Review

Chromatographic determination of non-ionic aliphatic surfactants of the alcohol polyethoxylate type in the environment

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Abstract

The chromatographic procedures available for the determination of alcohol polyethoxylates (AE) in environmental samples are reviewed. The proposed methods are classified according to their routine or confirmatory (i.e., structurally oriented) potential application. The routine analysis of AE is shown to depend crucially on the formation of derivatives amenable to UV-absorption or fluorescence detection in normal- or reversed-phase high-performance liquid chromatography (HPLC), or to volatile compounds suited for gas chromatography (GC) coupled to FID. The confirmatory methods are those requiring the mass spectrometric detection operating in the selected ion monitoring mode. Only HPLC–MS is currently capable of analysing unaltered AE. HPLC and GC separations are described together with the extraction/enrichment procedures used for the isolation of these compounds from aqueous and solid environmental matrices.

Keywords: Reviews; Environmental analysis; Surfactants; Alcohol polyethoxylates; Polyethoxylates; Nonylphenol polyethoxylates

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1. Production, chemical structure and environmental behaviour

The aliphatic alcohol polyethoxylates (AE) are the most important non-ionic surfactant accounting for ca. 55% of the 1.15×10^6 ton world production of non-ionic surfactants [1]. In Western Europe, however, this percentage increases to about 75% due to the regulatory and/or voluntary ban of the aromatic polyethoxylated alkylphenols which were shown to biodegrade through the formation of toxic intermediates [2].

AE are synthesized industrially via the base-catalysed addition of ethylene oxide to mixtures of aliphatic alcohols of oleochemical or petrochemical origin [3].

The oleochemical alcohols, produced by saponification and subsequent hydrogenation of vegetal oil fatty acid triglicerides, are linear and primary, and only contain even C_{12} to C_{18} homologs (Fig. 1). The oleochemical AE mixtures account for ca. 40% of the total AE production. The petrochemical mixtures (the remaining 60%), produced mainly by oxo-synthesis-reduction-polyethoxylation, comprise even and odd homologs, the structure and composition of which depend on both the feedstock (linear or branched olefins) and the reaction conditions of the industrial oxo-synthesis process. Oxo-AE from linear

olefins are partly linear and partly 2-alkyl substituted (so-called monobranched AE) with 11 to 15 alkyl carbons (Fig. 1). Highly branched oxo-AE (Fig. 1), resulting from branched olefins produced by oligomerization of propene and butene, represent not more than 10% of total production. The hydrophilic moiety of AE used in the formulation of detergents, representing the majority of the industrial production, is typically composed of 1 to 15-25 ethoxy units with an average ethoxylation number of 6-11. A residue of unreacted alcohol always occurs in commercial AE, accounting for 5-15% (on a molar basis) of the total oligomers [3].

The commercial AE blends are therefore very complex. Taking into account the typical ethoxylation range for detergent AE and the wide rank of possible linear, mono- or multi-branched hydrophobic moieties, more than 2500 individual chemicals are expected to be present in washing machine liquor and then in the raw sewage. The levels of AE in untreated municipal sewage are in the 0.5-5 mg/l concentration range [4-6]. Much lower concentrations (0.015-0.040 mg/l) are expected and found in wastewaters after activated sludge [5] or trickling filter [6] treatments as well as in natural waters [5], depending on the extent of the biotic and abiotic removals of AE. In result, environmentally oriented analytical procedures for the determination of AE must cope typically with <10 to 100 μg/l concentration levels and be able to distinguish the various homologs and oligomers, since the acute and chronic toxicities [7] as well as the biodegradation rates [8,9] of AE depend on the length of both the alkyl and the polyethoxylic chains.

Moreover, the possible concurrent presence in the environmental samples of the AE and the other structurally similar nonionic surfactant alkylphenol polyethoxylates (APE), particularly the nonylphenol polyethoxylates (NPE) [4,5], introduces an additional analytical difficulty, i.e., the separation of AE from APE [10].

Finally, a desirable capability offered by analytical protocols for the environmental determination of AE is the identification and quantification of possible biotransformation products. The literature on AE biodegradation [11,12] and recently acquired results [13] indicate the poly(ethyleneglycol) (PEG) family

Oleochimical AE
AE from Ziegler alcohols

R: C12-C18
(Even omologs only)
n: 0-25 (average=5-10)

Oxo-AE from linear olefins

R+R': C11-C15
(Both even and odd homologs)
R': H-, methyl-, ethyl, etc.
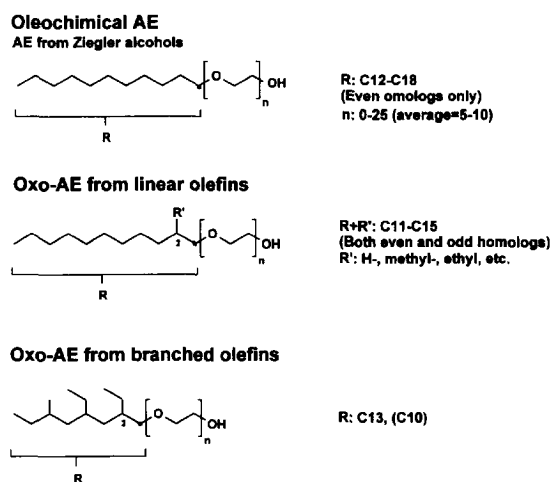
Oxo-AE from branched olefins

R: C13, (C10)

Fig. 1. Chemical structure of manufactured AE.

as primary key intermediates of the aerobic biodegradation of AE.

2. Chromatography-based procedures for the environmental analysis of AE

The high number of components of AE surfactant mixtures span a wide range of molecular mass (180–1400 Da), polarity and volatility. This variability makes the development of effective environmental extraction–enrichment procedures and the choice of the most suitable chromatographic technique for AE separation very challenging. None of the most important chromatographic techniques (HPLC, GC, supercritical fluid chromatography (SFC)) gives a complete separation of such potentially complex mixtures. Furthermore, the final detection of AE molecules by most of the common devices is often affected by limitations in terms of detectability, selectivity and/or sensitivity. So far, the most used chromatographic techniques for determining AE in environmental samples, as well as in biodegradation test liquors are HPLC and GC. Thin-layer chromatography (TLC) was formerly applied in biodegradadation studies [14,15] but appeared unsuitable to the environmental determination of AE because of its poor separation efficiency.

