Methods for the Determination of Cyclopropenoid Fatty Acids. VII. The Dilution-HBr-Titration Technique as a General Method[1]

FRANK C. MAGNE, JAMES A. HARRIS, ROBERT A. PITTMAN and EVALD L. SKAU,
Southern Regional Research Laboratory,[2] New Orleans, Louisiana

Abstract

The stepwise HBr titration method for the cyclopropenoid analysis of cottonseed oils is subject to serious inaccuracies when applied to samples containing higher cyclopropenoid concentrations, particularly if they contain appreciable amounts of alumina-adsorbable materials. A modification of the method is described which eliminates these sources of error. Its validity has been established by the analysis of a wide range of synthetic compositions including compositions containing massive amounts of interfering HBr-reactive substances and other alumina-adsorbable materials. The method with further modification can be used to analyze glycerides with the same high degree of accuracy.

Introduction

THE STEPWISE HBr titration method (1) for the determination of cyclopropenoid fatty acids in cottonseed oil is based upon the following facts and principles: (a) Cyclopropenoids give no titration at 3C. (b) They can be titrated quantitatively at 55C. (c) All oils or fatty methyl esters, whether they contain cyclopropenoids or not, usually contain interfering substances which give a small HBr titration at 3C and a further titration at 55C. (d) These interfering substances can be removed by selective adsorption on activated alumina. (e) If a sample gives a HBr titration at 3C it can be assumed that it contains interfering substances contributing to the titration at 55C.

Thus, the procedure for cottonseed oils, or the methyl esters of cottonseed oil fatty acids, involves pretreatment with activated alumina followed by titration with standard HBr in glacial acetic acid, first at 3C and then at 55C. If the 3C titration is negligible the titration at 55C is equivalent to the cyclopropenoid present. The precision of these determinations is approximately $\pm 0.01\%$, i.e., one part in 10,000 parts of sample, when a 7-g sample is titrated.

The validity of this method is based upon the assumption that the cyclopropenoid concentration is not changed appreciably by the alumina treatment. This assumption is valid only when the percentage of cyclopropenoids and of interfering substances is small, as in cottonseed oils. The method is subject to serious inaccuracy, however, when applied to samples containing high concentrations of alumina-adsorbable materials.

Interfering substances fall in two categories: (a) epoxides, peroxides, hydroperoxides, and α,β-conjugated dienols, all known to be HBr-reactive (2,3), which were found by preliminary experiments to be completely removed by the alumina treatment, and (b) non-HBr-reactive substances such as free fatty acids, oxygenated fatty esters, monoglycerides, and diglycerides, which may be completely or only partially removed. The cyclopropenoid content of samples containing high concentrations of such substances will obviously be markedly increased by the alumina treatment.

The present report deals with the development of a modification of the HBr titration procedure which can be used for the accurate determination of the cyclopropenoid content of virtually any methyl ester mixture regardless of the percentage of cyclopropenoid or alumina-adsorbable components. It involves the stepwise titration of the methyl ester sample at 3C and 55C after it has been diluted with a known amount of methyl oleate and subjected to the alumina pretreatment. The method can be used to analyze glycerides with the same accuracy but they must first be converted to methyl esters.

Experimental

Materials

Sterculia foetida oil was the chief source of cyclopropenoid component used in making up synthetic mixtures of known concentrations. It was prepared as previously described (4) by the hexane extraction of the fresh meats of *S. foetida* seeds. *Hibiscus syriacus, Lavatera trimestris* and *Dimorphotheca sinuata* seed oils were prepared similarly by extraction of the whole seed. The oils were converted to methyl esters by methanolysis. One part by weight of oil is added to 3 parts of absolute methanol in which 0.003 parts of metallic sodium in excess of the free fatty acid equivalent of the glyceride has been dissolved and the mixture is refluxed for 30 min beyond the attainment of homogeneity or at most a total of 45 min. The cooled mixture is stirred into a slight excess of aqueous acetic acid and the esters are taken up in petroleum ether, water-washed, dried over anhydrous sodium sulfate and filtered, and stripped free of solvent on a rotary evaporator below 60C under reduced pressure with a small nitrogen sweep. The epoxy ester used was a methyl ester prepared by the methanolysis of a commercial epoxidized linseed oil.

