Modification of AOCS Chromatographic Procedure for Neutral Oil Determination

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Abstract

The AOCS chromatographic procedure for neutral oil determination, Ca 9f-57, has been modified through use of a newly designed flask. This modified procedure increases the efficiency and standardization of neutral oil determination by: permitting automatic transfer of weighed crude oil samples onto alumina columns; converting column operation to a semiautomatic level thus reducing cost of operation; and allowing the chromatographic procedure to be performed in laboratories which are not air-conditioned. As a result of collaborative studies the AOCS Fats and Oils Subcommittee for neutral oil investigation has recommended that this procedure be adopted officially by the Uniform Methods Committee.

Introduction

A translation of the International Chemical Union Method for the determination of neutral fatty material by alumina chromatography was published in this Journal in 1949 (1). Since that time the vegetable oil industry in this country has extensively investigated (2–6) this laboratory procedure for possible correlation with commercial refining losses of crude vegetable oils.

Collaborative studies by the AOCS Commercial Fats and Oils Subcommittee for neutral oil loss led to modification and adoption of this chromatographic procedure as a standard method of analysis in 1957 (7). Later in 1963 the method was adopted for trading of crude soybean oil by the National Soybean Processors Association (8).

In our investigation of free fatty acids in crude vegetable oil by conductivity measurements this method was used as a standard reference for neutral oil value of crude oils. Greater precision in the procedure was essential, however, in order to compare it reliably with conductivity results.

Preliminary investigation revealed that the precision of analysis was increased by decreasing the activity of the alumina columns.

Alumina was deactivated by increasing its moisture content from about 5%, as specified in the AOCS method, to about 8% by the addition of 3% water.

The AOCS Neutral Oil Subcommittee was at this time also investigating means of improving the precision of the method. At the 1964 Fall Meeting of the Subcommittee, the Chairman, E. H. Tenent, Jr., requested that our laboratory continue its investigation of the chromatographic procedure using the less active alumina columns. J. W. McEwan, of Central Soya, a member of the Subcommittee, corroborated our findings by obtaining better chromatographic results with alumina columns of about 10% to 11% moisture content (9). At these higher moisture levels phospholipids are more completely adsorbed by the alumina columns.

A collaborative study was made by the Subcommittee to determine the optimum moisture level of the alumina columns. The alumina was prepared by Leifield and Kneip of Mallinckrodt Chemical Company. The results of the study indicated that the precision was significantly improved by using alumina columns ranging from 8% to 11% moisture.

Neutral oil results were compared among industrial laboratories using alumina columns of 11% (±1%) by including this analysis in the 1965–66 Smalley Check Series. The results from 75 laboratories were analyzed statistically (10). Precision increased 25% with alumina columns of 11% moisture as compared with columns of 5%. Modification of the procedure was recommended by the Subcommittee and adopted by the Uniform Methods Committee in 1966 (9).

This paper describes further modifications in the procedure which increase the efficiency and standardization of the neutral oil determination.

Experimental Procedures

Apparatus

Chromatographic equipment: Chromatographic column (Scientific Glass Apparatus Company No. JC1075 or equivalent); Velasco flask, 20 ml, with base and extension tube (Ace Glass Company No. F3731); solvent reservoir, 175 ml (Scientific Glass Apparatus Company No. 580294 or equivalent). Soxhlet flask, 250 ml (Corning No. 5160 or equivalent). Separatory funnel, 125 ml, with glass stopcock (Teflon stopcock not suitable). Wash bottle, polyethylene, with fine tip, capacity 8 oz. Funnels. Stirring rods of suitable size, 5 × 150 mm is convenient. Forced Draft Oven (AOCS Specification H 1-39).

Reagents

Ether-solvent, prepared by mixing 25 ml of methanol (ACS grade) with 975 ml of anhydrous diethyl ether (ACS grade). *n*-Hexane, Commercial grade, AOCS specification H 16–56 or better.

