Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information for

Simultaneous Determination of Intracellular Reduced and Oxidized Glutathione by the König Reaction

Ryu Mochizuki, Akane Kobayashi, Hiromitsu Takayama, Toshihiko Toida, and Yasumitsu Ogra*

Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260–8675, Japan

*Correspondence to Y.O. (ogra@chiba-u.jp)

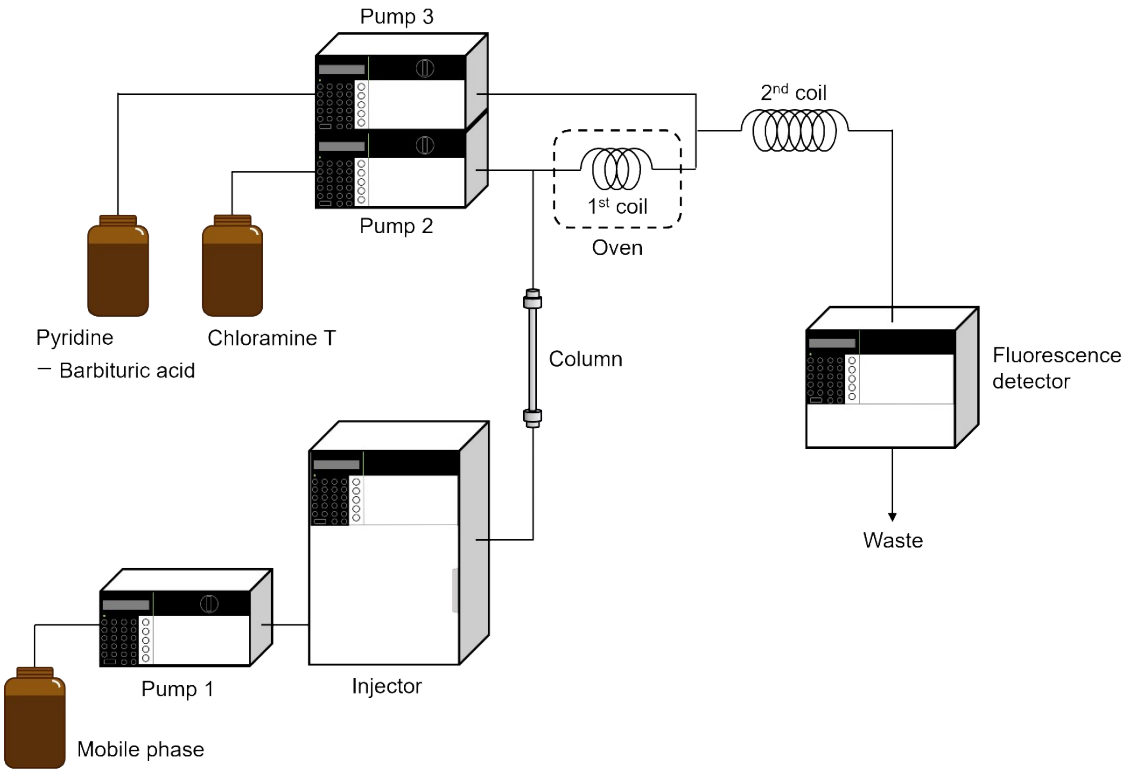


Fig. S1. Schematic diagram of HPLC.

