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Analysis of alcohol ethoxylates and alkylamine ethoxylates in agricultural soils using pressurised liquid extraction and liquid chromatography–mass spectrometry

Abstract Nonionic surfactants e.g. alcohol ethoxylates (AEOs) and alkylamine ethoxylates (ANEOs) are commonly utilised as adjuvants in pesticide formulations to enhance their effectiveness. In this study, analytical methods for AEO and ANEO determination in soil samples using pressurised liquid extraction (PLE) were developed and used in connection with LC–MS. The recovery of the method, which was highly dependent on the soil properties, varied in the range 47–106% for AEO and 27–109% for ANEO. Detection limits (LOD) were 7–13 μg kg^{-1} for AEO and 24–43 μg kg^{-1} for ANEO. The developed method has been applied to determine AEOs and ANEOs in surface soil samples from fields sprayed with glyphosate herbicides. Tallowalkylamine ethoxylates (an ANEO) were detected in the soil before and after pesticide application, with increasing concentrations after treatment. The highest concentration in the soil samples was observed for the ANEO homologues with the longest ethoxy chains; in the clay soil the concentration decreased with the length of the ethoxy chain. ANEOs added to pesticide formulations as a technical mixture will, as demonstrated in this study, behave as individual homologues, which is reflected in their behaviour in the environment.

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Introduction

Nonionic surfactants e.g. alcohol ethoxylates (AEOs) and alkylamine ethoxylates (ANEOs) (Fig. 1) are widely used as adjuvants in pesticide formulations [1, 2]. Environmental concern regarding pesticides has previously focused mainly on the active ingredients in the formulation. Only few papers [3, 4, 5] consider the use of adjuvants as contamination source of soil and waters. The load on the agricultural areas depends on the crop, frequency of treat-
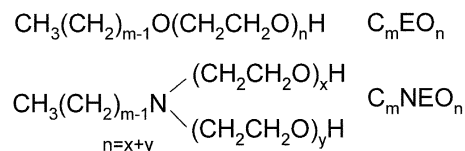

Fig. 1 Chemical structure and molecular formula of alcohol ethoxylates (AEOs) and alkylamine ethoxylates (ANEOs)

ment, and the specific pesticide used, with an average load around 0.3–0.4 kg surfactants per ha per year [4].

Until now no data on environmental concentrations in soil have been published, even though these compounds may, according to their physicochemical properties, become adsorbed on soil particles after application of the pesticide on the agricultural land [5]. Surfactants are present as technical mixtures in pesticide formulations. This implies that they are not one single compound but constitute a whole range of compounds with side chains of varying length present in different ratios. Each of these groups of compounds, besides having the overall ability to distribute between different phases, also possesses some single compound behaviour. These differences in adsorptive behaviour are presumably reflected in the concentrations of the different compounds in soil. The fact that the surfactants are technical mixtures (i.e. consist of the numerous compounds), is besides being a challenge in predicting the environmental fate likewise a challenge when analysing these compounds in environmental matrices.

For the determination of nonionic surfactants in complex matrices such as soils, suitable analytical methods including efficient and reliable extraction methods are essential. Several publications have presented different extraction techniques used to extract nonionic surfactants, mainly alkylphenol ethoxylate (APEO), from soil, sediments [6, 7, 8, 9] and sewage sludge [10]. Extraction techniques consist of ultrasonic extraction [6, 10], Soxhlet extraction [7, 11], microwave-assisted extraction (MAE) [11]) and pressurised liquid extraction (PLE), also called accelerated solvent extraction (ASE) [8, 9, 11]. Heise and Litz (1999) compared Soxhlet extraction, PLE, and MAE in the extraction of, among others, APEO [11]. Soxhlet and PLE give the highest recoveries for the tested soils and sediments. PLE is, compared to Soxhlet, less time consuming, requires less extraction solvent, and less time is subsequently needed for solvent evaporation.

The most important parameters to optimise for PLE methods are temperature, polarity of the solvent, extraction time, and water content of the matrix [12]. Besides the extraction technique, the choice of solvent is an essential parameter in improving the effectiveness of the method. The extraction solvent is highly dependent on the soil characteristics, hence binding potential of the soil surface to the analytes. Pressure is of minor importance for dry samples whereas for moisturised samples pressure becomes a critical parameter and elevated pressure is needed [13]. Studies have shown that increasing water content in soil samples leads to decreasing extraction efficiency [13, 14].

The purpose of the present work is to propose a method for simultaneous extraction of AEOs and ANEOs from soil samples using PLE and subsequently LC–MS analysis. The developed method is applied on soil samples collected from agricultural soils of an area in Northeastern Spain. The results are evaluated by comparing the ratio between the compounds in the matrix with the ratio of the compounds in the formulations.

Materials and methods

Solvents, standards, and materials

All solvents were obtained from Merck. Acetonitrile, methanol and dichloromethane were LiChrosolv grade, while sodium hydroxide, triethylamine (TEA) and acetic acid (100%) were of analysis grade (p.a.). De-ionised Milli-Q water (Millipore) was used in all experiments.

