Separation of Instrumental and Chemical Errors in the Analysis of Oils by Gas Chromatography—A Collaborative Evaluation

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Thirty-five analysts studied the concept that, in the gas chromatographic (GC) analysis of fatty acid composition, errors can be separated into those caused by poor chromatograph optimization and those related to inefficient conversion of triacylglycerols (TAG) to fatty acid methyl esters (FAME). A primary standard mixture of FAME was used to determine how well the participants had optimized their chromatographs. A primary standard of the equivalent TAG was used to determine total error of analysis. "Chemistry error" was calculated as the difference between the absolute errors found for the FAME and the TAG standards. Grades of analysis were computed for the FAME and TAG results and for the chemistry errors calculated from these analyses.

Only four analysts achieved grades of analysis for the FAME standard that can be considered excellent or good. These four analysts used different injector/column configurations, indicating that, when properly optimized, a GC with a flame ionization detector is an extremely accurate instrument. Conversely, it is evident that there is the potential for most analysts to improve their instrumental optimization. In agreement with published information, AOCS method Ce 2-66 and AOAC method 969.33 gave low chemistry grades, but a number of analysts used modifications of these methods, and some achieved much better grades. It would appear that many of the standard methods that are in common use are capable of producing improved results, but that critical parameters need to be better specified to ensure minimization of error. The concept of separating errors into those of instrument origin and those caused by the chemical component of the total method would appear to be a useful concept for the validation of analytical methods.

KEY WORDS: Chemistry error, collaborative evaluation, GC analysis of fatty acid methyl esters, instrumental error, primary FAME and TAG standards, separation of analytical errors.

Soon after the inception of gas chromatography as a new technique for the separation of low-molecular weight fatty acids (1), Cropper and Heywood (2) demonstrated its applicability to the longer-chainlength fatty acid methyl esters (FAME). Since that time, there have been many publications that have dealt with the conversion of fatty acids and of lipid esters into methyl esters (FAME), the most commonly prepared volatile analyte. In spite of this plethora of papers on methylation, there remain many differences of opinion, even between experienced analysts, as to the merits and disadvantages of many of these published methods. It is not within the scope of this paper to review this literature. For those who may need further information, the topic has been treated by Christie (3) and, from references in this publication, further detail may be obtained from the original literature.

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Of the many published methods, a small number have been tested collaboratively and adopted as standard methods by organizations such as AOCS (4), AOAC (5), ISO (6), IUPAC (7) and others. In spite of the care that these organizations take to validate their official methods, they are not necessarily accepted without reservation. Bannon *et al.* (8) demonstrated that for the AOCS (4) and ISO (6) methods, FAME of chainlength below 10 were not extracted quantitatively into the analyte solution. They also showed that the quantitative accuracy could be much improved simply by shaking vigorously at the extraction step.

In recent years, there have been many changes in methodology and instrumentation that ought to have led to improvements in the accuracy and reliability of analysis, but there have been few attempts, by way of collaborative trials, to determine whether, and to what extent, progress has been made. Ackman *et al.* (9–11) have carried out three collaborative trials. While the major focus of these trials was the determination of erucic acid or of long-chainlength ω-3 FAME in edible oils, the third trial (11) covered the range of FAME from 14:0 to 24:1. Each year, a more general collaborative trial, the Smalley trial, is organized by AOCS. In this trial, participants are required to analyze a number of oils and fats representative of those that are commonly used in commerce. The results of the Smalley trial are submitted to a rigorous statistical analysis, but the trial design suffers from the problem that the true analysis of the oils is not known, so the assumption must be made that the most probable true analysis is the average of all results submitted, after elimination of statistical outliers.

During the years 1982 to 1988, Bannon, Craske and their colleagues (8,12–19) published a series of papers in which they dealt with a number of individual facets of the total process of analyzing oils by gas chromatography (GC). This work led to the development of a total system for the production of highly accurate and reliable results (20). In this work, primary standards of FAME and triacylglycerols (TAG) were used, so the exact errors were known for each of the facets studied.

As part of this work it was demonstrated that the concept of theoretical relative response factors, first proposed by Ackman and Sipos (21), was valid for saturated FAME, and proof was given that it also applied to a number of unsaturated FAME (15).

One concept that was developed during this program of work was that of separating the errors that arose because the gas chromatograph was not optimized (instrument errors) from those that occurred because the technique of FAME preparation was either not stoichiometric, or was otherwise not optimized (chemistry errors). The major aim of the present work was to evaluate this concept of separation of error source by way of collaborative process, with the consequent aim of developing an improved technique for the justification of methods for the preparation of FAME for GC analysis. A secondary aim was that of improving the logic of designing primary

standards that would more stringently test the processes of optimizing both the instrumental and chemical facets of the analysis of FAME.

EXPERIMENTAL PROCEDURES

Collaborative study. Thirty-five laboratories participated in the study. Participants were asked to provide details of the chromatograph, column and integrator used, together with operating parameters, details of injection technique and the method of conversion of TAG to FAME.

Each collaborator was provided with one ampoule that contained 1 mL of 3% solution of a primary standard mixture of FAME in isooctane, and two vials, each of which contained 250 mg of a mixture of TAG at 100% concentration. The percentage compositions of the standards were for FAME and TAG, respectively: 8:0, 8.55, 8.63; 10:0, 6.62, 6.42; 12:0, 46.73, 46.90; 14:0, 18.94, 18.67; 16:0, 9.13, 9.28; and 18:0, 10.03, 10.10.

The standards used were similar to those that had been used by Bannon *et al.* (8), and the method used for validating their composition is detailed in Reference 12.

Collaborators were asked to analyze the FAME standard three times, using the technique that they normally use to optimize and operate their gas chromatograph. TAG were to be twice converted to FAME, again using the method that they normally use for this operation. Each FAME preparation so obtained was to be analyzed in triplicate, making nine analyses in total for each set. Raw peak areas were to be submitted. The appropriate theoretical relative response factors, first advocated by Ackman and Sipos (21) and tabulated by Craske and Bannon (22), were applied to the above figures. These were 8:0, 1.1927; 10:0, 1.1233; 12.0, 1.0771; 14:0, 1.0440; 16:0, 1.0193 and 18:0, 1.0000.

From these corrected peak areas, the following information was calculated: composition of the FAME and TAG standards found by the analyst; absolute error for each component, for both the FAME and TAG standards; chemistry error for each component; grade of analysis, in a manner similar to that used for the calculation of Smalley results (23), for the FAME, TAG and chemistry errors; average and standard deviation of each of these results; and a fractionation index for the FAME standard, where the chemistry error = average TAG error − average FAME error (for each chainlength):

$$\text{grade of analysis} = 100 - \Sigma|\%\ \text{found} - \%\ \text{known}| \quad [1]$$

$$\text{fractionation index for linear error trends} = \text{average FAME error 8:0} - \text{average FAME error 18:0} \quad [2]$$

$$\text{fractionation index for nonlinear error trends} = \text{difference between greatest positive and negative errors} \quad [3]$$

Participants were invited to analyze the standards by two or more instruments, or to compare different techniques, and a number of analysts submitted more than one set of results.

Each participant was given a report on the figures calculated from their raw results. If possible, suggestions were made as to how the analyst might optimize procedures, but there were many occasions when it was not possible to give definitive comment.

RESULTS AND DISCUSSION

Selection of standards. The standards chosen for analysis comprised the even-numbered FAME or TAG from 8:0 to 18:0, the content of each being an approximate match of the chainlength distribution of the fatty acids of coconut oil, *i.e.,* 18:0 content of the standard approximated the content of 18:0 and 18:1 of coconut oil. The rationale of this choice is discussed in detail in reference 12 but, briefly, the principal reasons are: (i) As the components are saturated, they can be obtained at high purity; their exact composition can be determined, hence the composition of the prepared standard also can be determined; and they are stable against autoxidative degradation. (ii) The chainlength range is wide enough that it is a challenge to the analyst to optimize the chromatograph for linear operation. (iii) For many methods of conversion of TAG to FAME, extraction of low-molecular weight FAME into the analyte solution is not quantitative, so the technique of FAME preparation is also challenged.

