

Combined in-beam electron impact-B/E-linked scan mass spectrometry of oxazoline derivatives for the structure determination of long-chain unsaturated fatty acids¹

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Summary Long-chain unsaturated fatty acids (UFA) having up to six double bonds are derivatized to 2-substituted 4,4-dimethyloxazolines (DMOX) and then analyzed by combined in-beam electron impact (IBEI)-B/E-linked scan mass spectrometry. This technique provides highly characteristic mass spectra and may serve as an auxiliary means for direct structure determination of individual UFA in mixtures.—Yang, Y. M., J. Y. Zhang, and Z. H. Huang. Combined in-beam electron impact-B/E-linked scan mass spectrometry of oxazoline derivatives for the structure determination of long-chain unsaturated fatty acids. *J. Lipid Res.* 1989. 30: 127-133.

Supplementary key words double-bond location

It has been a generally accepted view that the direct determination of site of unsaturation in fatty acids (FA) is impossible by conventional mass spectrometry due to the facile migration of the double bond under electron impact (1). Many efforts have been made so far to disclose the position of the double bond by MS or GLC-MS combined with chemical derivatization (2, 3) at the double bond (on-site modification) or, alternatively, at the carboxyl function (remote site modification). The former approach is commonly associated with the conversion of the unsaturated fatty acid (UFA) to vicinal diols or carbonyl compound mixtures, and therefore was restricted only to the analysis of simple olefinic acids owing to the formation of highly polar derivatives or rather complex mixtures of degradation products. Another more elegant way was undertaken by measuring

the FA as their N-acyl pyrrolidides (4) or β -pyridylmethyl esters (5). These derivatives are able to induce preferential charge localization at the nitrogen-containing part of the molecules, and thus show distinctive mass spectra, which are frequently consistent with the distribution pattern of double bonds in the chain.

A new way to increase the speed and versatility so that unusual acids can be studied would be to develop a method for directly analyzing fatty acid mixtures without using GLC. The need for newer techniques is especially obvious in the study of high molecular weight FA in lipid components of certain bacteria and marine sponges. Such analysis is indeed possible by fast atom bombardment (FAB) in combination with tandem mass spectrometry (MS-MS). Jensen, Tomer, and Gross (6) have reported that free fatty acids can be readily desorbed either as anions $(M-H)^-$ or as alkali metal-activated cations $(M + Li)^+$ or $(M - H + 2Li)^+$ (7) and collisionally activated. The highly specific structural information thus obtained is potentially a useful complement to GLC-MS analysis of these substances. Furthermore, this technique has been successfully applied to pyridylmethyl esters of FA samples (8). Usually, the determination of chain structure is possible for PUFA derivatives of this type with up to four double bonds, but becomes more difficult for molecules with higher degrees of unsaturation.

Recently, a new derivatization through 2-alkenyl-4,4-dimethyloxazolines (DMOX) has been developed in this laboratory (9). An extended study of the component FA from marine fish oil and rat testis lipids (10) showed that this method may serve as a valuable alternative to the

Abbreviations: MS, mass spectrometry; GLC, gas-liquid chromatography; FAB, fast atom bombardment; MS-MS, tandem mass spectrometry; DMOX, dimethyloxazolines; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; FA, fatty acid; IBEI, in-beam electron impact; AMP, 2-amino-2-methylpropanol; DCC, dicyclohexylcarbodiimide.

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pyridylmethyl ester and the pyrrolidide technique for the GLC-MS of long-chain UFA. It has been found that DMOX exhibit more abundant diagnostic peaks which allow definite determination of the unsaturation points in polyenoic acids having up to six double bonds. Besides, these derivatives show promising GLC characteristics, being comparable to that of simple esters in volatility and separating ability. They will be of potential value for the analysis of UFA with longer chains. In this report, the structure determination of UFA by combined in-beam EI (IBEI)-linked scanning (B/E = constant) of oxazoline derivatives is described and the factors influencing the use of this method are discussed briefly.

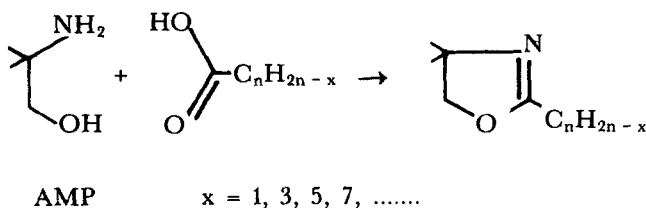
EXPERIMENTAL SECTION

Reagents

All UFA were obtained from Sigma Chemical Co. with purity higher than 99%. 2-Hydroxystearic acid was prepared according to a literature method (11). 2-Amino-2-methylpropanol (AMP) and dicyclohexylcarbodiimide (DCC) were purchased from Aldrich Chemical Company with purity of 99%.

