A Beginner's Guide to Mass Spectrometry of Fatty Acids

Part 1. Methyl Esters

William W. Christie

Formerly of the James Hutton Institute and the MRS Lipid Analysis Unit (Dundee)

At its simplest, mass spectrometry (MS) is a technique in which organic molecules are bombarded by electrons or other ionic species causing them to ionize and fragment for separation by a magnetic field. We use the fragmentation patterns as a means of identification. If you pick up a journal dealing with mass spectrometry, there will be a host of technical terms dealing with methods of ionization especially, such as "electrospray ionization", "chemical ionization", "fast-atom bombardment" and others, but in the more basic mass spectrometers linked to gas chromatography (GC-MS), electron impact ionization is encountered most often. It is the one most relevant to this specific topic and is the only one considered here. The various ionic species produced from a given organic compound by electron impact ionization are separated according to mass (strictly speaking mass/charge (m/z) ratio in which z = 1) in a magnetic field in the mass spectrometer, and a spectrum is obtained that in effect is a bar diagram showing the masses of the fragment ions and their abundances relative to the most abundant ion (base ion), which is usually normalized to 100% for record, comparison and publication purposes.

In this and Part 2, I discuss the basic principles only of identification of fatty acid structures by mass spectrometry. More detailed information with many more spectra illustrated is available in the other articles in the mass spectrometry pages of this website, and two review articles [1,2] or a book [3] can be recommended.

Jigsaw Puzzles and Bricks

Interpretation of a mass spectrum is often compared to doing a jigsaw puzzle. We have many different pieces or fragments, and we must try to put them together in a sensible way to find the picture or to describe the molecule. I prefer an analogy, in which mass spectrometry is compared to demolishing and re-assembling a brick wall. If we use a sledgehammer to create a pile of rubble, we will know the total mass present but reassembling the wall is impossible. If we can take the wall apart cleanly a few bricks at a time, it will be possible to reassemble it more easily. We will know the correct dimensions and where any door or window should be placed. With a fatty acid derivative in comparison, we need to confirm that it is indeed a fatty acid, determine the molecular weight and then locate any double bonds or other functional groups in the aliphatic chain.

Methyl esters are the derivative of choice in most applications to the analysis of fatty acids, but when we subject them to electron impact ionization in a mass spectrometer, the charged aliphatic chain breaks up into so many fragments that a branch-point or other structural feature cannot be identified easily. A double bond can carry the charge and become mobile; it moves up and down the chain, so that its original position cannot be determined. This is especially troublesome with mono- and dienoic fatty acids. Using the above analogy, we have taken a sledgehammer to break up the molecule. This is something of an exaggeration, as we can usually settle on a restricted number of possible structures from mass spectra of methyl esters, although we may not obtain a definitive answer. An important detail is the molecular weight of the fatty acid ester, which is obtained from the molecular ion as this tells us the number of carbon, hydrogen and oxygen atoms. We therefore know if the fatty acid is saturated or unsaturated, and often an experienced eye can tell

from close examination of the spectrum if there is a branch point or other substituent and where that substituent is located in the aliphatic chain. It helps that there are many authentic mass spectra of methyl esters available in the scientific literature for comparison purposes, not least in the main website.

Interpretation of the mass spectrum of a simple saturated isomer such as methyl palmitate is straight-forward (**Figure 1**). There is a clear molecular ion at the end of the high mass range at *m/z* = 270. The ion at *m/z* = 239 represents a loss of 31 mass units (CH_3O) as shown, and this confirms that we are dealing with a methyl ester, while the ion at *m/z* = 74 is also diagnostic for a methyl ester and is most abundant with saturated fatty acids. The latter is the so-called 'McLafferty ion' and is displayed as a simple cleavage here, although in reality it is formed by a rearrangement involving the ester moiety (more detail in the main website). The long homologous series of related ions (14 amu apart) at *m/z* = 87, 101, 115, 129, 143, 157, 199, etc. of general formula $[CH_3OCO(CH_2)_n]^+$ is evidence that there are unlikely to be other functional groups in the chain. Unfortunately, the spectrum of a comparable branched chain derivative can look very similar to this, although there can be minor but significant differences.