Comments by collaborators. A number of collaborators commented that, as they analyze only soybean and similar oils, the selection of a lauric-type standard was not relevant to their operation. This is not a valid criticism, for the reasons listed above, and also because the submission of a standard that simulated oils of this type would not have been an adequate challenge of analytical technique.

Other participants suggested that a standard that contained C20 and C22 acids would have been relevant for those who need to analyze fish oils. This is a valid comment, and the collaborative analysis of such a standard might well be worth consideration for a future trial. However, it may be noted that although fish oils present a challenge in optimizing the chromatograph for linearity at least equal to that of the standard selected, they may not present the same challenge in the preparation of FAME.

One analyst suggested that it would be valuable to have a standard that covers the whole range of FAME available from C4 of butterfat to C22 of fish oils. In view of the problems that were encountered in the analysis of a standard of much narrower range, this would seem to be too ambitious a proposal.

Statistical analysis of results. In many collaborative trials, the exact composition of the analyte is not known, hence the most probable true answer is taken to be the average figure, after elimination of outliers. In the present case, this approach was not relevant, as the samples analyzed were primary standards whose composition was accurately known. By separation of errors into those of instrumental and those of chemical origin, it is possible for the analyst to determine the source of inaccuracies and, by an examination of the error trends, to optimize technique so as to improve the accuracy and reliability of analyses. It is as valid for a single analyst to do this for his/her own results as it is for an independent third party to evaluate the results of many analysts, as in the present case. Statistical analysis comprised only an assessment of the repeatability of techniques by determination of the average and standard deviation of each set of triplicate results. The determination of the average value of triplicate analyses is a valid exercise. However, the determination of the standard deviation of three values is probably of doubtful validity. It was done with full recognition of

this lack of statistical relevance and solely for the purpose of reducing the information content to a single figure for ease of comprehension.

Instrumentation used by participants, and operational parameters. Details of the chromatograph, integrator and column used by participants are collected in Table 1. Injection technique is recorded in Table 2, and instrumental parameters in Table 3.

With respect to the primary purpose of the study, *viz.*, evaluation of the concept of error separation, the main use of most of these figures was as supplementary information when trying to suggest ways that accuracy might be improved, but there are two points that are germane to the compilation of appropriate standard methods of chromatographic analysis: (i) All analysts used a computing integrator or computer for determination of peak areas. In our summary paper (20), we noted that accurate determination of peak areas can be obtained only by way of a computing integrator or computer and suggested that it might be appropriate for Societies to build into their standard method the recommendation to use this type of equipment for improved accuracy. In view of the present ubiquity of this equipment, it would appear appropriate to reiterate this injunction. (ii) Of the 35 participants, 28

TABLE 1

Instrumentation Used by Collaborative Study Participants

Analyst number	Gas chromatograph	Integrator	Column supplier[a]	Phase	Column dimensions
1	HP5880A	HP5880A	J&W	DB-23	30 m × 0.25 mm × 0.25 μm
2	Shimadzu GC 9A	Shimadzu C-R3A	Supelco	SP2310/SP2300	1.7 m × 3 mm; 3%/2%; 100/120 Chromosorb WAW
3	HP5890 Ser. II	HP3396 Ser. II	J&W	DB-225	30 m × 0.25 mm × 0.25 μm
4	Varian 3700/SGE CCS-4/NC	HP3390A	SGE	BPX-70	25 m × 0.25 mm × 0.25 μm
5	HP5890	HP3365 Chem Stat	SGE	BPX-70	50 m × 0.22 mm × 0.25 μm
6	HP5890	HP3396A	Supelco	Supelcowax 10	30 m × 0.75 mm × 1.00 μm
7	HP5880A	HP5880A Level 4	Alltech	Carbowax	30 m × 0.25 mm × 0.25 μm
8	PE Auto System	SP4290	J&W	DB-23	30 m × 0.32 mm × 0.25 μm
9a	HP5890 Ser. II	HP3396 Ser. II	Supelco	Supelcowax 10	30 m × 0.32 mm × 0.25 μm
9b	HP5890 Ser. II	HP3396 Ser. II	Supelco	Supelcowax 10	30 m × 0.32 mm × 0.25 μm
9c	HP5890 Ser. II	HP3396 Ser. II	Supelco	Supelcowax 10	30 m × 0.32 mm × 0.25 μm
10	HP5890 Ser. II	Shimadzu C-R4AX	J&W	DB-23	30 m × 0.32 mm × 0.25 μm
11	HP5890	PE Turbochrome 3	Supelco	Supelcowax 10	30 m × 0.53 mm × 1.00 μm
12	HP5890	HP3392A	Supelco	SP2310/SP2300	3.1 m × 2 mm; 3%/2%; 100/120 Chromosorb WAW
13	HP5890	VG Minichrom Data	J&W	DB-23	30 m × 0.32 mm × 0.25 μm
14	HP5890	HP DOS Chem Stat	J&W	DB-225	30 m × 0.25 mm × 0.25 μm
15	HP5890A	HP3292A	J&W	DB-225	30 m × 0.25 mm × 0.25 μm
16	HP5890A	HP DOS Chem Stat	Supelco	SP2340	60 m × 0.25 mm × 0.20 μm
17	Varian 2740-10	HP3292A	Chrom Spec	DEGS	1.8 m × 3.2 mm; 10%; 100/120 Chromosorb WAW
18	Varian 3700	HP3394A	Supelco	SP2310/SP2300	1.8 m × 3.2 mm; 3%/2%; 100/120 Chromosorb WAW
19	Varian 3700	SP4100	J&W	DB-225	30 m × 0.53 mm × 1.00 μm
20	HP5840	HP5840 Terminal	Supelco	SP2330	30 m × 0.32 mm × 0.20 μm
21	Varian Vista Series	Varian CDS 401	Chrompack	EGA	2 m × 2 mm; 10%; 80/100 Chromosorb WAW DCMS
22	HP5890	HP3292A	J&W	DB-23	30 m × 0.32 mm × 0.25 μm
23	HP5890	HP3393A	Supelco	SP2330	3.3 m × 2 mm; 10%; 100/120 Chromosorb
24	HP5890 Ser. II	HP3396A	Supelco	Omegawax 320	30 m × 0.32 mm × 0.25 μm
25	HP5880A	HP3357/3350	J&W	DB-WAX	15 m × 0.25 mm × 0.25 μm
26	HP5890	HP3365 Chem Stat	Supelco	Supelcowax 10	15 m × 0.32 mm × 0.25 μm
27	HP5890	HP1000/18625 A/D	J&W	DB-WAX	15 m × 0.25 mm × 0.25 μm
28	HP5890A	HP3393A	Chrompack	CP Wax 58 CB	25 m × 0.25 mm × 0.2 μm
29	PE8500	Trivector Trio	Supelco	Supelcowax 10	15 m × 0.25 mm × 0.25 μm
30	HP5890 Ser. II	HP3396 Ser. II	Restek	Stabilwax	30 m × 0.32 mm × 0.25 μm
31a	HP5713	HP3353	Supelco	DEGS-PS	1.8 m × 2 mm; 10%; 100/120 Supelcoport
31b	HP5880	HP3350	Supelco	SP2310/SP2300	1.8 m × 2 mm; 3%/2%; Chromosorb WAW
32	HP5890 Ser. II	HP3365 Chem Stat	Restek	Stabilwax	60 m × 0.53 mm × 1.5 μm
33a	Siemens SICHROMAT 2-8	SP4400	Supelco	Supelcowax	30 m × 0.25 mm × 0.25 μm
33b	Siemens SICHROMAT 2-8	SP4400	Supelco	Supelcowax	30 m × 0.25 mm × 0.25 μm
34	Shimadzu GC 14A	Shimadzu Chromatopac CR5A	J&W	DB-WAX	30 m × 0.25 mm × 0.25 μm
35a	HP5880A	HP5880A	Quadrex	Bonded CPS-2	50 m × 0.25 mm × 0.25 μm
35b	HP5880A	HP5880A	Quadrex	Bonded CPS-2	50 m × 0.25 mm × 0.25 μm

[a]More information on suppliers, etc., can be found in Reference 12.

