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Improved high performance liquid chromatographic analysis of omeprazole in human plasma

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Abstract

A simple high-performance liquid chromatographic method was developed for the determination of omeprazole in human plasma. Omeprazole and the internal standard, chloramphenicol, were extracted from alkalinized plasma samples using dichloromethane. The mobile phase was 0.05 M Na_2HPO_4–ACN (65:35, v/v) adjusted to pH 6.5. Analysis was run at a flow rate of 1.0 ml/min at a detection wavelength of 302 nm. The method was specific and sensitive with a detection limit of 2.5 ng/ml at a signal-to-noise ratio of 4:1. The limit of quantification was set at 5 ng/ml. The calibration curve was linear over a concentration range of 5–1280 ng/ml. Mean recovery value of the extraction procedure was about 96%, while the within and between day coefficient of variation and percent error values of the assay method were all less than 14%.

Keywords: Omeprazole; Reversed phase HPLC assay

1. Introduction

Omeprazole, a substituted benzimidazole, is a potent inhibitor of gastric acid secretion by interacting with H^+/K^+ ATPase in the parietal cells [1]. This drug is effective in controlling the gastric acidity in Zollinger–Ellison syndrome patients not responding satisfactorily to histamine H_2-receptor antagonist [2].

A review of the literature revealed that several high performance liquid chromatographic (HPLC) methods have been reported for the determination of omeprazole in plasma and urine, one using a normal phase [3] and the others [4–7] reversed phase systems. While the normal phase system described by Lagerström and Persson [3] involved a simple sample preparation step and had relatively good sensitivity (approximately 7.0 ng/ml), the precision of the method was determined using only one concentration value. Moreover, the concentration used was relatively high (approximately 1.7 μg/ml), being much higher than those normally determined in bioavailability/pharmacokinetic studies.

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On the other hand, the reversed phase systems reported by Amantea and Narang [4], Macek et al. [5] and Kobayashi et al. [6] employed a mobile phase with relatively high pH values of 7.4, 7.8 and 8.6, respectively, which may cause rapid deterioration of conventional silica based columns. Thus, an alkaline-resistant, but more expensive polymeric column may be required for the analysis [6]. Mihaly [7] reported a procedure using a mobile phase with pH of 7, but the method was not validated for its accuracy, while the precision was carried out using a single high concentration value of 104.7 ng/ml. Also, in many of the methods [3,5,7], the internal standards used are not commercially available, while the method of Kobayashi et al. [6] had a relatively long run time of about 18 min.

In this paper, we report a simple, sensitive and specific HPLC method for the determination of omeprazole in human plasma using ultraviolet detection. The assay method was evaluated for accuracy, precision, recovery and linearity. The method employed a mobile phase of pH 6.5, permitting the use of a cheaper silica-based column. Also, a widely available compound, chloramphenicol, was used as the internal standard. The applicability of the method was demonstrated by applying it to analyze plasma samples obtained from a bioequivalence study.

2. Experimental

2.1. Materials

Glacial acetic acid, methanol and dichloromethane were of AR grade or HPLC grade purchased from Merck (Darmstadt, Germany). Disodium hydrogen orthophosphate was purchased from Fisher Scientific (NJ). Omeprazole and the internal standard, chloramphenicol, were obtained from United States of Pharmacopoeia (MD). Acetonitrile (ACN) of AR grade was purchased from Pharmitalia Carlo Erba (Italy).

2.2. Instrumentation

The LC system comprised a Jasco PU-980 pump (Jasco, Tokyo, Japan), a Gilson 119 uv/vis detector (Gilson, Villiers-Le-Bel, France), a Rheodyne 7125 sample injector fitted with a 20 μl sample loop (Rheodyne, CA) and a Hitachi D-2500 Chromato-integrator (Hitachi, Tokyo, Japan). The analytical column was a Crestpak C18, 150 × 4.6 mm i.d, 5 μm (Bioscience, Kuala Lumpur, Malaysia) preceded by a refillable guard column (Upchurch Scientific, Oak Habour, WA) packed with Perisorb RP-18 (30–40 μm, pellicular). The mobile phase was disodium hydrogen orthophosphate (0.05 M)-ACN (65:35, v/v) (pH* 6.5). The pH of the mobile phase mixture was adjusted with glacial acetic acid. The flow rate was set at 1.0 ml/min and the detection wavelength was 302 nm with a sensitivity range of 0.005 aufs.

2.3. Standard solutions

Stock solutions of omeprazole were prepared by dissolving 60 mg of omeprazole with 1000 ml methanol followed immediately by further 40 × dilution with 0.01 M Na_2HPO_4 of pH 9.3. Blank plasma was buffered to a pH of around 8.0 prior to spiking with omeprazole in the preparation of the plasma standards for construction of the calibration curves. Calibration curves were constructed at the following concentrations: 5, 10, 20, 40, 80, 160, 320, 640 and 1280 ng/ml. The plasma standards were kept frozen until analysis.

