Published in final edited form as:

Analyst. 2016 February 21; 141(4): 1325–1330. doi:10.1039/c5an02116b.

Chemical cytometry of thiols using capillary zone electrophoresis-laser induced fluorescence and TMPAB-*o*-M, an improved fluorogenic reagent

Xiao-Feng Guo^{1,2}, Jennifer Arceo¹, Bonnie Jaskowski Hugel¹, Katelyn R. Ludwig¹, and Norman J. Dovichi^{1,*}

¹Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556

²Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China

Abstract

Low molecular weight thiol compounds play crucial roles in many physiological processes. Most methods for determination of thiol compounds are population-averaged; few methods for quantification of thiol compounds in single cells have been reported. We report an ultrasensitive method for determination of thiol compounds in single cells by use of 1,3,5,7-tetramethyl-8-phenyl-(2-maleimide)-difluoroboradiaza-s-indacene (TMPAB-*o*-M), a fluorogenic probe with good properties, coupled with capillary zone electrophoresis and laser induced fluorescence detection using a post-column sheath flow cuvette. TMPAB-*o*-M provides low background, high sensitivity, and excellent reactivity. After optimization of the separation method, we achieved baseline separation of labeled glutathione (GSH), cysteine (Cys), homocysteine, and γ -glutamylcysteine within 11 min, and produced concentration limits of detection from 10 to 20 pM and mass LODs of 65 to 100 zmol. The method was applied for analysis of thiol containing compounds in both cell homogenates and in single HCT-29 and MCF-10A cells. GSH was the main thiol, and Cys was also detected in both cell types. Cells were treated with N-ethylmaleimide, which significantly attenuated thiol levels.

Keywords

chemical cytometry; thiol; TMPAB-*o*-M; CZE-LIF; sheath flow cuvette

1 Introduction

Endogenous low molecular weight thiol-containing compounds, such as glutathione (GSH), cysteine (Cys), homocysteine (Hcy), and γ -glutamylcysteine (GluCys), play crucial roles in a variety of physiological processes.^{1, 2} For example, GSH is endogenously synthesized in all cells and helps to maintain the redox state of cells,^{3, 4} which is closely related to many other functions including antioxidant defense, detoxification of electrophilic xenobiotics, modulation of redox regulated signal transduction, storage and transport of cysteine,

*Corresponding author. ndovichi@nd.edu.

regulation of cell proliferation, synthesis of deoxyribonucleotides, regulation of immune responses, *etc.*³ Cys, a standard proteinogenic amino acid, is a critical substrate for protein synthesis and is vital in determination of the tertiary structure of proteins through the formation of disulfide bonds. Also, Cys is an important precursor in the production of GSH, and can act as an antioxidant, much like GSH.⁵ Other common low molecular weight thiols such as homocysteine (Hcy) and γ -glutamylcysteine (GluCys), are components of the biosynthetic or metabolic pathways of GSH and Cys.⁶ The concentrations of these thiols are closely related to cell state and function.

There have been many studies that analyze thiols in various biological samples using spectrofluorimetric analysis^{7, 8} and fluorescence imaging.^{9, 10} Other analytical methods employ separations using high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) coupled with fluorescence,^{11, 12} mass spectrometry,^{13, 14} and electrochemical detection.¹⁵ Most of these studies focus on the quantification of thiols in biological fluids, tissues, or cell lysates, which provides information on the average composition of the ensemble.^{7, 8, 11, 12, 14–16}

These population-averaged measurement methods obscure any information on the cell-to-cell variation of intracellular thiols.^{17–18} For instance, the redox status may vary between cells due to heterogeneous generation of reactive oxygen and nitrogen species. In addition, pretreatment of cell homogenates is required to prevent interferences from proteins and other sample components, and this pretreatment may degrade the highly active thiol group in trace-level targets. Therefore, a simple method for analysis of different thiols simultaneously with high sensitivity and good selectivity would be valuable in single cell analysis.