TABLE 2

Injection Technique of Collaborative Study Participants[a]

Analyst	Inlet design	Syringe supplier	Type	Filling technique	TAG (%)	FAME (%)	Volume (μL)	Manual/ automatic	Injection rate
1	Split	Hamilton	Barrel	Sample only	0.12	0.12	2	Auto	Fast, immediate withdrawal
2	N/S	Hamilton	Barrel	Sample only	0.4	0.3	3	Manual	Fast, immediate withdrawal
3	Split packed	HP	Barrel	Sample only	1.5	1.5	1	Auto	Fast, immediate withdrawal
4	No insert	Ito Corp	Barrel	Solvent, air; sample, air in needle	8	3	0.4	Manual	Fast, immediate withdrawal
5	Jennings Cup; packed 10% OV101	Hamilton	Barrel	Air space	0.6	0.5	1	Auto	Fast, immediate withdrawal
6	Capillary glass liner	Unimetric	Barrel	Sample only	2	2	1	Auto	Fast, immediate withdrawal
7	HP 19251-60540	HP	Barrel	Sample only	5–7	3	1	Auto	Fast, immediate withdrawal
8	Capillary split/splitless	SGE/PE	Barrel	Sample only	N/S	3	1	Auto	Information not clear
9a	Jennings Cup; packed 10% OV101	Hamilton	Barrel	Sample only	N/S	0.3	3	Auto	Fast, immediate withdrawal
9b	Jennings Cup; packed 10% OV101	Hamilton	Barrel	Sample only	N/S	0.3	3	Auto	Fast, immediate withdrawal
9c	Jennings Cup; packed 10% OV101	Hamilton	Barrel	Sample only	N/S	0.3	3	Auto	Fast, immediate withdrawal
10	Nozzle cup diffuser	HP	Barrel	Sample only	0.5	3	1	Auto	Fast, immediate withdrawal
11	Split	Hamilton	Barrel	Sample only	0.75	0.75	2	Auto	Fast, immediate withdrawal
12	On column (packed)	SGE	Barrel	Sample only	3	3	1	Manual	Fast, immediate withdrawal
13	On column (capillary)	HP	Barrel	Sample only	N/S	0.015	0.5	Auto	Slow, immediate withdrawal
14	HP 19251-60540 Glass wool	Hamilton	Barrel	Sample only	0.25	0.25	1	Auto	Fast, immediate withdrawal
15	Split insert	Hamilton	Barrel	Sample only	0.3	0.3	1	Auto	Fast, immediate withdrawal
16	Straight tube; 1 cm glass wool	HP	Barrel	Sample only	3.5	3	1	Auto	Fast, immediate withdrawal
17	1/4″ flash vaporization	Hamilton	Barrel	Solvent push	N/S	3	0.08	Manual	Fast, immediate withdrawal
18	N/S	Hamilton	Needle	Sample only	0.6	3	0.2	Manual	Fast, immediate withdrawal
19	0.53 mm direct liner	Hamilton	Barrel	Solvent; sample; air in needle	1	0.5	0.2–0.5	Manual	Fast, immediate withdrawal
20	N/S	Hamilton	Barrel	N/S	N/S	0.5	1	Auto	Slow, left in
21	1/8″ packed liner	SGE	Barrel	Air space	N/S	3	0.8	Manual	Fast, immediate withdrawal
22	Split	Hamilton	Barrel	Solvent push	2.5	1.5	1	Auto	Fast, immediate withdrawal
23	On column	HP	Barrel	Sample only	1.5	1.5	2	Auto	Fast, immediate withdrawal
24	Capillary split/splitless	SGE	Barrel	Sample only	2.9	3	1	Auto	Fast, immediate withdrawal
25	Split/glass wool	Hamilton	Barrel	Sample only	1.25	3	1	Auto	Fast, immediate withdrawal
26	Supelco Cat No 2-4986	Hamilton	Barrel	Sample only	1	3	1	Auto	Fast, immediate withdrawal
27	Jennings cup; packed	Hamilton	Barrel	Sample only	2	2	1	Auto	Fast, immediate withdrawal
28	HP jet & Jennings cup	Hamilton	Barrel	Sample only; needle dried	1	0.6	1	Manual	Fast, immediate withdrawal
29	N/S	SGE	Barrel	Solvent push	2	3	0.6	Manual	Slow (*ca.* 4 s)
30	Capillary split/splitless	Hamilton	Barrel	Air space	0.75	0.5	6	Auto	Fast, immediate withdrawal
31a	On column (packed)	Hamilton	Barrel	Sample only	2–2.5	2	2	Auto	Fast, immediate withdrawal
31b	On column (packed)	Hamilton	Barrel	Solvent; air; sample, air in needle	2–2.5	2	2	Manual	Fast, immediate withdrawal
32	Purge packed	Hamilton	Barrel	Sample only	1.5	3	1	Auto	Fast, immediate withdrawal
33a	Jennings cup; 1 cm 1% JXR on GC-Q	Nil - Syringeless autosampler			0.8	1	0.5	Auto	Fast, immediate withdrawal
33b	Jennings cup; 1 cm 1% JXR on GC-Q	Hamilton	Barrel	Solvent + air push	0.8	1	1	Manual	Fast, immediate withdrawal
34	Split/splitless	Hamilton	Barrel	1 μL air; 1 μL sample; air	2.5	3	1	Manual	2 s in injector; fast inject; hold 1 s
35a	1 cm glass wool in straight tube	HP	Barrel	Sample only	0.4–0.8	0.3	1	Auto	Fast, immediate withdrawal
35b	1 cm glass wool in straight tube	HP	Barrel	Sample only	0.4–0.8	0.3	1	Auto	Fast, immediate withdrawal

[a]More details can be found in Reference 12. TAG, triacylglycerols; FAME, fatty acid methyl esters; auto, automatic; N/S, not stated; N/A, not applied.

TABLE 3

GC Parameters Used by Collaborative Study Participants[a]

