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HPLC Determination of Penicillamine in Human Urine Applying a Chemiluminescent Detection System

Z. D. Zhang, W. R. G. Baeyens,* X. R. Zhang and G. Van der Weken

University of Ghent, Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Analysis, Lab. Of Drug Quality Control, Harelbekestraat 72, B-9000 Ghent, Belgium

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INTRODUCTION

D-Penicillamine (β,β-dimethylcysteine; 3-mercaptovaline) is used as a medicinal agent for the treatment of Wilson's disease, cystinuria, lead poisoning, and rheumatoid arthritis. Recently, it was reported that D-penicillamine selectively inhibits the replication of the human immunodeficiency virus, the acquired immune deficiency syndrome (AIDS) infective agent. In previous work, a rapid flow injection method with chemiluminescence (CL) detection was proposed by the present authors for determining penicillamine in a commercial preparation (Zhang *et al.*, 1995). Based on these results, a simple and fast high-pressure liquid chromatography (HPLC) method with CL detection was developed for the determination of penicillamine in human urine.

EXPERIMENTAL

Urine samples (24 h) from healthy subjects were collected and measured after an oral dose of 250 mg of penicillamine. Acidification of the samples was done with 2 M H_2SO_4 so as to adjust the pH to 2. The urine sample was then passed through a Sep-Pak C18 cartridge for de-proteinization.

Penicillamine was separated on a Rosil C18 column (150×4.6 mm, particle size 5 μm) (Alltech, Belgium) with a C18 guard column (Valco Europe, Schenken, Switzerland). Acetate buffer solution of 0.2 M (pH 3) containing 1 g/L of octanesulphonic acid and 150 mg/L of EDTA was used as mobile phase. The flow-rate of mobile phase was 1.5 mL/min. The eluent from the HPLC column was mixed with CL reagents of 2 mM Ce(IV) and 10 mM quinine (both in 0.1 M H_2SO_4) at the flow-rates of 4 mL/min. The CL generated was monitored with a Bio-Orbit 1250-Luminometer (Bio-Orbit Oy, Turku, Finland) equipped with a flow cell as CL detector. Figure 1 shows the schematic diagram of the applied HPLC–CL detection system.

RESULTS

CL responses were linearly related to the concentration of penicillamine in the range 2 μM–2 mM, with a detection limit of 1 μM. The regression equations were $h=0.45$ (penicillamine) – 0.31, and $S=0.01$ (penicillamine) – 0.06, where h is the peak height in mV; S is peak area in s•mV and penicillamine concentrations are expressed in μM; the correlation coefficients were of 0.9994 and 0.9997, indicating excellent linearity. The precision of the method was checked at three different concentrations. The RSD ($n=10$) was less than 5%. The recoveries of penicillamine from human urinary samples were 93–98%.

The method was applied to determine penicillamine in urine of three volunteers after an oral dose of 250 mg of the drug. Urine collected was immediately acidified upon collection with 2 M sulphuric acid so as to avoid penicillamine degradation. The penicillamine content in urine samples at different times of delivery was successfully detected. The highest urinary concentrations of penicillamine were found about 5 h after oral administration (Fig. 2).

CONCLUSION

The HPLC method described for the detection of penicillamine in urinary samples has the advantages of wide linear dynamic range including the entire clinical urine concentration range. The sample preparation is simple and no precolumn derivatization was involved.

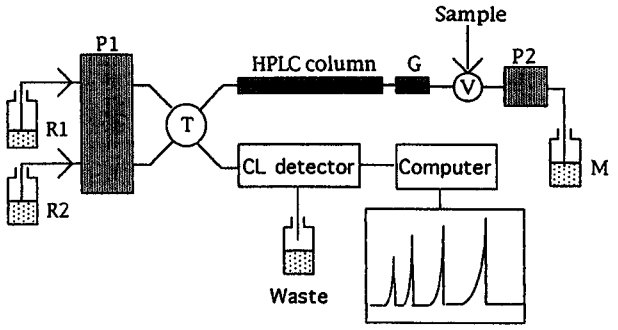

Figure 1. Schematic diagram of the HPLC–CL detection system for the penicillamine determination. R1, 2 mM (Ce(IV) in 0.1 M H_2SO_4 at a flow-rate of 4 mL/min; R2, 10 mM quinine in 0.1 M H_2SO_4 at a flow-rate of 4 mL/min; M, mobile phase, 1 g/L octanesulphonic acid and 150 mg/L EDTA in 0.2 M acetic buffer at a flow-rate of 1.5 mL/min. P1, peristaltic pump; P2, HPLC pump; T, mixing tee; V, injection valve (100 μL); G, C18 guard column.

