

Analysis of Amoxicillin using the LCMS-2010EV and the LCMS-IT-TOF

Amoxicillin is a drug belonging to a class of compounds known as β -lactam antibiotics. Amoxicillin, a member of the penicillin family, is used most often to treat a number of bacterial infections including *H. influenzae*, *N. gonorrhoea*, *E. coli*, *Pneumococci*, *Streptococci*, and some strains of *Staphylococci*. It is thought that these penicillin-derived compounds work to stop the bacteria from multiplying by inhibiting its cell wall synthesis.

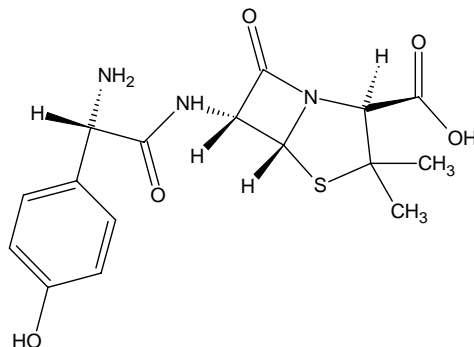


Figure 1. Structure of Amoxicillin, $C_{16}H_{19}N_3O_5S$; $[M+H]^+ = 366.1118$

Methods

Sample preparation. For LCMS-2010EV analysis, ~ 1mg/mL solution of amoxicillin was prepared in a mixture of MeOH and ACN (4:1). For LCMS-IT-TOF analysis a 1.25 mg/mL solution was used for analysis.

Table I. Analytical Conditions for LCMS-2010EV.

Column	Shim-pack VP-ODS (4.6 mm x 150 mm)
Mobile phase A	95% H ₂ O (0.1% Formic Acid) + 5% ACN
Mobile phase B	95% ACN + 5% H ₂ O (0.1% Formic Acid)
LC Time program (linear gradient)	0% B (0-1min); 25% B (15-23min); 50% B (23.01-25min); 5% B (25.01-30min)
Flow rate	0.3 mL/min
Injection volume	1 μ L
Probe voltage	+4.5 kV (+ ESI)
CDL temperature	250 °C
Block heater	200 °C

temperature	
Nebulizing gas flow	1.5 L/min
CDL voltage	-5 V
Q-Array	DC 50 V; RF 150 V
Scan range	100 – 500 <i>m/z</i>

Table II. Analytical Conditions for LCMS-IT-TOF

Column	Shim-pack VP-ODS (4.6 mm x 150 mm)
Mobile phase A	95% H ₂ O (0.1% Formic Acid) + 5% ACN
Mobile phase B	95% ACN + 5% H ₂ O (0.1% Formic Acid)
LC Time program (linear gradient)	0% B (0-1 min); 25% B (15-23 min); 50% B (23.01-25 min); 5% B (25.01-30 min)
Flow rate	0.3 mL/min
Injection volume	0.5 µL
Probe voltage	4.50 kV
CDL temperature	200 °C
Block heater temperature	200 °C
Nebulizing gas flow	1.5 L/min
Ion accumulation time	50 msec
MS	100 – 1000 <i>m/z</i> ; event time 200 msec
MS ²	101 – 400 <i>m/z</i> ; event time 188 msec; precursor 366.1000 <i>m/z</i>
MS ³	97 – 400 <i>m/z</i> ; event time 257 msec; precursor 349.0600 <i>m/z</i>
CID parameters	Energy 100 %; collision gas 100%; time 30 msec

Results

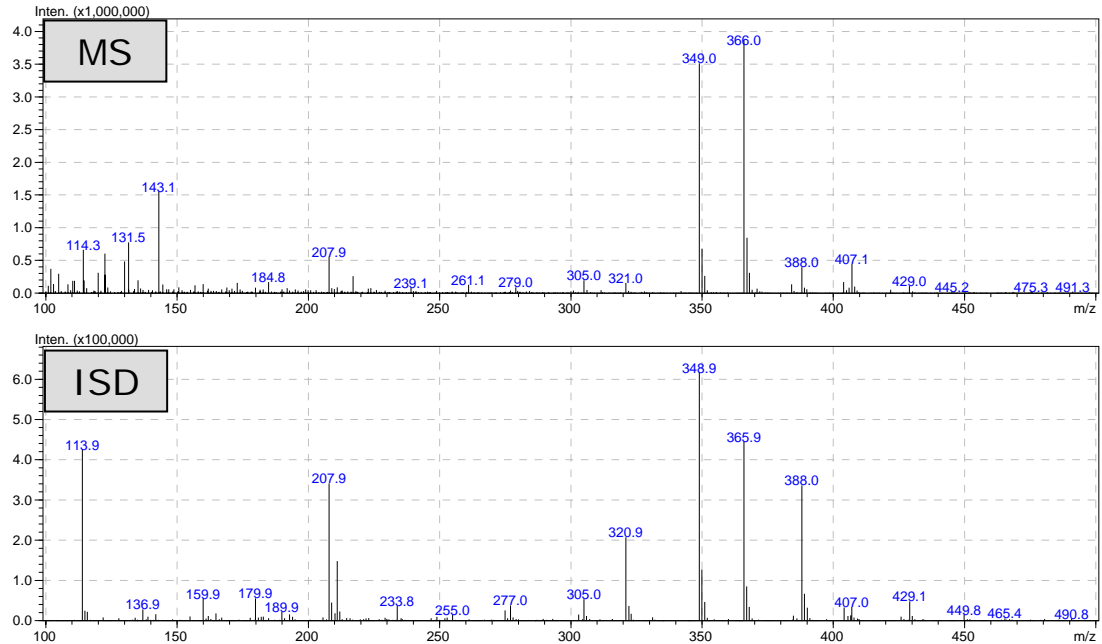


Figure 2. Top: Analysis of amoxicillin $[(M+H)_{thr}^+ = 366.11]$ on LCMS-2010EV. Observed parent ion (366 m/z) and loss of NH_3 (349 m/z). Bottom: Fragmentation of amoxicillin using increased Q-Array Voltage (+ 5 to + 50 V) leading to in source dissociation (ISD).

