

A Satisfactory GLC Column for the Determination of Epoxyoleic Acid in Seed Oils¹

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2036

Abstract

A number of stationary phases were evaluated for their suitability for the analysis of methyl epoxyoleate. Ethylene glycol succinate polyester, Carbowax 20M and Apiezon "L" all showed evidence of decomposition or alteration of the compound and also a loss of area on the chromatogram. A silicone rubber (SE-30) column did not exhibit this tendency and was found satisfactory for the determination of methyl epoxyoleate.

A limitation of the use of an SE-30 column in the analysis of mixed esters is its inability to separate effectively fatty acid esters of the same chain length. However, by chromatographing the sample on a polyester column as well as on an SE-30 column, all of the fatty acid components of the oil can be determined quantitatively.

Introduction

BECAUSE OF increasing industrial use of epoxy-fatty acids and their derivatives, there is growing interest in reliable methods for determining the composition of naturally occurring and chemically prepared epoxy oils. Previous work at this laboratory (1) demonstrated that alteration of methyl epoxyoleate occurred during the analysis of *Vernonia anthelmintica* seed oil (GLC) with a polyester stationary phase. Satisfactory values for the fatty acid composition of the oil were obtained only by employing an internal standard and applying correction factors.

The further study of the alteration of the methyl esters of epoxy-fatty acids during GLC analysis with the objective of finding more satisfactory column conditions and stationary phases for analysis of oils containing epoxy fatty acids is discussed in this paper.

Experimental

The procedures for the preparation of methyl esters of *Vernonia anthelmintica* and the isolation of methyl epoxyoleate have previously been described (1).

Gas-Liquid Chromatography. GLC analyses were performed with the apparatus described previously (2,3) and with one of our own design which could be temp programmed. Both employed a 4-filament thermal conductivity cell. Two columns were used for analyses. The first was a column of 2 ft x 3/16 in. (ID = 0.124 in.) stainless steel tube packed with 42-60 mesh acid and base washed Chromosorb "W" coated with 15% silicone polymer SE-30 (General Electric) and heated to 200C for isothermal operation or from 175-260C at a rate of 4°/min for temp programmed operation. The other column was an 8 ft x 1/8 in. (ID = 0.095 in.) stainless steel tube packed with 42-60 mesh acid and base washed Chromosorb "W" coated with 20% ethylene glycol succinate polyester (EGS) heated isothermally at 207C.

Helium flow was 40 ml/min and 30 ml/min, measured at the exit, for the silicone and polyester columns, respectively. Other columns employed in this study are described as part of the appropriate figure or table.

Thin Layer Chromatography. Thin layer chromatoplates were prepared with Silica Gel G according to the procedure of Stahl (4). The solvent system to develop the chromatograms was essentially that described by Morris (5). The developed plates were sprayed with sulfuric acid and then heated in order to visualize the components.

Oxirane. The percentage of oxirane oxygen was determined by the method of Durbetaki (6).

Results and Discussion

Pertinent portions of the chromatograms of methyl epoxyoleate are shown in Figure 1 which demonstrate the alteration that occurs on several different GLC substrates. The chromatogram in Figure 1,A was obtained when the 18:1 epoxy was chromatographed on Apiezon L. The solid line is a tracing of the first injection of sample. Each succeeding injection exhibited a more symmetrical curve until finally, after six or seven injections, the trace appeared as shown by the dashed line. However, the area of the epoxy compound relative to that found

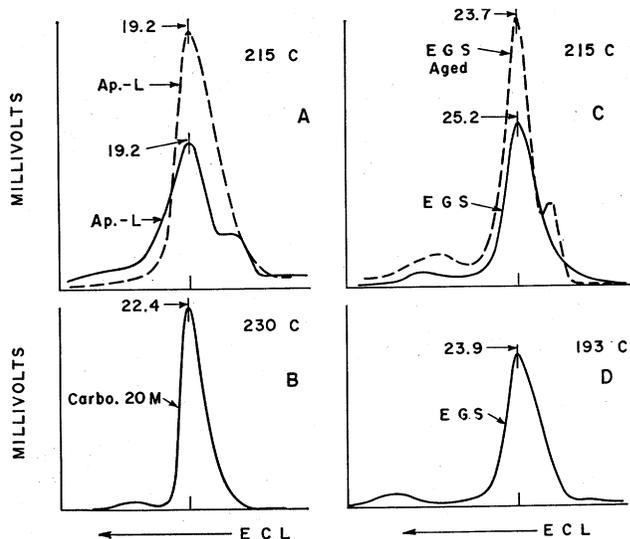


FIG. 1. Chromatograms of methyl epoxyoleate.

