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# Determination of aromatic amines in water samples by capillary electrophoresis with electrochemical and fluorescence detection

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**Abstract**

Two capillary electrophoresis methods have been compared for the determination of aniline derivatives in environmental water samples. With the first method the anilines were separated as cations by free zone electrophoresis at low pH, and detected by amperometry. For this, the separation capillary was connected through a palladium field decoupler to an electrochemical detection cell which had been modified to match the volume scale of the separation. Most anilines tested, except chlorinated compounds, could be detected with full sensitivity at a detection potential of +0.7 V. Detection limits with this detection scheme were on a low μg/l level. The alternative method involved the derivatization of the anilines with fluorescamine, the separation of the derivatives formed by micellar electrokinetic chromatography, and fluorescence detection. For detection a lamp-based, fibre optics instrument was used. Detection limits with fluorimetry were comparable with those obtained with amperometric detection (in the order of 1 μg/l). Still, this method was preferred since it gave a higher separation efficiency and shorter analysis times (approximately 4 min). The most important argument, however, was its higher reliability and ease-of-handling. Preliminary experiments with water samples collected in areas where pollution with anilines may be expected showed that the method is highly specific, with few interferences showing up in the electropherograms. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Detection, electrophoresis; Water analysis; Environmental analysis; Amines, aromatic; Anilines

## 1. Introduction

Aromatic amines such as aniline and its derivatives are an important class of environmental water pollutants. Anilines are used in the manufacturing of rubbers and plastics, dyes, agrochemicals and pharmaceuticals [1]. The anilines can be released into the environment directly as industrial effluent from, e.g., the chemical, textile or leather industry, or indirectly as breakdown products of herbicides and pesticides. Due to their high solubility in water, anilines can easily permeate through soil and contaminate ground water, and therefore they can be present at trace levels in drinking water. Several aromatic amines are strongly toxic and suspected carcinogens [2]. Moreover, aniline compounds may be converted into carcinogens in the environment or in the body.

Several methods have been developed for the determination of anilines in environmental samples. Gas chromatography (GC) is a classical method [3,4], and presently GC–mass spectrometry (MS) is often used [5,6]. For non-volatile aniline compounds high-performance liquid chromatography (HPLC) may be used with fluorescence detection after derivatization [7,8], or with amperometric detection [9,10].

In our laboratories we have been studying the applicability of capillary electrophoresis (CE) for

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environmental analysis [11–13]. CE is a method that offers a high separation speed and efficiency [14]. An additional advantage in our context is that CE can be performed with relatively simple instrumentation, and with very low running costs. The main problem in the application of CE for the analysis of, e.g., environmental water samples is the low detection sensitivity. With the most common detection method, UV absorbance detection, only in selected cases sensitivities can be obtained that are relevant in environmental studies [15,16]. However, we have found that with an amperometric detector [11,12] or with a lamp-based fluorescence detector [17,18] detection limits in the order of 1 μg/l are possible in CE. Such a sensitivity would be adequate for our purpose: the screening of river water in areas with a large textile or leather industry on the presence of aniline derivatives. Anilines are electrochemically active and they are also easily derivatized to fluorescent compounds.

In the study presented in this paper we have compared the performances of two possible electrokinetic methods for the separation and determination of anilines. The first method is capillary zone electrophoresis (CZE) of the free amines with amperometric detection. The second method involves the derivatization of the anilines with fluorescamine [19], the subsequent separation of the (neutral) derivatized compounds by micellar electrokinetic chromatography (MEKC) and their detection by fluorimetry. The methods will be compared in respect to separation performance, sensitivity, repeatability and robustness.

## 2. Experimental

### 2.1. Apparatus

A Prince CE instrument with programmable injector and high-voltage source was obtained from Prince Technologies (Emmen, The Netherlands). Samples were injected by pressure (40 mbar, 6–24 s). Fused-silica capillaries with an O.D. of 375 μm and an I.D. of 75 μm were obtained from Composite Metal Service (Hallow, UK). Capillaries were etched daily by flushing for 10 min with a 0.1 *M* sodium hydroxide solution before use. All CE experiments were performed at ambient temperature (20±1°C).

