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Separation of isomers of nonylphenol and select nonylphenol polyethoxylates by high-performance liquid chromatography on a graphitic carbon column

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Abstract

p-Nonylphenol (NP) is a ubiquitous degradation product of nonylphenol polyethoxylate (NPE) surfactants and has been reported to be an endocrine disrupter. It is composed of numerous structural isomers resulting from the various branching patterns of the C-9 group. Twenty-two isomers in a technical mix of NP have been identified with high-resolution capillary GC–MS. In most HPLC analyses, nonylphenol elutes as a single, broad peak. In the method described here, HPLC using a graphite carbon column resulted in the resolution of a technical mixture of NP into 12 peaks or groups of isomers. This method was also applied to select NPEs with one to 10 ethoxy units with similar results. Separation was achieved by gradient elution with 1% acetic acid in water and acetonitrile. Elution of individual isomers 4-butylphenol and 4-propylphenol under the same gradient conditions indicate that increased branching of an alkyl group results in shorter retention times than for the less substituted alkyl groups. This method can be used to fractionate NP based on structure and assess the potential for different isomers (or groups of structurally similar isomers) to act as endocrine disrupters. Published by Elsevier Science B.V.

Keywords: Nonylphenol; Nonylphenol polyethoxylates; Ethoxylates; Surfactants

1. Introduction

Nonylphenol polyethoxylate (NPE) surfactants, one of the most commonly used surfactants worldwide, degrade in wastewater treatment systems to form a variety of metabolites including shorter chain NPE analogs and nonylphenol (NP) by sequential deethoxylation [1,2].

Nonylphenol and short-chain NPEs (~1–3 ethoxy units) are more toxic than their parent compounds and it has been reported that NP has endocrine disrupting characteristics [3–7]. In Europe NPE surfactants have been banned for household use and are being phased out for industrial use. In the United States, the use of NPE surfactants is under extreme scrutiny [1,8].

Nonylphenol, as found in most environmental situations, is not a single compound but a mixture of several isomers due to the branching of the C-9

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group (Fig. 1). Recent papers have reported high-performance liquid chromatography (HPLC) methods to separate NP and NPEs based on the number of ethoxy units using normal-phase [9–13], and reversed-phase [14–18] and carbon column [19] chromatography, but did not further separate the homologues into individual isomers based on the alkyl side chain. In addition, many of these methods do not resolve NP and the shorter chain NPEs (1–4 ethoxy units) as effectively as the longer chain NPEs.

Gas chromatography (GC) and GC–mass spectrometry (MS) have also been used extensively for NP analysis [20,21]. High-resolution GC–MS has resolved a technical mixture of *p*-NP into 22 distinct isomers [22]. With the exception of the recent work of He and Lee [23] who used micellar electrokinetic chromatography, individual isomers have of NP have not been separated by nondestructive methods. Wheeler et al. [22] proposed structures for many of the isomers separated by GC–MS. At present, authentic, individual standards are not available to confirm the proposed structures.

