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SPE/LC/ESI/MS with phthalic anhydride derivatisation for the determination of alcohol ethoxylate surfactants in sewage influent and effluent samples

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Abstract

A new method for the analysis of alcohol ethoxylates (AEs) using electrospray ionisation liquid chromatography/mass spectrometry (ESI LC/MS) is described. The procedure incorporates a novel derivatisation step with phthalic anhydride for the analysis of $EO_{0–20}$ ethoxylates in a single analysis. The derivatives obtained have proved to be very stable and the negative ion spectra show reduced background ions and competing adduct formation as compared to positive ion spectra. An automated solid phase extraction (SPE) step is used to allow both pre-concentration and clean-up of the environmental samples. The method provides more efficient recovery of AEs across the C_{12}–C_{18} range than previously reported in the literature. Recoveries from final effluent spiked at 100 μg/L total AE, for the 126 species analysed, were found to be in the range 55–117%, with approximately 100 of the individual analytes having recoveries of 90–105%. An LOD of 0.02 μg/L for individual ethoxylate components is reported with the instrument operated in scan mode over the range *m*/*z* 300–1300. The method was applied to sewage effluent and influent samples, with AEs determined at approximately 7 and 5000 μg/L, respectively.

Keywords: Alcohol ethoxylates; Solid phase extraction; LC/MS; Phthalic anhydride derivatisation; Environmental samples

1. Introduction

Alcohol ethoxylates (AEs) are widely used with 560,000 metric tonnes consumed in Western Europe in 1997 [1]. AEs are synthesised industrially by base catalysed addition of ethylene oxide to aliphatic alcohols from oleochemical and petrochemical sources. The oleochemical-derived alcohols are linear and primary. They contain only an even number of carbon atoms in the homologous chains, between C_{12} and C_{18}. The petrochemical mixtures account for 60% of the production of AEs [2]. These mixtures contain even and odd numbers of carbon atoms in the homologues and are either linear or branched depending on the composition of the olefin feedstock. These so-called oxo-AEs from linear olefins are primarily linear but contain also a degree of 2-alkyl substitution and typically contain 11–15 carbon atoms. The alcohols are then reacted with ethylene oxide in industrial processes resulting in a Poisson-like ethoxymeric distribution of each AE homologue. The general structure of AEs used in this work is represented in Fig. 1. A commonly used abbreviation of AE structure is C_nEO_m, where *n* and *m* denote the number of carbon atoms (C) in the alkyl chain and ethoxylate groups (EO), respectively.

Household laundry detergents are the single largest end use for AEs, with linear primary AEs being preferred due to their rapid biodegradability [3]. This widespread use and disposal down the drain has led to a need to monitor levels of these surfactants in the environment, a responsibility which has been co-ordinated by the Environmental Risk Assessment and Management Committee (ERASM) on behalf of a number of detergent industries and raw materials

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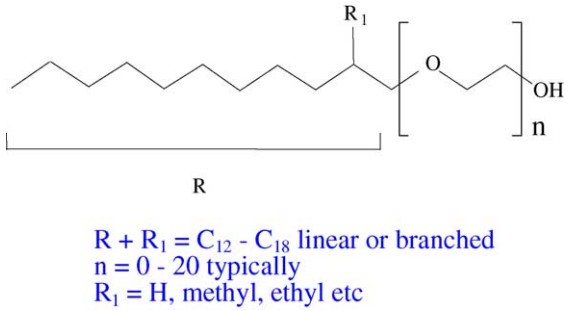

Fig. 1. Structure of alcohol ethoxylates.

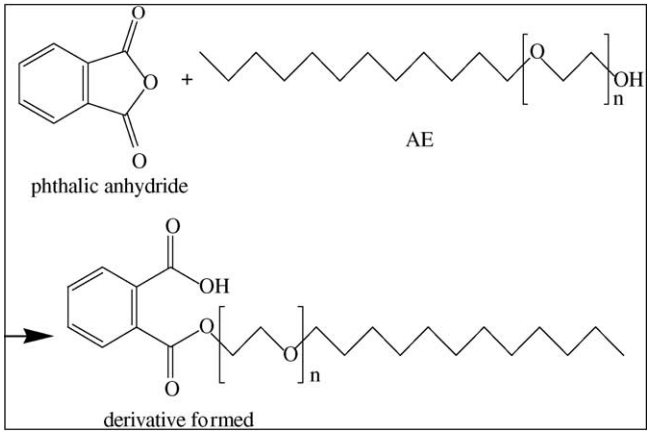

Fig. 2. Reaction scheme for the derivatisation of alcohol ethoxylates using phthalic anhydride.

producers. Due to the efficiency of removal of AEs in activated sludge treatment plants (97%) [4] environmentally orientated analytical procedures for the determination of these compounds must be capable of quantitation at concentrations of <10 to 100 μg/L in receiving waters [2]. There is also a need of speciation of the individual homologues and oligomers as toxicity and biodegradation of AEs depend on the length of both the alkyl and polyethoxylate chains [2]. A recent review discussing the occurrence, fate and effect in the aquatic and terrestrial environment of AEs used as adjuvants in pesticide formulations has been conducted by Krogh et al. [5].

