Supplementary Information (SI)

Operando isotope selective ammonia quantification in nitrogen reduction studies via gas chromatography-mass spectrometry

Davide Ripepi,[a] Riccardo Zaffaroni,[a] Martin Kolen,[a] Joost Middelkoop[a] and Fokko M. Mulder*[a]

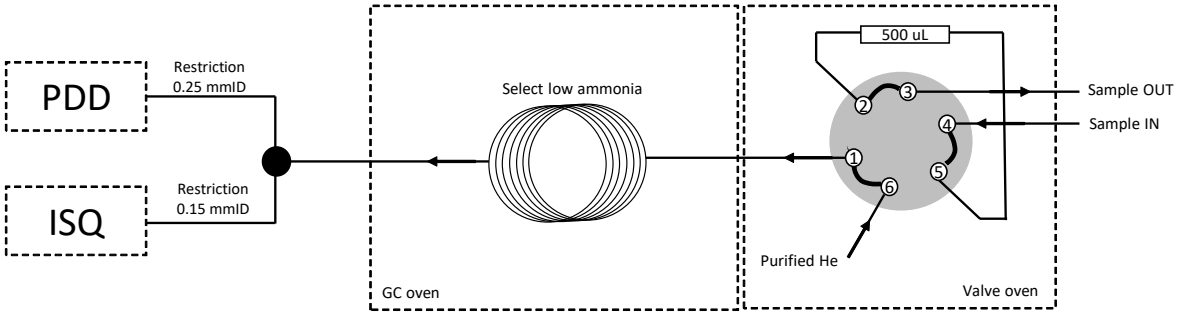
[a.] *Faculty of applied sciences, department of chemical engineering at Delft University of Technology, 2629HZ Delft, The Netherlands.*

