



ELSEVIER

Journal of Chromatography A, 925 (2001) 297–301

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Recovery of 4-nonylphenol and 4-nonylphenol ethoxylates from river sediments by pressurised liquid extraction

Sara Valsecchi^{a,*}, Stefano Polesello^a, Silvano Cavalli^b

^aCNR-IRSA, via Mornera 25, I-20047 Brugherio, Italy

^bDionex, via Tulipani 5, I-20090 Pieve Emanuele, Italy

Received 2 April 2001; received in revised form 6 June 2001; accepted 8 June 2001

Abstract

The pressurised liquid extraction (PLE) of 4-nonylphenol (4-NP) with methanol (100°C and 100 atm) from river sediments was compared with methanolic Soxhlet extraction, the standard method for the sediment analysis. The PLE method showed a precision (average RSD ranged from 6 to 33%) and an accuracy (average recovery 85 and 87% for 4-NP and 4-NPE, respectively) comparable to those of Soxhlet. The extraction was performed on river sediments and no organic carbon content influence was found. The comparative study presented in this paper demonstrates that PLE is an alternative suitable extraction method for 4-nonylphenol and 4-nonylphenol ethoxylate determination in sediments. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Pressurised liquid extraction; 4-Nonylphenol; 4-Nonylphenol ethoxylate

1. Introduction

Nonylphenol ethoxylates (NPE) are a class of the broader group of compounds known as alkylphenol ethoxylates (APE). NPE have been used for more than 40 years as detergents, emulsifiers, wetting agents and dispersing agents. NPE-containing products are used in many sectors, including textile processing, pulp and paper processing, paints, resins and protective coatings. A variety of cleaning products, degreasers and detergents are also available for institutional and domestic use [1]. APE can be biodegraded in the environment through a mechanism of stepwise loss of ethoxy groups to form lower

ethoxylated congeners, carboxylated products and alkylphenols (AP). The latter derivatives are more persistent than their parent compounds and may accumulate in sediments under anaerobic conditions. As nonylphenol (NP) has been reported to cause a number of estrogenic responses in a variety of aquatic organisms [2], a certain number of studies have been devoted to determine the environmental occurrence and fate of NP and its parent compounds [1–3]. The field studies have shown that concentration of NP is generally low in treated effluents, as it degrades and sorbs to sludge particles. Adsorbed NP tends to deposit and accumulate in river sediments: the extraction of NP and parent compounds from sediments has been carried out by conventional systems such as Soxhlet extraction with non-polar [4–5] or polar solvents [6], or by ultrasonication in static [7] or flow-through mode [8]. The growing

*Corresponding author. Tel.: +39-039-200-4303; fax: +39-039-200-4692.

E-mail address: valsecchi@irsa.rm.cnr.it (S. Valsecchi).

interest in the simultaneous determination of NPE and their degradation product, NP, has induced to explore the feasibility of less solvent and time consuming methods such as dynamic supercritical fluid extraction (SFE) with methanol modified carbon dioxide [9], pressurised liquid extraction (PLE) with methanol in a SFE apparatus [10] or PLE with non-polar solvents [11]. PLE was described to be very promising in simultaneous NP and NPE extraction from marine sediments, but the authors gave only little information on method validation parameters. Another advantage of PLE is the ease of transfer from Soxhlet method to PLE, because the same solvent used in the Soxhlet extraction can be chosen for PLE. In our laboratory we routinely determine 4-NP and 4-NPE using Soxhlet extraction with methanol and reversed-phase–high-performance liquid chromatography (RP–HPLC) with fluorimetric detection, following a previously described method [6]. The aim of the present work was to compare PLE with Soxhlet extraction in the determination of moderately polluted river sediments.