For electrochemical detection a Unijet cell from Bioanalytical Systems (BAS, West Lafayette, IN, USA) with a 1-mm glassy carbon working electrode and a Ag/AgCl reference electrode was used, in conjunction with a BAS LC-3C battery-operated potentiostat. The separation capillary was connected to the detection cell through a palladium decoupler [20,21] and a polyether ether ketone (PEEK) tube (50 mm×63.5 μm I.D.). The cell was modified in such a way that the connecting PEEK tubing could be mounted directly in the cell block facing the working electrode. To compensate for the back-pressure of the connecting tubing, a pressure of 20 mbar was applied to the inlet vial during runs.

For fluorescence detection, an ARGOS 250 fibre-optics instrument (Flux Instruments, Pfaffenhofen, Germany) equipped with a 75 W mercury–xenon lamp was used. The excitation light was filtered through a Schott glass UG 11 filter, and a 495 nm cut-off filter was applied for the emitted light. With this detector, a 15-mm detection window was created on the capillary by burning off the coating. For the decoupling of the emitted light glycerol was applied between the capillary and the optical cone.

For data processing Caesar 40.0 software (Prince Technologies) was used.

### 2.2. Chemicals and solutions

Fluorescamine {4-phenylspiro[furan-(3H),1-phthalan]-3,3′-dione} was obtained from Fluka (Buchs, Switzerland). As derivatization reagent a 0.01 *M* solution in HPLC-grade acetone was prepared daily. Other chemicals, obtained from standard suppliers, were of analytical-reagent grade quality and were used as received. Stock solutions of the anilines were prepared in water or methanol and stored in the dark at 4°C.

## 3. Results and discussion

### 3.1. CZE with electrochemical detection

In previous work on CZE with electrochemical detection it had been found that a commercially

available detector cell can be used after only minor modifications regarding the connections [12]. In this study it was shown that the extra-column contribution to zone broadening could be kept within reasonable limits by a careful positioning of the capillary and other connections. Most problems were encountered with cationic analytes migrating in the same direction as the electroosmotic flow (EOF). For such compounds the zone volumes are relatively low and extra-column effects will be more pronounced than for late-eluting zones. Therefore, to suppress the influence of the extra-column zone broadening, in the present application a relatively long (1 m) separation capillary was used. To compensate the backpressure of the connecting tubing and the detector cell a small overpressure was applied to the inlet vial during the run. With an applied voltage of 25 kV the highest separation efficiency was obtained with a 20 mbar pressure. Under these conditions plate numbers in the order of 50 000 to 70 000 were obtained for the various anilines tested. As in the previous study [12], it appeared to be essential for the performance of the system in terms of plate numbers to carefully align the connecting PEEK tubing in the detector cell.

A separation of a standard mixture of exemplary anilines could be obtained in a background electrolyte (BGE) with a pH of 5.0 (10 m*M* sodium acetate and 5 m*M* acetic acid). The separation in this BGE is shown in Fig. 1. At this pH the anilines, which have p$K_a$ values in the order of 4–5, are partly protonated, and separation is based on differences in effective charge and size, with the diamine compound (1) having the highest mobility. Although at the pH of the BGE used the electroosmotic mobility is relatively low, the run time is only 12 min, since all analytes elute before the EOF peak. The repeatability of migration times was in the order of 3% (relative standard deviation, RSD). For calculated mobilities, corrected for the variation in the EOF velocity, a repeatability of ≤1.3% could be realised.

The optimum detector setting was determined by measuring peak areas for the anilines at applied potentials in a range from +0.1 to +0.7 V, with intervals of 0.1 V. The relative responses measured at different potentials are shown in Fig. 2. Chlorinated anilines gave low sensitivities in this potential range, and were therefore not included in the test set. For

