Determination of triacylglycerol regiosomers using electrospray ionization-quadrupole ion trap mass spectrometry with a kinetic method

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The kinetic method was applied to differentiate and quantify mixtures of regioisomeric triacylglycerols (TAGs) by generating and mass selecting alkali ion bound metal dimeric clusters with a TAG chosen as reference (ref) and examining their competitive dissociations in a quadrupole ion trap mass spectrometer. This methodology readily distinguished pairs of regioisomers (AAB/ABA) such as LLO/LOL, OOP/OPO and SSP/SPS and consequently distinguished sn-1/sn-3, sn-2 substrates on the glycerol backbone. The dimeric complex ions [ref, Li]TAG(AAB and/or ABA)+ generated by electrospray ionization mass spectrometry were subjected to collision induced dissociation causing competitive loss of either the neutral TAG reference (ref) leading to [Li(AAB and/or ABA)]+ or the neutral TAG molecule (TAG(AAB and/or ABA)+) leading to [ref, Li]+. The ratio of the two competitive dissociation rates, defined by the product ion branching ratio (Rsn), was related via the kinetic method to the regioisomeric composition of the investigated TAG mixture. In this work, a linear correlation was established between composition of the mixture of each TAG regioisomer and the logarithm of the branching ratio for competitive fragmentation. Depending on the availability of at least one TAG regioisomer as standard, the kinetic method and the standard additions method led to the quantitative analysis of natural TAG mixtures.

1. Introduction

In the biosynthesis of triacylglycerols (TAGs) of vegetable oils, each fatty acid (FA) occupies a preferred position on the glycerol backbone [1]. The regiospecific positioning of distinct FA has been indicated to be responsible for specific effects on lipoprotein metabolism and atherosclerosis which also affect lipid metabolism in humans. For the first time, the regiospecific distinction was reported by Barber and Merren [2]. Until now, the differentiation between TAG isomers (AAB/ABA) has been based on the ratio of characteristic fragment ions in mass spectrometry. Thus, Mottram and Evershed [3] and Byrdwell [4] demonstrated that the loss of the acyl chain from the sn-1 or sn-3 position was energetically favoured over loss from the sn-2 position. The APCI mass spectra of pure TAG isomers reported by the above authors, demonstrated that the ion abundances [AA]++[AB]+ were expected to change with the regiosomeric composition of a mixture of TAG regioisomers. However, discordant results were published concerning the identification of regioisomers using APCI. Although Mottram and Evershed [3] could identify the positional isomers, Segall et al. [5] showed that analysis of sodium adduct molecular ions did not provide differences in the relative abundance of regioisomeric triacylglycerols ions. Duffin et al. [6] reported that the relative abundance of product ions resulting from the dissociation of fatty acid chains from TAGs was not significantly different, while Hvattum [7] found a smaller signal due to the neutral loss of the sn-2 fatty acid residue. Both experiments of Duffin et al. [6] and Hvattum [7] were conducted using ESI ionization. In a previous work [8] we showed that it was possible to distinguish regiospecifically some pairs of TAGs thanks to silver adducts and MS3 analysis. However, this MS approach was long and needed much quantity of TAG to allow regiospecific distinction of TAGs by LC–MS3. Therefore, a new simple, faster MS and reliable MS method for regiospecific ratio determination is desirable.

In an attempt to resolve the problem of regiospecific distinction of TAGs, we explore the applicability of the kinetic method, a procedure for thermochemical determinations which has seen growing use for chiral recognition [9–15] and some use in isomeric differentiation [16]. The kinetic method [10,16,17] uses a transition metal ion to form complex ions involving both an analyte and a reference compound, thereby allowing multiple point interactions between these ligands. In the absence of solvent in the mass spectrometer, all interactions are between the ligands and this increases the importance of the reference compound as well as the strength of the metal–ligand interactions. Most of the kinetic method’s literature deals with trimeric complexes [11,15–21] and more rarely it was also proved that the kinetic method was applicable with dimeric clusters [20,22–24].
The complex ions were mass-selected and allowed to undergo collision-induced dissociation (CID), which occurred by competitive loss of either the neutral analyte or the neutral reference. The competitive reactions are illustrated in Eq. (1):

\[
\text{ABA or AAB} \rightarrow \text{ESI} \rightarrow ((\text{ref}) \text{ C (ABA or AAB)})^+ \rightarrow [(\text{ref}) \text{ C}^+] + (\text{ABA or AAB})
\]

In these reactions, C^+ is the central ion, ABA and AAB are two regioisomeric TAGs, (ref) is a reference compound and ESI means electrospray ionization.