AEOs were pure standards (purity between 97 and 99%), with well-defined alkyl and ethoxylate chains, obtained from Fluka, the standards being $C_{10}EO_6$, $C_{12}EO_4$, $C_{12}EO_5$, $C_{12}EO_6$, $C_{12}EO_7$, $C_{12}EO_8$, $C_{14}EO_6$, $C_{16}EO_6$, and $C_{18}EO_6$. ANEOs were two technical mixtures, Berol 907 (Tallowalkylamine ethoxylate 70%, ethylene glycol 1–5%) and Ethomeen C/12 (Cocosbis(2-hydroxyethyl)amine, purity ca. 100%), kindly provided by Akzo Nobel, Stenungsund, Sweden. In these mixtures both the alkyl and the ethoxylate chain varied (Table 1). Stock solutions (1000 mg L^{-1}) 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 were dissolved in 100% methanol.

Glassware was cleaned in a dishwasher using Neodisher FLA (an alkaline cleansing agent) and neodisher Z (neutralisation cleansing agent) neither of these products contains surfactants (Claus Dam A/S, Humlebaek, Denmark). Subsequently, the glassware was dried in an air oven at 105 °C for 2 h. In addition, glassware used at concentration levels below 10 mg L^{-1}, was heated at 450 °C for 6.5 h to ensure removal of possible contaminants.

A Dionex ASE 200 extractor (Dionex, Salt Lake City, US) equipped with stainless steel extraction cells (33 mL) was applied for the extractions.

Soil sampling and handling

Samples were collected in November and December 2000 from three different fields (Fields I, II, and III) near the towns, Artesa de Segre and Agramunt in the province of Lleida in Northeastern Spain. From each field ten surface core samples (0–20 cm depth) were collected randomly in a zigzag path. Samples were taken before pesticide application (control sample) and twice after pesticide application of glyphosate (Eranca, LZ Ltd, Nicosia) (4 and 14 days after treatment) (Table 2). Samples were kept on ice after sampling. All samples were thoroughly mixed and aliquots of about 2 kg were taken out for analysis. The samples were frozen and later freeze-dried. Dried soils were crushed using a mortar and sieved through a 1 mm sieve. The samples were kept in the freezer (at –20 °C) until extraction and analysis.

Soil characteristics

Soil characterisation of the fine fraction (<2 mm ∅) was done by soil standard methods (Table 2). Analysis of the organic content,

Table 1 Molecular formulae of the homologous alkylamine ethoxylates in the technical mixtures, and the ions used for identification by mass spectrometry [m+H]$^+$

Ethomeen C/12	Berol 907
$C_{12}H_{25}N(CH_2CH_2OH)_2$ [274]	$C_{16}H_{33}N(CH_2CH_2O)_{16}H_2$ [946.6]
$C_{14}H_{29}N(CH_2CH_2OH)_2$ [302]	$C_{16}H_{33}N(CH_2CH_2O)_{17}H_2$ [990.5]
$C_{16}H_{33}N(CH_2CH_2OH)_2$ [330]	$C_{16}H_{33}N(CH_2CH_2O)_{18}H_2$ [1034.6]
	$C_{18}H_{37}N(CH_2CH_2O)_{13}H_2$ [842.5]
	$C_{18}H_{37}N(CH_2CH_2O)_{14}H_2$ [886.6]
	$C_{18}H_{37}N(CH_2CH_2O)_{15}H_2$ [930.7]
	$C_{18}H_{37}N(CH_2CH_2O)_{16}H_2$ [974.8]
	$C_{18}H_{37}N(CH_2CH_2O)_{17}H_2$ [1018.6]

Table 2 Soil properties and field characteristics

Field name	Field I	Field II	Field III
Fertiliser/Manure		Fertiliser (NPK 8.15.15) 450 kg ha^{-1}	Pig manure (25 tons ha^{-1})
Area	1.25 ha	10 ha	1 ha
Crop	Barley	Barley	Barley
Pesticide product and concentration applied		1.25 L Eranca ha^{-1}	1.25 L Eranca ha^{-1}
Organic content (%)	1.43	1.69	1.28
pH (H$_2$O)	7.51	7.63	8.26
pH (KCl)	7.28	7.28	7.72
Conductivity (E.C.) at 25 °C (dS m^{-1})	1.76	2.12	0.30
Particle size distribution (%)	5/30/73/0	4/13/86/0	24/37/29/11
CEC (cmol kg^{-1} dry soil)	23.44	20.15	20.71

Particle size distribution in %: Coarse sand/fine sand/silt/clay

total loss of carbon and easily oxidised organic carbon follow the methods described by Nelson and Somers [15]. Soil pH was measured in soil:water (2:5) or potassium chloride slurry [16] using a Crison pH meter (MicropH2002). Electrical conductivity was measured by means of Crison Conductivitymeter 525 equipment in a 1:5 soil/water extract obtained by shaking for 60 min on a horizontal shaking plate, followed by filtration using Whatman 43 (110 mm ⌀). It was necessary to filter the samples to remove the insoluble salts, which might interfere. Particle-size distribution was determined using the Pipette method [17]. Cationic exchange capacity (CEC) was measured using the method of Polemio and Roades (1977), which determines exchangeable sodium as a final step [18]. A flame photometer was used for sodium quantification.