The lack of chromophors in the AE molecule and the low volatility of the higher AE oligomers require suitable derivatisation or alteration when conventional detectors such as UV-absorption and fluorescence (FL) for HPLC and FID for GC are employed. The combination of HPLC and detectors such as refractive index, evaporative light scattering [16,17] and flame ionization [18,19] does not require derivatisation, but the sensitivity and selectivity necessary for detecting environmentally occurring AE are by no means reached. Unaltered AE in environmental samples can be analysed in a substance-specific way only by coupling HPLC and mass spectrometric detection.

The recent development of the atmospheric pressure interfaces (API) has prompted the effective hyphenation of liquid chromatography and mass spectrometry [20]. However, the current high cost of the interfacing systems still severely limits the spread of MS detection in liquid chromatography.

SFC, which provides a resolution similar to that of GC without being affected by the analyte volatility (i.e., no derivatisation is needed), would at first glance appear to be the chromatographic technique of choice [21]. However, the drawback of SFC is currently due to the problems encountered in developing suitable interfaces for the hyphenation of SFC with MS detection. The current availability of poorly selective detectors, such as FID, restricts the application of this technique to the analysis of commercial mixtures containing few AE homologs, since only in this case the AE individual components can be effectively separated, thus avoiding any peak overlap.

The choice of the suitable extraction/enrichment technique for the recovery of AE in traces from environmental matrices must take into account not only the separation capabilities of the selected chromatographic technique, the sensitivity and selectivity of detection amenable (or available) and the complexity of the sample matrix, but also the high complexity of the considered analytes in terms of both number of different compounds and physicochemical characteristics. Solid-phase extraction (SPE) and sublation were mainly used to recover AE from aqueous matrices. SPE exploited the hydrophobicity of the AE molecule. Employed sorbents were macroreticular styrene–divinylbenzene resins such as XAD-2 [10,22–24], bonded silica (C_1, C_8, C_{18}) [6,10,25,26], as well as graphitized carbon black (GCB) [5,13]. GCB allowed the selective elution of nonionic surfactants and did not require further purification. SPE by other sorbents (i.e., alkyl bonded silica) usually required additional extract clean-up by cationic/anionic exchange to remove charged interfering molecules (i.e., anionic and cationic surfactants). Sublation [27] was successfully applied to the cumulative extraction of polyethoxylated non-ionic surfactants (AE and APE) even though many other surface-active substances (including cationic and anionic surfactants) were extracted as well, and subsequent ion-exchange clean-up was necessary [10,26]. Liquid–liquid extraction, based on the partitioning of AE at levels below the critical micellar concentration, was also used [10], but

sublation, and particularly SPE, appeared much more efficient, time- and chemical saving.

Extraction of AE from solid samples such as sewage sludge was carried out using traditional Soxhlet liquid–solid continuous extractor, as well as by supercritical fluid extraction (SFE), which efficiently recovered AE in a wide range of polarity [26].

Depending on the requirements of the adopted chromatographic technique, further clean-up of environmental extracts by ion-exchange [10,23,26,28], alumina [10,26] or silica [10] columns were also reported [10,23].

Comments on performance of cited extraction/enrichment techniques in terms of selectivity, accuracy (recovery) and precision are given in the sections dealing with the major chromatographic approaches proposed for the environmental analysis of AE.

2.1. High-performance liquid chromatography

HPLC has been widely used for the development of analytical methodologies designed for the determination of AE in environmental aqueous samples [10,23,28–32] and, noticeably, is the only technique which has been routinely applied to field investigations, particularly in sewage treatment plants.

2.1.1. Derivatisation and absorption/fluorescence detection

Detecting trace concentrations of AE in environmental sample extracts by either UV absorption or fluorescence detection after HPLC separation requires pre- or post-column derivatisation by UV-absorbing or fluorescent agents, since AE exhibit neither appreciable UV absorption (above 200 nm) nor fluorescence activity. The choice of the derivatising agent is affected by parameters such as the absorption and/or fluorescence activity and the chromatographic behaviour of the resulting adduct, the reaction conditions required for quantitative conversion of AE, as well as by the chemical stability, commercial availability and safety handling of the derivatising agent. A broad variety of AE derivatising agents for pre-injection derivatisation is reported in the literature, while no information regarding post-column derivatisation is yet available. Fig. 2 lists the proposed derivatising agents together

Fig. 2. Fluorescent and/or UV-absorbing reagents proposed for AE derivatisation.

with the corresponding adducts and detection conditions.

Among the various derivatising agents presented in Fig. 2, the UV-absorbing agent phenyl isocyanate (PIC) [33], though not fluorescent, was the most extensively used for developing suitable analytical protocols for the environmental determination of AE so far [10,23,28]. Adducts of AE with other UV-absorbing derivatising agents, such as benzoyl chloride and 3,5-dinitrobenzoyl chloride, exhibited a performance similar to that of PIC in terms of both reactivity and molar absorptivity [32,34,35].

Derivatisation with fluorescent agents such as 1-anthroyl nitrile [36], 1-naphthyl isocyanate [30,32] and 1-naphthoyl chloride, however, led to a significant enhancement of sensitivity and selectivity.

2.1.2. Chromatographic separation of AE adducts by HPLC

The chromatographic behaviour of the AE adducts shows remarkable differences depending on both the chromatographic conditions (type of stationary phase, composition of mobile phase) and the derivatising agent used. Under normal-phase conditions, both unaltered and derivatised AE behave

similarly to the aromatic polyethoxylated non-ionics (APE), which have been investigated much more extensively than AE [37–39]. By separating the PIC adducts of a mixture of C_{12}–C_{18} linear AE (average ethoxylation number 8) on a silica column, Allen [33] obtained a complete ethoxymer-by-ethoxymer separation (i.e., co-elution of different AE homologs with the same ethoxylation degree) for the oligomers with more than 3 ethoxy units. However, literature on both AE and APE suggests that adsorption chromatography does not provide a satisfactory separation of AE mixtures with a wide ethoxymeric distribution [18,19,40]. Better separation of poly-ethoxylated surfactants according to the length of polyethoxy chain was provided by normal-phase partition chromatography using aminopropyl [23], cyano- [41–43] and dyol-bonded silica [44,45] stationary phases. In particular, Schmitt [23] separated, using aminopropyl silica, the PIC derivatives of a mixture of AE homologs according to only the ethoxymeric distribution and applied successfully the separation to the analysis of wastewater extracts (Fig. 3).

