應用手冊

LC-MS Analysis of Pyrene Glucuronide, An Environmental Pollutant

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Abstract

This application note demonstrates LC-MS analysis of pyrene glucuronide, an environmental pollutant.

Introduction

Pyrene is one of many polyaromatic hydrocarbon compounds (PAH) that is generated as a byproduct of industrial processes. It then contaminates the environment, including bodies of water. The fish and other marine species ingest the pyrene which is metabolized to the glucuronide and concentrated in the fish bile. It can be used as an index of contamination of the water and the food chain.

To be useful, low levels (5 ng/µL) must be detected and the compound identified by mass spectrometry. Using positive and negative switching, electrospray and atmospheric pressure chemical ionization, and photodiode array detection a lot of information can be gained from a small amount of sample (100 µL) for identification of pyrene glucuronide.

Experimental

Experimental Conditions

LC-MS:	Waters Alliance System: 2690 Separations Module, 996 Photodiode Array Detector, Platform LC Mass Detector
Compound:	Pyrene glucuronide extracted from fish bile. Matrix was removed by solid phase extraction.
Column:	Symmetry C_{18}, 2.1 x 150 mm at 60 °C
Mobile phases	Linear gradient from 100% water to 99% acetonitrile both containing 0.1% formic acid in 25 minutes
Flow rate:	0.15 mL/min
PDA detection:	200–400 nm at 1.2 nm resolution

MS detection:	ESI^+ and ESI^- 150–500 *m/z* or APCI+ and APCI- 150–500 *m/z*
Fragmentation:	CID at cone voltages of 15, 40, and 80V

Results and Discussion

The total wavelength chromatogram from 200–400 nm (top) and the electrospray negative, ESI^- (middle) and electrospray positive ESI^+ (bottom) chromatograms are shown. The data were obtained from a single injection with the PDA and mass spectrometer connected in series and the rapid switching between positive and negative ionization modes. The ESI^- response is much greater for analytes that were retained on the column.

The electrospray negative mass spectra of selected peaks are shown. The peaks in Figure 1 labeled A to E are shown. The base peak at 20.4 minutes has a *m/z* 393.11 which is $[M-H]^-$ for pyrene glucuronide (spectrum D).

Mass spectra can also be obtained for the ESI^+ mode (data not shown).