Sample preparation

The developed and optimised PLE extraction method for ANEO and AEO consisted of two successive extraction steps (1 and 2) with different solvents leading to two separate extracts. For both extraction steps, the optimised settings were: preheat (2 min), heat (7 min), static (5 min), and flush (180 s) time, flush volume (60%), temperature (150 °C), pressure (1500 psig), and the number of cycles (3). The two extraction solvents were A: Methanol 100% followed by B: Hexane:acetone (1:1), 75 mmol L^{-1} CH$_3$COOH and 100 mmol L^{-1} TEA. These extracts were kept separately, evaporated under N$_2$ at 30 °C and reconstituted in 100% methanol and filtered using a 0.2 µm nylon syringe filter (Titan Filtration Systems, no. 42213-NC) before analysis by LC–MS.

Prior to the soil extraction, the extraction cells were cleaned. After rinsing the cells with distilled water, they were filled with Ottawa sand (Fisher Chemicals, S/0365/63) and extracted by ASE using a special washing method involving the same two extraction solvents (A and B) and extraction performed at a higher temperature (200 °C) and with the time settings: preheat (1 min), heat (9 min), static (1 min), flush (60 s) time and one cycle, the remaining settings were as described above. After this extraction method the cells were clean and ready for extraction.

For the spiking of the soil samples 1.00 mL spiking solution and 3.0 mL methanol (to wet the soil and thereby improve mixing) were added to 10.0 g soil samples. The samples were mixed for 1 h by rotation of the samples, kept at 5 °C for 24 h and then left open to the atmosphere at room temperature for 24 h to dry. The air-dried soil samples were weighed into the extraction cells (10.0 g), which were filled with Ottawa sand (ca. 30 g) before extraction.

In order to improve the sensitivity of the method and reduce possible matrix effects, a further cleanup step, solid-phase extraction (SPE), was introduced in the sample-preparation procedure. For this extraction, the ASE extracts A and B were mixed prior to evaporation under N$_2$. The evaporated extracts were reconstituted in 500 mL water and extracted on Porapak RDX SPE cartridges as described by Krogh et al. [19]. Four spiked replicates at concentration level 2A (Table 3), together with duplicated blank samples, were extracted using the SPE method whereas other four spiked replicates and two blanks were analysed without SPE cleanup. The mean recovery was determined for the samples with and without SPE cleanup.

Four replicate samples spiked at level 2A were applied to determine the limit of detection (LOD) of the method (Table 3). LOD was calculated as 3 times the standard deviation (SD) of the average concentrations in soil.

To investigate the relation between the spiking concentration and the recovery replicate samples were spiked at three levels 1, 2, and 3; the spiking concentrations are shown in Table 4. Furthermore, the method was validated by spiking, extracting and analysing samples on different days and by different persons. The exact spiking levels and the obtained recoveries are listed in Table 3.

Table 3 Mean recovery, standard deviation [SD] and limit of detection (LOD) of spiked soil samples (spiking level 2 or 2A) from field II extracted and analysed on different days

Compound	Spiking concentration level (µg kg^{-1})		Mean recovery (%)		LOD (µg kg^{-1})
	2A	2	2A (n=4)	2 (n=3)	2A (n=4)
C$_{10}$EO$_6$	25	25	93 [12]	86 [6]	9
C$_{12}$EO$_4$	25	25	88 [9]	73 [7]	7
C$_{12}$EO$_5$	25	25	100 [15]	86 [4]	10
C$_{12}$EO$_6$	25	25	106 [13]	92 [12]	9
C$_{12}$EO$_7$	25	25	99 [13]	88 [13]	11
C$_{12}$EO$_8$	25	25	87 [15]	63 [16]	12
C$_{14}$EO$_6$	25	25	91 [15]	89 [8]	13
C$_{16}$EO$_6$	50	25	79 [9]	76 [6]	7
C$_{12-16}$N(EO)$_2$[a]	50	500	81 [18]	79–87 [11–19]	27
C$_{18}$N(EO)$_{16-18}$[a]	50	50	63–88 [19–33]	30–109 [11–23]	24–43

[a]Recovery range representing the recovery obtained for the different homologues
n is the number of samples

Table 4 Recovery and 95% confidence intervals for replicate analysis of soil samples (field II) spiked at three different concentration levels