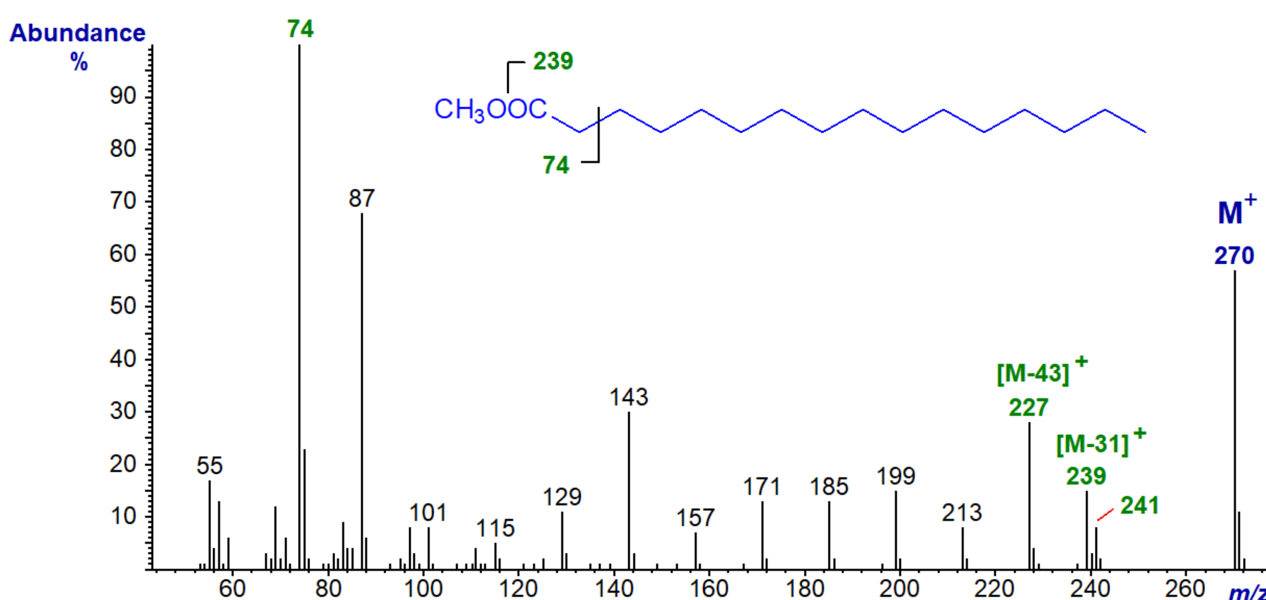


Figure 1. Mass spectrum of methyl palmitate

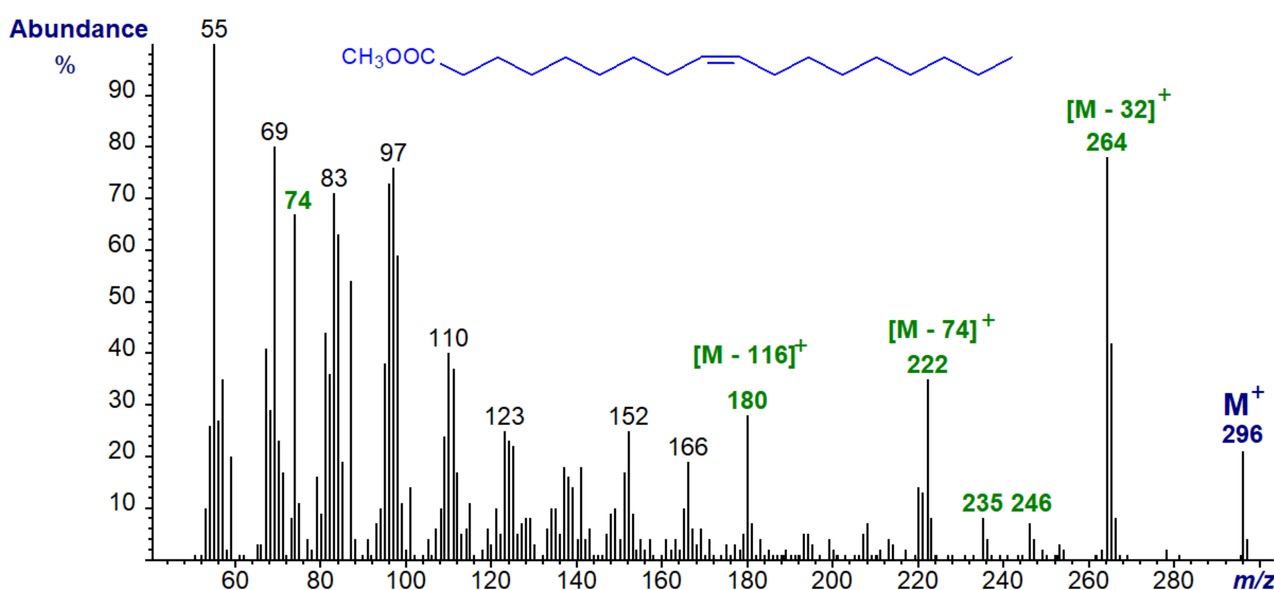


Figure 2. Mass spectrum of methyl oleate

The mass spectrum of methyl oleate is illustrated next (**Figure 2**). The molecular ion is at *m/z* = 296, i.e., two less than for methyl stearate (or -2H). We usually lose one or two 'bricks' neatly, and an ion representing loss of 32 mass units (methanol from the ester moiety) and another of 74 units (both *m/z* = 74 per se and $[M-74]^+$ at *m/z* = 222) confirm that we really do have a methyl ester, although there are no ions that help us to determine the position or configuration (*cis/trans*) of the double bond.

Strictly speaking, as the molecular weight is two less than for a fully saturated fatty acid derivative, we could have either a double bond or more rarely a cyclic structure present, but the correct assignment is usually possible from close examination relative to standards or from simple experiments, for example by attempting hydrogenation. The spectrum is 'busier' than for the methyl ester of a saturated fatty acid, i.e., there are ion clusters rather than relatively solitary ions.

Further invaluable information that we should have as an aid to identification is the retention time relative to standards of the fatty acid methyl ester (or equivalent chain-length value) on the GC column of the GC-MS system. This together with the molecular weight and mass spectrometry data will usually suggest that some structures are more plausible than others.

Double Bond Location in Polyunsaturated Fatty Acid Methyl Esters

We usually wish to know where double bonds are located in an aliphatic chain, and the mass spectrum of the methyl esters are not always of assistance. Although we cannot do this directly with monoenes and dienes, it is fortunate that with the conventional series of polyunsaturated fatty acids, i.e., with three or more methylene-interrupted double bonds, we have some useful characteristic ions of diagnostic value. These can be seen in the mass spectrum of methyl 6,9,12-octadecatrienoate (γ-linolenate) illustrated in **Figure 3**.

