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# Large-volume sample stacking for on-capillary sample enrichment in the determination of naphthalene- and benzenesulfonates in real water samples by capillary zone electrophoresis

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***Received 28th March 2001, Accepted 7th June 2001***
***First published as an Advance Article on the web 23rd July 2001***

We investigated the on-line preconcentration of a test mixture of 15 substituted and unsubstituted naphthalene- (NSs) and benzenesulfonates (BZSs) by large-volume sample stacking (LVSS). Analyses were carried out by capillary zone electrophoresis (CZE) with on-column UV detection. In particular, we focused on how experimental variables such as the inside diameter of the capillary, the volume of sample introduced and polarity switching influenced the enrichment procedure. The best results were obtained when 300 nl were injected and stacked using a bubble cell capillary. Under these conditions, LVSS increased the detector response of conventional hydrodynamic injection by a factor of 40. The limits of detection of the method were between 5 and 10 μg $l^{-1}$. Determinations were reproducible, in terms of peak area and migration time, under such conditions. The performance of the method was examined by determining NS and BZS in real samples, such as tap, river and surface waters and inflow/outflow waters from a water treatment plant. Real samples were injected directly into the CZE column with little or no preparation.

## Introduction

Benzene- and naphthalenesulfonate isomers are xenobiotic, highly water soluble compounds that are used in the production of many consumer products and in various industrial applications. They are introduced into the environment mainly by industrial or municipal waste water which may or may not have been treated. Most of them have only a slight tendency to adsorb on organic material. Because of this, they are considered to be very mobile in the aquatic environment. Although they are not very toxic and have no genotoxic or carcinogenic effects, the microbiological persistence of some compounds is a potential environmental risk.[1] The occurrence and fate of these compounds in the aquatic environment at concentrations which range from a few μg $l^{-1}$ to several mg $l^{-1}$ have been reported by several groups.[2–4]

Capillary electrophoresis (CE) is a highly efficient technique for separating benzene- and naphthalenesulfonate isomers.[5–8] However, it has two major limitations: the sample injection volume is low and the optical pathlength for on-capillary UV detection is short. It usually provides detection limits in the low mg $l^{-1}$ range,[7] but these are not sufficient to determine aromatic sulfonates, which in some cases are in the low μg $l^{-1}$ range, in real samples.

Some designs have been developed to increase the on-capillary detection window, *i.e.*, extended pathlength capillaries, high-sensitivity detection cells, *etc*. These designs can increase the sensitivity of most common UV detectors by up to 10-fold.[9,10] Nowadays, modern fluorescence detectors can give better detection limits than UV absorption detection. Laser-induced fluorescence (LIF) detection is highly appropriate for the compounds studied because they show native fluorescence. However, expensive UV lasers have to be used because the absorption wavelengths of naphthalenesulfonates are relatively short.[11,12]

The sensitivity can also be improved by preconcentrating the sample before the CE separation. Sample preconcentration can be performed either off-line, by solid phase extraction (SPE), or on-line (or on-capillary), directly in the CE column.[13]

Some groups have described the use of SPE for preconcentrating aromatic sulfonates with CE. Kok *et al*.[14] used a three-step SPE procedure to clean up and preconcentrate spiked river water samples. They used ion-pair SPE with $C_{18}$ material to concentrate some naphthalenesulfonates. Recently, Loos *et al*.,[15,16] showed that polymeric sorbents such as LiChrolut EN and Isolute ENV+ or more recently developed sorbents such as HR-P and Oasis HLB can also be used to enrich aromatic sulfonate isomers.

A more straightforward way of preconcentrating samples is to use an on-line enrichment procedure. In conventional CE, separation efficiency can only be maintained if the injected sample plug is short enough for the separation to be performed in diffusion-controlled zones. However, several approaches have been designed by which the volume of sample injected into the capillary can be increased, thus improving the detection sensitivity without losing separation efficiency.[17,18]

In 1992, Chien and Burgi[19,20] performed several in-depth studies on the injection of large sample volumes. Since then, numerous techniques based on sample stacking procedures have shown that these enrichment procedures can significantly improve the detector response, thus reducing sample handling and cost.[17,21,22]

Pollutants in water samples are often preconcentrated by extraction techniques.[23] Very few studies have been reported on the suitability of stacking procedures for preconcentrating organic pollutants in real samples, and up to now these procedures have not been applied to aromatic sulfonates.

