

An Apparatus for the Rapid Preparation of Fatty Acid Esters from Lipids for Gas Chromatographic Analysis

IN APPLYING THE METHOD of Metcalf, Schmitz and Pelka (1), I have found the apparatus shown in Figure 1 useful, especially when only a few milligrams of lipid are available for analysis. The apparatus (all glass) consists of a ground glass joint, a capillary and a bulb. The ground glass joint is a type 10/30, the capillary has an inside diameter of 3 mm and a length of 15 mm. The volume of the bulb can be varied to meet the needs of the individual researcher, but I prefer 5 ml. The overall length of the flask is 90 mm. It was designed to fit into a No. U 6878, International Clinical Centrifuge.

Because of the capillary, it is necessary to introduce all samples and reagents directly into the bulb with a syringe (1 or 5 ml) fitted with 10 cm long needles. It is desirable to blunt the tips of the needles.

The reagents as described in (1) were used, except 0.5 N methanolic potassium hydroxide was used instead of 0.5 N sodium hydroxide. Approximately 25 mg of fatty material is introduced on to the bottom of the flask using the 1 ml syringe. One milliliter of 0.5 N methanolic potassium hydroxide is then introduced using the 5 ml syringe. The mixture is heated on a steam bath until the fatty globules go into solution. This step takes about 2 min. Two milliliters of boron-trifluoride reagent is added, again using a syringe, and the mixture boiled for 2 min. During both the saponification and esterification steps, a condenser can be attached to the top of the flask to return low boiling fatty material. After esterifying with boron-trifluoride sufficient saturated sodium chloride solution is added to the flask to raise the level of the liquid to about halfway up into the capillary. Again, the syringe is used. Addition of the saturated salt solution too rapidly will cause an air bubble to form in the capillary which will be difficult to remove. The flask is then centrifuged for 2 min.

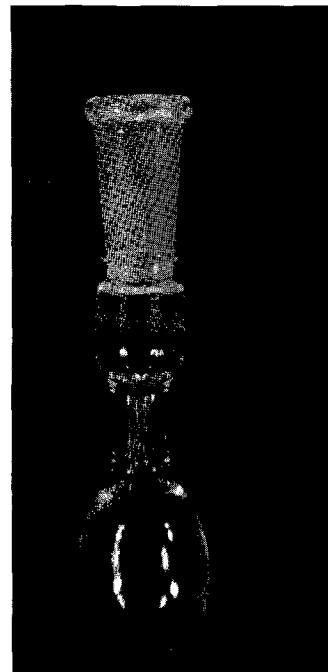


Fig. 1. Apparatus for the preparation of fatty acid esters.

The methyl esters float to the surface and are concentrated in the capillary, providing easy availability of the material.

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REFERENCES

1. Metcalf, L. O., A. A. Schmitz and J. R. Pelka, *Anal. Chem.* **38**, 514 (1966).

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The Determination of Organically Combined Sulfuric Anhydride In Sulfated Tall Oil Fatty Acid Esters

In the determination of organically combined sulfuric anhydride by the AOCS method (F2a-44) the procedure calls for heating under reflux for 1.5 hr or until the layers are clear. Use of this method for sulfated tall oil fatty esters gives poor results. We have found that a 4- to 6-hr reflux period is required with sulfated tall oil fatty acid esters before the oil and water layers are clear. With longer periods under reflux the combined sulfuric anhydride assay increases. Sulfated tall oil fatty acids, on the other hand, are clear after boiling for one-half to one hour and give a very satisfactory analysis.

A study of the time under reflux versus SO_3 content was made using sulfated tall oil fatty acid n-butyl ester to determine the time necessary for complete removal of combined SO_3 . The results are shown in Figure 1. Both layers were clear after 4 hr of boiling, corresponding to 6.18% SO_3 ; however, the maximum value of 6.54% was obtained after 8 hr.

A variety of other sulfated materials was analyzed using a 2- to 24-hr period to determine the minimum time required for complete hydrolysis. The results are shown in Table I.