

ENVIRONMENTAL ANALYSIS

EVALUATION OF AN ACCURATE-MASS QUADRUPOLE TIME-OF-FLIGHT (Q-TOF) LC/MS SYSTEM FOR THE DETERMINATION OF TRACE-LEVEL NONYLPHENOL POLYETHOXYLATES IN EFFLUENTS FROM NON-ACTIVATED SLUDGE BIOFILM REACTORS



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ABSTRACT

The ability of a contemporary accurate-mass Q-TOF instrument coupled to a UHPLC to detect trace levels of nonylphenol polyethoxylates (NPEOs) in complex wastewaters has been investigated. NPEOs are environmentally hazardous substances that affect aquatic organisms. The study was motivated by the “confirmation of contaminants” concept, which is based on the use of identification points (IPs) as proposed in the European Commission Guidelines (EU Commission Decision 2002/657/EC) for the identification and qualification of organic residues and contaminants.



INTRODUCTION

Alkylphenol polyethoxylates (APEOs) are widely used as non-ionic surfactants in industrial formulations in the textiles, tannery, paper and metal working industries. They include octylphenol polyethoxylates (OPEOs), nonylphenol polyethoxylates (NPEOs) and dinonylphenol polyethoxylates (DNPEOs) (1). APEOs are degraded in wastewater treatment plants (WWTPs) and in the environment (rivers, lakes) leading to the loss of their ethoxy (EO) groups (2). Metabolites originating from OPEOs and NPEOs can mimic natural hormones by interacting with estrogen receptors in aquatic organisms (3).

Commercial APEOs usually contain between 1 and 23 EO-units and may have different degrees of branching in their alkyl moieties. APEOs are banned or restricted in Europe. Two of their degradation products, nonylphenol (NP) and octylphenol (OP), are included in the EU's Water Framework Directive (decision 2455/2001/CE) as priority hazardous substances.



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Gas chromatography mass spectrometry (GC-MS) is widely used for the analysis of APEOs with short ethoxy chains such as AP, AP1EO and AP2EO. However, the limited volatility of APEOs with longer ethoxy chains means they are not readily amenable to GC-MS analysis even when derivatization procedures are used. A HBr fission procedure has been developed that hydrolyzes NPEOs and DNPEOs to NP and DNP respectively, thereby facilitating GC-MS analysis of APEOs with chains having > 2 ethoxy units (4).

Liquid chromatography mass spectrometry (LC-MS) is regarded as the analytical technique of choice for the analysis of APEOs. Electrospray ionization (ESI) is preferred to atmospheric pressure chemical ionization (APCI) as it often provides better sensitivity (5,6), although some authors recommend APCI as it is less sensitive to matrix interference (5,7). Reversed-phase liquid chromatography (RP-LC) is commonly used but is incapable of separating oligomers with different numbers of EO-units that can be separated using normal phase liquid chromatography (5). Thus, if RP-LC is used, APEOs are eluted in single peaks. This facilitates the application of integration procedures in LC-UV and LC-fluorescence detector based methods (1, 8). Ultra-high performance liquid chromatography (UHPLC) using columns with sub-2 μ m particles is often recommended for environmental analysis as the analytes elute in narrow concentrated bands, providing superior peak resolution and peak capacity (9). Moreover, these methods use smaller volumes of the organic mobile phase than the alternatives as they have lower flow rates than HPLC. MS analyses of APEOs are typically performed using single quadrupole (Q) or triple quadrupole (QqQ) mass analyzers. However, other instrument types have also been used, including time of flight (TOF) and ion trap (IT) analyzers (5, 10).

