Quantitative determination of octylphenol, nonylphenol, alkylphenol ethoxylates and alcohol ethoxylates by pressurized liquid extraction and liquid chromatography-mass spectrometry in soils treated with sewage sludges

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Abstract

Surfactants have one of the highest production rates of all organic chemicals. Non-ionic surfactants, especially alkylphenol ethoxylates, received most attention as precursors of estrogenic metabolic products generated during wastewater treatment. Alkylphenols (octyl and nonylphenol), alkylphenol polyethoxylates (APEOs), and alcohol ethoxylates (AEOs) have been determined in a Mediterranean forest soil (Mediterranean Rendzic Leptosol) amended with sludges from six waste water treatment plants (WWTPs) located in the Valencian Community. These compounds were isolated from soil by pressurized liquid extraction (PLE) using a mixture acetone-hexane (50:50 v/v), the extracts were cleaned up by solid-phase extraction (SPE) with C_{18}, and determined by liquid chromatography atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) using analytical standards for quantification. The method enabled high-reliable identification by monitoring the corresponding ammonium adduct $[M+NH_3]^+$ for AEOs and APEOs, and the deprotonated molecule $[M-H]^-$ for octyl and nonylphenol. Recoveries, determined spiking soil samples at different concentrations, ranged from 89 to 94 %, with limits of quantification from 1 to 100 µg kg^{-1}. Data obtained from a soil sample mixed with biosolids in the laboratory showed that these compounds are present at concentrations ranging from 0.02 to 5 mg kg^{-1}. According to these concentrations, levels of possible risk can be concluded for the presence of non-ionic surfactant in soil. However, further assessment will be necessary to establish the relation-ship between exposure and effect findings.

Keywords: Soil contamination, Amended soils, Surfactants, Non-ionic detergents, LC-APCI-MS.

1. Introduction

In the Mediterranean Europe one of the critical factors regarding soils is the decline of the organic matter content. It is mainly due to intensive agriculture and the increasing incidence of forest fires, under an extreme and changing climate. Most of the agricultural soils of this area are of the Cambisol, Calcisol and Regosol types (FAO-UNESCO, 1988). These soils, under the particular climatic characteristic of water stress of the Mediterranean, are usually poor in organic matter and show conditions, which are not very favourable for an acceptable productivity or profitability.

To mitigate this problem, the use of sewage sludge from wastewater treatment plants as organic amendment has become usual in Europe during the last decade. Sewage treatment plants in Europe produce each year eight tons of sludge. Applying sludge to soil as organic amendment is one of most common ways of getting rid of it. In 2005 up to 50%, 54%, 65% and 71% in Germany, Spain, France, and United Kingdom, respectively, was handled this way (Margoarou, 2000).

The composition of the sludge is, however, barely known, and it may contain chemical substances which are potentially toxic, like heavy metals and persistent organic chemicals such as various surfactants (Generalitat Valenciana, 1995; Generalitat Valenciana, 1998). At the European level, this fact increasingly raises concern (Directive EEC/86/278). These pollutants may have an adverse effect on the soil microbial communities, threaten ground water aquifers, and also alter the natural solubility equilibrium for clays by ion exchange and complexation mechanisms (Lee et al., 2002; Abu-Zreig et al., 1999; Adeel and Luthy, 1995; Rosen, 1989).

Surfactants are classified in different groups: anionics, non-ionics, cationics and zwitterionics. Non-ionic surfactants, such as nonylphenol ethoxilates (NPEOs) and AEOs, are

63 applied as detergents, emulsifiers, humidifiers, stabilizers, skimmers and intermediates in the
64 synthesis of a great variety of industries. Recent data reports about an annual production of
65 $800 \cdot 10^6$ kg of AEOs in Western Europe, indicating that they are still the most widely used
66 non-ionic surfactants (Castillo et al., 2000).

67 The European Commission (EU, 2000), elaborated a draft "Working Document on
68 Sludge" that proposes maximum levels for some micro-contaminants among which AEOs
69 and APEOs are present (the maximum value for their concentration in sludges for agricultural
70 use is of 50 mg kg^{-1} of dry matter, and includes the nonylphenol and nonylphenol ethoxylate
71 with one or two groups ethoxy).

