

Determination of nonylphenol ethoxylates in leather by GC-MS pretreated with cleavage of hydroiodic acid and the feasibility

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Abstract: Nonylphenol ethoxylates (NPEO_n) residue is considered to be a ubiquitous pollutant in leather. The related test techniques have attracted wide attention. However, no popular methods have been developed for NPEO_n test in leather. Therein, the test method of NPEO_n is one of the main research directions in analytical field. In this paper, an analytical method for the determination of NPEO_n in leather was studied. The method combined an ultrasonic assisted extraction procedure, an enrichment step onto C₁₈ solid phase extraction (SPE) treatment and cleavage process with hydroiodic acid prior to analysis using gas chromatography mass spectrometry (GC-MS). Method validation of each process was carried out by recovery evaluation. 5 g leather samples were extracted assisted by sonication in two consecutive steps of 30 min using 100 ml methanol. 10 ml of the obtained extracts were mixed with 10 ml water and loaded onto C₁₈ cartridges and analytes were eluted with 3 × 2 ml methanol. The elution was evaporated and treated by 20 ml hydroiodic acid (37%) with 4 g sodium hypophosphite at 100°C for 24 h. Finally, NP in the cleavage resultants was extracted with hexane and analyzed by GC-MS. The results presented that the procedures performed low quantitative recoveries mainly due to the fact that NPEO_n was not transformed to NP with equal moles during the cleavage process. Further improvement and optimization of the cleavage conditions should be done for the application of the proposed method to quantitatively determine NPEO_n residues in leather by GC-MS.

Key words: leather; nonylphenol ethoxylates; GC-MS; cleavage; hydroiodic acid

1 Introduction

Nonylphenol ethoxylates (NPEO_n, n = number of ethoxy units) are a major group of nonionic surfactants widely used as emulsifiers, dispersive and auxiliary agents in fine chemicals [1]. The use of NPEO_n has been banned by European Directive 2003/53/EC which establishes restrictions on the marketing and preparation of NPEO_n because their metabolites, as nonylphenol (NP) and short-chain nonylphenol ethoxylates isomers (NPEO₁, NPEO₂), have been included among the group of so-called “emerging contaminants” due to their disrupting the endocrine systems of living organisms [2]. The general structures of these compounds are presented in Fig. 1. Although the possible estrogen-mimicking characteristics of these metabolite are well known, many leather processing auxiliaries such as fatliquors and pigments are very likely to contain NPEO_n as a major ingredient due to its low price and excellent characteristics. So the determination of NPEO_n is desirable and necessary for monitoring and restricting NPEO_n residues in leather.

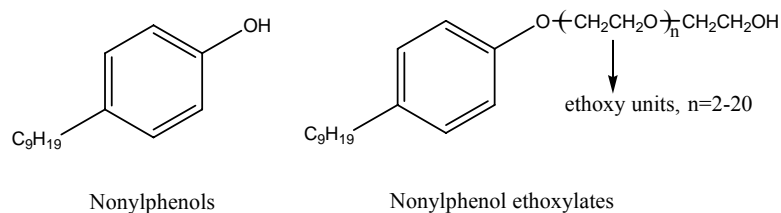


Fig. 1 Chemical structures of nonylphenol and nonylphenol ethoxylates

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For an efficient monitoring of these compounds in leather and verification of the compliance with regulations, a reliable and rugged analytical method is crucial, while literatures about NPEO_n test in leather are quite fewer than these in environmental samples such as river water, sludge and soil. Reviews [3, 4] on the analytical methods of NPEO_n in some environmental samples have been widely documented. Liquid chromatographic analysis (HPLC) coupled to fluorescence or MS detector has been recognized as the preferred technique because many of these chemicals are not directly amenable to gas chromatography (GC). However, no chromatographic separation has been reported that can simultaneously separate NPEO_n on the basis of alkyl and ethoxy chain length. In addition, fluorescence detection lacks the specificity inherent to the mass spectrometer, and interferences from complex matrixes may complicate the analysis. Electrospray (ES) HPLC-MS can provide the sensitive and selective analysis of NPEO_n, but the high cost prevents its wide application in the normal analytical laboratories. The analysis of NPEO_n is still challenging.