	Temperatures (°C)				Gas parameters								
Analyst	Inject	Detect	Column	Program	Carrier gas	Carrier cm/s[b]	Purge mL/min	Split mL/min	Split ratio	Make-up gas	Make-up mL/min	Fuel mL/min	Air mL/min
1	250	250	120–230	Hold 0; 6°C/min to 230°C	Helium	42	2.5	23.5	19	N/S	N/S	25	300
2	275	275	150–225	Hold 6; 3.5°C/min to 225	Nitrogen	20–25[b]	N/A	N/A	N/A	N/A	N/A	N/S	N/S
3	200	250	180–220	Hold 2; 4°C/min to 220	Hydrogen	54	1	450	280	Nitrogen	30	30	300
4	240	280	170–225	Hold 0; 5°C/min to 225	Hydrogen	32	0	80	260	Nitrogen	25	30	250
5	250	280	195	Isothermal	Helium	31.2	5	N/S	60	Helium	30	42.8	375
6	225	275	125–205	Hold 2; 10°C/min to 205	Helium	30.2	6	100	12.5	Helium	20	30	250
7	220	270	125–185	Hold 2; 10°C/min to 185	Helium	37	3	247.5	225	Helium	30	30	400
8	230	300	120–240	Hold 1.5; 6°C/min to 240; hold 5	Nitrogen	42	2	14.3	8	Nil	Nil	40	400
9a	285	285	75–220	Hold 1.5; 10°C/min to 170;	Helium	25	2.2	26.6	22	Nitrogen	30	30	400
9b	285	285	75–220	3°C/min to 200; 8°C/min to 220	Helium	25	2.2	26.6	22	Nitrogen	30	30	400
9c	285	285	75–220	Hold 22 min	Helium	25	2.2	26.6	22	Nitrogen	30	30	400
10	250	275	180–225	Hold 1; 1.5°C/min to 225; hold 2	Helium	(?)	0	150	(?)	Nil	0	38	300
11	275	260	245	Isothermal	Helium	27	2.5	90	25	Helium	35	32	390
12	240	260	190–210	Hold 10; 10/min to 210; hold 1.7 min	Nitrogen	30[b]	N/A	N/A	N/A	N/A	N/A	30	400
13	Cool	260	90–200	Hold 3; 4°C/min to 180; hold 2; 8°C to 200; hold 12.5	Helium	20	N/A	N/A	N/A	N/A	N/A	30	300
14	230	300	120–220	Hold 2; 8°C/min to 220	Helium	87	4.7	99	38	Nitrogen	9	29	284
15	175	300	80–220	Hold 0; 8°C/min to 220	Helium	29	1	53	57	Helium	29.4	29.7	400
16	250	250	150–200	Hold 0; 1.3°C/min to 200; Hold 10	Helium	15	3	140	317	Helium	30	30	350
17	240	260	180	Isothermal	Nitrogen	N/S	N/A	N/A	N/A	N/A	N/A	30	300
18	300	260	120–215	Hold 0; 5°C/min to 215; hold 10	Helium	N/S	N/A	N/A	N/A	N/A	N/A	N/S	N/S
19	220	220	140–185	Hold 0; 2°C/min to 185	Helium	75.5	0	0	N/A	Helium	25	40	310
20	250	300	50–240	Hold 0.5; 7.5°C/min to 240	Helium	36	N/S	N/S	N/C	Nitrogen	30	40	240
21	250	275	150–200	Hold 0; 10°C/min to 200; hold 45	Nitrogen	50[b]	N/A	N/A	N/A	N/A	N/A	40	20(?)
22	225	280	100–225	Hold 2; 4°C/min to 225; hold 5	Helium	41	2.5	400	200	Helium	29	33	400
23	250	250	150–210	Hold 7; 4°C/min to 210	Nitrogen	54[b]	N/A	N/A	N/A	N/A	N/A	27.6	545
24	260	260	160–210	Hold 0; 3°/min to 210; hold 18	Helium	26.5	1	13	10	Helium	29	30	400
25	210	230	110–220	Hold 1; 15°C to 170; 6°C to 200; 10°C to 220; Hold 8	Helium	60	1.5	100	57	Helium	30	30	300
26	250	250	115–240	Hold 2; 10°C/min to 240; hold 3	Hydrogen	58.1	5.8	52.6	19	Nitrogen	7.8	50	260
27	210	255	110–235	Hold 1; 15°C to 170; 2°C to 190; 10°C to 235; hold 8	Helium	43	1.6	150	118	Helium	30	30	400
28	200	250	100–240	Hold 2; 10°C to 240; hold 4	Helium	30	2	80	90	Nitrogen	30	30	260
29	290	300	100–240	Hold 0; 8.7°C to 240; hold 0	Helium	N/S	N/S	N/S	N/S	Nitrogen	N/S	N/S	N/S
30	250	300	180–254	Hold 0; 3°C to 210; 10°C to 230; 2°C to 254; hold 0	Helium	70	5.5	34.9	10	Helium	26.9	33.2	420
31a	220	250	170	Isothermal	Helium	N/S	N/A	N/A	N/A	N/A	N/A	47	320
31b	220	250	150–225	Hold 6; 3°C/min to 180; 10°C/min to 225; hold 2	Helium	35	N/A	N/A	N/A	N/A	N/A	35	300
32	250	250	100–240	Hold 1; 10°C/min to 240; hold 7	Helium	47	1.5	Nil	0	Nitrogen	19.2	34	500
33a	260	320	140–185	Hold 0; 5°C/min to 185; hold 5	Helium	24	1	108	153	Nitrogen	30	35	405
33b	260	320	140–185	Hold 0; 5°C/min to 185; hold 5	Helium	24	1	108	153	Nitrogen	30	35	405
34	275	275	165	Isothermal	Helium	N/S	10	80	N/C	Helium	N/S	N/S	N/S
35a	250	250	55–220	Hold 0; 5°C/min to 100; 10°C to 170; 1.5°C to 182;	Helium	27	N/S	50	63	Helium	30	40	400
35b	250	250	55–220	1°C to 220; no intermediate holds	Helium	27	N/S	50	63	Helium	30	40	400

[a]More details can be found in Reference 12. Abbreviations as in Table 2. [b]Carrier flow for packed columns recorded in mL/min.

used capillary columns, all of which were of fused silica construction. As capillary systems are now dominant, and will no doubt continue to take over from packed columns, it would appear appropriate for Societies to update their standard methods to cater more specifically for the needs of analysts who use capillary columns.

Analysis of the FAME standard. Analytical results obtained by participants for the analysis of the FAME standard are collected in Table 4. Three types of information can be obtained from an examination of these results, *viz.*, an estimation of the repeatability of the analyst's injection technique, information as to whether or not the chromatograph has been optimized, and the trend of individual errors, which can be used to identify the probable reason(s) for low grade of analysis, to guide the analyst toward optimum conditions.

All possible error trends are exemplified by the results of participants, *viz.*, negative linear (−8:0, +18:0, *e.g.*, Analyst No. 5); positive linear (+8:0, −18:0, *e.g.*, Analyst No. 4); negative bowed (+8:0, −12:0, +18:0, *e.g.*, Analyst No. 21); positive bowed (−8:0, +12:0, −18:0, *e.g.*, Analyst No. 24); and mixtures of positive and negative errors that do not appear to follow a definable trend. For linear error trends, one normally expects to find a single parameter that has not been optimized. For bowed trends, it is common to find two parameters that require attention, and there may be multiple problems when the trend is unpatterned.

It is not within the scope of this paper to detail the approaches that have been used to optimize chromatographs. Various factors that may be associated with nonlinear splitting have been discussed by Bannon *et al.* (16), Grob and Neukom (24), Purcell (25), Marshall and Crowe (26), Munari and Trestianu (27), Bruderreck *et al.* (28), Schomburg *et al.* (29) and Bayer and Liu (30).

No comment is necessary on the figures for repeatability except to note that, as might have been expected, those who used auto injectors achieved a generally better level of repeatability. However, as those who used manual injection achieved much the same grades of analysis, it