[.] *Corresponding author*

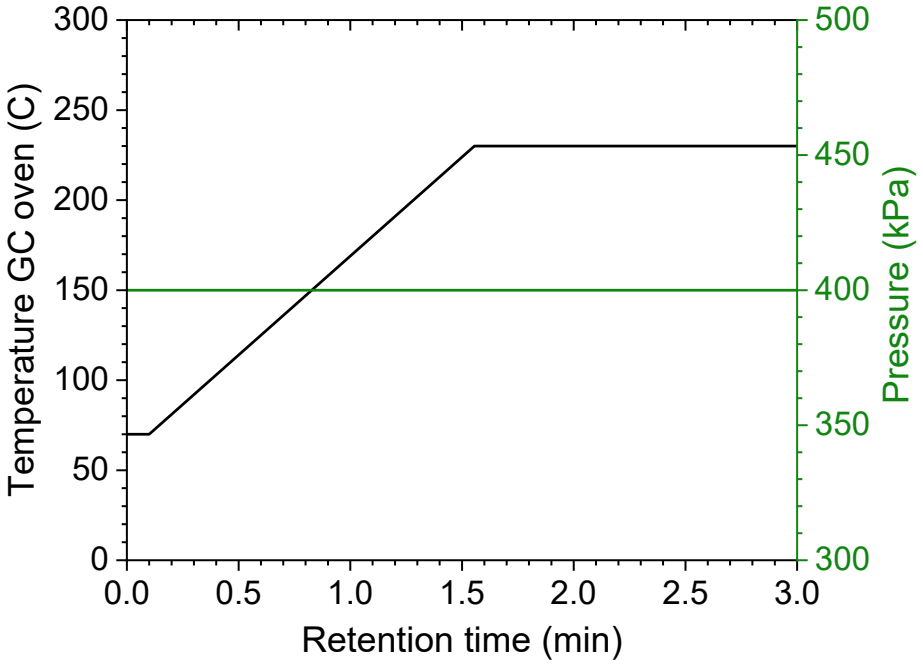
Email: F.M.Mulder@tudelft.nl

S.1 – GC-MS layout and experimental method

A simplified representation of the presented GC-MS system is shown in Supplementary Fig. 1. The sample is first introduced in a switching multiport VICI Valco diaphragm valve, kept at a constant temperature of 80°C. A sample loop of 500 µL is filled with the analyte and then, at the moment of the injection, transported with a purified He carrier gas through two Select Low Ammonia (Agilent) capillary columns. Temperature and pressure profiles are provided in Supplementary Fig. 2. This ensures the best water/ammonia separation and an optimal peak shape. Once eluted out of the chromatographic column, the sample is partitioned between the pulse discharge detector (PDD) and the single quadrupole mass spectrometer (ISQ) via two restriction lines of one meter each and with internal diameter (ID) of 0.25 mm and 0.15 mm, respectively. Importantly, all the surfaces in contact with the analyte are inert or carefully passivated with inert coating (SilcoNert® 2000) to minimize any possible ammonia physisorption. The system was assembled by Interscience BV (Breda, The Netherlands).



Supplementary Fig. 1 Simplified GC-MS layout.



Supplementary Fig. 2 GC oven temperature (black) and He carrier gas pressure (green) settings as function of retention time.

S.2 – Estimated experimental time for cumulative detection methods

The required minimum time for an electrochemical nitrogen reduction reaction (NRR) experiment can be defined as the time required to reach the limit of detection of the analytical method used to quantify ammonia. NH_3 detection methods, as Berthelot method, ^{1}H NMR or UPLC-MS, rely on extensive NH_3 accumulation in a liquid medium (electrolyte or external acid trap).

As today, most reported ammonia electrochemical synthesis rate (r_{NH_3}) are on the order of 10^{-10} mol_{NH3} cm^{-2} s^{-1},[1] and electrochemical H-cells have a typical catalyst area to electrolyte volume ratio $\left(\frac{A}{V}\right)$ of 0.02-0.04 cm^2 mL^{-1}. Therefore, considering a practical limit of quantification (LOQ) of 10 µM,[2] the minimum time strictly necessary for a NRR experiment (t_{LOQ}) to reach such limit would be between 0.7 and 1.4 h, as calculated from equation 1. Additional time is then required for sampling, analyte preparation and analysis for multiple data points. Therefore, a single NRR experiment based on indirect cumulative detection methods can take several hours.

$$t_{LOQ} = \frac{LOQ}{r_{NH_3} \times \frac{A}{V}} \quad (1)$$

S.3 – Ammonia calibration measurements

Supplementary Table 1 – Data set of integrated MS peak area (in 10^3 counts x min), average and standard deviation (STD) of mass-to-charge ratio 16 and 17 used to build the calibration curve of Fig. 2.

