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IDENTIFICATION OF NITROPHENOLS IN RAIN-WATER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTODIODE ARRAY DETECTION

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SUMMARY

Nitrophenols were extracted from rain-water with dichloromethane in two steps. Isocratic separation by high-performance liquid chromatography was achieved with methanol–phosphate buffer (40:60) at pH 3.25. A photodiode array detector was employed to detect the individual nitrophenols at their optimum wavelengths and to identify these compounds by comparison of their UV spectra with those of reference compounds. Depending on the individual compound and the extraction method, the recoveries ranged from 26 to 100% while the detection limit was 0.1–0.5 μg/l. 4-Nitrophenol, 2,4-dinitrophenol, 2,6-dimethyl-4-nitrophenol, 4,6-dinitro-2-methylphenol and most likely 3-methyl-4-nitrophenol were identified in rain-water.

INTRODUCTION

Nitrophenols and in particular dinitrophenols are toxic compounds[1]. They uncouple the oxidative phosphorylation and are used in part as pesticides. 4-Nitrophenol, 2-nitrophenol, 2,4-dinitrophenol (DNP) and 4,6-dinitro-2-methylphenol (DNOC) are listed as priority pollutants by the US Environmental Protection Agency[2]. Although nitrophenols have been rarely identified in surface water[3], they were found recently in relatively high concentrations in rain-water[4–7]. It is most likely that nitrophenols in the atmosphere are formed by photochemical reaction of aromatic hydrocarbons such as benzene and toluene with NO_x and OH radicals as demonstrated by smog chamber studies[8,9].

So far, nitrophenols in rain-water have been determined by gas chromatography–mass spectrometry (GC–MS) without prior derivatization. However, although adequate GC behaviour of phenols is observed if new fused-silica colums with immobilized stationary phases are used[10,11], considerable tailing in particular of nitrophenols is observed after repeated use of the column due to adsorption of these polar compounds at the active centres of the column. Thus, phenols are usually derivatized prior to their determination by GC[12].

Alternatively, phenols in water can be analyzed by high-performance liquid

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chromatography (HPLC)[13–23]. While phenols have been determined as their 4-aminoantipyrine derivatives[13–15], most often the underivatized compounds are used and an UV detector is employed. In these studies only a very limited number of nitrophenols and only spiked samples were analyzed. We report here the analysis of sixteen nitrophenols by HPLC with photodiode array detection. The method was applied to the analysis of rain-water. It is demonstrated that the use of a photodiode array detector is particularly valuable for an unambiguous identification of these compounds in real environmental samples.

EXPERIMENTAL

Instruments

The analysis was performed with a Varian Model 5000 liquid chromatograph and a Waters Model 990 photodiode array detector using a Merck LiChrosorb RP-18 column (250 × 4 mm, 5-μm particles). Methanol–phosphate buffer (40:60) was used as the mobile phase. The phosphate buffer was prepared from a 0.05 *M* potassium dihydrogenphosphate solution adjusted to pH 3.25 with orthophosphoric acid. Flow-rates: 0–20; min, 1 ml/min; 20–21.5 min, linear gradient to 1.2 ml/min; > 20 min, 1.2 ml/min.

Sampling

Rain-water was collected on the roof of the institute using a stainless-steel sampler with a sampling area of 1 m^2. The sampler can be closed with a lid to prevent dry deposition. Mercury dichloride was added to the sample to avoid biological degradation. The sample was stored at 4°C until analyzed.

Extraction

Two extraction methods were used.

A 1-l volume of rain-water was extracted three times with 50 ml dichloromethane (shaking for 1 min). The pooled organic phases were shaken with 10 ml 1 *M* potassium hydroxide. The organic phase was analyzed for neutral and basic compounds. The aqueous phase was diluted in 40 ml water, adicified with 1.4 ml 6 *M* hydrochloric acid to pH < 2 and extracted three times with 20 ml dichloromethane (shaking for 1 min). (When fatty acids were to be determined, the pooled organic phase was treated further with boron trifluoride–methanol). The pooled organic phases were dried over sodium sulphate and reduced in volume with a rotary evaporator. After exchange of the solvent (methanol instead of dichloromethane), the volume was adjusted to exactly 250 μl

The second method was similar but here the water sample was first adjusted to pH > 12 with 20 ml 1 *M* potassium hydroxide prior to extraction with dichloromethane. After acidification to pH < 2, the aqueous phase was again extracted with dichloromethane as described above.