The relative branching ratio R for the two competitive dissociations is given by:

\[
R_{\text{ABA or AAB}} = \frac{I[(\text{ABA or AAB})]^+}{I[(\text{ref}) \text{ C}]^+}
\]

with \(I[(\text{ABA or AAB})]^+\) and \(I[(\text{ref}) \text{ C}]^+\) the intensity of the ion [ABA or AAB]^+ and [(ref) C]^+ respectively.

\(R_{\text{ABA or AAB}}\) corresponds to the branching ratio when only one of the two regioisomers (ABA or AAB, respectively) is present in the sample. The ratio \(R_{\text{iso}}\), measured the efficiency of regioisomer distinction under the particular experimental conditions chosen:

\[
R_{\text{iso}} = \frac{R_{\text{ABA}}}{R_{\text{AAB}}}
\]

The more different the \(R_{\text{iso}}\) value is from unity, the higher the degree of isomeric recognition. This approach offers the main advantage of a large isomeric differentiation resulting from the logarithmic relationship between \(R_{\text{iso}}\) and the difference in free energy. Also this method does not require isotopic labelling [15].

In the present study, we applied the kinetic method to the analysis of regioisomeric mixtures of TAG pairs such as LLO/LOL, OOP/OPO and SSP/SPS. The cluster ion included the analyte (a TAG, either as a single isomer or a regioisomeric mixture), a reference compound (ref), and a metal ion (C). The thermochemical and kinetic basis for this new approach to regioisomer differentiation of TAGs was investigated. The first part of the work consisted of choosing the alkali ion (C) and the reference compound from common and commercially available TAGs (ref). The next step of the work consisted of evaluating the capability of the kinetic method for the differentiation and quantification of regioisomeric TAG pairs.

2. Experimental

All experiments were performed using a commercial LCQ quadrupole ion trap mass spectrometer (Thermo Fisher, San Jose, CA) equipped with an ESI source operated in the positive ion mode under the following conditions: source voltage 4.5 kV, capillary temperature 250 °C, capillary voltage 25 V and tube lens offset 20 V. Nitrogen was used both as sheath gas and as an auxiliary gas at a flow rate of 75 and 50 (arbitrary units, instrument settings), respectively. In the full-scan MS2 modes, the precursor ion of interest was first isolated by applying an appropriate waveform across the end cap electrodes of the ion trap to resonantly eject all trapped ions, except those ions of the m/z ratio of interest. The isolated ions were then subjected to a supplementary ac signal to resonantly excite them in order to cause CID. The Mathieu qv values for resonance excitation and resonance ejection were 0.25 and 0.83, respectively. The excitation time used was 30 ms. The excitation amplitude can be varied from 0 to 100% relative collision energy corresponding to 0–2.5 V zero-to-peak resonant excitation potential; this value was optimized in each experiment, but kept constant for measurements of the regioisomers. Spectra shown represent the average of 25 scans, each requiring 0.1 s.

Acetone and acetonitrile were HPLC grade from Carlo Erba (Rodano, Italy). Synthetic triacylglycerols (TAGs) standards were obtained from Sigma (St. Louis, MO, USA); 1,2-dioleoyl-3-palmitoyl-glycerol (OOP); 1,3-dioleoyl-2-palmitoyl-glycerol (OPP); 1,2,3 trioleyl glycerol (OOS); 1,3-dioleoyl-2-stearoyl-glycerol (OSO); 1,2,3 trilinoleoyl-glycerol (LLL); 1,2,3 trilinolenoyl-glycerol (LnnLn); 1,2 dipalmitoyl-3-oleyl-glycerol (PPO); 1,3 dipalmitoyl-2-oleyl-glycerol (POP); 1,2-stearoyl-3-palmitoyl-glycerol (SSP); 1,3-stearoyl-2-palmitoyl-glycerol (SSPS); tri-palmitoyl-glycerol (PPP); 1,3 dilinoleoyl-2-oleyl (LOL) and 1,2 dilinoleoyl-3-oleyl (LLO) were purchased from Larodan (Malmö, Sweden).

