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GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF AQUEOUS SOLUTIONS OF HYDROXYPYRIDINES AND HYDROXYQUINOLINES

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SUMMARY

A method for the quantitative analysis of aqueous solutions of certain hydroxy derivatives of pyridine and quinoline is described. The method requires extraction into *n*-butanol followed by O-trimethyl silylation and separation of the derivatives by gas–liquid chromatography.

The technique yields linear calibration plots using an internal standard procedure. Concentrations as low as 10^{-7} g·ml^{-1} can be detected. Retention data for the derivatives on XE-52, Apiezon L and neopentylglycol sebacate are reported.

INTRODUCTION

In connection with a proposed study of the microsomal oxidation of N-heteroaromatic compounds, a method for the detection and estimation of hydroxypyridines and hydroxyquinolines was required. Previous qualitative studies on the metabolism of quinoline[1], have relied upon thin-layer chromatographic (TLC) methods for separation and detection of the metabolites. Those of the metabolites which could be identified were found to be hydroxyquinolines. Since a quantitative method of analysis was required, it was decided to explore the possibilities of a gas–liquid chromatographic (GLC) method for the determination of this type of compound. Consideration of the solubilities of hydroxypyridines and hydroxyquinolines suggested that *n*-butanol was the most suitable solvent for extraction. Also, these hydroxy compounds are not amenable to direct GLC estimation and therefore preliminary derivatisation is necessary. The choice of derivative was limited by the solvent used for extraction. Of the derivatives available the O-trimethylsilyl ether was most convenient since its preparation could be carried out in *n*-butanol and furthermore it has been shown previously[2] that aliphatic alcohols catalyse the reaction between hexamethyldisilazane and phenols. Recently[3] a method has been reported for the

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GLC analysis of the trimethylsilyl derivatives of certain halogenated 8-hydroxyquinolines.

Although theoretically a mixture of O- and N-trimethylsilyl derivatives could result in the case of those hydroxy compounds capable of a prototropic shift, it has been demonstrated that for 2- and 4-hydroxypyridines the O-trimethylsilyl derivative is formed exclusively[4]. Consequently a single chromatographic peak is to be expected for each of the singly substituted compounds studied.

Three stationary phases of different polarity, *viz.*, Apiezon L, XE-52 and neopentylglycol sebacate (NPGS) were investigated.

EXPERIMENTAL

Materials

The commercially available hydroxy compounds (2-, 4-, 5-, 6-, 7- and 8-hydroxyquinolines, 2-, 3- and 4-hydroxypyridines, and 2,6- and 2,3-dihydroxypyridines) were recrystallised from ethanol–water. 3-Hydroxyquinoline was prepared from commercial 3-aminoquinoline by the method of Mills and Watson[5].

Pyridine was redistilled from KOH pellets.

Columns were 5 ft. in length made of $\frac{1}{4}$ in. O.D. glass tubing with the following packing: column I, 10% Apiezon L; column II, 10% XE-52; column III, 5% XE-52; column IV, 2.5% XE-52; column V, 5% NPGS. All on 100–120 A.S.T.M. Chromosorb G, DMCS treated.

The stationary phases were deposited from solution under a stream of nitrogen.

Procedure

Preliminary attempts at silylation were performed at 25° in reaction vials fitted with PTFE lined caps using 1% solutions of the hydroxy compounds in dry pyridine (1 ml) with a silylating mixture (1 ml) consisting of 9 parts of pyridine, 3 parts of hexamethyldisilazane, 1 part of trimethylchlorosilane. 0.1-μl samples were directly injected into a Pye 104 gas chromatograph fitted with dual columns and flame ionization detectors. The silylating reagent was completely eluted before the derivatives even at the lower temperature (150°) and moreover single derivative peaks were observed in all cases. When the reaction mixture was allowed to stand overnight or heated at 90° for 40 min, the peak heights did not increase. The derivative peaks were subjected to mass spectrometry analysis and gave a molecular ion corresponding to the monotrimethylsilyl ether. Attempts were then made to carry out derivatisation at 25° in *n*-butanol using 1% solutions of the hydroxy compounds (1 ml) and the silylating mixture (3 ml). Again the solvent and reagents eluted well before the appearance of the derivative peak. In each case a single peak resulted which corresponded to the retention volumes obtained in the experiments using pyridine solutions. Again the silylation reaction appeared to be complete within 15 min but overnight standing of the 6-hydroxyquinoline derivative in the reaction mixture resulted in total disappearance of the derivative peak. In the case of 3-hydroxypyridine the derivative peak height remained constant when samples were examined using column I at 150° (Table I). The stability of the other derivatives varied between these two extremes. GLC analysis carried out within 2 h of derivatisation gave reproducible results.