2.4. Extraction procedure

A 1 ml aliquot of the plasma was accurately measured into a glass tube with a teflon lined screw cap, followed by the addition of 100 μl of 0.5 M Na_2HPO_4, 100 μl of internal standard solution (3 μg/ml of chloramphenicol in methanol) and 5 ml of dichloromethane. The mixture was vortexed for 30 s on a vortex mixer before centrifuging at 2000 × g for 10 min. The organic layer was transferred into a reactivial and evaporated to dryness at 35°C under a gentle stream of nitrogen gas. The residue was reconsti-

tuted with 100 μl of mobile phase and 20 μl was injected onto the HPLC system.

2.5. Assay validation

Samples were quantified using peak height ratio of omeprazole over the internal standard. Extraction recovery, within day and between day precision and accuracy ($n = 5$) of the method were determined using the plasma standards. The recovery of the extraction procedure for omeprazole and the internal standard were calculated by comparing the peak height obtained after extraction with that of aqueous drug solution of corresponding concentrations without extraction. The accuracy was expressed as percentage error, obtained by calculating the percentage of difference between the measured and the spiked concentration over that of the spiked value, whereas the precision was denoted using the coefficient of variation.

3. Results and discussion

A polymeric column, 150 × 4.6 mm i.d., 5 μm (Polymer Laboratories, Amherst, USA) was first used during the development of the assay method because the initial mobile phase employed had a high pH value of 8. A mobile phase with pH of 7 or more tends to shorten the life of common reversed phase columns [8]. However, under this chromatographic condition, significant interference to the omeprazole peak was observed in the blank plasma and could not be resolved by lowering the pH of mobile phase to 7.5 and 7.0. Attempts to reduce ACN content of the mobile phase resulted in unsatisfactory long run time. However, the interference could be resolved by using a conventional reversed phase C18 column, while reducing the pH of the mobile phase to 6.5. Moreover, omeprazole could be eluted faster compared to using the polymeric column. The retention time of omeprazole obtained was 6.3 min. The shorter run time allows for more samples to be analyzed per day.

The wavelength of 302 nm used for detecting omeprazole, posed a problem in selecting a suitable and yet readily available internal standard. Several drug candidates which included ranitidine, famotidine, cimetidine and chloramphenicol were evaluated as the internal standard. Chloramphenicol was found most suitable, as it not only possess satisfactory absorbance at 302 nm but was also well resolved from omeprazole and has a high recovery value of approximately 92.8%. The retention time obtained was around 4.5 min. Ranitidine, famotidine and cimetidine were found to have very short retention times under the chromatographic conditions used, thus confirming the findings of Lagerström et al. [3], that these drugs do not cause interference in the assay, if present in the sample.

Chromatograms obtained with blank plasma and plasma spiked with omeprazole and chloramphenicol are shown in Fig. 1A and B while Fig. 1C is a chromatogram of a plasma sample of a healthy volunteer obtained 3 h after dosing with 20 mg omeprazole. Omeprazole and chloramphenicol were well resolved and free from interference by endogenous compounds in the plasma.

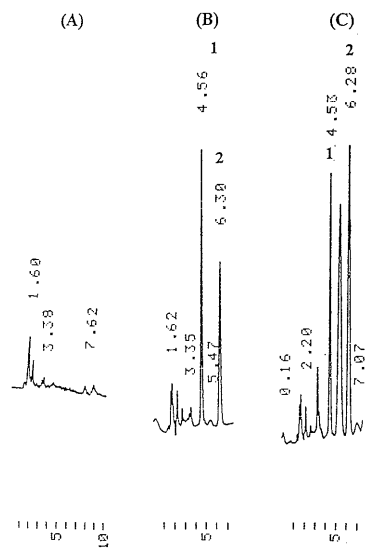

Fig. 1. Chromatograms for the analysis of omeprazole in plasma: (A) blank plasma, (B) blank plasma spiked with 80 ng/ml omeprazole and 3 μg/ml chloramphenicol, (C) a volunteer plasma containing 164.5 ng/ml omeprazole 3 h after oral administration of 20 mg omeprazole. *Y*-axis, attenuation = 5; *X*-axis, chart speed = 2.5 mm/min; 1, choramphenicol, 2, omeprazole.