The different salts (LiI, NaCl, AgNO3, KCl, CsCl) were obtained from Sigma (St. Louis, MO, USA).

Gas phase metallic cation complexes with TAGs were generated by electrospraying 50/50 acetone/acetonitrile solutions containing a mixture of the TAG (100 μM) and a reference compound (100 μM), together with 25 μM of the salt.

The developed kinetic method presented in this following work was applied to the soya oil. In order to do this work, the chromatographic conditions already published [8] were used. Briefly, the oil was injected into an HPLC system composed of a Nucleosil 100 A C18 column (150 mm × 2.1 mm i.d., 3 μm, Macherey Nagel (Düren, Germany)) and a mobile phase composed of acetonitrile/acetone (50/50, flow rate = 0.25 mL min⁻¹). In this work LiI and OOO or LLL were used as post-column reagent (flow rate 0.05 mL min⁻¹, LiI at 25 μM and OOO or LLL at 100 μM in acetone/acetonitrile (50/50)). Therefore, the ratios of [ABA] C⁺ over [ABA] C⁺ allowed the calculation of \(R_{\text{iso}}\) in infusion as well as in LC–MS, thereby allowing the drawing of calibration curves as standard addition method.

3. Results and discussion

3.1. Selection of the central metal and the reference compound

To make the choice of the central metal, we tested different metal salts (LiI, NaCl, KCl, AgNO3, CsCl) as they were reported to readily form complexes with oxygen containing compounds [17]. Abundant alkali metal cluster ions were observed for LLO/LOL, OOP/OPO and SSP/SPS in the ESI mass spectra of mixtures of TAGs with only Li⁺. Therefore, the ion was used as the central metal for our clusters.

The reference compound was selected to allow regioisomeric distinction and also help to get a \(R_{\text{iso}}\) value quite different from unity. The choice of appropriate references, mainly from TAGs, was based in part on their assumed abilities to produce steric interactions, as well as their structural similarity to the analyte. The latter characteristic allowed ready complex formation and accurate measurements of relative abundance ratios. Otherwise dissociation proceeds overwhelmingly to the more stable product. The reference compounds selected here, LLL, OOO and SSS, were the best to achieve accurate and large distinctions as the \(R_{\text{iso}}\) values were the most different from unity (\(R_{\text{iso}} = 1\)) compared to those found with the other reference compounds for LLO/LOL, OOP/OPO and SSP/SPS, respectively (Table 1). Therefore, LLL, OOO and SSS were retained as reference compounds in the following experiments for LLO/LOL,
Table 1
Selection of the reference compound used for the distinction of (a) OOP/OPO, (b) SSP/SPS and (c) LLO/LOL.

<table>
<thead>
<tr>
<th>Reference</th>
<th>$R_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td></td>
</tr>
<tr>
<td>SOO</td>
<td>1.29</td>
</tr>
<tr>
<td>OSO</td>
<td>1.33</td>
</tr>
<tr>
<td>OOO</td>
<td>2.21</td>
</tr>
<tr>
<td>LLL</td>
<td>1.30</td>
</tr>
<tr>
<td>LnLnLn</td>
<td>1.40</td>
</tr>
<tr>
<td>PPO</td>
<td>0.98</td>
</tr>
<tr>
<td>POP</td>
<td>1.19</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
</tr>
<tr>
<td>SOO</td>
<td>1.05</td>
</tr>
<tr>
<td>OSO</td>
<td>1.07</td>
</tr>
<tr>
<td>PPP</td>
<td>1.36</td>
</tr>
<tr>
<td>LLL</td>
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</tr>
<tr>
<td>SSS</td>
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</tr>
<tr>
<td>PPO</td>
<td>0.95</td>
</tr>
<tr>
<td>POP</td>
<td>1.01</td>
</tr>
<tr>
<td>(c)</td>
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</tr>
<tr>
<td>SOO</td>
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</tr>
<tr>
<td>OSO</td>
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<tr>
<td>OOO</td>
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<td>LLL</td>
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<td>LnLnLn</td>
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<tr>
<td>PPO</td>
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<tr>
<td>POP</td>
<td>0.94</td>
</tr>
</tbody>
</table>