Two grades of methyl oleate were employed as diluent. One was a highly pure sample prepared from the methyl esters of olive oil fatty acids by fractional distillation through a Podbielniak column. It was subjected to alumina treatment immediately before use. The other was prepared from a commercial oleic acid, Emersol 233LL, purchased from Emery Industries. One part by weight of the acid was refluxed for 6 hr with one part of absolute methanol and 0.075 parts of 95% sulfuric acid. The product was washed free of mineral acid, taken up in petroleum ether, dried, and freed from alumina-adsorbable components by percolation through a col-

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[2] So. Utiliz. Res. Dev. Div., ARS, USDA.

umn of activated alumina. Either butylated hydroxyanisole (BHA) or hydroquinone, 0.005 to 0.01% based on the esters, was added to the percolate before stripping. The stabilized product gave no appreciable hydrogen bromide titration at 3C or 55C and required no further alumina pretreatment even after standing for two months at room temperature.

Alorco 80–200 mesh, F-20 grade activated alumina from Aluminum Ore Company of America was used as received. The standard 0.1N HBr solution, Durbetaki reagent (2), was prepared from the HBr-acetic acid concentrate, obtainable from Distillation Products Corporation, by diluting with glacial acetic acid. It was standardized daily against standard anhydrous sodium carbonate. All titrations were performed in a closed system as in the Durbetaki oxirane titration using a 10-ml automatic reservoir semimicro burette graduated to 0.05 ml.

Procedures

Alumina Treatment. For a 25-g sample, 100 g of the activated alumina are slowly poured into a chromatograph tube (22 mm inside diameter, 500–600 mm length) containing a plug of absorbent cotton and a sufficient volume of petroleum ether (bp 30–60C) to completely cover the entire charge of alumina. The packing density is that obtained by gravitational settling without tamping or tapping. If a plug forms at the top of the solvent, this can be broken by the use of a thin glass rod without disturbing the lower part of the column. The 25-g sample dissolved in an equal volume of petroleum ether is poured onto the column when the solvent has drained to within about 0.5 cm of the top of the packing. It is allowed to percolate by gravity and eluted by controlled addition of 250 ml of solvent from an automatic siphoning separatory funnel. Although recovery is not complete, more exhaustive elution is unnecessary. The combined percolate is filtered and the solvent removed on a rotary evaporator under reduced pressure at a temperature below 60C. The stripping flask is then cooled and the vacuum broken with nitrogen. Further precaution can be taken against autoxidation by addition of 0.005 to 0.01% of antioxidant to the percolate before removal of the solvent. Proportionally smaller amounts of alumina and solvent are used for smaller samples, maintaining about the same ratio of packing height to column diameter. In general 4 g of alumina are used per gram of sample.

Titration Procedure. Duplicate 0.25 to 7.0 g specimens of the alumina-treated sample, the weight depending upon the cyclopropenoid concentration, are accurately weighed into 50-ml Erlenmeyer flasks and immediately dissolved in 5 ml of benzene and 15 ml of glacial acetic acid. If a series of samples is weighed, addition of the acetic acid is delayed and the flask is stoppered until ready for titration. After adding 4 to 5 drops of the crystal violet indicator (0.1% in glacial acetic acid) and a stirring bar the flask is attached to the titration burette, the magnetic stirrer started, and the solution cooled to 3C by a surrounding ice-water bath. It is then titrated with standard HBr reagent to a blue-green end point of at least 30 seconds' duration. Duplicability of the end point is improved by use of a fluorescent titration illuminator. The ice bath is then replaced with a water bath maintained at 55C by a thermostatically controlled hot-plate equipped for magnetic stirring and the solution is titrated again to the same blue-green end point.

