Notes

CHROM. 4660

Gas chromatography: a method of solid injection

In gas chromatography, test materials are usually dissolved in a suitable solvent and an aliquot applied to the column by means of a microsyringe. Even when the concentration of the solute is high, the solvent is usually the largest amount of material introduced into the column. The recorder tracing may show a large solvent peak with a long tailing edge which often interferes with early peaks. To minimize this problem it is general practice to limit the volume injected to 1–5 μl, and to avoid working at high sensitivity. Many methods have been described for reducing or eliminating the troublesome solvent peak. These include the use of an inlet splitter as described by CLARKE[1] and solid injection by means of a needle[2], platinum spiral[3] or gauze[4]. A different system consists of a precolumn inlet having both a cool and hot zone[5] and this enables the solvent peak to be well separated from the first sample peak. Occasionally it is possible to dissolve the sample in a non-volatile solvent such as silicone oil[6].

The method described here is a development of earlier work when it was shown that trifluoroacetylated amino acid methyl esters could be concentrated without loss[7]. It relies on the application of a liquid sample to a precolumn and the solvent being evaporated without loss of solutes. The precolumn is then placed in the heater zone of the apparatus for gas chromatography. Up to 95% of the final test solution can be injected on to the column, thus making better use of the potential sensitivity offered by gas chromatography[8,9].

Apparatus

A gas chromatograph Pye Series 104, Model 24, fitted with two flame ionization detectors (W. G. Pye Ltd., Cambridge, Great Britain) was used in conjunction with a Honeywell 10 mV 1 sec strip chart recorder (Honeywell Controls Ltd., 411 Taunton Road, Greenford, Middx., Great Britain). Integration of peak areas was carried out with the Kent Chromalog 2 digital integrator (Kent Instruments Ltd., Luton, Beds., Great Britain). Nitrogen (99.9% "white spot" from British Oxygen Co. Ltd., and "high purity oxygen-free" from Air Products Ltd.) was used as carrier gas.

Modification of the instrument. The hole below the septum (B in Fig. 1) in the injection head (C) was enlarged by drilling to a diameter of 3.18 mm. When fitting the chromatography column into the injection head (C) by means of the usual O-ring (E) and back-nut (F) an additional silicone rubber O-ring (D) with 6.3 mm O.D. and 2.7 mm I.D. (Griffin & George Ltd., Alperton, Wembley, Middx., Great Britain) was inserted into the recess as shown.

Precolumn. The precolumn (see Fig. 1) consisted of an 8 cm long glass tube with a 3.1 mm O.D. and wall thickness 0.8 mm. A disc of Whatman glass fibre paper (H. Reeve Angel and Co. Ltd, London, Great Britain) was fused to the bottom end of the tube. This was done by heating the tip of the tube to red heat and pressing the tip against the filter paper placed on an asbestos sheet. This tube was filled loosely to a

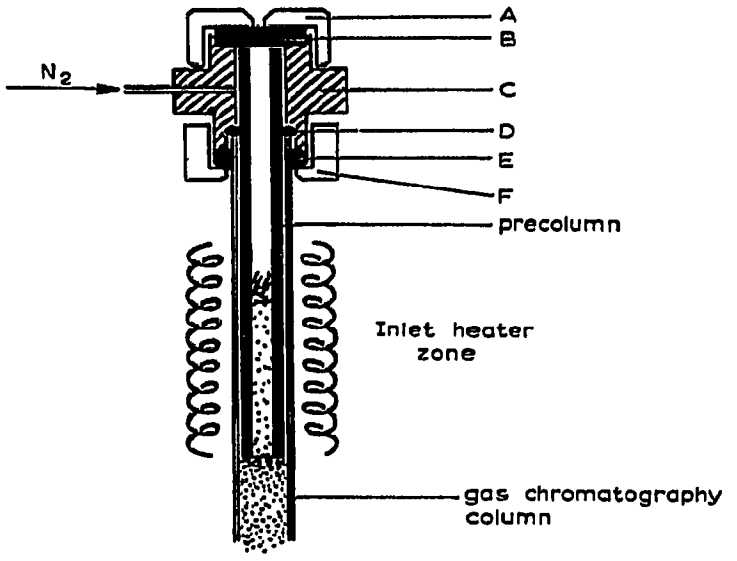

Fig. 1. Injection head of gas chromatograph showing precolumn in position. A = knurled nut, B = septum; C = inlet injection block; D, E = silicone O-rings, F = back-nut.