Derivatization

2-Alkenyl-4,4-dimethyloxazolines were prepared according to the procedures reported in our previous paper (9) by a) heating a mixture of FA with excess AMP (170°C, 1-2 hr, as shown below) or b) treating the FA and AMP with DCC (in CH₂Cl₂, room temperature, 2 hr) followed by the action of SOCl₂ (0°C, 0.5 hr). The conversion was better than 90% (estimated by GLC).



Mass spectrometry

All of the IBEI and IBEI-B/E-linked scan spectra were recorded on a MAT 711 double focusing mass spectrometer equipped with an SS-166 data system. The experiments were carried out under the following conditions: accelerating voltage 8 kV, electron energy 70 eV, emission current 0.8 mA, source temperature 20°C, resolution 1000. The samples were dissolved in chloroform (1 µg/µl). For measurements under IBEI mode, 1 µl of solution was placed on the stainless-steel target of the FAB direct inlet probe and then inserted into the ion source 3-4 mm away from the electron beam. The IBEI-linked scanning was accom-

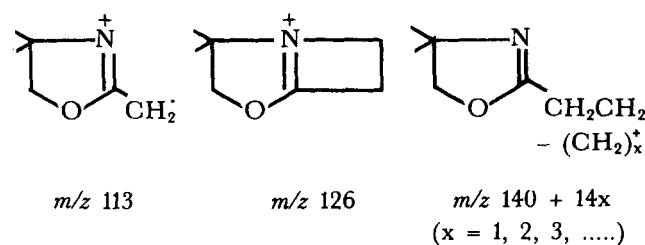
plished by operating the Finnigan-MAT Metascan unit in the B/E mode at a linear scanning speed of 128 sec/decade and the spectra were recorded by using an UV recorder. The measured mass of the product ion m_2 was calculated from the apparent mass of the marker, m^* , and the mass of the precursor ion, m_1 , according to the equation $m_2 = (m^* \times m_1)^{1/2}$.

RESULTS AND DISCUSSION

In-beam EI

The EI and IBEI spectra of oxazoline derivatives prepared from linolenic acid, 18:3(9c, 12c, 15c), are given in Fig. 1.

Both spectra exhibited the same fragmentation pattern with an intense peak at m/z 113 (usually as the base peak formed through the McLafferty rearrangement), m/z 126 (often as the second large peak, due to cyclization-displacement), and an even-mass homologous ion series at m/z 140 + 14x (x = 1, 2, 3,) continuing up to the molecu-



lar ion region. The fragmentation is perturbed in a highly specific manner, whereby the location of a double bond at carbon n is indicated by a mass separation of 12 units instead of the regular 14 units between the most abundant peaks of two neighboring homologous clusters containing n-1 and n carbon atoms of the original acid moiety (9). Thus, the ion pairs at m/z 196 (C₈), 208 (C₉); m/z 236 (C₁₁), 248 (C₁₂); and m/z 276 (C₁₄), 288 (C₁₅) are typical of a methylene-interrupted unsaturated system of $\Delta^{9,12,15}$.

Nine oxazoline derivatives of UFA containing one to six double bonds at different positions have been measured under IBEI. The intensities of the molecular ion in each spectrum under EI and IBEI are listed in Table 1.

Generally, IBEI gives significantly higher total ion current values than that produced by conventional EI on the same quantity of samples. It can be seen from the data that when going from EI to IBEI, a predominating increase in relative intensities occurs for the molecular ion peaks (200-1000% enhancement), while the change in relative intensities for fragment peaks is insignificant (10-30% enhancement). The differential feature of these results now assumes a new possibility of double bond location by IBEI coupled with linked scanning of DMOX derivatives of UFA, either as pure samples or in complex mixtures.

