CHROM 3823

THE USE OF A FLAME-IONISATION DETECTOR TO DETECT COMPONENTS SEPARATED BY THIN-LAYER CHROMATOGRAPHY

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SUMMARY

Lipids were separated by thin-layer chromatography on a thin silica glass rod coated with adsorbent. After the chromatogram was developed the separated compounds were detected by quickly passing the glass rod through the flame of a flame-ionisation detector. The apparatus built for this purpose is described and the factors affecting response have been evaluated.

A standard mixture of cholesterol stearate, methyl palmitate and triolein was analysed ten times. The standard deviation, S.D., was 1.22 and the 95% confidence limits are given by $\bar{x}_n \pm (2/\sqrt{n}) \cdot 1.22$, where $\bar{x}_n$ is the average value of n readings of x.

INTRODUCTION

The quantitative evaluation of thin-layer chromatograms can be accomplished by a variety of procedures either directly on the plate or on material extracted from the plate[1–3]. Components are estimated most rapidly by photodensitometry or spot area measurements. Both techniques have the disadvantage that a reasonable number of standard mixtures must be run alongside the unknown to obtain quantitative results. We have recently described a method[4,5] which promises to offer a rapid and reproducible way of estimating components separated by thin-layer chromatography*.

The method involves first a thin-layer chromatographic separation performed on a thin silica glass rod coated with Silica Gel G. The rod is then passed directly through the flame of a flame ionisation detector and as the separated components enter the flame they burn and are detected. The technique is reminiscent of the liquid–liquid chromatographic column detector devised by JAMES, RAVENHILL AND SCOTT[6] but differs from it by eliminating a "cracking procedure" prior to detection. COTGREAVE AND LYNES[7] have recently described a method of detecting components on a TLC plate by cracking or vaporising the separated components and passing them to a suitable detector.

This paper describes our most recent work using a more precise instrument than the meccano device used previously.

* The instrument is the subject of British Patent No. 18448/67 licensed to Shandon Scientific Co, London.

EXPERIMENTAL

Apparatus

The general objective was to design an instrument which would allow us to pass a coated rod horizontally through the flame of an F.I.D. We knew that the position of the rod in the flame affected detection and by suitable design we hoped to minimise any variations in the distance between the rod and the flame nozzle during detection.

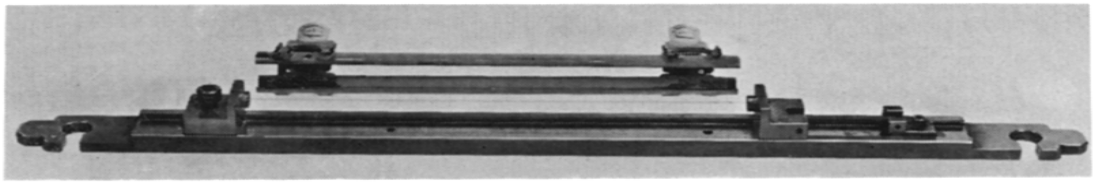


Fig. 1. Tension bar holding the glass rod, and divided tube to assist chromatographic development.

The apparatus consisted essentially of a detachable tension bar which holds the rod (Fig. 1) and this moved on ball bushings along a set of horizontal bars (Fig. 2). The tension bar could be driven at various speeds (10–60 cm/min) by a synchronised motor attached to a Kop variator gear box.

The glass rod was held in the tension bar by placing the ends of the rod in narrow slots at each end of the bar. One slot was fixed, the other could slide along the bar under the tension of a spring. Glass beads at both ends of the rod fitted into counter-sunk holes, and a slight tension was placed on the rod by the spring attached to the movable slot.

