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Characterization and Confirmation of Ampicillin with LC-MS on a Single Quadrupole Mass Spectrometer

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Abstract

This application note demonstrates characterization and confirmation of ampicillin with LC-MS on a single quadrupole mass spectrometer.

Introduction

We have used LC-MS with a single quadrupole mass spectrometer to characterize and quantitate six antibiotics of the penicillin family. Ampicillin is shown in the Applications Note. “In-source” collision-induced dissociation (CID) was used to produce characteristic fragmentation spectra of each antibiotic. Use of “in-source” CID allowed us to investigate in detail the general mechanisms by which the antibiotics in this study fragment. In addition, confirmation of identity was accomplished by monitoring for parent and characteristic fragment ions in multiple SIR channels at customized cone voltages. Limits of detection as low as 16.5 pg on-column were obtained, with confirmation from the characteristic fragment ions.

Experimental

Analytical Conditions

LC-MS system:	Waters 2690 Separations Module and a Waters/Micromass ZMD Single Quadrupole Mass Spectrometer
Column:	Waters XTerra C_{18}, 2.1 x 150 mm
Gradient:	Water-methanol containing 0.1% acetic acid. Linear gradient was from 5-95% organic for 7 minutes, followed by a 3 minute hold at 95%.

SIR:	To maximize detection of parent or fragment ions 15V or 40V were used. Dwell times were minimized to 0.1–0.2 sec to maximize the number of data point to maintain high sensitivity.
Sample:	Ampicillin, 20 ng/µL, 5 µL injection

Results and Discussion

The full scan electrospray positive mass spectrum for ampicillin at low cone voltage, 17V, is shown. The background spectra have been subtracted. The molecular ion $[M+H]^+$ 350 with its isotopes is observed as the base peak with few fragments.

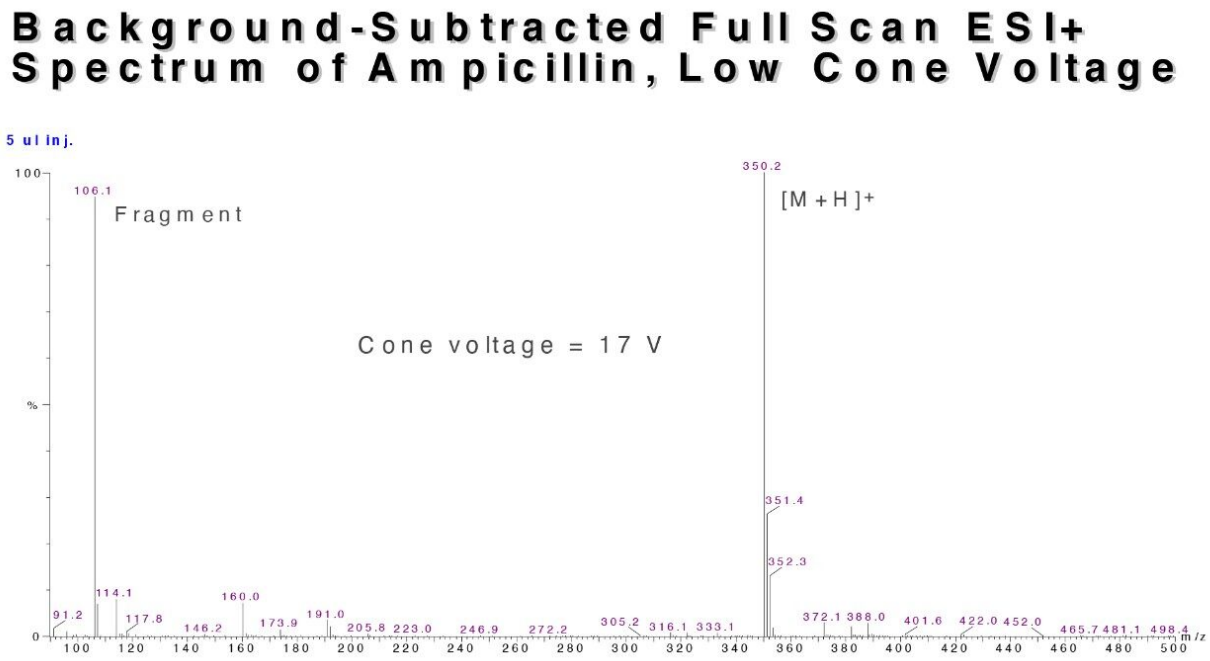

Figure 1. Mass Spectrum at Low Cone Voltage.

