THE SEPARATION OF FATTY ACID METHYL ESTERS (INCLUDING "CRITICAL PAIRS") BY THIN-LAYER PARTITION CHROMATOGRAPHY

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Mixtures of saturated higher fatty acids and of unsaturated higher fatty acids, or their methyl esters, may be separated by partition chromatography, but it has been observed that the introduction of a double bond into a molecule gives a change in partition coefficient roughly equivalent to that produced by a reduction in chain length of two methylene groups[1,2]. Thus in the partition chromatography of fatty acids or their methyl esters using a hydrocarbon[3,4] or silicone oil[5] stationary phase, palmitic and oleic acids or their esters, for example, run together and appear as a single spot on the developed chromatogram. Such acids, or their esters, have been termed "critical pairs"[3,6,7]. Numerous methods have been suggested in order to decide whether a spot is composed of saturated or unsaturated material, or both, but these usually require the running of a second chromatogram (or the original chromatogram in the second dimension) after hydrogenation[2,4], oxidation[2,7], or complexing[6] of any unsaturated species present. MICHALEC[8], however, has separated palmitic and oleic acids by two-dimensional paper partition chromatography, development in the second direction being carried out at —8°. APARICIO[9] has shown that lauric acid may be separated from linolenic acid and that myristic may be separated from linoleic acid by paper chromatography, using undecane as stationary phase and aqueous acetic acid as mobile phase.

We now wish to report the separation of critical pairs by thin-layer partition chromatography of fatty acid methyl esters. The identification of the components is facilitated by the use of a detecting agent which gives different coloured spots for saturated and unsaturated methyl esters.

EXPERIMENTAL

Thin layers of Kieselgur G (Merck) (300 μ) were prepared and dried at 100°. After cooling, the layers were impregnated by immersion in 10 % v/v liquid paraffin B.P. in petroleum spirit (b.p. 60–80°); the solvent was allowed to evaporate at room temperature for 0.5–24 h.

Methyl esters were prepared from fatty acids (L. Light & Co.) using 12 % BF_3 in methanol[10]. Aliquots of solutions of the esters in petroleum spirit (b.p. 60–80°) were applied to the layer surfaces using a Hamilton Microliter Syringe and chromatograms were developed at ambient temperature using a nitromethane–acetonitrile–

acetic acid (75:10:10) mixture over a 10 cm run. It was not found necessary to equilibrate the mobile with the stationary phase. The developed chromatogram was dried and the resolved materials were detected by spraying with a saturated aqueous solution of ferric chloride followed immediately by a 0.1 *M* aqueous solution of sodium molybdate[11], and heating at 140° for about 3–5 min. A very fine spray is required for this operation and the spray jar described by KIRCHNER *et al*[12] was found to be suitable. The detection of unsaturated material with iodine vapour was occasionally found to be advantageous; this in no way affected the result obtained by subsequent spraying with the ferric chloride–sodium molybdate reagent.

RESULTS AND DISCUSSION

Various loadings of liquid paraffin, vaseline and silicone oil (MS200) were used as stationary phase in conjunction with different combinations of acetonitrile, acetic acid, nitromethene, ethanol, acetone, dioxan and water as mobile phase. Best results were obtained using the solvent system specified above together with layers impregnated with 10% liquid paraffin in petroleum spirit (giving a loading of 30 g/100 g Kieselgur G) but small variations in the acetonitrile and acetic acid contents of the mobile phase had no appreciable effect on the result. The chromatograms took 20–25 min to develop in this solvent system.

After treatment of the chromatograms with the ferric chloride–sodium molybdate detecting agent the saturated methyl esters gave orange spots and the unsaturated methyl esters blue-purple spots on a brown background. It was found desirable to

