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Determination of free and ethoxylated alkylphenols in leather with gas chromatography–mass spectrometry

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ABSTRACT

An analytical approach was developed to determine nonylphenol (NP), octylphenol (OP), nonylphenol ethoxylates ($NPEO_n$) and octylphenol ethoxylates ($OPEO_n$) in leather samples involving the conversion of $NPEO_n$ and $OPEO_n$ into the corresponding NP and OP. The four targets were extracted from samples using ultrasonic-assisted acetonitrile extraction. NP and OP in the extracts were directly isolated with hexane and quantitatively determined with 4-n-nonylphenol as internal standard by gas chromatography–mass spectrometry (GC–MS). For $NPEO_n$ and $OPEO_n$ in the extracts, they were first converted into NP and OP with aluminum triiodide as cleavage agent, and the yielded NP and OP were determined by GC–MS. The contents of $NPEO_n$ and $OPEO_n$ were calculated by normalizing to $NPEO_9$ and $OPEO_9$, respectively. This method was properly validated and the real sample tests revealed the pollution significance of leather by $NPEO_n$ and $OPEO_n$.

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1. Introduction

In recent years, restricted substances in consumer products have been an important issue for both manufacturers and consumers [1,2]. Their use is limited for a number of reasons including consumer safety, worker safety and environmental grounds. Alkylphenol ethoxylates ($APEO_n$, n = average number of ethoxy units), the widely used industrial surfactants, have been listed as restricted substances by legislation due to their potential to form estrogenic substances as alkylphenol (AP) and mono- or di-ethoxylated alkylphenols [3,4]. In most cases, $APEO_n$ refer exclusively to nonylphenol ethoxylates ($NPEO_n$) and octylphenol ethoxylates ($OPEO_n$) because both are by far the most commonly used and encompass more than 98% of $APEO_n$ market. The most influential legislation relating to $APEO_n$ is the European Union (EU) Directive 2003/53/EC which especially restricts the use of $NPEO_n$ and nonylphenol in substance or preparations for leather and textile processing. Although the legislation is not intended as a restriction of $APEO_n$ in the final product, it provides a basis for many Eco-labels and brands to introduce their own limits for the presence of $APEO_n$ in the consumer products made of leather or textile.

Reports about $APEO_n$ in consumer products have been published by Greenpeace, and one particular report highlighted that "toxic gender bending chemicals" including $APEO_n$ were found in the toys, textiles and baby care products [5]. Although this publicity has caused the concern of Eco-labels and brands on $APEO_n$, only few people are aware that $APEO_n$ are used in consumer goods we buy and use everyday. These commonly include clothes, bags and shoes made of leather or textile. The consequence of $APEO_n$ presented in consumer products is that the user is constantly exposed to these chemicals and that they will enter the environment during or after use of the products. Thus, $APEO_n$ in the products should be checked in order to demonstrate due diligence and corporate social responsibility whether they are potentially harmful to the environment or to the user.

For efficient determination of $APEO_n$ in the consumer products and verification of the compliance with the legislation, a reliable analytical method is crucial to screen these compounds in the raw materials such as leather or textile. However, publications about analysis of $APEO_n$ in these samples were few compared with them in environmental samples as water, sludge and soil [6–8]. Moreover, there is currently no officially authorized method for the determination of $APEO_n$. This could result from the fact that $APEO_n$ and their metabolites are homologous, non-volatile, polarity-variable and hydrophilic–hydrophobic [9], placing an enormous burden on the analytical technology. Typically, the analysis of $APEO_n$ is done by high performance liquid chromatography (LC) coupled with fluorescence detection, mass or tandem mass spectrometry [6]. However, determination of these compounds based on LC techniques suffers from poor separation due to their extreme ranges in polarity [10–12]. Therefore, an alternative methodology needs to be developed that can adequately screen for the potential formation of these estrogenic compounds.

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This paper reports the possibilities of using a totally new approach for determining $APEO_n$. In the experiments, leather samples were chosen to be investigated due to that goods made of leather are preferred and common in the consumer market, as well as that $NPEO_n$ and $OPEO_n$ used in the preparations for leather processing have been proved [13–15]. The analytical procedure included ultrasonic-assisted acetonitrile extraction followed by aluminum triiodide (AlI_3) cleavage process. Based on the AlI_3 cleavage reaction, $NPEO_n$ and $OPEO_n$ were qualified by detecting the yielded products—nonylphenol (NP) and octylphenol (OP) with GC–MS and quantified by normalizing to $NPEO_9$ and $OPEO_9$. In addition, the NP and OP contained in leather samples were also monitored along with analyzing $NPEO_n$ and $OPEO_n$ by the procedure, to meet the requirements of the consumer market and environment.