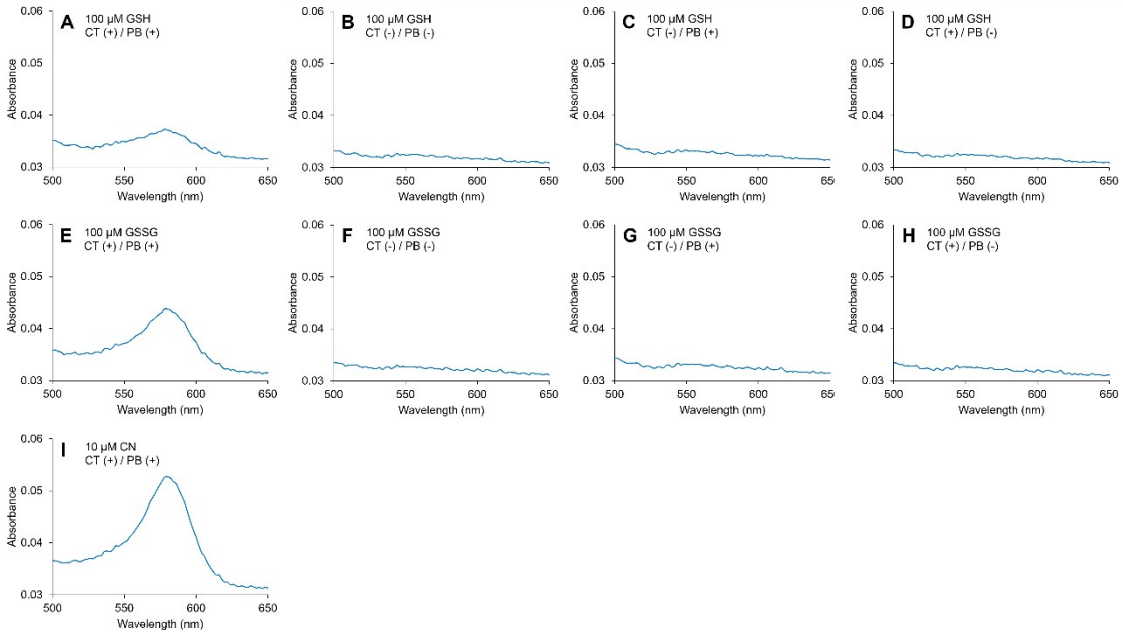


Fig. S2. Absorption spectra of reaction mixtures containing GSH, GSSG, or CN^- and post-column derivatizing reagents. (A), (E), and (I): complete mixtures. (B) and (F): mixtures without the derivatizing reagents. (C) and (G): mixtures without chloramine T. (D) and (H): mixtures without the pyridine–barbituric acid reagent. CT: chloramine T, PB: pyridine–barbituric acid reagent.

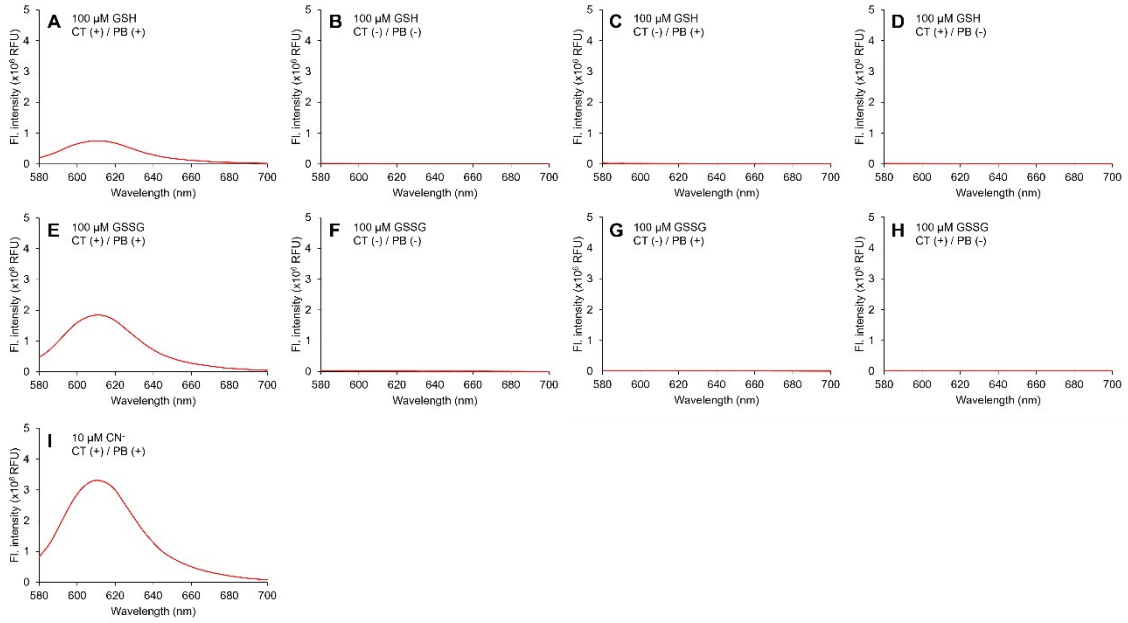
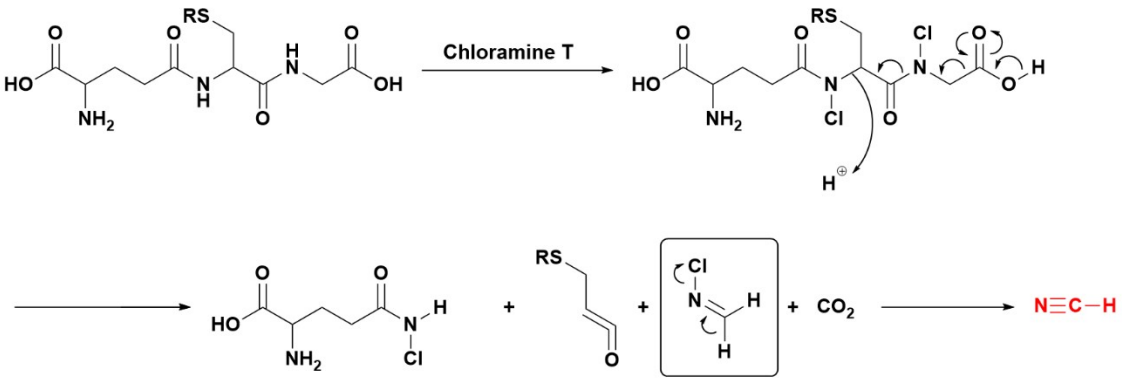


