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Dual parallel electrospray ionization and atmospheric pressure chemical ionization mass spectrometry (MS), MS/MS and MS/MS/MS for the analysis of triacylglycerols and triacylglycerol oxidation products

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Two mass spectrometers, in parallel, were employed simultaneously for analysis of triacylglycerols in canola oil, for analysis of triolein oxidation products, and for analysis of triacylglycerol positional isomers separated using reversed-phase high-performance liquid chromatography. A triple quadrupole mass spectrometer was interfaced via an atmospheric pressure chemical ionization (APCI) interface to two reversed-phase liquid chromatographic columns in series. An ion trap mass spectrometer was coupled to the same two columns using an electrospray ionization (ESI) interface, with ammonium formate added as electrolyte. Electrospray ionization mass spectrometry (ESI-MS) under these conditions produced abundant ammonium adduct ions from triacylglycerols, which were then fragmented to produce MS/MS spectra and then fragmented further to produce MS/MS/MS spectra. ESI-MS/MS of the ammoniated adduct ions gave product ion mass spectra which were similar to mass spectra obtained by APCI-MS. ESI-MS/MS produced diacylglycerol fragment ions, and additional fragmentation (MS/MS/MS) produced $[RCO]^+$ (acylium) ions, $[RCOO + 58]^+$ ions, and other related ions which allowed assignment of individual acyl chain identities. APCI-MS of triacylglycerol oxidation products produced spectra like those reported previously using APCI-MS. APCI-MS/MS produced ions related to individual fatty acid chains. ESI-MS of triacylglycerol oxidation products produced abundant ammonium adduct ions, even for those molecules which previously produced little or no intact molecular ions under APCI-MS conditions. Fragmentation (MS/MS) of the $[M + NH_4]^+$ ions produced results similar to those obtained by APCI-MS. Further fragmentation (MS/MS/MS) of the diacylglycerol fragments of oxidation products provided information on the oxidized individual fatty acyl chains. ESI-MS and APCI-MS were found to be complementary techniques, which together contributed to a better understanding of the identities of the products formed by oxidation of triacylglycerols.

Since the first report of atmospheric pressure chemical ionization mass spectrometry (APCI-MS) as a detector for the reversed-phase high-performance liquid chromatographic (RP-HPLC) separation of triacylglycerols (TAGs),[1] APCI-MS has become increasingly popular for analysis of TAGs. Two reasons for its popularity are that (i) it is one of the few ionization techniques that is easily interfaced to existing high performance liquid chromatography (HPLC) separation methodologies, and (ii) it is capable of producing structurally definitive ions from this class of large, neutral molecules. APCI-MS of normal triacylglycerols produces mostly protonated molecules and diacylglycerol (DAG) fragment ions, with the proportions of these being dependent on the degree of unsaturation within the TAG. This dependence of fragment ratios on unsaturation had distinct implications for the quantitation of TAGs.[2,3] Furthermore, the abundance ratios of the diacylglycerol fragments have been shown to depend on the positional placement of the fatty acyl chains on the glycerol backbone, and have been demonstrated to be useful for qualitative identification of positional isomers.[4–6] APCI-MS has also been shown to produce protonated molecules, diacylglycerol fragment ions, and diagnostically important fragment ions from TAG oxidation products (TAGOX).[7–9] Different TAGOX functional groups give different fragmentation patterns, and many of these have been characterized thus far. Applications of APCI-MS to normal TAG and TAGOX have been recently reviewed.[10]

The increasing number of reports in which authors have utilized APCI-MS for triacylglycerol analysis demonstrates that this technique has been found to be a useful and valuable tool for analysis of these large, neutral molecules.

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