72 There is a need of reliable tools for environmental monitoring as regards a series of
73 pollutants introduced by anthropogenic impacts. The chemical complexity of APEOs and
74 AEOs, which are mixtures of numerous isomers and oligomers, needs a quite sophisticated
75 analytical methodology for its isolation, identification and quantification in soils that,
76 generally, is based on their determination by gas chromatography-mass spectrometry (GC-
77 MS) or liquid chromatography- mass spectrometry (LC-MS) (la Guardia et al., 2001; Bruno
78 et al., 2002; Krogh et al., 2002; Krogh et al., 2003). The degradation products are
79 alkylphenols, alkylphenol monoethoxylates and alkylphenol diethoxylates, which are
80 recognized as endocrine disruptors (Magoarou, 2000).

81 The objective of this study is to develop and tune an analytical method, such that it is
82 sensitive enough for the simultaneous determination of octylphenol, nonylphenol,
83 alkylphenol ethoxylates and alcohol ethoxylates in soils treated with organic amendments.
84 Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), will
85 be used to identify unequivocally the compounds by means of LC-MS. Concentrations of
86 these types of compounds in samples from forest soils treated with organic amendments
87 coming from different WWTPs of the Valencian Community, are also compared.

2. Material and Methods

2.1. Reagents

Octylphenol (OP), nonylphenol (NP), Nonidet 40 ($NP_{6-15}EOs$), Triton X-100 ($OP_{6-15}EOs$), nonylphenol monoethoxylate (NP_1EO), the technical mixtures of nonylphenols ($NP_{1-5}EOs$) and octylphenols ($OP_{1-5}EOs$) with low ethoxylate grade, hexaethylene glycol monooctadecyl ether (C_8EO_6), hexaethylene glycol monodecyl ether ($C_{10}EO_6$), hexaethylene glycol monododecyl ether ($C_{12}EO_6$), hexaethylene glycol monotetradecyl ether ($C_{14}EO_6$) and hexaethylene glycol monohexadecyl ether ($C_{16}EO_6$) were provided by Aldrich (Madrid, Spain) and Symta (Madrid, Spain). Methanol and dichloromethane were HPLC grade and the deionised water was obtained with a MilliQ system. The solid phase used, C_{18}, was acquired from Análisis Vínicos (Tomelloso, Spain). Ammonium acetate and anhydrous sodium sulphate were of analytical grade.

2.2. Soils and sludges

Six samples of the superficial horizon (A) of a Mediterranean forest soil (Rendzic Leptosol) (FAO, 1988), developed on Jurassic limestone, were taken from a hillside degraded by forest fire and further erosion. The previous vegetation of the area belonged to a sparse Pine (*Pinus halepensis*) forest with a dense shrubland stratum characterized by *Rosmarinus officinalis*, *Ulex parviflorus, Quercus coccifera, Rhamnus lycioides, Stipa tenacissima*, *Globularia alypum*, *Cistus clusii* and *Thymus vulgaris* (Gimeno-García et al., 2000).

The soil had a total carbonate content of 45.2%, pH of 7.1 and a sandy-loam texture. Once in the laboratory the soil was left to dry at room temperature for 24 hours, passed through a 2 mm sieve, homogenised and stored in polyethylene boxes sealed until analysis. Laboratory standard analytical methods were applied for the determination of the most important physical and chemical characteristics of these samples.

Each soil sample was added 10% sewage sludge from a different WWTP and after 24 hours, analyzed. The selected WWTPs were located in distinct areas of the Valencian Community and used biological treatment. Samples 1-3 were added of sludges from WWTPs that receive mainly industrial effluents and samples 4-6 of sludges from WWTPs treating at equal domestic and industrial waste water (mainly from tanneries and textile industry).

2.3. Extraction

Five grams of soil were homogenized with anhydrous sodium sulphate, placed in a cylindrical cell (22 ml), and extracted using a Dionex Accelerated Solvent Extractor (Model 2000, Salt Lake City CA, USA). Each extraction began with 3 min preheating time, followed by 5 min static extraction with acetone-hexane (50:50 v/v). Static extraction was performed at constant temperature and pressure (60 ºC and 550 psi). Two extraction cycles were used. The 40 ml extract was evaporated to dryness using rotary evaporation, re-dissolved in 100 ml of water and extracted with a glass column that contained 500 mg of previously activated C_{18} with 10 ml of methanol and 10 ml of water. The retained surfactants were eluted with 10 ml of methanol. The extract was evaporated to 1 ml.