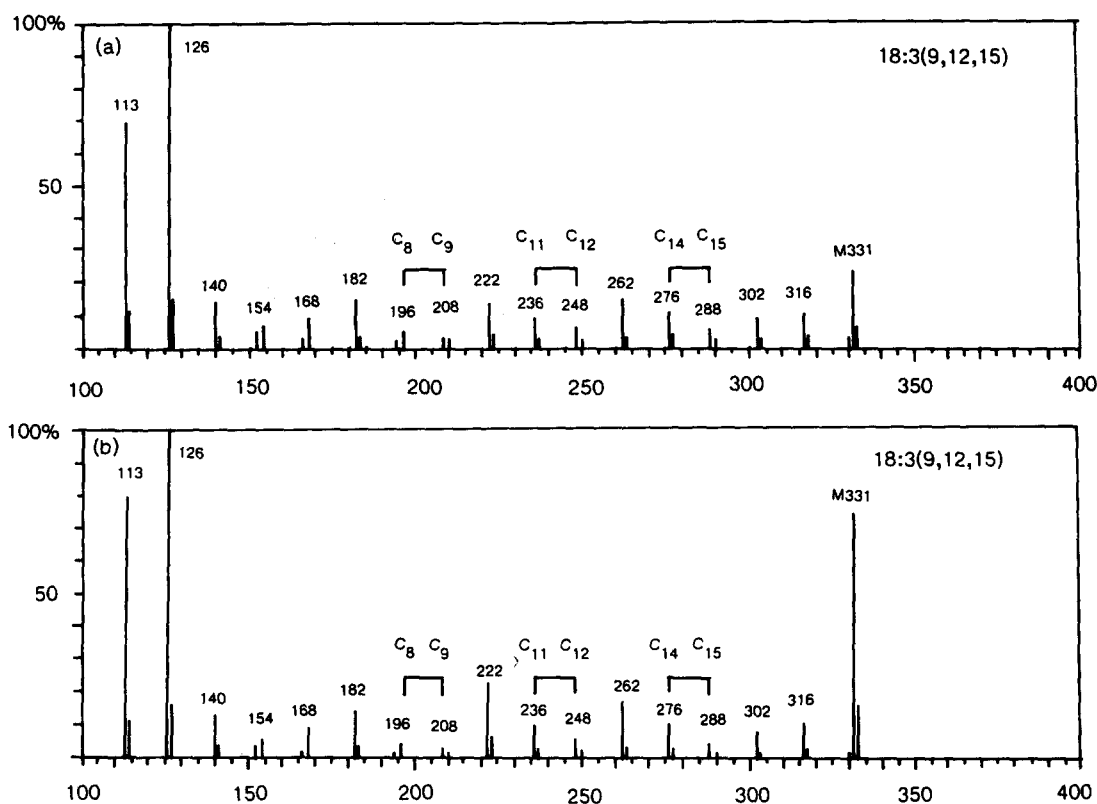


Fig. 1. Mass spectra of oxazoline derivatives of 18:3 (9_c, 12_c, 15_c) under (a) EI and (b) IBEI.

Combined IBEI-linked scan

Samples were then subjected to IBEI coupled with linked scan analysis of the molecular ions, while keeping the ratio B/E constant. Thus, the mass spectra of all the product ions originating from one precursor were obtained. The product ion spectra thus produced from 5 to 8 are given in Fig. 2.

The sites of unsaturation in all cases were located readily by applying the "12 mass difference rule" (9). For certain UFA with the first double bond at carbon 4, 5, or 6, in addition to the normally occurring characteristic ions m/z 138 (C_4), 152 (C_5), and 166 (C_6), the corresponding oxazolines furnish a strong satellite peak at m/z 139, 153, and 167, respectively (9). Such unique fragmentation is important

TABLE 1. Relative intensities of molecular ions in the spectra of oxazoline derivatives under EI and IBEI using equal amounts of test samples (1 μ g each)

No.	Structure	Molecular Weight	% EI	% IBEI	(IBEI)/(EI)
1	18:1(6)	335	13.7	25.0	1.8
2	18:1(9)	335	14.3	28.7	2.0
3	20:1(11)	363	14.5	83.0	5.7
4	22:1(13)	391	26.0	100.0	3.8
5	24:1(15)	419	27.6	99.1	3.6
6	18:2(9,12)	333	12.0	56.3	4.7
7	18:3(9,12,15)	331	22.2	72.9	3.3
8	20:4(5,8,11,14)	357	5.0	33.2	6.6
9	22:6(4,7,10,13,16,19)	381	3.9	39.4	10.1

