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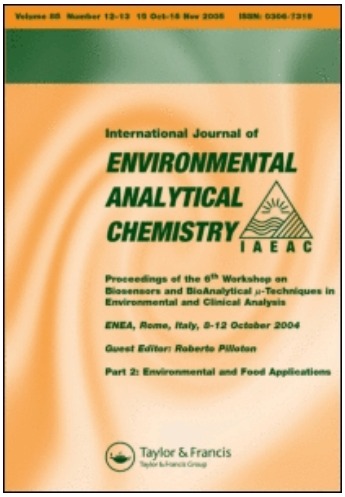

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Liquid Chromatographic Determination of Cationic Surfactants in Environmental Samples using a Continuous Post-Column Ion-Pair Extraction Detector with a Sandwich Phase Separator

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Liquid Chromatographic Determination of Cationic Surfactants in Environmental Samples using a Continuous Post-Column Ion-Pair Extraction Detector with a Sandwich Phase Separator

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Dedicated to Professor W. Haerdi on the occasion of his 60th birthday

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A new detection technique is described for the quantitative analysis of cationic surfactants by HPLC via post-column ion-pair formation. A new sandwich type phase separator, as part of the extraction detector, was successfully introduced. The method was used to determine ditallowdimethylammonium chloride (DTDMAC) in various

environmental samples. Detection limits of DTDMAC in river water were about 2 μg/l (60 ng absolute; S/N = 5) and 10 ng/l (260 pg absolute; S/N = 5), using methyl orange and 9,10-dimethoxyanthracene-2-sulphonate (DAS) as ion-pairing reagents, respectively. The environmental concentration of DTDMAC found on random samples from two Belgian rivers range from 30 to 40 μg/l. The reproducibility of the determination of DTDMAC in river water was 4.2% (RSD) ($n = 20$).

KEY WORDS: Cationic surfactants, liquid chromatography, post-column extraction detection, sandwich phase separator.

INTRODUCTION

Ditallowdimethylammonium chloride (DTDMAC) is a major cationic surfactant used in consumer products as a fabric softener and antistatic agent. It is removed efficiently from sewage in wastewater treatment plants (>90%). Trace amounts escaping sewage treatment are released in the aquatic environment.

Currently, only a limited number of analytical methods exist for the determination of DTDMAC in environmental samples. A simple colorimetric procedure based upon the formation of an ion-pair with the intensely colored disulfineblue anion (DBAS) which is subsequently extracted into a suitable organic solvent, has been described by Waters[1] and by Osburn.[2] This method, however, is not selective for DTDMAC since all long chain ammonium compounds respond as well.

Wee and Kennedy[3] presented a selective analytical procedure for the quantification of cationic surfactants by coupling HPLC with conductivity detection. A unique feature of this method is that non-UV-absorbing long alkyl chain quaternary compounds are determined in sub-microgram quantities without any form of derivatization. This procedure allows to determine the major cationic surfactants in aqueous environmental samples with a relative standard deviation of about 10%. Although the procedure is very efficient for influents, effluents and river water, its conductometric detection requires the use of an isocratic chromatographic system.[4] This limits its possibility to resolve DTDMAC from interferences in complex matrices, and increases the analysis times.

The ability of quaternary ammonium surfactants to form an extractable ion-pair with an anionic dye has been described.[1,2,5–8]

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The concept of ion-pairing has also been extended to HPLC.[9–11] In continuous flow post-column extraction detectors, the column effluent is segmented by an immiscible solvent, containing a UV/Vis absorbing or fluorescent counter-ion. Ion-pair formation takes place in an extraction coil. Before detection is possible, the two phases must be separated by means of a phase separator, which is the most critical part of a post-column extraction detector. Many of the phase separator designs, recently reported in the literature, make use of a hydrophobic (PTFE) membrane.[12–15] A major drawback of these membrane phase separators in the analysis of surfactants is their very short lifetime. Therefore, a novel separation design is applied, based on wetting of individual solvents on suitable materials, and gravity. This sandwich phase separator, without a membrane, is very reliable, gives only a small contribution to band broadening and has no lifetime problems.[16–18]

In this paper, a detection technique is described for the analysis of cationic surfactants by HPLC with a post-column extraction detector, based on the on-line ion-pair extraction of the cationic surfactant with a counter ion of methyl orange or 9,10-dimethoxyanthracene-2-sulphonate sodium salt (DAS) into the organic eluent, which is monitored by a UV or a fluorescence spectrophotometer, respectively. This technique has been applied to the analysis of DTDMAC in environmental samples. Other pairing ions, such as applied in standard colorimetric and titrimetric methods for surfactants would probably serve equally well and provide alternatives in specific applications.

