

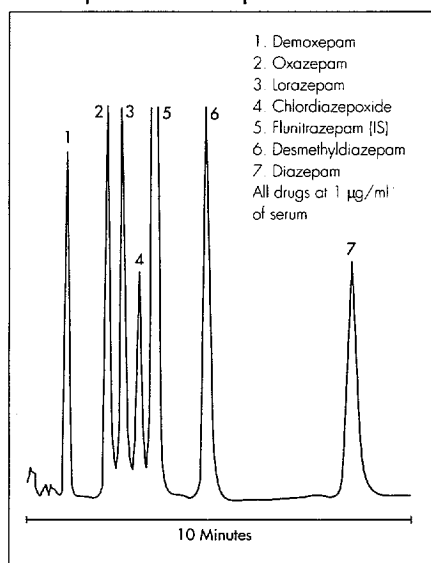
HPLC Analysis of Benzodiazepines from Serum.

Instrumentation,
Chemistries, and
Expertise for
Bioseparations

Rapid analysis of multiple benzodiazepines in a single system.

The benzodiazepines are the major treatment modality for anxiety. They are among the most commonly dispensed drugs and frequently appear in drug overdose screens. This method provides the quantitation of the parent drugs and their metabolites in a rapid (under five minutes) assay.

Figure 1: The simultaneous analysis of multiple benzodiazepines and metabolites from a spiked serum sample.



Determine the presence of alprazolam and clonazepam.

Alprazolam and clonazepam are benzodiazepines that are used chiefly as an antidepressant (alprazolam) and an anticonvulsant (clonazepam). When used therapeutically, they are typically found in serum at lower levels than other benzodiazepines. They are also found at high levels in overdose screens. With this method you have the additional compatibility of detecting alprazolam and clonazepam at overdose levels.

Sample preparation.

1. To a 16 x 100 screw cap tube add (in the order given): 0.1 ml internal standard (flunitrazepam 10 µg/ml); 0.5 ml serum, standard or control; 0.1 ml 1M Na₂CO₃. Vortex 5 seconds.
2. Add 5 ml hexane:ethylacetate (70:30). Shake 5 minutes. Centrifuge 5 minutes at 5000 RPM.
3. Transfer organic layer to a 16 x 100 tube. Evaporate to dryness at 30°C.
4. Redissolve in 0.25 ml of mobile phase.
5. Inject 20 µl into chromatograph.

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

Chromatographic conditions.

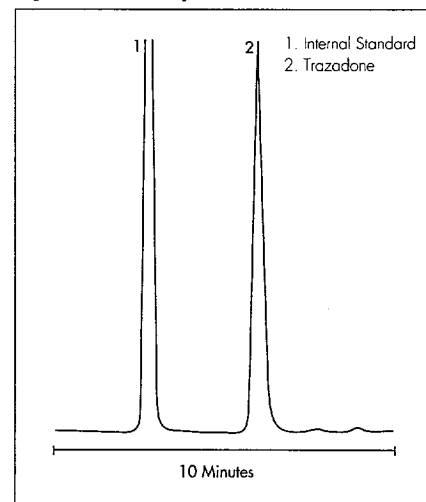
Column: Nova-Pak™ C₁₈ (3.9mm x 150mm or 3.9mm x 7.5mm)
Mobile Phase: CH₃CN/CH₃OH/
K₂HPO₄ 30 mM pH 6.3, 29.2/
9.8/61

Flow Rate: 2.0 ml/min

Detection: 214 nm

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

Figure 2: The analysis of Trazadone in serum.



The same system can be used for the analysis of trazadone and to screen for overdoses of alprazolam and clonazepam.

A versatile analysis system.

By simply switching to a Waters Resolve™ C₁₈ 8mm x 100mm cartridge, the system can perform the analysis of anticonvulsant drugs. This reduces assay changeover time and decreases turnaround time. Using the standard assay conditions, the antidepressant Trazadone which cannot be assayed in typical tricyclic antidepressant assay systems can be quantitated, eliminating the need for a separate chromatographic system.

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.

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