Article title.

Evaluation of Performance and Validity Limits of Gas Chromatography electron ionization – Orbitrap Detector for fatty acid methyl esters analyses.

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Abstract

RATIONALE

While the GC-Orbitrap, marketed in 2015, represents a technological breakthrough in terms of sensitivity, resolution and mass stability, many studies have reported ion ratio modification in mass spectra using the standard 70 eV electron ionization.

METHODS

We studied herein the influence of the acquisition and sample parameters leading to these modifications on FAMEs.

RESULTS

Fatty acid methyl esters (FAMEs) showed that these variations in relative intensities of the ions were related to the acquisition parameters such as the mass range and the offset values of the C-TRAP, but also directly related to the column concentration of the sample, and especially that it was molecule-dependent. Advantageously, it is possible to use this feature to promote the molecular ions of FAMEs sometimes not present in a spectrum under electron ionization at 70 eV.

CONCLUSIONS

The 70 eV electron ionization mass spectra from GC-Orbitrap was cleary molecule-dependent and could be due to metastable ion during storage states in C-TRAP.

Keywords

GC-Orbitrap, EI, Q-Exactive, FAME

1. Introduction

Marketed since June 2015, Orbitrap detection coupled with gas chromatography remains a relatively recent technique. Peterson et al [1,2] for the first time described the beginnings of an instrument before it was marketed. The first works using this instrument studied pesticides [3], essential oils [4,5], fatty acids in the form of methyl esters (FAMEs) [6,7] or 3-hydroxymethyl pyridine ester [8] or metabolomic analysis [9,10]. The ionisation is either performed by electron ionization (EI) at 70 eV or by chemical ionisation (CI). EI spectrum at 70 eV is known for its robustness and is independent of the mass detector [11]. However, most of these studies report changes in mass spectra using GC-Orbitrap, either on pesticides, terpenoids or fatty acid esters. In an attempt to explain these changes, we have explored various acquisition parameters in order to verify their possible contribution to these spectral modifications. Having observed a significant modification on FAMEs, our work is mainly focused on this molecular class. The determination of FA profile is generally performed by gas chromatography (GC) with flame ionisation detector (FID) [12] or by mass spectrometry (MS) [13]. To characterise FA by GC, their derivatization to fatty acid methyl esters (FAMEs) [14] is necessary. Even if FAMEs are preferentially used, in spite of the fact that it does not allow to remove all the structural ambiguities [8], other derivatization methods can be used to have more structural information [15]. On the other hand, GC-FID can rapidly give quantification information. For identification, it is essential to compare retention times of standards with samples [16] and/or use mass spectrometry (MS). The equivalent chain length (ECL) [17] method makes it possible to confirm the order of elutions of the FAMEs for a given chromatography column. GC-EI-MS methods allow structural identification for most FAMEs. GC-CI-MS, performed with ammonia, methane, or a mixture of the two gases, allow access to pseudo molecular ion of FAMEs [18,7]. GC-MS method for FA qualitative analysis is often performed with quadrupole (Q) [19], ion trap [18], TOF and recently with Q-TOF analysers [20]. More recently, Orbitrap technology has been coupled with GC [9,3,21] and used for the characterization of FA *via* FAMEs [19] or *via* 3-pyridylcarbinol esters [8]. Although a low-resolution detector is able to determine the electronic ionization spectrum of FAMEs, the contribution of the high resolution provides a gain in sensitivity to highlight species in trace amounts by exact mass filter application on particular ions. The contribution of the exact mass with an accuracy of less than 2 ppm also makes it possible to highlight the isobaric ions and to determine the formula or for study of isotopologues. In order to understand how the GC-Orbitrap works, we have varied parameters of injections and acquisitions.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, n-heptane, sulphuric acid and standard FAME37 were purchased from Sigma-Aldrich (St-Quentin-Fallavier, France). Methyl 15-methylhexadecanoate (isoC16:0) and cis-9,10-methyleneoctadecanoic acid (C19:1cycloΔ9) were purchased from Matreya (State-College, USA) and Larodan (Solna, Sweden) respectively. Deuterated Methanol was purchased from eurisotop (Saint-Aubin, France).

2.2. Plant material

Crocus sativus L oil was obtained following the protocol of Ksouda et al.[7] FAMEs were prepared by transesterification of oil triglycerides according to Bligh and Dyer[22], with some modifications according to Ksouda et al.

2.3. Methylation of C19:1cycloΔ9 acid

C19:1cycloΔ9 methyl ester was prepared by Christie's methanolic-H_2SO_4 [23] transesterification with methanol or deuterated methanol.

2.4. GC-full scan on ion trap analyzer

FAMEs were identified using a TRACE GC 2000 with Polaris IT (Thermo Fisher Scientific, Villebon, France). The mass spectrometer was controled by Xcalibur software. Data was acquired in the range of 40-400 m/z, 70 eV for EI. The spectrometer was tuned and calibrated externally. The GC was equipped with a BPX70 capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, SGE, Bellefonte, USA). Helium was used as carrier gas at a flow rate of 0.9 mL/min. The injector port was heated to 200 °C and the transfer line to 270 °C. One microliter of diluted sample was injected in split mode (20:1). The initial oven temperature was 140 °C for 3 min, then ramped at 10 °C/min to 190 °C and held for 6 min before ramping at 20 °C/min to 260 °C and held for 10 min. The MS source temperature was maintained at 280 °C.

2.5. GC-full scan on triple quadrupole analyzer

FAMEs were identified using a GC Quantum GC-MS/MS (Thermo Fisher Scientific, Villebon, France). The mass spectrometer was controlled by Xcalibur software. Data was acquired in the range of 50-500 m/z, 70 eV for EI. The spectrometer was tuned and calibrated externally. The GC was equipped with a BPX70 capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, SGE, Bellefonte, USA). Helium was used as carrier gas at a flow rate of 1.2 mL/min. The injector port was heated to 230 °C and the transfer line to 270 °C. One microliter of diluted sample was injected in split mode (100:1). The initial oven temperature was 100 °C for 4 min, then ramped at 3 °C/min to 240 °C and held for 10 min before ramping at 30 °C/min to 250 °C and held for 5 min. The MS source temperature was maintained at 280 °C.

