

## **Supplementary Information**

# **Isotopically Selective Quantification by UPLC-MS of Aqueous Ammonia at Submicromolar Concentrations using Dansyl Chloride Derivatization**

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## Experimental

**Materials:** NaCO<sub>3</sub> (anhydrous powder, ≥99.5%, ACS grade, EM Science), <sup>14</sup>NH<sub>4</sub>Cl (≥99.7%, ACS Reagent, Baker Analyzed), <sup>15</sup>NH<sub>4</sub>Cl (<sup>15</sup>N ≥ 99%, Cambridge Isotope Laboratories, Inc), sulfuric acid (1.0 N, laboratory reagent, VMR Chemical), acetonitrile (UPLC grade, Fischer Chemical), dansyl chloride (≥99.0%, for HPLC, Sigma Aldrich). High-purity deionized water (measured resistivity ≥ 18.2 MΩ cm at room temperature, by Milli-Q purification system) was used in all experiments.

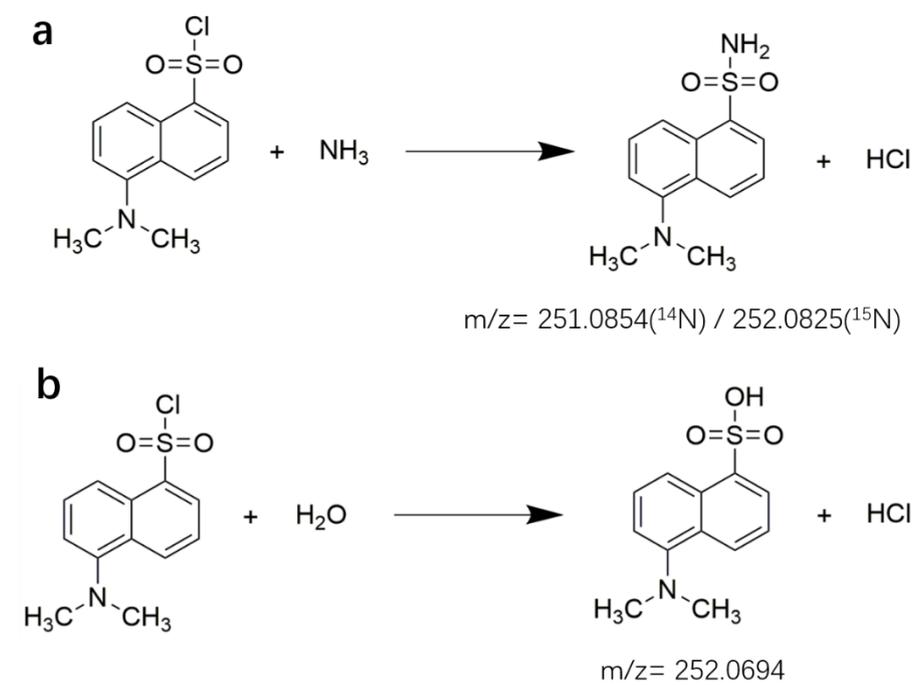
**UPLC-MS analysis:** UPLC-MS analyses were performed on an Acquity I-Class UPLC coupled to a Xevo G2-S Quadrupole Time-of-Flight (QToF) mass spectrometer (both from Waters Corporation, Milford MA). 3 μL of the final derivatized solution was injected for separation on a Waters BEH C18 column (2.1 x 100 mm, 1.7 μm) with mobile phase flowing at 0.300 mL/min. Initial composition was 90 % water. At 0.25 minutes acetonitrile was increased linearly to 90 % at 3.25 minutes and maintained for 0.5 minutes. Acetonitrile was linearly decreased back to 10 % at 4.00 minutes and held for 0.2 minutes before the next run was started. Ionization was performed in a Waters atmospheric pressure ionization source operated in electrospray ionization mode. The capillary voltage was 3.0 kV, sampling cone 40 V, source temperature 120 °C, source offset 80 V, desolvation temperature 450 °C, cone gas flow 30 L/hr and desolvation gas flow 800 L/hr. The TOF was operated in resolution mode using the 120.0813 m/z fragment of leucine enkephalin as a lock mass ion and data was recorded from 50 to 500 m/z.

