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Note

High-performance liquid chromatographic analysis of naphtho- and anthranilohydroxamic acids*

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In the course of a photochemical study of aromatic hydroxamic acids[1,2], it became necessary to develop an efficient method for the analysis of these and related compounds. A literature survey revealed that a number of paper, thin-layer and gas chromatographic methods had been applied for the measurement of hydroxamic acids[3]. We found these methods unsatisfactory for our purposes; a direct gas chromatographic method, for example, was unreliable as hydroxamic acids had a tendency to undergo the Lossen rearrangement on heating.

There are only a few reports on high-performance liquid chromatographic (HPLC) analysis, dealing with the determination of less polar N-(2-fluorenyl)acetohydroxamic acid[4,5] and its derivatives[6,7]. As hydroxamic acids are biologically important compounds[8,9], it seemed of general interest to establish a HPLC technique for their analysis.

EXPERIMENTAL

Chromatography

All analyses were performed on a Waters Model ALC/GPC 244 HPLC system (Waters Assoc., Milford, MA, U.S.A.). A 1 ft. × $\frac{1}{4}$ in. I.D. column of μBondapak/C_{18} was employed with flow-rates of 1.5–2 ml/min. The eluents were monitored with a Type 440 UV absorbance detector operating at 254 nm. The column temperature was not controlled but was generally kept at *ca.* 25°C.

Reagents

2-Naphthoic acid, 2-naphthoyl chloride, 2-naphthamide, anthranilamide and methyl N,N-dimethylanthranilate were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). 2-Naphthohydroxamic acid, its N- and O-methyl derivatives were prepared by the reaction of 2-naphthoyl chloride with the corresponding hydroxylamines[1,2]. Anthranilohydroxamic acid and N,N-dimethylanthranilohydroxamic acid were prepared as described in the literature[10]. All of the prepared samples gave satisfactory

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elemental analyses and their physico-chemical properties agreed well with those in the literature.

Solvent preparation

Mobile phases for HPLC were prepared by mixing methanol with 0.1 *M* sodium phosphate buffer solution. The latter was obtained by dissolving disodium hydrogen phosphate (Merck, Darmstadt, G.F.R.) in distilled water and adjusting the pH to 3.5 (for systems A, B and C_b) or to 5 (for system C_a) with phosphoric acid