2.6. GC-full scan high resolution mass spectrometry (HRMS) analysis

FAMEs were identified using a QExactive™ GC Orbitrap™ GC-MS/MS (Thermo Fisher Scientific, Villebon, France). The mass spectrometer was controled by Xcalibur software (Version 4.0.27). By default, Orbitrap system acquired data in the range of 50-500 m/z at 60,000-resolution mode and 70 eV for EI. C-TRAP

tune value was set to 0 V, automatic gain control (AGC) target to 1^{E6} ions with an automatic filling limit. The spectrometer was tuned and calibrated externally. The GC was equipped with a BPX70 capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, SGE, Bellefonte, USA). Helium was used as carrier gas at a flow rate of 1.2 mL/min. The injector port was heated to 280 °C and the transfer line to 280 °C. One microliter of diluted sample was injected in split mode (100:1). The initial oven temperature was 150 °C for 2 min, then ramped at 15 °C/min to 280 °C and held for 4 min. The MS source temperature was maintained at 280 °C.

2.7. GC Orbitrap parameter evaluation

A standard mixture of 37 FAMEs at 10mg/mL each from Supelco served as reference. The sample was injected under varying conditions of Orbitrap detector acquisition settings (mass range, resolution, AGC target, acquisition time) and C-TRAP tune values (Table 1). The evaluation of the impact of the concentration on the profile of the mass spectra was carried out by injection of samples at different concentrations (dilution level) and split ratio (SSL), as reported in Table 1.

2.8. Data processing

Mass spectral processing was carried out using Xcalibur software (Version 4.0.27). The ion quantification was performed by applying a mass filter of 5 ppm for each ion. The ion peak area allows comparing the abundance of ions. Spectral identification was performed by 2014 NIST mass spectral library and a homemade HRMS database created with controlled parameters of mass range and C-TRAP energy offset values.

3. Results

3.1. Mass accuracy and isotopic pattern evaluation

The mass accuracy of GC-Orbitrap was evaluated during one week without calibration after initial calibration. During this period, we reported the mass of ion $C_4H_7O_2^+$ (87.0441 uma). The mass accuracy was less than 1 ppm after 7 days. An excellent mass accuracy for both saturated and unsaturated ions was observed in accordance with the study of Qui [24]. At the same time, we can observe for the same chromatographic peak, a variation of isotopologues relative intensity. These variations can be up to ±30% compared to the theoretical value, where the manufacturer gave reliabilities of the order of 0.5% for time of flight coupled to quadrupole (Q-TOF) technologies. Krätschmer reported similar results during analysis of chlorinated paraffins with a variation ranging from -7.5% to +2.5% and up to 49% for samples close to the limit of detection [25]. Because of this uncertainty about the isotopic pattern, Qui [24] has preferred to use the mass precision and resolution of the orbitrap detector to differentiate the isotopologues regardless of theoretical abundances.

3.2. Sensibility and linearity

The sensitivity of GC-Orbitrap was evaluated by injecting a series of dilutions of isoC16: 0 in heptane. For each point, the area of peak was evaluated for 3 ions, the base peak ion ($C_4H_7O_2^+$, 87.0441 uma), the molecular ion ($C_{17}H_{34}O_2^{+\cdot}$, 270.2553 uma) and a specific ion ($[M\text{-}C_3H_7]^+$, 227.2009 uma, $C_{14}H_{27}O_2^+$) to isoC16: 0, to evaluate the contribution of mass accuracy for quantification (Figure S5). It is possible to obtain a linear regression curve with a correlation index close to 1 for the 3 ions over 4 orders of magnitude (10-1000 ng/ml for $C_4H_7O_2^+$, r= 0.9999, 20-1000 ng/ml for the other two ions with r= 0.998 and 1 for $C_{17}H_{34}O_2^+$ and $C_4H_7O_2^+$). By using the ion 227 as a quantifier ion, it is possible to obtain an LOD of 10 ng/ml from a spectrum acquired in the full scan mode(Figure S4). The best LOD obtained on a QQQ detector in the same mode is 400 ng/ml and 200 ng/ml for an IT detector [26]. At the same time it is possible to obtain a quality mass spectrum at the concentration of 10 ng/ml, which had already been shown previously to identify a 3-PCE ester, even if the signal-to-noise ratio remains insufficient to quantify the molecule [8]. The EI 70 eV spectra of isoC16:0 and C16:0 are too similar to allow differentiation based solely on the mass spectrum. The use of the ECL method or derivatization remains essential here.

3.3. EI mass spectrum profile

During FAMEs analyzes, it was found that the Orbitrap GC-Qexactive™ detector modified the mass spectra made by electron ionization at 70 eV. The same kind of modifications was previously reported for pesticides [3]. The changes in the intensity of the generated ions, lead to the alteration of the profiles of the mass spectra. These modifications concern both the relative ratios of the ions and the production of a different base peak ion, except for polyunsaturated FAMEs with 3 (Figure 1) or more unsaturations (Figure S2). For a chromatographic peak, this directly affects the identification of the molecules by comparison with common databases grouping 70 eV mass spectra from different analyzers (NIST, Wiley). The modifications generated are also of a different nature than with an ion trap (IT) (Figure 2). With IT analyser, ion 74 is the base peak, like with QQQ, but with IT, the intensities of ions of higher masses are decreased compared to QQQ. Changes in spectra are molecules- dependent. Some compounds are poorly affected like some pesticides and essential oils [21]. On the other hand, FAMEs (Figure 3) or 3-hydroxymethyl pyridine esters [8] have an important inversion of major peaks. Normally, the most abundant ion is ion m/z 74 ($C_3H_7O_2$) and the second is m/z 87 [27] from McLafferty rearrangements [28]. We can observe that the base peak is common for saturated FAMEs ($C_4H_7O_2$, 87.0443 uma), mono- and di-unsaturated FAMEs (C_6H_9, 81.0700 uma) and tri-unsaturated FAMEs (C_6H_7, 79.0544). This particularity allows to apply an exact mass filter, with a 5 ppm window on chromatograms to rapidly identify saturated and unsaturated FAMEs (Table S1). This information was validated for common FAMEs and cannot be generalized for unusual FAMEs with atypical fragmentations. It can also be applied to exclude non FAMEs compounds or to identify degradation of molecules like methoxy with one more oxygen atom [8]. By realizing the derivation of C19: 1cycloΔ9 with deuterated methanol, we can easily compare the mass spectra of the two FAMEs and observe the addition of two molecules of methanol on the fatty acid and thus validate the C19:1cycloΔ9 degradation hypotheses during the methylation phase. The ion which characterises the methoxy from C19:1cycloΔ9, at 201.1487uma ($C_{11}H_{15}O_3$) is increased by 6.0377 uma (Figure 4), the difference between 6 ^{1}H and 6 D ((2.014102 – 1.007825)*6).

