Electronic Supplementary Material (ESI) for Analyst.

Electronic Supporting Information

Quinones as novel chemiluminescent Probes for sensitive and selective determination of biothiols in biological fluids

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Abstract

Supplementary material for the new HPLC-CL analytical method for simultaneous determination of aminothiols in plasma includes experimental section, figures of method preparation and optimization, and table of analytical regression.

Table S-1. Analytical parameter and regression characteristic of studied aminothiols by the proposed method

Aminothiols	Range (nM)	Calibration equation[a]	Correlation coefficient (*r*)	Detection limit (nM) [b] (fmol/injection)
GSH	**2.5-500**	**Y = 23.87x - 0.32**	**0.995**	**0.2 (3.8)**
NAC	**5-500**	**Y = 22.34x - 0.23**	**0.995**	**0.2 (4.2)**
Hcys	**10-1500**	**Y = 7.08x - 0.19**	**0.990**	**0.4 (8)**
Cys	**20-2000**	**Y = 6.23x - 0.37**	**0.991**	**0.8 (16)**

[a] Peak height versus concentration (nM)

[b] Detection limit (S/N=3)

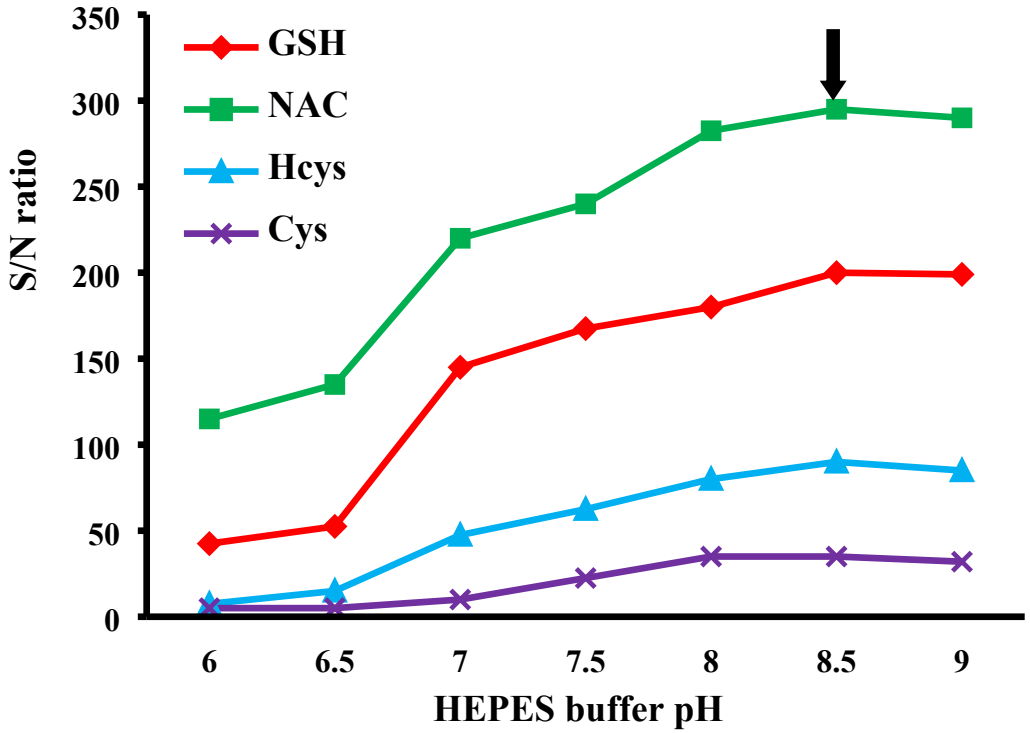

Figure S1. Effect of HEPES buffer pH on S/N ratio under optimal derivatization condition

100 µl calibration solutions, 10 µl TCEP (10 mM), and 170 µl HEPES (0.5M) of various pH were mixed and incubated for 15 min at RT, then 20 µl represented quinones (600 µM) was added. The mixture was spinned for 5 min at RT, and 20 µl was diluted with mobile phase and injected to HPLC-CL system.

