Journal of Chromatography, 257 (1983) 170–173
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,432

Note

Separation and determination of 2,4-dinitrophenyl thioethers by reversed-phase high-performance liquid chromatography

H. VAN LANGENHOVE*, M. VAN ACKER and N. SCHAMP
Laboratory of Organic Chemistry, Faculty of Agricultural Sciences, State University Ghent, Coupure Links 653, B-9000 Ghent (Belgium)
(Received October 7th, 1982)

2,4-Dinitrophenyl thioethers (DNPTs) were first described by Bost *et al.*[1], who demonstrated that they can be used to identify mercaptans by their characteristic melting points. Mercaptans are very powerful odorants, occurring naturally as flavour compounds[2,3] and as malodorants[4]. Odour thresholds of mercaptans are at sub-parts per billion levels and the concentrations at which they occur are often too low to permit collection of sufficient derivative for melting point determinations. In spite of this, DNPTs exhibit some properties that make them attractive as derivatives for mercaptan analysis. In DNPTs, the sulphydryl group is protected against conversion to sulphides and disulphides. Also, DNPTs exhibit maximum absorption at 335 nm and molar absorptivities of 13,000 l mol^{-1} cm^{-1} (ref. 5) which makes them particularly suitable for UV detection. So far DNPTs have been separated by column[6], paper[5] and gas chromatography (GC)[7,8].

In this paper the reversed-phase (RP) high-performance liquid chromatographic (HPLC) analysis of 2,4-dinitrophenyl thioethers is described.

EXPERIMENTAL

Preparation of 2,4-dinitrophenyl thioethers

A mixture of mercaptan (0.012 mole), sodium hydroxide (0.4 g) and 2,4-dinitrophenyl chloride (2 g) in methanol (13 ml) is shaken for 15 min, water (10 ml) is added, the thioethers are extracted with diethyl ether and the ether is evaporated. After drying, the purity is checked by HPLC.

Chromatographic conditions

A Varian 5000 instrument with a UV 100 detector was used for HPLC analyses. The column (30 × 4 mm I.D.) was packed with RP-C_{18} (5 μm). The eluent was water–acetonitrile at a flow-rate of 1 ml/min, the temperature was 38°C, the detector wavelength was 335 nm and the sample loop volume was 10 μl.

A Tracor 560 provided with a flame-photometric detector was used for GC analyses. The glass capillary column (30 × 0.5 mm I.D.) was coated with SE-30 (4 mg/ml) by the static method. The carrier gas was helium at a flow-rate of 4 ml/min. The detector temperature was 250°C, the injector temperature was 230°C and the oven temperature was programmed from 150 to 220°C at 4°C/min.

0021-9673/83/0000–0000/$03.00

TABLE I

RETENTION TIME OF 2,4-DINITROPHENYL THIOETHERS OF C_1–C_{12} LINEAR ALIPHATIC MERCAPTANS

2,4-Dinitrophenyl-thioether of	*Retention time (min)*	
	Isocratic elution	*Gradient elution*
Methanethiol	3.3	5.4
Ethanethiol	3.5	6.5
Propanethiol	3.8	7.6
Butanethiol	4.1	9.1
Pentanethiol	4.9	10.7
Hexanethiol	5.7	12.6
Heptanethiol	6.6	14.4
Octanethiol	8.1	16.1
Nonanethiol	10.2	18.0
Decanethiol	12.8	19.4
Undecanethiol	16.7	20.6
Dodecanethiol	22.0	21.5