Okada [46] demonstrated that ion-exchange chromatography can be alternatively used to separate mixtures of polyethoxylated compounds including PEG, APE and AE, according to the length of the polyethoxy chain. Under reversed-phase conditions, however, either co-elution of the AE ethoxymers presenting the same alkyl chain length (so-called "homolog-by-homolog" separation) or separation according to both alkyl and polyethoxy chain length can be achieved, depending primarily on the nature of stationary and mobile phases, but also on the chemical structure of the AE derivatives. The homolog-by-homolog separation of AE derivatives has to be considered as the most suitable one for environmental analysis (Fig. 4B), since the elution of each individual AE according to both hydrophilic and hydrophobic moieties is feasible only for simple AE mixtures such as those shown in Fig. 5 [32,34–36]. It cannot be applied to the analysis of environmental samples where much more complex AE mixtures are expected.

The homolog-by-homolog separation was obtained for PIC [23], NIC [32,47] and 3,5-dinitrobenzoyl chloride derivatives of AE [35,46] by using C_{18}-bonded silica or polymer-based [46] stationary
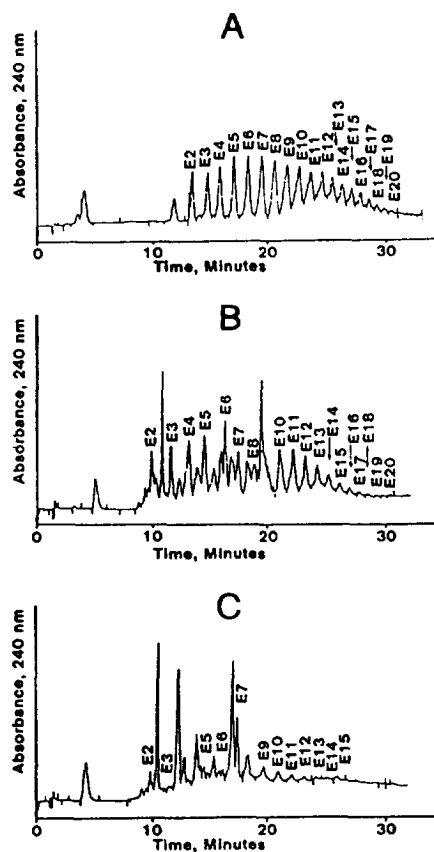

Fig. 3. Normal-phase separation of AE in a standard mixture (A) and raw (B) and treated (C) wastewater extracts after derivatisation by PIC [23].

phases and methanol–water, methanol–acetonitrile or acetonitrile–water mixtures as mobile phases. The homolog-by-homolog elution represents a particular chromatographic condition which can be reached more or less easily (when feasible) depending mainly on the mobile-phase components, and, to a lower extent, on the type of derivatising agent and alkyl-bonded stationary phase. Among the various solvents, methanol and dioxane were found to favour the co-elution of ethoxymers belonging to a given AE homolog [32,35], while acetonitrile [32,34–36,47], THF and, to a minor extent, acetone [35] enhance the effect of the ethoxylation degree on the differentiation of retention times. The latter were generally found to be inversely proportional to the ethoxylation degree [32,34–36], in the case of acetonitrile–water mobile phases. A gradual shift
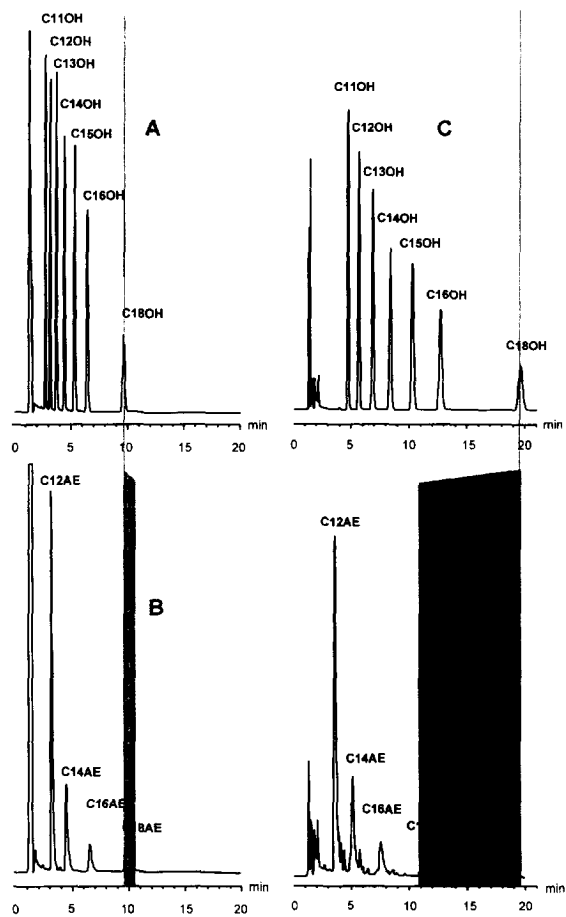
Fig. 4. Reversed-phase HPLC chromatograms of 1-naphthyl isocyanate (A, B) and 1-naphthoyl chloride (C, D) derivatives of a C_{11}–C_{18} linear primary alcohol standard mixture and a C_{12}–C_{18} linear primary AE oleochemical mixture, respectively. The shaded areas outline the elution region of the ethoxymers of C_{18}AE homolog derivatives. Stationary phase: C_{18}-bonded silica; mobile-phase: methanol–acetonitrile; detection: fluorescence [32].

towards the opposite behaviour was also observed for NIC derivatives of AE by progressively changing the content of the phase modifier [47]. The type of derivatising agent appears to affect remarkably the chromatographic behaviour of the AE adducts [32]. Fig. 4 shows the homolog-by-homolog separation of 1-naphthyl isocyanate and 1-naphthoyl chloride derivatives of a C_{12}–C_{18} AE commercial mixture under reversed-phase conditions. By using a C_{18}-bonded silica stationary phase and methanol–acetonitrile binary mixtures, only the 1-naphthyl iso-

cyanate derivatisation method allowed full homolog-by-homolog separation (Fig. 4B), as can be seen by comparison with the chromatogram of the corresponding alcohol derivatives (Fig. 4A). For the 1-naphthoyl chloride derivatives (Fig. 4C,D), a significant increase of the retention times of the lower ethoxymers (maximum for the alcohol) of each AE homolog could not be avoided.

