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Note

Gas chromatography of samples in dilute solution

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In gas–liquid chromatography, accurate quantification of trace components on the tail of large peaks is difficult (see *e.g.* ref. 1). An example of this is the component eluted on the solvent tail. This paper describes a simple technique for the removal of solvent before it interferes with the chromatography. Other methods have also been described which attempt to solve this problem[2–5].

EXPERIMENTAL AND RESULTS

A method developed in this laboratory for the estimation of 5-hydroxytryptamine (5-HT) in brain tissue[6] involves the partial purification of 5-HT using solvent extraction procedures. The final extract is evaporated to dryness and the residue taken up in methyl cyanide: trifluoroacetic anhydride is then added in order to convert the 5-HT to its fully trifluoroacetylated derivative. This derivative is then chromatographed on a 1.5-m (4-mm-bore) glass column of 3.6% OV-17 on 100–120 mesh Gas-Chrom Q with a carrier gas flow-rate of 20 ml/min. It has a retention time of 12.5 min when injected onto the column at a temperature of 120°, which is first held for 6 min and then increased at a rate of 0.5°/min. The instrument is fitted with a standard ^{63}Ni source electron capture detector. Under these conditions only 1 μl from a total sample volume of over 500 μl is injected onto the column in order to prevent the large solvent tail from interfering with the 5-HT derivative peak. In attempts to increase the overall sensitivity of the assay procedure, larger amounts were injected with the result that the 5-HT derivative was eluted on the rapidly sloping baseline of the solvent tail.

The large solvent tail is caused by the excess trifluoroacetic anhydride in the reaction mixture and the obvious solution to this problem is to evaporate the excess reagent from the sample and reconstitute it in an appropriate solvent. However, this is not possible since the trifluoroacetyl derivative of 5-HT is very labile and evaporation either under vacuum or with a stream of dry nitrogen results in considerable decomposition. The reaction mixture must therefore be used as the solvent for the chromatography and a device has now been developed which allows the excess solvent to be largely removed, before it interferes with the chromatography.

The technique now adopted is to introduce the sample onto a short pre-column which carries out the initial separation of the bulk of the volatile solvent and the excess reagent from the derivative being studied. The solvent is eluted first from

this pre-column and is vented to the atmosphere through a valve; the valve is closed before the derivative of interest arrives at the splitter and thus it passes onto the main part of the column. The system is similar to that described by Zumwalt *et al.*[4,5], though the components of the apparatus described here are all commercially available and it can be readily constructed in the laboratory.

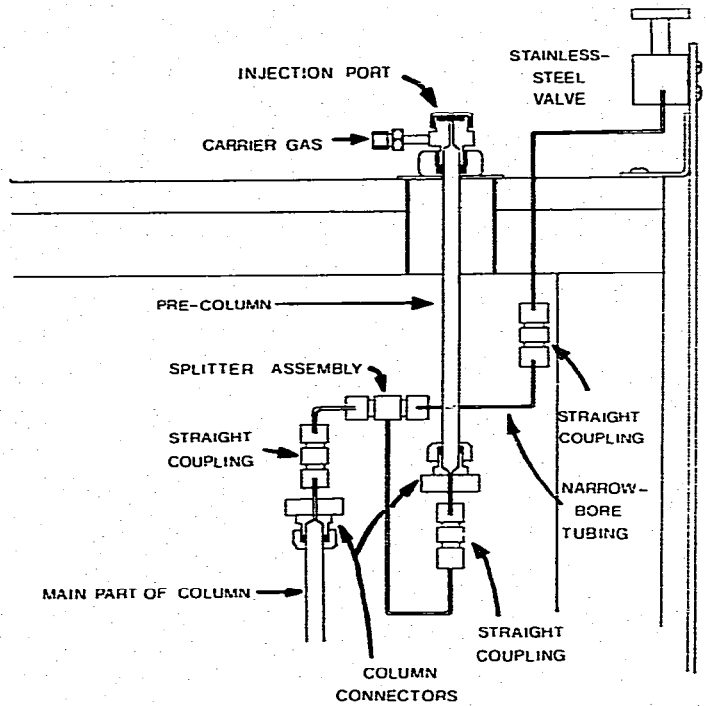

Fig. 1. Diagrammatic representation of the apparatus described in the text (not to scale).

The system is built onto a standard Pye 104 gas chromatograph, and the numbers given in brackets refer to the Pye catalogue numbers except where stated. Alternative instruments can obviously be used, the only limitation being the column connector for the 6.4-mm-O.D. columns. A diagrammatic representation of the apparatus is shown in Fig. 1. A pre-column of 13 cm in length (4 mm bore) is connected via a Vitonseal-type column connector (No. 717041) (if temperatures in the region of 240° are to be used, the GC "O" rings (No. 15500) supplied by Applied Science Labs. are less susceptible to leakage) and a straight coupling (No. 653853 for 1.6-mm-O.D. pipe) to the splitter assembly (No. 719440). One side of the splitter assembly is then connected to the main part of the column in a similar manner with stainless steel tubing (No. 111661, 1.6 mm O.D., 0.8 mm I.D.) and the other side of the splitter is connected to a straight coupling with 2.5 cm of small-bore stainless-steel tubing (No. 111641, 1.6 mm O.D., 0.25 mm I.D.) and thence to a stainless-steel shut-off valve (Nupro Type SS-2H2, available through Phase Separations, Queensferry, Great Britain) with stainless-steel tubing (No. 111661). The fine-bore tubing ensures that, when the valve is open, there is a small residual flow of carrier gas through the column to prevent damage.

Purge gas is supplied to the detector when the column flow is decreased in order to prevent detector damage.

The chromatography is carried out as follows. Purge gas to the detector is turned on at about 40 ml/min and the shut-off valve is opened. The sample (5 μl) is then injected onto the pre-column and after 15 sec the shut-off valve is closed and the purge gas to the detector is turned off. The chromatography then continues as normal. Under these conditions the majority of the volatile trifluoroacetic anhydride and methyl cyanide is vented from the chromatographic system through the valve. The derivative of interest remains on the pre-column for about 20 sec and hence, when the valve is closed, it continues down to the main part of the chromatographic column.

The peaks are slightly broader than in a typical full-column chromatogram, presumably due to the diffusion which takes place in the dead space of the splitter. The results using this device are reproducible and we have found no evidence of decomposition on the metal parts of the system. It has allowed an increase in the overall sensitivity of 5 times though we have found that even larger volumes can be applied to the pre-column.

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