![Graph](https://example.com/graph1)

**Fig. 1.** ESI-MS spectrum of 1:1 acetonitrile:acetone solution containing OOP, OOO and Li+. The presence of the complex [(OOO)Li(OOP)]$^+$ is indicated.

OOP/OPO and SSP/SPS, respectively. It can be deduced from these results that the similarity of the reference compound to the analyte is essential to improve the regioisomeric distinction as it avoids the production of the most stable product after dissociation of the complex. These results are in good accordance with those found in literature [16], especially the work done by Tao et al. [16] in which they demonstrated that selecting a dipeptide as a reference allowed the isomeric distinction of dipeptide pairs.

A typical ESI-MS spectrum of a mixture of pure OOP, OOO and Li in acetonitrile/acetone (50/50, v/v) is illustrated in Fig. 1. The formation of the complex [(OOO)Li(OOP)]$^+$ (m/z 1751.6, isowidth 5) was observed and indicated in this spectrum. Other intense ionic adducts [(OOP)Li]$^+$ (m/z 865.7) and [(OOP)H]$^+$ (m/z 860.7) were also detected. The same ions were observed with a mixture OPO, OOO and Li.

One limitation of the proposed method described below is that dimer adducts formation is instrument dependent. As it was previously demonstrated by Pan [25] and Jiang et al. [26] the precursor ion formation is related to surface catalytic properties of the materials used in construction of the sources or ionization needles of the instruments. As a consequence, the presented results could not necessary be replicated in other laboratories. Nevertheless, this new approach is repeatable and it works for different TAG pairs such as OOP/POP, LLO/LOL and SSP/SPS.

3.2. First case: the two regioisomers were available as standards

**Fig. 2** shows the product ion (MS$^2$) spectrum of a typical dimeric cluster ion [(OOO)Li (TAG)]$^+$, where TAG is either OOP or OPO, which was mass-selected and dissociated. Fragmentation occurred simply by competitive loss of one or the other intact neutral ligand to form a pair of cationized monomers. The results indicated that the branching ratio ($R_{OOP}$ or $R_{OPO}$) for these fragmentations depended strongly on the regiochemistry of the TAG when the same reference ligand, OOO, was used. When TAG was pure OOP, the branching ratio $R_{OOP}$ was 0.34 (Fig. 2a), whereas $R_{OPO}$ was 0.75 for pure OPO (Fig. 2b). The regioisomeric selectivity, $R_{reg}$, was therefore 2.21 for this case under these conditions (Table 1a).

**Fig. 3** shows the typical MS$^2$ spectrum of the dimeric complex [(SSS)Li(SSP)]$^+$ and [(SSS)Li(SPS)]$^+$. $R_{SPS}$ was 2 (Fig. 3a) and $R_{SPS}$ was
1.16 (Fig. 3b). The regioisomeric selectivity \( R_{\text{iso}} \) was then 0.58 for SPS/SSP (Table 1b).

Fig. 4 shows the \( \text{MS}^2 \) spectrum of \([\text{LLL}][\text{Li}][\text{LLO}]^+\) and \([\text{LLL}][\text{Li}][\text{LOL}]^+\). The \( R_{\text{LOL}} \) was 0.42 and \( R_{\text{LLO}} \) was 0.85. Consequently, \( R_{\text{iso}} \) was 0.49 (Table 1c).

