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# Liquid chromatography–mass spectrometry method to determine alcohol ethoxylates and alkylamine ethoxylates in soil interstitial water, ground water and surface water samples

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**Abstract**

Alcohol ethoxylates (AEs) and alkylamine ethoxylates (AMEs) are used as adjuvants in pesticide formulations. Analytical procedures for these compounds in environmental aqueous samples using LC–MS are presented. Sample preparation uses solid-phase extraction with Porapak Rdx cartridges. Detection limits and recoveries in ground water and surface water are, respectively, AEs: 16–60 ng/l, 35–93% and AMEs: 0.3–6 μg/l, 28–96%. The lower recoveries are obtained for the apolar surfactants. The procedure was employed on samples of ground water and soil interstitial water collected from farming areas. The individual AEs were detected at concentration levels ranging from 33 to 189 ng/l water. 

*Keywords:* Water analysis; Environmental analysis; Extraction methods; Alcohol ethoxylates; Alkylamine ethoxylates; Ethoxylates; Surfactants; Pesticides

## 1. Introduction

Pesticide formulations contain beside the active ingredients nearly always several adjuvants. Adjuvants can either be added as ingredients of the pesticide formulations or used as spray adjuvants, which are added to the spray solution. These chemicals are added to enhance the effectiveness of the pesticide. Adjuvants in pesticides include solvents, surfactants, spreaders, dispersants, emulsifiers, antifoam agents, wetting agents, antifreezing agents, preservatives, etc. [1]. Until recently, research on pesticides has focused mainly on the environmental problems of the active ingredients in the formulation. Only one paper [2] considers the use of pesticide additives as source of contamination of soil and waters.

Surfactants make up the largest group of adjuvants beside the solvents [3–5]. The mean load of surfactants from pesticide application, on the Danish cultivated areas, is between 0.3 and 0.4 kg surfactants per ha per year depending on the crop, frequency of treatment and the specific pesticide used [6]. Nonionic surfactants constitute the most utilised

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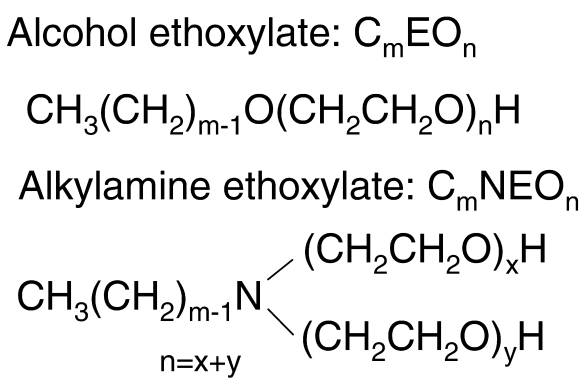


Fig. 1. Chemical structures and abbreviations of alcohol ethoxylates and alkylamine ethoxylates.

group of surfactant [5]. Alcohol ethoxylates (AEs) (see Fig. 1), alkylphenol ethoxylates (APEs) and alkylamine ethoxylates (AMEs) (see Fig. 1) are the primary used nonionic surfactants in pesticide formulations [7,8].

Several papers have reviewed suitable analytical methods used in the analysis of nonionic surfactants (e.g., AE) in environmental samples [2,9–17]. These papers discuss different methods of sample preparations, chromatographic separation systems and detection techniques. Various detection techniques, utilising derivatisation of AEs followed by UV or fluorescence detection, are described including examples of detection using mass spectrometry (MS). Chromatographic methods applying reversed phase (RP) or normal-phase (NP) systems are often considered, where separation are performed either on behalf of the hydrophobic, alkyl chain or the hydrophilic, ethoxy chain. For RP conditions, many analytical methods for AEs apply $C_8$ or $C_{18}$ columns and mobile phase mixtures of methanol and water [18–20], acetonitrile and water [21], acetone and water [22] or tetrahydrofuran and water [23–25]. Others use mixtures of methanol, acetonitrile and water as mobile phase [26] and with a Hypersil Green ENV column [27,28]. The method by Castillo et al. [27] is a multi-method for surfactants including AE in wastewater. However, only AEs with shorter alkyl chains ($C_{10}$–$C_{14}$) are analysed using this method.

Considering analysis of AMEs, only three papers have been published [29–31]. All the described analytical methods are used for characterisation of the technical mixtures. Schreuder et al. [29] extended the analysis of AMEs to determinations of AMEs in pesticide formulations. Their method presented two separation approaches: one using a cyano column and the other using an amino column to determine the alkyl and ethoxy distribution of the AMEs, respectively. Detection was performed applying post column ion pairing followed by fluorescence detection. Including steps of derivatisation and ion paring in the analytical method are not always optimal, instead direct analysis with a selective detection method like MS is often preferred. Considering the previously published methods, it would be advantageous to have one common method of clean up and analysis of the two groups of surfactants for environmental aqueous samples. A common method is needed especially, because these two groups often are included as adjuvants in pesticide formulations and tank mixtures.

The purpose of the present work is thus to develop and optimise an LC–MS method, including a preconcentration clean-up step and chromatographic separation followed by MS detection of both AEs and AMEs applicable to aquatic environmental samples. For the validation, the recovery and the precision indicated by the reproducibility and repeatability were tested for ground water. Furthermore, the method is applied to the analysis of samples collected from agricultural areas in Denmark.

## 2. Materials and methods

### *2.1. Solvents and standards*

All solvents were obtained from Merck (Darmstadt, Germany). Acetonitrile, methanol and dichloromethane were LiChrosolv grade, while sodium hydroxide, triethylamine and acetic acid (100%) were of analytical-reagent grade. Deionised Milli-Q water (Millipore, Bedford, MA, USA) was used in all experiments.

AEs are pure standards (purity between 97 and 99%) with well-defined alkyl and ethoxylate chains obtained from Fluka, the standards are $C_{10}EO_6$, $C_{12}EO_3$, $C_{12}EO_4$, $C_{12}EO_5$, $C_{12}EO_6$, $C_{12}EO_7$, $C_{12}EO_8$, $C_{12}EO_9$, $C_{14}EO_6$, $C_{16}EO_6$ and $C_{18}EO_6$.