2.1.3. HPLC coupled to mass spectrometric detection

The coupling of HPLC and mass spectrometric detection (HPLC–MS) allows the separation and detection of unaltered AE. Moreover, the MS operating in selected ion monitoring (SIM) mode does not require the optimization of homolog-by-homolog or ethoxymer-by-ethoxymer separations. The interfaces/ion sources most commonly used to introduce liquid flows into the MS analyser are: thermospray (TSP), frit-fast atom bombardment-secondary ion mass spectrometry (frit-FAB-SIMS) and electrospray (ESI). TSP operates at 0.5–1.0 ml/min and can be coupled to conventional HPLC systems, whereas frit-FAB-SIMS and ESI require lower flow-rates (1–50 µl/min) such as those typical of capillary HPLC or obtainable by splitting properly the mobile-phase flows from micro- or conventional HPLC columns.

Evans [25] proposed the following HPLC–TSP-MS-based method to determine AE in surface waters and effluents from sewage treatment plant in the concentration range 26–100 µg/l. After extraction/enrichment by SPE using octadecyl silica, extracts underwent HPLC separation on a C_{18} column. A continous post-column addition of aqueous ammonium acetate was done before TSP-MS. Individual AE were identified by both protonated molecular ions ($[M+H]^+$) and $[M+NH_4]^+$ adduct ions, and quantified by means of external calibration plots obtained by using standard solutions of a commercial mixture of AE. The sum of signal intensities of $[M+H]^+$ and $[M+NH_4]^+$ was considered for quantitation purposes. Aliquots of a C_{11}AE standard (lack of C_{11}AE from analysed samples was previously assessed) were added to both calibration standards and samples, and the signal intensities of each AE were normalized by the signal of the corresponding $C_{11}E_n$ ethoxymers. Such a procedure minimized the fluctuation of both the ionization efficiency and
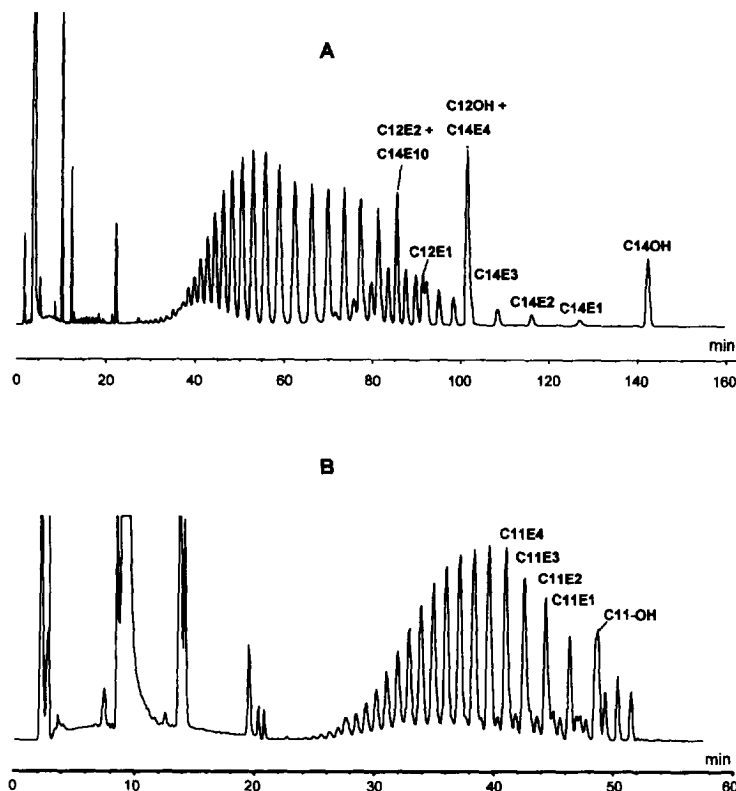
Fig. 5. Reversed-phase HPLC chromatograms of the 1-naphthoyl chloride derivatives of a C_{12}–C_{14} linear primary AE oleochemical mixture (A), and a C_{11}-oxo-AE containing ca. 50% 2-alkyl-branched isomers (B). Stationary phase: C_{18}-bonded silica; mobile-phase: acetonitrile–water gradient; detection: fluorescence [48].

response factors depending on the ethoxylic chain length. By this method, interfering compounds with the same m/z value and different isomers of the same AE may be distinguished, as inferrable from the chromatograms shown in Fig. 6.

In particular Fig. 6A, showing the $C_{13}E_n$ $[M+H]^+ + [M+NH_4]^+$ single ion chromatograms resulting from the analysis of a standard mixture of linear and branched $C_{13}AE$, highlights the capability to discriminate between linear and branched AE characterized by the same formula weight on the basis of the higher retention of linear alkyl chains under reversed-phase conditions. In Fig. 6B, the $[M+H]^+ + [M+NH_4]^+$ SIM chromatograms of $C_{12}E_n$ from an extract of sewage treatment plant (STP) effluent spiked with a C_{12}–$C_{15}AE$ mixture, clearly show the occurrence of interfering compounds in the profile of the $C_{12}E_2$ signal.

HPLC separation coupled to FAB-MS was used

[49,50] for the analysis of commercial AE blends and provided an identification of individual components similar to that obtained by HPLC–TSP-MS. Environmental applications of TSP-MS were proposed by Schröder [51,52] for the analysis of organic micropollutants, including AE and their metabolites, in aqueous samples. The comparison between the results obtained by a prior HPLC separation and those from the direct injection (FIA) [51–53] of the environmental extracts, led Schröder to conclude that the chromatographic separation does not contribute significantly to the information attainable by the direct introduction of the sample extract in FIA–TSP-MS. Also discontinuous ionization techniques such as desorption chemical ionization (DCI) exhibited sensitivity suitable for environmental determination of AE [54,55].