When the volume of sample introduced into the capillary is greater than the optimum in the conventional injection mode, the sample matrix must be pumped out from the capillary if the

 DOI: 10.1039/b102868p

separation efficiency is to be preserved.[19] This sample stacking mode is known as large volume sample stacking (LVSS). There are two main factors that can affect the analyte detection: the narrowing of the analyte bands in the column and the amount of sample that can be loaded into the column.

The main aim of this study was to develop a method for determining aromatic sulfonates [benzene- (BZS) and naphthalenesulfonates (NSs)] in water samples using an on-line preconcentration technique. We used a stacking procedure (LVSS) as the on-capillary enrichment step to lower the limits of detection of a BZS–NS test mixture. The experimental conditions for LVSS were optimised and the results obtained were compared with those obtained when conventional hydrodynamic injection was used. The method was applied to real water samples. The method developed is particularly useful because it provides concentration factors that are sufficient for analysing some real samples and it reduces the sample handling and analysis time.

## Experimental

### Instrumentation

All separations were performed with a Hewlett-Packard (Palo Alto, CA, USA) $^{3D}$CE capillary zone electrophoresis (CZE) system equipped with a UV diode array detector. Unless indicated otherwise, 225 nm was selected as the absorbtion wavelength.

Two conventional straight uncoated fused-silica capillaries of 75 μm id (Supelco, Bellefonte, PA, USA) and 100 μm id (Polymicro Technologies, Phoenix, AZ, USA) were used. Also, a 50 μm id uncoated fused-silica capillary with an extended pathlength of 150 μm (50-μm BF3, Hewlett-Packard) was used. This will be referred to as the bubble cell capillary. The total length of all capillaries was 64.5 cm and the effective length was 56 cm. Alignment interfaces containing optical slits matched to each capillary id were used. The capillaries were thermostatted using a Peltier system at 30 or 35 °C. Electrophoretic separations were performed at 30 kV. Electropherograms were analysed using the HP Chemstation chromatographic data system (version A:04:02).

### Reagents and chemicals

The BZSs and NSs used in this study were purchased from Acros Organics (Geel, Belgium), Aldrich (Beerse, Belgium) and Fluka (Buchs, Switzerland). The abbreviations, the peak assignments and the wavelengths of the compounds at the maximum in the UV spectra ($\lambda_{max}$) are given in Table 1. The way in which stock standard solutions were prepared was described in a previous paper.[7] Working standard solutions were prepared daily by diluting the stock standard solutions with water [Milli-Q, tap, river and water treatment plant (WTP) water]. Sodium tetraborate was obtained from Fluka. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). The background electrolyte (BGE) was prepared daily, de-gassed by ultrasonication and filtered through a 0.45 μm nylon syringe filter (Tracer, Teknokroma, Barcelona, Spain) before use.

### Samples and sample pre-treatment

Tap water samples were collected from the local water supply (Tarragona, Spain). River water samples were taken from the river Ebro (Tortosa, Spain). Surface water was collected near a chemical plant in Tarragona (Spain) that sometimes uses aromatic sulfonates and other dye intermediates in its production processes. The inflow/outflow waters were obtained from the water treatment plant (WTP) of Vilanova del Camí (Barcelona, Spain), which collects both urban and industrial waste with a significant proportion of tannery effluents.

Samples were collected in dark glass bottles, which were stored at 4 °C. Real samples (tap, river and surface waters) were filtered through a 0.45 μm nylon membrane filter (Whatman, Maidstone, UK) before analysis. WTP waters were previously ultracentrifuged and filtered before being injected into the CE system. No further sample preparation was carried out because the tested matrices were compatible with sample stacking, which made direct injection possible and prevented further sample handling.

### Operating procedure

Before each session, the capillary was conditioned by flushing for 10 min each with 0.1 M NaOH and water, and then with BGE for 15 min. In order to remove material adsorbed on the capillary walls when surface and WTP waters were analysed, the capillary had to be conditioned between runs by flushing for 3 min each with 0.1 M NaOH and water, and then with BGE for 5 min. Using this procedure, it was found that the run-to-run reproducibility was maintained. For the other samples and the standards, the capillary was conditioned between runs by flushing it only with the BGE for 3 min.