Aluminum oxide, activated alumina, grade F-20 (Aluminum Company of America, East St. Louis, Illinois) or equivalent. The alumina as received must be further processed so that 100% will pass a 100-mesh U.S. Standard Sieve and 70–90% is held on a 200-mesh sieve. The moisture content is adjusted to grade 4 on the Brockmann-Schodder scale by addition of water. The water content is determined by the loss on ignition (LOI) of about 1 g of alumina, accurately weighed, in a tared, covered platinum or porcelain crucible for 2 hr at 600 C. The loss on ignition is regulated to 11% ± 1%. Mallinckrodt Chemical Works, Cat. No. 0065 Aluminar CC-10 or equivalent.

Preparation of Sample

The sample container must be shaken vigorously and the sample mixed thoroughly in order to incorporate and uniformly distribute meal or other sediment. In order to acheive this, the oil must be brought to at least 20 C (50 C for soybean oil and 38 C until completely melted for coconut oil) before shaking.

Preparation of Alumina Column

Fill glass chromatographic column to about ⅔ capacity with ether-solvent. Add 20 g (±1 g) alumina to column in slurry form. The slurry may be added from a beaker or in the following manner. Add the weighed alumina, with the aid of a small polyethylene funnel, to a 125 ml separatory funnel (glass stopcock) containing about 50 ml of ether solvent.

Dispense the alumina into the glass column by opening separatory funnel stopcock. Maintain a high solvent level in the column by adjusting the flow of solvent from the funnel and column. Wash down standard joint and sides of glass column with ether solvent. Tap glass column to pack and level the alumina. Drain column until the ether solvent is almost flush with top of alumina. Add hexane to column until level rises to about ½ in. from bottom edge of the standard joint.

Procedure

Place a tared 250 ml Soxhlet flash under delivery tube of column (to prevent splashing place a glass rod in flask and position it to receive the column flow).

Weigh a sample of approximately 5 g to an accuracy of ±0.0002 g in Velasco flask. Remove the flask from its weighing base and attach the extension tube to the lower inner joint of the flask using hexane to wet the joint and a slight twisting pressure to seat it.

Place the flask with extension tube on the glass column wetting joint with hexane. Wash down neck and sides of flask with ether solvent from a polyethylene wash bottle. Carefully direct the fine stream from the bottle into the center of the oil pool to dilute and mix the oil. Continue dilution until the level rises to almost the top of the siphon arm. Give the flask a final wash down with a minimum of solvent so that the level is just above the siphon arm.

Add 125 ml of ether solvent to reservoir. Place reservoir on flask using ether solvent to wet the joint. Partially open reservoir and column stopcocks and adjust column flow rate to about 4–6 ml/min with column stopcock. The liquid level in the flask should be a little above the siphon arm. This level can be adjusted by closing column stopcock and turning reservoir stopcock to deliver more solvent into the flask. If the solvent from the reservoir does not flow into the flask, tap reservoir to start flow. The column should now operate without further attention. Allow column to drain, then wash down any traces of oil from delivery tube of the column into the Soxhlet flask.

Evaporate the ether solvent solution in a steam or water bath with the aid of a gentle stream (2–5 liters/min) of clean, dry air or nitrogen. After all but the last traces of ethyl ether fumes have disappeared, remove the sample from the steam bath and immediately place it in a 105 C forced-draft oven for 1 hr. Remove from the oven, cool and weigh the flask and contents. Calculate the neutral oil content of its loss.

Results and Discussion

One of the critical steps in the chromatographic procedure for neutral oil determination is the manual transfer of a weighed crude oil sample onto a column of alumina. This step requires careful handling in order to assure a quantitative transfer. Since the nature of this transfer involves a considerable chance of error, duplicate determinations must be made in