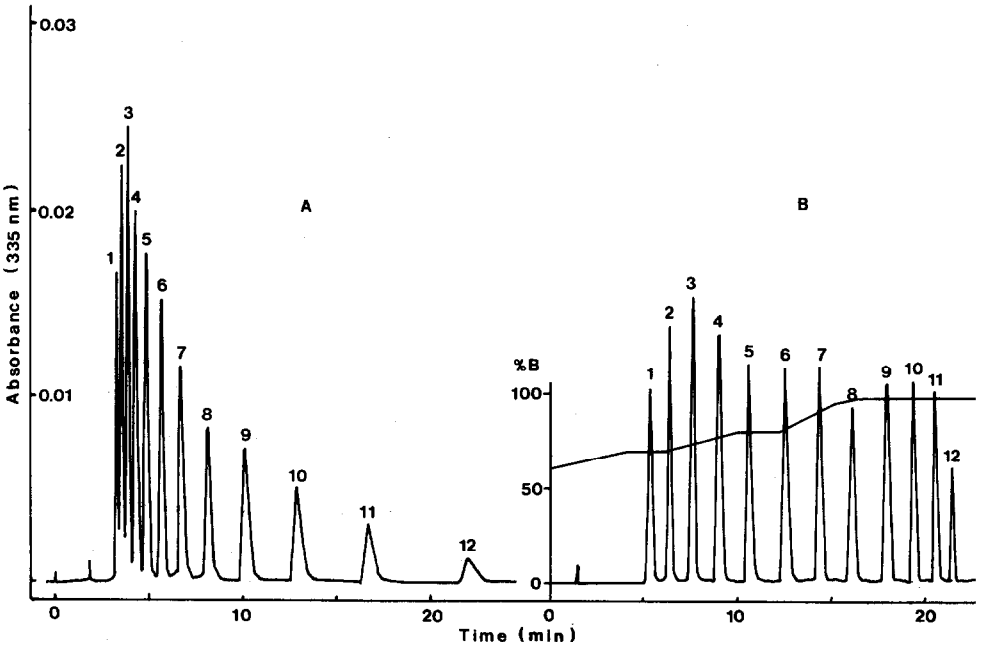

Fig. 1. HPLC separation of 2,4-dinitrophenyl thioethers: (A) isocratic elution, water–acetonitrile (20:80); (B) gradient elution (gradient indicated as %B = acetonitrile). Compounds: 2,4-dinitrophenyl thioethers of (1) methanethiol, (2) ethanethiol, (3) propanethiol, (4) butanethiol, (5) pentanethiol, (6) hexanethiol, (7) heptanethiol, (8) octanethiol, (9) nonanethiol, (10) decanethiol, (11) undecanethiol, (12) dodecanethiol.

Peak area integration was performed with an Infotronics CRS-208 digital integrator.

RESULTS AND DISCUSSION

Isocratic analysis (20% water–80% acetonitrile) of DNPTs of linear C_1–C_{12} aliphatic mercaptans resulted in baseline separation of C_5 and higher derivatives. Complete separation of all derivatives was achieved with gradient elution. The retention times for both chromatographic conditions are given in Table I and chromatograms are shown in Fig. 1. The gradient (% acetonitrile) is indicated in Fig. 1.

DNPTs of chain isomers of butanethiol were separated with water–acetonitrile (47:53) (Fig. 2). The derivative of *tert.*-butylmercaptan was completely separated. For peaks of equal area the derivatives of 1- and 2-methylpropanethiol were separated to the extent of 98% (resolution, $R = 1$) and derivatives of 2-methylpropanethiol and butanethiol to the extent of for 96% ($R = 0.9$). These separations permit quantitation of the derivatives. All samples were injected five times and the maximum coefficient of variation of the retention times was 1.5%.

The repeatability of the analysis was evaluated by injecting samples containing derivatives of C_3, C_6, C_9 and C_{12} linear aliphatic mercaptans (0.1 μg/μl each). Results for five consecutive injections are shown in Table II, from which it is clear that the repeatability of the HPLC analysis is excellent.

The GC separation of DNPTs has been described by others[7,8] but the repeatability was not evaluated. Therefore, the sample used for testing HPLC repeatability was analysed by GC. The separations were good but during five consecutive injections the detector sensitivity changed by a factor of 10. Therefore, no further GC analyses of DNPTs were performed.