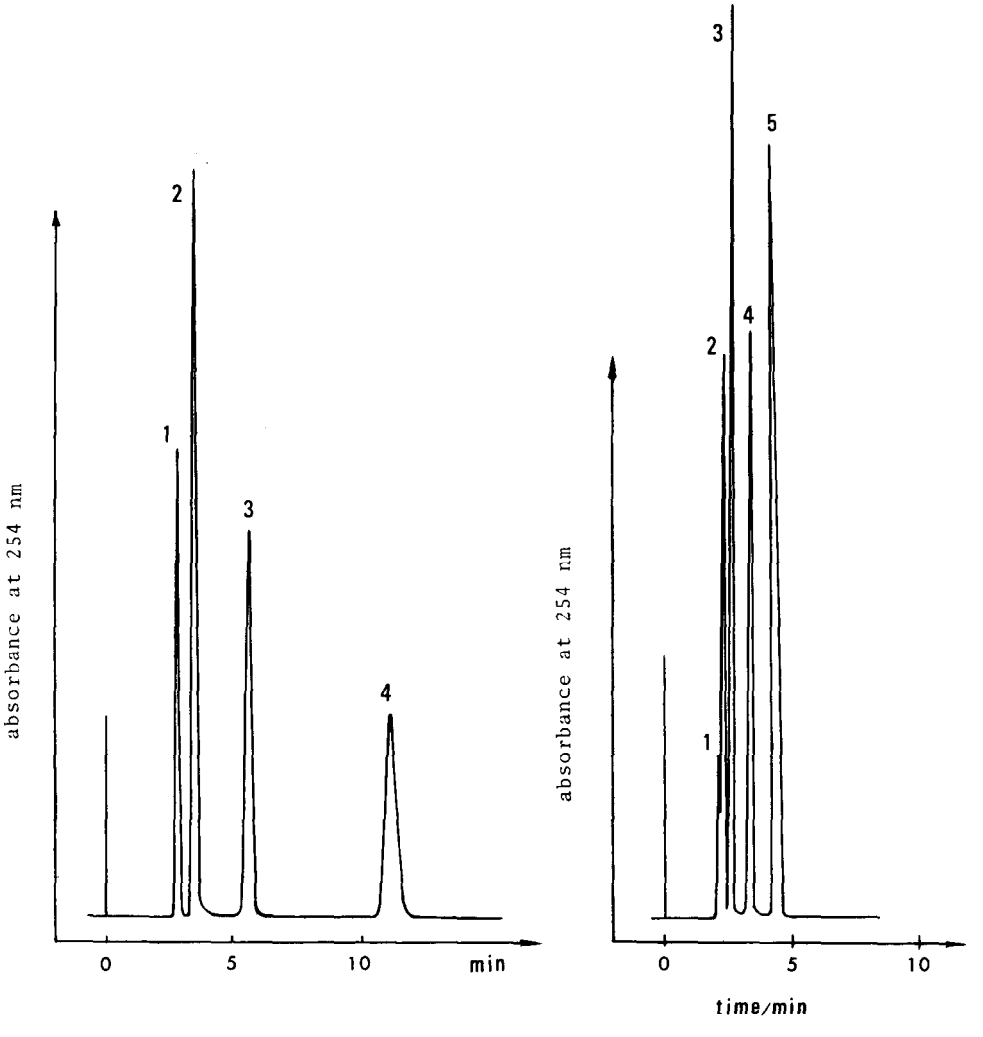

Fig. 1. HPLC trace of a mixture of (1) 2-naphthohydroxamic acid, (2) N-methyl-2-naphthohydroxamic acid, (3) 2-naphthoic acid and (4) 2-naphthoyl chloride. Elution with system A at 1.7 ml/min.

Fig. 2. HPLC trace of a mixture of (1) 2-naphthohydroxamic acid, (2) N-methyl-2-naphthohydroxamic acid, (3) 2-naphthoic acid, (4) O-methyl-2-naphthohydroxamic acid and (5) naphthoyl chloride. Elution with system B at 2.0 ml/min.

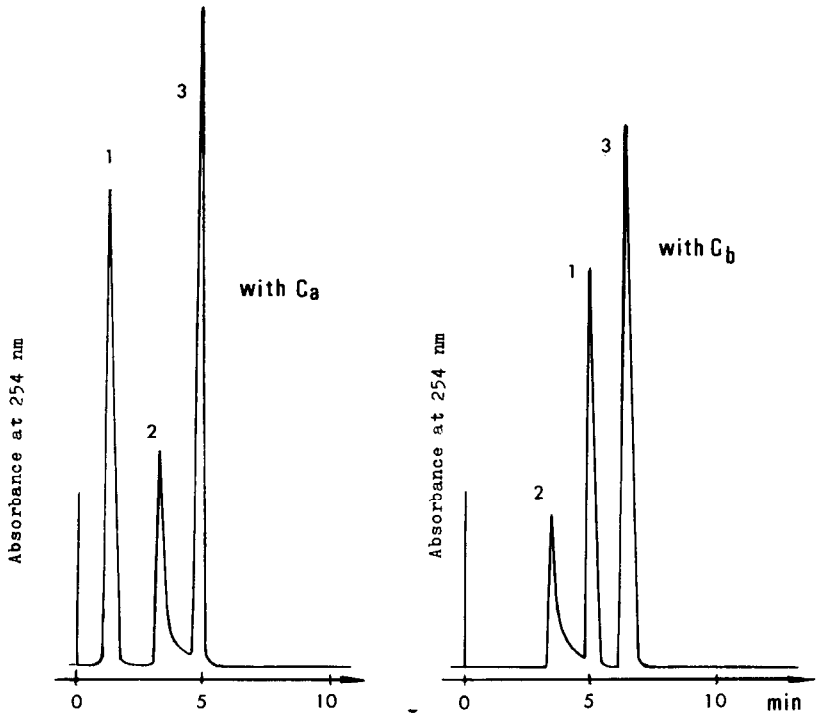

Fig. 3. HPLC trace of a mixture of (1) anthranilamide, (2) anthranilohydroxamic acid and (3) anthranilic acid. Elution with systems C_a and C_b at 2.0 ml/min.