2.4. Liquid chromatography- mass spectrometry

LC-MS analyses were performed using an Agilent (Palo Alto, CA, USA) HP-1000 Series LC system consisting of an autosampler (volume injected was 25 µL) and a binary solvent pump, and a single quadrupole mass selective detector.

The chromatographic separation was carried out with a column Luna C_{18} (150 x 4.6 mm), 5 µm Phenomenex (Madrid, Spain), using a methanol-water gradient (both solvents contain 5 mM ammonium acetate to obtain the ammonium adduct) with a flow of mobile phase of 1 ml min^{-1}. The gradient begins with 70% methanol in water during 16 min and next the methanol percentage increases to 95% up to 30 min, and remains for 15 min more. Detection was

138 carried out using a mass spectrometer equipped with an APCI source. The conditions of the
139 APCI were for the alkylphenol ethoxylates and the alcohol ethoxylates: source in positive
140 ionization mode, capillary voltage of 4000 V, corona current 4 μA, fragmentor voltage 140
141 V, temperature of the APCI 400 ºC and, temperature and flow of the drying gas, 350 °C and 3
142 l min^{-1} respectively. Nonylphenol and octylphenol, were detected in negative ionization mode
143 and with corona current of 25 μA. The remainder conditions were identical.
144
145 **3. Results and Discussion**
146 *3.1. Extraction procedure*
147 For sample preparation, PLE followed SPE clean-up procedures have been developed and
148 optimised. Pressurized liquid extraction has already been successfully used for recovering
149 OPEAs, NPEOs and AEOs from soils and sludges (Petrovic et al. 2002; Krogh et al. 2003;
150 Loyo-Rosales et al. 2003). In this study, different extraction solvents (methanol,
151 dichloromethane and mixtures of hexane:acetone) have been tested. The recoveries obtained
152 varied from 65-126% for methanol; from 70-170% for dichloromethane, and from 89-102%
153 for the hexane/acetone 50:50 mixture. Therefore, the hexane:acetone 50:50 mixture was
154 chosen as extraction solvent.
155 Regarding the extraction conditions, Petrovic et al. (2002) reported about problems with
156 degradation at temperatures above 60 ºC, for APEOs and their degradation products. In
157 contrast, AEOs are fairly stable even at temperatures up to 150 ºC. For simultaneous
158 determination of the APEO and AEO, 60 ºC was used as extraction temperature. The
159 extraction pressure have no influence when the samples are dry (Petrovic et al. 2002). Thus
160 the pressure, the static time and the number of extraction cycles was chosen according to
161 Krogh et al. (2003).
162 *3.2. LC-MS analysis*

As can be observed in Figure 1, OP, NP, OPEOs, NPEOs and AEOs were well resolved using the chromatographic conditions previously described. Retention times were repeatable and reproducible. When the studied compounds were analyzed simultaneously, OP and OPEOs eluted earlier than the respective NP and NPEOs. However, the individual homologues of OPEOs and NPEOs are not resolved co-eluting in the same peak. In addition, co-elution of the AEO $C_{10}EO_6$ with the OPEOs was observed. In these cases, the compounds were distinguished based on their mass spectra. The various masses of the NPEOs are shown in the insert of Figure 1. The resulting mass spectra display a series of masses with 44 (-CH_2CH_2O-) mass units of difference between each compound making it possible to know the length of the ethoxylate chain. The same pattern was observed for OPEOs. On the contrary, AEOs showed mass spectra with 28 mass units of difference between each homologue.

Preliminary experiments were performed to differentiate between positive and negative ionization modes (PI or NI). The studied AEOs and APEOs gave response in PI but not in NI. In contrast, the OP and NP gave response only in NI mode providing the deprotonated molecule $[M\text{-}H]^-$ at m/z= 205.1 and 219.1, respectively.

The optimal ionization depends as well on the LC mobile phase composition. Addition of acetic acid and ammonium acetate to enhance the signal of the studied compounds was tested. Acetic acid in the mobile phase provided the highest intensities of the $[M+H]^+$ ion while ammonium acetate increased the intensity of the $[M+NH_3]^+$. Ammonium adducts are a good choice for quantitative analysis of APEOs (Cohen et al., 2001) and addition of low NH_4^+ concentrations to the mobile phase served to stabilize the adduct ion signals in PI, and did not adversely affect in NI. Therefore, the different compounds were identified by the presence of the adduct with ammonium $[M+NH_3]^+$ (AEOs and APEOs) or the deprotonated molecule $[M\text{-}H]^-$ (OP and NP).

