CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Imipramine, Desipramine, And Metabolites

Purpose

The tricyclic antidepressants, used in the treatment of endogenous depression, have been widely prescribed in recent years. Since there is a strong correlation between the plasma levels of these tricylic amines and their therapeutic outcome, there is a great demand for routine laboratory procedures to assay plasma of patients for the tricyclic antidepressants (TCA's) and their metabolites.

The mode of interaction of tricyclic antidepressants is theorized to occur by enhancing noradrenergic activity through the blockade of norepinephrine reuptake in peripheral and central noradrenergic neurons. This results in an increase in the availability of norepinephrine at post- synaptic receptor sites and also in the concentration of its metabolite, normetanephrine.

One of the problems in monitoring plasma levels of patients receiving tricyclic antidepressants is that some of the drugs produce active metabolites that have somewhat different pharmacological actions (1). The inhibition of the uptake of norepinephrine by tricyclic antidepressants is competitive in nature, and generally, secondary amines have been found to be more potent than the corresponding tertiary amines in blocking the uptake of norepinephrine through neuronal membranes. Tricyclic antidepressants also inhibit the amine uptake mechanism for 5-HT in platelets. Structure-activity relationships reveal that tertiary amines of tricyclic antidepressants are more potent inhibitors of 5-HT uptake than the corresponding secondary amines (2). The hydroxy metabolites of imipramine have strong cardiovascular activity and are essentially equipotent to the parent compound in the blockade of norepinephrine and 5-HT receptors in rat brain slices and isolated synaptosomal fractions (3).

The clinical outcome of tricyclic antidepressant therapy - whether an effect is required on

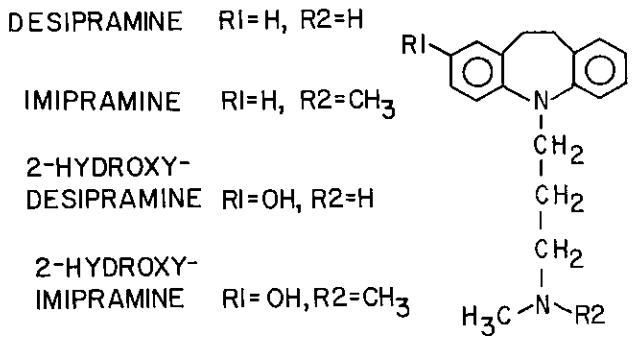

Figure 1. Structures of the tricyclic antidepressants and their 2- hydroxy metabolites.

noradrenergic neurons or serotoninergic neurons - is not a simple problem to overcome. Generally, it appears that secondary amines predominantly cause an increase in the level of psychomotor activation and drive, whereas tertiary amines exert predominantly mood elevating properties (2).

Another problem associated with tricyclic antidepressant therapy is the adjustment of therapeutic plasma levels. There is still controversy on the therapeutic ranges of the tricyclic antidepressants. However, most agree that 20-200 ng/mL (plasma) is a satisfactory therapeutic range. For 1 milliliter samples, this requirement stipulates that an analytical technique capable of very low nanogram detection limits is desirable.

This Capsule outlines an analytical protocol for the determination of desipramine, imipramine, and their 2-hydroxy metabolites in plasma. The protocol utilizes liquid chromatography with electrochemical detection. Following a simple solvent extraction based on the acid-base characteristics of these amines, the extracts are injected into the LCEC analyzer and separated into component compounds. Detection is accomplished amperometrically, through the oxidation of the aromatic amine at a glassy carbon electrode. The procedure is a modification of an

assay originally reported by Raymond Suckow and Thomas Cooper of the Rockland Research Institute, Orangeburg, N.Y. (3).

Reagents

Tricyclic Antidepressant Stock Solution. Dissolve 25 mg of each tricyclic amine in 50 mL methanol. 2-hydroximipramine and 2-hydroxydesipramine were donated by Dr. Albert A. Manian, National Institute of Mental Health, Rockville, Maryland. Desipramine and imipramine were donated by Dr. Mark Walter, Waterbury Hospital, Waterbury, Connecticut.

Working Standard Solutions. Dilute the stock solution with appropriate volumes of mobile phase.