Compound	Mean recovery [95% confidence interval]					
	Level 1 ($n=3$)		Level 2 ($n=3$)		Level 3 ($n=3$)	
	Concentration ($\mu g\,kg^{-1}$)	Mean recovery (%)	Concentration ($\mu g\,kg^{-1}$)	Mean recovery (%)	Concentration ($\mu g\,kg^{-1}$)	Mean recovery (%)
$C_{10}EO_6$	15	93 [88–98]	25	86 [79–93]	75	86 [82–90]
$C_{12}EO_4$	15	72 [66–78]	25	73 [65–81]	75	68 [67–69]
$C_{12}EO_5$	15	95 [85–106]	25	94 [89–99]	75	86 [85–88]
$C_{12}EO_6$	15	95 [84–107]	25	92 [79–105]	75	92 [81–102]
$C_{12}EO_7$	15	92 [91–94]	25	88 [73–103]	75	99 [95–104]
$C_{12}EO_8$	15	71 [45–98]	25	63 [44–81]	75	88 [74–102]
$C_{14}EO_6$	15	97 [94–99]	25	89 [80–99]	75	94 [85–103]
$C_{16}EO_6$	30	71 [62–81]	50	76 [69–82]	150	90 [78–103]
$C_{18}EO_6$	75	100 [100]	125	88 [81–94]	375	98 [87–109]
$C_{12–16}N(EO)_2$[a]	200	93–95 [83–103]	500	79–87 [57–104]	1500	88–93 [71–107]
$C_{16–18}N(EO)_{13–18}$[a]	25	<LOD	50	30–109 [8–135]	150	29–88 [20–108]

[a]Recovery range representing the recovery obtained for the different homologues
n is the number of samples

LC–MS analytical conditions

Determination of the surfactants was performed applying two distinct LC–MS methods for the two types of surfactant, as described by Krogh et al. [19]. The liquid chromatographic system used was a Waters system consisting of a Waters 717 Autosampler and a Waters 600 MS System Controller. For both methods, a Hypersil BDS C18 (250 mm×2 mm i.d., 5 μm) with a similar guard column (10 mm×2 mm) was applied. The mobile phases used were for the AEO analysis A: methanol:acetonitrile (1:1), B: Milli-Q water, both containing 20 mmol L^{-1} CH$_3$COOH, and for the ANEO analysis A: methanol:acetonitrile (1:1) and B: Milli-Q water, both containing 20 mmol L^{-1} CH$_3$COOH, 25 mmol L^{-1} 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 flow rate was set to 0.2 mL min^{-1} at 30 °C and the injection volume was 50 μL. All standards and samples were analysed by double injection.

MS determination was performed using a Finnigan TSQ 700 with atmospheric pressure chemical ionisation (APCI) and the following optimised instrumental settings: capillary temperature: 220 °C, vaporiser temperature: 500 °C, corona voltage: 3 mA, sheath gas: 40 psig and auxiliary gas: 0 psig. Nitrogen was used as sheath and auxiliary gas. The ions were detected using selected ion monitoring (SIM) in positive ion mode. The ions used to quantify the AEO were [m+H]$^+$ or [m+H$_2$O+H]$^+$ (m, molecular ion); the ions used for ANEOs are listed in Table 1.

Application of the analytical method

The analytical method was applied in a study to monitor ANEO and AEO in soil samples collected in Spain. Both blank samples and two spiked samples from different fields were extracted and analysed. In addition, four blank samples obtained from fields II and III were extracted, of which two samples were reconstituted in 100% methanol after evaporation, while the other two were reconstituted in the spiking standard to investigate the matrix effect. The duplicated soil samples were extracted and analysed.

Results and discussion

Extraction and analysis

In development and optimisation of the PLE method several parameters need to be considered such as extraction solvent, temperature, the static time, the number of extraction cycles, etc. The extraction pressure is said to have no influence when the samples are dry [9]. In this study, many different extraction solvents, such as methanol, acetonitrile, ethyl acetate, cyclohexane, acetone and various mixtures of these have been tested. Addition of a mixture of acetic acid and TEA, which has shown to be successful in eluting ANEO from the C$_{18}$ LC-column [19], was evaluated. The optimum conditions were found to be a two step extraction method with first a polar solvent (A) and subsequently an apolar acidic solvent (B) being A: Methanol 100% and B: Hexane:acetone (1:1), 75 mmol L^{-1} CH$_3$COOH and 100 mmol L^{-1} TEA. Extract A contained all the AEO, the polar ANEO (Ethomeen C/12) and some of the more apolar ANEO (Berol 907). The remaining amount of Berol 907 was found in B. Combining extract A with B resulted in lower recoveries compared to when the extracts were kept separately, so they were not mixed. Reasons for the negative effects of mixing are probably higher noise or reduced resolution due to dirty samples. Consequently, the recovery was obtained as the sum of the two contributions for Berol 907.

