Poster #43 This is great poster for chemists dealing with cystine analysis. Its provides a very clean and effective method for the quantitation of cysteine in peptide/protein preparations. This poster contains a level of sophistication that should sit well with all protein/peptide chemists. It also lends to our creditability as investigators, not just hardware jocks. The Quickblot fraction collector, was also used for this application.

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Improved Cysteine Analysis Using Disulfide Interchange - 07

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Amino acid analysis has become a simple and effective tool for quantitation of protein and peptide amino acids. However, under standard hydrolysis conditions, unprotected cysteine is highly susceptible to thermal degradation and is characteristically difficult to quantify. A recently reported method uses disulfide interchange reagents for cysteine protection [1]. Cysteine reacts with each dithio compound to form a thermally stable mixed disulfide. Recently, we have used this method in conjunction with the novel derivatizing agent, 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate, for sensitive amino acid analysis of proteins [2,3]. This poster will compare the analyses of several protein samples hydrolyzed in the presence of three dithio-compounds dithiodiglycolic acid (DTDGA), dithiodipropionic acid (DTDPA), and dithiodibutyric acid (DTDBA). As expected, the three cysteine disulfide adducts were detected. When necessary, modifications were made in the normal hydrolysate chromatographic conditions to improve resolution of the cysteine analog. In addition to the cys-derived peak, a smaller previously unidentified peak was detected, and has been tentatively identified for each of the three.

Protein fractions (β lactoglobulin and cytochrome C) were collected on a membrane fraction collector following separation by capillary electrophoresis. These proteins were then hydrolyzed on the membrane in the presence of DTDPA and analyzed using AQC derivatization.

[1] Barkholt, V., and Jenson, A.E., Anal. Biochem. 177 (1989) 318.
[2] Strydom, D.J., and Cohen, S.A., (1993) in Techniques in Biochemistry IV, (R.H. Angeletti, ed.), Academic Press, San Diego.
[3] Cohen, S.A., and Michaud, D.P., Anal. Biochem., 211 (1993) 279.