Report of the Instrumental Techniques Committee AOCS 1964-65[1]

Following its established procedure, the Instrumental Techniques Committee met twice during the past year. A single meeting was held on Tuesday, Oct. 13, 1964, in the Columbian Room of the Pick-Congress Hotel in Chicago, in conjunction with the 38th Fall Meeting of the Society. At the 56th Annual Meeting at the Shamrock-Hilton Hotel in Houston April 25–28, 1965, again following established practice, each Subcommittee held individual meetings, followed by a general meeting of the entire Committee later during this Convention.

At the meeting of the entire Committee, held in the Venetian Room of the Shamrock-Hilton Hotel in Houston on Wednesday, April 28, the Subcommittee Chairman agreed that holding individual meetings of each Subcomittee required attendance at an excessive number of committee meetings during a convention, that several of the discussions had to be repeated at the meeting of the Committee as a whole, and that prior attendance at a series of Subcommittee Meetings was a factor in curtailing attendance at meetings of the entire Committee. It was agreed that a single meeting of the entire Committee would very probably be more efficient. Reports and plans of each Subcommittee would be presented, permitting members of all groups to become informed of the activities of the entire Committee. Elimination of formal subcommittee meetings would simplify the task of resolving meeting conflicts and would reduce the duplication of report presentations. Accordingly, a single meeting of the entire Committee is planned for the next Annual (Spring) Meeting of the Society.

Special Task Group for Preparation of Methyl Esters

This Task Group was established to devise and recommend a method for the preparation of methyl esters from either the free acids or from the triglycerides, which would be rapid, reliable, simple, and widely applicable. The method was to be designed especially for the preparation of methyl esters as required for analyses be either infrared spectrophotometry or by gas chromatography.

As a result of collaborative testing previously reported (1) this special group has drafted a procedure for the preparation of methyl esters of long-chain fatty acids which has been submitted to the entire committee for approval. Results of a letter ballot from the 31-member committee were: approved, 16; approved with qualifications, 5; disapproved, 2; abstained from voting, 5; and no reply to ballot, 3. In accordance with this letter ballot, the method has been submitted to the Uniform Methods Committee with recommendations for its adoption as a tentative method of the Society. The recommended procedure is attached to and made a part of this report.

Gas Chromatography Subcommittee

E. M. Sallee has resigned as Chairman of this Subcommittee to devote his activities of the Society more intensively to the Uniform Methods Committee and the publication and editing of the Society's tentative and official methods. He has been replaced, as Subcommittee Chairman, by S. F. Herb. The new Chairman presented a revision of the present AOCS Tentative Method Ce 1-62 on gas chromatography of fatty acid methyl esters. Certain instructions are made more specific in the expectation of reducing discrepancies in results reported from various laboratories analyzing identical samples. Definite arrangements were announced by the Chairman for collaborative study of the procedure described in the revised draft. Included are tests to determine if the proposed revisions will have the desired effect on precision and tests to determine whether the procedure should be extended to detectors other than the thermal conductivity type. If the anticipated improvement in precision is realized, the revised procedure, attached to and made a part of this report, will be submitted to the Uniform Methods Committee for consideration as a revision of the present AOCS Tentative Method Ce 1-62.

Spectroscopy Subcommittee

The Spectroscopy Subcommittee has been considering the reported small positive errors in the determination of isolated *trans* isomers according to AOCS Official Method Cd 14-61, caused, apparently, by small absorption of saturated triglycerides at 10.3μ and negative errors reported in the analysis of methyl esters by this method. As these errors are in opposite directions, a triglyceride analyzed without modification against triglyceride standards and then reanalyzed after conversion to methyl esters against methyl ester standards will result in appreciably different values. A study of the AOCS Method Cd 14-61 by the Association of Official Agricultural Chemists indicates that more accurate results could be obtained if a correction were applied. The Subcommittee will investigate the improvement in accuracy of analyses with the use of an appropriate correction factor, by means of collaborative effort.

