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# SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF OMEPRAZOLE AND ITS SULPHONE AND SULPHIDE METABOLITES IN HUMAN PLASMA AND URINE

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## SUMMARY

Omeprazole, a substituted benzimidazole which suppresses gastric acid secretion, and its sulphone and sulphide metabolites were simultaneously measured in human plasma and urine using a selective, reversed-phase, high-performance liquid chromatographic method with a sensitivity of 5 ng/ml for omeprazole, 30 ng/ml for omeprazole sulphone, and 50 ng/ml for omeprazole sulphide. The coefficients of variation for within-day assays were 4.4, 7.5, and 17.5%, respectively. In a pilot pharmacokinetic study, 40 mg of omeprazole (encapsulated enteric-coated granules) were administered to two healthy volunteers. Peak plasma concentrations for omeprazole of 240 and 520 ng/ml, and for omeprazole sulphone of 320 and 400 ng/ml, were reached between 3 and 4 h post-dose. Omeprazole concentrations fell rapidly with apparent half-lives of about 40 min, and concentrations of both omeprazole and the sulphone metabolite were below the minimal detectable level by 6—8 h. Omeprazole sulphide could not be detected in this study.

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## INTRODUCTION

Substituted benzimidazoles are novel inhibitors of gastric acid secretion in animals and man [1, 2]. They appear to act by selectively inhibiting the hydrogen/potassium ($H^+ + K^+$)-ATPase [3] in the secretory canalicular membrane of the parietal cell [4]. This ($H^+ + K^+$)-ATPase is thought to be involved in gastric hydrogen ion transport from the cell to the lumen [5]. One of these substituted benzimidazoles, omeprazole, is currently being investigated in man. This drug is effective in the control of gastric acidity in Zollinger–Ellison syndrome patients not responding satisfactorily to histamine $H_2$-receptor antagonists [6]. At present there are no published methods for measuring this drug in biological samples.

This report describes a high-performance liquid chromatographic (HPLC) assay for the determination of omeprazole in human plasma and urine, which is also suitable for the simultaneous measurement of the sulphone and sulphide metabolites. This method allowed analysis of plasma and urine samples obtained from pilot pharmacokinetic studies in two volunteers.

## EXPERIMENTAL

### *Reagents*

Pure samples of omeprazole (H168/68, 5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl] sulphoxide}-1H-benzimidazole), omeprazole sulphone (H168/66, 5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl] sulphone}-1H-benzimidazole), omeprazole sulphide (H168/22, 5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulphide}-1H-benzimidazole), and internal standard (H168/24, 5-methyl-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl] sulphoxide}-1H-benzimidazole) were provided by Astra Pharmaceuticals (North Ryde, Australia) (Fig. 1). The HPLC mobile phase contained analytical grade methanol, triethylamine, phosphoric acid, and glass-distilled water.

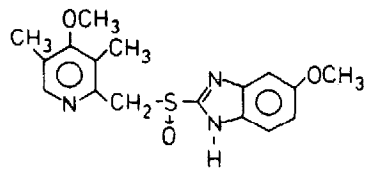
### *Instrumentation*

A constant-flow high-pressure liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) was used for all assays. This consisted of a solvent delivery system (Model 6000A), a universal injector (Model U6K), and a variable-wavelength ultraviolet absorbance detector at 302 nm (Model 480). The reversed-phase plastic column was obtained prepacked (Rad Pak A; $C_{18}$ 10-$\mu$m particles, 100 mm × 8 mm I.D.; Waters Assoc.) and was housed in a radial compression module (RCM-100; Waters Assoc.) which maintained external column pressure at approximately 370 kPa.

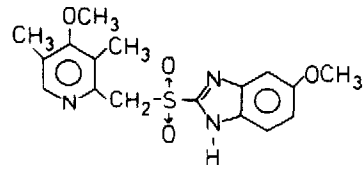
### *Calibration standards*

Samples of pooled drug-free plasma were spiked with a mixture of pure omeprazole, omeprazole sulphone, and omeprazole sulphide (1000 ng/ml, 500 ng/ml, and 500 ng/ml respectively) dissolved in polyethylene glycol 400. Plasma was buffered by adding 4 $\mu$l of 1.0 *M* sodium carbonate per

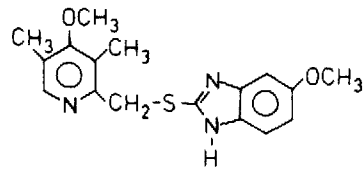
Omeprazole



Omeprazole-sulphone



Omeprazole-sulphide



Internal Standard H168/24

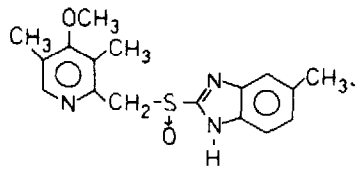


Fig. 1. Structural formulae of omeprazole, omeprazole metabolites and internal standard.