The first analysis of GSH in single cells was reported by Yeung's laboratory twenty years ago using monobromobimane as a fluorogenic reagent.¹⁹ This reagent requires excitation in the mid-UV portion of the spectrum, which tends to generate relatively large fluorescent background signals. Since that time, many thiol-specific fluorescent reagents have been developed with improved properties. For instance, several maleimide-based fluorescent reagents have been reported; these reagents typically show more than 100 fold enhancement after reacting with thiol groups.^{20, 21} In addition, fluorescent reagents based on other derivatizing moieties have been reported, including 2, 4-dinitrobenzenesulfonate derivatives, galactose moiety based probes, *etc.*^{22–23} In these reagents, the thiol compounds are not conjugated to the fluorophore and can not be coupled to a separation method to quantify several thiol compounds simultaneously. Recently, we have reported the use of 1,3,5,7-tetramethyl-8-phenyl-(2-maleimide)-difluoroboradiaza-s-indacene (TMPAB-*o*-M) as fluorogenic reagent for thiol determination.^{8, 16} TMPAB-*o*-M has many useful properties, including good cell membrane permeability, high reactivity with thiols (~2 min reaction time for intracellular thiols), good stability of the derivatives at room temperature, and 350-fold enhancement of fluorescence after derivatization with thiols.¹⁶ Moreover, the BODIPY fluorophore in TMPAB-*o*-M generates a strong fluorescence signal with excitation near 490 nm, which matches the wavelength of commonly used lasers operating at 488 nm.

We have reported the use of high-sensitivity CZE-LIF instruments for chemical cytometry, which is the use of modern instrumental methods for analysis of the composition of single

cells.^{20–21} Our CZE-LIF instruments rely on a post-column sheath flow cuvette for fluorescence detection. In this experiment, TMPAB- α -M is used to label intracellular thiols in intact cells. The treated cell is injected into the separation capillary by applying vacuum to the distal end of the separation capillary. After injection, the cell is lysed, the cellular contents are separated by zone electrophoresis, and analytes migrating from the separation capillary are entrained by a pure sheath buffer in a square cuvette that has optically flat windows. Fluorescence is excited by a focused laser beam. Emission is collected at right angles with a high numerical aperture objective, passed through spectral filter to block scattered light, and detected with a high quantum yield photodetector operating in the photon counting mode. The signal is reported in photons per second and has units of Hz.

2 Experimental method

2.1 Materials and chemicals

Unless otherwise stated, all reagents were of analytical grade and purchased from Sigma-Aldrich (St Louis, MO). Deionized water was obtained with a Barnstead NANOpure deionization/sterilization unit (Thermo Scientific Model 7146). Gibco Dulbecco's phosphate buffered saline (DPBS) was purchased from Thermo Fisher Scientific Inc. Cys (97%), Hcy (95%), GSH (98%) and GluCys (80%, HPLC) were dissolved in deionized water to give 10 mM stock solutions. TMPAB- α -M (Fig 1) was synthesized as described earlier;¹⁹ a 2 mM solution was prepared in dimethyl sulfoxide (DMSO). 50 mM N-ethylmaleimide (NEM) stock solution and 1% Triton X-100 stock solutions were prepared in water and diluted to appropriate concentrations before use. Running buffer was prepared by adjusting 10 mM sodium citrate (Na₃Cit) to the required pH using 1.0 M HCl. NaH₂PO₄–Na₂HPO₄ buffers were prepared by mixing 0.1 M NaH₂PO₄ and 0.1 M Na₂HPO₄ to the required pH value.

2.2 Instrument

The capillary electrophoresis instrument was described earlier (Fig 1).^{22–24} Briefly, the system was constructed on a 4 ft × 4 ft optical breadboard, with a diode pumped solid state laser operating at 473 nm with 2.5 mW output power (Model BML473-100FEE, Lasermate Group Inc., Walnut, CA, USA). The beam from this laser was reflected from a mirror, centered on a 6.3×, 0.2 NA microscope objective (22.5 mm focal length, Melles Griot, Rochester, NY), and then focused into the sample stream within the cuvette. The cuvette and the holder have been described elsewhere.²³ Fluorescence was collected with a 60×, 0.7 NA microscope objective (Universe Kogaku), passed through a FB510-10 bandpass interference filter (Omega Optical), and imaged onto a fiber-optic coupled single-photon counting avalanche photodiode module (SPCM-AQ, PerkinElmer). All separations were performed in an uncoated fused silica capillary (50 cm long, 50 μ m i.d., 150 μ m o.d., Polymicro, Phoenix, USA). Single cell injection was performed on an Olympus IX70 inverted microscope (Olympus America INC., USA) using an injection block.²⁴ Data were recorded at either 20 Hz (for cell analysis) or 50 Hz (all other experiments). Data were treated with a five point median filter to remove noise spikes due to scattered light from particles.