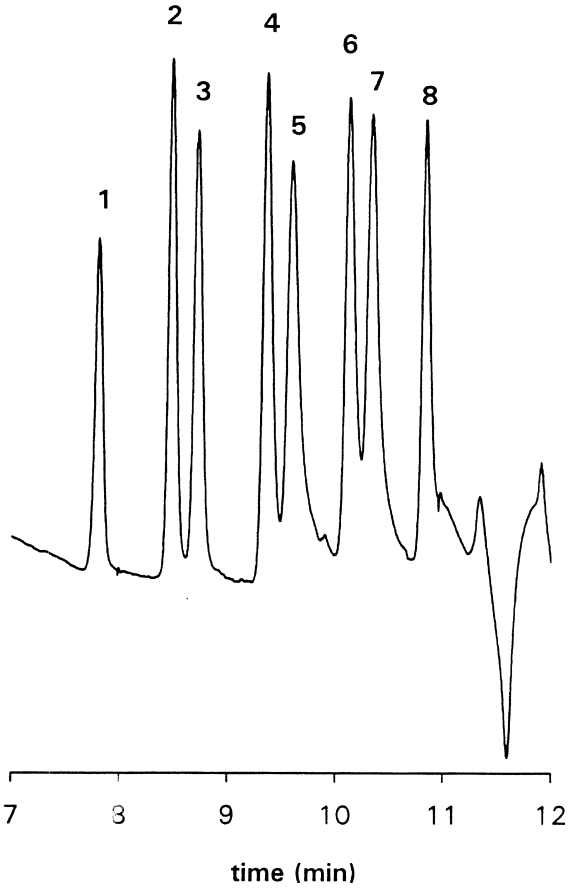


Fig. 1. Separation of a standard mixture of anilines with amperometric detection. BGE: 10 m*M* sodium acetate, 5 m*M* acetic acid and 1 m*M* sodium chloride, pH 5. Capillary: 1 m×75 μm I.D. Separation voltage: 25 kV. Detection potential: +0.7 V vs. Ag/AgCl. Sample concentration: $10^{-6}$ mol/l for each compound. (1) 1,3-Phenylenediamine; (2) 2-methoxy aniline; (3) 4-ethoxy aniline; (4) 4,4′-diaminobiphenyl; (5) 2-methylaniline; (6) 2,4-dimethylaniline; (7) 2-ethylaniline; (8) 2,6-dimethylaniline.

most other compounds tested a maximum sensitivity was found at a working electrode potential of +0.7 V. The limiting-current potential of +0.7 V was used in further experiments.

In order to maximise the sensitivity, the volume loadability of the system was studied. Standard solutions of the anilines diluted in the BGE were injected at 40 mbar headpressure with various injection times. It appeared that injection times of up to 18 s, corresponding to an injection volume of 60 nl, could be applied without deterioration of the plate

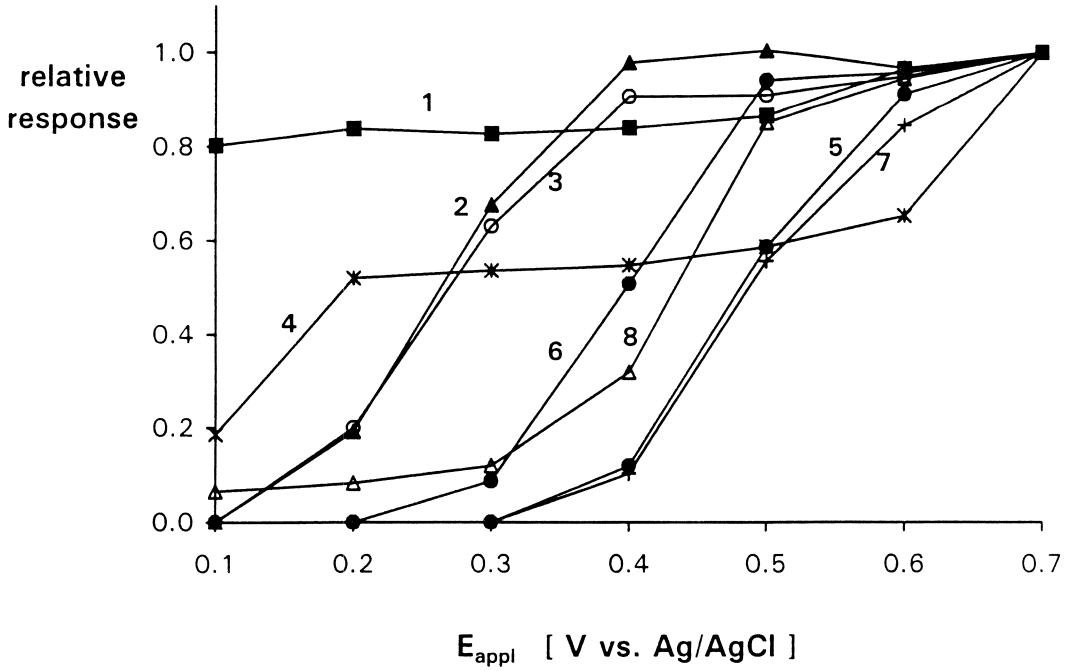


Fig. 2. Hydrodynamic voltammograms of anilines. Responses (peak areas) are given relative to those obtained at +0.7 V. For experimental conditions and numbering of compounds see Fig. 1.

numbers. It must be noted, however, that the efficiency of the system was already low compared to other CE systems, because of the zone broadening effect of the off-column detector cell.