Chemicals

Nitrophenols were obtained from Fluka and Aldrich. Orthophosphoric acid, potassium dihydrogenphosphate, acetic acid, citrate buffer and heptanesulphonic acid were obtained from Merck, methanol and dichloromethane (HPLC grade) from

Rathburn. Twice distilled water, potassium hydroxide and hydrochloric acid were extracted with dichloromethane prior to use.

RESULTS AND DISCUSSION

HPLC separation

Binary mixtures of methanol–water or acetonitrile–water are not useful as mobile phases for the separation of phenols, as partial dissociation of the phenols leads to peak tailing. Thus seventeen different isocratic mixtures of methanol–water acidified with acetic acid, a citrate buffer (pH 2), heptanesulphonic acid or a phosphate buffer were tested. Methanol–0.05 *M* K_2HPO_4 (H_3PO_4) (40:60) proved to be best. Fig. 1 shows the separation of sixteen nitrophenols (and three non-nitrated phenols). 2-Nitrophenol/3-methyl-4-nitrophenol as well as 5-methyl-2-nitrophenol/4-methyl-2-nitrophenol are not resolved. The use of a gradient, *i.e.*, from 20 to 40 min, to shorten the analysis time was not attempted as the reproducibility of isocratic mixtures is better. Retention times and capacity factors, k', are summarized in Table I.

The selective detection of nitrophenols from a complex matrix (containing, *e.g.*, also other non-nitrated phenols) is facilitated by the fact that nitrophenols usually show absorption maxima also at > 300 nm. Fig. 1 shows the suppression of non-nitrated phenols at 317 nm.

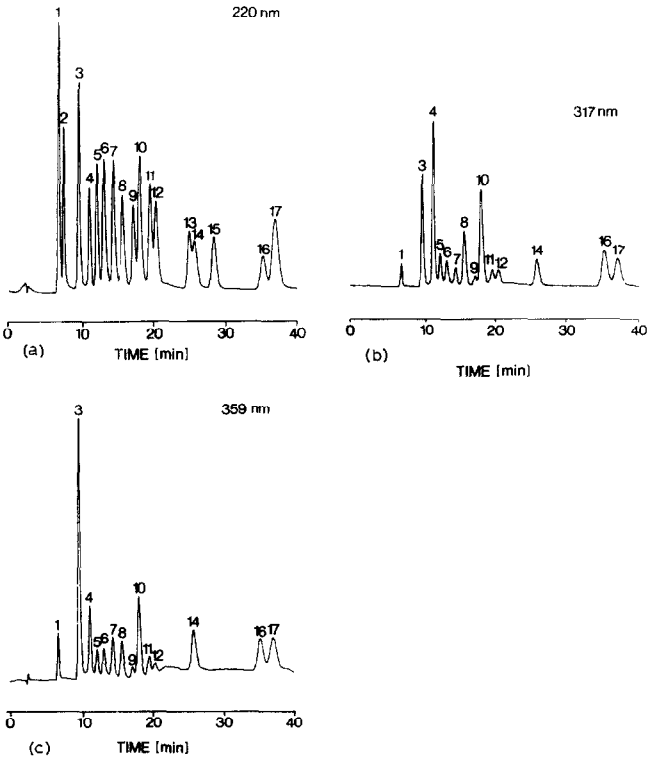


Fig. 1. HPLC chromatogram of nineteen phenols (sixteen nitrophenols) (see Table I for peak assignment)

