Discrimination Among Geometrical Isomers of α-Linolenic Acid Methyl Ester Using Low Energy Electron Ionization Mass Spectrometry

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There is a consensus that electron impact ionization mass spectrometry is not capable of discriminating among geometrical isomers of unsaturated fatty acid methyl esters (and in general olefinic compounds). In this paper, we report the identification of all eight geometrical isomers of α-linolenic acid, one of the few essential ω-3 fatty acids that has attracted great attention, using low-energy electron ionization mass spectrometry. Three electron energies 70, 50, and 30 eV were studied and the mass spectrum of each isomer was obtained from the analysis of different concentrations of a standard mixture of α-linolenic acid methyl ester geometrical isomers to ensure the robustness of the method. Principal component analysis was employed to model the complex variation of *m/z* intensities across the isomers. Only using the data of 30 eV energy was complete differentiation among geometrical isomers observed. The unique cleavage pattern of the α-linolenic acid methyl ester isomers leading to a benzenium ion structure is discussed and general fragmentation rules are derived using the mass spectra of over 300 compounds with different kinds and levels of unsaturation. Application of the proposed method is not limited to α-linolenic acid. It can potentially be used to identify the geometrical isomers of any compounds with an olefinic chain. (J Am Soc Mass Spectrom 2009, 20, 1272–1280) © 2009 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

Unsaturated fatty acids possess one or more double bonds, which can appear in different positions along the hydrocarbon chain and in different geometries (*cis* or *trans*). While the naturally occurring isomers have mainly cis configuration, trans fatty acids appear naturally in milk [1, 2] and can be artificially produced by heating and hydrogenation of vegetable oils [3]. Investigation of function and possible adverse effects of trans fatty acids on human health have been carried out extensively in recent years [4, 5]. A major challenge confronting researchers is unambiguous identification and differentiation of geometrical isomers of polyunsaturated fatty acids (PUFA) [6]. For a methylene-interrupted fatty acid with n double bonds at fixed positions there are 2^n geometrical isomers. *Cis* and *trans* isomers of fatty acids with one or two double bonds differ significantly in polarity but geometrical isomers with three or more double bonds can have very similar polarities. As an example α-linolenic acid methyl ester isomers 9-*cis*, 12-*cis*, 15-*trans*, and 9-*trans*, 12-*trans*, 15-*cis* cannot easily be separated by gas chromatography (GC) even using a highly polar column such as BPX-70 [7]. The similarity of isomers and complexity of the chromatograms increase in fatty acids with four, five, and six double bonds giving rise to 16, 32, and 64 geometrical isomers. The ultimate challenge is when a mixture of positional and geometrical isomers with $m \times 2^n$ isomers is to be analyzed where m is the number of positional methylene interrupted isomers.

Gas chromatography-mass spectrometry (GC-MS) is a powerful method for analysis of fatty acids. As a standard procedure, fatty acids are derivatized to methyl esters that are less polar and more volatile, therefore suitable for analysis by GC [8]. MS of fatty acid methyl esters (FAMEs), on the other hand, are believed to provide little structural information such as the position and geometry of double bonds [9]. Pyrrolidide, picolinyl, and dimethyl oxazoline derivatives [10], double-bond deuteration, silylation [11], and preparation of dimethyl disulfide adducts [12] are among the derivatization methods used for the determination of double-bond positions. While many studies have been made on the determination of the position of double bonds, there are very few reports on identification of the geometry of double bonds in fatty acids using mass spectrometry [9, 13–21]. These methods include fast atom bombardment (FAB) [13, 19], collision induced MS of fatty acids cationized by copper [14], acetonitrile chemical ionization [15], resonance electron capture [16], electron

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impact ionization (EI) of picolinyl derivatives and chemometrics [17], EI of 2-alkenyl-4,4-dimethyl-oxazolin derivatives [18], and EI of Diels-Alder adducts using 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene [20, 21]. This is because geometrical isomers exhibit very similar mass spectra. There is no diagnostic ion to be used to distinguish cis geometry from *trans* and identifications are done only by comparing the relative abundances of some *m/z* (note that retention time data are also used for identification but this aspect is not explored in this study). While it may be possible to construct few *m/z* intensity ratios to identify two (in a monoene) or four (in a diene) geometrical isomers, it is nontrivial for trienes (2^3 isomers) and higher unsaturated fatty acids. In those cases a multivariate classification technique such as principal component analysis (PCA) is required to model the complex (and rather small) variation of *m/z* intensities across the large number of isomers [9]. This may be the reason that so far, except for the work by Mjos et al. [9], only discrimination among the geometrical isomers of monoene and diene fatty acids have been reported using mass spectrometry.

