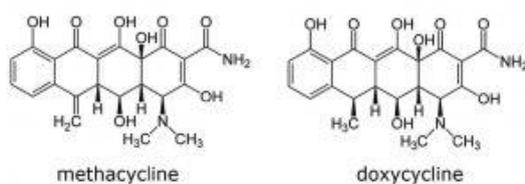
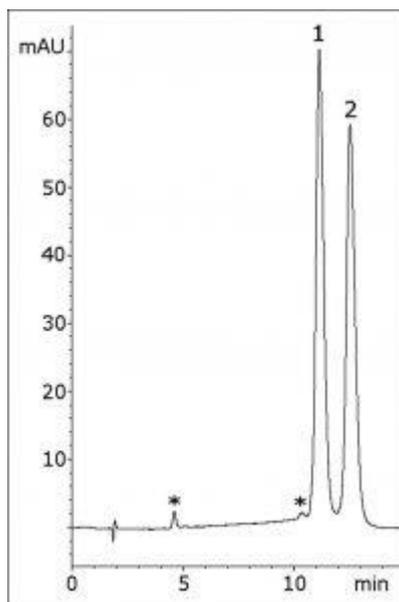


## Doxycycline and Methacycline Analyzed with HPLC - AppNote

### Simple Isocratic Method for API and Main Impurity

Formulations of Doxycycline may contain a Methacycline Impurity. As such, it is crucial for analytical methods to be able to separate these two peaks. Using this simple isocratic Method, the baseline resolution is obtained for this pair.



#### Peaks:

1. Methacycline, 2. Doxycycline

\* Impurities in doxycycline standard

#### Method Conditions

Column: Cogent UDC Cholesterol™, 4 µm, 100Å

Catalog No.: [69069-15P](#)

Dimensions: 4.6 x 150 mm

#### Mobile Phase:

A: 73% DI Water / 0.1% TFA

B: 27% Acetonitrile / 0.1% TFA

Injection vol.: 10 µL

**Flow rate:** 1.0 mL / minute

**Detection:** UV @ 350 nm

**Temperature:** 25°C

**Samples:** 1.0 mg / mL stock Solutions of Doxycycline HCL and Methacycline HCL reference standards were prepared in a diluent of 50/50 Solvent A / Solvent B. 100µL of each solution was transferred to a new vial and diluted with 800µL diluent. Individual standard dilutions were also prepared for peak identity confirmation.

**t<sub>o</sub> :** 1.8 minutes

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**Note:** Doxycycline is an antibiotic used for treatment of various bacterial infections such as Lyme disease, Rocky Mountain Spotted Fever, and others. Methacycline is a synthetic precursor to Doxycycline and therefore is a known impurity in Doxycycline formulations.

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