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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 $OPEO_n$.

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