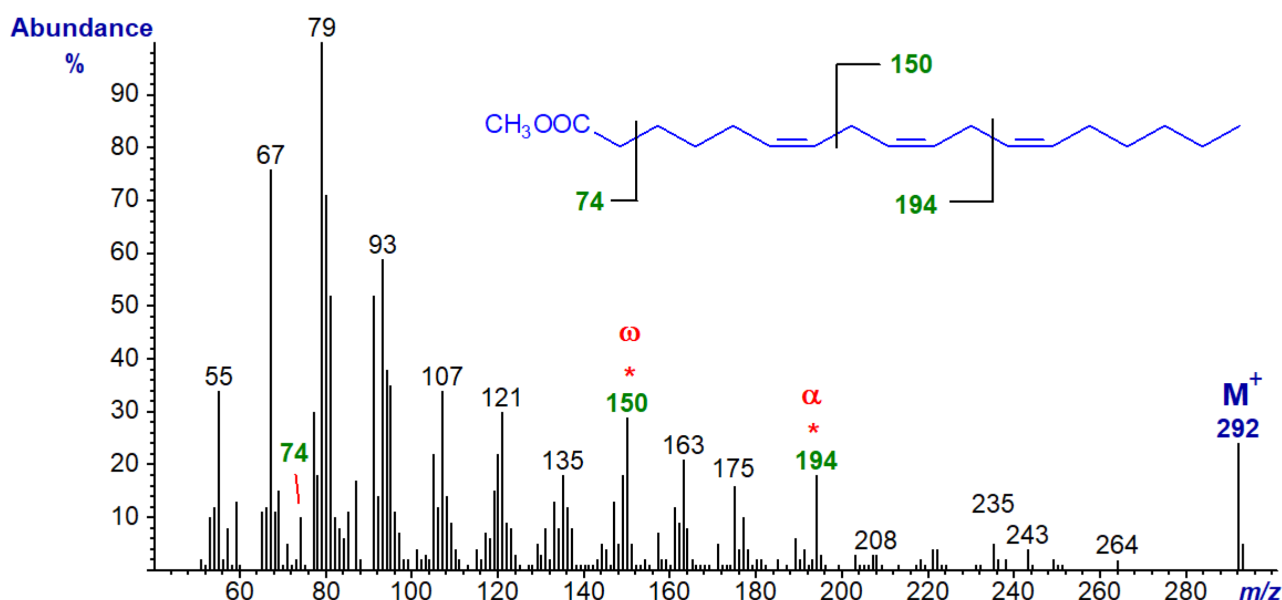


Figure 3. Mass spectrum of methyl 6,9,12-octadecatrienoate (γ-linolenate).

First, the molecular ion at *m/z* = 292 is 6 units less than for methyl stearate (18:0), which tells us that there are three double bonds, while the ion at *m/z* = 74 again confirms that we do indeed have a methyl ester. These ions tend to be much smaller than in spectra from more saturated esters. Ions from the hydrocarbon part of the molecule of general formula $[C_nH_{2n}-5]^+$ tend to dominate the spectrum with that at *m/z* = 79 as the base ion, but these tell us little about the detailed structure.

However, an ion at *m/z* = 150 ('omega' ion) formed by a fragmentation at the terminal end of the molecule is characteristic of methyl esters of all polyunsaturated fatty acids from the *n*-6 biosynthetic family [4]. To completes the identification, there is a fragment ('alpha' ion) from the carboxyl end of the molecule at *m/z* = 194, although the position of this ion will vary according to the position of the first double bond in fatty acids of the *n*-6 family. The latter ion was best defined in a paper by Brauner and coworkers [5] (more details hereon the main webpage).

In mass spectra of methyl esters of fatty acids of the *n*-3 family, the omega ion stands out at *m/z* = 108 (while that at *m/z* = 150 is negligible), and for the minor (*n*-9) and (*n*-4) biosynthetic families of fatty acids, the relevant ions are at *m/z* = 192 and 122, respectively. It should be stressed that these ions are valuable but not infallible guides, and they are not reliable when the double bonds are not methylene interrupted.

That said, it is surprising how often mass spectra from methyl esters of different polyunsaturated fatty acids are sufficiently distinctive to be considered as 'fingerprints', which can be identified by comparison with the spectra of standards. Unfortunately, when there are five or more double bonds, the molecular ion can be of low abundance and difficult to identify.

Chemical Derivatization for Double Bond Location

If we want to have definitive information on double bond positions, we must get away from the sledgehammer approach. There are two ways this can be done. One is to prepare particular nitrogen-containing derivatives of fatty acids, and this is the topic of the Part 2 of my "Beginner's Guide". Alternatively, with monoenoic fatty acids, it is possible to "fix" the double bonds by reacting them with appropriate reagents to give chemical derivatives that give distinctive fragmentations in the mass spectrometer.

A host of such derivatives were described, usually involving oxygenation followed by further derivatization, but most of these fell by the wayside when dimethyl disulfide adducts were described [6]. This is a simple single-step (if odoriferous) reaction involving reaction of dimethyl disulfide with an unsaturated ester in the presence of iodine as catalyst. The nature of the reaction is shown in **Figure 4**.

