application

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Multisignal LC/MS Analysis for Compound Screening

Christine Miller

Introduction

Acquiring MS information using different acquisition modes within a single sample analysis is a powerful way to improve productivity in LC/MS compound screening and method development. It speeds analyses and makes it easier to use a single, generic method for LC/MS screening.

The enhanced Agilent 1100 LC/MSD provides the ability to cycle through as many as four different acquisition modes on a scan-by-scan basis within a single sample analysis. Each acquisition mode is user-definable and can be customized for specific needs. This flexibility allows many combinations of acquisition modes including high/low energy in-source collision-induced dissociation (CID), positive/negative polarity switching, and selected ion monitoring (SIM)/scan modes.

This note shows examples of using positive/negative polarity switching to confirm compound identification and to screen mixtures.

Experimental

All experiments were done using an Agilent 1100 Series LC/MSD system that was comprised of a binary pump, vacuum degasser, autosampler, thermostatted column compartment with column-switching valve, diode-array detector, and an enhanced LC/MSD. The LC/MSD was used with either the electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source. Complete system control and data evaluation were done on the Agilent ChemStation for LC/MS.

Reagent grade chemicals and HPLC grade solvents were used in preparing mobile phases and standards.

Tuning the LC/MSD is the process of adjusting parameters for sensitivity, mass resolution, and mass accuracy. A commercially available stable mixture of compounds (p/n G2421A and G2422A) is used for tuning. The autotune process on the LC/MSD automatically delivers the tune mix, optimizes parameters in both positive and negative ionization modes, and then creates a tune file that contains optimized parameters for both ionization modes. Therefore, data can be acquired in positive/negative polarity switching mode with a single tune file.

Results and Discussion

Analysis parameters were developed with conditions that would allow the formation of both negative and positive ions. In the electrospray ionization process, the mobile phase emerging in the electric field is charged and then the charged liquid is sprayed into droplets. Analyte ions in solution migrate to the droplet surfaces and, as the droplets are evaporated, gas phase ions are released. To maximize ion formation, the mobile phase needs to be conductive so that the liquid can be highly charged. In addition, the mobile phase should be at a pH that will promote analyte ion formation. Because the evaporation process will cause changes in the droplet pH that affect ionization,[1] volatility of mobile phase components is also an important consideration. For example, sulfamethizole, which has a pKa of 5.45, gives a better negative mode response with 0.1% acetic acid (pH 4.5) than with 10 mM ammonium acetate (pH 5.5). Both acetic acid and ammonium acetate will make the droplets conductive and promote ionization. However, because acetic acid is more volatile, the pH in the droplets will increase as the acetic acid concentration is reduced, producing a condition that is more favorable for the formation of negative ions of sulfamethizole.

In APCI, ions are generated in the gas phase. Solvent and analytes are vaporized; the solvent is ionized by corona discharge; and the charge is transferred from the solvent to the analyte molecules. Using a protic solvent such as methanol will generally aid in the ionization process.

Under the appropriate LC/MS conditions, some molecules will produce both positive and negative ions. The ESI-LC/MS analysis of a mixture of four sulfonamide antibiotics shows a response in both ionization modes (Figure 1). The mass spectra for sulfamethizole show the protonated molecular ion $[M+H]^+$ at *m/z* 271 and the deprotonated molecular ion $[M-H]^-$ at *m/z* 269 (Figure 2). Having results from both positive and negative ionization in a single analysis provides confirmation of peak molecular weight without increasing analysis time or sample use.

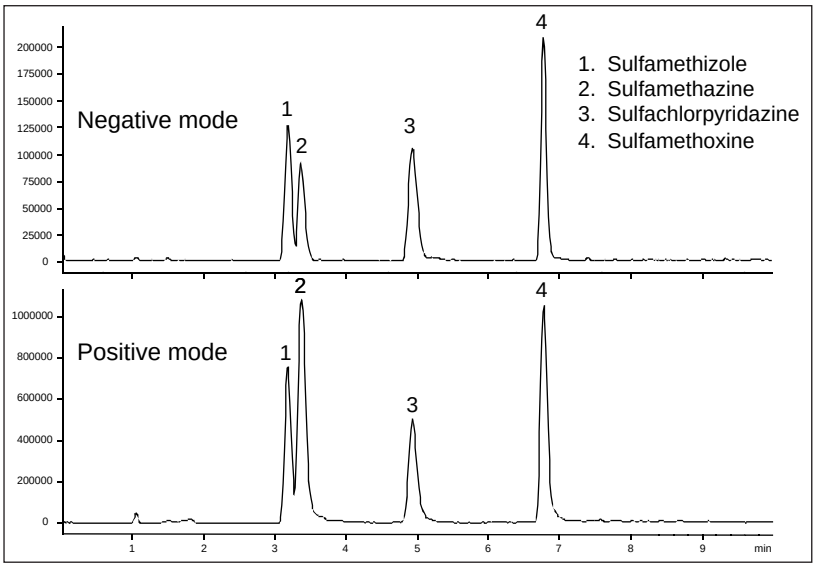

Figure 1. ESI-LC/MS analysis of sulfonamide antibiotics using positive/negative switching mode.

