CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Tetracycline Formulations

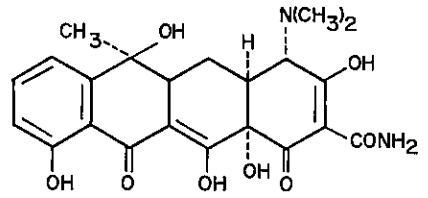
Purpose
Determination of tetracycline in pharmaceutical preparations.

Tetracycline [4-(Dimethylamino)-1,4,4a,5,5a,6,11,-12a-octahydro-3,6,10,12-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, F1] is a broad spectrum antibiotic used to treat bacterial and rickettsial infections. Therapeutic concentrations range from 0.5 - 5 µg/mL blood. Side effects may include renal toxicity, hepatotoxicity, gastro-intestinal disturbances, and opportunistic infections from resistant microbes (e.g., *Candida* in the ano-genital area).

Existing Methods
Microbiological assay, which does not discriminate among the various related compounds; TLC, which is time consuming; and LC.

Conditions
System: BAS 400 Liquid Chromatograph
Detector: BAS UV-108 variable wavelength (274 nm)
Column: BAS 3 µm Phase II ODS reverse-phase (100 x 3.2 mm) (PN MF-6213)
Mobile Phase: 500 parts 0.05 M ammonium phosphate adjusted to pH 2.5 with phosphoric acid, 100 parts acetonitrile, 30 parts dimethylformamide and 2.5 parts ethanolamine. Flow rate was 0.9 mL/min.
Detection Limit: 100 pg (S/N = 3)
Linear Range: 100 pg to 500 ng

Sample Preparation
A calibration curve was prepared by injecting appropriate amounts of tetracycline standards diluted in mobile phase. Injection volume was 20 µL.



TETRACYCLINE

Figure 1. Structure of tetracycline.

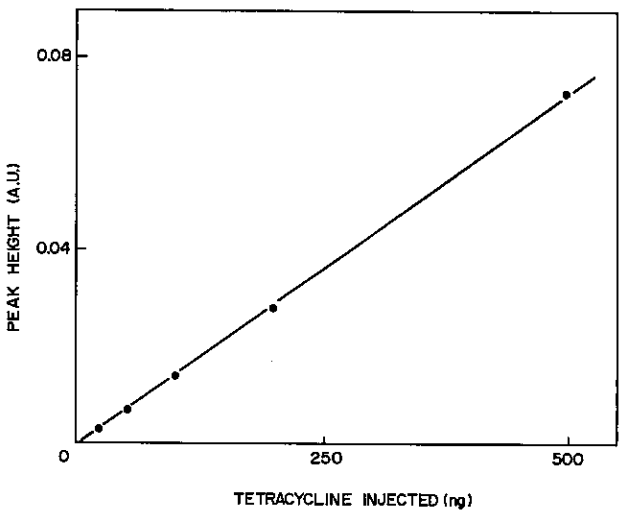


Figure 2. Calibration curve for injected standards. Each point represents the mean of two determinations.

Notes
A calibration curve for tetracycline standards is depicted in F2 and a sample chromatogram is shown in F3.

A similar mobile phase has been used to separate tetracycline and the related compounds 4-epitetracycline, chlortetracycline, 4-epianhydrotetracycline and anhydrotetracycline [1].

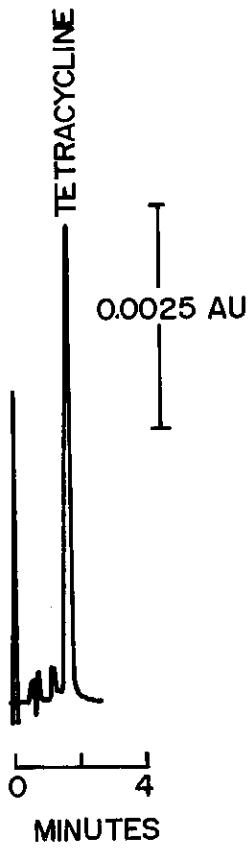


Figure 3. Chromatogram of 50 ng tetracycline standard.

Although the absorption maximum of 274 nm was used here, tetracycline can be monitored over much of the UV range with only moderate loss of sensitivity. Peak height at 254 nm, for example, was only 25% lower than at 274 nm.

The determination of tetracycline presented above can be duplicated using the BAS 200 Problem Solver.

Reference

1. Determination of Tetracycline and Related Compounds by High-Performance Liquid Chromatography, J.Y.C. Hon and L.R. Murray, J. Liq. Chromatogr. 5 (1982) 1973-1990.