CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Plasma Chlorpromazine Monitoring Therapeutic Levels

Purpose

The phenothiazines have been prescribed for the treatment of psychotic patients in recent years. These antipsychotic drugs produce improvement in the mood and behavior of psychotic patients-emotional quieting, psychomotor slowing, decrease of anxiety and delusions - without causing addiction or excessive sedation. Chlorpromazine, an aliphatic phenothiazine, is used most frequently in the treatment of schizophrenia. It is also useful in the prevention of nausea and vomiting.

There is still controversy on the mode of action of phenothiazines. The phenothiazines are theorized to inhibit dopamine sensitive adenylate cyclase activity, an effect that is probably related to their blocking action on dopamine receptors (1). The phenothiazines are also thought to predominantly act by blocking the effective interaction of norepinephrine with its receptors (2).

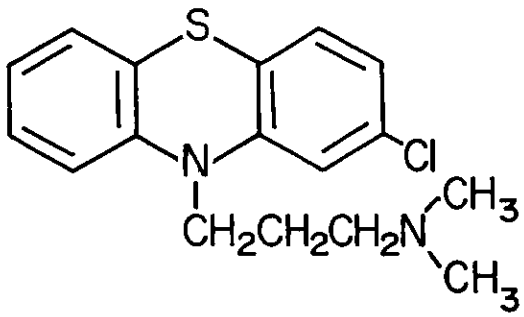

Figure 1. Structure of chlorpromazine.

Existing Methods

There is a strong correlation between plasma concentrations of phenothiazines and their therapeutic outcomes. However, the concentrations of phenothiazines in plasma are quite low, measured in nanograms per mL, and highly sensitive techniques are required to assay plasma concentrations. This has stimulated great interest in the development of

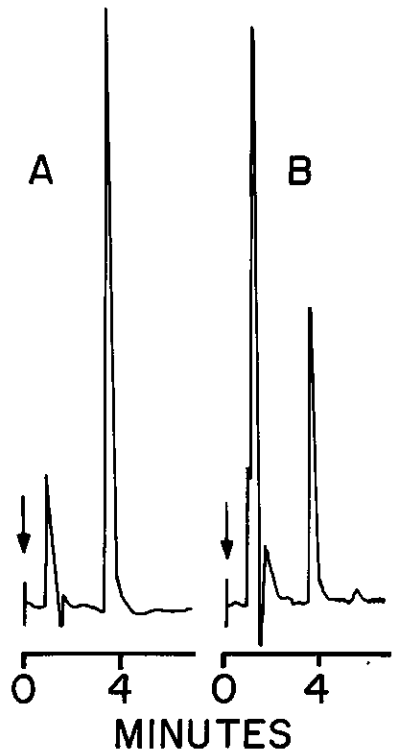

Figure 2. Spiked plasma extract chromatograms. (A) 60 ng chlorpromazine injected, corresponds to 120 ng/mL plasma, 20 nAFS. (B) 7.2 ng chlorpromazine injected, corresponds to 14.4 ng/mL plasma, 5 nAFS.

assays that can be used on a routine basis to determine plasma phenothiazine levels. Methodologies that have evolved include: GC/MS (3), GLC (4), LCUV (5,6), and LCEC (7). GC/MS has the advantage of measuring low plasma levels (1-5 ng/mL), however, assays are long and tedious, requiring the synthesis of derivatives, and the instrumentation is quite expensive. A GLC method is described for the assay of seven phenothiazines; however, the detection limits were rather high: 50 ng/mL plasma. (4) An LCUV method demonstrated poor peak symmetry with severe peak tailing for five phenothiazines. The detection limits for chlorpromazine were much too high, determined to be 5.08 µg/mL plasma (5).

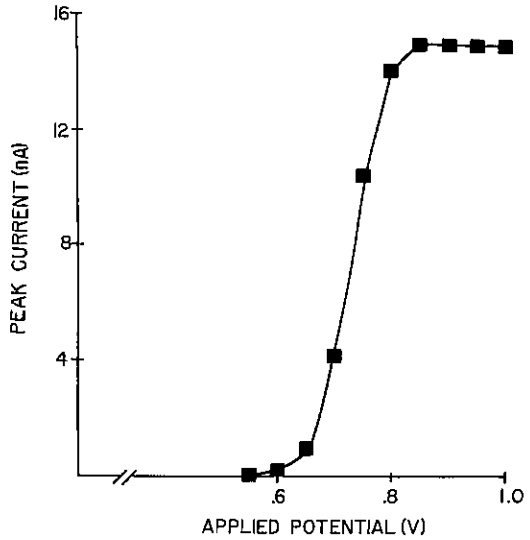

Figure 3. Hydrodynamic voltammogram of repetitive 60 ng injections of chlorpromazine.