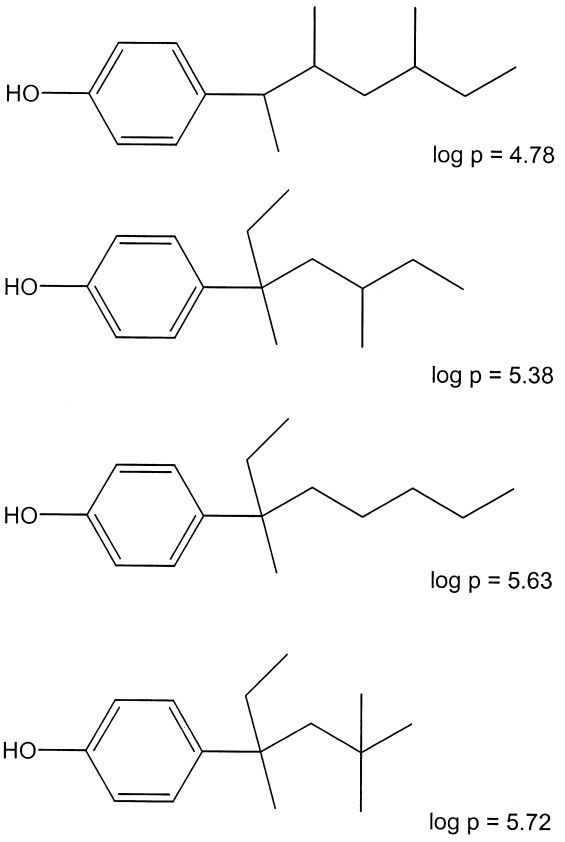

Fig. 1. Representative structures of *p*-nonylphenol (based on Wheeler et al. [22]). Log *P* estimates were calculated using Cambridge Software (Cambridge, MA, USA) ChemDraw Pro Version 4.5, 1997 using ''best method'' calculation (Broto's method).

Comparison of the proposed structures with estrogen, suggests that some isomers may be more effective mimics than others. Additionally, preliminary estimates of log *P* for representative NP structures shows that they range through almost 1 log *P* unit – between 4.7 and 5.6 (Fig. 1), indicating that bioconcentration of isomers will most likely differ. To date, toxicity of individual isomers has not been reported. This is due, in large part, to the inability to effectively separate individual isomers from the mixture for further testing.

Graphite carbon chromatography has been used extensively to separate planar polychlorinated biphenyls (PCBs) from nonplanar congeners and has also been used in the separation of chiral compounds and other structurally similar organic compounds [24–28]. This technique has now been extended to an alkylphenol and related ethoxylates. This paper reports the first separation of nonylphenol and select NPEs into component isomers by HPLC and offers a potential method to separate isomers for further biological and toxicological testing as well as environmental monitoring.

2. Experimental

2.1. Materials

Nonylphenol (technical grade, CAS No. 84852-15-3) was kindly supplied by Dr. R. Yunick of Schenectady Chemical (Schenectady, NY, USA). Nonylphenol ethoxylates [NPE(1–2) (trade name: Igepal CO-210, CAS No. 9016-45-9), NPE(4) (trade name: Igepal CO-430, CAS No. N/A) and NPE(9–10) (trade name: Igepal CO-630, CAS No. 68412-54-4)] were from ChemService (West Chester, PA, USA). [NPE(1–2) indicates NPE with one to two ethoxy units]. 4-*sec*.-Butylphenol, 4-*tert*.-butylphenol, 4-isopropylphenol and 4-propylphenol were from Aldrich (Milwaukee, WI, USA). 4-*n*-

Butylphenol was from TCI America (Portland, OR, USA). Acetonitrile (CH_3CN) was HPLC grade (Burdick and Jackson, Muskegon, MI, USA). Deionized water (Milli-Q) was prepared in the laboratory. Glacial acetic acid was from Aldrich.

2.2. Instrumentation

Separation was performed on a Waters (Milford, MA, USA) Alliance HPLC system with a Model 440 scanning fluorometer (set at 277 nm excitation/300 nm emission with a slit width of 1.2 nm) equipped with a Hypersil (Cheshire, UK) HyperCarb 100 mm, 7 μm mesh graphite carbon column. An ODS-AQ guard column (120 Å, 5 μm, 10×2 mm; YMC, Wilmington, NC, USA) was used in-line with the Hypersil column. Eluents were CH_3CN (A) and 1% (v/v) glacial acetic acid in water (B) and were degassed prior to use. Flow-rates in all cases were 1.0 ml/min. Nominal concentration of each standard was 1 mg/ml in acetonitrile. Injection volumes were 0.1 to 2.0 μl, based on response. The column was thermostated at 30°C. Butyl- and propylphenols were analyzed under the same gradient conditions as NP.

