A METHOD FOR THE INTRODUCTION OF SAMPLES OF LONG CHAIN FATTY ACID METHYL ESTERS ON TO GAS CHROMATOGRAPHY COLUMNS

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One of the problems encountered in the application of gas chromatography to the study of animal lipids is finding a satisfactory way of introducing the sample to be analysed on to the column. The difficulties are caused mainly by the small amounts of material available for analysis. These amounts are difficult to handle and make it necessary to use the very sensitive β-ionization detector for their measurement. With this detector quantitative analysis may be very inaccurate if traces of water, air or a large amount of volatile solvent are admitted into the chromatograph with the sample.

Most sample introduction devices use a capillary micropipette to measure and transfer the sample. Although many modifications of the micropipette exist, it is inconvenient in operation and may introduce air or solvent into the system. It is also subject to other errors since the solution of material for analysis must be highly concentrated to get a sufficiently big sample into the micropipette (see PERFORMANCE, point 3).

The present system was developed to overcome these difficulties. It does not involve measurement and handling of very small volumes of concentrated material. It has been applied satisfactorily to the chromatography of methyl esters of long chain fatty acids (C_{12}–C_{24}) at column temperatures 170–200°.

The principle is as follows: A volume of up to 0.2 ml of solution of fatty acid methyl esters in a volatile solvent is pipetted into a metal trough at the end of a

Fig. 1. The trough at the end of the sample introduction device (the "spoon"). The dimensions of the "spoon" are given in the legend to Fig. 2.

metal rod (the "spoon", Fig. 1) and the solvent is allowed to evaporate at room temperature. The trough containing the dry esters is then introduced into the chromatograph without admitting air or interrupting the flow of argon and comes into contact with the hot packing phase of the chromatographic column. This causes the

esters to volatilize almost instantaneously and they are carried into the column by the stream of argon as a narrow band.

A detailed description of the apparatus* and its use and some data on its performance are given in the following sections.

DESCRIPTION

Apparatus

The device is shown in Fig. 2. The details of construction can be varied considerably to suit individual requirements without affecting the performance of the system.

The central component of the apparatus is the "spoon" (Fig. 1; G, Fig. 2). This is a stainless steel trough capable of holding 0.1–0.2 ml of liquid, at one end of a steel rod. A soft iron cylinder at the other end of the rod enables the "spoon" to be manipulated by means of a magnet.