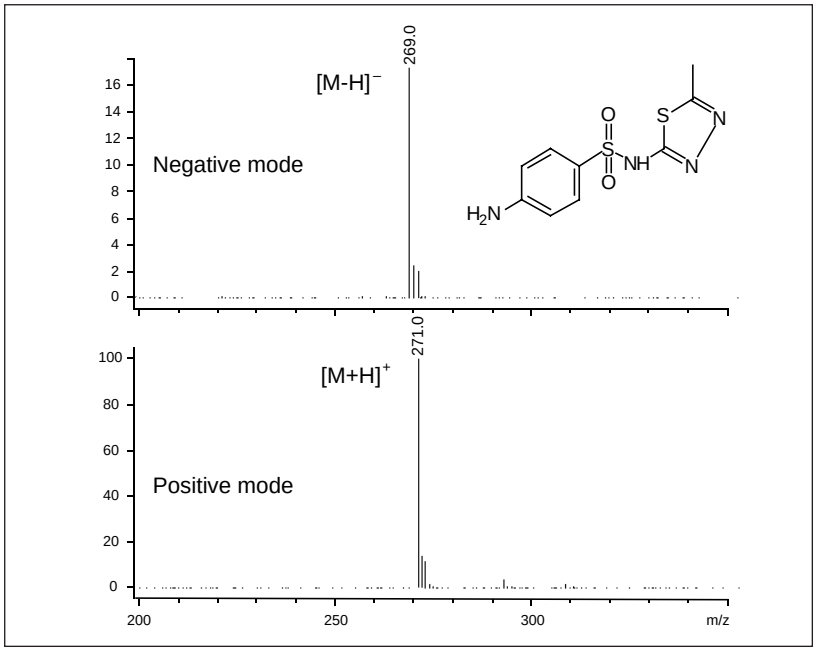

Figure 2. Mass spectra of sulfamethizole from the chromatogram in Figure 1.

ANALYSIS METHOD:

Chromatographic Conditions

Column:	15 × 3 mm Zorbax® SB-C18, 3.5 µm (p/n 863954-302)
Mobile phase:	A = 0.1% acetic acid in water B = 0.1% acetic acid in acetonitrile
Gradient:	start with 20% B at 3 min, 20% B at 5 min, 50% B
Flow rate:	0.6 ml/min
Column temperature:	40°C
Injection volume:	5 µl
Diode-array detector:	signal 270, 10 nm

MS Conditions

Source:	ESI
Drying gas flow:	11 l/min
Nebulizer:	45 psig
Drying gas temperature:	350°C
Vcap:	3000 V (positive); 2250 V (negative)
Stepsize:	0.1
Peakwidth:	0.09 min
Time filter:	On
MS Signal 1:	Ion mode: Negative Scan: 150–400 amu Fragmentor: 50 V
MS Signal 2:	Ion mode: Positive Scan: 150–400 amu Fragmentor: 50 V

Because some molecules may respond in only the positive or negative ionization mode, screening methods that incorporate both modes are very useful. Figure 3 shows the analysis of a mixture of polymer additives by APCI-LC/MS. Some of the polymer additives ionize in both modes as with the sulfa drugs, but butylated hydroxytoluene (BHT) only responds well in negative mode. Moreover, some of the additives that respond in