3.4. Evaluation of influence of acquisition parameters, tune values and sample concentrations.

Orbitrap technology is based on ion oscillation frequency measurements around a central electrode, detected on a receiver plate [29]. The mass spectrum is obtained after Fourier Transform of oscillation signal with a two point calibration. The longer the signal acquisition time, the better is the resolution [30].

During this time, ions generated in EI source are stored in an ion trap, the C-TRAP. The storage of ions in C-TRAP plays a vital role in prematurely analyzing discontinuous ions produced continuously by the chromatographic separation and the ionization source. The ions introduced into the C-TRAP lose their energy in contact with N_2 at approximately 1 mTorr [29]. During an acquisition in full scan mode, it is possible to set the detector with different parameters: acquisition mass range, resolution, AGC target and the tune values. The tune files gather the different settings of the mass spectrometer aiming to produce the ions of the electron ionization source, but also the different voltages applied to the lenses in the ion path from the source to the C-TRAP. It is also possible to change the value of the C-TRAP energy offset (-5V to +5V). For this study, we used standard conditions of electron ionization (70 eV) in order to be in the ideal conditions of reproducibility of the spectra. By default, Orbitrap system acquires data in the range of 50-500 m/z at 60,000-resolution mode and 70 eV for EI. C-TRAP tune value was set to 0V, AGT target to 1^{E6} ions with an automatic filling limit. In order to study the influence of different settings and injection conditions, we compared the relative intensities of the discriminant peaks for 3 linear saturated FAMEs (C12:0, C16:0, C20:0) and 4 linear unsaturated FAMEs (C18:1n9c, C18:2n6c, C18:3n6, C18:3n3). The ions selected for the study of saturated FAMEs are m/z 87, 74 and 101; and for the unsaturated FAMEs 67 and 81 for C18:1 and C18:2, and 67 and 79 for C18:3 (Table 1). We can observe an base peak inversion respectively between ions m/z 87 and 74 for C16:0 and between ions m/z 81 and 55 for C18:1. At the same time, C18:3 is less affected and retains ion m/z 79 as base peak. During a chromatographic peak, the ratio between ion 74 and 87 can vary from ±3% (Figure 5). In Table 2, we can show the relative variation of ions ratio for the 7 FAMEs. With regard to the variation intra scan, we can consider that a variation of less than 5% is not significant. It would then be clear that the resolution, and therefore the time of presence of ions in the cell of the Orbitrap, does not have a noticeable effect on the appearance of the mass spectra. It is the same with the manual filling of numbers of ions in the C-TRAP during a fixed time and the number of ions inside it. On the other hand, a decrease in the number of ions in the automatic mode suggests a slight variation for C12:0 and C18:2n6c, but does not show significant modifications for the other molecules. However, when the concentration of the sample is increased, a change in the ion ratio is observed. This change is more pronounced for saturated FAMEs. Finally, we can observe a significant variation of ion ratios in play on the value of C-TRAP energy offset. At the same time, this parameter has a significant influence on the sensitivity. For extreme values, +5 V, there is an important signal decrease. Negative values promote ions of low masses whereas positive values, ions of high masses. For a +3 V value, the molecular ion can be strongly favoured. It is possible to use a C-TRAP energy setting to promote high molecular ions in chemical ionization [25]. In Figure 3, we can observe that ion 101.0598 ($C_5H_9O_2$) was the base peak on C-TRAP energy offset of -5 V whereas the latter has a mass greater than the ion 87.0442 ($C_4H_7O_2$). At the same time, ion 115.0745 ($C_6H_{11}O_2$) decreases when ion 87.0442 increases, when the C-TRAP energies offset increase. These variations go against the expected values and suggest that we observe at the same time other phenomena related to fragmentation by electron ionization. Indeed, the ion 115.0745 can produce the ion 87.0442 as shown in Figure 6.

4. Discussion

The GC-Qexactive™ analyzer presented by Thermo Scientific in June 2015, represents a technological breakthrough in the field of high-resolution mass spectrometry coupled with gas chromatography. Until 2015, only time-of-flight technologies, TOF and Q-TOF, were available coupled with GC. Indeed, due to the high resolution of GC, the acquisition frequency of the detector is an essential parameter in order to obtain sufficient points per peak to correctly define a Gaussian peak. TOF technology requires reducing the number of pulses to increase the acquisition frequency. This directly impacts on the

sensitivity of the analyzer. The same applies to the resolution in Orbitrap technology. The GC-Orbitrap will accumulate enough ions in C-TRAP to send it in a second time on the Orbitrap cell which will carry out the mass measurements. Previously, the speed of detection was one of the limiting points for Orbitrap technologies in order to couple them with a GC. In the 2015's version, Orbitrap detector of Q-Exactive allows to have frequencies in the order of 20 Hz, acquisition rate equivalent to Q-TOF technologies, without losing in sensitivity [24]. Only the resolution is decreased, while remaining acceptable (15k). The result is a sensitive, resolute and precise analyzer that makes it a competitive challenger to TOF technologies. However, it was found that this detector altered the isotopic masses pattern, in, one scan, with variations of more than ±30% [1] of the isotopic masses compared to the monoisotopic ion, where the manufacturer gives reliabilities of the order of 0.5% for Q-TOF technologies. This inaccuracy is also observed with LTQ/Orbitrap [31] and could be attributed at the "isotope beating", like in FTICR [32]. More recently, Eiler et al [33] showed that the isotopic pattern error could decrease to 0.015‰ after 24h, an incompatible time with a GC chromatographic peak duration. During our FAMEs analyzes, it was found that the Orbitrap detector modified the mass spectra made by electron ionization ion source at 70 eV. These changes in the intensity of the ions generated, lead to alterations of the profiles of the mass spectra. These modifications concern both the relative ratios of the ions and the production of a different base peak ion. This directly affects the identification of the molecules by comparison with databases grouping 70 eV mass spectra on different analyzers (NIST, Wiley). The modifications generated are of different nature than those of an ion trap. Changes in spectra are molecules-dependent and more certainly dependent of the structure of the molecule. In order to overcome the lack of recognition of the 70 eV EI spectra made in GC-Orbitrap, it is interesting to develop databases specific to this analyzer. In Table 3, the *Crocus sativus L* oil analysis shows improved FAMEs recognition using the HRMS database.. The score was greater than 900 for most FAMEs but it could be lower then for PUFAs with 20 carbons and more. However, it remains to verify the portability of these databases from one device to another. Concerning FAMEs, it is possible to make databases made with both C- TRAP energy offsets, 0V and -3V to maximize the identifications. The 0V database is used to supplement the NIST database and to improve peaks recognition. On the other hand, the database performed at C-TRAP energy value of +3V can help to enhance recognition by promoting molecular ions often can abound in FAMEs. However, it is imperative to cross-reference the identifications with the equivalent chain lengths [17] in order to ensure the correct identification of molecules. In order to be able to use these databases in an optimal way, it is necessary to work with correctly diluted samples in order not to modify the mass spectra, but also to fix the values of C-TRAP energy offsets and the mass range.

