HPLC Separation of Antibacterial Drugs with Tetracycline Structure

Application

Drug Development

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Tetracyclines occur naturally in some streptomyces species. Besides being used in human and veterinary medicine, they are fed as nutritional antibiotics in pig and poultry farming. Because of their long half-life and resistance, there is a high restriction on their usage in some European countries, such as Germany. Figure 1 shows the HPLC separation of three common tetracycline analogs on a Zorbax SB-C18 reversed phase column.

This application demonstrates separation without ion pairing and the use of an alternative mobile phase to TFA in separating antibacterial drugs.

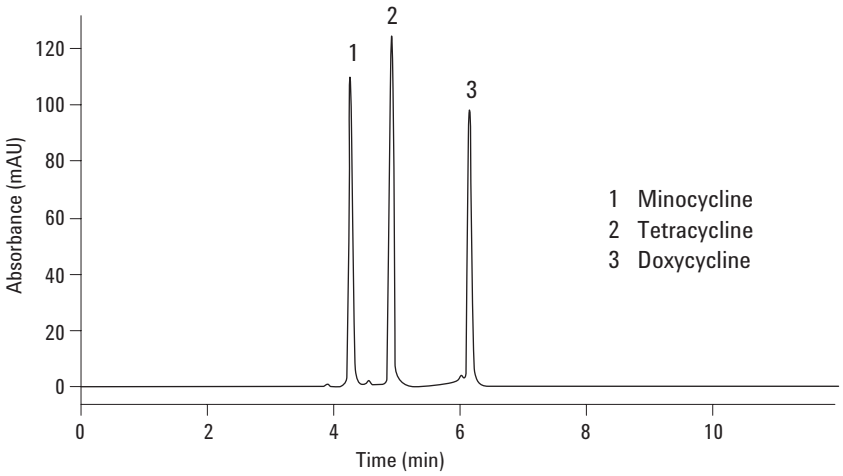

Figure 1. Separation of three antibacterial drugs with tetracycline structure.

Highlights

- The SB-C18 column provides excellent peak shape and selectivity for basic antibacterial drugs.
- The SB-C18 column shows excellent stability at low pH.
- The SB-C18 column shows excellent and rapid resolution of antibiotics at low pH and buffer concentration.
- 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, 350 nm, standard cell; **Column:** Zorbax SB-C18, 3. 5 µm, 4.6 × 75 mm (part number 866953-902); **Mobile phase:** A = 0.025 M KH_2PO_4 in water (pH = 3), B = acetonitrile; **Injection volume:** 5 µL; **Temp:** 25 °C; **Flow rate:** 1.0 mL/min; **Gradient:** at 5% B to 60% B in 10 min; **Column wash:** 60% B to 5% B in 2 min

Table 1. HPLC Method Performance of Antibacterial Drugs with Tetracycline 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)*
Minocycline	0.1	0.06	0.14
Tetracycline	0.1	0.05	0.13
Doxycycline	0.1	0.04	0.21

*Injection volume: 5 µL

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