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Determination of Omeprazole in Rat Plasma by HPLC with Column Switching

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A new high-performance liquid chromatographic method with column switching has been developed for the determination of omeprazole in plasma. The plasma samples were injected onto a Bondapak phenyl/corasil (37-50 μm) precolumn and polar plasma components were washed with 0.06 M borate buffer. After valve switching, the concentrated drug were eluted in the back-flush mode and separated on a μ-Bondapak C18 column with acetonitrile-phosphate buffer as the mobile phase. The method showed excellent precision, accuracy and speed with detection limit of 0.01 $\mu g/ml^{-1}$. Total analysis time per sample was less than 20 min and the coefficients of variation for intra and inter-assay were less than 5.63%. This method has been successfully applied to plasma samples from rats after oral administration of omeprazole.

Key words: HPLC, Omeprazole in plasma, Column switching

INTRODUCTION

Omeprazole, 5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl] sulphoxide}-1H-benzimidazole, is a prodrug which is converted to its active form only at the site of action, namely the parietal cell. There it binds irreversibly with H^+K^+-ATPase (the gastric proton pump), which causes an effective and long-lasting inhibition of gastric acid secretion. The use as an anti-ulcer agent in upper gastro-intestinal tract ulcers and in the treatment of the Zollinger-Ellison syndrome is being evaluated (Oosterhuis *et al.*, 1989; Howden, 1991; Clissold *et al.*, 1986; Regård et al., 1985). A review of the published literature reveals that few methods are currently being employed for the quantitation of omeprazole in biological samples (Lagerstrom *et al.*, 1990; Grundevik *et al.*, 1986). The major disadvantages of these methods are multi-step sample preparation, a disposable extraction column apparatus and lengthy assay times (Arvidsson *et al.*, 1991; Amantea *et al.*, 1988; Mihaly *et al.*, 1983). A rapid, specific, and sensitive HPLC method with direct injection of plasma samples was therefore, developed using a column-switching technique for on line sample clean-up. This enabled the determination of omeprazole without an extraction procedure.

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MATERIALS AND METHODS

Reagents

Omeprazole was obtained from Hanmi Pharmaceutical Co. (Seoul, Korea). All other reagents were of HPLC grade. Standard solution was prepared by dissolving compound in methanol and diluting to the appropriate concentrations with methanol.

Chromatographic System

The HPLC system comprised two Waters Model 510, 600 pumps (Milford, MA, USA), a Rheodyne 7125 injector (Cotani, CA, USA), a ten-port multifunction valve (Valco, Houston, TX, USA) and Model 486 UV detector. Data handling was performed by a Waters 746 computing integrator. The instrument arrangement for a ten-port column-switching system is shown in Figure 1.

The precolumn (40×2.0 mm i.d.) was tap-filled with phenyl/corasil (37-50 μm, Waters, Milford, USA) and was changed after injection of 70-80 samples. A Co: Pell ODS guard column (40×4.6 mm i.d., 37-53 μm) was inserted before the analytical column which was packed with μ-Bondapak C18 (300×3.9 mm, i.d., 10 μm, Waters, Milford, USA).

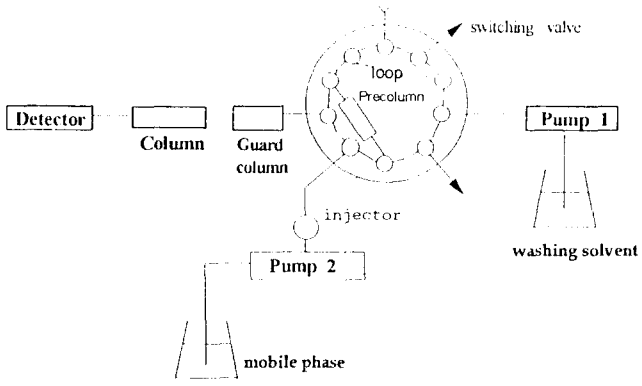

Fig. 1. Schmatic diagram of ten-port column switching system.
— load, --- inject

The washing solvent was 0.06 M borate buffer (pH 8.6) and the mobile phase consisted of acetonitrile: 0.05 M phosphate buffer (pH 7.5) (35 : 65 v/v) at a flow rate of 1.0 ml/min ^{1}, respectively. The column temperature was ambient and detection was at 302 nm.