2.3 Derivatization procedure

10 μL of 2.0 mM TMPAB-*o*-M stock solution and 20 μL of the mixed thiol standard solution or lysed cell solution were transferred into a 0.5 mL tube and diluted to 250 μL using pH 7.40 $\text{NaH}_2\text{PO}_4\text{--Na}_2\text{HPO}_4$ buffer. Solutions were incubated at 37°C for 6 min. After the derivatization was completed, the solution was diluted with the running buffer to appropriate concentrations and injected into the capillary by pressure of 2 psi for 1 s.

2.4 Electrophoretic procedure

Before use, the new capillary was conditioned by rinsing with methanol, water, 1.0 M NaOH solution, H_2O , 1.0 M HCl solution, and H_2O for 30 min in succession. The capillary was rinsed at the start of each day with H_2O for 5 min, 0.1 M NaOH solution for 5 min, H_2O for 5 min, 0.1 mol/L HCl solution for 5 min, and H_2O for 5 min. Between runs, the capillary was rinsed with running buffer for 3 min. Sample solutions were introduced into the capillary from the anodic side by pressure of 2.0 psi for 1 s. CZE was performed at room temperature at 15 kV.

2.5 Sample preparation

Human colon cancer (HCT-29) and breast (MCF-10A) cell lines were cultured using standard conditions.^{25, 26} The cells were resuspended in DPBS and transferred into 500 μL Eppendorf tubes ($1.5 \times 10^5/100 \mu\text{L}$ for each tube). NEM was used to change the thiol concentrations in the cells.⁷ 2 μL of different concentrations of NEM was added followed by incubation for 5 min at room temperature. Next, 3 μL of 2 mM TMPAB-*o*-M was added, mixed, and incubated at 37 °C for 5 min.

For cell lysis, 10 μL of 2% Triton X100 was added and vortexed, and the resulted solution was stored at -20°C . Before injection, the thawed sample solutions were diluted with running buffer as appropriate. For single cell analysis, cells were incubated with TMPAB-*o*-M for 5 min, the cell suspension was mixed with the same volume of running buffer, and single cell analysis was performed immediately.

2.6 Single cell analysis

10 μL of cell suspension was transferred on to a 21-well Teflon-printed microscope slide (Electron Microscopy Sciences, Washington, USA). Single cells were injected into the separation capillary using an Olympus IX70 inverted microscope by negative pressure. After cell injection, a plug of 0.1% Triton X100 solution was aspirated at 2 psi for 1 s to perform on-column cell lysis. The procedure was completed within 2 min. CZE was performed as described in Section 2.4.

3 Results and discussion

3.1 Optimization of separation conditions

Many low molecular weight thiols (GSH, Cys, Hcy, and GluCys) are zwitterions, and their isoelectric points are below pH 7.0. As a result, the pH of running buffer should be higher than 7.0 to ensure that the thiols are negatively charged to minimize electrostatic interaction with the negatively charged capillary walls.

However, maleimide is the moiety reacting with thiol group, and maleimide may be hydrolyzed at high pHs. Therefore, the effect of pH value of running buffer was studied in the range of 7.0–8.4. As shown in Fig 2, Hcy and Cys migrate near 7 min, and GSH and GluCys migrate near 10 min. We observe the resolution of Hcy and Cys is improved as the pH value of running buffer increased from 7.0 to 8.4, but the resolution of GSH and GluCys degrades at pH 8.4. pH 7.5 was selected as the final pH value of running buffer.

We tested two background electrolytes for the separation, Na_3Cit and $\text{NaH}_2\text{PO}_4\text{--Na}_2\text{HPO}_4$. In $\text{NaH}_2\text{PO}_4\text{--Na}_2\text{HPO}_4$ electrolyte, we observed four more peaks with longer migration time following incubation of the derivatized analytes 60 min; we assume that these peaks hydrolytic products from the derivatized analytes. In contrast, the derivatized analytes were more stable in Na_3Cit , and no other peaks were found. Therefore, pH 7.5 Na_3Cit was used for further experiments. We also investigated the concentration of Na_3Cit in the range of 5–20 mM (Fig 3). From the results, the resolution of the four analytes remain nearly constant when the concentration of Na_3Cit is in the range of 10–20 mM, and a slight tailing of the Cys peak was observed when the concentration of Na_3Cit in the buffer is 5 mM. Finally, 10 mM pH 7.5 Na_3Cit was selected as the running buffer, and all the four analytes are baseline separated within 11 min. A typical electropherogram of the thiol derivatives obtained under the optimal and separation conditions is as shown in Fig. 3, trace b.