TABLE I

RETENTION TIMES AND CAPACITY FACTORS

Compound		*Retention time (min)*	*Capacity factor, k'*
1	2,6-Dinitrophenol	6.62	1.57
2	Phenol	7.31	1.83
3	2,4-Dinitrophenol	9.43	2.66
4	4-Nitrophenol	10.96	3.25
5	3-Nitrophenol	11.97	3.64
6	2,3-Dinitrophenol	12.91	4.00
7	2,5-Dinitrophenol	14.20	4.50
8	3,4-Dinitrophenol	15.46	4.99
9	3-Methyl-2-nitrophenol	17.03	5.60
10	2-Nitrophenol/3-methyl-4-nitrophenol	17.91	5.94
11	4-Methyl-3-nitrophenol	19.43	6.53
12	2-Methyl-3-nitrophenol	20.27	6.86
13	2,5-Dimethylphenol	25.02	8.70
14	4,6-Dinitro-2-methylphenol	25.71	8.97
15	2,6-Dichlorophenol	28.32	9.98
16	2,6-Dimethyl-4-nitrophenol	35.12	12.61
17	5-Methyl-2-nitrophenol/4-methyl-2-nitrophenol	36.85	13.28

The use of a photodiode array detector is particularly valuable as it allows the recording of chromatograms at the optimum wavelength for each nitrophenol. Furthermore, comparison of the UV spectra with those of reference compounds in gener-

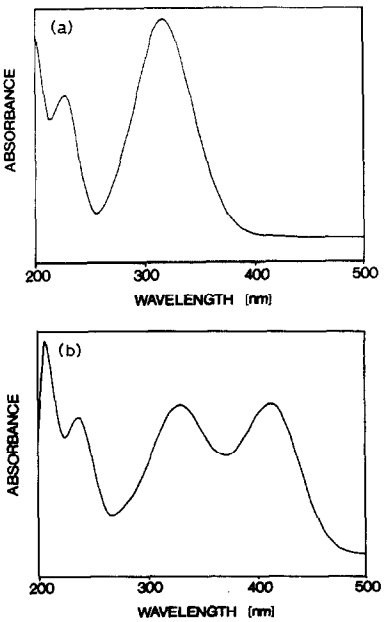


Fig. 2. UV spectra of 4-nitrophenol at (a) pH 3.25 and (b) 7.

TABLE II

ABSOLUTE DETECTION LIMITS OF THE PHOTODIODE ARRAY DETECTOR (S/N = 3)

Compound	*Wavelength (nm)*	*Detection limit (ng)*
2,6-Dinitrophenol	220	0.4
	435	0.9
2,4-Dinitrophenol	220	0.4
	435	1.1
4-Nitrophenol	220	0.6
	317	0.9
2,5-Dinitrophenol	220	0.8
3-Methyl-2-nitrophenol	220	1.0
2-Methyl-3-nitrophenol	220	0.9

al allows unambiguous identification of nitrophenols. In particular, most nitrophenols show unique UV spectra with several pronounced absorption maxima between 200 and 500 nm. It should, however, be kept in mind that the UV spectra strongly depend on the pH value as shown in Fig. 2 for 4-nitrophenol. Thus reference spectra must be recorded at the pH at which the actual analysis is carried out (UV spectra of the various nitrophenols are available upon request from the authors).

The linearity of the detector was tested for the concentration range of 4 to 2000 ng with five nitrophenols. Good linearity was observed in each case. The reproducibility was tested by four injections of a mixture of five standard compounds at 4–2000 ng (injected volume = 20 μl). The coefficient of variation is < 1% at 2000 ng. 1–6% at 8 ng and 1–14% at 4 ng. The detection limit is shown in Table II for six nitrophenols (signal-to-noise ratio, S/N = 3).