Analytical methodology for the analysis of AEs has progressed rapidly over the years. Non-specific spectrophotometric and titration methods have been reviewed by Holt et al. [6]. However, these methods are not suitable for the low concentration of non-ionic surfactants found in most surface waters. The lack of a chromophore in the AE molecule means that high-performance liquid chromatography (HPLC) using conventional detectors depends on the formation of derivatives amenable to ultra violet (UV) absorption or fluorescence detection. Examples of derivatives include phenyl isocyanate, widely used in environmental analysis, providing a UV chromophore [7]. Derivatisation with 1-naphthoyl chloride and 1-naphthyl isocyanate [8] yield fluorophores, adding more sensitivity and selectivity. The separation of AEs by HPLC methods can be based on ethoxymeric distribution using normal phase HPLC, or as is more commonly used in environmental analysis the separation is based on the resolution of the hydrophobic homologues under reversed phase HPLC conditions. The combination of HPLC with evaporative light scattering detection [9] permits the analysis of AEs without the requirement of derivatisation, but lacks the sensitivity required for environmental analysis. Application of gas chromatography (GC) has been limited to compounds with less than five ethoxy units, due to high polarity, low volatility and thermal instability associated with the higher oligomers [10]. However, biodegradation of AEs has been studied by GC–flame ionisation detection (FID) of the alkyl bromides produced by acid cleavage of the ether linkages with hydrogen bromide [11]. To provide detail of homologues, oligomers and isomers the current methodology has focussed on liquid chromatography/mass spectrometry (LC/MS). LC/MS methods utilising thermospray [12] and electrospray ionisation (ESI) [13,14] have been used to provide such information. However, these methods suffer fundamental flaws in that the ionisation efficiency of the individual ethoxylates vary greatly and $EO_{0–2}$ are not normally detected or produce a weak signal. LC/MS methods utilising atmospheric pressure chemical ionisation (APCI) have been successfully employed for the analysis of AEs in environmental samples [15–17]. However, APCI was also shown to have low sensitivity with the lower ethoxymers and some thermal degradation of higher ethoxymers [18]. Method development has now done the full circle with derivatisation strategies converting alcohols to ionic or solution ionisable compounds to aid the LC/MS process [19]. The work of Dunphy et al. [20] utilised the reaction of the terminal hydroxyl group of each surfactant species with 2-fluoro-*N*-methylpridinium *p*-toluenesulphonate, imparting a cationic charge allowing all species including the free alcohol and EO_1 to be determined by ESI–MS.

This paper details an alternative derivatisation approach for the analysis of AEs by LC/MS, which utilises the reaction of the hydroxyl group with phthalic anhydride (Fig. 2). The derivatisation has its origins as a classical wet chemical procedure for the determination of hydroxyl number [21]. Phthalic anhydride derivatives have previously been used as a UV chromophore for HPLC [22] and also capillary electrophoresis (CE) [23,24]. In this paper, phthalic anhydride derivatisation of AEs is used to facilitate detection of all ethoxylates and the free alcohol by negative ion ESI–MS. The derivatisation and LC/MS methodology is linked to solid phase extraction (SPE) for the determination of environmental levels of AE in influent and effluent samples. The SPE procedure has been optimised in an attempt to give better recovery of the more hydrophobic AE species present in samples, particularly $C_{16,18}EO_{0–10}$, which have traditionally been difficult to quantify.

2. Experimental

2.1. Chemicals and reagents

Solvents (methanol, methyl *tertiary*-butyl ether (MTBE), dichloromethane (DCM), acetonitrile (ACN), tetrahydrofuran (THF) and pyridine) of LC quality were supplied from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Phthalic anhydride (>99%) was supplied from Fisher Scientific (Loughborough, UK) and ammonium hydroxide (33%, extra pure) was obtained from Fluka Ltd. (Gillingham, UK). The Ultrapure water used in the preparation of mobile phases was Milli Q Plus (Millipore, Watford, UK).

Three commercial ethoxylates were used. These were Genapol C100 (a linear AE with C_{12} and C_{14} alkyl chains and an average of 10 ethoxy (EO) units), Genapol T110 (a linear AE with C_{16} and C_{18} alkyl chains and average of 11 EO units), both supplied by Clariant (Frankfurt, Germany). Lutensol A07 (a linear AE with C_{13} and C_{15} alkyl chains and average of 7 EOs) was supplied by BASF (Ludwigshafen, Germany). The purity of the commercial samples was >98% as determined by nuclear magnetic resonance spectroscopy (NMR) [25] and equal amounts were dissolved in either methanol or pyridine for spiking or calibration standards, respectively. The C-chain distribution in the mixed standard was C_{12} (19.91), C_{13} (23.34), C_{14} (7.04), C_{15} (9.99), C_{16} (14.30) and C_{18} (25.42) % w/w as determined by MS [25]. The internal standard used in the quantitation of SPE samples was *n*-hexadecyl-d_{33} alcohol, supplied by Qm_x Laboratories Limited (Thaxted, UK).