TABLE 4

Analytical Results for Fatty Acid Methyl Ester (FAME) Standard

Analyst	Errors for individual FAME (SD)						Grade	Fractionation index
	8:0	10:0	12:0	14:0	16:0	18:0		
1	−0.50 (0.10)	−0.07 (0.01)	+0.11 (0.13)	−0.23 (0.06)	+0.22 (0.01)	+0.47 (0.03)	98.34 (0.21)	−0.97
2	−0.81 (0.20)	−0.27 (0.10)	+0.18 (0.30)	−0.15 (0.05)	+1.04 (0.32)	+0.00 (0.22)	97.19 (0.35)	2.85[a]
3	−0.62 (0.29)	−0.09 (0.02)	−0.27 (0.14)	+0.29 (0.06)	+0.31 (0.03)	+0.39 (0.03)	98.03 (0.25)	−1.01
4	+1.17 (0.28)	+0.66 (0.14)	+1.88 (0.24)	−0.72 (0.19)	−1.10 (0.18)	−1.89 (0.27)	92.59 (1.28)	+3.07
5	−0.81 (0.01)	−0.34 (0.00)	−0.99 (0.01)	+0.45 (0.01)	+0.67 (0.01)	+1.03 (0.01)	95.70 (0.04)	−1.85
6	−0.26 (0.15)	+0.22 (0.05)	+1.26 (0.14)	−0.14 (0.10)	−0.40 (0.08)	−0.69 (0.11)	97.03 (0.37)	1.95[a]
7	−0.37 (0.04)	−0.41 (0.02)	−1.36 (0.01)	+0.46 (0.02)	+0.67 (0.01)	+1.01 (0.02)	95.72 (0.10)	−1.39
8	−0.09 (0.04)	−0.03 (0.01)	+0.18 (0.02)	−0.01 (0.02)	−0.04 (0.01)	−0.01 (0.01)	99.62 (0.05)	0.27[a]
9a	+1.38 (0.02)	+0.85 (0.02)	+2.43 (0.06)	−0.88 (0.04)	−1.43 (0.03)	−2.35 (0.07)	90.67 (0.19)	+3.74
9b	+1.38 (0.02)	+0.85 (0.02)	+1.43 (0.06)	−0.88 (0.04)	−1.43 (0.03)	−2.35 (0.07)	90.67 (0.19)	+3.74
9c	+1.38 (0.02)	+0.85 (0.02)	+2.43 (0.06)	−0.88 (0.04)	−1.43 (0.03)	−2.35 (0.07)	90.67 (0.19)	+3.74
10	−0.63 (0.08)	−0.27 (0.05)	−0.58 (0.10)	+0.25 (0.05)	+0.41 (0.06)	+0.83 (0.14)	97.02 (0.45)	−1.47
11	0.00 (0.09)	−0.17 (0.14)	−1.62 (0.18)	−0.38 (0.16)	+0.60 (0.17)	+1.58 (0.28)	95.56 (0.33)	−1.58
12	−0.75 (0.07)	+0.19 (0.03)	+0.93 (0.10)	−0.22 (0.08)	−0.06 (0.05)	−0.08 (0.06)	97.76 (0.27)	1.68[a]
13	−0.13 (0.04)	−0.05 (0.01)	+0.13 (0.03)	+0.03 (0.01)	+0.03 (0.02)	−0.01 (0.01)	99.62 (0.09)	0.26[a]
14	−0.60 (0.01)	−0.19 (0.00)	−0.13 (0.02)	+0.37 (0.01)	+0.28 (0.01)	+0.27 (0.01)	98.14 (0.01)	−0.87
15	−0.26 (0.08)	−0.14 (0.04)	−1.24 (0.12)	−0.02 (0.08)	+0.53 (0.07)	+1.13 (0.09)	96.64 (0.36)	−1.38
16	−0.63 (0.06)	−0.28 (0.01)	−1.65 (0.07)	+0.54 (0.03)	+0.80 (0.01)	+1.22 (0.04)	94.88 (0.09)	−1.85
17	−3.89 (0.33)	−1.55 (0.01)	+5.81 (0.28)	+0.16 (0.02)	−0.13 (0.04)	−0.40 (0.11)	88.05 (0.58)	9.70[a]
18	−0.52 (0.05)	−0.23 (0.04)	+1.16 (0.19)	+0.10 (0.06)	−0.11 (0.06)	−0.39 (0.17)	97.48 (0.43)	1.68[a]
19	+1.12 (0.03)	+0.71 (0.04)	+1.99 (0.26)	−0.70 (0.09)	−1.10 (0.03)	−2.01 (0.25)	92.37 (0.47)	+3.13
20	−0.18 (0.27)	−0.25 (0.14)	+0.29 (1.55)	−0.38 (0.55)	+0.08 (0.29)	+0.45 (0.31)	97.01 (1.43)	−0.63
21	+0.31 (0.03)	+0.39 (0.01)	−1.63 (0.06)	+0.35 (0.05)	+0.41 (0.02)	+0.17 (0.11)	96.73 (0.12)	+1.94
22	+0.96 (0.14)	+0.69 (0.05)	+0.01 (0.13)	−0.28 (0.05)	−0.40 (0.09)	−0.97 (0.13)	96.58 (0.40)	+1.93
23	−0.07 (0.03)	+0.02 (0.01)	−0.17 (0.01)	+0.10 (0.02)	+0.12 (0.01)	+0.01 (0.03)	99.49 (0.04)	0.24[a]
24	−0.92 (0.00)	−0.25 (0.00)	+0.57 (0.01)	+0.62 (0.02)	+0.14 (0.01)	−0.16 (0.01)	97.35 (0.04)	1.54[a]
25	−0.46 (0.03)	−0.14 (0.05)	−0.49 (0.21)	+0.36 (0.05)	+0.33 (0.09)	+0.40 (0.14)	97.82 (0.55)	−0.86
26	−0.28 (0.06)	0.00 (0.06)	+0.68 (0.11)	+0.07 (0.03)	−0.14 (0.04)	−0.31 (0.07)	98.46 (0.19)	0.96[a]
27	−1.04 (0.07)	−0.31 (0.03)	+0.26 (0.03)	+0.53 (0.04)	+0.28 (0.02)	+0.27 (0.01)	97.31 (0.20)	−1.30
28	−0.65 (0.11)	−0.51 (0.00)	−0.01 (0.22)	+0.83 (0.35)	+1.25 (0.05)	−0.91 (0.23)	95.64 (0.65)	1.56[a]
29	+0.24 (0.10)	+0.10 (0.09)	+0.14 (0.13)	−0.54 (0.19)	+0.23 (0.20)	−0.16 (0.11)	98.54 (0.21)	+0.40
30	−1.08 (0.02)	−0.39 (0.01)	−0.89 (0.00)	+0.63 (0.01)	+0.69 (0.01)	+1.03 (0.01)	95.28 (0.05)	−2.11
31a	−0.27 (0.06)	−0.08 (0.03)	+1.13 (0.09)	−0.47 (0.05)	−0.12 (0.00)	−1.19 (0.02)	97.74 (0.18)	1.40[a]
31b	−0.50 (0.38)	−0.24 (0.28)	+2.11 (2.30)	−0.81 (0.79)	−0.26 (0.40)	−0.30 (0.44)	95.16 (3.95)	2.71[a]
32	−0.15 (0.00)	−0.04 (0.00)	+0.08 (0.00)	+0.01 (0.00)	+0.04 (0.00)	+0.05 (0.00)	99.64 (0.00)	−0.19
33a	+1.04 (0.06)	+0.40 (0.03)	+1.09 (0.09)	−0.67 (0.02)	−0.67 (0.06)	−1.19 (0.08)	94.94 (0.30)	+2.23
33b	+0.76 (0.23)	+0.56 (0.09)	+2.17 (0.08)	−0.65 (0.21)	−1.04 (0.08)	−1.80 (0.08)	93.01 (0.47)	+2.57
34	+0.31 (0.01)	+0.16 (0.00)	+1.29 (0.02)	−0.41 (0.00)	−0.52 (0.01)	−0.84 (0.02)	96.46 (0.06)	+1.15
35a	−0.65 (0.02)	−0.21 (0.02)	+0.12 (0.00)	+0.56 (0.02)	+0.30 (0.01)	−0.12 (0.01)	98.04 (0.06)	1.21[a]
35b	−0.14 (0.04)	+0.01 (0.02)	+0.45 (0.01)	+0.17 (0.03)	−0.05 (0.02)	−0.44 (0.01)	98.74 (0.03)	0.89[a]

[a]Nonlinear error trend, fractionation index = difference between greatest positive and negative errors.

would appear that needle fractionation was not the major cause of the poor grades of analysis that were so prevalent.

When capillary columns are used, the amount of sample that the column will accept is so small that the problem of detector overload is a rare phenomenon. However, when the column is of high efficiency, thereby producing tall, sharp peaks, and the sample contains a large amount of an early eluting component, it is possible to overload the detector. There was no evidence of detector overload for those participants who used capillary columns.

With the use of fused silica columns (the only material of construction that was used by the participants of this trial who used capillary columns), the problem of adsorptive loss on the column has been virtually eliminated. Thus, for the case of capillary column operation, optimization essentially involves the process of tuning the injection system so that a representative aliquot of the sample is applied to the column. Of the 28 analysts who used capillary columns for this trial, only three produced results that might be considered as very good or good (arbitrarily defined as grades of 99.50+ and 99.00–99.49, respectively).

Of the seven analysts who used a packed column, only one achieved a good grade of analysis. In the case of packed column operation, loss of grade can be attributed to failure to optimize the inlet system, to detector overload and/or to adsorptive loss on the column. For those who use packed columns, it is thus necessary to consider a wide range of possible problem areas when trying to improve grade of analysis. The results of Analyst No. 21 (Table 1) are consistent with detector overload, but there is no reason to suspect that detector overload was the cause of the low grades achieved by any of the remaining five analysts who used packed columns.