In this work, we investigated the capability of an Agilent 6550 iFunnel Q-TOF LC/MS system combined with a 1290 Infinity UHPLC to detect NPEOs at sub- μ g/L concentrations in sludge-containing wastewaters. The use of high resolution LC-MS in the analysis of harmful organic pollutants is motivated by the "confirmation of contaminants" concept, which is based on the use of identification points (IPs) as proposed by the European Commission Guidelines (EU Commission Decision 2002/657/EC) for the identification and qualification of organic residues and contaminants. The decision proposes a system of IPs, whereby four IPs are required to confirm a positive finding. When using low resolution MS instruments, it is necessary to monitor four ions (each of which is "worth" one IP) or one precursor ion and two product ions (using low resolution MS/MS instruments); each product ion is worth 1.5 IPs (10). If using a high resolution MS (HRMS) instrument, each precursor ion is worth 2 IPs and the product ions 2.5 IPs. HRMS is therefore the preferred technique. As QqQ instruments provide more sensitivity in quantitative applications than alternative MS technologies (11), the aim of this work was to determine whether the 6550 Q-TOF system could identify NPEO precursor ions and fragment them (MS/MS) at sub- μ g/L concentrations in the presence of a complex matrix. NPEOs in textile water have previously been quantitated using a Q-TOF LC/MS system coupled to a UHPLC with an ESI interface (12), with measured concentrations in the range of 0.93 – 5.68 mg/L. In addition, the precision, sensitivity, mass accuracy, and linearity of the system were also investigated, as well as the occurrence of ion suppression effects (13) and their potential impact on the observed EO distribution.

ANALYTICAL TECHNIQUE

Chemicals and Samples: 4-Nonylphenol-polyethylene glycol with an average of 10 EO-units (NP10EO) was purchased from Fluka, Switzerland. Milli-Q™ water from a Milli-Q Plus water filtration device (Millipore Corporation, USA) was used for sample preparation. The mobile phases were prepared with LC-MS grade water and acetonitrile (ACN) from JT Baker, USA and ammonium acetate LC-MS Ultra, ≥ 99.0% from Fluka, Switzerland.

Two samples of effluent from non-activated sludge biofilm reactors at GE Healthcare, Uppsala (2012-10-19, 2013-04-25) were analyzed.

Standard Preparation: NP10EO was dissolved in Milli-Q water to a concentration of 5.00 mg/L. Standards with concentrations of 1.00, 0.05, 0.01 and 0.005 mg/L were then prepared by further dilution.

Sample Preparation: Lyophilized sludge-containing water was extracted with ACN using pressurized fluid extraction (PFE). After SpeedVac™ evaporation, amino acids and peptides were removed by strong cation exchange (SCX). Finally, the samples were again evaporated with the SpeedVac and diluted to 1 mL with ACN (1).

Q-TOF LC/MS Operating Conditions

UHPLC Conditions	Agilent 1290 Infinity Binary LC System	
Column	ACE Excel 2 Phenyl, 150 x 2.1 mm id, 2 µm particles	
Injection Volume	7.4 µL	
Mobile Phase Solution A	Milli-Q water with 2 mM ammonium acetate*	
Mobile Phase Solution B	Acetonitrile	
Mobile Phase Gradient	Time (mins)	% B
	0	40
	3.2	64
	12.0	97
	15.33	97
	15.4	40
	23.4	40
Flow Rate	0.44 mL/min	
MS Conditions	Agilent 6550 Q-TOF LC/MS System	
Ionization Mode	Positive	
m/z range	100 - 1700	
Lock Masses	m/z 121.05087300 and m/z 922.00979800	
Quadrupole Isolation Window	Narrow, corresponding to an isolation width of ~ 1.3m/z	

The UHPLC-method parameters (gradient, injection volume and flow-rate) were recalculated from an existing HPLC-FLD-method (1) that uses an ACE 3 Phenyl column with 150 x 3 mm id and 3 µm particles.

*Initially, mobile phase A contained 0.1% formic acid. However, ammonium acetate was preferred as an additive because some of the target compounds showed a strong tendency to form ammonium adducts (see Results and Discussion).

Analyses were performed using the methodology outlined in Figure 1.

