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# Gas chromatographic behavior of fatty acid derivatives for mass spectrometry on low-polarity capillary columns

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**Abstract:**

The gas chromatographic properties of four derivatives of fatty acids (FA), namely fatty acid methyl esters, picolinyl esters, *N*-acyl pyrrolidides and 4,4-dimethyloxazoline derivatives, which contain various structural features (double bonds, branching, hydroxyl group) in their acyl chains have been compared on a low-polarity capillary column with a mass spectrometer as detector. Temperature programming rates yielding the highest resolution were optimized for each derivative by means of computer-assisted column temperature optimization software. Indeed, the Drylab software represents a valuable assistance for estimating the optimum analysis conditions. Time and efforts required for such method development can greatly be reduced. Different parameters (derivatization procedure, total run time, resolution and response factor) are discussed. *N*-Acyl pyrrolidides and picolinyl esters appear quite powerful for structure elucidation of polyunsaturated FA by GC-MS, and both these types of FA derivatives can be very well separated on a low-polarity phase.

**Keywords:** 4,4-Dimethyloxazoline • Fatty acid derivatives • GC-MS • Methyl esters • *N*-Acyl pyrrolidides • Picolinyl esters

# 1. Introduction

Gas chromatography linked to mass spectrometry (GC-MS) has been widely used for a long time for structural analysis of fatty acids (FA). However, identification and location of some structural features such as hydroxyl, epoxy, methyl branches, rings and double bounds can be problematic. To overcome these problems, the analyst has to choose among some appropriate derivatives.

Fatty acid methyl esters (FAME) are the most widely used derivatives for GC analysis. Indeed, they are simple in structure, volatiles, and have good chromatographic properties, and their preparation methods are now in routine at a small scale. FAME are sufficient for localization of some branching patterns (*iso*, *anteiso*, highly branched), or oxygenated functions (2-OH, 3-OH, O-Me). Unfortunately, they are not suitable for locating other methyl branches, double bonds or other unsaturations because the structural information obtained from the mass spectra of unsaturated and some functionalized FAME are frequently of limited value [1]. This is mainly due to the bond migration in unsaturated FA occurring during electron impact [2]. Thus, their mass spectra exhibit molecular ions but are usually devoid of diagnostic ions indicative of these structural features [3] and [4].

To overcome the problem of bond migration, the analyst can use the reaction of FA carboxyl group with nitrogen-containing compounds. Such resulting derivatives are highly favourable charge site, in that they capture the electronic charge during ionization and therefore minimize double bond ionization and migration. The first useful nitrogen-containing derivatives, i.e. *N*-acyl pyrrolidides, were described and discussed more than 35 years ago [4-7]. However, most analysts now prefer picolinyl esters or 4,4-dimethyloxazoline (DMOX) derivatives [8-13], which were respectively first described in 1982 [14] and 1988 [15].

A major challenge in the development of chromatographic separation methods is the rational selection of optimal experimental conditions that can provide an adequate resolution in a reasonable run time. It has been demonstrated that computer simulation can be a valuable tool in the systematic optimization of these separations, varying for example the initial temperature and programming rate [16]. By analogy with liquid chromatographic separations based on gradient elution, the use of segmented-temperature programs seems a potentially useful tool for maximizing overall sample resolution in GC separations.

In the present study, four derivatives have been investigated, namely FAME, picolinyl esters, *N*-acyl pyrrolidides and DMOX derivatives. Their elution conditions on low-polar column were optimized and their mass spectroscopic properties (response factor) were compared each other. A column coated with a low polarity silicone phase was used, because of its high thermal stability. Such columns lack the resolution of phases of higher polarity, but are adequate for many purposes especially when GC-MS is in view. We use it for FA longer than usual chain-lengths (especially as picolinyl esters), for FA with polar functional groups, e.g. hydroxyls, and for other lipid compounds of relatively high molecular weight, including sterols and waxes. Previous studies have compared such derivatives each other [1, 3, 7, 12, 13], but to the best of our knowledge, none has evaluated four FA derivatives simultaneously with the same chromatographic apparatus.

# 2. Material and methods

## 2.1. Materials

FA standards were all purchased as methyl esters from Sigma Chemical (St Louis, MO, USA) and from Supelco Inc. (Bellefonte, PA, USA). These quantitative commercial preparations (Table 1) of FAME included homologous series of normal straight-chain saturated, monounsaturated, and polyunsaturated FA, as well as *iso*-, *anteiso*-branched and hydroxylated acids, from 4 to 24 carbon atoms in length. Acetic acid, pyrrolidine, 3-hydroxymethyl pyridine, potassium tert-butoxyde, and 2-amino-2-methyl-1-propanol were purchased from Sigma Chemical Co. (St Louis, MO, USA). All solvents used were purchased from Carlo-Erba (Val-de-Reuil, France) and were of analytical-reagent grade.

## 2.2. Preparation of fatty acid derivatives

All standards of FA were used in this study as FAME. These mixtures were then further derivatized into three compounds, namely picolinyl esters, *N*-acyl pyrrolidides and DMOX derivatives.

*Picolinyl esters:* Picolinyl esters were prepared by treatment of FAME with a solution of potassium *tert*-butoxyde in tetrahydrofurane and 3-hydroxymethylpyridine for 45 min at 45°C as previously described [7-10, 18].

*N-Acyl pyrrolidides:* FAME were converted into *N*-acyl pyrrolidide derivatives by heating them directly with pyrrolidine and acetic acid under nitrogen atmosphere at 100°C for one hour [6, 17, 19-21].

*4,4-Dimethyloxazoline derivatives:* FAME were converted into DMOX by heating them with 2-amino-2-methyl-1-propanol (0.25 g) at 90°C overnight, after the flushing of the vessel with nitrogen [7, 21].

## 2.3. GC-MS analysis of FAME, picolinyl esters, *N*-acyl pyrrolidides and DMOX derivatives

### 2.3.1 GC-MS apparatus

Structural analysis of derivatives were performed by GC-MS on an Agilent model 6890 series II gas chromatograph linked to an Agilent model 5973N selective quadrupole mass detector. GC-MS operated in the electron impact mode at 70 eV with a scan frequency of 1.37 scan/sec, and was connected to a computer with Hewlett-Packard Chemstation. Scanning range varied regarding the derivatives: from 50 to 550 amu for FAMEs, DMOX and *N*-acyl pyrrolidides and from 50 to 800 amu for the picolinyl esters.