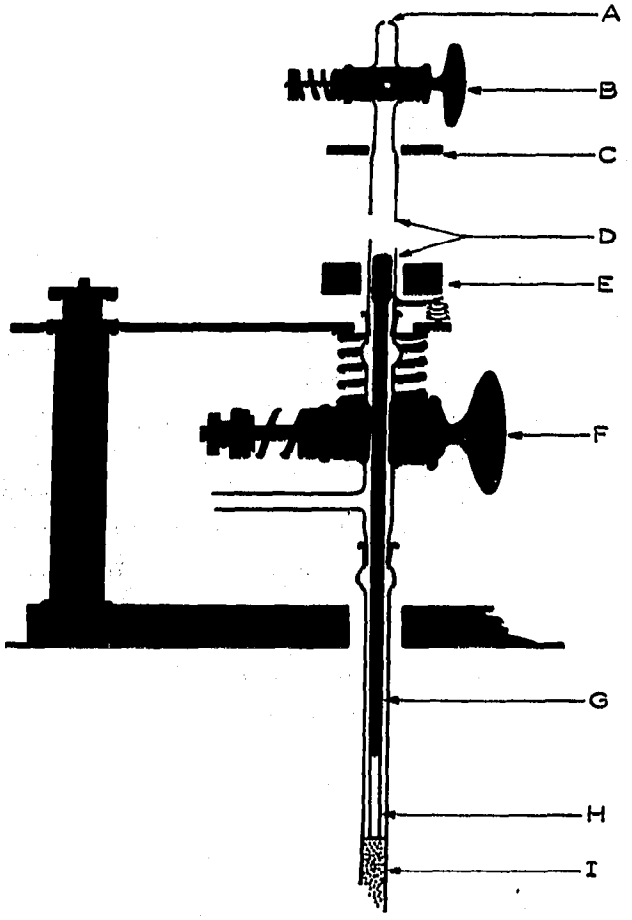

Fig. 2. The complete sample introduction apparatus as used with the "Pye" Argon Chromatograph. The apparatus is drawn in the position in which the sample is discharged into the column, with the trough of the "spoon" touching the packing phase. A: capillary gas leak; B: tap leading to the leak; C: soft iron ring; D: the holder of the "spoon"; E: circular magnet; F: wide bore tap with side arm; G: the sample introduction device (the "spoon"); H: the trough at the end of the "spoon"; I: gas chromatographic column. The figure is approx. 1/4 full size. The dimensions of the "spoon" (G) are: length 28 cm, length of trough (H) 3.5 cm, diameter 3 mm. The length of the holder between the soft iron ring (C) and the ground glass joint is 28.5 cm.

* An application for patent rights for the device described below has been lodged by the National Research Development Corporation, 1 Tilney Street, London W. 1.

During sample introduction the trough at the end of the "spoon" should come into contact with the packing phase of the column in a region of high temperature to cause rapid volatilization of the sample, as described in the introductory section, above. For this either a special heater may be used or the "spoon" must be lowered sufficiently far into the heating jacket surrounding the column. The length of the "spoon" will depend on what method of generating the high temperature to vaporize the sample is adopted. When using the device with the "Pye" Argon Chromatograph operating at 170–200° it was found that the top of the packing phase was at a sufficiently high temperature when columns were packed to within 12 cm from the top of the heating jacket.

The "spoon" rests in a holder (D, Fig. 2) which is a glass tube having a ground glass joint at one end, a tap (B, Fig. 2) and capillary gas leak (A, Fig. 2) at the other. The capillary leak permits the holder to be filled with argon before the "spoon" is lowered on to the column (see *Procedure* below) without exposing the sample to a rapid stream of gas. By filling the holder with argon, air is completely excluded from the chromatographic column during analysis and cannot affect the recorder base-line. The bore of the gas leak should be adjusted to the pressure of argon used, so that air is displaced from the holder in 3–8 sec. With our apparatus one leak has been found satisfactory for pressures from 5–20 p.s.i.

A circular magnet (E, Fig. 2) is used to move the "spoon" in and out of the holder. A soft iron ring (C, Fig. 2) is fixed to the holder near the tap so that when the magnet is held by this ring the spoon is also held completely inside the holder.

A wide bore tap (F, Fig. 2) with a side arm is fitted on top of the column with a ground glass joint. The side arm is connected to the argon supply and gives an uninterrupted flow of argon through the apparatus during the whole sample introduction. The bore of the tap must be larger than the diameter of the trough at the end of the "spoon". A ground glass joint connects the tap to the holder.

A bracket fixed to the cabinet of the chromatograph holds the tap in position and supports the whole device. Gas leaks are prevented by securing taps and joints with springs.

Before the sample is introduced the solvent is evaporated from the sample in the drying box (Fig. 3). The box has a transparent sliding lid and a hole through which