3.2 Analytical performance

Method validation encompassed linearity, limit of detection, and precision. Five different concentrations of analytes from 0.1 to 10 nM were tested for linearity, Table 1. The linear correlation coefficients for these calibration curves were in the range 0.993 to 0.998. The relative standard deviations for peak areas were evaluated by four replicate injections of mixed samples with concentrations 5 nM (each analyte), and the result is less than 4%, revealing excellent precision and repeatability. The concentration LODs (signal-to-noise ratio of 3) were from 13 pM for Hcy to 20 pM for GluCys. The injection volume was ~5 nL, and the mass LODs were from 60 zmol for Hcy to 100 zmol for GluCys. As demonstrated earlier, common amino acids and metal ions in biological samples do not interfere significantly in the derivatization procedure.²⁷ The only previous report of the analysis GSH in single cells was published 20 years ago.¹⁹ In that report, monobromobimane (mBrB) was used as fluorescence reagent for GSH. The sensitivity was not mentioned in that report, but from the signal to noise ratio of mBrB-GSH in the electropherograms, the sensitivity of our study appears to be significantly improved. More importantly, we report the analysis of three additional intracellular thiols in single cells.

3.3 Analysis of cell homogenate solutions

In this study, HCT-29 and MCF-10A cells were first incubated with TMPAB-*o*-M and then lysed to prepare homogenate solutions. NEM was used to change the thiol concentrations in the cells.⁷ As shown in Fig 4 and Table 2, Cys and GSH were found in the cell homogenate solutions. The concentrations of Cys and GSH in the samples decreased with increasing NEM concentration. Based on a cell diameter of ~15 μm , the intracellular concentration of Cys was estimated to be ~20 μM for HCT-29 and ~110 μM for MCF-10A; the intracellular

concentrations of GSH are estimated to be 1.1 mM for HCT-29 and 1.8 mM for MCF-10A. In both cases, the estimates are consistent with literature values.^{28,29}

3.4 Single-cell analysis of thiols

MCF-10A and HCT-29 cells were suspended in DPBS. After pretreated with NEM, the suspended cells were incubated with TMPAB-*o*-M. Single cells were then injected into the separation capillary using negative pressure and lysed with 0.1% Triton X100, followed by electrophoretic separation. As shown in Fig 5, GSH was the main thiol in both cell types. In addition, Cys was also detected in both cell types. GluCys was found in a MCF-10A cell (peak 4 of electropherogram c in Fig 5 A), and several small peaks were also found near the Cys peak, which were likely thiol-containing peptides.

Signals corresponding to labeled proteins were not observed. There are two likely reasons that proteins were not observed. First, we did not reduce disulfide bonds, so most cysteine residues were unavailable for reaction with TMPAB-*o*-M. Second, separation conditions were not designed for intact proteins. Top-down proteomic analysis is best performed with coated capillaries in acidic background electrolytes or with the use of surfactant in the background electrolyte.^{30–31} Proteins were likely adsorbed to the uncoated capillary under our separation conditions.

A modest variation in the amount of intracellular thiols was observed between cells. GSH ranged from ~1.3 to 1.9 mM and Cys ranged from ~50 to 140 μ M in MCF-10A cells. GSH ranged from ~0.60 to ~1.1 mM and Cys ranged from 10 to 130 μ M In HCT-29 cells. These results showed the cell-to-cell variation of intracellular thiols concentrations and redox status of different cells, which is consistent with population-averaged measurements, such as the analysis of cell homogenate solutions listed in Table 2 and discussed in Section 3.3. In addition, in this method the cells were lysed in the capillary just before electrophoresis, so the degradation of highly active intracellular thiol compounds could be prevented. Thus, compared to the reported population-averaged measurement methods, we believe our results are closer to the true value of concentrations of intracellular thiols.

4. Conclusions

This study demonstrates the quantitation of a number of intracellular thiols by use of a thio-specific fluorogenic reagent and chemical cytometry, where the labeled thiols within a single cell are separated by capillary electrophoresis and detected by laser-induced fluorescence. This technology should be compared with conventional flow cytometry, which uses a similar fluorescent probe but measures total fluorescence without the separation of the labeled components.³² Our data reveals that the total fluorescence signal is dominated by glutathione, and that any change in the abundance of other thiols is likely lost in conventional flow cytometry analysis. Chemical cytometry is required to assay for low-level thiols in single cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

XiaoFeng Guo acknowledges a fellowship from the China Scholarship Council. Jennifer Arceo and Bonnie Jaskowski Huge acknowledge support from a National Science Foundation Graduate Research Fellowship under Grant No. DGE-1313583. This work was supported by the National Institutes of Health (R01GM096767 – NJD).