The gradients used were as follows:

For nonylphenol, NPE(1–2) and butyl- and propylphenols: 0–15 min: A–B (50:50), 15–60 min: linear gradient to A–B (70:30), 60–100 min: hold at A–B (70:30).

For NPE(4) and NPE(9–10): 0–15 min: A–B (70:30), 15–60 min: linear gradient to 100% A, 60–140 min: hold at 100% A.

3. Results and discussion

Fig. 2 shows a chromatogram of *p*-nonylphenol eluted on the HyperCarb column equipped with an ODS-AQ guard column. Twelve distinct peaks are visible along with several shoulders. Most nonylphenol technical mixtures contain small amounts of octyl- and decylphenol contamination. Peaks in Fig. 2 eluting before 20 and after 40 min are presumed to be octyl- and decylphenol, respectively. At present, individual isomer standards are not available for comparison. Similarly, Figs. 3, 4 and 5 show chromatograms of NPE(1–2), NPE(4) and NPE(9–10), respectively.

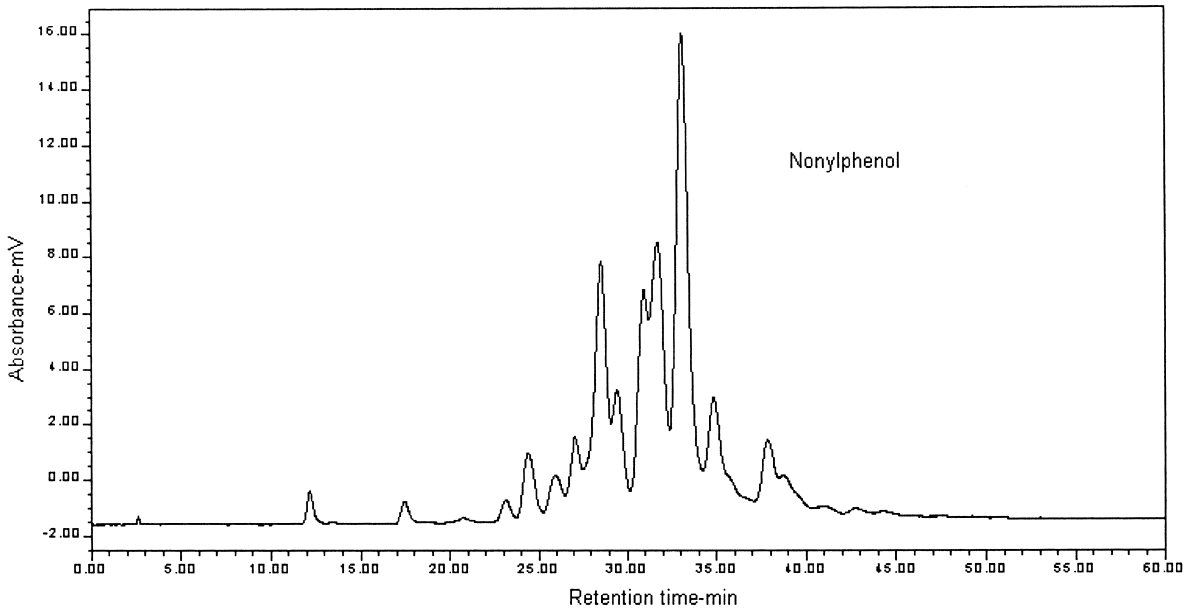

Fig. 2. HPLC chromatogram of nonylphenol. Column: ODS-AQ guard column (120 Å, 5 μm, 10×2 mm; YMC) with Hypersil HyperCarb 100 mm, 7 μm mesh graphite carbon column, thermostated at 30°C. Eluents: CH_3CN (A) and 1% (v/v) glacial acetic acid in water (B), flow-rate: 1.0 ml/min. Gradients: 0–15 min: A–B (50:50), 15–60 min: linear gradient to A–B (70:30), 60–100 min: hold at A–B (70:30).

