

Metabolite Analysis: Mouse Urine

Nuclear Magnetic Resonance Spectroscopy is an important technique for the analysis of biological fluids in life science applications such as metabolomics, metabolite identification, biomarker analysis etc. Flow-NMR techniques are ideal in these applications for high-throughput screening and hyphenation with other analytical methodologies such as chromatography and mass spectrometry. Enhanced mass sensitivity, superior fluidic performance, reduced cost, and greater speed are possible using capillary-scale, microflow NMR due to the reduced sample and solvent volumes required, enabling analysis of fluids from smaller animals or any fluid that is only available in small volumes. The direct NMR analysis of mouse urine using the Protasis/MRM MicroFlow NMR™ probe is shown in this application note.

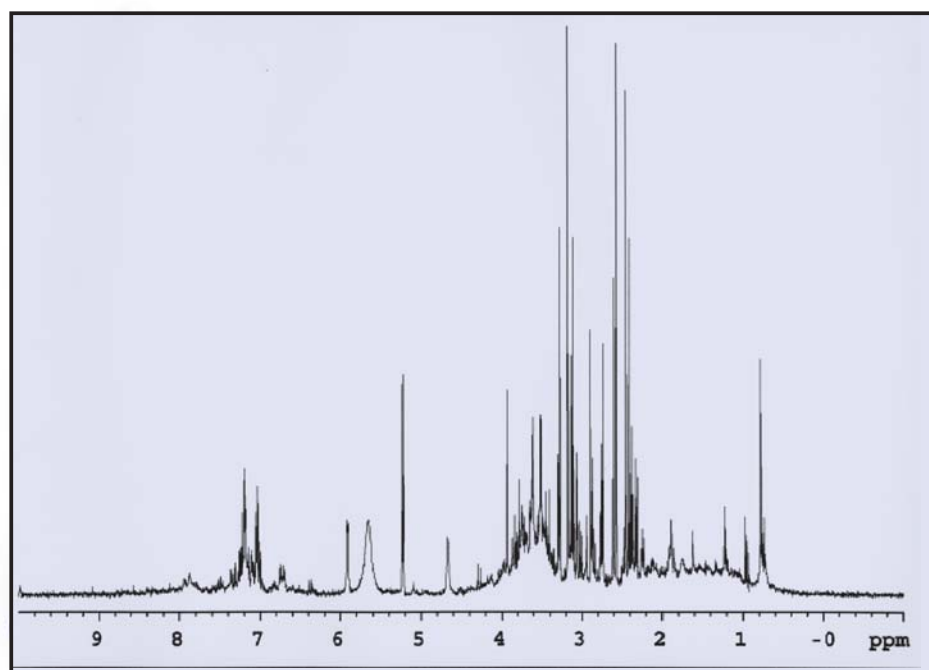


Figure 1. Mouse Urine - Flow Injection

- 1D Presat (500 MHz)
- Neat with 5% D₂O
- 5 μ l in flow cell
- 16 Scans / 41 seconds

The low volume of urine generated by small animals, such as the mouse, is a challenge for conventional NMR. The metabolic profile may need to be averaged across an excessive time period or the urine sample may have to be diluted, adversely impacting NMR acquisition time. The high-resolution proton spectrum of mouse urine shown in Figure 1 was obtained in 41 seconds using a 1.5 μ L (active cell volume) Protasis/MRM CapNMR™ probe installed on a Varian INOVA 500 MHz system. In this experiment, the entire probe (volume 7 μ L) was filled with the mouse urine. Sophisticated statistical analysis and data mining can be applied to the resulting high-resolution proton spectrum to identify metabolites or monitor a physiological effect.

Mouse Urine Analysis

Demanding, multiple-pulse two-dimensional experiments, such as TOCSY (proton-proton correlation), are also possible on small-volume biological samples using the CapNMR probe. The selective TOCSY of the same urine sample, acquired in one hour, is shown in Figure 2. The sequential TOCSY experiments may allow one to identify or time profile high-concentration metabolites present in the urine.

By interfacing MicroFlow NMR with capillary liquid chromatography biological samples can be simplified into less complicated fractions, enabling, for example, sample preparation to be combined with metabolite identification. The proton spectrum of a 1 μ L injection of neat urine onto a capillary HPLC column is shown in Figure 3, illustrating a clean-up or fractionation of the urine prior to NMR analysis. The urine sample was parked in the flow cell of the probe for two minutes while data was collected. This analysis highlights the sensitivity of capillary LC-NMR analysis and the capability to sift through a complex mixture in order to examine minor components.

In summary, the Protasis/MRM CapNMR probe is an easy-to-use probe for standard NMR experiments, extending NMR analysis to small-volume biological samples without dilution. It is an enabling technique for the study of physiological changes in the fluids of small animals. Enhanced mass sensitivity, reduced cost, greater speed of analysis, and the superior fluidics of the CapNMR probe technology make this possible.

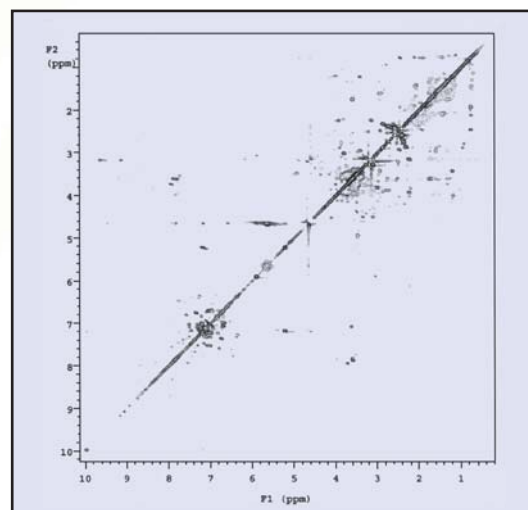


Figure 2. Mouse Urine - Flow Injection

- TOCSY (500 MHz)
- Neat with 5% D₂O
- 5 μ L in flow cell
- 4 scans / 256 increments
- 1 hour acquisition

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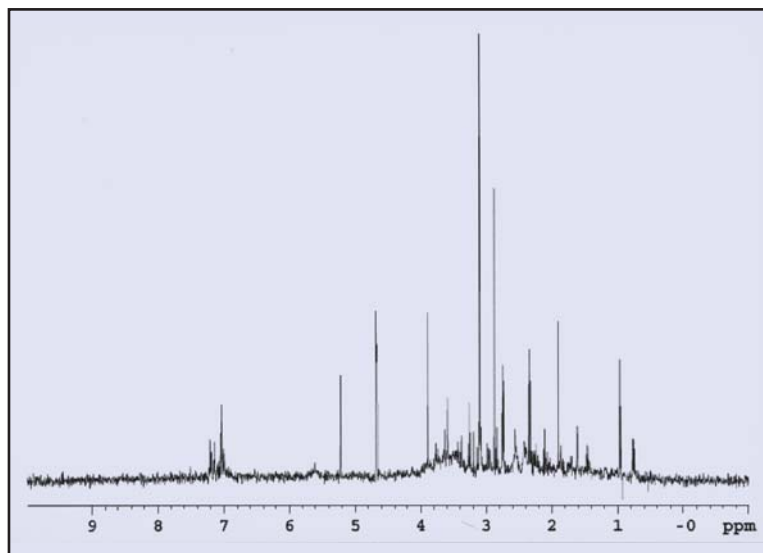


Figure 3

Sample Cleanup

- 1D Presat (500 MHz)
- 1 μ L inject CapLC
- 5 μ L in flow cell
- 32 Scans / 2 min