Separation was performed on a factorFour VF-5 MS capillary column (Varian; 60 m x 0.25 mm i.d., 0.25 µm film thickness) for all derivatives. The stationary phase was equivalent to 5% phenyl 95% dimethylpolysiloxane guaranteed low bleed phase, and the maximum temperature applied in run for this column was to 350°C. The temperature of injector and interface were held at 250°C and helium was used as carrier gas under constant flow (1.5 mL/min). All samples were prepared and injected in triplicate.

### 2.3.2 Gas chromatographic conditions

The optimum conditions for analysis can be estimated from chromatograms simulated by computer software. In this study, separations were simulated by use of Drylab GC software (LC Resources Inc., Lafayette, CA, USA). As the application of computer simulation requires an initial knowledge of the dependence of sample retention on temperature, two experimental runs using programmed temperature GC have been performed: oven temperature increased linearly at 1 and 4°C/min from 80°C to 300°C for FAME, 100°C to 350°C for *N*-acyl pyrrolidides and DMOX, and 150°C to 350°C for picolinyl esters. Sixty-one FA were directly injected as methyl esters, or further derivatized into *N*-acyl pyrrolidides, picolinyl esters or DMOX, before injection onto the column. For each derivative, retention times and peak areas were noticed (two preliminary injections at 1 and 4°C/min, and one injection with the optimized method, i.e. triplicate under three different injections = nine injections per derivative). The elution temperatures of the four derivatives were further calculated for each fatty acids under the same chromatographic conditions (i.e. 1°C/min and 4°C/min).

Equivalent chain lengths (ECL) were also calculated for each derivative using corresponding retention time (at 1°C/min and 4°C/min). For each fatty acid the ECL data was estimated by the mean of the triplicate. With linear temperature programs there usually a quite linear relationship

between chain length and retention time, and ECL can be calculated by the following equation which is a modification of the Van Den Dool and Kratz equation applied for Kováts indices:

$$ECL_{(x)} = n \frac{t_{R(x)} - t_{R(z)}}{t_{R(z+n)} - t_{R(z)}} + z$$

where $t_R$ is retention times of the compound of interest and two references (saturated FAME) eluting on each side of the compound [22]. $z$ represents the number of carbons in the reference eluting before $x$ and $n$ is the difference in carbons between the two references. For maximal accuracy, it is recommended that $n$ is one.

Calibration curves were established by injecting seven different concentrations (from 0 to 700 µg of initial FAME) and measuring the resulting surface areas under optimal chromatographic conditions (after Drylab optimization).

# 3. Results and discussion

## 3.1. Chromatographic properties

Chromatographic systems separate mixture of compounds into individual peaks, and the quality of separation can be estimated by some terms such as Resolution factor (Rs) between adjacent peaks, separation number (SN), or Plate number (N). Drylab software works with Rs factor. The most widely used method for Rs calculation is the tangent method, which was adopted by the United States Pharmacopoeia (USP), as shown in the followed equation

$$Rs = \frac{(t_2 - t_1)}{W_2/2 + W_1/2}$$

where $t_2$ and $t_1$ are the retention times of the two components, and $W_2$ and $W_1$ are the corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

In GC, Rs values and retention times depend on column parameters (stationary phase, length, and internal diameter), heating rate, starting temperature, temperature programming file (isocratic, linear or multilinear), … [22-25]. In this comparative study, only heating rate and starting temperature were modulated, while ending temperature was fixed to the maximum allowed by the column (350°C in programming step). Even with these two parameters (heating rate and starting temperature), a large number of experiments may be required to adequately explore the various separation methods. However, computer simulation can greatly reduces the time and effort required for such method development. As Rs is the key factor, Drylab software optimization procedure will tend to get the highest value between the two closest peaks (critical pair). However, most of the time, a compromise has to be found between Rs and total run time (which could reach several hours for optimized conditions). As baseline separation, normally with an Rs higher than 1.5, allows accurate integration of individual peaks and their quantification [25] we have decided to limit optimization to these value.

As some FA were present in different commercial standards (Tab. 1), the reproducibility of the overall analysis process (derivatization and chromatography) can be checked by using the two preliminary assays needed for computer optimization (1°C/min and 4°C/min between 80°C to 350°C).

As expected, both retention time and peak area were identical for a defined FA derivative under the same conditions. In Sigma standards used, all fatty acids presents were not observed in chromatograms. *Cis* and *trans* isomers for a given fatty acid give retention time very close, a

simple peak was observed for these two compounds. For example, this is the case for vaccenic acid. This compound was never observed, whatever the derivative analysed, and so was not cited in Tab. 1.

In this study, chromatographic separations were conducted on a low-polar phase, which implies that elution was mainly due to the boiling point of FA derivatives (however, some polar interactions exists notably with aromatic groups of the derivatives). Obviously, under the same chromatographic conditions and for a given FA, all derivatives were not eluted at the same temperature (Fig. 1).

Indeed, many authors have previously noticed that DMOX are only slightly less volatile than methyl esters which are the first eluted [1-3]. Moreover, our results are in accordance with those obtained by Fay and Richli [26] who have evaluated that typical elution temperatures of DMOX are about 10-15°C higher than those required for the corresponding FAME. In this study, this difference was estimated at 14.85°C with 1°C/min and 15.82°C with 4°C/min.

*N*-acyl pyrrolidides also have reasonable chromatographic properties with an elution temperature 49.5±1.5°C upper than FAME (34.2±1.0°C upper than DMOX). This is due to the three additional carbon atoms and the higher polarity of the amide group [27].

Picolinyl esters, on the other hand, seem to require column temperatures about 50°C higher than for methyl esters and 5°C higher than for *N*-acyl pyrrolidides [2, 3, 17] which is in accordance with our results (54.5±1°C and 4.3±0.2°C respectively).

The ECL system uses the saturated straight-chain derivatives as reference compounds, and the ECL values of the references are by definition equal to the number of carbons in the FA with saturated chain. This is done by plotting the retention times of a homologous series of straight-chain saturated FA derivatives against the number of carbon atoms of the fatty acid. However, this implies the linearity of the curve, which is not the case under 10 carbons (see ECL data for 6:0 and 8:0 in Tab. 1). During the optimization some FA peaks (although they were present into the mixture), were hided by peaks of others compounds. This is the reason why their ECL data were not calculated (i.e. 22:2 ω6, 20:4 ω3). These FA were noted in the Tab. 1 as "absent". Other cannot be derivatized into a type of derivative. This is for example, the case of hydroxylated FA, which were not converted into picolinyl esters.

