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Note

Determination of priority pollutant nitrophenols in water by high-performance liquid chromatography

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4-Nitrophenol, 2-nitrophenol, 2,4-dinitrophenol and 4,6-dinitro-2-methylphenol are listed as priority pollutants by the U.S. Environmental Protection Agency[1]. 4,6-Dinitro-2-methylphenol is used as an insecticide, 2,4-dinitrophenol as a wood preservative and in the manufacture of dyes, and 2-nitrophenol and 4-nitrophenol for the manufacture of a wide variety of industrial chemicals. 4-Nitrophenol is also formed by hydrolysis of the insecticide parathion.

Nitrophenols are toxic compounds[2]. They enter the environment in different ways, *e.g.* as degradation products from insecticides, wood preservatives etc., and in industrial effluents. The nitrophenols used as insecticides lead to soil contamination. Further, the insecticides and their degradation products are leached into both surface water and groundwater. Therefore methods for their determination at low concentrations are required. Gas chromatography has been used, but this requires a derivatization procedure, *e.g.* acetylation[3]. Recently high-performance liquid chromatography (HPLC) has proved useful[4–7]. This paper reports an HPLC method for direct determination of priority pollutant nitrophenols in water at micrograms per litre concentrations (ppb level). If this HPLC method is combined with a pre-extraction procedure, the nitrophenols can be determined at nanogram per litre concentrations (ppt level).

EXPERIMENTAL

Chemicals

The nitrophenols and propylene glycol were obtained from Fluka (Buchs, Switzerland), methanol from May & Baker (Dagenham, U.K.), dichloromethane from Merck (Darmstadt, F.R.G.) and $5 \cdot 10^{-3}$ *M* tetrabutylammonium phosphate buffered at pH 7.5 (PIC-A reagent) from Waters Assoc. (Milford, MA, U.S.A.). Standard solutions of the nitrophenols were prepared in millipore filtered water.

High-performance liquid chromatography

A liquid chromatograph consisting of a Waters Model 6000A pump, a Waters U6K injector and a Waters Model 440 ultraviolet absorbance detector (254 and 405 nm) was used. A Merck LiChrosorb RP-18, 5-μm column (250 × 4.6 mm I.D.) was used. The column was operated at ambient temperature with 30 ml of PIC-A reagent in 1 l of water–methanol (55:45) as the mobile phase at a flow-rate of 1 ml/min.

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UV spectra

UV spectra of the nitrophenols were obtained in water at a concentration of 10 μg/ml. The pH was adjusted with 1 *M* hydrochloric acid or 1 *M* sodium hydroxide solution. A Unicam SP 800 A spectrophotometer was used.

Extraction from water samples

Water samples (tap water) containing 500 ng/l and 50 ng/l of each of the four nitrophenols were preconcentrated. The acidity of a 500 ml sample was adjusted to pH 1.5 with concentrated sulfuric acid. The sample was extracted with 4 × 25 ml of dichloromethane. The combined extracts were evaporated to *ca.* 7 ml under reduced pressure at 30°C, after addition of 50 μl of propylene glycol as keeper to reduce loss of the nitrophenols during the evaporation procedure. The remaining dichloromethane was removed by evaporation under nitrogen. To the residue 1 ml of water was added, and finally 100 μl was analyzed by HPLC.

RESULTS AND DISCUSSION

In neutral and acidic media the nitrophenols have an absorption maximum between 360 and 420 nm; the maximum is shifted towards lower wavelengths in acidic media (Fig. 1). In this method measurements were carried out at 405 nm. At this