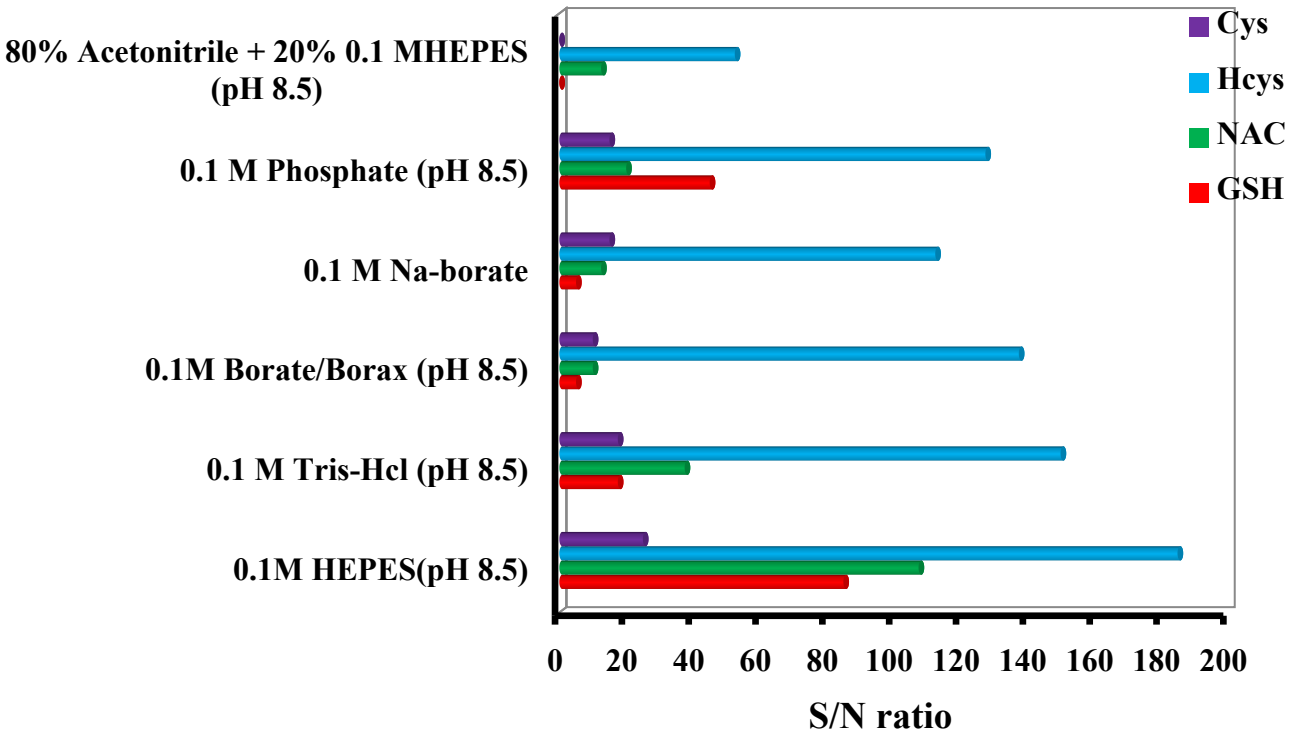

Figure S2. Effect of various buffers on S/N ratio under optimal derivatization condition

100 µl calibration solutions, 10 µl TCEP (10 mM), and 170 µl of various buffer (0.5M, pH 8.5) were mixed and incubated for 15 min at RT, then 20 µl represented quinones (600 µM) was added. The mixture was spinned for 5 min at RT, and 20 µl was diluted with mobile phase and injected to HPLC-CL system.