To our knowledge, this is the first time that a comparison of the ECL values of four FA derivatives is realized using the same chromatographic apparatus. Indeed, only Miralles *et al.* [28] have done such study but by comparing only ECL data for FAME and *N*-acyl pyrrolidides.

In our study, whatever the derivatives injected on the chromatograph, the resulting ECL is similar to the one obtained for the same FA derivatized otherwise (Tab. 1). Indeed, very few differences can be observed for the four derivatives for a same FA which confirms the usefulness of the ECL for identifying compounds. Thus, for a same number of carbon atoms on the linear chain, an evolution of the ECL value is observed with the number of double bound (conversely proportional) but also with the position of the double bond. The more the first double bond is close to the carbonyl group of the molecule, the higher the ECL will be. For example, the ECL of 18:1ω7 was estimated to be 17.81 while for the 18:1 ω12 it was 17.64. The similarity of ECL values determined for FAME and *N*-acyl pyrrolidides has been confirmed with the noticeable exception of highly branched FA such as 4,8,12-trimethyltridecanoic and 3,7,11,15-tetramethylhexadecanoic (phytanic) acids [28].

DMOX total ion current (TIC) has revealed the presence of many artefacts (data not shown), eluted later from GC columns and that interfered with DMOX. They were due to an incomplete conversion reaction (ring opening) due to traces of moisture on the glassware and/or solvent used [29]. Furthermore, these artefacts give mass spectra almost identical to that of the required derivatives [10, 12, 21, 29]. The only difference observed in mass spectra of these compounds was the intensity of the fragment ion at *m/z* 58. This ion was absent on the mass spectrum of the DMOX, whereas it was present at a major intensity on the artefacts mass spectra. Thus, these artefacts can masked peaks of DMOX and then disturbed analysis results. This is the reason why the optimization for DMOX was not conducted in this study. Nevertheless, these difficulties seem to be avoided if the DMOX are prepared in two steps via the acid chloride [30].

Optimal oven temperatures for FAME, *N*-acyl pyrrolidides and picolinyl esters, after Drylab optimization, are presented in Tab. 2.

For all these derivatives, a compromise has to be defined between an adequate resolution and a reasonable run time. For this reason, only FA with at least 10 carbons were studied for FAME and picolinyl esters, while only hexanoic acid was not studied under DMOX form.

Critical pair was defined as the pair of derivatives for which the resolution factor (Rs) was under 1.5. Whatever the chromatographic conditions or derivatives used, at least two critical pairs were observed (two for FAME and three for picolinyl esters and *N*-acyl pyrrolidides). Moreover, they were all due to 18 or 20 carbons chain length compound with 1 to 3 double bonds (Tab. 2). However, we can notice that they were not always the same. Indeed, for FAME and *N*-acyl pyrrolidides, two critical pairs were identified, but only one (18:3ω6/18:4ω3), is common. Three critical pairs were detected for picolinyl esters with one similar to FAME also (18:2ω6/18:1ω7). In general, authors considered that *N*-acyl pyrrolidides gave slightly better separations than did the more polar picolinyl esters [10]. Moreover, in addition to their lowest volatility compared to FAME, picolinyl esters and *N*-acyl pyrrolidides have also poorest resolution [2].

This study reveals that with an optimized chromatographic procedure, such inconvenient can be reduced and that regarding to the complexity of the sample to analysed, one derivative can be more appropriated to another leading to a better separation among FA. However, it should be emphasized that so called "optimal parameters" are optimal for the separation of the analysed references only and with this column.

Total ionic currents (TIC) of a standard mixture of 37 FAME (A) converted into *N*-acyl pyrrolidides (B) and picolinyl esters (C), are presented on Fig. 2.

As defined into the Material and Methods section, those TIC were obtained with the same chromatographic apparatus but with the different optimized temperature conditions. It can be observed that elution profile of the derivatives were different. Indeed, chromatograms begin in each case with a different peak. Fatty acids considered for the optimisation of migration conditions were 10:0 to 24:0. Short chain fatty acids were more or less masked by the solvent delay. On the other hand, the peaks' widths for FAME were low with a very short total run time (59 min). This is the reason why FAME are generally preferred by analysts for the FA analysis on GC. For picolinyl esters (C) peaks were wider and total run time was much more longer (125 min). For *N*-acyl pyrrolidides (B), the peaks' tight was identical to the one of FAME with an intermediate total run time (98 min).

As expected, whatever the derivative used, the elution order of the different FA was the same [19, 22]. Indeed, on low-polar phase unsaturated FA were eluted before the saturated component with the same chain length. Positional isomers were clearly resolved, although one abnormality was observed, i.e., 20:4ω6 was eluted before 20:5ω3. This reverse order was found for the four derivatives.

Drylab predictions were accurate and very few variations were observed between them and the experiments.


## 3.2. Response factor


Calibration curves of eicosanoic (20:0), gadoleic (20:1ω9), and EPA (20:5ω3), as FAME, picolinyl esters, and *N*-acyl pyrrolidides are presented on Fig. 3. Among the different FA analysed here, EPA was chosen to illustrate the PUFA as it is one of the most studied worldwide.

As expected, all were linear. Whatever the FA, the response factors obtained with the GC/MS were always higher for *N*-acyl pyrrolidides then for picolinyl esters, while FAME had the lowest. This is in accordance with our previous observations [18]. These observations can be extended to the 61 FA studied here, where the mass detector response, in electronic impact, was higher for *N*-acyl pyrrolidides and lower for FAME (data not shown).

Due to the linearity of the calibration curves, a comparison factor (CF) can be calculated. which represents the ratio between the area of a *N*-acyl pyrrolide peak divided by the one of the corresponding FAME. The Fig. 4 illustrates that this CF varies with the unsaturation status.

For a saturated FA, this CF between picolinyl esters and FAME was estimated to be around 5, which is in accordance with a previous study [18]. Moreover, the resulting CF between *N*-acyl pyrrolidide and FAME was about 8.5 and 1.7 between *N*-acyl pyrrolidide and picolinyl esters. With one double bound (monounsaturated FA), the CF between picolinyl esters and FAME has

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decreased to 2.9 and to 6.9 between *N*-acyl pyrrolidide and FAME (2.4 between *N*-acyl pyrrolidide and picolinyl esters). For three double bounds (polyunsaturated FA), the respective CF were 2.8 (picolinyl esters and FAME), 6.4 (pyrrolidide and FAME) and 2.3 (between pyrrolidide and picolinyl esters)

This confirms that determination of fatty acids proportions by GC-MS from a reconstructed chromatogram (here with electron impact) was not quantitative. Thus, the relative proportions of each component in natural samples should be determined by other means such as flame ionization detector (FID). This fact is well known as some authors related that the results obtained by FID were very similar for the four derivatives, but differed somewhat from those obtained from the TIC in the mass spectrometer [17]. This was probably due to the lower yields of ions from the polyunsaturated components.

