

## Analysis of penicillin analogs using LC-MS

The classical definition of an antibiotic is a chemical produced by a microorganism that kills or prevents reproduction of infectious microorganisms. More recently, chemically synthesized substances have also come to be called antibiotics. Antibiotics generally have selective effects that act on specific microorganisms only. For example, penicillins act on the cell wall of certain bacteria but not on the cell membrane of animals. Thus, the penicillins are able to hinder cell growth and kill susceptible bacteria without harming the host's cells. In terms of chemical structure, antibiotics are classified into over ten

types that are widely used in medicine, including  $\beta$ -lactams (penicillins, cephalosporins, etc.), macrolides, and tetracyclines.

This data sheet introduces an example of the analysis of typical synthetic penicillins (Figure 1). Using ESI ionization, amoxicillin, ampicillin, and ticarcillin were detected as positive ions with an added proton, and flucloxacillin was detected as a negative ion with a proton removed. In addition, solvent adduct ions were detected. Figure 2 shows the MS spectra for each standard.

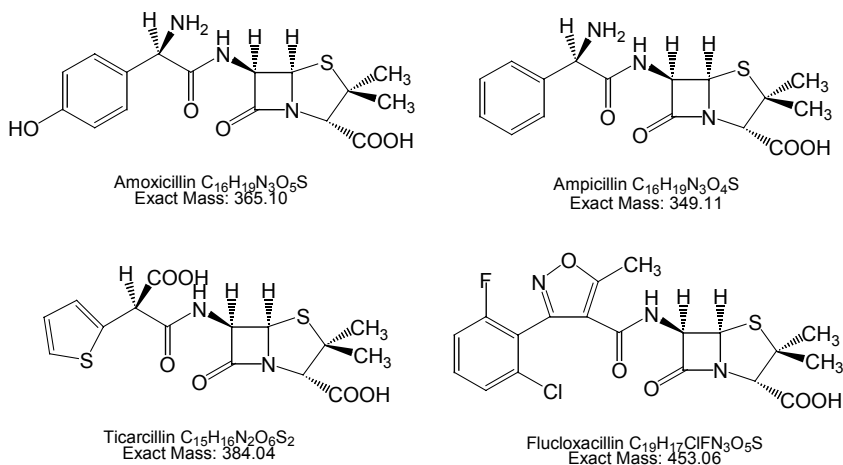


Fig. 1 Structures of synthetic penicillin analogue.

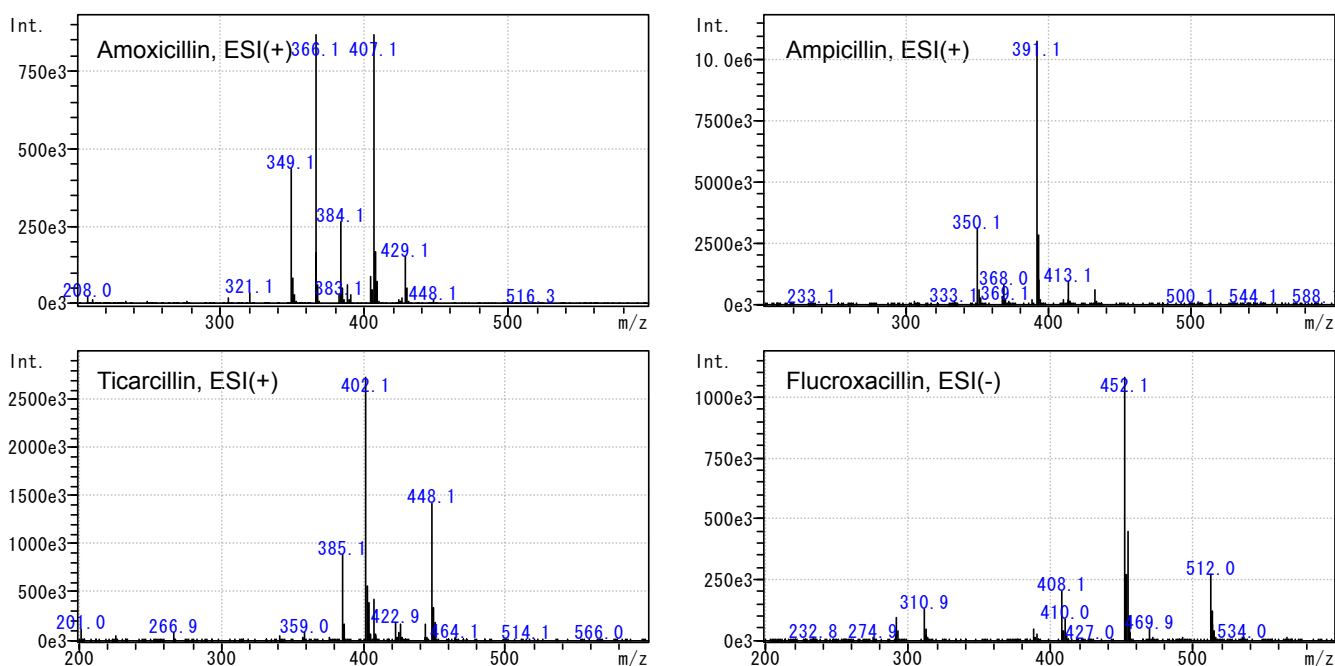


Fig. 2 ESI MS spectrum of penicillin analogue.

