Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 5, pp. 643–647, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Short Communication

Determination of glutathione in biological tissues by high-performance liquid chromatography with electrochemical detection

E. BOUSQUET, N. A. SANTAGATI and T. LANCETTA

Istituto di Chimica Farmaceutica e Tossicologica, Universita' di Catania, Viale A. Doria, 6, 95125 Catania, Italy

Keywords: *Glutathione; distribution in mouse tissues; reversed-phase chromatography; electrochemical detection.*

Introduction

Thiol compounds play a fundamental rôle in a multitude of biological functions. Glutathione (GSH), the tripeptide L-γ-glutamyl-cysteinylglycine, is widely distributed among living cells and is involved in several metabolic processes such as the transport of amino acids across membranes or intracellular reduction reactions. GSH serves as a coenzyme for a select number of enzymes and also plays an important rôle in the cellular protection from reactive drugs and carcinogen metabolites [1–4].

Several procedures for the determination of GSH in biological materials have been described. The most commonly used method was based on its reaction with the 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) to form a mixed disulphide liberating a product with maximum absorption at 412 nm [5].

More specific methods involve enzymatic [6, 7] or fluorimetric [8, 9] techniques.

HPLC methods have been reported [10–14] for the assay of GSH, but most of these involved pre-column or post-column derivatization reactions. In addition, the application of electrochemical detection for liquid chromatography has been described [15].

In the present paper we report a method of analysis for the accurate estimation of GSH levels in mouse tissue extracts by high-performance liquid chromatography (HPLC) using a dual electrode detection system.

Experimental

Apparatus and chromatographic conditions

The liquid chromatograph consisted of a Kontron LCS 620 Gradient System (Kontron A.G., Zurich, Switzerland) equipped with a Model 7125 injector with a 10-μl injection loop (Rheodyne, Berkeley, USA).

The electrochemical detector was a Model 5100A Coulochem (ESA, Bedford, USA) equipped with a Model 5011 detector that consisted of two porous graphite analytical electrodes (ESA, Bedford, USA) in series, as shown in Fig. 1, and included a Model 5020 single electrode cell (ESA, Bedford, USA) as a guard cell, which is placed before the HPLC injector. Chromatography was performed at room temperature on a stainless-steel column (25 × 0.4 cm, i.d.) packed with Hypersil ODS 10 μm (Policonsult Scientifica, Rome, Italy). Detector 1 was set at +0.25 V and detector 2 set at +0.75 V.

The flow-rate and chart speed were set at 1 ml min^{-1} and 0.25 cm min^{-1}, respectively.

The mobile phase consisted of aqueous phosphate buffer (NaH_2 PO_4 50 mM l^{-1}) containing 5 mM sodium pentasulphonate adjusted to pH 4 with 2 N NaOH/methanol (85:15, % v/v). All eluents were filtered through a 0.5-μm Millipore filter (Millipore, Bedford, USA) and degassed before use.

Sample preparation

Animal tissues were homogenized at +4°C in 5% trichloroacetic acid, the homogenate was centrifuged (800 *g*) for 10 min, the supernatant fraction was then filtered and injected (10 μl) into the chromatograph.

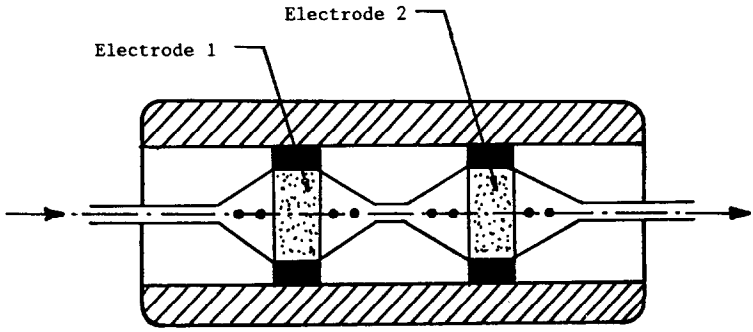

Figure 1
Schematic design of dual electrode cell.