One possible approach to identify PUFA isomers is to change the ionization conditions which lead to differences in the mass spectra of these compounds and exploit these differences to deconvolute the overlapping chromatographic peaks. Providing that there is sufficient difference in the mass spectra of isomers, chemometrics approaches of multivariate curve resolution [22] or parallel factor analysis [23] can be employed to deconvolute the chromatographic peaks and recover the mass spectra of overlapping geometrical isomers.

While the EI (70 eV) spectra of FAMEs are known to provide little information about the position and geometry of double bonds in monoene and dienes, they have been shown to be valuable in the cases of triene and higher unsaturated fatty acids [9]. Several rules based on the diagnostic ions in the EI mode have been developed for identification of the position of double bonds in FAMEs. For example, in methylene interrupted PUFAs ions $[C_{n+5}H_{2n+6}]^+$ can be used to identify the position of the first double-bond from the methyl end (*n* is the carbon number of the double-bond counted from the methyl end). Using this, $n-3$, $n-6$, and $n-9$ (also known as $\omega 3$, $\omega 6$, and $\omega 9$) can be identified from their intense peaks at *m/z* 108, 150, and 192, respectively [9]. Mjos et al. have shown that the geometry of the central double-bond(s) in trienes and tetraenes can be determined using the 70 eV EI spectra of FAMEs [9]. In that work a small difference between the *cis* and *trans* geometry of the terminal double-bond (closest to the methyl end) of trienes has also been observed. However, so far identification of the geometry of all double bonds, that is, discrimination among all geometrical isomers of PUFAs using mass spectrometry of FAMEs has not been possible.

In this paper we put forward and test the hypothesis that use of low-energy electron ionization can lead to information-rich differences in the mass spectra of geometrical isomers of FAMEs. A limitation to any approach relying on subtle changes in fragmentation patterns is the random variation of the relative ion abundances in mass spectra induced by factors such as instrumental and statistical noise. These contributions increase as the amount of analyte and the corresponding signal levels decrease. To test our hypothesis and the robustness of the proposed method we analyzed three different concentrations of a mixture of eight geometrical isomers of α-linolenic acid methyl ester (C18:3 $n-3$) using a gas chromatograph connected to an orthogonal acceleration time of flight mass spectrometer [24]. Three electron energies 70, 50, and 30 eV were used and the mass spectral data were analyzed by PCA [25], with display of the clusters of isomers in scores plots.

Selection of α-linolenic acid geometrical isomers in this work is motivated by the fact that accurate analysis and identification of these isomers is very important and challenging. They have been found in food products such as infant formulas [26]. It has also been shown that the enzymes involved in lipid metabolism are unable to discriminate between a *trans* isomer of α-linolenic acid (*cis*-9, *cis*-12, *trans*-15 C18:3), which is a $\omega 3$ triene and *cis*-9, *cis*-12 linoleic acid, which is a $\omega 6$ diene. Therefore, the former is incorporated (preferentially) into cardiolipins [27].

Experimental

A Supelco 47,792 α-linolenic acid methyl ester (C18:3 $n-3$) isomer mixture containing all eight geometrical isomers was purchased from Sigma Aldrich (Castle Hill, Australia). It contained a total FAME weight of 10 mg mL^{-1} in methylene chloride. The mixture composition and compound naming system are given in Table 1.

Three different dilutions of this mixture were prepared in hexane with dilution factors 10, 100, and 200 in 2 mL GC vials. This was done to investigate the robustness of the approach with diminishing signal to noise. Data were obtained with a GCT (Micromass, Waters Co.) mass spectrometer equipped with an Agilent HP6890 GC (Wilmington, DE). The GC was fitted with a highly polar column (BPX-70, 70% cyanopropyl polysilphenylene-siloxane, 60 m, 0.25 mm i.d., 0.25 μm film thickness from SGE, Ringwood, Australia); 1.0 μL of each sample was injected via a splitless injector at 240 °C at constant pressure of helium with column flow of 0.4 ml min^{-1} at an oven temperature of 140 °C. A starting oven temperature of 140 °C was used and was held for 2 min. The temperature was then increased by 2 °C min^{-1} to 180 °C where it was held for 30 min. The total run time was 50 min. The trap current was regulated at 250 μA and the ion source temperature was 180 °C. Ion detection and TOF measurement were facilitated by a time to digital converter (TDC) with a sampling rate of 3.6 GHz. With a 30 kHz pusher pulse, a full spectrum is generated every 33 μs. Data acquisition was performed for *m/z* 30–800 Da with 30,000 full

Table 1. Naming convention and mixture composition for α-linolenic acid methyl esters used in this study. The mixture and the mass fraction data have been obtained from Supelco