AMEs are two technical mixtures, Berol 907 (B907, Tallowalkylamine ethoxylate 70%, ethyleneglycol 1–5%) and Ethomeen C/12 (EC12, Cocosbis(2-hydroxyethyl)amine, purity ca. 100%), which kindly have been provided by Akzo Nobel, Stenungsund, Sweden. In these mixtures both the alkyl and the ethoxylate chain vary (see Table 1). Stock solutions were prepared once a year and stored at −20 °C, while diluted standards were freshly made once a month and kept at 5 °C. All standards are dissolved in 100% methanol.

All glassware, used at concentration levels below 10 mg/l, is, after a washing and drying procedure, heated at 450 °C for 6.5 h to ensure removal of possible contaminants.

### 2.2. Sample preparation

Five different types of solid-phase extraction (SPE) cartridges were tested. Three contained polymeric and two $C_{18}$-based packing materials. Sep-Pak Porapak Rdx (divinylbenzene–vinylpyrrolidone, Waters, Milford, MA, USA; 500 mg), Isolute ENV (cross-linked styrene–divinylbenzene copolymer, International Sorbent Technology, Mid-Glamorgan, UK; 500 mg), Oasis™ HLB (poly(divinylbenzene–co-*N*-vinylpyrrolidone) copolymer, Waters, 200 mg), Sep-Pak $C_{18}$ (Waters, 1 g) and Sep-Pak t$C_{18}$(trifunctional $C_{18}$ Waters, 1 g). The tests were performed using one blank and three spiked samples of 1 l drinking water. Spike concentrations of the AMEs were EC12: 10 000 ng/l and B907: 800 ng/l, and for each of the AEs: 100 ng/l.

The influence of pH on the extraction efficiency of the SPE cartridges was tested, by adjusting the pH of the drinking water samples to pH 4.5, 7 or 9. Three spiked water samples and a blank were loaded onto RDX and Oasis cartridges.

The SPE procedure after optimisation (as discussed in Section 3.1.1) was as follows: 1 l water samples were pH adjusted to pH 4.5 with 5 ml 25% sodium hydroxide and 6 ml acetic acid (100%). The samples were vacuum-filtered through an MN GF-4 (Machery–Nagel, Düren, Germany). To avoid loss of analytes, the filters were rinsed after filtration with 5 ml methanol, and added to the sample. The SPE cartridges were conditioned with 10 ml acetonitrile followed by 10 ml methanol and 20 ml Milli-Q

Table 1
Chemical structure of the ions used for identification and quantification of the alcohol ethoxylates (AEs) and alkylamine ethoxylates (AMEs)

| Alcohol ethoxylates (m) | *m/z* | Alkylamine ethoxylates (m) | *m/z* |
|---|---|---|---|
| $C_{10}H_{21}(CH_2CH_2O)_6H$ | 423[a] | Ethomeen C/12 | |
| $C_{10}H_{21}(CH_2CH_2O)_6H$ | 440[b] | $C_{12}H_{25}N(CH_2CH_2OH)CH_2CH_3$ | 256[c] |
| $C_{12}H_{25}(CH_2CH_2O)_3H$ | 336[b] | $C_{12}H_{25}N(CH_2CH_2OH)_2$ | 274[a] |
| $C_{12}H_{25}(CH_2CH_2O)_4H$ | 363[a] | $C_{14}H_{29}N(CH_2CH_2OH)_2$ | 302[a] |
| $C_{12}H_{25}(CH_2CH_2O)_4H$ | 380[b] | $C_{16}H_{33}N(CH_2CH_2OH)_2$ | 330[a] |
| $C_{12}H_{25}(CH_2CH_2O)_5H$ | 407[a] | | |
| $C_{12}H_{25}(CH_2CH_2O)_5H$ | 424[b] | Berol 907 | |
| $C_{12}H_{25}(CH_2CH_2O)_6H$ | 451[a] | $C_{16}H_{33}N(CH_2CH_2O)_{16}H_2$ | 947[a] |
| $C_{12}H_{25}(CH_2CH_2O)_7H$ | 495[a] | $C_{16}H_{33}N(CH_2CH_2O)_{17}H_2$ | 991[a] |
| $C_{12}H_{25}(CH_2CH_2O)_7H$ | 512[b] | $C_{16}H_{33}N(CH_2CH_2O)_{18}H_2$ | 1035[a] |
| $C_{12}H_{25}(CH_2CH_2O)_8H$ | 539[a] | $C_{18}H_{37}N(CH_2CH_2O)_{13}H_2$ | 843[a] |
| $C_{12}H_{25}(CH_2CH_2O)_9H$ | 600[b] | $C_{18}H_{37}N(CH_2CH_2O)_{14}H_2$ | 887[a] |
| $C_{14}H_{29}(CH_2CH_2O)_6H$ | 479[a] | $C_{18}H_{37}N(CH_2CH_2O)_{15}H_2$ | 931[a] |
| $C_{14}H_{29}(CH_2CH_2O)_6H$ | 496[b] | $C_{18}H_{37}N(CH_2CH_2O)_{16}H_2$ | 975[a] |
| $C_{16}H_{33}(CH_2CH_2O)_6H$ | 507[a] | $C_{18}H_{37}N(CH_2CH_2O)_{17}H_2$ | 1018[a] |
| $C_{16}H_{33}(CH_2CH_2O)_6H$ | 524[b] | | |
| $C_{18}H_{37}(CH_2CH_2O)_6H$ | 535[a] | | |
| $C_{18}H_{37}(CH_2CH_2O)_6H$ | 552[b] | | |

Note: m, molecular ion.
[a] $[m+H]^+$.
[b] $[m+H_3O]^+$.
[c] $[m-H_2O+H]^+$.

water. Water samples were loaded onto SPE cartridges through PTFE tubes (Visiprep Large Volume Sampler, Supelco, Bellefonte, PA, USA) using vacuum, at a flow-rate of 10–20 ml/min. A Waters 12-position Vacuum Manifold was used for the SPE extraction. Before and after each extraction the tubes were rinsed with water and ethanol to avoid contamination. After loading the cartridges were dried using vacuum and stored in the freezer at −20 °C until analysis. The cartridges were equilibrated to room temperature before elution with 5 ml methanol–acetronitrile (1:2) followed by 5 ml methanol–dichloromethane (1:4). The extract was evaporated to dryness under a gentle stream of nitrogen at 25 °C and re-dissolved in 1.00 ml 100% methanol before analysis.

