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ANALYSIS OF FATTY ACID METHYL ESTERS WITH HIGH ACCURACY AND RELIABILITY

I. OPTIMIZATION OF FLAME-IONIZATION DETECTORS WITH RESPECT TO LINEARITY

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SUMMARY

The effective linear range of a flame-ionization detector was increased by operating at a higher hydrogen flow-rate than that required for optimum sensitivity. Evidence of a "superlinear" response was seen at even higher hydrogen flow-rates and at high sample loads. Determination of the "grade" of analysis of a mixture which simulated coconut oil methyl esters was shown to be a rapid and simple technique for routinely checking that detector linearity is optimized. Under these conditions, excellent quantitative results were obtained for the mixture over a wide range of analyte concentrations. The value of the Ackman and Sipos theoretical response factors was confirmed.

INTRODUCTION

When it is noted that the first paper published on gas–liquid chromatography (GLC)[1] dealt with fatty acid analysis, and that since that time papers too numerous to list have been published on the analysis of fatty acid methyl esters (FAME), it might be supposed that there could be no further problems to resolve. Evidence that this is not so may be derived from a study of results such as those of the American Oil Chemists' Society (AOCS) Smalley Gas Chromatography Check Program for the fatty acid composition of fats and oils[2,3]. Thus, in the 1980–81 series, the best overall results were obtained by a panel of 81 for a sample of safflower oil for which "grades" of analyses (see Experimental) ranged from 99.63 down to 85.16%. At the upper end, the total error for all 10 FAME was only 0.37%, representing extremely good analytical performance. At the lower end, it is apparent that totally unacceptable errors are present. Approximately 40% of the panel had grades below 98%, representing a total error of more than 2%, which we consider to be unacceptable. In the same series, the worst results were obtained for a sample of coconut oil for which grades of analysis

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ranged from 99.30 down to 77.26%. Approximately 86% of the panel had grades below 98%, and 50% did not even exceed 96% grade, representing a total error for only nine FAME of more than 2% and 4%, respectively. The above results indicate an unacceptable frequency of poor quantitative work and/or state of knowledge amongst analysts who work routinely in this field. Given the high state of refinement of modern chromatographic and peak-area measurement instrumentation, we believe that it should be possible to achieve grades of no less than 99% routinely for easily resolved mixtures of the above kind containing about ten components. We have thus set this standard as a criterion of satisfactory results for the work described herein.

In an extensive programme to improve the accuracy and reliability of fatty acid analysis we have pinpointed two areas where error is likely to occur, *viz.*, failure to optimize the flame-ionization detector (FID) for quantitative performance and faulty preparation of FAME. This paper deals with FID optimization, and subsequent papers[4,5] will deal with FAME preparation.

The phenomenon that FIDs pass through a peak of sensitivity at a particular ratio of hydrogen to carrier gas flow-rate was one of the earliest recognized properties of these detectors[6,7]. In our experience, it is usual for chromatographers to work at or near this sensitivity optimum, even though Bruderreck *et al.*[8], in 1964, cautioned that "maximum signal and optimum performance, therefore, are two quite different concepts which must not be confused". While there is no doubt that the optimum for sensitivity will yield excellent results in many applications, we now show that, by increasing the flow-rate of hydrogen above that required to maximize sensitivity, we were able to increase the linear range of a detector with improvement in quantitative accuracy.

In the analysis of FAME, it must be stressed that a wide linear range is always necessary no matter what the type of oil, because it is necessary to ensure that small signals from the trace components can be detected, whilst the very large signals from the major components do not exceed the linear capability of the detector. An important consequence of increased linear range is that it gives the analyst a greater degree of freedom in a practical situation, as it leads to accurate results over a wide sample size range.

Coconut oil presents a special challenge because of the high content of methyl laurate, which elutes early at high mass flow-rate, thus placing particularly high demands on detector linearity. If coconut oil can be accurately analysed, detector linearity will be assured for all other fats and oils. In our study, we worked with a mixture of saturated FAME with a chain length distribution approximating that of coconut oil. Such a mixture is equivalent to coconut oil methyl esters in the demands it places on detector linearity and has the advantage that it can be prepared with high quantitative accuracy. It is thus an excellent model mixture for investigations of detector linearity.

The most common solution to the problem of analysing coconut oil methyl esters is to carry out a temperature-programmed analysis[9], but this is not always acceptable in a busy factory control laboratory, nor is it necessarily the best solution for high accuracy. Our solution has been to optimize the detector for linearity rather than for sensitivity. In particular, we have found that the linear range of the detector may be effectively increased at the upper end by operating at an increased hydrogen flow-rate. The dependence of linearity of response on hydrogen flow-rate at high

analyte concentrations has been observed by McWilliam[10]. Thus the phenomenon which we describe is not new, but its utilization appears to have been neglected.