With the maximum injection volume, detection limits in the order of $1–3 \cdot 10^{-8}$ mol/l (1–3 μg/l) were obtained. The peak area repeatability was ≤1.6% (RSD, $n=4$).

### *3.2. MEKC of fluorescamine derivatives*

The influence of the reaction conditions on the yield of the derivatization of the anilines with fluorescamine was investigated. To 1 ml of a sample solution 20 μl of a 0.01 mol/l fluorescamine solution in acetone was added, and peak areas were measured in the MEKC system used for separation (see below). It was found that a maximum yield was obtained when the anilines were derivatized in an aqueous buffer solution with a pH between 5 and 8. At lower or higher pH the yield was decreased. Best results were obtained at a pH of 5.5 (acetate buffer). It appeared that the amount of fluorescamine as used was sufficient for a fast derivatization; at higher reagent amounts the reaction mixtures became turbid. With a reaction time of 5 min the reaction was complete. For most anilines the reaction products were stable for at least 20 min; only for 2,6-dichloroaniline was some decrease of the concentration of the derivative with time found (see Fig. 3).

In preliminary experiments it was found that the fluorescamine derivatives of the anilines tested all migrated as anions against the EOF in a BGE with a pH of 9 (borate buffer). However, their mobilities were all approximately equal, so that no separation was obtained. Therefore, a variant of MEKC was tried. The addition of sodium dodecyl sulfate (SDS) to the BGE increased the effective mobilities differentially. As expected, the mobilities of the most hydrophobic anilines were affected most (see Fig. 4). A satisfactory separation of the test mixture was obtained with 20 mmol/l of SDS in the BGE. With this SDS concentration a 30 kV separation voltage could still be used without overheating the system. The migration time repeatability of the system was ≤1.0% (RSD, $n=5$). Plate numbers obtained ranged

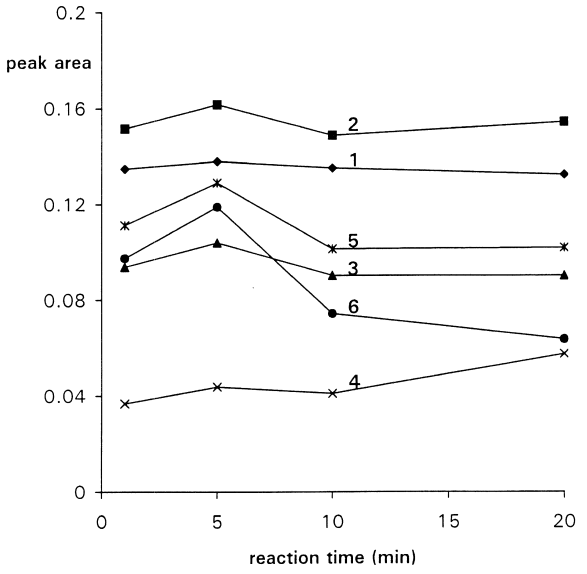


Fig. 3. Influence of the reaction time on the yield of the derivatization. For conditions see text. Compound numbering: (1) aniline; (2) 4-chloroaniline; (3) 2-methylaniline; (4) 2,6-dimethylaniline; (5) 2-ethylaniline; (6) 2,4-dichloroaniline.

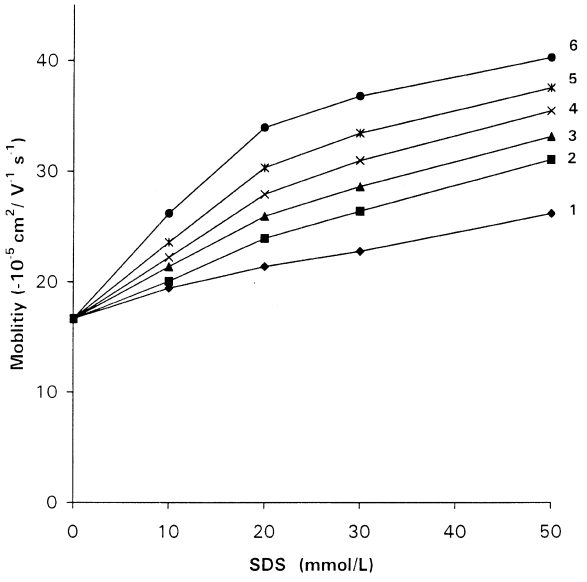


Fig. 4. Effect of the SDS concentration of the BGE on the apparent mobilities of the fluorescamine derivatives. BGE: 5 m*M* sodium tetraborate, 4.5 m*M* boric acid (pH 9) and SDS. Compound numbering as in Fig. 3.