It is relevant to compare some critical statistics of the four analysts who achieved good grades. Analyst No. 8 used auto-injection into a split/splitless insert, a split ratio of 8, a capillary column of 0.32 mm i.d., and achieved a grade of 99.62. Analyst No. 13 used auto direct on-column injection to a capillary column of 0.32 mm i.d., no split. The grade was 99.62. Analyst No. 23 used auto on-column injection to a 2 mm i.d. packed column, no split, to obtain a grade of 99.49. Analyst No. 32 used auto injection to a column of 0.53 mm i.d., no split. The grade obtained was 99.64.

The inescapable conclusion to be drawn from the number of grade results that were less than 99 is that few analysts have spent sufficient time to optimize their chromatograph. We may also conclude that, when properly optimized, a GC equipped with a flame ionization detector is an extremely accurate instrument. It is possible to obtain very high accuracy, no matter which of the four most common methods of operation are used, *viz.*, capillary/split, capillary/on-column, megabore/on-column or packed/on-column.

As the soundly based evaluation and development of methylation methodology is dependent upon accurate and repeatable operation of the chromatograph, it is evident that there is the need for analysts first to address this facet of the total analytical procedure.

Analysis of the TAG standard. The figures that participants achieved by analysis of the TAG standard are shown in Table 5. The presented errors are the sum of the errors that arise because of failure to optimize both the instrumental and the chemical components of the total analysis. They are intermediate figures in the calculation of the "chemistry error" and, when examined in isolation, give no information as to why a grade figure is less than optimum, nor are they any guide as to how to improve the grade. A further assessment of the repeatability of injection technique can be obtained and, while it is possible to obtain an indication of the repeatability of the methylation technique, this is better obtained from a comparison of duplicate or replicate chemistry grades.

Chemistry errors and methylation procedures. The methylation procedures used by participants are set out in Table 6, which includes the chemistry errors that were calculated from the analyses of both the standards.

The chemistry error for a particular FAME is defined as the difference between the absolute error, determined by analysis of the TAG standard, and that determined for the FAME standard. A chemistry error was calculated for the FAME of each chainlength in the standards. The grade of the chemistry procedure was determined in the usual way, by subtracting the sum of the absolute values of individual chemistry errors from 100.

The information that is obtained from the calculated chemistry errors is of the highest reliability when both the grades of analysis for the FAME standard and the repeatability of the determinations for both the FAME and TAG standards are high. When these conditions are met, the chemistry errors will be close in magnitude to those of the errors determined for the TAG standard, and the variation between replicates will be small.

It is still possible to obtain some useful information from the calculated chemistry errors if the grade of analysis of the FAME standard is more modest, but the repeatability of the analytical technique remains good. Clearly, the figures for the TAG standard will not match those calculated for the chemistry errors, and the differences will increase as the grade of analysis of the FAME standard decreases. It is reasonable to assume that confidence in the validity of the chemistry errors calculated from analyses of this lower quality will also be somewhat lower, as it is necessary to assume that, although the chromatograph was not performing optimally, its performance was constant.

As the repeatability of the analysis declines, the concept of calculating chemical error becomes decreasingly relevant and, ultimately, if both grade and repeatability are of inadequate quality, the analytical errors become of such magnitude that the calculation of chemical error becomes a meaningless exercise.

If we take, as a measure of repeatability, the sum of the standard deviations of the individual errors for the FAME standard, the 40 responses by the 35 participants can be assigned to three quality classes, *viz.*, 0.0 to 0.20 = good, 13 analysts; 0.21 to 0.40 = moderate, 10 analysts; above 0.41 = poor, 17 analysts. It is evident that much of the chemistry error information collected in Table 6 is of lower reliability than we might have hoped. In spite of this doubt, I have chosen to draw conclusions from the total body of information, because, were I to eliminate doubtful chemistry grades, the remaining database would be too small to allow comparisons to be made between the several methylation techniques that were used.

Of the 39 methylation techniques reported, 22 utilized boron trifluoride or trichloride, 16 were carried out by