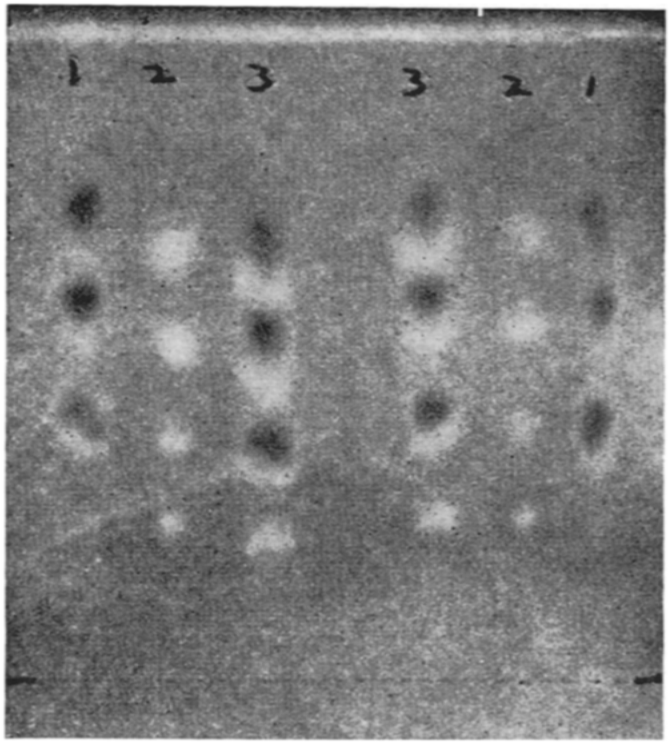

Fig. 1. Separation of fatty acid methyl ester mixtures. Mobile phase CH_3NO_2–CH_3CN–CH_3COOH (75:10:10). Detection: $FeCl_3$–Na_2MoO_4. Dark spots: unsaturated. Light spots: saturated. From top to bottom: (1) C_{18}''', C_{18}'', C_{18}'. (2) C_{12}, C_{14}, C_{16}, C_{18}. (3) C_{18}''', C_{12}, C_{18}'', C_{14}, C_{18}', C_{16}, C_{18}.

watch the chromatogram during heating and sometimes to ring the unsaturated spots when they appeared, as they tended to fade while the saturated spots were being intensified. Excess heating is to be avoided once all the spots are at maximum intensity. The blue-purple spot of methyl oleate was often found to be surrounded by a pale, light coloured halo; experience enables this to be distinguished from slight palmitate contamination.

The R_F values and detection limits for several fatty acid methyl esters are given in Table I, and Fig. 1 shows the separation obtainable for mixtures of unsaturated,

TABLE I

R_F VALUES AND DETECTION LIMITS FOR FATTY ACID METHYL ESTERS

Mobile phase CH_3NO_2–CH_3CN–CH_3COOH (75:10:10)

Methyl esters	R_F *(average of eight determinations)* ± 0.03	$FeCl_3$–Na_2MoO_4		I_2
		μg	*μg/cm²*	*μg*
C_{18}'''	0.69	11	100	1.0
C_{12}	0.62	6	40	
C_{18}''	0.56	11	110	1.0
C_{14}	0.49	4	30	
C_{18}'	0.39	21	270	1.5
C_{16}	0.35	2	20	
C_{18}	0.24	2	30	

C_{12} = methyl laurate
C_{14} = methyl myristate
C_{16} = methyl palmitate
C_{18} = methyl stearate
C_{18}' = methyl oleate
C_{18}'' = methyl linoleate
C_{18}''' = methyl linolenate

saturated, and saturated + unsaturated methyl esters. Methyl linolenate is completely separated from laurate and linoleate from myristate but we were unable to separate completely oleate from palmitate, though such separation was almost achieved using this solvent system.

The minimum quantity of one member of a critical pair which is definitely detectable in a mixture of both members is given in Table II. From this table it will be seen that it is often desirable to use both iodine and ferric chloride–sodium molybdate as detecting agents.

TABLE II

LIMITS OF DETECTION OF ONE MEMBER OF A MIXTURE OF CRITICAL PAIRS (%)

Critical pair mixture	*Detection method*		
	$FeCl_3/Na_2MoO_4$		I_2
	Saturated	*Unsaturated*	*Unsaturated*
C_{18}'''–C_{12}	25	6	1.5
C_{18}''–C_{14}	6	12	1.5
C_{18}'–C_{16}	1.5	50	3

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SUMMARY

The separation of critical pairs of fatty acid methyl esters has been carried out by thin-layer partition chromatography, complete separation of linolenate and laurate, linoleate and myristate, and almost complete separation of oleate and palmitate being achieved. The chromatograms took about twenty minutes to develop. A detecting agent was used which gives different coloured spots for saturated and unsaturated methyl esters.

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