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Quantitative Analysis of Fatty Acid Methyl Esters by Capillary Gas Chromatography with Flame-Ionization Detection: Quadrupole and Sector Mass Spectrometer

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ABSTRACT. The response of a flame-ionization detector and of two mass detectors, *viz.* a quadrupole mass spectrometer and a sector mass spectrometer, is described. A relationship between the amount of a fatty acid methyl ester and the relative response in the three detectors was found. The detectors were compared and their possible use for biological samples was discussed.

Gas chromatography of microbial fatty acids has already been used for more than 20 years (*e.g.*, Drucker 1981) and chromatographic analyses have proved their worth, *e.g.* in chemotaxonomy (Brondz and Olson 1986) or in studies of metabolism and physiological conditions of organisms (Kaneda 1977).

The recent vigorous capillary gas chromatography was made possible by commercially available fused silica capillary columns. As the capillary column separates and thus also quantifies individual fatty acid isomers better than a packed column, it is more useful tool for analyses; thus Brown *et al.* (1985), using a capillary column, could detect much higher numbers of fatty acids than Guzeva *et al.* (1973), using a packed column. Whatever the purpose of the study may be, the reproducibility of the results is of utmost importance. At present, the GC–MS (gas chromatography–mass spectrometry) combination is frequently used where the flame-ionization detector (FID) is replaced with the mass spectrometer detector (MS).

However, to our knowledge, no comparative paper on the response of FID and MS detectors in the case of methyl esters of fatty acids has so far been published.

This paper is an attempt to compare the response of the two in a natural mixture of fatty acid methyl esters from *Streptomyces virginiae*, producer of the antibiotic complex of virginiamycins.

MATERIALS AND METHODS

A mixture of fatty acid methyl esters prepared by hydrolysis (NaOH in 50 % MeOH, 1 mol/L) of *Streptomyces virginiae* total lipids was methylated with BF_3–MeOH. After hexane extraction the final concentration was adjusted to 10 mg of methyl esters in 1 mL and 1 μL of the mixture was injected.

The mixture was analyzed in the quadrupole GC–MS system Shimadzu model QP 1000 (*Shimadzu*, Japan) using silica capillary column SPB-1 (*Supelco*, USA), 60 m × 0.25 mm ID × 0.25 μm film thickness; carrier gas He (25 cm/s). Injector temperature 280 °C (split 1:50), column temperature programmed from 160 to 250 °C at 6 K/min, ionization voltage energy 70 eV.

An identical mixture was analyzed in the sector type GC–MS apparatus Finnigan MAT 90 (*Finnigan MAT*, USA) with a gas chromatograph Varian model 3400 and a capillary fused silica column HP-1 (*Hewlett-Packard*, USA) 25 m × 0.2 mm ID × 0.11 μm film thickness; carrier gas He (30 cm/s). Injector temperature 280 °C at 3 K/min, GC–MS interface temperature 279 °C; ionization voltage energy 70 eV.

The same mixture was further analyzed in the gas chromatograph Perkin-Elmer Sigma 3B (*P–E*, USA) with a flame-ionization detector (FID). A capillary fused silica column SBP-5 (*Supelco*, USA) (5 % phenyl – 95 % methylpolysiloxane) 30 m × 0.25 mm ID × 0.25 μm film thickness was used; carrier gas hydrogen (41 cm/s). Injector temperature and detector temperature was 280 °C (split 1:70). Column temperature was programmed from 150 to 270 °C at 6 K/min. The results were evaluated by the Perkin-Elmer 3600 Data Station (*P-E*, USA).

RESULTS AND DISCUSSION

Fig. 1 shows a record of the chromatogram on the SPB column. As compared with Figs. 2 and 3 (capillary chromatography on the sector and quadrupole MS, respectively) peak 6, *i.e.* the internal standard dibutylphthalate, is significantly shifted. Dibutylphthalate was selected intentionally, being a common component of almost all solvents. The mass spectrum of phthalates is also generally very different from mass spectra of fatty acid methyl esters. The increased retention time was caused by π–π electron interactions between the stationary phase – 5 % phenyl groups and benzene ring of phthalate as the chromatographed compound. This fact demonstrates clearly that compounds other than fatty acids can be detected even without using MS as the detector. In general, it is a long known and used phenomenon, *i.e.* chromatography of a single mixture on two or more columns of different polarity (Pörschmann *et al.* 1982). By using a different split, *i.e.* 1:50 and 1:70 in both GC–MS and GLC, respectively, the results are not distorted as the ratios are sufficiently similar (and high). Also the polarity of the SPB-1 and HP-1 colums is almost identical, always the same stationary phase (dimethyl-silicone) being involved. The polarity of the SPB-5 phase has been discussed above.