### 2.3. Liquid chromatographic system

The liquid chromatographic system used was a Waters system consisting of a Waters 717 Autosampler and a Waters 600 MS system controller. The optimised analytical methods for the analysis of AEs and AMEs consist of two distinct analytical methods for the two types of surfactants. Mobile phases used for the AE analysis: (A) methanol–acetonitrile (1:1) containing 20 m*M* acetic acid; (B) Milli-Q water containing 20 m*M* acetic acid and for the AME analysis: (A) methanol–acetonitrile (1:1) containing 20 m*M* acetic acid, 25 m*M* triethylamine (TEA) and (B) Milli-Q water containing 20 m*M* acetic acid, 25 m*M* TEA. LC conditions consisted of the same RP gradient for the two types of surfactants: 70% A for 5 min, linear change to 98% B in 10 min, hold for 30 min, linear change to 70% A in 3 min, and hold for 12 min, giving an analysis time of 60 min. The injection volume was set to 50 μl. For both methods a Hypersil BDS $C_{18}$ (250×2 mm I.D., 5 μm) column was used as analytical column with a guard column (10×2 mm) containing the same packing material. Analysis was run with a LC flow-rate of 0.2 ml/min at 30 °C. The analytical methods are summarised in Table 2. All standards and samples are analysed by double injection.

### 2.4. Mass spectrometry conditions

The mass spectrometer used is a Finnigan TSQ 700 equipped with an atmospheric pressure ionization interface. Analyses are performed using atmospheric pressure chemical ionisation (APCI) with the following optimised instrumental settings: capillary temperature: 220 °C, vaporiser temperature: 500 °C, corona voltage: 3 mA, sheath gas: 40 p.s.i. and auxiliary gas: 0 p.s.i. (1 p.s.i.=6894.76 Pa). Nitrogen is used as sheath and auxiliary gas.

The ions are detected using selected ion monitoring (SIM) in the positive ion mode. A scan window of ±0.3 $m/z$ and a scan time of 0.25 s are chosen. In Table 1, the ions used to quantify the compounds are listed. The LC–MS system is connected to a DEC station 5000/125 personal computer with ICIS software (from Finnigan) used for in-

Table 2
Details of the analytical method for the two groups of surfactants

| | AE | AME |
|---|---|---|
| SPE cartridges | Porapak RDX | Porapak RDX |
| LC column | Hypersil BDS $C_{18}$ (250×2 mm I.D., 5 μm) | Hypersil BDS $C_{18}$ (250×2 mm I.D., 5 μm) |
| Mobile phase[a] | (A) Methanol–acetonitrile (1:1), 20 m*M* acetic acid (B) Milli-Q water, 20 m*M* acetic acid | (A) Methanol–acetonitrile (1:1), 20 m*M* acetic acid, 25 m*M* TEA (B) Milli-Q water, 20 m*M* acetic acid, 25 m*M* TEA |
| MS[b] | APCI, SIM(+) | APCI, SIM(+) |

[a] TEA: Triethylamine.
[b] APCI: atoms; SIM: selected ion monitoring.

Table 3
Physico–chemical properties of the ground and surface waters

| Parameter | Ground water | Surface water |
|---|---|---|
| pH | 7.7 | 8.2 |
| Conductivity (mS/m) | 84 | 45.3 |
| Hydrogen carbonate (mg/l) | 361 | 252 |
| Hardness (dH) | 18 | 16.2 |
| Dissolved oxygen (mg/l) | 8.4 | 9.1 |
| BOD (mg $O_2$/l) | | 1.5 |
| COD (mg $O_2$/l) | | 25 |

BOD, biological oxygen demand; COD, chemical oxygen demand.

strumental control and data recording. For quantification, Xcalibur version 1.2 (from Finnigan) was used.

### 2.5. Ground water and surface water

Ground water, which had not been treated (Risø, Denmark) and surface water (Haraldssted Lake, Ringsted, Denmark) were used for validation tests of the developed analytical method. Physico–chemical properties of the ground and surface water are outlined in Table 3. To determine the limit of detection (LOD) and recovery, five spiked samples of both ground water and surface water were prepared. The replicated samples (1 l) were spiked at a concentration of 100 ng/l of each of the AE standards, 10 000 ng/l for EC12 and 800 ng/l for B907 in the water sample. All samples were together with one blank pre-concentrated using SPE (see SPE procedure described above) and analysed by LC–MS. The LOD was determined as three times the standard deviation of the average concentrations divided by the recovery and by a volume coefficient. The coefficient was 1000 because the the 1 l sample had become 1 ml.

Three sets of triplicates (1 l) of, respectively ground water and surface water were spiked at three different concentrations (see Table 4). These nine samples and one blank of each type of water were loaded onto SPE cartridges and analysed.

Furthermore, ground water was applied to validate the reproducibility and repeatability of the analytical method. Three samples of ground water (1 l) were spiked at concentration levels as for the LOD determinations. At three different days and by two persons, a set of these three samples and one blank were prepared and loaded onto SPE cartridges. The samples were analysed at different days. Standard deviations ($SD_{between}$ and $SD_{within}$) are calculated as the square roots of $s^2$ obtained by a single-factor analysis of variance (ANOVA, Excell). $SD_{within}$

Table 4
Recovery (%) in ground and surface water at three spiking levels (see below)

| Compound | Recovery (%) in ground water | | | Recovery (%) in surface water | | |
|---|---|---|---|---|---|---|
| | Spiking level A | Spiking level B | Spiking level C | Spiking level A | Spiking level B | Spiking level C |
| $C_{10}EO_6$ | 47 | 82 | 77 | 77 | 97 | 117 |
| $C_{12}EO_3$ | 62 | 69 | 60 | 60 | 53 | 91 |
| $C_{12}EO_4$ | 53 | 69 | 63 | 63 | 84 | 93 |
| $C_{12}EO_5$ | 46 | 67 | 62 | 62 | 92 | 102 |
| $C_{12}EO_6$ | 41 | 62 | 53 | 53 | 87 | 103 |
| $C_{12}EO_7$ | 45 | 53 | 57 | 57 | 76 | 93 |
| $C_{12}EO_8$ | 50 | 53 | 50 | 50 | 84 | 91 |
| $O_{12}EO_9$ | 39 | 52 | 48 | 48 | 89 | 84 |
| $C_{14}EO_6$ | 35 | 43 | 37 | 37 | 60 | 64 |
| $C_{16}EO_6$ | 28 | 39 | 29 | 29 | 49 | 56 |
| $O_{18}EO_6$ | 34 | 44 | 36 | 36 | 35 | 35 |
| $C_{12-16}NEO_2$ | 42–80 | 61–91 | 61–92 | 61–92 | 73–98 | 67–109 |
| $C_{16}NEO_{16}$ | 26–31 | 35–39 | 38–47 | 38–47 | 42–46 | 53–57 |
| $C_{18}NEO_{16}$ | 26–34 | 39–43 | 39–45 | 39–45 | 28–36 | 31–40 |