When using conventional hydrodynamic injection, samples were injected by pressure (50 mbar) and the injection times

**Table 1** Benzene- and naphthalenesulfonate compounds

| Compound | Abbreviation | Peak No. | $\lambda_{max}$/nm[a] (experimental) |
|---|---|---|---|
| 5-Amino-2-naphthalenesulfonate | 5-$NH_2$-2-NS | 1 | 213 |
| 1-Naphthalenesulfonate | 1-NS | 2 | 224 |
| 3-Aminobenzenesulfonate | 3-$NH_2$-BZS | 3 | 203 |
| 2-Aminobenzenesulfonate | 2-$NH_2$-BZS | 4 | 203 |
| Benzenesulfonate | BZS | 5 | 213 |
| 6-Amino-4-hydroxy-2-naphthalenesulfonate | 6-$NH_2$-4-OH-2-NS | 6 | 252 |
| 6-Amino-1-hydroxy-3-naphthalenesulfonate | 6-$NH_2$-1-OH-3-NS | 7 | 230 |
| 4-Hydroxy-1-naphthalenesulfonate | 4-OH-1-NS | 8 | 220 |
| 4-Hydroxybenzenesulfonate | 4-OH-BZS | 9 | 197 |
| 7-Amino-1,3-naphthalenedisulfonate | 7-$NH_2$-1,3-NDS | 10 | 247 |
| 3-Amino-2,7-naphthalenedisulfonate | 3-$NH_2$-2,7-NDS | 11 | 247 |
| 1,5-Naphthalenedisulfonate | 1,5-NDS | 12 | 225 |
| 2-Hydroxy-3,6-naphthalenedisulfonate | 2-OH-3,6-NDS | 13 | 237 |
| 8-Amino-1-hydroxy-3,6-naphthalenedisulfonate | 8-$NH_2$-1-OH-3,6-NDS | 14 | 235 |
| 1-Hydroxy-3,6-naphthalenedisulfonate | 1-OH-3,6-NDS | 15 | 219 |

[a] UV Spectrum 190-400 nm.

were adjusted for an identical injection volume (15 nl) for all the capillaries. The proper choice of injection time and injection volume was optimised. For a 50 μm id capillary, the injection plug length should not be greater than 15 nl. Sample overloading leads to poor peak shape, low separation efficiency and poor precision. This effect was also observed by other workers.[24]

When using LVSS as a previous enrichment step, samples were also injected by pressure. First, a large volume of sample was injected into the capillary. Second, a reversed voltage of 30 kV was applied to focus the zones and remove the sample matrix. When anionic compounds had been focused and most of the sample matrix removed, the voltage was stopped and the polarity reversed. This occurred when the observed current reached about 95% of the current when the capillary was filled with borate buffer. Third, a voltage of 30 kV was applied for CZE separation.

## Results and discussion

### Electrophoretic separation

CZE separation of the 15 aromatic sulfonates (Table 1) was optimised in accordance with previous studies,[5] and the optimum conditions are described in the Experimental section.

First, two straight capillaries of 75 and 100 μm id and a bubble cell capillary (50-μm BF3) were tested in order to evaluate how they influence the analytical signal and the resolution of the analytes.

The amount of sample injected depends on the pressure applied, the injection time, the capillary length, the inside diameter and the sample viscosity. To find out how the inside diameter affects the analytical signal, a standard solution (15 nl of 0.5 mg $l^{-1}$ NSs and 1 mg $l^{-1}$ BZSs) was hydrodynamically injected into each capillary. For the straight capillaries, the analyte response increased in proportion to the increase in the id, but the heating effects of Joule heat also increased the current and negatively affected the precision of the method. The experimental variables were modified to compensate for the heating effects but this was detrimental to separation. Therefore, to improve the method sensitivity further, a bubble cell capillary was used. The analytical signal was nearly twice as large as when the 75 μm id capillary was used. The separation, however, was largely unchanged and the current generated fell from 80 to 35 μA. Fig. 1(a) shows the electropherogram obtained by hydrodynamically injecting 15 nl of the test mixture into a bubble cell capillary.

