CHROM. 4573

Gas chromatographic estimations of compounds derived from acetylcholine

Gas chromatographic estimations of acetylcholine require pretreatment of this relatively involatile quaternary base to yield suitably volatile derivatives; procedures based on the estimation of ethanol[1], dimethylaminoethyl acetate[2,3], and acetic acid[4] have been described. Porous polyaromatic polymer beads have been reported to be particularly versatile stationary phases for the gas chromatography of such polar substances with a number of distinct advantages over conventional packings[5]. The present investigation examines the use of Porapak Q, an ethylvinylbenzene–divinylbenzene polymer, as a general stationary phase for the above estimations of acetylcholine.

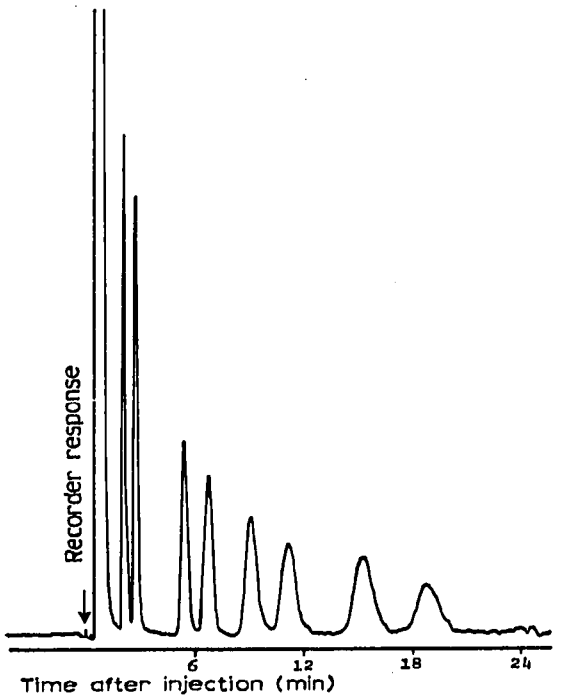
Materials and methods

A Varian Aerograph Model 600D gas chromatograph with a hydrogen flame ionization detector was used, with an Offner RS Dynograph recorder operating at 0.05 mV/cm or a Varian Aerograph Model 20, 1 mV f.s.d., recorder. Chromatography was isothermal, the oven temperature being maintained with a Varian Aerograph Model 325 Isothermal Temperature Controller. The injection port temperature was maintained approximately 25° higher than the oven temperature. The following flow rates were used: nitrogen carrier gas, 33 ml/min; hydrogen, 22 ml/min; and air, 300 ml/min. All three gases were dried using molecular sieves (Union Carbide type 5A). Injections of 2 μl (chloroform solutions) or 10 μl (aqueous solutions) were made with 10- and 30-μl syringes (S.G.E.). Peak areas were measured with a planimeter (Ott) on records obtained at a chart speed of 80 in./h. Glass columns (5 ft. × 1/8 in.) containing 50–80 mesh Porapak Q (Waters Assoc.) were used after purging for 1 h at 230°. For the gas chromatographic determination of acetylcholine by the method of STAVINOHA AND RYAN[1] stainless steel columns (10 ft. × 1/8 in.) packed with 20 % by weight Carbowax 6000 (Bronson and Jacobs) on 60–80 mesh HMDS-treated Chromosorb W (Johns-Manville) were used. Reagents were purchased from BDH except for the following: anhydrous acetic acid (Standard Laboratories), acetylcholine chloride (Merck), potassium borohydride (Metal Hydrides), and thiophenol (Fluka). The acetates, *n*-propionates and *n*-butyrates of dimethylaminoethanol and 1-dimethylamino-2-*n*-propanol were prepared by JOHNSTON *et al.*[6].

