Perphenazine in Serum

Purpose
Determination of perphenazine in serum by single-electrode EC, dual-series EC and UV detection.

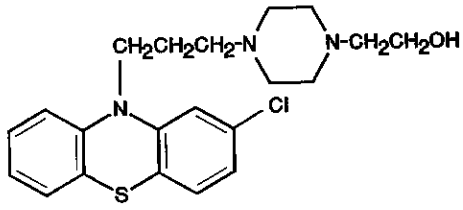

Figure 1. Structure of perphenazine.

Perphenazine (F1, 4-[3-(2-chlorophenothiazin-10-yl)propyl]-1- piperazineethanol) is a tranquilizer of the phenothiazine family. It is used for the treatment of psychotic disorders and for the control of nausea and vomiting. Side effects may include hypotension and liver toxicity. Therapeutic levels in blood range from 50-500 ng/mL.

Chromatography of this and other phenothiazines can be difficult because the piperazine side chain participates in the separation, producing split peaks and peak asymmetry. In the procedure below dibutylamine is included in the mobile phase as a competing base to improve peak shape.

We demonstrate 3 methods of detection here. First, perphenazine can be oxidized at a single electrode. Second, the product of this oxidation can be reduced by a second electrode downstream to the first. This dual-series combination can result in a much cleaner (more selective) chromatogram, since few interfering compounds will have electrochemical behavior like that of the analyte. Finally, perphenazine can be detected by UV absorbance.

Existing Methods
RIA and RRA, which won't distinguish among the compound and some of its metabolites, GLC and LC.

Conditions
EC Detector: BAS LC-44 dual Amperometric Detector
Electrode: BAS Dual Glassy Carbon
Potential: Upstream: + 0.775 V vs Ag/AgCl;
Downstream: 0.0 V vs Ag/AgCl
UV Detector: BAS UV-108 variable wavelength (254 nM)
Column: 3 µm, C 18 reverse-phase, 100 x 3.2 mm (PN MF-6213)
Mobile Phase: 65% (v:v) 0.3 M formic acid, 0.02 M dibutylamine, pH 3.2; 35% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: Upstream EC: 125 pg injected standard, 3 ng/mL serum. Downstream EC: 550 pg injected standard, 7 ng/mL serum. UV: 250 pg injected standard, 5 ng/mL serum. (All at S/N = 3.)

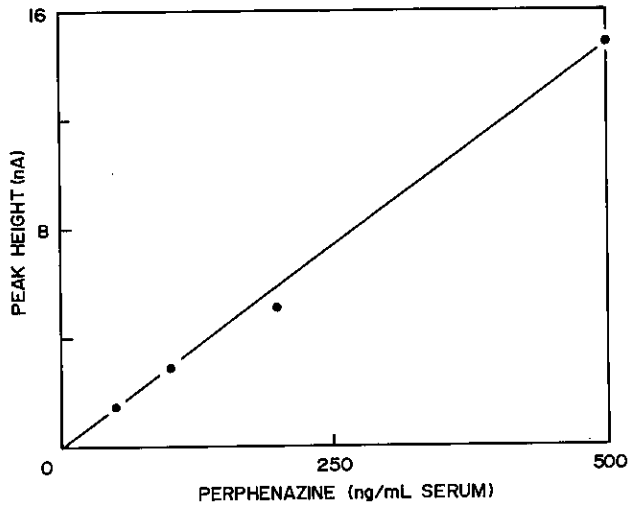

Figure 2. Calibration curve for spiked serum samples detected at the upstream (+ 0.775 V) electrode.

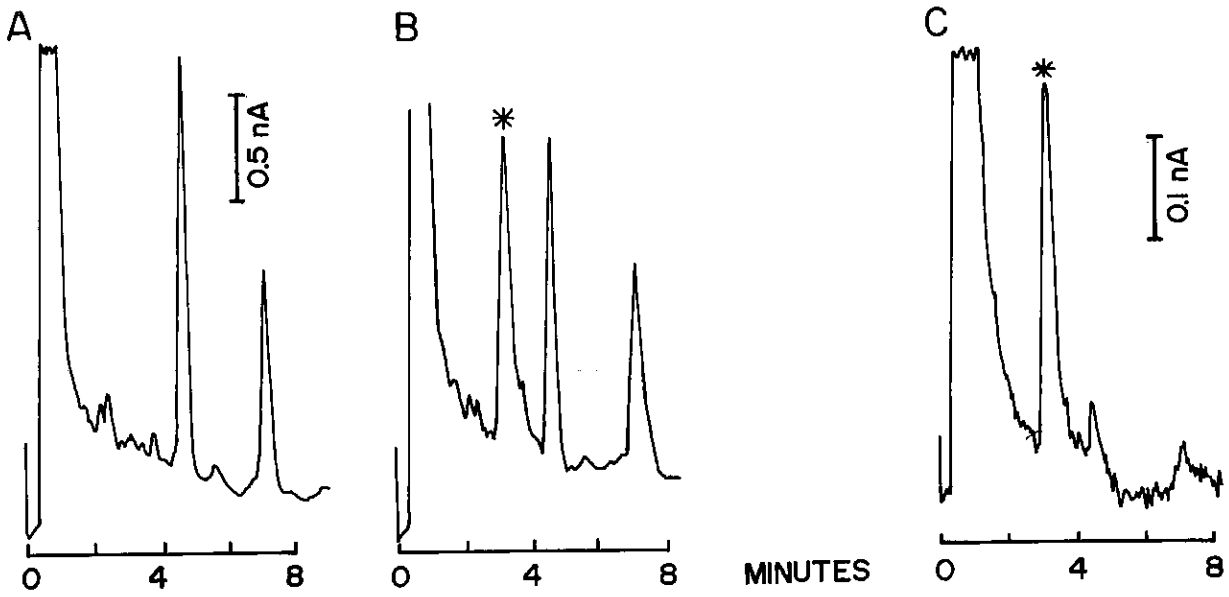

Figure 3. Sample chromatograms. A: Unspiked serum at upstream (+ 0.775 V) electrode. B: Serum spiked with 50 ng/mL perphenazine at upstream electrode. C: Downstream (0.0 V) detection of B. The perphenazine peak is marked with an asterisk in A and B.

Linear Range: All detectors: 1-100 ng injected standards, 50- 500 ng/mL serum.

Sample Preparation

1. Alkalinize serum samples by adding 10 µL of 1 N NaOH per mL of serum.

2. Prepare Bond-Elut® C_{18} solid-phase extraction columns by washing with 1 mL methanol followed by 1 mL water.

3. Load 1 mL serum and standards as appropriate onto each column and wash through.

4. Wash each column with 1 mL 0.05 M Na_2HPO_4, pH 7.5, followed by 1 mL 50% aqueous methanol.

5. Elute the samples with 1 mL methanol. Dry the samples in a stream of nitrogen and redissolve in 200 µL mobile phase. Inject 50 µL into the chromatograph.

Notes

A calibration curve for spiked serum samples is presented in F2, and sample chromatograms are shown in F3. Note that the downstream EC detector was relatively insensitive to the extraneous peaks in the sample (F3A and F3B vs F3C). However, the samples were sufficiently clean so that all three detectors produced similar results.

Recovery of perphenazine from spiked serum samples was 75%. An alternative liquid-liquid extraction (with 70% recovery) can be found in [1].

CPPZ (4-[3-(2,8-dichlorophenothiazin-10-yl)propyl]-1-piperazineethanol) has been used as an internal standard for the determination of perphenazine [1].

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

Reference

1. Larsen, N-E., J. Chromatogr. 342 (1985) 244-250.