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Development and validation of a liquid chromatography isotope dilution mass spectrometry method for the reliable quantification of alkylphenols in environmental water samples by isotope pattern deconvolution

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ABSTRACT

We present here a new measurement method for the rapid extraction and accurate quantification of technical nonylphenol (NP) and 4-t-octylphenol (OP) in complex matrix water samples by UHPLC-ESI-MS/MS. The extraction of both compounds is achieved in 30 min by means of hollow fiber liquid phase microextraction (HF-LPME) using 1-octanol as acceptor phase, which provides an enrichment (preconcentration) factor of 800. On the other hand we have developed a quantification method based on isotope dilution mass spectrometry (IDMS) and singly $^{13}C_1$-labeled compounds. To this end the minimal labeled $^{13}C_1$-4-(3,6-dimethyl-3-heptyl)-phenol and $^{13}C_1$-t-octylphenol isomers were synthesized, which coelute with the natural compounds and allows the compensation of the matrix effect. The quantification was carried out by using isotope pattern deconvolution (IPD), which permits to obtain the concentration of both compounds without the need to build any calibration graph, reducing the total analysis time. The combination of both extraction and determination techniques have allowed to validate for the first time a HF-LPME methodology at the required levels by legislation achieving limits of quantification of 0.1 ng mL^{-1} and recoveries within 97–109%. Due to the low cost of HF-LPME and total time consumption, this methodology is ready for implementation in routine analytical laboratories.

1. Introduction

Alkylphenols (AP) are chemical compounds that are mainly used to produce alkylphenol ethoxylates (APEs), a class of synthetic surfactants widely used in detergents and cleaning products. Among the AP ethoxylates, the nonylphenol ethoxylates represent an 80% while the remaining 20% are almost entirely octylphenol isomers [1]. After degradation, APEs are released as AP, mainly in the water environment. The main problem associated to AP is their ability to mimic the structure of natural hormones, specifically 17-β-estradiol, which confers on them endocrine disrupting capabilities. The widespread use of AP polyethoxylates coupled with the harmful effects of alkylphenols had led to include them in the list of the priority substances of the Water Framework Directive (WFD) [2] which contain t-octylphenol (OP) and branched nonylphenol (NP), a complex mixture of nonylphenol isomers which is known as "technical grade nonylphenol". Due to the method sensitivity required in international regulations (i.e. 0.1 μg L^{-1} for OP and 0.3 μg L^{-1} for NP according to EU Environmental Quality Standards, EQS), together with its ubiquitous presence as contaminant, the identification and quantification of AP still presents considerable challenges.

Contrary to the determination of OP, the determination of NP involves a particular analytical challenge because NP comprises a complex mixture of isomers. Since the legislation establishes the EQS for the sum of all forms of branched 4-nonylphenol (CAS 84852-15-3), most laboratories employed commercial technical nonylphenol mixtures as analytical reference standard to quantify NP. In GC–MS their identification is based on the peak pattern (fingerprint) and NP is quantified from the sum of all peaks belonging to the chromatographic pattern [3]. This can be a tedious and non-reproducible work since the analyst should use caution to include only those peaks from the analyte. On the other hand, in LC–MS/MS the overall NP isomers elute as a single chromatographic peak associated to transition 219 > 133. This transition suggests that the α-carbon of the nonylphenols is mainly a tertiary

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carbon [4]. Nevertheless, we always have to take into account that a complete separation is the only real way to assess both different disrupting capabilities and sensitivity in the MS detector for the different nonylphenol isomers. However, NP isomers can only be completely separated by GCXGC [4] which is not a feasible option in most routine laboratories. Moreover, neither all the chromatographic peaks are identified nor commercial standards exist for all possible isomers. Taking these reasons into account, a recent work of Rabouan et al. [5] proposes the isomer 353-NP ((3,5-dimethylheptan-3-yl)phenol) as a reference material representative of both, instrumental sensitivity and toxicity of NP.

In order to achieve the low quantification levels established by the EQS a preconcentration step before the determination of alkylphenols in water samples is mandatory. The most employed methodologies in AP determination include liquid–liquid extraction (LLE) or solid phase extraction (SPE) [6]. These methodologies are usually time-consuming and require the use of large solvent volumes. Nowadays there is an increasing demand of faster, cheaper and environmentally friendly isolation techniques [7]. These requirements can be fully accomplished by hollow fiber liquid phase microextraction (HF-LPME), a relatively new and unexploited extraction technique [8]. As a consequence, there are just a few HF-LPME methods ready to be implemented in routine laboratories. Briefly, a liquid membrane of organic solvent is supported in the pores of a hollow fiber which is in contact with an aqueous donor phase (the sample) and an acceptor phase. If the acceptor phase is the same organic solvent as the immobilized in the fiber, it is known as a two-phase system. On the other hand, if the acceptor phase is an aqueous solvent, the extraction is performed in a three-phase system. Liu et al. [9] have developed an extraction procedure for sampling OP and NP using 1-octanol supported on a microporous hollow fiber. Nevertheless, this procedure was not able to quantify both compounds at the required legislation levels since only a thin film of octanol, attached to the outer fiber surface, is employed to extract the compounds. Besides analytes need to be desorbed from the thin layer for analysis by HPLC, which increased the total analysis time. A recent work [10] proposed a three phase HF-LPME procedure for the extraction of the linear isomers n-OP and n-NP and later HPLC determination, which assures the compatibility between sample extract and chromatographic mobile phase and column, but provided lower enrichment factors than two phase configurations. However, incompatibility with LC solvents can be avoided by simple dilution with methanol [11].