In the light of the collected data, one can wonder about the reasons of the ions abundances modifications. Several reasons can be evoked: influence of the pressure in the source of ionization, source design promote another fragmentation, presence of neutral molecule ion reaction at the source or the C-TRAP, the generation of ions from metastable ions during their capture in C-TRAP. Fagerquist and Schwarz[27] concluded that the pressure of the carrier gas can have an effect, but essentially by proton transfer, without drastically modifying the fragmentation. Harrison and Cotter [34] reported that the design of the source may have an influence on ion/molecule reactions. To our knowledge, the drawing of the source shows the main lines of the other devices of the Thermo scientific range. Apart from a modification of possitionnement, the source is common with other devices. Another possibility is the collision of high molecular molecules with nitrogen contained in C-TRAP, which slows them down. It has been shown that the concentration of molecules has a notable effect. However, it is unimportant and may be due to ionization [35]. Finally, Takayama [36] suggested that ions 74 and 87 are formed after their residence in the source (~ 10^{-6} s) from metastable ions produced from the molecular ion. In this work, we have shown that the high residence time of ions in C-TRAP up

to 10^{-2} s, may favour the production of ion 87. Although the ion 87 can be generated by alpha cleavages of the n3 and n5 carbons, it can also be produced from a rearrangement from the molecular ion. This fractionation seems to be slower than the alpha breaking of the carbons of the aliphatic chain. The hypothesis of metastable ion generation [37] seems most likely for several reasons. First, we observed a dependence on the chemical structure of mass spectrum changes that is not observed in non-ion trap analyzers (Q-TOF, Q) and therefore with faster ion travel times. However, we cannot exclude that these modifications are multifactorial with collision phenomena induced in C-TRAP.

5. Conclusion

During this study, we have shown the versatility of the EI 70 eV spectra realized with the GC-Orbitrap detector. These variations are dependent on molecules but also affected by the acquisition parameters and the column concentration of the sample. In order to be able to compare the spectra acquired with the GC-Orbitrap, it is thus imperative to control the concentration of the sample mass range and to set the value of the C-TRAP offset. The application of C-TRAP positive value can also improve the detection of molecular ions sometimes scanty in IE. At the same time, we have shown the capacity of the GC-Orbitrap to facilitate molecular identification by its exact mass measurement stability.

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Figures

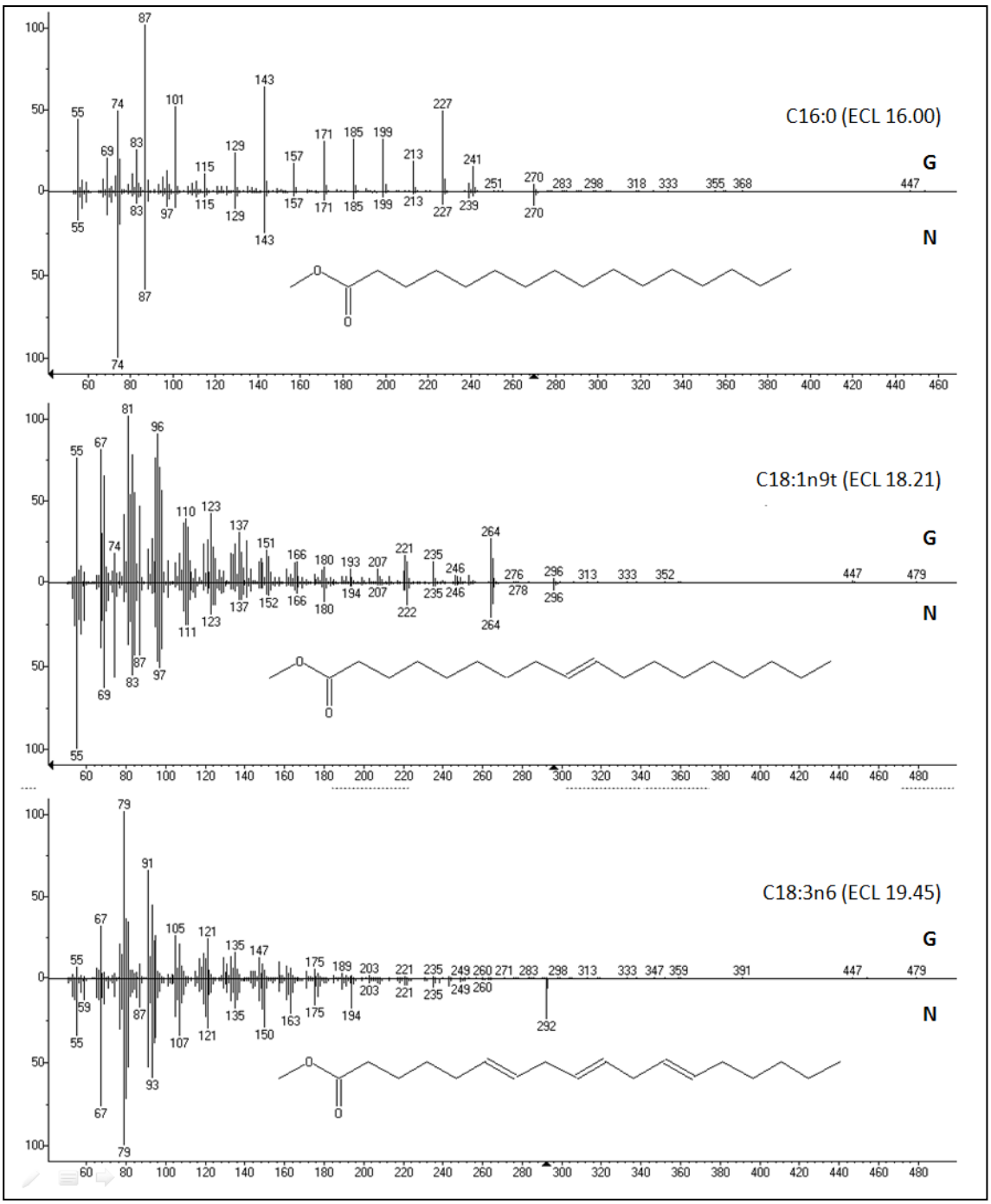

Figure 1. Comparison between (G) GC-Orbitrap EI spectrum at 70 eV and (N) the NIST reference, for C16:0, C18:1n9t and C18:3n6, under default conditions.