Results and discussion

Porapak Q proved to be a suitable stationary phase for the isothermal separation and quantitative estimation of ethanol and related primary alcohols, dimethylaminoethyl acetate and related esters, and acetic acid and related carboxylic acids, exhibiting low background noise under operating conditions of maximum sensitivity and affording well separated peaks of closely related derivatives. Separations of alcohols of low molecular weight and carboxylic acids on Porapak Q are already well documented[5]; Fig. 1 illustrates the separation of a variety of esters of dimethylaminoethanol and 1-dimethylamino-2-*n*-propanol that can be produced from the corresponding esters of choline and β-methylcholine by demethylation. Oven temperatures for the above separations were as follows: 120° and 140° for the alcohols, 200°

and 250° for the esters, and 150° and 180° for the acids. The higher temperatures quoted were used, as in Fig. 1, for essentially qualitative chromatograms to identify components in a mixture, while the lower temperatures were optimal for the quantitative estimation of ethanol, dimethylaminoethyl acetate and acetic acid. Relevant retention times were: ethanol, 4 min at 120°; acetic acid, 9 min at 150°; and dimethylaminoethyl acetate, 8 min at 200°.



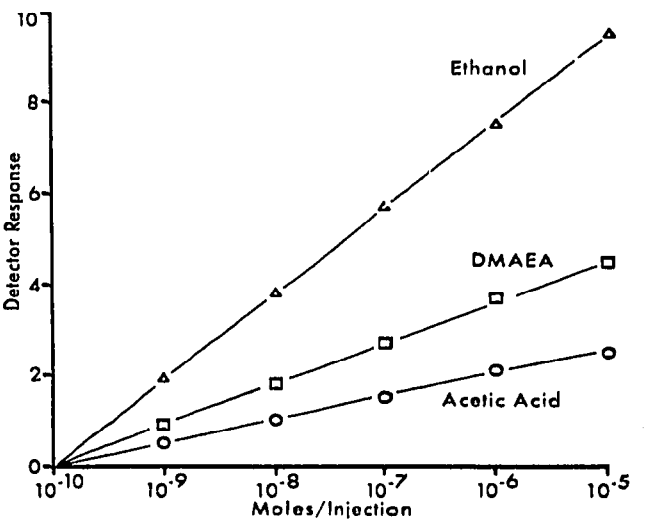


Fig. 1. Gas chromatogram of compounds derivable from choline, β-methylcholine and their esters by demethylation. Column: 5 ft. × 1/8 in. Porapak Q. Nitrogen carrier gas at 33 ml/min. Oven temperature: 250°. Peaks in order of elution after injection (arrow): chloroform solvent, dimethylaminoethanol, 1-dimethylamino-2-*n*-propanol, dimethylaminoethyl acetate, 1-dimethylamino-2-*n*-propyl acetate, dimethylaminoethyl *n*-propionate, 1-dimethylamino-2-*n*-propyl *n*-propionate, dimethylaminoethyl *n*-butyrate and 1-dimethylamino-2-*n*-propyl *n*-butyrate.

Fig. 2. Detector response to compounds derivable from acetylcholine. Column: 5 ft. × 1/8 in. Porapak Q. Nitrogen carrier gas at 33 ml/min. Oven temperature: 120° for ethanol, 150° for acetic acid, and 200° for dimethylaminoethyl acetate (DMAEA). The detector response is measured as peak area in cm^2 at attenuation 10 × 1.

The sensitivity of hydrogen flame ionization detectors to non-hydrocarbons is known to be not directly related to molecular weight[7]. The responses measured as peak areas, to equimolar amounts of dimethylaminoethyl acetate in chloroform solution, and of ethanol and acetic acid in aqueous solution, were determined under identical operating conditions (same column and gas flow rates) except for oven and injector port temperatures and are reported in Fig. 2. The detector response to each of these derivatives was a linear function of concentration over a wide range. For concentrations higher than 10^{-9} moles/injection the detector responses to equimolar amounts of ethanol, dimethylaminoethyl acetate and acetic acid were in the approximate ratio of 4:2:1, respectively. Below this concentration, however, factors such as

solvent blanks, septum and column bleed, and detector instability became important, and the lower concentration limits for quantitative estimation of these derivatives did not reflect this ratio. Least affected by these factors was dimethylaminoethyl acetate, which could be quantitatively detected down to 3×10^{-11} moles/injection. This was also the limit for ethanol estimation mainly due to the ethanol content of the solvent: the avidity of solvents and reagents to pick up traces of atmospheric ethanol has been noted elsewhere[8]. Tailing peaks and difficulty in washing out traces of acetic acid remaining on the column from previous injections resulted in 3×10^{-10} moles/injection being the lower limit for acetic acid estimation.