Electrospray Ionization

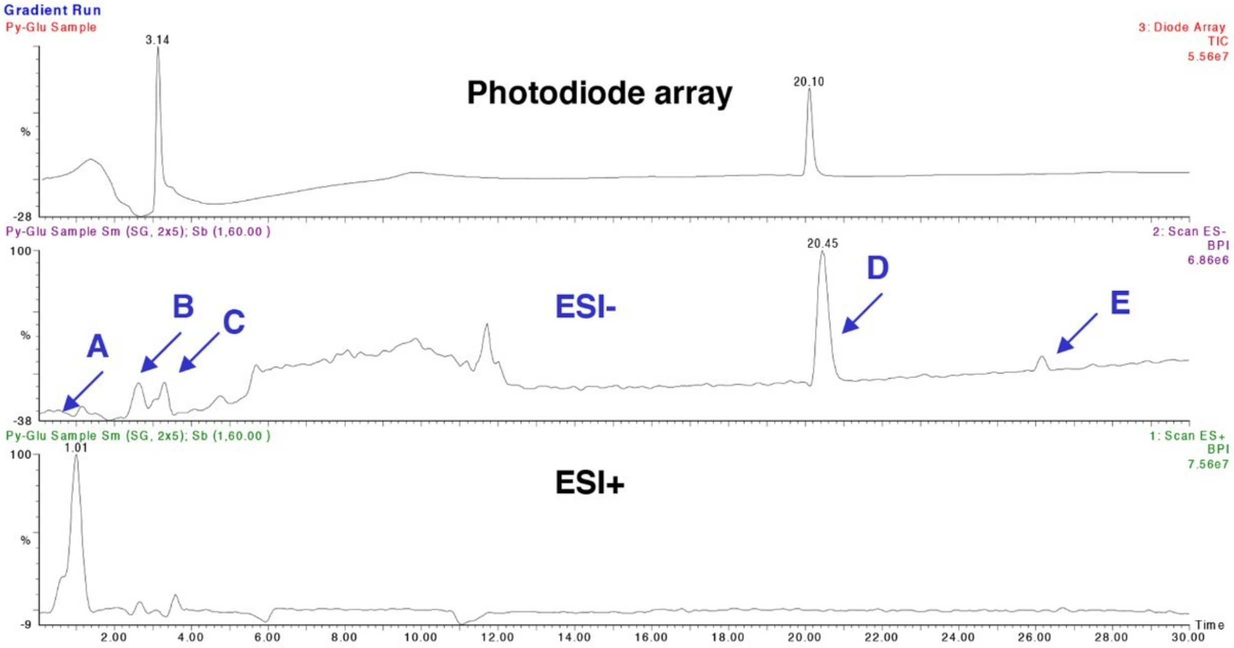

Figure 1. PDA and ESI Chromatograms.

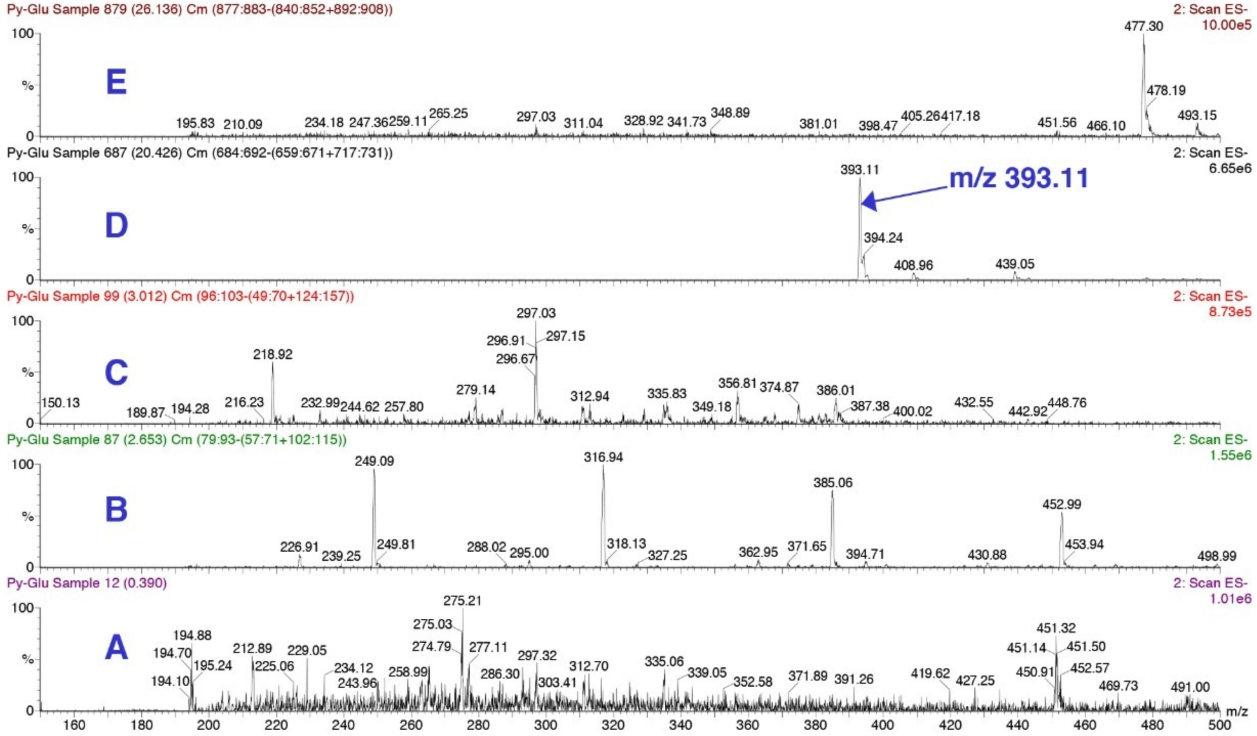

Figure 2. ESI- Mass Spectra.