2. Experimental

2.1. Chemical and reagents

Tergitol NP-9 ($NPEO_9$, mixture of $NPEO_n$ with $n \sim 9$) and Triton X-100 ($OPEO_9$, mixture of $OPEO_n$ with $n \sim 9$) were obtained from Sigma–Aldrich (Shanghai, China). Analytical standards of 4-NP (NP, CAS No. 84852-15-3, technical mixture), 4-tert-OP (OP, CAS No. 140-66-9) and 4-n-NP (4n-NP, CAS No. 104-40-5, used for internal standard) were purchased from Dr. Ehrenstorfer (Germany). Acetonitrile, hexane and methanol of HPLC grade were supplied by Merck (Germany). Organic free water was obtained using a Milli-Q system (Millipore). All other reagents used were of reagent grade.

Stock standard solutions of OP, NP, $NPEO_9$, $OPEO_9$ and 4n-NP were separately prepared in acetonitrile at a concentration of 1.0 mg/mL. These standard solutions were then diluted to appropriate concentration using acetonitrile. All solutions were stored in darkness at 4 °C.

2.2. Sample preparation

Cattle hide leathers were carefully prepared at the Research Center for Leather (Haining, China) according to the normal leather processing procedure [16]. After dyeing and waterproofing, the samples were air dried to moisture contents of ~9% (w/w) and stored in sealed beakers at 4 °C. Prior to the leather processing, all the chemical auxiliaries were carefully selected and only those without AP or $APEO_n$ were used, to ensure these samples were not contaminated by these pollutants. These samples were used for negative control.

A positive sample containing known amounts of the analytes was prepared by spraying the analytes spiked acetone solution to the both sides of the negative sample which lay on a glass plate. Then the sample was then placed in a ventilated chamber at room temperature for 24 h to allow the solvent evaporation. After that, it was sprayed with water for the second time until it was wet through but no drip occurred. The sample was dried under the room conditions for almost one week with a moisture content of 8.4% (w/w), and then stored in sealed beakers at 4 °C. The theoretical amounts of the analytes in the samples was calculated by taking into account the amounts of them used, as well as the remains on the glass plate surface after the glass were acetonitrile rinsed and the targets were detected according to analytical procedure described in the follows. 85.5% were found contained in the samples giving concentrations of OP, NP, $OPEO_9$ and $NPEO_9$ with 67.4, 135.8, 135.8 and 271.6 mg/kg, respectively. The distribution of the analytes in the positive sample was considered to be the same as genuine leather.

Prior to test, both the negative and positive samples were cut into pieces (~4 mm × 4 mm) and conditioned for 24 h at standard atmosphere of temperature 20 °C and relative humidity 65% (Temp. 20 °C/R.H. 65%). The moisture contents were near to 12% (w/w). The spiked samples were prepared by adding aliquots of OP, NP, $NPEO_9$ and $OPEO_9$ to the negative leather pieces, and conditioned for 24 h at the standard atmosphere to allow solvent evaporation.

2.3. Sample test

2.3.1. Sample extraction

Extraction of AP and $APEO_n$ from leather samples was carried out by ultrasonic-assisted acetonitrile extraction performed in a flask charged with 2 g of accurately weighted sample pieces spiked with 4n-NP. Both the positive and negative samples were used. After adding 5 g of Na_2SO_4 and 50 mL aliquot of acetonitrile, the flask was sealed and immersed into the ultrasonic water bath (40 kHz) and treated continuously at 45 °C for 60 min. The contents were then cooled to room temperature, and the extracts were transferred into the vacuum manifold with a 0.45 μm nylon filter, and elution was collected.

2.3.2. Analysis of AP

Isolation of AP in the sample extracts was performed by liquid–liquid extraction operation. A 10 mL aliquot of the extracts was mixed with 40 mL of water and then acidified (pH < 2) with concentrated HCl. The solution was treated with hexane 2 × 30 mL, and the hexane extracts were washed with distilled water 2 × 30 mL and anhydrated over Na_2SO_4. After evaporation of the solvent, the residues were reconstituted in 5 mL aliquot of hexane and filtered through 0.45 μm nylon filter. The isolated AP was analyzed by GC–MS based on the area response (A_1).

