QUANTITATIVE GRAVIMETRIC ANALYSIS OF FATTY ESTER MIXTURES BY THIN-LAYER CHROMATOGRAPHY

E. DUNN AND P. ROBSON
Procter & Gamble Limited, Basic Research Department, Newcastle upon Tyne (Great Britain)
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INTRODUCTION

The use of thin-layer chromatography (TLC) for the qualitative analysis of fatty acid esters is now widespread. MANGOLD[1] separated the acetoxy-mercuri methoxy derivatives of esters into classes according to their degree of unsaturation. After decomposition of the derivatives, the compounds in each group were analysed by gas–liquid chromatography (GLC).

The ability of olefins to form labile co-ordination compounds of varying stabilities with certain metals, particularly silver, has been used as the basis for separations of unsaturated compounds and also geometrical isomers. DE VRIES achieved separations of lipid materials by column chromatography[2,3] and TLC[4] using silica impregnated with silver nitrate and BARRETT *et al.*[5] separated glycerides by TLC using the same medium. MORRIS[6] has reported the separation of higher fatty acid isomers and vinylogues by TLC using silica plates sprayed with silver nitrate or boric acid. The main advantage of these methods is that the derivatives are formed *in situ* and do not have to be decomposed after chromatography.

Techniques and apparatus have been described for preparative TLC[7,8]. Most methods for quantitative TLC[9] have involved the use of very small samples (less than 100 γ) but a semi-quantitative gravimetric method for samples of 20 to 50 mg has been reported by WILLIAMS[10].

In the present work fatty acid methyl esters were prepared from fats by the method of LUDDY[12]. With the aid of preparative TLC using Silica Gel H impregnated with silver nitrate, a sample of about 60 mg of esters was separated into groups containing saturated, mono-unsaturated, di-unsaturated and poly-unsaturated compounds. These were extracted from the silica and their proportions were determined gravimetrically. Since this work was completed, KOMAREK has reported the quantitative recovery of lipids separated by TLC[11].

EXPERIMENTAL

Preparation of esters

Methyl esters were prepared from glycerides by methanolysis with a large excess of sodium methoxide in methanol[12]. A 20% solution of the esters in ethyl ether was prepared and stored under nitrogen in a refrigerator.

Preparation of chromatoplates

Merck Silica Gel H (100 g) was shaken up in a stoppered flask for about 1 min with a 12.5 % (w/v) aqueous solution of silver nitrate (200 ml). The resulting uniform slurry was sufficient to prepare five chromatoplates 20 cm × 20 cm and 0.5 mm thick. A Shandon Unoplan Leveller and a perspex spreader were used for the preparation of the plates.

Application of sample to chromatoplate

A sample applicator has been devised which can apply about 60 mg of the esters quickly and easily in a narrow continuous streak across the chromatoplate (see Fig. 1). It was made from two thin glass plates 3.5 in. × 2.5 in. held 0.003 in. apart by a piece of copper foil 3.5 in. × 1.5 in. There was, therefore, a space 3.5 in. × 1.0 in. × 0.003 in. between the plates. The edges of the plate were ground as shown in the diagram and then polished. The applicator was filled by dipping the open edge into the sample solution and, after wipping excess from the outside, it was discharged by gently touching onto the surface of the silica gel. Two such applications, end on,

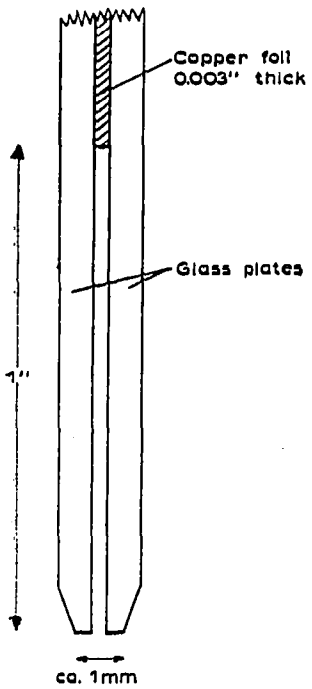

Fig. 1. Sample applicator.

with a 20 % solution of the sample gave a continuous streak of about 60 mg across the plate. The streak was applied to the plate in the position shown in Fig. 2. The plate was heated to about 70° before sample application so that the solvent evaporated quickly and the sample was contained in a narrow band.

Development of chromatoplate

A 10 % (v/v) solution of ethyl ether in 60–80° petroleum ether was used for development of the chromatogram. No equilibration period was necessary. Develop-

ment, which took about 20 min, was continued until the solvent front was about 1.5 in. from the top of the plate.

The developed plate was allowed to dry at room temperature under nitrogen, was sprayed with 0.2 % ethanolic solution of dichlorofluorescein and then was immediately examined under U.V. light. The separated compounds showed up as a series of light green bands.

Extraction and determination of the separated compounds

The bands observed corresponded to saturated, *trans*-mono-unsaturated, *cis*-mono-unsaturated, di-unsaturated and poly-unsaturated esters. The approximate positions of the bands are shown in Fig. 2. These were marked off with a fine knife blade and the silica gel in each section was transferred quantitatively to 50 ml centrifuge tubes. This was achieved by carefully scraping off with a spatula and then