In-source collision-induced dissociation is a way of fragmenting a molecule in a single quadrupole mass spectrometer. All data can be obtained in a single injection by switching between cone voltages, saving time and sample. At 15V (bottom) the analyte $[M-H]^-$ 393.3, a formic acid adduct, *m/z* 439.3, and a fragment at 193.1 are seen. Increasing the sample cone voltage generates additional ions. The ion at 157.1 *m/z*, was the result of a loss in 18 *m/z* from the 175.1 *m/z* fragment ion.

Two fragmentation structures are proposed that yield some of the common masses to those detected by the mass spectrometer (Figure 3). The molecular ion $[M-H]^-$ and its fragments can be used to confirm the identity of a compound like pyrene glucuronide.

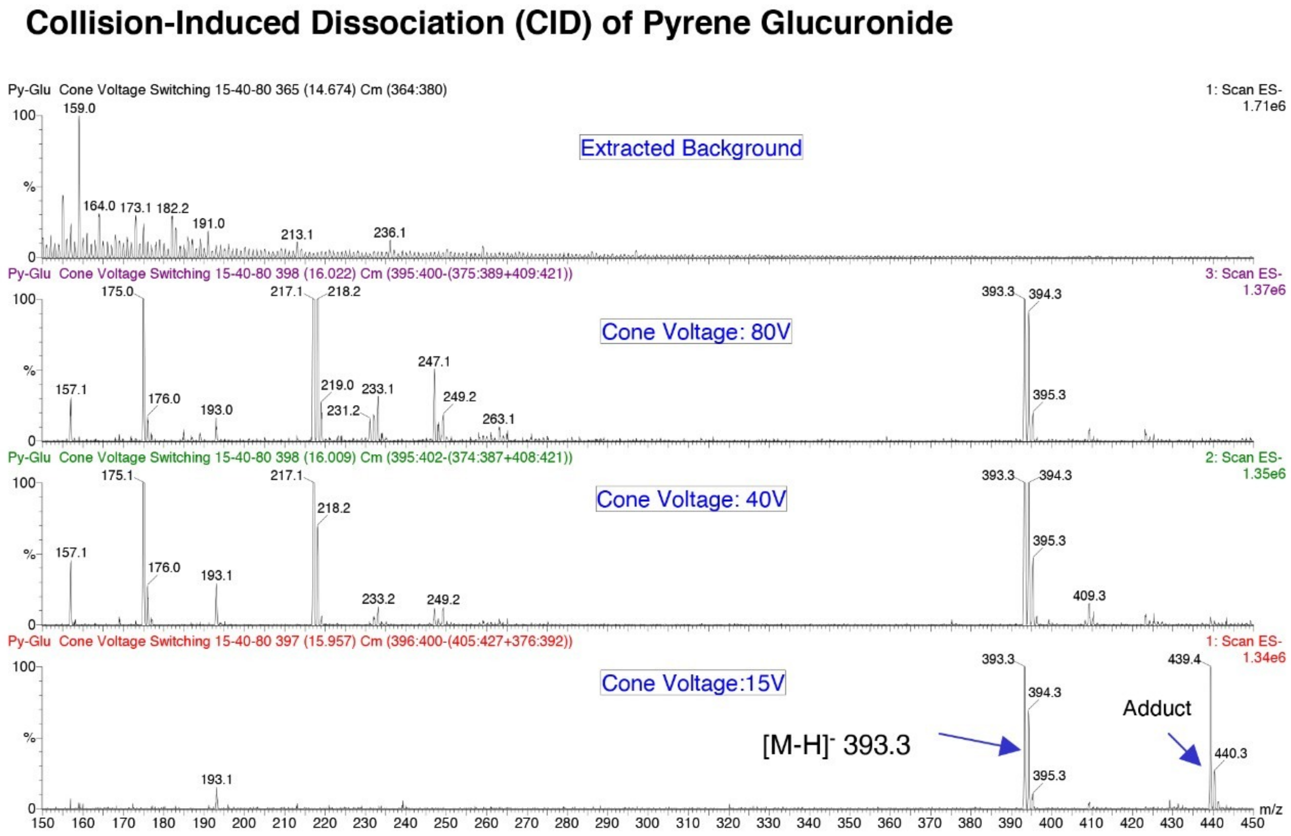

Figure 3. Mass Spectra at Different Cone Voltages.