Spiking levels: level A: AE 50 ng/l, $C_{12-14}NEO_2$ 5000 ng/l, $C_{16-18}NEO_{16}$, 400 ng/l; level B: AE 100 ng/l, $C_{12-14}NEO_2$ 10 000 ng/l, $C_{16-18}NEO_{16}$, 800 ng/l; level C: AE 200 ng/l, $C_{12-14}NEO_2$20 000 ng/l, $C_{16-18}NEO_{16}$, 1600 ng/l.

represent the SD obtained within samples pre-concentrated and analysed the same day. $SD_{between}$ was calculated as SD between the mean recoveries for the three data sets obtained from the different days of extraction.

### 2.6. Sampling areas

Samples have been collected monthly from agricultural areas in Denmark. Ground water was collected from sampling wells, respectively, 1–2, 2–3 and 3–4 m below the maximum ground water table and samples of soil interstitial water were taken 1 or 2 m below terrain. All samples were pre-concentrated using the developed SPE method within 2–4 days after sampling, in the intervening days the samples were stored at 5 °C. The sampling areas are research fields located in Silstrup and Tylstrup in the Northern part of Denmark.

## 3. Results and discussion

### 3.1. The analytical system

#### 3.1.1. Sample preparation

SPE was chosen as the preferred sample preparation method. The sample preparation consisted of a four-step off-line SPE procedure: first preparation of the water sample, then conditioning of the SPE cartridges, sample loading and finally elution of the extract. All four steps have been optimised individually to improve the recovery of the analytes.

The best recoveries were obtained with Oasis and Rdx testing the five different types of packing material [32]. These two types of cartridges were subsequently used in the optimisation experiments. Varying the acidity, of the water samples to pH 4.5, 7 or 9, the influence of the pH on the loading and thereby the retention abilities were tested. Due to the $pK_a$ values around 6–7 of the AMEs, it is important to investigate the influence of pH. For the AEs, the difference in pH did not, as expected, lead to any appreciable difference in recovery. However, for the AMEs effects of varying the pH were observed. At pH 7 and 9 all ions had very low recoveries (below 50%) whereas adjusting the water samples to pH 4.5 led to higher recoveries. Especially for the more polar analytes recoveries were increased to above 80%. When comparing the influence of the pH on the two kinds of cartridges, the overall picture showed similar recoveries.

Additionally, the retention capacity of the analytes on the packing material was checked by loading the samples on two cartridges, which were connected in sequence and subsequently, eluted as well as analysed separately. For the second cartridge nothing could be detected. It can, therefore, be concluded that the loading capacity of the analytes was not exceeded and the reason for the low recoveries (20–40% for the more apolar compounds) was not poor retention of the analytes to the packing material. These findings indicate therefore that the low recoveries might be due to an insufficient elution of the SPE columns.

In optimising the recovery abilities of the analytes from SPE, the overall problem to deal with was the fact that the analytes with the shorter alkyl chains gave good recoveries, while for the ones with the longer alkyl chains (i.e., higher log $K_{ow}$ between 5 and 7, where $K_{ow}$ is the octanol–water partition coefficient) poor recoveries were obtained. Especially, for the AMEs with long alkyl chains the problem of poor recovery was pronounced. Several experiments have been carried out to improve the recovery abilities of the analytes including many different combinations of elution solvents going from the more polar range to the more apolar, and with varying pH. Furthermore, addition of acetic acid and TEA, as used in the LC mobile phase for the AMEs, was tried alone and together to investigate if it could have a positive impact on the recovery. Recoveries were found to be more dependent on polarity than on addition of acetic acid or TEA. Using an elution solvent with a medium polarity was not as effective as a combination of two solvent mixtures for elution. Especially, when applying first a rather polar and then a more apolar elution solvent the highest recoveries were seen. The recoveries, when using Oasis and Rdx cartridges, were similar; however, Oasis tended in several cases to clog during loading. Hence, Rdx was preferred.

#### 3.1.2. LC separation

The LC–MS method implemented here is a modification of a previous work by Castillo et al. [27]. As

earlier indicated, their method was developed to analyse AEs with only alkyl chains from $C_{10}$–$C_{14}$. However, analysis of AEs ranging from $C_{10}$–$C_{18}$ was required for our work. Furthermore, the possibility of expanding the method for AEs to include the analysis of AMEs was examined. In this study, a column with a narrower diameter and lower flow-rate was applied, which decreases the amount of waste, compared to the method of Castillo et al. [27]. The applied Hypersil BDS $C_{18}$ column was chosen due to its base deactivated surface (BSD), which removes the active silanols, and makes this column suitable for basic analytes. The analysis time was prolonged to increase the length of the gradient applying the more apolar mobile phase, where all the compounds are eluted. Furthermore, time was needed to return to the initial conditions as well as to ensure stabilisation before the next injection. The method developed provides a separation according to the length of the alkyl chain.

However, the method was not suitable for AMEs, since they become retained on the LC column. After having studied the papers published so far on analysis of AMEs [29–31], new strategies were tried out. Many different NP and RP systems with LC columns packed with cyano, amino, diol and silica were tested together with combinations of mobile phases implying hexane, 2-propanol, acetone, acetonitrile, methanol and water. No improvement was seen, since the AMEs were either eluted without any retention or had infinite retention. However, addition of TEA to the mobile phase led to acceptable retention, this effect has previously been demonstrated by Lang et al. [31]. However, TEA was found to suppress the signal of AEs especially the $[m+H]^+$ ion; therefore, it is required to have two different LC methods for the analysis of AEs and AMEs to obtain a sufficient low LOD for the AEs. Previously, published analytical methods apply $C_{10}E_6$, as internal standard [19]. For analysis of surfactants origina-