ml of plasma, and plasma standards were obtained by double dilutions with drug-free plasma. The concentrations of these standards were 500 ng/ml, 250 ng/ml, and 125 ng/ml for omeprazole and 250 ng/ml, 125 ng/ml, and 62.5 ng/ml for omeprazole sulphone and omeprazole sulphide. Similarly, drug-free urine (buffered with 4 $\mu$l of 1.0 *M* sodium carbonate per ml of urine) was spiked with pure omeprazole (10 $\mu$g/ml), omeprazole sulphone (5 $\mu$g/ml) and omeprazole sulphide (5 $\mu$g/ml). Urine standards were then prepared by dilution. The final concentrations were 5 $\mu$g/ml, 2.5 $\mu$g/ml and 1.25 $\mu$g/ml for omeprazole, and 2.5 $\mu$g/ml, 1.25 $\mu$g/ml, and 0.625 $\mu$g/ml for omeprazole sulphone and omeprazole sulphide. All standards were stored at −20°C. Calibration curves were prepared by plotting the relationship between the peak height ratio of omeprazole, omeprazole sulphone, or omeprazole sulphide to the internal standard (H168/24).

*Analytical recovery*

Recoveries of omeprazole, omeprazole sulphone and omeprazole sulphide were estimated by comparing the peak height of an extracted plasma sample containing a known amount of the substance with the peak height of an aqueous solution containing the same amount of each compound.

*Extraction of plasma*

Internal standard (H168/24, 40 μg/ml, 50 μl), and dichloromethane (10 ml) were added to 1.0 ml of plasma in a 30-ml glass tube. After vortex mixing (60 sec) and centrifugation (1500 *g*, 10 min) the organic layer was carefully transferred to a second tube and evaporated under a gentle stream of nitrogen at 45°C. The residue was reconstituted in 100 μl of the chromatographic mobile phase, and 40 μl were injected into the liquid chromatograph. The samples were chromatographed on the day of extraction.

*Extraction of urine*

The internal standard (H168/24, 40 μg/ml, 250 μl) and dichloromethane (10 ml) were added to 1.0 ml of urine in a 30-ml glass tube, and the sample was treated as described for plasma. Reconstitution and injection were also the same as for plasma.

*Chromatography*

The mobile phase was methanol—water (60:40) containing 1% triethylamine, and was adjusted to pH 7 with phosphoric acid (85%, v/v). The flow-rate was 3 ml/min at a back pressure of approximately 340 kPa. The approximate retention times were omeprazole 3.6 min, omeprazole sulphone 3.1 min, omeprazole sulphide 7.7 min, and internal standard 4.9 min.

*Patient sampling*

Two healthy male volunteers (25 and 22 years, 74 and 73 kg) were given single oral doses of omeprazole (40 mg) as two 20-mg capsules containing enteric-coated granules. Food and beverages were witheld for 8 h prior to and 3 h after dosage. Venous blood samples (10 ml) were withdrawn immediately before dosage, and at intervals up to 24 h. Each sample was collected in a heparinized tube, cooled for at least 5 min, and then centrifuged for 10 min. The plasma phase was transferred to plastic tubes containing 20 μl of 1.0 *M* sodium carbonate, and stored at −20°C. Urine was also collected pre-dose and serially to 4, 8, 12, and 24 h. Urine samples were buffered after collection by the addition of 4 μl of 1.0 *M* sodium carbonate per ml of urine, and stored at −20°C.

*Pharmacokinetic calculations and statistical analysis*

Coefficients of variation were calculated from the ratio of values of the standard deviation to the mean. Correlation coefficients of calibration curves were determined by least-squares regression analysis. Plasma omeprazole concentration versus time data were subjected to non-linear least-squares regression analysis [7].