ppm	1.02		1.58		2.07		2.20		2.91		4.80		5.33		10.98		13.80	
injection	m/z=17	m/z=16	m/z=17	m/z=16	m/z=17	m/z=16	m/z=17	m/z=16	m/z=17	m/z=16	m/z=17	m/z=16	m/z=17	m/z=16	m/z=17	m/z=16	m/z=17	m/z=16
1	0.5151	0.4173	0.8614	0.6752	1.0232	0.8671	0.9905	0.7831	1.4987	1.1971	2.3667	1.8549	2.6616	2.2712	6.0567	4.7315	7.1335	5.9952
2	0.4918	0.2998	1.0098	0.8801	1.0784	0.9503	1.1647	0.9026	1.6058	1.2825	2.3656	1.856	2.7801	2.3808	5.9546	4.5521	7.1356	5.8901
3	0.3401	0.2603	0.9248	0.7373	0.9938	0.8497	1.0825	0.8385	1.4853	1.1666	2.4330	1.9978	2.5300	2.2380	6.1308	4.7897	7.6352	6.0109
4	0.3677	0.3993	0.9384	0.7576	1.122	0.9138	1.0667	0.8605	1.4140	1.1262	2.3390	1.8539	2.4715	2.1899	5.6930	4.4488	7.3728	6.0930
5	0.4399	0.3669	0.9529	0.7200	1.002	0.8423	1.1948	0.9220	1.4628	1.1863	2.2645	1.7746	2.4380	2.0834	5.8011	4.5390	7.3083	6.0388
6	0.5059	0.4273	1.0150	0.8019	0.9796	0.8895	1.1998	0.9001	1.4134	1.1206	2.3348	1.8384	2.5988	2.2299	5.8816	4.5911	7.2882	6.2093
7	0.3985	0.3487	0.8835	0.7195	1.0764	0.9413	0.9984	0.7395	1.6755	1.3400	2.4714	1.91084	2.6001	2.2223	5.9064	4.7146	7.2499	6.1071
8	0.4125	0.3284	0.9044	0.7181	1.0552	0.8328	1.1000	0.8527	1.5300	1.2343	2.3464	1.8059	2.4765	2.1408	5.8939	4.6967	7.1981	5.9489
9	0.4459	0.3627	0.9118	0.7306	1.1125	0.9059	1.1084	0.8795	1.5546	1.2411	2.4215	1.8466					7.4258	6.1070
10					0.9921	0.8106			1.5445	1.2684	2.4070	1.8623					7.5660	6.2535
11									1.4893	1.1951							7.2374	5.9150
12									1.5819	1.2649							7.3217	6.1320
13									1.6245	1.2955							7.4380	6.0713
14									1.5881	1.2344							7.3047	6.0369
15																	7.2964	6.0305
Average	**0.4353**	**0.3567**	**0.9336**	**0.7489**	**1.0435**	**0.8803**	**1.1006**	**0.8532**	**1.5335**	**1.2252**	**2.3750**	**1.8601**	**2.5696**	**2.2195**	**5.9148**	**4.6329**	**7.3274**	**6.0560**
STD	**0.0579**	**0.0516**	**0.0494**	**0.0564**	**0.0497**	**0.0449**	**0.0722**	**0.0560**	**0.0750**	**0.0613**	**0.0565**	**0.0570**	**0.1071**	**0.0827**	**0.1285**	**0.1096**	**0.13766**	**0.09696**

S.4 – Additional GC-MS measurements and calculations

To further clarify the peak identification and ammonia quantification, we hereby report a series of measurements performed under the following different conditions: $^{14}NH_3$ alone, from certified calibration gas in dry and humidified conditions (Supplementary Fig. 3); $^{15}NH_3$ alone, from aqueous solution headspace (Supplementary Fig. 4); $^{14}NH_3/^{15}NH_3$ mixture from aqueous solution headspace (Fig. 3); ultrapure water headspace without ammonia (Supplementary Fig. 5) and direct analysis of purified (Agilent OT3-4 filter) N_2 gas (Supplementary Fig. 6).

When present in the analyte, ammonia (^{14}N or ^{15}N) is detected in the chromatograph as a peak with a retention time of 1.83 min, in the absence of interferences from water vapour, which is eluted at a later chromatographic elution time of about 2 min. Similarly, excellent water/ammonia separation is established at the MS detector for the m/z 16, 17 and 18. The outsized amount of water vapour (compared to typical ppm levels of ammonia produced in electrochemical nitrogen reduction experiments) carried during sampling of aqueous headspace is affected by several factors and therefore can largely vary over time, making unfeasible to reliably quantify NH_3 without a proper water/ammonia separation (e.g. in MS detectors without chromatographic separation).

When both $^{14}NH_3$ and $^{15}NH_3$ are present in the analyte, the contributions from ^{14}N and ^{15}N ammonia are calculated from the respective electron ionization mass spectra (Supplementary Table 2) and the integrated peak areas of m/z 16, 17 and 18 (Supplementary Table 3), using the equations 2-4. The estimated error based on the difference between the measured and calculated peak area at m/z=16 is about 1.6% (equation 5).

Supplementary Table 2 - The electron ionization mass spectrum of ^{14}N and ^{15}N ammonia.[3] The relative intensities of the corresponding fragments are reported between brackets.