The tension bar was fixed to the rest of the apparatus by self-centring nuts which ensured that the rod was directly above the nozzle of the flame. The distance

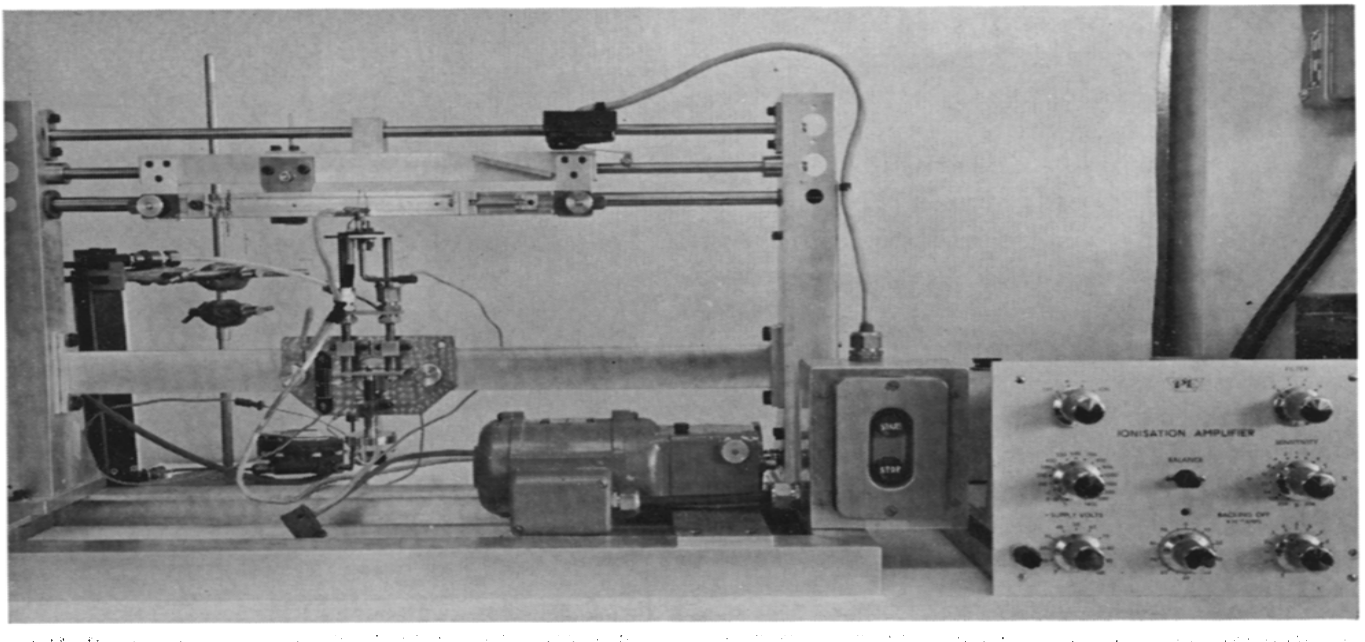


Fig. 2. Flame-ionisation thin-layer detector.

between the nozzle and the rod could then be adjusted with an accuracy of approximately 25 μm (1/1000 in.) by using a screw-driven attachment which moved the detector (and therefore the nozzle).

The flame-ionisation detector and amplifier were taken from a gas–liquid chromatograph* and required little or no modification. Pure hydrogen was fed to the detector, however, and the flame was allowed to burn in air.

Thin-layer chromatography

This was performed on a thin silica glass rod (0.45 mm diameter) held in the tension bar (Fig. 1). The rod was removed from the tension bar and coated by dipping it in a slurry of silica gel (50 g) in acetone (80 ml) and water (10 ml). The slurry was stored in a stoppered cylinder and could be re-used after shaking. The consistency of the slurry had to be adjusted slightly to ensure that an adequate layer ($\approx$ 100 μ thick) was placed on the rod. The coated rod was quickly placed in the tension bar to prevent any damage to the silica layer by vibration of the rod. The silica layer was then activated and cleaned by passing the coated rod at $\approx$ 20 cm/min through the flame of the F.I.D.

The mixture to be separated ($\approx$ 5γ) was placed approximately 2 cm from the