from 300 000 for early eluting compounds to 70 000 for compounds strongly absorbed in the SDS micelles.

Since the conductivity of the sample solution was low compared to that of the BGE, sample stacking could be applied. Up to 120 nl of sample solution could be introduced into the 75 μm capillary (24 s at 40 mbar) without appreciable deterioration of the separation efficiency. Together with the high sensitivity of the fluorescence detector applied, this led to low concentration detection limits, which ranged from 0.5 to 2 μg/l. A representative electropherogram is shown in Fig. 5. The peak area repeatability was in the order of 1–2% (RSD), except for the disubstituted anilines. With these compounds the yield of the derivatization procedure was less well reproducible.

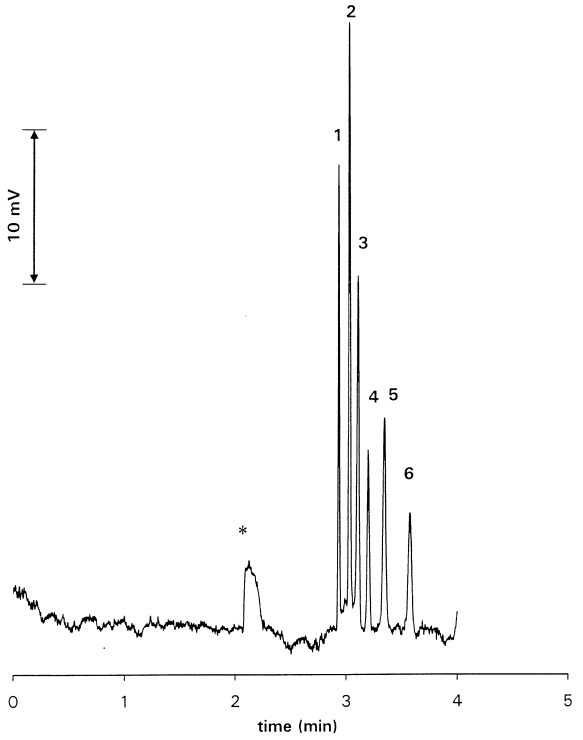


Fig. 5. Separation of a standard mixture of derivatized anilines by MEKC with fluorescence detection. Capillary: 0.68 m (0.53 m effective length)×75 μm I.D. Applied voltage: 30 kV. BGE: 5 m*M* sodium tetraborate, 4.5 m*M* boric acid and 20 m*M* SDS (pH 9). Sample concentration: $10^{-7}$ mol/l of each compound. Compound numbering as in Fig. 3.

Table 1
Comparison of the analytical performance of the two alternative techniques for the determination of aniline compounds

| Performance parameter | CZE–electrochemical detection | MEKC–fluorescence detection |
|---|---|---|
| Typical LOD[a] | 2 μg/l | 1 μg/l |
| Plate number | 70 000 | 200 000 |
| Run time | 12 min | 5 min |
| Migration time repeatability | 3% | 1% |
| Peak area repeatability | 2% | 2–6% |
| Robustness | − | + |
| Ease of use | − | + |

[a] Limit of detection, $S/N=2$.