Electrospray interface (ESI), potentially quite powerful for determining polymeric substances, until
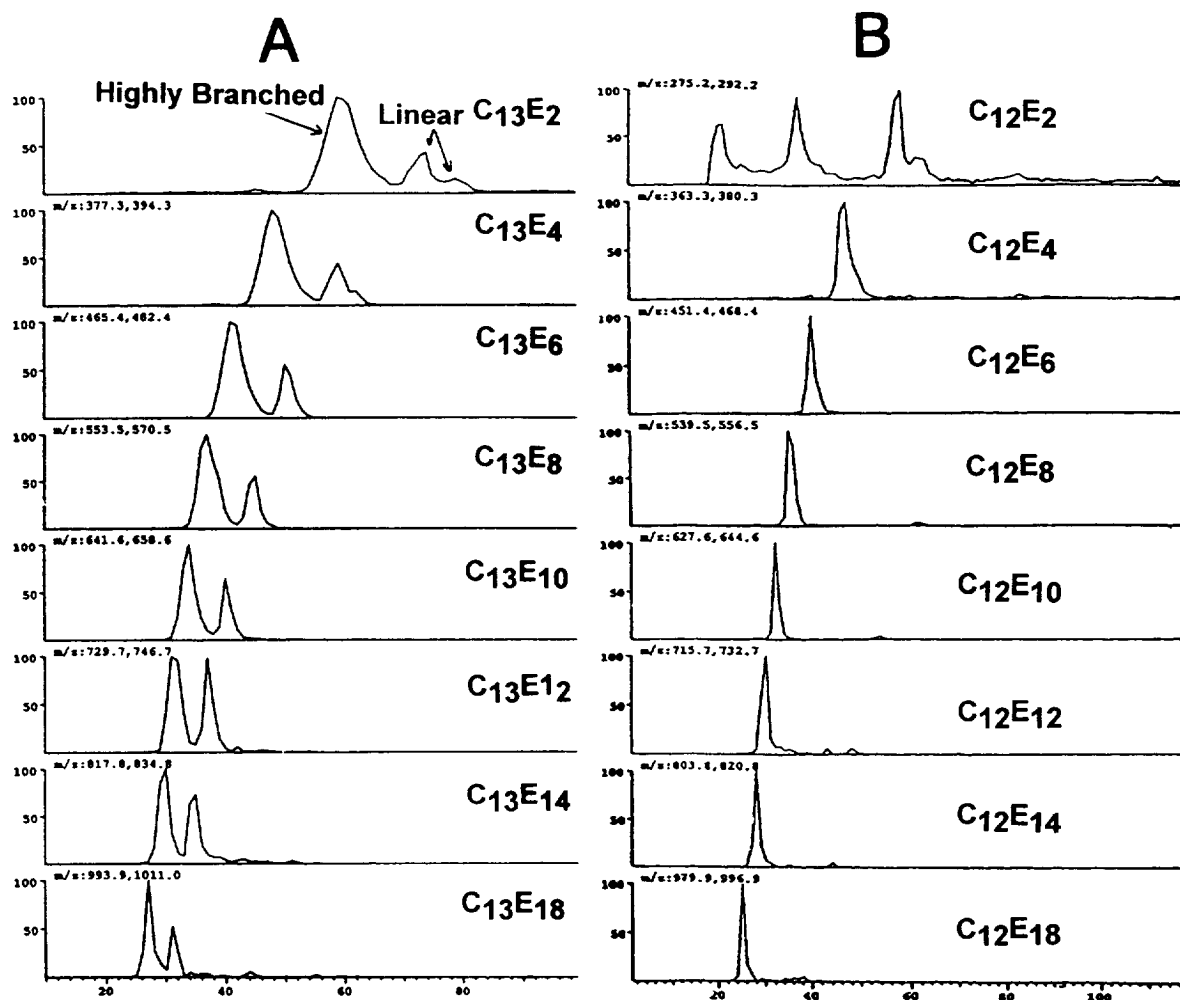
Fig. 6. TSP-MS chromatograms of a mixture of linear and branched $C_{13}AE$ (A) and of an extract from a STP effluent spiked with a $C_{12}-C_{15}AE$ mixture (B) obtained by selected ion monitoring (SIM) of $[M+H]^+ + [M+NH_4]^+$ ions of $C_{13}E_n$ and $C_{12}E_n$, respectively. Analytical conditions: see Fig. 7 in Ref. [25].

recently suffered from a lack of stability, signal reproducibility and reliability. However, newly designed ESI interfaces provided remarkably improved sensitivity, stability and control of operating conditions [20]. Neutral compounds can be detected in the positive ion mode, provided they are able to form stable ionic species by binding protons or inorganic cations. A former limitation of ESI interface was the that upper flow-rate could not exceed 10 μl/min. Pneumatically assisted electrospray [54] circumvented this limitation by facilitating the nebulization of the liquid effluent by a turbulent gas flow. Under

this condition, the ESI is capable of handling flow-rates up to 50–60 μl/min. The application of HPLC–ESI–MS to the determination of AE in influents and final effluents of sewage treatment plants, as well as environmental aqueous samples has been reported recently [5]. The column effluent was split and 40 μl/min (corresponding to 20% of the effluent) were diverted to the ESI and then to the MS operating in positive ion mode. The observed day-to-day fluctuations in the sensitivity of the MS detector were overcome by using $C_{10}E_6$ as an internal standard whose chromatographic peak was extracted

from the total ion current (TIC) chromatogram. Calibration curves for the various AE homologs were employed for the quantitation of AE in the environmental samples. The estimation of the linear dynamic range of the ESI-MS instrumentation is of basic importance for quantification purposes. In this study, a fairly good linear response was found for 1–20 ng of $C_{11}E_n$ injected into the column. The linear response was maintained even when lower amounts of $C_{11}-E_n$ down to the limit of detection were injected; partial saturation of the MS detector took place on injection of 400–500 ng $C_{11}E_n$. The detector response increased exponentially as the number of ethoxy units for each homolog increased from 1 to 6, and then did not increase significantly [5].

The extraction/enrichment of AE from the environmental aqueous samples involved the use of a GCB cartridge which, by taking advantage of the presence on its surface of positively charged active centres, allowed the fractionation of the complex mixture of the analytes by selective elution according to the differences in their acid strengths [56]. The AE components were fractionated together with the aromatic surfactant nonylphenol polyethoxylate (NPE). The homolog-by-homolog separation of the two surfactants was attained by the use of C_8-bonded silica as stationary phase and aqueous methanol as mobile phase. Besides simplifying the quantitation, by lumping all of the ethoxymers into a single peak, this method offered the advantage of increasing the peak intensity. This provided a means of measuring very low concentrations of AE such as those encountered in some types of environmental samples. The resulting limits of detection (signal-to-noise ratio 3) for each single homolog of AE as well as for NPE in influents and effluents of sewage treatment plants, river water, and drinking water, were estimated to be 0.6, 0.02, 0.002 and 0.0002 $\mu g/l$, respectively. The recoveries of AE from 10, 100, 1000 and 4000 ml of raw sewage, treated sewage, river water and drinking water after passing through 1 g GCB, ranged between 85 and 97%.

