

# The Bottom-Up Solution to the Triacylglycerol Lipidome Using Atmospheric Pressure Chemical Ionization Mass Spectrometry

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**ABSTRACT:** Presented here is an approach to representing the data from atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) of triacylglycerols (TAG) using a set of one, two, or three Critical Ratios. These Critical Ratios may be used directly to provide structural information concerning the regioisomeric composition of the triacylglycerols (TAG), and about the degree of unsaturation in the TAG. An AAA-type, or Type I, TAG has only one Critical Ratio, the ratio of the protonated molecule,  $[M + H]^+$ , to the DAG fragment ion,  $[AA]^+$ . The Critical Ratio for a Type I TAG is  $[MH]^+/\Sigma[DAG]^+$ , and the mass spectrum of a Type I TAG can be reproduced from only this one ratio. An ABA/AAB/BAA, or Type II, TAG has two Critical Ratios, the  $[MH]^+/\Sigma[DAG]^+$  ratio and the  $[AA]^+/[AB]^+$  ratio. The  $[AA]^+/[AB]^+$  ratio for a single TAG or TAG mixture can be compared with the  $[AA]^+/[AB]^+$  ratios of pure regioisomeric standards, and the percentage of each regioisomer can be estimated. The abundance of the protonated molecule and the abundances of the two  $[DAG]^+$  fragment ions can be calculated from the two Critical Ratios for a Type II TAG. To calculate the abundances, the Critical Ratios are processed through the Bottom-Up Solution to the TAG lipidome. First, Critical Limits are calculated from the Critical Ratios, and then the Critical Ratios are classified into Cases by comparison with the Critical Limits. Once the Case classification is known, the equation for the abundance of each ion in the mass spectrum is given by the Bottom-Up Solution. A Type III TAG has three different FA and three Critical Ratios. The  $[MH]^+/\Sigma[DAG]^+$  ratio is the first Critical Ratio, the  $[AC]^+/( [AB]^+ + [BC]^+ )$  ratio is the second Critical Ratio, and the  $[BC]^+/[AB]^+$  ratio is the third Critical Ratio. The second critical ratio for a Type III TAG can be compared with regioisomeric standards to provide an estimate of the percentage composition of the regioisomers. The three Critical Ratios for a Type III TAG can be processed through the Bottom-Up Solution to calculate the four ion abundances that make up the APCI-MS mass spectrum. The Critical Ratios constitute a reduced data set that provides more information in fewer values than the raw abundances.

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Work over the last decade has proved that HPLC combined with atmospheric pressure chemical ionization (APCI) MS is

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Abbreviations: APCI, atmospheric pressure chemical ionization; BUS, Bottom-Up Solution; CID, collision-induced dissociation; EIC, extracted ion chromatogram; ESI, electrospray ionization; ITMS, ion-trap MS; L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; RP, reversed-phase; S, stearic acid; SFC, supercritical fluid chromatography; TIC, total ion current chromatogram; TSQ, triple-stage quadrupole.

an instrumental technique capable of accomplishing qualitative and quantitative analyses of complex mixtures of many classes of lipids. HPLC/APCI-MS is useful for qualitative and/or quantitative analysis of FA (1–4), TAG (5–11), phospholipids (12–16), ceramides (17), carotenoids (18–22), steroids (23–25), and others. Reviews of the applications of APCI-MS for lipid analysis have been published in recent years (26–29).

An important aspect of TAG analysis is the determination of the positional placement of the FA on the glycerol backbone (30). Plants synthesize lipids with structural specificity, namely, saturated FA are most often preferentially located on the *sn*-1 and *sn*-3 positions of the glycerol backbone, and PUFA are preferentially found in the *sn*-2 position. TAG are metabolized by enzymes in the human digestive system with structural specificity, with FA in the *sn*-1 and *sn*-3 positions being removed from the glycerol backbone first. Furthermore, enzymatic synthesis of TAG can be used to produce structured TAG with particular FA located in regiospecific locations (31). Therefore, knowledge of the composition of molecular species in a mixture of TAG, and of the regioisomeric configurations of selected TAG for which standards are available, could provide valuable information to be considered in the planning of dietary, nutritional, metabolic, and related studies. It has been demonstrated by a growing literature precedent that APCI-MS, preceded by a variety of HPLC or supercritical fluid chromatography (SFC) techniques, or simply by direct infusion, provides much of the information sought by those engaged in dietary studies of natural and/or synthetic TAG.

We have been interested in the qualitative and quantitative analysis of TAG. In 1995, we reported the first applications of reversed-phase (RP)-HPLC/APCI-MS to a mixture of synthetic TAG (6). In that initial work, we described basic characteristics of APCI-MS mass spectra of TAG and showed that APCI-MS mass spectra of TAG molecules exhibited primarily two types of ions. One is the protonated molecule and the other is DAG-like ions. We noted that the amount of the protonated molecule depends on the number of sites of unsaturation in the molecule. TAG with more sites of unsaturation form larger protonated molecule abundances (and small  $[DAG]^+$  abundances), whereas those TAG with few sites of unsaturation form only small abundances of the protonated molecule (and large abundances of  $[DAG]^+$ ). In summary, the abundance of the protonated molecule in a mass spectrum obtained by APCI-MS is proportional to (increases with) the amount of unsaturation in a TAG molecule and is inversely proportional to the abundances