Analysis of Glutathione and other (di-)sulfides

L.M. van Heerwaarden, H.J Brouwer, M. Eysberg and N.J. Reinhoud

Antec Leyden, Industrieweg 12, 2382 NV Zoeterwoude, The Netherlands

Introduction

Glutathione (GSH) is a tripeptide composed of glutamate, cysteine and glycine that has numerous important functions within cells. GSH is involved in detoxification and serves as antioxidant in the highly oxidizing environment of the erythrocyte. The sulfhydryl of GSH can be used to reduce peroxides formed during oxygen transport. The resulting oxidized form of GSH consists of two molecules disulfide bonded together (GSSG). The measurement of GSH, GSSG, Cysteine, Cystine and other thiol and disulfides levels in biological samples is essential to determine the redox and detoxification status of cells and tissue. In this publication the analysis of GSH, GSSG and several other (di-) thiols is described using a DECADE II electrochemical detector with Dual Cell Control (DCC). The DCC option makes it possible to control two electrochemical cells and use them for data acquisition.

An ALEXYS 100 micro LC-EC system has been used with a reactor cell for reduction of disulfides to thiols.

$$R'\text{-}S\text{-}S\text{-}R'' + 2\,H^+ + 2\,e^- \rightarrow R'SH + R''SH$$

In the second cell thiols are detected by oxidative amperometric detection. Thiols as well as the oxidised free disulfides can be quantified this way, which is not possible when a pre-column reduction of disulfides is applied.

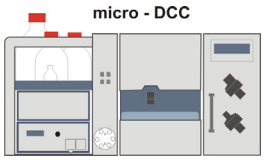

Fig. 1. ALEXYS 100 Disulfides I

Method

HPLC	ALEXYS 100 Disulfides I (p.n. 180.0068) with DECADE II Dual Cell Control
Column	ALB-115, 150 x 1 mm (ID), particle size 3 µm (p/n 250.1096)
Flow cell 1	Reactor cell with GC electrode vs. Hy-REF reference electrode
Flow cell 2	FLEXCELL with gold WE and Hy-REF
Mob. phase	50 mM phosphoric acid, 50 mM citric acid, 500 mg/L octane sulphonic acid, brought at pH 3.0 with 19.2 M NaOH
Flow rate	50 µL/min
VInjection	2 µL, partial loop-fill with 10 µL pre-flush
Toven	35 °C (separation and detection)
Range	2 mA/V (cell 1), 100 nA/V (cell 2)
Ecell 1, RED	-1400 mV vs. HyREF (Icell 1 -25 – -30 mA)
Ecell 2, OX	250 mV vs. HyREF (Icell 2 5 – 15 nA)
ADF	off

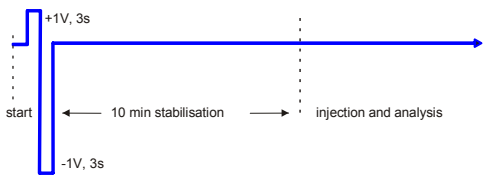

Fig. 2. Before every run a short cleaning pulse (+1 V and -1 V for 3 s) was executed to remove contaminants and regenerate the gold (Au) surface.

Hydrodynamic voltammogram

For oxidative analysis of glutathione, cysteine and homocysteine a working potential of 250 mV vs. HyREF was used. The optimum working potential for the reactor cell was found to be -1.4 to -1.6 V (Fig. 3).

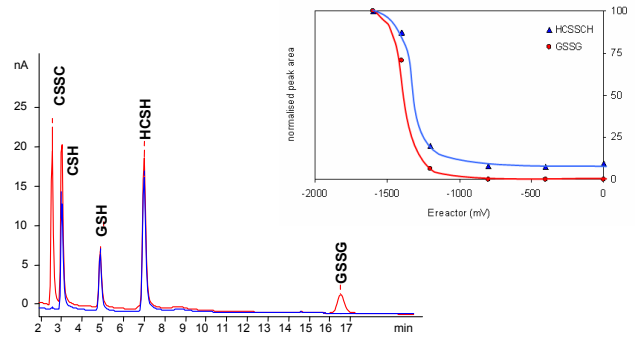

Fig. 3A. Analysis of 2 µL 1 µM cystine (CSSC), cysteine (CSH), GSH, homocysteine (HCSH) and GSSG in mobile phase with reactor on (red) or off (blue). Fig. 3.B (right) Normalised IE-curves of homocystine (HCSSCH) and di-glutathione (GSSG) measured at the second cell after varying the working potential in the reactor cell.

Reproducibility, Linearity & LOD

Reproducibility (n=10) of 2 µL injections of 1 µM CSSC, GSH and HCSH.

	Retention		Height		Area	
	tr (min)	RSD (%)	h (nA)	RSD (%)	A (nA.s)	RSD (%)
CSSC	2.25	0.1	15.1	2.4	158.1	3.4
GSH	3.98	0.1	12	1.1	100	1.4
HCSH	4.51	0.1	33	1.3	392	4.2

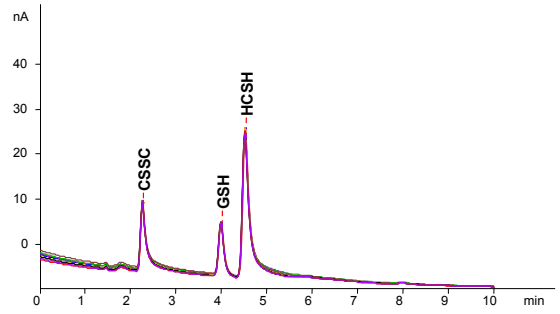

Fig. 5. Overlay of 10 chromatograms of 2 µL injections of 1 µM CSSC, GSH and HCSH in mobile phase.

The response for CSSC, GSH and HCSH was linear in the concentration range of 0.25 - 4 µmol/L. Correlation coefficients of 0.9997 – 1.000 were found for peak heights. A Limit of Detection (LOD) of 10 nmol/L for all three components was determined. The LOD was calculated as the concentration resulting in a signal that is 3 times the peak-to-peak noise of the baseline.

Conclusion

Thiols as well as the oxidised free disulfides can be quantified using the 'ALEXYS 100 Disulfides I'. A cleaning step for the gold working electrode improves the reproducibility. A detection limit down to 10 nmol/L can be achieved for CSSC, HCSH and GSH.