

## The thin-layer chromatographic separation of fatty acid methyl esters according to both chain length and unsaturation

In partition chromatography of fatty acids or their methyl esters, both direct and reversed-phase, critical pairs occur mainly because the presence of a double bond alters the partition coefficient to the same extent as a reduction in chain length by two methylene groups. In chromatography on silica impregnated with silver nitrate, resolution is based on the degree and type of unsaturation, all the saturates moving together.

A two-dimensional thin-layer chromatographic (TLC) method was reported<sup>1</sup> in 1964 for the separation of fatty acid methyl esters according to chain length, structure and configuration, using a reversed-phase separation in the first dimension, followed by impregnation with silver nitrate before the second development. HAMMONDS AND SHONE<sup>2</sup> attempted to separate the critical pairs of fatty acid methyl esters on Kieselguhr G plates impregnated with liquid paraffin, using nitromethane-acetonitrile-acetic acid (70:10:10) for development. Methyl linolenate was separated completely from laurate, and linoleate from myristate, but oleate was not fully resolved from palmitate. Recently VERESHCHAGIN<sup>3</sup> developed a paper chromatographic method for the separation of unsaturated fatty acid methyl esters as their silver complexes by reversed-phase partition chromatography in the solvent system 70-100% aqueous methanol saturated with silver nitrate and dodecane. The unsatu-

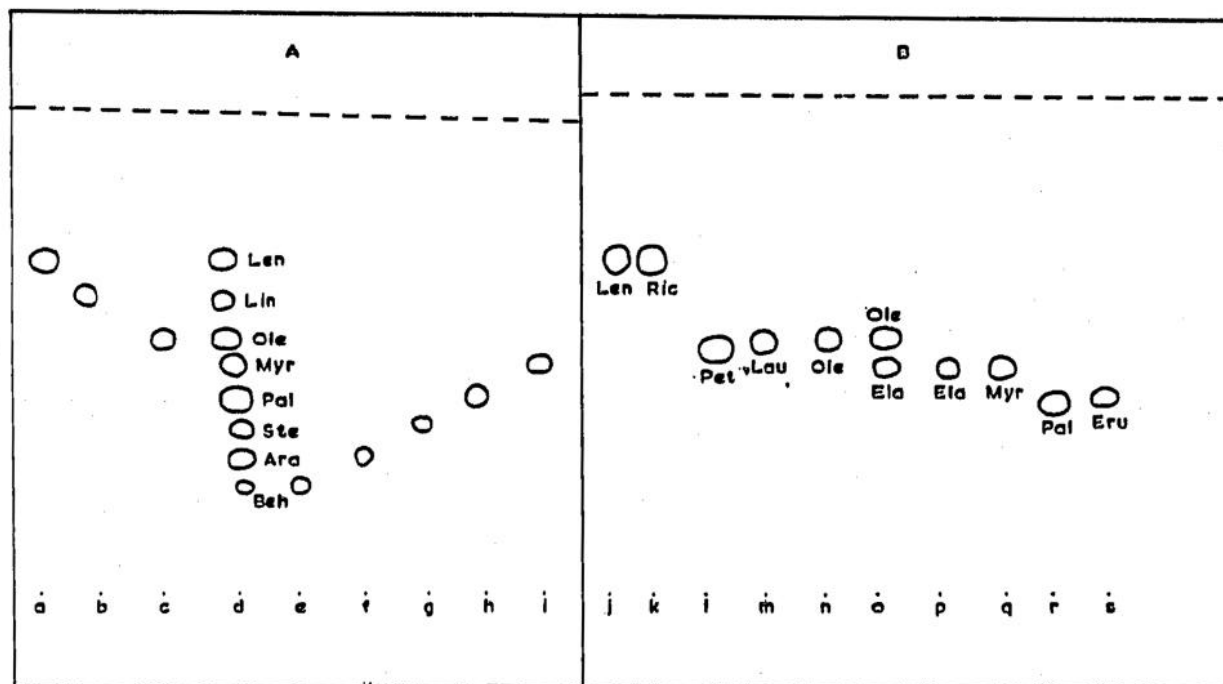


Fig. 1. TLC of fatty acid methyl esters. Plate: Silica gel G, impregnation with Dow Corning silicone fluid 200 followed by silver nitrate spray. Solvent: 95% (v/v) aqueous methanol saturated with silicone oil and silver nitrate. Detection: phosphomolybdic acid, hot plate at 200°. Compounds: Methyl ester of acid(s): a = linolenic, b = linoleic, c = oleic, d = mixture of a-c and e-i, e = behenic, f = arachidic, g = stearic, h = palmitic, i = myristic, j = linolenic, k = ricinoleic, l = petroselinic, m = lauric, n = oleic, o = mixture of n and p, p = elaidic, q = myristic, r = palmitic, s = erucic.

rated esters were resolved on the basis of chain length, and number and position of double bonds, but all the saturated esters remained at the baseline.

In the present communication, a TLC method is reported for the separation of fatty acid methyl esters according to both chain length and unsaturation on silica gel G impregnated with silicone oil and silver nitrate.