2.2. Sample collection and pretreatment

Effluent and influent samples were taken from Broardholme sewage treatment works (STW), Wellingborough, Ditchford, Northants, UK. Broadholme STW (Anglian Water) treats predominately domestic waste, with a trade flow of 6.2% and trade organic load of 12.4%. Samples were taken in borosilicate glass bottles, which had previously been rinsed with Ultrapure water and methanol and kept at 4 °C prior to preconcentration, which occurred no later than 24 h after sampling. Effluent was filtered through a Whatman GF/C filter (Maidstone, UK) until a volume of approximately 4 L was collected. Six 600 mL aliquots were dispensed into 1 L bottles, three were spiked at 100 μg/L total AE and three were left unspiked. To each of the six samples, 400 mL of methanol were added. One hundred and twenty millilitres of unfiltered influent were thoroughly mixed with 1680 mL of Ultrapure water and divided into six 300 mL aliquots. Three of these were spiked at 1 mg/L of total AE and three were left unspiked. To all six influent samples, 200 mL of methanol were added.

2.3. Extraction procedures

SPE was carried out on Isolute C_8 cartridges (1 g, 6 mL) from Argonaut (Hengoed, UK). The procedure was automated using the autotrace SPE Workstation from Zymark Limited (Runcorn, UK). The cartridges were conditioned with 10 mL elution solvent (methanol:MTBE:DCM, 2:1:1, v/v/v), followed by 10 mL of methanol and 10 mL of Ultrapure water. The samples were loaded at 10 mL/min, and dried for 60 min under nitrogen before being eluted with 15 mL of elution solvent. The extracts were initially concentrated to 1–2 mL under a gentle stream of nitrogen, at room temperature before being quantitatively transferred to an HPLC vial and taken to dryness on a Reacti-vap unit from Pierce and Warriner (Chester, UK) prior to derivatisation. Samples were resuspended in 990 μL of phthalic anhydride in pyridine solution (3 g in 50 mL) and 10 μL of internal standard (1000 μg/mL in pyridine) were added. The samples were then capped and heated at 85 °C for 1 h in an oven for derivatisation to occur. For calibration standards the required amount of pyridine stock solution was added to the derivatisation solution to give a calibration in the range 0–60 μg/mL total AE in derivatised form, using five data points.

2.4. LC/MS analysis

Samples were analysed on an 1100 LC/MS G1946B version MSD from Agilent Technologies Ltd. (Stockport, UK). AEs were separated under gradient elution conditions with a mobile phase of A, 0.1% (v/v) ammonia in Ultrapure water (v/v) and B, 0.1% (v/v) ammonia in 900/100 (v/v) ACN/THF. The mobile phase composition was 15% B at the beginning of the gradient and then linearly increased to 100% B in 25 min. It was then held for a further 10 min before re-equilibrating at the initial conditions for 20 min. The AE derivatives were separated on a Luna C_{18} (2) analytical column (150 mm × 2 mm i.d., 5 μm particle size) and 2 mm i.d. C_{18} (2) guard cartridge, both from Phenomenex (Macclesfield, UK). The flow rate was maintained at 0.25 mL/min and 10 μL of the pyridine extracts were injected onto the column which was thermostatically held at 40 °C. A divert time of 8 min was used to minimise source contamination with derivatisation agent.

The MS was operated in negative ion mode electrospray with a gas temperature of 300 °C, drying gas 7.0 L/min, nebuliser gas pressure 35 psi, and a capillary voltage of 4000 V. The MS was operated in full scan mode over the range *m/z* 300–1300 amu and a fragmentor voltage ramp was utilised to give optimal performance over this range. Method development was carried out on a Pump 11 syringe pump from Harvard Apparatus Ltd. (Kent, UK).

3. Results and discussion

3.1. Optimisation of the MS procedure

AE response was optimised by syringe infusion ESI–MS of a concentrated Lutensol A07 derivative (200 mg/5 mL derivatisation reagent) diluted 100-fold with mobile phase (50:50, v/v, A:B). A similar concentration of Lutensol A07,