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EXPERIMENTAL

Chemicals

Methanol, acetonitrile (HPLC grade) and chloroform (analytical grade) were obtained from J. T. Baker (Deventer, The Netherlands). Monotallowtrimethylammonium chloride (MTTMAC), ditallowdimethylammonium chloride (DTDMAC) and tritallowmethylammonium chloride (TTMAC) were obtained from Procter & Gamble E.T.C. (Brussels, Belgium). 9,10-Dimethoxyanthracene-2-sulphonate sodium salt (DAS) (~98%) was purchased from Fluka

(Buchs, Switzerland) and methyl orange from Janssen (Beerse, Belgium).

Demineralized water, treated in a Milli Q, (Millipore, Bedford, MD, USA) ultrafiltration system, was used.

All other chemicals used were analytical grade and obtained from J. T. Baker.

Liquid chromatography

A scheme of the apparatus used is shown in Figure 1. Injection of the samples and delivery of the mobile phase (flow rate, 2.0 ml/min) was done with a Hewlett–Packard (Waldbronn, F.R.G.) 1090 liquid chromatograph. The ion-pairing reagent was delivered by a Gilson (Villier-le-Bel, France) model 302 LC pump (flow rate, 0.75 ml/min) equipped with a home-made membrane pulse damper. The 250×4.6 mm I.D. analytical column was packed with Whatman (Clifton, NJ, USA) Partisil PAC 10 μm. A 7.8×2.35 mm I.D. home-made stainless-steel guard column, packed with Baker CN 40 μm material, was only used for the analysis of DTDMAC in sludge and sediment, since in all other cases, extensive contamination of the analytical column was not observed. However, the analytical column was flushed overnight with chloroform-methanol (20:80, v/v) at a flow rate of 0.1 ml/min. The post-column extraction system consisted of a Valco (Houston, TX, USA) T-piece with 0.25 mm bores, a

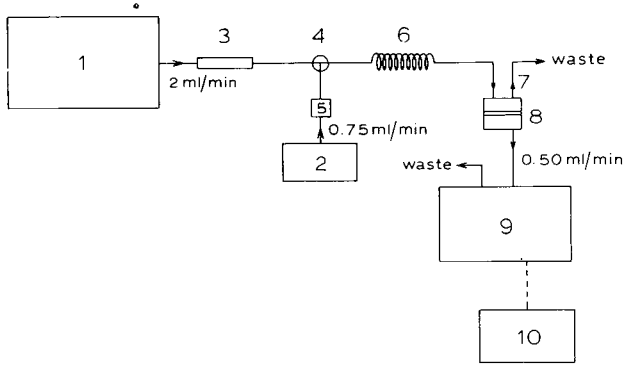

Figure 1 Diagram of the HPLC system: (1) HP 1090 liquid chromatograph; (2) reagent pump; (3) column; (4) T-piece; (5) pulse damper; (6) extraction coil; (7) restrictor; (8) sandwich phase separator; (9) detector; (10) recorder.

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1.5×0.8 mm I.D. stainless-steel (316) capillary (helix diameter, 40 mm) and a home-made sandwich phase separator.[17] With this phase separator, a purely organic phase can be obtained. The ratio of the organic phase through the detector to the total eluent flow was regulated by means of a PTFE capillary (0.8 mm I.D.) equipped with a restrictor. A ratio of about 0.25, corresponding to a flow of 0.50 ml/min through the detector was a good compromise in terms of ease of operation, signal-to-noise ratio and band broadening. For detection a Perkin–Elmer (Norwalk, CT, USA) LC 55 UV/Vis spectrophotometer ($\lambda = 420$ nm) or a Perkin–Elmer LS-4 fluorimeter set at $\lambda_{ex} = 383$ nm and $\lambda_{em} = 452$ nm (slits, 10 nm), was used. Chromatograms were recorded on a Kipp (Delft, The Netherlands) BD-8 recorder.