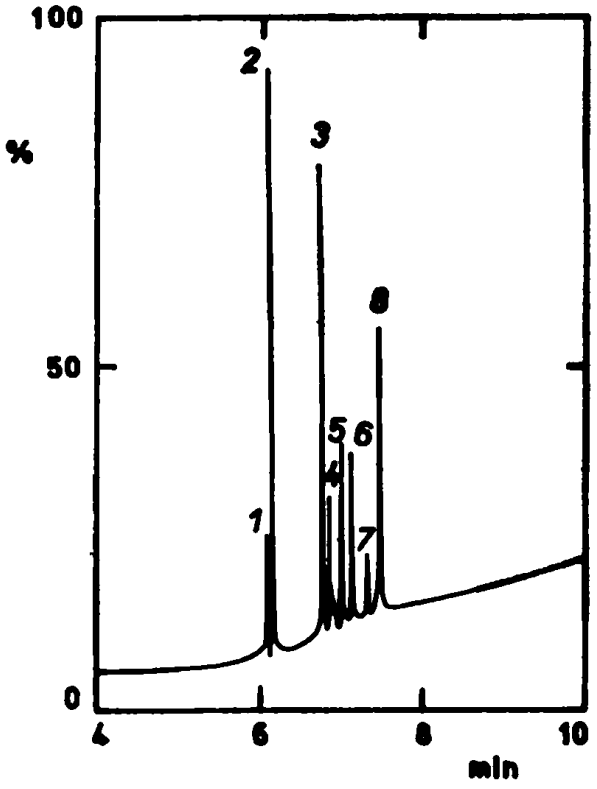

FIG. 1. Mixture of fatty acid methyl esters from *S. virginiae* – CGC–FID.

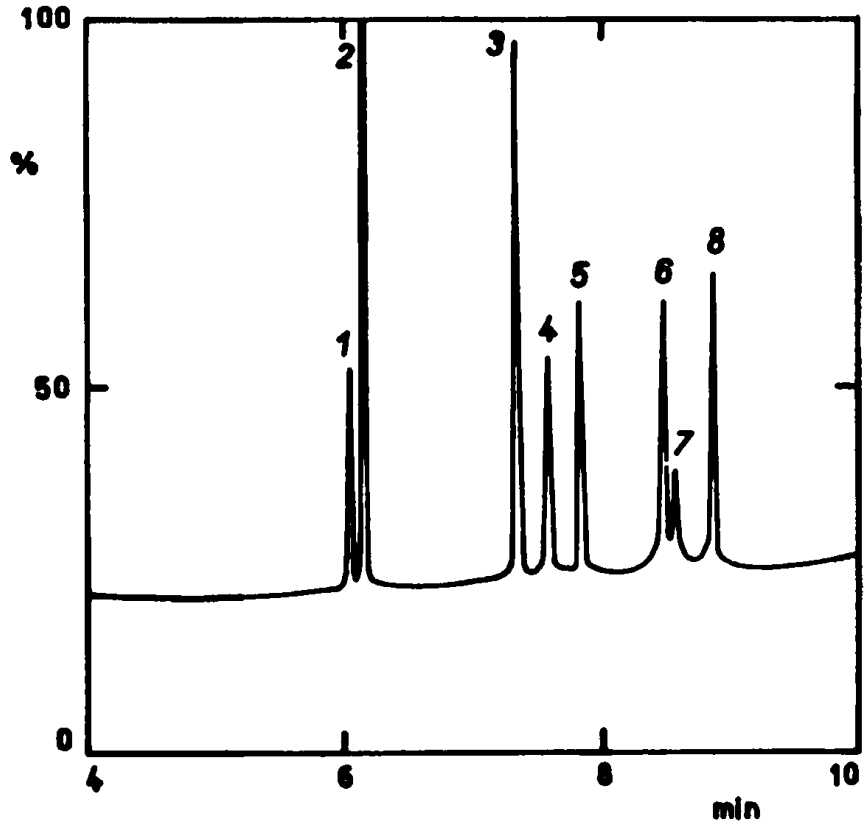

FIG. 2. Mixture of fatty acid methyl esters from *S. virginiae* – GC–sector MS.