* Correspondence to: W. R. G. Baeyens

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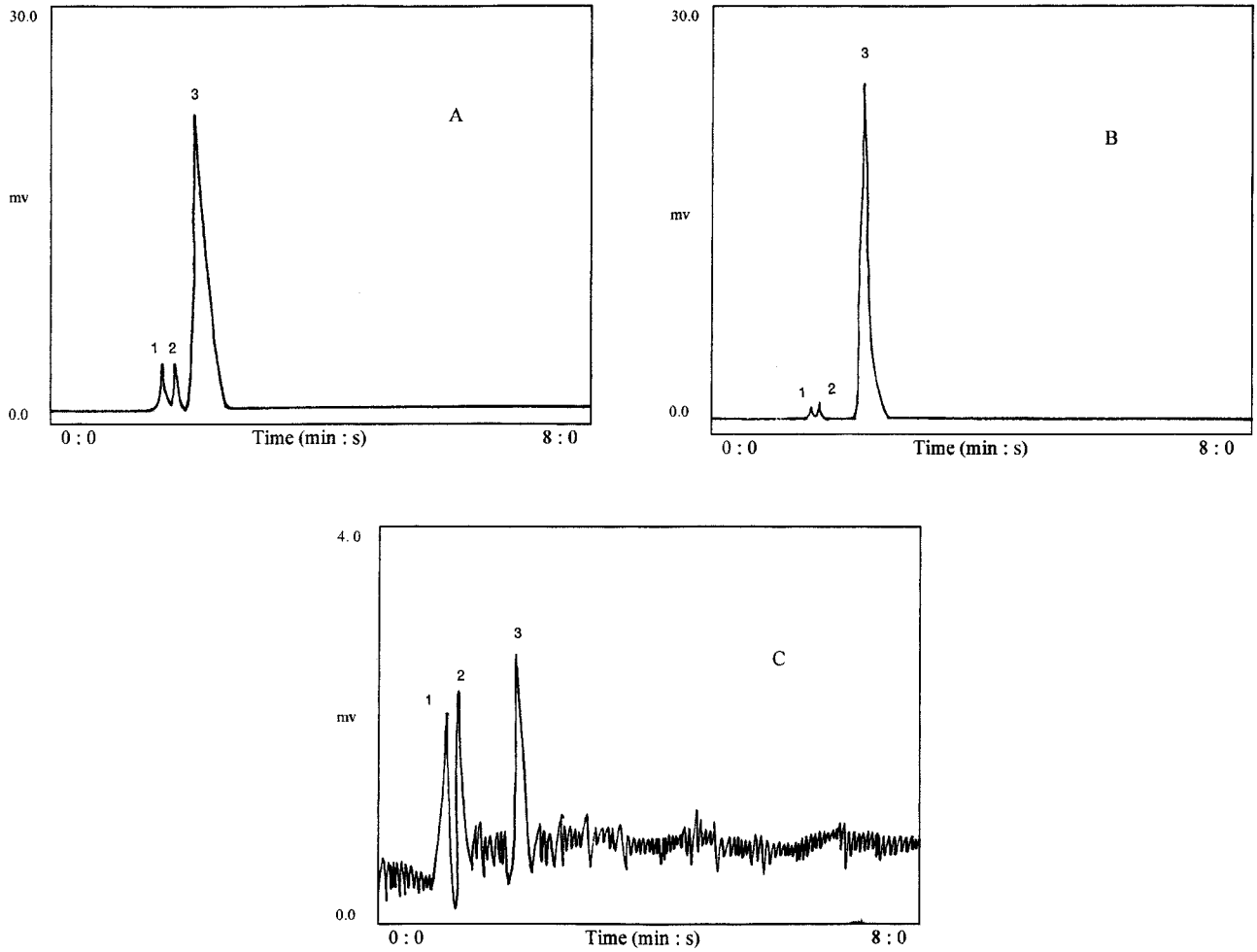

Figure 2. Chromatogram of (A) a mixed aqueous solution, (B) 7 h urine samples from a volunteer after an oral dose of 250 mg of penicillamine, (C)10 h urine samples from a volunteer after an oral dose of 250 mg of penicillamine. (1) Homocysteine; (2) cysteine; (3) penicillamine.

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