REVIEW

Atmospheric Pressure Chemical Ionization Mass Spectrometry for Analysis of Lipids

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ABSTRACT: Atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) has proven to be a very valuable technique for analysis of lipids from a variety of classes. This instrumental method readily produces useful ions with gentle fragmentation from large neutral molecules such as triacylglycerols and carotenoids, which are often difficult to analyze using other techniques. Molecules that are easily ionized, such as phospholipids, produce molecular ions and diagnostically useful fragment ions that are complementary to those produced by methods such as electrospray ionization MS with collision-induced dissociation. The simplicity and versatility of APCI-MS make it an ideal tool for use in solving hitherto very difficult analytical problems.

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Mass spectrometry (MS) analysis of liquid chromatographic effluent has long been a goal of chemists studying nonvolatile, often large molecules, which are not separated well on a gas chromatograph, or at all. Many approaches have been developed to meet this objective, with particle beam, thermospray (TSP), continuous-flow fast atom bombardment (or the similar liquid secondary-ion MS, LSIMS), electrospray ionization (ESI), and atmospheric pressure chemical ionization (APCI) interfaces becoming most widely used (1). The recent book by Niessen (1) contains an excellent summary of the development and history of each, some of which is reflected here. Each has achieved varying degrees of success in allowing analysis of large molecules, and each has its own set of inherent drawbacks as well as benefits. Environmental and pharmaceutical samples have been the primary subjects of analysis, but lipids have also been analyzed extensively over the years by using each of these technologies as they were developed. TSP was the most popular commercially available liquid chromatography (LC)/MS interface for a period because of its relative simplicity, low background noise, and high flow rates allowed. But in recent years, TSP has been supplanted in popularity by two atmospheric pressure ionization (API) methods, ESI-MS and APCI-MS (1,2).

The first API source was developed in the 1970s by Horning *et al.* (3–6) at the Baylor College of Medicine (Houston, TX). The method described an ionization interface at atmospheric pressure, external to the high vacuum chamber of a modified Finnigan Model 1015 quadrupole mass spectrometer. Initially, a ^{63}Ni foil was used as a source of electrons to perform ionization (3–5), but later a corona discharge electrode was used (5,6). This version with the corona discharge electrode became the model for modern commercially available APCI interfaces. Initially simply called API, this ionization method later become known as APCI to differentiate it from the other ionization source at atmospheric pressure, ESI. The full potential of APCI was not initially realized, and in the meantime the other emerging API source, ESI, was developed by Fenn and coworkers at Yale (New Haven, CT) (7). As ESI became recognized for its ability to identify the masses of large proteins (8), interest in API techniques increased. Improvements in commercial ESI sources, such as those made by Bruins *et al.* (9) to yield pneumatically assisted electrospray (called ionspray), allowed larger effluent flow rates than could previously be used. These improvements in ESI caused API methods to gain more widespread use. By the late 1980s and early 1990s, all major MS instrument manufacturers had introduced API sources, with most of them having both ESI and APCI interfaces. Although use of APCI is not yet as widespread as ESI, the number of reported applications of APCI-MS is burgeoning. A review of the applications of APCI-MS to analysis of lipids has appeared elsewhere (10). The number of recent applications reported in the literature warrants an updated chronicle of APCI-MS for lipid analysis.

APCI is a soft ionization technique, but unlike ESI, APCI usually does produce some degree of fragmentation that is useful for structural characterization. Very simple in its design, a typical APCI source has the following components, as shown in Figure 1: (i) a capillary out of which the LC effluent is sprayed through a nozzle by means of a concentric nebulizer gas surrounding the capillary, (ii) a heated vaporizer tube, concentric around the LC capillary outlet, which desolvates the analyte molecules, (iii) a corona discharge needle, which pro-

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Abbreviations: ACN, acyl carbon number; AMVN, azobis(2,4-dimethylvaleronitrile); APCI, atmospheric pressure chemical ionization; API, atmospheric pressure ionization; Br-MB, 3-bromomethyl-7-methoxy-1,4-benzoxazin-2-one; CID, collision-induced dissociation; cSFC, capillary supercritical fluid chromatography; DAG, diacylglycerol; ECN, equivalent carbon number; EIC, extracted ion chromatogram; ELSD, evaporative light-scattering detector; ESI, electrospray ionization; FA, fatty acid; FAME, fatty acid methyl ester; FI, flow injection; FID, flame-ionization detection; GC, gas chromatography; L, linoleic acid; LC, liquid chromatography; Ln, linolenic acid; LSIMS, liquid secondary ion mass spectrometry; MAG, monoacylglycerol; MS, mass spectrometry; MS/MS, tandem mass spectrometry; O, oleic acid; P, palmitic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; RP-HPLC, reversed-phase high-performance liquid chromatography; S, stearic acid; SFC, supercritical fluid chromatography; SIM, selected ion monitoring; TAG, triacylglycerol; THCA, 3α-,7α,12α-trihydroxy-5β-cholestanic acid; TSP, thermospray; UV, ultraviolet; vis, visible.