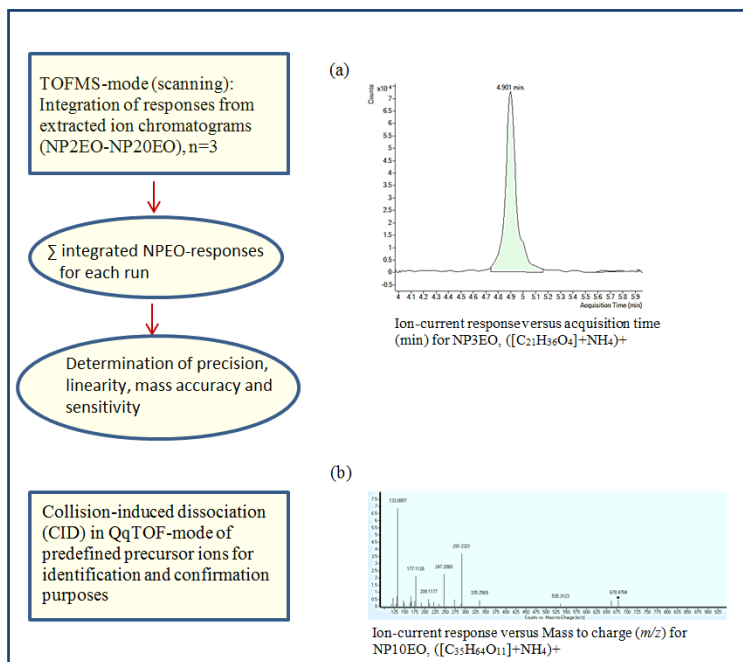


Figure 1. Methodology used to evaluate the Agilent 6550 Q-TOF LC/MS for the analysis of NPEOs when performing quantitation and identification using (a) TOFMS-mode and (b) QqTOF-mode, respectively.

RESULTS AND DISCUSSION

Choice of Polarity and Counter-ions: NPEOs with between 2 and 19 ethoxy units were successfully detected in all of the standard solution runs using positive mode. NP20EO was detected in two of the three replicates containing 0.005 mg NPEO/L. NP could only be detected in negative ion mode at high concentrations (1 and 5 mg/L). NP1EO could not be detected in either negative or positive ion mode. $[M+H]^+$, $[M+Na]^+$ and $[M+NH_4]^+$ ions were all detected. The protonated species were the least abundant ions and for some ethoxymers, no protonated ions could be detected. Sodium adducts were the most abundant species for NP2EO, NP3EO and NP4EO. For NP5EO, the abundance of ammonium and sodium adducts was almost equal. From NP6EO to NP20EO, the ammonium adducts were the most abundant species. Figure 2 shows the mean ethoxymer distributions (n = 3) for 0.005 and 5 mg NPEO/L standard solutions based on results for the corresponding $[M+NH_4]^+$ ions. There are some notable differences in the ethoxymer distributions for these species. In particular, NPEOs with low degrees of ethoxylation are more abundant in the low concentration standard, whereas those with higher degrees of ethoxylation are more abundant in the 5 mg NPEO/L standard.

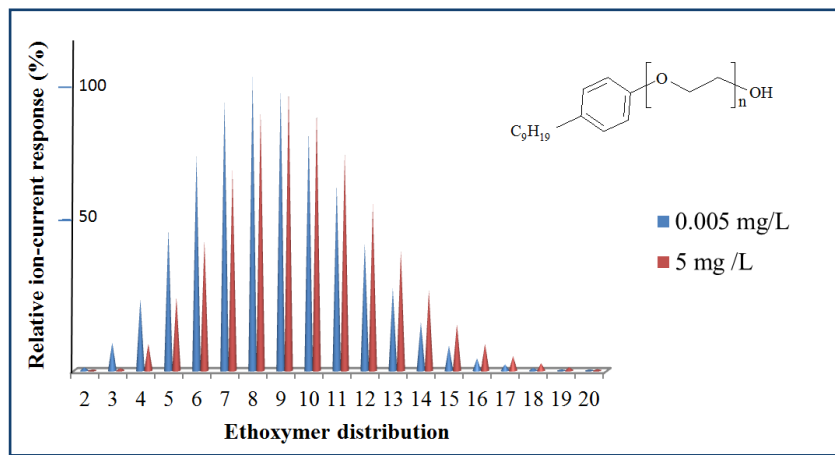


Figure 2. Ethoxymer distribution in two NPEO standard solutions containing a broad range of ethoxymers. $[M+NH_4]^+$ ion responses are shown.