TABLE 5

Analytical Results for Triacylglycerol Standard

Analyst	Errors for individual fatty acid methyl ester (SD)						Grade
	8:0	10:0	12:0	14:0	16:0	18:0	
1	−1.39 (0.07)	−0.38 (0.02)	−0.19 (0.13)	+0.23 (0.05)	+0.71 (0.04)	+1.02 (0.17)	96.04 (0.34)
2	−2.13 (0.20)	−0.87 (0.11)	−0.09 (0.15)	+0.49 (0.15)	+2.19 (0.45)	+0.41 (0.05)	93.63 (0.84)
3	−2.13 (0.02)	−0.54 (0.05)	+0.49 (0.02)	+1.00 (0.02)	+0.68 (0.07)	+0.50 (0.02)	94.66 (0.11)
4	+0.08 (0.20)	+0.15 (0.10)	+0.63 (0.23)	−0.06 (0.15)	−0.14 (0.15)	−0.67 (0.21)	97.99 (0.71)
5	−1.47 (0.02)	−0.51 (0.01)	−0.63 (0.02)	+0.81 (0.03)	+0.87 (0.01)	+0.93 (0.01)	94.77 (0.06)
6	+0.08 (0.06)	+0.29 (0.04)	+0.89 (0.22)	−0.11 (0.07)	−0.29 (0.08)	−0.87 (0.15)	97.46 (0.56)
7	−1.63 (0.02)	−0.80 (0.00)	−1.08 (0.01)	+1.10 (0.01)	+1.17 (0.00)	+1.24 (0.01)	92.99 (0.02)
8	+1.61 (0.06)	+1.73 (0.03)	+4.66 (0.04)	−2.41 (0.03)	−2.26 (0.02)	−3.34 (0.02)	83.99 (0.14)
9a	−1.03 (0.04)	−0.18 (0.02)	+2.90 (0.07)	+0.64 (0.03)	−0.58 (0.03)	−1.75 (0.08)	92.91 (0.18)
9b	−1.61 (0.04)	−0.39 (0.02)	+1.79 (0.17)	+0.95 (0.04)	0.00 (0.06)	−0.75 (0.14)	93.70 (0.18)
9c	−0.15 (0.05)	+0.29 (0.03)	+2.04 (0.11)	+0.09 (0.03)	−0.63 (0.06)	−1.64 (0.09)	95.03 (0.26)
10	−3.35 (0.02)	−1.16 (0.04)	−1.25 (0.08)	+1.58 (0.03)	+1.77 (0.04)	+2.40 (0.10)	88.48 (0.26)
11	−0.30 (0.15)	−0.20 (0.08)	−1.97 (0.23)	+0.32 (0.24)	+0.89 (0.13)	+1.25 (0.27)	95.05 (0.50)
12	−0.75 (0.14)	+0.03 (0.07)	+0.69 (0.19)	−0.01 (0.12)	+0.18 (0.09)	−0.14 (0.11)	98.04 (0.26)
13	−1.90 (0.01)	−0.32 (0.00)	+0.83 (0.03)	+0.69 (0.01)	+0.49 (0.01)	+0.21 (0.02)	95.55 (0.03)
14	−0.63 (0.02)	−0.17 (0.01)	+0.14 (0.05)	+0.47 (0.01)	+0.35 (0.01)	−0.16 (0.06)	98.08 (0.06)
15	+0.11 (0.07)	+0.28 (0.02)	+0.16 (0.09)	+0.09 (0.05)	−0.10 (0.02)	−0.53 (0.06)	95.99 (0.13)
16	−0.06 (0.34)	+0.12 (0.21)	+0.47 (0.80)	+0.55 (0.17)	+0.11 (0.40)	−1.19 (0.77)	95.81 (1.74)
17	−2.70 (0.16)	−0.69 (0.16)	+3.95 (0.52)	−0.29 (0.11)	+0.03 (0.13)	−0.30 (0.21)	91.90 (1.13)
18	−0.02 (0.11)	+0.03 (0.08)	+1.26 (0.08)	−0.02 (0.07)	−0.23 (0.06)	−1.02 (0.11)	96.45 (0.26)
19	+0.19 (0.36)	+0.65 (0.12)	+2.44 (0.72)	−0.38 (0.26)	−0.91 (0.28)	−1.99 (0.44)	92.95 (1.68)
20	+0.08 (0.15)	−0.01 (0.09)	−0.40 (0.24)	−0.13 (0.11)	+0.25 (0.13)	+0.20 (0.19)	98.67 (0.50)
21	−1.27 (0.07)	−0.08 (0.04)	−0.81 (0.06)	+1.06 (0.14)	+0.83 (0.07)	+0.28 (0.04)	95.67 (0.34)
22	−0.31 (0.14)	+0.59 (0.09)	+1.65 (0.27)	+0.06 (0.08)	−0.46 (0.14)	−1.54 (0.36)	95.34 (0.76)
23	−1.12 (0.02)	−0.22 (0.01)	+0.22 (0.03)	+0.56 (0.01)	+0.46 (0.01)	+0.11 (0.05)	97.31 (0.05)
24	−3.09 (0.03)	−0.87 (0.03)	+0.98 (0.16)	+1.64 (0.07)	+0.90 (0.07)	+0.44 (0.06)	92.08 (0.12)
25	−0.76 (0.05)	−0.24 (0.02)	−0.36 (0.06)	+0.47 (0.03)	+0.54 (0.04)	+0.36 (0.05)	97.27 (0.23)
26	+0.38 (0.06)	+0.07 (0.01)	+1.23 (0.28)	−0.05 (0.04)	−0.43 (0.10)	−1.19 (0.19)	96.63 (0.62)
27	−1.29 (0.09)	+0.11 (0.03)	+4.00 (0.08)	+0.83 (0.04)	−0.76 (0.02)	−2.89 (0.02)	89.97 (0.17)
28	−0.58 (0.00)	−0.21 (0.09)	−0.43 (0.06)	+1.26 (0.10)	+0.46 (0.00)	−0.51 (0.11)	96.55 (0.20)
29	−1.05 (0.25)	+0.78 (0.11)	+2.50 (0.29)	−0.66 (0.12)	+0.05 (0.07)	+1.61 (0.08)	93.04 (0.38)
30	−1.13 (0.03)	−0.42 (0.01)	−0.85 (0.02)	+0.76 (0.02)	+0.83 (0.01)	+0.81 (0.02)	95.20 (0.07)
31a	+0.13 (0.01)	−0.14 (0.01)	+1.49 (0.09)	−0.69 (0.04)	−0.21 (0.04)	−0.57 (0.03)	96.78 (0.20)
31b	+0.01 (0.18)	−0.05 (0.05)	−0.62 (0.05)	+0.19 (0.12)	+0.35 (0.06)	+0.13 (0.05)	98.47 (0.16)
32	−0.23 (0.01)	−0.08 (0.01)	−0.11 (0.02)	+0.22 (0.01)	+0.25 (0.00)	−0.05 (0.03)	99.06 (0.03)
33a	+0.99 (0.06)	+0.35 (0.04)	+1.43 (0.07)	−0.56 (0.05)	−0.64 (0.02)	−1.58 (0.10)	94.45 (0.31)
33b	+0.44 (0.18)	+0.43 (0.11)	+2.63 (0.39)	−0.35 (0.14)	−0.95 (0.21)	−2.20 (0.34)	92.99 (1.37)
34	+0.40 (0.27)	+0.13 (0.16)	+0.92 (0.15)	−0.48 (0.11)	−0.27 (0.12)	−0.70 (0.17)	96.80 (0.35)
35a	−0.64 (0.08)	−0.12 (0.03)	+0.59 (0.08)	+0.44 (0.06)	+0.21 (0.05)	−0.48 (0.08)	97.52 (0.14)
35b	−1.12 (0.03)	−0.32 (0.01)	+0.34 (0.07)	+0.83 (0.02)	+0.51 (0.02)	−0.25 (0.06)	96.62 (0.12)

alkaline catalysis and 1 by pyrolysis of tetramethyl ammonium hydroxide. Summarized information of the performance of these methylation techniques is shown in Table 7.

In preparing FAME, the most likely cause of error is that due to the difficulty of extracting low-molecular weight (MW) FAME from the water-diluted reaction mixture into the hydrocarbon analyte solution. This is due to the decreasingly favorable partition coefficient as the chainlength is decreased, and to the fact that many methods recommend mild or no agitation, with the result that a part of the low-MW FAME that should partition into the analyte phase does not do so. Of the 39 responses shown in Table 6, there were 27 cases where the low-MW FAME were less than theory. There is a high probability that most of these errors were caused by failure to extract the whole of the low-MW FAME into the analyte phase.

Under some reaction conditions, the longer-chainlength fatty acids can react at a slower rate than do those of low MW (14,17), and this can give rise to the converse error. The results of 11 analysts followed this pattern, and it is possible that they can be explained by this phenomenon. However, the conditions that can generate a differential rate of reaction are unusual and, given that many of the results were of lower grade and reliability than is desirable, it is at least as likely that this converse trend can be attributed to the fact that many of these calculated chemistry errors are themselves in error.

Bannon *et al.* (8) showed that the AOCS method (4), which calls for no agitation at the work-up step, allows a significant loss of low-MW FAME, and they obtained grades of 94.2 when analyzing a standard that contained fatty acids from 6:0 to 18:0. The grade would have been about 95.0 had the standard contained only the range of FAME used in the present trial, *i.e.,* 8:0 to 18:0. The figure of 93.0 obtained by the panel is consistent with this published figure.

The same paper showed that by changing to the ISO method (6), in which slight agitation is specified, the grade increased by about one point (6:0 again calculated out of the published result). The AOAC method (5) is similar to the ISO method, and it should be noted that the trial participants achieved slightly better results with this method.

TABLE 6

Methylation Procedures Used by Collaborative Study Participants[a]