Because of the need to have routine checks on the analytical system so developed, we illustrate the usefulness of the concept of "grade" of analysis[3] of a suitable mixture as a criterion (though not a measure) of detector linearity.

Our studies also revealed evidence of a further example of "superlinear response", a phenomenon recently discussed by Bromly and Roga[11], under conditions of high sample load, together with hydrogen flow-rate above that required for optimum linearity. Finally, we present evidence that the superlinear response behaviour could be general for flame-ionization detectors.

Throughout the work we have used solutions with a total ester concentration of 1.25–20%, which should adequately cover the range of 5–10% generally resulting from use of the standard methylation procedures[12].

EXPERIMENTAL

Chemicals

Isooctane (2,2,4-trimethylpentane) was of Baker Analyzed Reagent grade (J. T. Baker, Phillipsburg, NJ, U.S.A.). A purity check by GLC under conditions similar to those used throughout the experiments showed that there were no peaks on the solvent tail.

Reference esters were methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitate and methyl stearate (puriss grade, Fluka, Buchs, Switzerland), and all were individually checked for purity by GLC under the same conditions used throughout the experiments, except that they were allowed to run long enough for the corresponding free fatty acids to be detected. In the one instance where free fatty acid was detected, a temperature-programmed run from 150 to 200°C at 2°C/min was carried out to obtain better quantitation.

Chromatography

GLC for the main series of experiments was carried out on a Varian Model 2700 chromatograph fitted with an FID to which two modifications had been made. First, the standard flame tip, which had a silica bead insulator, was replaced with a tip with a ceramic insulator and a 0.25 mm jet (Varian, Palo Alto, CA, U.S.A., Part No. 02-001875-00). Second, the standard collector, which is a cylindrical tube of approximately 16 × 6 mm O.D., was replaced with a tubular section of dimensions approximately 23 × 8 mm O.D. cut from the collector electrode of a Pye Model 104 chromatograph. The glass column (2 m × 4 mm I.D.) was packed with 10% DEGS-PS on 80–100-mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The carrier gas was high-purity nitrogen at a flow-rate of 30 ml/min, which was the Van Deemter optimum for the column. High-purity hydrogen was supplied to the detector from a General Electric Model 15EHG 2B 1 hydrogen generator and compressed, oil-free laboratory air was supplied at a flow-rate of 500 ml/min. Analyses were carried out isothermally at 165°C, with injection port and detector temperatures of 200°C. Samples were injected using a Hewlett-Packard Model 7670A automatic liquid sampler set at a nominal injection volume of 2.5 μl. The output signal from the detector was amplified at an electrometer sensitivity of 10^{-9} A/mV, and the electrometer signal

was attenuated by a 1:10 voltage divider before A/D conversion. Peak areas were measured using a Hewlett-Packard Model 3354 Laboratory Automation System operating in a tangent skim mode for the shorter chain fatty ester peaks on the solvent tail. The apparent weight-% composition of the fatty methyl ester solutions was determined by the same automation system after applying the theoretical relative response factors of Ackman and Sipos[13], which were as follows: methyl caprylate 1.1927, methyl caprate 1.1233, methyl laurate 1.0771, methyl myristate 1.0440, methyl palmitate 1.0193 and methyl stearate 1.0000.

Additional experiments were carried out on the same Varian instrument fitted with a 0.5-mm jet diameter ceramic-type flame tip (Varian part No. 02-001938-00) and on a Hewlett-Packard Model 5880A chromatograph fitted with 0.28- or 0.46-mm jet diameter flame tips. All other experimental conditions were as given above.

Primary standard mixture

A primary standard mixture with a chain length distribution which simulated that of coconut oil methyl esters was made from the reference esters. The actual composition of the mixture was calculated after applying corrections indicated by the purity checks. These corrections took into account first, the absolute purity of the ester which was determined allowing for all minor peaks detected in the purity checks. The weight contribution of an ester to the standard mixture was reduced accordingly. Second, if an impurity coincided in the chromatogram with another of the reference esters, the weight contribution of this ester was increased accordingly. A corrected composition for the standard mixture was calculated by normalizing the corrected weight contributions of the reference esters, but ignoring all other impurities which did not coincide with the reference esters. Such impurities were similarly ignored in the subsequent analyses.

Standard solutions of the primary mixture were prepared by first making 10 ml of 20% (w/v) solution in isooctane with respect to the total weight of esters. Successive accurate dilutions of 5 ml to 10 ml with isooctane were then made to prepare standard solutions of 10, 5, 2.5 and 1.25% (w/v).