Table 5
Analytical data of the LC–MS methods for alcohol ethoxylates and alkylamine ethoxylates

| Compound ($m/z$) | $t_R$ (min) | Linear concentration range[a] (μg/l) | Correlation coefficient ($R^2$)[c] | RSD (%) ($n=4$[d]) |
|---|---|---|---|---|
| $C_{10}EO_6$ (423) | 28.0 | 0.5–1000 | 0.9583 | 6–26 |
| $C_{12}EO_3$ (336) | 30.6 | 0.1–1000 | 0.9949 | 2–15 |
| $C_{12}EO_4$ (363) | 30.6 | 0.1–1000 | 0.9740 | 2–17 |
| $C_{12}EO_5$ (407) | 30.6 | 0.1–1000 | 0.9632 | 5–20 |
| $C_{12}EO_6$ (451) | 30.6 | 0.1–1000 | 0.9755 | 3–18 |
| $C_{12}EO_7$ (495) | 30.6 | 0.1–1000 | 0.9765 | 5–24 |
| $C_{12}EO_8$ (539) | 30.6 | 0.1–1000 | 0.9729 | 5–27 |
| $C_{12}EO_9$ (600) | 30.6 | 0.1–1000 | 0.9684 | 3–36 |
| $C_{14}EO_6$(479) | 34.3 | 0.1–1000 | 0.9464 | 1–28 |
| $C_{16}EO_6$ (507) | 37.8 | 1–1000 | 0.9242 | 8–24 |
| $C_{18}EO_6$(535) | 43.2 | 10–1000 | 0.9312 | 8–32 |
| $C_{12}NEO_2$ (256) | 29.7 | 500–100 000 | 0.9216 | 5–34 |
| $C_{12}NEO_2$ (274) | 29.7 | 500–50 000 | 0.9344 | 2–27 |
| $C_{14}NEO_2$ (302) | 29.7 | 500–50 000 | 0.9545 | 4–24 |
| $C_{16}NEO_2$ (330) | 33.0 | 500–100 000 | 0.9414 | 4–32 |
| $C_{16}NEO_{18}$ (1035) | 35.1 | 1–1000[b] | 0.9414 | 5–27 |
| $C_{16}NEO_{17}$ (991) | 35.2 | 1–1000[b] | 0.9450 | 2–29 |
| $C_{16}NEO_{16}$ (947) | 35.2 | 1–500[b] | 0.9561 | 8–29 |
| $C_{18}NEO_{17}$(1018) | 39.5 | 1–500[b] | 0.9615 | 3–32 |
| $C_{18}NEO_{16}$ (975) | 39.6 | 1–1000[b] | 0.9575 | 4–26 |
| $C_{18}NEO_{15}$ (931) | 39.6 | 1–500[b] | 0.9690 | 2–31 |
| $C_{18}NEO_{14}$ (887) | 39.8 | 1–1000[b] | 0.9578 | 3–20 |
| $C_{18}NEO_{13}$ (843) | 39.9 | 1–500[b] | 0.9552 | 6–39 |

[a] Standard solutions made in 100% methanol.
[b] Concentration range for the $C_mNEO_n$ refer to the concentration of the total technical mixture.
[c] Number of injections $C_mEO_n$: $n=28$–44; $C_mNEO_n$: $n=32$–36.
[d] Range of relative standard deviation (RSD) with four injections of each concentration.