Analytical Procedure

A 50 µl sample of the spiked rat plasma was injected. The sequence of sample analysis included the following steps and required about 20 min for completion.

Step I (0-5 min): Diluted plasma sample was injected onto the precolumn. Polar interfering plasma components were eluted to waste. The guard column and analytical column were equilibrated with mobile phase.

Step II (6-10 min): The washing solvent passed directly to waste. Retained components were eluted in the back-flush mode from the precolumn to guard column-analytical column by mobile phase.

Step III (11-20 min): The eluted drugs were separated on the analytical column. Meanwhile the precolumn was reequilibrated with the washing solvent ready for the next injection.

RESULTS AND DISCUSSION

Chromatography

The chromatographic conditions for omeprazole on reverse-phase material have been reported previously (Arvidsson *et al.*, 1991; Howden *et al.*, 1984; Aoki *et al.*, 1991; Andersson *et al.*, 1990). However, the determination of omeprazole by column switching technique has not been reported. Use of the mixture of acetonitrile-0.05 M phosphate buffer (pH7.5) (35:65, v/v) as mobile phase permitted the determination of omeprazole in plasma with better resolution in column switching technique.

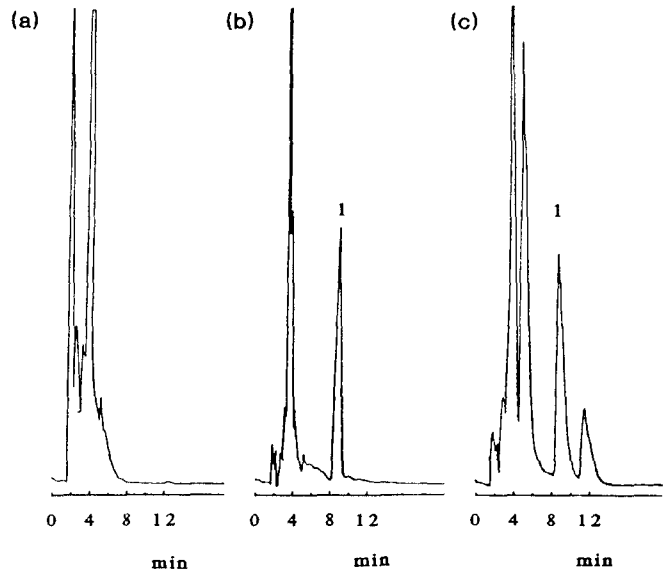

Fig. 2. Chromatograms of (a) rat blank plasma, (b) blank plasma spiked with omeprazole (1.0 µg/ml ^{1}), (c) plasma sample from a rat 0.5 h after oral administration of 10 mg/kg ^{1} omeprazole (peak 1-omeprazole).

Typical chromatograms illustrating the separation of omeprazole are shown in Figure 2. The eluted peaks in the plasma are clean, sharp and symmetric. Retention time of omeprazole was 8.3 min.

Column-Switching Procedure

In the column-switching technique, it was necessary to choose the precolumn packing material, washing solvent and washing time so that omeprazole would be completely adsorbed but none of the interfering components in plasma on the precolumn. Bondapak phenyl/corasil (37-50 µm), a nonpolar adsorbent, is a suitable precolumn packing because of its strong adsorptivity and ready availability. To obtain sharp peak and clean chromatogram, the clean-up process was completed in less than 5 min by washing the precolumn at a flow-rate of 1.0 ml/min^{-1} with 0.06 M borate buffer.