**Dansyl chloride derivatization:** dansyl chloride derivatization processes were performed by preparing solution mixtures in 2 mL glass or plastic vials in the following order: 0.4 mL of ~290 μM dansyl chloride dissolved in acetonitrile; 0.3 mL of sodium carbonate stock solution (2 g/100 mL); and 0.4 mL of a NH<sub>4</sub>Cl solution in 50 mM H<sub>2</sub>SO<sub>4</sub>(aq). The vials were capped and vortexed for 10 s before

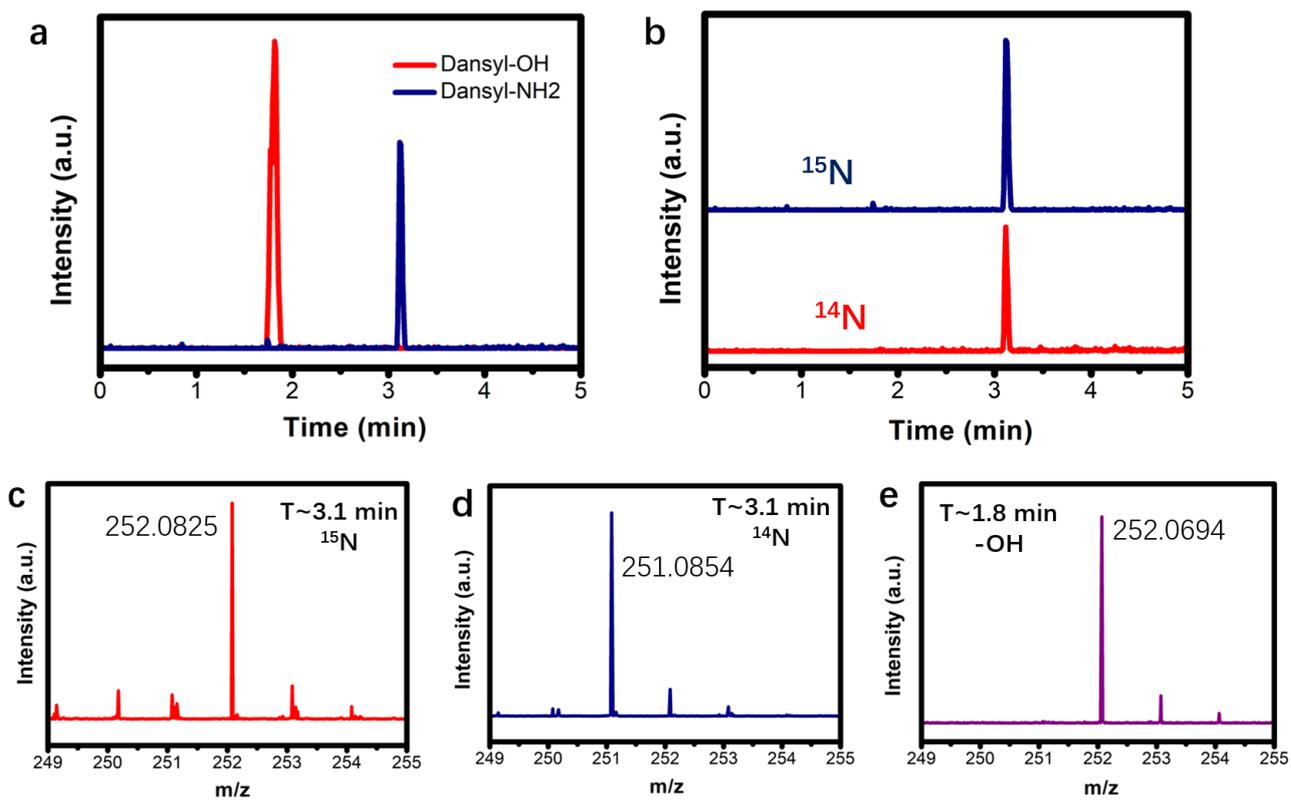
being loaded onto the autosampler plate for analysis. The derivatization conditions were extensively optimized; for example, the optimal pH value of the resulting mixtures was  $\sim 9.5 \pm 0.5$ , and no heating was required. The derivatization products (DNS-NH<sub>2</sub>) were separated by reverse-phase chromatography using a binary water/acetonitrile solvent with a steady gradient. Each sample was measured three times consecutively. The concentration of the derivative was quantified by the MS peak area at specific mass positions ( $m/z=251.0854$  for <sup>14</sup>N and  $m/z=252.0825$  for <sup>15</sup>N). The mass windows were 0.01  $m/z$  for this instrument. Data analysis was performed using Quanlynx software (Waters Corporation).

It is important to note that the product molecule containing <sup>14</sup>N from ammonia and one <sup>13</sup>C atom appears at  $m/z$  252.0888, or 0.0063  $m/z$  away from <sup>15</sup>N. Care must be taken that adequate mass resolution is maintained, especially if the concentration of <sup>14</sup>N ammonia is more than several times the <sup>15</sup>N concentration.

