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Quantitative micro thin-layer chromatography of fatty materials

Quantitative separation by gas–liquid chromatography (GLC) has become an almost indispensable analytical tool in many fields including lipids. For certain fatty materials, such as those containing hydroxy or epoxy groups or conjugated systems, there is a risk of alteration of the structure at the high temperatures necessary for resolution by GLC. Thin-layer chromatography (TLC) is a mild, room-temperature technique with remarkable powers of resolution, and its quantitation could overcome some of these limitations of GLC. Macro-quantitation of TLC has been achieved by several workers (for a review, see MANGOLD[1]). In the present paper a procedure is reported for quantitation on microscope slides (microchromato plates) with several variations of the TLC technique, *viz.* direct, reversed-phase, boric acid-coated, silver nitrate-coated, etc., which are useful for specific separations. The technique matches GLC in speed, requiring only 3–5 min for the separation and about 30 min for quantitation thereafter.

Experimental

Materials. The materials used in this investigation were prepared and purified by conventional methods.

Method. A slurry of silica gel G (30 g) was prepared in a solution of chloroform–methanol (80:20, v/v)[2]. Two glass microchromatoplates (2.5 × 7.5 cm) held flat together were dipped in the slurry and withdrawn. After drying at room temperature, they were activated by heating at 110° for 30 min.

These plates were further treated for the special separations. Reversed-phase plates were obtained by impregnation by the ascending technique with a 5 % silicone oil–ether solution[3]. Spraying with a 6.25 % solution of silver nitrate yielded argentated plates. Boric acid plates were obtained by upward development in a clear saturated aqueous solution of boric acid. The plate was spotted with a chloroform solution of the mixture to be analysed *ca.* 1 cm from the bottom at the centre. Development was conducted in small covered glass jars or glass beakers for *ca.* 3 min.

Solvent systems and techniques used in the separation of various mixtures are shown in Table I. After development, the plates were dried at room temperature, sprayed lightly with chromic–sulphuric mixture (a saturated solution of potassium dichromate in 80 %, by wt., sulphuric acid[4]), and heated for 30 min at 170–180°. The microchromatoplate containing the charred spots was cooled to room temperature and scanned on the stage of an automatic densitometer ('Chromoscan', model 64/7/CSC, Joyce, Loebel & Co. Ltd, Princesway Team Valley, Gateshead-on-Tyne, England) with the slit opening at 1 × 10 mm. In this instrument the integrated numbers of the peak area are automatically recorded on a counter.

Results and discussion

Table I shows the mixtures separated, the plates and systems used and the quantitative estimation of the components. The separation of methyl palmitate and methyl stearate by reversed-phase TLC using an acetonitrile–acetic acid–water (70:10:20, v/v) system serves as an example. The peaks obtained for the charred spots on the Chromoscan were smooth and well separated. The integrated numbers were all corrected to allow for differences in the carbon density using stearate as standard (any relative standard will suffice) and the % wt. of the individual components calculated as in Table II.

The correction factor for methyl palmitate is given by carbon density of stearate/carbon density of palmitate, or 76.50/75.55, which is 1.013. Correction factors for some other methyl esters used in the present work are: methyl oleate 0.9933; methyl linoleate 0.9865; methyl 9,10-epoxystearate 1.047. Triplicate readings of the charred spots gave identical integrated numbers. As the length of the slit is 10 mm, the spread of the spot should not exceed this figure, and the amount of material spotted must be adjusted accordingly. The quantitations shown in Table I were similarly obtained. The values by densitometry agree with the calculated values to within ± 2 % for all the systems and plate coatings examined.

Reversed-phase TLC separates saturated homologues, and argentation TLC resolves according to degree of unsaturation. Application of these procedures to various combinations of the usual saturated (methyl palmitate and stearate) and unsaturated (methyl oleate and linoleate) esters are shown in examples 1, 2 and 3 of Table I. Quantitation is seen to be satisfactory. Separation of acetylenic compounds of C_{18} and C_{22} chain length (Item No. 4, Table I) using reversed-phase TLC and a solvent system of acetonitrile–acetic acid–water (70:10:20, v/v) can also be quantitated.

