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GAS CHROMATOGRAPHIC SEPARATION OF *cis–trans* ISOMERS: METHYL OLEATE/METHYL ELAIDATE

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SUMMARY

The factors which contribute to the separation of methyl elaidate/methyl oleate are examined. These factors include the nitrile content of the stationary phase, the type and nature of the support surface as well as the amount of stationary phase and the column temperature. Baseline separation of the elaidate/oleate pair is obtained with either 15% SP-2340 or OV-275 on Chromosorb P AW-DMCS.

INTRODUCTION

Although the separation of methyl elaidate (*trans*-9) and methyl oleate (*cis*-9) was reported by James and Martin[1], this separation has been a very difficult one to obtain. The conventional packed columns employing the various polyester stationary phases which are generally used to separate mixtures of fatty acid methyl esters do not readily separate this pair. With the advent of high-resolution capillary columns, this difficult separation has been more readily accomplished[2–5]. More recently a packed column employing a special silver complex has been reported by Magidman[6] for the gas-chromatographic separation of *cis–trans* alkene and the elaidate/oleate pair.

The object of this paper is to study the separation of the elaidate/oleate pair evaluating a number of cyanosilicone stationary phases and to determine those factors such as concentration of stationary phase, type of support and support treatment as well as column temperature and carrier gas flow-rate which contributes to the separation.

EXPERIMENTAL

The apparatus used was a Hewlett-Packard Model 7610 gas chromatograph (Hewlett-Packard, Avondale, Pa., U.S.A.) equipped with flame ionization detectors. The columns were 6 ft. × 2 mm I.D. × 1/4 in. O.D. glass U tubes and 20 ft. × 0.085 in. I.D. × 1/8 in. O.D. stainless steel.

All of the columns were evaluated with a standard synthetic mixture of 50% methyl elaidate and 50% methyl oleate consisting of 5 mg each in 1 ml of isooctane. A second test mixture consisted of 95% methyl oleate and 5% methyl elaidate with a

total of 10 mg in 1 ml of isooctane. The sample size injected into the chromatograph was 0.3 μl, using a SGE-Type-B standard syringe with a capacity of 0.5 μl.

The chromatographic support used was 100–120 mesh Chromosorb W acid-washed (AW) and Chromosorb W AW-DMCS, Chromosorb P AW and Chromosorb P AW-DMCS. The stationary phases used were DEGS, SP-2300, SP-2310, SP-2330, SP-2340 and OV-275, all obtained from Supelco stock. The supports were coated by depositing the stationary phase on the support from a chloroform solution. The columns were conditioned with a nitrogen flow-rate of 10 ml/min at a temperature of 200° for DEGS and 225° for the other columns.

RESULTS

The various stationary phases were compared under the same conditions using a standard test mixture consisting of methyl elaidate–methyl oleate (1:1). The peak separation defined by Kaiser[7] and Ettre[8] was measured and listed in Table I. The OV-275 column gave both the most complete and most rapid separation of the elaidate/oleate pair, while SP-2340 was second. DEGS and SP-2300 columns showed no separation of the pair.

TABLE I

COMPARISON OF STATIONARY PHASE

Column: 6 ft. × 2 mm I.D.; 15% stationary phase on 100–120 mesh Chromosorb P AW-DMCS; flow-rate: 10 ml/min.

Stationary phase	*Column temperature (°C)*	*Retention time (min)*		*Separation factor*	*Peak separation (%)*
		Elaidate	*Oleate*		
DEGS	200	33.4	33.4	0	0
SP-2300	225	30.7	30.7	0	0
SP-2310	225	13.7	14.1	1.029	10.8
SP-2330	225	10.7	10.8	1.048	32.2
SP-2340	225	6.5	6.8	1.054	37.0
OV-275	225	5.4	5.7	1.059	54.0

A second study was carried out where both the amount of SP-2340 stationary phase and the treatment of the support surface were compared at a variety of temperatures. Concentrations of 10% and 15% SP-2340 were used with Chromosorb W AW and Chromosorb W AW-DMCS. The peak separation data are presented in Table II. With 10% SP-2340, Chromosorb W AW-DMCS gave greater separation than did Chromosorb W AW. These results were checked with three separate batches of packing for each support and the results were the same in each case. With 15% SP-2340 the Chromosorb W AW gave greater separation than Chromosorb W AW-DMCS. These results were checked with two separate batches of packing to insure the ability to duplicate the results.