Spiked samples of soil from field II were used to develop and optimise the different extraction parameters. These spiked samples contain both the added and previously more tightly bound surfactants. This implies that the optimised method is usable for aged samples as well as spiked samples

Fig. 2 Mean recoveries at different PLE extraction temperatures for ANEO (*n*=4)
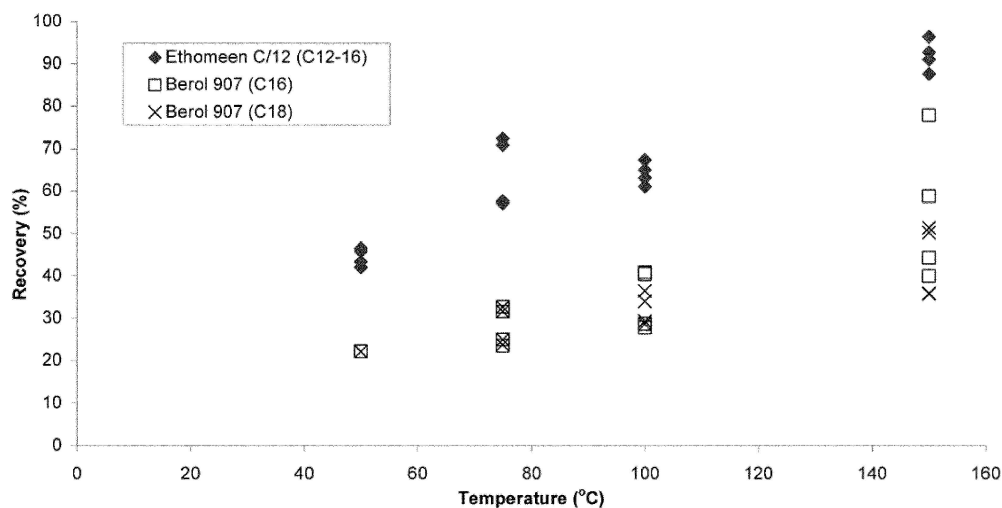
To optimise the extraction temperature, experiments were carried out in which four different extraction temperatures (50, 75, 100 and 150 °C) were tested. The spiking levels were, respectively, 1 and 0.1 mg kg^{-1} soil of the ANEO and AEO. The obtained recoveries at the different temperatures are shown for ANEO in Fig. 2. Similar trends of increasing recovery with increasing temperature were observed for AEO. In agreement with Zhu et al. (2000), elevated temperatures increase the solvating power of the solvent, the solvent diffusivity, and the mass transfer rate [13]. Extraction at 150 °C was subsequently used as extraction temperature. Problems with degradation at temperatures above 50 °C, as seen for a method of APEOs and their degradation products [9], have not been observed in this study with AEO and ANEO. This is in agreement with the findings of Evetts et al. (1995), who characterised AEOs as being fairly stable even at temperatures up to 150 °C [20].

When the effect of the number of cycles was evaluated, three cycles (i.e. three cycles for solvent A and three for solvent B, in total 6 cycles) gave higher recoveries than only one cycle.

Cross-contamination problems, especially for the more apolar ANEO, were observed between successive analyses of, e.g., spiked soil samples and blank Ottawa sand samples in the preliminary experiments. Consequently, a washing method was developed using ASE extraction. Satisfactory true blanks were obtained by applying first solvent A and then B together with an optimised ASE method; the specified settings are given in Materials and methods.

In several of the published PLE methods for APEO, the sample clean up included a SPE step, using a cartridge packed with aluminium oxide [11, 21], C$_{18}$ [9] or cyano [8]. Hence, an experiment was set up to investigate the necessity of an SPE cleaning step, using a SPE method analogous to the method published by Krogh et al. (2002) [19]. This cleanup step did not result in an overall improvement of the analytical method, so the SPE step was left out of the analytical procedure.

Chromatograms obtained from blind samples from field I and field II are shown together with a spiked sample from field II for both ANEO and AEO in Fig. 3. Furthermore, in Table 3 the LOD and mean recovery determined using four replicated soil samples (field II) are shown. The obtained LODs of the analytical method were for AEO 9–13 µg kg^{-1} and for ANEO 24–43 µg kg^{-1} soil. The recoveries of the ANEOs are represented as a recovery range for the different homologues present in the technical mixture. For the AEOs, the recoveries were 79–106% and 63–88% for ANEO. The SD was for the AEOs between 9–15, which was satisfactory regarding the low spiking concentration around twice the concentration of the LOD. For the ANEOs, the SD was higher (18–33), probably because the spiking concentration of the ANEOs was near the LOD, and small variations in the recovery of one homologue of the mixture imply differences of all the other recoveries of the remaining homologues.

Investigations of AEO loss during N$_2$ solvent evaporation have shown that for C$_{12–15}$EO$_{1–3}$ up to 50% was lost during solvent evaporation, while for C$_{12–15}$EO$_9$ the loss represented only slightly more than 1% [22]. Considering the AEO analysed in this study, of which the majority has EO chains longer than six, solvent evaporation is not believed to be an essential factor leading to reduced recovery.