Abbreviation	Compound	Approximate mass fraction (%) in solution
ccc	*cis*-9, *cis*-12, *cis*-15-Octadecatrienoic acid methyl ester	3
cct	*cis*-9, *cis*-12, *trans*-15-Octadecatrienoic acid methyl ester	7
ctc	*cis*-9, *trans*-12, *cis*-15-Octadecatrienoic acid methyl ester	7
ctt	*cis*-9, *trans*-12, *trans*-15-Octadecatrienoic acid methyl ester	15
tcc	*trans*-9, *cis*-12, *cis*-15-Octadecatrienoic acid methyl ester	7
tct	*trans*-9, *cis*-12, *trans*-15-Octadecatrienoic acid methyl ester	15
ttc	*trans*-9, *trans*-12, *cis*-15-Octadecatrienoic acid methyl ester	15
ttt	*trans*-9, *trans*-12, *trans*-15-Octadecatrienoic acid methyl ester	30

spectra being accumulated per s yielding the continuum GC-MS spectra with a mass resolution of 7000 FWHM. MassLynx 4.0 software (Manchester, UK) was used for data acquisition and manipulation. The continuum data were converted to centroid and further data analysis and processing was performed on centroid data.

Data Analysis

Multivariate curve resolution (MCR) is a chemometrics method that can be used to deconvolute co-eluting compounds. In this study, MCR using the PLS_Toolbox 4.1 [28] is used to recover the mass spectra and elution profiles of co-eluting isomers.

PCA is a data reduction method that is used to simplify the data using a number of so called "latent variables or principal components (PCs)" that are linear combinations of original variables (in this case *m/z* channels). Each PC consists of two sets of data (vector) called "scores" and "loadings." The scores vectors contain the information about the samples (geometrical isomers in this case) and the loadings vectors carry the information about the variables (*m/z* channels here). Samples (geometrical isomers) are represented by these new variables that explain significant and independent sources of variation in the data. PCA can be used as a pattern recognition and classification tool by plotting the values (called scores) of samples on significant PCs versus one another. If original variables (*m/z*) contain diagnostic features by which different geometrical isomers can be identified, this will emerge in the PC plots (plots of scores vectors versus one another) showing separate classes, one for each isomer.

Results and Discussion

Ionization by electrons having significantly less kinetic energy than 70 eV imparts less internal energy to the ionized molecule. At 70 eV, the ionization of different isomers is often believed to result in indistinguishable mixtures of rearranged ions. By minimizing this rearrangement by using lower electron energy while maintaining sufficient sensitivity in the analysis (ion yields drop significantly with electron energy) the double bonds in the molecule would more likely preserve their initial geometry. Hence, differences in the fragmentation pattern of ionized molecules with different geometry, which leads to a unique mass spectrum for each individual isomer, could be expected.

The chromatographic method separated six of the eight isomers (**ttt**, **ctt**, **tct**, **ctc**, **tcc,** and **ccc**). As shown in Figure 1a the isomers **ttc** and **cct** co-elute. The assignment of the peaks was based on the work by Mjos et al. [9]. For each separated isomer, a combined (averaged) mass spectrum was obtained using software MassLynx 4. To recover mass spectra and elution profiles of the co-eluted isomers MCR was applied. The mass spectra of these two isomers (**ttc** and **cct)** show large differences due to opposite geometry of the central double-bond. The influence of the geometry of the central double-bond of these compounds has previously been observed [9], and here the *m/z* and elution profiles were easily obtained by MCR as shown in Figure 1b–d. Mass spectra (*m/z* 50–294) of all eight isomers at three different concentrations with two replicates in each were scaled individually so that the intensity reading for the base peak was 100. An exception was the isomer **ccc**, which was studied only at two concentrations with two replicates in each. The concentration of **ccc** in the most dilute mixture was too close to its detection limit to provide useful data.

To quantify the variance of the *m/z* intensities from sample to sample (replicate injections and different dilutions) standard deviation (s) and relative standard deviation (%RSD) of the key ions were calculated. They were ranged on average (across all eight isomers) 0.4 to 5.1 and 4.2% to 37.4%, respectively. The details are given in Table S1 of the Supplementary Information, which can be found in the electronic version of this article. Note that because the data of only two replicates for each dilution is available in this study, the within concentration variance, which is small compared with the overall variance for most of the cases, is not reported.