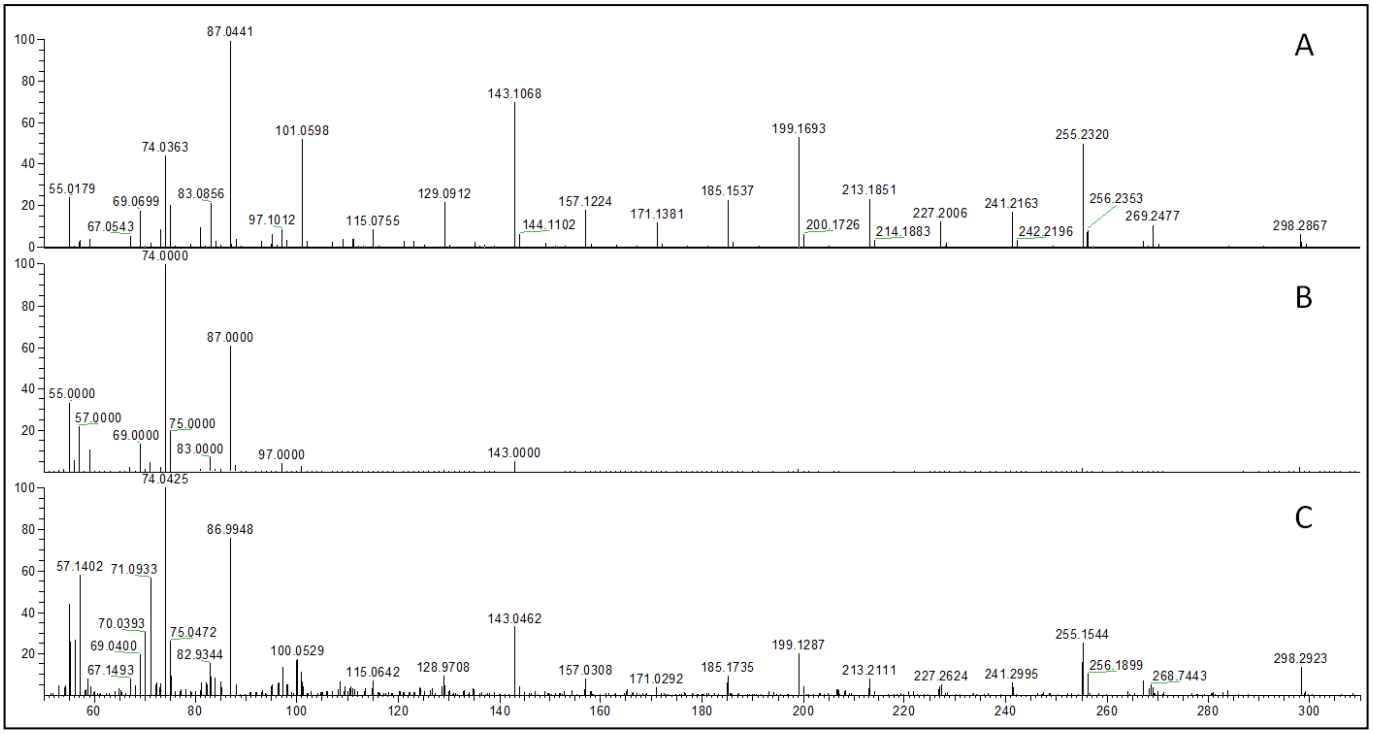

Figure 2. C18:0 EI 70eV mass spectrum recorded with A: GC-Orbitrap; B: GC-IT; C: GC-QQQ.

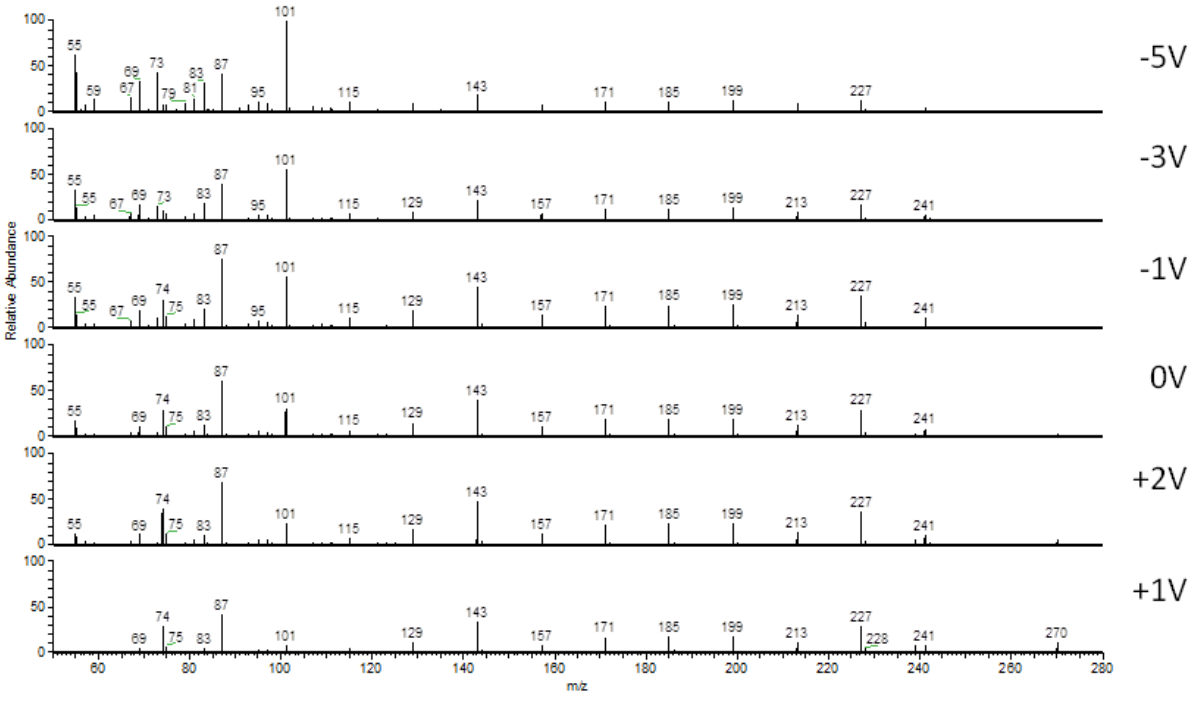

Figure 3. Mass spectra of C16:0 FAMEs for different C-TRAP energy offsets. Ions with relative abundance >10% were plotted.

