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Why LC/MS/MS and GC/MS/MS Are Required for the Analysis of Certain Pesticides

Author

Anthony Macherone
Agilent Technologies, Inc.
& John Hopkins University
School of Medicine

Introduction

Residual pesticide analysis in cannabis matrices is arguably the most challenging of all the mandated regulatory assays due to the complexity of the matrix and the very low action limits. Further complicating the issue is the inclusion of pesticides not amenable to electrospray ionization (ESI), the most common LC/MS/MS ionization source for this analysis. For example, California and Canada have included pentachloronitrobenzene (PCNB) in their target list. PCNB is a known bad actor when analyzed by LC/MS/MS using ESI. To overcome this issue, the relevant literature space has proposed using negative atmospheric pressure chemical ionization liquid chromatography/tandem mass spectrometry (Ni-APCI LC/MS/MS). The analysis of PCNB using Ni-APCI LC/MS/MS has been shown with a precursor ion of m/z 275.5 and a mechanism of ionization reported as the loss of HCl followed by formation of an ammonium ion adduct.[1] There are two intrinsic problems with this description: 1) the empirical formula for PCNB is $C_6Cl_5NO_2$, thus there is no hydrogen atom to lose as H-Cl, and 2) the formation of a positively charged ammonium ion in negative ionization mode is highly unlikely. This Application Brief demonstrates the correct ionization mechanism for PCNB using Ni-APCI LC time-of-flight mass spectrometry (Ni-APCI LC/Q-TOF), and evaluates the appropriateness of this analytical technique for this analyte.

Experimental

An Agilent 1290 Infinity II LC combined with an Agilent 6545 LC/Q-TOF system was used. The system was configured with an APCI source operated in negative ionization mode, and a phenyl-hexyl column. The mobile phases were 2 mM ammonium formate + 0.1% (v/v) formic acid in LC/MS-grade water on the A channel, and 2 mM ammonium formate + 0.1% (v/v) formic acid in LC/MS-grade methanol on the B channel. For each experiment, 1 ppm PCNB in solvent was injected, and both high-resolution accurate mass (HRAM) MS and MS/MS data were collected.

Results and discussion

Chlorinated nitrobenzene compounds such as PCNB form phenoxide ions under negative atmospheric pressure ionization conditions.[2] Using LC/Q-TOF, we have determined that this mechanism is true, and that the current literature[1] is mistaken. Figure 1 illustrates the HRAM spectrum of PCNB in Ni-APCI LC/Q-TOF mode. It is shown that no pseudomolecular ion of PCNB is formed, and the primary chemical species is in fact tetrachloronitrobenzyl phenoxide (TCNP), with a minor species (3.5:1 ratio) formed from a secondary pathway of TCNP. Neither of these chemical species are selective for PCNB, and any collision-induced dissociation (CID) product ion derived from these precursor ions is not selective for PCNB.

The above data demonstrate that using Ni-APCI LC/MS/MS for the analysis of non-ESI amenable compounds such as PCNB results in nonselective results not related to the parent compounds. In addition, the current literature[1] presents a four-point quadratic calibration curve with a regression fit (R^2) of 0.9755. The California Bureau of Cannabis Control requires a six-point calibration for quadratic curves, and regression fit of 0.99 for all analytes.
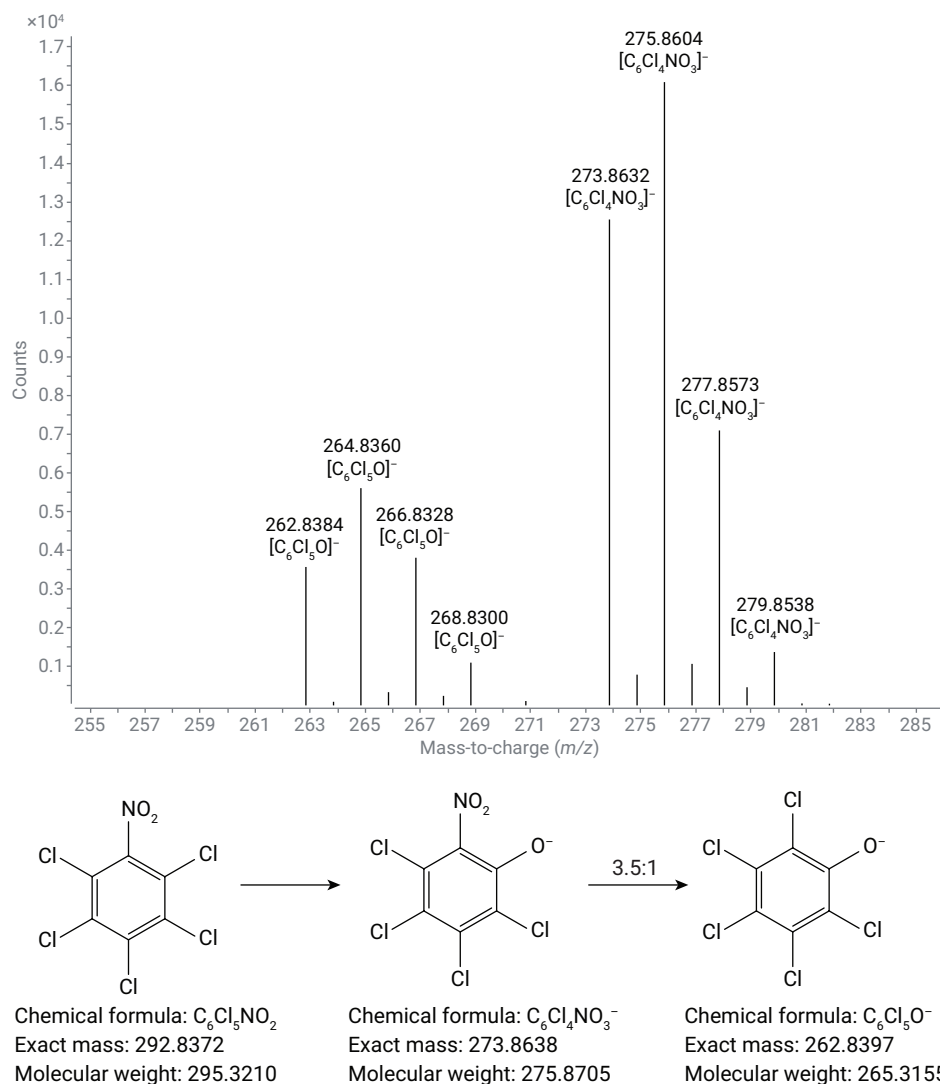

Figure 1. Accurate-mass spectrum and reaction scheme for PCNB in Ni-APCI LC/Q-TOF analysis.

Conclusion

The analysis of PCNB and several other polychlorinated pesticides using Ni-APCI LC/MS/MS results in nonselective precursor/product ion pairs in tandem mass spectrometry. Furthermore, the nonlinear response and poor coefficients of determination (R_2) shown for these compounds in Ni-APCI LC/MS/MS disqualifies this analytical methodology for these analyses in certain U.S. states such as California and Canada.

Acknowledgements

References

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2. Dzidic, I. *et al.* Atmospheric Pressure Ionization (API) Mass Spectrometry: Formation of Phenoxide Ions from Chlorinated Aromatic Compounds. *Anal. Chem.* **1975**, *47(8)*, 1308–1312.

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