## 4. Conclusions

It is essential to have simple rapid method for determination of FA structures. GC-MS is one of the most widely used due to its usefulness (readily available reagents, simply glassware requirements, no need of pure compound isolation). FA are usually analysed by GC as FAME, but their mass spectra do not contain enough ions indicative of structural features. In the most useful approach to structure determination, the carboxyl group is derivatized with a reagent containing a nitrogen atom. In this study, three derivatives with nitrogen moiety are compared to FAME and each other.

DMOX derivatization procedure had always lead to artefactual components perturbing the identification of FA. Thus, they did not appear very convenient and were not further studied here.

As previously noticed, FAME, even if they present several interests such as an easy derivatization procedure and very good chromatographic properties (reduced elution temperature allowing the use of highly polar column, good separation on polar and non polar column, short run time) do not allow identification in case of branched-chain FA and PUFA.

Picolinyl esters appear quite powerful for structure elucidation of FA by GC-MS. Indeed, up to six double bonds have been localized without any doubt (DHA) but, none of the five hydroxyl-FA studied here, have been detected under their picolinyl ester form (this was also the case for the cyclo 19:0). This can suggest some limited uses of those derivatives for particular FA. In addition, picolinyl esters are eluted at higher temperature than the corresponding FAME (54°C higher which forbids the use of highly polar column) and even with an optimized chromatographic procedure, some separation problems remain (critical pairs).

Except one FA (4:0) which is not detected due to the chromatographic conditions used, all others FA (60) were identified under their *N*-acyl pyrrolidide form. Moreover, after a computerized chromatographic optimization, only two critical pairs are remaining. This indicates that even if such derivatives need higher elution temperature (+44°C compared to FAME), they can be very well separated on a low-polar column such as the one used here.

In this study, among the four FA derivatives tested on GC-MS (61 FA), the *N*-acyl pyrrolidides appears the most powerful and useful as they are easy to prepare (less than 2 hours), and well separated each other leading to FA identification without doubt (even on a low-polar column). However, a longer chromatographic run time is required compared to FAME (99 min instead of 59 min), which can constitute the unique limitation of their development in analytical laboratories.

**Conflict of interest statement**

The authors have declared no conflict of interest.

## References

[1] G. Dobson, W. W. Christie: Spectroscopy and spectrometry of lipids (Part 2) - Mass spectrometry of fatty acid derivatives. *Eur J Lipid Sci Technol.* 2002, **104**, 36-43.

[2] V. Spitzer: 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.* 1997, **35,** 387-408.

[3] W. W. Christie: Some recent advances in the chromatographic analysis of lipids. *Analusis Magazine* 1998, **26**, 34-40.

[4] B. A. Andersson, R. T. Holman : Pyrrolidides for mass spectrometric determination of the position of double bond in monounsaturated fatty acids. *Lipids* 1974, **9**, 185-190.

[5] B. A. Andersson, W. H. Heimermann, R. T. Holman : Comparison of pyrrolidides with other amides for mass spectral determination of structure of unsaturated fatty acids. *Lipids* 1974, **9**, 443-449.

[6] B. A. Andersson: Mass spectrometry of fatty acid pyrrolidides. *Prog Chem Lipids Fats other Lipids* 1978, **16**, 279-308.

[7] W. Vetter, W. Walther, M. Vecchi: Pyrrolidide als Derivate für die Strukturaufklärung aliphatischer und alicyclischer Carbonsäuren mittels Massenspektrometrie. *Helvetica Chimica Acta* 1971, **54**, 1599-1605.

[8] G. Dobson, W. W. Christie: Structural analysis of fatty acids by mass spectrometry of picolinyl esters and dimethyloxazoline derivatives. *Trends Analyt Chem.* 1996, **15**, 130-136.

[9] F. Destaillats, P. Angers : One-step methodology for the synthesis of fatty acid picolinyl esters from intact lipids. *J Am Oil Chem Soc.* 2002, **79**, 253-256.

[10] J. T. G. Hamilton, W. W. Christie : Mechanisms for ion formation during the electron impact-mass spectrometry of picolinyl ester and 4,4-dimethyloxazoline derivatives of fatty acids. *Chem Phys Lipids*, 2000, **105**, 93-104.

[11] G. Dobson, J. L. Sebedio : Monocyclic dienoic fatty acids formed from $\gamma$-linolenic acid in heated evening primrose oil. *Chem Phys Lipids*, 1999, **97**, 105-118

[12] J. L. Garrido, I. Medina : Identification of minor fatty acids in mussels (*Mytilus galloprovincialis*) by GC-MS of their 2-alkenyl-4,4-dimethyloxazoline derivatives. *Anal Chim Acta*, 465, 2002, 409-416.

[13] D. L. Wetzel, J. E. Reynolds : Definitive identification of fatty acid constituents in marine mammal tissues. *Can J Fish Aquat Sci.* 2004, **61**, 554-560.

[14] D. J. Harvey : Picolinyl esters as derivatives for the structural determination of long chain branched and unsaturated fatty acids. *Biomed mass spectrum.* 1982, **9**, 33-38.

[15] J. Y. Zhang, Q. T. Yu, B. N. Liu, Z. H. Huang: Chemical modification in mass spectrometry IV. 2-alkenyl-4,4-dimethyloxazolines as derivatives for doubles bonds location of long chain olefinic acids. *Biomed environm mass spectrum.* 1988, **15**, 33-44

[16] D. E. Bautz, J. W. Dolan, W. D. Raddatz, L. R. Snyder: Computer simulation (based on a linear-elution-strength approximation) as an aid for optimizing separations by programmed-temperature gas chromatography. *Anal Chem.* 1990, **62**, 1560-1567.

[17] W. W. Christie, E. Y. Brechany, S. B. Johnson, R. T. Holman: A comparison of pyrrolidide and picolinyl ester derivatives for the identification of fatty acids in natural samples by gas chromatography-mass spectrometry. *Lipids* 1986, **21**, 657-661.