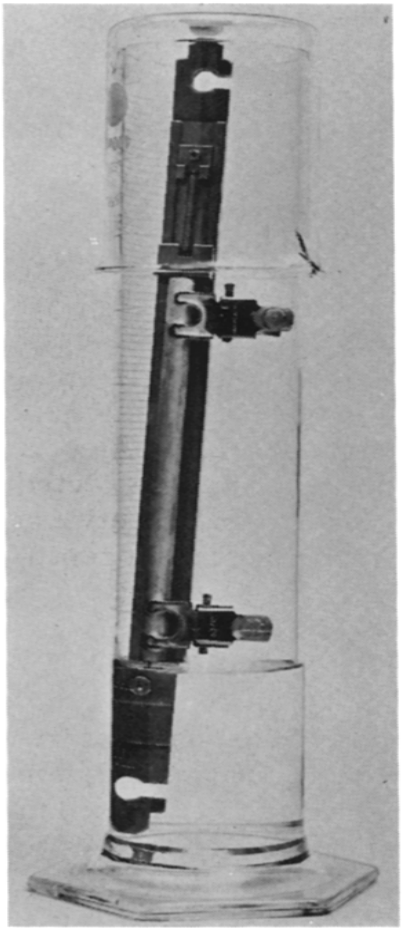


Fig. 3. Chromatographic assembly.

* Perkin Elmer 541 Fractometer but other detectors of suitable design would do.

end of the rod which was then surrounded by a narrow chromatographic chamber consisting of a stainless steel tube split lengthways and held together by two spring clips (Fig. 1). The split tubes were also coated on both sides with silica by dipping in a less concentrated slurry of Silica Gel G. The tension bar was then placed in a large cylinder containing sufficient developing solvent to reach the lower end of the chromatographic rod (Fig. 3). Development of the rod took approximately 15 min and was assisted by the solvent developing up the walls of the coated stainless steel tubes. (Glass had been used for this purpose originally but was not as robust.)

Compounds were separated using solvent systems similar to those used in conventional thin-layer chromatography. For example cholesterol stearate, methyl palmitate and triolein were separated using a mixture of light petrol (95%) and ether (5%).

Detection

After development the tension bar and rod were attached to the instrument. The nozzle height, flame size and rod speed were adjusted and the compounds then

TABLE I

STANDARD CONDITIONS FOR DETECTION

Rod diameter	0.45 mm
Thickness of SiO_2 layer	$\approx$ 100 μ
Distance between nozzle and rod	0.5 mm
Hydrogen flow	80 ml/min
Size of flame	$\approx$ 5 mm diameter
Rod speed	35 cm/min
Chart speed	40 cm/min

detected by passing the rod through the flame. In Table I typical conditions for the analysis are given, which indicate that detection takes approximately 30 sec to complete.

To determine the effect of certain variables on the response of the detector known amounts of material were applied directly to the coated rod and then detected directly without chromatography. By varying rod speed, flame size, distance between rod and nozzle etc., we were able to determine what factors were important for reproducible detection.

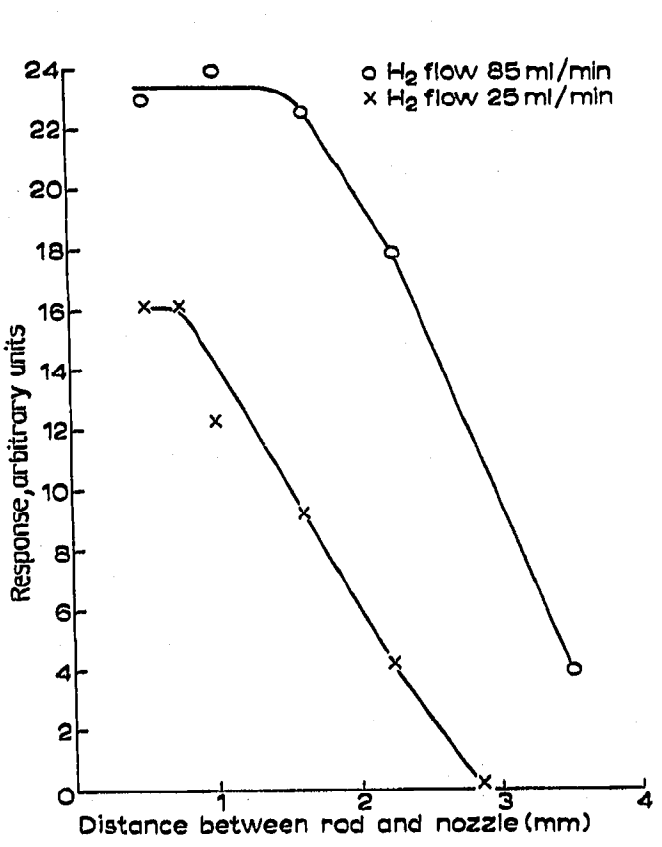
RESULTS AND DISCUSSION

We have attempted to evaluate the effect that known variables have on response. One of the most important factors is the volatility of the compounds being detected and it was soon discovered that most of the common organic solvents used in chromatography are not detected by the present system. It is only when one comes to compounds such as acetic acid that any detection is observed. It is to our advantage that the developing solvents do not interfere with detection. The detection of fairly volatile components in a mixture, however, would necessitate further modifications in the design of the present instrument.