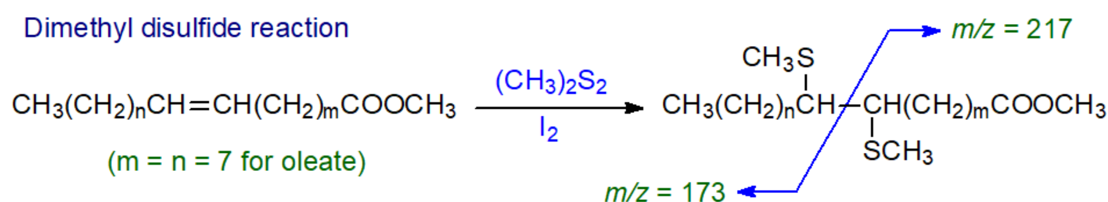


Figure 4. Reaction of methyl oleate with dimethyl disulfide.

With methyl oleate as illustrated, the molecular weight increases substantially (from 296 to 390) but this is still in a comfortable range for GC analysis. The mass spectrum gives a substantial molecular ion but the most abundant ions represent cleavage at carbon atoms that were originally linked by the double bond (*m/z* = 173 and 217 as shown), and this is located unequivocally (the full spectrum is available in the main website). This technique has now been applied to identify many monoenoic fatty acids (and other aliphatic compounds) in natural samples in numerous laboratories, and new applications continue to appear in the scientific literature. (This is the best test of any method - often a new procedure is described with one or two model compounds and then falls into oblivion).

The technique is less straight-forward if there is more than one double bond in the molecule, as bis-adducts are only formed quantitatively if there are more than three carbons between double bonds, although the reaction can be used usefully even with methylene-interrupted dienes, such as linoleic acid, if the reaction is carried out under mild conditions. Two mono-adducts (one double bond derivatized and one not) are then formed and these are easily separable by GC for analysis by MS [7]. There is a more substantial article on this aspect of the topic on the main website.

Such methods add an extra complication to analysis, and it would obviously be much easier if a single derivative could be used for all purposes, as discussed in Part 2.

Be Wary of Computerized Identification Techniques!

Most GC-MS systems are sold with software that enables comparison of spectra of unknowns with a library of spectra of standards, and this can indeed be very useful. That said, beginners should be very careful of how this facility is used for many reasons. While the library may contain 100,000 spectra or more, this is still a small number in comparison to the number of organic compounds in general and fatty acid derivatives in particular that might be encountered. Then, the identification is made from the intensities of a relatively small number of ions, and there can be some variability depending on such factors as the make and model of the instrument and its condition/age. A comparison with the spectrum of methyl oleate, for example, may suggest many possible positional or configurational isomers, all with a confidence of 99%. The computer does not recognize that isomeric methyl octadecenoates of this kind have spectra that are identical for all practical purposes. Analysts should have a sufficient understanding of the principles of mass spectrometry to know when to ignore the computer and make informed decisions.

Quantification

I do not discuss quantification by GC-MS in these pages, mainly because I believe that GC with flame-ionization detection and quantification is robust methodology with an appreciable linear range and with a relatively simple requirement for standards [3]. While GC-MS can be used for the purpose, it requires careful calibration with a wide range of standards [8]. I view quantification and structure determination as separate tasks.

Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS with electrospray ionization and other soft ionization methods is being used increasingly for determination of fatty acid compositions with derivatives of various kinds, including methyl esters. In my opinion, which is given with the caveat that I have no direct practical experience, it offers no advantages over GC-MS (when this is available) for analysis of the common types of fatty acid that are likely to be encountered in animal and plant tissues. However, it is undoubtedly the best approach for many oxylipins, which are relatively unstable chemically.

Part 2. 3-Pyridylcarbinol, 4,4-Dimethyloxazoline and Pyrrolidine Derivatives

In Part 1 of this topic, I used an analogy in which mass spectrometry was compared to demolishing and re-assembling a brick wall. If we take it apart a few bricks at a time, it is possible to reassemble it easily; we will know the correct dimensions and where any door or window should be placed. With a fatty acid derivative, in comparison, we need to use the fragment ions to confirm that it is indeed a fatty acid, determine the molecular weight and then locate any double bonds or other functional groups. Methyl esters give satisfactory identifications in a limited range of circumstances only, and most often in simple samples where no unusual fatty acids are expected. However, there are invaluable alternative derivatives, all of which contain nitrogen atoms close to the carboxyl group. With such compounds the nitrogen atom carries the charge when the molecule is ionized in the mass spectrometer, rather than the aliphatic chain (as with methyl esters). Rather uniform fragmentation then occurs along the aliphatic chain, and with a little experience, it is easy to pick out functional groups such as double bonds, methyl branch points or cyclic structures. Many spectra are available for comparison purposes in this website in the various tutorials and in the Archive sections, and most of these spectra are not available in commercial computerized data bases.

Three types of fatty acid derivative have been used most often for structure determination: in order of the original publications, acyl pyrrolidides, 3-pyridylcarbinol (incorrectly termed ‘picolinyl’ in most publications) esters and 4,4-dimethyloxazoline (DMOX) derivatives (**Figure 5**).

