# Report of the Instrumental Techniques Committee, AOCS, 1966-1967[1]

## Introduction

THE INSTRUMENTAL TECHNIQUES COMMITTEE met twice during the past year with its Subcommittees represented at each meeting. The first meeting was held in the Gold Room of the Bellevue-Stratford Hotel, Philadelphia, Pa., Monday, Oct. 3, 1966, in conjunction with the 40th Fall Meeting of the Society. The second meeting was held during the 58th Annual Meeting in the Southern Pine Room of the Roosevelt Hotel, New Orleans, Louisiana, Monday, May 8, 1967.

## Special Task Group for Preparation of Methyl Esters

Following discussions during the meeting in Philadelphia in October 1966, this Subcommittee decided to investigate two methods for the preparation of methyl esters from either fatty acids or triglycerides which had appeared in the technical literature since the adoption by the Society of a tentative method recommended by this Subcommittee. These two methods appear to be both simpler and much shorter than the method accepted as a tentative method by the AOCS. Investigation revealed that one of the suggested methods was not capable of being scaled up to yield quantities of material sufficient for routine analysis by infrared absorption spectroscopic techniques, and this method was not further investigated.

The second method, proposed by Metcalfe, Schmitz, and Pelka (1) was tested in collaborative testing by the Subcommittee using two samples mailed about the first of February 1967, with a modification of the method of Metcalfe et al. scaled up to handle 1 g of fat.

To facilitate evaluation, fats containing fatty acids with structural features that are reasonably sensitive to harsh treatment and that can be determined accurately in the original fat and in the methyl esters were selected. These were: ME 67–1, dehydrated castor oil (courtesy W. E. Link, Archer-Daniels-Midland Company, Chemical Division) containing conjugated diene unsaturation, and ME 67–2, a fat containing isolated *trans*-unsaturation and free of interfering conjugated diene (courtesy J. V. Luck, Durkee Famous Foods).

Each collaborator was requested to obtain the reaction products in a dry, solvent-free state, and to calculate his total product recovery. All ester preparations were returned to The Hormel Institute for evaluation.

A. *Total product recovery.* This was to detect any losses resulting from mechanical transfers, inefficient extractions, removal of solvents and other deficiencies which do not originate in the esterification reaction itself and which could be corrected by supplying more specific instructions.

B. *Ester yield.* Determined by David Firestone (Food and Drug Administration). This is a measure of the monomer content of the reaction product by a microsublimation technique, to determine if the method gives complete transformation of the glycerides into methyl esters.

C. *Diene conjugation.* (The Hormel Institute.) Conjugated diene was determined on the dehydrated castor oil, methyl esters prepared by the collaborators and samples of methyl esters prepared at The Hormel Institute by the methanol sulfuric acid Method Ce 2–66. AOCS Method CD 7–58 (preformed conjugation) was employed.

D. *Isolated trans unsaturation.* (The Hormel Institute.) This was determined on the original glyceride fat and all methyl esters using the AOCS infrared Method CD 14–61 corrected as suggested by Firestone and LaBouliere (2).

E. *Fatty acid composition.* (The Hormel Institute.) This was determined on all methyl esters by gas chromatography on a 6 ft, ¼ in., 20% DEGS column, stabilized with 2% phosphoric acid at 190C using a flame ionization detector.

Methyl esters were prepared by 10 of the 11 collaborators, but one set of samples was lost during shipment and only nine methyl esters from each fat were available for evaluation.

The results are summarized in Table I.

Individual results from the 11 laboratories participating in the collaborative gas chromatographic analysis are given for the two samples in Tables II and III.

The Subcommittee, from an analysis of these data reached the following conclusions:

A. *Product recovery.* One laboratory reported recovery for both samples of only about 50%. Inquiry revealed that this was due solely to failure of the analyst to strive for quantitative recovery and did not, in any way, indicate a deficiency in the method. Excluding this value from the average, the results indicate that a recovery of 95% or better is easily attainable.

B. *Methyl ester content.* The methyl ester content of the dehydrated castor oil esters ranged from 58 to 82% and averaged 73%. These low and variable values are due to the high content of conjugated material which polymerizes readily at the temperature used for the analysis. A similar low value was obtained from a sample of methyl esters prepared by the methanol-sulfuric acid method. The excellent agreement between the conjugated diene content of the original triglycerides and of the methyl esters indicates that the esterification procedure itself has no effect on diene conjugation. Esters prepared from the *trans* glycerides contain more than 96% monomers, indicating essentially complete transformation of glyceride into methyl esters.

