

## Molecular speciated isotope dilution mass spectrometric methods for accurate, reproducible and direct quantification of reduced, oxidized and total glutathione in biological samples

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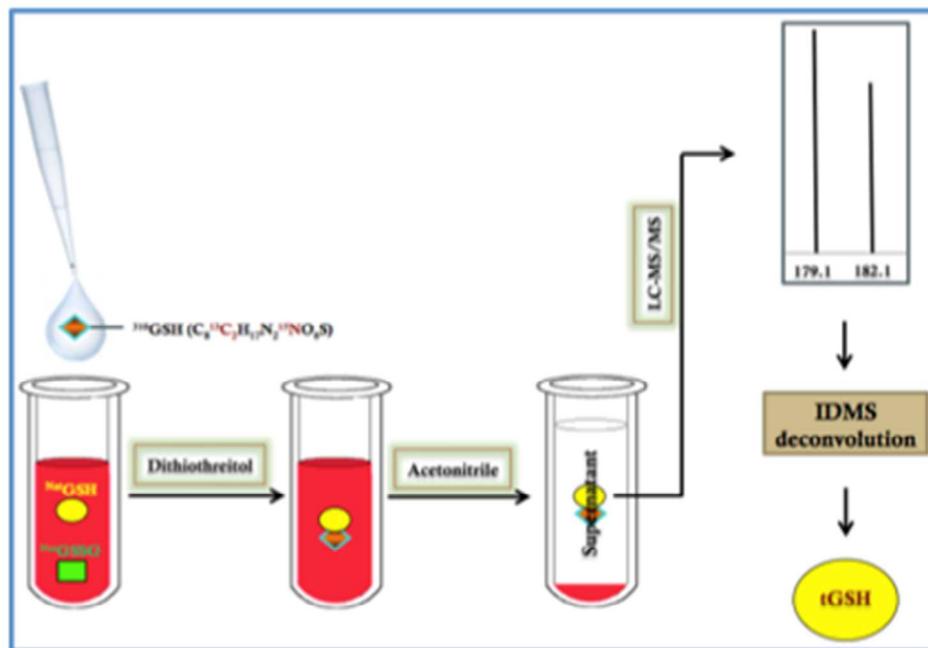
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### Supporting Information

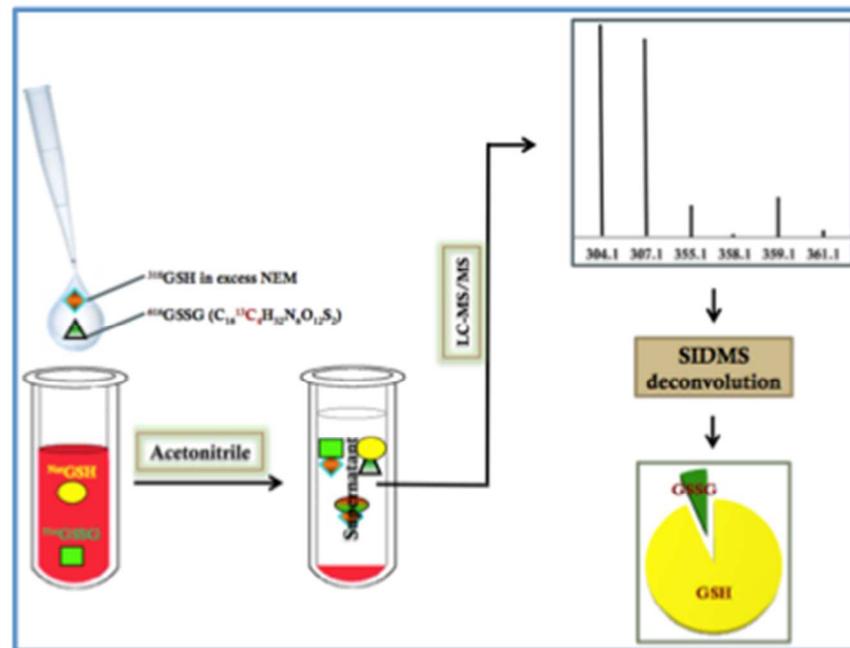
The material contains a pictorial presentation of the method and additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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#### tGSH by IDMS



#### GSH and GSSG by SIDMS



### Optimum conditions of the LC-MS/MS method

Condition	tGSH	GSH and GSSG
Mobile phase	(a) 10 mmol/L ammonium formate, pH 3.8, and (b) acetonitrile	(a) 15 mmol/L ammonium formate, pH 3.8, and (b) acetonitrile
Elution	Isocratic: 70% B	0 min: 75% B, 10 min: 50% B, 16 min: 50% B, 20 min: 75% B, 25 min: 75% B
Run time	10 min	25 min
Flow rate	0.30 mL/min	0.35 mL/min
Column temp	45 °C	45 °C
Injection volume	5 µL <sup>a</sup> , 25 µL <sup>b</sup>	10 µL <sup>a</sup> , 40 µL <sup>b</sup>
Nebulizer gas flow and temperature	11.0 L/min, 300 °C	11.0 L/min, 300 °C
Sheath gas flow and temperature	8.0 L/min, 250 °C	8.0 L/min, 250 °C
Collision energy	12 V	12 V (for GS-NEM) and 25 V (for GSSG)
Capillary voltage	3500 V	3500 V
Fragmentor voltage	135 V	135 V
Dwell time	50 ms	50 ms (0–6.5 min), 5 ms (6.5–25 min)
Resolution	Unit (0.7 m/z)	Unit (0.7 m/z)

<sup>a</sup> RBC and whole blood

<sup>b</sup> Saliva

**Chromatograms showing detection of (a) tGSH, and (b) GSH and GSSG in a whole blood sample by LC-MS/MS**

(a) Sample was spiked with  $^{310}\text{GSH}$  followed by treatment with DTT. Endogenous and spiked GSH were detected at  $m/z$  179 and 182, respectively, after elimination of a pyroglutamate moiety from each molecule. (b) Sample was spiked with  $^{310}\text{GSH}$  and  $^{616}\text{GSSG}$  along with excess NEM. Endogenous and spiked GSH (as GS-NEM) were detected at  $m/z$  304 and 307, and GSSG at 355 and 359, respectively, after elimination of two pyroglutamate moieties from each molecule. See Table 1 for the experimental conditions.