Analyst	Sample wt (mg)	Methylation procedure	Analyte (%)	Chemistry errors 8:0	10:0	12:0	14:0	16:0	18:0	Chemistry grade
1	N/S	BF_3/MeOH/isooctane; 40 min @ 100°C	0.12	−0.89	−0.30	−0.30	0.46	+0.49	+0.54	97.02
2	400	Saponify KOH/MeOH; BF_3	0.4	−1.32	−0.60	−0.27	+0.65	+1.14	+0.40	95.60
3	N/S	AOCS Method Ce 2-66	1.5	−1.51	−0.45	+0.77	+0.71	+0.37	+0.11	96.09
4	N/S	Bannon & Craske, *J. Chrom. 247*:71 (1982)	8	−1.09	−0.50	−1.24	+0.66	+0.96	+1.22	94.33
5	N/S	Similar to AOCS Method Ce 2-66	0.6	−0.66	−0.17	+0.36	+0.36	+0.20	−0.10	98.15
6	N/S	React 25% NaOMe/MeOH; neut $KHSO_4$; extr pet ether	2	+0.34	+0.07	−0.37	+0.03	+0.11	−0.18	98.86
7	*ca.* 50	4 drops TAG; 0.5N NaOMe, 88°C for 2 min; 30–40 mL 0.1N HCl; 2 mL pet ether	5–7	−1.26	−0.39	+0.28	+0.64	+0.50	+0.23	96.71
8	N/S	AOCS Method Ce 2-66	N/S	+1.70	+1.76	+4.48	−2.39	−2.22	−3.32	84.13
9a	25	AOAC 15th edn. 969.33 (NaOH/BF_3) (times modified)	N/S	−2.41	−1.03	+0.47	+1.52	+0.85	+0.60	93.12
9b	25	10 mL 25% NaOMe/MeOH + 10 mL heptane; mix; wash	N/S	−2.99	−1.24	−0.64	+1.83	+1.44	+1.61	89.82
9c	25	10 mL 25% NaOMe/MeOH + 10 mL heptane; reflux; wash	N/S	−1.53	−0.56	−0.39	+0.96	+0.80	+0.72	95.04
10	N/S	AOCS Method Ce 2-66	0.5	−2.72	−0.88	−0.67	+1.34	+1.37	+1.57	91.46
11	N/S	Tetramethyl ammonium hydroxide	0.75	−0.29	−0.03	−0.34	+0.70	+0.30	−0.33	97.91
12	N/S	Sodium methoxide	3	0.00	−0.16	−0.24	+0.21	+0.24	−0.06	99.07
13	N/S	MeOH/BF_3; partitioned to heptane	N/S	−1.77	−0.27	+0.70	+0.67	+0.46	+0.23	95.90
14	10	1 mL hex; 1 mL MeOH; 1 mL BF_3; 45 min @ 100°C sealed; 3 mL H_2O; centrifuge	0.25	−0.03	+0.02	+0.27	+0.09	+0.07	−0.43	99.09
15	N/S	Seal; saponify 2 h, 80°C, 0.5N NaOH/MeOH; 3 mL BF_3, 2 h @ 80°C; extr heptane	0.3	+0.36	+0.42	+1.40	+0.10	−0.63	+1.66	95.21
16	N/S	AOCS Method Ce 2-66	3.5	+0.58	+0.39	+2.12	+0.01	−0.69	−2.41	93.65
17	N/S	AOCS Method Ce 2-66	N/S	+1.19	+0.85	−1.86	−0.45	+0.16	+0.11	95.30
18	N/S	KOH/MeOH/hex/boil to clear; neut AcOH/NaCl; shake well; sep; dry Na_2SO_4	0.6	+0.51	+0.26	+0.11	−0.12	−0.13	−0.63	97.86
19	50	5 mL hex/NaOH/MeOH; 15 min reflux;	1	−0.92	−0.06	+0.45	+0.32	+0.19	+0.03	97.89
20	N/S	NaOH/MeOH	N/S	+0.26	+0.24	−0.68	+0.25	+0.18	−0.25	98.14
21	N/S	AOCS Method Ce 2-66	N/S	−1.58	−0.47	+0.82	+0.70	+0.42	+0.10	95.90
22	N/S	AOAC 15th edn. 969.33 (NaOH/BF_3)	2.5	−1.27	−0.10	+1.64	+0.34	−0.05	−0.57	96.04
23	N/S	AOAC 15th edn. 969.33 (NaOH/BF_3)	1.5	−1.05	−0.24	+0.39	+0.47	+0.34	+0.10	97.42
24	N/S	AOCS Ce 2-66	2.9	−2.17	−0.62	+0.41	+1.02	+0.76	+0.60	94.42
25	125	10 mL NaOMe; mix 8 m @ 65°C; 7 mL NaCl-1.6% HCl; mix 0.5 m; 10 mL hexane; mix 2 m	1.25	−0.30	−0.11	+0.13	+0.11	+0.21	−0.05	98.82
26	50	5 mL isooctane; .5 mL .5N NaOMe, 10 min; .3 mL N HCl; 1 mL .15N Na_2CO_3; 7 mL H_2O	1	+0.66	+0.07	+0.55	−0.12	−0.29	−0.88	97.42
27	200	25 mL 0.13N NaOMe; 25 mL NaCl-0.5% HCl; extr. hexane; dry Na_2SO_4; evap 10 mL	2	−0.25	+0.42	+3.73	+0.29	−1.04	−3.16	90.77
28	N/S	Sodium methoxide	1	+0.07	+0.30	−0.41	+0.43	−0.79	+0.40	97.60
29	N/S	Boil 15 min 0.5N KOH; 2 min BF_3; 1 min hexane	2	−0.74	+0.10	+0.70	+0.30	+0.17	−0.54	93.44
30	N/S	Saponify; BF_3; satd NaCl; extr isooctane	0.75	−0.05	−0.03	+0.04	+0.13	+0.14	−0.23	99.38
31a	N/S	BF_3	2–2.5	+0.39	−0.06	+0.36	−0.21	−0.10	−0.38	98.50
31b	N/S	BF_3	2–2.5	+0.51	+0.19	−2.73	+1.00	+0.61	+0.43	94.54
32	100	Reflux 30 min 0.5N NaOH/MeOH; reflux 30 m 5 mL 5% H_2SO_4; 10 mL PE + 30 mL NaCl; shake vigorously; dry Na_2SO_4	3	−0.08	−0.04	−0.20	+0.21	+0.21	−0.10	99.16
33a	N/S	Bannon & Craske, *J. Chrom. 242*:71 (1982)	1	−0.05	−0.05	+0.35	+0.12	+0.03	−0.39	99.01
33b	N/S	Bannon & Craske, *J. Chrom. 247*:71 (1982)	1	−0.32	−0.13	+0.46	+0.30	+0.09	−0.40	98.29
34	50	2 mL benzene; 1 mL MeOH; 1 mL BCl_3; flush N_2; 100°C for 1 h; cool; 2 mL H_2O; extr 2 × 2 mL hexane; dry Na_2SO_4; evap to 2 mL	2.5	+0.09	−0.04	−0.38	−0.07	0.25	+0.14	98.38
35a	200	4 mL NaOH/MeOH & reflux 5–10; 5 mL BF_3 & reflux 2;	0.4–0.8	−0.50	−0.13	+0.13	+0.28	+0.26	−0.04	98.66
35b	200	25 mL heptane & reflux 1; 25 mL H_2O; shake, sep.; dry Na_2SO_4	0.4	−0.47	−0.10	+0.22	+0.28	+0.21	−0.13	98.58

[a]More details can be found in Ref. 12. Abbreviations as in Table 2.

TABLE 7

Summary of Grade of Analysis for Methylation Techniques[a]

Method	No. of analysts	Grade of analysis			
		Average	SD	Maximum	Minimum
Catalysis by boron trifluoride/trichloride					
AOCS 2-66	7	93.0	4.2	96.09	84.13
Similar AOCS 2-66	6	96.7	2.3	99.38	93.44
AOAC 969.33	3	95.5	2.2	97.42	93.12
BF_3/MeOH	5	97.0	1.9	99.09	94.54
BCl_3/MeOH; 2 × extractions	1	98.4	N/A	N/A	N/A
Alkaline catalysis					
NaOH/MeOH, neutralized	4	98.3	0.6	99.16	97.86
NaOMe/MeOH, neutralized	5	96.5	3.3	98.86	90.77
Bannon *et al.* Methoxide[a]	3	97.2	2.5	99.01	94.33
Similar Bannon *et al.*[a]	4	95.4	4.1	99.07	89.82
Tetramethyl ammonium hydroxide					
Pyrolysis	1	97.9	N/A	N/A	N/A

[a]Reference 13. N/A, not applied.

Finally, Bannon *et al.* (8) showed that the grade could be improved significantly by employing vigorous shaking to promote equilibrium partition. Six analysts used methods that were stated to be similar to AOCS Method Ce 2-66 (4). However, while the average grades were again slightly improved, none recorded whether they had shaken vigorously, so it is not possible to determine the reason for their improved performance.

Five analysts used boron trifluoride/methanol, and one used boron trichloride/methanol without prior alkaline transesterification, and they obtained grades comparable with the best of those already discussed.

Analysts who used one of the four alkaline-catalyzed methods achieved average grades within the range 95 to 98.

For most of the methods, the variation of grade within any method was wide. This indicates that the wording of important steps to be followed when using the methods is not sufficiently explicit, thereby allowing analysts to introduce unwarranted variation in methodology.

Five analysts achieved good chemistry grades, arbitrarily defined as better than 99. One good result was achieved when each of the following methods was used: (i) similar to AOCS Ce 2-66; (ii) BF3/MeOH; (iii) NaOH then neutralized; (iv) Bannon *et al.* methoxide (13); and (v) similar to Bannon *et al.* (13) methoxide. As this is a wide spectrum of method types, it would appear that many of the methods that are commonly used would be capable of better performance if the critical parameters were better identified and specified.

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