Analyses of the standard solutions were carried out in duplicate at hydrogen flow-rates of 60, 50, 45, 40, 35 and 30 ml/min.

Maximum mass flow-rates through the detector for each of the reference esters under the various analytical conditions were calculated by dividing the estimated mass (micrograms) of ester introduced on to the column by the peak width at half-height (seconds). The mass of ester introduced on to the column was estimated from the calculated concentration of the ester in a standard solution and the volume of solution injected. Whereas the nominal volume of solution injected was 2.5 μl, the actual volume, which included a contribution from the needle contents, was estimated by difference when the sample and residue were drawn into the syringe barrel before and after injection, respectively. This volume was approximately 3.0 μl and was assumed to be constant for the purposes of the detector load calculations. The peak width at half-height was determined by the Hewlett-Packard Laboratory Automation System to a resolution of 0.125 sec.

Determination of hydrogen flow-rate optima

The optimum hydrogen flow-rate for sensitivity was established for each ester

in each standard solution, by determining the flow-rate which consistently gave the highest raw peak area.

The optimum hydrogen flow-rate for linearity was determined from two criteria. First, the slope of the response, Φ, for methyl laurate was determined as a function of sample size according to the equation

$$\log R = \log k + \Phi \log C \tag{1}$$

which is derived from the response equation

$$R = kC^{\Phi} \tag{2}$$

where R is detector response, C is the sample concentration and k is the detector constant. The raw peak areas for methyl laurate were corrected for minor sample size variations using the methyl myristate peak as an internal standard, it having been demonstrated that no discrimination of sample composition had occurred during injection or as a result of any adsorption in the chromatographic process, and that the methyl myristate peak never exceeded the linearity range of the detector. The rationale of this correction procedure is explained under Results and Discussion. The hydrogen flow-rate which consistently gave a slope closest to unity was taken as optimum.

Second, the "grade" of analysis of the standard mixture was determined for each of the standard solutions as a function of hydrogen flow-rate. The grade of analysis of a mixture was adapted from the Smalley Gas Chromatography Check Program for Fatty Acid Analysis[3] and is defined as

$$\text{Grade} = 100 - \Sigma|C_i - C_i'|$$

where C_i = % content of ester determined and C_i' = % content of ester known.

The hydrogen flow-rate which consistently gave the highest grade was taken as the optimum for linearity.

RESULTS AND DISCUSSION

Composition of the standard mixture

The results of the purity checks on the reference esters are given in Table I and the uncorrected and corrected compositions of the standard mixture in Table II. Impurities in the esters which did not coincide with a major peak are not included in Table I, but were nonetheless allowed for in calculating the corrected composition given in Table II.

Most of the esters were of excellent purity, and minimal corrections to their weight contributions were required. The methyl palmitate, however, contained 2.75% of palmitic acid and a significant correction to both its weight and percentage contributions to the mixture was required. As no account was taken in the experimental runs of the palmitic acid impurity, or of any other impurity which did not coincide with a major ester, this correction was further reflected in an increase in the percentage contribution of the other esters to the composition of the mixture (except

TABLE I

PURITY CHECKS ON REFERENCE ESTERS

Reference ester	*Composition by GLC analysis (%)*					
	Fatty acid methyl ester					
	8:0	*10:0*	*12:0*	*14:0*	*16:0*	*18:0*
Methyl caprylate	99.96	0.03				
Methyl caprate	0.04	99.72		0.08		
Methyl laurate		0.13	99.80			
Methyl myristate			0.62	99.06	0.07	
Methyl palmitate				0.18	96.46*	0.02
Methyl stearate				0.06	0.38	99.55

* Also contained 2.75% palmitic acid.

methyl myristate, which showed a small net decrease because of its own relatively low purity). This increase, however, was significant only in the case of the major component, methyl laurate.

Optimum hydrogen flow-rate for sensitivity

The mean raw peak areas for all component esters of the standard solutions are given as a function of total ester concentration and of hydrogen flow-rate in Table III, and the maxima are indicated in italics.

There are two points of interest in these results. First, it was apparent that the response curves were all very flat in the hydrogen flow-rate range 30–40 ml/min and that differences between neighbouring values were generally within experimental error. Collectively, however, the maximum response occurred most commonly at 35 ml/min. It was concluded, therefore, that the true optimum was close to this flow-rate.

