Journal of Chromatography, 195 (1980) 435–440
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,828

Note

Quantitative analysis of polynitrophenols in water in the micro- to nanogram range by reversed-phase ion-pair liquid chromatography

JOHN C. HOFFSOMMER*, DONALD J. GLOVER and CONSTANCE Y. HAZZARD
Naval Surface Weapons Center, White Oak Laboratory, Silver Spring, MD 20910 (U.S.A.)
(First received January 23rd, 1980; revised manuscript received March 11th, 1980)

Environmental concerns dictate the need for reliable methods for the separation, identification and analysis of specific contaminants that might be present in process and related waters. This is certainly true in the explosives industry. In this regard, remarkable success has been achieved by several workers for the thin-layer chromatographic (TLC) separation of a wide variety of explosives[1]. Yasuda's work on the two-dimensional TLC separation and identification of trinitrotoluene impurities[2], the nitroso- and nitro-derivatives of diphenylamine[3] and ethyl centralite[4] are especially noteworthy. These procedures have depended on TLC separations on silica gel plates with organic solvents. The low-level detection of explosives in water has relied on solvent extraction followed by gas chromatographic (GC) analysis with a sensitive electron capture detector[5,6], or combination TLC–GC[7], and TLC–visible spectrometry[8]. Difficulties arise, however, with both the TLC and GC analyses of strongly ionizable compounds such as picric and styphnic acids. Extraction efficiencies from water into organic solvents are usually quite low, and often, these types of compounds cannot be analyzed by GC and either remain at the origin or tail on TLC plates, *e.g.*, trinitrobenzoic acid[2] and hexanitrodiphenylamine[3].

Reversed-phase paired-ion liquid chromatography (PIC) offers the possibility of directly analyzing water solutions containing these strongly ionizable compounds such as the polynitrophenols. This approach has recently been outlined in an excellent review by Tomlinson *et al.*[9]. These authors cite the work of Culbreth *et al.*[10] on the analysis of 4-nitrophenol in admixture with 4-nitrophenyl phosphate. We wish now to report the separation and analysis by PIC of synthetic mixtures of several types of polynitrophenols in water containing up to nine components. One compound, 2-amino-4,6-dinitrophenol, has recently been reported[11] as a biotransformation product from picric acid. A preconcentration step is outlined for the analysis of polynitrophenols at ng/ml concentrations.

EXPERIMENTAL

Polynitrophenol solutions

All nitrophenols were purified by recrystallization from the appropriate solvents, and melting points compared with known literature values. Standard aqueous solutions of each nitrophenol varying from 15 to 350 mg/l were made by

0021-9673/80/0000–0000/$02.25

dissolving the nitrophenol directly in water. Synthetic mixtures of nitrophenols were made from these standard solutions.

Chromatographic conditions

A high-performance liquid chromatograph (Hewlett-Packard, Model 1084A) equipped with a variable-wavelength detector (HP Model 1030) and variable-volume injector was used with a 10-μm RP-8 column (25 cm × 4.6 mm) maintained at 40°C. For isocratic elution, column flow was 2.0 ml/min, methanol–water (50:50, v/v) containing $5 \cdot 10^{-3}$ *M* tetrabutylammonium phosphate buffered at pH 7.5 (Pic-A reagent; Waters Assoc., Milford, MA, U.S.A.). Thirty milliliters of Pic-A reagent were dissolved in 1 l of distilled water, diluted with another liter of HPLC grade methanol, and filtered through a 0.45-μm filter (Millipore). For gradient elution, column flow was 1.0 ml/min, methanol–water (45:55, v/v) containing Pic-A reagent for 11 min, then increased to methanol–water (50:55, v/v) from 11 to 16 min. Solutions were degassed at 35°C for 30 min before establishing column flow. Standard injections were 100 μl.

Preconcentration of nitrophenols

The acidity of a 50–100 ml aqueous sample containing from 1 to 15 ng/ml each of a mixture of 2-amino-4,6-dinitrophenol and 2,4,6-trinitrophenol was adjusted to pH 2.0–2.5 with 0.1 *N* HCl. The acidified solution was passed in increments through a Sep-Pak C-18 Cartridge (Waters Assoc.) by means of a 10-ml glass syringe with Luer end-fitting. The adsorbed nitrophenols were extracted from the cartridge with 1–2 ml HPLC grade methanol and collected in a 10-ml beaker to which was added 0.5 ml distilled water. Most of the methanol was removed on a water-bath maintained between 60°C and 70°C. The residual volume was measured and analyzed by liquid chromatography (LC).