If the titration value at 3C, corrected for the 3C solvent blank, is negligible (one drop or less) the titration value at 55C, corrected for the 55C solvent blank, is equivalent to the cyclopropenoid moiety. If not, the sample should be reanalyzed using a larger portion of alumina per gram of sample in the alumina pretreatment.

General Dilution-HBr-Titration Procedure. Appropriate amounts of the methyl ester sample to be analyzed and methyl oleate, depending upon the degree of dilution required, are accurately weighed into a small Erlenmeyer flask. The mixture as a whole, preferably about 25 g, containing a maximum of 4% of cyclopropenoids calculated as sterculic acid, is subjected to the alumina treatment. After removal of the solvent accurately weighed 6–7 g specimens are titrated in duplicate at 3C and 55C. The observed cyclopropenoid concentration is then multiplied by the appropriate dilution factor to determine the cyclopropenoid content of the original sample. For higher cyclopropenoid concentrations proportionately smaller samples are required for alumina treatment and titration.

Theory of the Dilution Method

Consider a sample of methyl esters for which

x = per cent of cyclopropenoids
y = per cent of alumina-adsorbable components.

After dilution to D times its weight with methyl oleate the mixture will have the following composition:

x/D = per cent of cyclopropenoids
y/D = per cent of alumina-adsorbable components.

The percentage of cyclopropenoids, $(x/D)_{obs}$, determined with a precision of 0.01% by HBr titration after alumina treatment (based on the titration of a 7-g sample) will be

$$(x/D)_{obs} = 100\left(\frac{x/D}{100 - y/D}\right) \pm 0.01$$

$$= x\left(\frac{100}{100D - y}\right) \pm 0.01$$

Multiplying by the dilution factor, D, to adjust for the dilution:

$$x_{obs} = x\left(\frac{100D}{100D - y}\right) \pm 0.01D \qquad [1]$$

and the deviation, Δ, from the true value, x, will be

$$x_{obs} - x = x\left(\frac{y}{100D - y}\right) \pm 0.01D$$

Thus the inherent error in the percentage of cyclopropenoids as determined by the dilution-HBr-titration technique can be represented by the general relationship:

$$\Delta = x\left(\frac{y}{100D - y}\right) \pm 0.01D \qquad [2]$$

The error, i.e., the increase in cyclopropenoid concentration resulting from the alumina pretreatment, is represented by the term $x\left(\frac{y}{100D - y}\right)$. Its magnitude depends upon both x and y and is decreased markedly by increasing D since the function $\frac{y}{100D - y}$ decreases very sharply as D is increased

(see Fig. 1). For example, if $y = 20\%$ this function decreases from 0.25, when $D = 1$ (i.e., no dilution), to 0.07, 0.04, 0.02, and 0.01 as D is increased to 3, 5, 10, and 20, respectively. The precision error, on the other hand, represented by the term $\pm 0.01D$, shows a very slow linear increase with an increase in D and is relatively insignificant except at high dilutions or when x is very small.

Optimum Degree of Dilution

The first term on the right hand side of Equation [2] will always be positive. The second term may be positive or negative. The optimum dilution factor, D_{opt}, to insure the maximum reliability of the results will be the value of D when $d\Delta/dD = 0$; i.e., when

$$\frac{d}{dD}\left(\frac{xy}{100D - y} + 0.01D\right) = 0$$

That is, $$D_{opt} = \sqrt{xy} + \frac{y}{100} \qquad [3]$$

Selection of Suitable Degree of Dilution

Actually only the order of magnitude of D_{opt}, and consequently only rough estimated values of x and y, need be known to select a suitable degree of dilution for a given sample. For example, if $x = 20\%$ and $y = 20\%$, the values of x_{obs} when $D = 10$. 20, and 30 calculated from Equation [1] would be 20.4 ± 0.10, 20.2 ± 0.20, and $20.12 \pm 0.30\%$, respectively. All these values are comparable to that obtained when D_{opt}, i.e., 20.2 ,is used. Proportionally less leeway is permissible for samples having lower D_{opt} values, especially when x is less than about 2%.

