

CapNMR™ Probe Fluidic Option: Manual Sample Injection

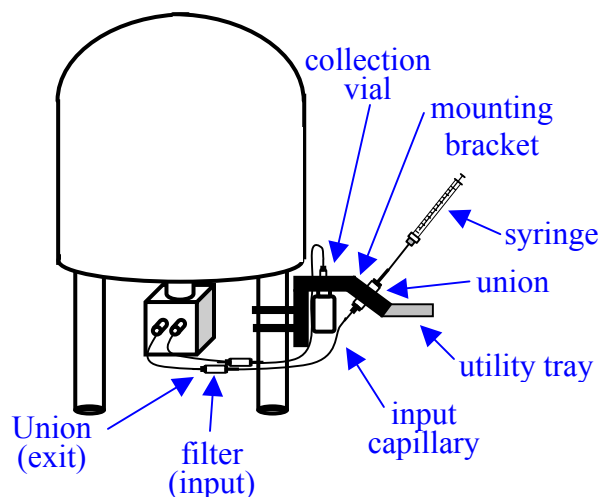
Protasis/MRM Technical Applications Note NMR-10

Overview

This application note pertains to manual sample injection (MSI) into the CapNMR probe using a hand-held syringe. This method of sample management is both simple and economical to implement allowing a novice user to readily infuse 10 μL of sample. Sample volumes as small as a few microliters (1-3 μL) can be accommodated with only a slight change in the loading technique. Protasis/MRM offers well plate or vial format (VAST™, BEST™) automation and chromatographic hyphenation sample management options for users who desire greater system versatility.

Hardware

Protasis/MRM offers kit MSI-01 to accommodate manual sample injection. The kit includes a non-magnetic and easily transportable mounting bracket that attaches via quick connect/disconnect straps to the leg of the magnet. The bracket houses ports for syringe injection, sample recovery and waste collection following NMR analysis. The components included in the kit are detailed in a table on the reverse side of this application note.



Injection¹ of Sample into CapNMR Probe

For beginning users, Protasis/MRM recommends use of 100 μg of sample in 10 μL of solvent². This quantity completely fills the probe and provides ample S/N to carry out single and multi-dimensional NMR experiments. Sample is drawn into a gas-tight (Teflon-tipped plunger) syringe, after which the sample vial is secured onto the mounting bracket for re-collection. The syringe is inserted into manual sample injection port. With the needle securely in the manual sample injection port, the sample is slowly infused into the probe. A safe target injection rate of approx. 1 $\mu\text{L}/\text{sec}$ should be used, requiring 10 sec for full injection of 10 μL . An in-line filter is used on the input capillary to prevent particulates from entering the CapNMR probe. The exit capillary is routed to the original sample vial for full sample recovery (or to waste, at the user's discretion). Capillary input and exit unions facilitate easy mechanical disconnection of the probe and capillary feed lines from the probe upon probe removal. NMR data acquisition can now begin.

Injection of Sample Volumes < 10 μL that do not completely fill the probe

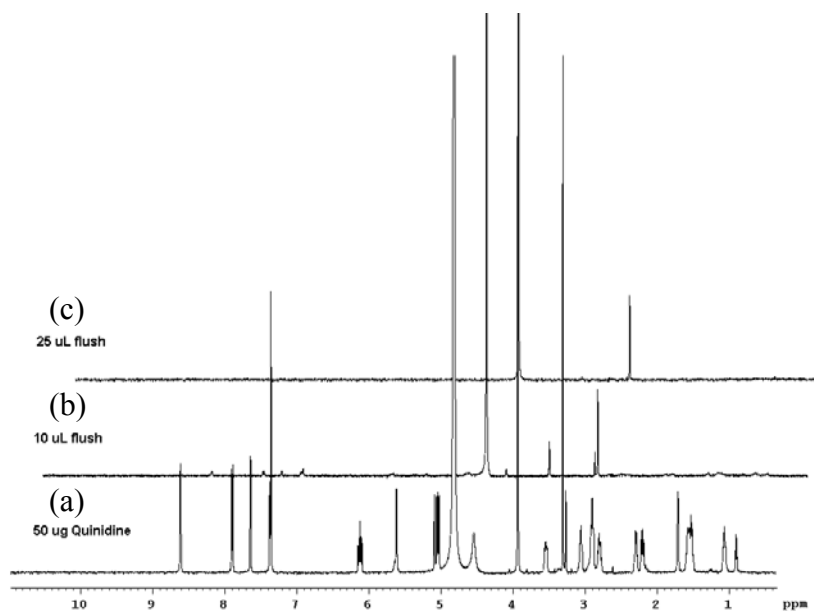
Two 10 μL gas-tight syringes are needed. Fill one 10 μL syringe with 8-9 μL of solvent, being sure to expel all bubbles. This will be used as the "push" syringe. Rinse the other 10 μL syringe with solvent, expelling all bubbles, and retain 0.5 μL of solvent in the syringe. This will be used as the "sample" syringe. Draw up 5 μL of sample into this syringe. Inject contents into the probe using techniques previously described. Using the push syringe, inject an additional 4 μL of solvent. This will place the sample into the flow cell.