Precision: The NPEO standard solutions were injected in triplicate and the ion responses for each ethoximer were integrated and summed (see Figure 1). Mass selection was facilitated by comparison to tables of exact masses of the compounds of interest. All of the tested ethoxymers (NP2EO-NP20EO) were present in sample 2012-10-19. The summed ion response for this sample was lower than that for the 0.005 mg NPEO/L sample (see Figure 3).

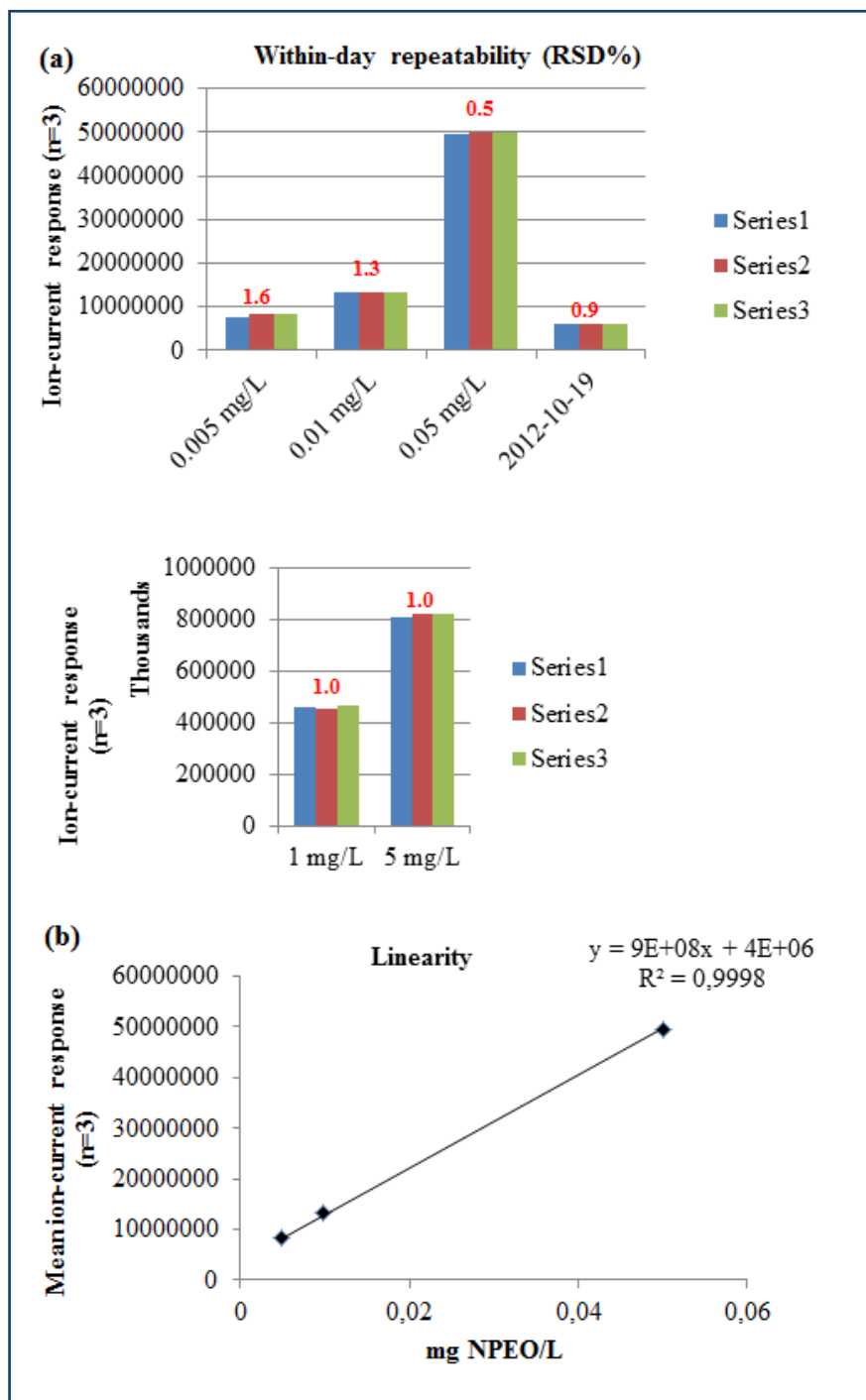


Figure 3. Precision (within-day repeatability) for NPEO standard solutions and sample 2012-10-19 (a) and linearity determination (b).