Second, although it was difficult to define the precise optimum, it was clear that, except for methyl laurate in the most concentrated solutions, the response de-

TABLE II

UNCORRECTED AND CORRECTED COMPOSITION OF THE STANDARD MIXTURE

Fatty acid methyl ester	*Weight of reference ester (mg)*	*Nominal composition (%)*	*Corrected weight of ester (mg)*	*Corrected composition (%)*
8:0	180.58	9.05	180.56	9.08
10:0	130.93	6.56	131.85	6.63
12:0	949.58	47.60	949.83	47.78
14:0	348.02	17.45	345.31	17.37
16:0	186.15	9.33	180.56	9.08
18:0	199.85	10.01	199.87	10.05
	1995.11	100.00	1987.98	99.99

creased significantly above 40 ml/min and that the optimum did not lie in this region of the curve, which was later shown to be the optimum for linearity. With methyl laurate in the 1.0 and 20% solutions, the flat region of the response curve extended to 50 ml/min of hydrogen. In this instance, the expected sensitivity loss due to increasing hydrogen flow-rate was counteracted by the superlinear response, which is discussed later.

Optimum hydrogen flow-rate for linearity

Linearity assessment based on peak area/sample size analysis of the methyl laurate peak. Two basic techniques may be used to assess the linearity of an FID. Ideally, a constant mass flow-rate of substrate in the carrier gas is fed directly into the detector without passing through the chromatographic system, and the ion current is plotted against a range of values of the mass flow-rate. More commonly, the chromatographic system is utilized in the normal manner and peak area plotted against sample size. Bruderreck *et al.*[8] have rightly pointed out that peak area/sample size techniques reflect the linearity, not only of the FID itself, but also of the entire chromatographic system. As the ion current/constant mass flow-rate technique is not practical in most situations, it is necessary to ensure that, when using a peak area/sample size method, systematic errors[14] such as sample discrimination or sample size are negligible or corrected for. Having obtained valid data, the results may be expressed in a number of ways[15]. Commonly the peak area/sample size plots will be done on a log–log scale and detector linearity expressed as the gradient of the corresponding multiple linear regression equation. A perfectly linear detector has a gradient of unity.

As we used a peak area/sample size method for our experiments, we first checked the results for systematic errors. To this end, it proved highly effective to classify all systematic errors as either those which give rise to sample discrimination, or those which give rise to sample size errors. By sample discrimination we mean any mechanism which gives rise to chain length disproportionation of the sample, by way of differential vapour pressure effects (*e.g.*, fractionation in the syringe needle), differential adsorption, etc. By sample size errors we mean any mechanism which gives rise to the introduction into the chromatograph or detector of a sample of representative composition, but of inaccurate size. We have examined our results and found that, whereas sample discrimination was negligible, sample size errors of small magnitude were present, but could be corrected for. All other deviations in the results from the known composition of the standard mixture have thus been attributed to detector non-linearity.

A stringent check for sample discrimination may be made by assuming that the detector remains perfectly linear for all of the reference esters except the major peak, methyl laurate, for all sample sizes and hydrogen flow-rates. If this assumption is valid, and sample discrimination is absent, the normalized results of all esters except methyl laurate should remain constant under all experimental conditions. The results in Table IV indicate that this was indeed true in spite of the wide range of maximum mass flow-rates of the esters concerned (Table V), confirming the validity of the assumption. Hence there was good agreement between the theoretical and determined values for all the components considered, together with low standard deviations, indicating that sample discrimination was negligible under all experimental con-

TABLE III

MEAN RAW PEAK AREAS OF REFERENCE ESTERS IN STANDARD SOLUTIONS AS A FUNCTION OF TOTAL ESTER CONCENTRATION AND HYDROGEN FLOW-RATE