	NH_3^+ (100%)	**NH_2^+ (80.1%)**	**NH^+ (7.5%)**	**N^+ (2.2%)**
m/z [$^{14}NH_3$]	17	16	15	14
m/z [$^{15}NH_3$]	18	17	16	15

Supplementary Table 3 – Integrated peak area of the m/z equal to 16, 17 and 18 at the ammonia elution time (1.83 min) from Fig. 3c. The data refers to the GC-MS analysis of the headspace of a $^{15}NH_3/^{14}NH_3$ (0.21/0.68 mM) aqueous solution in a sealed vial, with a N_2 gas flow of 2.5 mL min^{-1}.

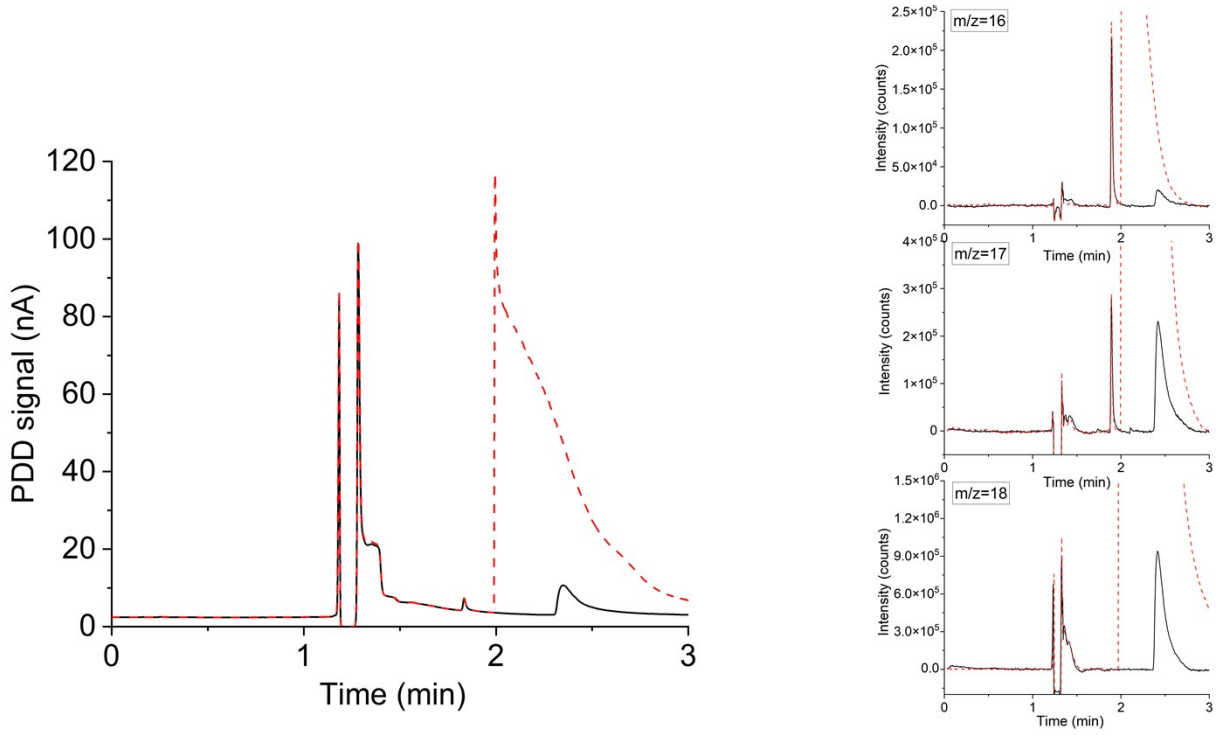
Retention time	**Peak area m/z=16**	**Peak area m/z=17**	**Peak area m/z=18**
(min)	(counts*min)	(counts*min)	(counts*min)
1.83	652.563	2305.459	2090.715

$$^{15}NH_3 = \left(Peak\ area\ {}^{m}/_{z18}\right) \times 0.8 \times 527.72 \quad (2)$$