GC-MS has been widely applied for the direct determination of NP and short chain NPEO_n [5-7]. The high resolving power of capillary GC allows separation of many of the alkyl isomers of NP and results in compositional information. The test procedure of NP in river water with GC-MS has been standardized [8]. Then, if NPEO_n are transformed to NP isomers with equal moles by some cleavage technique (presented in Fig. 2), the analysis will be easy and be widely accepted because of popularization of GC-MS in normal laboratories now.

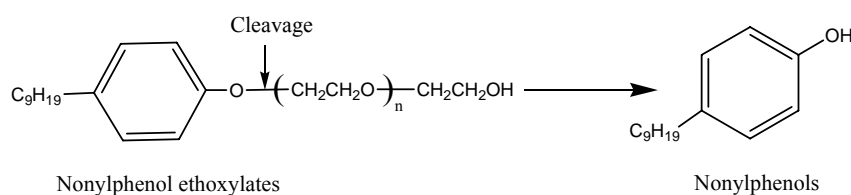


Fig. 2 Scheme of cleavage reaction of nonylphenol ethoxylates

Recently, IULTCS circulates a draft [9] for the determination of NPEO_n residues in leather by GC-MS pretreated with hydroiodic acid as cleavage reagent. The cleavage mechanism is based on the chemoselective reaction occurred in the phenyl ether site as shown in Fig. 2. According to the draft content, a modified method by GC-MS with cleavage of hydroiodic acid was investigated. The validity of the whole determination procedure, especially the cleavage process, was evaluated by comparing with these results supplied by reverse HPLC-fluorescence method [10, 11] and other laboratories, to demonstrate the feasibility of the methodology proposed.

2 Experimental

2.1 Reagents

Nonylphenols (NP, technical mixture) were supplied by Igepal. Nonylphenol ethoxylates (NPEO_n, $n \approx 9$) were purchased from Sigma-Aldrich. Methanol of HPLC grade and sodium hypophosphite of analytical grade was supplied by Alfa Aesar. Hydroiodic acid of analytical grade with concentration 37% (m/m) was purchase from EHSY. Water was produced using a MilliQ Plus system (Millipore, USA). All the other reagents used were of analytical grade.

2.2 Instruments and materials

2.2.1 Extraction equipment

The ultrasonic water bath (290 W, 50/60 Hz) was supplied by KunShan Ultrasonic Co.Ltd (JiangSu). A normal vacuum manifold was employed to collect the extraction solvent. Nylon filters (0.45 μ m) were

supplied by DIKMA. Rotary evaporator with thermostatic float and vacuum system were purchased from IKA (German). Constant temperature oil-bathing (HH-SD) with range between room and 160°C were purchased from Nanjing. The C₁₈ SPE cartridges (6 ml, 500 mg Accubond) were obtained from Supelco.

2.2.2 GC-MS analysis

Agilent 7890A GC instrument equipped with 5975C MS detector and autosampler was used for the measurement of NP. A DB-5MS capillary column (30 m × 0.25 mm I.D., 0.25 µm film, J & W Science, USA) was applied. After 2 min of holding the injector temperature at 280°C, the GC temperature program began as follows: 80°C for 2 min, followed by a temperature ramp at 10 °C/min to 280°C, and hold for 10 min. The transfer line was set at 280°C. Full scan EI data was acquired under the following conditions: mass range 50-350 m/z, scan time 1 s, solvent delay 5 min, ionization energy of EI 70 eV. temperature of MS source 230°C, temperature of MS quadrupole 150°C.

2.2.3 HPLC-fluorescence analysis

Agilent 1200 LC instrument equipped with programmable fluorescence detector was used for the measurement of NPEO_n. The chromatographic separation was carried out using Eclipse XDB-C₁₈ HPLC column (5 µm × 250 mm × 4.6 mm) thermostatted at 20°C, injection volumes of 20 µL, flow rate of 1 ml/min and isocratic elution with 40% water and 60% acetonitrile during 25 min. Analytes were monitored by fluorescence detection (λ_{ex} : 222 nm, λ_{em} : 305 nm) and quantified by external calibration using peak area measurements.

2.3 Procedures

2.3.1 Sample preparation

Six pieces of leather sample were selected from the tested samples of our laboratory, which were supplied by tanneries of china for NPEO_n test. These leather samples have been determined by other laboratories such as SGS (Shanghai), ITS (Shanghai) and STC (Shanghai), and NPEO_n contents were verified. Prior to test, all the samples were conditioned under 20°C/65% RH for 24 h to allow equilibration, and then cut into pieces with size of 2 mm × 2 mm.