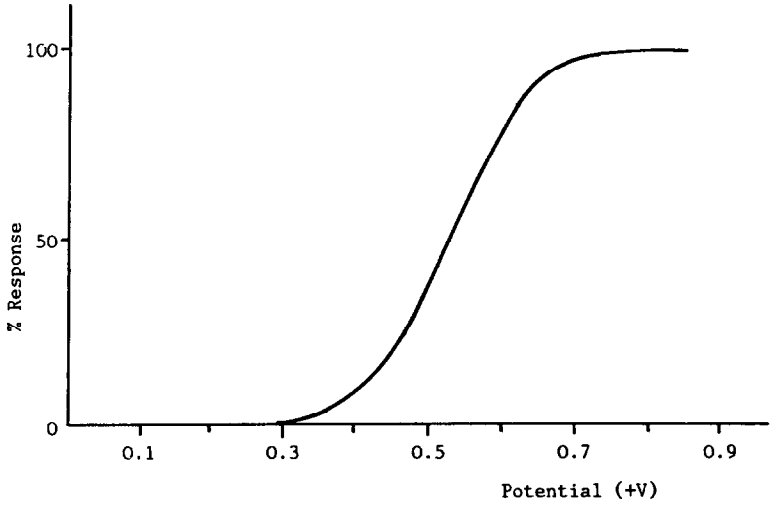

Figure 2
Voltammogram of GSH at the potential of the second electrode.

Results and Discussion

Figure 2 shows the response–voltage curve of GSH obtained using chromatographic conditions described. At positive potential GSH undergoes the following anodic reaction:

$$2\ GSH \rightarrow GSSG + 2\ H^+ + 2\ e^-.$$

Detector 1 (+0.25 V) removes GSH from any interferences, whilst detector 2 (+0.75 V) detects GSH in the injected sample. Figure 3 shows typical chromatograms for GSH standard, GSH in kidney homogenate and blank sample.

The precision of the assay was determined by analyses of 30 samples of kidney homogenate containing, for every 10 samples, the amounts of 15, 30 and 45 μg of

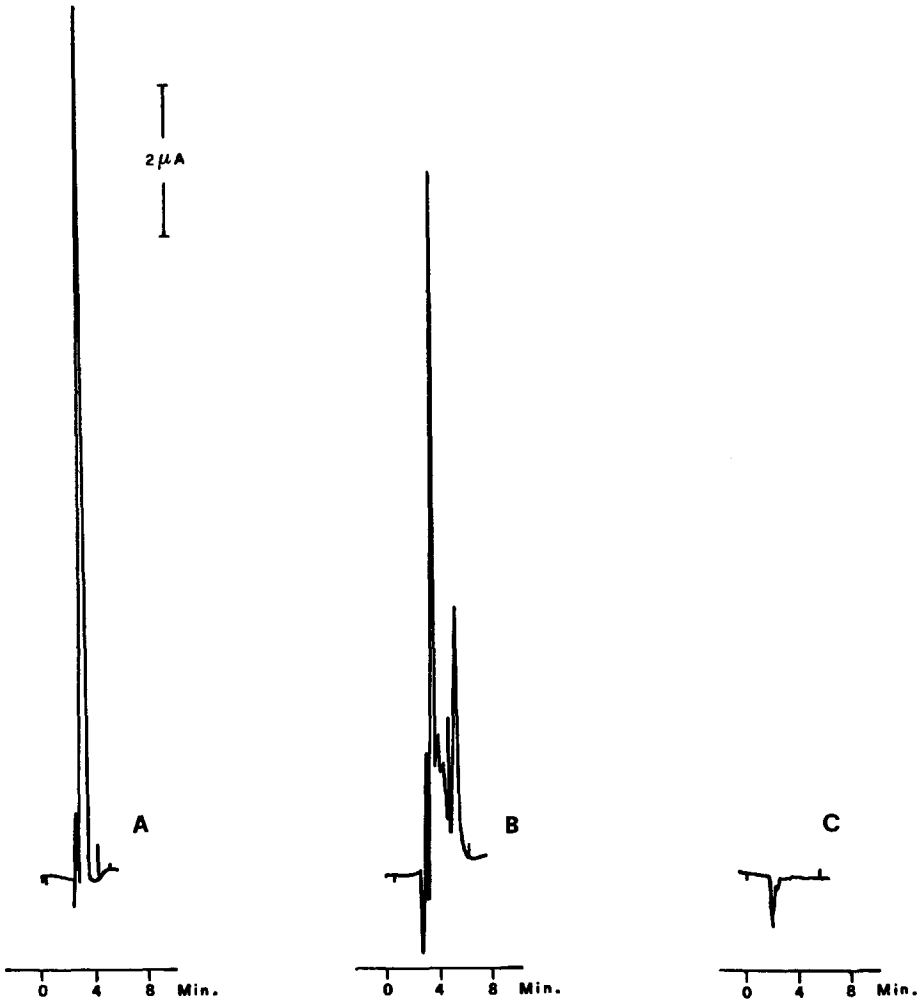

Figure 3
Chromatograms of 2 nmol of GSH standard (A), GSH in kidney homogenate (B) and blank sample (C). Sample size: 10 μl.

