Push-through Direct Injection NMR: an optimized automation method applied to metabolomics†

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†Electronic Supplementary Information (ESI) available.

Electronic Supplementary Information

Table 1(ESI). Tissue extraction procedure

1.	Add bearing (3.2 mm, BioSpec Products, Bartlesville, OK, USA) to 17 Eppendorf tubes (Safelock®, Eppendorf, Hauppauge, NY, USA).
2.	Add ~ 200 mg chicken liver to each tube in a cold rack.
3.	Add 400 μL ice cold methanol (HPLC grade) and 85 μL ice cold deionized water (dH_2O) to each tube.
4.	Place tubes in a tissuelyzer (Qiagen, Valencia, CA, USA) and run 10 minutes at 15 Hz.
5.	Remove and centrifuge at 1,000 g briefly (~1 min.) at 4 ºC to bring solvents down from cap.
6.	Place tubes back into the cold rack.
7.	Add 200 μL ice cold chloroform (HPLC grade) and run 20 minutes at 15 Hz.
8.	Centrifuge briefly again.
9.	Add 200 μL ice cold chloroform and 200 μL ice cold dH_2O, and run 10 minutes at 15 Hz.
10.	Centrifuge at 1,000 g for 15 minutes at 4 ºC. The solutions separate into an upper methanol/water phase (polar metabolites) and a lower chloroform phase (lipophilic metabolites) separated by protein debris.
11.	Using a pipette, carefully remove upper phase leaving a small amount behind (to avoid protein contamination) and dispense into a labeled 2 mL cylindrical microfuge tube.
12.	Dry samples in a vacuum concentrator (Thermo Scientific, Asheville, NC, USA) without radiant heat for 6 hours.