The analysis of OP and NP by LC techniques is preferred over GC because there is no need to perform any derivatization step, which increases the total analysis time and may show low yields in complex matrices [12]. On the contrary, extracts obtained after a two phase configuration in HF-LPME are directly compatible with GC analyzers. This advantage has been applied in the analysis of NP and n-OP by GC–MS [13,14].

A relevant problem with the use of electrospray ionization source (ESI) is the matrix effect [15–18]. Signal suppression or enhancement can affect drastically to sensitivity, precision and accuracy of the analytical results. Regarding alkylphenols, for example, Chen et al. [19] found around 50% signal reduction in river water for nonylphenol and other endocrine disruptor compounds, and Vega-Morales et al. [20] observed signal suppression for alkylphenols ranging from 9% to 24% in different wastewater treatment plant samples. Different approaches have been assayed to minimize matrix effect, being the use of Stable Isotope Labeled Internal Standard (SIL-IS) the most robust approach [16–18]. Thus, matrix-effects associated to complex matrices can be properly overcome using a quantification methodology based on isotope dilution mass spectrometry (IDMS). Classical IDMS, based on the use of methodological calibration curves requires the use of multiple labeled compounds to avoid overlapping in mass spectra. However, these multiple labeled compounds can induce isotopic effects, notably when deuterium isotopes are used. According to González-Antuña et al. [21,22] isotopic effects are not observed by the use of singly labeled analogs with ^{13}C. To avoid the overlapping problem in the mass spectra, isotope pattern deconvolution (IPD) quantification tool can be used. IPD do not requires the construction of any calibration graph and has been tested satisfactorily for rapid quantifications in complex matrices [23–25]. Briefly, IPD permits to isolate distinct isotope signatures from mixtures of natural abundance and enriched tracer and the corresponding molar fraction for each compound. From the ratio of the molar fractions between natural and labeled compounds the concentration of the analyte in the sample can be directly obtained [21].

The aim of this study is the development and validation of a HF-LPME-UHPLC–MS/MS method for the determination of alkylphenols in complex water samples in a single run. OP and NP, the two most ubiquitous EU-regulated alkylphenols, are selected. Sample treatment has been minimized to avoid contamination and a HF-LPME in a two phase configuration has been developed using octanol as extraction phase. Quantification of OP and NP is based on the combination of minimal labeling and IPD. To this end, a minimal labeled $^{13}C_1$-4-(3,6-dimethyl-3-heptyl)-phenol and $^{13}C_1$-t-octylphenol isomers were synthesized.

2. Materials and methods

2.1. Reagents and materials

4-tert-Octylphenol (purity grade 99.0%) was obtained from Supelco (Bellefonte, PA, USA). In-house synthesized [25] $^{13}C_1$-4-(3,6-dimethyl-3-heptyl)phenol ($^{13}C_1$-NP) (purity 99% and $^{13}C_1$-enrichement 98%) was also employed. In order to obtain the apparent concentration relative to technical nonylphenol of in-house synthesized $^{13}C_1$-NP by reverse IDMS, we acquired two technical nonylphenol mixtures: technical nonylphenol (Pestanal, purity grade 95.4%) from Riedel de Haen (Seelze, Germany) and technical nonylphenol (purity grade 100.0%) by Dr. Ehrenstorfer (Augsburg, Germany). Methanol (analysis grade), ammonium acetate (reagent grade) and hydrochloric acid (37%, reagent grade) were provided by Scharlau (Barcelona, Spain). For the extraction, 1-octanol (reagent grade, 99%) was obtained from Sigma–Aldrich Co. (Madrid, Spain). The pH of the mobile phase was adjusted approximately to 7 by adding ammonium hydroxide from Fluka (Buchs, Switzerland). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q gradient A10 (Millipore, Bedford, MA, USA). Drinking bottled water stored in polyethylene terephthalate (PET) bottles was also employed to test the effect of mobile phase composition in analyte sensitivity.

Individual stock solutions of alkylphenols were prepared by dissolving 50 mg, accurately weighted, in 50 mL of methanol. An intermediate mixed solution of OP, NP, $^{13}C_1$-NP and $^{13}C_1$-4-tert-octylphenol ($^{13}C_1$-OP) at a concentration of 0.5 mg L^{-1} was prepared after mixing individual stock solution and dilution with methanol. An equivalent mixed solution with only the singly $^{13}C_1$-labeled compounds was also prepared. Working solutions were subsequently prepared from the mixed solution by dilution the appropriate volume with methanol. All standard solutions (stock, intermediate and working solutions) were stored in amber glass bottles at −20 °C in a freezer.

Accurel® Q3/2 polypropylene hollow fibers (600 μm i.d., 200 μm wall thickness and 0.2 μm pore size) were purchased from Membrana (Wuppertal, Germany).

For the synthesis of 4-tert-octylphenol, boron trifluoride (BF_3) diethyl etherate, hexane, 2,4,4-trimethylpent-1-ene (also known