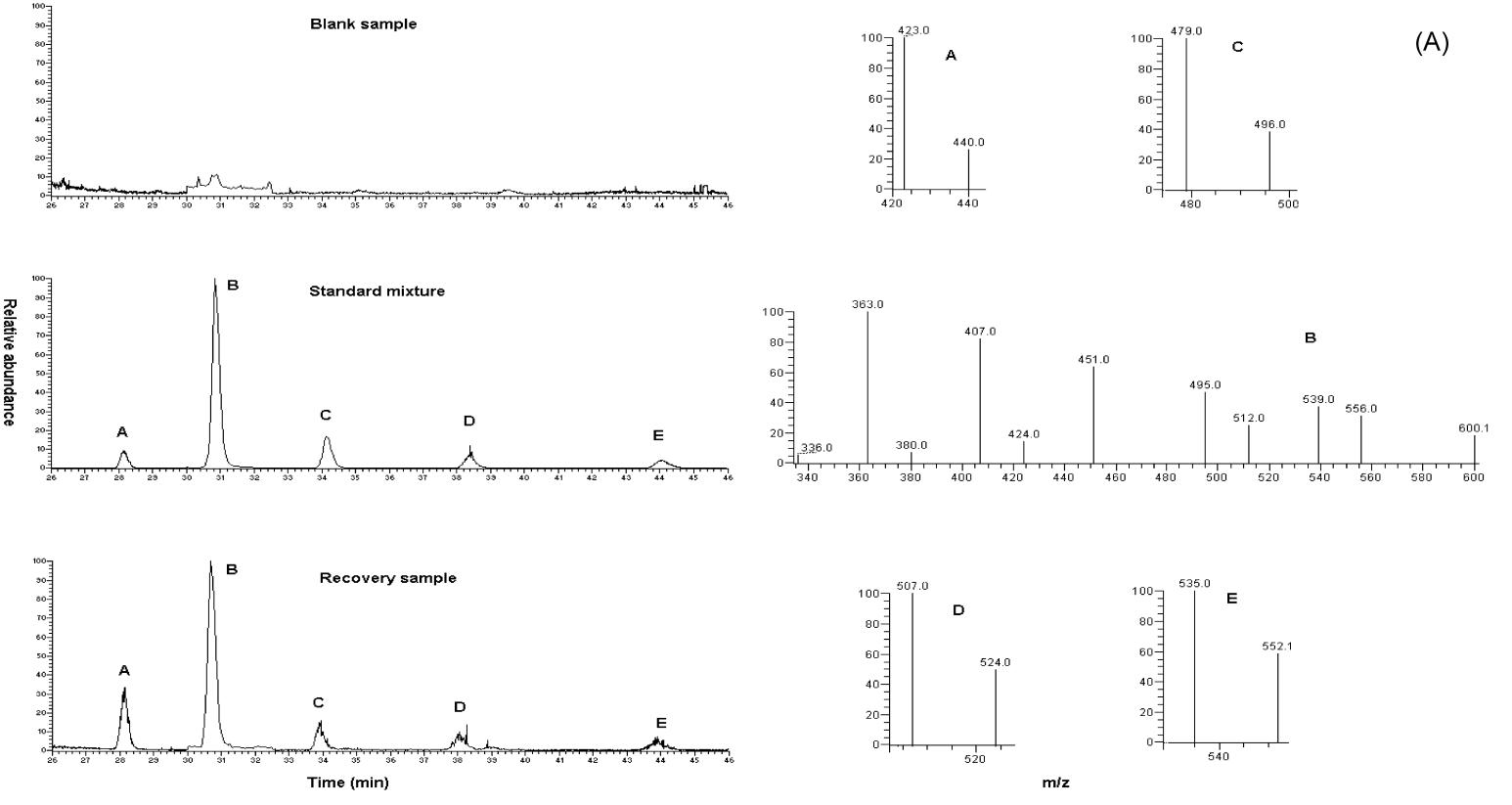


Fig. 2. (A) For the alcohol ethoxylates: LC–MS chromatograms of blank (non-spiked) ground water sample, standard mixture, spiked ground water sample and spectra of the different selected ions of the peaks A = $C_{10}EO_6$, B = $C_{12}EO_{3-9}$, C = $C_{14}EO_6$, D = $C_{16}EO_6$ and E = $C_{18}EO_6$. For alkylamine ethoxylates: LC–MS chromatograms of blank (non-spiked) ground water sample, standard mixture, spiked ground water sample and spectra of the different selected ions of the peaks: A = $C_{12-14}NEO_2$, B = $C_{16}NEO_2$, C = $C_{16}NEO_{16}$ and D = $C_{18}NEO_{16}$.

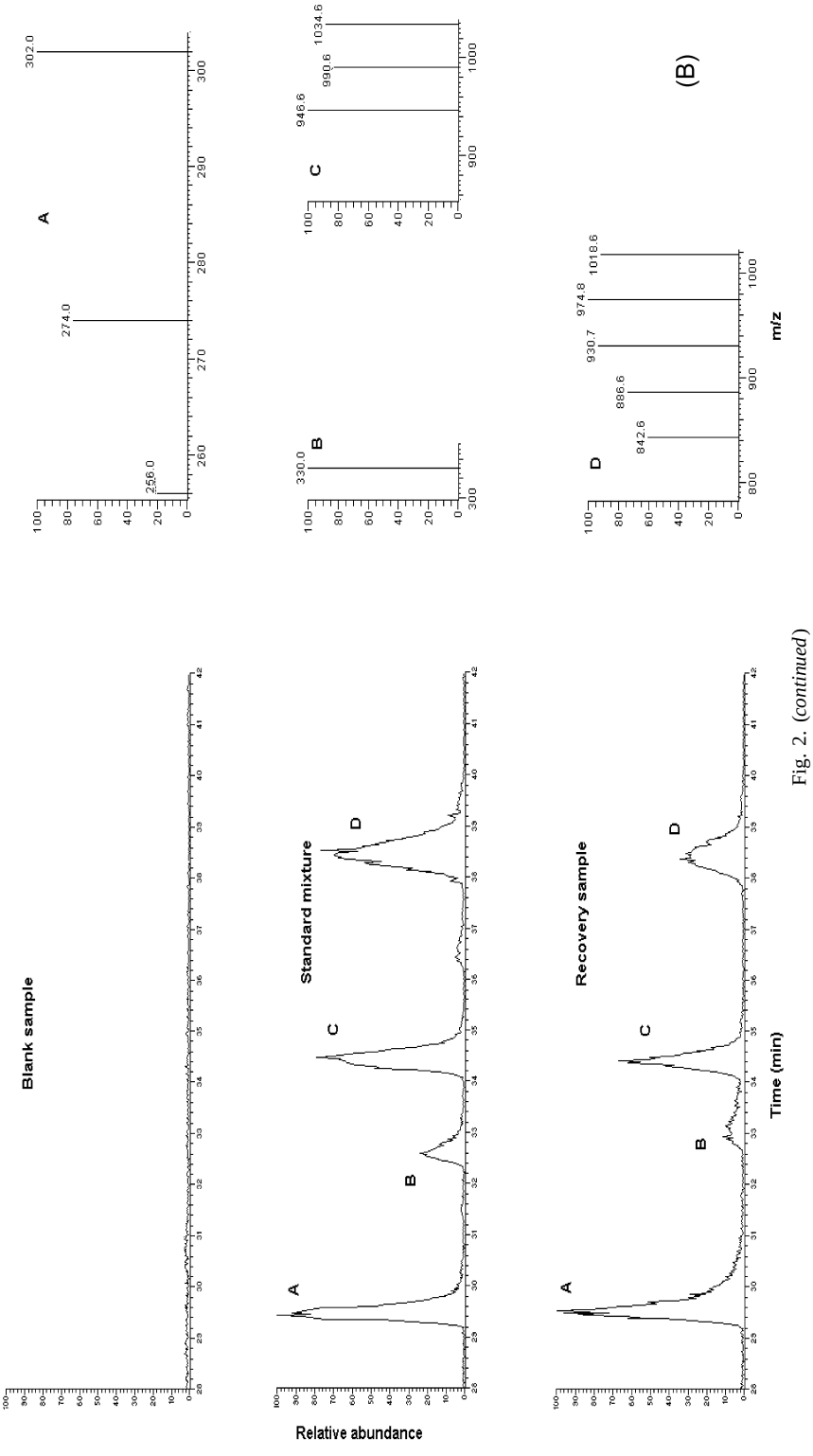


Fig. 2. (*continued*)

ting from pesticide, this compound cannot be used as internal standard, because it might occur in the technical mixture of the surfactants used as adjuvant. It has not been possible to find an alternative and suitable internal standard therefore no internal standard has been used.

Retention times for the different peaks and linearity range of the developed chromatographic system are presented in Table 5. The standards were analysed by double injection in a sequence of increasing followed by decreasing concentration, i.e., each standard was injected in total four times. Dependent on detection limits between 28 and 44 injections make up the standard curves. The correlation coefficient ($R^2$) for the standard curves was mostly above 0.95 with linear ranges varying from a few μg/l to mg/l with 50 μl as injection volume.