Reference ester	*Hydrogen flow-rate (ml/min)*	*Raw peak are (μV sec $\times 10^{-4}$)*				
		Total ester concentration of standard solution (%)				
		1.25	*2.5*	*5*	*10*	*20*
Methyl caprylate	60	2.26	4.50	8.68	18.0	36.0
	50	2.82	5.58	11.0	21.8	44.0
	45	2.92	5.76	11.2	22.2	45.1
	40	3.13	6.27	12.2	23.6	45.9
	35	*3.14*	*6.36*	12.2	23.4	*46.2*
	30	3.12	6.30	*12.4*	*23.8*	*46.2*
Methyl caprate	60	1.78	3.54	6.81	14.1	27.5
	50	2.24	4.39	8.74	17.3	34.5
	45	2.32	4.54	8.97	17.6	35.4
	40	*2.46*	4.91	9.70	18.8	36.5
	35	2.45	*5.00*	*9.72*	18.6	*37.3*
	30	2.43	4.93	9.69	*18.9*	37.2
Methyl laurate	60	13.2	26.6	52.2	113	222
	50	17.0	32.8	66.3	133	258
	45	17.6	33.8	67.8	136	*263*
	40	*18.6*	36.4	*71.6*	*138*	260
	35	18.4	*37.2*	71.1	134	253
	30	18.2	36.6	71.0	136	254
Methyl myristate	60	4.94	10.0	19.3	40.2	79.3
	50	6.40	12.5	25.0	49.2	99.2
	45	6.62	12.8	25.7	50.4	101
	40	*7.00*	13.8	27.4	53.1	104
	35	6.90	*14.3*	*27.6*	53.1	*106*
	30	6.75	14.0	27.4	53.5	*106*
Methyl palmitate	60	2.60	5.36	10.4	21.5	41.8
	50	3.38	6.66	13.4	26.4	52.9
	45	3.49	6.84	13.8	27.1	54.2
	40	*3.66*	7.32	14.6	28.6	56.1
	35	3.64	*7.56*	*14.8*	28.6	*57.7*
	30	3.53	7.32	14.6	*28.7*	57.4
Methyl stearate	60	2.94	6.04	11.6	24.3	47.6
	50	3.82	7.52	15.2	29.9	60.2
	45	3.93	7.72	15.5	30.8	61.6
	40	4.10	8.22	16.5	*32.4*	63.7
	35	*4.11*	*8.52*	*16.6*	*32.4*	*65.6*
	30	3.96	8.23	16.4	*32.4*	65.1

ditions. In contrast, the anomalous behaviour in this respect of the methyl laurate peak is reflected in the high standard deviation for all values of this component as shown in Table VI. This behaviour was assigned to departures from the linear range

TABLE IV

MEANS AND STANDARD DEVIATIONS FOR NORMALIZED ANALYTICAL RESULTS FOR ALL ESTERS EXCLUDING METHYL LAURATE

Fatty acid methyl ester	*Composition (%)*		*Standard deviation*	*Relative standard deviation (%)*
	Known	*Mean for all analyses*		
8:0	17.39	17.07	0.259	1.52
10:0	12.70	12.69	0.099	0.78
14:0	33.27	33.50	0.134	0.40
16:0	17.39	17.43	0.146	0.84
18:0	19.25	19.31	0.224	1.16
Grade		99.3		

of the detector at the generally much higher maximum mass flow-rates for this component compared with the other components.

Having demonstrated that sample discrimination was negligible, it was now possible to check for sample size errors by relating the raw peak areas of esters other than methyl laurate to the respective total ester concentrations in Table III. As small deviations from direct proportionality may be seen, it was concluded that small variations in sample size (*i.e.*, volume of sample injected) were occurring, and corrections were therefore made to the raw peak areas of methyl laurate. For this purpose, the methyl myristate peak was selected as an internal standard because of the very low relative standard deviation seen in Table IV. Using the 10% ester solution as an arbitrary reference, the corrected peak areas for methyl laurate were obtained according to the equation

$$A'_{LC} = CA_{M10}A_{LC}/10A_{MC} \tag{3}$$

TABLE V

ESTIMATED DETECTOR MAXIMUM MASS FLOW-RATES FOR ALL ESTERS IN ALL STANDARD SOLUTIONS

Fatty acid methyl ester	*Maximum mass flow-rate (μg/sec)*				
	Total ester concentration of standard solution (%)				
	1.25	*2.5*	*5*	*10*	*20*
8:0	1.0	2.0	3.9	7.8	14
10:0	0.5	1.0	2.0	3.8	7.0
12:0	1.9	3.8	7.5	15	29
14:0	0.52	1.0	2.1	3.9	7.4
16:0	0.16	0.32	0.65	1.3	2.4
18:0	0.10	0.21	0.42	0.80	1.5

TABLE VI

STANDARD DEVIATIONS FOR STANDARD MIXTURE COMPONENTS BASED ON RELATIVE STANDARD DEVIATIONS FOR ESTERS IN TABLE IV AND ON ALL RESULTS FOR METHYL LAURATE

Fatty acid methyl ester	*Known standard composition (%)*	*Standard deviation*
8:0	9.08	0.138
10:0	6.63	0.052
12:0	47.78	0.903
14:0	17.37	0.069
16:0	9.08	0.076
18:0	10.05	0.117

where

C = total ester concentration;
A'_{LC} and A_{LC} = corrected and raw peak areas of methyl laurate at C% total ester concentration;
A_{M10} and A_{MC} = raw peak areas for methyl myristate at 10% and C % total ester concentrations

The corrected peak areas for methyl laurate were used to calculate the slopes of response as a function of sample size (eqn. 1) and the correlation coefficients. The results are presented in Table VII.