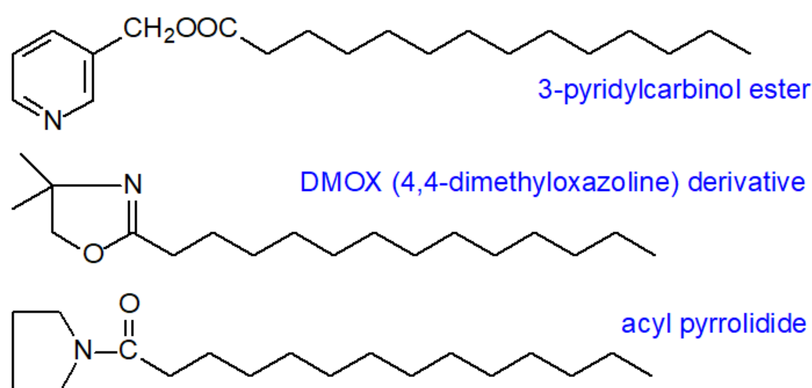


Figure 5. Nitrogen-containing derivatives for mass spectrometry

Pyrrolidides were the first derivative of this type to be described in 1971, but they have been overtaken by alternatives, probably because their chromatographic properties are less than ideal. 3-Pyridylcarbinol esters have better mass spectrometric properties than pyrrolidides, and DMOX derivatives are better for analysis by gas chromatography (GC), so these two are now favoured in most laboratories, but pyrrolidides derivatives should not be forgotten. Alternative derivatives have occasionally been proposed, but they are unlikely to be accepted unless a substantial body of sample spectra is made available for comparison or reference purposes.

In addition to the review articles cited in Part 1 of this topic, definitive reviews on the use of pyrrolidides [9], 3-pyridylcarbinol esters [10] and DMOX derivatives [11] have been published. Detailed protocols for the preparation of these derivatives are available on the main website.

Please note that I have not considered the use of mass spectrometry for quantitative analysis of fatty acids here. Then, quite different problems arise, and methyl ester derivatives may be as good as any other for the purpose. I will leave that topic to someone else to discuss, but readers should be aware that GC with flame-ionization detection is by far the simplest and most robust approach to quantitative analysis [3].

3-Pyridylcarbinol ('picolinyl') Esters

3-Pyridylcarbinol esters are not as popular now as they once were, in part because their preparation can be tedious, but they remain a good choice for structural analysis of fatty acids. To illustrate this, the mass spectrum of the 3-pyridylcarbinol ester of γ-linolenic acid (6,9,12-18:3) is illustrated (**Figure 6**) -

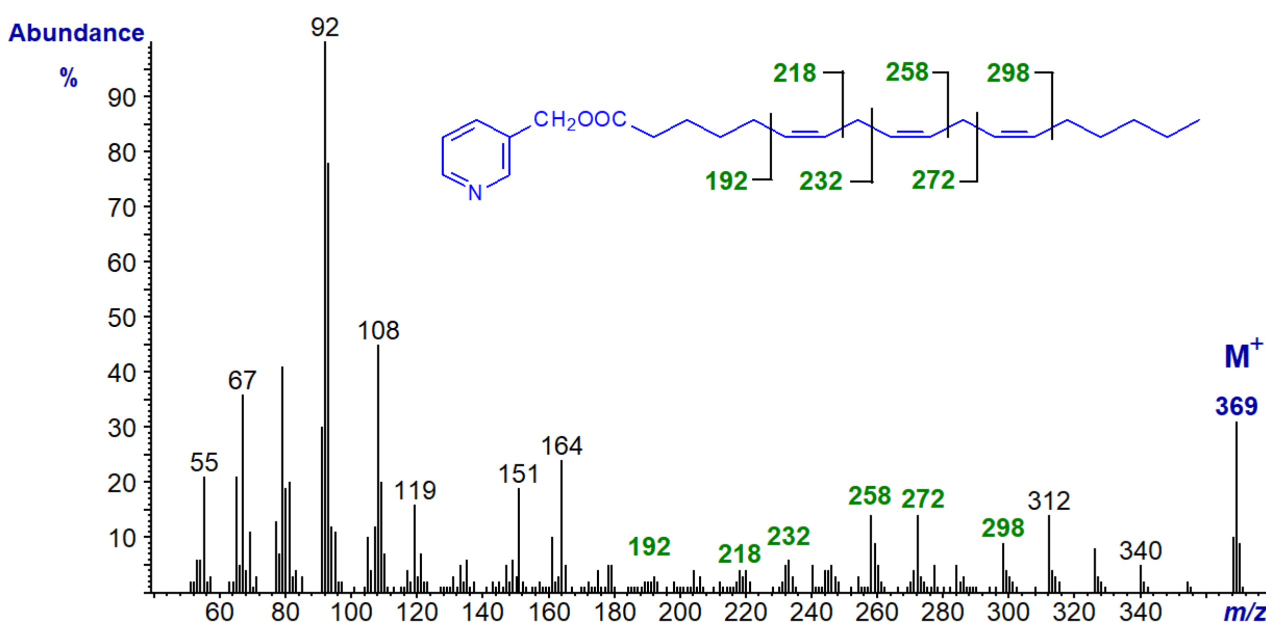


Figure 6. Mass spectrum of the 3-pyridylcarbinol ester of γ-linolenic acid (6,9,12-18:3).

There are large ions at *m/z* = 92, 108, 151 and 164, which contain the pyridine ring and various elements of the carboxyl group and serve mainly to indicate that the compound is indeed a 3-pyridylcarbinol ester. To locate functional groups such as the double bonds here, it is necessary to consider the higher molecular weight region of the spectrum. There is an abundant molecular ion (*m/z* = 369), which is always odd-numbered and is useful confirmation that we have a C_{18} fatty acid with three double bonds. Then there is a uniform series of ions 14 atomic mass units (amu) apart, representing loss of each successive methyl and methylene group from the terminal end of the molecule, until we reach the ion at *m/z* = 298. There is a gap of 26 amu for the carbons constituting the terminal double bond to *m/z* = 272, a further gap of 14 amu for the methylene group at carbon-11, then another gap of 26 amu between *m/z* = 234 and 258, a gap of 14 amu for the methylene group at carbon-8, and so forth.

