

## Quantitative Mass Spectrometric Analysis of Mixtures of Unsaturated and Saturated Fatty Acids

BO HALLGREN, EINAR STENHAGEN  
and RAGNAR RYHAGE

Department of Medical Biochemistry,  
Institute of Medical Chemistry, University of  
Uppsala, and

Laboratory for Mass Spectrometry,  
Biochemical Institute, Karolinska Institutet,  
Stockholm

It has been shown<sup>1</sup> that with the aid of a high-resolution mass spectrometer it is possible to obtain excellent mass spectra of methyl esters of saturated long chain carboxylic acids. Two series of peaks are prominent, *viz.* ionized hydrocarbon fragments and methoxycarbonyl-containing fragments, respectively. The latter type of fragments dominates quantitatively over the former. The spectra show comparatively large parent peaks due to the ionized unfragmented molecules. These allow a direct determination of the molecular weights of the compounds studied. In mixtures, the peak heights of the parent peaks can be used for the quantitative analysis of mixtures of normal chain fatty acids of high molecular weight (*cf.* Ref.<sup>1</sup>). The general principles of the analysis of mixtures in the mass spectrometer are outlined in standard texts (*cf.*, *e.g.*, Refs. 2-4).

If we have a mixture of a saturated methyl ester and methyl esters of mono- or poly-ethenoid acids having the same number of carbon atoms the mass spectrum will show a series of parent peaks corresponding to the different molecular weights. The high-mass range of the mass spectrum for a mixture of  $C_{18}$  acids is reproduced in Fig. 1. The saturated ester (methyl stearate) has a parent peak at  $m/e = 298$ , the mono-ethenoid ester (methyl oleate) at 296, the di-ethenoid ester (methyl linoleate) at 294 and the tri-ethenoid ester (methyl linolenate) at 292 (*cf.* Figs. 1 and 2). The strong isotope peaks ( $m/e = M+1$ ) are at odd mass numbers and do not overlap the parent peaks. The sensitivity coefficients of the parent peaks in the mass spectrum of the different esters studied are not the same, and in order to derive the quantitative composition of the mixture it

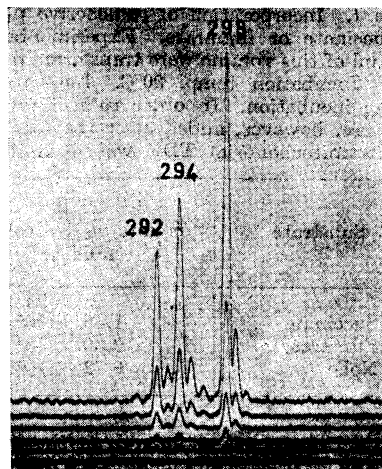


Fig. 1. High-mass end of the mass spectrum of a mixture containing 25 % methyl stearate, 38 % methyl linoleate, 36 % methyl linolenate and approximately 1 % methyl oleate.

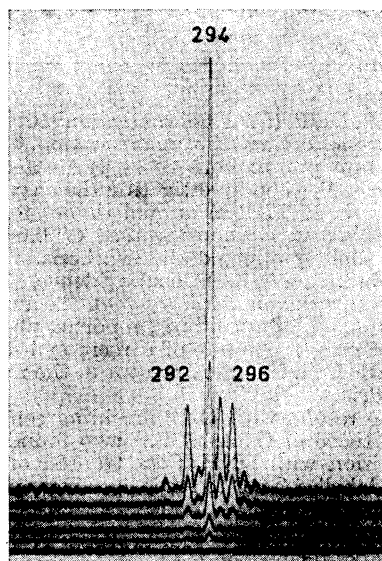


Fig. 2. High-mass end of the mass spectrum of a mixture containing 31 % methyl oleate, 55 % methyl linoleate and 14 % methyl linolenate.

is necessary to apply empirical corrections. If this is properly carried out it is possible to determine the percentage of the various components of the mixture directly. If pure components are available it is easy to check the results by running artificial mixtures and thus to get a very high accuracy in the quantitative analysis.