2.3.3. Analysis of $APEO_n$

Prior to analysis of $APEO_n$, the cleavage reagent AlI_3 was prepared. AlI_3 is commercially available, and can also be easily obtained in the laboratory according to the publication [17]. Briefly, 0.4 g of aluminum, 3.2 g of iodine and 10 mL of acetonitrile were submitted into a 50 mL flask. The contents were then heated at 90 °C with refluxing until the iodine color disappeared (~4 h), yielded ~2 g of white AlI_3 (weight analysis) in acetonitrile, which were used for cleavage treatment.

After getting AlI_3, another 10 mL aliquot of the sample extracts was added into the flask. The contents were then refluxed at 90 °C for 5 min. The reaction was quenched with water (40 mL) and the contents were cooled to room temperature. Being acidified (pH < 2) with concentrated HCl, the yielded targets were extracted with hexane 2 × 30 mL, de-iodinated with $Na_2S_2O_3$ (saturated solution, ~2 mL) and washed with distilled water 2 × 30 mL. After evaporation of the solvent, the residues were anhydrated with Na_2SO_4, reconstituted in 5 mL of hexane and filtered through 0.45 μm nylon filter. The isolated AP was then detected by GC–MS giving the area response (A_2).

It was noted that AP was stable during the cleavage process. Thus, the area response (A_1) of AP in the sample extracts contributed to the area response (A_2) of the isolated total AP from the cleavage reaction, because the sample extracts were directly submitted for cleavage without removing the free AP. Accordingly, ($A_2 - A_1$) was used when calculating the contents of AP yielded from $APEO_n$. Then, the contents of $APEO_n$ were expressed as $APEO_9$ followed the yielded AP using a 1:1 molar conversion with Tergitol NP-9 and Triton X-100 as calibration substances.

2.3.4. Calibration curves

The internal calibration curves for AP were prepared by testing five levels of increasing concentrations of AP standards with 4n-NP as internal standard. For $APEO_n$, the internal calibration curves were made by plotting five pairs of the amounts of given $APEO_9$ and

Table 1
Recoveries (R%) and relative standard deviations (RSD%) obtained for NP, OP and 4n-NP in the AlI_3 cleavage process[a].

Compounds	Spiking level[b]		Spiking level[c]		Spiking level[d]	
	R%	RSD%	R%	RSD%	R%	RSD%
NP	101.5	9.9	98.3	5.4	99.6	4.7
OP	97.3	6.7	99.6	3.7	99.1	3.5
4n-NP	99.4	6.2	100.4	3.6	99.3	3.4

[a] Six replicates was carried for each spiking level.
[b] Concentrations: 0.4, 0.2 and 0.2 mg/L for NP, OP and 4n-NP, respectively.
[c] Concentrations: 2, 1 and 1 mg/L for NP, OP and 4n-NP, respectively.
[d] Concentrations: 4, 2 and 2 mg/L for NP, OP and 4n-NP, respectively.

the contents of the yielded AP with 4n-NP as internal standard. The curves were fitted by linear-ship and the correlation coefficient r^2 was calculated from the linear regression, which was expected to be greater than 0.99.

2.4. Instrumental analysis

Agilent 7890A GC equipped with 5975 MS operated in the positive electron impact mode (EI+) was used for analysis. The GC separation was performed on a fused silica capillary column (DB-5 ms; film thickness, 0.25 μm; 30 m × 0.25 m i.d. (J&W Science)). The GC–MS conditions were as follows: carrier gas, helium; septum purge flow rate, 3 mL/min; average linear gas velocity, 40 cm/s; injector temperature, 250 °C; and transfer line temperature, 280 °C. The ion source temperature was adjusted to 150 °C. For analysis, 1 μL splitless injections with purge time 0.8 min were made by the autosampler. GC conditions were programmed as follows: initial oven temperature 80 °C, held for 1 min, followed by a temperature ramp of 10 °C/min to 280 °C, and held for 10 min.

For the identification of NP, OP and 4n-NP, mass spectrums of the available authentic standards at the same retention times were used, as well as comparison with mass spectrums in other publications [9,18]. Confirmation of analytes was carried out in full scan mode, and data were acquired from m/z 50 to 300. For the quantitation purposes, chromatograms were registered using selective ion monitoring (SIM) of the main characteristic fragment ions. The selected ions for OP were at m/z 135 and 206 with dwell time of 100 ms, for NP at m/z 107, 121, 135 and 149 with dwell time of 60 ms, and for 4n-NP at m/z 107 and 220 with dwell time of 100 ms. Instrumental detection limits in the experiment were calculated by signal-to-noise of 3:1 obtained in the analysis of spiked negative leather sample extract. They were approximately 0.5, 0.2 and 0.3 mg/L for NP, OP and 4n-NP, respectively.