2. Experimental

2.1. Materials

Dichloromethane for residue analysis and HPLC grade methanol (Fluka Chemie, Buchs, Switzerland) were used without further purification. Technical grade 4-nonylphenol (4-NP) (Aldrich Chemie, Steinheim, Germany), 4-nonylphenol ethoxylate with 9 and 10 ethoxy units (4-NPE [9,10]), (Chem Service, West Chester, PA, USA) and technical *t*-octylphenol ethoxylate with an average number of 9.5 ethoxy units (Triton X-100, Fluka) were used for standard preparation and spiking experiments. Neutral aluminium oxide for chromatography was purchased from Fluka. Water for chromatography was purified (18 M Ω cm⁻¹ quality) by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Sediment pretreatment and spiking

Sediments, with different organic carbon and alkylphenol content, were collected by a grab from the Po river (Northern Italy), upstream (sample

called “Po1”) and downstream (sample “Po2”) the confluence of its most polluted tributary, the Lambro river, and one from the Lambro river itself (sample “Lambro”). The day after the collection, sediments were homogenised, freeze-dried and stored in a tightly closed brown glass bottle in a desiccator at room temperature until the extraction. The recovery experiments were performed using recently spiked sediments. A volume of 1 ml of a 20 mg l⁻¹ 4-NP and 4-NPE [9,10] methanolic solution were added to about 6 g sediments, stirred for half an hour and kept at 40°C for 1 day.

Total organic carbon was determined by high temperature oxidation using a Carlo Erba (Milano, Italy) NA1500 series 2 C/H/N/O/S analyser according to a published procedure [12].

2.3. Soxhlet extraction and PLE

Six grams of freeze-dried sediment were Soxhlet extracted with 400 ml of methanol for 10 h (9 cycles/h) [6]

PLE extraction was performed loading a 11 ml stainless steel extraction cell with the freeze-dried sediment (about 6 g). Two cycles of extraction with methanol (about 15 ml) at 100°C, 100 atm, in static mode (flush 60%, purge 90 s) for 5 min were carried out [10]. Five samples and one blank were simultaneously run in each set of experiments, both in PLE and Soxhlet procedures. No memory effect between consecutive analyses of real samples was detected.

2.4. Clean-up

Soxhlet and PLE extracts were subjected to the same clean-up procedure. After the addition of 1 ml of the surrogate solution (20 mg ml⁻¹ Triton-X in methanol), extracts were concentrated by rotary evaporation to 1–2 ml under vacuum and transferred onto a 15% water deactivated neutral alumina column (about 8 g in a 1.5 cm I.D., 4.5 cm h glass column) which was previously conditioned with methanol. The column was eluted with 10 ml dichloromethane. The purified extract was concentrated to 0.5 ml under a gentle stream of nitrogen and filtered through a 0.45 μ m PTFE filter (Millipore).

2.5. Analysis

Analytical separations were performed by an HPLC system consisted of a 9012 Q ternary pump (Varian, Walnut Creek, CA, USA), with a 10 μl sample loop, and a FP-920 fluorescence detector (Jasco, Tokyo, Japan), set at 230 nm excitation and 302 nm emission. The separation was achieved on a C_{18} LiChrospher 60 RP-select B, 250×4.6 mm, 5 μm , (Merck, Darmstadt, Germany) at 25°C.

Eluent solvents for gradient elution were water and methanol delivered at a constant flow-rate of 1 ml min^{-1} . The elution profile began with a 5 min isocratic step at 30% water and 70% methanol, followed by a 20 min linear gradient from initial condition eluent ratio to 20% water and 80% methanol and by another 5 min isocratic step at the final eluent ratio.

A Varian Star 4.51 chromatography workstation was used for data collection and processing.

Six-level linear calibration curves, ranging from 1 to 40 mg l^{-1} , were generated for Triton-X, 4-NP and 4-NPE [9,10]. Regressions were performed using means of the peak areas acquired on 17 consecutive days. The RSD of the peak areas ranged from 2% to 30% ($n=3-8$) for upper and lower standard levels, respectively. From RSD data, a LOD value of 1 mg l^{-1} was estimated corresponding to a LOD of 0.08 $\mu\text{g g}^{-1}$ in the sediments.

