

HPLC Analysis of Antibacterial Drugs with Penicillin-Like Structure Application

Drug Development

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Penicillins can be isolated from the culture medium of certain fungi-producing natural penicillin, such as *Penicillium notatum* and *P. chrysogenum*. Other penicillins can be synthesized semisynthetically or by precursor-indicated biosynthesis. Total synthesis would not be economical.

Penicillin inhibits the polymerization of murin, which is responsible for the stability of the bacteria's cell wall. Because many antibacterials are toxic, various countries regulate the level of antibacterial residues in agricultural, veterinary, dairy, and meat-based food products.

Figure 1 shows the HPLC separation of four common antibacterial drugs with penicillin-like structure (amoxicillin, ampicillin, penicillin G, and penicillin V) on an SB-C18 reversed phase column.

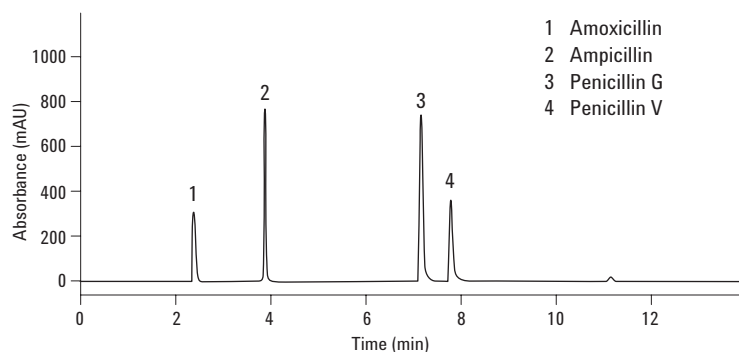


Figure 1. Separation of four penicillin analogs.

Highlights

- There is excellent resolution of penicillin analogs without ion pairing agent.
- There is rapid resolution of the penicillin analogs on the SB-C18 column at low pH and buffer concentration.
- Penicillins are eluted from the column with good and narrow peak shape.
- Extreme stability of sterically protected SB-C18 bonded phases allows for excellent separation at low pH.
- The SB-C18 column provides excellent peak shape and selectivity for antibacterial drugs.
- The HPLC method shows an easy but reliable and precise analysis of the antibacterial drugs.
- The values for limit of detection (LOD), precision of retention time (RT) and area show the good performance of the HPLC analysis.

Experimental Conditions

Equipment: Agilent 1100 Series HPLC; **UV Detector:** Variable wavelength detector, 204 nm, standard cell; **Column:** Zorbax SB-C18, 3.5 μ m, 4.6 \times 75 mm (part number 866953-902); **Mobile phase:** A = 0.025 M KH_2PO_4 in water (pH = 3), B = acetonitrile; **Injection volume:** 5 μ L; **Temp:** 40 $^\circ\text{C}$; **Flow rate:** 1.0 mL/min; **Gradient:** at 5% B to 60% in 10 min; **Column wash:** 60% B to 5% B in 2 min; **Stop time:** 12 min; **Post time:** 5 min



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Table 1. HPLC Method Performance of Antibacterial Drugs with Penicillin-Like Structure

Compound	LOD for S/N = 2 (mg/L)*	Precision of RT (RSD of 10 runs) (100 mg/L)*	Precision of area (RSD of 10 runs) (100 mg/L)*
Ampicillin	1.0	0.32	0.54
Amoxicillin	1.0	0.32	0.55
Penicillin G	1.0	0.32	0.49
Penicillin V	1.0	0.25	0.48

*Injection volume: 5 µL

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