C. *Conjugated diene and trans content.* All the values for the methyl esters are in excellent agreement with those obtained directly with the glycerides.

D. *Fatty acid composition.* The composition of the methyl esters prepared with boron fluoride was virtually identical to that of the corresponding esters obtained with the methanol-sulfuric acid procedure.

E. *Preparation of methyl esters.* Within the scopes

[1] Report of collaborative work from Government, Industrial, and Academic Laboratories by members of the ARS, USDA, Southern and Eastern Utilization Research and Development Divisions; Anderson, Clayton and Co., Food Division; The Hormel Institute, University of Minnesota; and Durkee Fine Foods.

TABLE I

| | Ester from | | | |
|---|---|---|---|---|
| | ME-67 1 Dehydrated castor oil | | ME-67-2 *trans* glycerides | |
| | $BF_3$ | $MeOH–H_2SO_4$ | $BF_3$ | $MeOH–H_2SO_4$ |
| Product recovery % | 93.5 (6.2 )[a] | ..... | 94.1 (2.4 ) | ..... |
| Methyl ester content % | 73.3 (9.9 ) | 79.0 | 97.9 (1.3 ) | 97.3 |
| Conjugated diene content: | | | | |
| Original glycerides | 28.08 | | ..... .. | |
| Methyl esters | 28.77(0.75) | 27.68 | ..... | ..... |
| Isolated *trans* content: | | | | |
| Original glycerides | ..... | | 40.28 | |
| Methyl esters | ..... | ..... | 40.03(0.79) | 37.94 |
| Fatty acid composition: | 0.26(0.03) | 0.30 | 0.52(0.08) | 0.56 |
| 12:0 | ..... | ..... | 0.72(0.04) | 0.74 |
| 14:0 | 1.19(0.12) | 1.15 | 11.90(0.20) | 11.85 |
| 16:0 | | | | |
| 16:1 | ..... | ..... | 0.54(0.04) | 0.54 |
| 18:0 | 1.52(0.14) | 1.46 | 9.95(0.06) | 9.55 |
| 18:1 | 4.53(0.34) | 4.36 | 73.59(0.32) | 73.58 |
| 18:2 | 48.43(1.48) | 48.04 | 3.11(0.07)[b] | 3.15[b] |
| 18:3 + 18:2 C,T-conj. | 21.99(1.18) | 22.46 | ..... | ..... |
| 18:2 C,C-conj. | 6.68(0.38) | 7.00 | ..... | ..... |
| 18:2 T,T-conj. | 15.32(0.97) | 15.27 | ..... | ..... |

[a] Average (Standard Deviation).
[b] Also includes some 19:0.

and limitations indicated in the method the boron trifluoride procedure is suitable for preparation of methyl esters directly from glycerides.

Based on these conclusions the Subcommittee recommended that the method be rewritten to include information for preparing methyl esters from samples varying in size from 100 mg to 1.0 g, specifying appropriate quantities of reagents for each sample size.

The proposed method as modified by the Subcommittee is attached and made a part of this report.

## X-Ray Subcommittee

We are happy to announce the reappointment of C. W. Hoerr, past President of the Society, as Chairman of the X-Ray Subcommittee. Mr. Hoerr resigned this position when elected to President of the Society. Subcommittee members have been contacted regarding a resumption of Subcommittee activities. Immediate plans include a collaborative investigation of the highly purified tristearin (courtesy, E. S. Lutton, Procter & Gamble Co., Miami Valley Laboratories), for a systematic compilation of its crystal characteristic in several laboratories.

Future plans of the Subcommittee include similar collaborative measurements of the X-ray diffraction of a series of triglycerides with the over-all objective of establishing standard nomenclature and symbols, fixing some degree of coordination among X-ray data published in the literature and resolving the chief causes of discrepancies among various investigators, arising mainly from the fact that some data are reported on highly purified glycerides, while other data are reported on mixtures such as vegetable oils, animal fats, etc. [See preceding Report of the Committee, 1965–66(3).]