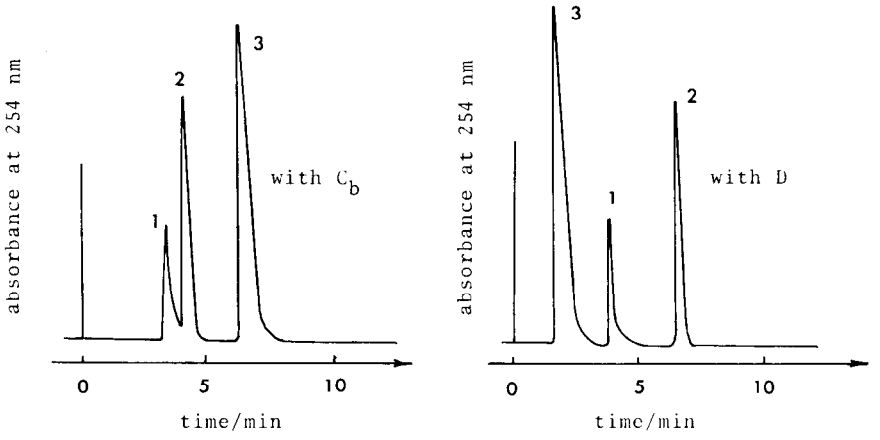

Fig. 4. HPLC trace of a mixture of (1) N,N-dimethylanthranilohydroxamic acid, (2) N,N-dimethylanthranilic acid and (3) methyl N,N-dimethylanthranilate. Elution with systems C_b and D at 2.0 ml/min.

(Merck, Suprapur). The mixed solvents were filtered through membrane filters (Fluoropore FP-045, pore size 0.45 μm; Sumitomo Electric, Osaka, Japan) and degassed by application of an aspirator. The buffer to methanol volume ratios were 1:1.5 (for system A), 1:4.2 (for system B), 4:1 (for systems C_a and C_b) and 7:1 (for system D).

RESULTS AND DISCUSSION

Satisfactory results could not be obtained when aqueous methanol at various pH values was used as the mobile phase, a serious tailing of the peaks due to hydroxamic acids occurred. The combination of phosphate buffer with methanol was satisfactory. 2-Naphthohydroxamic acid and its derivatives were well separated from possible impurities, *e.g.*, 2-naphthoxyl chloride as a residue of the starting material and 2-naphthoic acid as a hydrolysis product (Figs. 1 and 2). Solvent systems A and B allowed the determination of impurities at concentrations down to $10^{-3}\%$ in 2-naphthohydroxamic acids.

The more polar mobile phases C and D were effective in determining anthranilohydroxamic acids. The sequence of elution depended strongly on the pH (Fig. 3) and the ratio of the components in the buffer–methanol mixture (Fig. 4).

In conclusion, phosphate buffer–methanol systems are the mobile phase of choice for the analysis of hydroxamic acids.

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REFERENCES

1 E. Lipczyńska-Kochany and H. Iwamura, *Chem. Lett.*, (1982) 1825.
2 E. Lipczyńska-Kochany and H. Iwamura, in preparation.
3 L. H. Patterson, L. A. Damani, M. R. Smith and J. W. Gorrod, in J. W. Gorrod (Editor), *Biological Oxidation of Nitrogen*, Elsevier North-Holland Biomedical Press, Amsterdam, 1978, p. 213; and references cited therein.
4 H. R. Gutmann, *Anal. Biochem.*, 58 (1974) 469.
5 S. Thorgeisson, S. Snorri and W. L. Nelson, *Anal. Biochem.*, 75 (1976) 122.
6 H. R. Gutman, D. Malejka-Giganti and R. McIver, *J. Chromatogr.*, 115 (1975) 71.
7 I. D. Goodman, *Anal. Biochem.*, 70 (1976) 203.
8 E. Lipczyńska-Kochany and H. Iwamura, *J. Org. Chem.*, 47 (1982) 5277.
9 E. Lipczyńska-Kochany, H. Iwamura, K. Takahashi and Y. Kawazoe, *Mutat. Res.*, in press; and reference cited therein.
10 M. A. Stolberg, W. A. Mosher and T. Wagner-Jauregg, *J. Amer. Chem. Soc.*, 79 (1957) 2615.