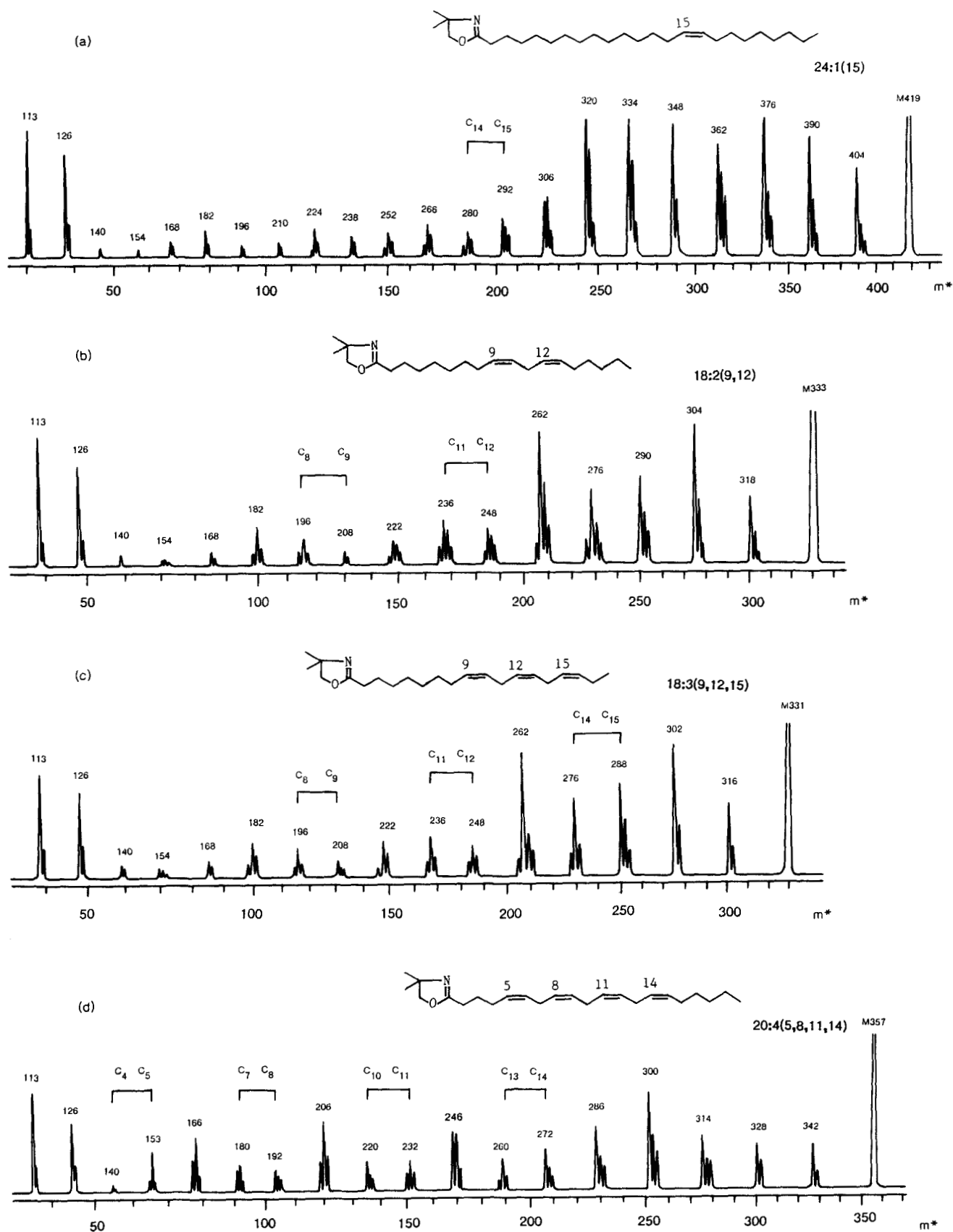


Fig. 2. IBEI-linked scan ($B/E = \text{constant}$) spectra of UFA derivatives: (a) 24:1 (15c); (b) 18:2 (9c, 12c); (c) 18:3 (9c, 12c, 15c); (d) 20:4 (5c, 8c, 11c, 14c).

