Supporting Information

# Automated Online Solid Phase Derivatization for Sensitive Quantification of Endogenous S-Nitrosoglutathione and Rapid Capture of Other Low-Molecular-Mass S-Nitrosothiols

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## Experimental Section

### Synthesis of GSNO, $^{13}C_2$,$^{15}N$-labeled G*SNO (internal standard) and derivatizing reagent[1]

Equimolar amounts of sodium nitrite (0.345 g, 5 mmol) and glutathione (1.53 g, 5 mmol) were mixed in 2N HCl (4 mL) under stirring and argon flux. After 40 minutes in an ice bath, the red solution was treated with acetone (20 mL) and stirred for an additional 10 minutes. The pink solid GSNO was filtered and rinsed successively with ice-cold water (5 mL), acetone (3 mL), and diethyl ether (3 mL); freeze-dried and stored in the dark at -20 °C. $^{13}C_2$,$^{15}N$-labeled G*SNO was prepared using $^{13}C_2$,$^{15}N$-labeled G*SH.

The 2-[1-(dimethylamino)ethanethioate]triphenylphosphine was selected as derivatizing reagent for the capture of RSNOs. A solution of 2-(diphenylphosphino)benzenethiol (300 mg) in dry DMF (25 mL) was added with N,N-dimethylglycine hydrochloride (171 mg), N,N'-dicyclohexylcarbodiimide (252 mg) and dimethylaminopyridine (149.5 mg). The resulting mixture was reacted at room temperature for 2 h, white solid formed in reaction (1,3-dicyclohexylurea) was then removed by filtration. The filtrate was concentrated under reduced pressure and purified by flash chromatography (Hexane/Ethyl acetate, 40/10) to give the desired derivatizing reagent.

The GSNO, $^{13}C_2$,$^{15}N$-labeled G*SNO and derivatizing reagent were all confirmed by LC/ESI-HR-MS analysis (Figure S-1).