depth of about 3 cm with the same deactivated support material used in the gas chromatography column and a little pretreated glass yarn[10] was inserted on top. When the precolumn was in position the packing was in the inlet heater zone and the top end close to the septum. No differences were observed whether our support material was coated with stationary phase or not. The sizes of the precolumn and the O-ring (D) were selected so that a push fit was obtained and during gas chromatography of the sample all the carrier gas passed through the precolumn.

Method of use. The liquid sample was applied to the packing in the precolumn and the solvent removed by evaporation (see below). The knurled nut (A) was slowly unscrewed to release the carrier gas pressure in the column. The precolumn was pushed into place and the knurled nut with its underlying septum was immediately screwed tightly back into position. The carrier gas flow controls were not altered. There was no observable blow-back of the packing when the pressure was slowly released at the inlet end of the column.

Experimental

An attempt was made to reduce the size of the solvent peak by injecting small volumes of various test solutions on to the column with a Hamilton microsyringe. But as the volume decreased from 0.5 to 0.1 μl so the HETP[11] values of the peaks increased. Furthermore, amounts of less than 1 μl were not easily reproducible. A needle-type micro-solids injector[2] and a gauze[4] were tried as methods of injection. Even when 1 μl of methylene chloride solvent was evaporated prior to injection considerable losses occurred of such test materials as bibenzyl, pyrene and trifluoroacetylated amino acid methyl esters, and low efficiencies were recorded.

A 1 μl aliquot of a standard mixture (dodecanoic, tridecanoic, tetradecanoic and hexadecanoic acid methyl esters in methylene chloride) was transferred by microsyringe to a precolumn, which was then immediately placed in position in the apparatus for gas chromatography. The HETP values and the peak areas obtained gave

comparable results with those obtained by direct injection of 1 μl on to the column thus showing that this new method of injection was satisfactory.

The same standard solution was diluted × 30 with methylene chloride and 30 μl of this diluted solution was applied by means of a syringe to the precolumn. Most of the solvent was removed by placing the precolumn in a B14 test tube, which was immersed in an ice–water bath at 0° whilst rotary evaporation was carried out, first with a water pump for 1 min and then with an oil pump for 0.5 min. The vacuum measured during the evaporation was 10 torr. Initial use of the oil pump caused bumping. Preliminary experiments are advisable[7]. The sample was then chromatographed.

A total of 100 μl of the original solution diluted × 100 was then applied to the precolumn for chromatography. Since the total capacity of the precolumn was 30–40 μl the sample was applied by means of three separate applications followed by solvent evaporation each time.

TABLE I

PEAK AREAS FOR DODECANOIC, TRIDECANOIC AND TETRADECANOIC ACID METHYL ESTERS RELATIVE TO HEXADECANOIC ACID METHYL ESTER TAKEN AS 1.0

Gas chromatography conditions as in Fig. 2. A = 6 replicates, 1 μl test solution injected through septum with Hamilton microsyringe (as in Fig. 2a), B = 6 replicates, 30 μl test solution diluted × 30 applied to precolumn and solvent evaporated (as in Fig. 2c), C = 4 replicates, 100 μl test solution diluted × 100 applied to precolumn and solvent evaporated.

