

Determination of triglyceride composition by horizontal thin-layer chromatography

Since thin-layer chromatography was developed¹ into a simple and reliable analytical technique, it has proved to be extremely useful in many fields of chemistry. In the chemistry of fats, for instance, the technique enables 100 mg amounts of complicated triglyceride mixtures to be separated and analysed. This has been achieved by using 1 mm thick layers of silica gel impregnated with silver nitrate, on 20 × 40 cm glass plates, which were covered by another plate in order to ensure the immediate saturation of the air space so enclosed with solvent vapour². The preparation of the AgNO₃-silica layers has been described elsewhere³; the apparatus was in essence identical to that described by BRENNER *et al.*², but adapted to the dimensions of the 20 × 40 cm glass plates.

100 mg triglyceride mixture, dissolved in *ca.* 1 ml light petroleum or carbon tetrachloride, was brought onto the adsorbent layer in a 16 cm long streak at a distance of *ca.* 4 cm from one of the short edges of the plate, and then developed with a suitable solvent mixture. Using this technique, the formation of droplets on the cover is generally observed⁴, but this could easily be prevented by covering the apparatus with a light-excluding cloth. After *ca.* 4 h the solvent front had run a distance of 35 cm. A good, visual indication of the progress of the separation during the chromatographic process was obtained by the introduction of small amounts of suitable dyes at the start. Decanal-DNPH behaves as glyceryl tristearate (SSS)* while Sudan-III (ex Fluka, Switzerland) contains two dyes that behave as triglycerides with 1 and 2 *cis* double bonds respectively. When the SSS fraction had reached the top of the plate the process was stopped and the fractions then obtained^{3,5} were examined by various techniques. Very good results have thus been obtained.

The following mixtures have been quantitatively analysed:

1. An interesterified mixture of equal amounts of glyceryl tristearate (SSS), trielaidate (EEE) and trioleate (OOO), in which the acyl groups are expected to be randomly distributed. Apart from positional isomers of the type SOO, OSO etc., in which the radicals are in a different position, ten different triglycerides are expected to be present in this mixture. Using benzene as eluant, this mixture was well separated into 7 fractions. The results are compiled in Table I.

Recoveries were at least 97%. The fatty acid composition of each fraction was used for identification, and was found to be in good agreement with the theoretical values.

2. *Natural fats.* Palm oil was easily separated into five fractions, and soyabean oil into seven fractions, using as eluants benzene-diethyl ether (95:5 and 85:15 v/v respectively). In view of the results of experiments with synthetic triglycerides^{5,6} using column chromatography on silica impregnated with silver nitrate, each fraction was assumed to contain only triglycerides of the same degree of unsaturation. The triglyceride composition of each fraction was further calculated from the total fatty acid composition determined by G.L.C. and from the fatty acid composition at the 2-positions determined by semi-micro lipase splitting⁷. The results allowed of complete and accurate triglyceride analysis of these very complicated fats. As one of the most

* S = stearate; O = oleate; E = elaidate.

TABLE I
TRIGLYCERIDE COMPOSITION OF INTERESTERIFIED SSS, EEE AND OOO

Fraction No.	Content	Amount (%)		Distance from start (cm)	
		Found	Calc.	to lower side of fraction band	to upper side of fraction band
1	OOO	5.6	3.7	2.2	4.5
2	EOO	9.7	11.1	5.1	7.0
3	SOO + EEO	20.7	22.2	7.9	11.5
4	EEE + SEO	25.6	25.9	13.0	18.5
5	SSO + SEE	21.4	22.2	19.7	24.6
6	SSE	11.8	11.1	25.6	30.0
7	SSS	5.1	3.7	33.0	36.0

important results the correctness of VANDER WAL's distribution theory⁸ was ascertained.

Other synthetic mixtures as well as natural and hardened fats are currently being analysed. Details of the results and of the experimental conditions will be published in due course.

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¹ E. STAHL (Editor), *Dünnschichtchromatographie*, Springer-Verlag, Berlin, 1962.

² M. BRENNER AND A. NIEDERWIESER, *Experientia*, 17 (1961) 237, cf. Ref. 1, p. 24.

³ B. DE VRIES AND G. JURRIENS, *Fette, Seifen, Anstrichmittel*, 65 (1963) 725.

⁴ P. M. REISTERT AND D. M. SCHUMACHER, *Experientia*, 19 (1963) 84.

⁵ G. JURRIENS, B. DE VRIES AND L. SCHOUTEN, *J. Lipid Res.*, in press.

⁶ B. DE VRIES, *J. Am. Oil Chemists' Soc.*, in press.

⁷ G. JURRIENS, B. DE VRIES AND L. SCHOUTEN, *J. Lipid Res.*, in press.

⁸ R. J. VANDER WAL, *J. Am. Oil Chemists' Soc.*, 37 (1960) 18.

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Beitrag zur Dünnschichtchromatographie von Barbituraten*

In einer früheren Arbeit beschäftigten wir uns mit der adsorptionschromatographischen Trennung von Barbituraten unter Verwendung von Aluminiumoxyd-Säulen¹. Inzwischen hat die Dünnschichtchromatographie vor allem durch die Untersuchungen von STAHL² eine starke Verbreitung erfahren. So wurde diese elegante Schnellmethode schon verschiedentlich für die Identifizierung von Barbituraten herangezogen³⁻⁸.

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