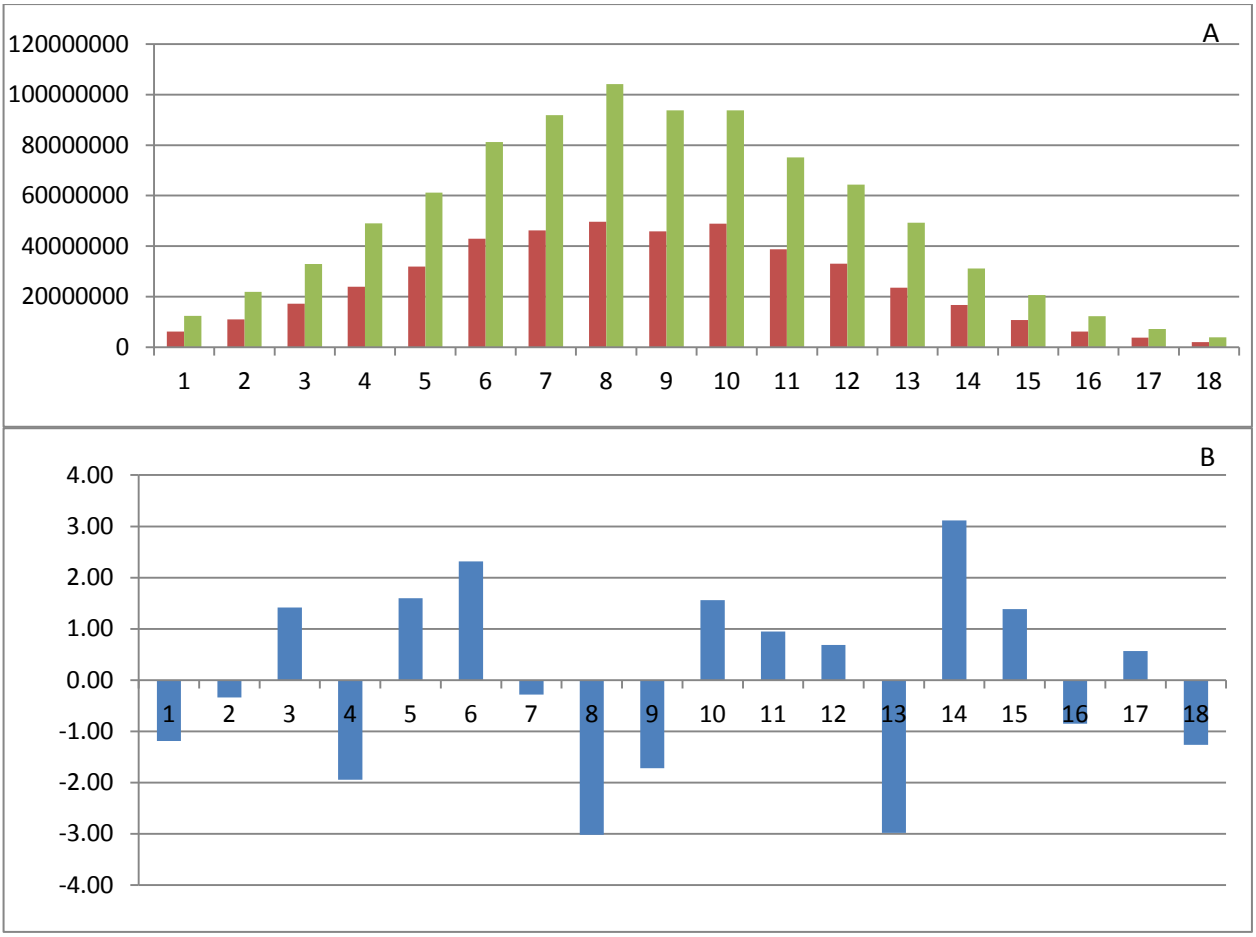

Figure 4. (A) Intensity of ions 74 (red) and 87 (green) versus ion scan for C16:0 during chromatographic separation; (B) variation of the percentage of the ion 87.0442 with respect to the ion 74.0363.

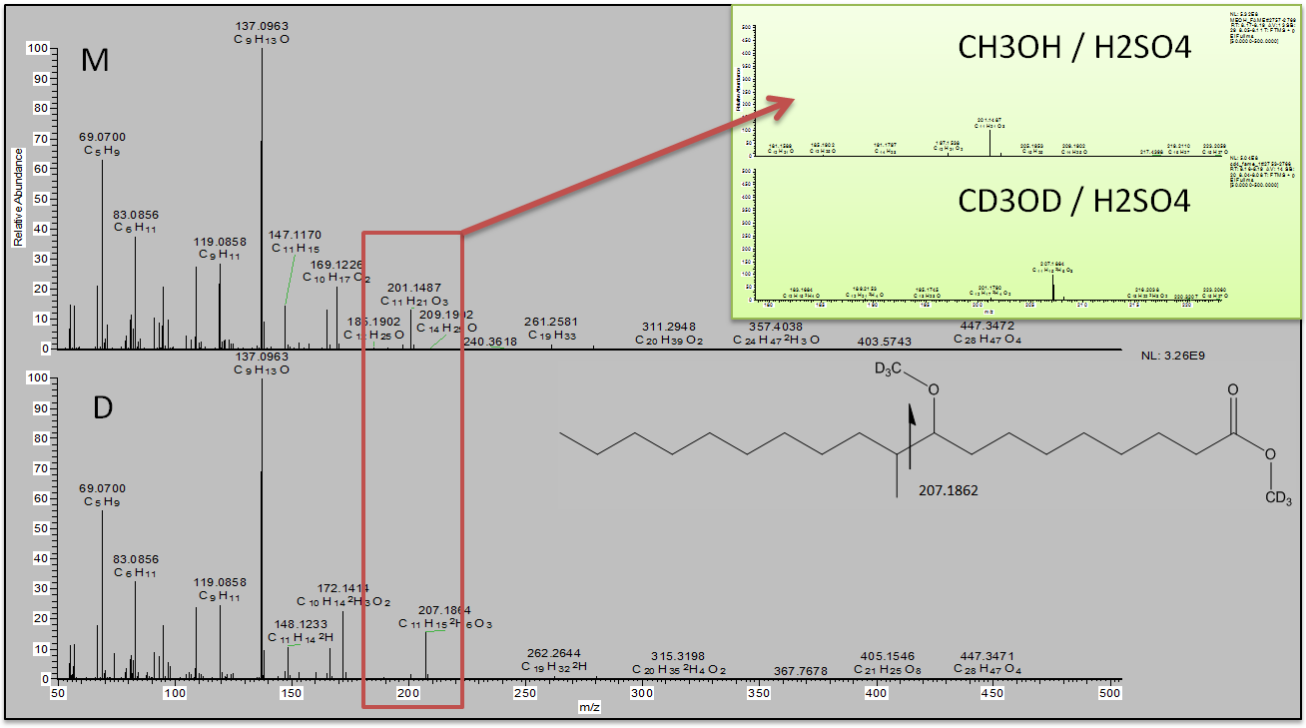

Figure 5. EI 70eV spectra of methoxy FAME after direct derivation of C19:1Δ9 with H_2SO_4 and (M) Methanol or (D) deuterated Methanol.