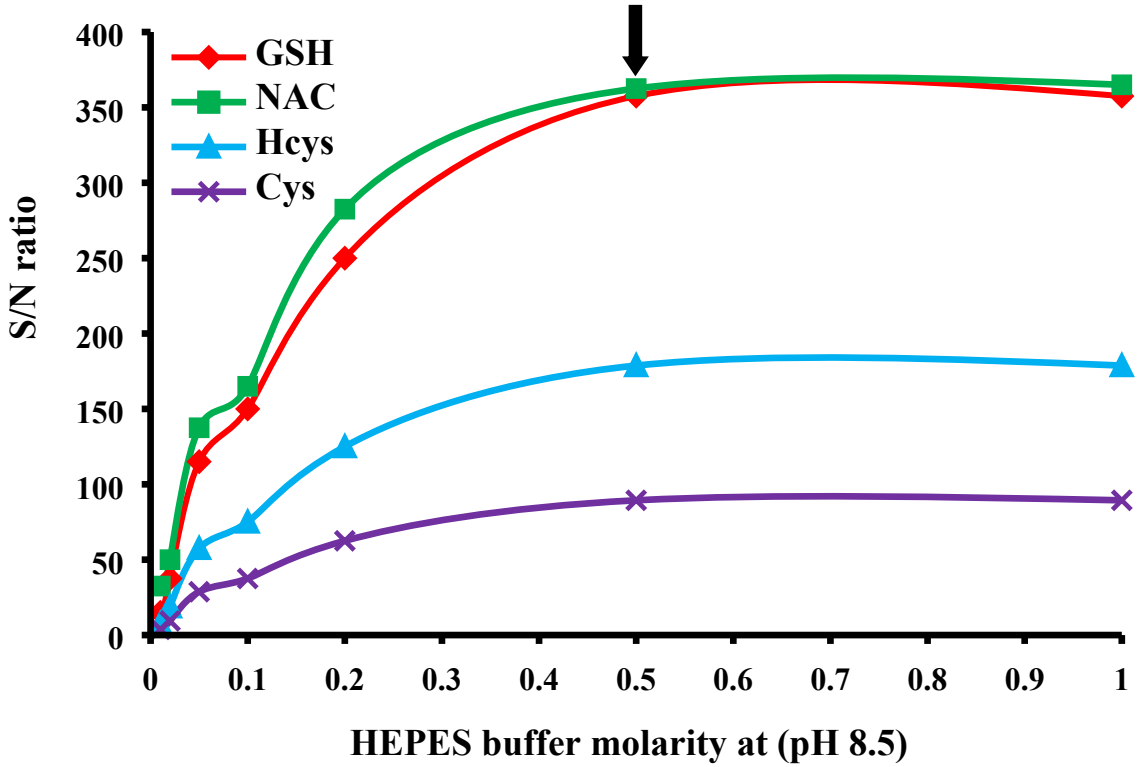

Figure S3. Effect of HEPES molarity on S/N ratio under optimal derivatization condition

100 µl calibration solutions, 10 µl TCEP (10 mM), and 170 µl HEPES (pH 8.5) at different molar ratio were mixed and incubated for 15 min at RT, then 20 µl represented quinones (600 µM) was added. The mixture was spinned for 5 min at RT, and 20 µl was diluted with mobile phase and injected to HPLC-CL system.