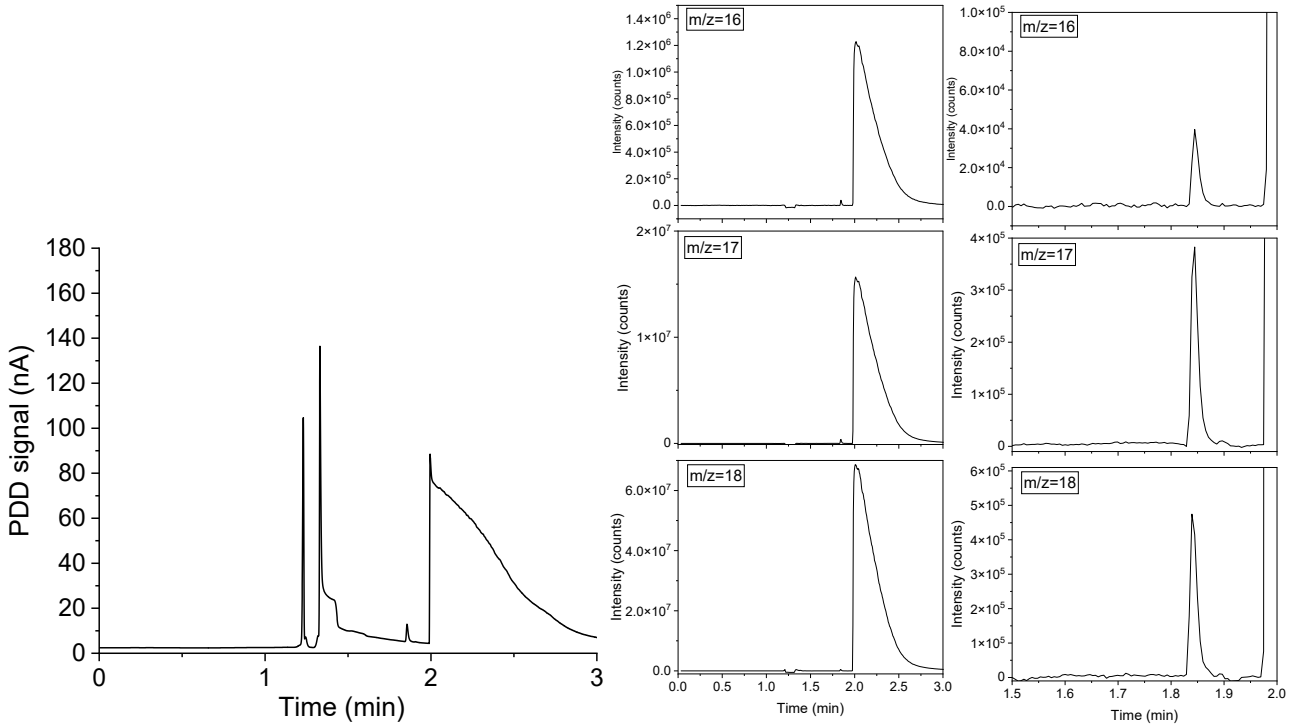
$$^{14}NH_3 = \left[\left(Peak\ area\ {}^{m}/_{z17}\right) - \left(Peak\ area\ {}^{m}/_{z18}\right) \times 0.8\right] \times 527.72 = {}^{14_N m}/_{z17} \times 527.72 \quad (3)$$

$$\left(Peak\ area\ {}^{m}/_{z16}\right)^{calc.} = \left[\left(Peak\ area\ {}^{m}/_{z18}\right) \times 0.075\right] + \left[\left({}^{14_N m}/_{z17}\right) \times 0.8\right] \quad (4)$$