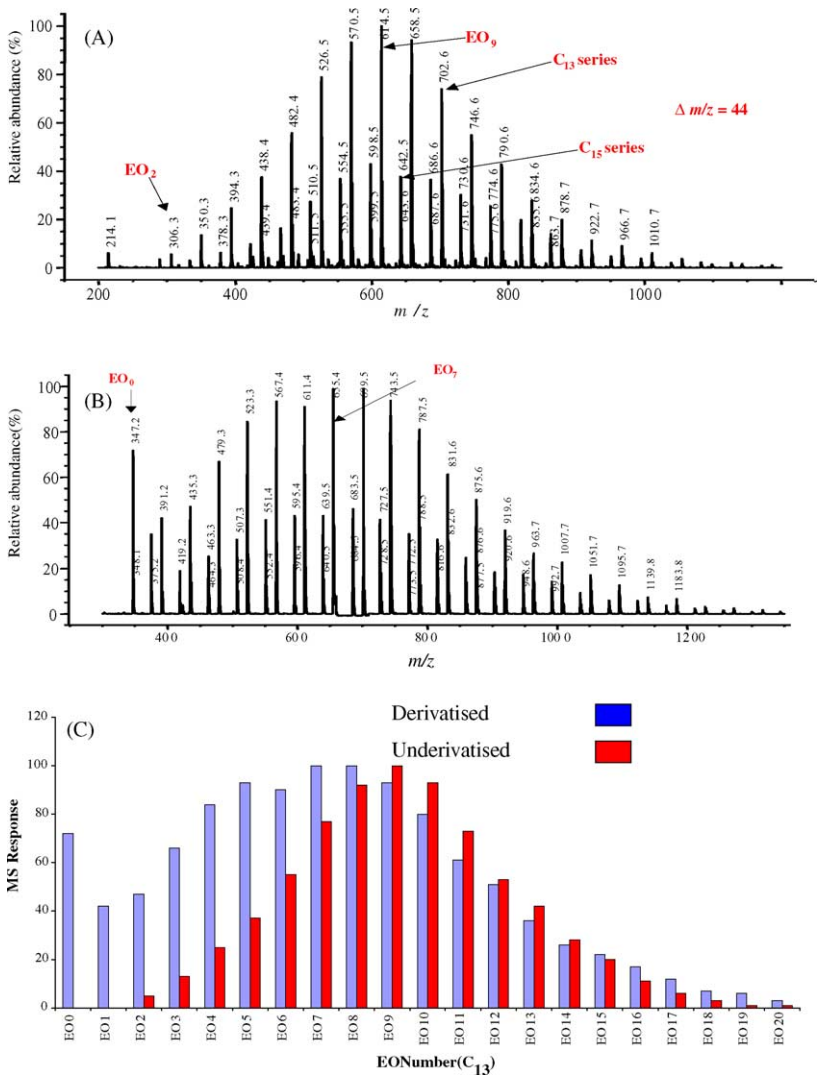

Fig. 3. Syringe infusion of Lutensol A07. (A) Underivatised form; (B) derivatised form; and (C) graphical comparison of underivatised and derivatised forms.

to that of the diluted derivative, was also infused in the same mobile phase mixture in an underivatised form to show the benefit imparted by the derivatisation process. The results of these experiments are displayed in Fig. 3. Without derivatisation there is no apparent ionisation of EO_0 or EO_1 for either the C_{13} or C_{15} alkyl series (Fig. 3(A)). The first peak visible in the C_{13} series, characterised by a difference in *m/z* of 44 units of the $[M + NH_4]^+$ adducts, is the *m/z* 306.3 ion which corresponds to $[C_{13}EO_2 + NH_4]^+$. Ionisation of EO_0 and EO_1 would give ions of *m/z* 218 and 262, respectively. The EO distribution maximises at approximately $C_{13}EO_9$, *m/z* 614.5 (Fig. 3(A)). However, for the derivatised sample the $C_{13}EO_0$ is clearly visible at *m/z* 347.2 (Fig. 3(B)). This peak corresponds to the C_{13} alcohol phthalic anhydride derivative with the loss of a proton, producing a negative ion (see Fig. 1, Table 1). The EO distribution maximises at approximately $C_{13}EO_7$ (Fig. 3(B)), which is in agreement with NMR data [25]. Fig. 3(C) shows a graphical comparison of the underivatised and derivatised 'fingerprint' forms of the AE. It can be clearly seen that with derivatisation a shift of MS response to lower EO distribution is made which is in keeping with theory and supporting NMR data. The derivatisation should therefore allow a more accurate environmental fingerprint of AE to be established. This is very important in allowing shifts in chemical composition found in environmental monitoring to be incorporated into risk assessment data obtained from testing of parent material in laboratory toxicity studies [26]. In addition, it is noted that the spectrum (Fig. 3(C)) is largely free from background interference when observed in negative ion mode, with ionisation of the derivatives not subject to competition from other adducts or doubly charged species as can occur in positive ESI data.

3.2. Calibration of LC/MS

A calibration over the range of 0, 7.5, 15, 30 and 60 μg/mL total AE was constructed for each of the ions listed in Table 1. The correlation coefficients for these curves are listed in

Table 1
Negative ions (*m*/*z*) used to generate extracted ion chromatograms for standards and spiked samples