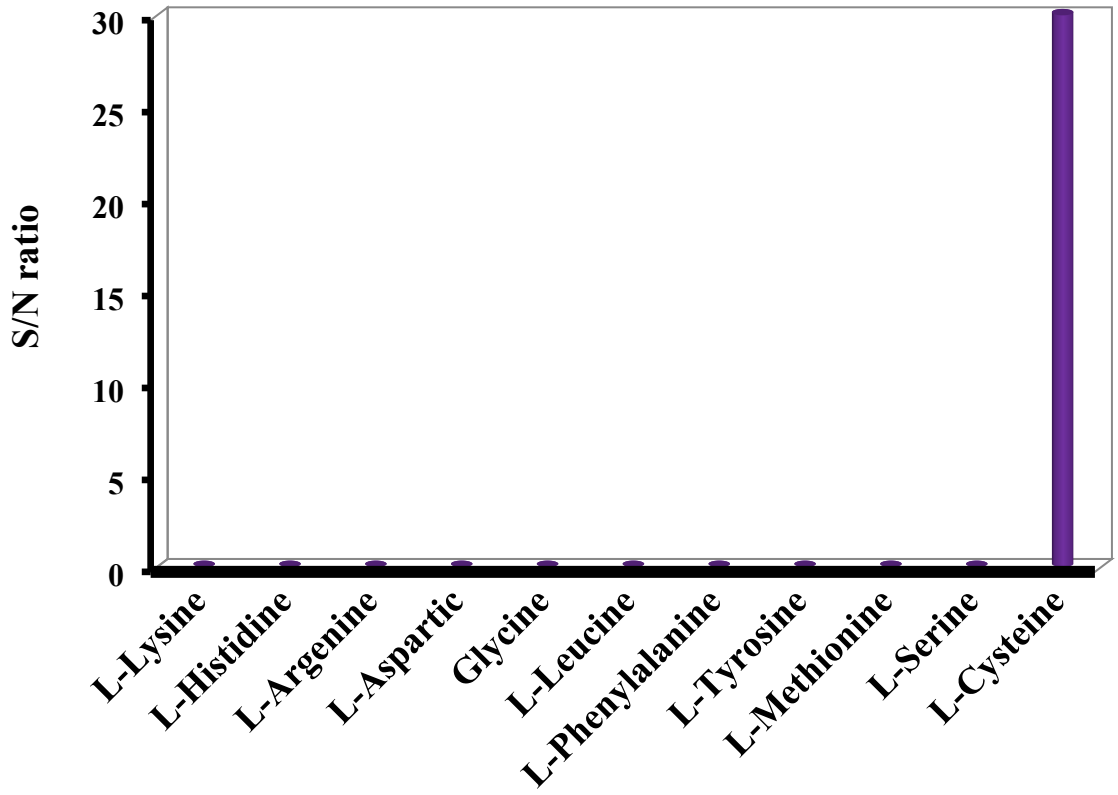

Figure S4. CL intensity of various amino acids after reaction with MQ under optimal derivatization condition

100 µl of amino acid solutions, 10 µl TCEP (10 mM), and 170 µl HEPES (0.5M, pH 8.5) were mixed and incubated for 15 min at RT, then 20 µl represented quinones (600 µM) was added. The mixture was spinned for 5 min at RT, and 20 µl was diluted with mobile phase and injected to HPLC-CL system.

