Waters Alliance® LC/MS System

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

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Key Words
Ampicillin
Antibiotics
Collision-induced dissociation, CID
Fragmentation
Penicillins
XTerra™ column

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.

Analytical Conditions

LC/MS system: Waters 2690 Separations Module and a Waters /Micromass ZMD single quadrupole mass spectrometer
Column: Waters XTerra™ $C_{18,}$ 2.1x150mm
Gradient: Water-methanol containing 0.1% acetic acid. Linear gradient wasfrom 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

Background-Subtracted Full Scan ESI+ Spectrum of Ampicillin, Low Cone Voltage

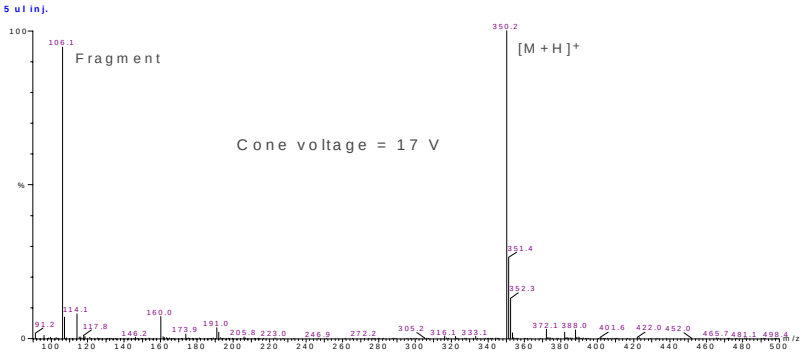

Figure 1: Mass Spectrum at Low Cone Voltage

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.

Background-Subtracted Full Scan ESI+ Spectrum of Ampicillin, High Cone Voltage

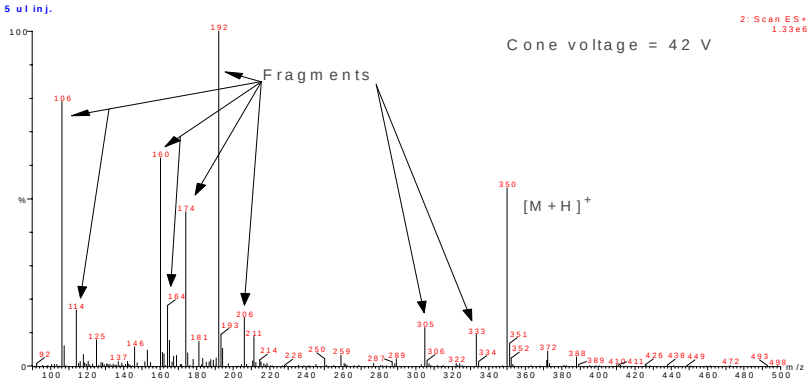

Figure 2: Mass Spectrum at High Cone Voltage

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.

General Fragmentation Scheme for Penicillins with 5-Membered Rings

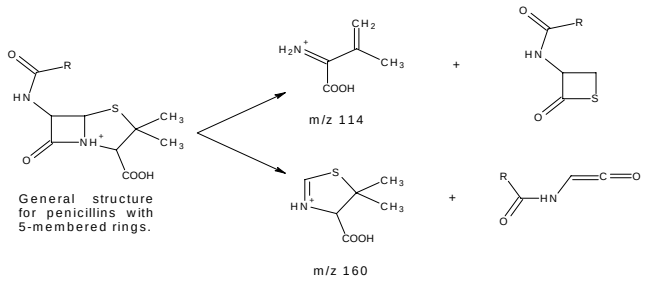

Figure 3: Fragmentation of Penicillins with 5-Member Rings

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

Confirmation for Presence of Ampicillin with Multiple SIR Channels. 33 pg on-column.

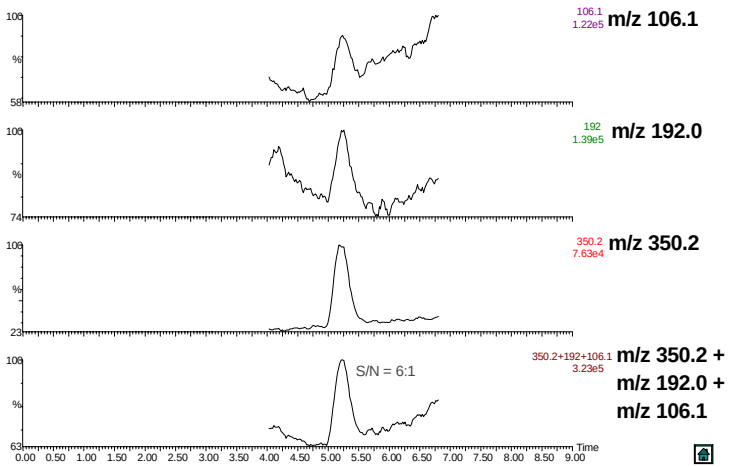

Figure 4: Single Ion Mass Chromatograms (SIR)

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 m/z, 192, and 350. The bottom panel is the "TIC" combined chromatogram.

Overlaid Chromatograms of Ampicillin (33 pg on-column) and a Blank Injection

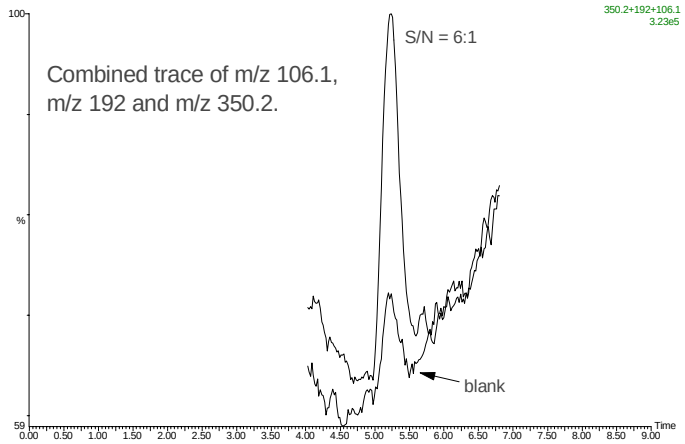

Figure 5: Overlaid Chromatograms of Ampicillin vs. Blank

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

Conclusions:

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 on-column*. 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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