Comparison of Methods for the Quantitative Determination of Tall Oil Fatty Acids by Gas Chromatography[1]

R. B. IDEN and E. J. KAHLER, Battelle Memorial Institute, Columbus, Ohio

A gas-chromatography procedure, using methyl margarate as an internal standard, has been applied to the quantitative determination of unsaturated tall oil fatty acids. Such an internal standard is necessary for accurate analyses, especially if components of the unknown are present which are not sufficiently volatile to be measured in the chromatograph. The acids are converted to methyl esters before being chromatographed. The preparation of reliable standards, methods of esterification, accuracy obtained on prepared standards using both thermal conductivity and beta-ray ionization detectors, and analyses of tall oil samples, are discussed.

THIS PAPER summarizes the results of work done to apply the internal standard method of analysis to tall oil fatty acids.

Most of the quantitative work reported in the literature is based on the total-area method of analysis, which assumes that all material in the original sample appears in the chromatogram. Hornstein, Alford, Elliott, and Crowe (1) added an internal standard to a meat fat before extraction of the free fatty acids, and carried this standard through the entire procedure. Gehke, Goerlitz, Richardson, and Johnson (2) used the internal-standard method to determine the fatty acid content of cow's milk, and verified the quantitative recovery of fatty acid esters by using standards which contained saturated methyl esters and methyl oleate.

Standards

Since the major components in tall oil fatty acids are oleic and linoleic acids, standard mixtures containing stearic, oleic, and linoleic acids were prepared and esterified. Methyl margarate was then added as the internal standard because its retention time is near that of the major components of tall oil. The concentration of each component of the standard was selected to be approximately the same as the corresponding component in tall oil fatty acids.

Because of the tendency for oxidative degradation of unsaturated fatty acids and their esters, the internal-standard method of analysis to determine the purity of components used in standards is imperative. Extreme precautions were used to prevent oxidation of these materials during preparation of standards, and of samples from tall oil acids. Standards and samples were refrigerated and stored under an inert gas. A stream of inert gas was directed over the materials during esterification at all times except during weighings.

A sample of methyl linoleate, which showed a purity of 98% by the internal-standard method, was stored under refrigeration and an inert gas for approximately one month. After storage, the total-area method of analysis indicated a purity of 97%, but the internal-standard method showed a purity of only 75%.

Each compound used in the standards was itself chromatographed by the internal-standard method; not only was the purity determined, but also the concentration of any impurity that would contribute to the chromatographic peak of another component of the standard. The concentrations of the components of the standard mixtures were then calculated, taking into account the contributions made by the impurities in the various compounds.

Peak areas were measured by the triangulation method. A significant source of error was introduced due to the inaccuracy of peak width measurements until a precision optical measuring instrument was used. Measurements were accomplished through a combination of a high-precision, 6 power magnifying lens equipped with a 0.5 in linear rule with 0.005 in graduations engraved on the lens.

Esterification

Three methods of esterification were evaluated: diazomethane, methanol-hydrochloric acid, and the boron trifluoride-methanol method of Metcalf and Schmitz (3).

The methanol-HCl method gave results comparable with the diazomethane method but required more time than the other two methods.

Results comparing the diazomethane method and the boron trifluoride-methanol method are shown in Table I. In this table, and in all subsequent tables, all percentages reported are weight %. The two methods show good agreement when the prescribed heating period of 2 min is used. Elimination of the heating period produced low results because of incomplete esterification. The boron trifluoride-methanol reagent is easily prepared and eliminates the hazards involved with diazomethane.

Correction Factors

A hot-wire filament detector may have a different sensitivity for each methyl ester because of individual compound differences in thermal conductivity; therefore, correction factors were obtained and applied to other standards and samples.

The sensitivities of methyl stearate, oleate, and linoleate to the thermal-conductivity detector were related to methyl margarate, the internal standard, which was arbitrarily assigned a correction factor of one. The correction factor values increased linearly with the elution time. The exception was methyl stearate, which gave a lower correction factor than would be expected.

