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Note

Direct gas–liquid chromatographic analysis of N-hydroxyamphetamine, a metabolite of amphetamine

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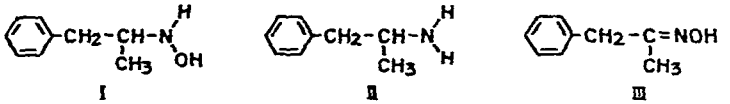
The thermal instability of N-hydroxyamphetamine, and hydroxylamines of arylalkylamines in general, presents a problem in gas–liquid chromatographic (GLC) analysis. N-Hydroxyamphetamine (I), for example, decomposes differently under different analytical conditions[1], and recently it has been shown to decompose to approximately equimolar ratios of amphetamine (II) and phenylacetone oxime (III) during GLC analysis[2]. The normal practice has been to form stable silyl or acyl derivatives before the GLC analysis[2–4].



I II III

Despite the thermolabile nature of N-hydroxyamphetamine, attempts to modify the GLC column so that the analysis can be performed at a much lower temperature, or to evaluate the role, if any, of the support material in causing this decomposition have not been reported. In previous work[2], diatomaceous earth type supports were used. Possibly decomposition can be prevented if the analysis can be performed at a much lower temperature. Glass beads were therefore chosen as the support material for the present work, since glass beads with small specific surfaces will allow the use of a very small quantity of stationary phase and a simultaneous reduction in column temperature.

EXPERIMENTAL

Glass beads were purchased from Field Instruments (Richmond, Great Britain); stationary phases from Perkin-Elmer (Norwalk, Conn., U.S.A.). N-Hydroxyamphetamine was synthesised using the method described by Morgan and Beckett[5] for the synthesis of N-hydroxy-3,4-dimethoxyamphetamine.

Preparation of glass bead packing material

A weighed amount (200 g) of 60–80 mesh glass beads, previously dried at 120° for 2 h, were added to a solution of 25 ml of dimethyldichlorosilane in 200 ml

of toluene. The beads were allowed to stand in the solution for 3 h with occasional stirring. The solution was decanted off, the beads were washed by decantation with 2 × 100 ml of toluene and transferred on to a filter funnel. The beads were washed with two further 100 ml portions of toluene followed by 2 × 100 ml of each of methanol and chloroform. After a preliminary air drying, the beads were dried at 100° for 15 min. 100 mg of the stationary phase were dissolved in 50 ml of chloroform and 50 g of DMCS-treated glass beads were added. The chloroform was removed at reduced pressure using a rotary film evaporator.

Gas–liquid chromatography

A Perkin-Elmer Model F11 and a Hewlett-Packard Model 5700A gas chromatograph both equipped with dual flame ionization detectors were used. For the Perkin-Elmer instrument, air and hydrogen pressures were 175 and 140 kPa, respectively. The injection port temperature was 100°. For the Hewlett-Packard instrument, air and hydrogen pressures were 168 and 210 kPa, respectively; this instrument did not incorporate a heated injection port. All columns were 1 m × 3 mm I.D. glass tubing. The following columns were used: A: 0.2% OV-17; temperature, 60°; carrier gas, nitrogen; pressure, 70 kPa; flow-rate, 1.5 cm^3/sec. B: 0.2% XE-60; temperature, 65°; carrier gas, nitrogen; pressure, 100 kPa; flow-rate, 1.66 cm^3/sec. C: 0.2% Carbowax 20M; temperature, 100°; carrier gas, nitrogen; pressure, 90 kPa; flow-rate, 1.5 cm^3/sec.

Gas–liquid chromatography–mass spectrometry

The mass spectrum of N-hydroxyamphetamine was obtained using a Perkin-Elmer Model 270 gas chromatograph-linked mass spectrometer and the following conditions: ionization potential, 70 eV; source temperature, 180°; manifold temperature, 120°; carrier gas, helium; pressure, 140 kPa. Column and injection port temperatures were both at 100°. A Carbowax 20M glass bead column (column C) was used.