In the absence of any information as to the values of x and y, a reasonably good estimate of the cyclopropenoid content may be obtained, with an (absolute) precision of $\pm 0.15\%$, by using a dilution factor of 15. It is apparent from Figure and Equation [2], for example, that at this degree of dilution the first term of Equation [2] will vary from zero to $0.014x$ as y varies from zero to 20%. Thus, using a dilution factor of 15, if $x = 1\%$ and $y = 1\%$, x_{obs} would be 1.00 ± 0.15; if $x = 1\%$ and $y = 20\%$, x_{obs} would be $1.01 \pm 0.15\%$; if $x = 30\%$ and $y = 1\%$, x_{obs} would be $30.01 \pm 0.15\%$; and if $x = 30\%$ and $y = 20\%$, x_{obs} would be $30.42 \pm 0.15\%$. More accurate and precise analyses can usually be obtained from data at two different degrees of dilution, as will be described later.

Results and Discussion

Stability of S. foetida Oil and Methyl Esters

Before undertaking any investigation on diluted or deliberately adulterated test compositions of the cyclopropenoid standards, it was important to know that no change in cyclopropenoid concentration would occur during the period of investigation as a result of instability of the standard itself. Data were therefore obtained by direct HBr titration, without methyl oleate dilution, on the following samples stored in cork-stoppered vials for various periods at about 4C: *S. foetida* oil without alumina treatment, and the corresponding methyl esters (a) without alumina treatment, (b) after alumina treatment, and (c) after alumina treatment and addition of antioxidant, 0.01% BHA. The results, see Table I, show that *S. foetida* oil, the methyl esters without alumina treatment, and the BHA-stabilized alumina-treated methyl esters are sufficiently stable for use in the preparation of standard mixtures of known cyclopropenoid concentration. It was also found that the oil shows no decrease in cyclopropenoid content during storage for 30 days at room temperature and can therefore be used as an interlaboratory standard.

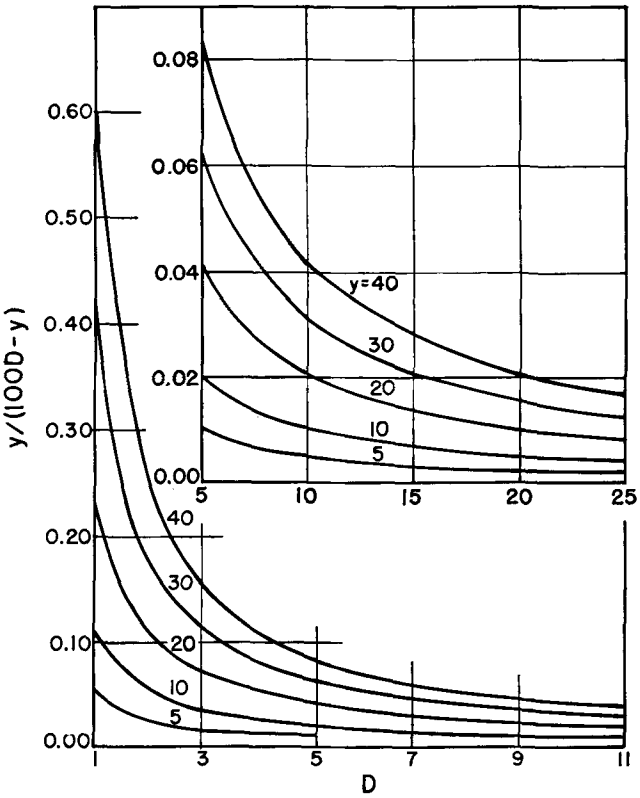

FIG. 1. Relationship between the values of $y/(100D\text{-}y)$ and D for various values of y.