187 For AEOs and APEOs detection, some studies utilise electrospray ionisation (ESI)
188 (Ferguson et al., 2000; Cohen et al., 2001) while others use atmospheric pressure chemical
189 ionization (APCI) (Krogh et al., 2002). In this study, both ESI and APCI, in negative and
190 positive ion mode, have been investigated. The different instrumental settings, such as
191 capillary and vaporiser temperature, corona current, sheath and auxiliary gas for APCI and
192 spray voltage, sheath and auxiliary gas for ESI, were optimised; APCI and ESI in the positive
193 ion mode gave similar signals. However, lower matrix and more stable analyte response were
194 observed using APCI, which was the preferred ionisation mode.

195 *3.3. Analytical parameters*

196 The analytical parameters of the method are listed in Table 1. For the chromatographic
197 procedures a linear relation is observed. This linearity was evaluated by analyzing standard
198 solutions in triplicate at six different concentration levels, in the range from the LOQ to
199 hundred times the LOQ (although the linearity interval is wider, it is difficult to find real
200 samples with higher content of surfactants). The correlation coefficient was in all cases
201 >0.994.

202 The limits of detection (LODs) were calculated by using a signal-to-noise ratio (S/N) of
203 3. The LODs were in the range of 0.3 to 1 μg kg^{-1} for the OPEOs and NPEOs; 3 μg kg^{-1} for
204 the AEOs and 30 μg kg^{-1} for the OP and NP. These values are in agreement with those
205 reported in the literature (Cohen et al., 2001). The LOD for each individual compounds in
206 wastewater were typically 1 μg l^{-1}, whereas the detection limits in sludge samples were
207 typically 100 μg kg^{-1}, because it is a more complicated matrix. The limits of quantification
208 (LOQs) were estimated by using a S/N of 10. The analytical method was validated at LOQs
209 level and 10 times LOQ level (data not shown).

210 In validating the analytical method, concerning recovery and precision three sets of three
211 replicated samples were extracted at three different days and subsequently analysed at

212 different days. The results are shown in Table 1. Satisfactory recoveries were achieved for all
213 target analytes ranging from 89 to 102% with RSDs ≤ 20%.

214 Figure 1, shows the chromatograms obtained injecting extracts of a soil that does not
215 contain surfactants, and the same soil spiked at 0.1 mg kg^{-1} with the different surfactants. Soil
216 extracts are clean without interfering substances. In the same Figure, the mass spectrum
217 corresponding to the studied nonylphenol ethoxylates (n=1-15) is inserted. The current
218 method provides the highest available sensitivity for comprehensive AEOs and APEOs
219 analysis in environmental samples. This sensitivity was more than sufficient for the
220 determination of AEOs and APEOs in sewage amended soils.

221 *3.4. Analysed surfactant concentrations in soil samples treated with sludge*

222 The method was applied to the determination of NP, OP, AEOs and APEOs in soil treated
223 with sludges from six WWTPs of the Valencian Community. Results presented in Table 2
224 demonstrate that these compounds are incorporated in the treated soils. In all of them, the
225 presence of OP, NP and their ethoxylates was detected in significant amounts. The presence
226 of AEOs is scarce, although it is also detected in two samples. The homologues found in the
227 samples were all included in the present study. The most abundant compound present in soil
228 was NP, which agrees with previous findings in previous studies (Loyo-Rosales et al., 2003).
229 The octylphenol ethoxylate and nonyl ethoxylate homologues found in the samples have been
230 mainly mono-, di, tri-, tetra-, and pentaethoxylates. However, in sample S3 the entire pattern
231 of homologues selected for the method validation was found. These results are in agreement
232 with the proposed degradation pathway of these surfactants, which is through the breakdown
233 of the ethoxylate groups (Petrovic et al., 2002; Loyo-Rosales et al., 2003). AEOs and APEOs
234 concentrations detected in the soil treated with the sludges are always lower than 1 mg kg^{-1},
235 which evidence their low persistence in the environment.