It is apparent in the mass spectra of Figure 1c and d that for many *m/z* channels, in particular *m/z* 150–290, the ion signals are at the noise level and nonsignificant. This is particularly so when the isomers are dilute. Therefore, data were filtered to include only the *m/z*

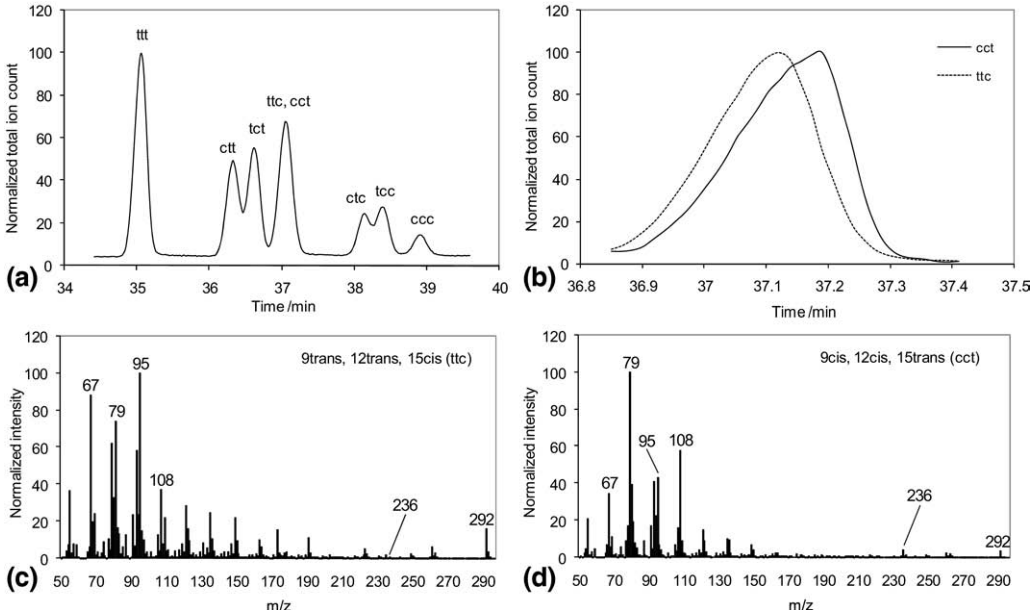

Figure 1. (**a**) Normalized total ion chromatogram of geometrical isomers of α-linolenic acid methyl ester (C18:3 $n-3$); (**b**) deconvoluted chromatograms of α-linolenic acid geometrical isomers of 9-*cis*, 12-*cis*, 15-*trans* (**cct**) and 9-*trans* 12-*trans* 15-*cis* (**ttc**) using multivariate curve resolution; (**c**) an (**d**) mass spectra of ttc and cct isomers obtained by multivariate curve resolution. Assignment of the peaks was based on the previously published paper by Mjos et al. [9].

channels corresponding to the 45 most abundant ions (over all spectra). These data were collected in a matrix of size 46 (sample) × 45 (*m/z*). The rows in this matrix represent the mass spectra of different concentrations and replicates of geometrical isomers of α-linolenic acid methyl ester. Three different electron energies (30, 50, and 70 eV) were used and a data matrix, as described above, was obtained for each electron energy. Each data matrix was mean-centered column-wise and PCA was applied to each dataset individually.

For all three electron energies, the first two principal components described at least 85% of the variance in the data. Figure 2 shows a joint plot of scores (isomers) and loadings (*m/z*) of PC1-PC2 for the data obtained at 70 eV. This "biplot" is a joint plot of scores and loadings. In the scores plot each close circle is a geometrical isomer with its coordinates being the scores of the first and second PCs. In the loadings plot each open triangle is a *m/z* channel with its coordinates being the loadings of the first and second PCs. Two major classes separated by PC1, which represents the geometry of the central double-bond (double-bond at carbon number 12) are observed. This pattern, which has been reported for EI at 70 eV by another group [9] was also seen here in the PC1-PC2 plots of 50 and 30 eV data. All the isomers with *trans* geometry in the central double-bond, that is, isomers **ttt**, **ctt**, **ttc,** and **ctc** returned positive scores on PC1 and the ones with a cis central double-bond, that is, **tct**, **cct**, **tcc**, and **ccc**, showed negative scores on PC1. Figure 2 also shows the distribution of *m/z* in the PC1-PC2 space. As shown *m/z* 95, 81, and 67 have high positive values (loadings) on PC1, therefore are diagnostic for *trans* geometry in the central double-bond, while *m/z* 79 and 108 have an opposite sign on PC1, therefore are diagnostic for *cis* geometry of a central double-bond. A similar pattern of *m/z* was also observed when data of 50 and 30 eV were analyzed by PCA. Figure 3 shows the mass spectra of the two isomers all-*trans* (**ttt**) and all-*cis* (**ccc**) as representatives