2.2. Gas chromatography

Although capillary column GC, compared to HPLC, in principle offers a higher resolution capa-

bility, its application to the separation of AE is severely limited by the low volatility of the higher molecular weight oligomers. Derivatisation is necessary to increase the volatility of these oligomers, but, to the best of our knowledge, only the silyl derivatives with up to 15–17 [21,26] or 20 [57] ethoxy units could be separated by GC. Moreover, the gas chromatographic separation, which is based essentially on the boiling temperature of the analytes, is unable to provide the useful homolog-by-homolog co-elution of AE attainable by HPLC. The poorly resolved GC separation of the AE derivatives, showing a high extent of peak overlapping, requires a mass spectrometer operating in SIM mode in order to obtain reliable quantitation.

The MS detection can be avoided if AE are altered by prior off-line HBr cleavage or on-line pyrolysis followed by GC separation.

2.2.1. GC after AE cleavage by HBr

The cleavage of the AE molecule by HBr [58–60] leads to the formation of the corresponding alkyl bromides and dibromoethane according to the following equation:

$$R^-[O^-CH_2^-CH_2^-]_n\,OH + 2n + 1\,HBr \overset{150°C}{\underset{3\,h}{\rightarrow}} R^-Br +$$

$$nC_2H_4Br_2 + n + 1H_2O$$
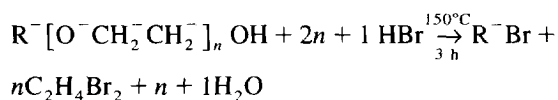
Comprehensive information on the homolog composition of analysed AE is attainable, while, out of the hydrophilic moiety, only the average degree of ethoxylation can be inferred from the concentration of dibromoethane. However, because the alkyl bromides and dibromoethane generated can also result from the reaction of HBr with fatty alcohols, poly-(ethylene glycol) species, monoethylene glycol, aromatic nonionics, polyethoxylated anionics, etc., environmental samples must undergo accurate clean-up in order to remove the interferences before the final reaction of AE with HBr.

The procedure proposed by Matthijs [30] consists of a series of liquid–liquid extractions, cationic and anionic exchange to remove anionic and cationic surfactants, respectively, clean-up on silica gel, HBr cleavage and extraction by CS_2 before the GC-FID analysis. The gas chromatograms obtained by applying this procedure to influent and effluent of a sewage treatment plant are presented in Fig. 7.
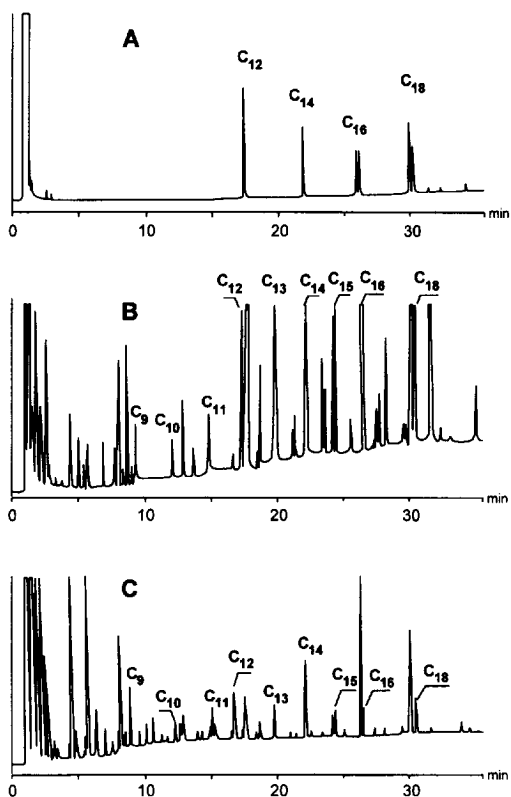
Fig. 7. Gas chromatograms of a standard C_{12}–C_{18} AE mixture (A) and of extracts of influent (B) and final effluent (C) of a sewage treatment plant after HBr cleavage [30].

The interlaboratory precision, in terms of relative reproducibility, for this procedure was reported to be in the 20–40% range. The accuracy, evaluated by the recovery of AE standard solutions, for raw and treated sewage, activated sludge and river water was 79, 94, 67 and 147%, respectively, by using the liquid–liquid extraction, 56–103% and 59–82% for treated and untreated sewage, respectively, after extraction by sublation. The limit of detection, in terms of amount of AE injected, was ca. 100 pg per homologue [30].

A similar method for determining AE in surface and wastewaters was recently proposed by Fendinger and co-workers [6]. AE are recovered from samples (50–200 ml aliquots) by SPE on C_1 stationary phase. After removal of potential ionic interferences by passing the extract trough strong anionic and cationic exchange columns, AE were reacted with HBr to form the corresponding alkyl bromide derivatives

that were analysed by GC coupled MS operating in SIM after electron impact (EI) ionization. Because the EI spectra for alkyl chain bromides are similar, the ions monitored for all chain lengths were 135 and 137. The former was used for quantification, the latter as a qualifier ion present at approximately the same abundance as the 135 ion. The minimum alkyl bromide detectable quantity with a 1 μl injection was ca. 500 pg for each homolog with a signal to noise ratio of ca. 5. The resulting chromatograms for all matrices were similar to those shown in Fig. 7. Recovery of AE from influent, treatment plant effluent and river water ranged from 78 to 102%. Concentrations less than 0.037 μg/l were measured in the receiving stream below the STP outfall.

