



## Supplementary Materials

# Hydrophilic Interaction Liquid Chromatography–Hydrogen/Deuterium Exchange–Mass Spectrometry (HILIC-HDX-MS) for Untargeted Metabolomics

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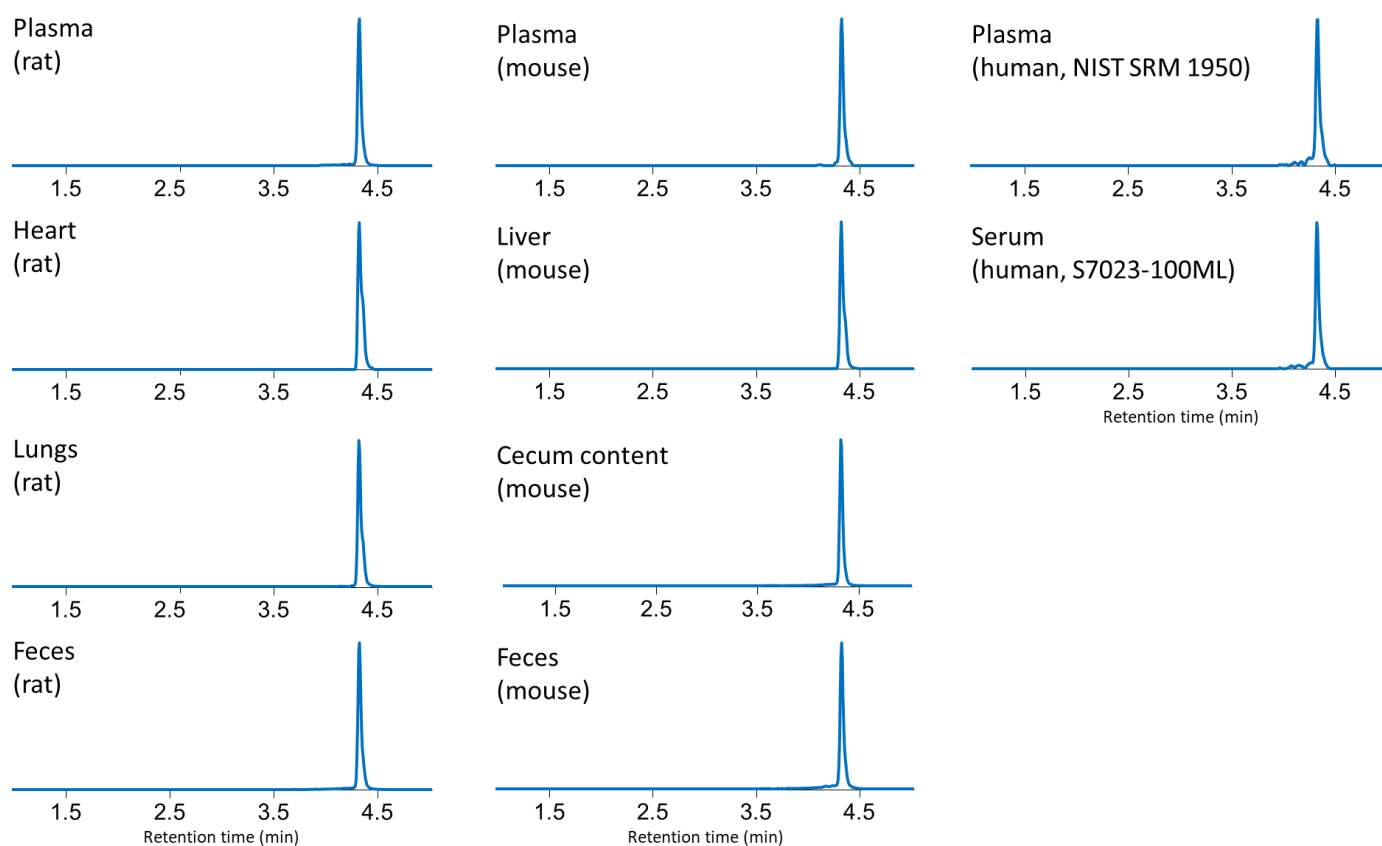
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**Figure S1.** Extracted ion chromatograms of an unknown metabolite (4.3 min;  $m/z$  188.1757) detected in different biological matrices and of different origins (rat, mouse, human).