It was concluded from these results, first, that the optimum hydrogen flow-rate for linearity was 45–50 ml/min because the slope was closest to unity in this range. Second, it was concluded that, at the optimum flow-rate for sensitivity (*ca.* 35 ml/min) appreciable depression of linearity was apparent. Finally, at the highest flow-rate, the slope exceeded unity and afforded evidence of a superlinear response effect under these conditions.

TABLE VII

CORRECTED MEAN RAW PEAK AREAS, SLOPES OF RESPONSE AND CORRELATION COEFFICIENTS FOR METHYL LAURATE AS A FUNCTION OF HYDROGEN FLOW-RATE

Hydrogen flow-rate (ml/min)	*Corrected raw peak area of methyl laurate (μV sec $\times 10^{-4}$)*					Φ	r
	Total ester concentration of standard solution (%)						
	1.25	*2.5*	*5*	*10*	*20*		
60	13.4	26.7	54.4	113	226	1.023	0.99995
50	16.3	32.3	65.2	133	256	0.999	0.99992
45	16.7	33.3	66.5	136	262	0.997	0.99993
40	17.6	35.0	69.5	138	266	0.980	0.99997
35	17.7	34.5	68.4	134	253	0.963	0.99992
30	18.0	35.0	69.3	136	256	0.962	0.99992

The effect of deviations from linearity on the accuracy of the analytical results obtained can be seen in Table VIII, from which it was concluded that appreciable errors may occur outside of the optimum hydrogen flow-rate range of 45–50 ml/min. Thus, at the higher sample concentrations, a superlinear response gave rise to falsely high results for methyl laurate, whereas depression of linearity at lower flow-rates gave rise to falsely low results. Of particular relevance are the large errors that occur at the optimum hydrogen flow-rate for sensitivity (35 ml/min) for the concentrated solutions. The results for methyl laurate are illustrated in Fig. 1.

A further conclusion from these results is that they support the accuracy of the theoretical response factors of Ackman and Sipos[13] and underline the necessity of using them for work of the highest accuracy. Without their use, all of the above results would have incurred severe errors.

It further follows that the results support our belief that it is necessary to adjust the system performance so that correct answers are obtained when these factors are applied, rather than to apply arbitrary factors to correct system errors, which is the usual practice.

Detector linearity assessment based on grade of analysis. For the purpose of routine standardization, it is not always possible to make an instrument available for enough time to check detector linearity according to the procedure already described, which requires multiple analyses of a number of solutions.

We have, therefore, investigated the parameter "grade of analysis" as a practical technique for rapid routine checks of detector linearity. In Table VIII are included the grades of analysis of the standard solutions as a function of hydrogen flow-rate.

From these results it can be seen that the highest grades of analysis were consistently obtained at the hydrogen flow-rates corresponding to the previously determined optimum for linearity, *viz.*, 45–50 ml/min. In particular, excellent grades were obtained at the highest sample concentrations (10 and 20%), indicating that the detector had maintained good linearity under these conditions (see Fig. 2). It was concluded that the results not only confirmed the earlier finding, but further indicated that grade of analyses at a high sample concentration could be used as a means of checking detector linearity. To this end it has the following advantages. First, only a single analysis may be necessary to give an assurance that this optimum is maintained. Second, if required, adjustments to the system can be made and their effect evaluated with minimum effort. Third, grade also reflects total system performance. In our experience, an analyst can often distinguish deterioration of grade due to loss of detector performance from that due to other systematic errors, and closer inspection of the results commonly leads to the cause of the problem. Fourth, there is not a critical dependence upon actual sample size injected, in contrast to the case with peak area/sample size determinations. Finally, the calculation of grade is a computer-amenable task, and an assessment of the status of the system may thus be obtained almost immediately after the completion of the analysis.

Further investigation of superlinearity

In an attempt to determine if the superlinear response observed is likely to be general, we carried out a limited number of experiments with other detectors. For this, the 20% standard solution was analysed under conditions as close as possible to

TABLE VIII

MEAN RESULTS AND GRADES OF ANALYSES OF STANDARD SOLUTIONS AS A FUNCTION OF HYDROGEN FLOW-RATE AND TOTAL ESTER CONCENTRATION