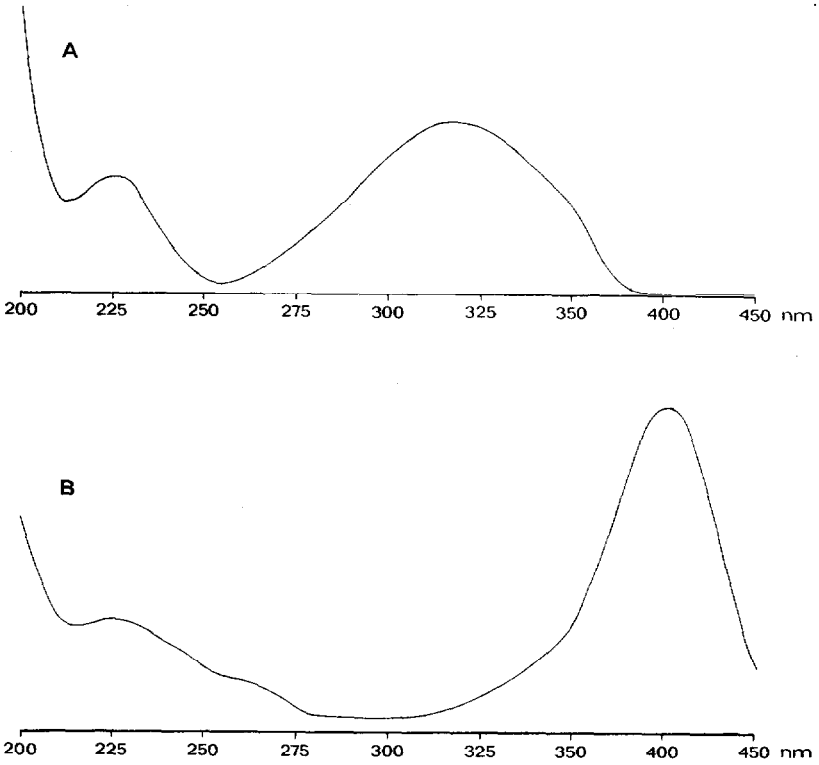

Fig. 1. UV spectra of 10 μg/ml solutions of 4-nitrophenol in water at pH 5.0 (A) and pH 7.0 (B).

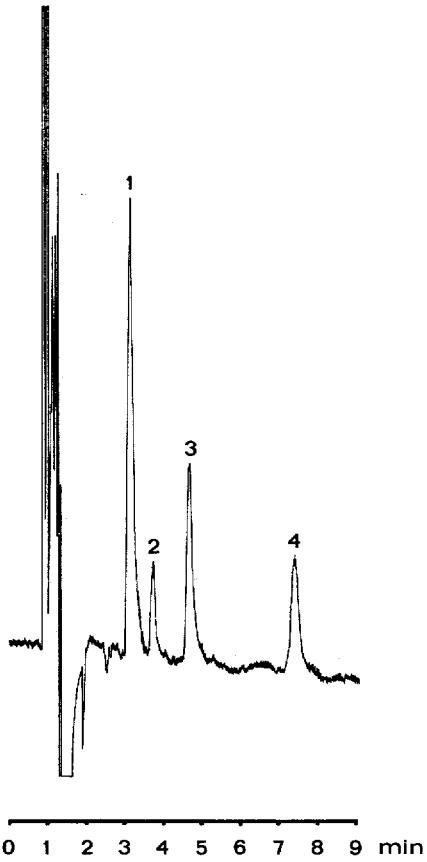

Fig. 2. High-performance liquid chromatogram of 1000 μl of tap water spiked with 10 μg/l of 4-nitrophenol (1), 2-nitrophenol (2), 2,4-dinitrophenol (3) and 2,4-dinitro-2-methylphenol (4). Column, LiChrosorb RP-18, 5 μm (250 × 4.6 mm I.D.); mobile phase, 30 ml of PIC-A reagent in 1 l of water–methanol (55:45); flow-rate, 1 ml/min; absorption, 405 nm; a.u.f.s., 0.005; recorder setting, 10 mV.

TABLE I

DETECTION LIMITS AND REPRODUCIBILITY FOR DIRECT DETERMINATION OF NITROPHENOLS IN WATER

Compound	*Detection limit* (μg/l)*	*R.S.D. (%) at 5 μg/l (n = 6)*
2-Nitrophenol	2	6.2
4-Nitrophenol	0.5	2.7
2,4-Dinitrophenol	1	2.7
4,6-Dinitro-2-methylphenol	2	5.1

* Sample size 1000 μl.

TABLE II

REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS FOR NITROPHENOLS IN THE 5–100 ng RANGE

Compound	*Regression equation**	*Correlation coefficient*	*Number of data points*
2-Nitrophenol	$y = 0.199 + 0.827x$	1.0	6
4-Nitrophenol	$y = -0.0362 + 0.985x$	1.0	6
2,4-Dinitrophenol	$y = 0.448 + 0.797x$	1.0	6
4,6-Dinitro-2-methylphenol	$y = -0.230 + 1.52x$	1.0	6

* x = amount injected (ng); y = top height (mm).

wavelength there are practically no interference problems, and highly specific detection of nitrophenols at low concentrations is possible.