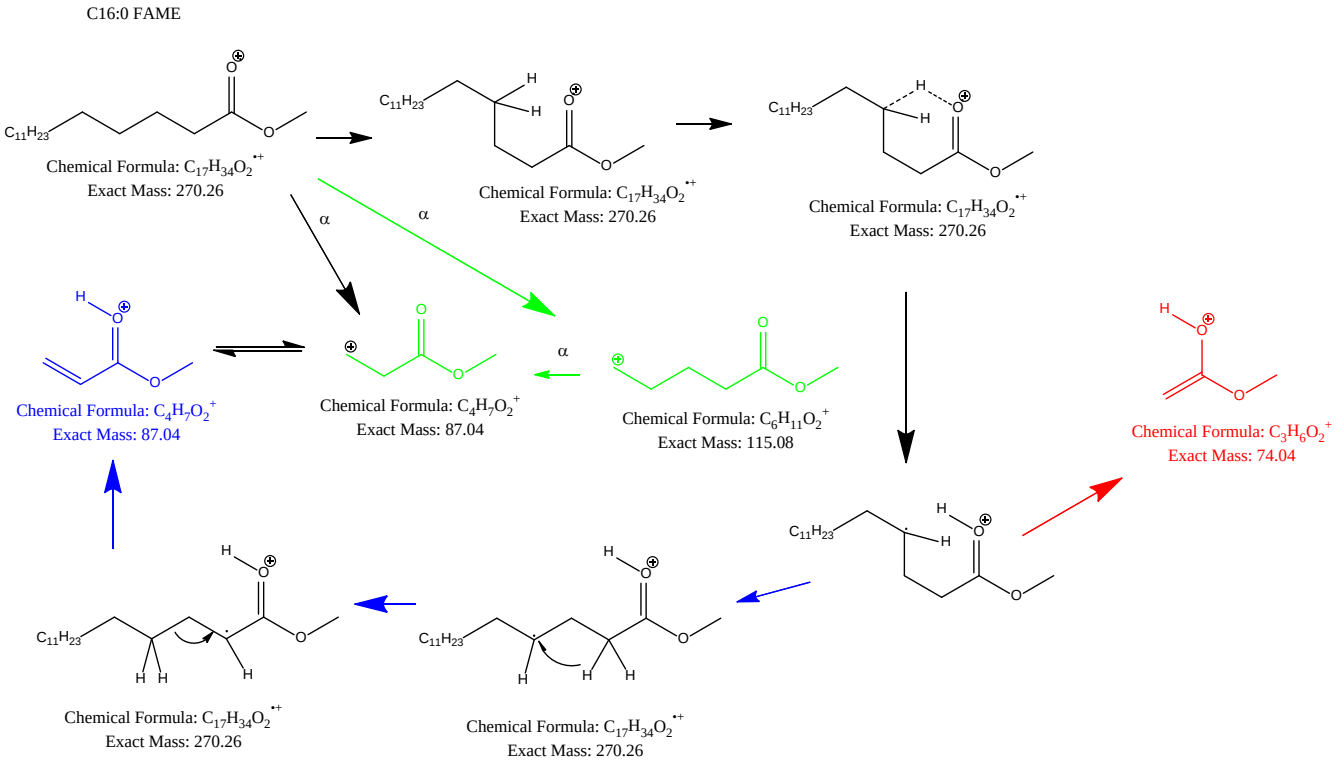

Figure 6. Fragmentation scheme of C16:0 FAME. The formation of ion 87.04 can come from several pathways of fragmentation, either from the alpha fragmentation of the carbon n3 or n5 from the ion 115.08 (green), or from the rearrangement of the molecular ion leading to the formation of the ion 74.04 (red) then 87.04 (blue).

Tables

Table 1. Experimental plan and ions ratio for FAMEs analyses on GC-orbitrap. Concentration level (Exp 1,3,5), automatic gain control (AGC) target (3,4), AGC filling mode (15,16,17), mass range (2,3), Orbitrap resolution (OR.) (12,13,14,15) and C-TRAP values (6,7,8,9,10,11) parameters, were evaluated.