The Spectroscopy Subcommittee has determined that there is a real need to develop a method for the determination of *trans-trans* and *cis-trans* isomers in conjugated double bond systems. Methods for these determinations have been proposed, but standards are not available for their use in most laboratories. The Subcommittee will attempt to establish secondary standards for these analyses, which can be made readily available as are standards for the determination of isolated *trans* isomer content by AOCS Method Cd 14-61. However, progress on the proposed collaborative work required to establish such secondary standards has been delayed as the Subcommittee has not been able to obtain samples of pure fatty acids, esters and triglycerides with the required conjugated double bond systems for use as primary standards. Attempts to obtain the required standards will be continued. If any member has information as to a possible source, he can advance the Committee work of the Society by furnishing such information to the Spectroscopy Subcommittee Chairman or to the Chairman of the Instrumental Techniques Committee. Plans whereby the required collaborative testing can be made once the required primary standards are made available, have been considered by the Subcommittee.

X-Ray Diffraction Subcommittee

This new Subcommittee held its inaugural meeting in the Nile B Room of the Shamrock-Hilton Hotel in Houston on Tuesday, April 27, in conjunction with the Society's 56th Annual Meeting. Chairman C. W. Hoerr has established a Subcommittee which includes several individuals who have established national and international reputations in the field of applications of x-ray diffraction to lipid chemistry and structure. The 13-member Subcommittee roster contains the names of 3 members from outside the United States.

One of the early objectives of this new Subcommittee is to establish some degree of coordination among the x-ray data published in the literature. Of particular concern to the Subcommittee will be the problem of increased uniformity in the matter of characterizing and naming various crystal forms of the glycerides.

Mr. Hoerr has announced that one of the first efforts of the Subcommittee might be to resolve the chief causes of discrepancy among various investigators, which arises 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.

[1] Report of collaborative work of numerous groups from Government, Industrial, and Academic Laboratories reported by members of the United States Department of Agriculture, Agricultural Research Service, Southern Utilization Research and Development Division; Eastern Utilization Research and Development Division; Anderson, Clayton and Co., Food Division; The Hormel Institute; University of Minnesota; and The Glidden Company, Durkee Famous Foods Division.

It has been known for a long time that the presence of impurities can exert a profound influence on polymorphic transformations. Attempts will be made to establish collaborative data on highly purified glycerides of known structure as soon as sufficiently adequate samples of such highly purified glycerides can be made available. Other plans for the Subcommittee include an attempt to become thoroughly familiar with the existing literature concerned with the application of x-ray diffraction to lipids, to more clearly delineate the problems and suggest paths toward their solution.

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

REFERENCE

1. O'Connor, R. T., E. M. Sallee, R. R. Allen, W. T. Coleman and J. R. Chipault, JAOCS *42* 347–351 (1965).

Preparation of Methyl Esters of Long-Chain Fatty Acids

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 containing no fatty acids with less than 12 carbon atoms (Note 1). Unsaponifiables are not removed and, if present in large amounts, they may interfere with subsequent analyses.

The procedure will 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. Methanol, absolute, analytical reagent, ACS grade.
2. Benzene, analytical reagent, ACS grade.
3. Sulfuric acid, sp gr 1.84, analytical reagent, ACS grade.
4. Petroleum ether, bp 30–60C, AOCS specifications, redistilled.
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. Prepare a sulfuric acid solution in methanol by adding cautiously, 2.0 ml of concentrated sulfuric acid to 230 ml of anhydrous methanol.

   Weigh 2 g of fatty acids in a 125 ml boiling flask and dissolve in 60 ml of the methanolic sulfuric acid solution. Attach the condenser to the flask and reflux for 1 hr. Cool, transfer to a 250 ml separatory funnel and add 100 ml of water. Extract twice with 50 ml portions of redistilled petroleum ether (bp 30–60C). Wash the combined extracts with 20 ml portions of water until free of acids (test the wash water with methyl red indicator), dry with sodium sulfate, and evaporate the solvent under a stream of nitrogen on the steam bath (Note 1, Note 2).
2. For Glycerides. Prepare the esterification reagent by adding, cautiously, 2.0 ml of concentrated sulfuric acid to 230 ml of a mixture consisting of 3 volumes of methanol and 1 volume of benzene.