The double bond nearest to the carboxyl group is not always easily spotted from first principles, but with a little experience it is not too difficult to define. Of course, it always helps to have access to spectra of standards for comparison purposes as in the Archive pages of this website.

After checking the ions for the pyridine ring, spectra of all other 3-pyridylcarbinol esters are thereafter interpreted in the same way, by starting with the molecular ion and working backwards one methylene group at a time until a functional group is reached. Thus, if there is a methyl branch, for example, there will be a gap of 28 amu for loss of -CH- with its attached methyl group.

3-Pyridylcarbinol esters have most often been prepared from unesterified fatty acids, and if such methods are to be used, it is necessary to first hydrolyse an intact lipid sample or methyl ester. The original method for preparation of the derivative involved dissolving the free fatty acid in an excess of thionyl chloride to form the acid chloride, which was then reacted with a 1% solution of 3-hydroxymethylpyridine in acetonitrile to form the 3-pyridylcarbinol ester for direct analysis by GC-MS [12]. Alternatively, a mild quantitative method may be preferred that involves formation of an imidazolide by reacting the fatty acid with 1,1'-carbonyldiimidazole in dichloromethane prior to reaction with the 3-pyridylcarbinol reagent in triethylamine in the presence of 4-pyrrolidinopyridine as a catalyst [13]. A newer method now permits direct preparation from methyl esters or intact lipids with 3-pyridylcarbinol in tetrahydrofuran and potassium tert-butoxide as catalyst [14], but again see practical details on the main website.

4,4-Dimethyloxazoline (DMOX) Derivatives

DMOX derivatives have good chromatographic and mass spectrometric properties, and they can be prepared in a relatively simple one pot reaction, both from unesterified fatty acids or those in ester form, so they are now the most widely used derivative for structural analysis of fatty acids. The mass spectrum of the DMOX derivative of oleic acid is illustrated next (**Figure 7**) -

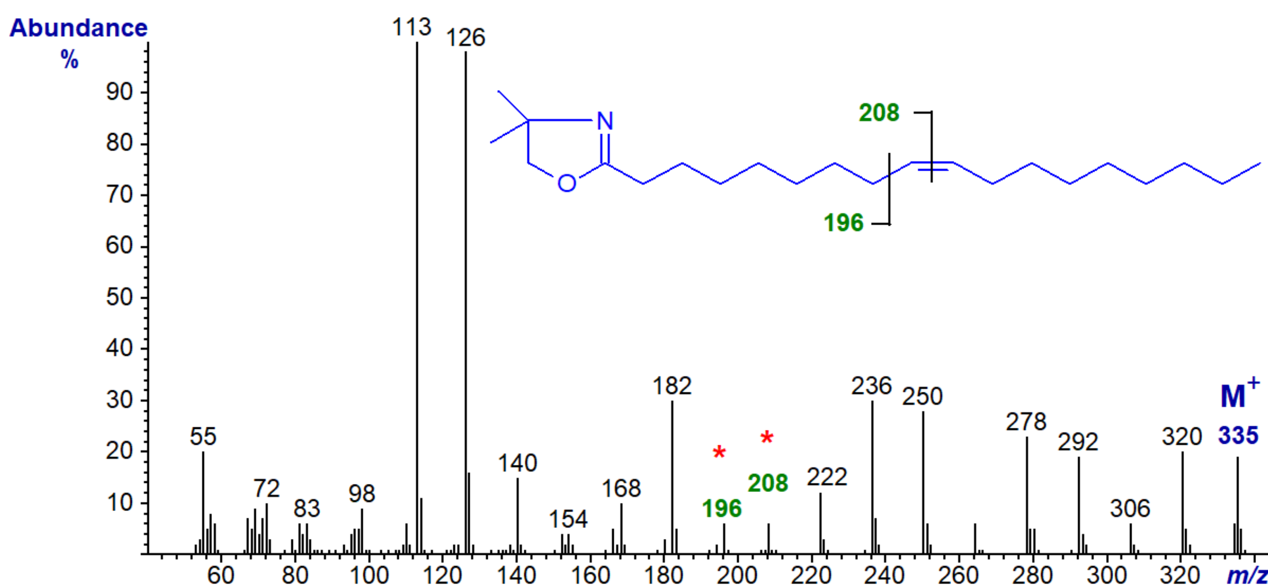


Figure 7. Mass spectrum of the DMOX derivative of oleic acid.

In this instance the ions at *m/z* = 113 and 126 confirm that we have indeed formed the DMOX derivative. Again, there is a clear molecular ion at m/z = 335, followed by gaps of 14 amu for the loss of each successive methylene group (*m/z* = 320, 306, 292, 278, etc.), until we find a gap of 12 amu which is indicative of the presence of the double bond, between *m/z* = 196 and 208. To locate this precisely, we must use the "12 mass rule", i.e.,

> "if a there is an interval of 12 amu between the most intense peaks of clusters of ions containing n and n-1 carbon atoms, there is a double bond between carbon n and n+1 in the molecule".

This may seem rather strange (it was first identified for pyrrolidide derivatives), but it works well in practice. Other functional groups, such as branch points or ring structures, are located as with 3-pyridylcarbinol esters. Problems of interpretation can arise when functional groups are near either end of the molecule, when it helps to have access to spectra of standards for comparison purposes as in the Archive pages of this website.