TABLE II

GLC Analysis
ME-67-1 dehydrated castor oil

| Laboratory | Peak | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12:0 | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 18:2 CT-Conj. | 18:2 CC-Conj. | 18:2 TT-Conj. |
| 1 | 0.28 | 1.15 | 1.46 | 4.38 | 48.15 | 22.51 | 6.91 | 15.13 |
| 2 | 0.28 | 1.14 | 1.50 | 4.43 | 47.57 | 22.62 | 6.86 | 15.56 |
| 3 | 0.31 | 1.20 | 1.50 | 4.44 | 48.17 | 22.36 | 6.88 | 15.10 |
| 4 | 0.27 | 1.15 | 1.46 | 4.45 | 47.87 | 22.69 | 6.86 | 15.20 |
| 5 | 0.21 | 1.14 | 1.51 | 4.47 | 48.21 | 21.95 | 6.93 | 15.54 |
| 6 | 0.28 | 1.23 | 1.53 | 4.49 | 47.64 | 21.62 | 5.91 | 17.26 |
| 7 | 0.23 | 1.49 | 1.87 | 5.43 | 52.30 | 19.02 | 6.16 | 13.44 |
| 8 | 0.28 | 1.16 | 1.51 | 4.44 | 47.77 | 22.44 | 6.91 | 15.46 |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 11 | 0.28 | 1.06 | 1.39 | 4.26 | 48.22 | 22.77 | 6.73 | 15.24 |
| Average | 0.26 | 1.19 | 1.52 | 4.53 | 48.43 | 21.99 | 6.68 | 15.32 |
| S.D. | 0.03 | 0.12 | 0.14 | 0.34 | 1.48 | 1.18 | 0.38 | 0.97 |
| $MeOH–H_2SO_4$ Esters[a] | 0.29 | 1.14 | 1.45 | 4.36 | 48.04 | 22.49 | 6.92 | 15.27 |
| GC No. 15[b] | 0.31 | 1.16 | 1.46 | 4.36 | 47.91 | 22.42 | 7.08 | 15.27 |
| Average | 0.3 | 1.3 | 1.5 | 4.9 | 48.2 | 21.1 | 7.2 | 13.4 |
| S.D. | 0.24 | 0.46 | 0.81 | 1.46 | 5.55 | 1.98 | 2.62 | 2.19 |

[a] Two analyses on one sample of esters by Gas Chromatography Subcommittee.
[b] Dehydrated castor oil methyl esters (11 values) included here for comparison purposes only.

## Spectroscopy Subcommittee

The Spectroscopy Subcommittee's problems in obtaining a suitable primary standard upon which to base collaborative effort with the aim of establishing an AOCS standard method for the determination of conjugated *cis-trans* and *trans-trans* isomers in fatty acids, esters or triglycerides, analogous to the present method for the determination of isolated *trans* isomers (AOCS Cd 14–61) appear to be nearing a solution. The Executive Committee of the Society has agreed to the purchase of a quantity of these highly purified compounds for the Committee's collaborative study if they cannot be obtained from any other source. The primary standards, pure *cis-trans,* and *trans-trans* compounds, will be used to establish a procedure based on secondary standards, fatty acids, esters, and glycerides, containing known quantities of these constituents, in a manner similar to the present AOCS method for determination of isolated *trans* isomers (AOCS Cd 14–61).

The Spectroscopy Subcommittee voted at the meetings referred to, in the introduction to this Report, to conduct a collaborative investigation of the back-

TABLE III

GLC Analysis
ME 67-2 *trans* glycerides

| Laboratory | Peak | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2–19:0 |
| 1 | 0.55 | 0.72 | 11.87 | 0.53 | 9.52 | 73.66 | 3.11 |
| 2 | 0.58 | 0.77 | 12.34 | 0.59 | 9.45 | 73.18 | 3.05 |
| 3 | 0.56 | 0.74 | 11.99 | 0.56 | 9.58 | 73.34 | 3.19 |
| 4 | 0.54 | 0.72 | 11.83 | 0.53 | 9.53 | 73.73 | 3.07 |
| 5 | 0.47 | 0.67 | 11.68 | 0.46 | 9.68 | 74.01 | 3.01 |
| 6 | 0.57 | 0.77 | 11.90 | 0.58 | 9.59 | 73.31 | 3.24 |
| 7 | 0.34 | 0.67 | 11.77 | 0.53 | 9.54 | 74.00 | 3.11 |
| 8 | 0.57 | 0.76 | 12.01 | 0.57 | 9.55 | 73.31 | 3.18 |
| 9 | | ..... | ..... | ..... | ..... | ..... | ..... |
| 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 11 | 0.54 | 0.71 | 11.72 | 0.52 | 9.56 | 73.82 | 3.09 |
| Average | 0.52 | 0.72 | 11.90 | 0.54 | 9.55 | 73.59 | 3.11 |
| SD | 0.08 | 0.04 | 0.20 | 0.04 | 0.06 | 0.32 | 0.07 |
| $MeOH:H_2SO_4$ ester | 0.56 | 0.74 | 11.85 | 0.54 | 9.55 | 73.58 | 3.15 |

ground correction to AOCS Cd 14–61 when used for the determination of isolated *trans* isomer content of methyl esters as suggested by Firestone and LaBouliere (2).