   Weigh 1 g of fat in a 125 ml boiling flask and dissolve in 60 ml of the esterification reagent. Attach a condenser to the flask and reflux for 2.5 hr. Cool and proceed with the extraction and isolation of the methyl esters as described for the fatty acids.

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 hr. For longer storage they should be sealed in a glass ampule under vacuum and placed in a freezer.

AOCS Tentative Method Ce 1-62, Revised 7-27-65

Fatty Acid Composition by Gas Chromatography

Definition

Methyl esters of fatty acids are separated and determined by gas chromatography.

Scope

The method is applicable to animal and vegetable oils, methyl esters, and fatty acids having 8–24 carbon atoms. Saturated and unsaturated acids are determined separately. The conditions specified in this method are not suitable for determining epoxy or oxidized fatty acids.

A. Apparatus
1. The gas chromatographic instrument should have the following characteristics. Several makes are commercially available.
   (a) Sample inlet port about 50C higher temperature than column temperature.
   (b) Column—4–10 ft ¼ in. O.D. glass, stainless steel, aluminum, or copper, packed with 20% polyester (polydiethylene glycol succinate is recommended) on 60–80 mesh acid washed Chromosorb P or W and operated at a constant temperature between 190 and 210C.
   (c) Detector of the thermal conductivity type and if separately thermostated maintained at column temperature or up to 25C hotter.
   (d) Recorder—0–1 mv range, 1 second full scale deflection with a chart speed of ½ in. per minute. Attenuator switch to change the recorder range.
   (e) Helium carrier gas.
2. Syringe for injecting sample. Hypodermic syringe with 10 μl capacity. A Hamilton fixed needle syringe is satisfactory.

B. Procedure
1. With helium gas flowing through the apparatus adjust to operating temperature and record a base line to check for stability of the instrument. A new column should be conditioned by holding at operating temperature with helium flowing for 24 hr.
2. Proper gas flow rate will permit elution of methyl linolenate and shorter chain esters in at least 30 min. The inlet pressure and gas flow necessary to accomplish this will vary between columns and instruments used but will be relatively constant for a single apparatus. The inlet gas pressure should not exceed 40 lb per square inch. A constant gas flow should be maintained throughout the analysis. The gas flow should be measured with a soap bubble flow meter or other device.
3. Measure the methyl ester sample (0.5–4 μl) in the syringe. Pierce the septum of the sample inlet port and quickly discharge the sample. Withdraw the needle and note on the recorder chart the small peak

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due to air which marks the sample introduction reference point. Sample size must be adjusted so that the major peak is not attenuated more than eight times, preferably less.

4. Watch the recorder pen to see that peaks do not go off scale. Change the setting of the attenuator as necessary to keep the peaks on the chart paper. Mark setting on the chart.
5. After all the peaks have been traced and the pen has returned to base line remove the chart for calculation.

C. Calculations

1. Identify the peaks by relative position on the chart. The esters appear on the chromatogram in order of increasing number of carbon atoms and of increasing unsaturation for the same number of carbon atoms. That is, C_{16} is ahead of C_{18} and the C_{18} esters appear in the order stearate, oleate, linoleate, and linolenate. The C_{20} saturated (arachidic) ester usually appears after $C_{18:3}$ (linolenic) ester but may be reversed on some columns, or the positions may change with column usage. With constant operating conditions, the retention times (or chart distances) from the air peak to various sample component peaks can be used for identification of the peaks. However, relative retentions are more reproducible. Relative retentions are determined by dividing the observed retention time for each peak by the retention time observed for the peak of methyl palmitate (or other peak if some other basis is desired). Compare the observed retention times or relative retention times with those calculated from known mixtures run periodically on the same column under the same conditions.
2. Determine the area of each peak. If the instrument is equipped with an electro-mechanical or electronic integrator, the area is best measured by following the manufacturer's instructions. Otherwise, the area is obtained by drawing lines tangent to the sides of the peak and intersecting the base line.