By plotting the correction factors for methyl margarate, oleate, and linoleate versus retention time, extrapolations were made to obtain correction fac-

TABLE I

Comparison of Diazomethane and Boron Trifluoride-Methanol Methods of Esterification

Methyl ester	Diazomethane	Boron trifluoride-methanol	
		Heated	Not heated
Stearate	2.4	2.2	2.0
Oleate	46.6	47.2	41.1
Unknown	0.4	0.5	0.6
Linoleate	32.3	33.5	29.8
Unknown	1.3	1.3	1.1
Linoleate	1.6	1.5	1.4

[1] Presented at the 52nd Annual Meeting, American Oil Chemists' Society, St. Louis, Missouri, April 30–May 3, 1961.

tors for the small amounts of unidentified compounds and methyl linolenate which are eluted after methyl linoleate.

Correction factors for the hot-wire filament instruments were reproducible over a period of several months and also reproducible on the two different makes of instruments with a precision of approximately 1% relative. Correction factors used for the sample analyses are shown in Table IV.

Correction factors were not necessary for the beta-ray ionization detector because any differences in sensitivity of the methyl esters to this type of detector were less than the reproducibility of the method.

Analyses of Standards Using Hot-Wire Filament Detector

Analyses of a standard mixture using an Aerograph (Wilkins Instrument & Research, Inc.) and a Chromacon (Podbielniak, Inc.) are shown in Table II. Both of these instruments were equipped with thermal-conductivity detectors. Instrument conditions were as follows:

Column	12 ft x ¼ in, 15% ethylene glycol succinate on 60–80 mesh firebrick.
Temperatures	Column 192C, injector 240C, exit 225C
Filament current	220 ma
Sample size	0.5 to 0.8 μl

In Table II and all subsequent tables, the maximum relative deviations were calculated by (1) finding the largest weight % differences between actual and analyzed contents for each of the esters and (2) multiplying these differences by 100 and dividing by the actual ester content. The average relative deviation was obtained in the same manner, except that the average of all runs was used in place of only the runs with the maximum deviation.

Analyses in duplicate or triplicate showed an average relative deviation of 1% or less for the major constituents, and 4% for the stearate, which was present in a concentration of 2.5%.

If sample sizes of 1.5 μl or greater were used, the accuracy of results diminished.

Analysis of Standards Using Ionization Detector

A modified Barber-Colman Model 10 instrument was used which was equipped with a strontium 90 beta-ray detector and with a conventional packed column. Instrument conditions were as follows:

Column	10 ft x 3/16 in, 15% ethylene glycol succinate on 60–80 mesh firebrick
Temperature	Column 193C, injector 260C, cell 215C
Column pressure	40 lb (argon flow 60 ml/min)
Scavenger pressure	12 lb (argon flow 60 ml/min)
Gain	30
Volts	750
Balance current	16
Sample size	0.09 to 0.11 μl

Table III shows results obtained on a standard analyzed a number of times over a period of 7 days. The % average deviation is in the same range as found with instruments equipped with the thermal-conductivity detectors. The accuracy of results diminished if sample sizes of 0.2 μl or greater were used.

TABLE II

Analysis of a Standard Using Instruments Equipped with Hot-Wire Detectors

Methyl ester	Actual composition, %	Composition by analysis, %						
		Aerograph			Chromacon			
		Run 1	Run 2	Avg.	Run 1	Run 2	Run 3	Avg.
Stearate	2.5	2.3	2.6	2.4	2.3	2.3	2.6	2.4
Oleate	52.4	52.9	51.8	52.3	53.0	52.3	53.4	52.9
Linoleate	45.1	46.0	44.9	45.4	45.5	44.6	46.4	45.5

Accuracy

Methyl ester	Aerograph		Chromacon	
	Max. rel. dev., %	Avg. rel. dev., %	Max. rel. dev., %	Avg. rel. dev., %
Stearate	8.0	4.0	8.0	4.0
Oleate	1.1	0.2	1.9	1.0
Linoleate	2.0	0.6	2.8	0.9