2.2.2. High-temperature GC

The transformation of AE into the corresponding trimethylsilyl derivatives by reaction with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA), tri-methylchlorosilane (TMCS), 1,1,1,3,3,3-hexa-methyldisilazane (HMDS) [21,29,57] or into tert.-butyldimethylsilyl derivatives by N-(tert.-butyldimethylsilyl) - N - methyl - trifluoroacetamide (MTBSTFA) [26] allows the GC elution of AE with up to 20 ethoxy units even though temperatures up to 400°C are required. Such thermic conditions, well above the uppermost operating temperature of stationary phases (ca. 350°C), lead to fast deterioration of the columns. Thus, short columns (length 10 m) and high flow-rates of carrier gas (ca. 5 ml/min) have to be used to minimize the time of analysis in order to slow down the detriment of the column [26]. Detection by FID [12,21,57] is only feasible for simple AE mixtures which can be completely separated into their individual components by GC (i.e., characterization of commercial detergents or monitoring biodegradation experiments where selected AE are tested). The wide variety of AE potentially encounterable in environmental samples absolutely requires coupling GC with mass spectrometry detection. Detecting a huge number of different molecules needs ionization processes leading to little or no primary fragmentation since identification and quantitation based on molecular ions is highly preferable. Furthermore, commercially available GC–MS interfaces are not designed to operate at the high temperature required (400°C) and need proper ad-

justment [26]. Scheidegger determined AE in waste-waters and sludge from STP by means of GC–MS [26]. After recovery of AE by sublation (liquid samples) or by supercritical fluid extraction (solid samples) and removal of ionic interferences by cationic/anionic exchange resins, AE were silylated by MTBSTFA before undergoing GC–CI-MS. A significant reduction of AE fragmentation under chemical ionization (CI) mode was obtained by using ammonia as reagent gas, even though ionization efficiency and pathways were found to depend strongly on the operating conditions of the source. Still ionization efficiency was affected by the degree of ethoxylation of AE. A mixture of $C_{12}E_1-E_n$ ethoxymers characterized by fully deuterated poly-ethoxylic chain was added to the samples before analysis. Quantitation of each ethoxymer was based on the sum of $[M+H]^+$ and $[M+NH_4]^+$ signals, normalized by the corresponding signals of the deuterated $C_{12}E_n$ characterized by the same degree of ethoxylation. In Fig. 8 two chromatograms of the same STP influent extract obtained by FID (Fig. 8A) and CI-MS detection (TIC of $C_{12}E_0-C_{12}E_{15}$ [M+H]$^+$ and $[M+NH_4]^+$ ions, Fig. 8B) are reported, which well illustrate the insufficiency of FID as far as environmental samples are concern. MS detection

allowed the identification of all individual ethoxy-mers (up to 15 ethoxy units) of each AE homolog occurring in the sample (C_{12}AE in Fig. 8B), and to discriminate between linear (higher, sharp peaks) and branched isomers (the envelopes of shorter peaks eluting just before each linear isomer). Contrarily, non-specific detection by FID yielded very poor qualitative and quantitative information due to the extent of peak overlapping.

In contrast to the relevant capability to identify AE and despite of the refined quantitation procedure proposed, accurate and precise quantitation by CI-MS could not be obtained.

2.2.3. GC after pyrolysis

The coupling of pyrolysis and gas chromatography is a well-established hyphenated technique for the analysis of high-molecular-mass organic chemicals. It was applied also to AE [30,61] but the results obtained are far less useful than those attainable by the previous GC-based methods. The AE pyrolysis (600°C, in 10 s, under inert atmosphere) led to the formation of molecular fragments which provided a fingerprint upon separation through the GC column. Only spiking experiments were carried out which showed the possible identification of the AE
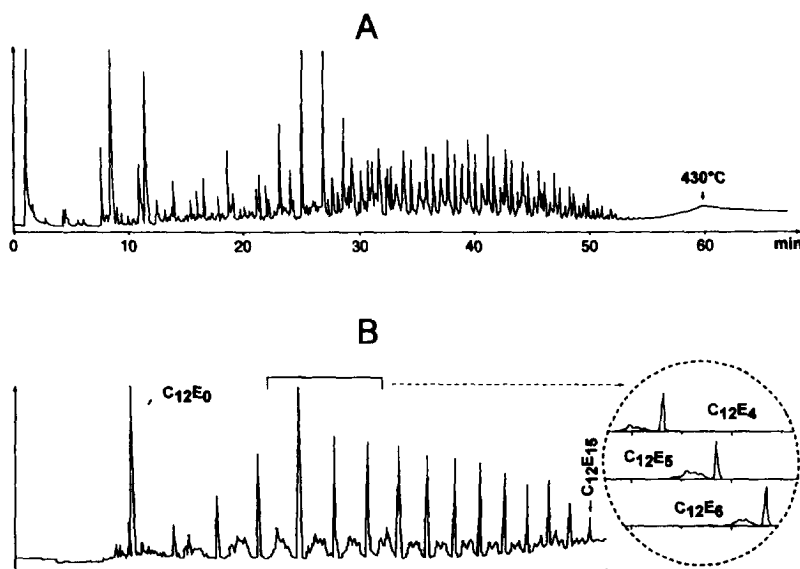

Fig. 8. Chromatograms of the extract of a sewage treatment plant influent obtained by HTGC–FID (A) and HTGC–MS (SIM: $[M+H]^+ + [M+NH_4]^+$) (B) [26].

homologs in aqueous samples (Fig. 9) [30]. No attempts were made, however, to identify and quantify AE in unspiked samples.

2.3. Supercritical fluid chromatography

Analysis of AE by SFC [17,21,57,62] does not require any derivatisation, since neither binding chromophoric groups nor increasing volatility are required. Attempts to separate AE mixtures or similar polyethoxylated/polypropoxylated compounds were done by both capillary [17,21,57] and packed SFC [62]. SFC shows separation capabilities very

