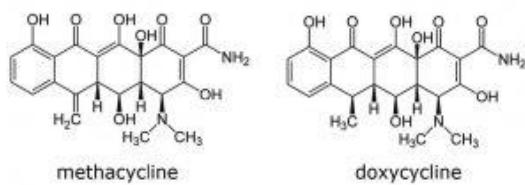
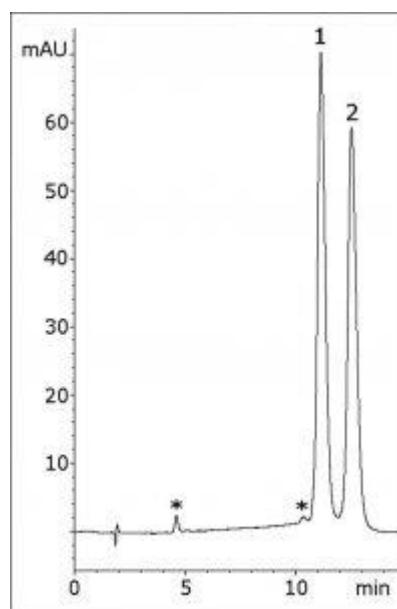




Doxycycline and Methacycline Analyzed with HPLC - AppNote

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™, 4µm, 100Å

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

Dimensions: 4.6 x 150mm

Mobile Phase:

A: 73% DI Water / 0.1% TFA

B: 27% Acetonitrile / 0.1% TFA

Injection vol.: 10 µL

Flow rate: 1.0mL / minute

Detection: UV @ 350nm

Temperature: 25°C

Samples: 1.0mg / 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.

t₀: 1.8 minutes

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.



Attachment No 311 Doxycycline and Methacycline Analysis by HPLC pdf 0.3 Mb

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