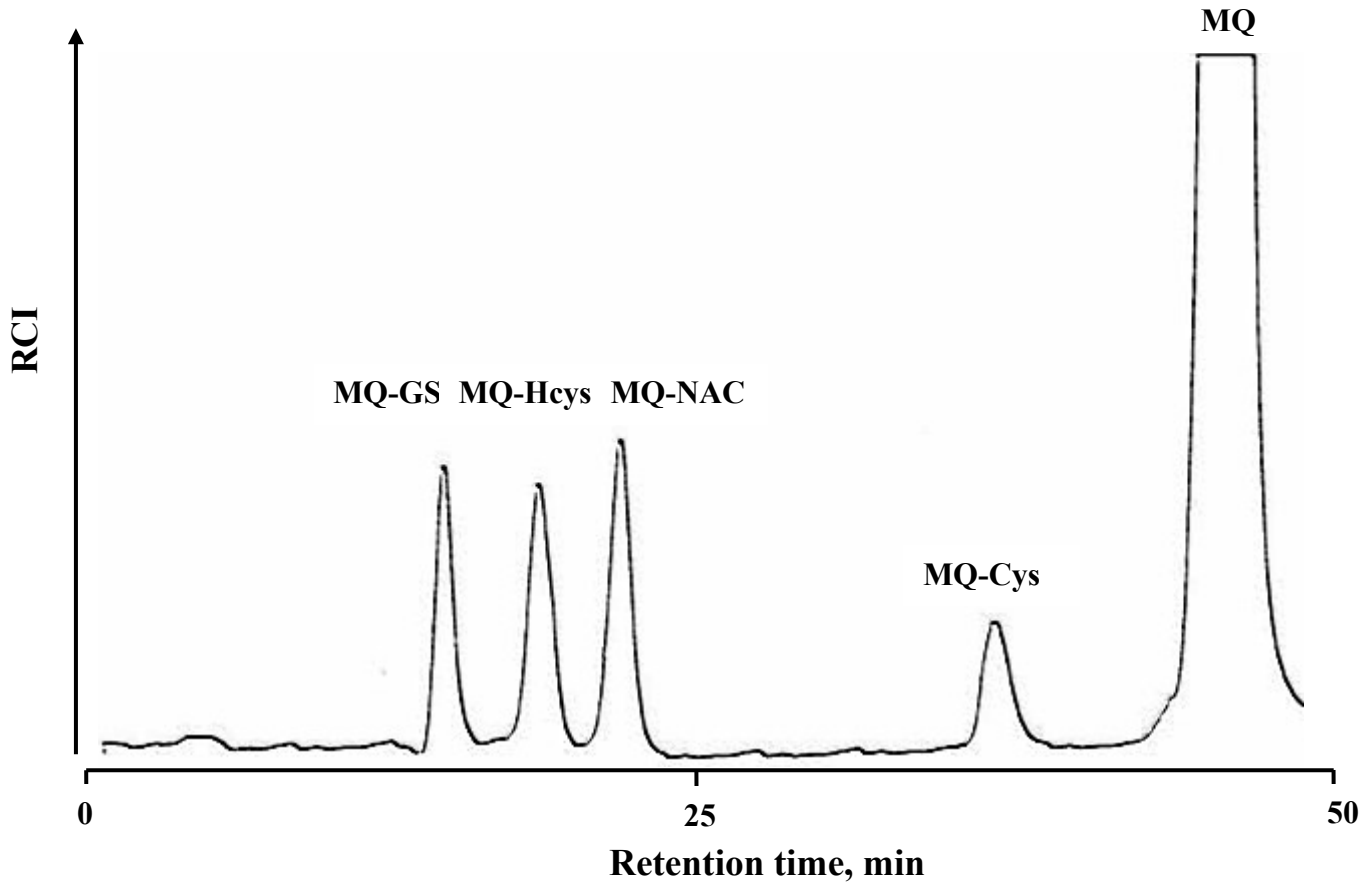

Figure S5. Chromatogram of aminothiols in HEPES buffer treated with TCEP and MQ

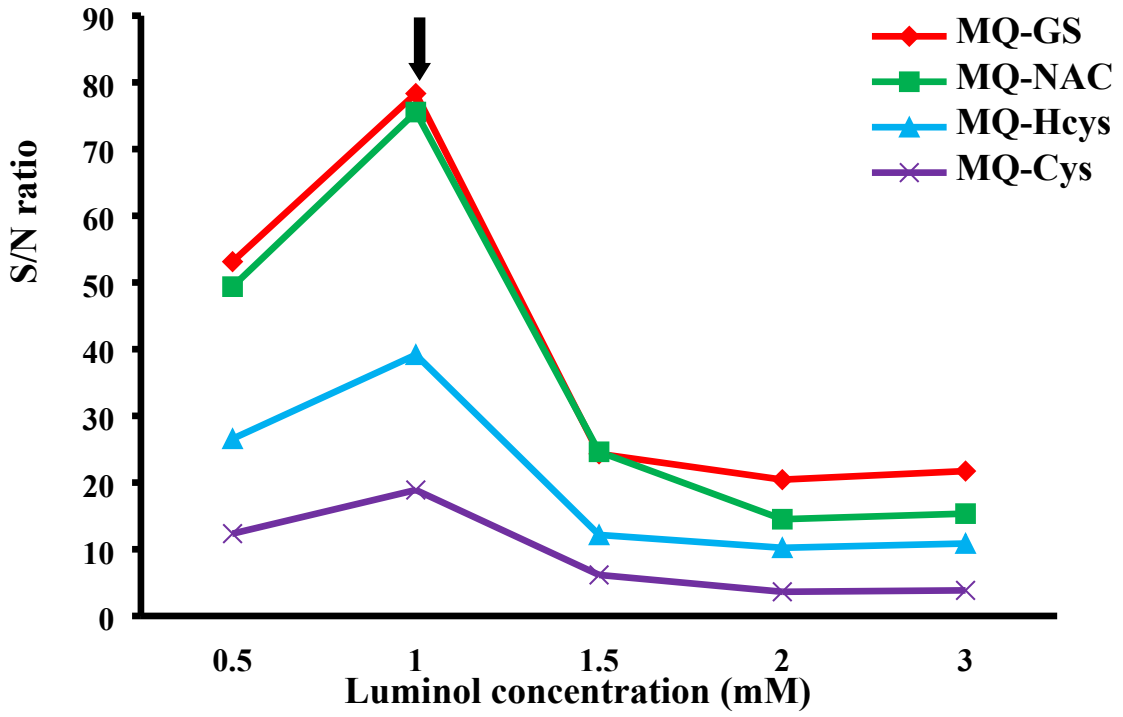