The eluent used was a chloroform-methanol-acetonitrile gradient (see Results and Discussion). The solvents were constantly degassed under a stream of helium. The reagent solutions were prepared as follows; methyl orange: 100 mg methyl orange were ultrasonically dissolved in 25 ml methanol. To this solution 975 ml Millipore water were added. DAS: 34 mg DAS were dissolved in 1000 ml Millipore water. Reagent solutions were degassed using an ultrasonic bath for at least 30 min.

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Procedures

Sample preparation Aqueous samples from sewage treatment plants and river water samples were treated by a combined procedure taken from the methods by Osburn[2] and Wee.[3] The procedure has a recovery in the range of $90 \pm 10\%$. In a first step, aliquots of 100, 400 and 1300 ml of influent, effluent and river water samples, respectively, were evaporated to dryness on a steambath under a stream of nitrogen. The cationic surfactants were isolated from the evaporated samples by a series of three consecutive extractions with 20 ml of hot acidified methanol (4% HCl v/v). The extracts were centrifuged and the combined supernatants were then evaporated on a steambath. The residue of the previous step was transferred into a separatory funnel using 100 ml of distilled water and 5 ml of concentrated hydrochloric acid. Prior to extraction with 50 ml chloroform, an excess of 2 mg of a linear alkylbenzenesulphonate were added to enhance the extraction of the quaternaries into the non-polar phase.

The combined chloroform layers of three successive extractions were then evaporated to about 10 ml and transferred quantitatively into a vial. The chloroform was evaporated and the residue was dissolved in 10 ml methylene chloride. Following dissolution, a series of three back-extractions with 5 ml water each were performed. The water layers were discarded and the remaining methylene chloride layer in the vial was evaporated to dryness on a steambath under a stream of nitrogen. The vial was kept in a dry state until analysis by HPLC. The residue was redissolved in chloroform prior to the HPLC separation.

Solid samples were extracted directly with acid methanol. Repetitive extractions were made with clean solvent until a colourless extract was obtained. Further sample pretreatment proceeded as described above. Recommended sample volumes are 1 g for dried sludge and 10 g for dried sediment.

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Quantification of DTDMAC in environment samples Quantification of DTDMAC in environmental samples was done by the standard addition method. Before the analysis could be started it was necessary to inject three high DTDMAC standards (10 μg) to ensure a constant system performance.

When using methyl orange as reagent, the injection volume was 100 μl of sample to which up to 10 μl of a DTDMAC standard in chloroform (180.3 ng/μl) were added. When using DAS as reagent, the injection volume was 10 μl of sample to which up to 10 μl of a DTDMAC standard solution in chloroform (15.0 ng/μl) were added. The addition of the standards was done automatically using the HP 1090 liquid chromatograph. Quantification of DTDMAC in the various environmental samples was done by plotting measured peak heights against the amount of DTDMAC standard added, using the ratio intercept/slope of the resulting curve after linear regression.

RESULTS AND DISCUSSION

Optimization of chromatography

Chromatography of DTDMAC was accomplished using a Whatman Partisil PAC (cyano-amino bonded phase) column and, initially, isocratic chloroform-methanol mixtures as the mobile phase, accord-

ing to Wee and Kennedy.[3] However, to achieve short analysis times, we had to develop an optimal mobile phase gradient. A major problem was the fact that the retention of DTDMAC on different columns of the same type from the same company, using the same mobile phase gradient, could considerably differ. In addition, when using chloroform-methanol gradients on a new column, separation of DTDMAC from major matrix interference present in river waters, was not possible. Therefore, we added acetonitrile as a ternary mobile phase solvent.

The use of acetontrile in the mobile phase had a dramatic effect on the retention of DTDMAC; going from 0% to 4% acetonitrile in the mobile phase (chloroform-methanol) resulted in a change in k' from about 15 to 5. A scheme of the applied gradient for the determination of DTDMAC in environmental samples is shown in Figure 2. Using this gradient, the analysis time is reduced by a factor of 3 compared to that of Wee and Kennedy.[3] The use of more than about 12.5% total modifier content is not recommended since above this value breakthrough of aqueous segments via the phase separator to the detector is observed, causing major disturbances.