## Supplementary Figures

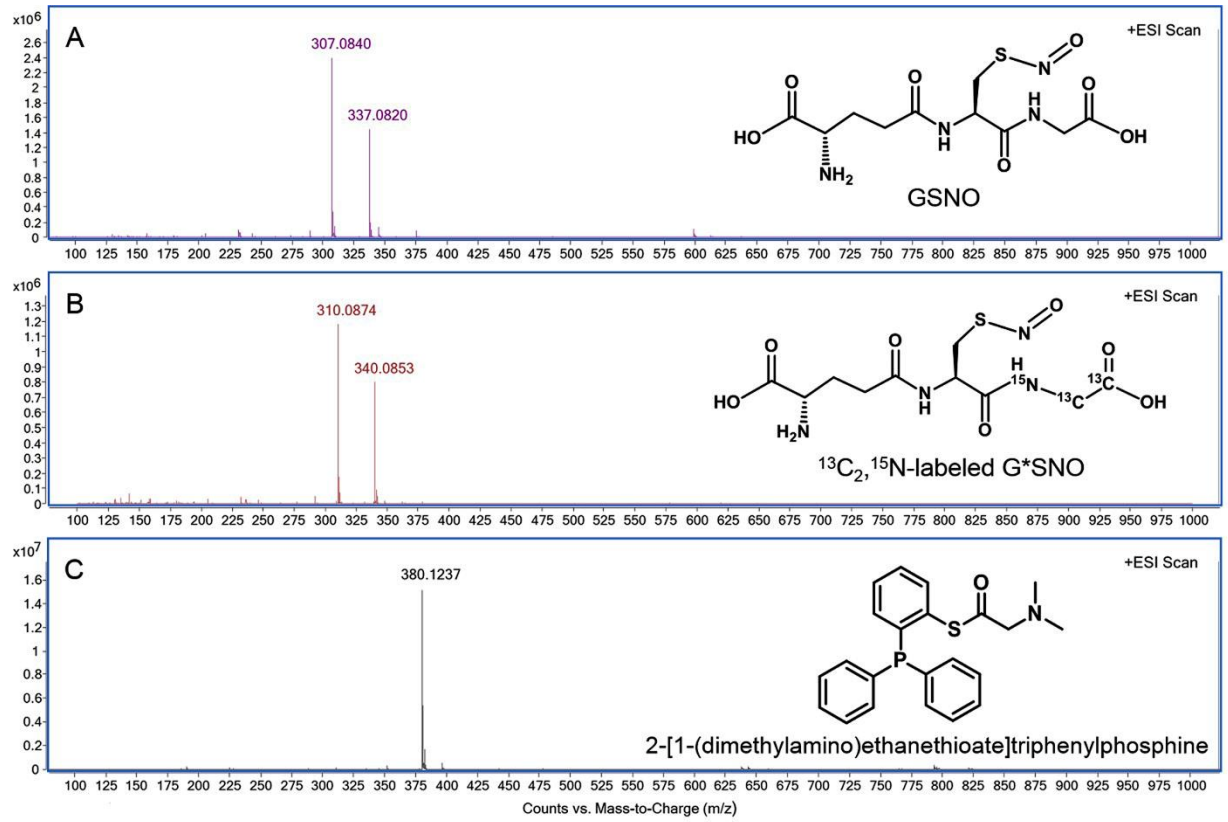


**Figure S-1.** High-resolution MS analysis of (A) the prepared GSNO, (B) the $^{13}C_2$,$^{15}N$-labeled G*SNO, and (C) the derivatizing reagent.

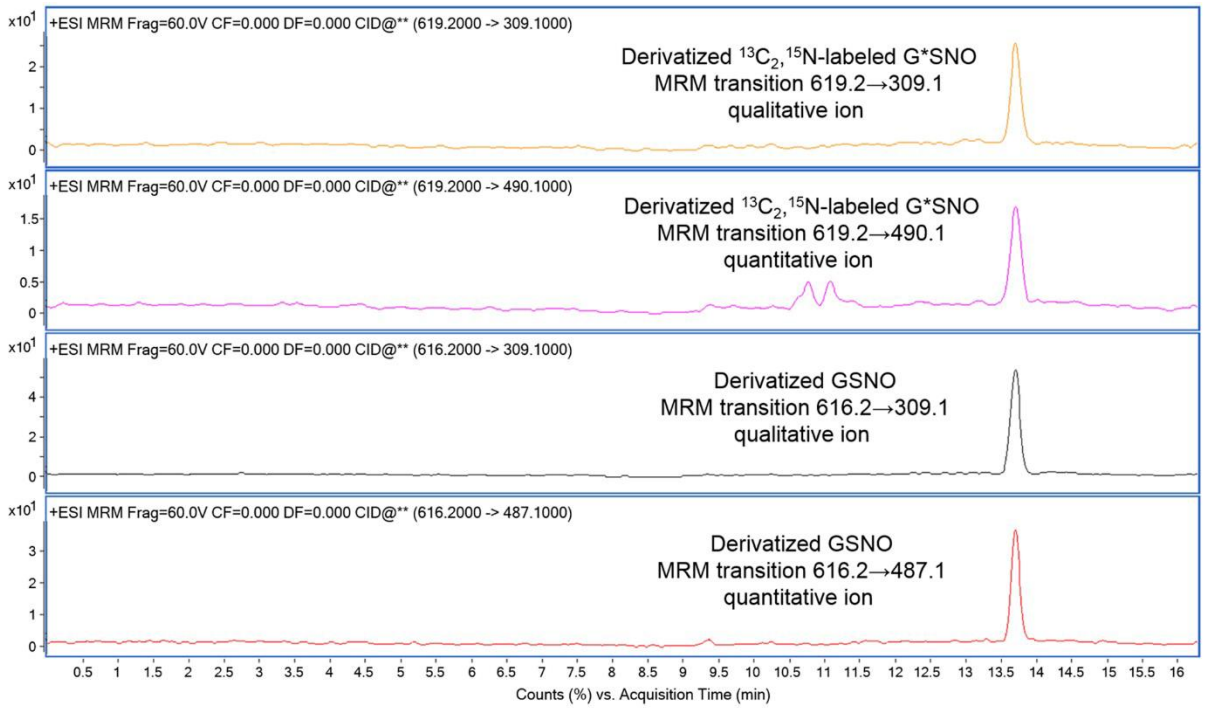


**Figure S-2.** Typical MRM chromatograms of the derivatized GSNO and derivatized $^{13}C_2$,$^{15}N$-labeled G*SNO analyzed by online SPD-LC-MS method.

