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Atmospheric Pressure Photoionization (APPI) for LC–MS: Analysis of Lipids

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In this Application Notebook we report on the enhanced capability for monitoring lipids by LC–MS using APPI versus APCI and ESI.

Atmospheric Pressure Photoionization (APPI) has been rapidly adopted by liquid chromatography–mass spectrometry (LC–MS) users since its commercial introduction in year 2000 (1–3). Its popularity is due to the success in ionizing compounds not readily ionized by ESI and APCI, such as nonpolars, weak acids, and halogenated organic compounds to mention a few. APPI also leads to cleaner spectra and is less susceptible to ion suppression.

The rapidly growing field of lipidomics, a branch of metabolomics, seeks to understand how lipids interact with genes and proteins to regulate cellular functions. Lipidomics involves the study of nonwater-soluble metabolites, which can present challenges for analysis by electrospray ionization (ESI). Normal phase LC–MS is often the better choice for nonpolar lipids and other compounds.

In this work we compare the quantitative accuracy and sensitivity of analyzing lipids by APPI+, APCI±, and ESI±. These results demonstrate the benefits of using APPI for normal-phase LC–MS for the analysis of lipids compounds.

Experimental Conditions

This work was conducted on a Waters ZQ LC–MS using four selected lipid compounds: EPA methyl ester (fatty acid group), monoarachidin and diarachidin (saturated glyceride) and trielaidin (unsaturated glyceride). APPI and APCI analyses used normal phase solvents such as pure hexane or 1:1 Isooctane:IPA. The mobile phase for ESI was 1:1 Isooctane:IPA with and without 10 mM ammonium formate, and 10:15:1 Isooctane:IPA:H_2O with 15.4 mM sodium acetate.

Results

Figure 1 shows the full scan MS spectra for EPA methyl ester by APPI+, APCI+, and by ESI+ with and without modifier. The APPI+ and APCI+ MS spectra give similar peaks with $[M+H]^+$ being the major ion detected for EPA methyl ester and $[M+H-H_2O]^+$ also giving strong ion signal for the other lipids. The ESI MS spectra give major ions of $[M+Na]^+$ or $[M+NH_4]^+$ using sodium acetate or ammonium formate additive in mobile phase.

Figures 2 and 3 show linearity plots for APPI+ and ESI+, respectively. The APPI curve shows greater than five decades of linear dynamic range. The ESI+ signals were less stable and gave a greatly reduced linear range compared to APPI+. A similar non-linear response was observed for ESI−. The linearity of APCI± is similar to that of APPI+; however, APPI is about of 2–4 × more intense than APCI with regard to area count and S/N, which translates into better detection limits.

Conclusions

APPI+ performed better than APCI± and ESI± for the analysis of lipids by generally offering higher signal intensity, S/N, linear dynamic range, and better detection limits. Furthermore, the APPI+ sensitivity was relatively constant over the flow rate range of 100 to 600 mL/min that was tested. Finally, improved detectability was observed by APPI relative to APCI and ESI for nonpolar lipids in free fatty acid and in methyl ester forms, as well as for triglycerides in fish oil samples.

References

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(3) K. A. Hanold, S. M. Fischer, P. H. Cormia, C. E. Miller, and J. A. Syage, *Anal. Chem.* **76**, 2842–2851 (2004).

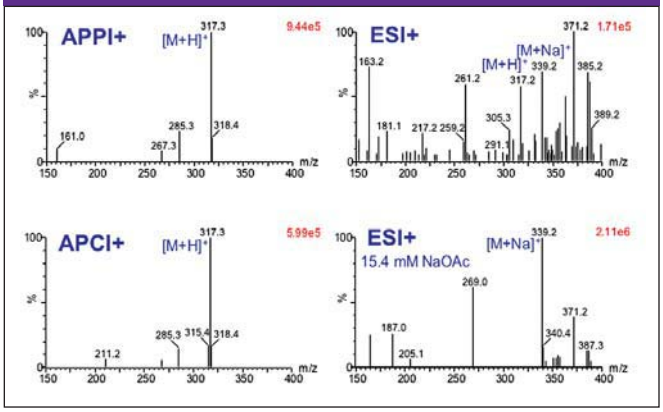

Figure 1: Full scan MS spectra of EPA methyl ester (MW = 316) by APPI, APCI, and ESI positive ion mode.

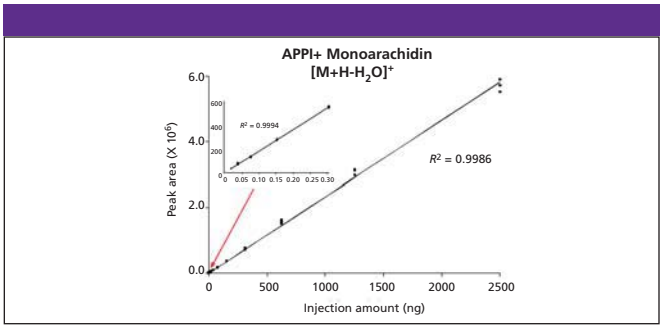

Figure 2: APPI+ linearity plot for monoarachidin with 1:1 isooctane/IPA mobile phase.

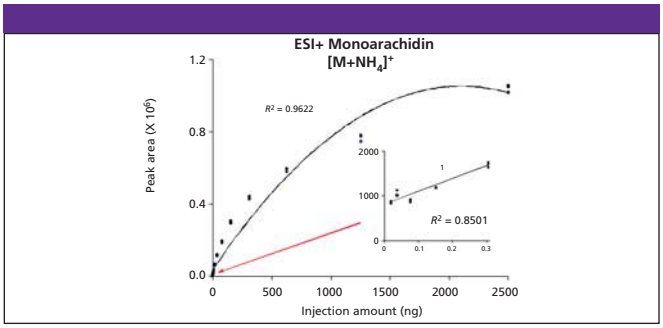

Figure 3: ESI+ linearity plot for monoarachidin with 10 mM ammonium formate in mobile phase.

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