[18] N. Dubois, C. Barthomeuf, J. P. Bergé : Convenient preparation of picolinyl derivatives from fatty acid esters. *Eur J Lipid Sci Technol.* 2006, **108**, 28-32.

[19] W. W. Christie (editor), Gas Chromatography and Lipids. A Practical Guide, The Oily Press, Dundee, Scotland, 1989, pp. 64.

[20] W. W. Christie: Preparation of ester derivatives of fatty acids for chromatographic analysis. Advances in Lipid Methodology, II (W. W. Christie ed), The Oily Press, Dundee 1993, pp. 69-111.

[21] W. W. Christie: Gas chromatography-mass spectrometry methods for structural analysis of fatty acids. *Lipids* 1998, **33**, 343-353.

[22] H. Van Den Dool, P. D. Kratz: A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr.* 1963, **11**, 463-471.

[23] G. Castello: Retention index systems: alternatives to the *n*-alkanes as calibration standards (review). *J Chromatogr A.,* 1999, **842**, 51-64.

[24] S. A. Mjøs, S. Meier, S. Boitsov : Alkylphenol retention indices. *J Chromatogr. A,* 2006*,* **1123**, 98-105.

[25] D. Song, J. Wang: Modified resolution factor for asymmetrical peaks in chromatographic separation. *J Pharm Biomed Anal.* 2003, **32**, 1105-1112.

[26] L. Fay, U. Richli: Location of double bonds in polyunsaturated fatty acids by gas chromatography-mass spectrometry after dimethyloxazoline derivatization. *J Chromatogr* 1991, **541**, 89-98.

[27] W. Vetter, W. Walther: Pyrrolidides as derivatives for the determination of the fatty acids of the triacylglycerols by gas chromatography. *J Chromatogr A.* 1994, **686**, 149-154.

[28] J. Mirallès, G. Barnathan, R. Galonnier, T. Sall, A. Samb, E. M. Gaydou, J. M. Kornprobst: New branched fatty acids from the Senegalese Gorgonian *Leptogorgia piccola* (white and yellow morphs). *Lipids* 1995, **30**, 459-466.

[29] W. W. Christie: Preparation of derivatives of fatty acids. Lipid Analysis, Third Edition (W. W. Christie ed), The Oily Press, Dundee 2003, pp. 217.

[30] W. W. Christie: Mass spectrometry of fatty acids with methylene-interrupted ene-yne systems. *Chem Phys Lipids* 1998, **94**, 35- .

# Tables

**Table 1.** Equivalent Chain Length (ECL) of all fatty acid derivatives
47080-U = bacterial acid methyl esters ; 47085-U = polyunsaturated fatty acid of omega 3 family ; - = absent ; nd = not derivated

| Peaks number | Fatty acid formula | References | | FAME | Picolinyl esters | Pyrrolidides | DMOX |
|---|---|---|---|---|---|---|---|
| | | SIGMA | SUPELCO | | | | |
| 1 | 4:0 | 189-19 | | - | - | - | - |
| 2 | 6:0 | 189-19 | | - | - | 5.52 | - |
| 3 | 8:0 | 189-19 | | - | - | 7.73 | 8.09 |
| 4 | 10:0 | 189-19 | | 10.00 | 10.00 | 10.00 | 10.00 |
| 5 | 11:0 | 189-19 | 47080-U | 11.00 | 11.00 | 11.00 | 11.00 |
| 6 | 12:0 | 189-19 | 47080-U | 12.00 | 12.00 | 12.00 | 12.00 |
| 7 | 13:0 | 189-19 | 47080-U | 13.00 | 13.00 | 13.00 | 13.00 |
| 8 | 14:0 | 189-19 | 47085-U, 47080-U | 14.00 | 14.00 | 14.00 | 14.00 |
| 9 | 15:0 | 189-19 | 47080-U | 15.00 | 15.00 | 15.00 | 15.00 |
| 10 | 16:0 | 189-19 | 47085-U, 47080-U | 16.00 | 16.00 | 16.00 | 16.00 |
| 11 | 17:0 | 189-19 | 47080-U | 17.00 | 17.00 | 17.00 | 17.00 |
