A Note on
POST-COLUMN REACTION WITH FERRIC CHLORIDE
FOR THE DETECTION OF D-PENICILLAMINE

D. Witts and I.D. Wilson

Laboratory of Toxicology
University College Medical School, Rayne Institute
London WC1E 6JJ, U.K.

Department of Drug Metabolism
Hoechst UK Ltd.
Walton Manor, Walton
Milton Keynes MK7 7AJ, U.K.

D-Penicillamine (β,β-dimethylcysteine) is used in the treatment of a wide variety of diseases, including rheumatoid arthritis (RA), cystinuria, Wilson's disease and heavy-metal poisoning. Many toxic side-effects have been observed during the treatment of RA with penicillamine, and there is a wide variability in therapeutic effect. A simple, rapid and specific assay is required to aid in the investigation of the toxic side-effects, to provide pharmacokinetic data and to monitor patient compliance. In the past many approaches have been tried in an effort to develop suitable assays, including GC, colorimetry and amino-acid analyzer methods [1]. More recently, HPLC methods have been developed. These have used electrochemical (EC) detection [2], pre-HPLC derivative formation [3] or post-column reaction in order to detect the drug [1] (which lacks a suitable UV chromophore and does not fluoresce). However, all of these methods lack specificity in that they are general methods detecting either thiols or sulphur-containing compounds. We have attempted to develop an assay with a higher degree of specificity for penicillamine based on the intense blue complex formed by the reaction of penicillamine with ferric chloride. In this method ferric chloride is mixed with the eluent from the HPLC column in a simple packed-bed post-column reactor. The method has been compared with our pre-existing assay which uses post-column reaction with Ellman's reagent to detect thiols [1].

The column was 150 x i.d. 4.6 mm, slurry-packed with Whatman SCX cation-exchanger. Mobile phase (phosphate-citrate buffer, pH 5.0, 0.015 ionic strength) and reagent (usually 50 mM ferric chloride) were each pumped at 1 ml/min using LDC Constametric III pumps (LDC...
Fig. 1. HPLC of a mixture of glutathione (GSH), cysteine (CYS) and penicillamine (PEN), with post-column detection by use of: A, ferric chloride reagent; B, Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)].

Initially we tried the same ion-pair RP-HPLC system as used in our earlier work with detection by Ellman's reagent [1]; but the substitution of ferric chloride resulted in the formation of a flocculent brown precipitate (presumably ferric hydroxide) when mobile phase and reagent were mixed. The conditions used for ion-exchange HPLC did not cause precipitation, and were adopted for these studies. Firstly the effects of ferric chloride concentration on peak height and reproducibility were investigated. These were unaffected by concentration within the range 25-100 mM but were both impaired with below 25 mM. With the chosen concentration of 50 mM, the assay was linear for penicillamine with on-column loads of 30 ng to 15 μg (as high as tested). With a 20 μl injection this corresponds to concentrations of 1.5-750 μg/ml. Fig. 1 shows results with a standard