except for TBM in river water (60%), and there is no significant difference between the recoveries obtained at pH 10.1 and 10.8.

The influence of the acceptor buffer concentration on the recovery of the herbicides from river water was studied by using 0.25, 0.5, and 0.8 mol l^{-1} $NaCO_3$–$NaHCO_3$ (pH 10.1 or 10.8). The 0.5 mol l^{-1} $NaCO_3$–$NaHCO_3$ buffer (pH 10.8) provided the best recoveries and was used in further work.

3.4. Analytical performance date

Under the above-optimized conditions, the extraction efficiency of the system was determined by using mixed standard solutions (Table 2). Except for TBM (84–85%), the extraction efficiencies of sulfonylureas were 91–108%; i.e. the analytes are completely extracted. Table 2 shows that essentially the same extraction efficiencies were obtained when the volume and concentration of the mixed standard solutions were 100 ml and 0.05 μg l^{-1}, and 60 ml and 0.5 μg l^{-1}, respectively; thus the extraction

Table 2
Extraction efficiencies (%) of five sulfonylurea herbicides in standard solution

Compound	0.05 μg l^{-1}, 100 ml ($n=4$)	0.5 μg l^{-1}, 60 ml ($n=2$)
MSM	104±8	99±11
SMM	108±7	104±10
TBM	84±15	85±4
EMS	91±7	96±15
BSM	97±17	91±7

Table 3
Recovery (%, mean±SD, $n=4$) and detection limit (LOD, ng l^{-1}) of five sulfonylurea herbicides from water (100 ng l^{-1} spiked; enrichment volume, 120 ml)

Analyte	Tap water		Reservoir water		River water	
	Recovery	LOD[a]	Recovery	LOD[a]	Recovery	LOD[a]
MSM	109±15	45	107±13	40	111±12	35
SMM	91±4	10	93±8	25	95±8	25
TBM	43±16	50	65±15	45	69±17	50
EMS	87±7	20	95±6	20	103±9	25
BSM	83±9	25	86±14	40	80±10	30

[a] LOD defined as 3SD calculated at the spiked level considered.

efficiency is independent of the sample concentration in this range.

Analytical recoveries, repeatability and detection limits of the proposed method were assessed by repeatedly analyzing 120 ml of 100 ng l^{-1} spiked tap, reservoir and river water. Results are reported in Table 3, and typical chromatograms of a tap water blank and a 100 ng l^{-1} spiked tap water are shown in Fig. 3.

Table 3 indicates that the recoveries were in the range of 83–111% for MSM, SMM, EMS and BSM. However, the recovery of TBM in 100 ng l^{-1} spiked tap water was relatively low (43%). As the extraction efficiency of TBM is 84–85% (Table 2), the low recovery of TBM should result from its degradation or reaction in sample matrix. Further experiments showed that good recovery (108%) was obtained for TBM at 400 ng l^{-1} spiking levels. This probably is because TBM hydrolyzed rapidly when acidified [1] and the chlorine present in tap water

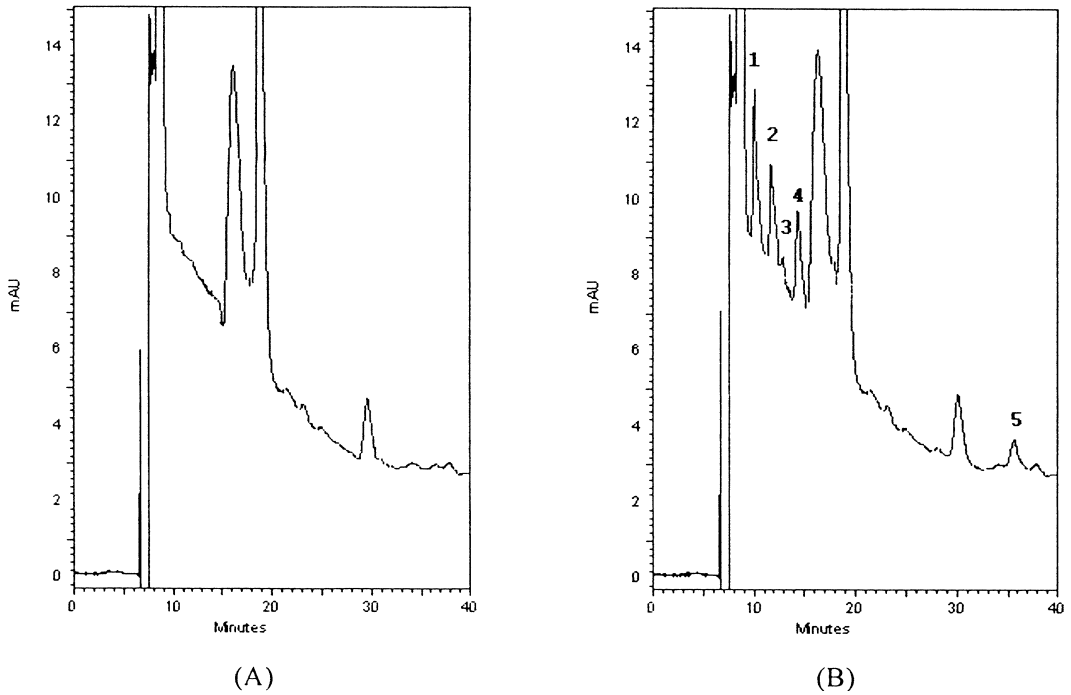


Fig. 3. Typical chromatograms obtained after enriching 120 ml of blank tap water (A) and 100 ng l^{-1} spiked tap water (B) by the proposed method. Peak numbers: 1, MSM; 2, SMM; 3, TBM; 4, EMS; 5, BSM.

reacts with the degraded TBM more easily [9]. In our experiment it was observed that TBM standard solution at low concentration is much less stable than the other ones. The precision at the studied spiking level was 4–17% and there is no significant difference between different aqueous matrixes. The calculated detection limits of these five compounds, defined as three times the standard deviation calculated at the spiking level considered [7], were 10–50 ng l^{-1} when 120 ml of water samples were enriched for determination. Obviously, the detection limits are dependent on the sample matrix and volume enriched. Lower detection limits should be obtained if a larger volume of sample was enriched. The throughput is typically one sample per hour.