#### *3.1.3. MS conditions*

In a paper by Di Corcia [15], an application of MS using different interfaces in the analysis of surfactants has been discussed. For AE detection some studies utilise electrospray ionisation (ESI) [19], while others used APCI [27]. In this study both ESI and APCI, in negative and positive ion modes, have been investigated. The different instrumental settings, such as capillary and vaporiser temperature, corona current, sheath and auxiliary gas for APCI and spray voltage, sheath and auxiliary gas for ESI, were optimised. APCI and ESI in the positive ion mode gave similar signals. However, lower matrix interference is usually obtained in environmental samples when using APCI; therefore, this was the preferred ionisation mode. The ionisation agents, acetic acid and ammonium acetate, were both tested. Adding acetic acid to the mobile phase gave the highest intensities of the $[m+H]^+$ ion for the different compounds, while ammonium acetate increased the intensity of the $[m+H_3O]^+$ compared to the $[m+H]^+$. Furthermore, when adding TEA to the mobile phase of the AE analysis, the ions $[m+Na]^+$ and $[m+H_3O]^+$ were more intense than the $[m+H]^+$.

The ions, used for quantification of the technical mixtures, were selected according to the presence and sensitivity of these ions. For Ethomeen C/12, the identified ions are the four major $m/z$ values, while for Berol 907 eight of the major $m/z$ values were selected for quantification (see Table 1).

In Fig. 2A, LC–MS chromatograms of a blank (non-spiked) ground water sample, a standard mixture and spiked ground water sample are shown for AEs. Spectra of the selected ions representing five different peaks are likewise shown in Fig. 2A. Similarly, three LC–MS chromatograms and spectra of the selected ion for AMEs are illustrated in Fig. 2B. In the figures, the same scale on the *y*-axis is used in the three chromatograms. When comparing the intensity proportions between the peaks in the standard mixture and the recovery sample in Fig. 2B, it is visible that the proportions are not identical. Especially for peaks C and D, the intensity is lower in the recovery sample, this is due to the lower recovery for the compounds of these peaks.

### *3.2. Application*

#### *3.2.1. LOD and recovery in water samples*

The LOD was determined both for samples of ground water and surface water. For each of the five replicated samples an average concentration of the results from the double injected sample, was calculated. In Table 6, the results are shown for ground water and surface water. For the AEs, the recoveries are in the same range for the two waters, between 48 and 88% in ground water and 35–93% in surface water. A high content of ochre in the non-treated ground water might be one of reasons of the slightly lower recovery obtained for the $C_{12}$–$C_{14}$ ethoxylates in this matrix. Recoveries for AMEs are similar, with recoveries, respectively, between 52 and 84% and 58–96% in ground water and surface water for the short-chained AMEs and between 27 and 41% and 28–42% for the AMEs with the longer alkyl and ethoxy chains. Both for AEs and AMEs, the surfactants with the longer alkyl chain length have a lower recovery, because these hydrophobic compounds become tightly bound to the packing material of the SPE cartridges and are therefore hard to elute. For the AEs, LODs are between 16 and 62 ng/l and are independent on the type of water matrix. For the AMEs, the LODs are higher in the ground water than in surface water. In ground water, the LODs are 6 μg/l for $C_{12-16}NEO_2$ and 0.5 μg/l for $C_{16-18}NEO_{16}$ while in surface water the LODs are 3 μg/l and 0.3 μg/l, respectively.

The influence of concentrations on the recovery has also been investigated. Recoveries of three levels

Table 6
Recovery (%) and limits of detection (LODs) for ground water and surface water samples

| Compound | Spiking (ng/l) | Recovery (%) | | LOD (ng/l) | |
|---|---|---|---|---|---|
| | | Ground water | Surface water | Ground water | Surface water |
| $C_{10}EO_6$ | 100 | 88 | 85 | 38 | 38 |
| $C_{12}EO_3$ | 100 | 68 | 80 | 31 | 46 |
| $C_{12}EO_4$ | 100 | 77 | 88 | 33 | 50 |
| $C_{12}EO_5$ | 100 | 73 | 93 | 48 | 30 |
| $C_{12}EO_6$ | 100 | 66 | 87 | 50 | 62 |
| $C_{12}EO_7$ | 100 | 60 | 80 | 28 | 41 |
| $C_{12}EO_8$ | 100 | 52 | 85 | 47 | 60 |
| $C_{12}EO_9$ | 100 | 57 | 88 | 37 | 29 |
| $C_{14}EO_6$ | 100 | 48 | 59 | 51 | 32 |
| $C_{16}EO_6$ | 100 | 48 | 48 | 34 | 29 |
| $C_{18}EO_6$ | 100 | 49 | 35 | 48 | 16 |
| $C_{12-16}NEO_6$ | 10 000[a] | 52–84 | 58–96 | 6150[b] | 2750[b] |
| $C_{16-18}NEO_{16}$ | 800[a] | 27–41 | 28–42 | 525[b] | 250[b] |

[a] Concentrations for AMEs are the total concentration of the technical mixture.
[b] LODs for the AMEs are represented by the ion with the highest LOD, where the concentration refers to the total concentration of the technical mixture.

of concentrations have been determined for both ground water and surface water (see Table 4). For more of the AEs and AMEs, the recoveries are lower at the lowest concentration level, compared to the recovery at the higher concentrations. For the AEs, the recoveries in ground water seem not to be concentration dependent, since recoveries at the lower and higher concentration are in most cases similar, while the middle concentrations show higher recoveries. However, in surface water recovery of the AEs increases with increasing concentration, especially from 50 to 100 μg/l. For most AMEs over the tested concentrations, the recovery increases slightly with increasing concentrations.

In validating the analytical method concerning repeatability and reproducibility the following set of experiments were set up. Three sets of three replicated samples were extracted at three different days and by different persons and subsequently analysed at different days, this was done to estimate the robustness of the methods. In Table 7, recoveries and SDs are given. $SD_{within}$ are between 11 and 20% except for $C_{18}E_6$ with $SD_{within}$ equal to 27%. Whereas the reproducibility between series ($SD_{between}$) is considerably higher between 6 and 49%, with most being around 6–30%. Including all replicates of the 3 days, the total SD is given as $SD_{total}$, which varies from 10 to 28%.