The full scan electrospray positive mass spectrum for ampicillin at high cone voltage, 42V is shown. The background spectra have been subtracted. The molecular ion $[M+H]^+$ 350 intensity is lower than in Figure 1. There are a number of fragments formed.

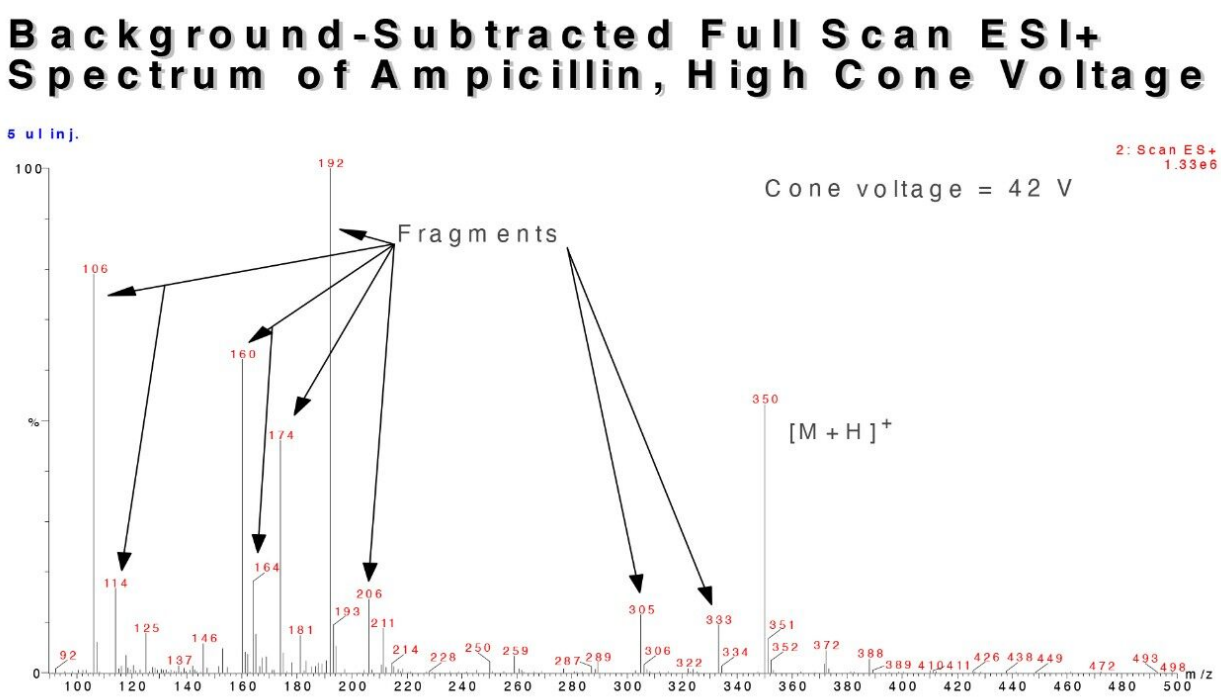

Figure 2. Mass Spectrum at High Cone Voltage.

General fragmentation patterns for these antibiotics can be elucidated from the results. The *m/z* 114 and 160 can be accounted for.

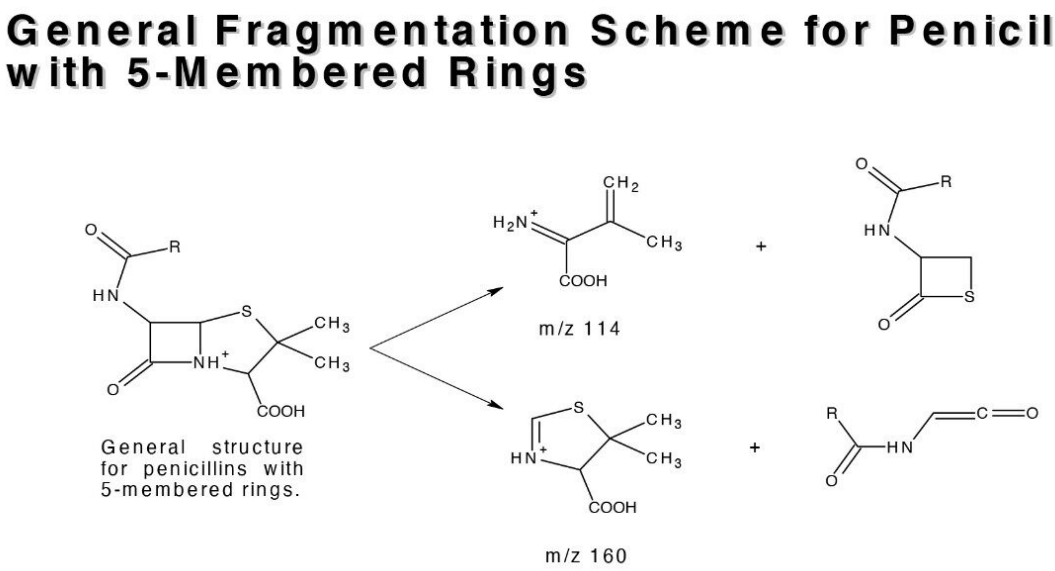

Figure 3. Fragmentation of Penicillins with 5-Member Rings.