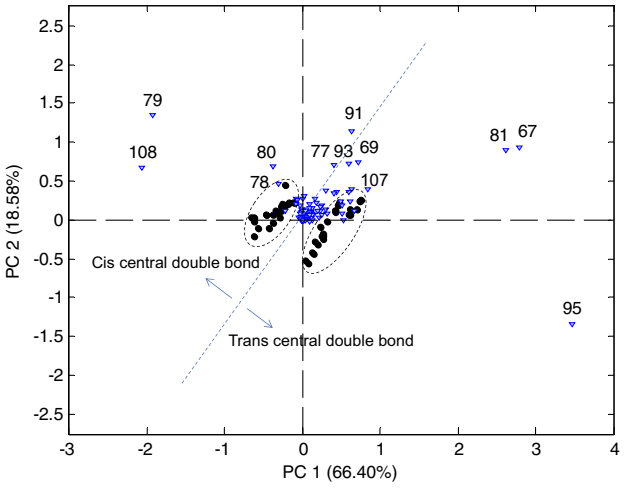

Figure 2. PC1-PC2 biplot of principal component analysis applied to the 70 eV EI mass spectral data of α-linolenic acid methyl ester geometrical isomers in different concentrations. The filled circles show the α-linolenic acid methyl ester isomers and the triangles are the *m/z* channels. The values of some of the *m/z* channels that are diagnostic for the *cis* and trans geometry of central double-bond are shown. The percentage of the variance in the data explained by each PC is shown in the axes titles.

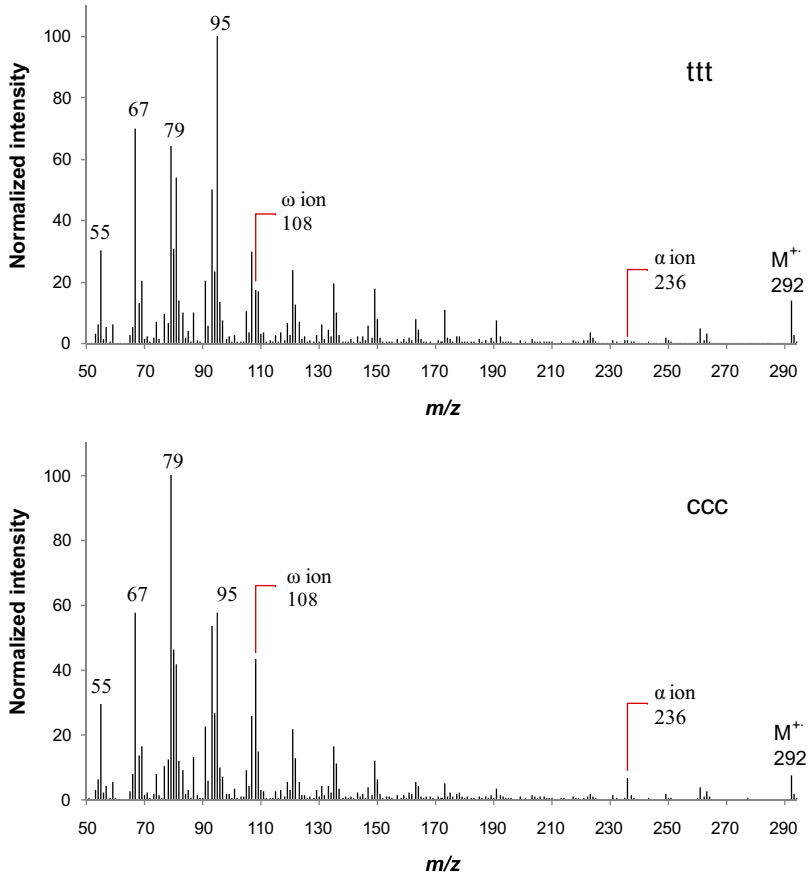

Figure 3. 30eV EI spectra of α-linolenic acid methyl ester isomers **ttt** and **ccc**.

for the isomers with *trans* and *cis* central double-bond, respectively.

There is no strong evidence about the mechanism of formation of *m/z* 79. Mjos et al. have suggested that α (*m/z* 236) and ω (*m/z* 108) ions formed by cleavages allylic to the central double-bond may further breakdown to produce *m/z* 79 [9]. Figure 4 shows these two cleavage processes as well as that leading to *m/z* 95. The *cis* geometry in the central double-bond is believed to facilitate the formation of *m/z* 79, which corresponds to the stable ion $[C_6H_7]^+$, the base peak in all the isomers with a *cis* central double-bond. The intensity of *m/z* 79 decreases in isomers having a trans central double-bond, and *m/z* 95 $[C_7H_{11}]^+$ becomes the base peak. This suggests the trans geometry in this position interferes with the formation of $[C_6H_7]^+$ [9]. Mass spectrometry studies of different cyclic and noncyclic compounds, including methyl-substituted cyclopentadienes [29], cyclohexadienes, and hexatriene [30] have indicated that cyclic $[C_6H_7]^+$ is the base peak, regardless of the structure of the compounds. The formation of $[C_6H_7]^+$ in these compounds requires considerable rearrangement of the original structure; therefore it was concluded that the driving force for the reaction must be the large stability of this ion.

