T131

Essentials in bioresearch

Instrumentation, Chemistries, and Expertise for Bioseparations

910461

The Analysis of Fluoxetine in Serum by HPLC.

Rapid analysis of a new antidepressant.

A new antidepressant Fluoxetine, introduced in December of 1987, (Prozac® Eli Lilly & Co.) has rapidly become the most prescribed antidepressant drug. It has a therapeutic effect similar to the tricyclic antidepressants: imipramine, amitriptyline, and doxepin. It is believed to function through a similar mechanism by the selective inhibition of presynaptic serotonin reuptake. However, it has fewer side effects, a wider therapeutic window and a broader range of use than other common antidepressant drugs. Despite fluoxetine's rapid success, therapeutic ranges have not been established. A potential problem is the disparity between high blood levels and onset of action that may lead to discontinuation of the drug before achievement of therapeutic effect. This can be minimized through the use of therapeutic drug monitoring.

No need to wait for an assay.

The versatility and ease of methods development inherent in HPLC means that you don't have to wait to set up an assay for fluoxetine. A simple isocratic system (single solvent) with UV detection is all that is needed to perform the assay as described here. Cost per tests are low because of the use of inexpensive easily available reagents. You can now respond to physician test requests without increasing sendouts.

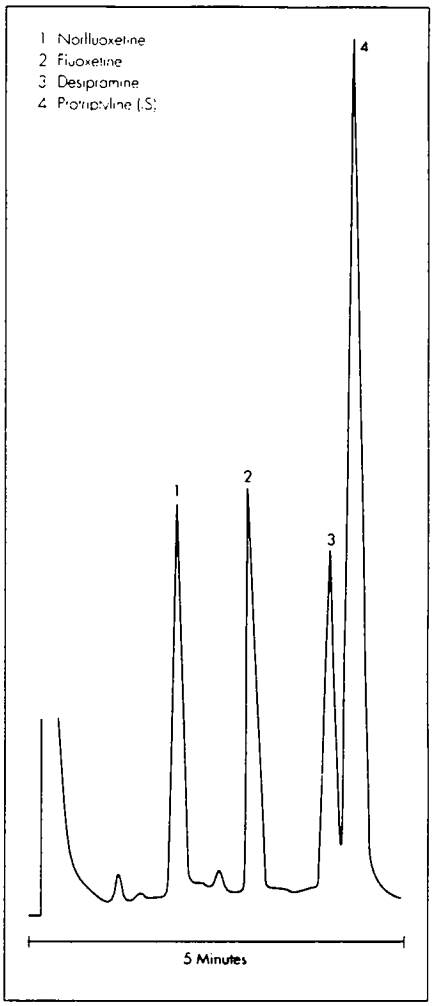
Rapid analysis of multiple antidepressants.

The simple single step extraction along with rapid chromatography (Figure 1) provides fast analysis. The same system can be used to assay the tricyclic antidepressants without switching column or reequilibration. It is a virtual random access antidepressant assay.

Positive identification of drugs and metabolites.

Fluoxetine, along with many other antidepressants, has a psychoactive metabolite, norfluoxetine, that is also quantitated with this method. When photodiode array detection is employed in the system, fluoxetine and its metabolite can clearly be differentiated from other molecules (Figure 2), in this case desipramine and protriptyline. By then running the spectra through the built-in library search routine, you can be assured of reporting the right drug.

Figure 1: The Analysis of Fluoxetine in Serum.



Fluoxetine can be easily quantitated in under seven minutes in the same system that can be used to monitor tricyclic antidepressants such as desipramine

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Sample preparation.

1. To a 16x25 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 Na_2CO_3. Vortex 5 seconds.
2. Add 5.0 ml hexane, shake 15 minutes, centrifuge 5 minutes (3500 RPM).
3. Transfer hexane layer to a 15x85 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 tubes be acid washed.

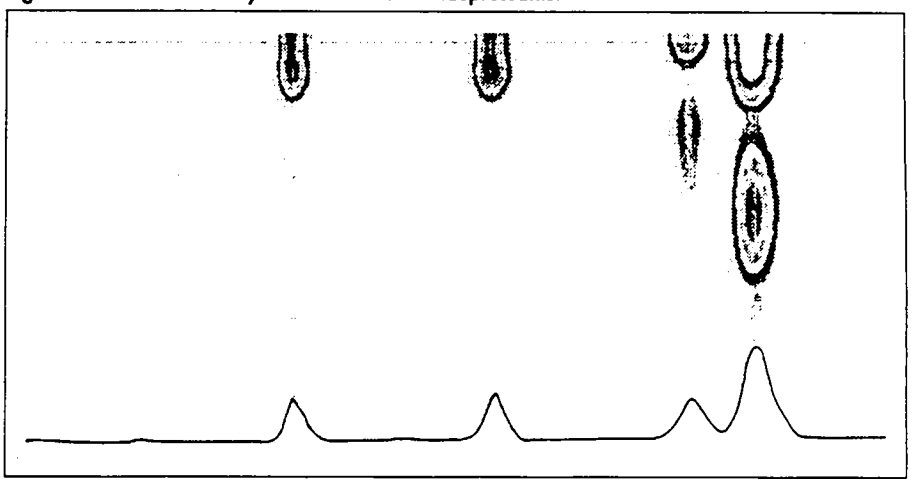
Chromatographic conditions.

Column: CN
Temperature: 33°C.
Mobile Phase: Acetonitrile/Methanol/K_2HPO_4 10 mM pH 7.0, 60/15/25
Flow Rate: 2.0 ml/min.
Detection: 214 and 254 nm or
Waters™ Photodiode Array Detector
Sampling Time: 24 msec.x16
Wavelength Range: 198-352 nm
Sense: high 3
Internal: 0.6 seconds

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

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

Figure 2: Photodiode Array Contour Plot of Antidepressants.



When the chromatogram in Figure 1 is displayed in a contour plot, it is easy to distinguish fluoxetine from desipramine and to identify norfluoxetine by its spectral similarity to fluoxetine.