DMOX derivatives can be prepared simply by reacting the free fatty acid (or the methyl ester or even an intact lipid) with 2-amino-2-methyl-1-propanol in a micro-reaction vial at 180°C for 16 hours in a nitrogen atmosphere [15]. Practical details are available on the main website, where it is noted that the product must be stored under strictly anhydrous conditions otherwise partial hydrolysis can occur.

Pyrrolidides

Pyrrolidides are less used nowadays, although they still have their devotees, and they should not be forgotten. They are prepared from the methyl ester derivatives in a simple one-pot reaction with pyrrolidine in the presence of acetic acid at 100°C for 1 hour (see the main website for practical details). In spite of the marked differences in structure, pyrrolidides have exactly the same molecular weight as the corresponding DMOX derivatives and they give very similar fragmentation patterns with electron impact ionization, although the diagnostic ions are often of lower abundance. They can be preferable to DMOX derivatives for fatty acids with near-terminal functional groups. Their GC properties are intermediate between those of 3-pyridylcarbinol esters and DMOX derivatives. As an example, the mass spectrum of the pyrrolidide of 14-methylhexadecanoic acid is illustrated (**Figure 8**) -

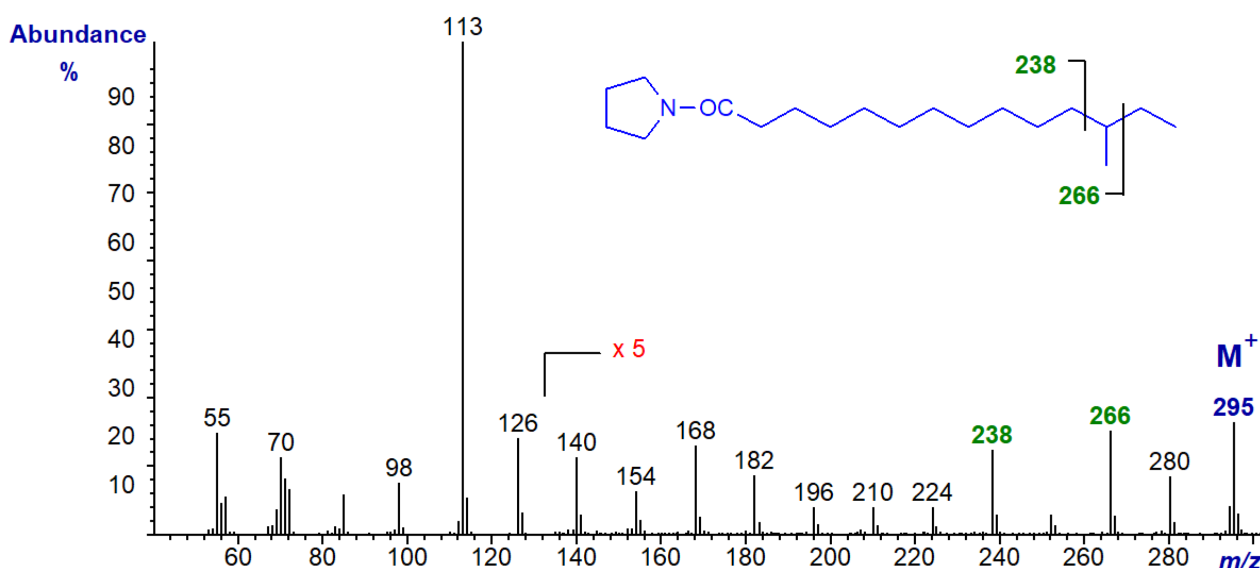


Figure 8. Mass spectrum of the pyrrolidide derivative of 14-methylhexadecanoic acid.

The ions for the carboxyl moiety at *m/z* = 113 and 126 are the same (other than intensity differences) as for the DMOX derivative for this fatty acid, as is the molecular ion at *m/z* = 323. The gap of 28 amu between *m/z* = 266 and 294 locates the methyl group, but there is no such diagnostic feature in the spectrum of the DMOX derivative of this fatty acid. It helps if the ions in the high mass region are magnified relative to the base ion to see this.

Which is Best?

How do we decide when to use 3-pyridylcarbinol esters and when DMOX or pyrrolidide derivatives for structural analysis of fatty acids? DMOX derivatives have excellent properties for gas chromatography, so they can be resolved easily on all the common polar stationary phases used in GC analysis. In my opinion, the mass spectral characteristics in the high mass range are not as good as with 3-pyridylcarbinol esters in general, although DMOX derivatives do appear to be especially useful for some fatty acids with internal ring structures and cyclic and conjugated double bonds, but of less value when structural features are near the terminal carbon of a fatty acid. 3-Pyridylcarbinol esters require much higher

temperatures than the equivalent DMOX or methyl ester derivatives to elute them from GC columns, and at first, they could only be analysed on non-polar stationary phases. Later, we used the thermally stable polar BPX-70 and Supelcowax 10 columns with some success with 3-pyridylcarbinol esters of fatty acids with up to 22 carbon atoms. I tend to use pyrrolidides for confirmatory purposes only, although they do have some distinctive GC properties.

Alternative GC-MS methods, such as that involving acetonitrile-chemical ionization, look interesting, as they may permit both the location of double bonds and their geometry (*cis/trans*), but they appear to be more limited in their potential range of application than the derivatives described above [16], and other 'soft' ionization methods are available for LC-MS [17].