Method of injection	*Fatty acid methyl ester*	*Mean peak area*	*% coefficient of variation*
A	Dodecanoic	0.366	2.13
	Tridecanoic	0.454	1.56
	Tetradecanoic	0.675	1.45
B	Dodecanoic	0.348	1 22
	Tridecanoic	0.441	0.82
	Tetradecanoic	0.658	0.40
C	Dodecanoic	0.355	2 70
	Tridecanoic	0.448	0.84
	Tetradecanoic	0.664	0.67

Table I presents the mean peak areas obtained for dodecanoic, tridecanoic and tetradecanoic acid methyl esters relative to hexadecanoic acid methyl ester. A comparison can be made of the results obtained after injecting 1 μl, 30 μl and 100 μl of test solution. In each case the percentage coefficient of variation for each injection decreases with increased retention time, but the quantitative results are similar.

Instead of applying the sample by syringe an alternative method was tried. This consisted of lowering the precolumn into the test solution and allowing the solution to be drawn up by capillary attraction. This method was satisfactory provided that the total liquid in the tube was less than the amount needed to saturate the packing. Approximately 90% of the total sample was taken up but the exact amount taken up could not be determined.

The advantages of a method, whereby the solvent peak is greatly reduced or even eliminated entirely from the chromatogram and a large volume of test solution may be used, are seen by comparison of Figs. 2a, b and c. In Fig. 2a 1 μl of a solution