2.3.2 Sonication-assisted extraction

Extraction of NPEO_n from leather samples was carried out by ultrasonic-assisted extraction which was performed in a 250 ml Erlenmeyer flask with 5 g of sample and 50 ml aliquot of methanol. The flask was immersed into the ultrasonic water bath and treated in two consecutive steps of 30 min at 50 Hz. Then, the solution was treated with the vacuum manifold and the extracts were collected and filtered through a 0.45 µm nylon filter. Finally, the extracts were fit to 100 ml with methanol before solid-phase extraction treatment.

2.3.3 Solid phase extraction (SPE) treatment

SPE treatment is a common and necessary method to reduce the interferences from matrix such as dyestuffs and lipids (fats), as well as enrich NPEO_n from the extracts. The SPE procedure follows the process described in the literature [10, 12] with minor modification. Briefly, 10 ml aliquot of the obtained extracts was pipetted and mixed with 10 ml of water, then the mixture was loaded onto C₁₈ cartridge previously conditioned with 2 × 2 ml methanol and 1 × 2 ml mixture H₂O:methanol (50/50, v/v). Subsequently, the analytes were eluted with 3 × 2 ml methanol, and collected into a 250 ml distilled bottle. Finally, the extracts were evaporated to dryness under a nitrogen stream and prepared for cleavage treatment.

2.3.4 Cleavage with hydroiodic acid

The cleavage procedure has been circulated [9] by IULTCS for its validation. Briefly, the dry residues were dealt with 20 ml hydroiodic acid (35%), along with 4 g sodium hypophosphite to avoid the

iodination of the nonylphenols and oxidation of iodine ion. The treatment is continued for 24 h at 100°C with reflux. Subsequently, the mixture was diluted with 100 ml water and dealt with 3×20 ml hexane. The organic phase was washed with 2×50 ml water and dehydrated with 2 g anhydrous sodium sulphate. After evaporating the hexane, the residues were resumed in 5 ml aliquot of dichloromethane and analyzed by GC-MS.

3 Results and discussion

3.1 Determination of nonylphenol

Typical GC-MS (EI) extracted mass chromatograms and corresponding mass spectra of NP have been presented in the literature [5, 6]. Fig. 3 demonstrates the total ion current (TIC) and typical selected ions mode (SIM) traces of NP standards, which consists of almost eleven separated peaks corresponding to the isomers which are quantified on different ion traces, indicating the various branching pattern in the nonyl substituent. To determination the response factors for individual NP isomers, pure standard compounds of these isomers are necessary, but currently not available. Therefore, the quantitations of NP were based on the sum of the SIM, and a preferred SIM ions ($m/z = 121, 135$ and 149) were chosen based on the ratio of the response factors with TIC for all isomers [13], and NP contents were calculated from the five point calibration curve, as indicated by the response factors. Calibration curves of these analytes were constructed with the standard solutions (1, 5, 10, 15, 20 $\mu\text{g/ml}$) and showed high linearity ($R^2 \geq 0.99$).

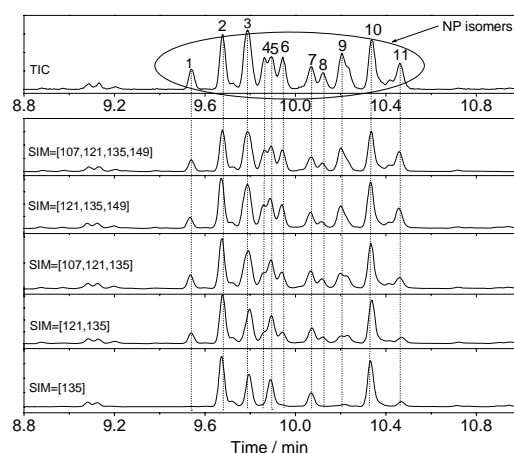


Fig. 3 Total ion current chromatogram and SIM traces of characteristics ions of NP standards by GC-MS (EI) in the SIM mode. Numbers are referring to the different NP isomers.