Validation results

The dependence of the extraction efficiency on the spiking concentration was investigated by determining the recovery of triplicate spiked samples at three different concentrations. The obtained results, i.e. mean recovery and 95% confidence intervals, are shown in Table 4. For both AEO and ANEO, the confidence intervals overlap at the different concentrations; this implies that the recoveries are independent of the spiking concentration within the investigated concentration range.

Comparing the recovery for the AEO and ANEO obtained in soil from field II spiked, extracted and analysed at different days and by different persons (Table 3), no statistical significance was observed between the two recoveries (*t*-test, *P*<0.05).
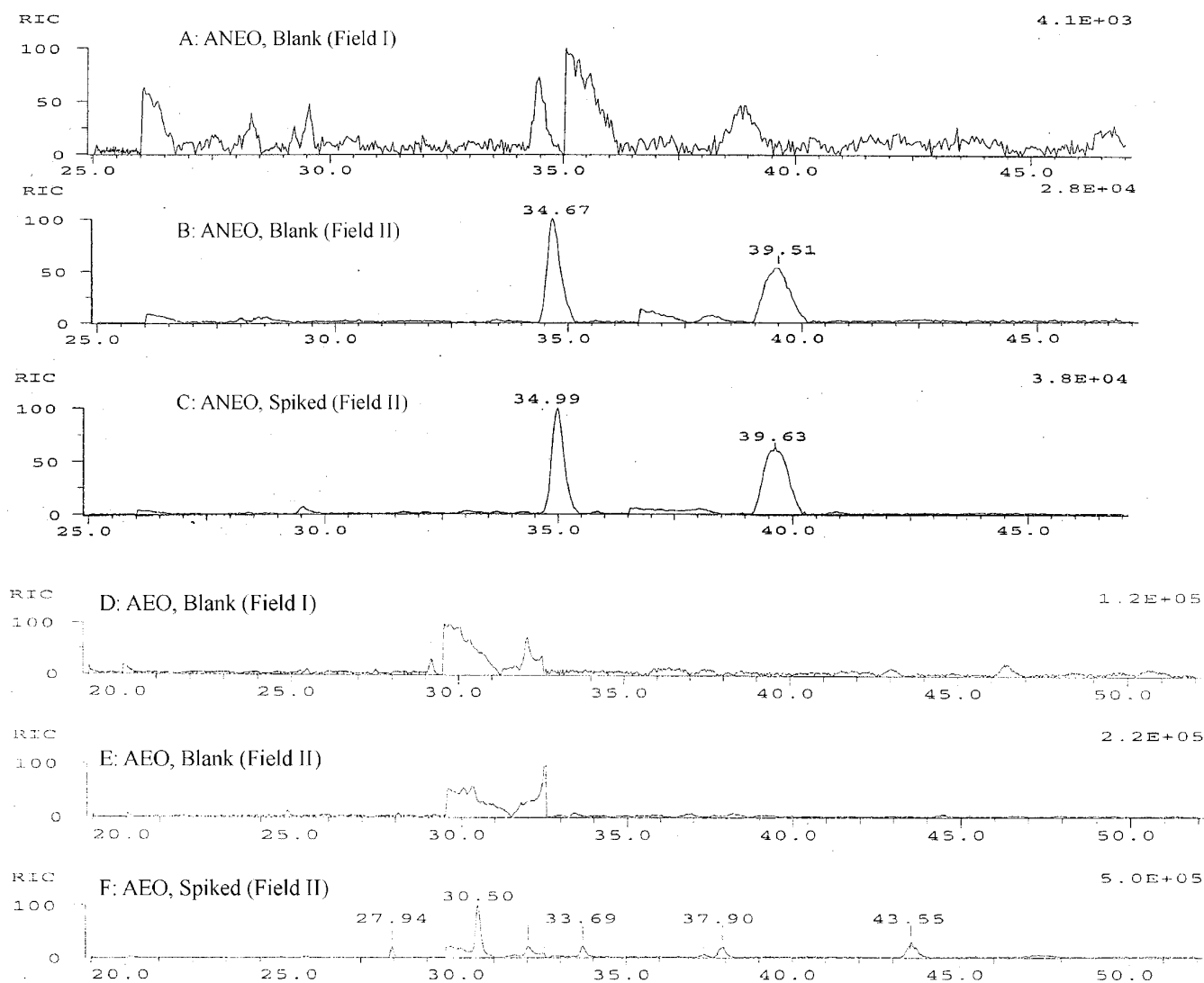
Fig. 3 For ANEO: LC–MS chromatograms obtained from blank (non-spiked) soil sample collected from Field I (**A**), Field II (**B**) and spiked (level 2) sample from Field II (**C**). For AEO: LC–MS chromatograms obtained from blank (non-spiked) soil sample collected from Field I (**D**), Field II (**E**) and spiked (level 2) sample from Field II (**F**)

Blank and spiked samples were extracted and analysed from each field. In order to evaluate the influence of the matrix on the recovery, blank samples from field II and III were reconstituted in the same standard mixture as used for spiking (concentration level: 2). The recoveries of these re-dissolved samples are shown in Table 5. For the ANEO with the longer alkyl chain, the recoveries in extract A were considerably lower than in extract B. The matrix effect is more prevalent in the more polar extract, as expected due to the presence of, e.g., humic acids. The lower recoveries, especially for extract A, of both AEOs and ANEOs in the re-dissolved samples in soil from field III compared to soil from field II, might be caused by differences in the texture composition; clay minerals are only present in field III.