RESULTS AND DISCUSSION

Tables I and II show the detector responses for the PIC separation of five- and nine-component synthetic mixtures of polynitrophenols in water, respectively.

TABLE I

RETENTION TIMES AND DETECTOR RESPONSES FOR PIC SEPARATION OF FIVE POLYNITROPHENOLS

Concentrations, 1–15 mg/l; 100 μl injected. Isocratic elution. Detector wavelength, 254 nm.

Synthetic mixture	*Retention (min)*	*Detector response**	
		Area counts/ng	*mm/ng***
(water)	1.5	—	—
2-Amino-4,6-dinitrophenol	3.06	133 ± 3 (8)	1.56 ± 0.04 (8)
2,4-Dinitrophenol	4.26	104 ± 2 (8)	0.86 ± 0.02 (8)
2-Methyl-4,6-dinitrophenol	6.34	92 ± 4 (8)	0.54 ± 0.01 (8)
2,4,6-Trinitrophenol	7.67	103 ± 2 (6)	0.51 ± 0.01 (9)
3-Methyl-2,4,6-trinitrophenol	11.5	89 ± 3 (6)	0.30 ± 0.01 (8)

* Values in parentheses are number of determinations; ± values are standard deviations.

** Sensitivity, $8 \cdot 10^{-5}$ a.u./mm.

TABLE II

RETENTION TIMES AND RELATIVE RESPONSES FOR PIC SEPARATION OF NINE POLYNITROPHENOLS

Concentrations, 2–6 mg/l; 100 μl injected. Gradient elution. Detector wavelength, 254 nm.

Synthetic mixture	*Retention (min)*	*Relative response*	
		Area	*Height*
(water)	3.0	—	—
3-Hydroxy-2,4-dinitrophenol	5.32	0.44	0.49
3-Hydroxy-2,4,6-trinitrophenol	6.11	0.44	0.55
2-Amino-4,6-dinitrophenol	7.13	1.0*	1.0**
3-Hydroxy-4,6-dinitrophenol	8.52	0.32	0.28
2,6-Dinitrophenol	9.46	0.79	0.57
2,4-Dinitrophenol	10.5	0.79	0.50
2-Methyl-4,6-dinitrophenol	16.8	0.69	0.31
2,4,6-Trinitrophenol	20.0	0.78	0.39
3-Methyl-2,4,6-trinitrophenol	25.8	0.69	0.29

* 255 area counts/ng.

** 1.63 mm/ng at a sensitivity of $8 \cdot 10^{-5}$ a.u./cm.

The data from Table I indicated that integrated area or peak height responses could be used with equal analytical accuracy with a standard deviation of $3 \pm 1\%$ for repeated injections without internal standard. The somewhat better analytical results for the synthetic mixture shown in Table III were obtained with an internal standard and integrated areas of eluted phenols. Figs. 1 and 2 show actual LC traces for the phenol separations described in Tables I and II.

TABLE III

PIC ANALYSIS OF A SYNTHETIC MIXTURE OF POLYNITROPHENOLS

Synthetic mixture	*mg/l (Actual)*	*mg/l (Found)**	*%***
3-Hydroxy-2,4,6-trinitrophenol	4.84	4.81	99.4
3-Hydroxy-4,6-dinitrophenol	4.34	4.39	101
2,6-Dinitrophenol	3.84	3.86	101
2,4-Dinitrophenol	5.25	5.24	99.8

* Isocratic elution; 100 μl injected.

** Using integrated peak areas and 3-hydroxy-2,4-dinitrophenol as internal standard.

Detection limits

From the height responses given in Tables I and II, the detection limit for all the polynitrophenols was calculated to be 0.03 ± 2 mg/l. This limit was made assuming a signal/noise ratio of 2. Fig. 3 shows the LC separation of a two-component synthetic mixture of phenols at the 1 mg/l level.