3. Results and discussion

3.1. Cleavage of $APEO_n$ with AlI_3

The first step is to cleave $NPEO_9$ and $OPEO_9$ with AlI_3 for the formation of target phenols. As presented in Fig. 1, the GC–MS screening of the isolated products indicated that the yielded products were the corresponding NP and OP, which were identified with the mass spectrums of NP and OP standards. It is noted that these phenols were not detected in the intact $APEO_n$. So these phenols were generated by splitting the O-alkyl bond in the aryl-O-alkyl ether, as shown in Fig. 2. The similar reaction has been observed with various aromatic aliphatic ethers as substrates [17], and illustrated by a chelation mechanism [19]. These indicated that AlI_3 was a suitable reagent to be used for the equivalent conversion of $APEO_n$ to AP.

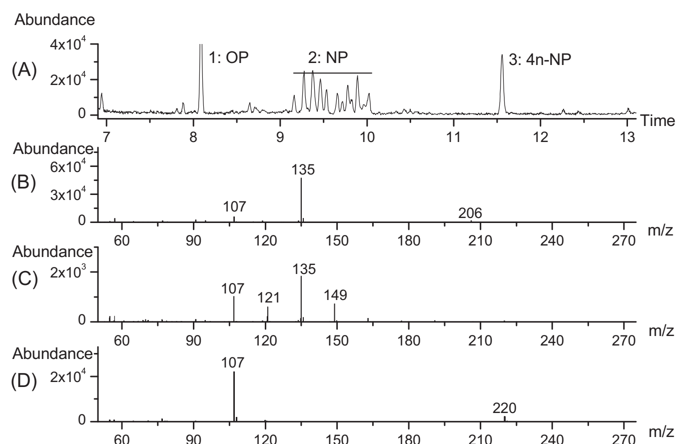

Fig. 1. (A) Total ion current chromatogram of OP (peak 1, retention time, 8.08 min) and NP (peaks 2, retention time, 9.16–10.03 min) yielded by AlI_3 cleaving $NPEO_9$ and $OPEO_9$. 4n-NP (peak 3, retention time, 11.56 min) was used as internal standard. (B) Mass spectrum of OP. (C) Mass spectrum of NP. (D) Mass spectrum of 4n-NP.

3.2. Stability of AP during the cleavage process

During the cleavage process, AP must be stable to obtain quantitative test for $APEO_n$. Then, the stabilities of NP, OP and 4n-NP were estimated according to the recoveries during the cleavage process. The experiments were performed by treating 10 mL aliquots of acetonitrile spiked with NP, OP and 4n-NP standards at three concentration levels under the cleavage conditions and six replicates of each concentration were prepared. After isolation and GC–MS analysis, the results were calculated and shown in Table 1. It can be observed that the recoveries were in the range of 97–102% with low RSD values. This indicated that the three targets were well recovered with stability during the cleavage process. In addition, these results revealed the suitability of the separation operation to recover the targets at a wide concentration level from the cleavage mixture, supplying a reliable technique coupled with the cleavage process.

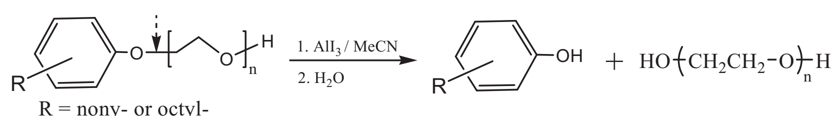

Fig. 2. Cleavage of $APEO_n$ to AP using AlI_3 as reagent.

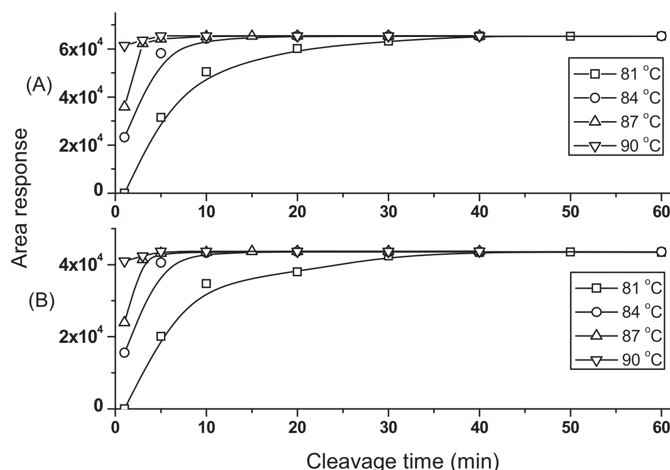

Fig. 3. Cleavage of (A) $NPEO_9$ and (B) $OPEO_9$ at different temperature. The initial concentrations of $NPEO_9$ and $OPEO_9$ in 10 mL aliquot of acetonitrile for cleavage were 10 and 5 mg/L, respectively.

