SPE-CapNMR™ Application Note

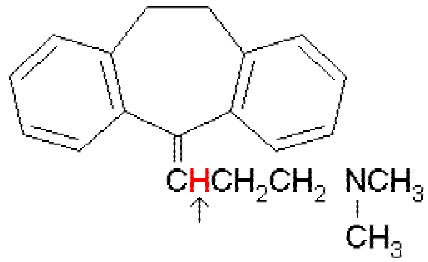
By: Aaron Wilson and David Detlefsen

A basic SPE process is described that can enrich and concentrate a sample in deuterated solvent for analysis by the CapNMR™ Probe.

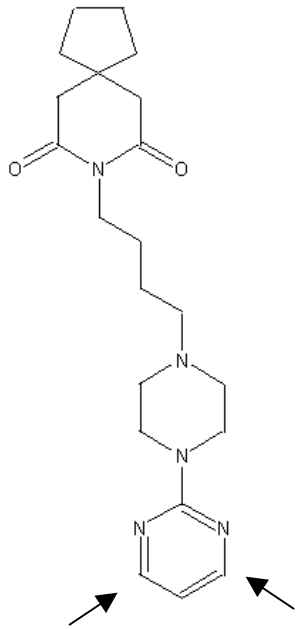
In the first case, Amitriptyline is used as a model compound in a basic SPE scheme to enrich the concentration of the sample. In the second case, Buspirone is used in a recovery scheme.

Sample Trapping Conditions

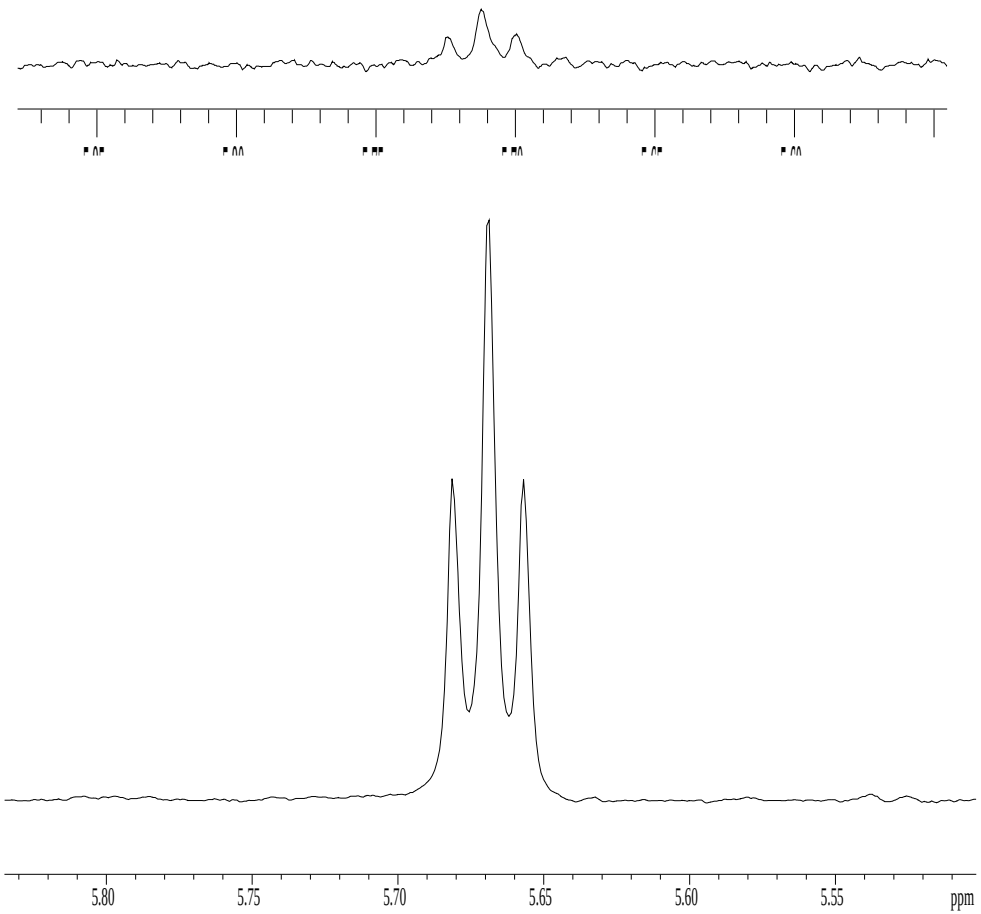
- Amitriptyline (MW = 314 g/mole)
  - Use Michrom BioResources microtrap
  - Pre-wash with 250 μL H_2O
  - Condition with 500 μL Methanol
  - Equilibrate with 500 μL of D_2O with 1.2% HFBA
  - Load 500 μL of Amitriptyline (20 ng/μL; 64 μM) in H_2O
  - Elute three 8-μL fractions of Methanol-d_4
  - CapNMR™ Analysis
    - Triplet at 5.7 PPM