A third study was carried out in which the support evaluated was Chromosorb P. In a preliminary study with SP-2340 concentrations used were 10, 15, 20, and 25%. The 15% concentration gave the best separation. Using 15% SP-2340 Chromosorb P AW and Chromosorb P AW-DMCS, columns were compared at a variety of tem-

TABLE II

COMPARISON OF CHROMOSORB W AW AND AW-DMCS COATED WITH SP-2340
Column: 6 ft. × 2 mm I.D.; flow-rate: 10 ml/min.

SP-2340 (%)	*Column temperature (°C)*	*Chromosorb W AW*		*Chromosorb W AW-DMCS*	
		Retention time elaide (min)	*Peak separation (%)*	*Retention time elaidate (min)*	*Peak separation (%)*
10	175	12.3	12.1	10.6	28.5
10	185	7.6	13.8	6.9	18.5
10	190	6.2	13.5	5.6	23.9
10	200	4.5	11.6	4.2	22.8
10	210	3.2	10.0	3.1	16.9
10	220	2.4	9.1	2.4	12.2
15	185	11.4	40	12.3	34.0
15	200	6.4	38	7.0	31.0
15	210	4.7	35	5.1	25.0
15	220	3.6	30	3.8	21.0
15	230	2.7	27	2.9	16.0

TABLE III

COMPARISON OF CHROMOSORB P AW AND AW-DMCS COATED WITH 15% SP-2340
Column: 6 ft. × 2 mm I.D.; flow-rate: 10 ml/min.

Column temperature (°C)	*Chromosorb P AW*		*Chromosorb P AW-DMCS*	
	Retention time elaidate (min)	*Peak separation (%)*	*Retention time elaidate (min)*	*Peak separation (%)*
200	15.2	27.0	14.5	33.5
210	10.6	30.0	10.0	39.7
220	7.8	31.7	7.4	38.1
225	6.8	31.4	6.4	37.0
230	5.7	31.1	5.5	36.0
240	4.4	30.2	4.3	35.2

peratures. The results are presented in Table III. This study showed that the AW-DMCS form gave better separation than the AW form.

Using 15% OV-275 on Chromosorb W AW, the peak separation obtained was relatively poor with very poor column efficiencies, therefore no additional work was carried out with OV-275 on this support. Additional work was done using Chromosorb P AW-DMCS coated with OV-275 in concentrations of 15, 20, and 25% with 15% found to give the most complete separation. The data for OV-275 at various temperatures are listed in Table IV.

The effect of flow-rate on separation was also studied using nitrogen as the carrier gas. With the 6 ft. × 2 mm I.D. glass columns the peak separation improved as the flow-rate was reduced from 30 to 10 ml/min, which was the optimum flow-rate. Below 10 ml/min column efficiency decreased significantly.

Sample concentration was also found to be important in the performance of the column. A sample concentration of 10 mg/ml of solvent gave good separation. For the 0.3-μl injection, if higher concentration of sample were used, the peaks broad-

TABLE IV

SEPARATION WITH OV-275 ON CHROMOSORB P AW-DMCS

Column: 6 ft. × 2 mm I.D.; flow-rate: 10 ml/min.

OV-275 (%)	*Column temperature (°C)*	*Retention time elaidate (min)*	*Peak separation (%)*
15	200	10.5	55.3
15	210	7.3	56.3
15	215	6.1	58.7
15	220	5.3	63.7
15	225	4.6	54.0
20	220	5.2	46.0
25	220	6.6	43.0

ened considerably and separation was lost. Isooctane proved to be a good solvent. Chloroform is not recommended as it showed considerable tailing.

A study was made to determine the column length necessary to obtain baseline separation of a 1:1 mixture of the methyl elaidate/oleate pair. Column lengths of 10, 15, and 20 ft. × 1/8 in. stainless steel with 15% SP-2340 on 100–120 mesh Chromosorb P AW-DMCS were evaluated and only the 20-ft. column was found to be adequate to obtain baseline separation. The separation of the pair is shown in Fig. 1, while the separation of a 5% elaidate–95% oleate mixture is shown in Fig. 2. In addition, a 20 ft. × 1/8 in. stainless-steel column with 15% OV-275 on 100–120 mesh Chromosorb P AW-DMCS was tested with the 1:1 mixture and this separation is shown in Fig. 3. Preliminary work comparing a 20-ft. column containing SP-2340 and OV-275 with samples containing the elaidate/oleate pair in nature products indicates