Additional conclusions can be derived from these data: (1) It is apparent that the oil can be converted to methyl esters with no change in cyclopropenoid content. (2) Alumina treatment of the methyl

TABLE 1

Stability Data for Refrigerated Samples of *Sterculia foetida* Oil and Methyl Esters

Days stored	*S. foetida* oil no Al_2O_3		Methyl ester					
			no Al_2O_3		Al_2O_3		Al_2O_3 + BHA	
	3C[a]	55C[b]	3C[a]	55C[b]	3C[a]	55C[b]	3C[a]	55C[b]
0	0.00	53.03	0.00	53.21	0.00	54.16	0.00	54.16
1	.....	.....	0.00	53.26	0.00	53.77	0.00	54.11
2	.....	.....	0.00	53.20	0.00	53.38	0.00	54.13
6	.....	.....	0.00	53.24	0.00	53.14	0.00	54.13
15	.....	.....	0.00	53.39	0.15	50.86	0.00	54.18
52	0.00	52.79	0.00	53.46	1.68	26.21	0.00	54.16
240	0.00	52.87						
350	0.00	53.03						

[a] Noncyclopropenoid-HBr-titratable component expressed as methyl sterculate.
[b] Cyclopropenoid content expressed as methyl sterculate.

esters reduces the stability, probably because of removal of natural antioxidants.

Choice of Diluent

Since the theory of the method is premised upon the use of an ideal, noninterfering, inert and nonvolatile diluent its implementation depended upon finding such a diluent. The principal requirement is that the cyclopropenoid content of mixtures formed by diluting methyl ester samples containing no alumina-adsorbable components must be the same after the alumina treatment as before. Since the recovery from the alumina column is never complete, this means that there must be no selective adsorption of the diluent or the cyclopropenoid ester with respect to each other. A series of methyl esters of fatty acids ranging from capric to linoleic, as well as a few mixtures and a diester, were examined from this point of view.

Quantitative dilutions of an alumina-treated cyclopropenoid methyl ester standard with each diluent were analyzed by duplicate HBr titrations after retreatment with alumina and removal of solvent, and the analyses were compared with the known cyclopropenoid compositions. The values x and x/D, Columns 1 and 2 of Table II, are known cyclopropenoid concentrations of the original and the diluted samples, respectively. The concentrations obtained by stepwise HBr titration of the diluted samples after alumina treatment, $(x/D)_{obs}$, are shown in Column 3. These were multiplied by the appropriate dilution factor, D, to give the observed concentration, x_{obs}, for the original sample as determined with each specific diluent (Column 4). Every diluent evaluated with the exception of methyl oleate and myristate exerted in some degree either an enhancing or a reductive effect upon the cyclopropenoid analysis. When methyl oleate, either pure or prepared from commercial oleic acid (Emersol 233LL), was used as the diluent there was good agreement between the known composition and that ascertained by dilution analysis.

Two aditional analyses were made based upon alumina-treated methyl esters of *Hibiscus syriacus* and *Lavatera trimestris* acids, in which the predominent cyclopropenoid is methyl malvalate instead of methyl sterculate. The results prove that methyl oleate is an equally satisfactory diluent for either of these cyclopropenoids.