Studies of $[C_6H_7]^+$ (known as "benzenium ion", "protonated benzene", "cyclohexadienyl cation") in solution and in the gas phase using NMR [31, 32], IR

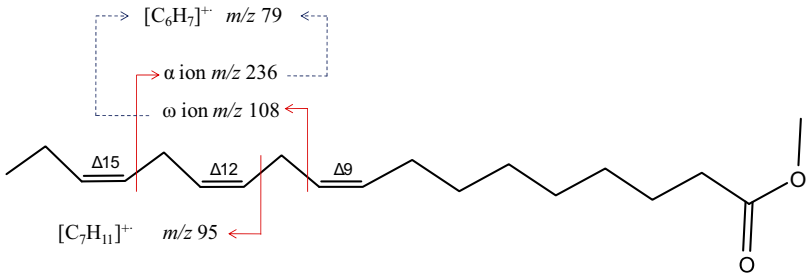

Figure 4. Cleavages leading to major fragment ions in α-linolenic acid methyl ester. Note: as will be explained later in this paper, the further decomposition of α and ω ions leading to the ion $C_6H_7^+$ are inconsistent. Contribution of all three double bonds is required for this ion to form.

[33–35], MS [36], and theoretical calculations [31, 33, 34, 37] have indicated a planar (C_{2v}) σ complex structure in which the extra hydrogen (to the benzene structure) is σ-bonded to a carbon. While there is a consensus on the σ complex being the most stable structure, Mason et al. [38] and Dewar [39] have proposed a π complex between hydrogen and the π system of the benzene ring. In the latter, it is argued that any other structure would not be aromatic and would suffer a significant decrease in resonance energy. The two structures of $[C_6H_7]^+$ are shown in Figure 5.

It has been shown that the σ complex of $[C_6H_7]^+$ (or its related isomer bicyclo[3.1.0]hexenyl cation) does not possess an aromatic or even homoaromatic character [32, 40–44]. Instead, it is a classic carbenium ion [40] with an allylic π system [32, 33] and minimal, if any, homoallylic delocalization nature [32]. In other studies, the benzenium ion is shown to be stabilized via hyperconjugation [40, 45–47], where the overlap between the σ bonds of CH_2 and the π bonds of remaining carbons in the ring creates cyclic delocalization. Muller et al. have reported a hyperconjugation contribution to the resonance energy of ~14 kcal/mol and an overall resonance energy that is larger than the conjugation energy of benzene [47]. Their calculations predicted two electronic transitions in the visible region, which was in good agreement with their experimental results. The benzenium ion is also stabilized by a 1,2-hydrogen shift (scrambling) [32–35] where at room temperature the hydrogens migrate on the six carbons at a high speed, thus delocalizing the positive charge. Interestingly, protonated polyaromatic hydrocarbons, including benzenium ions, have been found to be stable species in interstellar space [48].

A combination of the above mentioned processes creates a large delocalized, and therefore stabilized, positive charge system, which in turn drives the reaction toward the formation of *m/z* 79 $[C_6H_7]^+$ in an EI MS analysis of linolenic acid isomers. The *cis* geometry of the central double-bond creates a curvature in the molecule that facilitates the ring closure of $[C_6H_7]^+$. The *trans* geometry, however, hinders this process by straightening the molecule. To further investigate the stability and mechanism of formation of $[C_6H_7]^+$ we inspected the mass spectra of over 300 compounds having various types and levels of unsaturation. Except for very few cases with highly substituted double bonds, the following conditions are required so that *m/z* 79 $[C_6H_7]^+$ becomes the base peak:

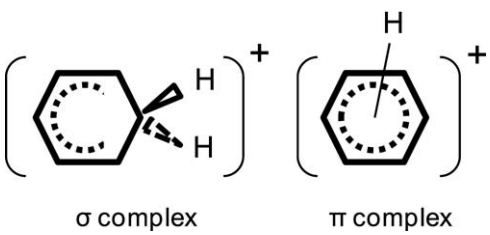

Figure 5. Two structures of benzenium ion, $[C_6H_7]^+$.