Linearity: The linearity of the mean responses for the three lowest standard concentrations was excellent (see Figure 3). However, the response over the entire studied concentration range (0.005 – 5 mg NPEO/L) was less linear, indicating a saturation effect. However, this is not considered a problem in the analytical application because the samples from the biofilm reactor effluents are typically expected to have NPEO concentrations of less than 0.01 mg/L.

Mass Accuracy: The following definition of mass accuracy was used:

$$\text{Mass accuracy (ppm)} = \frac{(\text{mass error})^*}{(\text{exact mass})} \times 10^6$$

*mass error = exact mass – accurate mass (from the analysis)

The mass accuracy was determined using the 0.05 mg NPEO/L standard. For all ethoxymers replicates (n=3), the mass accuracy was within 0.1 – 2.4 ppm when using the specified lock-masses.

Sensitivity: For the TOFMS runs, the within-day repeatability for the summed integrated ion-response obtained for sample 2012-10-19 was comparable to those for the standards. The tested method can thus be used to quantitate NPEOs at sub- $\mu\text{g/L}$ concentration levels without any loss of precision. In fact, it is possible to quantitate individual ethoxymers (NP2EO-NP20EO) that are present at ng/L concentration levels, with repeatabilities that are typically below 5% RSD, provided that an appropriate calibration method is used (see Summary and Conclusions).

Identification and Confirmation: The predefined precursor ions were fragmented by collision-induced dissociation (CID) and the fragment ions were recorded for the standards and sample 2012-10-19. Figure 4 shows some of the fragment ions including their chemical structures and exact masses.

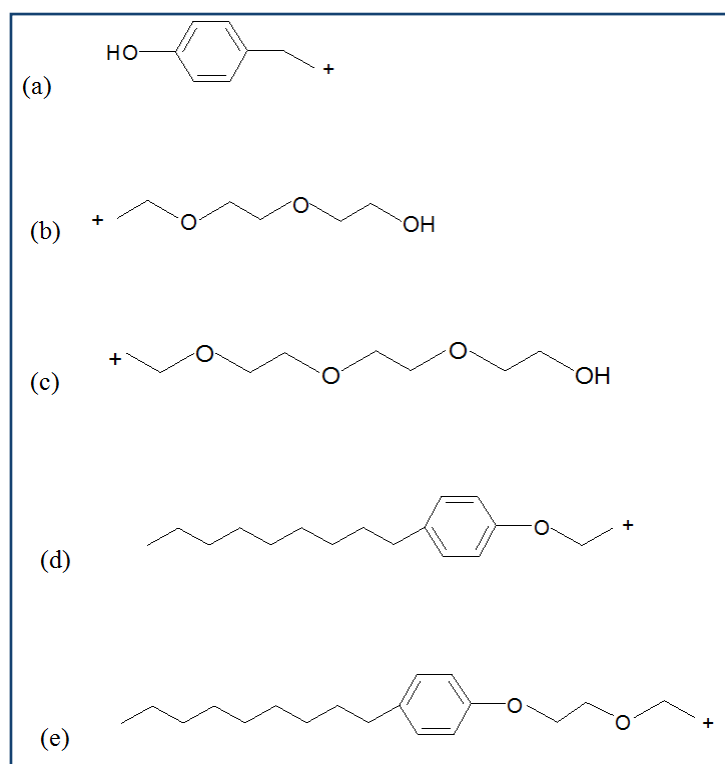


Figure 4. Common NPEO-fragment ions. Exact masses (m/z): (a): 121.0642, (b): 133.0858, (c): 177.1127, (d): 247.2053, (e): 291.2314

In the samples, fragments derived from the entire range of ethoxymers (NP2EO-NP20EO) were successfully detected. The observed fragmentation patterns depended on the degree of ethoxylation of the parent compound.