### *3.3. Comparison and application*

The performances of the two alternative analytical systems for aniline compounds, CZE with amperometric detection and MEKC with fluorescence detection after derivatization, are compared in Table 1. The run time with the first system was clearly longer than with the second. The electroosmotic mobility was relatively low because the separation had to be performed at low pH. Moreover, a long capillary and a lower separation voltage had to be used to limit the effect of extra column zone broadening. Even when the derivatization time is included in the time required for analysis (when derivatization and separation have to be carried out sequentially) the MEKC method performs better in

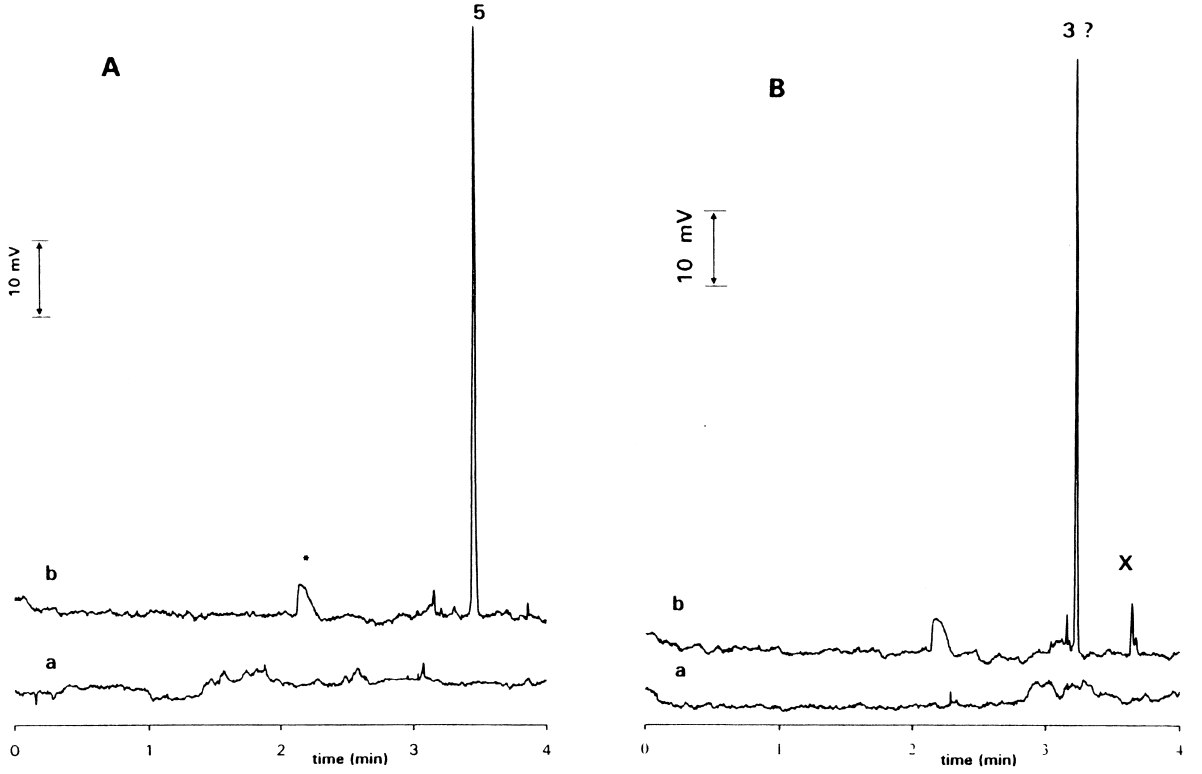


Fig. 6. Analysis of surface water samples. Conditions as in Fig. 5. (a) Without and (b) with fluorescamine derivatization. Samples taken in areas with (A) textile and (B) leather industries.

this respect. Moreover, with the MEKC system better separations can be obtained.

In terms of detection limits there is not much difference between the two methods. Applying a mild form of sample stacking with both systems sample concentrations in the order of one to a few μg/l can be measured. This would be adequate for our purpose: the screening of surface water samples for the occurrence of industrial discharges of pollutants. In respect to robustness, the MEKC–fluorescence detection system is clearly to be preferred. With the amperometric detector it appeared to be difficult to install or replace the separation capillary or the working electrode after cleaning in a reproducible way. Such problems were not encountered with the fluorescence detector. Even with the derivatization procedure as an additional source of variation the day-to-day reliability with this method was considerably better than with amperometric detection. Therefore, this method will be selected for further development and application.

Preliminary experiments have been carried out to study the applicability of the proposed method for real samples. Surface water samples have been analysed that had been collected in regions with a prominent textile industry (Fig. 6A) and one with a strong leather industry (Fig. 6B). In the first sample a compound has been found that gives a peak at the position of 2-ethylaniline; in the other sample 2-methylaniline could be present. In both cases no fluorescent compounds were found without derivatization. However, before any conclusions can be drawn a further study to the occurrence of (natural or industrial) interferences will be necessary.

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