To determine the capabilities of the present instrument we did a series of experiments in which we varied rod speed, distance between rod and nozzle etc., and detected a series of compounds of different volatility. In particular we chose a series of fatty acids from acetic (C_2) to stearic (C_{18}) and the relatively non-volatile compound tristearin. Known amounts (10 γ) of the compounds were applied as 1% solution as in hexane to the rod using a μl syringe. This was done within $\pm$ 10% due to the practical difficulties of ensuring that all the material was placed on the rod.

Rod dimensions

There was a tendency for the upper part of the rod to be shielded from the flame and a loss in sensitivity was observed when large-diameter rods were used. The most efficient detection was therefore obtained when the rod diameter was small compared to the size of the flame. From the chromatographic point of view the larger the rod diameter the greater the loading of material one can apply to the chromatogram. However too great a diameter will result in uneven development and poor resolution. With very narrow rods (0.1 mm) it was difficult to obtain a uniform coating and very difficult to obtain a chromatographic separation. No doubt these technical difficulties could be overcome but no advantage could be seen in using such narrow rods. For our particular detecting system a rod 0.45 mm in diameter was found to be suitable.



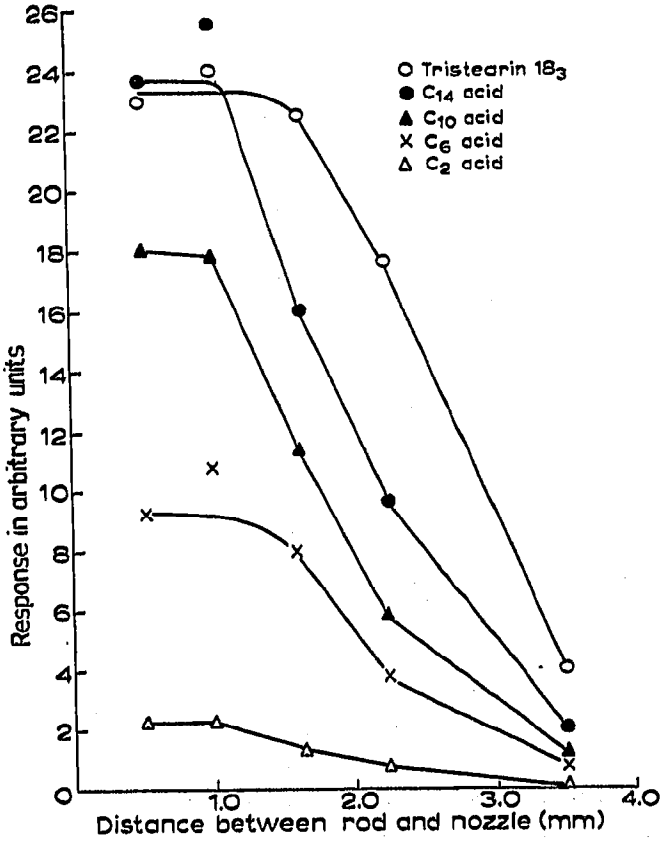


Fig. 4. Effect of rod position and flame size on the detection of tristearin. Conditions: rod speed, 34 cm/min; detector voltage, 200 V; rod diameter, 0.45 mm; coating, Silica Gel G.