In Table 6, the recoveries are shown for spiked soil samples prepared using soil collected from field III. Comparing the obtained recovery with the results of field II, it becomes obvious that the recoveries are highly dependent on the field, i.e. on the properties of the soil. The presence of clay minerals in the soil from field III seems to reduce the recovery of the spiked samples, as for the reduced recoveries found for the re-dissolved samples. Concerning ANEO, the recoveries are lower in soil from field III than from field II except for $C_{16}NEO_{16-18}$, for which the recoveries are fairly similar. For field III, the lower recovery is caused partly by the higher pH and higher clay content. At higher pH a larger fraction is deprotonated (pK_a=6–7 for Berol 907; unpublished results), i.e. increasing possibility of complex bonds to minerals in the soil [5], which might reduce the extraction capacity. In contrast to the findings for AEOs, the recoveries of ANEOs are highly dependent on the alkyl and ethoxy chain length. For $C_{16}NEO_{16-18}$ and $C_{18}NEO_{13-17}$ homologues, the recovery increases as the number of ethoxy groups decreases. Further, the recovery of the $C_{16}NEO_{16-18}$ was lower than for the $C_{18}NEO_{13-17}$ with an equal number of ethoxy groups. These data con-

Table 5 Mean recovery and recovery range [min–max] of blind soil samples ($n=2$) re-dissolved in a standard solution (concentration level 2) after the extraction procedure

ANEO A and B refer to re-dissolved extract A and B; n is the number of samples
[a] Recovery range representing the recovery obtained for the different homologues
[b] One replicate

Compound	Concentration ($\mu g\,L^{-1}$)	Mean recovery (%) [min–max]	
		Field II	Field III
$C_{10}EO_6$	250	94 [92–96]	66 [60–71]
$C_{12}EO_4$	250	80 [78–81]	49 [46–53]
$C_{12}EO_5$	250	90 [86–95]	80 [74–86]
$C_{12}EO_6$	250	94 [87–101]	66 [57–74]
$C_{12}EO_7$	250	95 [84–106]	71 [65–78]
$C_{12}EO_8$	250	73 [60–86]	74 [62–85]
$C_{14}EO_6$	250	96 [87–104]	71 [60–82]
$C_{16}EO_6$	500	79 [72–84]	74 [66–83]
$C_{18}EO_6$	1250	94 [93–94]	56 [45–68]
$C_{12–16}N(EO)_2A$	5000	108–117 [108–118][a]	32–55[a, b]
$C_{12–16}N(EO)_2B$	5000	95–99 [94–99][a]	85–86 [78–92][a]
$C_{16}N(EO)_{16–18}\,A$	500	67–76 [59–86][a]	23–54[a, b]
$C_{16}N(EO)_{16–18}\,B$	500	96–101 [95–101][a]	91 [77–105][a]
$C_{18}N(EO)_{13–17}\,A$	500	67–96 [60–103][a]	24–57[a, b]
$C_{18}N(EO)_{13–17}\,B$	500	97–102 [96–102][a]	87–92 [83–101][a]

Table 6 Mean recovery and recovery range [min–max] of spiked soil samples ($n=2$) from field III

Compound	Concentration ($\mu g\,kg^{-1}$)	Mean recovery (%) [min–max]
$C_{10}EO_6$	25	63 [62–63]
$C_{12}EO_4$	25	45 [42–48]
$C_{12}EO_5$	25	77 [76–78]
$C_{12}EO_6$	25	62 [60–64]
$C_{12}EO_7$	25	66 [65–67]
$C_{12}EO_8$	25	77 [70–85]
$C_{14}EO_6$	25	64 [63–65]
$C_{16}EO_6$	50	63 [59–66]
$C_{18}EO_6$	125	47 [37–57]
$C_{12–16}N(EO)_2$	500	37–55 [a]
$C_{16}N(EO)_{16–18}$	50	39–62 [a]
$C_{18}N(EO)_{13–17}$	50	27–62 [a]

[a] One replicate, recovery range representing the recovery obtained for the different homologues
n is the number of samples

firm as expected, that the recoveries depend on the properties of the soil such as texture and pH.

Analysed surfactant concentrations in soil

Field I has, in contrast to the other fields, not been sprayed with glyphosate herbicides during the last five years, and was therefore considered as a total blank. This was confirmed by the analytical data, since neither ANEO nor AEO were detected in the soil from field I.

