Short Communications

Gas–liquid chromatography: The introduction of samples, the preconditioning of polyester liquid phases and the measurement of R_F values in the analysis of fatty esters

The purpose of this communication is to describe some improvements in the operation of gas–liquid chromatography relating to preconditioning of polyester columns, the ease and accuracy of sample introduction, the consequences of detector overloading and the accurate measurement of R_F values. Some hitherto unpublished R_F values of methyl esters of fatty acids are also recorded.

The gas–liquid chromatograms used employ an ionising detector based on that of LOVELOCK, JAMES AND PIPER[1]. The columns were 254 cm × 6 mm o.d. 35 to 80 mesh "Celite 545" was used as solid support and argon as carrier gas, which was led into the top of the column via a Bio Quickfit joint.

Preconditioning of polyester columns

Reported methods for preconditioning of polyester columns appear to be confined to preheating of the column or the treatment of the polyester with ion exchange resins. In our experience neither method proved completely satisfactory. As an alternative it has been found better to heat the polyester to 260° and pass nitrogen through it for several days until the volatiles are completely removed. The residual polyester became dark and very viscous and was ready for immediate use at 200°.

Sample introduction

Most gas–liquid chromatograms use either micro-pipettes or an injection system for the introduction of non-gaseous samples. However, with both systems the size of samples is difficult to determine accurately. A system was therefore devised whereby the sample is introduced into the column on the inside of a short sampling tube (10 × 5 mm i.d.), which is open at both ends, with a ground inner surface to facilitate spreading of the sample and thus its rapid evaporation. The sample is introduced into the sampling tube with an "Agla" micro-syringe with square tip or preferentially with a fine glass capillary, in which case the relative amounts of sample can be determined by measuring the length of the liquid in the capillary. For quantitative work the sample is weighed and the areas compared with those of a weighed standard such as methyl stearate.

To introduce the sampling tube the argon is turned off and the tube dropped into the top of the column after half a minute when the backpressure in the latter is elim-

inated. The flow of argon is then resumed. The sampling tubes are recovered from the column at the end of the day with a piece of wire bent slightly at one end.

With this method of sample introduction reproducibility of peak areas, as determined with samples of methyl laurate varying from 40 to 140 μg, was 9.45 area units per 10μ g with a standard deviation S.D. = 0.52 units.

Detector overloading

The effects of overloading of ionising detectors have been described by FARQUHAR *et al.*[2]. This results in blunted or inverted peaks and is particularly apt to occur when low amplification and detector voltages are used with large samples containing relatively volatile esters which issue from the column in narrow bands of high concentration. The difficulty can be overcome by using detector voltages of 1,000 V or more, so that faulty peaks due to overloading exceed the height of the chart and are thereby excluded from normal quantitative treatment.

Table I shows the effect of varying degrees of overloading on the composition of an equimolar six-component mixture of methyl esters. These are denoted as by FARQUHAR *et al.*[2]. The table shows the percent recovery of each ester as determined by the peak areas.

TABLE I

THE EFFECT OF DETECTOR OVERLOADING ON THE COMPOSITION OF AN EQUIMOLAR MIXTURE

Overloading	*Methyl ester*					
	12:0	*14:0*	*16:0*	*18:1*	*20:0*	*22:0*
	% of possible peak area					
Slight	95	100	100	100	100	100
Medium	53	76	88	88	100	100
High	46	61	73	84	89	100
Very high	6	37	64	81	86	100

The errors due to overloading increase with sample size and are such as to completely invalidate the analysis. They are greatest in the more volatile components.

The measurements of R_F values

With the equipment described above, nearly symmetrical peaks are obtained with the exception of the negative air peaks, which are asymmetric. However, under other conditions asymmetric peaks may be obtained (*cf.* HAWKE, DUNKLEY AND HOOKER[3]; BEERTHUIS *et al.*[4]) and tailing may also take place. Under such conditions neither the use of peak apices nor of the midpoints of the base give an accurate R_F value. The method adopted in this laboratory is to extrapolate the straight portion of the sides of the peak to intersect the baseline and to bisect the base of the triangle obtained. The same procedure is carried out with the air peak. The distances between the midpoints thus obtained are used in the calculation of R_F values. This system

gave excellent reproducibility of R_F values as determined with 5% APL on "Celite 545" columns (Table III) and when R_F values obtained with 20% polydiethylene glycol adipate (DEGA) on "Celite 545" are determined, they closely resemble the values of FARQUHAR *et al.*[2] extrapolated to 207°. This is shown in Table II. The R_F values were independent of the shape of the peaks.