With RP-HPLC five-point calibration graphs for DNPTs, with amounts injected ranging from 50 ng to 1 μg, correlation coefficients greater than 0.998 were obtained for all derivatives. The detection limit under the given conditions is 0.8 ng of derivative (Fig. 3). Taking into account that the smallest volume in which the deriva-

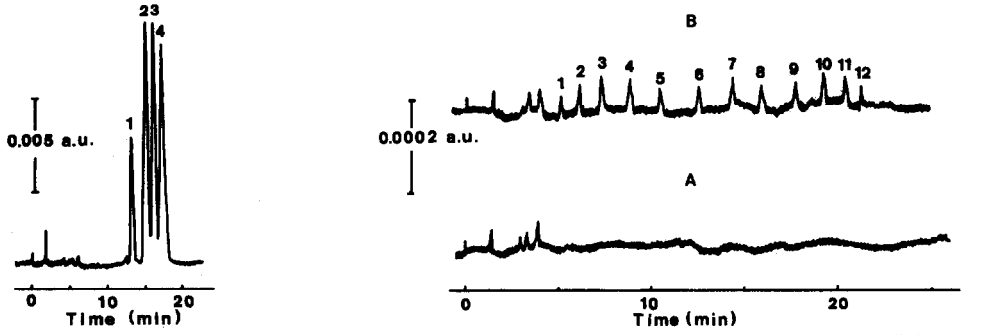

Fig. 2. HPLC separation of 2,4-dinitrophenyl thioethers of butanethiol isomers. Compounds: 2,4-dinitrophenyl thioethers of (1) *tert.*-butylmercaptan, (2) 1-methylpropanethiol, (3) 2-methylpropanethiol, (4) butanethiol.

Fig. 3. Detectivity of HPLC analyses for 2,4-dinitrophenyl thioethers. (A) Blank run, 10 μl of acetonitrile injected; (B) sample containing 0.8 ng each of 2,4-dinitrophenyl thioethers of C_1–C_{12} linear aliphatic mercaptans. Numbers on peaks indicate number of carbon atoms in the mercaptans.

TABLE II

REPEATABILITY OF 2,4-DINITROPHENYL THIOETHER ANALYSIS BY RP-HPLC (n = 5)

2,4-Dinitrophenyl-thioether of	*Mean counts*	*Standard deviation*	*Coefficient of variation (%)*
Propanethiol	10,332	90	0.9
Hexanethiol	5865	53	0.9
Nonanethiol	6268	30	0.5
Dodecanethiol	3713	49	1.3

tives can be dissolved after preparation is 200 μl and that the sample loop volume is 10 μl, amounts as low as 0.1 nmol of mercaptan can be detected as the 2,4-dinitrophenyl ether.

CONCLUSION

The RP-HPLC method described permits good separations and sensitive determinations of 2,4-dinitrophenyl thioethers. Excellent repeatability is achieved, which is an advantage of HPLC over GC analysis of DNPTs. The existence of a reliable method for the analysis of 2,4-dinitrophenyl thioethers will make these compounds useful as derivatives for mercaptan analysis.

REFERENCES

1 R. W. Bost, J. O. Turner and R. D. Norton, *J. Amer. Chem. Soc.*, 54 (1932) 1985.
2 H. E. Jansen, J. Strating and W. M. Westra, *J. Inst. Brew.*, 77 (1974) 154.
3 L. Schreyen, P. Dirinck, F. van Wassenhove and N. Schamp, *J. Agr. Food Chem.*, 24 (1976) 339.
4 R. L. Jenkins, J. P. Gute, S. W. Krasner and R. B. Baird, *Water Res.*, 14 (1980) 441.
5 E. A. Day and S. Patton, *Mikrochem. J.*, 3 (1959) 137.
6 J. F. Carson and F. F. Wong, *J. Org. Chem.*, 22 (1957) 1725.
7 L. Gasco and R. Barrera, *Anal. Chim. Acta*, 61 (1972) 253.
8 J. Kolrulczuk, M. Daniewski and Z. Mielniczuk, *J. Chromatogr.*, 100 (1974) 165.