Journal of Chromatography, 151 (1978) 396–400

CHROM. 10,643

Note

Separation of monounsaturated fatty acid methyl ester isomers by highly polar gas–liquid chromatographic stationary phases

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(Received September 27th, 1977)

The advent of highly polar gas–liquid chromatographic (GLC) stationary phases such as Silar 10C (Apolar 10C), SP-2340 and OV-275 has led to a renewed interest in studying the geometric and positional isomers of monounsaturated fatty acid methyl esters[1–10]. Although the use of glass capillary GLC columns to study the isomers of methyl esters is not new[11,12], the use of polar stationary phases with glass capillary GLC has greatly improved the resolution of these compounds. The state of the art, however, is less clearly defined than many authors and supply companies would have us believe.

Although most monounsaturated fatty acids occur in biological tissues in the *cis*-configuration, significant amounts of *trans*-acids have recently been reported[5,6,13,14]. Similarly, although most biologically produced monounsaturated fatty acids contain a limited number of positional isomers, normally with the double bond in the $\Delta 9$ or $\Delta 11$ position, recent studies have also shown significant amounts of additional positional isomers[5,6,13,14]. The presence of these unusual fatty acids has also been reported in sedimentary organic matter[7,8].

The problem that arises in the analysis of methyl esters on highly polar GLC phases is that increased resolution of the positional and geometric isomers is accomplished only at the expense of resolving various other methyl esters. In theory, the more polar the phase, the better the separation of the geometric and positional isomers. The McReynolds constants[15,16] for the most common phases presently used for methyl ester analyses are shown in Table I. The highly polar phases (SP-2340, Silar 10C and OV-275) have been those most commonly used for packed-column GLC work.

Our experience has shown that, although SP-2340 and Silar 10C give adequate separation of *cis–trans* isomers, the best separation on packed columns is achieved with OV-275. We have experimented with packed columns 2 to 14 m long and have found that the most acceptable compromise between resolution and analysis time is achieved by using a stainless-steel column (6.1 m × 2.2 mm I.D.) packed with 15% of OV-275 on 100–120-mesh Chromosorb P AW DMCS. Nevertheless, this column will separate only total *cis*-isomers from total *trans*-isomers for a given carbon-chain length and

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TABLE I

McREYNOLDS CONSTANTS FOR GLC STATIONARY PHASES USED IN FATTY ACID METHYL ESTER ANALYSES[15,16]

Solvents: 1 = benzene; 2 = butanol; 3 = pentan-2-one; 4 = nitropropane; 5 = pyridine.

Phase	*ΔI*					
	1	*2*	*3*	*4*	*5*	Σ_1^5
OV-101	17	57	45	67	43	229
Carbowax 20M	322	536	368	572	510	2308
FFAP	340	580	397	602	627	2546
DEGS	499	751	593	840	860	3543
SP-2340	520	757	659	942	800	3678
Silar 10C	523	757	659	942	801	3682
OV-275	781	1006	885	1177	1089	4938

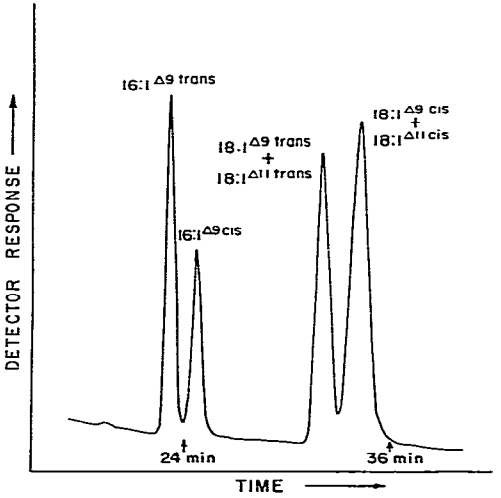

Fig. 1. Gas chromatogram of various isomers of 16:1 and 18:1 fatty acid methyl esters separated on a stainless-steel column (6.1 m × 2.2 mm I.D.) packed with 15% of OV-275 on 100–120-mesh Chromosorb P AW DMCS. Flow-rate, (nitrogen) 10 ml/min; column temperature, 210° (isothermal).

will not separate any positional isomers (see Fig. 1). In addition, separation of the *cis–trans*-isomer pairs from the saturated methyl esters cannot be adequately attained with such a column. In general, the 16:1 (carbon-chain length: number of double bonds) *cis*-isomers will overlap with any 17:0 methyl esters present, and the 18:1 *cis*-isomers will overlap with any 19:0 esters present. This may be significant in studies where odd-chain acids are present[17–19].