Table III comprises R_F values (relative to methyl stearate and adjusted to the air

TABLE II

R_F VALUES OFFATTY ACID ESTERS (RELATIVE TO METHYL STEARATE) ON DEGA COLUMNS AT 207° AND VALUES OBTAINED BY FARQUHAR *et al.*[2] EXTRAPOLATED TO 207°

Determination	*Methyl ester*			
	14:0	*16:0*	*18:1*	*20:0*
1	0.324	0.565	1.12	1.76
2	0.321	0.568	1.13	1.72
3	0.335	0.579	1.14	1.75
4	0.333	0.579	1.13	1.78
5	0.322	0.570	1.12	1.75
6	0.326	0.575	1.12	1.77
Mean	0.327	0.573	1.13	1.76
FARQUHAR *et al.*[2] at 207°	0.321	0.570	1.12	1.75

TABLE III

MEAN R_F VALUES OF METHYL ESTERS OF FATTY ACIDS DETERMINED ON A 5% APL ON "CELITE 545" COLUMN AT 207° AND STANDARD DEVIATIONS

Methyl ester	R_F *(air)/* $207°$ *18:0*	*S.D.*	*Methyl ester*	R_F *(air)/* $207°$ *18:0*	*S.D.*
11:0	0.061	0.004	18:3 (linoleic)	0.839	0.004
12:0	0.088	0.003	18:2 (linolenic)		—
13:0	0.140	0.003	18:1 (oleic)	0.879	0.004
14:0 iso	0.164	0.003	18:1 (elaidic)	0.901	0.001
14:0	0.203	0.007	18:0 iso	0.860	0.008
15:0 iso	0.263	0.005	18:0	1.000	—
15:0 anteiso	0.277	0.003	19:0	1.48	0.02
15:0	0.308	0.010	20:4 (arachidonic)	1.51	0.01
16:0 iso	0.390	0.006	20:0	2.17	0.05
16:1	0.394	0.005	21:0	3.20	0.05
16:0	0.456	0.007	22:1 (erucic)	4.28	0.07
17:0 iso	0.579	0.005	22:0	4.58	0.07
17:0 anteiso	0.603	0.003	23:0	6.87	0.18
17:0	0.681	0.011	24:0	10.2	0.3
			26:0	21.5	0.7

peak) of fatty acid methyl esters obtained with a 5 % APL on "Celite 545" column at 207°. The values quoted represent the means of 6 to 20 determinations. The esters are abbreviated as by FARQUHAR *et al.*[2].

It is well known that there is a linear relationship between log R_F and the number of carbon atoms in a homologous series of fatty acids. It is thus possible to derive simple empirical equations for the calculation of log R_F of any ester. Thus for:

n-saturated acid esters : $\log R_F n = 0.169n - 3.05$
(+)-anteiso acid esters : $\log R_F n = 0.169n - 3.10$
iso acid esters : $\log R_F n = 0.169n - 3.12$ and
cis monoenoic acid esters : $\log R_F n = 0.129n - 2.99$

where n represents the number of carbon atoms in the acid.

Similarly, linear relationships have been observed between R_F values and temperature (*cf.* FARQUHAR *et al.*[2]). Some changes of R_F value as determined with 5 % APL columns are shown in Table IV.

TABLE IV

CHANGES OF R_F WITH TEMPERATURE USING 5 % APL ON "CELITE 545" COLUMNS

Methyl ester	*R_F (air)/18:0 at*			
	*150°**	*180°**	*207°*	*225°**
12:0	0.056	0.076	0.088	—
14:0	0.146	0.170	0.203	0.222
16:0	0.379	0.398	0.456	0.474
18:0	1.000	1.000	1.000	1.000
20:0	—	2.22	2.17	2.06

* Determined on PYE Argon Chromatogram.

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