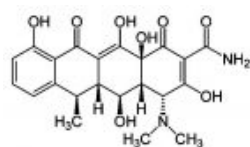
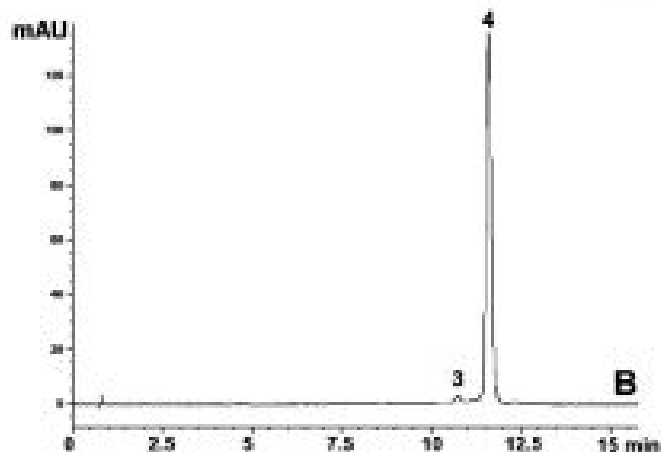
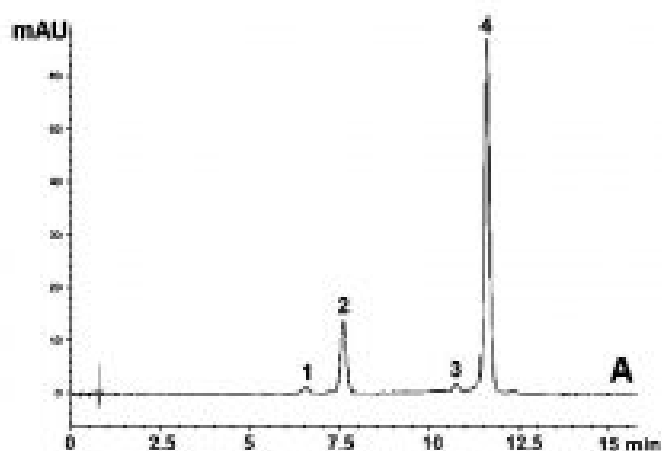


# Doxycycline Analyzed with HPLC – AppNote

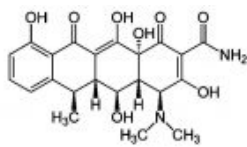
## Forced Degradation Method Using Shape Selectivity

Doxycycline is known to epimerize at the C4 and C6 positions in acid conditions. Epimers can be difficult to separate in Reversed Phase due to subtle differences in hydrophobicity. However, this Method differentiates based on shape as well as hydrophobicity and is therefore well suited to this Isomer separation.

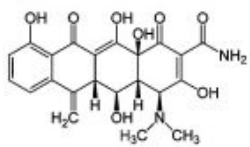
The method not only meets the USP System Suitability requirements for resolution between the API and both Epimer Degradants, but also demonstrates resolution from the Methacycline impurity.



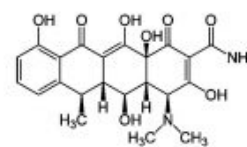
4-epidoxycycline



6-epidoxycycline



methacycline



doxycycline

**Peaks:**

1. Epimer degradant
2. Epimer degradant
3. Methacycline
4. Doxycycline

## Method Conditions

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

**Catalog No.:** 69069-7.5P

**Dimensions:** 4.6 x 75mm

**Mobile Phase:**

A: DI Water / 0.1% Trifluoroacetic Acid (TFA)

B: Acetonitrile / 0.1% Trifluoroacetic Acid (TFA)

**Gradient:**

Time (minutes)	%B
0	5
12	30
13	5

**Temperature:** 25°C

**Post Time:** 3 minutes

**Flow rate:** 1.5mL / minute

**Detection:** UV @ 350nm

**Injection vol.:** 20µL

**Sample Preparation:**

Figure A. Doxycycline forced Degradation extract

Figure B. Doxycycline non-Degraded extract

100 mg strength doxycycline Hyclate tablets were ground and added to 50mL volumetric flasks. Portions of diluent were added and the flasks were sonicated for 10 minutes. After diluting to mark, portions were filtered with a 0.45µm Nylon Syringe Filter and each diluted 1:200. The diluents used were 50% 1N HCl / 50% Solvent B for *Figure. A* and 50% Solvent A / 50% Solvent B for *Figure. B*. The extract used in Figure A was also heated at 80°C for 30 minutes.

**t<sub>0</sub>:** 0.8 minutes

**Note:** Doxycycline is a member of the tetracycline class of antibiotics. It can be used to treat various kinds of bacterial infections.

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