| 12 | 18:0 | 189-19 | 47085-U, 47080-U | 18.00 | 18.00 | 18.00 | 18.00 |
| 13 | 19:0 | | 47080-U | - | 19.00 | 19.00 | - |
| 14 | 20:0 | 189-19 | 47080-U | 20.00 | 20.00 | 20.00 | 20.00 |
| 15 | 21:0 | H 3265, 189-19 | | 21.00 | 21.00 | 21.00 | 21.00 |
| 16 | 22:0 | 189-19 | | 22.00 | 22.00 | 22.00 | 22.00 |
| 17 | 23:0 | T 9900, 189-19 | | 23.00 | 23.00 | 23.00 | 23.00 |
| 18 | 24:0 | 189-19 | | 24.00 | 24.00 | 24.00 | 24.00 |
| 19 | 2-OH-10:0 | | 47080-U | 10.84 | nd | 10.81 | nd |
| 20 | 2-OH-12:0 | | 47080-U | 12.82 | nd | 12.84 | nd |
| 21 | 3-OH-12:0 | | 47080-U | 13.44 | nd | 13.42 | nd |
| 22 | 2-OH-14:0 | | 47080-U | 14.81 | nd | 14.86 | nd |
| 23 | 3-OH-14:0 | | 47080-U | 15.47 | nd | 15.42 | nd |
| 24 | i- 15:0 | | 47080-U | 14.62 | 14.60 | 14.63 | nd |
| 25 | a- 15:0 | | 47080-U | 14.72 | 14.70 | 14.73 | nd |
| 26 | i- 16:0 | | 47080-U | 15.63 | 15.59 | 15.62 | nd |
| 27 | i- 17:0 | | 47080-U | 16.62 | 16.60 | 16.62 | nd |
| 28 | cyclo 19:0 | | 47080-U | 18.83 | nd | 18.86 | nd |
| 29 | 14:1ω5 | 189-19 | | 13.87 | 13.87 | 13.87 | 13.85 |
| 30 | 15:1ω5 | 189-19 | | 14.87 | 14.86 | 14.88 | 14.88 |
| 31 | 16:1ω7 | 189-19 | 47085-U, 47080-U | 15.77 | 15.77 | 15.72 | 15.76 |
| 32 | 17:1ω7 | 189-19 | | 16.78 | 16.79 | 16.78 | 16.80 |
| 33 | 18:1 trans ω6 | O 2633 | | 17.82 | 17.83 | 17.83 | 17.82 |
| 34 | 18:1ω7 | V 1256 | 47085-U | 17.81 | 17.80 | 17.77 | 17.79 |
| 35 | 18:1ω9 trans | 189-19 | 47080-U | 17.71 | 17.74 | 17.72 | 17.76 |
| 36 | 18:1ω9 cis | 189-19 | 47085-U, 47080-U | 17.73 | 17.78 | 17.80 | 17.79 |
| 37 | 18:1 cis ω12 | P 9125 | | 17.64 | 17.66 | 17.65 | 17.63 |
| 38 | 18:1 trans ω12 | P 5526 | | 17.66 | 17.65 | 17.65 | 17.68 |
| 39 | 20:1 ω7 | E 3512 | | 19.85 | - | 19.82 | 19.82 |
| 40 | 20:1ω9 | 189-19 | 47085-U | 19.71 | 19.75 | 19.72 | 19.76 |
| 41 | 20:1 ω12 | E 2765 | | 19.60 | - | 19.65 | 19.60 |
| 42 | 22:1ω9 | 189-19 | | 21.72 | 21.73 | 21.73 | 21.74 |
| 43 | 24:1ω9 | 189-19 | | 23.73 | 23.73 | 23.75 | 23.74 |
| 44 | 16:2ω4 | | 47085-U | 15.63 | 15.66 | 15.61 | 15.65 |
| 45 | 18:2ω6 | 189-19 | 47085-U, 47080-U | 17.67 | 17.66 | 17.64 | 17.65 |
| 46 | 20:2ω6 | E 7877, 189-19 | | 19.69 | 19.72 | 19.68 | 19.68 |
| 47 | 22:2ω6 | 189-19 | | 21.65 | - | 21.66 | 21.69 |
| 48 | 16:3ω4 | | 47085-U | 15.56 | 15.62 | 15.54 | 15.55 |
| 49 | 18:3ω3 | L 2531, 189-19 | 47085-U | 17.56 | 17.56 | 17.59 | 17.56 |
| 50 | 18:3 ω6 | 189-19 | | 17.52 | - | 17.43 | 17.45 |
| 51 | 20:3ω3 | E 6001, 189-19 | | 19.50 | 19.50 | 19.58 | - |
| 52 | 20:3 ω6 | 189-19 | | 19.48 | 19.46 | 19.48 | 19.47 |
| 53 | 20:3 ω9 | E 6013 | | 19.46 | - | 19.51 | - |
| 54 | 22:3 ω3 | D 3909 | | 21.55 | - | 21.58 | 21.55 |
| 55 | 18:4ω3 | O 5130 | 47085-U | 17.48 | 17.43 | 17.44 | 17.45 |
| 56 | 20:4ω3 | | 47085-U | 19.44 | 19.43 | 19.42 | - |
| 57 | 20:4ω6 | A 9298, 189-19 | 47085-U | 19.32 | 19.33 | 19.33 | 19.33 |
| 58 | 22:4 ω6 | D 3534 | | 21.35 | - | 21.34 | 21.33 |
| 59 | 20:5ω3 | 189-19 | 47085-U | 19.27 | 19.23 | 19.26 | 19.22 |
| 60 | 22:5ω3 | D 5679 | 47085-U | 21.27 | 21.30 | 21.29 | 21.23 |
| 61 | 22:6ω3 | D 4034, 189-19 | 47085-U | 21.03 | 21.03 | 20.96 | 21.03 |