Compounds were identified by comparison with commercial standards and nonylphenol was confirmed by selected GC–MS–SIM (selected masses: 220, 191, 135 m/z) analyses carried out on the same extracts, according to the de Voogt et al. procedure [6]. GC–MS system was composed by a gas

chromatograph HP-6890 (Hewlett–Packard, Palo Alto, CA, USA), equipped with a HP-5 capillary column (30 m, 0.25 mm I.D., 0.25 μm f.t.) and a mass detector HP 5973, operating in EI mode at 70 eV. A volume of 1 μl of the sample was injected in a split–splitless mode (injector temperature 250°C), and the temperature oven was programmed from 100°C to 280°C at 10°C min^{-1} .

3. Results and discussion

Soxhlet extraction is the conventional standard method for the analysis of sediment but it takes a long time and needs high quantities of solvent. For this reason we tested the feasibility of the PLE to extract alkylphenol from sediment matrix.

Three river sediments with different organic carbon and alkylphenol and their ethoxylates contents (Table 1) were PLE and Soxhlet extracted with the same solvent (methanol) and the extracts were subjected to the same procedure: after the addition of a Triton-X solution as internal standard, and clean-up on an alumina column, extracts were analysed by RP–HPLC with direct fluorescence detection at 230 nm excitation and 302 nm emission [6,9,13]. RP–HPLC with fluorimetric detection has become the favoured method of routine analysis of alkylphenols and their ethoxylates in environmental samples because it is able to separate and quantitate the various homologues and oligomers by length of the alkyl chains. Since RP–HPLC does not baseline resolve the ethoxylate oligomers, nonylphenol ethoxylate content was determined as the sum of the partially coeluted peaks.

Table 1
Organic carbon content, 4-NP and 4-NPE concentrations in Soxhlet and PLE extracts of river sediments

	Organic carbon %		4-Nonylphenol $\mu\text{g g}^{-1}$	4-Nonylphenol ethoxylate $\mu\text{g g}^{-1}$
Lambro river sediment	3.49	PLE	2.9 \pm 0.6 (5)	5.7 \pm 0.7 (5)
		Soxhlet extraction	4.7 \pm 0.3 (3)	4.1 \pm 0.1 (3)
Po2 river sediment	2.54	PLE	2.9 \pm 0.6 (5)	10.4 \pm 0.6 (5)
		Soxhlet extraction	2.8 \pm 0.8 (4)	11.2 \pm 2.5 (4)
Po1 river sediment	1.08	PLE	0.33 \pm 0.11 (5)	1.5 \pm 0.5 (5)
		Soxhlet extraction	0.45 \pm 0.30 (4)	2.9 \pm 0.8 (6)

Results expressed as mean \pm SD. Number of replicates in brackets.

Triton-X was chosen as the surrogate compound because preliminary sample extraction showed that octylphenol ethoxylates were less than the method detection limit ($0.08 \mu\text{g g}^{-1}$). The mean recovery of added Triton-X in all analysed samples was $77 \pm 18\%$ ($n=62$). Fig. 1 shows HPLC chromatograms of a