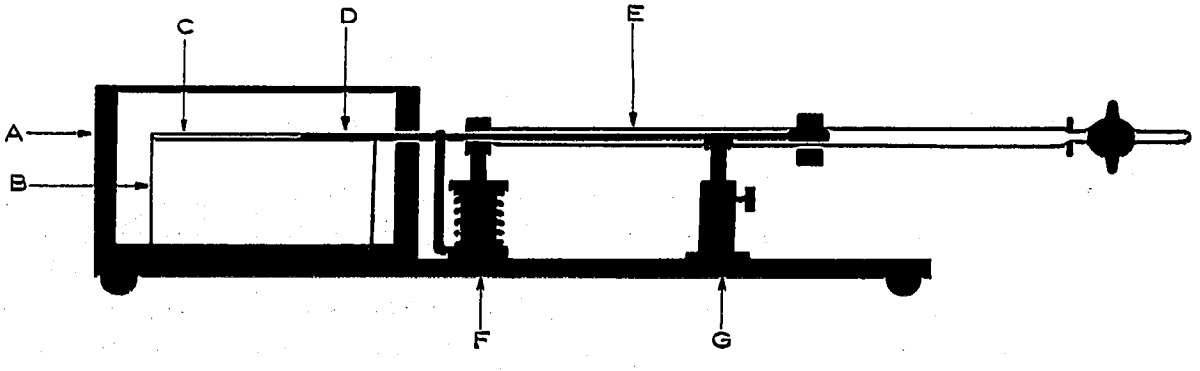

Fig. 3. The drying box for removal of solvent from sample prior to introduction. The box is shown with the "spoon" in the position in which it is held during solvent removal (see *Procedure*). A: the box with a transparent sliding lid; B: aluminium block with a V-shaped slot C; D: the "spoon" with the trough (*cf.* Fig. 1) inside the slot C; E: the holder of the "spoon"; F and G: supports which hold the "spoon" in contact with the block.

the "spoon" may be inserted as shown in the figure. It contains an aluminium block with a V-shaped depression into which the "spoon" fits. An adjustable stand and a spring outside the box can hold the "spoon" inside the depression in firm contact with the block.

Procedure

The "spoon" is placed on the aluminium block as shown in Fig. 3. 0.1–0.2 ml of a solution of the sample in hexane is pipetted into the trough at the end of the "spoon". The lid of the drying box is then closed and solvent is allowed to evaporate. The aluminium block supplies heat to evaporate the solvent. Without the block the temperature of the evaporating solvent may drop sufficiently to cause condensation of water vapour on the trough. To avoid condensation, it is also inadvisable to use very volatile solvents such as diethyl ether or light petroleum (b.p. 40–60°).

As soon as the solvent has evaporated the spoon is withdrawn into the holder where it is held by the soft iron ring and the circular magnet. The holder is then placed on top of the wide-bore tap and secured with a spring.

The wide-bore tap is then opened and the holder is filled with argon by opening the tap leading to the capillary gas leak. After air has been displaced from the holder the tap leading to the leak is closed and the "spoon" is lowered by means of the magnet so that the trough passes through the wide-bore tap and touches the packing phase as shown in Fig. 2. This causes the sample to volatilize and be carried into the column by the stream of argon.

PERFORMANCE

The advantages and limitations of the present system are discussed in this section together with results of experiments carried out to test the performance of the system.

(1) The sample may be put into the evaporating trough in a relatively large volume of solvent. This allows complete transfer of sample into the trough when the total amount of material available for analysis is very small.

(2) A volume of 0.1–0.2 ml can be accurately measured. Thus the new method permits the reproducible introduction of exactly known amounts of material for analysis. This opens new possibilities of study of detector response to different compounds. It also simplifies routine analysis, since the size of each sample can be adjusted to give peaks of suitable magnitude on the chromatogram.

(3) Samples for introduction with the present method are taken from a dilute solution of the material to be analysed. When the micropipette is used, however, the solution must be concentrated or even freed from solvent by evaporation before sampling.

Several sources of error may be introduced during this evaporation. Less soluble esters may crystallize out and the more mobile, shorter chain esters may spread on the sides of the containing vessel. Finally evaporation of more volatile, shorter chain esters may occur when solutions are concentrated at an elevated temperature, under reduced pressure or in a stream of gas. Any of these processes may cause a sample taken with the micropipette to be unrepresentative of the original mixture. The new procedure is not subject to these errors, since no evaporation is required before sampling (see also point 6, below).

(4) The "spoon" is introduced into the gas chromatographic apparatus without

stopping the flow of argon or introducing air, water-vapour or volatile solvent. The sample can thus be applied without disturbing the base-line on the recorder of the chromatograph, so that even esters which pass through the chromatographic column within the first five minutes (*e.g.* methyl laurate, C_{12}) can be estimated accurately.

(5) An important requirement of a sample introduction system is that the sample should leave the introduction device as quickly as possible and enter the column as a narrow band. Otherwise broad peaks will appear on the chromatograms and the overall efficiency of resolution (number of theoretical plates) of the system will be less than the actual efficiency of the column itself.