Extraction of N-hydroxyamphetamine from aqueous solution

N-Hydroxyamphetamine (10 μmole) was extracted with freshly distilled diethyl ether from a solution of its oxalate in phosphate buffer (pH 7.4). The ethereal extract was concentrated to a small volume (0.1 ml) in a water-bath (45°) and an aliquot was examined by GLC and GLC–mass spectrometry.

Extraction of N-hydroxyamphetamine from biological fluids

N-Hydroxyamphetamine (0.25 μmole) was extracted from urine from different human subjects and from liver homogenates of rabbit as described previously[2].

Calibration curves

N-Hydroxyamphetamine (0.05–1.0 μmole) was extracted with freshly distilled diethyl ether (3 × 8 ml) from a solution of its oxalate in phosphate buffer (pH 7.4). To the combined ethereal extract 1.0 ml of internal standard (0.4 μmole/ml of 1,1-dimethyl-1-nitro-2-phenylethane in *n*-pentane) was added, and the mixture was concentrated in a water-bath (45°) and with nitrogen to about 25 μl. An aliquot (3 μl) was injected on GLC column C. Peak height ratios of N-hydroxyamphetamine to internal standard were plotted against concentration of N-hydroxyamphetamine.

RESULTS AND DISCUSSION

N-Hydroxyamphetamine did not show any decomposition when examined on either column A or column B. However, both of these columns produced tailing peaks without adequate separation between N-hydroxyamphetamine and phenylacetone oxime. Although the surface of the glass beads is regarded as inert compared with the diatomaceous earth supports, it has some surface activity which may or may not be deactivated by silanization. Furthermore, the calcium ions on the surface of the normal soda lime glass beads act as weak Lewis acid sites to provide adsorption sites for molecules containing lone pair donor atoms[6].

To overcome the adsorption effect, column C with the more polar Carbowax 20M as the stationary phase, was used. Excellent results were obtained, as shown in Fig. 1. This column was necessarily operated at a higher temperature than columns A and B, but practically no decomposition of N-hydroxyamphetamine occurred. However, partial decomposition of N-hydroxyamphetamine to the corresponding amine and oxime occurred when the injection port temperature was raised from 100 to 200°. Also, certain batches of diethyl ether were found to cause partial decomposition of N-hydroxyamphetamine during extraction. With one particular batch of