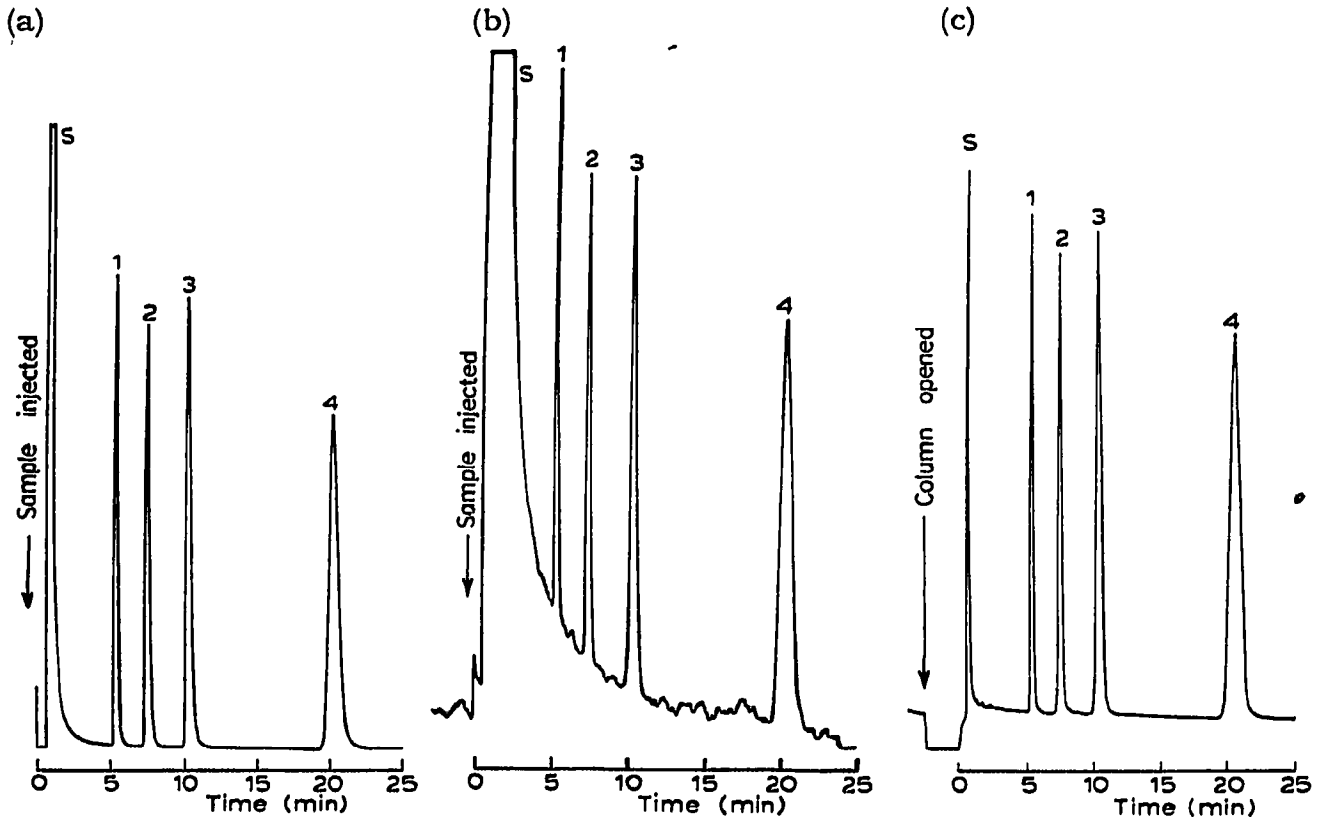

Fig. 2. (a) Gas chromatogram of a mixture of dodecanoic, tridecanoic, tetradecanoic and hexadecanoic acid methyl esters in methylene chloride. Conditions: glass column, at inlet end 10 cm × 3.2 mm I.D. joined to remainder of column 2 m × 2.5 mm I.D. packed with 10% (w/w) diethylene glycol succinate on Diatoport S 80–100 mesh; inlet heater block temperature, 190°; oven temperature, 150°; nitrogen gas flow, 30 ml/min, amplifier setting 5 × 10^{-10}A for f.s.d. Method of sample injection: 1 μl by Hamilton microsyringe through septum. 1 = methyl dodecanoate; 2 = methyl tridecanoate, 3 = methyl tetradecanoate; 4 = methyl hexadecanoate; S = solvent.
(b) Gas chromatogram of the same solution as in (a), except that it was diluted × 30 with methylene chloride. Conditions as in (a) with amplifier setting 20 × 10^{-12}A for f.s.d. Method of sample injection: 1 μl by Hamilton microsyringe through septum.
(c) Gas chromatogram of the same diluted solution as in (b). Conditions as in (a) and (b). Amplifier setting 5 × 10^{-10}A for f.s.d. Method of sample injection: 30 μl applied to a precolumn and the solvent removed before chromatography.

of fatty acid methyl esters in methylene chloride was injected on to the column with a microsyringe by injection through the septum. The sensitivity of the amplifier setting was attenuated to 5 × 10^{-10}A for full scale deflection and the ester peaks are well resolved clear of the solvent peak. In Fig. 2b 1 μl of the same sample diluted × 30 with methylene chloride was injected in the same manner with amplifier setting at 20 × 10^{-12}A for f.s.d. This illustrates the general problem of gas chromatography at high sensitivity. The baseline is "noisy", the solvent peak is large and interferes with the early peaks although the retention times remain the same. In Fig. 2c, 30 μl of the sample (as in Fig. 2b) was transferred to a precolumn and the solvent evaporated before chromatography. Note that the residual solvent gives a narrow peak, the size of which varies from injection to injection but does not interfere with the sample peaks. The drop in the baseline before the solvent peak is due to a temporary decrease

of nitrogen flow to the detector when the precolumn is inserted into position, but this does not interfere with the subsequent separation.

A methylene chloride solution of the lower fatty acid (octanoic, nonanoic, decanoic and dodecanoic) methyl esters was evaporated from a precolumn using temperatures from 0° to −20° with evaporation times of 1 to 4 min. Some differential losses usually occurred, ranging from about 6% octanoate down to 0–1% in the case of decanoate, so that our method cannot be recommended for these lower fatty acid methyl esters. It is successful with all the trifluoroacetylated amino acid methyl esters[7]. Although there are serious limitations to the use of the method, we believe that it could be useful in many cases where the solvent is very volatile and the test materials relatively non-volatile.

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