• TECHNICAL

Quantitative Analysis for Oleic and Petroselinic Acids in Glyceride Oils[1]

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Abstract

A rapid quantitative method for the determination of oleic (9-cis-octadecenoic) and petroselinic (6-cis-octadecenoic) acids was required for nutritional research. The methyl esters of these two acids have coincident emergence in GLC separations using polyester columns. A method involving oxidation and GLC analysis of the resultant monocarboxylic acids has been developed.

Introduction

SEPARATION OF POSITIONAL isomers of the monoenoic acids is not readily obtained by gas-liquid chromatography (GLC) using the polar polyester columns. The permanganate-periodate oxidation developed by von Rudloff (5,6) has been used in a number of investigations where positional isomers of the unsaturated acids are involved. The scission products including both mono- and dicarboxylic acids can be analyzed by GLC (3). The proportions of the resultant mono- and dicarboxylic acids can be related to the proportions of mono-, di- and triene acids which are found by GLC analysis of the original esters.

Oxidation of oleic and petroselinic acids would yield pelargonic and lauric acids, respectively. The proportions of these two acids applied to the octadecenoic content of the original ester would give the proportions of these two acids in the original mixture. Most of the vegetable oils and animal fats contain minor quantities of lauric acid which would not contribute to a major experimental error.

Experimental

The oxidation procedure of von Rudloff (5,6) modified by Youngs (7) was adapted further to eliminate transfers of sample and to reduce the volumes of solutions. Fifteen milliliters of standard oxidant, 1 ml of sodium carbonate (5 g per 100 ml) and 20 ml of tertiary butanol were added to a 50 ml glass stoppered (19/38 ST) Erlenmeyer flask and mixed. The sample (25 mg) of methyl esters was added and the mixture allowed to stand at room temperature for 15 min after mixing. The solution was refluxed for 1 hr under an air condenser and cooled to room temperature. One pellet of potassium hydroxide (100 mg) was dissolved in the solution and ethylene gas was bubbled into the flask until the permanganate color had disappeared. The contents of the flask were evaporated to dryness on a steam bath under a stream of air and the resultant solid was broken up with a spatula.

Eight milliliters of methanol, 0.75 g of sodium bisulfite and a drop of bromphenol blue indicator were added to the dry contents and the flask was connected to a water condenser. Acetyl chloride (0.3 ml) was pipetted through the condenser to acidify the soaps and alkali and the mixture was shaken at room temperature until the iodine color disappeared. Boron trifluoride etherate complex (2–3 drops) was added, the mixture was refluxed for 1 hr and cooled to room temperature. The mixture was transferred to a separatory funnel using 25 ml of water to dissolve the salts. The flask was rinsed with 20 ml of ethyl ether which was added to the separatory funnel. Sodium sulfate (0.50 g) was then added and the mixture was shaken to extract the methyl esters of the scission products. Twenty milliliters of petroleum ether (Skellysolve "F" bp 30–40C) was added, the separatory funnel was shaken again and the two clear layers were allowed to separate. The water layer and small excess of undissolved sodium sulfate were withdrawn and the ethereal layer was transferred to a 50 ml round bottom flask equipped with a conical tip. The solvent was carefully evaporated at 60C to a volume of approximately 50 μl using a 30 in. air condenser to provide partial reflux. An aliquot was injected onto a GLC unit equipped with a T/C detector. The column (10 ft by $\frac{3}{16}$ in. O.D. copper) packed with o-phthalic ethylene glycol polyester (1,2) 60–80 mesh, on acid washed C-22 firebrick (1:4.5 w/w) was temperature programmed from 160–225C at a rate of 2.25C per min with a helium flowrate of 60 ml per min. The detector system was maintained at 240C.

The peak areas of methyl pelargonate, laurate and palmitate were measured by triangulation. The necessary correction factors for the three esters were established with weighed proportions in mixtures. The proportions of oleic and petroselinic acids in the original mixture of esters were calculated by two methods as follows:

A. The molar ratios of methyl pelargonate and laurate were applied to the "percentage methyl oleate" from the GLC analysis of original methyl esters.