1. Non-cyclic compounds: at least three π bonds (three double bonds or a triple and a double-bond) distributed over carbons not separated by more than a carbon (maximally methylene interrupted).
2. Cyclic compounds: two π bonds distributed over carbons not separated by more than a carbon. π Bonds can be either both endocyclic (ring of any size) or one endocyclic and the other exocyclic.

Interestingly, in the cyclic compounds with one double-bond and noncyclic compounds with two double bonds (or a triple bond) *m/z* 79 has a very low intensity. This implies that in the case of α-linolenic acid methyl ester, fragments such as α and ω ions, which embrace two but not three double bonds, cannot further cleave to produce *m/z* 79. Thus, the two step cleavages of α and ω ions [9] leading to *m/z* 79 $[C_6H_7]^+$ as shown in Figure 4 are inconsistent. The question, however, is how three double bonds distributed over eight carbons in linolenic acid methyl ester forms a ring of six carbons, $[C_6H_7]^+$? A possible mechanism can be the migration of side double bonds toward the central position through methylene radical delocalization as seen in auto-oxidation of polyunsaturated fatty acids [13] followed by a ring closure. It is noteworthy that fragmentation of dienes and polyenes has been shown to be quite complex [50], and any concrete answer to the questions of this kind requires further studies, which are in progress in our laboratory.

A large portion (>66%) of the variance in these datasets is due to the difference of the geometry of the central double-bond of α-linolenic acid methyl ester isomers. This large variation can overwhelm the smaller variations caused by the difference of the geometry of the two side double bonds (double bonds on carbons 9 and 15). Therefore, to investigate the possibility of identification of *cis* and *trans* geometry in these two positions, the large variation caused by the central double-bond was removed by analyzing each of the two groups separated by PC1 in Figure 2 individually, that is, two applications of PCA were undertaken, one on each group representing molecules with a *cis*- or *trans*-central double-bond. 3-D PC plots of 70, 50, and 30 eV data for isomers with a *trans*- and *cis*-central double-bond are shown in Figure 6.

As indicated, complete separation of isomers is only possible at 30 eV, that is, at the lowest electron energy studied. The reason for this is likely to lie in the propensity of neutrals to undergo structural rearrangement as they ionize at 70 eV (where substantial internal energy is imparted). Thus, a similar distribution of different ion structures is created in the population of ionization events. These structures are associated with a correspondingly indistinguishable distribution of observed molecular or fragment ions irrespective of which isomer is ionized, thus confounding any ability to correlate the fragmentation pattern to a particular isomeric structure. When the electron energy decreases, the structural differences are more likely to be preserved and be reflected in differences in the mass spectra. In the present