Estimation of unsaturated, epoxy and dihydroxy esters after resolution is shown in Items No. 5 and 6 of Table I. This separation could be useful in the identification of epoxy compounds in vegetable oils. It has also been applied in other work to the estimation of the products of epoxidation of unsaturated fatty acids with peracids, which consist of the unreacted material along with monoepoxy, monohydroxy, hydroxyacetoxy and dihydroxy esters.

The satisfactory quantitation of separations on boric acid-coated plates (Item No. 7: *erythro* from *threo* dihydroxy esters) has found practical application in analysis of the products of reduction of diketo fatty acids with sodium borohydride, which was shown to furnish almost equal amounts of both *erythro* and *threo* dihydroxy fatty acids.

Finally in the preparation of epithio from epoxy compounds[5], the extent to

J. Chromatog., 26 (1967) 286–289

TABLE I

QUANTITATIVE ESTIMATION OF FATTY MATERIALS BY MICRO TLC

Item No.	*Mixture*	*TLC technique*	*Solvent system*	*Taken wt. %*	*Found*		
					Integrated number	*Integrated number corrected to stearate*	*wt. %*
1	Methyl palmitate	Reversed-phase	Acetonitrile–acetic acid–water	36.7	92	93.2	36.1
	Methyl stearate		(70:10:20)	63.3	165	165.0	63.9
2	Methyl palmitate	Argentated	Ether–*n*-hexane–acetic acid	20.2	17	17.2	20.4
	Methyl oleate		(3:96:1)	40.2	33	32.8	38.8
	Methyl linoleate			39.6	35	34.5	40.8
3	Methyl stearate	Argentated	Ether–*n*-hexane–acetic acid	8.0	12	12.0	8.9
	Methyl oleate		(3:96:1)	44.5	60	59.6	44.2
	Methyl linoleate			47.5	64	63.2	46.9
4	Methyl stearolate	Reversed-phase	Acetonitrile–acetic acid–water	61.9	81	78.6	61.9
	Methyl behenolate		(70:10:20)	38.1	49	48.3	38.1
5	Methyl oleate	Direct	Ether–petroleum ether–acetic acid	62.8	144	143.0	60.8
	Methyl 9,10-epoxystearate		(15:84:1)	37.2	88	92.2	39.2
6	Methyl 9,10-epoxy-stearate	Direct	Ether–petroleum ether–acetic acid	74.1	96	100.6	73.3
	Methyl *threo*-9,10-dihydroxystearate		(40:59:1)	25.9	33	36.6	26.7
7	Methyl *threo*-9,10-dihydroxystearate	Boric acid-coated	Ether–petroleum ether–acetic acid (50:49:1)	40.6	44	48.7	40.4
	Methyl *erythro*-9,10-dihydroxystearate			59.4	65	72.0	59.6
8	*cis*-6,7-Epoxystearic acid	Direct	Ether–petroleum ether–acetic acid (20:79:1)	74.4	115	121.3	73.2
	cis-6,7-Epithiostearic acid			25.6	40	44.5	26.8

TABLE II

Fatty ester	*Taken wt. %*	*Found*		
		Integrated number	*Integrated number corrected to stearate*	*wt. %*
Methyl palmitate	36.7	92	93.2	36.1
Methyl stearate	63.3	165	165.0	63.9
			258.2	

which the reaction has proceeded can be followed by this method (Item No. 8, Table I).

Quantitative micro TLC has wide-ranging possibilities in the analysis of fatty compounds. The same results have been obtained on longer 2.5 × 20 cm plates; the longer resolving path is advantageous when there are four or more components in the mixture.

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