In contrast to field I, glyphosate herbicides have been applied on the field II and III during the past years. AEO and the short-chained ANEO were not detected in the soil from field II and III before or after pesticide application. However, the long chained ANEO were detected in the soil from II and III both before and after pesticide application (Table 7). As indicated by the data, the content of $C_{16-18}NEO_{13-18}$ increases after pesticide application, as expected, because glyphosate herbicides are known to contain tallowalkylamine ethoxylates [23]. The exact content of surfactants in the pesticide formulation (Eranca) is not known due to the fact that this information is considered as a business secret. In the soil from field II the concentration increased after 4 days, while at day 14 the concentration was lower than at day 4, but higher than before application. As reflected in the high standard deviation of day 4, a large variation was observed between the two replicates, this might be the reason for a high mean concentration. This large variation of the two replicates found in the soil after 4 days is believed to be due to limited dilution down the soil column, which results in high demands on careful mixing of the soil samples. For the soil from field III, the concentration was higher after 4 days and even higher after 14 days. The concentration after 4 and 14 days were expected to be similar considering the degradation rates of these compounds [5]. However, heterogeneous dissipation of the surfactants from the topsoil, probably due to variation in the neighbouring soil texture, might lead to the observed concentration differences. These differences are expected to decrease over time.

Considering the differences in the concentration between the different homologues of the $C_{16-18}NEO_{13-18}$, a tendency of increasing concentration with increasing ethoxy chain length is observed. Increasing the ethoxy chain length from 16 to 17 ethoxy groups results in considerable increase in the concentration, this is particularly evident for the homologues in the soil of field III, in which the concentration increased nearly by a factor two. This dependence of the ethoxy chain length in the soil from field III agrees with the knowledge of binding properties of polyethoxylates, which binds to clay minerals [5]. Likewise, the difference in the concentration of the ANEO in between the two soils might be ascribed to the difference in soil properties (Table 2). ANEO are believed to bind to clay minerals, therefore concentration in the clayey soil from field III is higher than in the soil from field II. This im-

Table 7 Mean concentration ($\mu g\,kg^{-1}$) and concentration range [min–max] in the soil samples (n=2) collected before and after pesticide application

Compound	Field II			Field III		
	Before	4 days	14 days	Before	4 days	14 days
$C_{10}EO_6$	n.d.[a]	n.d.	n.d.	n.d.	n.d.	n.d.
$C_{12}EO_{4–9}$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$C_{14}EO_6$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$C_{16}EO_6$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$C_{18}EO_6$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$C_{12–16}N(EO)_2$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$C_{16}N(EO)_{18}$	135[b]	411 [229–594]	301 [273–329]	282[b]	197 [186–207]	722 [646–788]
$C_{16}N(EO)_{17}$	128[b]	398 [235–561]	297 [273–320]	243[b]	276 [271–280]	788 [713–863]
$C_{16}N(EO)_{16}$	97[b]	320 [211–428]	205 [189–221]	123[b]	157 [153–162]	391 [354–428]
$C_{18}N(EO)_{17}$	97[b]	287 [196–377]	216 [199–234]	329[b]	441 [383–499]	1023 [939–1105]
$C_{18}N(EO)_{16}$	81[b]	255 [188–322]	194 [185–202]	124[b]	208 [185–231]	391 [362–419]
$C_{18}N(EO)_{15}$	85[b]	264 [208–320]	199 [196–202]	89[b]	162 [148–177]	303 [282–324]
$C_{18}N(EO)_{14}$	81[b]	226 [180–273]	182 [3181–184]	90[b]	155 [142–168]	298 [276–319]
$C_{18}N(EO)_{13}$	69[b]	208 [189–227]	163 [1162–163]	78[b]	144 [135–152]	279 [259–298]
Mean $C_{16–18}N(EO)_{13–18}$	97	296	220	170	218	524

n is the number of samples
[a] n.d.: not detected
[b] One replicate

plies that the ratio of the different homologues in the soil matrix differs from the ratio in a typical technical mixture used in pesticide formulations.

Conclusions

An analytical method including extraction using PLE and LC–MS analyses has been developed to analyse ANEO and AEO in soil samples. The LOD in soil was $7–13\,\mu g\,kg^{-1}$ for AEO and $24–43\,\mu g\,kg^{-1}$ for ANEO. Differences in soil texture, composition and pH lead to differences in recovery in soil from different fields. AEOs and the short chained ANEOs ($C_{12–16}NEO_2$) were not detected in soil from fields sprayed with the pesticide glyphosate, whereas the longer chained ANEOs ($C_{16–18}NEO_{13–18}$) were found in the soil both before and after pesticide application. The concentration of these ANEO varied among the fields, and between the different ANEO homologues, however the concentration seemed to increase after pesticide application. The concentration of the different homologues increased with increasing ethoxy chain length especially in the clay soil. This is in agreement with the knowledge of binding properties of polyethoxylates, which binds to, among others, clay minerals. ANEOs added to pesticide formulations as a technical mixture will, as demonstrated in this study, behave as individual homologues, this means their fate in the environment might differ considerably.

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