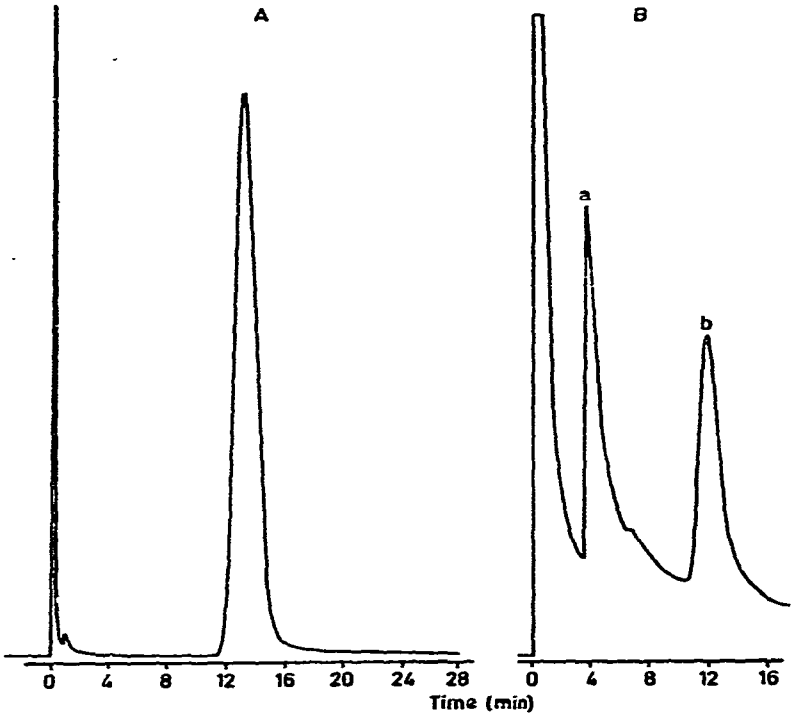


Fig. 1. Chromatograms of N-hydroxyamphetamine using (A) glass bead column C and (B) conventional diatomaceous earth support column (1 m × 4 mm I.D. glass, packed with 3% OV-17 on 80–100 mesh Chromosorb G AW DMCS; carrier gas, nitrogen; flow-rate, 1.66 cm^3/sec. Both column and injection port at 100°). a = Amphetamine; b = phenylacetone oxime. The retention times in column C of amphetamine and phenylacetone oxime are 0.8 and 23.5 min, respectively.

diethyl ether, N-hydroxyamphetamine could not be detected (thin-layer chromatographic and GLC evidence) in the ethereal extract; instead GLC peaks having retention times similar to amphetamine and phenylacetone oxime were obtained. N-Hydroxyamphetamine was stable on column C and produced symmetrical peaks when examined on several occasions over a period of 12 months. The retention time of N-hydroxyamphetamine can be reduced with consequent improvement in the peak sharpness by using a higher flow-rate, since the efficiency of columns packed with glass beads remains constant over a wide range of gas velocity[7].

When N-hydroxyamphetamine was extracted from liver homogenates of rabbit and from urine from different human subjects and then analysed on column C, the extracted constituents of the liver homogenates or of the urine did not interfere with the above analysis.

Linear and reproducible calibration curves were obtained in the range 0.05–1.0 μmole; regression analysis of the data gave correlation coefficients of not less than 0.999.

The identity of the GLC peak of N-hydroxyamphetamine was confirmed by combined GLC–mass spectrometry as shown in Fig. 2. The characteristic base peak was observed at m/e 60 corresponding to the ion $CH_3CH{=}\overset{+}{N}HOH$. A small molecular ion peak (0.3%) was also observed; this is of interest, since N-hydroxyamphetamine could not be directly identified hitherto by combined GLC–mass spectrometry because of its complete conversion to phenylacetone oxime[8].

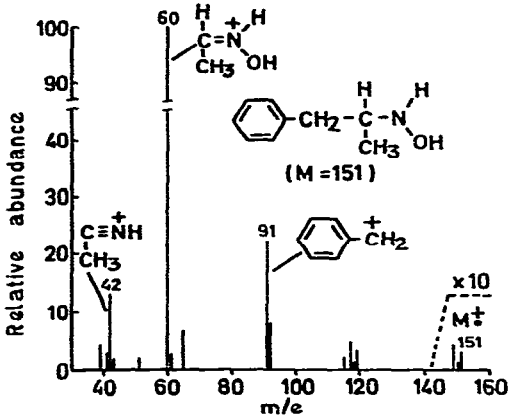


Fig. 2. GLC–mass spectrum of N-hydroxyamphetamine using the glass bead column.

These results show that the GLC decomposition of N-hydroxyamphetamine is primarily due to the catalytic activity of the solid support rather than that due to the column or injection port temperature or to oxidation in the column[9], because, under similar conditions, decomposition of N-hydroxyamphetamine occurred on columns packed with the conventional diatomaceous earth supports (see Fig. 1). Using the glass bead column, direct GLC analysis of N-hydroxyamphetamine is possible without decomposition and without the need for prior conversion to various derivatives. Being a "low bleed" column, it can be operated at a high electrometer

sensitivity without distortion of the baseline. One disadvantage of columns packed with glass beads is their high phase ratio and the associated low capacity; however, it is particularly suitable for metabolic studies where only very small concentrations of samples are involved.

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