References

1. Zhang H, Zhang C, Liu R, Yi L, Sun H. Chem. Commun. 2015; 51:2029–2044.
2. Ma T, Ding H, Xu H, Lv Y, Liu H, Wang H, Tian Z. Analyst. 2015; 140:322–329. [PubMed: 25406724]
3. Kerksick C, Willoughby D. J Int. Soc. Sport. Nutr. 2005; 2:38–44.
4. Sen CK, Packer L. Am. J. Clin. Nutr. 2000; 72S:653S–669S. [PubMed: 10919972]
5. Darkwa J, Olojo R, Chikwana E, Simoyi RH. J Phys. Chem. A. 2004; 108:5576–5587.
6. Meister A. Science. 1983; 220:472–477. [PubMed: 6836290]
7. Yang Y, Guan X. Anal. Chem. 2015; 87:649–655. [PubMed: 25423115]
8. Guo X, Wang H, Guo Y, Zhang H. Anal. Chim. Acta. 2009; 633:71–75. [PubMed: 19110118]
9. Ma T, Ding H, Xu H, Lv Y, Liu H, Wang H, Tian Z. Analyst. 2015; 140:322–329. [PubMed: 25406724]
10. Lee MH, Han JH, Kwon P, Bhuniya S, Kim JY, Sessler JL, Kang C, Kim JS. J Am. Chem. Soc. 2012; 134:1316–1322. [PubMed: 22171762]
11. Kono Y, Iizuka H, Isokawa M, Tsunoda M, Ichiba H, Sadamoto K, Fukushima T. Biomed. Chromatogr. 2014; 28:589–593. [PubMed: 24500895]
12. Andre C, Guillaume YC. Talanta. 2012; 93:274–278. [PubMed: 22483910]
13. Karazniewicz-Lada M, Danielak D, Tezyk A, Zaba C, Tuffal G, Glowka F. J Chromatogr. B. 2012; 911:105–112.
14. Park JB, Bae SH, Jang S, Noh WJ, Hong J, Yoon KD, Kang HC, Bae SK. J Sep. Sci. 2013; 36:2306–2314. [PubMed: 23686964]
15. Shanmugarajah D, Ding D, Huang Q, Chen X, Kochat H, Petluru PN, Ayala PY, Parker AR, Hausheer FH. J Chromatogr. B. 2009; 877:857–866.
16. Guo X, Chen J, Wang H, Zhang H, Huang W, Guo J. Talanta. 2012; 99:1046–1050. [PubMed: 22967661]
17. Ye X, Rubakhin SS, Sweedler JV. Analyst. 2008; 133:423–433. [PubMed: 18365109]
18. Sweedler JV, Arriaga EA. Anal. Bioanal. Chem. 2007; 387:1–2.
19. Wong KS, Yeung ES. Microchimica Acta. 1995; 120:321–327.
20. Dovichi NJ, Hu S. Curr. Opin. Chem. Biol. 2003; 7:603–608. [PubMed: 14580565]
21. Cohen D, Dickerson JA, Whitmore CD, Turner EH, Palcic MM, Hindsgaul O, Dovichi NJ. Annu. Rev. Anal. Chem. 2008; 1:165–190.
22. Essaka DC, Prendergast J, Keithley RB, Palcic MM, Hindsgaul O, Schnaar RL, Dovichi NJ. Anal. Chem. 2012; 84:2799–2804. [PubMed: 22400492]
23. Dada OO, Huge BJ, Dovichi NJ. Analyst. 2012; 137:3099–3101. [PubMed: 22606689]
24. Krylov SN, Starke DA, Arriaga EA, Zhang Z, Chan NW, Palcic MM, Dovichi NJ. Anal. Chem. 2000; 72:872–877. [PubMed: 10701276]
25. Yue X, Hummon AB. J Proteome Res. 2013; 12:4176–4186. [PubMed: 23927012]
26. Bauer KM, Watts TN, Buechler S, Hummon AB. J Proteome Res. 2014; 13:4910–4918. [PubMed: 25096929]
27. Guo X, Zhao P, Wang H, Zhang H. J Chromatogr. B. 2011; 879:3932–3936.
28. Horan AD, Chan CY, Pletcher CH, Menon C, Evans SM, Moore JS, Koch CJ. Cytometry. 1997; 29:76–82. [PubMed: 9298814]
29. Silva TM, Fiuza SM, Marques MPM, Persson L, Oredsson S. Amino Acids. 2014; 46:339–352. [PubMed: 24363201]