**Table 2.** Optimal chromatographic parameters for FAME, picolinyl ester and *N*-acyl pyrrolidide derivatives analysis on factourFour capillary column (Varian; 60 m x 0.25 mm i.d., 0.25 µm film thickness) after injection at 250°C. Helium was used as carrier gas (1.5 mL/min). Chromatographic parameters optimisation was not realised for DMOX derivatives

| | Oven temperature [°C] | | Gradient [°C/min] | Run time [min] | Critical pairs |
|---|---|---|---|---|---|
| | Initial | Final | | | |
| FAME | 140 | 270 | 2.2 | 59.0 | 18:2ω6/18:1ω7 18:3ω6/18:4ω3 |
| N-acyl pyrrolidide | 150 | 310 | 1.6 | 98.5 | 15:0/2-OH 14:0 18:3ω6/18:4ω3 |
| Picolinyl esters | 220 | 270 | 0.4 | 125.0 | Iso 16:0/16:3ω4 18:2ω6/18:1ω7 20:2ω6/20:1ω9 21:0/22:6ω3 |

# Figures



**DMOX**

+15.3 ± 0.5°C

+34.2 ± 1.0°C

+49.5 ± 1.5°C

**1. FAM**

**Pyrrolidide**

+38.3 ± 0.5°C

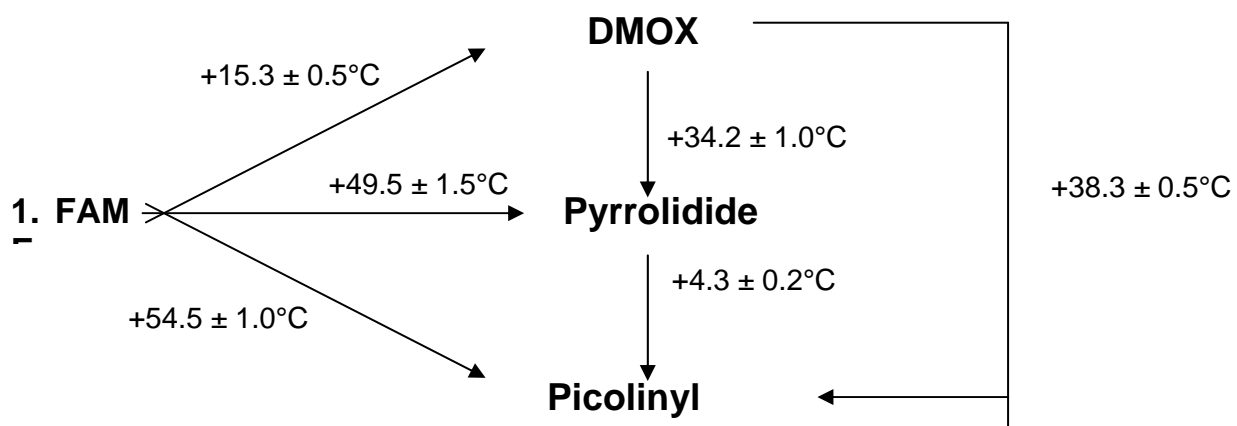+4.3 ± 0.2°C

+54.5 ± 1.0°C

**Picolinyl**

**Fig. 1 Dubois et al.**

**Figure 1.** Differences observed between retention times of all derivatives (FAME, DMOX, *N*-acyl pyrrolidide and picolinyl ester). Lower value correspond to a gradient temperature of 1°C/min while the higher value correspond to a gradient temperature of 4°C/min
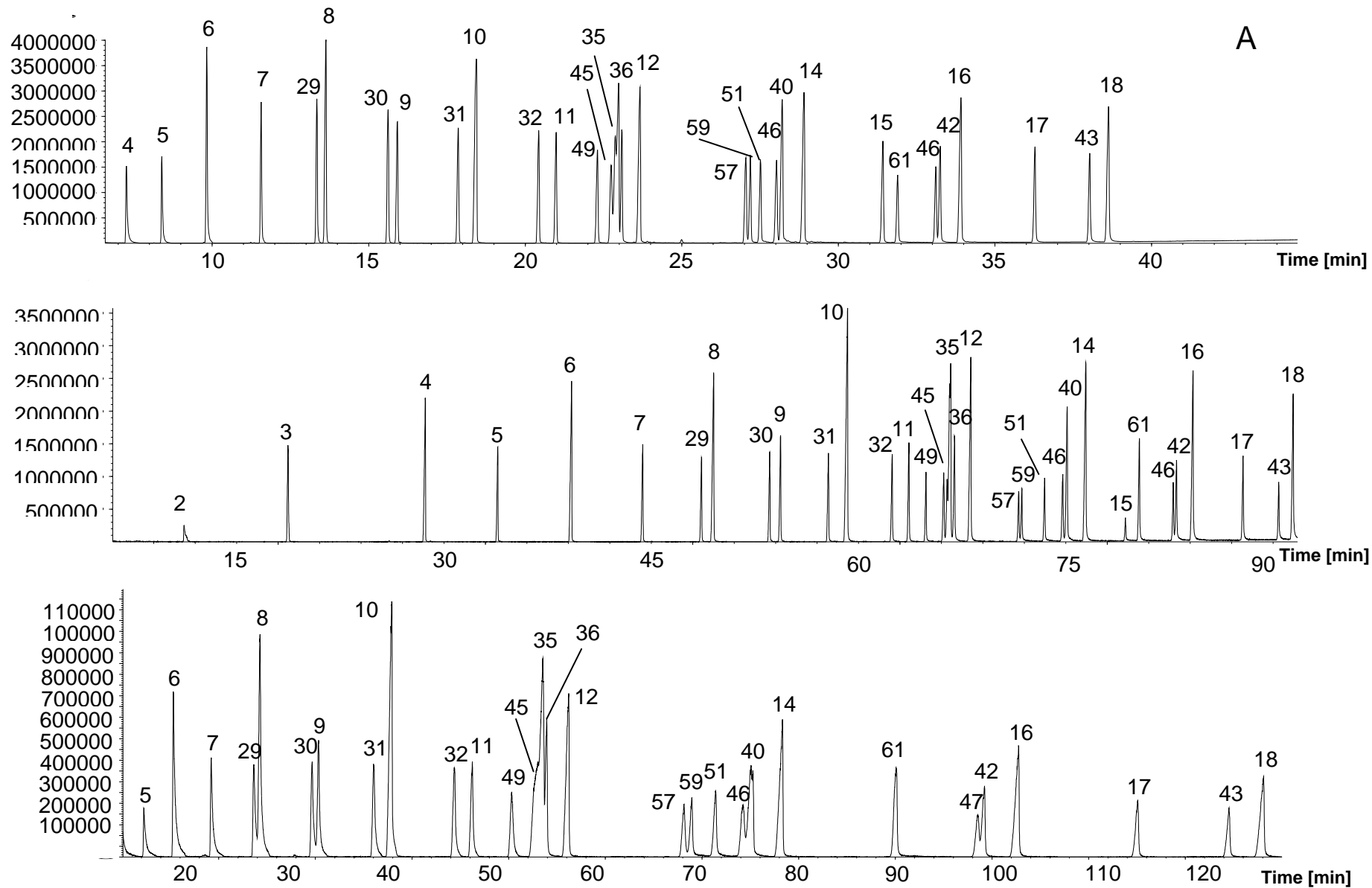
**Fig 2. Dubois et al**