TABLE III

Analysis of a Standard Using a Modified Barber-Colman Model 10

Date	Sample size, μl	Methyl ester, %		
		Stearate	Oleate	Linoleate
Dec. 16	0.08	5.2	55.2	37.8
Dec. 16	0.10	5.0	57.2	39.6
Dec. 19	0.09	5.8	57.7	39.7
Dec. 20	0.11	5.1	57.5	39.7
Dec. 23	0.11	5.1	56.8	37.4
Average		5.2	56.9	38.8
Actual composition		5.4	56.3	38.3
% Avg. deviation		3.7	1.1	1.3

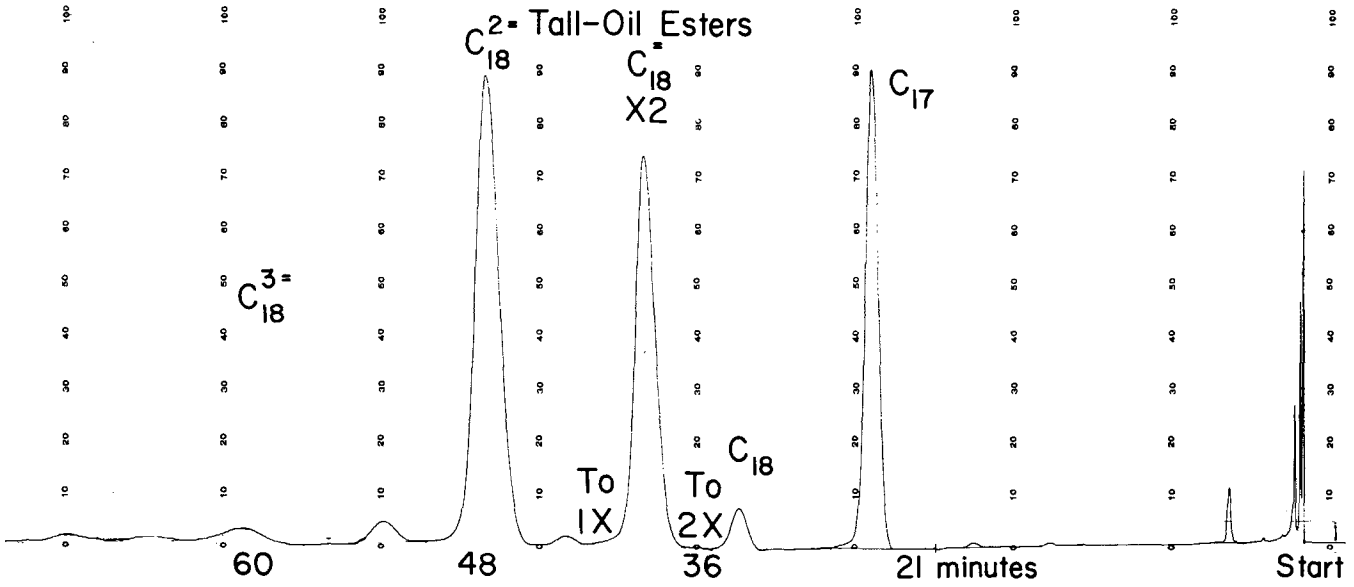

FIG. 1. Gas chromatographic analysis of tall oil fatty acid esters with methyl margarate added as an internal standard.

Analyses of Samples

A chromatogram of tall oil fatty acid esters using a hot-wire filament instrument is shown in Fig. 1. The complete separation of the stearate from the oleate peak should be noted, as well as the appearance of an unknown peak between oleate and linoleate. As columns of ethylene glycol succinate age, this excellent degree of separation is lost. However, continuous operation of such columns at 190C has been found satisfactory for about 1 mo.

Certainly, a more stable column material with the resolution of EGS, would be very helpful for the analysis of fatty acid methyl esters. The peaks which are not identified in the chromatogram are compounds for which positive identifications have not been made. Preliminary results from identification work indicate that all peaks with retention times longer than for methyl stearate are probably isomeric C_{18} compounds. Many samples of tall oil fatty acids contain a third unknown compound eluted after the two unknowns past methyl linolenate.

Analyses of the same tall oil fatty acid samples using both the hot-wire filament detector and the beta-ray ionization detector are shown in Table IV. The sample was esterified and then 31.3 parts of methyl margarate per 100 parts of sample were added. The instrument conditions used were the same as those used for the standards.