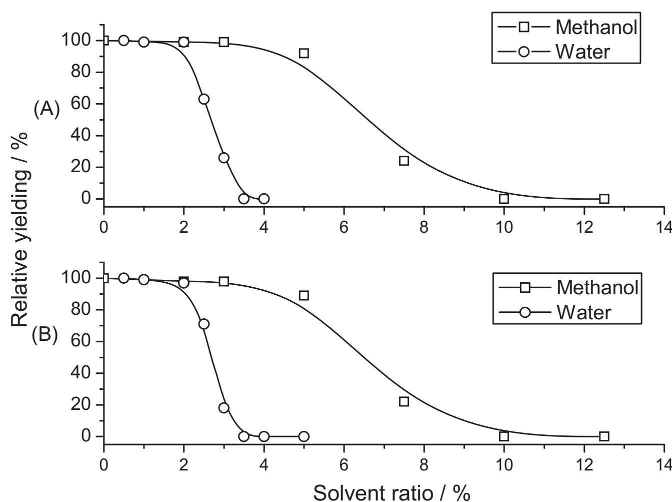

Fig. 4. Influence of methanol and water on the yields of NP and OP during the AlI_3 cleavage process (A) $NPEO_9$ and (B) $OPEO_9$.

3.3. Optimization of cleavage conditions

3.3.1. Temperature

Variables that could influence the cleavage process were evaluated to obtain a reliable process. The effects of temperature on the cleavage process were firstly examined by performing the reaction at 81, 84, 87 and 90 °C, respectively. These temperatures are just higher than the boiling point of acetonitrile (~81 °C), supplying a relatively mild refluxing condition. The amounts of the yielded AP, reflected by the peak area response, were analyzed at regular intervals to obtain the time dependence of cleavage process at each temperature, as shown in Fig. 3. Interestingly, $NPEO_9$ and $OPEO_9$ presented parallel trends under the identical cleavage conditions, indicating similar reaction activity of AlI_3 with the two substrates. The complete reaction time is reached when a further increase of the time does not result in a significant increase in the area response, which was established within 40 min for all the temperatures. As temperature increased, the time to reach the complete yielded is obviously shortened. It was almost 35 min to reach the complete yields at temperature 81 °C, while it was reduced to less than 5 min at temperature 90 °C. Accordingly, the definitive cleavage conditions were set in 5 min at 90 °C, presenting an efficient way for the conversion of $APEO_n$ to AP quantitatively.

3.3.2. Solvent

It has been demonstrated that solvent can play a significant role during ether-cleavage process [17]. Considering its wide application in $APEO_n$ analysis [8], methanol was selected to investigate the affects on the target yields. At the same time, water was also investigated for its influence on the cleavage process because it is everywhere in the normal experimental conditions. After treating a 10 mL aliquot of different ratios of solvent/acetonitrile solutions spiked with $NPEO_9$, $OPEO_9$ and various ratios of methanol and water, the relative yields of the NP and OP were calculated based on the results with acetonitrile spiked at the same concentration level, as shown in Fig. 4. The parallel trends were again observed for $NPEO_9$ and $OPEO_9$. Surprisingly, a decline in relative yields for increasing amounts of methanol and water was presented. It was apparent that the presence of 3.5% water or 10% methanol in the reaction mixture reduced the yields to a useless assay, indicating the considerable inhibition of the two solvents on the cleavage reactions.

It is well known that aluminum (III) halides (except AlF_3) are strong Lewis acids [20], which are quite sensitive to the basic moiety as OH^- contained in the molecular of water and methanol. These can be easily observed by the fume occurrence when putting $AlCl_3$ or AlI_3 in air. So the existence of trace water or methanol in the cleavage system would lead to the exhausting consumption of AlI_3, and result in failure of the cleavage reaction. Thus, water and methanol should be possibly removed prior to cleavage reaction. For methanol, it is unnecessary to be concerned because its use can be avoided during the whole test procedure. But for water, an essential technique must be adopted for its removal due to the fact that acetonitrile is hydrophilic and hygroscopic, and more than 10% (w/w) of water can be contained in leather and might be mixed in the extracts during the sample extraction process. Here, avoidance of water in the extraction step was attempted by addition of 5 g of Na_2SO_4 to the tested samples and satisfactory results were observed in the following sample extraction experiments as shown in Table 2.