Analyses at nanogram levels

The analyses of aqueous solutions containing 1–15 ng/ml concentrations of the polynitrophenols required a preconcentration step. In order to avoid solvent extrac-

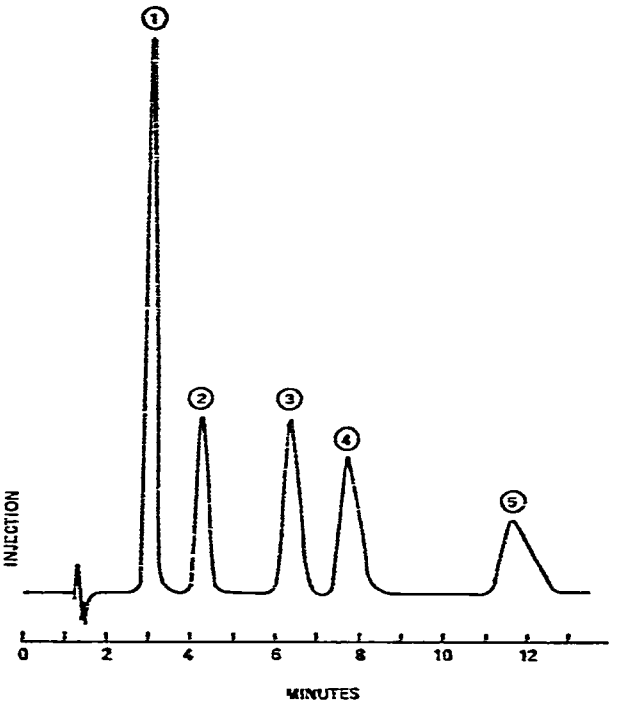

Fig. 1. PIC separation of 2-amino-4,6-dinitrophenol (1), 2,4-dinitrophenol (2), 2-methyl-4,6-dinitrophenol (3), 2,4,6-trinitrophenol (4) and 3-methyl-2,4,6-trinitrophenol (5).

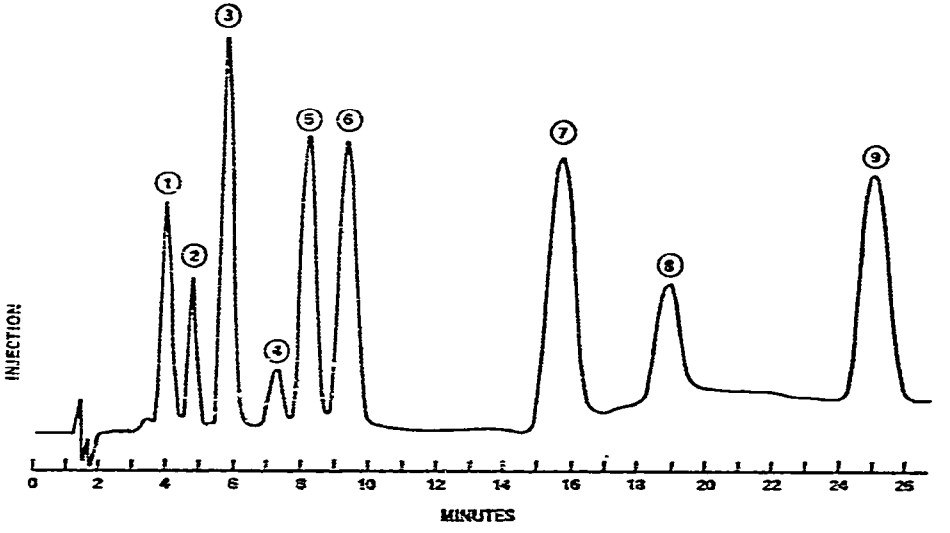

Fig. 2. PIC separation of 3-hydroxy-2,4-dinitrophenol (1), 3-hydroxy-2,4,6-trinitrophenol (2), 2-amino-4,6-dinitrophenol (3), 3-hydroxy-4,6-dinitrophenol (4), 2,6-dinitrophenol (5), 2,4-dinitrophenol (6), 2-methyl-4,6-dinitrophenol (7), 2,4,6-trinitrophenol (8) and 3-methyl-2,4,6-trinitrophenol (9).

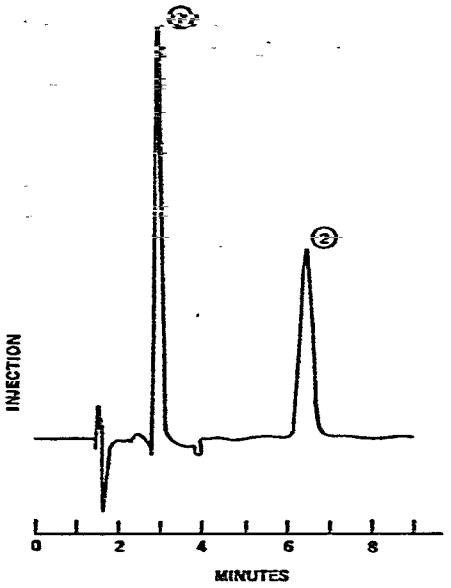

Fig. 3. PIC separation of 2-amino-4,6-dinitrophenol (1) and 2,4,6-trinitrophenol (2) at 1 mg/l concentration level.