To determine the linearity of the response, 15 nl of standard solutions were injected hydrodynamically. The linearity was good between 0.5 and 25 mg $l^{-1}$ for the NSs and between 1 and 50 mg $l^{-1}$ for the BZSs, with determination coefficients higher than 0.998. The limits of detection (LODs), the repeatability and the reproducibility were also studied. The LODs, based on a signal-to-noise ratio of 3, were between 0.30 and 0.15 mg $l^{-1}$. The precision of the method was evaluated by determining its repeatability and reproducibility. The repeatability values (RSD) obtained for five successive injections of standard solution containing 5 mg $l^{-1}$ of each compound were $<0.5\%$ and $<5\%$ for migration times and normalised peak areas, respectively. Reproducibility values were determined in the same way as repeatability but on different days. It was observed that the day-to-day precision values were only slightly higher than the within-day precision values.

### Optimization of LVSS

Aromatic sulfonates have been determined by CE at low mg $l^{-1}$ levels.[7] However, as explained above, they are present at very low concentration levels in environmental waters. Therefore, a previous enrichment step is usually needed which, up to now, has been carried out by SPE. Unfortunately, the efficiency of ion-pair extraction or more recently developed polymeric sorbents is limited for very hydrophilic compounds (substituted amino and hydroxy isomers) or for compounds with more than one sulfonate group.[14–16] This limitation encouraged us to use a sample stacking procedure (LVSS) because it enables all compounds present in a sample to be preconcentrated. For the determination of aromatic sulfonates, this is important because it enables those compounds that cannot be recovered by SPE to be preconcentrated. Furthermore, LVSS enables the sample to be enriched on-line and handling is minimized.

The capillary selected for the optimization of the LVSS procedure was the bubble cell capillary since it improved the detector response two-fold for conventional hydrodynamic injection and lower current intensities. The main parameters that can affect the LVSS procedure, sample injection volume and polarity switching, were optimized. Standard solutions

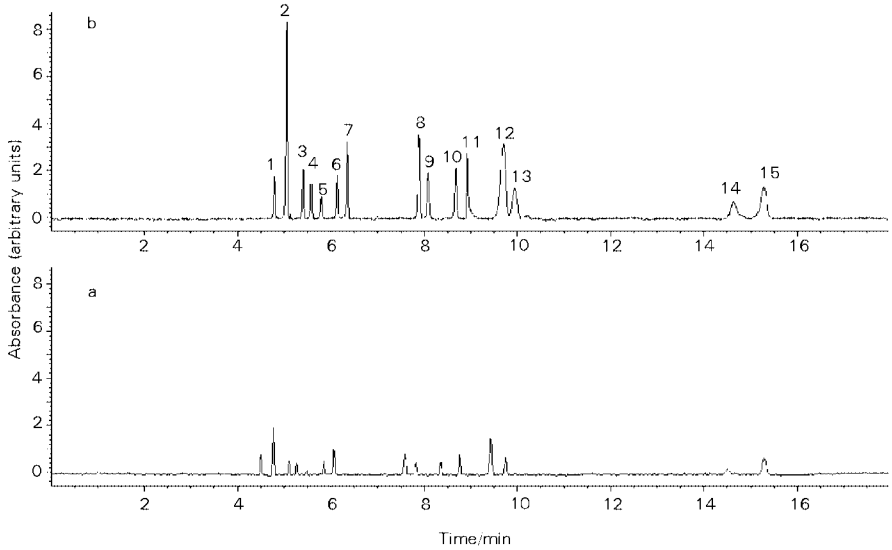


**Fig. 1** Electropherograms of standard solutions using (a) conventional hydrodynamic injection (500 μg $l^{-1}$ of NSs and 1000 μg $l^{-1}$ of BZSs) and (b) the LVSS enrichment procedure (50 μg $l^{-1}$ of NS and 100 μg $l^{-1}$ of BZS). Separations were performed using 20 mM borate buffer at 30 kV and 30 °C. Detection at 225 nm. Peak identification as in Table 1.

containing 50 μg $l^{-1}$ of NSs and 100 μg $l^{-1}$ of BZSs in Milli-Q water were used to evaluate the effect of these parameters.

