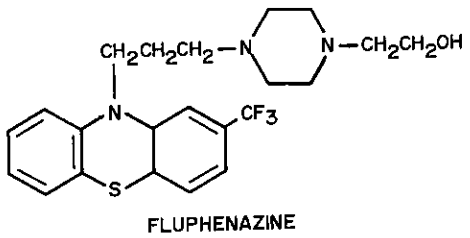
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Fluphenazine in Serum

Purpose

Determination of fluphenazine in serum by reverse-phase LC with UV, EC, and dual-series EC detection.



FLUPHENAZINE

Figure 1. Structure of fluphenazine.

Fluphenazine (F1, 4-[3-[2-trifluoromethyl)-10H-phenothiazin-10-yl]propyl]-1-piperazineethanol) is a phenothiazine (tricyclic) antidepressant used in the treatment of psychiatric disorders. Therapeutic concentrations of these compounds in blood typically range from 50-300 ng/mL. Chromatography of this phenothiazine is difficult because the piperazine side chain participates in the separation, producing split peaks and peak asymmetry [1]. In the procedure below, dibutylamine is included in the mobile phase to improve peak shape.

Existing Methods

GCECD, GCMS, and TLC. Normal phase LC also has been used.

Conditions

Detectors: BAS dual channel LC-44 amperometric detector and BAS UV-108 variable wavelength UV detector (254 nm)
Electrode: BAS dual-series glassy carbon
Potential: + 0.850 V upstream and 0.0 V downstream vs Ag/AgCl
Column: 3 µm, C 18 reverse-phase, 100 x 3.2 mm (PN MF-6213)
Column Temperature: 40°C
Mobile Phase: 62.5% 0.3 M formic acid, 0.02 M dibutylamine, pH 3.2; 37.5% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: UV: 400 pg injected standard, 15 ng/mL serum (S/N = 3). EC: 200 pg injected standard, 5 ng/mL serum (S/N = 3).
Linear Range: 1-100 ng injected standards, 50-350 ng/mL serum.

Sample Preparation

1. Prepare C-8 Bond Elut® extraction columns by washing with 1 mL methanol followed by 1 mL water.

2. Combine the following and load onto each extraction column: 0.5 mL serum, 0.5 mL 0.05 M Na_2HPO_4 pH 7.5, and standards as appropriate.

3. Wash each column sequentially with 1 mL of each of the following: 0.05 M phosphate buffer pH 7.5, water, water, 50% aqueous methanol.

4. Elute the samples with 0.25 mL methanol. Inject 20 µL of this eluent onto the chromatograph.

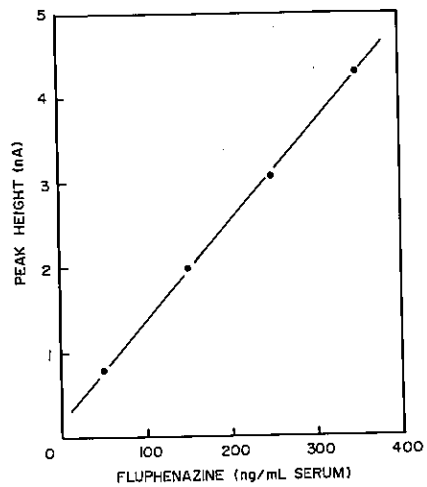


Figure 2. Calibration curve for upstream (+ 0.850 V) EC detection of serum spiked with fluphenazine.

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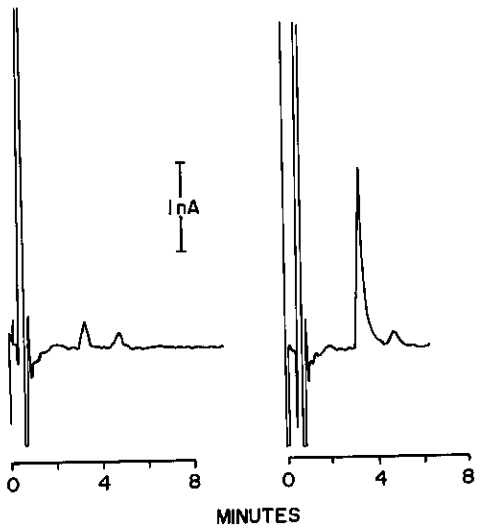

Figure 3. Representative upstream (+ 0.850 V) chromatograms of unspiked (left) and spiked (150 ng fluphenazine/mL) serum samples.

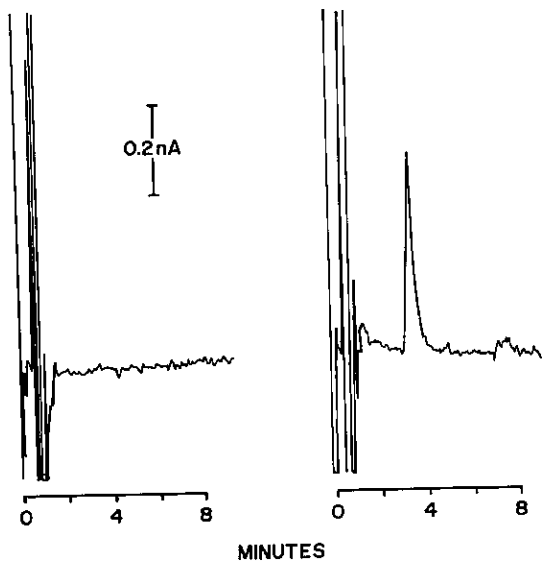

Figure 4. Representative downstream (0 V) chromatograms of unspiked (left) and spiked (150 ng fluphenazine/mL) serum samples.

Notes

A calibration curve for spiked serum samples is presented in F2. The results for UV detection were similar. Sample chromatograms for single-electrode EC detection (+ 0.850 V) are shown in F3. The blank serum chromatogram shows an interfering peak that coelutes with fluphenazine. This peak did not affect the linearity of the determination (F2).

The interfering peak was eliminated by the use of dual-series EC detection. The upstream electrode was set at + 0.850 V to oxidize compounds eluting from the column, and the downstream electrode was set at 0 V to selectively reduce some of these products. The results demonstrate the selectivity of dual- series EC detection (F4); the interfering peak was not detected.

Recovery of standard fluphenazine from spiked serum samples was 93 %.

The determination of fluphenazine presented above also can be performed on the BAS 200 Problem Solver.

Reference

Hoffman, D.W., R.D. Edkins, S.D. Shillcutt and A. Salama, J. Chromatogr. 414 (1987) 504-509.