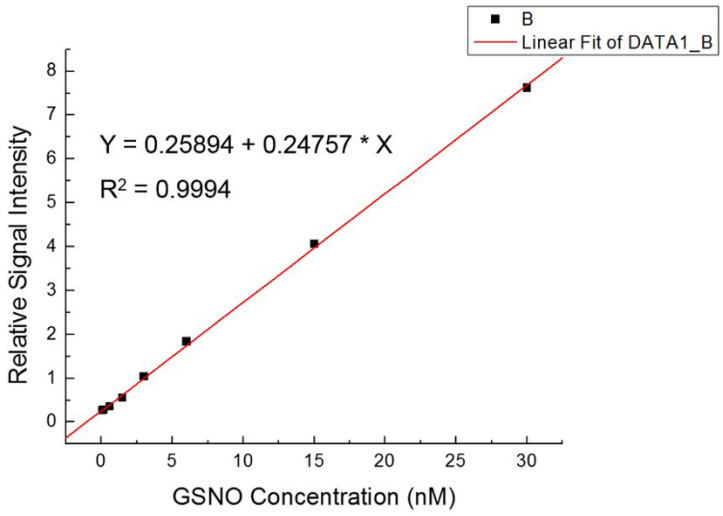


**Figure S-3.** Calibration Curve: 3 nM of $^{13}C_2$,$^{15}N$-labeled G*SNO is the isotope-labeled internal standard for compensating signal fluctuations and unavoidable matrix effects. The matrix-free calibration curve is constructed by plotting the signal intensity versus concentration, relative signal intensity to the internal standard is used for better reproducibility. Satisfactory linearity is obtained in the range of 0.06-30 nM GSNO with a linear coefficient of $R^2 = 0.9994$. The limit of detection (LOD, S/N = 3) and limit of quantification (LOQ, S/N = 10) are 0.015 nM and 0.054 nM, respectively.

**Table S-1.** The precision and recoveries of GSNO analysis using automated online SPD-LC-MS method

| Spiking Concentration | Intra-day precision RSD (%, n = 4) | Inter-day precision RSD (%, n = 4) | Recovery (% ± RSD, n = 4) |
|---|---|---|---|
| 0.15 nM (low) | 5.04 | 9.69 | 90.4 ± 5.7 |
| 1.5 nM (medium) | 7.44 | 9.56 | 95.8 ± 7.8 |
| 15 nM (high) | 5.45 | 8.17 | 103.6 ± 7.0 |

**Table S-2.** Summary of references for endogenous GSNO/RSNOs detection in plasma samples and mouse tissues

| Analytes | Real Samples | Detection | Endogenous concentration | Ref. |
|---|---|---|---|---|
| GSNO | Mouse plasma | SPD-LC-MS | 138 ± 13.2 nM | Proposed method |
| GSNO | Human plasma | LC-MS | N.D. (maybe less than 2.8 nM) | Ref. 2 |
| GSNO | Human plasma | Offline derivatization and GC-MS | 157-257 nM | Ref. 3 |
| GSNO | Human plasma | P-hydroxymercury benzoate derivatization fluorescence detection | 320 ± 60 nM | Ref. 4 |
| RSNOs | Human plasma | EPR spectrometry | 90 nM | Ref. 5 |
| LMM RSNOs | Mouse plasma | Spectrophotometer (Griess) | 5000 nM | Ref. 6 |
| RSNOs | Rat plasma | Chemiluminescence | 51 ± 6 nM | Ref. 7 |
| RSNOs | Rat plasma | NO electrode | 200 nM | Ref. 8 |
| RSNOs | Rat plasma | NO electrode | 50-1000 nM | Ref. 9 |
| RSNOs | Rat plasma | Chemiluminescence | 1780 ± 760 nM | Ref. 10 |
| LMM RSNOs | Swine plasma | Nitric Oxide Detection | 1500 ± 1000 nM | Ref. 11 |
| RSNOs | Porcine plasma | Amperometric Detection | About 3000 nM | Ref. 12 |
| GSNO | Mouse liver, kidney, heart, muscle, and brain (hippocampus, striatum, cerebellum, cortex) | SPD-LC-MS | Liver: 64.8 ± 11.3 pmol/mg protein<br>Kidney: 47.2 ± 6.1 pmol/mg protein<br>Heart: 8.9 ± 1.8 pmol/mg protein<br>Muscle: 1.9 ± 0.3 pmol/mg protein<br>Hippocampus: 5.3 ± 0.9 pmol/mg protein<br>Striatum: 6.7 ± 0.6 pmol/mg protein<br>Cerebellum: 31.4 ± 6.5 pmol/mg protein<br>Cortex: 47.9 ± 4.6 pmol/mg protein | Proposed method |
| GSNO | Rat cerebellum | LC-MS | Cerebellum: 15.4 ± 1.4 pmol/mg protein | Ref. 13 |
| GSNO | Mouse cortex, hippocampus and cerebellum | Offline derivatization and LC-MS | Hippocampus: 2.0 μM<br>Cerebellum: 3.1 μM<br>Cortex: 1.8 μM | Ref. 14 |