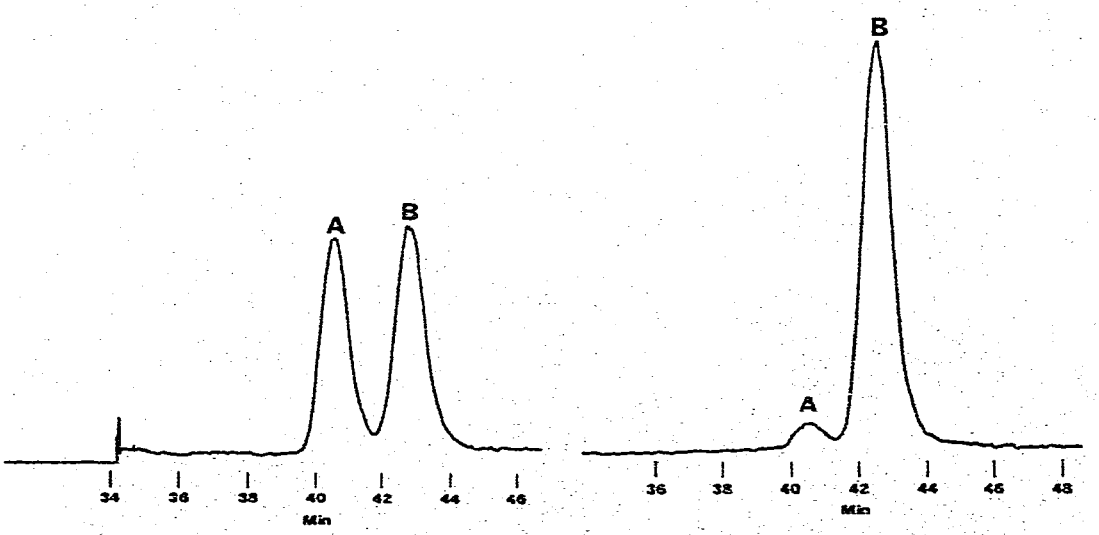


Fig. 1. Separation of a 1:1 mixture of methyl elaidate (A) and methyl oleate (B) using a 20 ft. × 1/8 in. stainless-steel column with 15% SP-2340 on 100–120 mesh Chromosorb P AW-DMCS. Column temperature, 225°; nitrogen flow-rate, 10 ml/min at 53 p.s.i; detector, FID at 8×10^{-10} a.f.s.

Fig. 2. Separation of a 5% elaidate (A)–95% oleate (B) mixture. Operating conditions, as in Fig. 1.

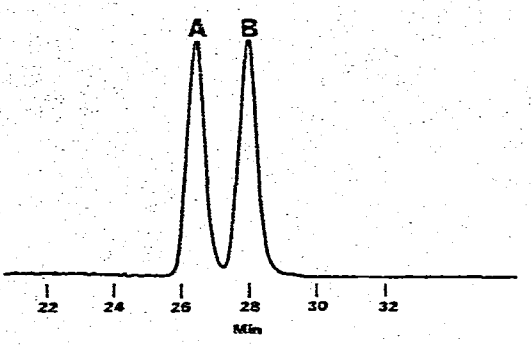


Fig. 3. Separation of methyl elaidate (A) and methyl oleate (B) using a 20 ft. × 1/8 in. stainless-steel column with 15% OV-275 on 100–120 mesh Chromosorb P AW-DMCS. Column temperature, 220°; nitrogen flow-rate, 10 ml/min at 40 p.s.i.; detector, FID at 8×10^{-10} a.f.s.

that the OV-275 is the superior column in terms of resolution and speed of analysis. With the 20-ft. column, the sample size was increased to 0.5 μl.

DISCUSSION

The inability to separate the elaidate/oleate pair has been due to the lack of either the suitable selectivity or inadequate thermal stability of the stationary phase. As an example, the highly polar DEGS, though capable of operating at 200° will not readily separate the pair. Litchfield *et al.*[2] were able to separate the pair with a capillary column coated with DEGS with a separation factor of 1.02. Cyanosilicone such as XE-60 or OV-225 can be operated at 250–275° but their nitrile content is too low to cause the separation. Stationary phase with a high cyano content such as 1,2,3-tris(2-cyanoethoxy)propane or tetracyanoethylated pentaerythritol do not have the necessary thermal stability to operate at column temperature necessary for the separation.