### 3.2.1. Concentration effects on \( R_{\text{iso}} \) values

When unknown samples were analysed, total concentrations of analytes were unknown. Therefore, the effect of the analyte and TAG reference concentrations as well as the metal level on \( R \) values was an important point to take into consideration. As shown in Table 2a, the \( R_{\text{LOL}} \), \( R_{\text{OPQ}} \), and \( R_{\text{SPS}} \) values were insensitive to significant changes to the ratio of the analyte and reference compounds concentrations, varying from 10:1 to 1:10. The \( R_{\text{LOL}} \), \( R_{\text{OPQ}} \) and \( R_{\text{SPS}} \) values were also invariable as Li concentrations (Table 2b) were varied. The above results indicated that the present ESI-\( \text{MS}^2 \) method was rather insensitive to changes in concentration conditions and suggested that regioisomeric selectivity using ESI-\( \text{MS}^2 \) was robust and required minimal sample preparation.

### 3.2.2. Quantitative analysis of isomeric mixtures

According to the kinetic method [17], \( \ln R_{\text{iso}} \) is proportional to the differences between the free energy changes for the competitive dissociations to yield the two monomeric product ions \([\text{ref}][\text{Li}]^+\) and \([\text{Li}([\text{ABA}])^+]\) as shown in Eq. (1):

\[
\ln R_{\text{iso}} = \frac{\Delta(\Delta G)}{RT_{\text{eff}}} \tag{4}
\]

where \( R \) is the gas constant, \( T_{\text{eff}} \), the effective temperature and \( \Delta(\Delta G) \) is the difference in the free energies in reactions (5) and (6) whose reversed barriers are considered negligible.

\[
[\text{ref}[\text{Li}][\text{ABA}]]^+ \rightarrow [\text{ref}][\text{Li}]^+ + \text{ABA} \tag{5}
\]

\[
[\text{ref}[\text{Li}][\text{ABA}]]^+ \rightarrow [\text{Li}][\text{ABA}]+^+ + \text{ref} \tag{6}
\]

For a regioisomeric OOP/OPO mixture in which the molar fraction of one regioisomer was given by \( \alpha \) we could write:

\[
\Delta(\Delta G) = \alpha \times \Delta(\Delta G)_{\text{OPO}} + (1 - \alpha) \times \Delta(\Delta G)_{\text{POO}} \tag{7}
\]

\[
= \Delta(\Delta G)_{\text{POO}} + \Delta(\Delta G)_{\text{OPO}} - \Delta(\Delta G)_{\text{POO}} \times \alpha \tag{8}
\]

and therefore the relationship between \( R_{\text{iso}} \) and \( \alpha \) can be expressed by combining Eqs. (4) and (8) to obtain:

\[
\ln R_{\text{iso}} = \frac{\Delta(\Delta G)_{\text{POO}}}{RT_{\text{eff}}} + \frac{\Delta(\Delta G)_{\text{OPO}} - \Delta(\Delta G)_{\text{POO}}}{RT_{\text{eff}}} \times \alpha \tag{9}
\]

Eq. (9) predicts a linear relationship between \( \ln R_{\text{iso}} \) and \( \alpha \). In practice, the intrinsic linear relationship between \( \ln R \) and \( \alpha \) was demonstrated in a series of measurements made using OOP and
Table 2a

Relative abundance ratio \([\text{Li(ABA)}]^+ | (\text{ref}) \text{Li}^+\) for various ratios of ABA and ref with ABA=LOL or OPO and ref=LLL or OOO respectively.

| [ABA]: [ref] | \(R_{\text{LOL}} = \left[ \text{Li} \right] (\text{LOL})^+ | ([\text{LLL}] \text{Li})^+ \) | \(R_{\text{OPO}} = \left[ \text{Li} \right] (\text{OPO})^+ | ([\text{OOO}] \text{Li})^+ \) | \(R_{\text{SSS}} = \left[ \text{Li} \right] (\text{SSS})^+ | ([\text{SSS}] \text{Li})^+ \) |
|-------------|-----------------|-----------------|-----------------|
| 10:1        | 0.83            | 0.75            | 1.16            |
| 8:1         | 0.85            | 0.76            | 1.16            |
| 5:1         | 0.85            | 0.76            | 1.17            |
| 3:1         | 0.84            | 0.75            | 1.17            |
| 1:1         | 0.85            | 0.76            | 1.16            |
| 1:3         | 0.86            | 0.76            | 1.15            |
| 1:5         | 0.85            | 0.76            | 1.16            |
| 1:8         | 0.86            | 0.77            | 1.16            |
| 1:10        | 0.85            | 0.76            | 1.17            |