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Performance of the post-column extraction detector

The performance of the post-column extraction detector was tested by flow injection analysis of a DTDMAC standard solution. The

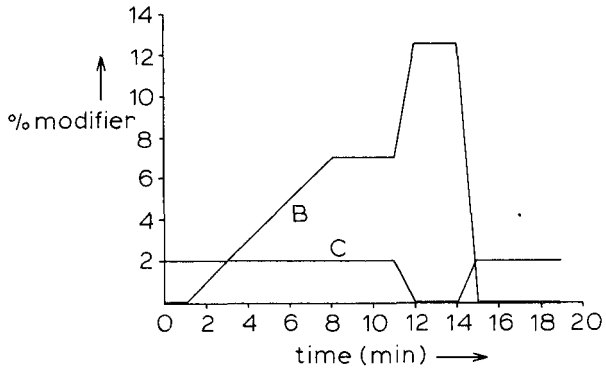

Figure 2 Mobile phase gradient: A = chloroform $(100\% - (B + C))$; B = methanol; C = acetonitrile.

total system as described in Figure 1, with the exception of the column, was used. The eluent was chloroform (flow rate, 2.0 ml/min), the aqueous reagent flow rate was 0.75 ml/min and the organic flow through the detector 0.65 ml/min. Using methyl orange as the reagent, 62 replicate injections of 12 μg DTDMAC showed a relative standard deviation (RSD) of 2.3%. For DAS, 24 replicate injections of 90 ng DTDMAC gave a RSD of 1.8%. These results indicate that the post-column extraction detector with the new sandwich phase separator is very reliable.

Calibration curves

Quantitation was carried out by peak-height measurement. Using methyl orange as reagent, calibration curves, for three different cationic surfactants were;

MTTMAC: $y = 19.95 + 12.01 \times \quad r = 0.999\ (n = 5)$
investigated range: 0.6–19 μg (amount injected)

DTDMAC: $y = -0.8 + 27.46 \times \quad r = 0.999\ (n = 5)$
investigated range: 0.8–23 μg

TTMAC: $y = 2.22 + 24.91 \times \quad r = 0.9999\ (n = 5)$
investigated range: 0.8–18 μg

The rather high intercept in the case of MTTMAC was due to a constant system interference as a result of the applied gradient.

Determination of DTDMAC in river water samples

The concentration of DTDMAC was determined in two rivers from Belgium, whereas from one of the rivers, water samples at two different locations were taken. The analysis was done using methyl orange as well as DAS as the reagent. Typical chromatograms, obtained from the same river, as well as the resulting data are shown in Figures 3 and 4 and Tables 1 and 2. From these figures it is clear that DTDMAC is well separated from matrix interferences in less than 20 min. The detection limit of DTDMAC in river water, using methyl orange as reagent is about 2 μg/l (60 ng absolute; S/N = 5),