3.2. Recovery of NPEO during extraction

Many literatures [3, 4, 10-12] have presented the liability of methanol extraction to NPEO_n in various samples. The recoveries from the extraction were evaluated with HPLC-fluorescence by spiking known amounts of NPEO_n into leather samples which are free of NPEO_n. Three sample replicates of 5 g leather samples were spiked to yield the final content of NPEO_n from 100 mg/kg to 1000 mg/kg. The number and duration of the extraction cycles were studied and optimized as shown in Tab. 1. The results revealed that the number of extraction cycles had a significant effect on the extraction efficiency. Extraction in two consecutive recoveries of 30 min instead of one cycle of 60 min, satisfactory recoveries for NPEO_n (> 94%) could be achieved. Accordingly, the extraction procedure was set in two consecutive

extraction cycles of 30 min and an efficient extraction allowing quantitative recoveries was obtained.

Tab. 1 Recoveries (R%) and relative standard deviations (RSD) during extraction process

Spiking level (mg/kg)	1 cycle		2 cycles	
	R (%)	RSD (%)	R (%)	RSD (%)
100.0	73.5	11.7	94.6	9.8
500.0	67.4	8.6	97.7	10.2
800.0	75.9	7.2	98.6	8.7
1000.0	70.4	4.9	95.3	7.2

3.3 SPE enrichment efficiency

Retention of NPEO_n by C₁₈ cartridges has been verified and widely applied for the analytes in environmental samples [3] in order to reduce the number of evaporation steps of the analytical method. Methanol was often used for the desorption due to its high solubility to NPEO_n. The effect of the addition of water to the extracts to increase the retention of NPEO_n by C₁₈ cartridges was studied. Different amount of water up to 15 ml was added to spiked methanol with concentration level of 10 mg/L and 50 mg/L NPEO_n. Three sample replicates of each concentration were prepared and loaded into the cartridge. The cartridges were eluted with 3 × 2 ml methanol. The recoveries were tested with HPLC-fluorescence. The results obtained are given in Tab. 2. An increase in the recoveries was obtained with the addition of water, and quantitative recoveries (> 97%) were achieved when 10 ml water was introduced. Therefore, this volume of water was chosen as the optimum amount to be added to the methanol extracts (10 ml). Finally, the elution was evaporated under a nitrogen steam and prepared for cleavage treatment.

Tab. 2 Recoveries (R%) and relative standard deviations (RSD) during SPE process ^a

Added water (ml)	Spiking level ^b		Spiking level ^c	
	R (%)	RSD (%)	R (%)	RSD (%)
1.0	32.1	5.8	33.7	5.6
2.0	57.4	6.3	55.4	4.7
5.0	87.6	7.2	84.9	3.4
10.0	97.3	4.9	98.3	6.2
15.0	98.4	6.1	97.8	4.4

^a The final elution was fit to 5 ml with methanol.

^b Concentration 10 mg/L of NPEO_n.

^c Concentration 50 mg/L of NPEO_n.

3.3. Cleavage with hydroiodic acid

Ether cleavage is a versatile transformation in organic chemistry. A variety of nucleophilic methods for the cleavage of aryl ethers have been investigated in the literature [14, 15]. Among the nucleophilic reagents, hydroiodic acid is usually used for the cleavage of epoxide ether to test the ethoxyl units in the surfactant or hydroxyethyl cellulose [16]. However, the cleavage experiments with hydroiodic acid were all carried out under high temperature (> 100°C) and acid condition [14]. The strenuous conditions might not lead to chemoselective cleavage and the NP isomers might not be transformed with equal moles. Thus the efficiency of the proposed reaction shown in Fig. 2 requires to be confirmed.

Accordingly, cleavage experiments were carried out in triplicate with various amounts (μg) of NPEO_n standards, to evaluate the cleavage efficiency of the method as described in 2.3.4 and the results were shown in Tab. 3. As can be observed, the resultant NP is detected and increased with increasing NPEO_n standard, implying that predicted cleavage reaction of NPEO_n occurred. However, the relationship between NPEO_n standard and resultant NP is nonlinear and all the recoveries were random and low (42.4% even in the best case), as well as high relative standard deviations (RSD > 14%). These indicate the cleavage efficiency was not acceptable. So the cleavage conditions described in 2.3.4 were not suitable, which required further optimization or modification to achieve satisfactory results.