Ion-suppression Effects: Even though the effluent samples were extensively cleaned up prior to analysis, it was suspected that the complex matrix might cause some ion-suppression effects. Therefore, a minor investigation was performed using another sample (2013-04-25) that was analyzed without dilution and at dilutions of 1:5 and 1:10 by volume in Milli-Q water following the procedure outlined in Figure 1(a). The mean summed integrated ion-responses for the 1:5 and 1:10 dilutions were multiplied by factors of 5 and 10 respectively, to enable comparison of the results. The ion-response was found to increase with the dilution of the sample: the responses for the 1:5 and 1:10 dilutions were 3.4 and 5.9 times stronger respectively, than that for the undiluted sample. This indicates that the sample matrix caused ion-suppression during the analysis. As shown in Figure 5, the severity of this effect varied among the different NPEO ethoxymers.

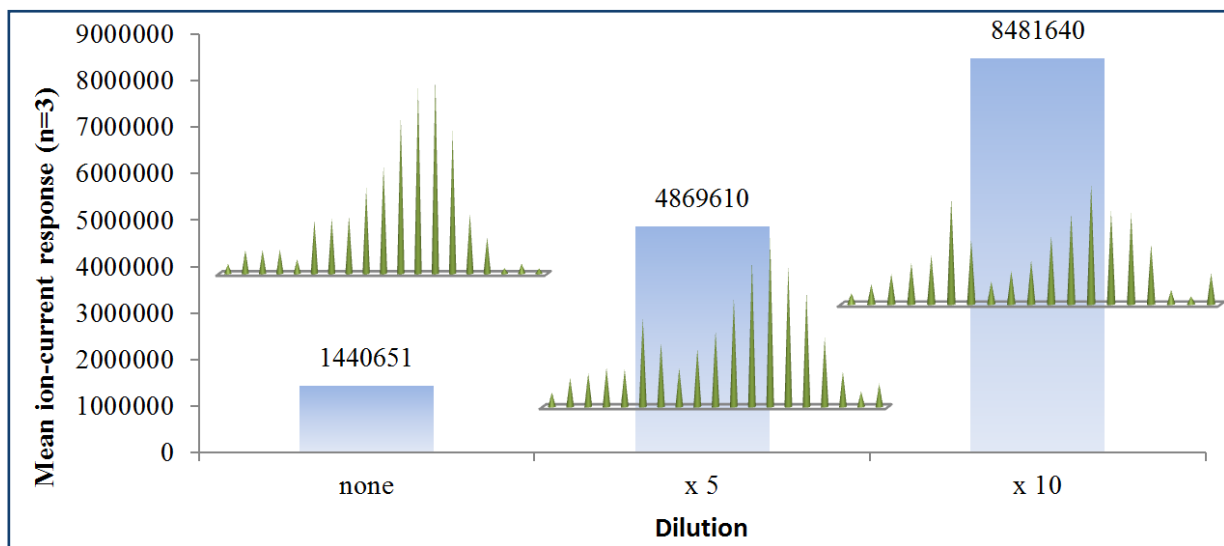


Figure 5. Ion Suppression effect measured by diluting sample 2013-04-25 5-fold and 10-fold. Ethoxymers are shown (NP20EO – NP2EO, left to right).

CONCLUSIONS

When coupled to a 1290 Infinity UHPLC, the iFunnel Q-TOF LC/MS system from Agilent Technologies proved to be suitable for the analysis of NPEOs at sub- $\mu\text{g/L}$ concentrations in a complex matrix. Using the described protocol, this instrumental setup provides acceptable precision, sensitivity, mass accuracy, linearity and identification of fragment ions using MS/MS. Various measures could be taken to mitigate the observed ion-suppression effects including (a) dilution, (b) the development of an improved sample preparation protocol, (c) the development of a method with superior chromatographic selectivity that would reduce the co-elution of target molecules with interfering substances, and (d) reduction of the injected sample volume. If the matrix effects cannot be eliminated, various calibration protocols could be used to compensate for them (13). The standard addition method and the use of internal standards with ionization properties similar to the analyte (ideally isotopically labeled analogs) should both be appropriate for this purpose.

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