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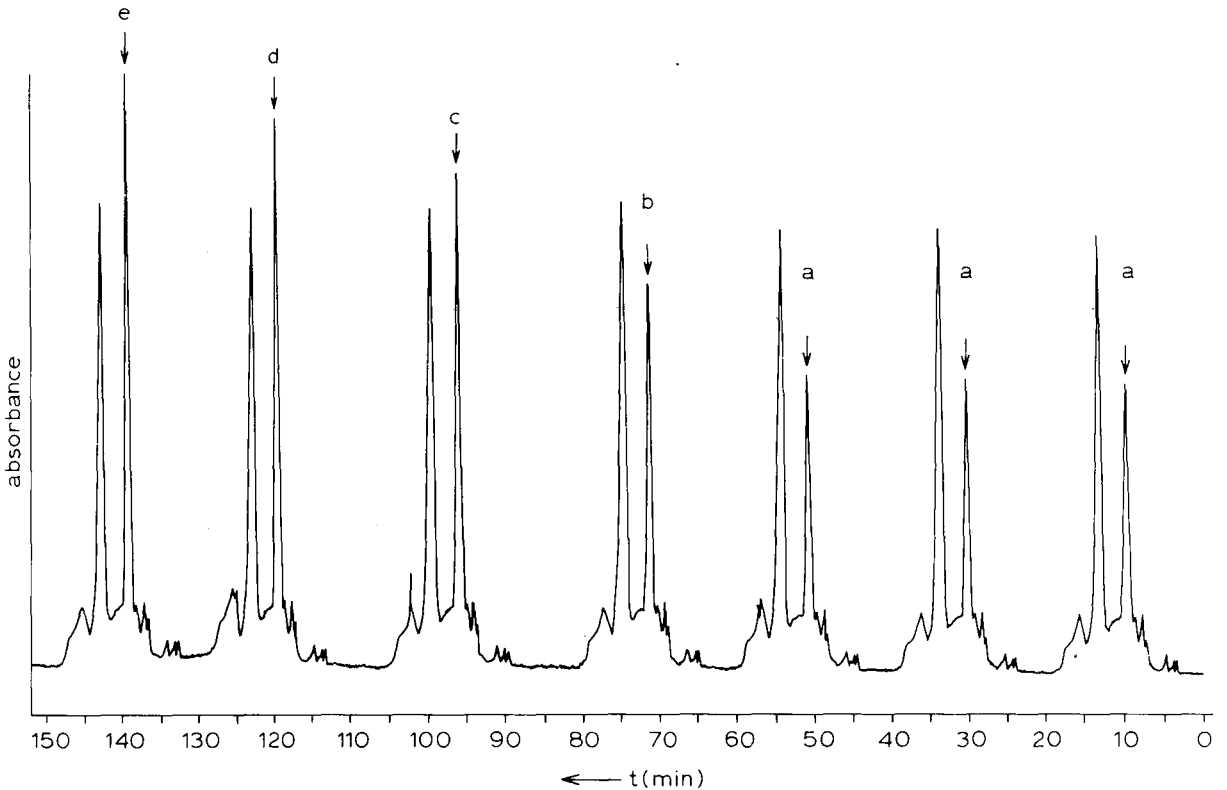

Figure 3 Chromatograms of river water sample (river A, at location 2): (a) sample as is; (b–e) with addition of DTDMAC standard (for data, see Table 1). Conditions: column, 250 × 4.6 mm I.D. Whatman PAC 10 μm; mobile phase gradient as described in Figure 2, flow rate 2.0 ml/min; reagent, methyl orange, flow rate 0.75 ml/min; flow through the detector, 0.50 ml/min; detector, UV/Vis, $\lambda = 420$ mm, abs. 0.1; initial sample volume, 1300 ml.

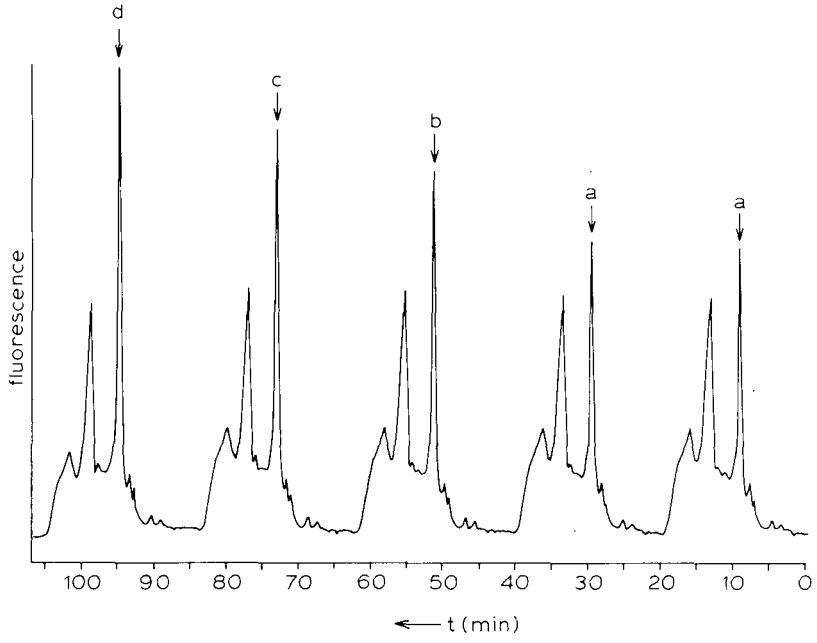

Figure 4 Chromatograms of river water sample (river A, at location 2): (a) sample as is; (b–d) with addition of DTDMAC standard (for data, see Table 2). Conditions as described under Figure 3, except: reagent, DAS; detector, fluorescence, $\lambda_{ex} = 383$ nm and $\lambda_{em} = 452$ nm (slits, 10 nm).

based on 100 μl injected (out of 5 ml) and a 1300 ml sample analyzed. Using DAS as the reagent, the detection limit is much lower. With an injection volume of 10 μl (which can be enlarged to 100 μl as in the case of methyl orange as the reagent), and the large sample volume worked up (1300 ml), a very low detection limit of about 10 ng/l (260 pg absolute; S/N = 5) is achieved.