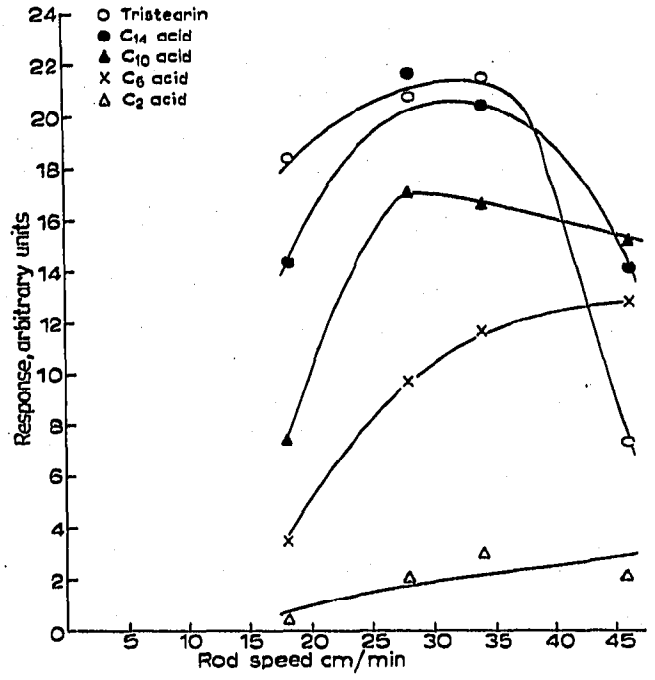
Fig. 5. Effect of rod position in flame on response. Conditions: H_2 flow, 85 ml/min; rod speed, 34 cm/min; detector voltage, 200 V; rod diameter, 0.45 mm; coating, Silica Gel G.

Thickness of silica layer

Operating under the typical conditions described previously no very significant differences in response were observed when the series of fatty acids were detected on widely different thicknesses of silica. There was possibly a slight tendency for the non-volatile compounds *e.g.* tristearin to show a higher response both on thin silica layers or using very narrow rods.

Position of the rod in the flame

The distance between the rod and the nozzle was varied by raising or lowering the detector using the screw attachment. The response of the detector to tristearin varied as the rod–nozzle distance altered (Fig. 4). When the rod to nozzle distance was large the rod was almost out of the flame and only a small proportion of the tristearin



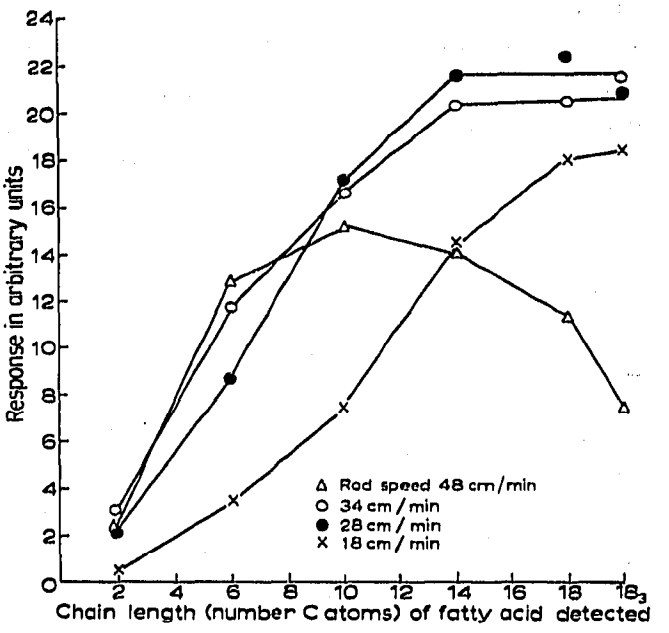


Fig. 6. Effect of rod speed on response. Conditions: H_2 flow, 50 ml/min; rod–nozzle distance, 0.6 mm; rod diameter, 0.45 mm; coating, Silica Gel G.

Fig. 7. Effect of rod speed on response. Conditions: H_2 flow, 50 ml/min.; rod–nozzle distance, 0.6 mm; rod diameter, 0.45 mm; coating, Silica Gel G.

was detected. As the rod approached the nozzle the response increased rapidly reaching a fairly constant value. However the distance over which a constant response was obtained decreased as the size of the flame decreased (Fig. 4). The relative response of compounds with different volatilities was also affected by the position of the rod. Whereas the acids from C_{14} upwards gave a similar response when the rod was close to the nozzle, a difference in response was noted as the distance increased (Fig. 5). The position of the rod in the flame is therefore fairly critical but provided that a relatively large flame is used and the rod is placed close to the nozzle the variations in response can be minimised. Flame size can also affect response when it is varied in conjunction with rod speed and this effect is discussed later.