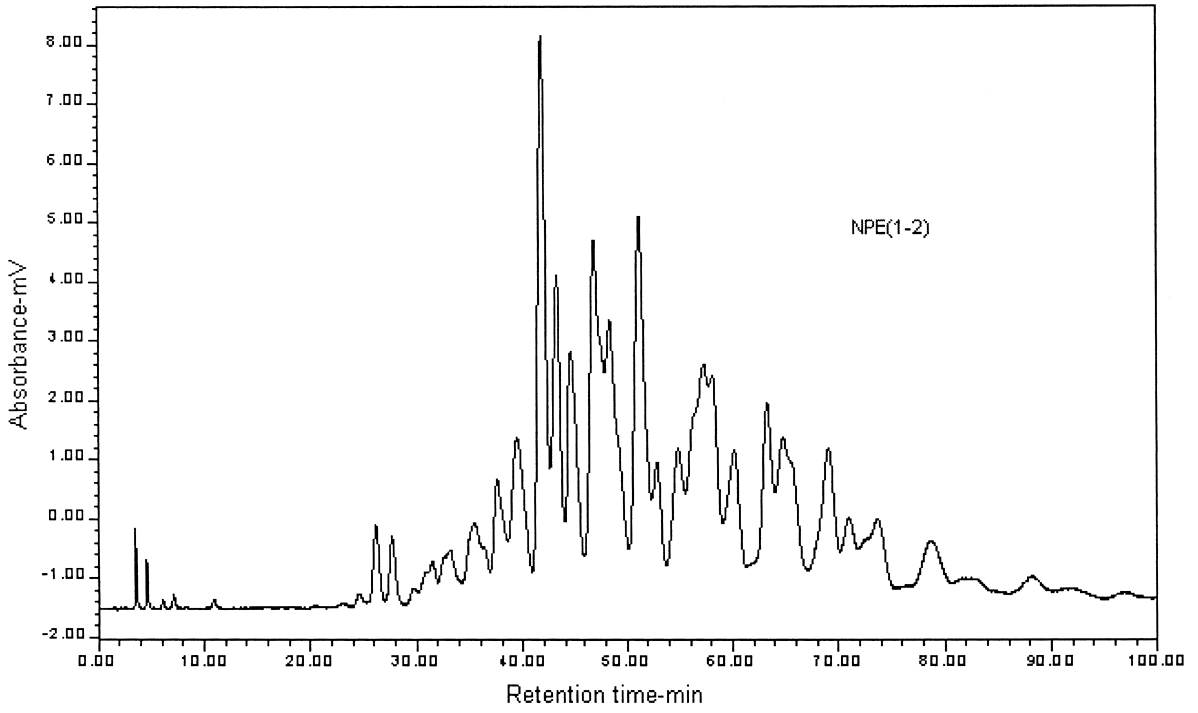

Fig. 3. HPLC chromatogram of NPE(1–2). Column: ODS-AQ guard column (120 Å, 5 μm, 10×2 mm; YMC) with Hypersil HyperCarb 100 mm, 7 μm mesh graphite carbon column, thermostated at 30°C. Eluents: CH_3CN (A) and 1% (v/v) glacial acetic acid in water (B), flow-rate: 1.0 ml/min. Gradients: 0–15 min: A–B (50:50), 15–60 min: linear gradient to A–B (70:30), 60–100 min: hold at A–B (70:30).

Retention times for the propyl- and butylphenols are listed in Table 1. These were run to assess the effect of branching on retention time. Isobutylphenol was not commercially available. The unsubstituted isomers (4-*n*-propyl- and 4-*n*-butylphenol) associated most strongly with the planar graphite in the column and were thus retained longer than the more branched isomers like 4-*tert*.-butylphenol. This implies that the early eluting nonylphenol peaks are more highly branched while less substituted NP isomers elute later. Fractionation and mass spectral analysis are in progress to confirm this.