The PTFE membrane used in the SLM device has long-term stability. Experiments demonstrated that there is no significant difference between the peak areas of the five herbicides obtained with a PTFE membrane used for 2 months and those obtained with a new membrane. The C_{18} precolumn used in this procedure is also very stable, no significant difference was observed after hundreds of uses during 2 months of experiments.

3.5. Comparison with column-switching

The proposed CFLME method was compared with column-switching (on-line SPE) procedure in respect of sensitivity and selectivity. Though Carbograph 4 [7] and RP-102 [4,9] were reported to be better sorbents for extracting sulfonylureas from aqueous samples, C_{18} sorbent was used for comparison in this study as it is more commonly used and is available in this laboratory. Fig. 4A and B shows typical chromatograms obtained after enriching 120 ml of 200 ng l^{-1} spiked river water by the proposed CFLME and the column-switching procedure, respectively. As can be seen, while good separated peaks of the five analytes were obtained by the proposed CFLME procedure, only the peak of BSM can be identified and the other four peaks were

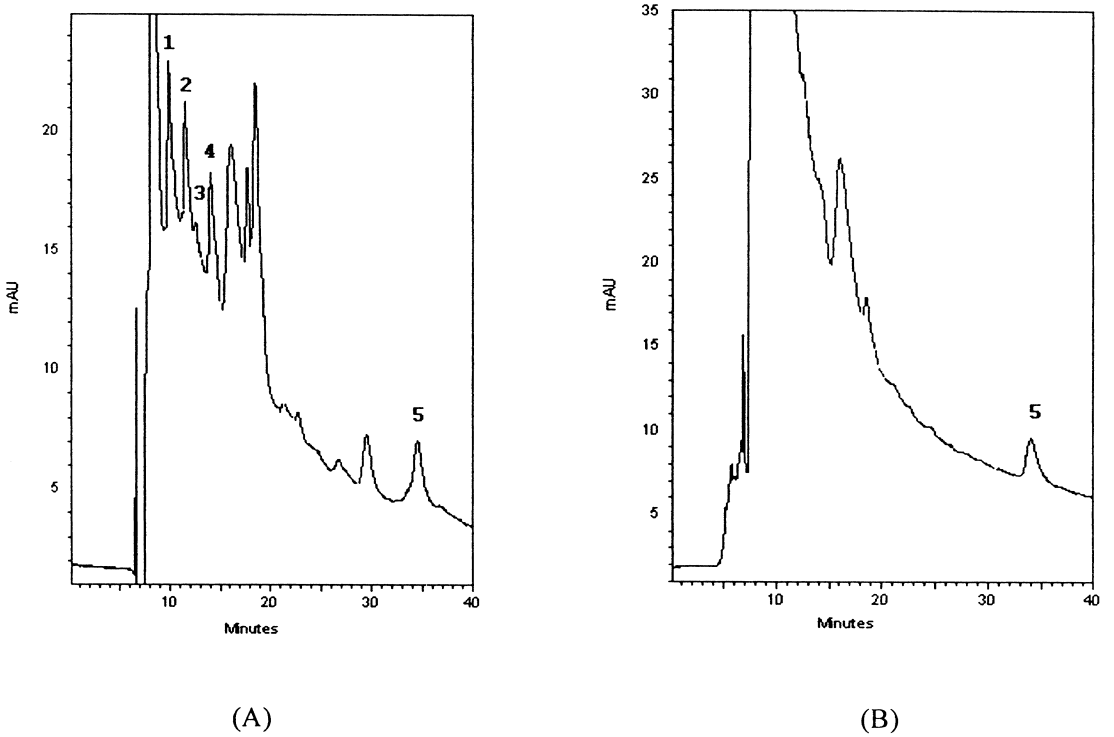


Fig. 4. Typical chromatograms obtained after enriching 120 ml of 200 ng l^{-1} spiked river water by the proposed CFLME (A) and column-switching (B) procedure. Peak numbers: 1, MSM; 2, SMM; 3, TBM; 4, EMS; 5, BSM.

overlaid by the huge sample matrix peak in the chromatogram of the column-switching procedure. Fig. 4 showed that this proposed CFLME procedure presents a higher degree of sample clean-up and thus lower detection limits than C_{18}-based column-switching.

4. Conclusion

This work demonstrated the feasibility for determining trace sulfonylurea herbicides in water samples with an on-line coupled CFLME–C_{18}-HPLC system. Sulfonylureas can be detected at about 50 ng l^{-1} levels in natural waters. CFLME provides more sample clean-up and thus lower detection limits than a C_{18}-based column-switching technique. The long-term stability and sample pre-treatment time of both approaches are similar. The proposed procedure was applied to determine sulfonylureas in several types of water samples.

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