Table 1
Extraction recovery, within day and between day precision and accuracy ($n = 5$)

Concentration (ng/ml)	Recovery		Within day		Between day	
	Mean (%)	CV (%)	Precision (CV%)	Accuracy (% error)	Precision (CV%)	Accuracy (% error)
5	106.9	10.6	13.8	−4.1	10.0	−3.9
10	107.4	8.7	6.9	−3.0	8.5	−6.7
20	–	–	6.6	−2.8	4.9	−0.9
40	–	–	11.0	+1.4	7.7	−3.6
80	91.6	10.2	10.2	−7.1	5.5	−2.6
160	94.4	3.6	5.2	−0.5	3.6	−2.2
320	–	–	9.6	+6.8	4.7	+0.1
640	87.6	5.5	7.1	1.0	3.3	−1.5
1280	90.8	7.4	5.6	−2.6	7.7	+1.5

The peak between chloramphenicol and omeprazole shown in Fig. 1C was a carryover peak from the previous injection. Each injection was timed as such to ensure this carryover peak would not interfere with either omeprazole or chloramphenicol peaks without the need to extend the total run time. The standard calibration curves ($n = 6$) were linear ($r = 0.9997$) over the concentration range used. A slope of 149.39 with an intercept of 0.02 was obtained. Addition of 0.5 M Na_2HPO_4 during the extraction process was found to increase the recovery of omeprazole from approximately 80% to about 96%. The extraction recovery, within day and between day precision and accuracy values of the assay method are presented in Table 1. The coefficient of variation (CV) values of both the within day and between day were all less than 14%, whereas the percentage error were less than 8%. It is interesting to note that, in seven out of nine concentrations determined, the between-day CV values appeared to be smaller than those of the within day, suggesting that the precision was not compromised during between-day analysis. The limit of detection was approximately 2.5 ng/ml at a signal-to-noise ratio of 4:1. However, the limit of quantification was set at 5 ng/ml being the lowest concentration used in the construction of the standards curve, but could be further improved by using a larger sample loop. This value is comparable to that reported by Amantea and Narang [4] but more sensitive that those of Macek et al. [5] and Kobayashi et al. [6] who reported a value of 9.7 ng/ml and 10 ng/ml, respectively. While Mihaly et al. [7] reported a sensitivity of 5 ng/ml, the limit of quantification was not given.

The method was applied to analyze plasma samples obtained from a bioequivalence study of a generic product of omeprazole versus the innovator preparation Losec®. During the clinical study, the plasma samples collected were buffered at a pH of about 8.0 by incorporating 50 μl of 1.0 M Na_2HPO_4 pH 9.3 into 5 ml volume (approximately 1:100) of the plasma. At this pH value, omeprazole was found to be stable and there was negligible or no change in drug concentration of samples stored at −20°C on repeated weekly measurements over 1 month. Fig. 2 shows the mean plasma concentration-time profiles of the volunteers ($n = 18$) obtained with the two preparations. It can be seen from the plasma profiles of both preparations that omeprazole could still be detected at 12 h after dosing and in all cases, the last detectable level was less than 8.0% of the peak plasma concentration.

4. Conclusion

The present LC method is sensitive, simple, specific and appropriate to be used for determination of plasma omeprazole in pharmacokinetic/bioavailability studies. The pH of the mobile phase used was well within the practical working

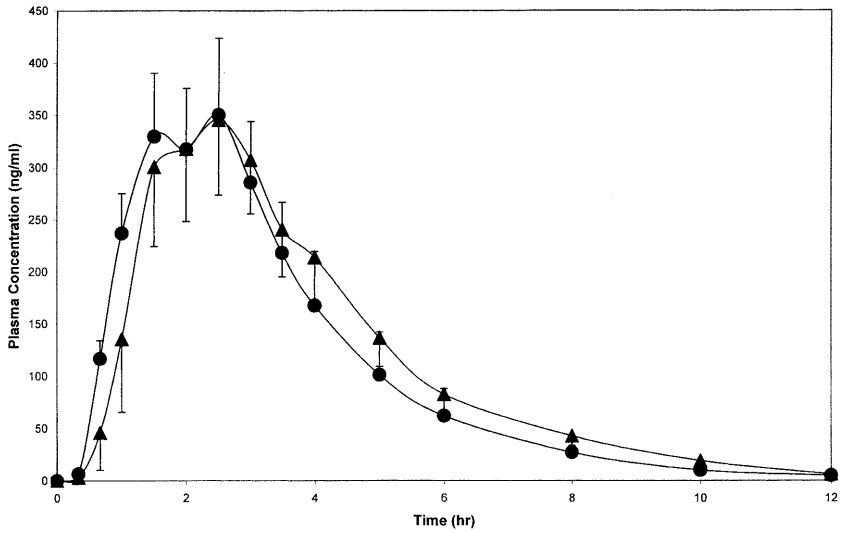

Fig. 2. Mean plasma omeprazole concentration versus time profiles of Losec® (●) and Omesec® (▲). Mean ± SEM, $n = 18$.

pH range of common reversed phase C18 columns, which is typically between pH 3–7, thus avoiding the use of more expensive pH resistant columns. Moreover, the assay method was well evaluated compared to other reported methods.

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