The sample injection volume was studied by monitoring the analyte response as a function of injection time. The pressure was 50 mbar and the standard solution was injected for times which ranged from 30 to 230 s. The stacking percentage was set at 95% of the original current. The maximum sample injection volume that could be injected without separation efficiency being lost was *ca*.. 300 nl (50 mbar for 200 s). When a higher volume of sample was injected, 1,5-NDS and 2-OH-3,6-NDS (peaks 12 and 13) tended to overlap and the compounds that eluted last tended to broaden.

The polarity switching was the next parameter to be optimised. The effect of the amount of sample matrix pumped out from the capillary was studied by monitoring the observed current during the stacking process until it reached 90–98% of the original current (current observed when the capillary is filled with borate buffer only). The results obtained show that the peak area of the analytes increases when this percentage increases until the observed current is 95% of the original current. If the volume of matrix pumped out from the capillary is increased, the analytes become more focused. However, at levels above 95% of the original current there is a decrease in the peak area of the compounds that eluted first, and above 98% they were almost completely lost.

The electropherogram in Fig. 1(b) shows the analyte response with LVSS. It was 40 times better than when conventional hydrodynamic injection was used [Fig. 1(a)].

Once the parameters had been optimised, the method was checked by analysing 300 nl of Milli-Q water samples spiked with the compounds studied. The linearity of the response in Milli-Q water was checked between 15 and 250 μg $l^{-1}$ for the NSs and between 30 and 500 μg $l^{-1}$ for the BZSs, with determination coefficients higher than 0.997. The LODs were between 5 and 10 μg $l^{-1}$. The repeatability values (RSD) obtained for five successive injections of a standard solution containing 30 μg $l^{-1}$ of each compound were $< 5\%$ and $< 10\%$ for migration times and normalised peak areas, respectively. Reproducibility values were determined in the same way as repeatability but on different days. They were between 7 and 17% for normalised peak areas.

## Analysis of real samples

The performance of the method was tested with some real water samples. For these studies seven compounds, 5-$NH_2$-2-NS (**1**), 1-NS (**2**), 3-$NH_2$-BZS (**3**), 2-$NH_2$-BZS (**4**), BZS (**5**), 6-$NH_2$-4-OH-2-NS (**6**) and 6-$NH_2$-1-OH-3-NS (**7**), were chosen. UV detection for real samples was monitored by using time-scheduled selected wavelengths: 210 nm for compounds **1**–**5** and 230 nm for compounds **6** and **7**. The background signals of the matrix compounds were recorded at 230 nm. The identities of the peaks appearing in the real samples were studied by comparing migration times and UV spectra and by further spiking the samples with the reference compounds.

When dealing with real samples, a sample preparation step (*e.g*., dilution or extraction) is often performed before injection. This is sometimes necessary because real samples often contain substances that increase the conductivity of the matrix, which may interfere with the stacking process. Previous experiments with diluted samples showed that the compounds of interest could be efficiently separated, thus allowing sample stacking with direct injection. Instead of diluting the real samples, we chose to inject lower sample volumes. We decided to inject a sample injection volume of 100 nl for tap and river waters, 60 nl for surface waters and 40 nl for WTP waters because for these volumes the analyte response was maximum with no decrease in separation efficiency.

The linearity of the method was checked with tap and Ebro river water samples. Analytical data were calculated in the same way as for Milli-Q water. The linearity of the response was checked in the range between 50 and 250 μg $l^{-1}$ with determination coefficients higher than 0.994. The LOD was 20 μg $l^{-1}$. The repeatability and reproducibility of the method were similar to those for Milli-Q water. Fig. 2 shows the electropherograms obtained when unspiked Ebro river water and Ebro river water spiked with 50 μg $l^{-1}$ of each compound were analysed. No NS or BZS peaks were observed in tap and Ebro river water electropherograms and the peak that appeared could not be identified by the UV spectra database.

Once the method had been validated, it was tested by analysing real samples with a high content of organic matter: a surface water sample collected near a chemical plant and inflow/outflow water samples from a WTP.