TABLE II

Effect of Diluent on Methyl Sterculate Analysis

Methyl ester diluent	% Cyclopropenoid as methyl sterculate				
	Known		Observed		Deviation
	Original x	Diluted (x/D)	Diluted $(x/D)_{obs}$	Original x_{obs}	$\Delta x_{obs}-x$
Capric	53.35	0.699	0.73	55.7	+2.4
Myristic	53.35	0.512	0.51	53.1	−0.3
Palmitic	53.35	0.592	0.54	48.7	−4.6
Palmitic-stearic [a]	26.16 [b]	0.688	0.62	23.6	−2.5
Pure oleic	26.68 [b]	0.523	0.52	26.5	−0.2
Commercial oleic	26.14 [b]	0.895	0.89	26.0	−0.1
Commercial oleic	54.19	0.764	0.76	53.9	−0.3
Commercial oleic	54.19	0.764	0.77	54.6	+0.4
Commercial oleic	21.90 [c]	0.580	0.58	21.9	0.0
Commercial oleic	6.78 [d]	0.435	0.43	6.7	−0.1
Linoleic	54.19	1.026	1.32	69.7	+15.5
Peanut oil acids	26.63 [b]	0.550	0.58	28.2	+1.6
Di-2-ethylhexyl adipate	53.35	1.872	2.27	64.7	+11.4

[a] Eutectic acid mixture.
[b] Epoxidized linseed oil methyl ester-*S. foetida* methyl ester mixture.
[c] Alumina-treated methyl ester mixture prepared from *Hibiscus syriacus* seed oil.
[d] Alumina-treated methyl ester mixture prepared from *Lavatera trimestris* seed oil.

TABLE III

Reproducibility of Analyses of *Sterculia foetida* Methyl Esters by Dilution Method at Two Levels of Dilution

Mixture I ($D = 97.582$)		Mixture II ($D = 25.239$)	
Diluted sample % [a]	Original sample % [a]	Diluted sample % [a]	Original sample % [a]
(0.546) [b]	(53.28) [b]	(2.111) [b]	(53.28) [b]
0.55	53.7	2.12	53.5
0.55	53.7	2.12	53.5
0.54	52.7	2.12	53.5
0.54	52.7	2.13	53.8
0.54	52.7	2.12	53.5
0.53	51.7	2.12	53.5
Mean	(52.87)		(53.55)
Stand. dev.	0.75		0.12

[a] Calculated as methyl sterculate.
[b] Known value based upon direct titration of cyclopropenoid ester standard 0.00% at 3C and 53.28% at 55C.

Reproducibility of Analyses

Table III shows the reproducibility of analyses obtained for a sample of methyl esters of *S. foetida* acids by the dilution technique using two levels of dilution. The diluted mixtures, prepared by mixing accurately weighed amounts of the sample and methyl oleate, had known cyclopropenoid concentrations of 0.546% and 2.111%, respectively, calculated as methyl sterculate. The corresponding dilution factors were 97.582 and 25.239, respectively. Sextuplicate specimens of each of the mixtures were subjected to the alumina treatment and titrated. The standard deviation of the analyses, 0.75 for Mixture I and 0.12 for Mixture II, and the corresponding deviations of the mean from the true values, 0.41 and 0.27%, respectively, are in agreement with the expected precision and accuracy of the method and demonstrate the disadvantage of using too high a dilution.

Analysis of Methyl Ester Samples Containing Alumina-Adsorbable Constituents

The dilution technique was applied to a number of synthetic methyl ester mixtures containing known percentages of cyclopropenoid and HBr-titratable alumina-adsorbable esters (see Table IV). The percentage of methyl sterculate in the *S. foetida* sample (Sample A), which gave no 3C titration, was increased from 53.4 to 54.2% by the alumina treatment. This increase was confirmed by results obtained by the dilution method on the original and the alumina-treated sample, 53.5 and 54.0%, respectively. Substitution of these values in Equation [1] indicates that the original methyl esters contained about 1% of non-HBr-titratable alumina-adsorbable materials.

The sample of epoxidized linseed oil methyl esters (Sample B) gave an HBr titration at 3C equivalent to about 30.4% of epoxyoleic acid and contained no constituents titratable at 55C. The original oil gave a 3C titration corresponding to 34.0% and a zero titration at 55C, showing that methanolysis caused a slight loss of oxirane oxygen.

