T97

Essentials in bioresearch

910459

HPLC Analysis of Tricyclic Antidepressants from Serum.

instrumentation, chemistries, and expertise for bioseparations

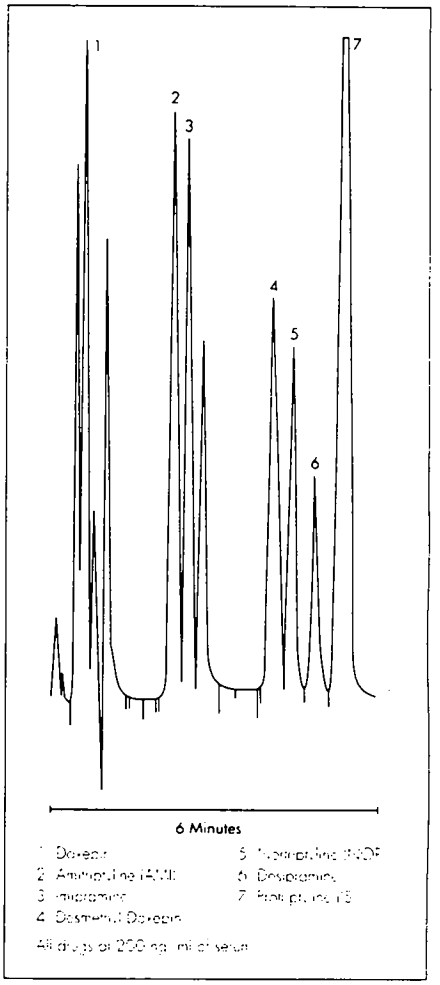
Simultaneous analysis of parent drug and psychoactive metabolites.
The tricyclic antidepressants are used in the treatment of depression. They are believed to act by inhibiting the reuptake of the neurotransmitters serotonin and norepinephrine in the central nervous system. The tricyclic drugs amitriptyline and imipramine have demethylated metabolites, nortriptyline and desipramine respectively, that are also psychoactive and are themselves used as antidepressants. With this method, the parent drugs and psychoactive metabolites are assayed simultaneously, thus eliminating the added cost for separate assays (Fig. 1).

Easier, higher throughput assay.
Short chromatographic analysis time linked with a single step, one solvent liquid/liquid extraction procedure provide a simplified assay for tricyclics. Requiring less technologist time, the rapid assay reduces turnaround time and increases throughput, and provides a lower cost per test than other HPLC assays.

Simple verification of peak identity.
The use of dual wavelength response ratioing can be used to increase the confidence level in determining peak identity. Pure parent drug is analyzed producing a characteristic dual wavelength response ratio. By comparing the ratio of a peak of an unknown to that of the standard, the identity of the unknown can be made by more than just retention time. The use of this technique for identification of cyclobenzaprine which coelutes with amitriptyline is well documented[1] (see Table 1).

The same system for tricyclic metabolites.
There is growing evidence that the hydroxy metabolite levels of tricyclics are an indicator of potential cardiotoxicity. As a result, some laboratories now report relative levels of hydroxymetabolites in addition to active drug levels. A simple change of extracting solvent (hexane with 3-5% isoamyl alcohol) along with the standard chromatographic system provides the analysis of these hydroxy metabolites.

Figure 1: Tricyclic antidepressant standards from spiked serum.



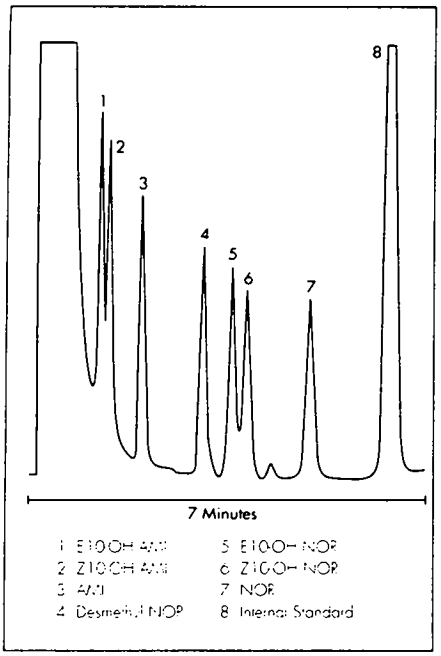
One method provides the rapid, simultaneous analysis of parent drug and psychoactive metabolites

Waters. The absolute essential for bioresearch.

Waters Chromatography Division
Millipore Corporation
34 Maple St.
Milford, MA 01757
(508) 478-2000

Waters
Division of MILLIPORE

Figure 2 Tricyclic antidepressants and metabolites in a single run.



A slight change in extraction solvent allows the same chromatography system to be used for the analysis of the hydroxylated metabolites of the tricyclics

Table 1: Dual wavelength ratios.

Drug	Retention Time	Ratio 214/25
E-10-OH Amitriptyline	2.49	4.16
Z-10-OH Amitriptyline	2.62	4.14
Cyclobenzaprine	2.88	1.83
Amitriptyline	2.84	4.22
Nortriptyline	5.03	4.43

The use of dual wavelength ratios increases confidence in peak identification. The ratio is determined for the pure drug and is used to verify the identity of a drug at the retention time of the tricyclic antidepressant.

Sample preparation.

1. To a 16 x 25 acid washed screw cap tube add: 0.1 ml internal standard (protriptyline 4 mg/L), 1.0 ml serum, standard or control, 0.25 ml 1M Na3CO3. Vortex 5 seconds.
2. Add 5.0 ml hexane, shake 15 minutes, centrifuge 5 minutes (3500 RPM).
3. Transfer hexane layer to a 15 x 85 acid washed tube. Evaporate to dryness at 37°C under nitrogen.
4. Redissolve in 0.5 ml of mobile phase with vortexing. Allow to stand 15 minutes and revortex.
5. Inject 50 µl into chromatograph.

Note: It is essential that the tube be acid washed.

Chromatographic conditions.

Column: CN
Temperature: 33°C
Mobile Phase: CH_3CN/CH_3OH/ 10 mM, K_2HPO_4 pH 7.0, 60/15/25
Flow Rate: 2.0 ml/min
Detection: 214 and 254 nm

Method Courtesy of Dr. James Flood and Patricia Puopolo Mass General Hospital Manuscript in preparation

This assay is not intended for in vitro diagnostic use without appropriate verification of performance characteristics.

System configuration.

A variety of analytical HPLC configurations are appropriate for this analysis. Your Waters representative can help you determine which system is best suited to your assay needs.

1. Ref. Clin. Chem. 33/6, 819-820 (1987).