- 1st sample injection, Apiezon "L" (10%) coated on Chromosorb "W" (42-60 mesh), column 5-1/2 ft x 3/16 in. OD.
----- 7th sample injection, same column and conditions.
- Carbowax 20M (15%) coated on Gas Chrom "P" (60-80 mesh), column 5-1/2 ft x 3/16 in. OD.
- freshly prepared ethylene glycol succinate polyester (20%) coated on Chromosorb "W" (42-60 mesh), column 8 ft x 1/8 in. OD.
----- same column after use for some time (aged); same conditions.
- Freshly prepared ethylene glycol succinate polyester (12%) coated on Chromosorb "W" (42-

¹ Presented at the AOCS Meeting, Toronto, Canada, 1962.

TABLE I
GLC Analysis of Known Mixtures of Methyl Esters by Two Procedures

Mixture	Procedure ^a	Esters					
		16:0	18:0	18:1	18:2	18:1 epoxy	22:1
A.....	Known (wt %)	3.42	1.67	3.63	7.70	73.7	9.91
	SE-30 (isothermal) }	4.02	1.82	3.93	7.65	72.5(72.2) ^b	10.0
	EGS (isothermal)						
	SE-30 (programmed) }	3.98	1.81	3.90	7.59	73.1(74.8) ^b	9.69
EGS (isothermal)							
B.....	Known (wt %)	15.4	14.9	35.1	18.2	7.17	9.28
	SE-30 (isothermal) }	16.1	16.0	35.5	16.7	6.54(6.66) ^b	9.11
	EGS (isothermal)						
	SE-30 (programmed) }	16.5	16.0	35.4	16.7	6.72(7.20) ^b	8.66
EGS (isothermal)							
C.....	Known (wt %)	11.3	11.0	18.6	8.95	39.9	10.3
	SE-30 (isothermal) }	12.8	11.4	19.3	8.11	38.7(41.4) ^b	9.63
	EGS (isothermal)						
	SE-30 (programmed) }	12.8	11.7	19.9	8.34	38.9(39.3) ^b	10.0
EGS (isothermal)							

^a See text for columns and conditions.

^b Values in () were calculated from 22:1 as internal standard.

for normal straight chain methyl esters was 20–25% low. This loss was noted for the compound when chromatographed on each of the substrates shown in the figure. The degree of loss in area seemed related to the time of elution, a longer retention on the column resulted in a greater loss of area. Figure 1,B is the chromatogram of the epoxy ester on Carbowax 20M. Here the alteration is shown by the appearance of a peak following the major component. On ethylene glycol succinate polyester columns, the alteration is quite apparent. In Figure 1,C two chromatograms of the methyl epoxyoleate on an EGS column are shown. The curve represented by the solid line was obtained on a freshly prepared column where evidence of alteration is the small peak that follows the fairly symmetrical major peak (1). The dashed curve is the chromatogram of the epoxy compound after the column had been in use for some time, or aged. Here alteration is exhibited not only by the peak following the major component but also by a peak preceding it. To determine whether temperature played a part in the alteration, the temperature of the column was lowered 22 degrees. This tracing is shown in Figure 1,D and again the alteration occurred. No significant difference was observed when the column temperature was varied over the range of 190C–225C or the injection temperature varied between 195C–300C. The number on the peak in each chromatogram is the equivalent chain length (7) which will locate the peak on the chromatogram relative to the methyl esters of the normal saturated fatty acids. For example in Figure 1,C the methyl epoxyoleate would appear between the peaks of a C₂₅ and C₂₆ saturated methyl ester on the freshly prepared column while on the aged column, the epoxy ester would be found between the C₂₃ and C₂₄ saturated methyl ester. Here, the change in relative retention time as a result of aging of a polyester column is greater than generally found (3). In Figure 1,D the equivalent chain length of the 18:1 epoxy ester on a freshly prepared column is about the same as that found on the aged column in Figure 1,C. This can be attributed to the lower temperature and also to the reduction of amount of stationary phase from 25–15%.

Since it has been reported that a number of oxygenated compounds could be chromatographed quantitatively on silicone rubber columns (8,9,10,11), a column was prepared with silicone polymer SE-30 as the stationary phase. When methyl epoxyoleate was chromatographed on this column, a single symmetrical peak appeared which had an area comparable to that of esters of the normal fatty acids. Unfortunately, the saturated and unsaturated esters of the same chain length are not well separated on this

stationary phase. Therefore, in order to obtain a complete analysis of an oil containing epoxy compounds, it was necessary to chromatograph the sample on a polyester column as well as on the SE-30 column and calculate the composition in the following manner. The percentage of epoxyoleate and of each group of normal fatty esters of the same chain length was first determined from the area of the peaks on the chromatogram of the sample on the SE-30 column. The percentage of each group of normal esters was then distributed among the individual components of that group according to their ratios to each other as calculated from the chromatogram of the same sample on an EGS column. The epoxy ester on EGS was ignored since its percentage had been determined on the SE-30 column.