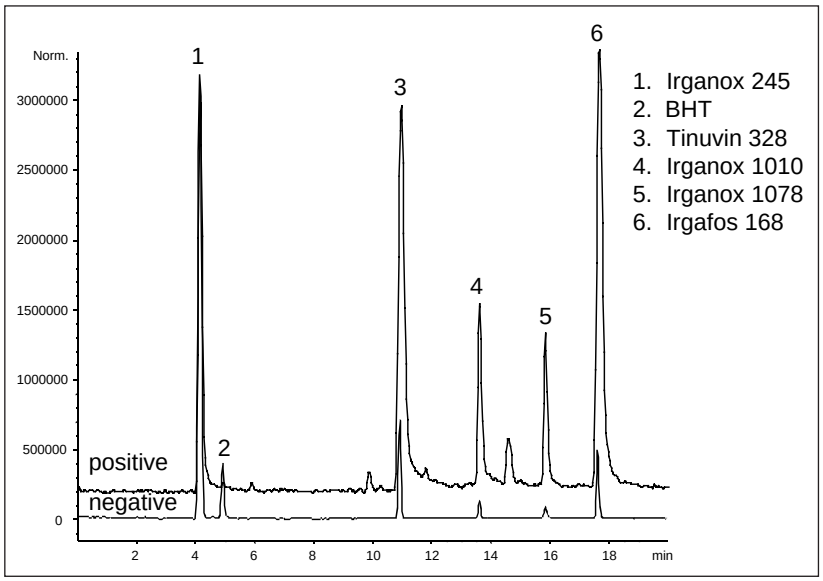

Figure 3. APCI-LC/MS analysis of a mixture of polymer additives.

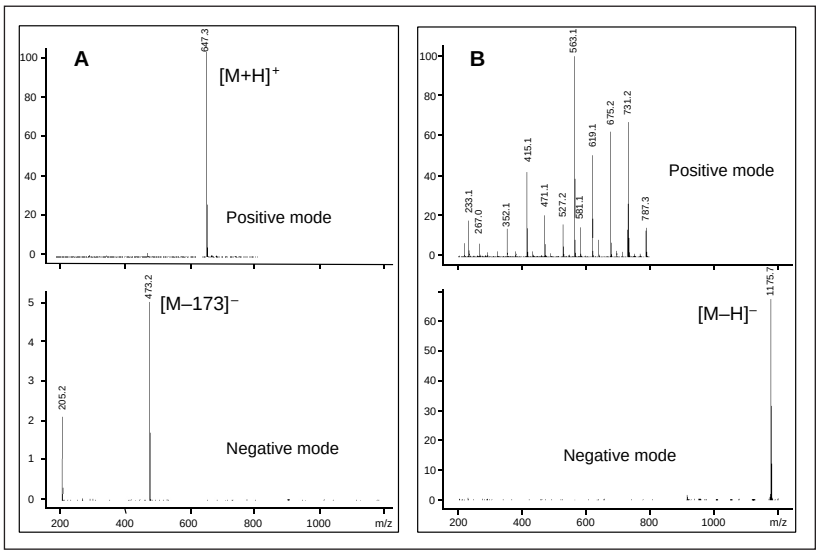

Figure 4. Mass spectra for (A) Irgafos 168 and (B) Irganox 1010 from chromatogram in Figure 3.

ANALYSIS METHOD:

Chromatographic Conditions

Column:	15 × 3 mm Zorbax® SB-C18, 3.5 µm (p/n 863954-302)
Mobile phase:	A = water B= methanol
Gradient:	start with 75% B at 5 min, 90% B at 14 min, 100%B
Flow rate:	0.8 ml/min
Column temperature:	50°C
Injection volume:	5 µl of 400 ppm per component
Diode-array detector:	Signal 220, 10 nm

MS Conditions

Source:	APCI
Drying gas flow:	5 l/min
Nebulizer:	60 psig
Drying gas temperature:	350°C
Vaporizer:	400°C
Vcap:	3000 V (positive); 3000 V (negative)
Corona:	4 µA (positive); 20 µA (negative)
Stepsize:	0.1
Peakwidth:	0.15 min
Time filter:	On
MS Signal 1:	Ion mode: Negative Scan: 200–1200 amu Fragmentor: 150 V
MS Signal 2:	Ion mode: Positive Scan: 200–1200 amu Fragmentor: 80 V

both modes show fragmentation in one of the modes. Irgafos 168 shows just the $[M+H]^+$ ion in positive mode, but in negative mode shows a fragment ion at *m/z* 205 and an ion from rearrangement of the remaining molecule at *m/z* 473 (Figure 4A). Similarly, Irganox 1010 shows just the $[M-H]^-$ ion in negative mode but shows extensive fragmentation in positive mode (Figure 4B).

This ability to acquire data using alternating positive and negative ionization modes maximizes the information from each analysis.

Reference

1. Zhou, S., Edwards, A. G., Cooke, K. D., and Van Berkel, G. J., Analytical Chemistry 1999, 71, 769–776.

Author

Christine Miller is an applications chemist at Agilent Technologies in Palo Alto, California.

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