From the observed fragmentation we determined the best fragment to monitor in SIR mode. Both the parent ion and the characteric fragment ions with multiple SIR channels at customized cone voltages. Limits of detection as low as 16.5 pg on-column, with confirmation from the characteristic fragment ions, were achieved. The figure shows the results of 33 pg on-column. The first three panels are the single ions at *m/z*, 192, and 350. The bottom panel is the "TIC" combined chromatogram.

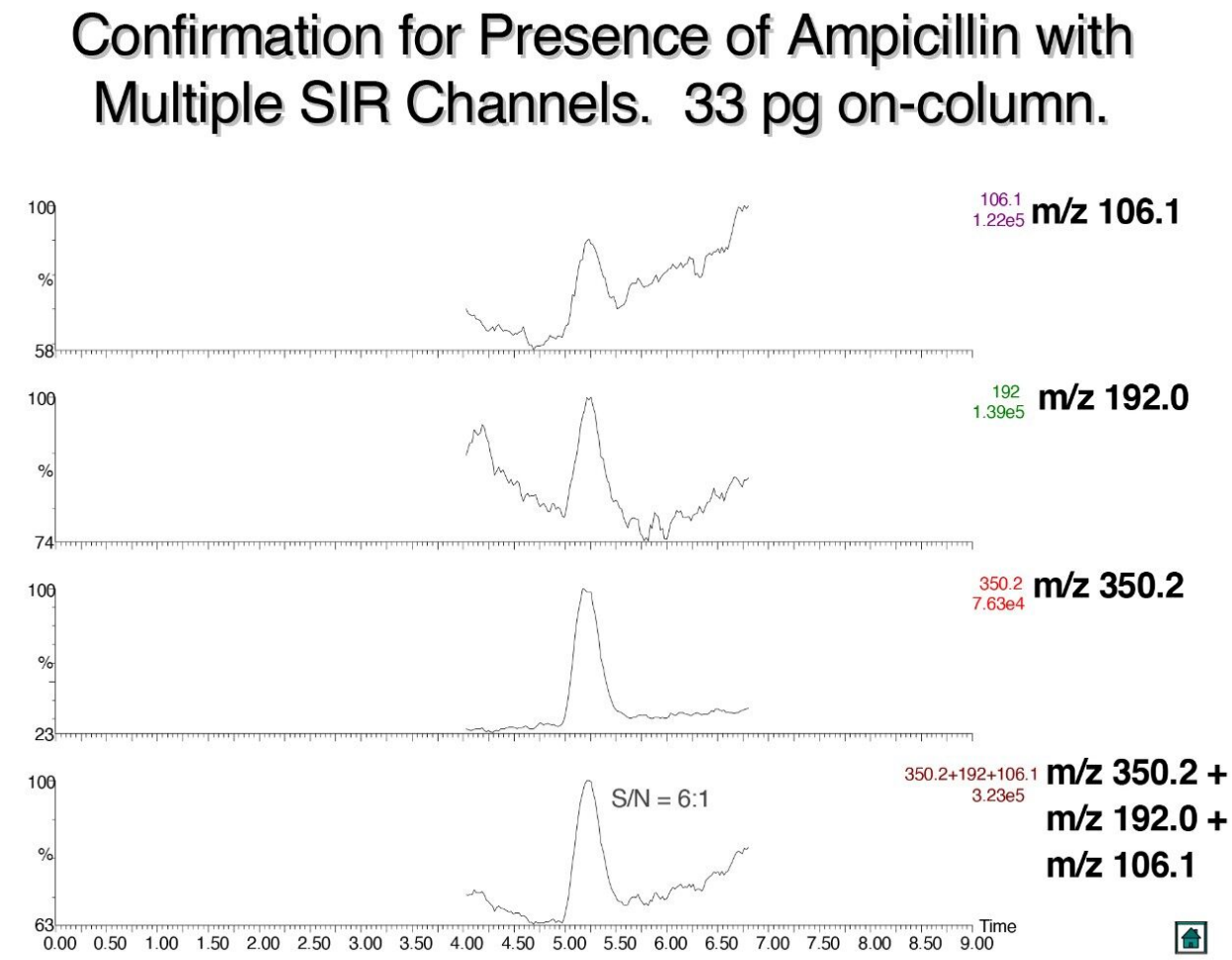

Figure 4. Single Ion Mass Chromatograms (SIR).