The total fatty acid content by the internal-standard method was 98.9 weight % for the hot-wire filament detector and 98.1 weight % for the beta-ray ionization detector. The relative deviations for the major components, methyl oleate and methyl linoleate, were 2.9% and 3.6%, respectively, based on the Aerograph results. The major difference in the analyses appeared in the methyl stearate results.

A number of tall oil samples from various sources was analyzed, and the total fatty acid contents ranged as low as 85 weight %. The use of the total-area method for samples with a low content of fatty acids leads to large errors. This is particularly true if the unsaturated acids have undergone any degree of oxidation.

TABLE IV

Gas Chromatographic Analyses of Tall Oil Fatty Acids
Comparison of Hot-Wire Filament and β-Ray Ionization Detector

Methyl ester	Hot-wire detector			Ionization detector		
	Correction factor	Retention time, min	Weight, %	Correction factor	Retention time, min	Weight, %
Margarate[a]	1.000	24.8	(31.3)	1.000	21.4	(31.3)
Stearate	1.001	32.2	2.6	1.000	28.9	1.6
Oleate	1.011	37.7	53.7	1.000	32.7	52.1
Unknown	1.016	42.0	0.7	1.000	35.7	0.7
Linoleate	1.020	46.7	37.1	1.000	39.8	38.5
Unknown	1.025	52.5	1.6	1.000	43.9	1.5
Linolenate*	1.032	60.5	1.8	1.000	51.8	2.1
Unknown	1.037	65.7	0.2	1.000	56.1	0.6
Unknown	1.042	70.5	1.2	1.000	60.3	0.9
Unknown	1.055	85.0	Trace	1.000	76.2	0.1
Total			98.9			98.1

[a] Internal standard.

* NOTE: Subsequent work on the peak material identified as methyl linolenate has been done. Sufficient material was obtained from a preparative gas chromatographic unit to permit infrared, ultraviolet, and nuclear magnetic resonance studies. This material is now identified as a conjugated, *cis-trans*-methyl linoleate. The position of the unsaturation has not been determined.

Discussion of Results

The rapid oxidation of unsaturated fatty acids and their esters makes their quantitative analysis difficult, regardless of the analytical method. The internal-standard method of analysis has the following advantages: (a) it is not necessary for all compounds in the sample to appear in the chromatogram to obtain reliable results, and (b) if unsaturated samples have oxidized, the analysis will indicate this by giving low results.

Based on analyses of the standard mixtures of fatty acids, the accuracy for oleic and linoleic acids, the major components of tall oil, was better than 2% relative on duplicate determinations. The accuracy for compounds present in low concentration was 4% relative, based on the stearic acid content of the standards.

The accuracy of the sample analysis is no doubt less than the accuracy obtained for standards, since the samples contain several unknown compounds in low concentration.

The relative accuracy for the hot-wire filament detector and beta-ray detector was the same. The beta-ray detector had the advantage of not requiring correction factors.

Acknowledgment

The authors wish to thank the Union Bag-Camp Paper Corporation for permission to publish this paper.

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[Received June 16, 1961]

Alkyd Resins Modified with Cyclic Fatty Acids. A Preliminary Evaluation[1]

W. R. MILLER, H. M. TEETER, A. W. SCHWAB, and J. C. COWAN, Northern Regional Research Laboratory,[2] Peoria, Illinois

Alkyd resins were modified to 50% oil length with crude, flash-distilled, and 78% pure cyclic fatty acids. These resins were compared with ones modified with naturally occurring fatty acids and with vegetable oils. Those modified with the cyclic acids process more rapidly than those prepared with linseed, safflower, or soybean fatty acids, and they also have good nonyellowing properties. Resins modified with 78% pure cyclic acids show definite improvement in drying time, hardness, and chemical resistance in air-dried films, and an almost equal improvement in baked films, over resins obtained with the other modifiers. Distilled cyclic acids also improve alkyd resins although not to the extent that pure acids do. Both give resins superior to commercial oil-modified resins under the test conditions. Resins with crude cyclic acids are as good in air-dried films as are the others, but are poorer in baked films.

[1] Presented at the American Chemical Society Meeting, St. Louis, Missouri, March 21–30, 1961.

[2] A laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, USDA.