Extraction

The extraction scheme was designed to allow not only the determination of nitrophenols but also of other compound classes which are present in rain. In view of the complexity of a rain sample in which usually a large variety of organic compounds are present at in part high concentrations[4,5], a separation of the acidic compounds (mainly phenols and fatty acids) and the neutral/basic compounds is desirable. Two extraction methods have been employed as described in the Experimental section. Recoveries are reported in Table III. It is apparent that the recoveries strongly depend on the individual compounds. Recoveries by the first method (three successive extractions at pH 7, > 12 and < 2) are particularly poor for 4-nitrophenol, 2,4-dinitrophenol and 2,6-dinitrophenol. When in the first step extraction occurred at pH < 2, identical recoveries were determined except for 2,6-dinitrophenol where a substantial improvement (103%) was observed. The second method is more straightforward. With this method the recovery was improved for the dinitrophenols, but became poorer for 4-nitrophenol (see Table II). The latter result is difficult to explain. Poor recoveries for 4-nitrophenol have also been reported by other authors[21,24,25]. Moreover, solid phase extraction with XAD-2, C_{18} or phenyl phases did not improve the recovery.

After extraction and solvent exchange, the extract is reduced to 250 μl using a

TABLE III

RECOVERIES OF NITROPHENOLS BY EXTRACTION WITH DICHLOROMETHANE FROM WATER

Triplicate determinations, 10 μg/l, 220 nm. First method: extraction at pH 7; extraction at pH > 12; extraction at pH < 2. Second method: extraction at pH > 12; extraction at pH < 2. n.d. = Not determined.

Compound	*Recovery (%)*	
	First method	*Second method*
2-Nitrophenol	73 ± 3	75 ± 7
3-Nitrophenol	48 ± 2	24 ± 1
4-Nitrophenol	33 ± 2	19 ± 3
2,4-Dinitrophenol	45 ± 2	99 ± 8
2,5-Dinitrophenol	84 ± 4	94 ± 4
2,6-Dinitrophenol	26 ± 5	84 ± 3
3-Methyl-2-nitrophenol	69 ± 8	n.d.
2-Methyl-3-nitrophenol	64 ± 2	69 ± 9
4-Methyl-3-nitrophenol	85 ± 3	n.d.

rotary evaporator at 40°C. This may lead to evaporation losses. To explore this effect a mixture of five nitrophenols was evaporated to dryness at 40°C. The recoveries are shown in Table IV. It is apparent that substantial evaporation losses are observed for 3-methyl-2-nitrophenol only.

The detection limit of the method was not determined systematically. Rather a mixture of five nitrophenols at 0.5 μg/l was extracted according to the first method and reduced in volume to 1 ml. The nitrophenols were adequately quantified, with recoveries shown in Table V. If reduced to 0.25 ml, 4-nitrophenol can still be quantified at a level of 100 ng/l as shown in Fig. 3 (recovery 29%, coefficient of variation 9%).

Rain samples

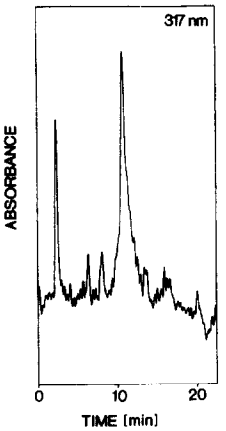
Rain samples were collected on the roof of our institute from May 1987 until December 1988 as described in the Experimental section and extracted as described above.

Fig. 4 shows the chromatogram of a rain sample from June 4th, 1987, recorded

TABLE IV

RECOVERIES OF NITROPHENOLS AFTER EVAPORATION TO DRYNESS

Compound	*Recovery (%)*
2,5-Dinitrophenol	81
2,6-Dinitrophenol	101
4-Nitrophenol	92
3-Methyl-2-nitrophenol	5
2-Methyl-3-nitrophenol	70



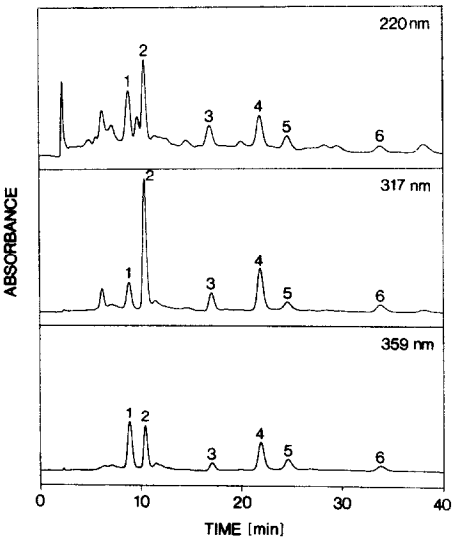
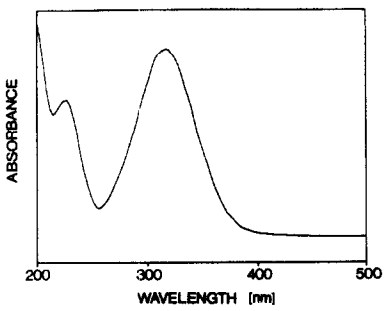