Conditions

System: LC-154T, a BAS 400 or BAS 200
Detectors: EC: LC-4 or LC4B UV: LC-6 or UV-8, UV-108 or UV-116 The detector cells were in series with the UV being first.
Electrode: Glassy carbon (BAS P/N MF-1000)
Potential: + 1.05 V vs Ag/AgCl
Wavelength: 254 nm

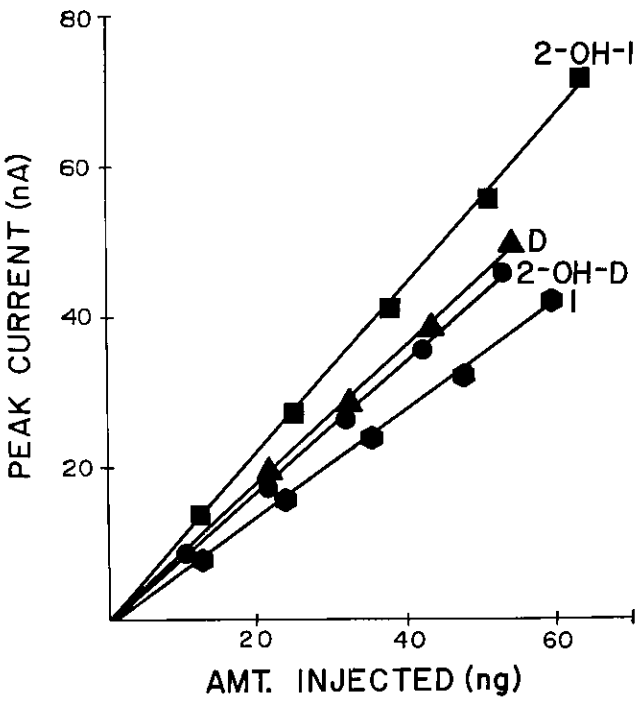

Figure 2. Linearity of LCEC for tricyclic amine standards. Legend: 2-OH-D = 2-hydoxydesipramine, 2-OH-I = 2- hydroxyimipramine, D = desipramine, I = imipramine.

Column: Biophase octyl, 5 µm, 250 x 4.6 mm (BAS P/N MF-6032).
Temperature: 30°C
Mobile Phase: 57.5% 0.2 M $NaClO_4$ - 0.005 M Sodium citrate, 32.5% acetonitrile, 10% methanol (pH 5.5). Flow rate was 2 mL/min.

Sample Preparation

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

This procedure was adapted from Suckow and Cooper (3). Per their suggestion, methyl-t-butyl ether was substituted for diethyl ether, yielding better recoveries and cleaner extracts.

1. To 1.0 mL plasma, add 10.0 mL of H_2O and 1.0 mL of 0.6 M K_2CO_3 (pH 11.3) in 15 mL conical centrifuge tubes with screw caps.

2. Add 8.0 mL methyl-t-butyl ether. Mechanically shake for 5 minutes, and centrifuge at 2000 rpm for 15 minutes.

3. Transfer the ether layer to a 15 mL conical centrifuge tube with screw cap containing 1.2 mL of 0.1 M HCl. Mechanically shake for 5 minutes, centrifuge at 2000 rpm for 10 minutes.

4. Aspirate the top layer to waste. Add 0.5 mL of 0.6 M K_2CO_3 (pH 11.3) and 10.0 mL methyl-t-butyl ether. Mechanically shake for 5 minutes. Centrifuge at 2000 rpm for 5 minutes.

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

6. Reconstitute the residue in 100 µL mobile phase.

7. Inject 50 µL.

Results and Discussion

Imipramine, desipramine, and their 2-hydroxy metabolites were quantitated simultaneously in plasma. Each chromatogram took approximately 16 mintues to elute all 4 compounds.

To determine the optimum potential for all 4 compounds used in this assay, a hydrodynamic voltammogram was generated by repetitive 11.0 ng injections of all 4 compounds. In order to reach the limiting current plateau, a high potential must be applied. However, with high applied potentials, oxidation of the mobile phase occurs, and high background currents result. The optimum operating potential for the assay of the 4 tricyclic amine compounds was chosen to be +1.05 V, a maximal limiting current response with minimal background current (typically 35-45 nA).

