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# Fast methodology for the reliable determination of nonylphenol in water samples by minimal labeling isotope dilution mass spectrometry☆

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ABSTRACT

In this work we have developed and validated an accurate and fast methodology for the determination of 4-nonylphenol (technical mixture) in complex matrix water samples by UHPLC–ESI-MS/MS. The procedure is based on isotope dilution mass spectrometry (IDMS) in combination with isotope pattern deconvolution (IPD), which provides the concentration of the analyte directly from the spiked sample without requiring any methodological calibration graph. To avoid any possible isotopic effect during the analytical procedure the in-house synthesized $^{13}C_1$-4-(3,6-dimethyl-3-heptyl)phenol was used as labeled compound. This proposed surrogate was able to compensate the matrix effect even from wastewater samples. A SPE pre-concentration step together with exhaustive efforts to avoid contamination were included to reach the signal-to-noise ratio necessary to detect the endogenous concentrations present in environmental samples. Calculations were performed acquiring only three transitions, achieving limits of detection lower than 100 ng/g for all water matrix assayed. Recoveries within 83–108% and coefficients of variation ranging from 1.5% to 9% were obtained. On the contrary a considerable overestimation was obtained with the most usual classical calibration procedure using 4-*n*-nonylphenol as internal standard, demonstrating the suitability of the minimal labeling approach.



## 1. Introduction

Nonylphenols (NPs) are degradation products of non-ionic surfactants, NP polyethoxylates, which have been widely used in the production of detergents, plastics, textiles, paper and agricultural chemical products. Since they are able to mimic the structure of the natural hormone 17β-estradiol, which confers on them endocrine disrupting capabilities [1], NPs have been included in the list of priority substances in the Water Framework Directive (WFD) [2] and in the Directive of Environmental Quality Standards (EQSD) [3]. The new proposal for a Directive amending the WFD and EQSD (COM(2011)876) [4] legislates the mixture of isomers nonylphenol (CAS 25154-52-3) including isomers 4-nonylphenol (linear) (CAS 104-40-5) and 4-nonylphenol (branched) (CAS 84852-15-3). Nevertheless, as far as we know the linear isomer has not been detected in water samples at significant concentrations and its estrogenic power is lower than in branched isomers [5]. Therefore, the evaluation of the harmful effects of NPs is focused on the determination of technical NP (NP), consisting mainly in a mixture of branched para-isomers (>90%) [5,6].

Due to the ubiquitous presence of NP [1,7] and the low levels required to assess the EQS for these compounds in a great variety of complicated matrices, the development of adequate analytical methods to determine NP is still a challenge [5,6,8,9].

Both GC–MS [6,8–10] equipped with a single quadrupole and LC–MS [11] with single quadrupole or LC–MS/MS [5,7–9,12–14] with a triple quadrupole (QqQ) have been widely used to quantify NP in environmental samples. Nevertheless, LC techniques are preferred over GC–MS because there is no need to perform any derivatization step, which increases the total analysis time and may show low yields in complex matrices [10]. On the other hand, in UHPLC–MS/MS the overall NP isomers elute as a single chromatographic peak, making possible the simultaneous quantification of NP with the same fragmentation pathway. Normally, an off-line preconcentration step by solid-phase extraction (SPE) is required to allow the determination of NP at the levels established in the legislation.

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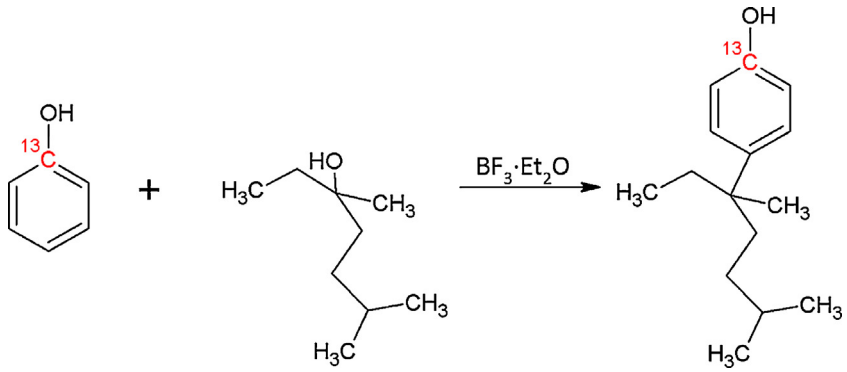