## RESULTS AND DISCUSSION

Chromatograms of drug-free plasma, spiked plasma, and plasma from a volunteer who had received omeprazole are shown in Fig. 2. Those of blank, spiked, and volunteer urine are shown in Fig. 3. These chromatograms show distinct, well-resolved peaks for omeprazole, omeprazole sulphone, omeprazole

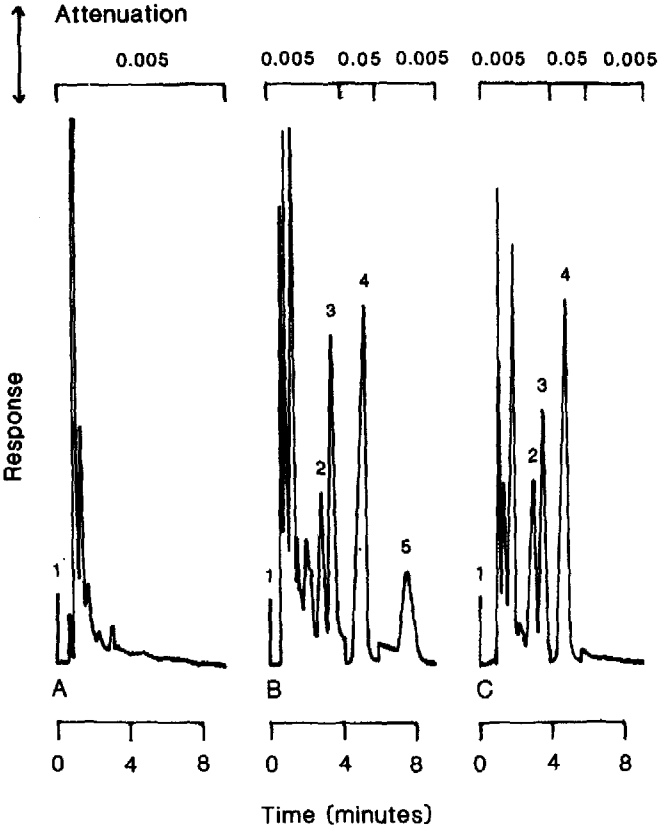



Fig. 2. Chromatogram of (A) a blank plasma extract, (B) a spiked plasma extract (omeprazole concentration = 125 ng/ml, omeprazole sulphone and omeprazole sulphide concentrations = 62.5 ng/ml), and (C) an extract of plasma sample from a patient who received a single dose of omeprazole (omeprazole concentration = 93 ng/ml and omeprazole sulphone concentration = 69 ng/ml). 1 = Injection; 2 = omeprazole sulphone; 3 = omeprazole; 4 = internal standard, H168/24; 5 = omeprazole sulphide.

sulphide, and internal standard. The minimum detectable level (defined as three times baseline noise) in plasma was 5 ng/ml for omeprazole, 30 ng/ml for omeprazole sulphone and 15 ng/ml for omeprazole sulphide.

The chromatogram of blank plasma shows a small endogenous component with a retention time similar to that for the metabolite, omeprazole sulphone. This peak was never seen to exceed 3—4% of full-scale deflection on the highest detector sensitivity used (× 0.005 a.u.f.s.), and did not interfere in the determination of omeprazole. Measurements of omeprazole sulphone in concentrations less than 30 ng/ml were unreliable due to its presence. However, it did not appreciably affect the accuracy or reproducibility of the determinations above this concentration (Table I).

Calibration curves of peak height ratios (i.e. peak height of drug to peak height of internal standard) against concentrations of omeprazole, omeprazole sulphone, and omeprazole sulphide, all showed linearity for both plasma and urine ($r \geqslant 0.999$ for all substances in plasma and urine). Analytical re-