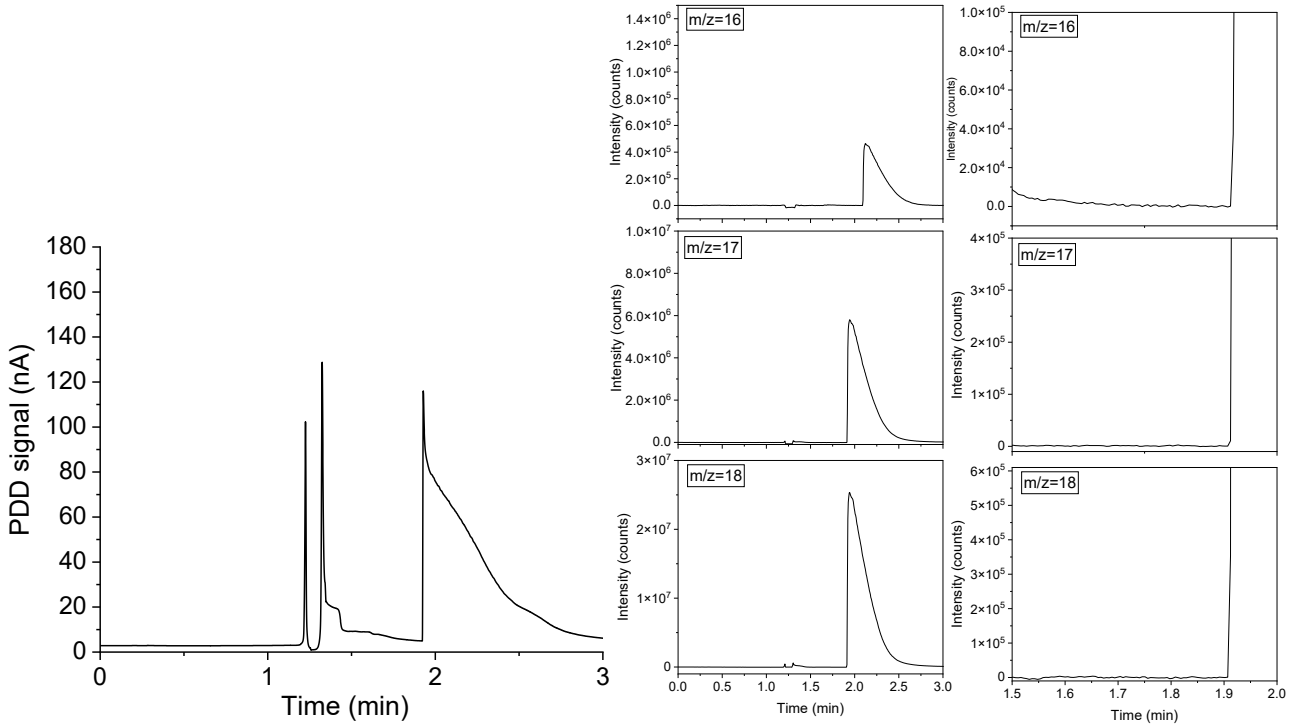
$$Error\ (\%) = \frac{\left[\left(Peak\ area\ {}^{m}/_{z16}\right)^{measured} - \left(Peak\ area\ {}^{m}/_{z16}\right)^{calc.}\right]}{\left(Peak\ area\ {}^{m}/_{z16}\right)^{measured}} \times 100 \quad (5)$$