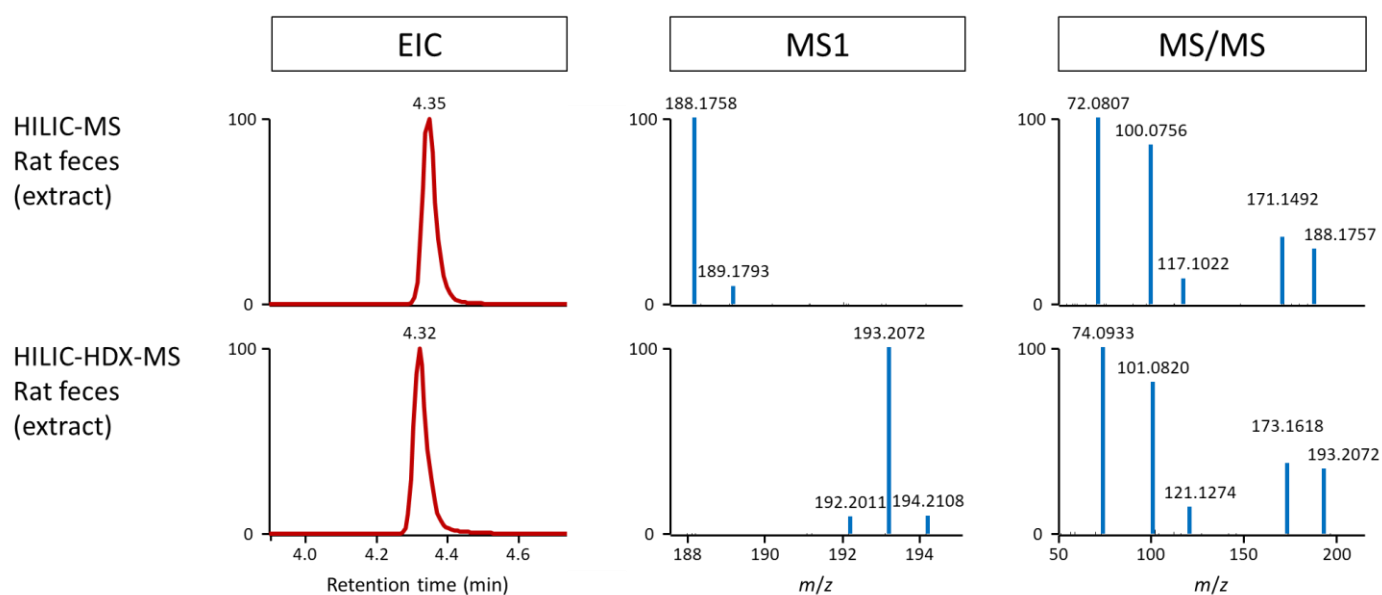
**Figure S2.** Extracted ion chromatograms (EICs) and MS1 and MS/MS spectra of  $N^1$ -acetylspermidine in rat feces under conventional HILIC-MS and full HILIC-HDX-MS conditions. In HILIC-MS, the EIC at  $m/z$  188.1757, corresponding to  $[M(H_4)+H]^+$ , is displayed. Conversely, in HILIC-HDX-MS, the EIC at  $m/z$  193.2071, corresponding to  $[M(D_4)+D]^+$ , is shown. MS/MS spectra were acquired at stepped normalized collision energies of 20, 30, and 40%.

**Figure S3.** Extracted ion chromatograms (EICs) and MS1 and MS/MS spectra of  $N^1$ -acetylspermidine in human plasma (NIST SRM 1950) under conventional HILIC-MS and full HILIC-HDX-MS conditions. In HILIC-MS, the EIC at  $m/z$  188.1757, corresponding to  $[M(H_4)+H]^+$ , is displayed. Conversely, in HILIC-HDX-MS, the EIC at  $m/z$  193.2071, corresponding to  $[M(D_4)+D]^+$ , is shown. MS/MS spectra were acquired at stepped normalized collision energies of 20, 30, and 40%.

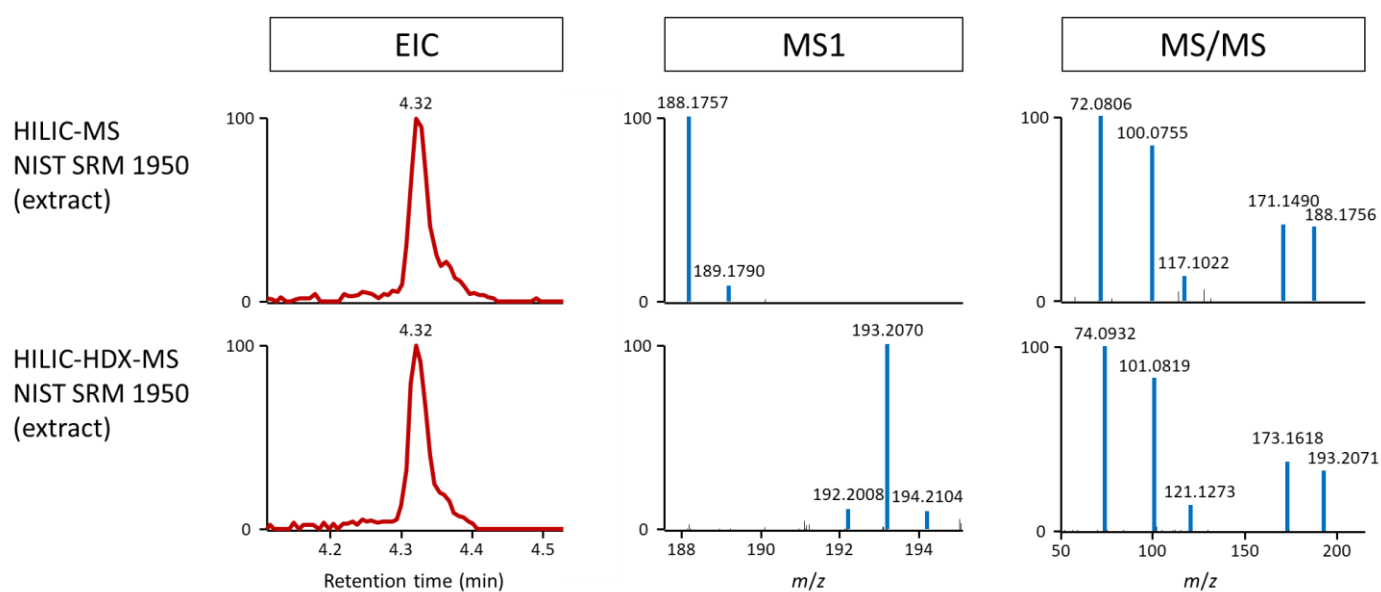
**Figure S4.** MS/MS spectra of (a) unknown metabolite (precursor ion  $m/z$  188.1757) in mouse feces extract, (b)  $N^1$ -acetylspermidine standard (precursor ion  $m/z$  188.1757), and (c)  $N^8$ -acetylspermidine standard (precursor ion  $m/z$  188.1757). MS/MS spectra were acquired at stepped normalized collision energies of 20, 30, and 40%.