236

4. Conclusions

The method presented in this work is capable of analysing a wide range of octylphenol polyethoxylates, nonylphenol polyethoxylates and alcohol polyethoxylates in samples of soils treated with sludges from WWTPs. The determination of neutral surfactants by PLE, SPE and LC-MS requires a small amount of sample, provides satisfactory recoveries, repeatability and reproducibility, and is a sensitive, selective and reliable analytical method.

The application of the reported method to soil treated with organic amendments demonstrates that the presence of these compounds may present a risk in agricultural and restoration zones. Nonylphenol, octylphenol, and their mono-, di-, tri-, tetra-, and pentaethoxylates were detected in significant amounts. Although less frequent, highly ethoxylate NPs and OPs as well as AEOs were also found in some samples. Further studies are needed to establish to which extent these surfactant residues pose a risk to soil functions and its macro and micro-fauna.

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310

311 Table 1. Mean Recoveries (R, %), relative standard deviation (RSDs,%), linear concentration
312 range, correlation coefficient (*r*) , and limit of detection (LODs) of the different alcohol
313 ethoxylates (AEOs) obtained for spiked soil samples (n=3).

	Recovery and precision			Linearity		LODs ($\mu g\ kg^{-1}$)
AEOs	Concentration ($mg\ kg^{-1}$)	R (%)	RSDs (%)	Range ($mg\ kg^{-1}$)	*r*	
C_8EO_6	0.010	90	16	0.01-1	0.997	3.0
$C_{10}EO_6$	0.010	94	14	0.01-7.4	0.999	3.0
$C_{12}EO_6$	0.010	91	14	0.01-1.0	0.999	3.0
$C_{14}EO_6$	0.010	96	12	0.01-1.0	0.998	3.0
$C_{16}EO_6$	0.010	96	12	0.01-1.0	0.998	3.0
NP	0.100	97	19	0.1-10	0.997	30.0
NP_1EO	0.010	89	13	0.01-1.0	0.998	3.0
NP_2EO	0.005	90	15	0.005-0.5	0.997	1.0
NP_3EO	0.005	94	12	0.005-0.5	0.999	1.0
NP_4EO	0.005	91	17	0.005-0.5	0.999	1.0
NP_5EO	0.005	89	13	0.005-0.5	0.994	1.0
NP_6EO	0.005	92	13	0.005-0.5	0.997	1.0
NP_7EO	0.001	89	15	0.001-0.1	0.998	0.3
NP_8EO	0.001	90	16	0.001-0.1	0.997	0.3
NP_9EO	0.001	94	15	0.001-0.1	0.999	0.3
$NP_{10}EO$	0.001	91	14	0.001-0.1	0.999	0.3
$NP_{11}EO$	0.001	95	10	0.001-0.1	0.995	0.3
$NP_{12}EO$	0.001	94	9	0.001-0.1	0.998	0.3
$NP_{13}EO$	0.001	89	19	0.001-0.1	0.998	0.3
$NP_{14}EO$	0.001	90	16	0.001-0.1	0.997	0.3
$NP_{15}EO$	0.001	94	17	0.001-0.1	0.999	0.3
OP	0.100	91	15	0.1-10	0.999	30.0
OP_1EO	0.010	89	18	0.01-1.0	0.998	3.0
OP_2EO	0.005	90	17	0.005-0.5	0.997	1.0
OP_3EO	0.005	94	12	0.005-0.5	0.999	1.0
OP_4EO	0.005	91	12	0.005-0.5	0.999	1.0
OP_5EO	0.005	97	11	0.005-0.5	0.994	1.0
OP_6EO	0.005	99	12	0.005-0.5	0.996	1.0
OP_7EO	0.001	89	19	0.001-0.1	0.998	0.3
OP_8EO	0.001	90	16	0.001-0.1	0.997	0.3
OP_9EO	0.001	94	14	0.001-0.1	0.999	0.3
$OP_{10}EO$	0.001	91	14	0.001-0.1	0.999	0.3
$OP_{11}EO$	0.001	102	11	0.001-0.1	0.997	0.3
$OP_{12}EO$	0.001	99	10	0.001-0.1	0.996	0.3
$OP_{13}EO$	0.001	89	19	0.001-0.1	0.998	0.3
$OP_{14}EO$	0.001	90	16	0.001-0.1	0.997	0.3
$OP_{15}EO$	0.001	94	14	0.001-0.1	0.999	0.3

314
315

316 Table 2. Concentration (μg kg^{-1}) of the studied compounds in soil amended with the different

317 sewage sludges.