B. The methyl palmitate in the original and oxidized samples was used as the internal standard and the proportions of oleate and petroselinate were calculated from the proportions of pelargonate, laurate and palmitate. This method of calculation should be used only if the methyl palmitate is present in the mixture in reasonable proportion (e.g. $>10\%$).

Results and Discussion

A sample of coriander seed oils was converted to methyl esters and analyzed according to the outlined procedure (A) with the following results shown in duplicate:

Fatty acid ester	Per cent composition of original methyl esters		
	I	II	Mean
Methyl oleate	9.3	8.7	9.0
Methyl petroselinate	73.1	73.7	73.4

A mixed oil made up from coriander seed oil, sunflowerseed oil and palm oil in weighed proportions gave the following results on duplicate analysis of methyl pelargonate and laurate on two different days:

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	First day			Second day			
	I	II	Mean	I	II	Mean	Calculated[a]
Methyl pelargonate	42.3	43.2	42.8	43.8	42.0	42.9	43.3
Methyl laurate	57.7	56.7	57.2	56.2	58.0	57.1	56.7

[a] Calculated from proportions of seed oils in the mixture and the fatty acid compositions of each.

A sample of body fat from mice fed on the mixed oil was analyzed for petroselinic acid content according to the two procedures and duplicate analyses gave the following values:

Method	Petroselinic %	
	I	II
A	15.3	15.7
B	15.4	15.6

The results show excellent agreement between the two methods for calculating the proportions of methyl oleate and petroselinate and a very good precision in the analysis. The addition of sodium bisulfite at the acidification step is necessary to reduce the free iodine which is liberated. If this is neglected byproducts are produced which interfere in the subsequent GLC analysis. The combination of extraction solvents was effective for both the monocarboxylic and dicarboxylic methyl esters. Petroleum ether in the solvent mixture reduced the water content and eliminated the necessity for using a drying agent, such as sodium sulfate.

The method requires about 3 hr for the preparation of the sample and has the advantage that no transfers are involved, which eliminates the possibility of losses and subsequent errors.

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The Simultaneous Determination of Both the Quantity and the Fatty Acid Composition of the Triglycerides in Three to Ten Microliters of Plasma[1]

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Abstract

An integrated system using chromatography on silica gel-impregnated glass paper, an internal standard and gas-liquid chromatography (GLC) makes possible the isolation of triglycerides, the determination of triglyceride, fatty acid composition, and the quantitative determination of triglyceride.

Recoveries are good and values for total triglyceride obtained by this system compare favorably with values obtained by application of the hydroxamate colorimetric method to the triglyceride eluate from silicic acid columns. The fatty acid composition of the cholesteryl ester (CE) fraction is obtainable at the same time by GLC of methyl esters prepared from the CE fractions which also appears as a distinct spot on these chromatograms.

Also, a densitometric method is described for triglyceride determination by photometry of the charred spots obtained after chromatography on the gel-paper.

THE LIMITED NUMBER of reports on serum triglyceride levels of animals and humans under various conditions of health and disease has been due at least in part to difficulties in measuring this lipid. Silicic acid column chromatography has been one of the more valuable methods of isolating triglycerides from serum extracts. Since this method requires constant monitoring of the physical and chemical conditions to effect a separation of the triglycerides from other components of the serum, it has gradually been replaced by methods using thin-layer and paper chromatography. An additional advance in the analysis of triglycerides has been the determination of their fatty acid composition by gas-liquid chromatography.

A detailed review of the glass paper chromatography of lipids has been published by Hamilton and Muldrey. These authors reported their experience and the advantages in separating various serum lipids by this method (1,2). Recently, a method has been described by Muldrey, Bowers, Miller and Hamilton (3), for the microassay of serum cholesteryl ester fatty acid (CEFA) patterns by densitometry of the charred spots produced after chromatography on silica-gel impregnated glass paper. Since the individual CEFA can be determined in 1–3 μl of plasma, it has been possible to perform serial measurements in single rats for several months (4).

In this study, a method was developed for measurement of serum triglycerides in 3 to 10 μl of plasma by use of a system consisting of chromatography on glass paper impregnated with silica gel (gel-paper) combined with an internal standard and gas liquid chromatography (the integrated system). By using

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