	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{18}
EO_0	333.2	347.2	361.3	375.3	389.3	417.3
EO_1	377.3	391.3	405.3	419.3	433.3	461.4
EO_2	421.3	435.3	449.3	463.3	477.4	505.4
EO_3	465.3	479.3	493.4	507.4	521.4	549.4
EO_4	509.4	523.4	537.4	551.4	565.4	593.4
EO_5	553.4	567.4	581.4	595.4	609.4	637.5
EO_6	597.4	611.4	625.4	639.5	653.5	681.5
EO_7	641.4	655.5	669.5	683.5	697.5	725.5
EO_8	685.5	699.5	713.5	727.5	741.5	769.6
EO_9	729.5	743.5	757.5	771.6	785.6	813.6
EO_{10}	773.5	787.6	801.6	815.6	829.6	857.6
EO_{11}	817.6	831.6	845.6	859.6	873.6	901.7
EO_{12}	861.6	875.6	889.6	903.6	917.7	945.7
EO_{13}	905.6	919.6	933.7	947.7	961.7	989.7
EO_{14}	949.7	963.7	977.7	991.7	1005.7	1033.8
EO_{15}	993.7	1007.7	1021.7	1035.7	1049.8	1077.8
EO_{16}	1037.7	1051.7	1065.8	1079.8	1093.8	1121.8
EO_{17}	1081.8	1095.8	1109.8	1123.8	1137.8	1165.9
EO_{18}	1125.8	1139.8	1153.8	1167.8	1181.9	1209.9
EO_{19}	1169.8	1183.8	1197.8	1211.9	1225.9	1253.9
EO_{20}	1213.8	1227.9	1241.9	1255.9	1269.9	1297.9

The ions listed are $[M-H]^-$ for the derivatised AE species.

Table 2. Good results were obtained with correlation coefficients principally >0.99 obtained for the majority of the ethoxylate species. The interference noted in Table 2 was an isobaric interference at *m*/*z* 845.6 for $C_{14}EO_{11}$. The AE concentration in samples was quantified against the response of standard checks, which were placed at regular intervals in the analytical sequence.

Table 2
Correlation coefficients for extracted ion data over the concentration range 0–60 μg/mL total AE ($n = 5$)

	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{18}
EO_0	0.9992	0.9990	0.9952	0.9951	0.9956	0.9959
EO_1	0.9894	0.9989	0.9995	0.9740	0.9943	0.9987
EO_2	0.9994	0.9959	0.9957	0.9902	0.9943	0.9979
EO_3	0.9972	0.9937	0.9917	0.9929	0.9984	0.9993
EO_4	0.9983	0.9945	0.9971	0.9983	0.9975	0.9995
EO_5	0.9969	0.9725	0.9975	0.9970	0.9989	0.9962
EO_6	0.9901	0.9948	0.9961	0.9936	0.9984	0.9965
EO_7	0.9953	0.9964	0.9973	0.9733	0.9943	0.9966
EO_8	0.9972	0.9961	0.9968	0.9977	0.9960	0.9929
EO_9	0.9954	0.9928	0.9954	0.9963	0.9928	0.9921
EO_{10}	0.9944	0.9943	0.9990	0.9949	0.9984	0.9916
EO_{11}	0.9912	0.9933	Int[a]	0.9973	0.9962	0.9889
EO_{12}	0.9942	0.9968	0.9983	0.9973	0.9867	0.9821
EO_{13}	0.9960	0.9949	0.9932	0.9983	0.9912	0.9835
EO_{14}	0.9981	0.9988	0.9928	0.9955	0.9902	0.9870
EO_{15}	0.9909	0.9909	0.9993	0.9925	0.9978	0.9880
EO_{16}	0.9914	0.9911	0.9896	0.9812	0.9952	0.9913
EO_{17}	0.9966	0.9923	0.9658	0.9873	0.9966	0.9996
EO_{18}	0.9973	0.9993	0.9876	0.9996	0.9833	0.9794
EO_{19}	0.9978	0.9480	0.9939	0.9536	0.9904	0.9999
EO_{20}	0.9679	0.9931	0.9983	0.9232	0.9917	0.9802

[a] Int: isobaric interference.

A method detection limit (MDL) of 0.02 μg/L for each ethoxylate component was estimated from the data. This was obtained by utilising the Poisson-like distribution of ethoxylates within the commercial samples, which results in certain ethoxylates being present at low concentrations in the standard mix and effluent spikes, and peak to peak signal-to-noise ratios calculated within the ChemStation software. A signal-to-noise ratio of 3 was then used to define the MDL for such components and this was applied as a general MDL for all ethoxylates based on the similar ionisation response of the derivatives across the mass range. The % w/w of each ethoxylate was known from previous characterisation of the commercial samples [25].

3.3. Optimisation of the SPE procedure

Initial work showed that a simple one solvent elution system using methanol was ineffective in AE recovery. It was also noticed that if methanol was not added to the aqueous sample significant losses of hydrophobic components ($C_{18}EO_{0–10}$) occurred, an effect that was more apparent at lower concentrations of AE. Experiments with different mixed elution solvents, e.g. combinations of methanol:MTBE:DCM, improved recoveries, but problems were still witnessed with $C_{18}EO_{0–10}$. It was shown that the poor recovery of these ethoxymers could be attributed to retention on glassware and the flow tubing of the Autotrace SPE system. Losses were minimised by the addition of 40% (v/v) methanol to the sample prior to loading, without any adverse effect being observed on the recovery of the polar ethoxylate species (e.g. $C_{12}EO_{10–20}$).