Considering intactness of AP during the cleavage system, increasing AlI_3 usage should be another efficient way to reduce the interference of water on the cleavage reaction. This means has been applied due to the fact that 2 g of AlI_3 was prepared for the cleavage use. The amount of the cleavage reagent was far in excess in the

Table 2
Recoveries (R%) in the spiked negative leather under different temperature and times.

Compounds	Room/30 min		35 °C/40 min		45 °C/40 min		55 °C/40 min	
	R%	RSD%	R%	RSD%	R%	RSD%	R%	RSD%
NP	62.9	6.8	84.6	7.1	95.8	7.4	103.1	6.4
OP	68.4	7.9	81.7	7.3	97.2	6.9	95.5	7.2
$NPEO_9$	64.3	9.7	76.5	9.3	95.1	8.4	96.3	8.1
$OPEO_9$	69.8	8.4	73.8	9.6	96.4	5.3	95.2	7.9
4-n-NP	65.2	6.6	83.4	6.7	97.5	3.6	97.8	5.4

Spiking concentrations: 100, 50, 250, 100 and 25 mg/kg for NP, OP, $NPEO_9$, $OPEO_9$ and 4n-NP, respectively.

Table 3
Effects of Na_2SO_4 used in the extraction process on the test results the sample[a].

Compounds	With Na_2SO_4		No Na_2SO_4	
	R%	RSD%	R%	RSD%
NP	94.6	7.5	91.5	6.7
OP	90.2	4.9	92.3	5.7
$NPEO_9$	92.9	8.1	62.6	32.2
$OPEO_9$	94.0	8.3	67.5	21.4

[a] Concentrations of NP, OP, $NPEO_9$ and $OPEO_9$ in the positive leather were 135.8, 67.4, 271.6 and 135.8 mg/kg, respectively. Six replicates were extracted for each test at 45 °C for 40 min.

investigation based on the conclusion of 1:1 stoichiometry in the AlI_3-ether adducts [19], because the molar ratio of AlI_3:$APEO_n$ was more than 10^4 according to their mass weights. Combining with the water removal operation, the dosage of the prepared AlI_3 was considered to be sufficient to counter the extra consumption in the cleavage process and ensure the complete conversion of $APEO_n$ to AP.

3.4. Sample extraction

Acetonitrile was selected as the extraction solvent due to its solubility to both AP and $APEO_n$, as well as the compatibility with the cleavage system. The spiked negative samples were initially performed by using 50 mL aliquot of acetonitrile and ultrasonic-assisted extraction times of 30 min at room temperature with 5 g of Na_2SO_4. The results are shown in Table 2. As can be observed, the quantitative recoveries obtained for the analyzed targets were not so satisfactory (<70%) at room temperature. However, the results were significantly improved by increasing the temperature (to 55 °C) and duration (to 40 min). It was observed that increasing extraction temperature up to 45 °C and using extraction duration longer than 40 min were sufficient to achieve quantitative recoveries as high as 95% for the four targets. It was noted that no loss of acetonitrile was found during the extraction because the operation was carried in a sealed container. Accordingly, the extraction procedure was set in one continuous extraction of 40 min at 45 °C, each using 50 mL of acetonitrile. This procedure resulted in an efficient extraction by avoiding a number of evaporation steps, as well as favoring the desired cleavage process.

The optimized time and temperature were further verified with positive samples which contained known amounts of NP, OP, $NPEO_9$ and $OPEO_9$. The analytes in the leather were sure to be fully adsorbed by matrix due to the ageing of one week. As illustrated in Table 3, the average recoveries were all at the range of 90–95% with RSD less than 9% for the four targets. These values were quite close to these obtained at 45 °C as listed in Table 2, validating the proposed sample extraction time and temperature.

