COMPARISON OF COLUMN, THIN-LAYER AND PAPER CHROMATOGRAPHY OF OXIDIZED ALKYL OLEATES

J. POKORNÝ AND J. HLADÍK

Faculty of Food Technology, Institute of Chemical Technology, Technical University, Prague (Czechoslovakia)

SUMMARY

Oxidized oleic acid esters are separated by means of column chromatography into several fractions, the purity and composition of which may be determined either by chromatography on silicic acid impregnated paper or by thin-layer chromatography. The two methods are considered equally suitable.

Autoxidized unsaturated fatty acid derivatives are complex mixtures of products. The analysis is very difficult as other oxidation products of the mixture interfere with the determination of individual components. A separation of the mixture into simple fractions prior to the analysis is, therefore, the method of choice.

Oxidized lipids are usually separated by column chromatography[1–4] on polar or non-polar adsorbents. The composition of the fractions thus obtained is best determined by paper or thin-layer chromatography[5–8]. The subject of this study was to compare the suitability of these chromatographic methods, pure oleic acid esters being used as model substances.

EXPERIMENTAL

Chemicals

Methyl and isopropyl oleates were prepared by direct esterification of oleic acid (Koch-Light Laboratories, Colnbrook); they were oxidized by atmospheric oxygen in 5 mm layers at 20 and 60°. Silica Gel CH (Spolana, Neratovice) for column and thin-layer chromatography was purified by the usual procedure[9], Silica Gel G for thin-layer chromatography was prepared according to STAHL. Phosphomolybdic acid was used in 6% ethanolic solution. A 0.001% Rhodamine B solution in 0.25 *M* sec potassium phosphate was used.

Column chromatography

The size of silica gel columns was 1.2 × 16 cm and for each 7 g of silica gel an amount of 50 mg of sample was weighed out. The elution was carried out by a modified procedure of HIRSCH AND AHRENS[9] as shown in Table I. The identification of the fractions has been described in ref. 10. The reproducibility of the separation was good and the individual fractions were contaminated only by traces of neighbouring

TABLE I

ELUTION OF FRACTIONS IN THE CASE OF COLUMN CHROMATOGRAPHY

Fraction No.	*Hexane (%)*	*Diethyl ether (%)*	*Volume of fraction (ml)*
1	100	0	100
2	98	2	200
3	95	5	250
4	90	10	200
5	70	30	200
6	0	100	150

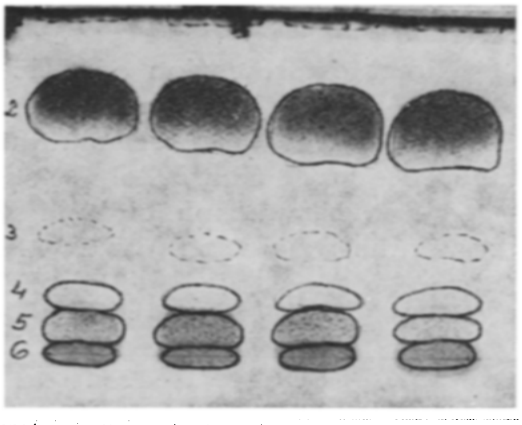
fractions, if at all. It is necessary to analyze the sample immediately or to protect it against a further autoxidation or other various spontaneous secondary reactions of peroxides by storage in an inert gas or at low temperatures.

Thin-layer chromatography

The Shandon apparatus was used for the preparation of thin layers; the Silica Gel A layers were activated by heating to 105° for 20 min. The solvent mixture consisted of 80% heptane and 20% diethyl ether with the possible addition of 1% acetic acid. The spots were detected by spraying with phosphomolybdic acid followed by heating to 115°.

Paper chromatography

Whatman No. 3 filter papers impregnated with silica gel according to MICHALEC and co-workers[11] were used. The solvent mixture contained 98.5% hexane and 1.5% diethyl ether, the development time being 30 min at 20° and the running distance 12.5 cm. Detection was with iodine vapour until yellow spots appeared (exposure time about 10 min); this was followed by treatment with the Rhodamine B solution as described by ROUSER and collaborators[12] and washing with tap water until the background becomes white.



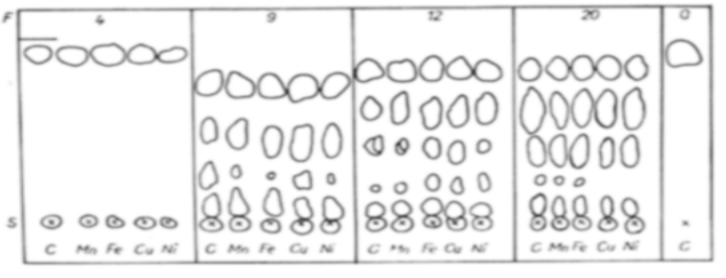


Fig. 1. Separation of oxidized isopropyl oleate on silicic acid impregnated paper. The sample was isopropyl oleate oxidized for 6 days at 60°.

Fig. 2. Separation of oxidized methyl oleate on Silica Gel CH thin layers. F = Front, S = start; 4, 9, 12, 20, 0 = days of oxidation at 60°.