30. Hu S, Jiang J, Cook LM, Richards DP, Horlick L, Wong B, Dovichi NJ. Electrophoresis. 2002; 23:3136–3142. [PubMed: 12298085]
31. Zhao Y, Sun L, Champion MM, Knierman MD, Dovichi NJ. Anal. Chem. 2014; 86:4873–4878. [PubMed: 24725189]
32. Dachary-Prigent J, Freyssinet JM, Pasquet JM, Carron JC, Nurden AT. Blood. 1993; 81:2554–2565. [PubMed: 8490169]

Figure 1. Sheath flow cuvette used for fluorescence detection, and structure of TMPAB- ϕ -M before and after reaction with a thiol.

Figure 2. Effect of pH of the background electrolyte on the separation. Running buffer: 10 mM H_3Cit - Na_3Cit . Injection: pressure, 2 psi, 1s. Separation voltage: 15 kV. (a) pH 8.4; (b) pH 7.5; (c) pH 7.0. Peaks: (1) Hcy (1.0 nM); (2) Cys (2.0 nM); (3) GSH (3.0 nM); (4) Glu-Cys (1.5 nM). Traces offset for clarity.

Figure 3.

Effect of running buffer concentration on the separation. Running buffer: pH 7.5 H_3Cit - Na_3Cit . Injection: pressure, 2 psi, 1s. Separation voltage: 15 kV. Electropherograms: (a) 5 mM; (b) 10 mM; (c) 15 mM; (d) 20 mM. Peaks: (1) Hcy (0.5 nM); (2) Cys (1.0 nM); (3) GSH (1.50 nM); (4) Glu-Cys (0.75 nM). Traces offset for clarity.

Figure 4.

Electropherograms of cell homogenate solutions. Insert shows a 5 $\times$ expansion of the electropherogram near the Cysteine peak. Running buffer: 10 mM pH 7.5 $\text{H}_3\text{Cit-Na}_3\text{Cit}$. Injection: pressure, 2 psi, 1s. Separation voltage: 15 kV. Electropherograms: (a) MCF-10A cells; (b) MCF-10A cells incubated with 8 μM NEM; (c) MCF-10A cells incubated with 16 μM NEM; (d) MCF-10A cells incubated with 32 μM NEM; (e) HCT-29 cells; (f) HCT-29 cells incubated with 16 μM NEM. Electropherograms offset for clarity.

Figure 5.

Electrophoretic analysis of thiols in of single cells. Running buffer: 10 mM pH 7.5 H_3Cit - Na_3Cit . Injection: pressure, 2 psi, 1s. Separation voltage: 15 kV. (A) MCF-10A cells; (B) HCT-29 cells. (a–c) electropherograms from individual cells incubated with TMPAB- α -M; (d–e) cells from the same line treated with NEM before incubated with TMPAB- α -M. Peaks: (2) Cys; (3) GSH; (4) Glu-Cys.

Table 1Analytical performance of TMPAB-*o*-M derivatives.

Thiols	Linear range (nM)	Slope of calibration curve (arbitrary units)	r ²	Concentration LOD (nM) ^a	Mass LOD (nmol) ^{a,b}
Hcy	0.1–10	1.0	0.9941	0.013	60
Cys	0.1–10	1.0	0.9974	0.015	70
GSH	0.1–10	1.7	0.9983	0.016	75
γ -GluCys	0.1–10	1.2	0.9928	0.021	100

^a : signal-to-noise ratio = 3.^b : injection volume ~4.7 nL.

Table 2

Analytical results for thiols in cell homogenates.

Samples	NEM Added (μ M)	Hcy (μ M) <i>n</i> =3	Cys (μ M) <i>n</i> =3	GSH (μ M) <i>n</i> =3	GluCys (μ M) <i>n</i> =3
MCF10A	0	- ^a	1.87 $\pm$ 0.08	30.4 $\pm$ 1.8	-
	8	-	0.56 $\pm$ 0.05	18.3 $\pm$ 1.3	-
	16	-	0.14 $\pm$ 0.07	11.8 $\pm$ 0.8	-
	32	-	-	-	-
HCT-29	0	-	0.33 $\pm$ 0.06	18.9 $\pm$ 1.2	-
	16	-	NQ ^b	13.9 $\pm$ 1.0	-

Results reported $\pm$ 1 standard deviation of the distribution

^a - not detected.

^b - NQ – not quantified