The results of the determination of DTDMAC in river waters are summarized in Table 3. These data clearly demonstrate that the results, obtained with methyl orange and DAS as the reagent, are in good agreement.

The reproducibility of the determination of DTDMAC in river water was good, i.e., 4.2% (RSD) for 20 replicate injections of river A (at location 2) sample, using DAS as reagent.

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Table 1 Quantification of DTDMAC in river water sample A (at location 2), by standard addition[b], using methyl orange as reagent

Chromatogram Figure 3	*DTDMAC[a] addition (μl)*	*Peak height (mm)*
a	0	62.4
	0	62.5
	0	63.8
b	2	85.9
c	4	114.4
d	5	129.0
e	6	141.3

[a]DTDMAC standard solution: 180.3 ng/μl.

[b]Standard addition curve: $y = 61.518(\pm 1.355) + 13.289(\pm 0.337)x$; $r = 0.999$. DTDMAC concentration found:

$$\frac{61.518}{13.289} \cdot 180.3 \cdot 50 \text{ (dilution factor)} \cdot \frac{1}{1.3} \text{(sample volume)} = \mathbf{32 \pm 1\ \mu g/l.}$$

Table 2 Quantification of DTDMAC in river A (at location 2), by standard addition[b], using DAS as reagent

Chromatogram Figure 4	*DTDMAC[a] addition (μl)*	*Peak height (mm)*
a	0	65.4
	0	67.5
b	2	87.0
c	3	97.3
d	4	115.7

[a]DTDMAC standard solution: 15.0 ng/μl.

[b]Standard addition curve: $y = 64.213 \ (\pm 3.307) + 11.015(\pm 1.289)x$; $r = 0.99$. DTDMAC concentration found:

$$\frac{64.213}{11.015} \cdot 15.0 \cdot 500 \text{ (dilution factor)} \cdot \frac{1}{1.3} \text{(sample volume)} = \mathbf{34 \pm 4\ \mu g/l.}$$

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Table 3 Environmental concentration of DTDMAC in river waters

River	*DTDMAC concentration found (μg/l) with*	
	Methyl orange	*DAS*
A, location 1	33±2	31±2
A, location 2	32±1	34±4
B	37±1	41±2

Determination of DTDMAC in sewage influent and effluent

Figure 5 presents the chromatograms of the determination of DTDMAC in sewage influent and effluent, from the same treatment plant, showing that DTDMAC is well resolved from matrix interferences. The DTDMAC concentrations found were 710±65 μg/l for the influent and 136±8 μg/l for the effluent.

Determination of DTDMAC in other environmental samples

In order to explore whether the scope of the analytical method, developed for the analysis of DTDMAC in river water and sewage influent and effluent can be extended to other environmental samples, duplicate analyses of aerobic sludge, digested sludge and sediment samples were performed. The results in Figure 6 indicate that the HPLC system, in combination with the post-column extraction detector used, provides adequate selectivity and sensitivity for the determination of DTDMAC in all cases.

CONCLUSION

A continuous flow ion-pair extraction detector has been successfully introduced for the analysis of DTDMAC in extracts from river water-, sediment-, sewage- and sludge-samples by gradient elution HPLC. An essential part in the instrumental design is a newly

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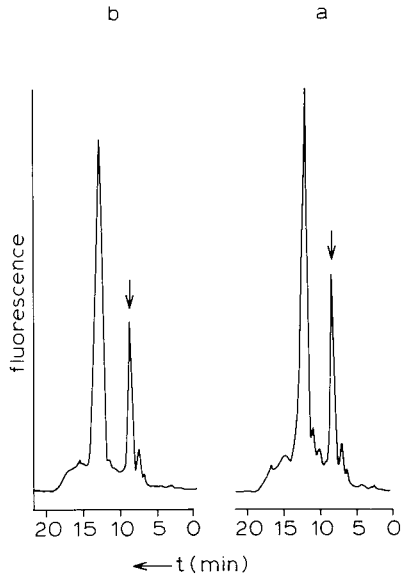