Two other guard columns were tested in conjunction with the HyperCarb column. Neither the YMC carotenoid (C_{30}, 120 Å, 5 μm, 10×2 mm, YMC) nor a Waters Bondpak C_{18} (125 Å, 10 μm; Waters) improved chromatography. Use of the ODS-AQ guard did improve separation slightly over use of the HyperCarb column alone. The carotenoid column (C_{30}, 120 Å, 5 μm, 150×3 mm; YMC) and ODS-AQ (C_{18}, 120 Å, 5 μm, 100×3 mm; YMC) columns were also investigated in-line with the HyperCarb column, the results were the same as with the respective guard column, but resulted in longer retention times and somewhat higher backpressures. Gradient elution with these solvents provided separation that was far superior to isocratic conditions.

While this method was developed primarily as a preparatory method, injections of 10 ng on-column (based on 0.1 μl injections of a 0.10 μg/μl NP standard) still showed all 12 NP peaks with at least a 3:1 signal-to-noise ratio. With injections of less than 10 ng, the leading and trailing peaks of NP are lost to baseline noise, but the largest peaks remain easily distinguished.

Separation of NP isomers with the HyperCarb column was not as effective as that of Wheeler et al. [22] with high-resolution GC–MS, but this nondestructive method shows promise as a method for separating NP into component isomers for further testing. This method may be useful to examine sorption of different isomers to sediments, bioavail-

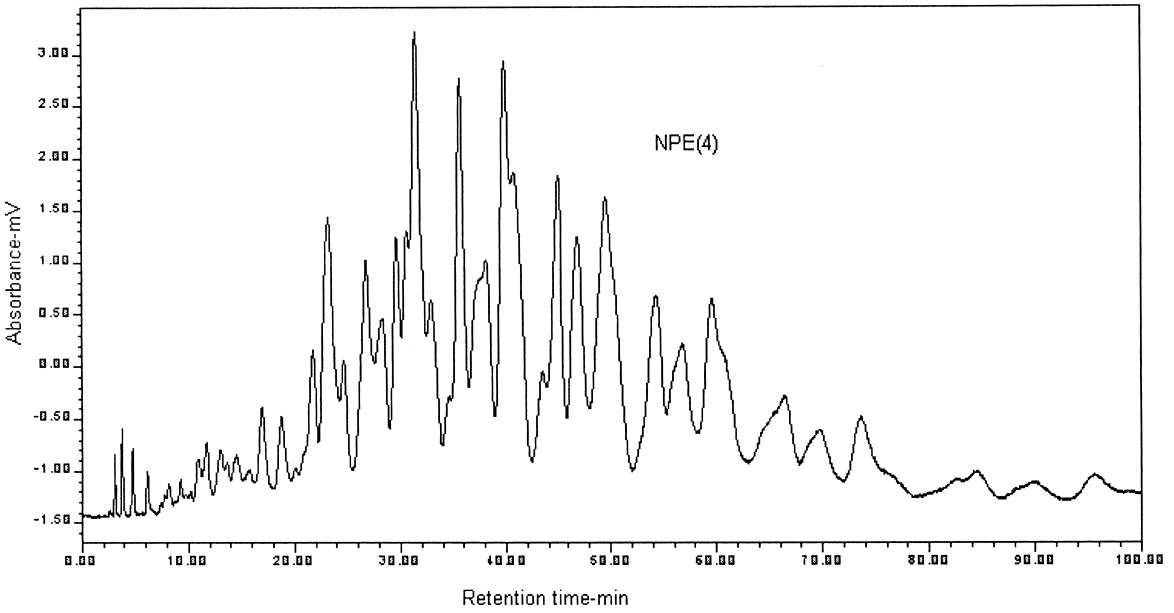

Fig. 4. HPLC chromatogram of NPE(4). Column: ODS-AQ guard column (120 Å, 5 μm, 10×2 mm; YMC) with Hypersil HyperCarb 100 mm, 7 μm mesh graphite carbon column, thermostated at 30°C. Eluents: CH_3CN (A) and 1% (v/v) glacial acetic acid in water (B), flow-rate: 1.0 ml/min. Gradients: 0–15 min: A–B (70:30), 15–60 min: linear gradient to 100% A, 60–140 min: hold at 100% A.