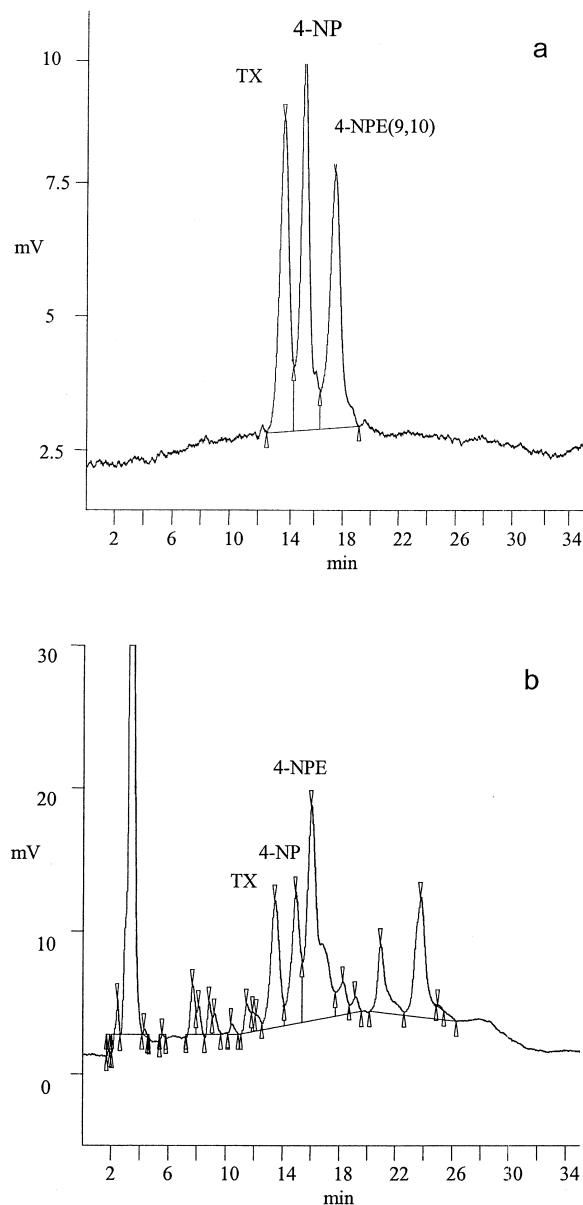


Fig. 1. Chromatograms of (a) standard solution (10 mg l^{-1}) and (b) a river sediment (sample Po2) extracted with PLE and spiked with Triton-X. Conditions as in the experimental section.

standard solution and of a PLE sediment extract spiked with 20 mg l^{-1} Triton-X.

The precision of the PLE and Soxhlet methods was evaluated by the RSDs of a series of extractions ($n=3-5$) carried out on different days (Table 1). RSDs, for both compounds, ranged from 2 to 67% for the Soxhlet and from 6 to 33% for the PLE, the worst values corresponding to the lowest concentration ($0.3-0.4 \mu\text{g g}^{-1}$). These values are an estimate of the total analytical variance, including sample homogeneity, extraction and chromatographic determination, the latter having coefficients of variation of 2% at the highest level and 30% at the lowest one.

The comparison of the 4-NP and 4-NPE concentrations determined in sediments (Table 1), characterised by different organic carbon content, by parallel PLE and Soxhlet extraction, showed that the PLE method was in a satisfactory agreement with the conventional Soxhlet extraction method in the explored alkylphenol ($0.3-3 \mu\text{g g}^{-1}$) and organic carbon content range (1–3.5%). The discrepancies between the two methods can be attributed, especially in the low concentration range, to different interferences from extracted matrices, but are much less than those reported by Ding and Fann [10]. Those authors, given scarce information about Soxhlet procedure and solvent, showed that PLE is more effective in extracting 4-NP from sediment samples than Soxhlet extraction, since the concentrations of 4-NP in a single sample extracted by PLE and Soxhlet were 18 and $7 \mu\text{g g}^{-1}$, respectively.

In order to evaluate the accuracy of the PLE method, recovery experiments were carried out by spiking sediments with methanol solutions of 4-NP and 4-NPE [9,10], either as a single compound or as a mixture (Table 2). The mean recoveries obtained with PLE ($85 \pm 22\%$ ($n=13$) and $87 \pm 13\%$ ($n=15$) for 4-NP and 4-NPE, respectively) were comparable to Soxhlet data ($79 \pm 24\%$ and $82 \pm 12\%$ for 4-NP and 4-NPE, respectively).