### Gas Chromatography Subcommittee

As described in the preceding report (3), this Subcommittee last year completed an extensive collaborative study of a revision of AOCS Tentative Method for the analysis of fatty acid methyl esters by gas chromatography (Ce 1–62). The revision was designed to improve the precision among laboratories using the method mainly by tightening the parameters, some of which were more or less arbitrary, and by requiring analyses based on a reference standard of known methyl esters, in the approximate proportions found in the unknown. Collaborative tests of the revised method revealed a marked increase in precision, the standard deviation being reduced by more than half that obtained when the present AOCS Tentative Method was used.

As of this time last year the Subcommittee had voted to recommend to the entire Committee adoption of the revised procedure as a substitute for the present AOCS Tentative Method Ce 1–62. During the past year the entire Instrumental Techniques Committee voted on this proposal, the final vote was:

| | |
|---|---|
| Members contacted | 42 |
| voting | 40 |
| approve | 25 |
| approve with qualifications | 4 |
| abstain | 11 |
| disapprove | 0 |

Several, but of course not all, of the suggestions of the four approving with qualifications were met involving editorial changes in the newly proposed revision.

On the basis of this vote the revised edition of the method was submitted to the Uniform Methods Committee with the usual data showing the precision from collaborative testing. At a meeting of the Uniform Methods Committee held, during the 58th Annual Meeting in New Orleans, on Tuesday, May 9, 1967, the recommendations of the Instrumental Techniques Committee, that the revised procedure be substituted for the present AOCS Tentative Method Ce 1–62, was accepted. The change will appear in the next publication of the changes in the Society's "Methods of Analysis."

The revised method was tested during the past year in collaboration with the Smalley Check samples. Each member of the Gas Chromatography Subcommittee was invited to analyze by the revised procedure three methyl esters containing polyunsaturated acids and three triglycerides, a cottonseed safflower, and a rapeseed oil which were being submitted to the Smalley Committee. Results obtained from over fifty participating laboratories further support the precision of the revised method. These results are not reported here, as they will presumably be included in a report of the Smalley Committee.

A final recommendation of the Gas Chromatography Subcommittee is contained in a statement from the Subcommittee Chairman: "I firmly believe that better precision than has been attained is possible if everyone would calibrate as carefully when employing GLC as they do when performing most analyses in the laboratory. Methyl ester mixtures are now available commercially that approximate the composition of many oils. Their use is an aid in obtaining greater precision (3) and should also result in greater accuracy."

With the completion of this task the Subcommittee has no definite plans for further collaborative investigations. A proposal that the Subcommittee be inactivated has been held in abeyance pending a study by a special Committee appointed by President Raymond Reiser of the scopes and overlap of scopes of the technical committees of the Society.

### Atomic Absorption Spectroscopy

From correspondence and contacts, interest in the establishment of a method for the quantitative determination of elemental contents of various oils and fats by means of atomic absorption spectroscopy continues to be high. Plans toward such an objective include the following:

a) Working out of a procedure which can be submitted for Subcommittee collaborative testing and possible creation of a method which could be recommended to the Uniform Methods Committee as an AOCS Tentative Standard Method.
b) Establishing a Subcommittee to investigate, by collaborative study, the merits of any procedure submitted to it.
c) Collaborative testing by Atomic Absorption Spectroscopy once such a Subcommittee is created and a procedure is available for it to investigate.

Activities now indicate that a procedure will be available, hopefully by the next Annual Meeting of the Society, which could be given to an Atomic Absorption Spectroscopy Subcommittee for evaluation. Once a subcommittee is established this procedure could be turned over to them for the proper collaborative investigation. Establishment of a subcommittee depends mainly upon not only the amount but the degree of interest in this activity. We are, therefore, repeating the invitation which closed our previous report (3) modified, however, to indicate that we need members interested in working to devise a suitable AOCS Standard Method, not merely in the idea of having one.

