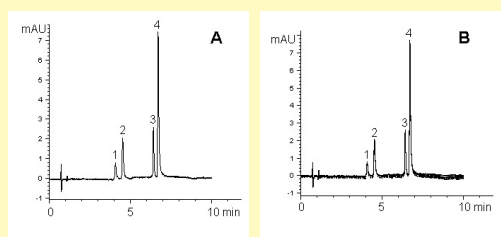
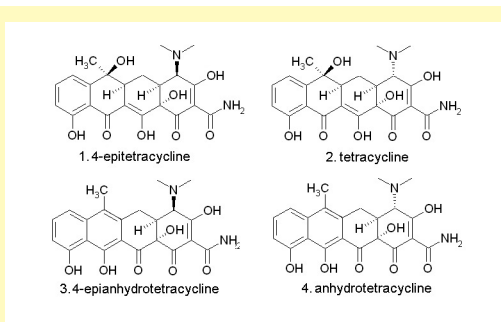


Stability Testing of Tetracycline HCl Capsules

Robust, high-throughput separation of API from its degradation products



- Peaks:**
1. 4-epitetracycline
 2. tetracycline
 3. 4-epianhydrotetracycline
 4. anhydrotetracycline

Note: Tetracycline is a broad-spectrum antibiotic widely used in both human and veterinary medicine. It is known to degrade primarily by two pathways: Dehydration and epimerization. Epimerization at the carbon-4 position leads to an inactive and non-toxic degradation product [1]. However, the anhydro form and its epimer are reported to be toxic in vivo and have been implicated in the development of Fanconi Syndrome [2, 3]. Therefore, it is crucial to have a reliable analytical method for discriminating between tetracycline and its major degradation products. The degradation procedure used here was adapted from Pena et al. [4].

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 2. M. Kühne, G. Hamscher, U. Körner, D. Schedl, S. Wenzel, *Food Chem.* 75 (2001) 423–429.
 3. J. Montoliu, M.T Carrera, A. Darnell, L. Revert, *Brit. Med. J.* 283 (1981) 1576–1577.
 A. Pena, L.P. Pallis, C.M. Lino, M.I. Silveira, A.C. Calokerinos, *Anal. Chim. Acta.* 405 (2000) 51–56.

Method Conditions

Column: Cogent Bidentate C18™, 4µm, 100Å
Catalog No.: 40018-75P
Dimensions: 4.6 x 75 mm
Solvents: A: DI water + 0.1% formic acid
 B: acetonitrile + 0.1% formic acid

Gradient:		Gradient:	
time (min.)	%B	time (min.)	%B
0	10	6	80
4	30	7	10

Post time: 3 min
Flow rate: 1.0 mL/min.
Sample: USP grade tetracycline HCl capsule extract (degraded).

Stock Solution: 10.0 mg capsule contents were diluted with 10 mL 1.0 N HCl and sonicated for 10 min. 1 mL aliquot was diluted to 20 mL with DI water. Solution was heated at 80 °C for 30 min. Solution was then diluted to 100 mL with DI water and filtered through a 0.45 µm nylon membrane HPLC filter (MicroSolv Technology Corp. Eatontown, NJ, USA).

Working Solution: Stock solution was diluted 10x using 0.01 N HCl diluent.

Detection: UV 360 nm (0–5 min) 430 nm (5–7 min)

Discussion

Figure A shows the chromatogram obtained from a single injection of the degraded tetracycline capsule extract. Tetracycline is well-resolved from its three main degradation products, the identities of which were confirmed by individual standards under non-degrading conditions. Amine-containing analytes such as these often require the use of an ion-pairing agent in the mobile phase in order to reduce peak tailing from silanolic interactions. However, ion-pairing agents often lead to poor reproducibility and long equilibration times due to slow uptake and release of these agents from the column. TYPE-C Silica™ based columns have most of the surface silanols replaced, and therefore ion-pairing agents are not necessary to obtain good peak shapes. Figure B shows an overlay of five sequential injections of the degraded tetracycline solution, illustrating the good repeatability of the method. Retention time %RSDs for all of the analytes were < 0.1%. In addition, the post time was minimal (3 min).

For more information visit www.MTC-USA.com

Cat. No.	Description
40018-75P	Cogent Bidentate C18™ HPLC Column, 100A, 4µm, 4.6mm x 75mm