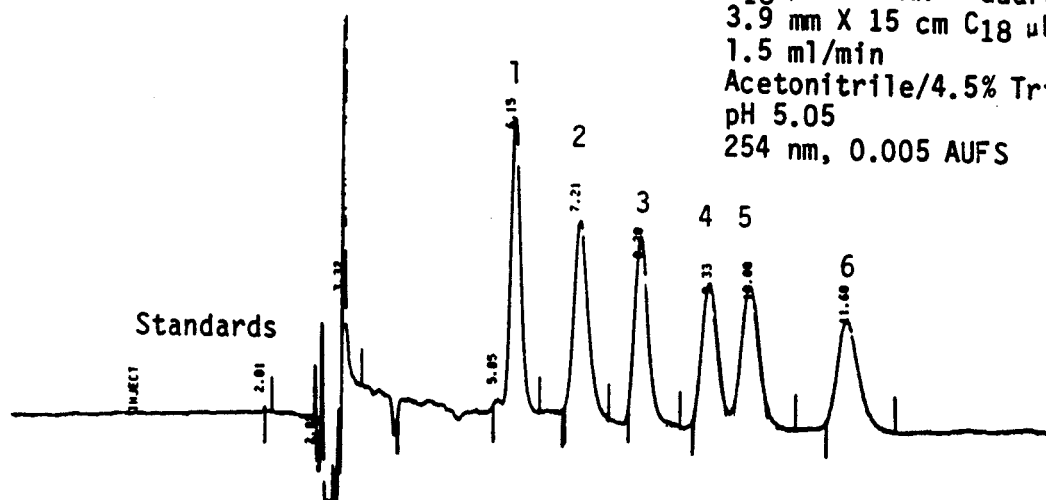


AUTOMATING ANALYSIS OF TRICYCLIC ANTIDEPRESSANTS ON μ BONDAPAK™ C₁₈ GUARD-PAK™ CARTRIDGE AND μ BONDAPAK™ C₁₈ COLUMN

The tricyclic antidepressants desmethyldoxepin, doxepin, desipramine, nortriptyline, imipramine, and amitriptyline are used in the treatment of endogenous depression. Optimal plasma levels for these drugs has not yet been established. The determination of these drugs in plasma has been performed but usually involves a liquid/liquid extraction. SEP-PAK® cartridges have been used to clean up plasma samples for tricyclic antidepressant analysis (1).

For high volume analysis, the whole process of incorporating the sample cleanup and preparation procedures used with SEP-PAK® cartridges onto a system providing automated sample cleanup and analysis can be set up using the WAVS™ Automated Valve Station.

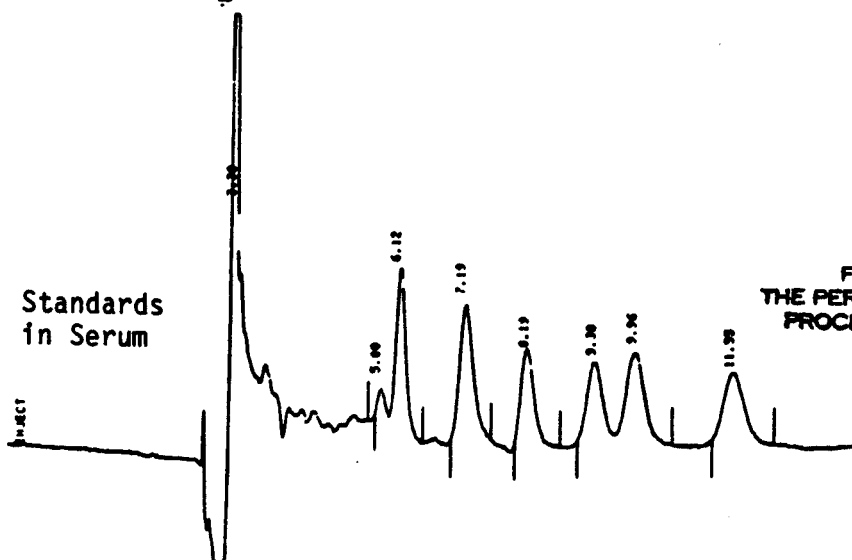
C₁₈ μ BONDAPAK™ Guard-PAK™
3.9 mm X 15 cm C₁₈ μ BONDAPAK™
1.5 ml/min
Acetonitrile/4.5% Triethylamine 33/67
pH 5.05
254 nm, 0.005 AUFS



Standards

1. Desmethyldoxepin
2. Doxepin
3. Desipramine
4. Nortriptyline
5. Imipramine
6. Amitriptyline

Standards
in Serum



FOR INVESTIGATIONAL USE ONLY.
THE PERFORMANCE CHARACTERISTIC FOR THIS
PROCEDURE HAS NOT BEEN ESTABLISHED.

Serum samples were spiked with the six tricyclic antidepressant standards, and 50 μ l injections were loaded onto a C18 μ BONDAPAKTM Guard-PAKTM precolumn insert in 100% water. The sample was eluted through the Guard-PAKTM precolumn insert and onto the analytical column using a 33/67 mixture of acetonitrile/4.5 triethylamine pH 5.05. During the analytical separation, the Guard-PAKTM precolumn insert was removed from the solvent flow for washing with 100% acetonitrile and then re-equilibrated with 100% water.

Recoveries of standards from serum exceeded 95% and retention times remained relatively constant for 16 runs (50 μ l each injection, 800 μ l total). After this amount, recovery suddenly dropped to 50% and retention times shifted. Replacing the Guard-PAKTM cartridge restored the chromatography. An increase in through-put might be accomplished by developing an appropriate washing procedure and/or using a larger capacity guard column.

The sample was injected with a weak solvent for retention on a Guard-PAKTM module or guard column (replaces the SEP-PAK^R cartridge). The retained sample can undergo the same wash cycle as a SEP-PAK^R procedure. The sample can be eluted through or backflushed off the Guard-PAKTM module or guard column and "loaded" onto the analytical column for final separation and analysis.

1. Narasimhachari, N., J. Chromatogr. 225 (1981) 189-195.