CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Measurement Of Tissue Sulfhydryls And Disulfides

Purpose
Measurement of tissue sulfhydryls (SH) and disulfides (SS) in protein and nonprotein fractions. This analysis was undertaken because of the possible role SH plays in diseases of the stomach.

Existing Methods
Colorimetric methods are not very specific or sensitive and do not allow simultaneous measurement of SH and SS. LC procedures with UV or fluorescence detectors require derivatization.

Reference
Measurement of Tissue Sulfhydryls and Disulfides in Tissue Protein and Nonprotein Fractions by High-Performance Liquid Chromatography Using Electrochemical Detection, D. Dupuy and S. Szabo, J. Liq. Chromatogr., 10(1987) 107-119.

Conditions
System: BAS LC-154
Detector: BAS Dual 4B/17
Electrode: dual series Au/Hg
Potential: upstream: -1.0 V; downstream + 0.15 V; vs Ag/AgCl
Column: BAS Biophase 5 µm (250 x 4.6 mm)
Mobile Phase: 96% (v:v) 0.05 M chloroacetic acid (pH 3.0); 4% methanol with 300 mg/mL sodium octyl sulfate. Temperature regulated at 40°C. Flow rate was 1.5 mL/min.
Detection Limit: 2.4 ng (glutathione) and 1.9 ng (cysteine)
Linear Range: Up to 307 ng (glutathione) and 500 ng (cysteine)

Sample Preparation
Fresh rat intestinal mucosa was homogenized in perchloric acid, frozen, thawed and centrifuged. The filtered supernatant was injected (20 µL) for determination of nonprotein SH. One portion of the protein pellet was hydrolized with HCl at 105°-110°C, dried, mixed with 0.05 M chloroacetic acid (pH 3.0), and injected (20 µL) to measure cystine and cysteine. A second portion of the pellet was reduced with 0.1% sodium borohydride for 30 min. at 37°C and centrifuged. The supernatant was injected (100 µL) to measure protein-bound SH concentration.

Notes
Recovery of standards added to the supernatants was 103% for glutathione, 122% for cysteine, and 106% for cystine.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by BAS technical staff.

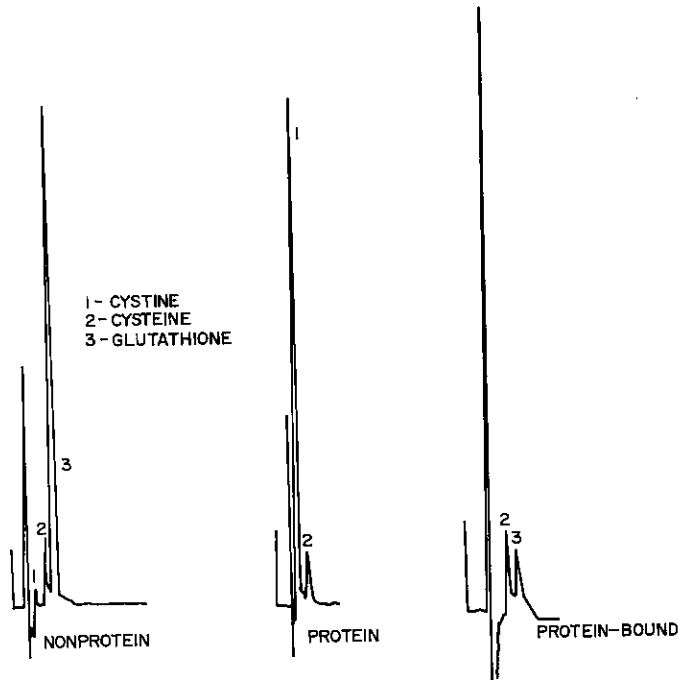

Figure 1. Chromatograms of cystine, cysteine and glutathione from rat intestinal mucosa. Gain not reported.

COPYRIGHT 1987, Bioanalytical Systems, Inc.