- Buspirone (MW = 386 g/mole)
  - Use Michrom BioResources microTrap
  - Clean trap with 100 μL acetonitrile
  - Equilibrate with 100 μL sample solvent with 0.05% HFBA
  - Load sample dissolve in solvent with 0.05% HFBA
  - Dry with 500 μL air injections
  - Elute with 15 μL Acetonitrile- d_3
  - CapNMR™ Analysis
    - Doublet at 8.39



Sample Trapping - Amitriptyline



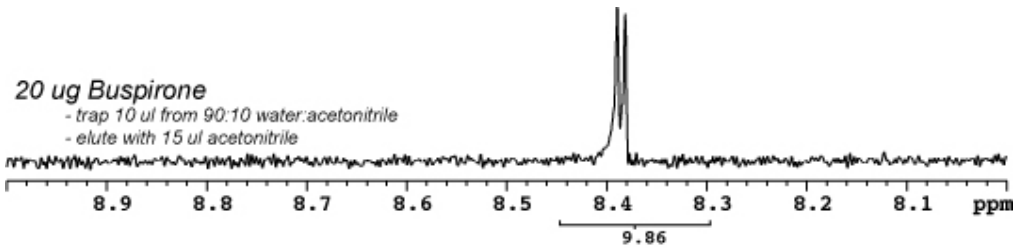
Before Trapping

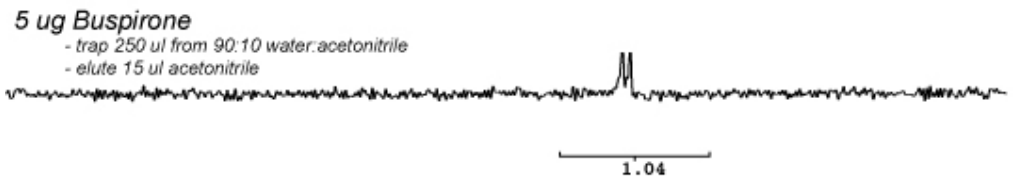
- Amitriptyline (256 Scans in 8.5 min)
- 20 ng/μL sample solution
- 8 μL manually injected into probe
- ~0.1 μg in V_{obs} of CapNMR™ probe
- S/N = 6.5

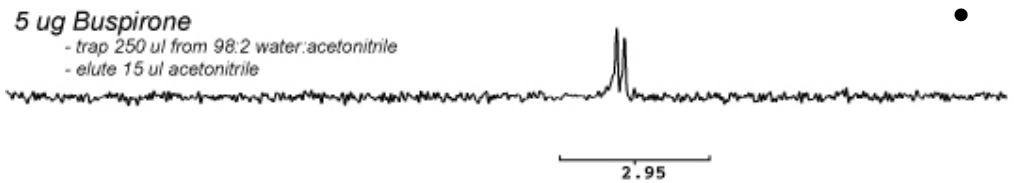
After Trapping

- Amitriptyline (256 Scans in 8.5 min)
- Other conditions same as above
- SPE fraction: 2nd 8-μL elution
- 8 μL manually injected into probe
- ~2 μg in V_{obs} of CapNMR™ probe
- S/N = 132 (20 fold enrichment)

Sample Trapping - Buspirone







- Reference: Trap from 90:10
  - 20 ug loaded onto trap
  - 10 μL of 5.2 mM solution
  - 128 scan in 4.5 min at 600 MHz
  - 10 μL manually injected into probe
  - Relative peak area 9.86 (100%)
- Trial 1: Trap from 90:10
  - 5 ug loaded onto trap
  - 250 ul of 52 uM solution
  - 128 scan in 4.5 min at 600 MHz
  - 10 μL manually injected into probe
  - Relative peak area 1.04 (42%of reference)
- Trial 2: Trap from 98:2
  - 5 ug loaded onto trap
  - 250 μL of 52 uM solution
  - 128 scan in 4.5 min at 600 MHz
  - 10 μL manually injected into probe
  - Relative peak area 2.95 (120% of reference)

Sample Trapping Summary

- Preconcentration simplifies trace NMR analysis
  - Presents opportunities for on-line preconcentration
  - Michrom Trap Cartridges
    - enable microscale sample preconcentration
- Efficient Sample Preconcentration
  - 20 fold enrichment
  - 50% or greater recovery
- Trapping Solution Dependencies
  - Aqueous:organic ratio affects recovery
  - HFBA ion pairing agent important