With complicated samples containing larger numbers of different fatty acids, such as those of marine origin, it can be advisable to prepare simpler fractions by the methods described in my web page on this topic or in my book [3] prior to analysis by GC-MS.

Finally in answering the question of which is best, I prefer not to take a rigid stance. 3-Pyridylcarbinol esters were long my favourites for samples containing novel fatty acid structures, although I almost always prepared DMOX derivatives for confirmation or for rapid screening of straight-forward samples. Indeed, I often prepare and analyse methyl esters and pyrrolidides also, partly as this may permit better chromatographic resolution of some components and partly to obtain reference spectra. My firm belief is that the various types of derivative should be considered as complementary to each other and not simply as alternatives. That said, the simplicity of the method of preparing DMOX derivatives together with the lower elution times on GC-MS means that they are often the first choice for definitive characterization of fatty acids.

References

1. Christie, W.W. Structural analysis of fatty acids. In *Advances in Lipid Methodology - Four*, pp. 119-169 (edited by W.W. Christie, Oily Press, Dundee) (1997).
2. Christie, W.W. Gas chromatography-mass spectrometry methods for structural analysis of fatty acids. *Lipids*, **33**, 343-353 (1998); **DOI**.
3. Christie, W.W. and Han, X. Lipid Analysis - Isolation, Separation, Identification and Lipidomic Analysis (4th edition), 446 pages (Oily Press, Woodhead Publishing and now Elsevier) (2010) - see **Science Direct**.
4. Fellenberg, A.J., Johnson, D.W., Poulos, A. and Sharp, P. Simple mass spectrometric differentiation of the n-3, n-6 and n-9 series of methylene interrupted polyenoic acids. *Biomed. Environ. Mass Spectrom*., **14**, 127-130 (1987); **DOI**.
5. Brauner, A., Budzikiewicz, H. and Boland, W. Studies in chemical ionization mass spectrometry. 5. Localization of homoconjugated triene and tetraene units in aliphatic compounds. *Org. Mass Spectrom.*, **17**, 161-164 (1982); **DOI**.
6. Francis, G.W. Alkylthiolation for the determination of double bond position in unsaturated fatty acid esters. *Chem. Phys. Lipids*, **29**, 369-374 (1981); **DOI**.
7. Yamamoto, K., Shibahara, A., Nakayama, T. and Kajimoto, G. Determination of double bond positions in methylene-interrupted dienoic fatty acids by GC-MS as their dimethyl disulfide adducts. *Chem. Phys. Lipids*, **60**, 39-50 (1991); **DOI**.
8. Quehenberger, O., Armando, A.M. and Dennis, E.A. High sensitivity quantitative lipidomics analysis of fatty acids in biological samples by gas chromatography-mass spectrometry. *Biochim. Biophys. Acta*, **1811**, 648-656 (2011); **DOI**.
9. Andersson, B.A. Mass spectrometry of fatty acid pyrrolidides. *Prog. Chem. Fats other Lipids*, **16**, 279-308 (1978); **DOI**.
10. Harvey, D.J. Mass spectrometry of picolinyl and other nitrogen-containing derivatives of fatty acids. In: *Advances in Lipid Methodology - One*, pp. 19-80 (W.W. Christie, ed., Oily Press, Dundee) (1992).
11. Spitzer, V. Structure analysis of fatty acids by gas chromatography - low resolution electron impact mass spectrometry of their 4,4-dimethyloxazoline derivatives - a review. *Prog. Lipid Res*., **35**, 387-408 (1997); **DOI**.
12. Harvey, D.J. Mass spectrometry of picolinyl and other nitrogen-containing derivatives of fatty acids. In: *Advances in Lipid Methodology - One*, pp. 19-80 (W.W. Christie, ed., Oily Press, Dundee) (1992).

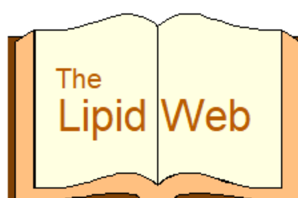
13. Balazy, M. and Nies, A.S. Characterization of epoxides of polyunsaturated fatty acids by mass spectrometry via 3-pyridinylmethyl esters. *Biomed. Environ. Mass Spectrom*., **18**, 328-336 (1989); **DOI**.

14. Destaillats, F. and Angers, P. One-step methodology for the synthesis of FA picolinyl esters from intact lipids. *J. Am. Oil Chem. Soc*., **79**, 253-256 (2002); **DOI**.

15. Yu, Q.T., Liu, B.N., Zhang, J.Y. and Huang, Z.H. Location of methyl branches in fatty acids: Fatty acids in uropygial secretion of Shanghai ducks by gas chromatography-mass spectrometry of 4,4-dimethyloxazoline derivatives. *Lipids*, **23**, 804-810 (1988); **DOI**.

16. Brenna, J.T. Structural analysis of unsaturated fatty acid methyl ester isomers with acetonitrile covalent adduct chemical ionization. In: Lipid Analysis and Lipidomics: New Techniques and Applications. pp. 157-172 (ed: M.M. Mossoba, J.K.G. Kramer, J.T. Brenna and R.E. McDonald, AOCS Press, Champaign, USA) (2006).

17. Chen, C., Li, R.J. and Wu, H. Recent progress in the analysis of unsaturated fatty acids in biological samples by chemical derivatization-based chromatography-mass spectrometry methods. *J. Chromatogr. B*, **1215**, 123572 (2023); **DOI**.



This and related document can be accessed online from - LIPID MAPS