Fig. 3(a) shows the unspiked surface water electropherogram. Two peaks appeared at the migration times for 5-$NH_2$-2-NS (**1**) and 1-NS (**2**), but only the spectrum of the first peak confirmed that it corresponded to 5-$NH_2$-2-NS. The other peaks that appeared in the electropherogram could not be identified. These results were also confirmed by spiking the surface water sample with 250 μg $l^{-1}$ of 5-$NH_2$-2-NS and 1-NS [Fig. 3(b)]. The response of the 5-$NH_2$-2-NS was calibrated. This compound was found at a concentration of 104 μg $l^{-1}$.

The method was also applied to evaluate the possible persistence of these compounds in water samples from the inflow and the outflow of a WTP. Fig. 4(a) shows the blank of the inflow WTP water electropherogram overlaid with the spiked sample. Various peaks appeared at the same migration time as the compounds studied. Some of these peaks (A–F) were tentatively identified as aromatic sulfonates by their migration times and by further spiking the samples with the standards. Peaks A and B were tentatively identified as isomeric amino-

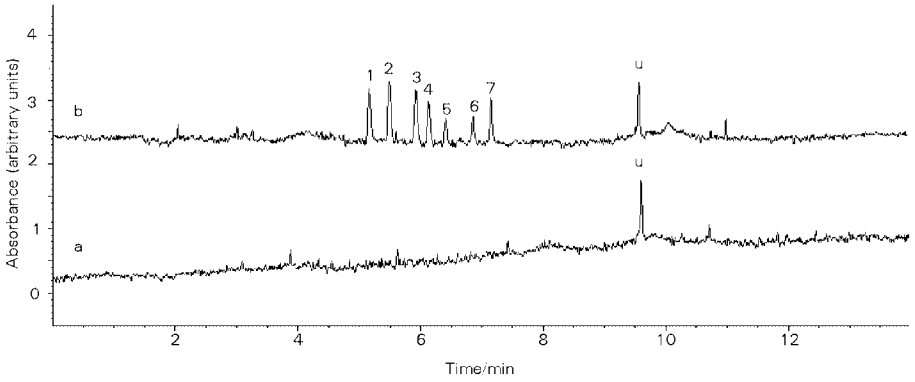


**Fig. 2** Electropherograms of a river Ebro water sample, (a) unspiked and (b) spiked at 50 μg $l^{-1}$ with the seven target compounds after the LVSS enrichment procedure. Separations were performed using 20 mM borate buffer at 30 kV and 35 °C. Time-scheduled selected wavelengths. Peak identification: (1) 5-$NH_2$-2-NS; (2) 1-NS; (3) 3-$NH_2$-BZS; (4) 2-$NH_2$-BZS; (5) BZS; (6) 6-$NH_2$-4-OH-2-NS; (7) 6-$NH_2$-1-OH-3-NS.

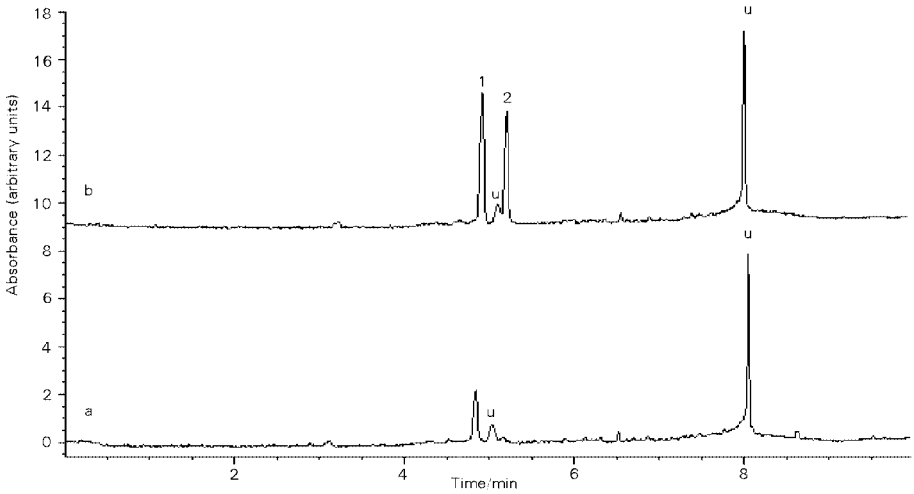


**Fig. 3** Electropherograms of a surface water sample, (a) unspiked and (b) spiked at 0.25 mg $l^{-1}$ with 5-$NH_2$-2-NS and 1-NS after the LVSS enrichment procedure. Separation conditions and peak identification as in Fig. 2.