Sample C was a mixture of equal parts by weight of Samples A and B and therefore was known to contain 26.68% of cyclopropenoid ester. When this sample, without dilution with methyl oleate, was subjected to the alumina column treatment the percolate still contained a small amount of the epoxy ester, as shown by the 3C titration, and had a cyclopropenoid content, x_{obs}, of 31.6%. This is in good agreement with the value predicted by substitution for x and y in Equation [1], i.e., 31.4%. The cy-

TABLE IV

Analysis of Cyclopropenoid Methyl Esters Containing Alumina-Adsorbable Components

Sample	Methyl ester mixture	Known composition[a]		Dilution factor D	Composition (exp.)[a]			Deviation Δ
					Diluted sample after Al_2O_3		Original sample	
					3C	55C		
		y[b]	x[b]		y[a]	$(x/D)_{obs}$	x_{obs}	$x_{obs}-x$
A	*S. foetida* ester	0	53.35	1[c]	0.00	54.2	54.2	+0.8
		0	53.35	25.239[d]	0.00	2.12	53.5	+0.2
A_1	*S. foetida* ester (after Al_2O_3)	0	54.19	71.001[d]	0.00	0.76	54.0	−0.2
B	Epox. linseed ester	30.39	0	1[c]	.....	.....	.....	.....
C	A + B (1:1)	15.19[e]	26.68[e]	1[c]	0.25[f]	31.6	31.6	+4.9
		15.19[e]	26.68[e]	16.855[g]	0.00	1.60	27.0	+0.3
		15.19[e]	26.68[e]	51.125[g]	0.00	0.52	26.6	+0.1
D	A + B (ca. 1:1)	15.57[e]	26.14[e]	29.214[d]	0.00	0.89	26.0	−0.1
E	*D. sinuata* ester + A (ca. 1:1)	[h]	26.95[i]	23.978[d]	0.00	1.12	26.8	−0.2

[a] All compositions expressed as % methyl sterculate.
[b] y = % HBr-titratable noncyclopropenoid components based on 3C titration; x = True % cyclopropenoid ester (55C titration).
[c] Undiluted sample.
[d] Diluent = methyl oleate from commercial acid.
[e] Calculated from A and B.
[f] All epoxy *not removed* by 4 g Al_2O_3 per g sample.
[g] Diluent = pure methyl oleate.
[h] Ca. 30% methyl dimorphecolate.
[i] Calculated from % of A.

clopropenoid analysis of Sample C obtained by the dilution technique using pure methyl oleate and dilution factors of 16.855 and 51.125 showed deviations of only 0.3% and 0.1% from the true value, respectively. Analysis of a similar mixture, Sample D, using the crude methyl oleate prepared from commercial oleic acid showed equally good agreement.

Sample E was a mixture of *S. foetida* methyl esters and the methyl esters of *Dimorphotheca sinuata* oil fatty acids. The latter contained about 60% of methyl dimorphecolate. The dimorphecolic acid moiety consumes HBr at 3C but cannot be titrated accurately because of side reactions (3). It differs from epoxy acid moieties in that its presence prevents the accurate subsequent titration of cyclopropenoids at 55C. The cyclopropenoid content of Sample E determined by the dilution technique was 26.8% compared to the true value, 26.9%.

Analysis of Glycerides

Since the dilution technique is applicable only to methyl esters it cannot be used directly for the cyclopropenoid analysis of glycerides. Glycerides must first be converted to methyl esters. This can be accomplished without loss of cyclopropenoids by the methanolysis procedure used in the preparation of methyl esters from *S. foetida* oil. The methyl esters are then analyzed by the dilution method. The analyses obtained by this procedure for a number of oils or oil mixtures containing considerable amounts of interfering HBr-titratable constituents are shown in Table V.