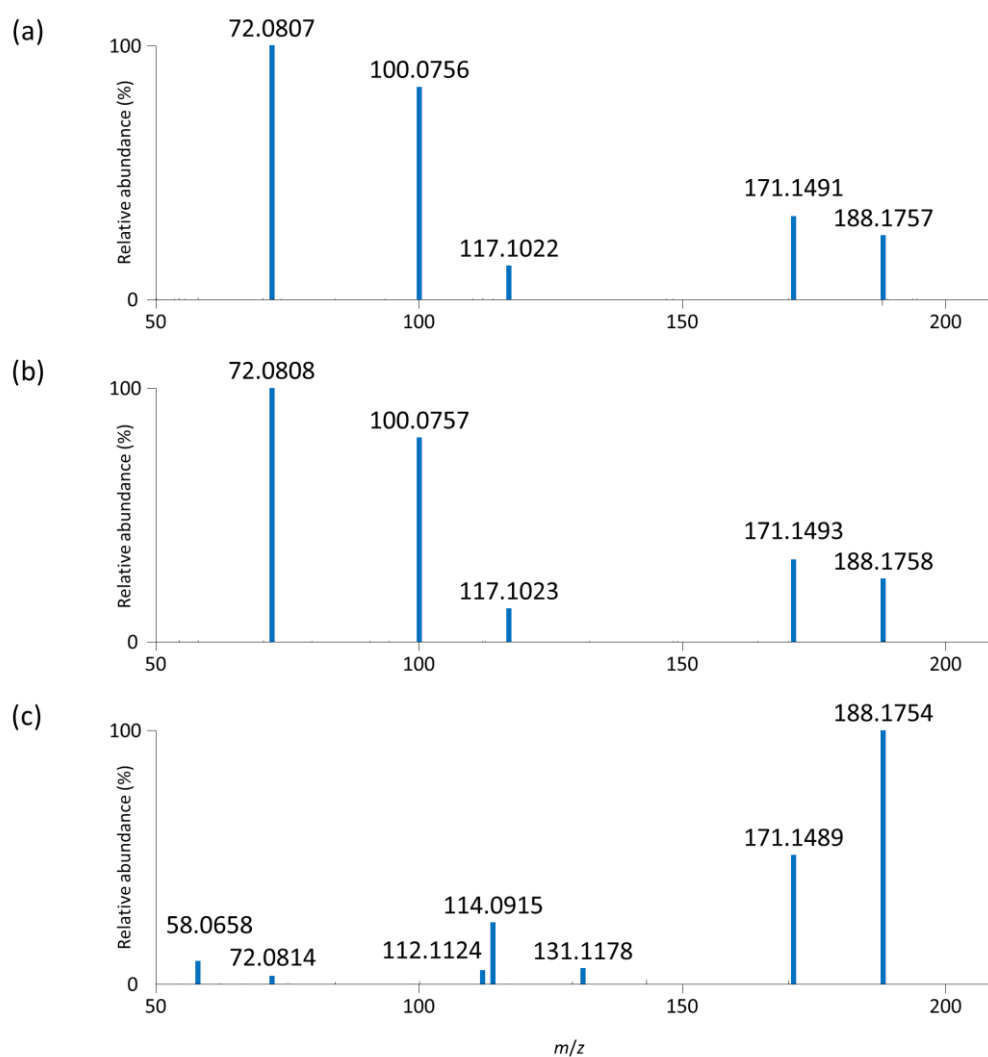
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