TABLE I

LOWER LIMITS OF GAS CHROMATOGRAPHIC ESTIMATIONS OF ACETYLCHOLINE

Procedure	*Lower limit of estimation (moles of acetylcholine/determination)*	
	As published	*Using Porapak Q columns*
Reduction to ethanol[1]	4×10^{-10}	6×10^{-11}
N-Demethylation with benzene thiolate[2]	2×10^{-11}	3×10^{-11}
Hydrolysis to acetic acid[4]	3×10^{-10}	3×10^{-10}

Acetylcholine could be estimated by the published procedures[1,2,4] substituting Porapak Q as the stationary phase for gas chromatography. The conversions of acetylcholine to ethanol[1], dimethylaminoethyl acetate[2], and acetic acid[4] proceeded in very high yield, and detector responses to these derivatives prepared from acetylcholine were close to the theoretical responses, except for ethanol at the lower limit of detection when the ethanol content of the reagents (particularly calcium chloride) gave rise to high blanks. The lower limits of quantitative estimation of acetylcholine by these procedures are recorded in Table I. The use of Porapak Q for the estimation of acetylcholine by the procedure of STAVINOHA AND RYAN[1] resulted in a significant decrease in the lower useful limit of detection. The published[1] limit of detection using Carbowax 600/HMDS-treated Chromosorb W columns was confirmed[9]: compared with Porapak Q columns these columns gave higher solvent and reagent blanks, required longer wash-out times and careful conditioning for maximum sensitivity.

Porapak Q appears to be a versatile and reproducible stationary phase for the gas chromatographic estimations of acetylcholine and related derivatives giving little or no tailing of polar volatiles and limited column bleed below 250°. For any particular procedure it probably offers only small advantages over the original packing, but it does enable the assay of acetylcholine by several different procedures to be done on the one column with a minimum of inconvenience. This could be very useful where absolute identification of a cholinomimetic[10,11] is required, since no physical method is currently available capable of absolutely identifying acetylcholine in trace amounts. It should be emphasised that gas chromatographic procedures even when combined with mass spectrometry[6] merely identify and estimate substances that can be produced from the biologically active agent. Thus identification and quantitative cross-correlation by several different biological and physical procedures of a cholinomimetic

activity are desirable to characterise the active agent(s). Using Porapak Q columns, comparison of three of the published procedures for the gas chromatographic estimation of acetylcholine[1,2,4] indicated that, while in general the procedure of STAVINOHA AND RYAN[1] gives the largest detector response to a given amount of acetylcholine, the procedure of JENDEN *et al.*[2] is sensitive to the lowest concentration of acetylcholine.

The authors wish to thank Mr. F. SZABO and Mr. B. MAHER for technical assistance. This investigation was supported in part by grant No. TW00294 from the U.S. Public Health Service.

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Received December 22nd, 1969

CHROM. 4563

Rapid measurement of therapeutic levels of glutethimide in plasma

Several gas–liquid chromatographic methods for the measurement of glutethimide in whole blood or plasma have been presented[1–6]. Those applicable to cases of overdosage have tended to sacrifice sensitivity for speed; others, developed specifically for therapeutic studies, are somewhat lengthy and involved. The following report illustrates how the criteria of speed and sensitivity may be simultaneously satisfied by the use of improved instrumentation.

Materials and methods

Chromatography. A Varian Aerograph Series 2100 Gas Chromatograph, fitted with a flame ionisation detector, was used. The metal column (6 ft. × ¼ in. O.D.) contained 5% SE-30 on 70–80 mesh AW Chromosorb W, and was conditioned at 200° over a 24-h period prior to use.