Fig. S3. Fluorescence emission spectra of reaction mixtures containing GSH, GSSG, or CN^- and post-column derivatizing reagents. (A), (E), and (I): complete mixtures. (B) and (F): mixtures without the derivatizing reagents. (C) and (G): mixtures without chloramine T. (D) and (H): mixtures without the pyridine–barbituric acid reagent. The excitation wavelength was 540 nm. CT: chloramine T, PB: pyridine–barbituric acid reagent.



Scheme S1. Speculated reaction mechanism. R=H: GSH, R=GS: GSSG.

Table S1. Linear equations of GSH and GSSG.

Compound	Equation
GSH	$y = 3070457x + 162155.7$
GSSG	$y = 6689536x + 770531.3$

Table S2. Comparison of LOQ values shown as a concentration and an absolute amount between the present method and previously reported LC-MS methods.

Reference	GSH	GSSG
The present method	18.3 nM 366 fmol (20 µL inj.)	33.9 nM 678 fmol (20µL inj.)
1	1.5 x 10^3 nM 1.5 x 10^4 fmol (10 µL inj.)	1 x 10^2 nM 1.5 x 10^4 fmol (15 µL inj.)
2	30 ng/mL (= 97.6 nM) 976 fmol (10 µL inj.)	1 ng/mL (= 1.63 nM) 16.3 fmol (10 µL inj.)
3	100 ng/mL (= 325 nM) 1.63 x 10^3 fmol (5 µL inj.)	100 ng/mL (= 163 nM) 816 fmol (5 µL inj.)

1. T. Moore, A. Le, A.-K. Niemi, T. Kwan, K. Cusmano-Ozog, G. M. Enns and T. M. Cowan, *Journal of chromatography B*, 2013, **929**, 51-55.
2. H. Liu, F. Xu, Y. Gao, Y. Pang, C. Xie and C. Jiang, *Analytical Chemistry*, 2020, **92**, 8810-8818.
3. Y.-F. Zhang, Y. Wang, K.-R. Zhang, H.-M. Lei, Y.-B. Tang and L. Zhu, *Journal of chromatography B*, 2020, **1148**, 122145.

Table S3. Precision and accuracy of the present method (n = 5).

Compound	Concentration (μM)	Precision (%RSD)	Accuracy (% of target)
GSH	0.5	3.69	80.6
	5	1.53	99.6
	50	0.84	99.4
GSSG	0.5	3.46	104.8
	5	0.73	99.0
	10	0.44	100.0