   Determine the area of the resulting triangle by multiplying the height by one-half the base. For an automatically attenuated peak the outer sides of the peak must be at least ⅔ full chart span and the tangents drawn intersecting the base line obtain the peak width. Determine the area by multiplying the height (corrected for attenuation) by one-half the base. Divide the area of each component by its calibration factor. The percentage of each component is calculated from the ratio of each area to the sum of the areas under all of the component peaks and reported as per cent by weight.
3. Calibration factors should be determined relative to methyl palmitate to correct for nonlinearity of instrument response and for molecular weight differences. Such factors may be determined by analyzing known mixtures preferably having composition similar to that of the unknown sample. Divide the area of each peak by the true weight percentage of that component; then by dividing each value by the value for methyl palmitate the calibration factors are obtained.
4. Instrument and column performance are monitored by noting the separation of the oleate and stearate ester peaks which is expressed as peak resolution.

$$\text{Peak Resolution} = \frac{2Y}{S + O}$$

where: Y is the distance between the peak maxima and oleate esters
S is the base width of the stearate peak
O is the base width of the oleate peak

These values should be determined on a sample containing approximately equal quantities of oleate and stearate esters using a sample size such that the hieght of these peaks are 25–50% of the chart width. If the Peak Resolution is equal to or greater than 1.0, the column and instrument are in satisfactory condition. All columns when used will show a gradual loss in peak resolution; when the value becomes less than 1.0, a new column should be installed.

D. Precision

1. Two single determinations of major components (>5%) performed in one laboratory shall not differ by more than 2.2%.
2. Two single determinations performed in different laboratories shall not differ by more than 6.1%.

[Received October 8, 1965—Accepted October 26, 1965]

• *New Products*

APPLIED SCIENCE LABORATORIES, INC., State College, Pa., announces the availability of the Di-Sil-Prep reagent. This mixture of tetramethyldisilazane, dimethylchlorosilane and pyridine has been found to be useful for the preparation of dimethylsilyl ether.

STEIN, HALL & CO., INC., has developed a new multi-purpose dextrine adhesive, Cart-N-Seel A-3911 V9, that can be used economically and efficiently in most industrial plant carton and case sealing operations. It is an accepted food packaging adhesive.

EDMUND SCIENTIFIC CO., Barrington, N. J., is offering a new, white foam expanded polystyrene tray, precision-moulded in one piece, as an attractive answer to manufacturing and industrial storage problems involving small parts. The trays come in two types, which fit interchangeably.

LABCONCO, Kansas City, Mo., has developed a new radioisotope glove box, made of corrosion-resistant, chemically inert fiberglass. With no gaskets or corners, it is easy to wash down and decontaminate.

ULTRA-VIOLET PRODUCTS, INC., San Gabriel, Calif., has a new Transilluminator, capable of achieving greater resolution and stronger contrast of both paper and thin-layer chromatograms.

• *New Literature*

TECHNICON CHROMATOGRAPHY CORP., Chauncey, New York, has available the Technicon Integrator Calculator (TIC), that analyzes any chromatogram from any liquid or gas system. Reading out directly in concentration terms, the instrument integrates at the rate of 30 seconds or less per peak.

WILL SCIENTIFIC, INC., Rochester, New York, has designed the Gyratherm II, a combination magnetic stirrer and hot plate, with each function available separately or in unison.

PENNSALT CHEMICALS CORPORATION, Philadelphia, Pa., has developed a water-powered proportioning pump, which will enable users of hypochlorite solutions and other chemicals, disinfectants and liquid fertilizers to dispense with these materials with a pump that can be attached to a garden hose or directly to a water line.