tion procedures altogether, concentration on a stationary column, such as a Sep-Pak C-18 cartridge, followed by solvent extraction seemed ideal. The analyses of synthetic mixtures of 2,4,6-trinitrophenol and 2-amino-4,6-dinitrophenol at the ng/ml level are shown in Table IV. Recovery efficiencies for both nitrophenols were found to be markedly decreased at pH > 2, while recoveries of 2-amino-4,6-dinitrophenol were decreased at pH < 2. There was also a certain amount of "wash-out" of the adsorbed nitrophenols with samples larger than 100 ml. The addition of a small amount of water to the methanol extract was found necessary to prevent loss of nitrophenol. In addition, aqueous injections of the polynitrophenols gave superior LC traces.

TABLE IV

PRECONCENTRATION AND ANALYSES OF SYNTHETIC POLYNITROPHENOL MIXTURES AT THE ng/ml CONCENTRATION LEVEL

Concentrations, 1–15 ng/ml. Overall recovery from 50 to 100 ml of aqueous solution after concentration on Sep-Pak C-18 cartridge. Isocratic elution.

Synthetic mixture	*Recovery (%)**
2-Amino-4,6-dinitrophenol	73 ± 12 (16)
2,4,6-Trinitrophenol	67 ± 10 (25)

* Values in parentheses are number of determinations; ± values are standard deviations.

Variable-wavelength analyses

2-Amino-4,6-dinitrophenol has two absorption maxima at 310 nm and 410 nm, while 2,4,6-trinitrophenol exhibits a single maximum at 355 nm in water. These maxima were not found to shift in the presence of Pic-A reagent. PIC separations of

a 1.0 mg/l synthetic mixture of these polynitrophenols were made with detector wavelength settings at 254 nm, 310 nm, 355 nm and 410 nm. Detector responses for 2-amino-4,6-dinitrophenol at 310 nm and 410 nm were found to be 0.81 and 0.91 times the responses at 254 nm, respectively. Detector responses for 2,4,6-trinitrophenol at 355 nm were 1.3 times the response at 254 nm. At wavelengths lower than 230 nm, absorption interferences with Pic-A reagent were observed. Although analyses of these compounds at wavelengths other than 254 nm did not markedly change their detection sensitivities, these measurable response differences could serve as a further means of phenol identification.

Application

A waste-water sample was filtered through a 0.45-μm filter and analyzed by the PIC procedure described. The LC traces showed the presence of a single peak corresponding to 40.7 $\pm$ 0.8 mg/l picrate ion. Spectrophotometric analysis (Cary 16) showed 41.6 $\pm$ 0.6 mg/l picrate ion. Since no other peaks were observed in the LC traces, it may be concluded that the sample contained none of the other nitrophenols reported in this study in concentrations greater than 0.03 $\pm$ 0.02 mg/l.

REFERENCES

1 J. G. L. Harthon, *Acta Chem. Scand.*, 15 (1961) 1401.
2 S. K. Yasuda, *J. Chromatogr.*, 13 (1964) 78.
3 S. K. Yasuda, *J. Chromatogr.*, 14 (1964) 65.
4 S. K. Yasuda, *J. Chromatogr.*, 16 (1964) 488.
5 J. C. Hoffsommer, *J. Chromatogr.*, 51 (1970) 243.
6 J. C. Hoffsommer and J. M. Rosen, *Bull. Environ. Contam. Toxicol.*, 7 (1972) 177.
7 J. C. Hoffsommer, D. J. Glover and J. M. Rosen, *Analysis of Explosives in Sea Water and in Ocean Floor Sediment and Fauna*, NOLTR 72-215, Naval Ordnance Laboratory, Silver Spring, MD, 1972 (available from NTIS, U.S. Dept. of Commerce, Springfield, VA; order No. AD-757778).
8 J. C. Hoffsommer and J. F. McCullough, *J. Chromatogr.*, 38 (1968) 508.
9 E. Tomlinson, T. M. Jefferies and C. M. Riley, *J. Chromatogr.*, 159 (1978) 315.
10 P. H. Culbreth, I. W. Duncan and C. A. Burtis, *Clin. Chem*, 23/12 (1977) 2288.
11 J. F. Wyman, H. E. Guard, W. D. Won and J. H. Quay, *Appl. Microbiol.*, 37/2 (1979) 222.