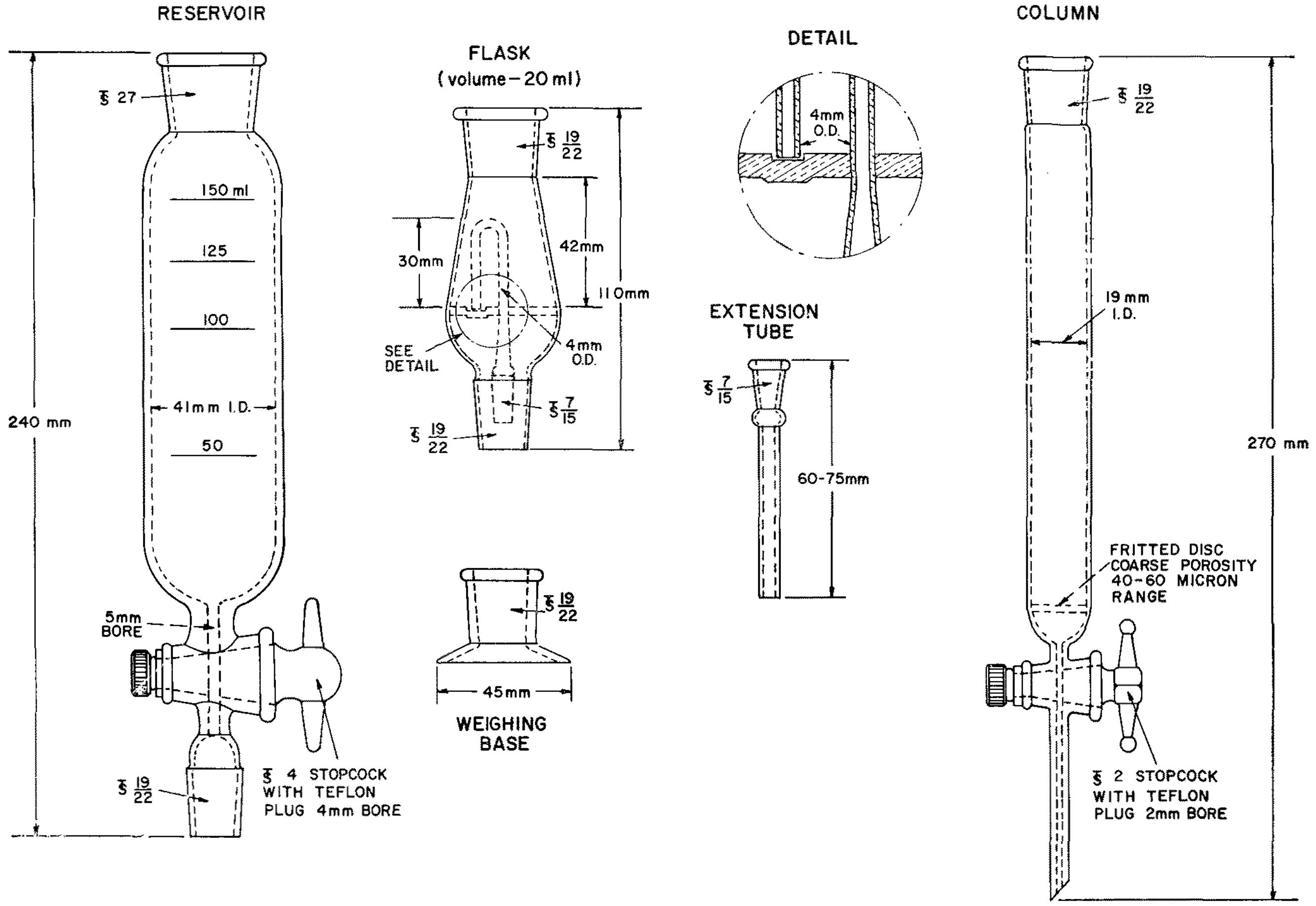

FIG. 1. Chromatographic apparatus for neutral oil determination.

order to assess the neutral oil loss of a sample with some degree of confidence.

Various attempts have been made by this and other laboratories to design some type of apparatus which would eliminate this manual transfer. Our latest design of such an apparatus, shown in Figure 1, allows this transfer to be made automatically. Figure 2 shows the apparatus assembled. This apparatus consists of a reservoir, flask, base and extension tube which is used with a glass column which is already a part of the official procedure.