RESULTS

An example of a separation of oxidized isopropyl oleate on silicic acid impregnated paper is shown in Fig. 1, while Fig. 2 shows one on silica gel thin layers. Both methods were suitable for the qualitative evaluation of the purity and composition of fractions. The reproducibility of R_F values was within 0.006–0.016 according to

TABLE II

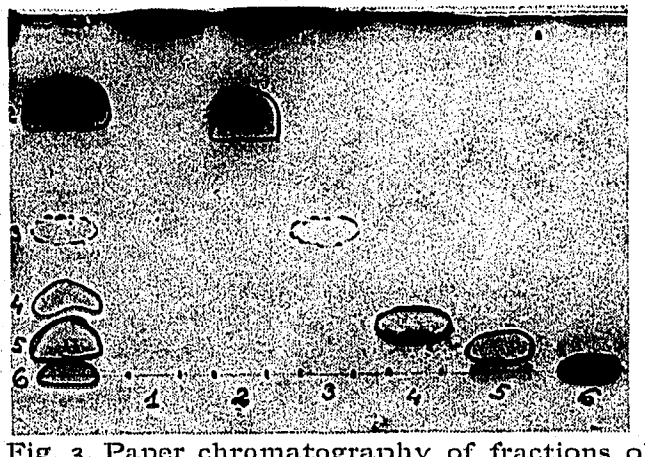
REPRODUCIBILITY OF R_F VALUES IN THE CHROMATOGRAPHIC SEPARATION OF FRACTIONS

$\bar{x}$ = average value, s = estimation of standard deviation, v = variation coefficient. The values were calculated on the basis of ten independent determinations.

Fraction No.	*Paper chromatography*			*Thin-layer chromatography*		
	$\bar{x}$	$s \times 10^2$	v (%)	$\bar{x}$	$s \times 10^2$	v (%)
1	0.98	0.65	0.67	0.98	0.44	0.50
2	0.66	1.62	2.46	0.90	1.85	3.14
3	0.28	1.62	5.81	0.81	2.02	7.20
4	0.18	0.98	5.24	0.67	1.02	5.66
5	0.11	0.65	5.90	0.25	0.56	4.90
6	0.00	—	—	0.05	0.01	0.01

the spot in the case of the paper chromatography, the values corresponding to thin-layer chromatography being very similar (see Table II).

An example of the paper chromatography of fractions obtained by a column chromatographic separation is given in Fig. 3, while that of thin-layer chromatography is given in Fig. 4. The separation on thin layers of Silica Gel CH was similar to that on silicic acid impregnated paper while that on thin layers of Silica Gel G



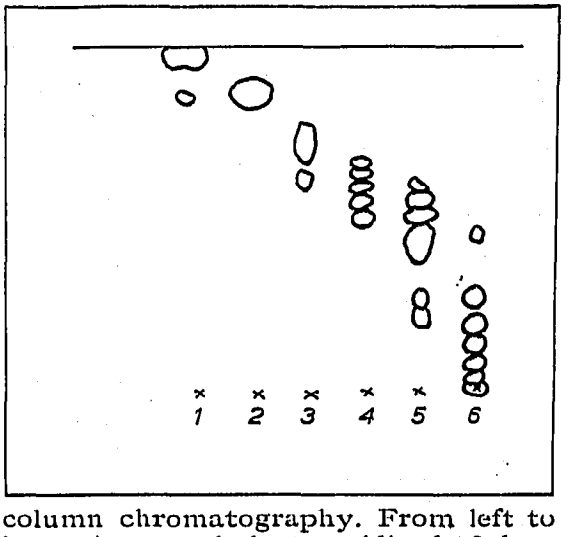


Fig. 3. Paper chromatography of fractions obtained by column chromatography. From left to right: the original sample and fractions No. 1–6; the sample was isopropyl oleate oxidized 28 days at 20°.

Fig. 4. Thin-layer chromatography of fractions obtained by column chromatography. From left to right: fractions No. 1–6; the sample was isopropyl oleate oxidized for 28 days at 20°; the adsorbent was Silica Gel C.

resulted in the isolation of a greater number of components in each fraction so that a more accurate determination of their composition was made possible.

CONCLUSIONS

Chromatography on silicic acid impregnated papers is equally as suitable for the control of fractions of oxidized esters of unsaturated fatty acids as thin-layer chromatography. The sensitivity of the two methods was approximately the same, the most suitable amount of sample being 200–500 μg when even the trace components are to be detected. The time needed for the separation is about the same, as the development time is longer in the case of thin-layer chromatography while the detection is longer in that of paper chromatography. The reproducibility of R_F values is also nearly the same.

REFERENCES

1 P. DESNUELLE AND M. BURNET, *Rev. Franc. Corps Gras*, 3 (1956) 325.
2 M. NAUDET, M. J. PERROT AND P. DESNUELLE, *Rev. Franc. Corps Gras*, 7 (1960) 429.
3 M. BURNET AND P. DESNUELLE, *Rev. Franc. Corps Gras*, 5 (1958) 194.
4 J. POKORNÝ AND H. ZWAIN, *Fette, Seifen, Anstrichmittel*, 69 (1967) 330.
5 H. P. KAUFMANN AND Y. S. KO, *Fette, Seifen, Anstrichmittel*, 63 (1961) 828.
6 H. P. KAUFMANN AND Z. MAKUS, *Fette, Seifen, Anstrichmittel*, 62 (1960) 1014.
7 R. SUBBARA, M. W. ROOMI, M. R. SUBBARAM AND K. T. ACHAYA, *J. Chromatog.*, 9 (1962) 295.
8 J. POKORNÝ AND E. DAVÍDKOVÁ, *Fette, Seifen, Anstrichmittel*, 68 (1966) 91.
9 J. HIRSCH AND E. H. AHRENS, *J. Biol. Chem.*, 233 (1958) 311.
10 J. HLADÍK, S. S. KONDRATENKO AND J. POKORNÝ, *Oléagineux*, in press.
11 Č. MICHALEC, Z. KOLMAN, M. ŠULC AND J. MĚŠŤAN, *J. Chromatog.*, 9 (1962) 237.
12 G. ROUSER, A. J. BAUMAN, N. NICOLAIDES AND D. HELLER, *J. Am. Oil Chemists' Soc.*, 38 (1961) 565.