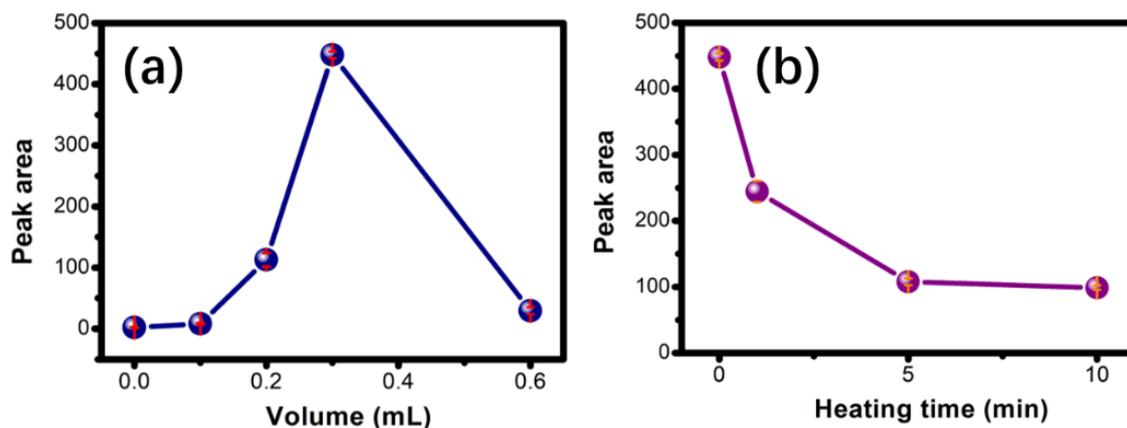
**Measurement of ambient ammonia contamination:** Ambient ammonia-trapping experiments were performed by leaving 0.4 mL of <sup>15</sup>N-labelled ammonium solutions ( $\sim 10 \mu\text{M}$ ) open for 6 h in vials placed in labs and/or restrooms. The derivatization procedures were then performed and the vials were capped and transferred for UPLC-MS analysis. During the course of ammonia-trapping experiments, a control sample was prepared by leaving the same volume of <sup>15</sup>N-labelled ammonia solution in place with the vial capped.



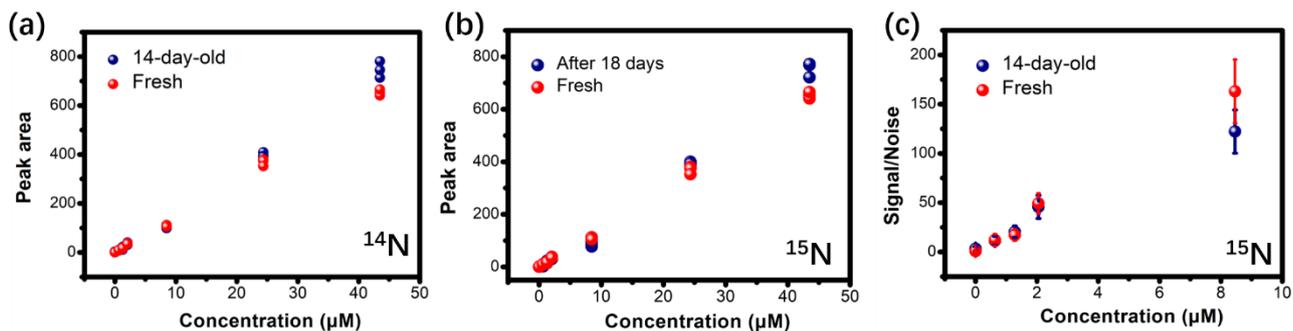
**Figure S1.** Reactions of dansyl chloride molecules with (a) ammonia to form DNS-NH<sub>2</sub> and (b) water to form DNS-OH and a comparison in molecular masses between DNS-NH<sub>2</sub>/DNS-OH



**Figure S2.** (a) Comparison of mass-selected chromatographs for DNS-OH (red) and DNS-NH<sub>2</sub> (blue) products; (b) comparison of mass-selected chromatographs for DNS-NH<sub>2</sub> labelled with <sup>14</sup>N or <sup>15</sup>N, respectively; (c-e) comparison of representative time-integral mass spectra for (c) DNS-<sup>15</sup>NH<sub>2</sub> (~3.1 min), (d) DNS-<sup>14</sup>NH<sub>2</sub> (~3.1 min), and (e) DNS-OH (~1.8 min). The shown values in the panel (c-e) represent the exact mass values for the major peaks.