Table 2b

Relative abundance ratio \([\text{Li(ABA)}]^+ | (\text{ref}) \text{Li}^+\) for various Li concentrations with ABA=LOL or OPO and ref=LLL or OOO or SSS respectively.

| Concentration of Li (µM) | \(R_{\text{LOL}} = \left[ \text{Li} \right] (\text{LOL})^+ | ([\text{LLL}] \text{Li})^+ \) | \(R_{\text{OPO}} = \left[ \text{Li} \right] (\text{OPO})^+ | ([\text{OOO}] \text{Li})^+ \) | \(R_{\text{SSS}} = \left[ \text{Li} \right] (\text{SSS})^+ | ([\text{SSS}] \text{Li})^+ \) |
|-------------------------|-----------------|-----------------|-----------------|
| 25                      | 0.85            | 0.75            | 1.17            |
| 50                      | 0.84            | 0.75            | 1.16            |
| 75                      | 0.85            | 0.76            | 1.17            |
| 100                     | 0.85            | 0.75            | 1.17            |
| 200                     | 0.85            | 0.76            | 1.16            |

Fig. 5. Calibration curve for quantitative analysis of OOP/OPO mixture TAGs using OOO as the reference and \text{Li}^+ as central cation (\(n = 3\)).

OPO. In our experiments, the analytes OOP, OPO were used in mixtures of various molar fractions with the pure reference OOO being used to generate the corresponding dimeric \text{Li}^+ clusters. Calibration curves were obtained by three consecutive measurements (Fig. 5). Note that the calibration curve should ideally be drawn before each set of measurements, although the data shown here represent daily calibrations. Similar work has been done with a mixture of SSP/SPS by using SSS as reference compound and \text{Li}^+ as the central metal ion. The calibration curve is shown in Fig. 6. Similar work has been done with LLO/LOL and the calibration curve obtained is shown in Fig. 7. Therefore, the linear relationships obtained in Figs. 5–7 were used as calibration curves and were consequently very useful for the recognition of regioisomers of triacylglycerols by the determination of the molar fraction of one isomer, \(\alpha\).

3.3. Second case: only one of the two regioisomers was available as standard-employment of the standard addition procedure

When only one of two regioisomeric TAGs was commercially available, then it was not possible to obtain calibration curves similar to the ones obtained in Figs. 5–7. We propose here a method when only one of the two regioisomers is available. This necessitates a standard addition procedure where the available TAG is used to determine an unknown mixture. To develop this method, we did the work as if only OPO was available as a commercial TAG. For demonstration two mixtures OOP/OPO (50/50) and OOP/OPO (25/75) were used as probes of natural mixtures. To 100 µL solution of each probe mixture was added 25, 50, 100, 200, 300 and 400 µL of OPO. Then \(\alpha\) was obtained by dividing the added volume by the total volume (i.e. if 25 µL were added to the 100 µL mixture solution, then \(\alpha\) equal 25/(25 + 100) = 0.2). On the curve of \(\ln R_{\text{iso}}\) vs \(\alpha\), the intersection between the \(x\)-axis and the curves (Fig. 8) allowed the determination of \(\alpha\) and consequently the regioisomeric determination. We obtained a composition mixture of 49% OPO/51% OOP...
for probe 1 (full line, Fig. 8). The dotted line showed that probe 2 was a mixture of 75% OPO and 25% OOP. These results were in good accordance with the expected results of the probe mixtures.

The proposed method could give the relative quantitative composition of any pair of regioisomeric TAGs, depending on the commercial availability of at least one regioisomeric TAG of a given TAG pair. This method was systematically validated with mixtures of known composition such as LLO/LOL and SSP/SPS (data not shown).