To separate positional isomers, one must use highly polar stationary phases in capillary GLC columns. Several authors have attempted this with various degrees of success (see, for example, refs. 2, 3 and 10). Unfortunately, as separation of the positional isomers begins, overlapping with the preceding or following *cis–trans* pairs occurs. The general rule for the order of elution of the *cis–trans* pairs is as follows: (1) with highly polar GLC phases such as Silar 10C, SP-2340 and OV-275, the *trans*-isomers emerge first, followed by the *cis*-isomers, for a given chain length. Thus, the

theoretical order of elution of the 18:1$^{\Delta 9}$ and 18:1$^{\Delta 11}$ *cis–trans*-series would be 18:1$^{\Delta 9}$ *trans*, 18:1$^{\Delta 9}$ *cis*, 18:1$^{\Delta 11}$ *trans*, 18:1$^{\Delta 11}$ *cis*; (2) with phases such as free fatty acid phase (FFAP) and others of lower polarity (such as OV-101), the *cis–trans*-pairs are eluted in reverse order. Thus, the above elution order would become 18:1$^{\Delta 9}$ *cis*, 18:1$^{\Delta 9}$ *trans*, 18:1$^{\Delta 11}$ *cis*, 18:1$^{\Delta 11}$ *trans*. In practice, however, with glass capillary columns up to 60 m long coated with highly polar GLC phases, the 18:1$^{\Delta 11}$ *trans*-isomer is generally co-eluted with the 18:1$^{\Delta 9}$ *cis*-isomer (see Fig. 2); hence this critical separation is not achieved. Similarly, the 18:1$^{\Delta 6}$ isomers cannot be separated from the 18:1$^{\Delta 9}$ isomers.

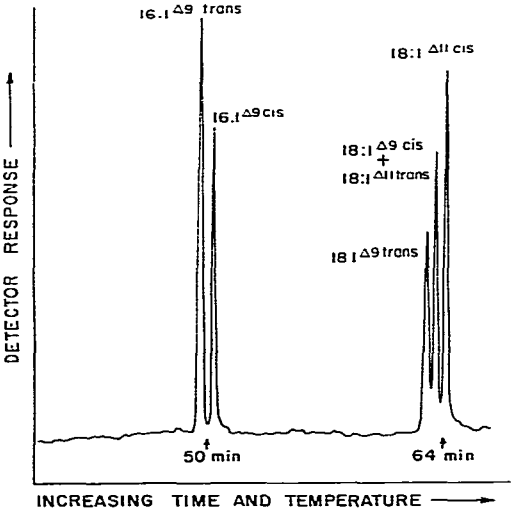

Fig. 2. Gas chromatogram of various isomers of 16:1 and 18:1 fatty acid methyl esters separated on a glass capillary column (60 m × 0.25 mm I.D.) coated with SP-2340. Flow-rate (nitrogen), 1.5 ml/min; temperature programmed from 100° to 170° at 1°/min and kept at 170° until end of run.

Most authors who claim separation of the positional isomers show only the separation of the $\Delta 9$ and $\Delta 11$ isomers, and even then do not show adequate separation of the respective *cis–trans*-pairs. Figs. 2 and 3 show the separation of various positional and geometric isomers attained on a 60-m glass capillary column coated with SP-2340 (J & W Scientific Inc., Orangevale, Calif., U.S.A.) and on a 100-m glass capillary column coated with FFAP (Quadrex Corp., New Haven, Conn., U.S.A.). On the FFAP column, resolution of the 18:1$^{\Delta 9}$ *cis–trans*-pair is compromised by increased resolution of the $\Delta 9$ and $\Delta 11$ positional isomers (see Fig. 3). Even on this column (400,000 theoretical plates calculated for 12:0 fatty acid methyl ester run isothermally at 150°), the 18:1$^{\Delta 6}$ isomers and 18:1$^{\Delta 9}$ isomers cannot be separated. Further, despite the excellent separation between the 18:1$^{\Delta 9}$ and 18:1$^{\Delta 11}$ isomers, the 16:1 *cis*- and *trans*-isomers and the 18:1$^{\Delta 11}$ *cis*- and *trans*-isomers are not separated. The elution time for the 18:1$^{\Delta 11}$ isomers is *ca.* 3 h, so that routine analysis of methyl esters on this column is impractical. Indeed, routine analysis of methyl esters in the hope of finding all possible *cis–trans* and positional isomers would also seem to be impractical if not impossible.