Hydrogen flow-rate (ml/min)	*Fatty acid methyl ester*	*Composition (%)*				
		Total ester concentration (%)				
		1.25	*2.5*	*5*	*10*	*20*
60	8:0	9.09	8.94	8.89	8.69	8.83
	10:0	6.72	6.63	6.57	6.40	6.37
	12:0	47.98	47.77	48.19	49.28	49.20
	14:0	17.37	17.51	17.32	16.96	17.04
	16:0	8.94	9.09	9.04	8.85	8.78
	18:0	9.90	10.06	10.00	9.82	9.79
	Grade	99.4	99.7	99.2	97.0	97.2
50	8:0	8.85	8.95	8.75	8:77	8.94
	10:0	6.60	6.64	6.58	6.54	6.60
	12:0	47.90	47.59	47.53	48.18	47.40
	14:0	17.56	17.57	17.52	17.32	17.61
	16:0	9.06	9.12	9.16	9.09	9.18
	18:0	10.02	10.11	10.15	10.10	10.26
	Grade	99.4	99.4	99.2	99.1	99.0
45	8:0	8.86	8.99	8.77	8.78	8.98
	10:0	6.60	6.66	6.59	5.56	6.64
	12:0	47.96	47.59	47.76	48.01	47.25
	14:0	17.55	17.56	17.55	17.38	17.66
	16:0	9.04	9.11	9.17	9.13	9.21
	18:0	9.98	10.09	10.16	10.15	10.27
	Grade	99.3	99.4	99.2	99.2	98.7
40	8:0	8.97	9.08	8.98	8.95	9.05
	10:0	6.63	6.70	6.69	6.70	6.78
	12:0	48.05	47.62	47.41	47.15	46.29
	14:0	17.54	17.58	17.61	17.64	17.90
	16:0	8.97	9.05	9.17	9.27	9.45
	18:0	9.85	9.97	10.14	10.28	10.52
	Grade	99.1	99.4	99.0	98.5	97.0
35	8:0	9.06	8.98	8.99	9.01	9.13
	10:0	6.67	6.65	6.71	6.78	6.93
	12:0	47.91	47.50	47.07	46.41	45.07
	14:0	17.44	17.65	17.75	17.90	18.29
	16:0	8.99	9.13	9.25	9.43	9.73
	18:0	9.94	10.10	10.23	10.46	10.85
	Grade	99.5	99.2	98.4	97.1	94.6
30	8:0	9.17	9.08	9.08	9.07	9.11
	10:0	6.72	6.69	6.72	6.79	6.92
	12:0	48.14	47.70	47.23	46.60	45.27
	14:0	17.36	17.58	17.68	17.83	18.24
	16:0	8.86	9.02	9.16	9.35	9.68
	18:0	9.74	9.94	10.12	10.35	10.77
	Grade	98.9	99.5	98.9	97.6	95.0

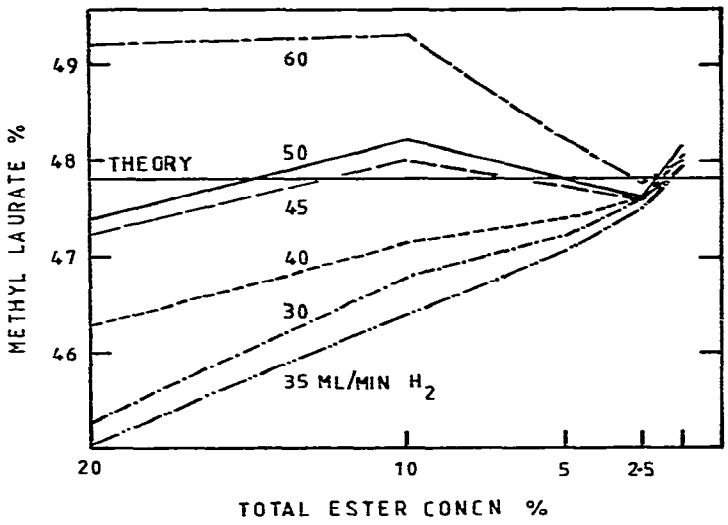

Fig. 1. Influence of hydrogen flow-rate and total ester concentration on determined methyl laurate content of simulated coconut oil methyl ester primary standard.

those used in the main experiment at hydrogen flow-rates of 60, 45 and 30 ml/min. The results are given in Table IX.

The elevated levels of methyl laurate in all the results at 60 ml/min hydrogen flow-rate indicate that a superlinear response occurred under these conditions. It was