Figure S6. Effect of luminol concentration on S/N ratio of studied aminothiols

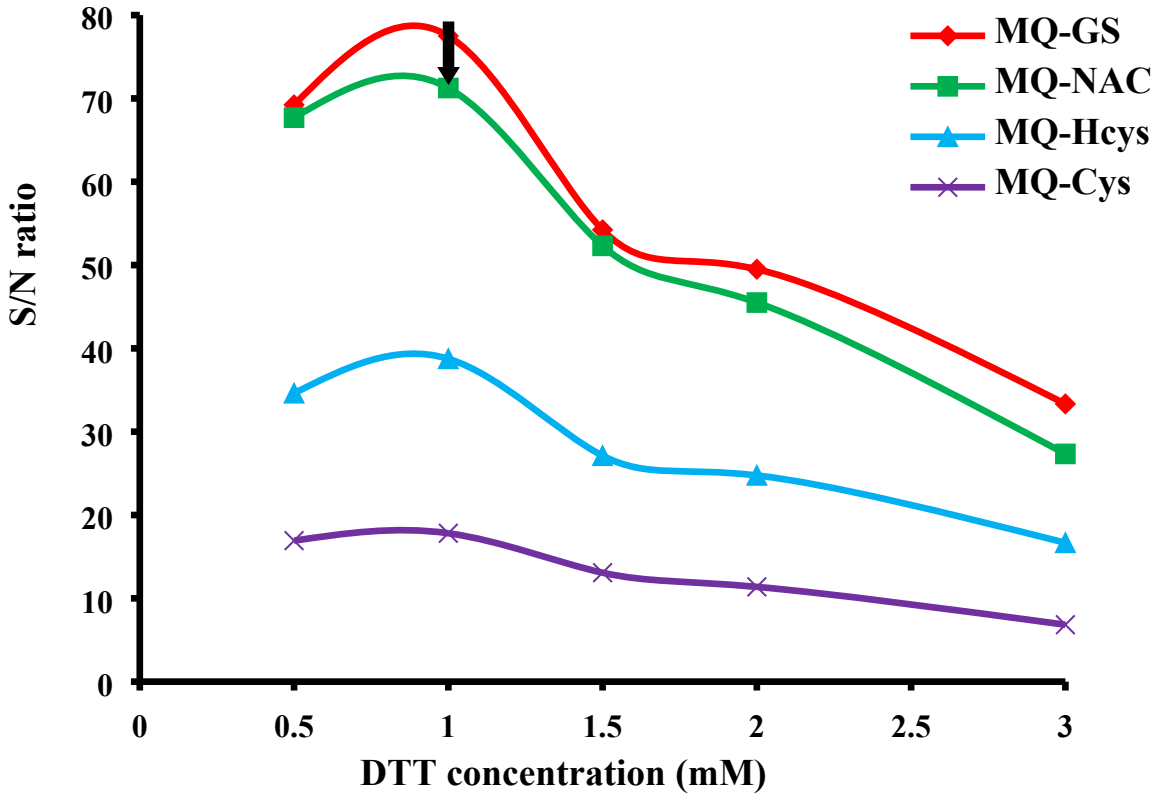

Figure S7. Effect of DTT concentration on S/N ratio of studied aminothiols