Another LCUV method reported 5-20% recovery of chlorpromazine (6). An LCEC method is described by Wallace, et al. for the determination of promethazine and other phenothiazines. The recovery of promethazine exceeded 75% from plasma samples, and the detection limits were very good - 0.2 µg/L, which was based on a sample signal equivalent to about 2% of the fullscale response (7).

The following outlines an analytical protocol for the determination of chlorpromazine in plasma. The protocol utilizes liquid chromatography/electrochemistry. Following a single extraction into hexane, the extracts are injected into the LCEC analyzer. The procedure is a modification of an assay originally reported by Wallace, Shimek, Stravchansky, and Harris (7).

Conditions

System: LC-154T, BAS 400 or BAS 200
Detector: LC-4 or LC-4B
Electrode: Glassy carbon (BAS P/N MF-1000)
Potential: + 0.85 V vs Ag/AgCl
Column: Biophase Octyl, 5 µm, 250 x 4.6 mn (BAS P/N MF-6032)
Temperature: 40°C
Mobile Phase: 60% 0.02 M H_3PO_4, 40% methanol, containing 2.5 mM tridecylamine, pH 2.5. Flow rate was 1.9 mL/min.

Sample Preparation

All test tubes, centrifuge tubes, and storage bottles were surface treated with PROSIL-28 (PCR Research Chemicals, Inc.) an organosilane surface treating agent.

1. To 1.0 mL plasma add 1.0 mL 0.5 M K_2CO_3 in 15 mL conical centrifuge tubes. Vortex 5-10 seconds.

2. Add 5.0 mL hexane. Mechanically shake for 15 minutes, centrifuge at 2000 rpm for 5 minutes.

3. Transfer hexane layer to a 5 mL test tube. Evaporate the hexane under a nitrogen stream at 40-45°C to dryness.

4. Reconstitute the residue in 100 µL mobile phase.

5. Inject 50 µL.

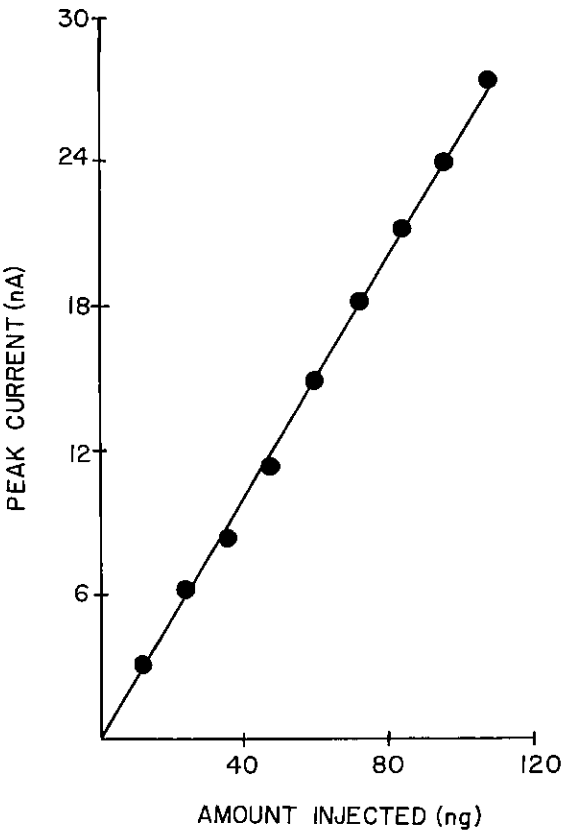

Figure 4. Linearity of LCEC analyzer for chlorpromazine standards.

Results and Discussion

Chlorpromazine was quantitated in spiked plasma specimens. Each chromatogram took approximately 4 minutes to elute chlorpromazine. Typical chromatograms obtained are shown in F2.