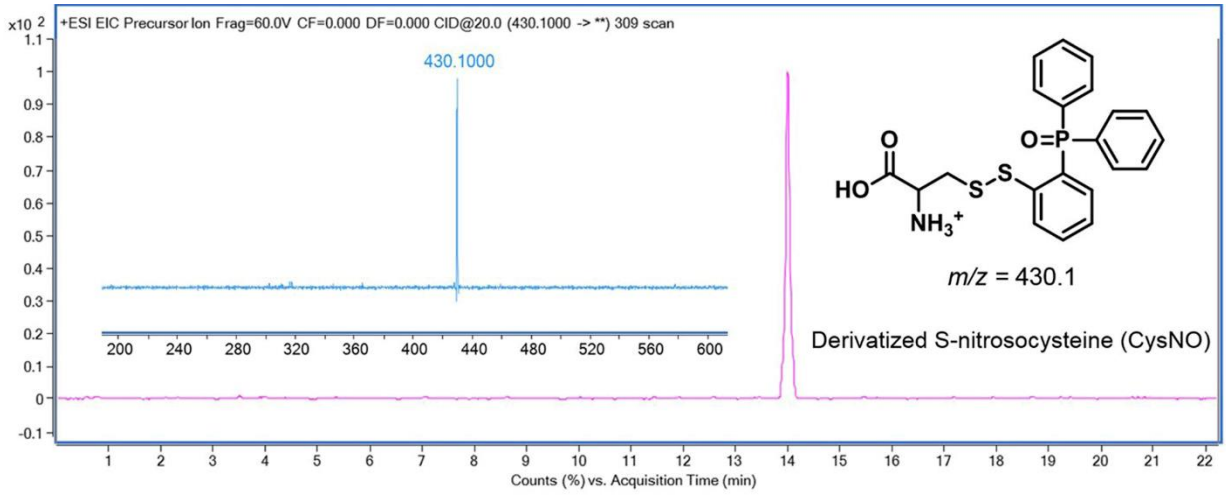


**Figure S-4.** Chromatogram and MS signal of the derivatized CysNO obtained by online SPD-LC-MS through precursor-ion scanning (*m/z* 309.1 is selected as the product ion with a precursor ion scan window of *m/z* from 100-1000).

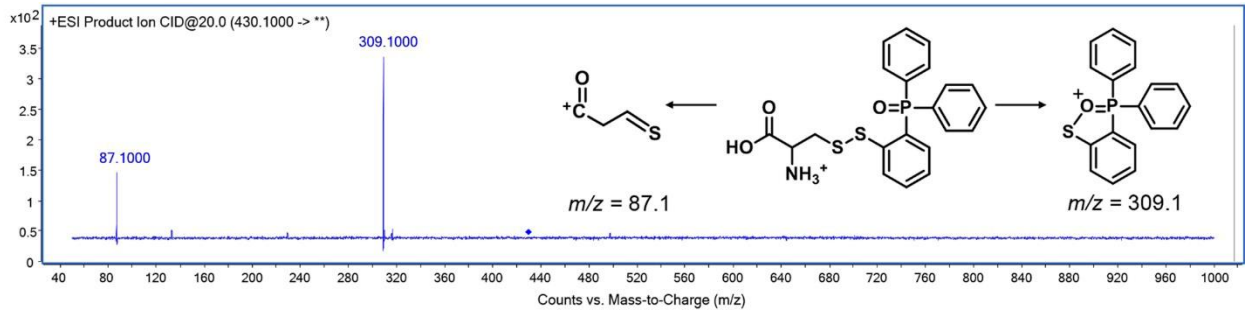


**Figure S-5.** MS/MS spectrum of the derivatized CysNO obtained by online SPD-LC-MS in positive mode; collision energy: 20 V.

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