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Application Note

Tetracyclines in Serum

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

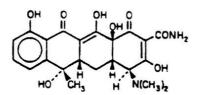
This application brief highlights the analysis of tetracyclines in serum using Symmetry Shield RP₈ Columns.

Introduction

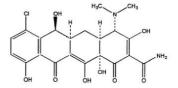
The compounds analyzed in this study are:

- 1. Minocycline
- 2. Tetracycline
- 3. Demeclocycline (I.S.)

1. Minocycline



2. Tetracycline



3. Demeclocycline (I.S.)

Experimental

HPLC Method

Column: Symmetry Shield RP8, 3.0 x 150 mm, 5 μ m

Guard column: Symmetry guard column, 3.9 x 20 mm, 5µm

Part numbers: Column - WAT094243, Guard - WAT200675

Mobile phase: 0.1% TFA in Water, Methanol, Acetonitrile 91:2:7

Flow rate: 0.90 mL/min

Injection volume: 20 μ L of reconstituted porcine serum extract

Oasis® HLB Extraction Method

Oasis® HLB 1 cc/30 mg Extraction Cartridge Part Number WAT094225

Condition

1 mL methanol/1 mL water

Load

1 mL spiked porcine serum with 20 µL phosphoric acid and 5.0 µg/mL demeclocycline (I.S.)

Wash

1 mL 5% methanol in water

Elute

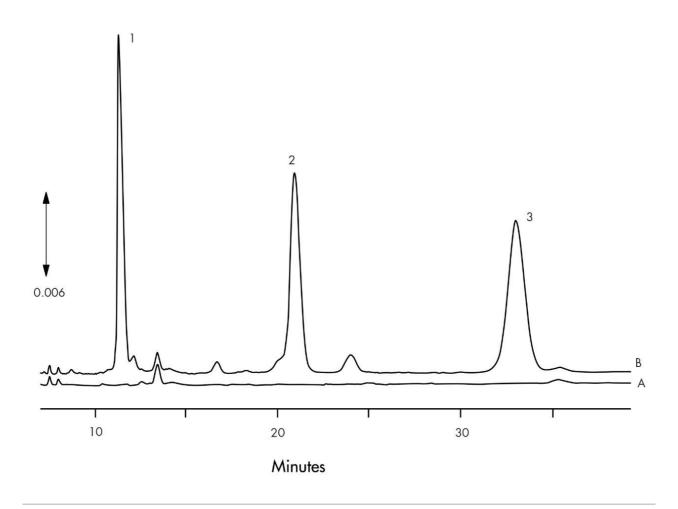
1 mL methanol

Evaporate and Reconstitute

40 °C under nitrogen stream 200 µL mobile phase

Results and Discussion

Compound	Concentration µg/mL	% Recovery	%RSD (n=6)
Minocycline	2.50	96.1%	1.3%
	0.500	88.0%	1.6%
Tetracycline	2.50	101.0%	0.5%
	0.500	97.8%	1.4%



Chromatogram of Serum Extracts: A) Blank B) Spiked Sample.

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