Figure 3 shows the selected-ion chromatogram (SIM) of the protonated molecules (M+H)<sup>+</sup> and deprotonated molecules (M-H)<sup>-</sup> of each component in a penicillin mixture. Figure 4 shows the 5-point calibration curves (5ng/mL to 1

μg/mL, n=5). Excellent linearity with a 0.999 coefficient of correlation was achieved across the entire measurement range for each component.

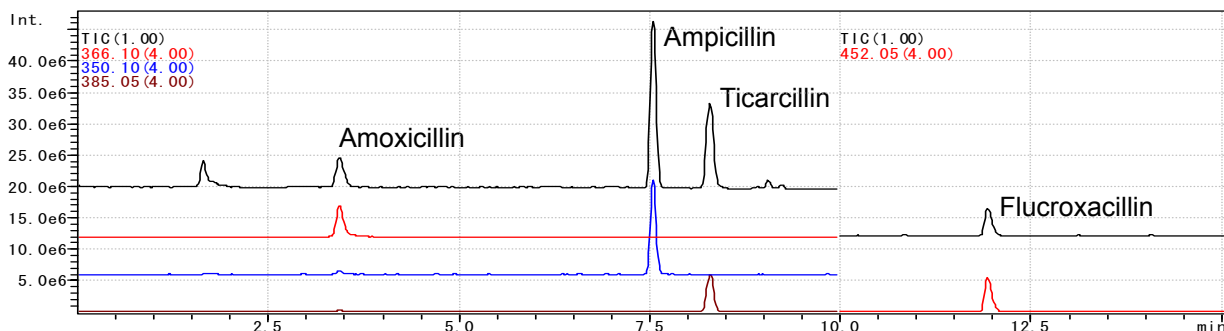


Fig. 3 SIM chromatogram of penicillin analogue.

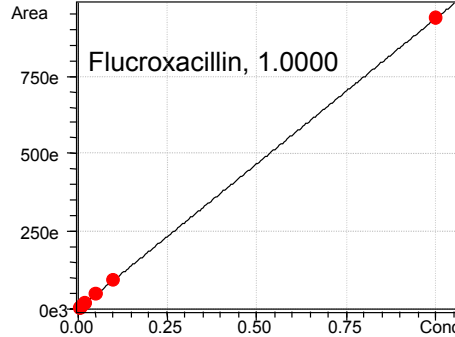
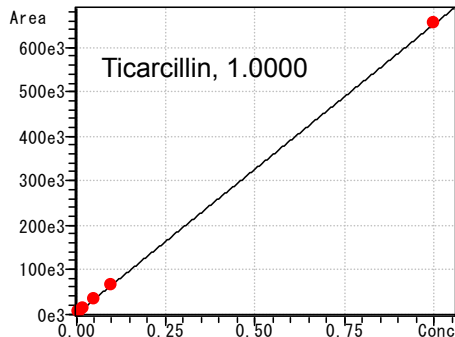
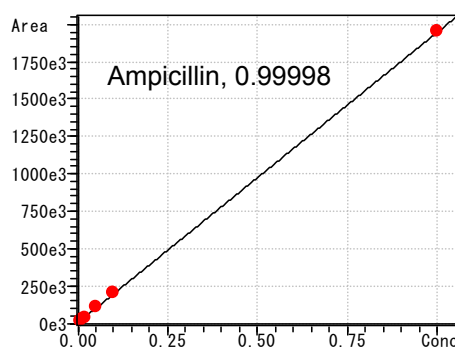
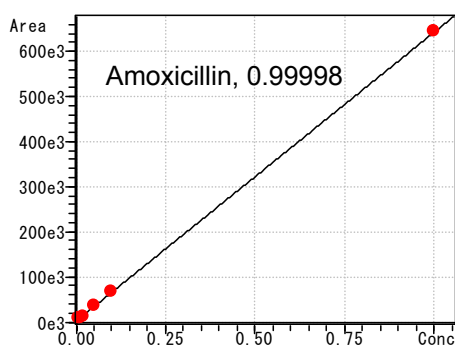


Table 1 Analytical conditions for LC-MS

Column	: Shim-pack VP-ODS (2.0mmI.D.x 150mmL.)	
Mobile phase A	: 10mM ammonium acetate buffer adjusted to pH 4.0 with acetic acid	
Mobile phase B	: acetonitrile	
Time program	: 5%B (0-1min) 80%B (12-15min) 80%B (15.01-25min)	
Flow rate	: 0.2 mL/min	
Injection volume	: 50uL	Column temperature : 40 °C
Probe voltage	: (1)+4.5 kV (0-10min, ESI positive); (2)-3.5kV (10-20min, ESI negative)	Block heater temperature : 200 °C
CDL temperature	: 200 °C	
Nebulizing gas flow	: 4.5 L/min	
CDL voltage	: (1) -20 V, (2) 20 V	Q-array 1, 2 DC : 0 V
Q-array 3 DC	: Scan mode	Q-array RF : Scan mode
Scan range	: m/z 200-600	

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