The mass spectra of the samples of methyl linoleate and methyl linolenate studied show a peak at  $m/e = 290$ . In the case of methyl linoleate the height of this peak is 1.7 % of the parent peak ( $m/e = 294$ ). For methyl linolenate the height of the  $m/e = 290$  peak is 18 % of the parent peak at  $m/e = 292$ . This peak appears to be the parent peak of the methyl ester of a  $C_{18}$  acid containing four double bonds. Whether this acid is present as an impurity in the original samples or whether it is formed by a dehydration reaction in the heated intake system of the mass spectrometer cannot be said at present. The present evidence, however, is in favour of the first mentioned possibility. It has been proposed that acids of this type may be formed from linoleic and linolenic acids by dehydrogenation between the existing double bonds and the carboxyl group in such a way that all unsaturation is methylene-interrupted and all *cis*. (cf. Ref.<sup>5</sup>).  $C_{18}$  tetra-ethenoid acids have been found in the fats of marine animals<sup>5, 6</sup>.

In a complex mixture it is not possible to distinguish between geometrical and positional isomers within each group of unsaturated esters. The peak at  $m/e = 296$  in the figure might thus be due to, e.g., methyl oleate (methyl *cis*- $\Delta^{9,10}$ -octadecenoate), methyl elaidate (methyl *trans*- $\Delta^{9,10}$ -octadecenoate), or methyl petroselinate (methyl *cis*- $\Delta^{6,7}$ -octadecenoate) or due to a mixture of mono-ethenoid esters. It may be possible to overcome this difficulty by examining suitable derivatives.

The high-mass, high-resolution mass spectrometer thus offers a convenient method for determining the fatty acid distribution ("fatty acid spectrum") of natural or synthetic fats. The analysis can be performed on a few milligrams of material.

The financial support of *Statens Tekniska Forskningsråd* and *Statens Medicinska Forskningsråd* is gratefully acknowledged. We are indebted to Dr. W. O. Lundberg, The Hormel Institute, Austin, Minnesota, for the gift of methyl esters of unsaturated fatty acids.

1. Asselineau, J., Ryhage, R. and Stenhagen, E. *Acta Chem. Scand.* **11** (1957) 196.
2. Brewer, A. K. and Dibeler, V. H. *J. Research Natl. Bur. Standards* **35** (1945) 125.
3. Robertson, A. J. B. *Mass Spectrometry*, London 1954, p. 84.
4. Barnard, G. P. *Modern Mass Spectrometry*, The Institute of Physics, London 1953, p. 192.
5. Holman, R. T. in Glick, D. *Methods of Biochemical Analysis*, N.Y. 1957, p. 99.
6. Cf. Hilditch, T. P. *The chemical constitution of natural fats*, 3rd. Ed., London 1956.

Received June 26, 1957.

## Note on the Crystal Structure of Niobium Monoxide

GEORG ANDERSSON and ARNE MAGNÉLI

*Institute of Chemistry, University of Uppsala, Uppsala, and Institute of Inorganic and Physical Chemistry, University of Stockholm, Stockholm, Sweden*

In connection with studies on transition metal compounds of defective sodium chloride structure, e.g. vanadium monoxide<sup>1</sup> and titanium monoxide<sup>2</sup>, the powder pattern of niobium monoxide was registered by means of an X-ray G.M. diffractometer (CuK radiation). The structure derived by Brauer<sup>3</sup> for this substance (a defective sodium chloride structure with the positions 000 and  $\frac{1}{2}\frac{1}{2}\frac{1}{2}$  vacant) is supported by our measurements, but as our investigation is based on quantitative data it may be worth mentioning.

The lattice parameter of the niobium monoxide sample as obtained from a Guinier powder photograph (internal standard potassium chloride  $a = 6.2919 \text{ \AA}$  at 20°C) was found to be  $4.210_8 \text{ \AA}$  in excellent agreement with the value given by Brauer  $4.2103 \pm 0.0004 \text{ \AA}$  (21°C). The density was found to be 7.3 likewise in perfect agreement with Brauer's value of 7.30 and the value 7.27 calculated for a cell content of three formula units of NbO.

Table 1 gives the  $F^2$  values for the first fourteen reflexions as obtained from the diffractogram. The alternative structures (2)–(4) are among those discussed by Brauer *viz.* 3 Nb in  $\frac{1}{2}\frac{1}{2}0$ ,  $\frac{1}{2}0\frac{1}{2}$ ,  $0\frac{1}{2}\frac{1}{2}$  for (2)