

# CAPSULES

preliminary notes and applications from Bioanalytical Systems, Inc.

## Temafloxacin In Biological Matrices

### PURPOSE

Separation and fluorescence detection of the antibiotic temafloxacin.

Temafloxacin (F1) is a quinolone antibiotic used for both gram-positive and gram-negative bacteria. About 98% of the dose is excreted as unchanged temafloxacin, while the remainder is metabolized to ethylenediamine derivatives (EDA, MEDA) and aminoquinolone (AQ). Typical therapeutic doses range from 800-1200 mg/day, with plasma levels averaging 4-6 µg/mL.

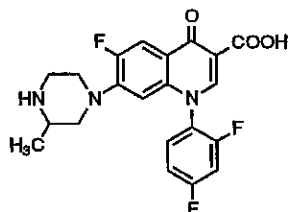


Figure 1. Structure of temafloxacin

### EXISTING METHODS

Temafloxacin, being an antibacterial, can be monitored by bioassay. But bioassays do not provide the speed, sensitivity or specificity required for pharmacokinetic and metabolic studies.

### REFERENCE

High-Performance Liquid Chromatographic Procedures for the Determination of Temafloxacin in Biological Matrices, G.R. Granneman and L.L. Varga, J. Chromatogr. 568(1991)197-206.

### CONDITIONS

Detector: Fluorescence, ex. = 280 nm, em. = 389 nm cutoff filter

Column: 7 µm, C<sub>18</sub>, 250 x 4.6 mm

Mobile Phase: Acetonitrile:water (53:47) containing 0.04 M H<sub>3</sub>PO<sub>4</sub>, 0.01 M NaH<sub>2</sub>PO<sub>4</sub>, 0.2% sodium dodecyl sulfate, 0.005 M N-acetylhydroxamic acid.

Flow rate was 1.5 mL/min

Detection Limit: 10 ng/mL (1 ng/mL using an alternative liquid-liquid extraction).

Linear Range: 0.05-10 µg/mL

### SAMPLE PREPARATION

Since temafloxacin is approximately 26% bound to plasma proteins, sodium dodecyl sulfate was used as a displacing reagent. Plasma samples (0.4 mL) were combined with buffer and the displacing reagent, then centrifuged through ultrafiltration membranes. Filtrates were directly injected onto the LC system. An alternative liquid-liquid extraction with methylene chloride and ethanol also was developed.

Urine samples were directly injected after a 20- to 100-fold dilution in mobile phase.

### NOTES

A sample chromatogram is presented in F2. The internal standard was a *p*-bromophenyl-substituted quinolone.

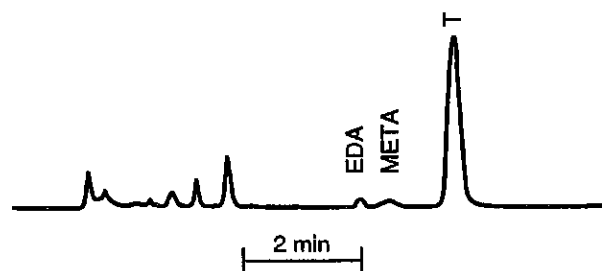


Figure 2. Chromatogram of plasma from a subject receiving 200 mg temafloxacin.

The separation of temafloxacin presented above can be duplicated on the BAS-480 or BAS-200 Liquid Chromatograph, equipped with a BAS FL-45 Fluorescence Detector.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by BAS technical staff.