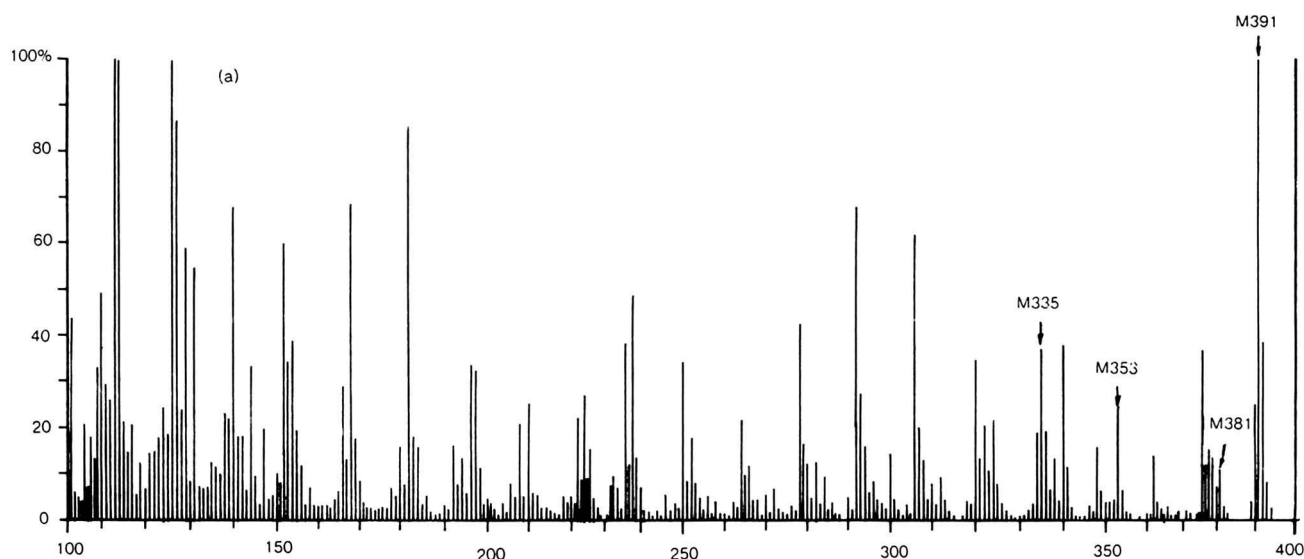


Fig. 3. (a) IBEI of 18:1 (9_c) + 2-HO-18:0 + 22:6 (4_c, 7_c, 10_c, 13_c, 16_c, 19_c) + 22:1 (13_c).

for interpretation purposes, although the explanation still remains unexplored at present.

One important implication in the use of this method for characterizing UFA is related to the analysis of mixtures. Fig. 3 gives the results of analysis of a simple mixture comprising nearly equal amounts of 18:1 (9_c) ($M = 335$), 2-HO-18:0 ($M = 353$), 22:6 (4_c, 7_c, 10_c, 13_c, 16_c, 19_c) ($M = 381$), and 22:1 (13_c) ($M = 391$), all derivatized as oxazolines. Since the relatively abundant molecular ions of these component FA are displayed as the only odd-mass peaks occurring in high mass region, they are easily distinguished in the general profile of the mixed spectrum (Fig. 3a). The daughter ion spectra produced thereby are presented (Fig. 3b-3e). The ions m/z 142 and 308 [($M-45$), loss of ($\text{HO}\dot{\text{C}}\text{HCH}_2 + \text{H}$)] in the spectrum of 2-HO-18:0 (Fig. 3c) are indicative of a hydroxyl-substitution at carbon 2 (11).

It is commonly considered that the accuracy in mass measurement is critical in B/E scanning. The main difficulty lies in visually observing and manually marking the peak top in the scanning operation and the experimental factors in defining the ratio B/E. In order to test the reliability of analysis, an examination of accuracy and reproducibility in mass measurement was undertaken by using the oxazoline derivative of oleic acid as a model compound. The spectra of seven B/E scans (m/z 335) were acquired and analyzed. The maximum standard deviation of the masses measured from those of the calculated values (except m/z 154, due to its broadened and lowered peak) was found to be less than ± 0.5 amu, a level comparable to that admitted for B scans. Considering the good reproducibil-

ity that has been obtained (deviation < 0.2 amu) in mass measurement, the present method proves to be practical for analytical purposes.

In summary, it appears likely that DMOX are superior in fragmentation-directing ability to most of the derivatives investigated earlier. The combined IBEI-B/E-linked scanning technique provides a potentially useful tool for the direct determination of structures of higher FA without prior separation. The derivatization is simple and efficient and the analysis can be performed easily on a relatively inexpensive two-sector instrument. It would be expected to be particularly useful for samples with long or very long chains ($> \text{C}_{22}$) that are not amenable to GLC-MS due to poor volatility or scanty MS information obtainable by other means. One limitation of this method is associated with the possible energy spread occurring in B/E scans. Consequently, artefact peaks may arise if the ions from other components in the UFA mixture fall within the mass window of $\pm 2.5\%$ of the focused precursor ion (12). Under such circumstances, ions due to unrelated compounds may be transmitted by the electrostatic analyzer during linked scan because of the limited energy resolution of MAT 711 instrument. However, this drawback can be overcome by using an MS-MS technique. **RL**