TABLE I

STABILITY OF THE SILYL DERIVATIVE OF 3-HYDROXYPYRIDINE AT 25° IN THE PRESENCE OF *n*-BUTANOL AND EXCESS SILYLATING REAGENT

Time (h)	*Peak height (mm)*
0	71
0.5	68
1.0	69
5.0	68
18.0	68

Successive extraction with *n*-butanol (1-ml portions) of the hydroxy compounds in solution in pH 7 buffer (5 ml) followed by evaporation of the combined extract and silylation gave results identical with those obtained above. Three and four extractions were sufficient to recover the hydroxyquinolines and hydroxypyridines, respectively.

For the quantitative studies tetradecane was selected as an internal standard since it could be incorporated in the silylating reagent and was clearly separated from the derivative peaks on all the columns used.

RESULTS AND DISCUSSION

The retention volumes relative to the first eluted of the silyl derivatives of the hydroxypyridines and the hydroxyquinolines studied are shown in Tables II–IV.

TABLE II

RETENTION DATA OF HYDROXYPYRIDINES FOR COLUMNS I, II AND V

Temperature, 150°.

Hydroxypyridine	*Retention volumes relative to silyl derivative of 2-hydroxypyridine*		
	Column I	*Column II*	*Column V*
2-OH	1.00	1.00	—
3-OH	1.15	1.79	—
4-OH	1.62	2.06	—
2,3-diOH	—	—	—
2,6-diOH	2.69	—	—

Hydroxypyridines

Column V was unsuitable for the analysis since the 4-hydroxypyridine derivative was not eluted. The results in Table II show that column II gives a better separation than column I, but it is not entirely satisfactory since the absolute retention volumes were undesirably large. Reference to Table III shows that column III gives the best separation and the absolute retention volumes are satisfactory. Therefore, column III was selected for the quantitative studies.

TABLE III

RETENTION DATA FOR COLUMNS II, III AND IV

Temperature, 150°.

Hydroxypyridines	*Retention volume relative to the silyl derivative of 2-hydroxypyridine*		
	Column II	*Column III*	*Column IV*
2-OH	1.00	1.00	1.00
3-OH	1.79	2.80	1.54
4-OH	2.06	3.10	2.62
2,3-diOH	—	6.55	3.08
2,6-diOH	—	7.30	3.46

Hydroxyquinolines

The results in Table IV show that no one of the columns allows the complete separation of all the derivatives. However, the derivatives of 2-, 4-, 5- and 8-hydroxyquinoline were separated by column I whilst on column V 3-, 6- and 7-hydroxyquinoline were separated. Thus all the isomers were separable using the two columns which were conveniently mounted in the same oven.

TABLE IV

RETENTION DATA OF HYDROXYQUINOLINES FOR COLUMNS I, II AND V

Temperature, 190°.

Hydroxyquinoline	*Retention volumes relative to that of the first eluted derivative*		
	Column I	*Column II*	*Column V*
2-OH	4.03	—	—
3-OH	1.95	1.09	1.52
4-OH	7.33	—	—
5-OH	1.80	1.00	1.40
6-OH	1.95	1.07	1.62
7-OH	2.00	1.10	1.00
8-OH	1.00	—	1.41

Quantitative results

Since an extraction process was involved calibration curves were drawn, plotting as abscissa the concentration of hydroxy compound in buffer pH 7 and as ordinate either the ratio of the derivative peak height to that of the internal standard or the ratio of the peak areas as determined by a Honeywell integrator. As can be seen in the typical example shown in Fig. 1, the lines were straight for each method and no real advantage is obtained by the use of relative areas.

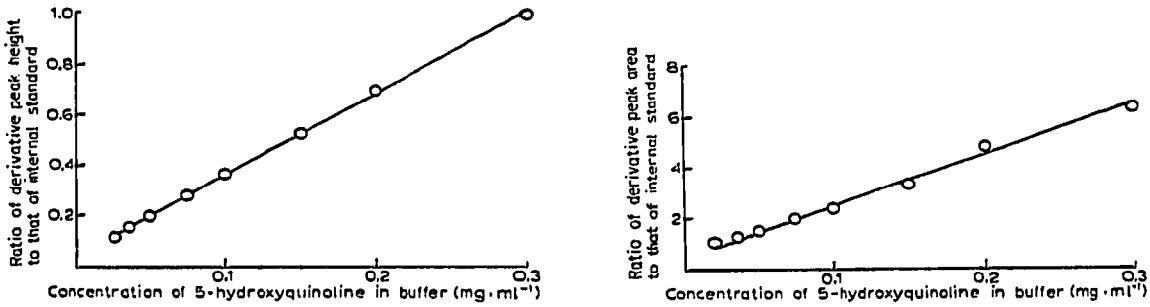

Fig. 1. Calibration lines for the analysis of 5-hydroxyquinoline in buffer pH 7. The silyl derivative eluted from column I (10% Apiezon L on 100–120 A.S.T.M. Chromosorb G, DMCS treated) at 190°.

The points on the graphs represent the mean of three determinations and the lines were found to be reproducible so that only a two-point calibration check was required before a series of analyses was carried out. The detection limits ranged from 5×10^{-7} g·ml^{-1} to 10^{-6} g·ml^{-1}.

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