F2 describes the linearity of the LCEC system for injections over the range of 10-65 ng of each tricyclic amine.

The recovery of this method was determined by comparing the current response of spiked plasma extracts to that of a standard solution of the tricyclic amines. The percent recoveries calculated for each tricyclic amine are reported in T1, where n = 14. The blank plasma extracts showed no interfering substances.

T2 compares the minimum detectable concentrations of each tricyclic amine using EC detection and UV detection with a signal-to-noise ratio of 5.5. This table clearly demonstrates the superiority of EC detection over UV detection in measuring low concentrations of each tricyclic amine in 1 mL plasma. F3 compares typical spiked plasma extract chromatograms obtained simultaneously by EC and UV detection. As can be seen, EC detection has less baseline noise and a better signal response than UV detection.

When following the extraction procedure, care must be taken when separating the organic and aqueous layers. A white precipitate forms at the interface of the two layers, and if this is transferred and saved during the extraction procedure, the sample will yield a chromatogram with many interfering substances, making it impossible to accurately quantitate the tricyclic amines. However, if this white precipitate is avoided, the chromatograms obtained are as shown in F3.

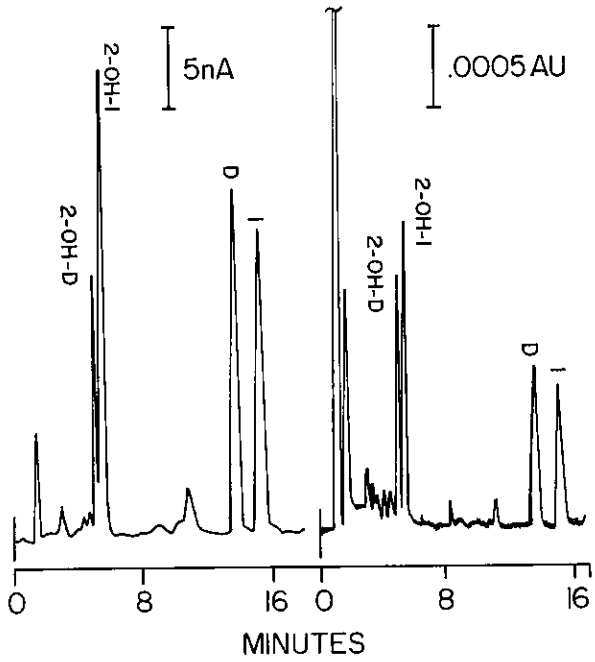

Figure 3. Comparison of spiked plasma chromatograms obtained by EC and UV detection. Legend: 2-OH-D = 2-hyroxydesipramine, 31.95 ng injected; 2-OH-I = 2-hydroxyimipramine, 38.10 ng injected; D = desipramine, 32.55 ng injected; I = imipramine, 35.70 ng injected.

Table 1. Recovery of Imipramine, Desipramine, 2-Hydroxyimipramine, and 2- Hydroxydesipramine from 1 mL Plasma.

Compound	Recovery %	SD	CV %
2-hydroxyimipramine	68.9	3.7	5.4
2-hydroxydesipramine	60.2	3.4	5.7
desipramine	79.9	5.1	6.4
imipramine	79.9	5.1	6.4

Table 2. Detection Limits in Plasma EC vs. UV Detection

Compound	EC Detection	UV Detection
2-hydroxydesipramine	5.6 ng/mL	18.0 ng/mL
2-hydroxyimipramine	3.8 ng/mL	18.0 ng/mL
desipramine	4.4 ng/mL	28.0 ng/mL
imipramine	5.4 ng/mL	17.5 ng/mL

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.

References

1. L.E. Hollister, Trends in Pharm. Sci., 2(1981) 89-92.

2. L.L. Iversen, S.D. Iversen, S.H. Snyder, Handbook of Psychopharmacology, Vol. 14 Affective Disorders: Drug Actions in Animals and Man, Plenum Press, New York, 1978.

3. R.F. Suckow, T.B. Cooper, J. Pharm. Sci., 70(1981) 257-261.