Our recovery data obtained with PLE using polar solvent do not differ from those obtained with PLE using a hexane/acetone mixture by Shang et al. [11], who reported recoveries of 65–93% with RSDs of 3.5–15.9% for all NPE oligomers. Similar data have been obtained also by supercritical fluid extraction [9] (57–90% for 4-NP and 50–90% for 4-NPE),

Table 2
Recovery results for 4-NP and 4-NPE

	4-Nonylphenol		4-Nonylphenol ethoxylate	
	Amount added $\mu\text{g g}^{-1}$	Mean spiked recovery %	Amount added $\mu\text{g g}^{-1}$	Mean spiked recovery %
<i>PLE</i>				
Lambro river sediment	1.9	91±16 (4)	9.5	86±10 (3)
Po2 river sediment	1.9	103±8.9 (5)	9.4	88±14 (6)
Po1 river sediment	1.9	58±10 (4)	4.7–9.4	86±15 (6)
<i>Soxhlet</i>				
Different river sediments samples	3.5	79±24 (4)	3.6	82±12 (4)

Results expressed as mean±SD. Number of replicates in brackets.

which in fact required a dynamic extraction step at very high pressure (≥ 450 atm).

Our results were rather worse than recovery (106%) and RSDs (4–5%) reported by Ding and Fann [10] for 4-NP determination in sediments by PLE, but in that case samples were spiked with a much higher concentration ($20 \mu\text{g g}^{-1}$) of 4-NP and a more sensitive and precise analytical method (GC–MS) was used.

4. Conclusion

The comparative study presented in this paper demonstrated that PLE is a suitable alternative extraction method for the 4-NP and 4-NPE determination in river sediments. The PLE method showed a precision (average RSDs ranging from 6 to 33%) and an accuracy (average recoveries of 85 and 87% for 4-NP and 4-NPE, respectively) comparable to those of Soxhlet, the standard method for the analysis of sediment. These data confirm that PLE can easily substitute Soxhlet in an already developed method, without the need of substantial changes in the analytical procedure.

A small amount of solvent consumption (15 ml), reduced extraction time (about 30 min) and a considerable improvement in operator safety are the most important advantages of the PLE method with respect to the Soxhlet apparatus. In spite of its high initial cost, PLE equipment shows to be a cost-effective alternative to Soxhlet in sediment and soil extraction methods.

Acknowledgements

Authors thank L. Viganò (CNR-IRSA Brugherio) for providing sediment samples and L. Patrolocco (CNR-IRSA Roma) for carbon content analyses

References

- [1] Environment Canada, Priority substances list assessment report: nonylphenol and its ethoxylates, Draft, March 2000.
- [2] M. Servos, *Water Qual. Res. J. Can.* 34 (1999) 123.
- [3] D.T. Bennie, *Water Qual. Res. J. Can.* 34 (1999) 79.
- [4] A. Marcomini, B. Pavoni, A. Sfriso, A. Orio, *Mar. Chem.* 29 (1990) 307.
- [5] D.Y. Shang, M.G. Ikonou, R.W. Macdonald, *J. Chromatogr. A* 849 (1999) 467.
- [6] P. de Voogt, K. de Beer, F. van der Wielen, *Trends Anal. Chem.* 16 (1997) 584.
- [7] M.A. Blackburn, S.J. Kirby, M.J. Waldock, *Mar. Pollut. Bull.* 38 (1999) 109.
- [8] P.L. Ferguson, C.R. Iden, B.J. Brownawell, *Anal. Chem.* 72 (2000) 4322.
- [9] A. Kreisselmeier, H.W. Dürbeck, *J. Chromatogr. A* 775 (1997) 187.
- [10] W. Ding, J.C.H. Fann, *J. Chromatogr. A* 866 (2000) 79.
- [11] D.Y. Shang, R.W. Macdonald, M.G. Ikonou, *Environ. Sci. Technol.* 33 (1999) 1366.
- [12] M. Pettine, L. Patrolocco, M. Camusso, S. Crescenzo, *Estuar. Coast. Shelf Sci.* 46 (1998) 127.
- [13] S.A. Snyder, T.L. Keith, D.A. Verbrugge, E.M. Snyder, T.S. Gross, K. Kannan, J.P. Giesy, *Environ. Sci. Technol.* 33 (1999) 2814.