

Separations of Tetracycline Antibiotics by Reversed Phase HPLC, Using Discovery Columns

Five tetracycline antibiotics were separated by reversed phase HPLC on Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. These columns offer excellent resolution and selectivity for this application.

Key Words

- antibiotics ● tetracycline antibiotics

There are more than 100 antibiotics on the market today, and many more are in development. Resistance to antibiotics is a significant problem (1,2): an antibiotic that takes a decade to bring to market can induce resistance within months of its introduction into clinical practice (3). The frequency of resistance in bacteria and the numbers of drugs to which they are resistant are increasing. Therefore, it is critical to monitor the level of antibiotics given to humans and animals.

HPLC is a powerful tool for isolation and quantification of antibiotics. In this application, five tetracycline antibiotics (Figure A) were analyzed by HPLC, using Discovery™ C18, Discovery C8, and Discovery RP-AmideC16 columns. Tetracycline antibiotics have a broad spectrum of activity, are relatively safe, and are effective against many infections caused by Gram-negative and Gram-positive bacteria (4,5).

Chromatographic separations were performed on a Waters Alliance HPLC system. All injections were made through an autosampler. A Waters 2487 dual wavelength UV detector was used to monitor the UV absorbance of samples at 260nm. The 15cm x 4.6mm ID Discovery C18, Discovery C8, and Discovery RP-AmideC16 reversed phase HPLC columns were used without guard columns or filters. The packing particles in all columns were 5µm in diameter.

Doxycycline, minocycline, tetracycline, chlortetracycline, and oxytetracycline were obtained from Sigma Chemical Co. All antibiotics were dissolved in 25mM KH₂PO₄ buffer, pH 3.

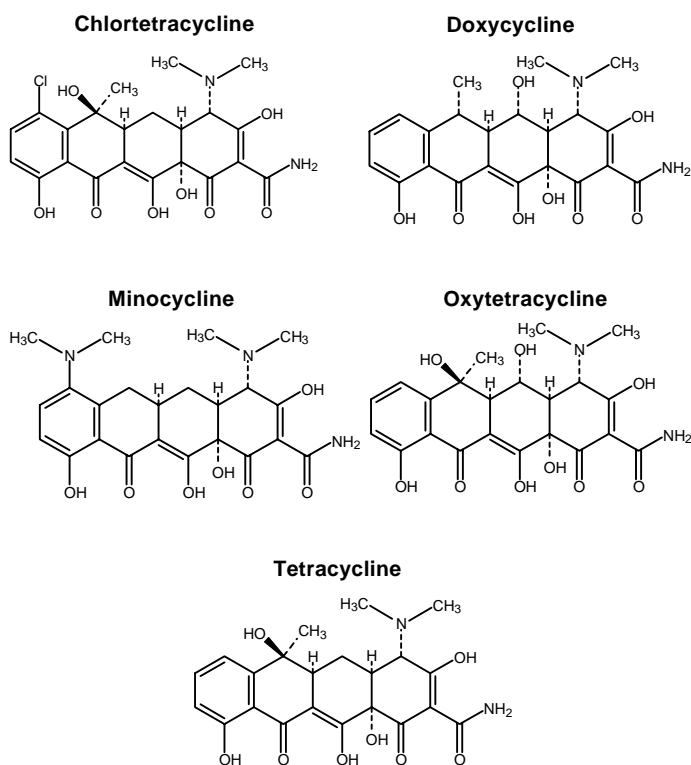
The five tetracycline antibiotics were separated by gradient elution. Column temperature was controlled at 35°C. Column pressure was below 1050psi in all cases. Detailed conditions for each analysis are presented with the corresponding chromatogram.

Separations of the five tetracyclines are illustrated in Figures B, C, and D. Minocycline and oxytetracycline will coelute from the C18 column, but are very well separated by the RP-AmideC16 and C8 columns (Figures C and D). The Discovery RP-AmideC16 column provides the best resolution of minocycline and oxytetracycline. The background around the chlortetracycline and doxycycline peaks is caused by impurities in the samples. Note that on-column quantities of analytes differed among Figures B, C, and D.

The ability of the RP-AmideC16 phase to separate minocycline and oxytetracycline might be explained by the hydrogen bonding between the amide functionality of the phase and the hydroxy functionality of oxytetracycline. Such differences in selectivity show the advantage of using the amide column for a difficult separation.