*Any members of the Society actively interested in joining in the establishment of an Atomic Absorption Subcommittee of the Instrumental Techniques Committee are urged to contact the Chairman. Only if sufficient interest in actually working with such a new Subcommittee is expressed will the Committee take the necessary steps to create the new Subcommittee.*

R. T. O'Connor, Chairman
R. R. Allen, Subcommittee Chairman
J. R. Chipault, Subcommittee Chairman
S. F. Herb, Subcommittee Chairman
C. W. Hoerr, Subcommittee Chairman

REFERENCES

1. Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka, Anal. Chem. *38*, 514–515 (1966).
2. Firestone, David and Pauline LaBoulier, J. Assoc. Off. Agr. Chem. *48*, 437–443 (1965).
3. O'Connor, R. T., R. R. Allen, J. R. Chipault and S. F. Herb, JAOCS *44*, 215–217 (1967).

### Preparation of Methyl Esters of Long-Chain Fatty Acids Using $BF_3$ Methanol

**Definition**

This method provides a means for preparing methyl esters of long-chain fatty acids for further analysis by methods such as gas-liquid chromatography

(Method Ce 1–62) and infrared spectroscopy (Method Cd 14–61).

**Scope**

The method is applicable to common fats, oils, and fatty acids. (Note 1.) Unsaponifiables are not removed and, if present in large amounts, they may interfere with subsequent analyses.

The procedure may result in partial or complete destruction of the following groups: epoxy, hydroperoxy, cyclopropenyl, cyclopropyl and possibly hydroxyl, and is not suitable for the preparation of methyl esters of fatty acids containing these groups.

A. *Apparatus*

1) 125 ml flat bottom boiling flask or Erlenmeyer flask with ST 24/40 outer neck.
2) Water cooled condenser, Liebig or West design, 30 cm jacket, with ST 24/40 inner joint.
3) 250 ml separatory funnels.
4) 200 ml boiling flask for solvent removal.

B. *Reagents*

1) *$BF_3$—Methanol Reagent* (125 g per liter of methanol). Available commercially or may be prepared using the gas and methanol [(see Section 3 (d) of ASTM Method D-1983-64T, or Anal. Chem. *38*, 514 (1966)].
2) *Sodium Hydroxide*, 0.5 N in methanol.
3) *Sodium Chloride*, saturated solution of NaCl in water.
4) *Petroleum Ether*, redistilled, bp 30–60C.
5) Sodium sulfate, anhydrous, analytical reagent, ACS grade.
6) Methyl red indicator, 0.1% in 60% ethanol.
7) High purity nitrogen gas.

C. *Procedures*

1) *For Fatty Acids*
Weigh one gram of fatty acid into the 125 ml conical flask. Add 10 ml of $BF_3$-methanol reagent and boil for two minutes. Cool. Add 5 ml of petroleum ether and boil for one minute longer. Add enough saturated salt solution to float the methyl esters up into the neck of the flask (about 100 ml salt solution). This petroleum ether solution may then be injected directly in a gas chromatography instrument. To recover dry esters, transfer the cool liquid to a 250 ml separatory funnel. Extract twice with 50 ml portions of redistilled petroleum (bp 30–60C). Wash the combined extracts with 20 ml portions of water until free of acids (test water with methyl red indicator), dry with sodium sulfate, and evaporate the solvent under a stream of nitrogen on a steam bath. (Note 1, Note 2)
2) *For Glycerides*
Weigh one gram of fat in a 125 ml boiling flask; add 10 ml of 0.5 N methanolic sodium hydroxide and add a boiling chip. Attach a condenser and heat the mixture on a steam bath until the fat globules go into solution. This step should take 5–10 minutes. Add 12 ml of $BF_3$-methanol reagent and proceed as described under the fatty acid section.

*NOTE* 1:
There is danger of losing low molecular weight esters if the solvent removal step is prolonged or if too vigorous a stream of nitrogen is used. For infrared spectroscopy, this step should be terminated as soon as all solvent is removed. For gas-liquid chromatography the method may be extended to fatty acids with 8-carbon atoms if the solvent is not completely removed.

*NOTE* 2:
The methyl esters should be analyzed as soon as possible. They may be kept in an atmosphere of nitrogen in a screw cap vial at low temperature for 24 hours. For longer storage they should be sealed in a glass ampoule under vacuum and placed in a freezer.