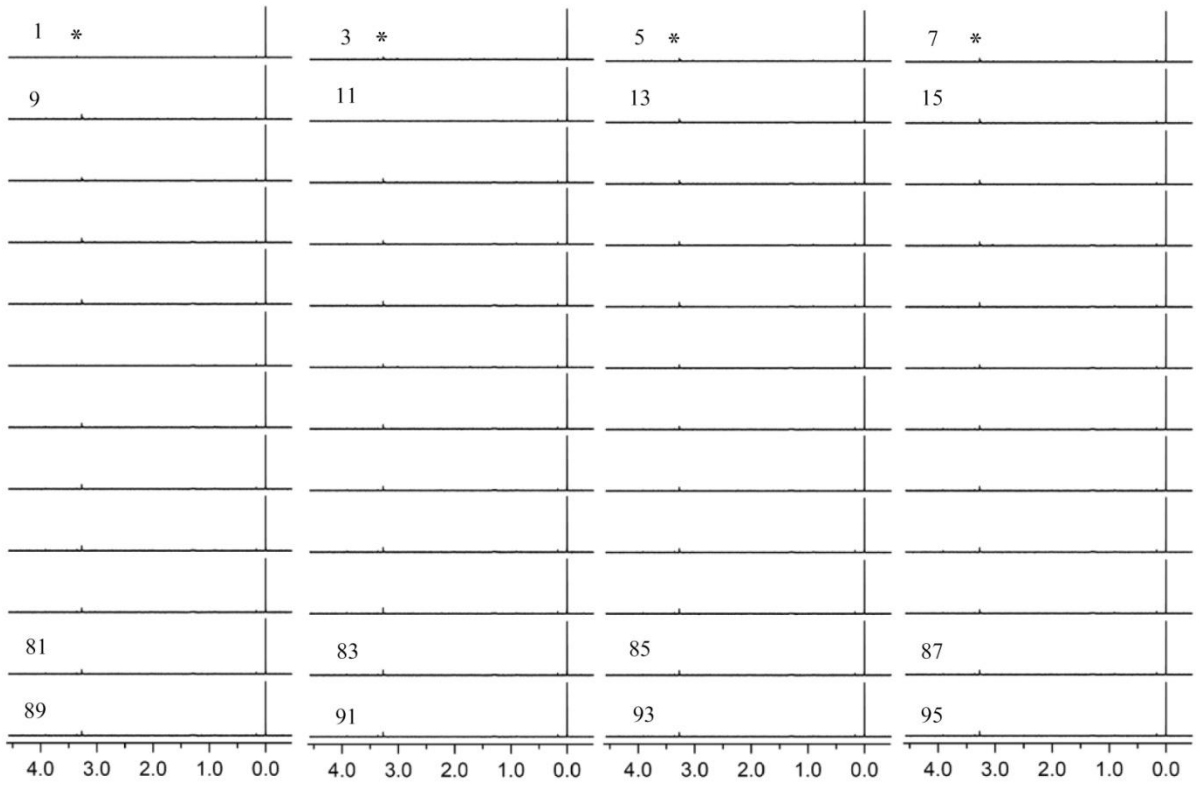

Figure 1(ESI). ^{1}H NMR spectra of all 48 phosphate buffer samples from well 1 to 95 as labeled. The peak at 0.0 ppm is from TSP (50 µM). None of the spectra showed detectable carryover peaks, indicating that there was no effective carryover from any tissue samples (in even number wells) to phosphate buffer samples. Note that the peak at 3.35 ppm (*) is due to a small but ubiquitous occurrence of methanol.

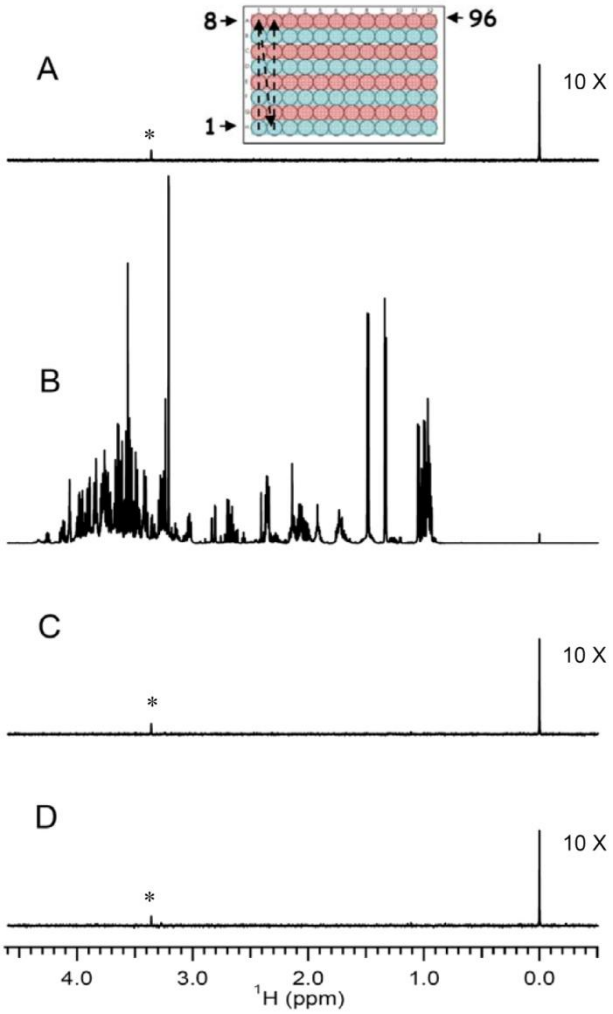

Figure 2 (ESI). ^{1}H NMR spectra of tissue extracts and phosphate buffer samples from the PT DI NMR method using syringe air (instead of compressed air) to flush out residual wash. A total of 1.3 mL $^{2}H_2O$ was used to wash the flow line (single wash) followed by injecting 3 mL air with the syringe in the final step of the wash procedure. This version of push-through DI NMR was not as efficient as the one using compressed air to clean the entire flow line, which required only 0.65 mL wash solvent. Spectra from: (A) buffer in sample well 1, (B) tissue extract aliquot in sample well 2, (C) buffer in sample well 3, and (D) last buffer in sample well 95. The vertical scale of buffer spectra was expanded by a factor of 10, relative to that in (B). The insert illustrates the sample run sequence of tissue extract aliquots and phosphate buffer samples in the 96-well plate, where odd numbered wells contained phosphate buffer samples and even numbered wells contained the tissue extracts. The peak at 3.35 ppm (*) is due to a small but ubiquitous occurrence of methanol.