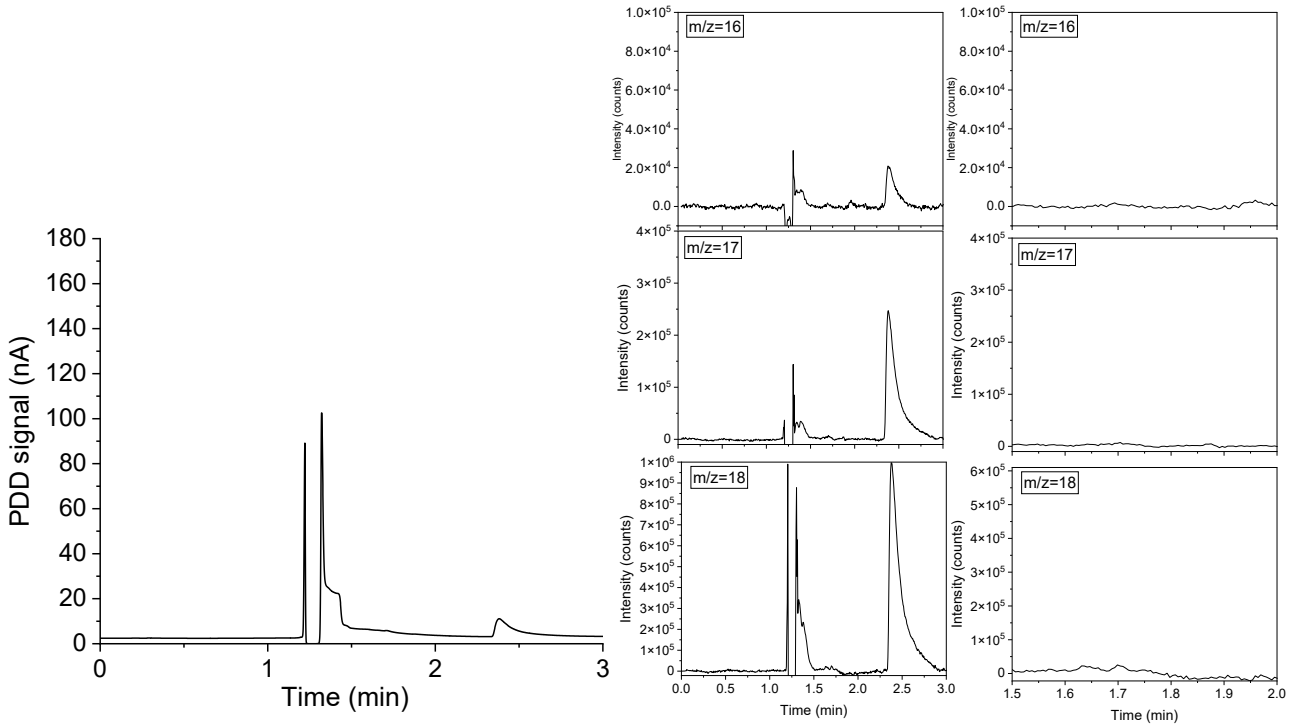
Supplementary Fig. 3 Comparison of two GC-MS measurements of 13.8 ppm of NH_3 in nitrogen, connected directly to the GC-MS (black) and feed into the headspace of a sealed vial containing Milli-Q water prior injection to the GC-MS (red). On the left, the full chromatograph. On the right, the MS signal corresponding to m/z 18, 17, 16. The significant increase in the water content of the analysed gas sample does not influence the ammonia quantification.



Supplementary Fig. 4 GC-MS analysis of the headspace of $^{15}NH_3$ ammonia aqueous solution (without $^{14}NH_3$) in a sealed vial, with a N_2 gas flow of 2.5 mL min^{-1}. On the left, the full chromatograph. In the middle, the MS signal corresponding to m/z 18, 17, 16. On the right, the closed up around the ammonia elution time (1.83 min) of the MS signal corresponding to m/z 18, 17, 16. The integrated peak areas at 1.83 min are respectively 7668.181 (m/z=18), 6218.650 (m/z=17) and 565.708 (m/z=16) counts per minutes, which correspond to the expected relative intensities of the electron ionization mass spectrum of $^{15}NH_3$ within measurement error (1.6%).



Supplementary Fig. 5 GC-MS analysis of the headspace of Milli-Q water (without added NH_3) in a sealed vial, with a N_2 gas flow of 2.5 mL min^{-1}. On the left, the full chromatograph. In the middle, the MS signal corresponding to m/z 18, 17, 16. On the right, the closed up around the ammonia elution time (1.83 min) of the MS signal corresponding to m/z 18, 17, 16. The absence of a peak at 1.83 min confirms that no ammonia is present in the analysed sample above the GC-MS detection limit.



Supplementary Fig. 6 GC-MS analysis of purified N_2 gas at 2.5 mL min^{-1} directly connected to the instrument inlet. On the left, the full chromatograph. In the middle, the MS signal corresponding to m/z 18, 17, 16. On the right, the closed up around the ammonia elution time (1.83 min) of the MS signal corresponding to m/z 18, 17, 16. The absence of a peak at 1.83 min confirms that no ammonia is present in the analysed sample above the GC-MS detection limit.