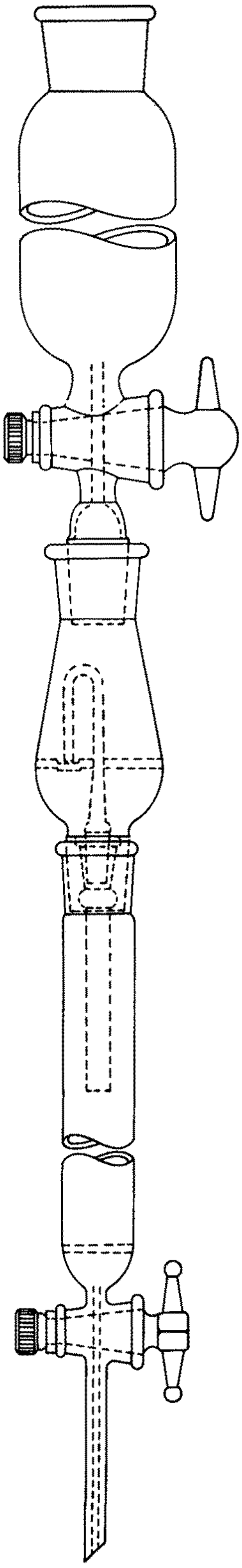

FIG. 2. Chromatographic apparatus assembled.

Essentially the flow of solvent, from a reservoir mounted on the oil flask, forces the crude oil out of the flask and onto the alumina column by means of a siphon arm and extension tube. The oil is carried through the alumina column which adsorbs the polar lipid fraction while allowing the neutral lipids to be passed into a tared flask.

In order to increase the rate at which the oil sample travels through the alumina column it is diluted in the flask with about twice its volume of solvent before the column flow is started.

To prevent continued dilution of the oil in the flask, the solvent from the reservoir is made to flow along the walls of the flask rather than allowing it to drip into the flask.

To make sure that no oil remains in the flask, all inside surfaces of the flask are exposed to the flow of solvent by adjustment of the liquid height in the flask to a level just slightly above the siphon arm. To make this adjustment, without disassembling the apparatus, the bore in the reservoir stopcock must be large enough, 4–5 mm, to allow the air trapped in the flask to escape past the downward flow of the solvent. The oil sample is transferred quantitatively from flask to alumina column, with a passage of 25–30 ml solvent.

The apparatus as designed should allow the column operation to become automatic once the flow rate is adjusted. The nature of the solvent, however, disrupts this flow arrangement. The solvent, which is 97.5% diethyl ether, creates an internal vapor pressure in the alumina column due to heat of adsorption, which forces the solution out of the column faster than it will allow it to come in. This necessitates the addition of solvent to the column at least once during each run to prevent it from running dry.

The flow problem was overcome by layering hexane above the alumina (commercial hexane is satisfactory). Since the vapor pressure of hexane is much lower than that of the ether solvent at these temperatures, the heat of absorption is not great enough to cause an interfering vapor pressure.

The lower vapor pressure of hexane also allows the column operation to be carried out at higher room temperatures. Columns have been operated at a room temperature of 93 F (34 C). This did not interfere with the column flow or cause separation of the alumina.

Even though hexane is freely miscible in oil or diethyl ether, hexane can be layered above these liquids by introducing them at the bottom of the hexane column. The lower specific gravity of hexane and the hydrostatic pressure of the hexane column prevent the oil or ether solution from mixing with hexane above the point of introduction. This restricts the contamination of the glass column with oil to a space between the top of the alumina and the end of the extension tube. This space is held to a minimum and is controlled by the length of the extension tube.

A collaborative study of this modified procedure was undertaken by the Fats and Oils Subcommittee for neutral oil investigation. The purpose of the study was to determine whether the precision of the proposed procedure was comparable to that of the official method.

Four crude soybean oil samples having low to high neutral oil losses were analyzed by two analysts from each laboratory. Samples were analyzed in duplicate

TABLE I

Collaborative Study of Neutral Oil Determination by Proposed Modified Method vs Official Method Ca 9f-57