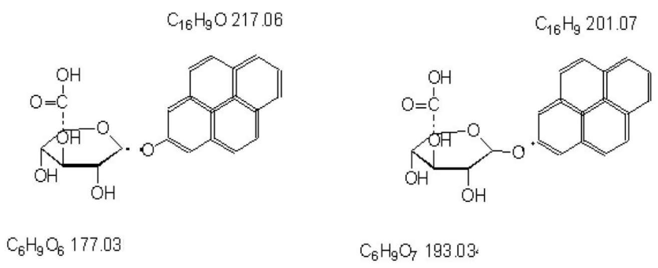

Figure 4. Proposed Fragmentation.

The total wavelength chromatogram (upper) and the APCI- (middle) and APCI+ (lower) chromatograms are shown. APCI mode detects different compounds than ESI, e.g. from 5 to 10 minutes in APCI+ (compare to Figure 1). There is a large peak at 16.37 minutes in the total ion chromatograms (TIC).

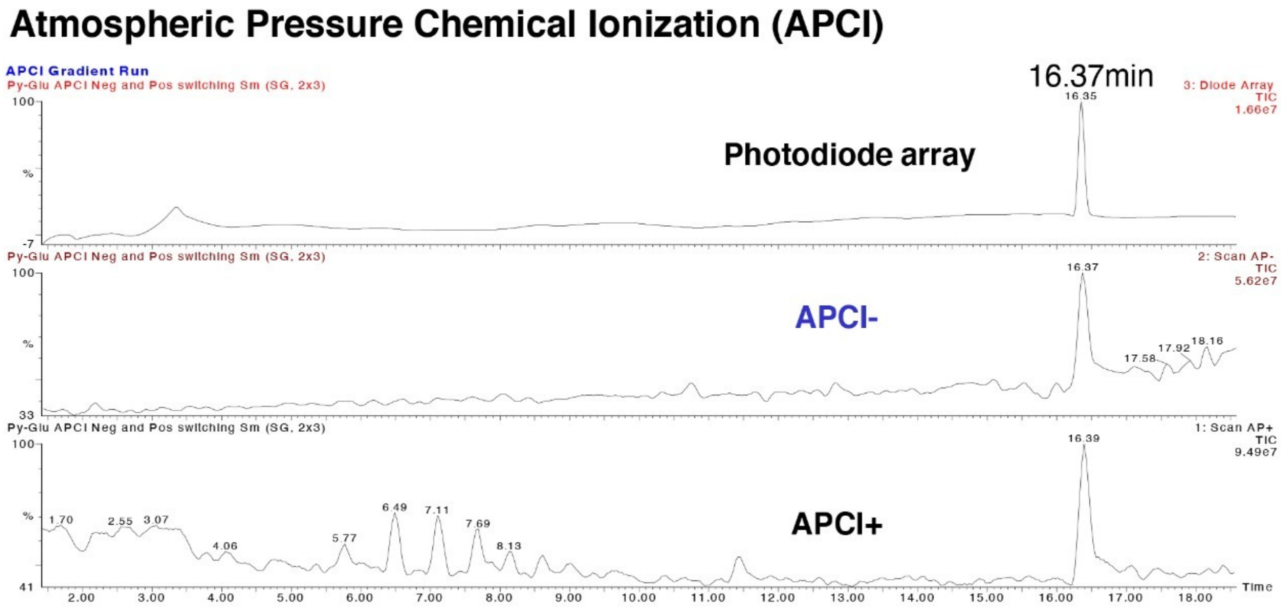

Figure 5. PDA and APCI Chromatograms.