Samples 1 and 2 were mixtures of about equal parts of *S. foetida* oil with epoxidized linseed oil and *Dimorphotheca sinuata* seed oil, respectively. The *S. foetida* oil was a standard sample which had been given an alumina treatment. The epoxidized linseed oil contained about 34% of epoxy acid moiety calculated as epoxyoleic acid and contained no 55C-titratable constituents. The *Dimorphotheca sinuata* oil contained about 60% of dimorphecolic acid moiety. The analysis of Sample 1 showed excellent agreement with the known cyclopropenoid value. Samples 3 and 4 were *Hibiscus syriacus* and *Lavatera trimestris* seed oil, respectively, both of which are known to contain epoxy moieties (5). Their cyclopropenoid contents, calculated as methyl malvalate, were found to be 19.5 and 5.8% as compared to the literature values, 19.3 and 8.3%, respectively, obtained by hydrogenation followed by gas-liquid chromatographic analysis (5).

TABLE V

Analysis of Glycerides and Glyceride Mixtures Containing Alumina-Adsorbable Components (via Methyl Esters)

Sample No.	Oil or oil mixture	Dilution factor	% Cyclopropenoid[a]		
			Known	Found	Deviation
1	*S. foetida* + epox. linseed[b]	20.137	27.1	27.5	+0.4
2	*S. foetida* + *D. sinuata*[c]	35.653	28.5[d]	29.1	+0.6
3	*Hibiscus syriacus*	20.091		19.5[e]	
		20.091		19.2[e]	
4	*Lavatera trimestris*	9.973		5.8[e]	
		9.973		5.9[e]	

[a] Calculated as methyl sterculate.
[b] Mixture contains 15.7% epoxy moiety calculated as methyl epoxyoleate.
[c] Mixture contains about 30% dimorphecolic moiety calculated as methyl ester.
[d] Assuming *D. sinuata* oil contains no cyclopropenoids.
[e] Calculated as methyl malvalate.

Advantage of Using Two Degrees of Dilution

By making determinations of the cyclopropenoid concentration at two dilutions, D_1 and D_2, one of which may be unity, the systematic (positive) error represented by the term $x\left(\frac{y}{100D-y}\right)$ in Equation [2] can be eliminated. This procedure is particularly useful when y is large.

From Equation [1], neglecting the precision term

$$(x_{obs})_1 = x\left(\frac{100D_1}{100D_1 - y}\right)$$

$$(x_{obs})_2 = x\left(\frac{100D_2}{100D_2 - y}\right)$$

Eliminating y and solving for x,

$$x = \frac{(x_{obs})_1\ (x_{obs})_2\ (D_2 - D_1)}{D_2(x_{obs})_1 - D_1(x_{obs})_2} \qquad [4]$$

Substitution of the experimental values for D_1, D_2 $(x_{obs})_1$ and $(x_{obs})_2$ in Equation [4] gives the correct value for x, regardless of the values of y or D, the precision being dependent upon the dilution factors used.

The values of x calculated by Equation [4] from the data for Sample C in Table I are in good agreement with the known value. Thus, for the combination $D_1 = 1$ and $D_2 = 16.855$, the calculated value of x is 26.75% and for the combination $D_1 = 1$ and $D_2 = 51.125$ it is 26.52%, as compared to the known value, 26.68%.

This procedure is particularly useful for the determination of low concentrations of cyclopropenoids in the presence of high concentrations of alumina-adsorbable materials. For example, consider a sample of methyl esters for which $x = 1.00\%$ and $y = 30\%$. It is apparent from Equation [1] that the value of x_{obs} by the dilution method at the optimum dilution, $D_{opt} = 5.8$, would be $1.05 \pm 0.06\%$. A more accurate and precise analysis would be obtained from the values of x_{obs} at $D = 1$ and $D = 2$, which by Equation [1] would be 1.43 ± 0.01 and $1.18 \pm 0.02\%$, respectively. By substitution of these values in Equation [4], the calculated value of x is 1.00 and the precision is approximately $\pm 0.02\%$.

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