Although the picture now seems less clear, we propose a rather tedious method for obtaining much of the above information. The first step is to separate the

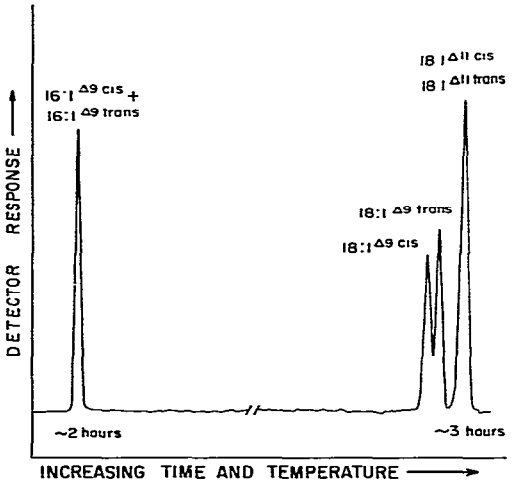

Fig. 3. Gas chromatogram of various isomers of 16:1 and 18:1 fatty acid methyl esters separated on a glass capillary column (100 m × 0.25 mm I.D.) coated with FFAP. Flow-rate (nitrogen), 1.0 ml/min; temperature programmed from 100° to 190° at 1°/min and kept at 190° until end of run.

saturated from the unsaturated fatty acid methyl esters. We have accomplished this by using a glass column (10 cm × 0.55 cm I.D.) packed with 0.6 g of 5% deactivated silica gel H-ADN coated with 20% silver nitrate (Applied Science, State College, Pa., U.S.A.). The methyl esters are loaded on the column in 1 ml of light petroleum (b.p. 30–60°)–toluene (4:1), then the saturated fatty acid methyl esters are eluted with 5 ml of light petroleum–toluene (4:1) and the monounsaturated methyl esters subsequently eluted with 20 ml of light petroleum–toluene (3:2). The saturated methyl esters can then be analyzed on a typical packed column, *e.g.*, one of stainless steel or glass (2 m × 2.2 mm I.D.) packed with 10% of DEGS on 80–100-mesh Chromosorb W AW. The monounsaturated fatty acids can be analyzed on a packed 6.1-m OV-275 column for the individual total *cis*- and total *trans*-isomers. If it is then necessary to separate all of the positional isomers, the most plausible approach might be to form the ozonide, mercuric acetate or O-isopropylidene derivative and proceed to analyze them by combined gas chromatography–mass spectrometry (GC–MS)[20]. The ability of GC–MS to differentiate between both geometric and positional isomers simultaneously, however, is still the subject of controversy; it is certainly not straightforward when the above-mentioned derivatives are used. Hence, one still has no measure of the individual positional *cis*- and *trans*-isomers. As an alternative, it is possible to use a glass capillary column of high polarity (such as SP-2340), as described above, to separate some of the positional isomers. Then, by comparing the ratios of these *cis*- and *trans*-isomers, it may be possible to observe changes from sample to sample. Another possibility is to separate total *cis*-isomers from total *trans*-isomers by argentation thin layer chromatography[7,21,22]; subsequent analysis of each isomer fraction would give more complete resolution of the positional isomers.

In conclusion, we have observed that the trend in separating positional and geometric fatty acid methyl esters is less well-defined than has been previously suggested. Increasing interest in this field, however, will assuredly produce new techniques for these analyses.

ACKNOWLEDGEMENT

This research was supported by a grant from the National Science Foundation (0CE76-08273).

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