318

Soil	OP	NP	OPEOs	NPEOs	AEOs
S1	238	500	369	200	n.d.
S2	215	200	232	149	152
S3	105	300	125	329	n.d
S4	125	150	124	134	37
S5	135	142	232	210	n.d.
S6	210	225	87	92	n.d.

319

320 OP: Octyl phenol. NP: Nonyl phenol. OPEOs: Octylphenol ethoxylates. NPEOs:

321 Nonylphenol ethoxylates. AEOs: Alcohol ethoxylates. n.d.: not detected.

322

Legend of Figure:

Fig.1. Chromatograms of (A) soil in which surfactants were not detected and (B) the same soil fortified with a mixture of the different NPEOs, OPEOs and AEOs at 0.1 mg kg^{-1}. The mass spectrum corresponding to the NPEOs is shown as an insert.

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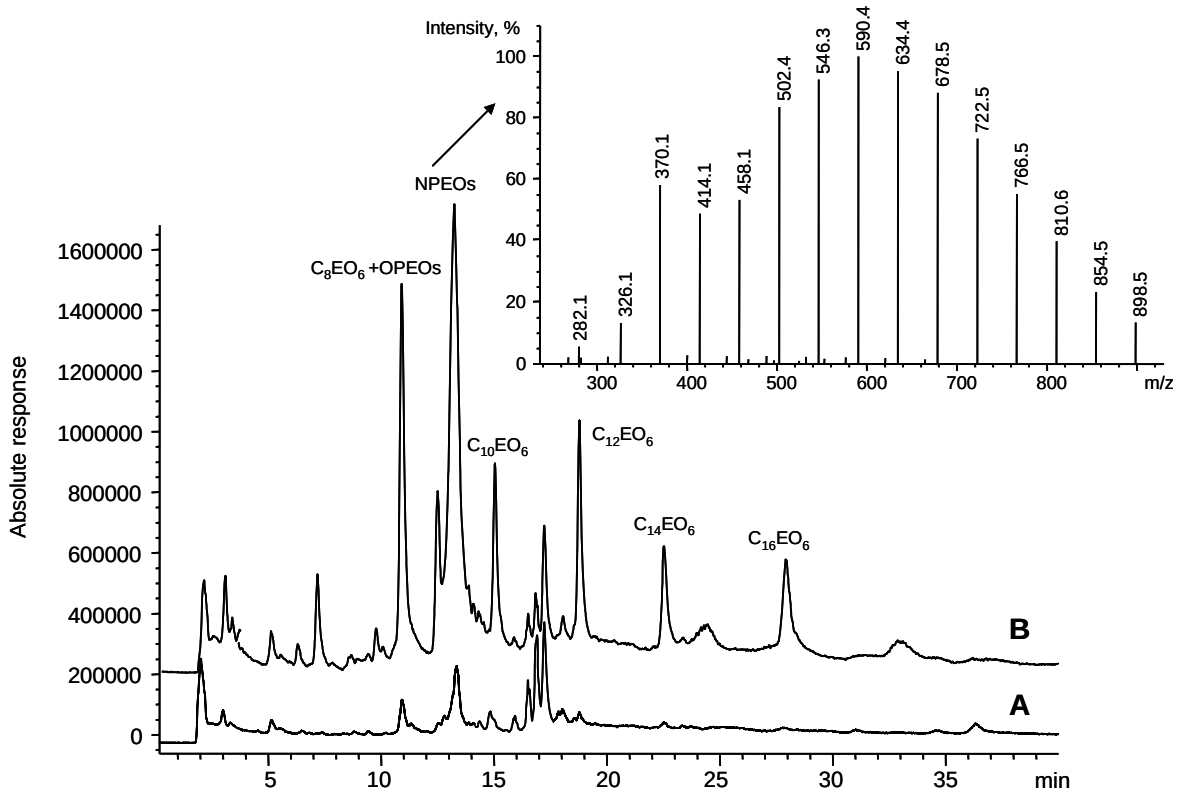
Intensity, %
NPEOs
282.1
326.1
370.1
414.1
458.1
502.4
546.3
590.4
634.4
678.5
722.5
766.5
810.6
854.5
898.5
m/z
Absolute response
C8EO6 +OPEOs
C10EO6
C12EO6
C14EO6
C16EO6
B
A
min