### *3.2.2. Surfactants in samples from farming areas*

Selected samples collected before and after the time of pesticide applications have been analysed to determine the occurrence of surfactants in soil

Table 7
Validation of recovery (%) and precision of the LC–MS method for ground water samples

| Compound | Recovery (%)[a] | $SD_{between}$ (%)[b] | $SD_{within}$ (%)[c] | $SD_{total}$ (%)[d] |
|---|---|---|---|---|
| $C_{10}EO_6$ | 82 | 20 | 11 | 10 |
| $C_{12}EO_3$ | 69 | 9 | 12 | 11 |
| $C_{12}EO_4$ | 69 | 23 | 12 | 14 |
| $C_{12}EO_5$ | 67 | 21 | 15 | 15 |
| $C_{12}EO_6$ | 62 | 28 | 16 | 19 |
| $C_{12}EO_7$ | 53 | 32 | 12 | 18 |
| $C_{12}EO_8$ | 53 | 17 | 13 | 13 |
| $C_{12}EO_9$ | 52 | 23 | 12 | 15 |
| $C_{14}EO_6$ | 43 | 32 | 16 | 21 |
| $C_{16}EO_6$ | 39 | 49 | 20 | 28 |
| $C_{18}EO_6$ | 44 | 30 | 27 | 22 |
| $C_{12-16}NEO_2$ | 61–91 | 5–29 | 14–18 | 12–21 |
| $C_{16}NEO_{16}$ | 35–39 | 34–44 | 12–17 | 20–26 |
| $C_{18}NEO_{16}$ | 39–43 | 6–15 | 11–18 | 12–16 |

Spiking concentration AE 100 ng/l, $C_{12-16}NEO_2$ 5000 ng/l, $C_{16-18}NEO_{16}$ 400 g/l.
[a] Mean recovery obtained by three analysis series.
[b] Variance in between days of extraction and analysis.
[c] Variance within days of extraction and analysis.
[d] Total variance of all samples.

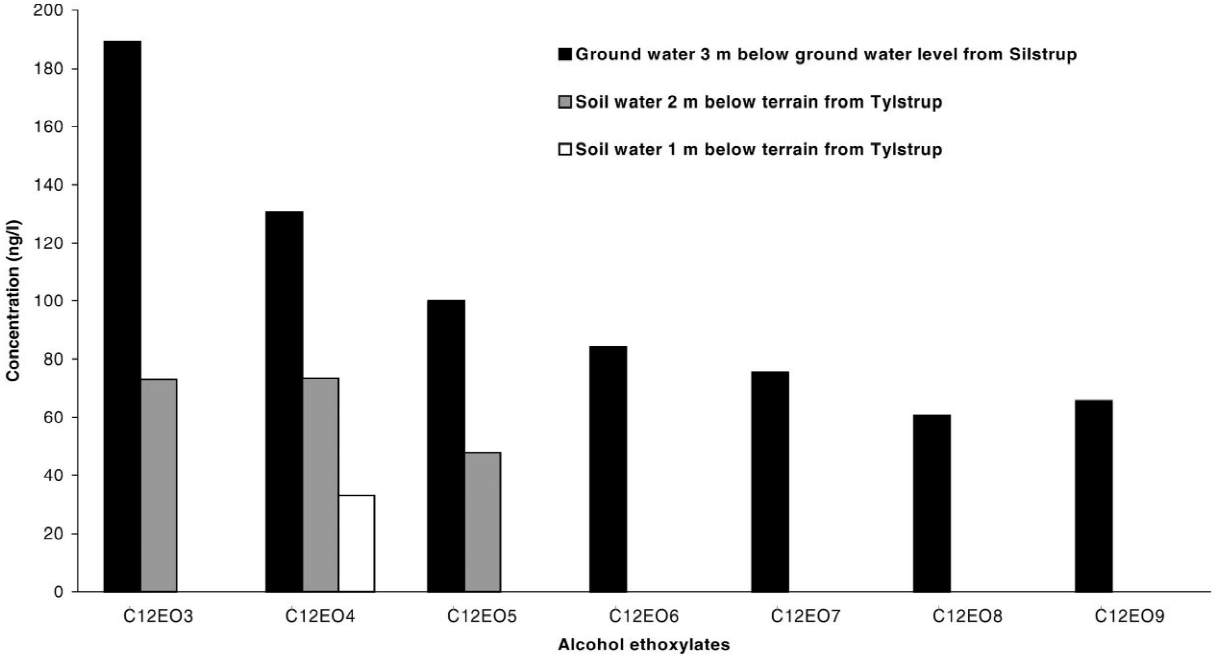


Fig. 3. Concentration of alcohol ethoxylates in water samples collected from farming areas in Denmark.

interstitial water and ground water. In all the soil interstitial water and ground water samples, the concentrations of AMEs were below the LODs, whereas, in three samples AEs have been detected (see Fig. 3). In one ground water sample (collected at 2–3 m below the ground water table) $C_{12}EO_{3-9}$ were detected at concentrations between 60 and 189 ng/l, giving a total concentration of 710 ng/l. In two samples of soil interstitial water from, respectively 1 and 2 m below terrain, $C_{12}EO_{3-5}$ were found at concentrations of 33–73 ng/l, giving a total concentration of 194 ng/l. The obtained distribution of the AEs in the real samples supports the fact that the findings cannot be due to laboratory contamination, because if they were, the same concentration should be expected for all AEs. The developed LC–MS method is therefore, useful for detecting AEs used as pesticide adjuvants, even at very low concentration.

## 4. Conclusions

Analytical procedures have been developed to determine the occurrence of AEs and AMEs in aquatic environmental samples at ng/l levels for AEs and μg/l levels for AMEs. For sample preparation, a SPE method using Sep-Pak Porapak Rdx cartridges, has been developed and optimised. Two different LC–MS methods have been developed for analysis of AEs and AMEs, respectively. The two methods have given acceptable recoveries (58–96% for the polar compounds and 28–49% for the more apolar) and LODs (AEs: 16–60 ng/l, AMEs: 0.3–6 μg/l) for the surfactants in ground water and surface water. Recoveries of AEs in ground water were independent of the concentration level (50–200 ng/l), while in surface water increasing concentration was followed by improved recovery. The recoveries of AMEs increased slightly with increasing concentration in the tested range. Repeatability and reproducibility were determined in ground water for the two methods. SD between days on extraction and analysis was from 6 to 49%, while SD within 1 day was on average 11–20%. This gives a total SD between 10 and 28%.

The developed methods have been applied to assess the content of surfactants in pesticides in the aquatic environment of farming areas. The results

obtained indicate that surfactants are present in soil interstitial water ($C_{12}E_{3-5}$ from 48–73 ng/l, total 194 ng/l) and ground water ($C_{12}E_{3-9}$ from 61–189 ng/l, total 710 ng/l) of two agricultural fields and that the method cover the concentration range expected of the AEs in ''real'' samples.

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