To determine the optimum potential for the assay of chlorpromazine, a hydrodynamic voltammogram was generated by repetitive 60 ng injections of chlorpromazine. F3 describes the HDV obtained. The optimum operating potential for this assay was chosen to be +0.85 V.

F4 describes the linearity of the LCEC Analyzer for injections over the range of 12-108 ng of chlorpromazine.

The recovery of this method was determined by comparing the current reponse of spiked plasma extracts to that of a standard solution of chlorpromazine. The percent recovery was calculated at a concentration of 120 ng chlorpromazine/mL plasma (n=11) and was found to be 90 ± 4.8%. The minimum detectable concentration, using a signal-to-noise ratio of 5.1, was determined to be 0.7 ng injected, corresponding to a concentration of 1.4 ng/mL plasma.

The addition of n-tridecylamine to the mobile phase is crucial for chromatography performance. The effect is dramatic, as can be seen in F5. It may be that the effect of n-tridecylamine in the mobile phase is due to an ion-repulsion mechanism. The protonated n-tridecylamine adsorbs onto the packing material and transforms it to an ion exchanger. As the chlorpromazine travels down the column, its positive charge repels the positive charge on the n-tridecylamine thus reducing the capacity factor.

Without n-tridecylamine, the chromatography of chlorpromazine yields a split peak. This is thought to be due to hydrogen bonding between chlorpromazine and exposed silanol groups on the packing material. The combination of several ill-defined and transient equilibria is probably the cause. These include mixing of the injection volume with the mobile phase and the differences in ionic strength and solvent composition between these two solutions.

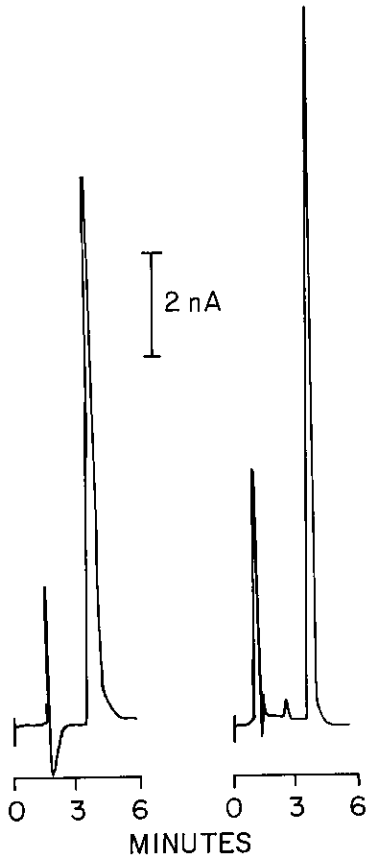

Figure 5. Chlorpromazine standard, 60 ng injected; 20 nAFS. A) Mobile phase without n-tridecylamine B) Mobile phase containing 2.5 mM n-tridecylamine.

This procedure provides the required precision and accuracy for assaying large numbers of samples per day. The detection limits are also sufficiently low to determine very low (1-10 ng) injectable amount.

References

1. Goth and Mosby, Medical Pharmacology Ninth Edition, Chapter 19, The C.V. Mosby Company,St. Louis, 1978.

2. Wilson, Gisvold, Doerge, Textbook of Organic Medicinal and Pharmaceutical Chemistry, Chapter 10, J.P. Lippincott Company, Philadelphia, 1977.

3. G. Alfredson, B. Wode-Helgodt, G. Sedvall, Psychopharmacology, 48(1976) 123-131.

4. E. Dinour, L. Goltschalk, B. Nandi, P. Geddes, J. Pharm. Sci., 65(1976) 667.

5. D.J. Smith, J. Chromatogr. Sci., 19(1981) 65.

6. J. Tekete, P. Del Castilho, J.C. Kraak, J. Chromatogr., 204(1981) 319-327.

7. J.E. Wallace, E.L. Shimek, Jr., S. Stavchansky, S.C. Harris, Anal. Chem., 53(1981) 960-962.

Liquid chromatography/electrochemistry is a highly useful tool for biomedical research. As with any instrumental technique, the precision and accuracy of the measurement depends on the instrumentation, the skill and knowledge of the operator and the integrity of the sample preparation procedure. Use of these techniques for medical diagnosis and accountability for the same rests entirely with the user of this equipment.

BAS