The chromatograms are the combined *m/z* of 106.1, 192 and 350.2. The signal-to-noise ratio is 6:1.

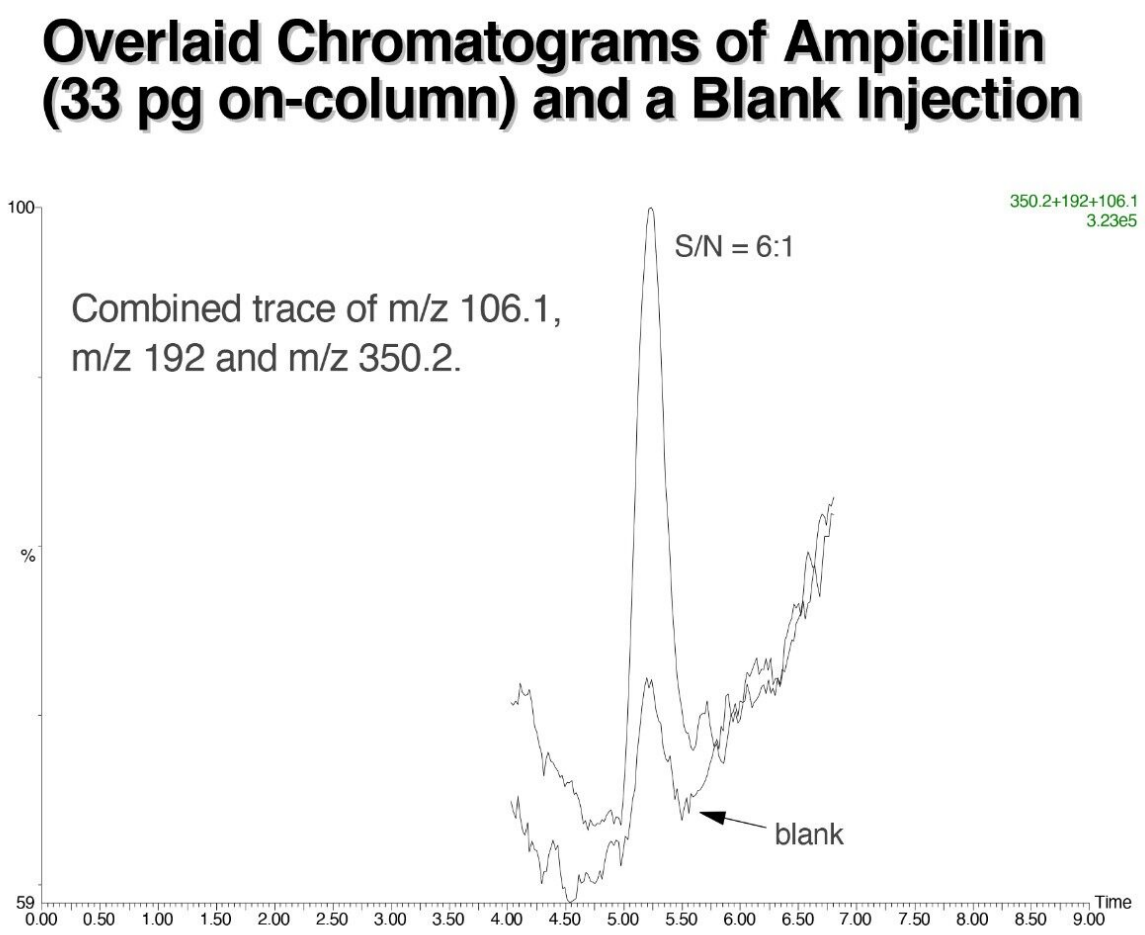

Figure 5. Overlaid Chromatograms of Ampicillin vs. Blank.

Conclusion

This work illustrates well how multiple SIR channels can be used to monitor both parent and characteristic fragment ions, allowing the quantitation of antibiotics with confirmation of identity on a single quadrupole mass spectrometer at levels as low as 15–35 pg oncolumn. It also illustrates how “in-source” CID allowed us to investigate in detail the general mechanisms by which the antibiotics fragment.

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