Litchfield *et al.*[3] noted that in working with capillary columns to separate *cis–trans* isomers, the separation was enhanced and the elution time reduced with increased nitrile content of the stationary phase. In their work, XE-60 with a nitrile content (% CN) of 13.6% and GE experimental polymer 238-149-99, with a nitrile content of 23% (our calculation), were evaluated. Even with a 200-ft. capillary they were unable to obtain baseline separation of the pair.

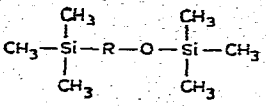
Working with packed columns to separate the elaidate/oleate pair we also see an improved separation and reduced retention time as the nitrile content increases using the newer cyanosilicone stationary phases. The structure of the SP-2300 series and their nitrile content are listed in Table V.

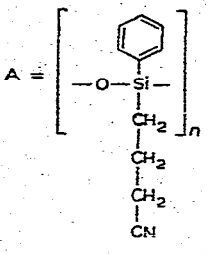
The OV-275 composition is not public knowledge as yet, but it is a cyanoethyl silicone with the highest cyano content of any of the available silicones.

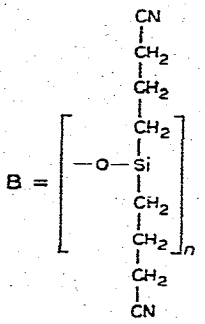
With respect to the column parameters necessary to optimize the elaidate/oleate separation, the amount of stationary phase, type of support and the nature of the support surface are important. Increasing the concentration of stationary phase improves the separation factor for the pair of isomers, but reduces the efficiency of the column. The 15% stationary phase concentration on both Chromosorb W and P appears to be the optimum in terms of separation factor and efficiency. Above 15%

TABLE V

STRUCTURE OF THE SP-2300 SERIES AND THEIR NITRILE CONTENT







	Structure	*% Nitrile*
SP-2300	R = A	12.4
SP-2310	R = AB	20.0
SP-2330	R = A9B	27.0
SP-2340	R = B	28.4

concentration, the decrease in efficiency off-sets any gain obtained by the improved separation factor. This is readily seen in Table VI, where the peak separation factor and column efficiency are listed for a number of the columns.

The use of Chromosorb P was considered because of its greater density and capacity to hold stationary phase than Chromosorb W. Using SP-2340 either support could be used with 15% concentration, but in the case of OV-275 Chromosorb P was the superior support.

A third factor contributing to the separation is the nature of the support surface. In a separate study[9] it has been found that the highly polar stationary phases such as SP-2340 and OV-275 exhibit poorer column efficiencies on a silanized (DMCS-treated) surface than on a non-silanized surface. The difference in efficiency is most noticeable above 5% and the difference in efficiency increases as the concentration is increased, as shown for the data on OV-275 in Table VI. This decrease in efficiency for the polar stationary phases on a silanized support is thought to be caused by inadequate wetting of the support surface.

A complicating issue appears to be the fact that the separation factor is improved by the silane-treated surface. This is shown in Table VI comparing the separation factors obtained with the silanized supports. In the case of 15% Chromosorb W AW the better column efficiency allows for a more complete separation compared to the 15% Chromosorb W AW-DMCS. In the case of 15% Chromosorb P AW-DMCS, the better separation factor was more important than the improved efficiency of the Chromosorb P AW.

TABLE VI

COMPARISON OF COLUMNS

Column: 6 ft. × 2 mm I.D.; flow-rate: 10 ml/min.

% Stationary phase	*Column temperature (°C)*	*Support*	*Peak separation (%)*	*Separation factor*	*Plates/ft.*
10% SP-2340	185	W AW	13.8	1.038	860
10% SP-2340	185	W AW-DMCS	18.5	1.047	702
15% SP-2340	210	W AW	38	1.052	814
15% SP-2340	210	W AW-DMCS	31	1.058	535
15% SP-2340	210	P AW	30	1.047	781
15% SP-2340	210	P AW-DMCS	40	1.057	638
15% OV-275	220	P AW-DMCS	63	1.05	841
20% OV-275	220	P AW-DMCS	47	1.06	492
25% OV-275	220	P AW-DMCS	43	1.07	424

CONCLUSIONS

The methyl elaidate/methyl oleate pair can be readily separated using 15% SP-2340 or 15% OV-275 on a 100–120 mesh Chromosorb P AW-DMCS, 20 ft. × 1/8 in. stainless-steel column operated with a flow of 10 ml/min nitrogen at a column temperature of 220°. A 0.5-μl sample containing approximately 10 mg/ml of isooctane is recommended.

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