It was worth stressing that Na_2SO_4 was additionally used during the extraction process, to prevent the water in sample entering the extracts because water was unfavorable to the cleavage process. To investigate the influence of Na_2SO_4 on the analytical procedure, the positive leather was used and the test results were compared, as listed in Table 3. Without Na_2SO_4, both NP and OP were successfully analyzed with high recoveries and low RSD values, while $NPEO_9$ and $OPEO_9$ were poorly analyzed with low recoveries as well as high RSD values (>21%). These indicated that it was necessary to use Na_2SO_4 for the analysis of $APEO_n$. The reason should lie in that the water mixed in the extracts which disturbed the cleavage reaction, not that the extraction efficiency was low because satisfactory results of NP and OP were achieved. Thus, it was considered that water removal was necessary during the sample extraction process and Na_2SO_4 was recommended because of its easy availability and low cost.

3.5. Method validation

3.5.1. Linearity

The linearity of the developed method was determined by performing internal calibration curves with negative leather sample spiked with increasing concentrations of NP, OP, $NPEO_9$ and $OPEO_9$, as well as a constant concentration of 4n-NP internal standard. Spiking levels were as follows: from 10 to 200 mg/kg for NP, from 5 to 100 mg/kg for OP, from 20 to 500 mg/kg for $NPEO_9$, from 10 to 200 mg/kg for $OPEO_9$ and 25 mg/kg for 4n-NP. The responses of NP and OP contained in sample, as well as the NP and OP yielded from the corresponding $NPEO_9$ and $OPEO_9$, were all normalized to 4n-NP. For the four analyzed targets, the relative responses were linear in the range of concentrations studied and the correlation coefficients (r^2) were all more than 0.995. The obtained calibration data for the selected targets are summarized in Table 4.

3.5.2. Precision

Precision of the method was assayed by repeatability studies for the peak area measurements of the negative leather samples spiked on two different days (six replicated each). Two different spiking levels close to the extremes of the previously determined operation range were selected for this investigation. Results of the average from both days for each level are presented in Table 4. The peak area relative standard deviations (RSD) obtained were all lower than 10% for the both spiking levels, confirming the good reproducibility of the developed method.

3.5.3. Limits of detection and quantification

Limits of detection (LOD) and limits of quantification (LOQ) of the developed method are included in Table 4. The LOD were determined by the above-metioned instrumental detection limits, as well as weight of the sample and volume of the extracts. The

Table 4
Calibration data, precision and detection limits for the analyzed compounds.

Compounds	Calibration data[a]			Precision (RSD)		Detection limits (mg/kg)[d]	
	k	p	r^2	Peak area[b]	Peak area[c]	LOD	LOQ
NP	0.467	0.262	0.9991	8.9	7.3	1.2	3.4
OP	0.344	0.051	0.9995	7.7	6.2	0.5	1.9
$NPEO_9$	1.213	0.570	0.9953	9.7	9.0	2.8	7.9
$OPEO_9$	1.047	0.152	0.9977	9.2	7.6	1.3	3.7
4n-NP				6.8	7.2		

[a] Calibration curves were fitted by linear regression according to: $w_i = k(A_i/A_{i'}) + p$, where w_i is the AP or $APEO_n$ content (mg/kg), A_i is the area response of AP, $A_{i'}$ is the area response of internal standard 4n-NP, p is the ordinate intercept and k is the slope. The correlation coefficient r^2 was calculated from the linear regression.
[b] Concentrations: 10, 5, 20, 10 and 25 mg/kg for NP, OP, $NPEO_9$, $OPEO_9$ and 4n-NP, respectively.
[c] Concentrations: 200, 100, 500, 200 and 25 mg/kg for NP, OP, $NPEO_9$, $OPEO_9$ and 4n-NP, respectively.
[d] Limits of detection (LOD) and limits of quantification (LOQ) achieved by the developed method.

Table 5
Concentrations of AP and $APEO_n$ found in genuine leather samples.

Sample	Sample number (n)	Number of targets detected				Conc. range (mg/kg)	
		$NPEO_9$	$OPEO_9$	NP	OP	$NPEO_9$	$OPEO_9$
Sheep-skin leather for garment	21	3	0	0	0	85–324	/
Goat-skin leather for garment[a]	24	6	3	0	0	11–128	21–1100
Cattle-hide for shoe	20	3	0	0	0	54–237	/
Cattle-hide for furniture	25	6	0	0	0	38–1500	/

[a] Three samples contain both $NPEO_9$ and $OPEO_9$.

results showed that the LOD of the analytical procedure were 1.2, 0.5, 2.8 and 1.3 mg/kg for NP, OP, $NPEO_9$ and $OPEO_9$, respectively. LOQ determined by considering signal-to-noise of 10:1, were 3.4, 1.9, 7.9 and 3.7 mg/kg for NP, OP, $NPEO_9$ and $OPEO_9$, respectively. These detection limits easily fulfil the purpose of monitoring AP and $APEO_n$ in leather samples.