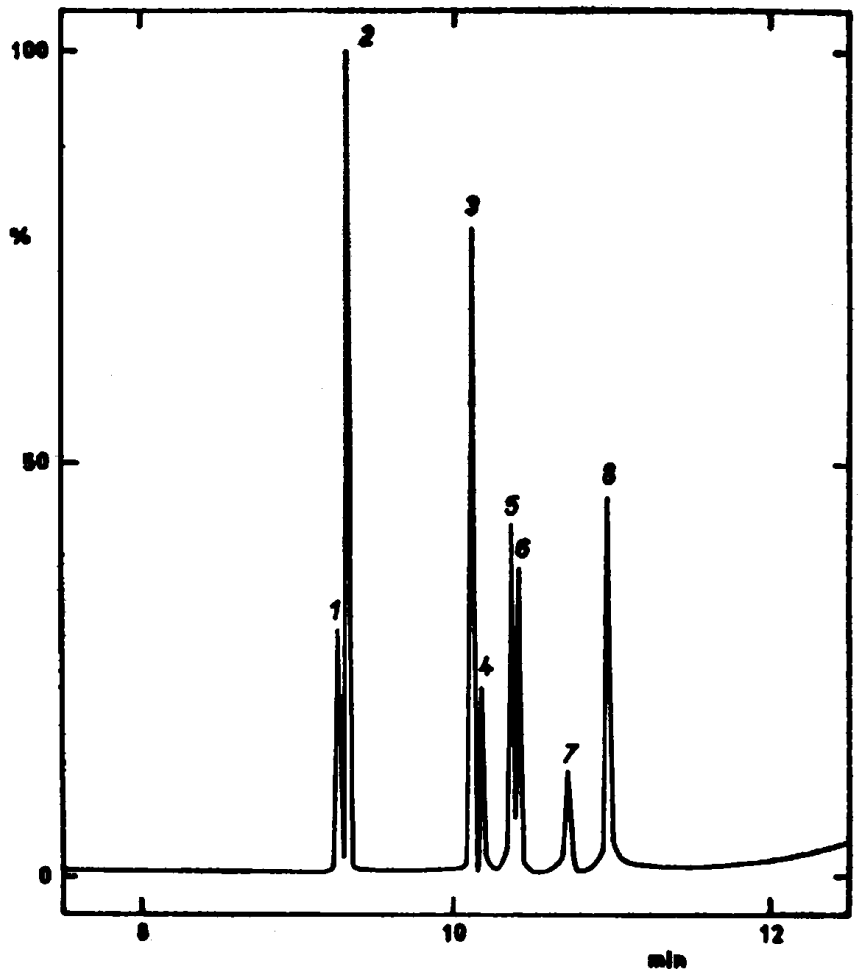

FIG. 3. Mixture of fatty acid methyl esters from *S. virginiae* – GC–quadrupole MS.

The responses of individual detectors (Table I) are in agreement in all three instruments. Occasional differences in percent representation of individual methyl esters can be explained by the fact that each apparatus utilized a different integration principle. Thus, a partial discrimination of minority peaks, *e.g. i*-15:0, 16:1 or 16:0,

TABLE I. Relative representation of individual fatty acid methyl esters from *Streptomyces virginiae* detected by CGC–FID and GC–MS in the sector and quadrupole MS, respectively.

Peak number	Methyl esters	CGC–FID	s-GC–MS	q-GC–MS
1	*i*-15 : 0	7.1	5.4	6.4
2	*ai*-15 : 0	27.4	31.0	30.1
3	*i*-16 : 0	20.0	23.9	22.5
4	16 : 1	7.6	5.6	6.8
5	16 : 0	13.9	10.4	9.5
6	n.s.	10.0	9.7	9.8
7	*i*-17 : 0	2.7	1.7	2.7
8	*ai*-17 : 0	11.3	12.3	12.2

can be observed, whereas an opposite tendency can be seen in the majority peaks *ai*-15:0 and *i*-16:0. Nevertheless, the data are in an excellent accordance and minor differences are statistically insignificant as compared for instance with the paper of Larsson *et al.* (1985) where CGC on three gas chromatographs of three laboratories differed several-fold, *e.g.* for the 16:1 methyl ester 1.6 % *vs.* 4.8 %.

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