similar or even superior to those of high temperature capillary GC (Fig. 10) [57]. An additional advantage of SFC over GC is that no limitations rise due to the low volatility of AE, and ethoxymers presenting more than 17–20 ethoxy units are not cut off. However, SFC seems to be able to furnish only "GC-like" separations of AE, not allowing "HPLC-like" co-elutions leading to homolog-by-homolog or the ethoxymer-by-ethoxymer separations. Thus SFC with non-specific FID detection is only suitable to analyse simple AE mixtures such as those occurring in detergent products. The significant peak overlap expected for AE from environmental matrices is
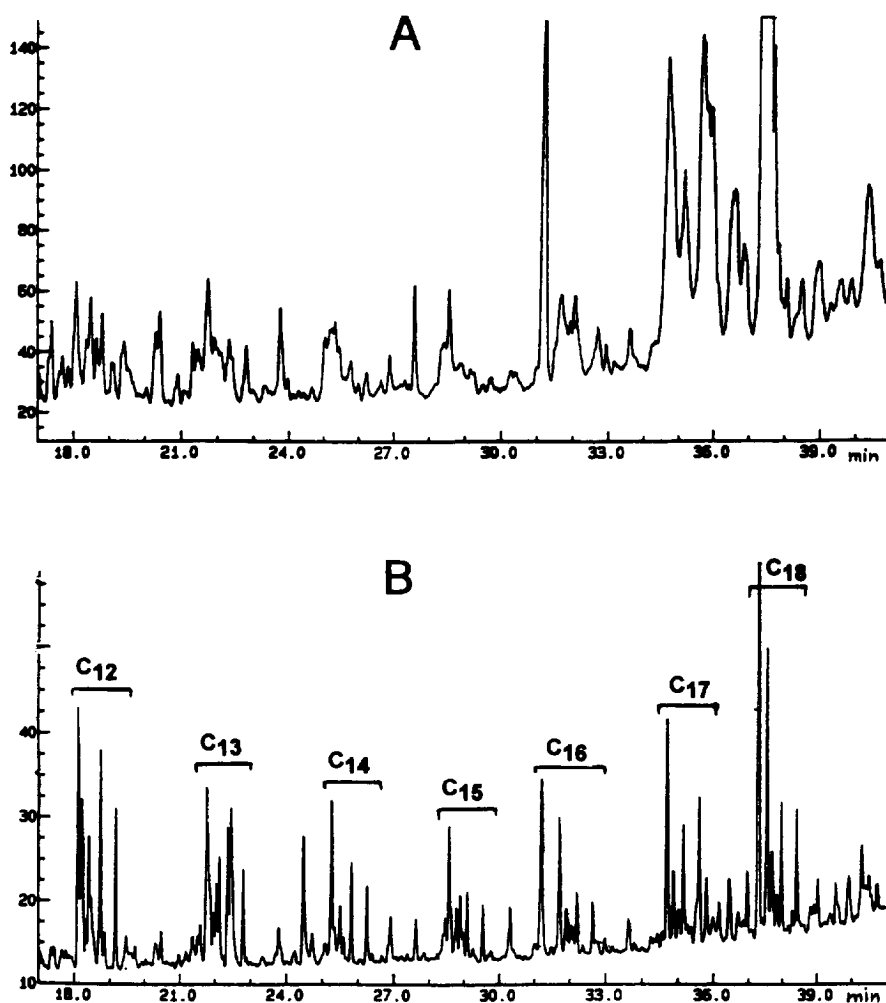

Fig. 9. Pyrograms of river water (A) and AE-spiked (40 µg/l) river water (B) extracts [30].
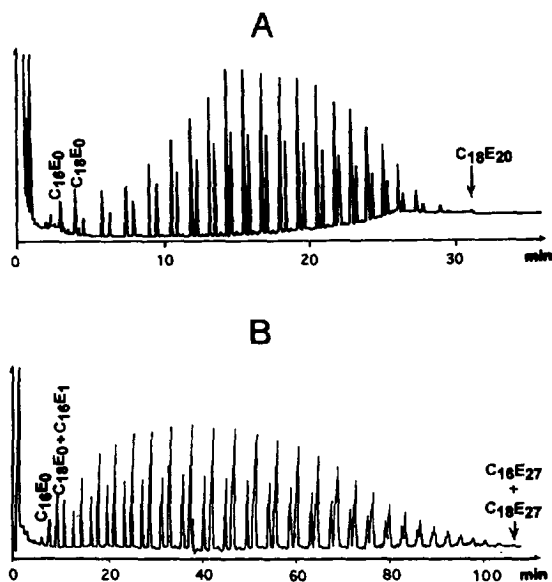
A

B

Fig. 10. GC–FID (A) and SFC–FID (B) chromatograms of a mixture of C_{16}–C_{18} AE (average ethoxy chain length: 11). Analytical conditions: (A) column, PS 090 (10 m×3 mm I.D., film thickness 0.2 μm); carrier gas, H_2 (5 ml/min); T program, from 100 to 390°C in 35 min; detector, FID (T 400°C); separation obtained after derivatization of AE with trimethylchlorosilane. (B) Column, Carbo-wax (10 m×50 μm I.D., film thickness 0.1 μm); supercritical fluid, CO_2; density program, from 0.15 to 0.73 g/ml in 100 min; T: 120°C; detector, FID (T 280°C) [57].

likely to prevent effective qualitative and quantitative results by FID detection.

Hyphenation of SFC and MS is still under development, and few commercial SFC–MS interfaces are currently available on the market. Recent efforts to adapt HPLC–MS atmospheric pressure interfaces (API) like FAB [62], ESI [63] and atmospheric pressure chemical ionization (APCI) [63,64] to couple packed or open tubular column SFC to MS led to significant improvement of MS detection, since pressure changes due to increasing volumetric flow during SFC separation exhibited little or no effect on API ionization processes [64]. In contrast, ionization by conventional reduced-pressure ion sources is affected by pressure fluctuations [65] and requires more complex adaptation. Although declared performance and sensitivity of SFC–MS would make this technique suitable for environmental analysis [66,67], very few applications regarding AE or similar polymeric compounds were found in the

literature [62,64,68], none of which concerning environmental samples.

3. Conclusions

The routine determination of AE in environmental samples can be, in principle, carried out either by HPLC or GC. Both techniques require modification of the AE molecules but the procedures based on HPLC appears more comprehensive, since separate information on the homolog and the ethoxymeric distributions can be obtained, whereas GC, after HBr cleavage, only provides the homolog-by-homolog separation. Moreover, the combination of HPLC with fluorescence detection ensures a simpler and faster sample handling compared with GC–FID.

Advanced, or confirmatory, environmental determination of AE is based on the coupling of GC and HPLC with mass spectrometry operating in selected ion monitoring mode. Only AE derivatives undergo GC–MS, and only the oligomers with up to 20 ethoxy units are amenable to GC separation. Vice versa, unaltered AE can be identified and quantified by HPLC–MS which greatly simplifies the sample preparation. However, the HPLC–ESI-MS response factors for non-ethoxylated alcohols as well as for mono- and diethoxylated AE which have been reported so far are very low compared with those of the remaining ethoxymers. The identification of suitable derivatising agents, yielding, with AE and alcohols electrically charged derivatives detectable by ESI-MS regardless the length of the polyethoxylic moiety, is highly desirable.

Several reliable extraction and enrichment procedures from both aqueous and solid environmental samples are available for AE but not for their biotransformation products such as poly(ethylene glycol). The foreseeable development of the methods for the environmental analysis of AE will include, in the same protocol, key biointermediates and other environmentally relevant surfactants.

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