Quantitative analysis of mixtures of glycerides

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SUMMARY The accuracy of a method for the estimation of glycerides separated by thin-layer chromatography has been tested and found to be high. Mono-, di-, and triglycerides separated on silica gel, and triglycerides of differing degrees of unsaturation separated on silver nitrate-impregnated silica gel, were found to give equally reproducible results.

Triglycerides can be separated by thin-layer chromatography (TLC) on silica gel impregnated with silver nitrate according to their degree of unsaturation (1). For instance, groundnut oil can be separated in one run into the groups SSS, SSO, SOO + SSL, OOO, SOL, OOL, SLL, OLL, and LLL[1] on a plate 20 x 40 cm (2), using the horizontal technique of Brenner and Niederwieser (3). Moreover, mixtures of triglycerides containing acyl groups with *cis* and/or *trans* double bonds can be separated into the groups SSS, SSE, SEE + SSO, SOE + EEE, etc. A triglyceride with two *trans* double bonds comes in the same group as a triglyceride with one *cis* double bond.

For quantitative determination, mixtures of 60–100 mg glycerides were separated on 1 mm thick layers of adsorbent. The triglycerides were made visible in ultraviolet light after spraying the plate with dichlorofluorescein and subsequently extracted from the adsorbent with diethyl ether. Each fraction was saponified with alcoholic alkali and after acidification the fatty acids were extracted with ether. The alcohol in the aqueous layer was removed and the solution neutralized using methyl red as indicator. Glycerol was determined by adding 20–100% excess of periodic acid to the solution and back-titrating with a sodium arsenite solution of known strength (4). For a blank determination about the same amount of adsorbent was extracted.

TABLE 1 Reproducibility of the Analysis of a Standard Mixture of Triglycerides

Components	Weight % in Mixture	Found	s_M*
		wt %	
PPP	10.60	10.05	0.07
PPE	11.00	10.40	0.06
PPO	20.85	20.15	0.06
EEE	15.05	15.15	0.08
StOO	20.65	20.95	0.09
OOO	21.85	21.65	0.04
Recovery		98.4	0.15

* Standard deviation of the mean.

A standard mixture of synthetic triglycerides, namely PPP, PPE, PPO, EEE, StOO, and OOO was analyzed four times: 83.2 mg of the standard mixture was separated on a 20 x 40 cm plate using petroleum ether (bp 40–60°)–benzene 1:4 (v/v) as eluent. The reproducibility and recovery are shown in Table 1.

Mono-, di-, and triglycerides were separated on 1 mm thick layers of silica gel (without silver nitrate) by the ascending technique using *iso*-octane–ether 1:1 (v/v) as developer. The silicic acid (Mallinckrodt 100 mesh) was purified beforehand by extraction with methanol–acetone 1:1 (v/v) and mixed with 8% $CaSO_4 \cdot {}^1/_2H_2O$. After the separation, the glycerides were detected in ultraviolet light after spraying with a 1% (w/v) Ultraphor WT solution[2] and extracted from the silica with ethanol–acetone mixtures. The mean recovery, obtained by analysis of three different mixtures of mono-, di-, and triglycerides, was 99.5%, s_M = 1.5. A technical monoglyceride has

TABLE 2 Reproducibility of the Analysis of a Technical Monoglyceride*

Component	Found	s_M
	wt %	
Monoglycerides	35.6	0.1
Diglycerides	50.9	0.2
Triglycerides	12.9	0.3

* The material was found to contain 0.7% glycerol.

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[1] S = saturated, St = stearoyl, P = palmitoyl, E = elaidoyl, O = oleoyl, and L = linoleoyl.

[2] Optical Division, BASF, Ludwigshafen/Rhein, Germany.

been analyzed four times to demonstrate the reproducibility of the method (see Table 2).

Both tables demonstrate that glycerides can be accurately estimated by means of TLC on layers of adsorbent 1 mm thick.

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