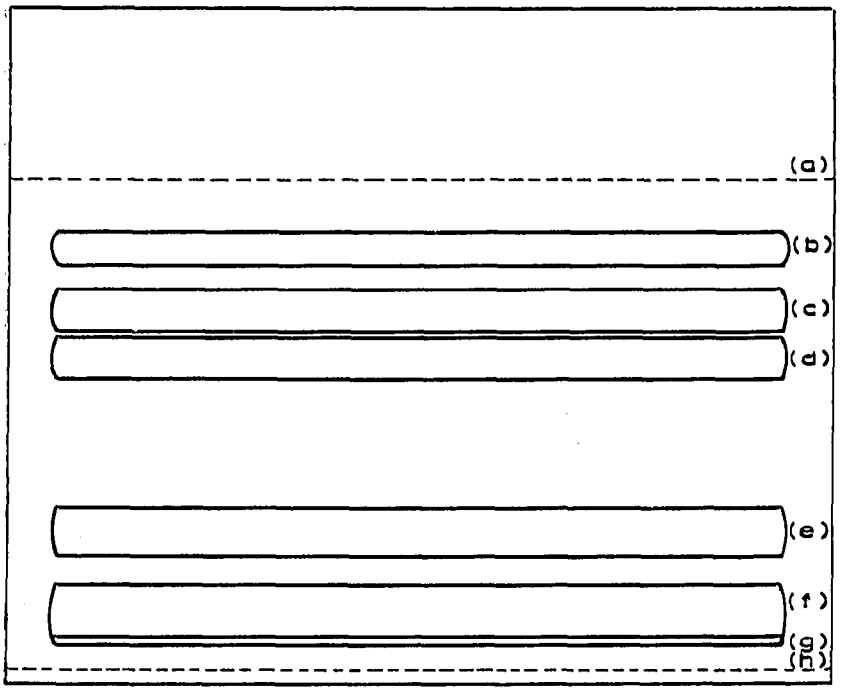

Fig. 2. Thin-layer chromatogram of fatty esters. (a) Solvent front; (b) saturated; (c) *trans*-mono-unsaturated; (d) *cis*-mono-unsaturated; (e) di-unsaturated; (f) poly-unsaturated; (g) sample streak; (h) lower edge of silica gel.

brushing off each area onto a piece of hard gloss paper before transferring to the centrifuge tube. The fractions containing *cis*- and *trans*-mono-unsaturated esters were combined. Each lot of silica gel was then extracted with three 25 ml portions of ethyl ether. The combined extracts from each section were evaporated to small bulk and transferred to 6 ml high speed centrifuge tubes. Centrifuging for 5 min at about 19.000 r.p.m. gave clear solutions which were transferred to 20 ml beakers which had been tared on a micro balance. The deposits of fine silica left in the centrifuge tubes were each extracted twice more with 4 ml portions of ether and after high speed centrifugation the extracts were added to the appropriate beakers. The combined extracts were then evaporated off under a stream of nitrogen on a steam

bath. Care was taken to prevent the beakers going dry on the steam bath. The safest procedure was to remove the bulk of the solvent on the steam bath and then blow off the last traces from the sample while it was still warm. After cooling, the beakers were re-weighed on the micro balance.

RESULTS AND DISCUSSION

A relatively simple mixture derived from linseed oil was used to test the method. The mixture was analysed by GLC alone and the results obtained are compared with values obtained from the TLC separation (Table I).

TABLE I

LINSEED OIL

Class of compound	*Percentages*	
	By TLC	*By GLC*
Saturated (C_{16} and C_{18})	10.3, 11.2, 11.2	10.2, 10.6, 9.3
Mono-unsaturated (C_{18})	20.8, 18.7, 19.8	20.3, 20.4, 20.1
Di-unsaturated (C_{18})	17.2, 16.4, 16.7	16.3, 15.1, 17.9
Tri-unsaturated (C_{18})	51.7, 53.6, 52.4	53.2, 53.9, 52.6

With mixtures of esters obtained from natural oils there is a considerable range of homologues within each group and the bands are a little wider. However, the separation between each group should be sufficient to allow for this and in work with herring oil and hardened herring oils, GLC analysis confirmed that the separated saturated, mono-unsaturated and di-unsaturated esters were free from contamination from adjacent groups. It also showed that they were not contaminated with conjugated unsaturates[4]. If any small overlaps of the groups did occur in the TLC separation, the contaminants in any group would easily be detected and estimated in the subsequent GLC analysis and allowance would be made for them.

Mono-unsaturated esters were separated into two groups containing the *cis* and *trans* isomers. In the present work they were recombined but it should be possible to extract them separately and determine their proportions.

Table II shows four sets of results obtained in the analysis of esters obtained

TABLE II

HERRING OILS

Class of compound	*Percentages*			
	Raw herring	*Hardened herring*		
		1	*2*	*3*
Saturated	24.2, 27.1, 26.4, 25.7	23.1, 23.7	37.7, 38.3	22.2, 21.2
Mono-unsaturated	60.2, 58.6, 58.1, 57.9	55.7, 55.1	50.4, 49.6	60.1, 59.0
Di-unsaturated	2.5, 4.7, 4.0, 3.1	11.1, 10.8	5.7, 6.5	14.8, 15.2
Poly-unsaturated	13.1, 9.4, 11.5, 13.3	10.0, 10.3	6.2, 5.3	2.9, 4.6

from raw herring oil. Also shown are the analyses of three samples of hardened herring oil.

Although there was no evidence of serious oxidation occurring in this work, the use of 4-methyl-2,6-di-*tert.*-butylphenol[13] would appear to be a worthwhile safeguard.

It may be possible to separate further the poly-unsaturates by using the TLC technique with a more polar mobile phase. The gravimetric method of determining compounds separated by TLC should be of use in many other problems.

SUMMARY

A thin-layer chromatographic technique, employing silica gel impregnated with silver nitrate as stationary phase, is used to separate esters into groups containing saturates, mono-unsaturates, di-unsaturates and poly-unsaturates. The proportions of these groups are determined gravimetrically and the constituent members of the first three groups can be separated, identified and determined by gas–liquid chromatography.

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