Table 1
Precision of the assay of GSH in kidney homogenate

GSH added ($\mu g\ ml^{-1}$)	GSH found* ±SD ($\mu g\ ml^{-1}$)	RSD	Recovery (%)
15	13.32 ± 0.30	2.25	88.80
30	27.32 ± 0.25	0.93	91.07
45	40.65 ± 0.61	1.50	90.35

*Each value is the mean of 10 determinations.

Table 2
GSH values in organs of mouse

Source	Number of samples	Amount of GSH ±SE ($\mu mol\ g^{-1}$ wet wt of tissue)
Liver	15	6.19 ± 1.32
Kidney	15	5.18 ± 0.95
Brain	15	2.51 ± 0.51
Lung	15	1.43 ± 0.36
Heart	15	1.03 ± 0.29

radioactive GSH, respectively. The measurements were performed using glutathione [glycine-2-^{3}H] (240 mCi $nmol^{-1}$) with a Packard Tri-Carb Model 4530 instrument.

The method was reproducible, the relative standard deviations (RSD) were from ±0.93 to 2.25%.

The average recovery was 90% (Table 1). The linearity is expressed by the relationship $y = 0.367x - 0.00245$ where x was the area ($\times 10^4$) under the peak; $r = 0.9998$. Table 2 reports GSH concentrations ($\mu mol\ g^{-1}$) in organs of mouse. The limit of detection was 20 pmol ml^{-1}. The sensitivity was defined as the amount giving a signal-to-noise ratio of 2.

The stability of GSH solutions (working standards) has been examined and no significant differences of concentrations were found when the tripeptide was allowed to stand at room temperature for 5 h. The analysis of working solutions after 14 h showed that the amount of degraded product was about 30%. GSH solutions were stable for at least 3 days after extraction if stored at −20°C.

The analytical method employed allows an accurate evaluation of levels of GSH in different kinds of tissues. Its application appears to be particularly convenient because the use of an electrochemical detector required small sample volumes. Furthermore, the method is highly reproducible and can be applied to routine analysis.

References

[1] W. E. Knox, in *The Enzymes*, p. 253. Academic Press, New York (1960).
[2] A. Lazarow, in *Glutathione*, p. 231. Academic Press, New York (1954).
[3] N. Kaplowitz, J. Kuhlenkamp, L. Goldstein and J. Reeve, *J. Pharmac. Exp. Ther.* **212**, 240–245 (1980).
[4] J. R. Bend, B. R. Smith, L. M. Ball and H. Muktar, in *Conjugation Reaction in Drug Biotransformation*, p. 3. Biomedical Press, North-Holland, Amsterdam (1978).
[5] G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70–77 (1959).
[6] F. Tietze, *Analyt. Biochem.* **27**, 502–522 (1969).
[7] E. Bernt and H. U. Bergmeyer, in *Methods of Enzymatic Analysis*, p. 1643. Academic Press, New York (1974).
[8] V. H. Cohn and J. Lyle, *Analyt. Biochem.* **14**, 434–440 (1966).

[9] P. J. Hissin and R. Hilf, *Analyt. Biochem.* **74**, 214–226 (1976).
[10] A. R. Buckpitt, D. E. Rollins, S. D. Nelson, R. B. Franklin and J. R. Mitchell, *Analyt. Biochem.* **83**, 168–177 (1977).
[11] J. Reeve, J. Kuhlenkamp and N. Kaplowitz, *J. Chromatogr.* **194**, 424–428 (1980).
[12] D. J. Reed, J. R. Babson, P. W. Beatty, A. E. Brodie, W. W. Ellis and D. W. Potter, *Analyt. Biochem.* **106**, 55–62 (1980).
[13] C. W. Tabor and H. Tabor, *Analyt. Chem.* **74**, 543–553 (1976).
[14] H. Nakamura and Z. Tamura, *Analyt. Chem.* **53**, 2190–2193 (1981).
[15] D. L. Rabenstein and R. Saetre, *Analyt. Chem.* **49**, 1036–1039 (1977).

[*Received for review 25 February 1988; revised manuscript received 3 January 1989*]