Fig. 3. Chromatogram of a water sample spiked with 100 ng/l of 4-nitrophenol; monitored at 317 nm.

Fig. 4. HPLC chromatogram of a rain-water sample at pH 3.25. Peaks: 1 = 2,4-dinitrophenol; 2 = 4-nitrophenol; 3 = 3-methyl-4-nitrophenol(?) + coeluting unknown component; 4 = unknown; 5 = 4,6-dinitro-2-methylphenol; 6 = 2,6-dimethyl-4-nitrophenol.

at 220, 317 and 359 nm; the wavelength 317 nm corresponds to an absorption maximum of 4-nitrophenol, 359 nm to one of 2,4-dinitrophenol. 4-Nitrophenol and 2,4-dinitrophenol can readily be identified by their retention times and UV spectra. Fig. 5 shows the UV spectrum of peak 2 (4-nitrophenol, compare with Fig. 2). Even minor peaks can be assigned. Thus Fig. 6 compares the UV spectrum of peak 5 with that of 4,6-dinitro-2-methylphenol (DNOC), Fig. 7 the UV spectrum of peak 6 with that of 2,6-dimethyl-4-nitrophenol. Similarly, peak 3 was assigned as 3-methyl-4-nitrophenol. The contour plot of this peak revealed, however, a second, minor coeluting component. Similarly, peak 4 must result from two coeluting components. A definite



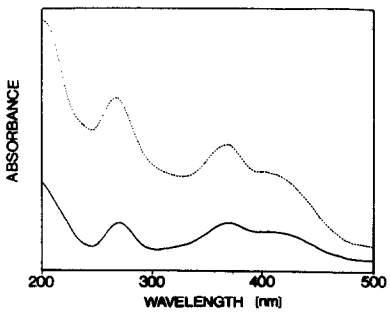


Fig. 5. UV spectrum of peak 2 in Fig. 4 (4-nitrophenol).

Fig. 6. Comparison of the UV spectrum of peak 5 in Fig. 4 (dashed line) with that of 4,6-dinitro-2-methylphenol (solid line).

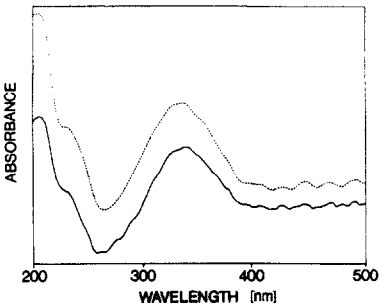


Fig. 7. Comparison of the UV spectrum of peak 6 in Fig. 4 (dashed line) with that of 2,6-dimethyl-4-nitrophenol (solid line).

TABLE V

RECOVERIES OF FIVE NITROPHENOLS FROM WATER AT A CONCENTRATION OF 500 ng/l

Triplicate determinations, 220 nm.

Compound	*Recovery (%)*	*Coefficient of variation (%)*
2,5-Dinitrophenol	79	15
2,6-Dinitrophenol	35	12
4-Nitrophenol	30	8
3-Methyl-2-nitrophenol	57	11
2-Methyl-3-nitrophenol	63	6

assignment was, however, not possible. The fact that this compound absorbs at long wavelengths (see 359 nm in Fig. 4) indicates the presence of a further nitrophenol. Unfortunately, not all isomers of methylnitrophenol and dimethylnitrophenol were commercially available to aid assignment of peak 4. The above assignments are corroborated by comparison of the retention times.

More details including the identification and quantitation of additional nitrophenols by other methods are reported elsewhere[26].

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