The efficiency given by the present system was compared with that of a widely used capillary micropipette—the "Pye"* closed injection system, and the results are

TABLE I

EFFICIENCY OF RESOLUTION OF METHYL STEARATE (C_{18}) AND BEHENATE (C_{22}) OBTAINED ON THE SAME COLUMN USING EITHER THE NEW PROCEDURE OR A "PYE" CLOSED INJECTION MICROPIPETTE FOR SAMPLE INTRODUCTION

Column temperature 190°. Flow rate of argon 33 ml/min. Measure of efficiency used is the number of theoretical plates $n = (4d/w)^2$ (see, for example PATTON[1]), where d = distance of intersection of tangents to the peak from the start of the chromatogram, and w = width of peak (intercept of base-line between tangents to the peak).

	Methyl stearate			*Methyl behenate*		
	Mean n	*S.D.**	*No. of runs*	*Mean n*	*S.D.**	*No. of runs*
Present procedure	2208	140	5	2354	171	5
"Pye" pipette	1884	162	5	2580	162	5

* S.D. = standard deviation.

shown in Table I for methyl stearate (C_{18}) and behenate (C_{22}). The new procedure gave considerably higher efficiency for methyl stearate, oleate, linoleate and shorter chain esters, but slightly lower efficiency for methyl behenate.

With both procedures, however, the efficiency of resolution increases with chain length, so that the slightly lower efficiency obtained with the new procedure relative to the micropipette does not diminish its usefulness for the introduction of samples of esters of chain length C_{22} or more. This slight loss of resolution efficiency is due to the slower vaporization of the long chain esters from the "spoon" at the column temperature used (190°) and indicates that higher column temperatures or an auxillary heater may be needed for introduction of samples of very involatile compounds (*e.g.* triglycerides or steroids).

(6) Before the sample is introduced into the chromatograph with the present procedure solvent must be removed from it by evaporation (see *Procedure* above) and erroneous results will be obtained if the more volatile components of the sample evaporate together with the solvent. Experiments such as the two described below indicate that for methyl laurate and higher fatty esters no detectable loss occurs. Further tests would be advisable, however, before the procedure is adopted for more volatile compounds.

In one experiment a mixture of methyl laurate (C_{12}), myristate (C_{14}), palmitate

* Pye Ltd., Cambridge, England.

(C_{16}) and stearate (C_{18}) was chromatographed fourteen times using alternately the present procedure or the "Pye" micropipette for sample introduction. The mean composition of the mixture measured on the chromatograms was similar for both systems. The scatter of individual results was slightly smaller for the present procedure.

In another experiment 0.1 ml samples of methyl laurate solutions of diminishing concentrations were chromatographed with the new procedure of sample introduction. The amounts of ester put on the column ranged from 0.2 μmoles to 0.002 μmoles. The lower limit of the range gave a 7% deflection of recorder pen at the highest useful sensitivity of the "Pye" instrument used (nominal detector voltage 1250 V, amplifier sensitivity $\times$ 3). Within this range a linear relationship was obtained between amount of laurate taken and area of corresponding peak on the chromatogram. A linear relationship would not be expected to apply over this range if appreciable evaporation of methyl laurate occurred.

The two experiments confirm the suitability of the present system for esters of chain length C_{12} and above.

(7) The new procedure of sample introduction is quick and simple in practice. The apparatus is robust and easy to clean. There are no capillaries, which are easily blocked.

(8) It is probable that the new procedure increases the stability of column packing by excluding air, water and large amounts of solvent from the system.

In conclusion the new procedure offers a fairly satisfactory way of sample introduction of long chain fatty acid methyl esters and is probably better than most micropipette systems for this group of compounds. It should be equally applicable to other compounds of similar volatility. The use of the method with much more or much less volatile compounds would require further development (see above, points 5 and 6).

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SUMMARY

A new method of introducing samples on to gas chromatography columns has been developed. A dilute solution of the sample in a volatile solvent is evaporated to dryness in a metal trough attached to a special manipulating device. The trough is introduced into the chromatograph without interrupting the flow of gas through the column or admitting air and is brought into contact with the hot packing phase of the column. This causes rapid volatilization of the sample which is then carried into the column by the gas stream.

The performance of the new procedure with long chain fatty acid methyl esters is discussed and compared with that of a capillary micropipette.

REFERENCE

1 H. W. PATTON, in R. L. PECSOK, *Principles and Practice of Gas Chromatography*, John Wiley and Sons, Inc., New York, 1959, p. 12.