**Fig. 1.** Synthesis pathway of labeled $^{13}C_1$-4-(3,6-dimethyl-3-heptyl)phenol ($^{13}C_1$-NP).

Nowadays there is still no consensus in the selection of a NP isomer which represents both the estrogenic power and the composition of NP in nature. Due to the complexity of the vast majority of environmental samples, the use of an internal standard (IS) during NP determination is almost mandatory. This selected IS should show the same behavior than NP, regarding sample treatment and instrumental measurement [5]. As far as we know, with the exception of Rabouan et al. [5], all published papers have used some commercial technical mixture as quantification standard. On the other hand several internal standards have been employed. In GC/MS, isomers $^{13}C_6$-363-NP (4-(3,6-dimethyl-3-heptyl)phenol-ring-$^{13}C_6$) [6] and 4-sec-NP(4-(2,6-dimethylhept-3-yl)phenol) [10] have been used and each one was compared with the linear isomer 4-*n*-NP, other usual internal standard. Both papers consider the linear isomer unsuitable due to the different behavior during SPE step [6] or to the different derivatization yield [10] compared to branched NP. Regarding LC–MS (or MS/MS), 4-*n*-NP-$d_8$ has been used satisfactorily by Loos et al. [7,12] although in older works [14] it has been considered as unsuitable due to low purity of the standard. Another deuterated isomer, 4-*n*-NP-$d_4$, has been also employed as surrogate [13]. Ferguson et al. [11] discuss the potential limitations of the method using the isomer $^{13}C_6$-4-*n*-NP as surrogate and 4-*n*-NP as internal standard. They conclude that the linear internal standard accounts for the matrix effect in the surrogate correctly since both isomers coelute. However, the branched NP elutes at different retention time, probably together with different matrix, and hence the quantification is approximate.

The use of an appropriated internal standard with identical retention time than the analyte might provide the true concentration of NP in complex matrix water samples. In this sense, according to González-Antuña et al. [15,16] a minimal labeling (e.g. a single $^{13}C$ label in the molecule) ensures the same physicochemical behavior between the analyte and the isotopically labeled internal standard. The problem associated with this choice is the non-linear isotope dilution calibration graphs owing to the spectral overlap. Nevertheless it can be overcome using isotope pattern deconvolution (IPD).

IPD permits the calculation of the molar fraction of natural and labeled compound in the spiked sample by multiple linear regression using the whole or a part of the mass isotopomer distribution [17]. In addition, this alternative approach does not require any methodological calibration graph, so the total analysis time is drastically reduced. Recently, IPD has been adapted to the determination of diclofenac by UHPLC–MS/MS, demonstrating its applicability to tandem mass spectrometry [18].

In this work, we propose a procedure based on minimal labeling and IPD for the determination of NP in water samples by SPE–UHPLC–MS/MS. For this purpose, the labeled branched isomer $^{13}C_1$-4-(3,6-dimethyl-3-heptyl)phenol ($^{13}C_1$-363-NP) has been synthesized in our laboratory and characterized in terms of isotope composition and concentration. Furthermore, possible matrix effect has been corrected since labeled NP surrogate, enriched in a single carbon atom, coelutes with NP. The method has been validated in bottled water, effluent wastewater and influent wastewater spiked at two concentration levels. Finally, the figures of merit provided by the developed methodology were compared with those obtained by the usual external calibration using 4-*n*-NP as internal standard.

## 2. Materials and methods

### 2.1. Reagents and materials

The technical 4-nonylphenol mixture (NP) of chain isomers (no. 290858) was purchased from Sigma–Aldrich (Steinheim, Germany) and 4-*n*-nonylphenol (4-*n*-NP) were delivered by Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions were prepared by dissolving the corresponding standards in dichloromethane. All stock solutions were stored at −20 °C and employed to prepare daily gravimetrically diluted working standard solutions in methanol. Methanol, acetonitrile and dichloromethane solvents (analysis grade) as well as formic acid (reagent grade) and ammonium acetate (reagent grade) were provided by Scharlau (Barcelona, Spain). Solid phase extraction (SPE) was performed using C18 cartridges Extrabond of 3 mL volume and 500 mg sorbent which were also provided by Scharlau. 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). Commercially bottled water stored in polyethylene terephthalate (PET) bottles was employed.

For the synthesis of 4-(3,6-dimethyl-3-heptyl) phenol, boron trifluoride ($BF_3$) diethyl etherate, *n*-hexane, 3,6-dimethyl-3-heptanol and phenol were purchased from Alfa Aesar (Karlsruhe, Germany). The $^{13}C_1$-4-(3,6-dimethyl-3-heptyl)phenol ($^{13}C_1$-NP) was synthesized using $^{13}C_1$-phenol from Cambridge Isotope Laboratories (Andover, MA, USA).

### 2.2. Synthesis of $^{13}C_1$-nonylphenol

The synthesis of $^{13}C_1$-4(3,6-dimethyl-3-heptyl)phenol (or $^{13}C_1$-363 NP) was based on a Friedel–Crafts alkylation of $^{13}C_1$-labeled phenol and a tertiary nonylalcohol employing $BF_3$–ether complex as catalyst [19,20]. The procedure is illustrated in Fig. 1. As can be observed, the selected labeling position corresponds to the carbon linked to the hydroxyl group, which remains in the main fragment ions measured by tandem mass spectrometry.