The analyses of several mixtures by the two column procedure show in Table I. Mixture A was prepared to approximate the composition of *Vernonia anthelmintica* seed oil. Mixture B would approximate seed oils with a low epoxy content and Mixture C contains an intermediate epoxy ester content. The ratios of C₁₈ esters were varied widely to demonstrate the applicability of the method to samples which differ considerably in composition. The analyses were made employing two columns, SE-30 and EGS. The EGS column was operated isothermally while the SE-30 column was operated both isothermally and temperature programmed. Both modes of operation gave essentially the same quantitation. No significant loss of area of the methyl epoxyoleate relative to normal C₁₈ saturated methyl esters is evident by comparing the percentage found by measurement of total area with that obtained by employing the 22:1 ester as an internal standard.

As further evidence that little or no alteration takes place during chromatography on a SE-30 column, the material represented by the peak of highly purified methyl epoxyoleate from this column and also from an EGS column was collected (3). The collected material from both columns and the original ester was chromatographed by TLC. The original ester and the ester collected from the SE-30 column appeared as a single spot while the material from the EGS column exhibited several spots, indicating alteration. The bleed from each column was also checked by TLC and at no time did spots occur in the region where the altered material was found.

Chromatograms of the methyl esters of *Vernonia anthelmintica* seed oil on two substrates show in Figure 2. The upper tracing was obtained from a temperature programmed heating of an SE-30 column and the lower curve from isothermal operation on an EGS column.

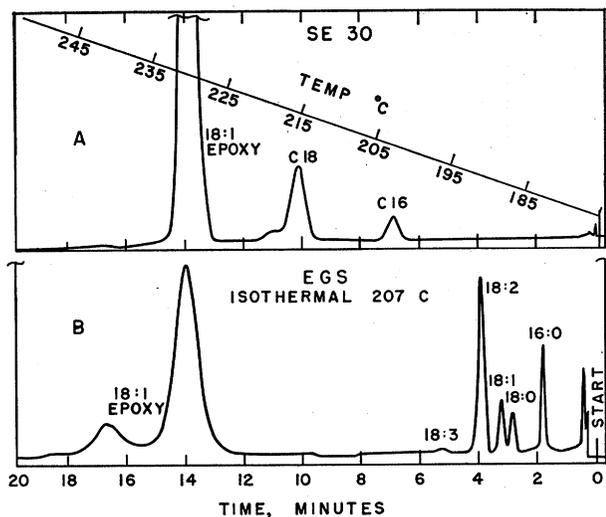


Fig. 2 Chromatograms of *Vernonia anthelmintica* seed oil methyl esters.

A. Temperature programmed, SE-30 stationary phase (see text).
 B. Isothermal, EGS stationary phase (see text).

The analysis of the *Vernonia anthelmintica* seed oil methyl esters by two procedures is given in Table II. One analysis was obtained by combining data from the SE-30 and EGS columns as described in this paper. The other was determined from data obtained from the EGS column only but where an internal standard and correction factors were applied as described in a previous publication (1). The agreement was good. However, the combined SE-30 + EGS procedure requires considerably less calculation and we believe the analysis can be more readily reproduced.

TABLE II
 GLC Analysis of *Vernonia anthelmintica* Seed Oil

Components	Method A ^a	Method B ^b
	%	%
16:0.....	2.52	1.94
18:0.....	1.37	1.40
18:1.....	1.88	2.05
18:2.....	8.10	7.86
18:3.....	0.26	0.30
18:1 epoxy ^c	76.7	76.4
Other fatty acids.....	1.35	2.31
Unsap.....	7.76	7.76

^a SE-30 (programmed) and EGS (isothermal); see text for columns and conditions.

^b EGS (isothermal) + internal standard (15:0) + correction factors (1).

^c Oxirane value = 3.87 = 75.1% as methyl epoxyoleate.

Preliminary studies indicate that the procedure may be applicable to the analysis of commercial epoxidized oils such as epoxidized soybean or linseed oils. We have had limited success with this type oil but find some unidentified peaks on the chromatogram when di- and possibly tri-epoxy compounds are present.

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[Received August 20, 1963—Accepted December 9, 1963]