Sample			Per Cent Neutral Oil Loss							
			VO 2		VO 4		VO 6		VO 8	
Laboratory	Analyst	Day	Official	Modified	Official	Modified	Official	Modified	Official	Modified
A	1	1	0.65	0.62	2.18	2.09	3.71	3.56	2.17	2.03
		2	0.68	0.66	2.08	2.07	3.65	3.52	2.16	2.02
	2	1	0.59	0.64	2.06	2.09	3.21	3.57	2.05	2.09
		2	0.57	0.61	2.07	2.09	3.44	3.59	2.07	2.07
B	1	1	0.62	0.74	2.01	2.06	3.05	3.19	1.92	2.14
		2	0.55	0.58	1.96	2.09	3.11	3.20	1.96	2.04
	2	1	0.72	0.79	2.05	2.31	3.19	3.05	1.97	2.15
		2	0.61	0.69	2.05	2.14	3.21	3.38	1.93	2.05
C	1	1	0.58	0.59	2.10	2.10	3.32	3.30	2.01	2.07
		2	0.60	0.59	2.06	2.08	3.34	3.30	2.03	2.10
	2	1	0.59	0.58	2.11	2.09	3.30	3.27	2.03	2.08
		2	0.61	0.58	2.11	2.05	3.31	3.30	2.00	2.10
D	1	1	0.60	0.56	2.07	1.97	[a]		2.01	1.99
		2	0.61	0.66	1.99	2.30			2.10	1.97
	2	1	0.56	0.65	2.01	2.18			1.98	2.11
		2	0.51	0.51	2.03	2.12			1.94	1.99
E	1	1	0.66	0.74	2.10	2.21	3.72	3.46	2.11	2.02
		2	0.70	0.79	2.18	2.26	3.60	3.55	2.19	2.16
	2	1	0.67	0.70	2.13	2.04	3.47	3.39	2.04	2.09
		2	0.78	0.75	2.32	2.19	3.62	3.44	2.13	2.16
F	1	1	0.66	0.60	2.04	2.20	3.78	3.72	2.02	2.04
		2	0.63	0.80	2.08	2.00	3.82	3.90	1.94	2.14
	2	1	0.69	0.65	2.12	2.15	3.76	3.82	2.10	2.18
		2	0.63	0.68	2.10	2.19	3.86	3.84	2.08	2.11
G	1	1	0.60	0.62	1.98	2.21	3.09	3.32	2.02	2.08
		2	0.53	0.82	2.06	2.06	3.27	3.15	1.97	1.98
	2	1	0.53	0.49	1.95	1.99	3.21	3.01	2.07	2.17
		2	0.63	0.42	1.96	2.09	3.25	3.20	1.90	2.25
H	1	1	0.61	0.63	2.03	1.78	3.36	3.25	2.17	2.30
		2	0.56	0.71	1.91	2.07	3.22	3.11	2.05	2.21
	2	1	0.63	0.59	2.03	2.09	4.12	3.13	2.12	2.14
		2	0.64	0.65	2.05	1.97	3.53	3.23	1.84	1.97
I	1	1	0.51	0.50	2.33	2.12	3.45	3.31	1.82	1.86
		2	0.65	0.41	2.06	1.89	3.53	3.39	1.94	1.79
	2	1	0.58	0.41	2.10	1.94	3.45	3.28	1.93	1.91
		2	0.42	0.37	1.98	1.93	3.55	3.31	2.02	1.95
Mean			0.61	0.62	2.07	2.09	3.45	3.37	2.02	2.07
Variance			0.0044	0.0128	0.0076	0.0124	0.0665	0.0513	0.0082	0.0109
Standard deviation			0.06	0.11	0.08	0.11	0.25	0.24	0.09	0.10
Official Modified										
Average variance			0.0216	0.0218						
Average standard deviation			0.15	0.15						

[a] Sample failed to dissolve completely upon heating.

by both methods and duplicates were taken on different days.

The Laboratories which participated in the study were: Ralston Purina, St. Louis, Missouri; Procter and Gamble, Cincinnati, Ohio; Central Soya, Decatur, Illinois; Hahn, Columbia, South Carolina; Archer Daniel Midland, Decatur, Illinois; Buckeye Cellulose, Memphis, Tennessee; Barrow Agee, Memphis, Tennessee; Woodson and Tenent, Memphis, Tennessee; U.S. Department of Agriculture, Washington, D.C.

The results of the four samples as determined by the nine laboratories are shown in Table I.

Statistical analysis of the data indicates that the precision of the modified procedure is essentially equal to that of the official method. There is no significant difference between these two methods at the 1% level, as determined by the calculation of their variance ratio: $F = 0.00218/0.00216 = 1.009$ with 140/140 degrees of freedom. A standard deviation of 0.15% was obtained by both methods on the four oil samples analyzed by the nine laboratories. The Neutral Oil Subcommittee recommended (12) that this procedure be adopted officially by the Uniform Methods Committee.

ACKNOWLEDGMENT

Thanks to Marion E. Whitten and T. L. Potts for encouragement.

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[Received November 12, 1968]