High Sensitive Thiols Detection using a Gold Working Electrode

AN11-0815

Biological thiols such as cysteine (Cys) and glutathione (GSH) play a crucial role in redox status control. Homocysteine (Hcys), one of the main thiols, has a close relationship with the pathogenesis of arteriosclerosis. Looking through the literature, several analytical methods for measuring these thiols have been reported. One of the most common procedures is to use post or pre-column derivatization with UV-Vis or fluorescence detection. However, derivatization methods do not always satisfy the required sensitivity and one is forced to use a complicated protocol. One of the ways to avoid the derivatization procedure is to use electrochemical detection using an amalgam working electrode. However, this procedure demands intensive maintenance of the working electrode. One of the best and most realistic analytical procedures involves using electrochemical detection with a gold working electrode as we describe below.

We introduce a new concise analytical procedure for measurement of biological thiols using a gold working electrode. Thiols have a high affinity for gold working electrodes and this can result in pollution of the working electrode and can lead to less reproducible results. Our new pretreated gold working electrode is coated with a material to prevent such pollution from thiols and other compounds. Thus, this allows highly sensitive and reproducible thiol detection using a simple methodology. As far as injecting protein-precipitated samples, this electrode could be used for one year without any maintenance. Users do not need to perform any treatments such as generating an amalgam layer or applying pulse potentials in order to clean the gold working electrode.

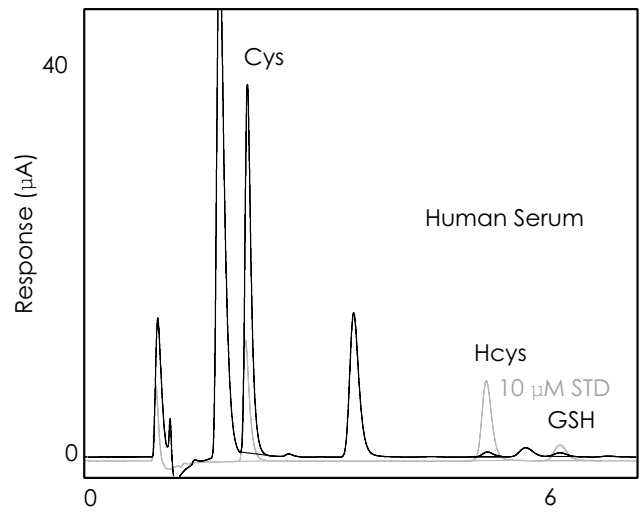

Fig 1. Hcys analysis in the human serum (black) and standard solution (grey)

The detection limit with this methodology is 1 fmol for GSH and 0.5 fmol for Cys at the conditions set when using Eicompak SC-3ODS column. Thiols (i.e. Cys, GSH, Glu-Cys, Cys-Gly and Hcys) as well as ascorbic acid can be analyzed using this procedure.

Chromatographic Condition

HPLC-ECD	Eicom HTEC-500
Column	Eicom SC-3ODS (3.0 ID x 100 mm)
M.P. flow rate	400 µl/min
Applied potential	+600 mV vs. Ag/AgCl
Working electrode	Eicom WE-AU
Time constant filter	1.5 sec
System Temperature	25°C
Mobile Phase	99% 0.1 M Sodium phosphate buffer (pH2.5), 1% MeOH and 50 mg/L Sodium Octansulfornate (SOS), 5 mg/L EDTA-2Na. (In the situation where the target compound is only GSH, the SOS concentration should be 100 mg/L)

Sodium octansulfate (SOS) increases the retention time of amine compounds but reduces it for other cations.

Figure 1 shows a typical chromatography from a human serum sample and standard solutions.

Reference

Y. Hiraku, M. Murata and S. Kawanishi; Determination of intracellular glutathione and thiols by high performance liquid chromatography with a gold electrode at the femtomole level: comparison with a spectroscopic assay. Biochimica et Biophysica Acta. 1570 (2002) 47-52.