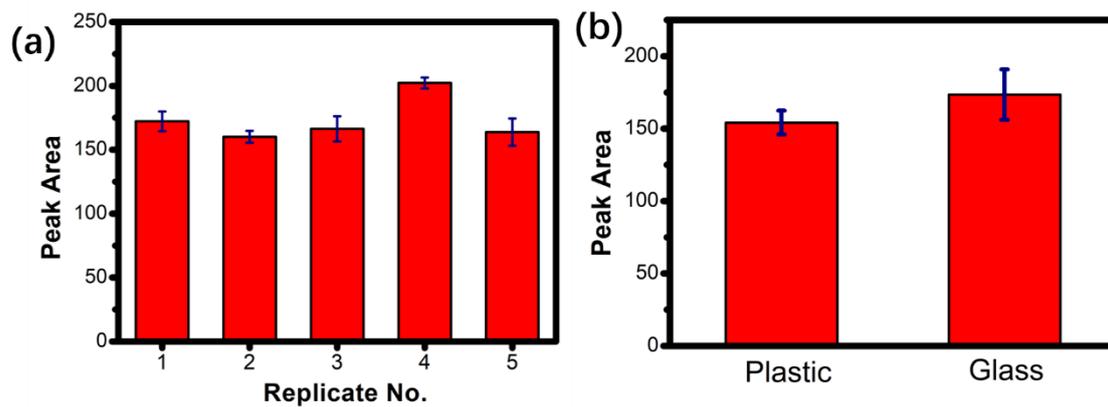
**Figure S3.** Optimizing ammonia derivatization conditions. (a) comparison of MS peak area (at  $m/z=252.0825$ ) for the same derivatized aqueous ammonia ( $^{15}\text{NH}_4^+$ ) sample ( $\sim 10 \mu\text{M}$ ) vs different added volumes of  $\text{NaCO}_3(\text{aq})$  stock solution (2g/100 mL), corrected by a volume factor; (b) comparison of the MS peak area vs different heating time ( $\sim 70 \text{ }^\circ\text{C}$ ) for the same aqueous ammonia sample in (a).



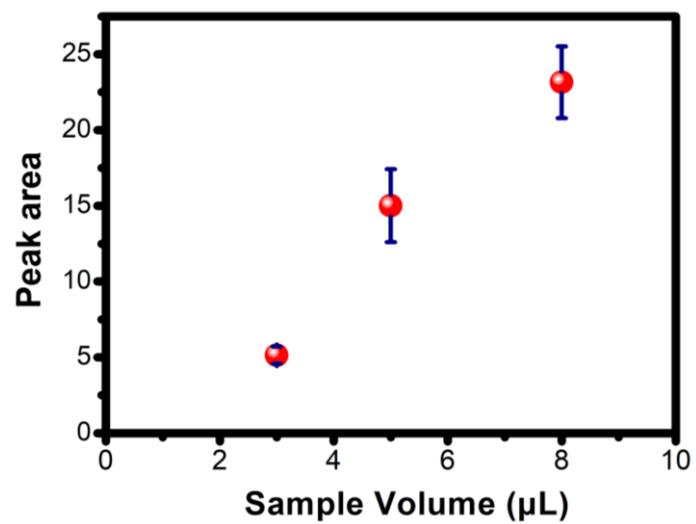
**Figure S4.** (a) comparisons of measured MS peak areas between a 14-day-old batch and a fresh batch of standard samples for generation of DNS- $^{14}\text{NH}_2$  calibration curves (the samples were measured from the same UPLC-MS run); (b) comparison of MS peak area for the same batch of DNS- $^{15}\text{NH}_2$  standard samples measured as-prepared and after storage of 18 days. (c) signal/noise ratios based on the MS signals for the DNS- $^{15}\text{NH}_2$  standard samples in the concentration range below 8.5  $\mu\text{M}$ .

**Table S1.** Comparison of linear-fitting results between a 14-day-old batch and a fresh batch of standard samples for  $^{14}\text{N}$ - or  $^{15}\text{N}$ -ammonia calibration curves measured over the same UPLC-MS run.

<b>Age</b>	<b>Curve</b>	<b>Slope</b>	<b>Intercept</b>	<b>R<sup>2</sup></b>
14-day	$^{14}\text{N}$	23.5	10.1	0.996
14-day	$^{15}\text{N}$	17.1	-9.1	0.995
Fresh	$^{14}\text{N}$	26.3	19.7	0.997
Fresh	$^{15}\text{N}$	15.1	-2.1	0.998



**Figure S5.** (a) Comparison of MS peak areas for five different samples obtained from the same aqueous ammonia solutions; (b) comparison of the use of glass and plastic vials for sample preparation



**Figure S6.** Comparison of peak areas from a 0.6- $\mu$ M ammonia sample as a function of the sample injection volume for UPLC-MS analysis.