In APCI- mode the large peak at 16.37 minutes corresponds with *m/z* 393.11, the $[MH]^-$ (upper). At 16.37 minutes in APCI+ mode there is no molecular ion middle). The lower *m/z* ions are different than the fragments seen in ESI- due to a different method of ionization.

The photodiode array spectrum (bottom) is a distinctive UV spectrum with four lambda maxima at 242, 266, 277, and 344 nm that can be used to help identify pyrene glucuronide.

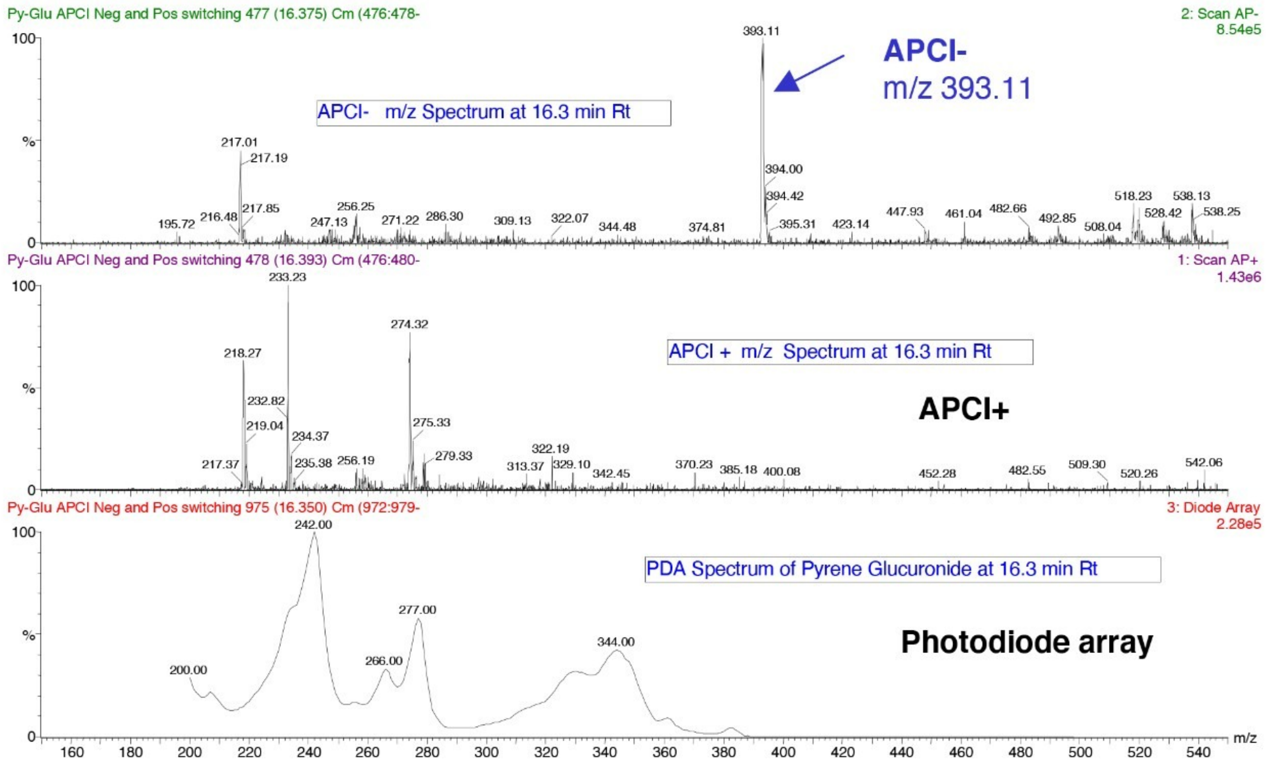

Figure 6. APCI and PDA Spectra.

Conclusion

The Waters LC-MS system can obtain UV/visible spectra, ESI or APCI spectra in the positive and negative mass spectra in the same chromatographic run. This is an efficient way of obtaining the maximum amount of information quickly with a limited amount of sample.

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