Figure 5 Chromatograms of sewage influent and effluent, from the same treatment plant: (a) influent, $710 \pm 65\,\mu g$ DTDMAC/l; initial sample volume, 100 ml. The standard addition curve obtained was $y = 54.188(\pm 2.665) + 5.723(\pm 0.439)\times$, $r = 0.99$. (b) effluent, $136 \pm 8\,\mu g$ DTDMAC/l; initial sample volume, 400 ml; standard addition curve, $y = 40.386(\pm 1.448) + 5.578(\pm 0.239)\times$, $r = 0.997$. Further conditions as described under Figure 4.

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developed sandwich phase separator, which showed a constant and reliable performance over several months.

The post-column extraction detection system allows a choice for the counter-ion. Two pairing ions were qualified, methyl orange for UV/Vis and DAS for fluorescence detection. Both yield comparable results in the analysis of DTDMAC in environmental samples. The detection limits are 60 ng and 260 pg, respectively. Hence, the use of DAS permits the work-up of much smaller sample volumes and could permit simplification of the rather time consuming sample preparation used in this study, i.e., by adopting solid phase extraction techniques.[19]

The HPLC system with the continuous flow extraction detector qualifies for mono-, di- and tri-alkyl ammonium quaternaries. The

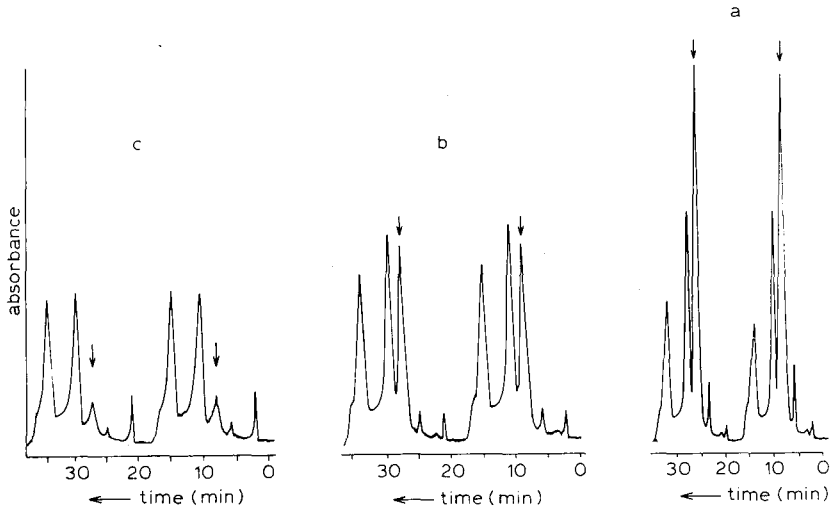

Figure 6 Determination of DTDMAC in other environmental samples. (a) duplicate analysis of aerobic sludge; initial sample, 0.13 g of dry sludge; (b) duplicate analysis of digested sludge; initial sample, 0.05 g of dry sludge; (c) duplicate analysis of sediment; initial sample, 1.0 g of dry sediment. Conditions: column, 250 × 4.6 mm I.D. Whatman PAC 10 μm; guard column, 7.8 × 2.3 mm I.D. column, packed with Baker CN 40 μm; mobile phase gradient as follows:

time (min)	%B	%C
0	0	4
1	0	4
6	5	4
11	5	4
12	12.5	0
14	12.5	0
15	0	4

A = chloroform (100% − (B + C))
B = methanol
C = acetonitrile

flow rate, 2.0 ml/min; reagent, methyl orange, flow rate 0.75 ml/min; flow through detector, 0.50 ml/min; detector, UV/Vis, λ = 420 nm, abs. 0.1.

method is expected to be applicable to all long chain quaternary compounds and long chain amines.

Acknowledgement

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Mr. D. van Ieperen, for construction of the phase separators. Mrs. C. de Jonge is thanked for typing the manuscript.

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