

From 600 to 100 MHz: Developing Reliable Benchtop NMR Protocols for Urine Metabolomics

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Metabolomics is the comprehensive analysis of low molecular weight metabolites in a biological system, providing insight into health and disease by reflecting genetic and environmental interactions(1). Among analytical techniques, Nuclear Magnetic Resonance (NMR) spectroscopy is valued for robustness, reproducibility, and minimal sample preparation. High-field NMR spectrometer operating at 600 MHz remains the gold standard(2), but its high cost and infrastructure requirements limit clinical use. Portable low-field benchtop NMR systems offer a promising cost-effective alternative(3).

We developed standardized protocols for urine metabolomics combining high-field (600 MHz) and low-field (100 MHz) NMR spectroscopy. To overcome solvent suppression issues and low sensitivity of low-field NMR instruments, urine samples were lyophilized and reconstituted in D₂O, enabling water removal and sample concentration.

This work presents optimized procedures for urine sample preparation, lyophilization, and NMR acquisition. Urine samples were systematically evaluated for lyophilization time, storage conditions, and reconstitution protocols to establish a robust and reproducible workflow.

Samples were acquired at both low- and high-field NMR to validate protocol reliability. Statistical analyses identified the most suitable strategy. Lyophilization for 18-24 h was sufficient, whereas 6 h was inadequate. A 3 M KH₂PO₄/K₂HPO₄ buffer in D₂O improved pH stabilization across samples. Vortexing followed by centrifugation under controlled conditions was optimal, and acquisition and spectral processing parameters were refined.

These results provide a reliable methodological framework for benchtop NMR metabolomics and support integrating low-field NMR systems into routine workflows.

References

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3. L. Fantato *et al.*, *Metabolites.* **16**, 3 (2025).