SHORT COMMUNICATION

Sample Size Influence on Boron Trifluoride–Methanol Procedure for Preparing Fatty Acid Methyl Esters

ABSTRACT

Sample size influences the yields of methyl esters of fatty acids by the boron trifluoride-methanol method. A study of the method revealed that best recoveries are obtained by using a minimum of 350 mg of lipid extract to prepare the methyl esters.

INTRODUCTION

The boron trifluoride-methanol method for preparing the methyl esters of fatty acids became the official method of the Association of Official Analytical Chemists (AOAC) in 1968 (1) to replace the sulfuric acid procedure (2). The adoption of the boron trifluoride method by AOAC was based primarily on the study of the AOCS Special Task Group for Preparation of Methyl Esters (3) and included data from a collaborative study. The Task Group recommended to the AOCS Uniform Methods Committee in 1968 that the boron trifluoride method (AOCS Method Ce 2-66) (4) be adopted as an official AOCS method. The special task group recommended that the method be rewritten to include information for preparing methyl esters from samples ranging in size from 100 mg-1.0 g and that appropriate quantities of reagents be specified for each sample size. The proposed method was made a part of the report, and the sample size specified is 1 g, whether the sample be fatty acids or glycerides. However, the AOAC adopted the sample size of 100 mg-1.0 g, specifying appropriate quantities of reagent, depending upon sample size.

When the new regulation pertaining to the labeling of foods with information on fat composition was proposed by the Food and Drug Administration (5,6) and finally adopted (7), it immediately became necessary to obtain suitable methodology. To expedite formulation of complete methodology, the official AOAC method (8) was utilized immediately without any additional testing, since it was based on the AOAC studies and it was a highly regarded method. However, after a large number of fatty acid analyses of food extracts had been made, it became apparent that the total fatty acid yields were abnormally low. All other aspects of the overall system had been checked thoroughly (9) before the series of analyses was begun. Thus, the BF_3-methanol method was the logical step to initiate further testing.

EXPERIMENTAL PROCEDURES

Large quantities of pure triglyceride were obtained commercially (99%, Nu Chek Prep, Elysian, Minn.) to test the method. All test samples were weighed on a semimicro analytical balance, and were kept under nitrogen and refrigeration whenever possible. Both gravimetric and gas liquid chromatographic (GLC) analyses (9) were carried out. The theoretical yields of methyl esters were calculated for each sample size of the individual triglycerides.

The first area of the method investigated was to determine whether or not the saponification step was complete. Potassium hydroxide was substituted for the specified sodium hydroxide with no noticeable effect. Next, longer saponification time (up to 1 hr) were tried, but the yields did not increase. The quantity of base was raised to 10 ml for the 100-250 mg range without effect. Qualitative thin layer analysis by the method of Malins and Mangold (10) indicated that the 10 min saponification was complete using either potassium or sodium hydroxide in the amounts specified for the base reagent as related to sample size (8); fatty acids were detected, but no triglyceride was found. The methyl esters were prepared as specified, and a GLC analysis was made on each sample prepared. The GLC quantitative results exhibited no significant fatty acid shifts within a specified sample wt (50 mg, 100 g, etc.), and the recovery was always considerably less than theoretically possible.

Since the triplicate results for 50 mg samples of the various triglycerides were so consistent (Table I) a possible alternative appeared feasible, namely, to prepare an equal wt mixture of triglycerides to be processed by the method and use the resulting fatty acid methyl esters to calibrate the gas chromatograph. A final test of this approach was made by preparing a composite mixture of 50 mg of the 7 triglycerides (Table I–fatty acid chain lengths) that had been tested individually to give a final sample wt of 350 mg. The methyl esters were prepared according to the method.

The results obtained by GLC analysis of the 350 mg mixture provided unexpected results. The recoveries of the theoretical yield of the fatty acid methyl esters from the 7 triglycerides were: trilaurin, 97.7%; trimyristin, 104.0%; tripalmitin, 95.3%; tristearin, 95%; triolein, 99.2%; trilinolein, 96.2%; trilinolenin, 96.6%. These results immediately led to an investigation of the influence of sample size on the results obtained by the method.

TABLE I

Wt Percent Recovery[a] of Triglyceride Fatty Acids

Triglyceride, mg	Triglyceride							Overall % recovery
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	
50	37.6±[b]1.5	52.2±3.2	67.2±0.8	65.8±2.8	74.8±0.8	61.1±1.5	66.1±1.4	60.7
100	77.3	94.5	95.6	92.8	96.6	98.3	89.8	92.1
200	93.5	96.6	99.0	93.5	98.8	93.6	95.8	95.8
350	98.6±1.4	97.0±0.1	99.8	96.5	99.7	100.0	97.5	98.4

[a]All data are mean values of two or more determination

[b]Standard deviation calculated only when triplicates used.

RESULTS

The results of the sample size study are summarized in Table I. In each situation, i.e. sample wt and individual triglyceride, 50, 100, 200, and 350 mg portions of each compound were weighed and determined by the method as outlined (8). It is readily apparent that sample size is a dominant factor in the recovery of the fatty acid methyl esters from the triglycerides. The recoveries throughout the chain length series are quite acceptable at the 350 mg sample size but become less acceptable as the sample size becomes smaller. Gravimetric data obtained throughout this latter experiment consistently followed the GLC data shown in Table I.

The results obtained in this investigation are the basis for the modifications of the Interim Methodology Instructions no. 1 (11), which specifies that a minimum of 350 mg of lipid extract be used for preparing the fatty acid methyl esters from foods, fats, and oils.

H.L. SOLOMON
W.D. HUBBARD
A.R. PROSSER
A.J. SHEPPARD
Division of Nutrition
Food and Drug Administration
Washington, D.C. 20204

REFERENCES

1. Firestone, D.J. Ass. Offic. Anal. Chem. 52:254 (1969).
2. Association of Official Analytical Chemists, "Official Methods of Analysis," Tenth Edition, Association of Official Analytical Chemists, Washington, D.C., 1965, sec. 26.052.
3. O'Connor, R.T., R.R. Allen, J.R. Chipault, S.F. Herb, and C.W. Hoerr, JAOCS 45:103 (1968).
4. AOCS, "Official and Tentative Methods of the American Oil Chemists' Society, Vol. 1 and 2, Third Edition, AOCS, Champaign, Ill., 1964 (revised to 1973).
5. "Federal Register" 36(No. 115): 11521 (1971).
6. Ibid., 38(No. 13): 2132 (1973).
7. Ibid., 38(No. 49): 6961 (1973).
8. Association of Official Analytical Chemists, "Official Methods of Analysis," Eleventh Edition, Association of Official Analytical Chemists, Washington, D.C., 1970, Section 28.058-28.062.
9. Sheppard, A.J., W.D. Hubbard, and A.R. Prosser, JAOCS 51:416 (1974).
10. Malins, D.C., and H.K. Mangold, Ibid. 37:576 (1960).
11. Sheppard, A.J., "Interim Methodology Instructions no. 1 for Implementing Requirements of Section 1.18 of Title 21, Chapter 1, Subchapter A, Part 1 ('Labeling of Foods in Relation to Fat, Fatty Acid, and Cholesterol Content')", Division of Nutrition (HFF-268), Bureau of Foods, Food and Drug Administration, Washington, D.C., March 29, 1973.

[Received May 24, 1974]