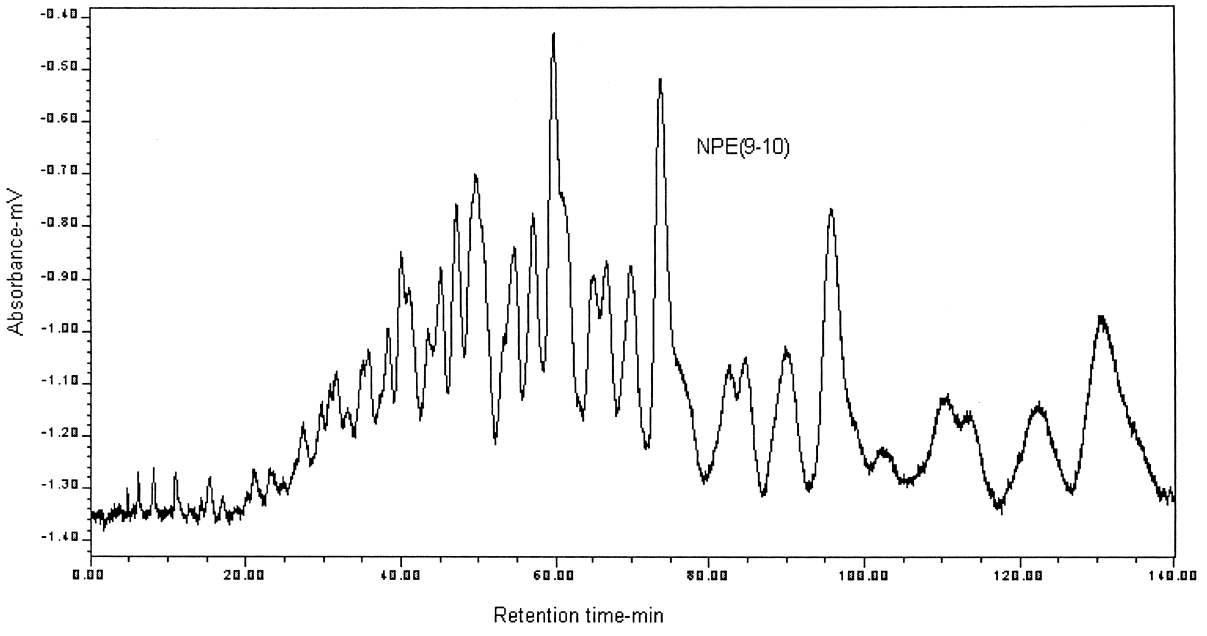

Fig. 5. HPLC chromatogram of NPE(9–10). Column: ODS-AQ guard column (120 Å, 5 μm, 10×2 mm; YMC) with Hypersil HyperCarb 100 mm, 7 μm mesh graphite carbon column, thermostated at 30°C. Eluents: CH_3CN (A) and 1% (v/v) glacial acetic acid in water (B), flow-rate: 1.0 ml/min. Gradients: 0–15 min: A–B (70:30), 15–60 min: linear gradient to 100% A, 60–140 min: hold at 100% A.

Table 1
Retention times of butyl- and propylphenols under the same elution conditions as *p*-nonylphenol

Compound	Retention time (min)
4-*n*-Butylphenol	12.69
4-*n*-Propylphenol	7.48
4-*sec*.-Butylphenol	6.67
4-*tert*.-Butylphenol	4.92
4-Isopropylphenol	4.78

ability based on structure or isomer specific estrogenicity. Future work is planned to improve chromatography by using a longer column with a smaller mesh. In addition, work is underway to obtain mass spectra and nuclear magnetic resonance spectra of the fractions for comparison with those of Wheeler et al. [22].

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