Effect of rod speed

The series of fatty acids and tristearin were detected over a range of rod speeds (18–46 cm/min), other conditions remaining constant (Fig. 6). One can see two effects occurring, *viz.* (1) the detection of the more volatile compounds (C_2 and C_6 acids) increases with rod speed, and (2) the detection of relatively non-volatile compounds reaches a maximum and then falls off. At low speeds the compounds are vaporised before entering the flame and are not detected and at fast speeds the non-volatile compounds pass through the flame so quickly that they remain on the rod and are undetected. The results have been presented in a different way in Fig. 7, where the

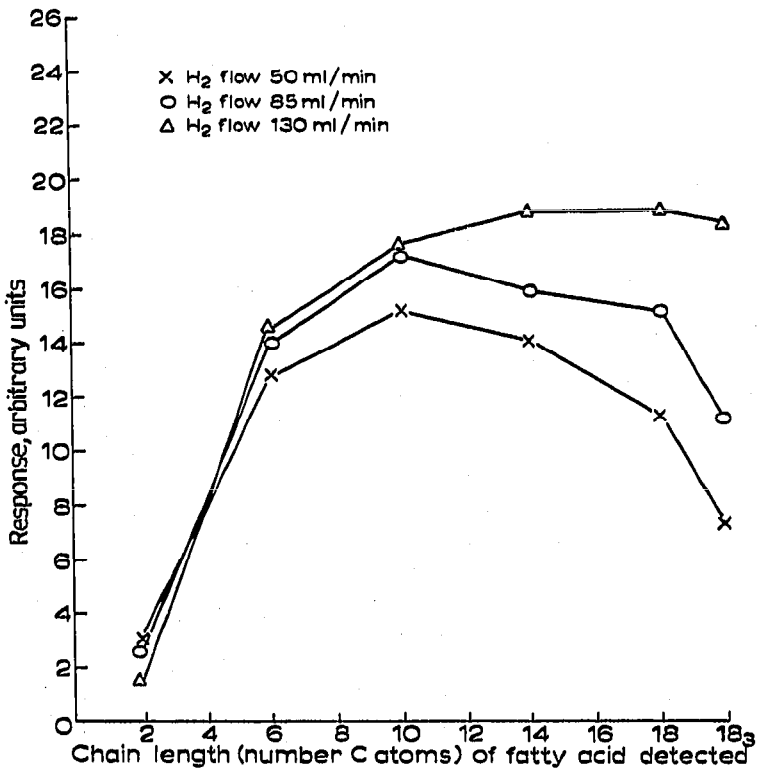


Fig. 8. Effect of flame size on response at high rod speeds. Conditions: rod–nozzle distance, 0.6 mm; rod speed, 46 cm/min; voltage, 200 V; rod diameter, 0.45 mm; coating, Silica Gel G.

effect of volatility on detection can be seen more readily. For compounds less volatile than myristic acid (C_{14}) a fairly uniform response will be shown for rod speeds in the range of 27–37 cm/min. The residence time of the rod in the flame, keeping the speed constant, was increased by increasing the flame size and as one might predict the response for the less volatile compounds (those passing through the flame without detection) was increased. The detection of compounds which vaporise before reaching the flame was not significantly altered however (Fig. 8). The design of the present detector limited the size of the flame we could use as excess noise was produced when the flow rate was increased above 80 ml/min ($\approx$ 1 cm flame diameter).

From these results we chose the standard conditions described in Table I for our analysis of a standard mixture of methyl palmitate, cholesterol stearate, and triolein.