A reversed-phase ion-pair HPLC system with PIC-A reagent as ion-pairing agent[6] was found to give good resolution of the priority pollutant nitrophenols. Fig. 2 illustrates a chromatogram of a tap water sample spiked with the nitrophenols at a concentration of 10 μg/l.

The detection limits for direct determination of the nitrophenols in water are in the low ppb range (Table I). Although injection volumes of 1000 μl are used, the chromatograms show no noticeable band broadening (Fig. 1). Detection limits are stated for a signal-to-noise ratio of 3. Table I lists the relative standard deviations (R.S.D.) obtained for direct determination of the nitrophenols. R.S.D. values between 2% and 6% are satisfactory for determination at 5 μg/l.

Regression analysis showed a linear correlation between injected amount and measured peak height for each of the nitrophenols in the 5–100 ng range (Table II). By combining the described HPLC method with a pre-extraction procedure it is possible to determine the nitrophenols in water at ppt level. The nitrophenols are extracted from the water sample with dichloromethane. To prevent loss of the nitrophenols under the evaporation procedure propylene glycol is used as a keeper. Detection limits of 25 and 50 ng/l were obtained in water (Table III). Fig. 3 shows a chromatogram of a tap water sample spiked with 50 ng/l of each of the priority pollutant nitrophenols. The chromatogram is almost free of interference owing to the high wavelength of detection (405 nm). The recoveries obtained by extraction of

TABLE III

DETECTION LIMITS AND RECOVERY DATA FOR ANALYSIS OF NITROPHENOLS IN WATER WITH PRE-EXTRACTION

Compound	*Detection limit (ng/l)*	*Recovery (%) at 500 ng/l**	*Recovery (%) at 50 ng/l**
2-Nitrophenol	50	67 ± 5	77 ± 16
4-Nitrophenol	25	51 ± 4	70 ± 13
2,4-Dinitrophenol	25	97 ± 1	88 ± 11
4,6-Dinitro-2-methylphenol	50	95 ± 2	97 ± 5

* ± values are standard deviations (n = 6); sample size, 100 μl.

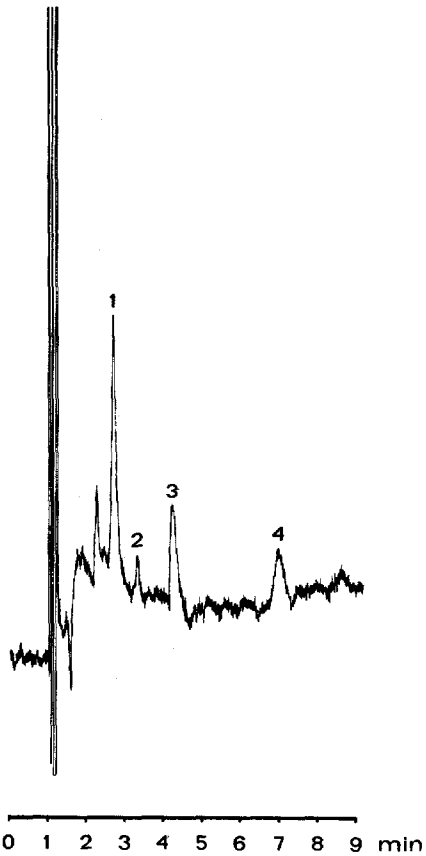

Fig. 3. High-performance liquid chromatogram of tap water spiked with 50 ng/l of 4-nitrophenol (1), 2-nitrophenol (2), 2,4-dinitrophenol (3) and 2,4-dinitro-2-methyl-phenol (4). Extraction procedure as described in text. Chromatographic conditions as in Fig. 2 except recorder setting of 5 mV.

the nitrophenols from spiked water samples are listed in Table III for concentrations of 500 ng/l and 50 ng/l, respectively. Considering the low concentration levels, the obtained recoveries are satisfactory. The losses of 2- and 4-nitrophenol are probably due to incomplete extraction of these very polar solutes.

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