3.4. Recovery from effluent samples

The extract from SPE was divided into two equal aliquots, with one being taken through the procedure described earlier. The other aliquot was retained for additional analysis if required. The blank effluent samples were found to have low concentrations of native AE present, but these concentrations were subtracted from AE concentrations measured in spiked samples in calculating recoveries. An internal standard was used to assess derivatisation efficiency and also to confirm that matrix suppression effects were not affecting quantitation. No matrix suppression was observed and results were not corrected for internal standard response. Results reported for recoveries of AE were assessed on the basis of the whole procedure, i.e. SPE and the preconcentration/derivatisation step. Losses of the more volatile AE components (e.g. C_{12} and C_{13} free alcohol) were reduced by careful control of the preconcentration step. The calibration standards were not subjected to blow down and hence any evaporative losses.

Typical total ion chromatogram (TIC) of a standard mixture of the commercial samples and extracted ion chromatograms (EICs) are shown in Fig. 4. Extracted ion chromatograms for selected AE ethoxymers for Genapol C100 and Lutensol A07 are shown in Fig. 4 (B and C,

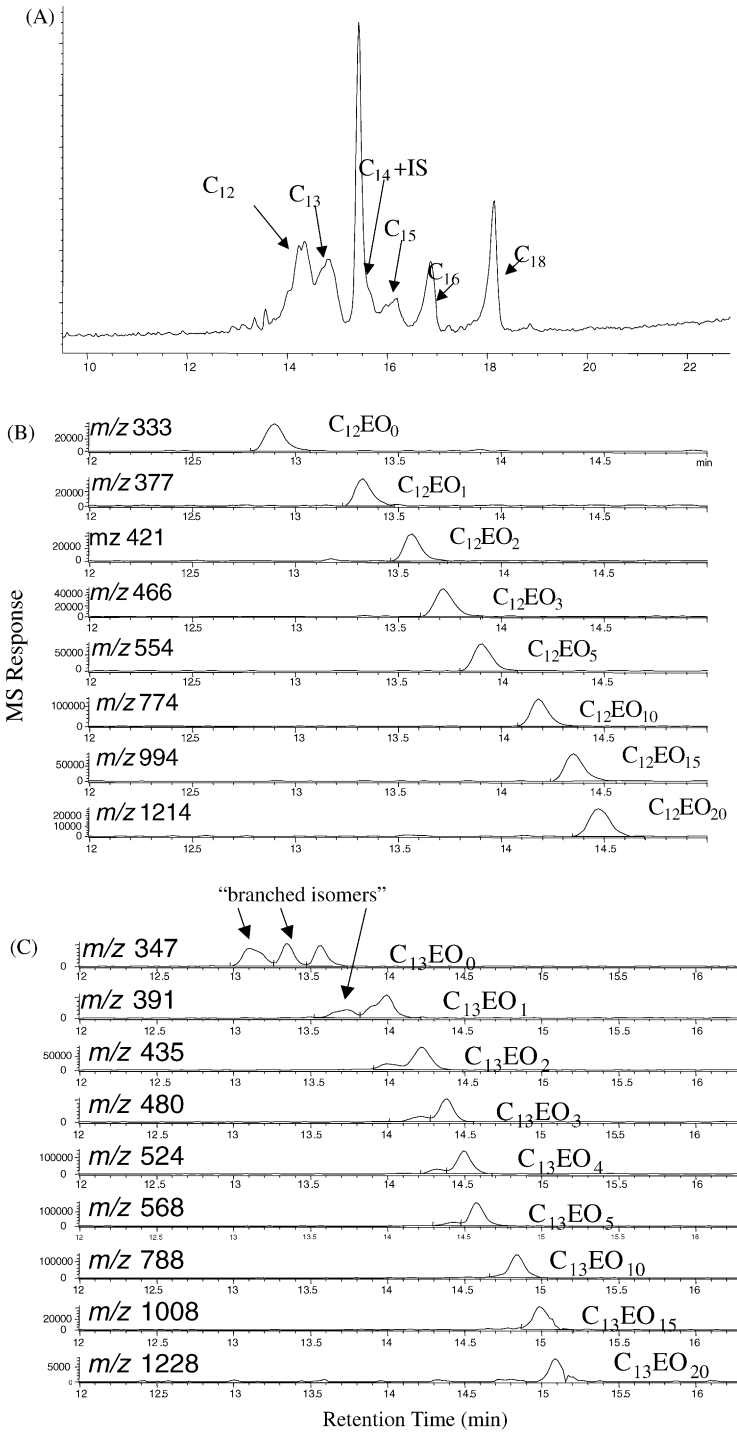

Fig. 4. LC/MS chromatograms for AE from a standard mixture containing equal amounts of Lutensol A07, Genapol C100 and Genapol T110. (A) Total ion chromatogram at a concentration of 60 μg/mL. (B) Extracted ion chromatograms for Genapol C100 showing selected C_{12} AE species. (C) Extracted ion chromatograms for Lutensol A07 showing selected C_{13} AE species.

respectively). Lutensol A07 is an "oxo"-AE, which contains β-methyl and β-ethyl isomers as well as linear species, whilst Genapol C100 is linear with no branched isomers. The EIC trace for Genapol C100 AE is shown in Fig. 4(B), whilst that for Lutensol A07 (Fig. 4(C)) clearly shows the resolution of branched and linear AE isomers, for EO_0 and EO_1, under the HPLC conditions employed. For recovery purposes the sum of the peak areas of all isomers present in each EIC was used.