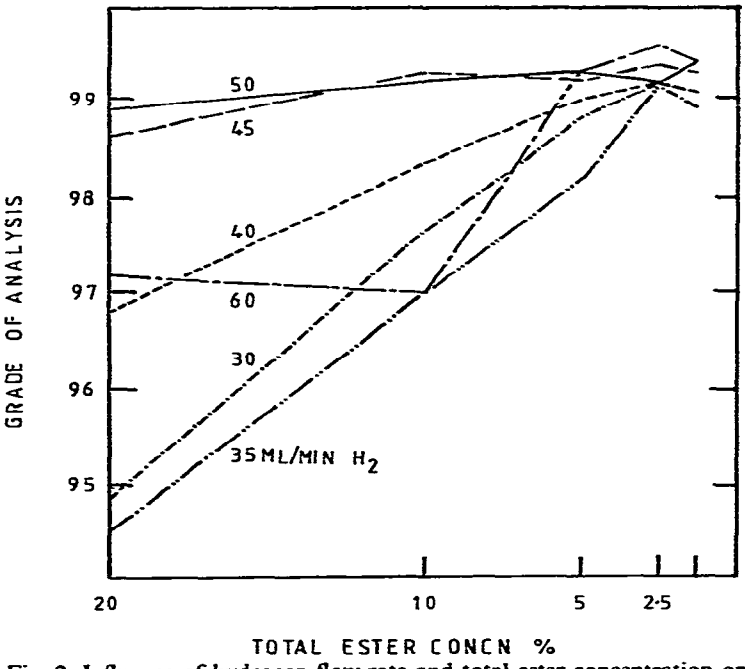

Fig. 2. Influence of hydrogen flow-rate and total ester concentration on grade of analysis of simulated coconut oil methyl ester primary standard.

TABLE IX

RESULTS OF FURTHER CHECKS FOR SUPERLINEAR RESPONSE WITH ALTERNATIVE FLAME-IONIZATION DETECTORS

Fatty acid methyl ester	*Known %*	*Hydrogen flow-rate (ml/min)*								
		60	*45*	*30*	*60*	*45*	*30*	*60*	*45*	*30*
		Varian 2700, 0.5-mm jet			*Hewlett-Packard 5880A*					
					0.28-mm jet			*0.46-mm jet*		
8:0	9.08	8.57	8.66	8.77	8.62	8.73	9.01	8.60	8.65	8.79
10:0	6.63	5.98	6.21	6.58	6.29	6.44	6.81	6.19	6.37	6.61
12:0	47.78	52.09	50.17	46.94	49.28	48.33	45.97	50.22	48.87	46.94
14:0	17.37	16.39	16.90	17.65	17.00	17.29	17.90	16.72	17.08	17.62
16:0	9.08	8.02	8.50	9.40	8.84	9.04	6.56	8.59	8.93	9.41
18:0	10.05	8.95	9.56	10.66	9.97	10.17	10.75	9.68	10.10	10.63

concluded, therefore, that the phenomenon was likely to be general. In detailed behaviour, these detectors varied from that used in the main body of the work and between each other. Elevated results for methyl laurate at 45 ml/min hydrogen flow-rate indicated that this flow-rate was not the optimum for linearity, and that the optimum lay between 30 and 45 ml/min in each instance. Jet diameter also influenced the magnitude of the superlinear response, which was greatest at the largest diameter size. As no attempt was made to determine the optimum hydrogen flow-rate for sensitivity, it is possible that it may be closer to or coincide with the optimum for linearity with these detectors. The important point to note is that, as detectors do vary, it is mandatory, for the highest accuracy, to determine and to work at the optimum for linearity rather than that for sensitivity.

The phenomenon of superlinear response has most commonly been attributed to geometric factors in the detector[11]. The effect which we have described appears similar to the "alternating effect" of Bruderreck *et al.*[8] which was observed for ethylene at high mass flow-rate. In this, the ion current–mass flow-rate curve showed a positive inflection at high mass flow-rates before a final downward inflection as the detector became saturated. They concluded that this effect was intrinsically involved in FID operation, probably because of the mechanism of ion generation in the flame, but the association of the effect with hydrogen flow-rate was not studied. In our observations, this alternating effect could not be seen at the lowest hydrogen flow-rates (30 and 35 ml/min). As a consequence of the alternating effect, the above authors concluded that the true linear range of a detector may be overestimated. While this is true in the strict sense, there remains the possibility of exploiting the behaviour to extend the practical linear range of the detector.

CONCLUSIONS

By increasing the hydrogen flow-rate above that required to give maximum sensitivity, the linear range of an FID was increased. Improved quantitative results were obtained by optimizing for linearity, rather than for sensitivity.

The value of the theoretical response factors of Ackman and Sipos[13] is confirmed. The concept is promoted that it is necessary to adjust the system performance so that correct answers are obtained when these factors are applied, rather than to apply arbitrary factors to correct system errors.

The determination of "grade" of analysis of a standard mixture is recommended as a rapid and simple method for monitoring the performance of a chromatographic system in practice.

Some evidence is presented of a "superlinear" response effect at hydrogen flowrates higher than that required to achieve optimum linearity of response.

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