**Figure 2.** Chromatograms of the 37 fatty acid methyl esters (SIGMA 189-19) (A), after conversion into pyrrolidides (B) or picolinyl esters (C). Peaks numbers correspond to compound numbers in Table 1. Chromatograms were obtained under optimal conditions (see Table 2)
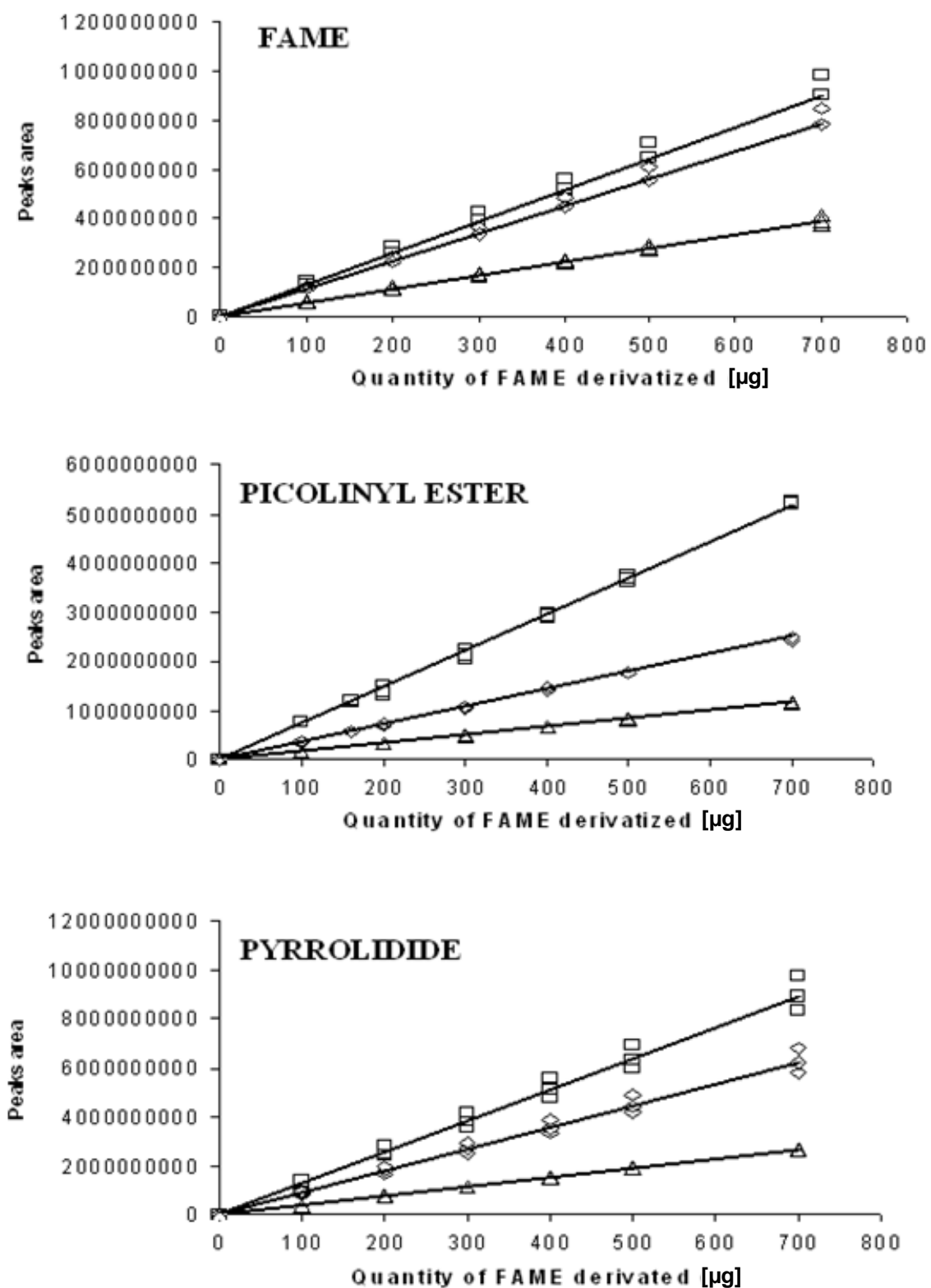
**Fig .3. Dubois et al.**

**Figure 3.** Calibration curve of derivatives according to the number of double bounds. (A) saturated, (B) monounsaturated, (C) polyunsaturated.
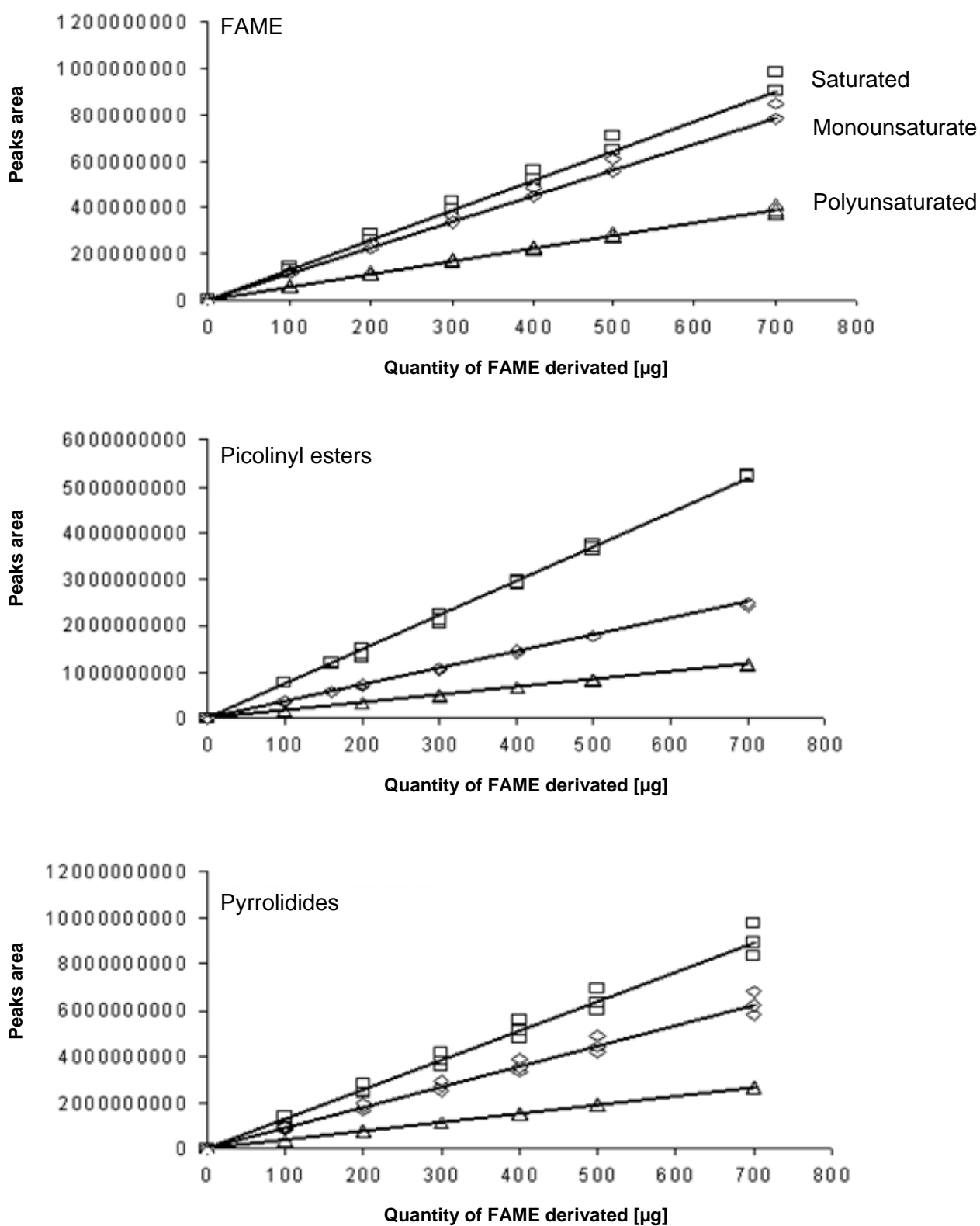
**Fig. 4. Dubois et al.**
**Figure 4.** Calibration curves of saturated, monounsaturated and polyunsaturated fatty acid derivatized in methyl esters, picolinyl esters and *N*-acyl pyrrolidides.