Table 3 shows spiked effluent recoveries. It is apparent that excellent recoveries and %R.S.D. figures were obtained across both the alkyl chain and ethoxymer chain ranges, albeit with some slightly lower recoveries being observed in the C_{12}

Table 3
Recovery of 100 μg/L of total AE spiked into final effluent ($n = 3$)

	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{18}
EO_0	55 (14)	63 (2)	84 (23)	88 (3)	108 (25)	102 (39)
EO_1	93 (6)	79 (8)	112 (4)	65 (36)	93 (10)	93 (4)
EO_2	110 (10)	93 (11)	116 (7)	95 (9)	100 (2)	90 (3)
EO_3	102 (3)	97 (5)	111 (5)	104 (9)	95 (8)	92 (4)
EO_4	101 (4)	91 (3)	105 (6)	81 (5)	93 (5)	90 (2)
EO_5	101 (4)	88 (5)	104 (8)	89 (4)	100 (3)	98 (2)
EO_6	102 (2)	90 (5)	100 (7)	83 (8)	92 (1)	100 (4)
EO_7	96 (2)	91 (3)	100 (2)	84 (15)	99 (3)	91 (2)
EO_8	102 (2)	89 (4)	101 (1)	74 (18)	98 (6)	91 (3)
EO_9	99 (2)	94 (1)	100 (3)	78 (12)	96 (4)	93 (2)
EO_{10}	96 (1)	96 (3)	92 (6)	79 (6)	95 (5)	93 (3)
EO_{11}	101 (1)	91 (2)	Int (Int)[a]	96 (2)	90 (3)	91 (3)
EO_{12}	97 (1)	91 (2)	100 (10)	75 (5)	95 (4)	95 (0)
EO_{13}	96 (3)	89 (3)	92 (6)	98 (7)	90 (5)	94 (7)
EO_{14}	91 (7)	98 (5)	87 (5)	92 (7)	93 (4)	96 (6)
EO_{15}	94 (4)	98 (6)	98 (2)	91 (2)	86 (6)	99 (2)
EO_{16}	94 (8)	91 (4)	91 (8)	107 (8)	101 (8)	98 (1)
EO_{17}	100 (7)	113 (5)	85 (8)	92 (6)	96 (9)	95 (2)
EO_{18}	95 (9)	105 (10)	99 (11)	99 (7)	95 (1)	90 (6)
EO_{19}	95 (9)	102 (7)	94 (9)	117 (11)	98 (13)	102 (3)
EO_{20}	94 (2)	82 (18)	90 (7)	76 (10)	111 (14)	90 (11)

Values quoted are mean (%R.S.D.).
[a] Int: isobaric interference.

and C_{13} free alcohols, probably due to evaporative losses. The addition of 40% methanol to samples prior to loading appears to improve recovery for apolar surfactants. This does not appear to have been used in other recent papers, which discuss the optimisation of AE recovery using SPE, and may account for some of the lower recoveries reported for these species [17,20].

Analogous data was obtained for influent samples (Table 4). However, as a result of the higher native levels of AEs in the influent, a much higher level of spike was required to give a measurable difference in response. A 10-fold dilution, made in derivatisation reagent, was required to bring the blank influent samples and spikes within the calibration range used.

Table 4
Recovery of 1000 μg/L of total AE spiked into influent ($n = 3$)

	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{18}
EO_0	60 (33)	64 (9)	42 (72)	84 (4)	100 (19)	78 (7)
EO_1	45 (38)	85 (3)	49 (44)	66 (4)	81 (7)	90 (5)
EO_2	34 (109)	89 (1)	88 (25)	107 (12)	100 (10)	88 (6)
EO_3	51 (58)	96 (2)	86 (18)	106 (10)	106 (13)	91 (7)
EO_4	61 (26)	93 (5)	88 (9)	92 (14)	108 (3)	98 (4)
EO_5	74 (16)	89 (2)	93 (11)	98 (8)	112 (4)	98 (5)
EO_6	91 (6)	96 (2)	104 (5)	101 (11)	103 (5)	93 (6)
EO_7	89 (5)	97 (10)	98 (3)	94 (8)	104 (2)	94 (2)
EO_8	99 (2)	97 (7)	113 (3)	99 (7)	99 (7)	92 (2)
EO_9	93 (4)	99 (10)	115 (1)	87 (16)	98 (5)	88 (3)
EO_{10}	98 (3)	92 (2)	107 (6)	95 (6)	102 (4)	98 (2)
EO_{11}	94 (1)	103 (5)	Int (Int)[a]	108 (5)	99 (3)	94 (4)
EO_{12}	97 (2)	94 (6)	106 (7)	100 (22)	93 (4)	100 (1)
EO_{13}	94 (2)	91 (9)	104 (15)	101 (5)	93 (3)	95 (1)
EO_{14}	92 (7)	97 (8)	86 (21)	97 (12)	94 (3)	94 (3)
EO_{15}	90 (4)	105 (2)	90 (8)	96 (10)	90 (8)	98 (3)
EO_{16}	98 (11)	106 (6)	88 (5)	113 (2)	95 (5)	98 (4)
EO_{17}	92 (3)	100 (2)	97 (5)	125 (3)	97 (2)	102 (7)
EO_{18}	107 (7)	100 (3)	103 (10)	125 (25)	104 (7)	105 (3)
EO_{19}	105 (9)	112 (6)	108 (8)	165 (5)	103 (13)	99 (5)
EO_{20}	95 (9)	100 (14)	102 (9)	156 (30)	93 (9)	93 (11)

Values quoted are mean (%R.S.D.).
[a] Int: isobaric interference.