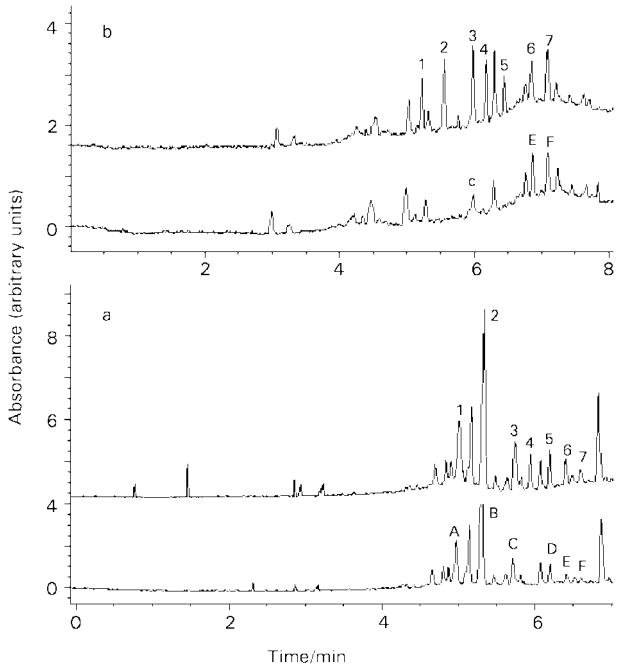


**Fig. 4** Electropherograms of (a) blank of the inflow WTP water overlaid with the spiked sample and (b) blank of the outflow WTP water overlaid with the spiked sample. Separation conditions and peak identification as in Fig. 2.

naphthalenesulfonate (like compound **1**) and isomeric unsubstituted naphthalenesulfonates (like compound **2**), because isomeric naphthalenesulfonates cannot be separated with a simple borate buffer. Peaks C and D were assigned to isomeric aminobenzenesulfonates (like compounds **3** and **4**) and the small peaks E and F were assigned to isomeric aminohydroxynaphthalenesulfonates (like compounds **6** and **7**). The other peaks that appeared in the electropherogram could not be identified. The response of the unsubstituted naphthalenesulfonate isomer was calibrated. This compound was found at a concentration of 166 μg $l^{-1}$. The other peaks could not be quantified because their concentrations were between the detection limit and the quantification limit of the method.

When the outflow WTP waters were analyzed, three of the peaks (C, D and E) which appeared in the inflow WTP water were found. This confirms the persistence of these compounds. Fig. 4(b) shows the blank of the outflow WTP water electropherogram overlaid with the spiked sample. Most peaks appeared in the outflow WTP water at low concentrations and so could not be identified by the UV spectra database. The availability of more selective detection systems such as an UV LIF detector or a MS detector would have enabled the compounds to be confirmed. In order to verify the persistence of these compounds, we compared the peak areas of the compounds tentatively found in the outflow WTP water with those in the inflow WTP water. The peaks were integrated and their normalized peak areas were compared. The normalized peak areas were similar for both samples. The above results are in line with other studies which showed the persistence of some aromatic sulfonate isomers in several real samples.[2,11,16]

In conclusion, this paper has described how an on-line preconcentration technique (LVSS) can be used to determine NSs and BZS by CZE. Stacking can give concentration factors that increase the sensitivity of the method to low μg $l^{-1}$ levels, which is sufficient for some real sample analyses. The combination of stacking, bubble cell capillaries or sensitive cells and sensitive detectors such as a UV LIF detector makes this technique a very promising procedure for on-line sample determination of organic pollutants in real waters at low levels.

## Acknowledgements

This work was supported by the Spanish Interministerial Commission for Science and Technology (CYCYT) (AMB98-0913). The authors are very grateful to Antoni Rius for providing the waste WTP water samples.

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