#	Injection		Acquisition setting				Tune setting		R 74/87			R 101/87				R67/81	R67/79	R67/79
	SSL	Dilution factor	AGC	value	Mass Range	OR.	C-TRAP		C12:0	C16:0	C20:0	C12:0	C16:0	C20:0	C18:1n9c	C18:2n6c	C18:3n6	C18:3n3
1	10	1	1.E+06	auto	**50-500**	60	0		52%	36%	34%	59%	100%	87%	77.93%	86.68%	30.22%	30.56%
2	10	100	1.E+06	auto	**50-500**	60	0		54%	49%	42%	39%	58%	53%	85.54%	92.03%	32.50%	33.11%
3	10	100	1.E+06	auto	60-95	60	0		66%	52%	52%	n/a	n/a	n/a	129.50%	116.00%	35.26%	37.91%
4	100	1000	1.E+05	auto	**50-500**	60	0		66%	48%	43%	37%	47%	47%	80.67%	102.00%	32.04%	34.11%
5	100	1000	1.E+06	auto	**50-500**	60	0		55%	49%	45%	39%	51%	51%	86.76%	91.42%	32.50%	33.51%
6	100	1000	1.E+06	auto	**50-500**	60	-5		24%	20%	16%	175%	252%	229%	116.60%	136.00%	28.50%	32.28%
7	100	1000	1.E+06	auto	**50-500**	60	-3		35%	30%	28%	101%	140%	140%	105.80%	116.00%	32.81%	34.19%
8	100	1000	1.E+06	auto	**50-500**	60	-1		51%	42%	39%	52%	76%	72%	98.98%	92.98%	32.17%	33.01%
9	100	1000	1.E+06	auto	**50-500**	60	1		64%	58%	55%	26%	51%	32%	72.39%	72.21%	32.95%	32.30%
10	100	1000	1.E+06	auto	**50-500**	60	3		78%	69%	65%	17%	18%	19%	41.63%	45.48%	28.39%	24.89%
11	100	1000	1.E+06	auto	**50-500**	60	5		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
12	100	1000	1.E+06	auto	**50-500**	15	0		53%	46%	43%	40%	53%	52%	84.54%	93.26%	30.45%	32.83%
13	100	1000	1.E+06	auto	**50-500**	30	0		56%	46%	44%	41%	53%	51%	79.23%	85.12%	31.30%	33.18%
14	100	1000	1.E+06	auto	**50-500**	60	0		57%	48%	43%	41%	54%	53%	84.49%	89.67%	31.71%	32.41%
15	100	1000	1.E+06	auto	**50-500**	120	0		58%	49%	45%	40%	52%	51%	84.17%	93.54%	33.43%	31.93%
16	100	1000	1.E+05	50ms	**50-500**	60	0		59%	46%	45%	35%	53%	42%	79.33%	95.15%	29.89%	33.57%
17	100	1000	1.E+05	100ms	**50-500**	60	0		57%	48%	43%	36%	53%	50%	74.41%	87.52%	31.46%	32.03%
18	100	1000	1.E+06	50ms	**50-500**	60	0		55%	49%	43%	40%	51%	51%	85.55%	86.45%	31.97%	32.57%
19	100	1000	1.E+06	100ms	**50-500**	60	0		54%	51%	44%	41%	57%	52%	83.69%	90.12%	31.19%	32.31%

N.S. : no signal ; n/a : not applicable

Table 2. Variation of ion ratios with respect to the default conditions of settings and concentrations (# 5).

#	Parameters	Values	Ratio m/z 74/87			Ratio m/z 101/87				R 67/81	R67/81	R67/79	R67/79
			C12:0	C16:0	C20:0	C12:0	C16:0	C20:0		C18:1n9c	C18:2n6c	C18:3n6	C18:3n3
1	concentration	SSL 10 df 1	-3%	***-12%***	***-11%***	**21%**	**48%**	**36%**		-9%	-5%	-2%	-3%
2	concentration	SSL 10 /df 1/100	-1%	0%	-3%	0%	7%	2%		-1%	1%	0%	0%
3	Mass Range	60-95	**11%**	3%	7%	-	-	-		**43%**	**25%**	3%	4%
4	AGC	1.E+05	**11%**	-1%	-2%	-1%	-5%	-4%		-6%	**11%**	0%	1%
5	Default		0%	0%	0%	0%	0%	0%		0%	0%	0%	0%
6	CTRAP	-5	***-31%***	***-29%***	***-29%***	**136%**	**200%**	**178%**		**30%**	**45%**	-4%	-1%
7	CTRAP	-3	***-20%***	***-19%***	***-18%***	**62%**	**89%**	**89%**		**19%**	**25%**	0%	1%
8	CTRAP	-1	-4%	-7%	-6%	**14%**	**25%**	**21%**		**12%**	2%	0%	-1%
9	CTRAP	1	9%	9%	**10%**	***-13%***	0%	***-19%***		***-14%***	***-19%***	0%	-1%
10	CTRAP	3	**23%**	**20%**	**20%**	***-21%***	***-34%***	***-32%***		***-45%***	***-46%***	-4%	-9%
11	CTRAP	5	-	-	-	-	-	-		-	-	-	-
12	Resolution	15	-2%	-3%	-2%	1%	2%	1%		-2%	2%	-2%	-1%
13	Resolution	30	1%	-3%	-2%	2%	2%	0%		-8%	-6%	-1%	0%
14	Resolution	60	2%	-1%	-2%	3%	2%	2%		-2%	-2%	-1%	-1%
15	Resolution	120	3%	0%	0%	2%	1%	0%		-3%	2%	1%	-2%
16	AGC manual mode	1E-5 / 50ms	4%	-3%	0%	-4%	1%	-9%		-7%	4%	-3%	0%
17	AGC manual mode	1E-5 / 100ms	2%	-1%	-2%	-2%	1%	-1%		***-12%***	-4%	-1%	-1%
18	AGC manual mode	1E-6 / 50ms	0%	0%	-2%	2%	0%	0%		-1%	-5%	-1%	-1%
19	AGC manual mode	1E-6 / 100ms	-1%	2%	-1%	2%	5%	1%		-3%	-1%	-1%	-1%

Df : Dilution factor, SSL : split factor:

Table 3 Identification of the molecules found after extraction and methylation of *Crocus sativus L* oil from seed.

Component Name	RT	ECL		Base peak (m/z)	% Area	NIST				DB FAME GC-HRMS			
		Calculated	Theorical			Match	R.MATCH	%	#	Match	RMATCH	%	#
FAME C14:0	5.42	13.75	14.00	87.0443	< 0.1%	630	741	3.87	7	736	850	60.6	1
FAME C16:0	6.54	16.00	16.00	87.0443	12.02%	753	765	47.80	1	961	964	86.5	1
FAME C18:0	7.54	18.00	18.00	87.0443	9.69%	770	776	59.70	1	946	948	87.2	1
FAME C18:1n9c	7.73	18.42	18.37	81.0701	14.41%	777	797	6.39	6	937	938	35.5	2
FAME C18:1n7c	7.77	18.48	nd	81.0701	< 0.1%	761	799	3.04	12	n/a			
FAME C18:2n6c	8.04	19.12	19.02	81.0701	59.26%	787	788	6.78	1	951	951	36.3	2
FAME C18:3n6c	8.41	19.94	19.78	79.0545	1.29%	-	-	-	-	-	-	-	-
FAME C20:0	8.43	20.00	20.00	87.0443	1.51%	752	777	41.50	1	900	908	73.3	1
FAME C20:1n9	8.62	20.45	20.39	81.0701	0.53%	760	805	16.70	1	549	631	0.14	13
FAME C20:2	8.92	21.18	21.06	81.0701	0.70%	672	718	2.50	8	705	822	34.4	1
FAME C22:0	9.25	22.00	22.00	87.0443	0.40%	696	743	20.00	2	734	767	8.97	5
FAME C24:0	10.00	24.00	24.00	87.0443	0.18%	693	739	38.30	1	817	884	83.9	1

n/a : not applicable.

C18:3n6c is practically coeluted at the same time as C20:0. Other analyzes with a slower temperature gradient made it possible to identify the molecule (not shown).