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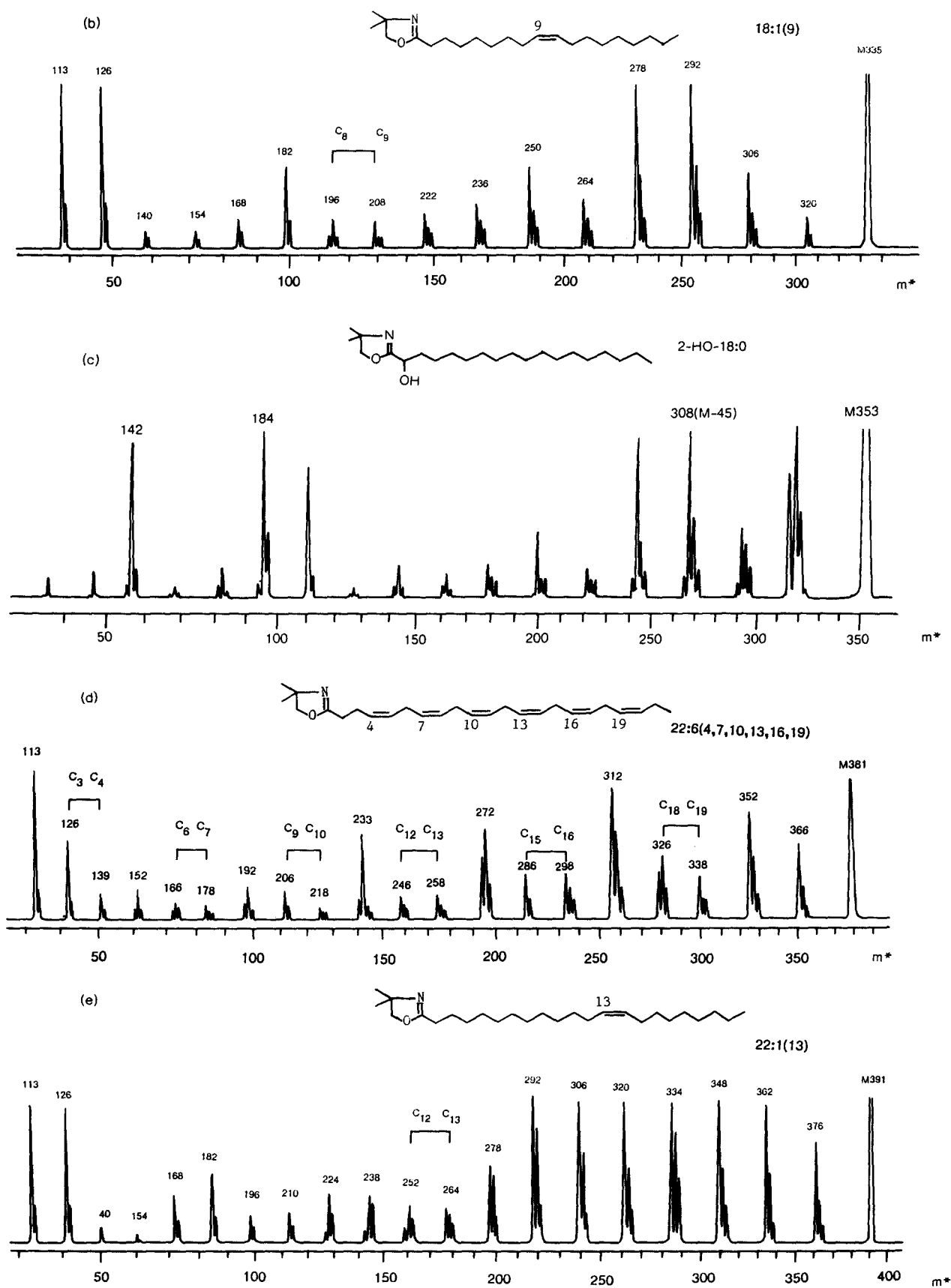


Fig. 3. (continued) (b) IBEI-linked scan of m/z 335 [18:1(9_C)]; (c) IBEI-linked scan of m/z 353 [2-HO-18:0]; (d) IBEI-linked scan of m/z 381 [22:6(4_C, 7_C, 10_C, 13_C, 16_C, 19_C)]; (e) IBEI-linked scan of m/z 391 [22:1(13_C)].

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