Linearity and Detectability

The correlation of peak-height ratio with the concentration of omeprazole in plasma was linear from 0.01 to 1.0 µg/ml^{-1}. The typical calibration line is shown in Figure 3. The correlation coefficient was better than 0.999. The detection limit was determined as the concentration of compound giving a signal-to-noise ratio greater than 2 : 1. The limit of detection of omeprazole

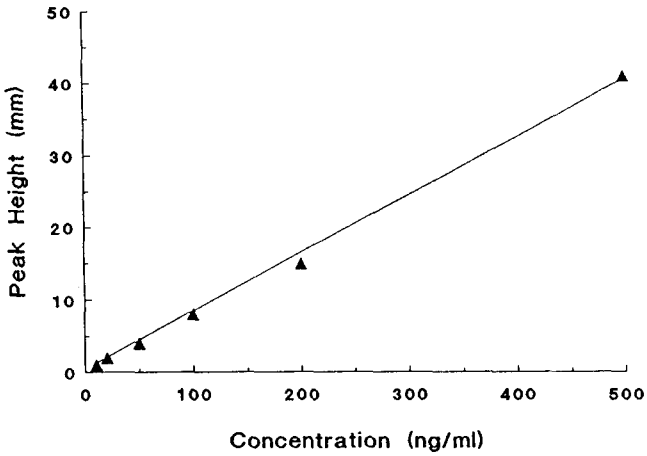

Fig. 3. Calibration of omerpazole concentration in the rat plasma (R>0.999).

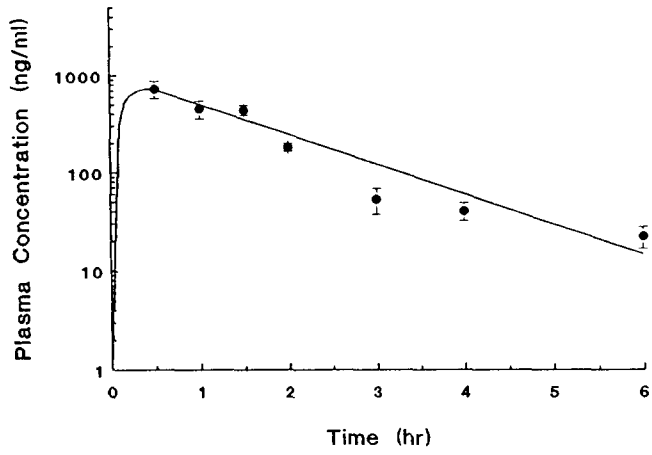

Fig. 4. Mean plasma concentration-time curve (Mean± S.E.M., n=5) in fasting SD rats following an oral administration of 10 mg/kg $^{-1}$ omeprazole.

Table I. Coefficients of variation for Intra-assay and Inter-assay reproducibility of omeprazole in plasma samples (n=5)

Omeprazole Conc. (μg/ml^{-1})		Coefficient of variation (%)	
Added	Found	Intra-assay	Inter-assay
0.05	0.05	3.97	4.01
0.10	0.11	3.45	5.63
0.20	0.22	1.85	2.98
0.50	0.49	2.38	1.08
1.00	1.00	0.60	1.33

was 0.01 μg/ml^{-1} after an injection of 50 μl of diluted plasma.

Recovery

The recovery of omeprazole from plasma was determined by the analysis of fixed amount of drug in plasma, followed by replicate injection of the same amount of a standard in 50 μl buffer directly onto the analytical column providing the 100% value. Mean absolute recovery of omeprazole in plasma was 94.35 ± 2.1% (Mean± S.D.).

Reproducibility

The precision (defined as the coefficient of variation of replicate analyses) and the accuracy (defined as the deviation between added and found concentrations) of the assay for omeprazole were evaluated over the plasma concentration range 0.01-1.0 μg/ml^{-1}. The results are shown in Table I. The coefficient of variation varied from 0.6% to 5.63% of the added amount in the spiked plasma samples.

Application to Biological Samples

The present method has been successfully applied to the analysis of more than 500 plasma samples from rats. The plasma chromatogram of a rat after oral administration of omeprazole is shown in peak of Figure 2(c). Figure 4 shows mean plasma concentration versus time plot of omeprazole following an oral administration of 10 mg/kg^{-1} omeprazole pellet to each of rats.

CONCLUSION

A new HPLC method with direct injection of small amount of diluted plasma samples was developed, using a column-switching technique, for the determination of omeprazole in plasma. The total analysis time is shorter and no transfer or evaporation of the sample is required. Reproducible retention times, recoveries and sensitivity show that the method is applicable to routine analysis.

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