S.5 – ^{1}H nuclear magnetic resonance analysis

Chemicals

Maleic acid (≥ 99%) and H_2SO_4 (≥ 99.999%) were obtained from Sigma Aldrich. Gadolinium(III) nitrate hexahydrate (99.9%) was obtained from Fisher Scientific. DMSO-d6 (99.9% D, 0.03 % V/V Tetramethylsilan) was obtained from Cambridge Isotope Laboratories. Ultrapure water was produced with a Milli-Q Advantage A10 water purification system (resistivity: 18.2 MΩ at 25°C).

Sample preparation

The concentration of $^{14}NH_3$ and $^{15}NH_3$ in solution was quantified using an ^{1}H NMR method with absolute quantification as previously described.[4] This method allows for quantification of $^{14}NH_3$ and $^{15}NH_3$ without requiring a calibration curve because the interscan delay is sufficiently long (>5T_1) to allow full relaxation of the analyte and the internal standard. To prepare the NMR sample, 510 µL sample solution, 45 µL 2M sulfuric acid (freshly prepared) and 45 µL detection solution were added to an NMR tube and stirred using a vortex mixer. The detection solution consists of 3.21 mM maleic acid (MA), 12.86 mM gadolinium nitrate hexahydrate diluted in DMSO-d6.

^{1}H NMR data acquisition

^{1}H NMR spectra were acquired using a 400 MHz Fourier transform NMR spectrometer equipped with an autosampler and an autotunable, temperature regulated Agilent OneNMR room temperature probe. The temperature was set to 25° C and the receiver gain was optimized automatically. The excitation sculpting pulse sequence “waterES” was used to suppress the resonance of water during acquisition. The “waterES” pulse sequence has the following structure:

waterES: d1-P90-G1-S180-P180-G1-G2-S180-P180-G2-aq

where, G1,G2 are z-gradients of different strengths, P90, P180 are hard pulses and S180 is a selective 180° pulse. The acquisition parameter were: 0.75 s acquisition time, 0.05 s recycle delay and 1024 number of scans.

^{1}H NMR data processing

The data was processed with the software package MestReNova (version: 12.0.1-20560) using the automated tools available with this software. Unless otherwise noted an apodization of 4 Hz was applied followed by phasing and baseline correction. The peaks of NH_4^+ (t/d ≈6.9 ppm, 4H) and MA (s, ≈6.21 ppm, 2H) were integrated using the line fitting tool (Lorentzian fit). The integrals of the $^{14}NH_4^+$ triplets (or $^{15}NH_4^+$ doublet) were added together to calculate the total $^{14}NH_4^+$ (or $^{15}NH_4^+$) integral (I_{NH4+}). The concentration of NH_4^+ was calculated with absolute quantification, from the ratio of the integral of NH_4^+ and MA (I_{std}), according to equation 6.

$$c_{NH_4^+} = \frac{I_{NH_4^+} \; N_{std}}{I_{std} \; N_{NH_4^+}} c_{std} \qquad (6)$$

where I, N and C are respectively the integral area, number of nuclei and concentration of NH_4^+ and internal standard maleic acid, respectively.

Line fitting data

Supplementary Table 4 – Line fitting data of the 1H NMR measurement shown in Fig. 3d.

	Chemical shift (ppm)	Intensity (a.u.)	Area (a.u.)	Compound
1	7.003	13.6	1410.11	$^{14}NH_4^+$
2	6.963	63.9	6113.98	$^{15}NH_4^+$
3	6.873	13.1	1276.26	$^{14}NH_4^+$
4	6.78	65.6	6417.14	$^{15}NH_4^+$
5	6.742	12.9	1170.5	$^{14}NH_4^+$
6	6.216	27.7	2207.35	$C_4H_4O_4$

Additional references

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