¹ Protasis/MRM recommends that the probe be fully evacuated before a new solvent is introduced into the probe. The procedure for evacuation is provided in this application note.

² Protasis/MRM recommends that sample concentrations be maintained at less than 25 $\mu\text{g}/\mu\text{L}$ unless additional rinsing is employed.

Flushing of Sample from CapNMR Probe

Protasis/MRM recommends the use of 50 μL of rinse solvent to fully evacuate the sample from the probe. This will result in less than 1% sample carry-over. Rinse solvent is drawn into a 50 μL gas-tight syringe and infused into the probe. If sample recovery is required, a recovery vial must be employed. Shown in the figure to the right are data from a 50 μg injection of quinidine into the NMR flowcell. NMR data was collected: a) with the sample in the flowcell, b) after 10 μL of methanol was used to flush the sample from the probe, and c) after an additional 15 μL of methanol was used. While trace amounts of sample can be observed after a 10 μL flush, virtually no sample ($\ll 1\%$) can be observed after a 25 μL flush.



Data courtesy of Mark O'Neil-Johnson, Sequoia Sciences

Solvent Changeover

When changing over from solvent #1 to solvent #2, Protasis/MRM recommends using the 50 μL syringe to:

- 1) Flush the probe with 50 μL of solvent #1 (expelling any residual sample).
- 2) Evacuate the probe using air (expelling solvent #1).
- 3) Flush the probe with 50 μL of deuterated acetone.
- 4) Evacuate the probe using air (expelling the acetone).
- 5) Flush the probe with 50 μL of solvent #2.

Sample injection can subsequently proceed.

Component Description

The components necessary for manual sample injection are included in kit #MSI-01 from Protasis/MRM. These components are detailed in the table below.

Component	Description	Qty.
<i>Syringes/Needles</i>		
Alltech 80065	Hamilton 10 μL gas-tight syringe with Teflon-tipped plunger and 22s 2'' blunt-tip point style 3 removable needle	2
Alltech 80930	Hamilton 50 μL gas-tight syringe with Teflon-tipped plunger	1
Alltech 80464 ³	22s 2'' blunt-tip point style 3 needle (to be used with 80930)	1
<i>Fittings/Filters</i>		
ZBU1XC	Valco Injection Union	1
P-779-01	Upchurch Union	2
F-331N	Upchurch 10-pk Nanotight nuts and ferrules for P-779-01	1
F-247	Upchurch 10-pk sleeve to engage 22s needle in ZBU1XC	1
F-242	Upchurch 10-pk sleeve to engage 360 capillary in F-331N and ZBU1XC	1
F-192	Upchurch 10-pk ferrule to secure F-247 in ZBU1XC	1
F-287	Upchurch 10-pk nut to secure F-247 in ZBU1XC	1
M-531	Upchurch filter	1
<i>Accessories</i>		
B-100	Mounting bracket for MSI	1
Capillary ⁴	50/360 fused silica capillary, 50cm length	2
Recovery Vial	Waters total recovery vial 0.8mL, P/N 186000384	1

³ The 80464 22s needle has a volume of approximately 0.9 μL

⁴ 50/360 capillary has a capacity of approximately 2 μL /meter of length.