Table 5
Concentration of AE found in sewage influent from Broardholme STW (μg/L)

	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{18}
Native AE in influent (μg/L)						
EO_0	146	106	196	68	110	520
EO_1	127	55	125	19	33	<6
EO_2	157	52	131	23	31	<6
EO_3	213	60	77	30	10	<6
EO_4	203	62	55	35	11	<6
EO_5	169	67	46	32	<6	<6
EO_6	145	47	40	36	<6	<6
EO_7	103	45	32	41	<6	<6
EO_8	90	47	26	38	<6	<6
EO_9	78	43	21	39	<6	<6
EO_{10}	58	42	20	35	<6	<6
EO_{11}	47	35	Int[a]	29	<6	<6
EO_{12}	35	26	8	21	<6	<6
EO_{13}	24	21	7	18	<6	<6
EO_{14}	16	11	7	11	<6	13
EO_{15}	11	<6	6	<6	<6	<6
EO_{16}	<6	<6	<6	<6	<6	<6
EO_{17}	<6	<6	<6	<6	<6	<6
EO_{18}	<6	<6	<6	<6	<6	<6
EO_{19}	<6	<6	<6	<6	<6	<6
EO_{20}	<6	<6	<6	<6	<6	<6
Totals	1652	756	829	510	290	648
Total			4685			

[a] Int: isobaric interference.

3.5. Analysis of sewage effluents and influents

Native concentrations of AEs in influent and effluent from Broardholme sewage treatment plant were determined using the procedures described above (Tables 5 and 6, respectively). Individual AE concentrations were calculated using characterisation data described earlier, giving % w/w of each ethoxymer present in the commercial mixtures used in this study. It was noted in the influent samples (Table 5) that significant levels of EO_0–EO_{15} were observed for C_{12}–C_{15} species, which is consistent for the average structure $C_{13.3}EO_{8.2}$ for European use [27] whereas C_{16} and C_{18} distributions were predominantly free alcohol. This is consistent with previous published monitoring data [20], where it was postulated that AE distributions may have contributions from related surfactants. In the case of effluent samples (Table 6) it is evident that the treatment works at Broardholme is functioning effectively in relation to AE removal, as observed AE concentrations were approximately 1000-fold lower than those observed in influent samples. Total AE in the influent samples was approximately 5000 μg/L whilst the concentration in effluent samples was

Table 6
Concentration of AE found in sewage effluent from Broardholme STW (μg/L)

	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{18}
Native AE in effluent (μg/L)						
EO_0	0.187	<0.04	0.216	<0.04	0.188	1.531
EO_1	0.053	<0.04	0.045	0.108	<0.04	<0.04
EO_2	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_3	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_4	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_5	<0.04	0.059	<0.04	<0.04	<0.04	<0.04
EO_6	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_7	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_8	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_9	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{10}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{11}	<0.04	<0.04	Int[a]	<0.04	<0.04	<0.04
EO_{12}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{13}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{14}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{15}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{16}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{17}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{18}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{19}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
EO_{20}	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Totals	1.000	0.859	0.981	0.908	0.988	2.331
Total			7.068			

[a] Int: isobaric interference.

approximately 7 μg/L. These figures include the contribution of MDL values for components not detected, which were corrected for the overall concentration step used for each type of sample. The MDL of 0.02 μg/L per individual ethoxylate equates to a MDL of approximately 2.5 μg/L of total AE (126 species analysed). The predicted no effect concentration for total AE has been calculated as 110 μg/L [27] and the method appears to be more than capable of providing data suitable for risk assessment and monitoring below this concentration.

The presence of native AE surfactants in the influent and effluent samples may also have contributed to the reduced precision and recoveries obtained for certain components in the spiked samples (Tables 3 and 4). Derivatised sample extracts were found to be stable for at least 18 days.

4. Conclusion

The derivatisation and LC/MS procedure described facilitate analysis of all AEs in the range $C_{12–18}EO_{0–20}$. ESI in negative ion mode for the phthalate derivatives gives a more accurate AE fingerprint, with ethoxylate distribution comparable to NMR analysis. The SPE procedure has been optimised to allow quantitative recovery of hydrophobic components by addition of 40% methanol to samples. The method has been validated for analysis of environmental levels of AEs in influent and effluent samples. Good recoveries of AEs across the wide polarity range, due to variation in both alkyl chain length and degree of ethoxylation, were obtained.

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