This study showed that mixtures of tetracycline antibiotics could be separated by reversed phase HPLC, using Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. Except for coelution of the tetracyclines minocycline and oxytetracycline on the Discovery C18 column, excellent resolution was achieved in every separation.

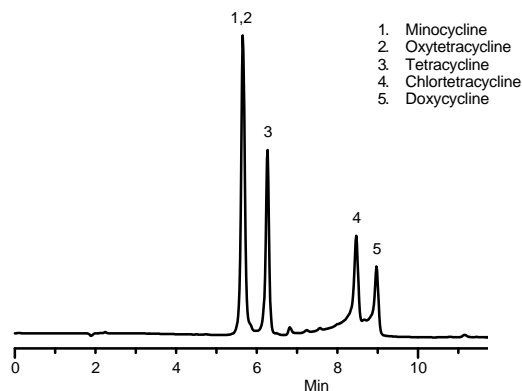
Figure A. Structures of Tetracycline Antibiotics



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Figure B. Tetracycline Antibiotics on a Discovery C18 HPLC Column

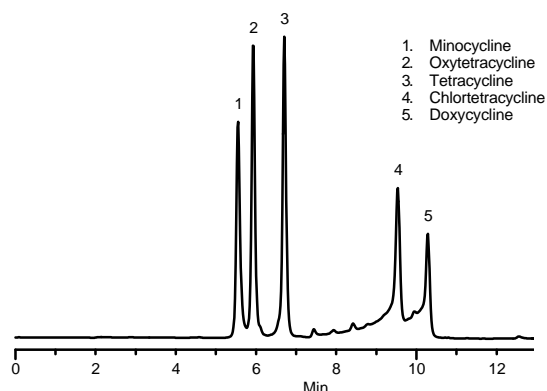
Column: **Discovery C18, 15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **504955**
 Mobile Phase: (A) 25mM KH₂PO₄, pH 3 (B) acetonitrile
 10% B to 50% B over 15 min
 Flow Rate: 1mL/min
 Pressure: <900psi
 Temperature: 35°C
 Detection: UV, 260nm
 Injection: 15µL 25mM KH₂PO₄, pH 3 containing 20µg/mL each analyte



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Figure D. Tetracycline Antibiotics on a Discovery C8 HPLC Column

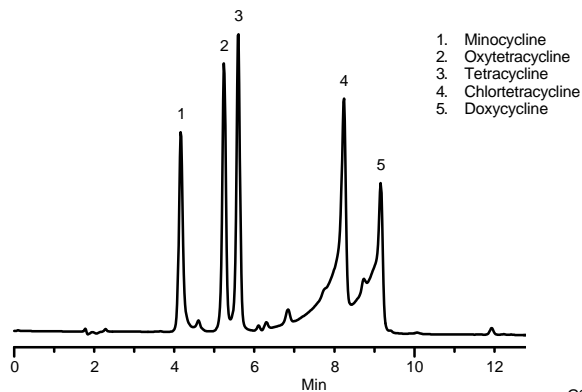
Column: **Discovery C8, 15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **59353-U**
 Mobile Phase: (A) 25mM KH₂PO₄, pH 3 (B) acetonitrile
 10% B to 40% B over 15 min
 Flow Rate: 1mL/min
 Pressure: <1030psi
 Temperature: 35°C
 Detection: UV, 260nm
 Injection: 5µL 25mM KH₂PO₄, pH 3 containing 100µg/mL each analyte



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Figure C. Tetracycline Antibiotics on a Discovery RP-AmideC16 HPLC Column

Column: **Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **505013**
 Mobile Phase: (A) 25mM KH₂PO₄, pH 3 (B) acetonitrile
 10% B to 40% B over 15 min
 Flow Rate: 1mL/min
 Pressure: <950psi
 Temperature: 35°C
 Detection: UV, 260nm
 Injection: 20µL 25mM KH₂PO₄, pH 3 containing 20µg/mL each analyte



G000925

Ordering Information:

Discovery Columns

15cm x 4.6mm ID, 5µm particles	
Discovery C8	59353-U
Discovery C18	504955
Discovery RP-AmideC16	505013

Discovery Selectivity Packs¹

5cm x 2.1mm ID Columns	55720-U21
15cm x 2.1mm ID Columns	55722-U21
5cm x 4.6mm ID Columns	55720-U
15cm x 4.6mm ID Columns	55722-U
25cm x 4.6mm ID Columns	55724-U

¹Four columns of equal dimensions, one of each Discovery phase (C18, RP-AmideC16, C8, Cyano).

References

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