### Experimental

Silica gel G (E. Merck) was coated in a 250  $\mu$  thick layer on glass plates (20  $\times$  20 cm). The plates were impregnated with silicone oil (Dow Corning silicone fluid 200) by allowing a 5% solution in petroleum ether (60–80°) to ascend the plate in a developing chamber<sup>4</sup>. A 10% solution of silver nitrate in 50% aqueous ethanol was sprayed on the plates, which were then dried at 105° for 15 min. About 3–5  $\mu$ g of pure fatty acid methyl esters were spotted. The plates were developed for about 2 h with 95% (v/v) aqueous methanol saturated with silicone oil and silver nitrate. For detection, the plates were sprayed with a 20% ethanolic solution of phosphomolybdic acid and heated on a large hot plate at 200°, when blue spots appeared on a yellow background.

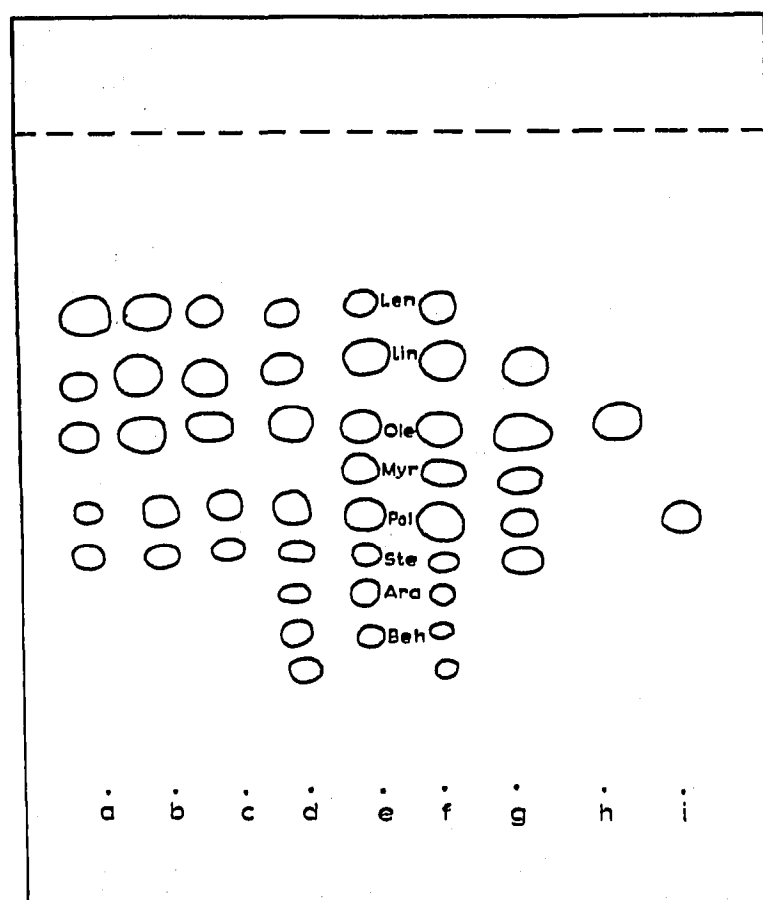


Fig. 2. TLC of vegetable oil mixed fatty acid methyl esters. Conditions: as for Fig. 1. Compounds: Methyl esters of acid(s): a = castor oil, b = linseed oil, c = safflower oil, d = groundnut oil, e = mixture of linolenic, linoleic, oleic, myristic, palmitic, stearic, arachidic and behenic, f = mustard oil, g = coconut oil, h = lauric, i = erucic.

*Results and discussion*

Fig. 1 shows the TLC separation of pure fatty acid methyl esters. The two sections depict separate runs, and the same compound may occur in both plates.  $R_F \times 100$  values were as follows: laurate 53, myristate 47, palmitate 41, stearate 34, arachidate 28, behenate 21, oleate 53, petroselinat 53, elaidate 47, linoleate 61, linolenate 70, ricinoleate 70, and erucate 41. Fig. 1A shows a clear separation of unsaturated esters of the same chain length differing in the number of double bonds, such as oleate, linoleate and linolenate, and of saturated esters differing in chain length by two carbon atoms. The common critical pairs, *viz.* linoleate and myristate, oleate and palmitate, are fully resolved.

Separation of a *cis* from a *trans* isomer, *viz.* oleate and elaidate, is also achieved (Fig. 1B). Positional isomers, such as oleate and petroselinat, are not resolved. The following esters also move together: linolenate and ricinoleate, oleate and laurate, elaidate and myristate, and erucate and palmitate. These pairs can of course be separated by ordinary reversed-phase TLC using silicone oil as the stationary phase<sup>5</sup>.

Fig. 2 shows the TLC separation of the methyl esters of the mixed fatty acids of six vegetable oils of known composition. A clear separation of the esters, with the exception of the pairs just noted, was achieved.

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Received July 16th, 1965

*J. Chromatog.*, 21 (1966) 141-143

## Dünnschichtchromatographische Trennung einiger Purinderivate einschliesslich Harnsäure

Über die Trennung von Xanthin- bzw. Purinderivaten liegen Literaturangaben vor.

BAEHLER<sup>1</sup> trennte Theophyllin, Theobromin und Coffein:

(1) Äthylacetat-Methanol-12 N HCl (18:2:0.05);

(2) Äthylacetat-Methanol-Eisessig (8:1:1);

(3) Chloroform-Methanol (19:1).

Der Nachweis erfolgte durch Sublimation an einer über das Chromatogramm gelegten gekühlten Glasplatte.

TEICHERT, MUTSCHLER UND ROCHELMMEYER<sup>2</sup>, verwandten mit Phosphat gepufferte Kieselgel G-Schichten und als Fließmittel Chloroform-Äthanol (96 %ig)

*J. Chromatog.*, 21 (1966) 143-145