Tab. 3 Recoveries (R%) and relative standard deviations (RSD) obtained from the cleavage process

NPEO _n standard added ^a (μg)	Average resultant NP ^b (μg)	Precision	
		Recovery (%)	RSD (%)
20.0	2.1	31.2	18.6
30.0	4.2	41.8	34.6
40.0	4.9	36.4	17.3
50.0	5.5	33.1	33.9
60.0	8.5	42.4	27.4
80.0	11.6	43.6	14.7
100.0	12.4	37.1	18.1

^a Concentration of standard solution of NPEO_n in methanol 1000 $\mu\text{g}/\text{ml}$.

^b Calculation based on the calibration curve of NP standard.

3.5. Cleavage resultants analysis

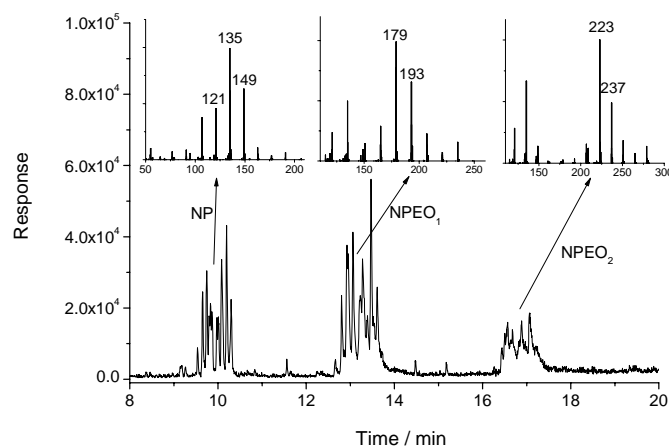


Fig. 4 TIC chromatogram and MS spectrum of cleavage resultants by hydroiodic acid (NP, NPEO₁ and NPEO₂)

Due to the poor recoveries presented above, the chromatographic profile and EI-MS spectrum of the cleavage resultants were reconstructed as shown in Fig. 4. Qualitative analysis of the marker peaks was performed based on their diagnostics ions, which provided indication of the presence of NP, NPEO₁ and NPEO₂ being easily extracted by organic solvent as hexane, toluene, acetic ester and dichloromethane. The predominant ions for NP, NPEO₁ and NPEO₂ have been documented [5]. These significant ions are

produced by benzylic cleavages, corresponding to the ions of m/z 149, 121 and 135 for NP isomers, ions of m/z 193 and 179 for NPEO₁ isomers, and ions of m/z 237 and 223 for NPEO₂ isomers. These confirm that NP isomers were not the only resultants under the cleavage conditions as described in 2.3.4 and the cleavage pathway did not follow that shown in Fig. 2. So it is not surprising that the poor results occurred as presented in Table 3.

3.6 Analysis of leather samples

Despite the low recoveries during the cleavage process, the proposed method was still applied to determine the content of NPEO_n in leather samples. The results obtained were summarized in Tab. 4, which were compared with these detected by HPLC-fluorescence and HPLC-MS method. It could be observed that all the results by the proposed method were much lower than these by HPLC-fluorescence and HPLC-MS method which represent the real content of NPEO_n residues in leather. The reason was mainly due to the resultants of short chain NPEO as NPEO₁, NPEO₂, which make it impossible to transform NPEO_n to NP with equal moles as shown in Fig. 2. The feasibility of the cleavage conditions might need to be further investigated and evaluated.

Tab. 4 Estimated average concentration in leather samples

Sample	NPEO _n content (mg/kg)		
	Proposed method ^a	HPLC- fluorescence ^b	HPLC-MS ^c
Leather-1 [#]	42.1	130.7	137.4
Leather-2 [#]	112.3	403.9	418.7
Leather-3 [#]	78.6	222.1	209.4
Leather-4 [#]	97.2	325.1	356.9
Leather-5 [#]	38.9	103.7	91.3
Leather-6 [#]	192.3	490.6	524.1

^a By GC-MS method described in 2.3.4.

^b According to reference [11].

^c Supplied by SGS, TUV and ITS laboratories.

4 Conclusions

The feasibility of a quantitative analysis method for NPEO_n was demonstrated. Both the extraction procedure and SPE enrichment step presented satisfactory recoveries, while the cleavage process showed a quite negative effect on the analytical results, which limited the application of the proposed method. Further improvement of the cleavage conditions should be developed to develop an acceptable procedure for GC-MS analysis of NPEO_n in leather.

Acknowledgements

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