Figure 2 shows the resulting mass spectra from the analysis of amoxicillin on the LCMS-2010EV yielding the protonated ion for amoxicillin at 366.0 m/z . Increasing the Q-array voltage from + 5 to + 50 V invokes considerable dissociation of the molecule as seen in the bottom spectrum.

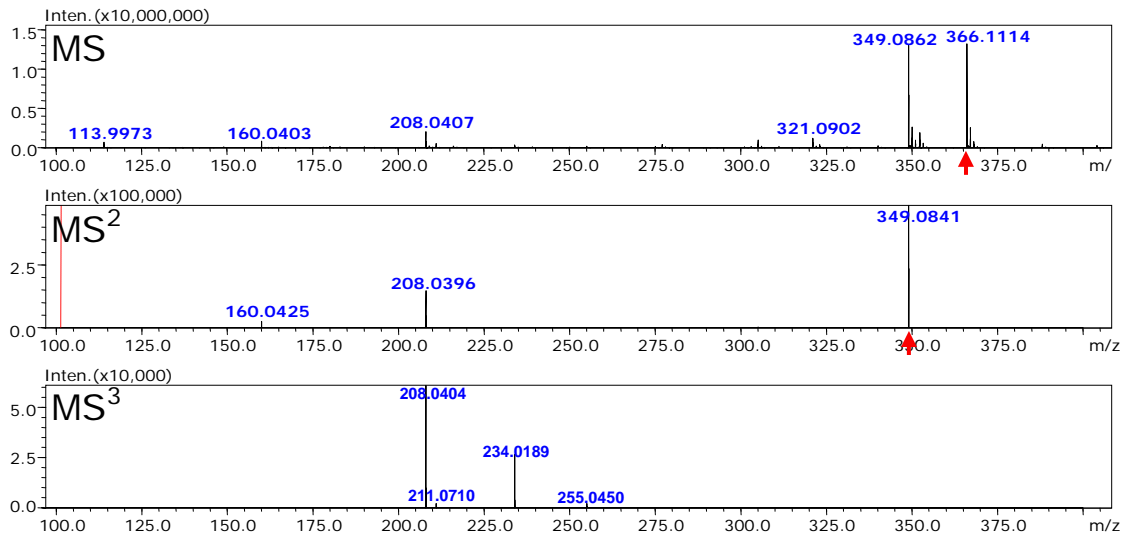


Figure 3. Analysis of amoxicillin $[(M+H)_{thr}^+ = 366.1118]$ on the LCMS-IT-TOF. Precursor for MS^2 - 366.1114 m/z ; Precursor for MS^3 - 349.0841 m/z (indicated by red arrows)

The LCMS-IT-TOF gave similar fragmentation of amoxicillin as the LCMS-2010EV with the added advantage of improved mass accuracy necessary for verifying fragment ion chemical composition.

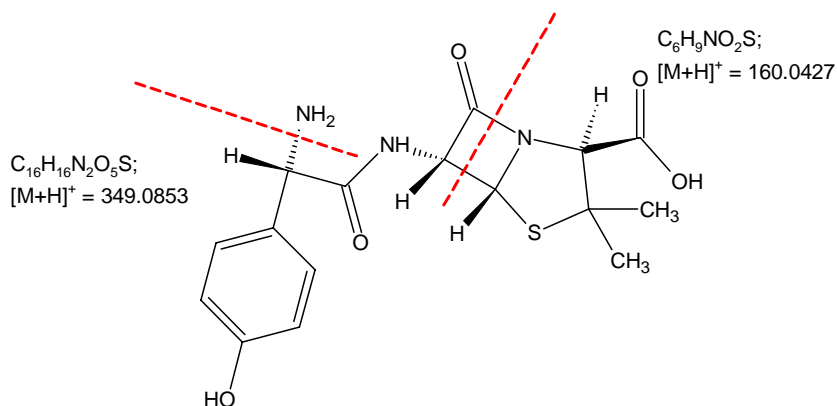


Figure 4. Fragmentation of amoxicillin

Table III. Mass accuracy values for amoxicillin and its CID fragments using the LCMS-IT-TOF. Note: Analysis performed without an internal standard.

Formula	Calculated mass ($M+H$) ⁺	Measured mass ($M+H$) ⁺	Mass accuracy (ppm)
C ₁₆ H ₁₉ N ₃ O ₅ S	366.1118	366.1114	1.1
C ₁₆ H ₁₆ N ₂ O ₅ S	349.0853	349.0841	3.4
C ₆ H ₉ NO ₂ S	160.0427	160.0425	1.3

Conclusions

Amoxicillin was successfully separated and detected under gradient conditions using a Shimadzu Prominence series LC coupled to both the LCMS-2010EV and the LCMS-IT-TOF. Both the LCMS-2010EV and the LCMS-IT-TOF could generate fragmentation data with the LCMS-IT-TOF allowing for MSⁿ and excellent mass accuracy. Mass accuracy data obtained from the LCMS-IT-TOF is comparable to data reported by other vendors requiring the use of an internal standard or dual-sprayer configuration.¹

¹ Nägele, E., Moritz, R. Structure elucidation of degradation products of the antibiotic amoxicillin with ion trap MSⁿ and accurate mass determination by ESI TOF. *J. Am Soc Mass Spectrom* **2005**, 16, 1670-1676.