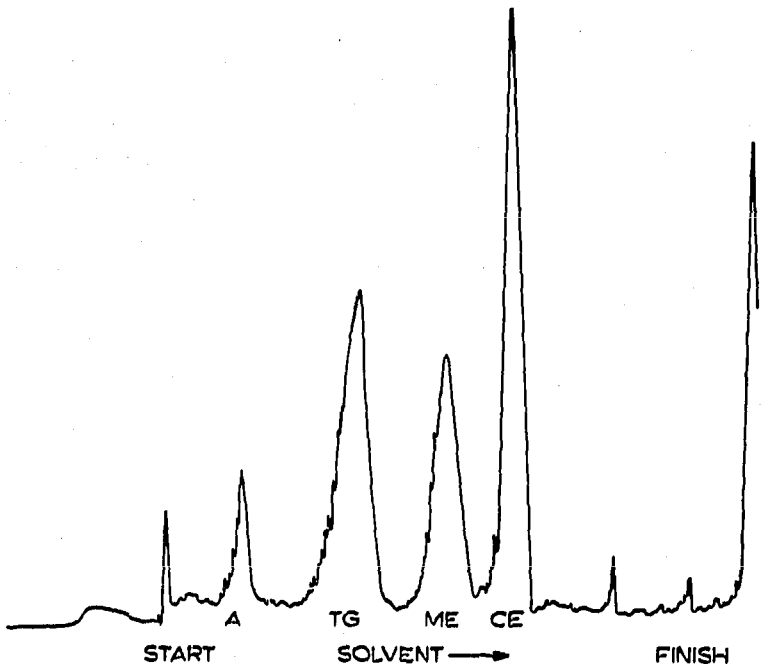


Fig. 9. Typical chromatogram of triglyceride (T.G.), methyl ester (M.E.), and cholesterol ester (C.E.). A is a polar impurity. Chromatogram obtained using standard conditions (Table I).

QUANTITATIVE ANALYSIS

The response of the detecting system to various compounds is largely a function of their volatility. Provided that the method is sufficiently reproducible, however, one could overcome this problem by applying suitable correction factors. The present instrument is, however, most suited to analyse relatively non-volatile compounds which will have the same response factors under the appropriate conditions.

The response of the instrument to different amounts of tristearin is reasonably linear (Table II) over a wide range of concentrations. This is important when one comes to analyse mixtures of unknown composition.

TABLE II

RESPONSE OF INSTRUMENT TO DIFFERENT WEIGHTS OF TRISTEARIN APPLIED TO THE ROD

Wt. (γ) × 150	*Response (arbitrary units)*
150	153
13.5	15
6.75	10

A standard mixture of methyl palmitate, cholesterol stearate and triolein was analysed ten times. The mixture was first separated by the chromatographic procedure described above and then detected using the standard conditions. A typical chromatogram is shown in Fig. 9. The results are given in Table III and have a standard deviation, S.D., of 1.22.

TABLE III

ANALYSIS OF STANDARD MIXTURE

Analysis	*Cholesterol ester (%)*	*Methyl ester (%)*	*Triglyceride (%)*
1	30	33	37
2	32	33	35
3	30	32	38
4	30	33	37
5	29	34	37
6	31	32	37
7	30	30	40
8	29	34	37
9	31	31	38
10	32	32	36
Known	33	31	36

TABLE IV

SIZE OF THE CONFIDENCE INTERVAL FOR DIFFERENT VALUES OF n

Number of readings	$\frac{1}{2}$ *confidence interval (95% level)*[a]
1	2.44
2	1.73
3	1.41
4	1.22
5	1.10
6	1.00
7	0.92
8	0.86
9	0.81
10	0.77

[a] Confidence limits are $\bar{x}_n \pm \frac{1}{2}$ (confidence interval)$_n$.

TABLE V

ANALYSIS OF MONOGLYCERIDE–TRIGLYCERIDE MIXTURE

Analysis	*Monoglyceride (%)*	*Triglyceride (%)*
1	7	93
2	6	94
3	6	94
4	7	93
Known composition (approximate)	10	90

The 95% confidence limits for n readings are given by $\bar{x}_n \pm (2/\sqrt{n}) \cdot 1.22$ where $\bar{x}_n$ is the average of n readings of x. The size of the confidence interval obtained by averaging the results of n readings is given in Table IV.

A mixture of monoglyceride and triglyceride was also analysed, with the results given in Table V.

The general conclusion from our work is that non-volatile lipids can be estimated quantitatively using a detection system of the type described. The method has the advantages of simplicity and speed over other methods in current use. Because the response for different lipids may vary with the operating conditions it is advisable to optimise these for each mixture of lipids being analysed. By suitably modifying the design of the instrument it should also be possible to improve the detection of volatile components.

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