3.6. Application to real samples

The described analytical method was used to determine the concentration of AP and $APEO_n$ in genuine leather samples including cattle-hide, sheep-skin and goat-skin designed for garments, furniture or shoes. Fig. 5 shows the typical GC–MS chromatograms obtained from a goat-skin leather after the cleavage treatment. The peaks of OP and NP were identified by retention times and mass spectrums as well as comparison with these shown in Fig. 1. It was obvious that SIM detection (Fig. 5b) allowed to easily disregard the matrix background observed in the TIC chromatograms (Fig. 5a), and helped the quantitation using area response. These indicated the high selectivity of the developed method.

A total of 90 different leathers were analyzed in this study. These samples were obtained from sixteen tanneries in China for quality control. 18 samples were found to contain $NPEO_n$ or $OPEO_n$, but no NP or OP was detected, as shown in Table 5. The detected concentrations ranged from 11 to 1500 mg/kg for $NPEO_9$ and 21 to 1100 mg/kg for $OPEO_9$. The highest concentration of $NPEO_9$ (1500 mg/kg) was in a cattle-hide leather designed for furniture. These results revealed the realistic levels of $APEO_n$ in leather as well as the ubiquitous occurrence of these restricted substances in the consumer products made of leather. The $APEO_n$ in these leather were mainly from the leather processing substances [15]. For pollution source control, an analytical method for monitoring these pollutants in the substances should be developed. Further studies for the purpose are now in process.

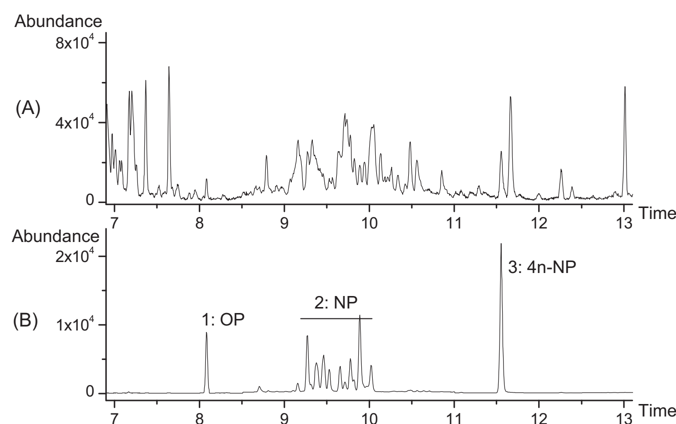

Fig. 5. (A) Total ion current and (B) selective ion monitoring chromatogram of the NP and OP yielded from the AlI_3 cleavage of $NPEO_n$ and $OPEO_n$ in leather sample extracts. The retention times of the peaks were consistent with these in Fig. 1.

3.7. Remarks on $APEO_n$ quantitation

In this study, the commercial Tergitol NP-9 and Triton™ X-100, each having an average of 9 ethoxy units, were selected as calibration substances in the investigation because the two products were the most commonly used surfactants [21]. The quantitation of $APEO_n$ in leather was based on the calibration curves of the two substances. However, it is well known that the ethoxy units (n) vary from 1 to 40 or more and $APEO_n$ with other average of ethoxy units might also be used for calibration. Therefore, the change of the calibration substances might lead to the deviation of the test results, and this problem was also faced by other developed methods based on LC techniques.

Considering the unification of the authentic standards of AP (OP and NP), as well as the successful conversion of $APEO_n$ to AP by the AlI_3 cleavage technique investigated in this paper, it is suggested that the quantitation of both AP and $APEO_n$ should be normalized to the total AP contents, which could make the test results more accurate and reliable. The concept has been proposed as "total potential alkylphenols", and was considered to be most suitable to fully assess the significance of samples contaminated by these pollutants [22,23]. Accordingly, the contents of the EU legislation should be modified as well, but adoption would first require broad discussion of this recommendation.

4. Conclusions

An analytical procedure for determining $APEO_n$ and AP in leather samples has been developed. The method combines an ultrasonic-assisted extraction and a cleavage process followed by GC–MS analysis. The sample extraction was performed in one step with successful recoveries. The cleavage process was accomplished by equivalently converting $APEO_n$ to AP using AlI_3 as cleavage reagent. Comparing with other proposed method for analyzing $APEO_n$, this methodology is completely independent of ethoxy chain length, offering a reliable and simple way for monitoring these pollutants.

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