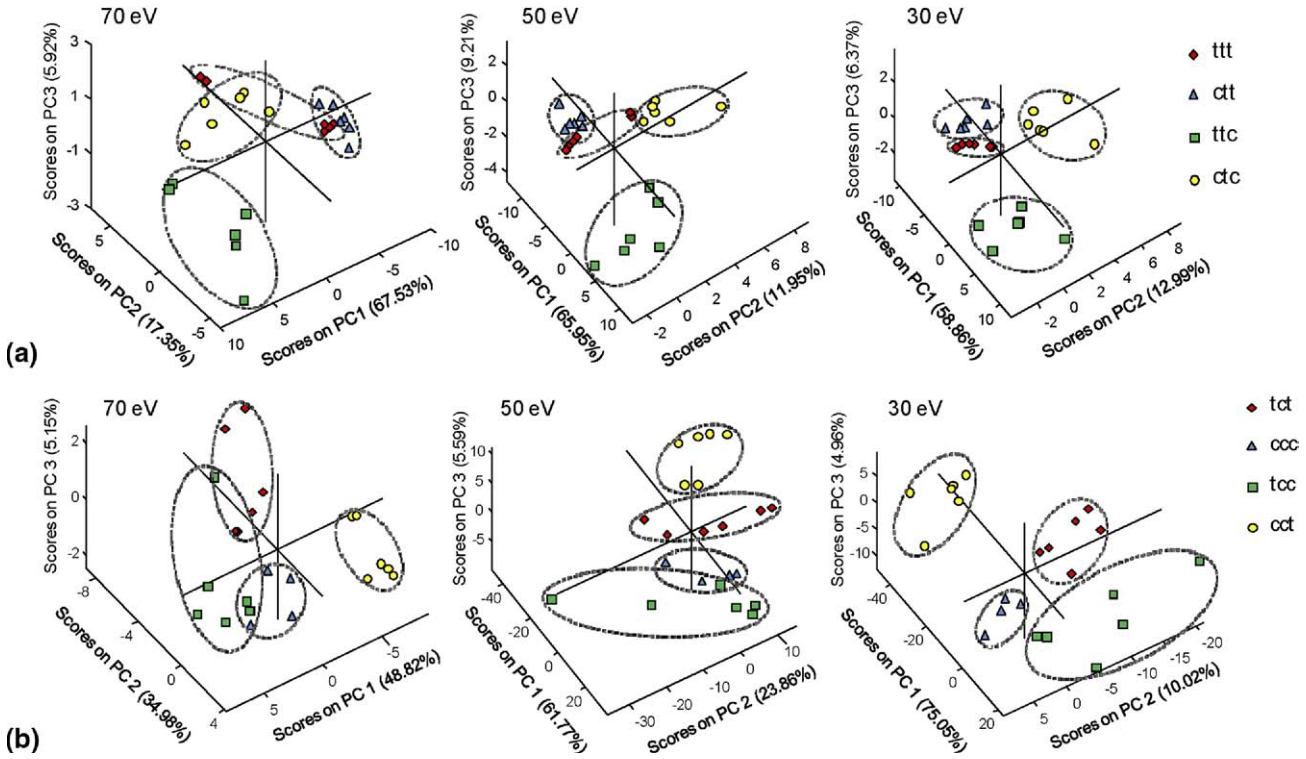

Figure 6. 3-D scores plot of the EI mass spectra of α-linolenic acid methyl ester geometrical isomers with (**a**) central *trans* and (**b**) central *cis* double-bond obtained at three different ionization energies. The percentage of the variance in the data explained by each PC is shown in the axes titles.

study, it is shown that using a 30 eV EI spectra and chemometrics it is possible to identify all eight isomers of α-linolenic acid methyl esters in a wide range of concentrations. It is worth noting that despite being milder than 70 eV, 30 eV generates most of the fragments observed in a 70 eV mass spectrum. Decreasing the energy from 70 to 30 eV causes differences in the relative abundances of fragment ions. Principal component analysis was used here to capture these diagnostic differences in an objective way. Successful classification at 30 eV despite using samples with very difference concentrations indicates the robustness of the proposed approach.

Using the method described above, identity of an unknown α-linolenic acid methyl ester can be established by the following steps:

1. Geometry of the central double-bond: A mass spectra with a base peak at *m/z* 79 indicates that the central double-bond is "*cis*." On the other hand a base peak at *m/z* 95 means that the central double-bond is "*trans*."
2. Geometry of side double bonds: Depends on the geometry of the central double-bond, one of the two models developed for *cis*-central and *trans*-central double-bond isomers is applied to the data (mass spectrum) of the unknown to determine the geometry of side double bonds. In this method, the mass spectra of the unknown, a vector of intensities with size "1× *m/z* channel", is projected onto the 3D principal component space of the model and three scores are obtained that determine the position of the unknown point in the PC space and therefore the isomer class it belongs to. All the calculations can easily be performed using the PLS_Toolbox for MATLAB or a SOLO standalone version from Eigenvector [28].

A disadvantage of using low-energy electrons is loss of sensitivity. To show this effect, the relative total ion current (TIC) peak areas of isomers at 70, 50, and 30 eV over those of 70 eV were measured in five independent runs. The average relative peak areas 1, 1/2, and 1/4 were obtained, respectively. This points to a trade-off in seeking an advantage in using even lower EI to resolve isomers. As the energy is further reduced, even if they lead to greater differences in fragmentation patterns, those differences become more difficult to measure reliably because the signal-to-noise ratio is decreased, and the variance may be due to this source rather than isomeric structure. It would be advisable, when lowering electron energy, also to adopt measures to preserve signal-to-noise. For example, using slower scan rates and higher concentrations when possible. Another drawback of lowering the energy below 30 eV is the loss of some diagnostic *m/z* channels. In the 16 eV EI spectra of α-linolenic acid methyl ester presented in the work of Brauner et al. [49], the ions with *m/z* 79, 95, 81, and 67, which are diagnostic for the geometry of the central

double-bond, almost vanish. Also a 1000-fold decrease in the ion yield for an ionization energy change from 100 to 16 eV is observed.

Application of the proposed method is not limited to the geometrical isomers of α-linolenic acid methyl ester. The method can potentially be used to identify geometrical isomers of unsaturated compounds, including fatty acids with various numbers and positions of double bonds. Examples are monoene and diene fatty acids for which no difference between the 70 eV EI spectra of *cis* and *trans* has been found, and tetraene geometrical isomers, among which only few have been chromatographically separated and identified [9].

Acknowledgments

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Appendix A Supplementary Material

Supplementary material associated with this article may be found in the online version at doi:10.1016/j.jasms.2009.02.027.

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