4. Application to the soya oil

To determine the proportion of OOP and OPO in the soya oil, LC–MS calibration curves as described in Section 3.3 were drawn. Similar calibration curves in Fig. 8 were also drawn. Then the analysis of the soya oil was done three times and this allowed the determination of the relative quantity of OOP/OPO (Fig. 9). The method described in this paper was used and our results demonstrated that the peak at 101.9 min is composed of a mixture of OOP/OPO (95/5) in the soya oil. Our experimental results are in good accordance with previous published paper [27]. According to Lisa et al. [27], the unsaturated fatty acids preferentially occupy the sn-2 position in plant oils, whereas it is the reverse for animal fats, where unsaturated fatty acids are found mainly in sn-1/3 positions [27]. The knowledge of sn-2 preference in TAGs is very important due to the bioavailability of particular fatty acids, because human lipases first cleave fatty acids in sn-1/3 positions and therefore fatty acids present in the sn-2 position can be least accessible for the human organism [27]. Therefore, in this work we demonstrated that in soya oil OOP was mainly present with oleic acid, an unsaturated fatty acid, in sn-2 position. Concerning LLO/LOL in the soya oil, similar work has been done with the standard addition procedure. This mixture is composed of 96% of LLO and 4% of LOL. This result is in a good accordance with those published by Karupaiah and Sundram [28].

Therefore, this paper describes a new approach to determine TAGs regioisomers. However, one limitation of this method is that the dimer formation is concentration dependant. Nevertheless, the $R_{ABA}$ value, which allows the determination the TAG regiospecificity, is insensitive to the concentration conditions. So, in this paper, we demonstrated that this new method is applicable to high concentration mixtures, but we still have to demonstrate that it can also work for smallest quantity of TAGs.

The main analytical separative techniques used for regiospecific analysis of TAGs are silver-ion HPLC and non aqueous reversed phase liquid chromatography (NARP-LC). The ability of NARP-LC to separate TAG regioisomers has already been demonstrated by various authors [29–33]. However, NARP-LC usually has long analysis time especially for complex mixtures analysis such as oils. Silver ion HPLC can also be applied to separate TAGs in oils [34–36]. When considered separately, neither NARP-LC nor silver-ion HPLC is capable of resolving the entire complex TAG mixtures of vegetable oils. Therefore, depending on the sample, the two separation mechanisms are complementary. Concerning MS techniques, HPLC/APCI-MS is now often used for characterization of prevailing FAs in the sn-2 position in complex natural mixtures [37]. Other ionization techniques, like electrospray ionization [4] or matrix-assisted laser desorption/ionization [38], can be used for the same purpose as well with the assistance of adduct formation using small cations (e.g., ammonium, sodium or silver). All MS approaches rely on the fact that the neutral loss of FA from the middle sn-2 position is less energetically favoured in comparison with cleavages from the side sn-1/3 positions [37]. This knowledge is often used for the estimation of prevailing FA in the sn-2 position, but regiospecific standards are essential for the quantitative determination [39].

In this study, a new method, which could be a new approach for TAG regioisomers distinction, has been proposed using dimeric species consisting of a TAG and a reference TAG cationized by Li⁺ under ESI conditions in the gas phase. It is necessary to point out that this new method, just as the other references cited in this paper, allowed the differentiation between AAB and ABA regioisomers but is still not able to distinguish AAB and BAA. To the best of our knowledge, only biochemical method is capable of making this distinction [40].
5. Conclusion

We report here a new method to differentiate and quantify TAGs in a complex matrix. The development was based on the kinetic method which is based on the competitive dissociations of alkali metal ion bound dimeric cluster in a quadrupole ion trap mass spectrometry. This methodology was efficient and allowed recognition and quantification of LLO/LOL, OOP/OP and SSP/SP ions isomers' pairs. The regioisomeric selectivity was improved by using Li as metal ion. Thus, the present study based on the kinetic method provided a significantly different approach compared to the current mass spectrometry methods for the analysis of TAG isomers. The spectra were simple and therefore easy to interpret. Although the information content was low, the fragment ion abundances were dependant on the regioisomeric interactions that were very sensitive to regiosomers forms. The next step of this work will consist of transferring this method onto UHPLC – quadrupole ion trap mass spectrometer to decrease the running time and consequently to gain time.

References