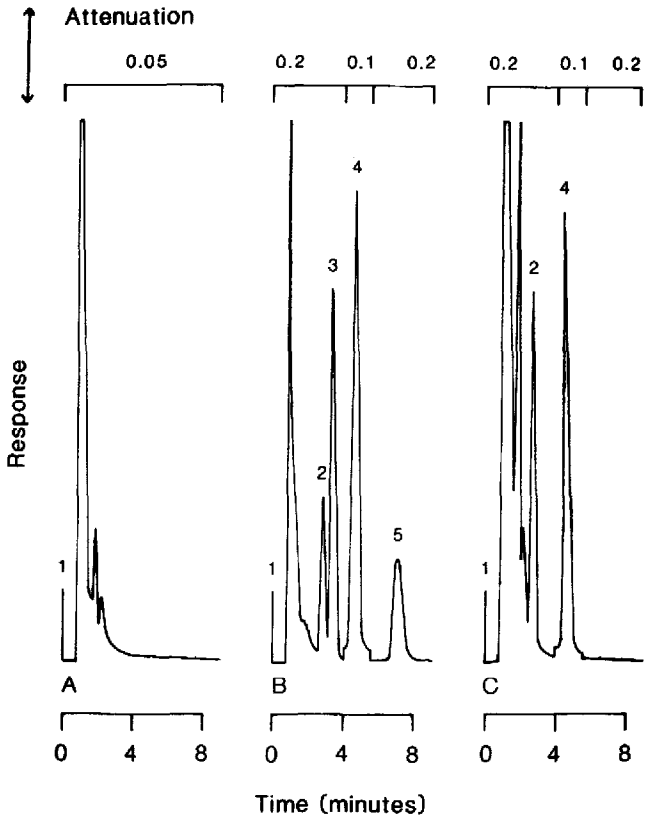



Fig. 3. Chromatogram of (A) a blank urine extract, (B) a spiked urine extract (omeprazole concentration = 5 μg/ml, omeprazole sulphone and omeprazole sulphide concentrations = 2.5 μg/ml), and (C) an extract of a urine sample from a patient who received a single dose of omeprazole (omeprazole sulphone concentration = 6.1 μg/ml). 1 = Injection; 2 = omeprazole sulphone; 3 = omeprazole; 4 = internal standard, H168/24; 5 = omeprazole sulphide.

coveries from plasma were 95% for omeprazole, 99% for omeprazole sulphone, and 82% for omeprazole sulphide.

The within-day, and day-to-day precision of the assay was determined for each of the substances by replicate assays of aliquots of the same sample (Table I). There was little variability in the assays for omeprazole and omeprazole sulphone, with coefficients of variation below 8%. By contrast the results for replicate assays of the sulphide metabolite were more variable at the concentrations tested. The accurate determination of sulphide levels is therefore limited to concentrations above 50 ng/ml. There was no appreciable deterioration of substances in either plasma or urine with storage at −20°C for two months.

The assay method was applied to the analysis of samples obtained from a pilot pharmacokinetic study in two healthy volunteers. The plasma concentration—time profiles of omeprazole and omeprazole sulphone are shown for each subject in Fig. 4 and 5. In no samples were the plasma levels of ome-

TABLE I

COEFFICIENTS OF VARIATION FOR WITHIN-DAY AND DAY-TO-DAY ASSAYS FOR OMEPRAZOLE, OMEPRAZOLE SULPHONE AND OMEPRAZOLE SULPHIDE IN HUMAN PLASMA AND URINE

| Compound | Mean drug concentration ($n$ = 5) | Coefficient of variation (%) | |
|---|---|---|---|
| | | Within-day | Day-to-day |
| *Plasma* | | | |
| Omeprazole | 104.7 ng/ml | 4.4 | 2.7 |
| Omeprazole sulphone | 77.5 ng/ml | 7.5 | 5.5 |
| Omeprazole sulphide | 47.5 ng/ml | 17.5 | 23.4 |
| *Urine* | | | |
| Omeprazole | 1.25 μg/ml | 1.7 | 2.7 |
| Omeprazole sulphone | 0.64 μg/ml | 1.8 | 1.8 |
| Omeprazole sulphide | 0.63 μg/ml | 2.0 | 3.3 |

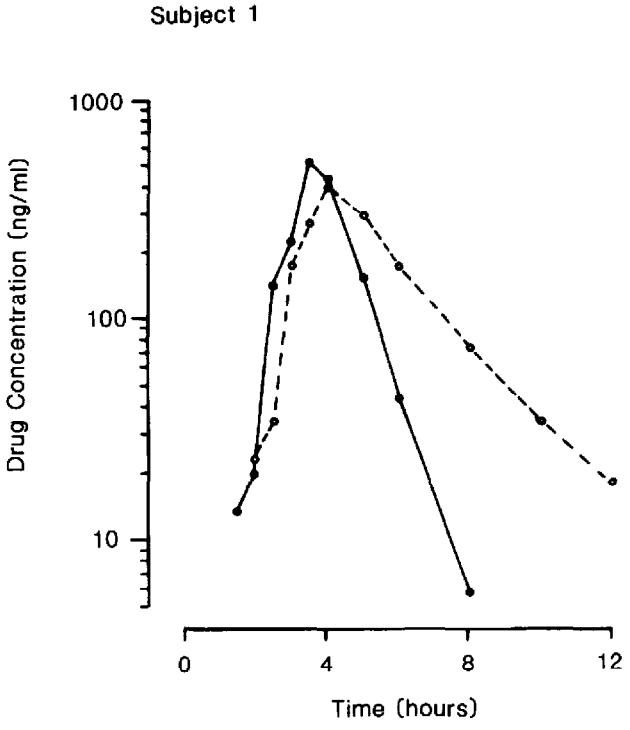



Fig. 4. Plasma levels of omeprazole (•) and omeprazole sulphone (○) following 40-mg oral dosage (subject 1).

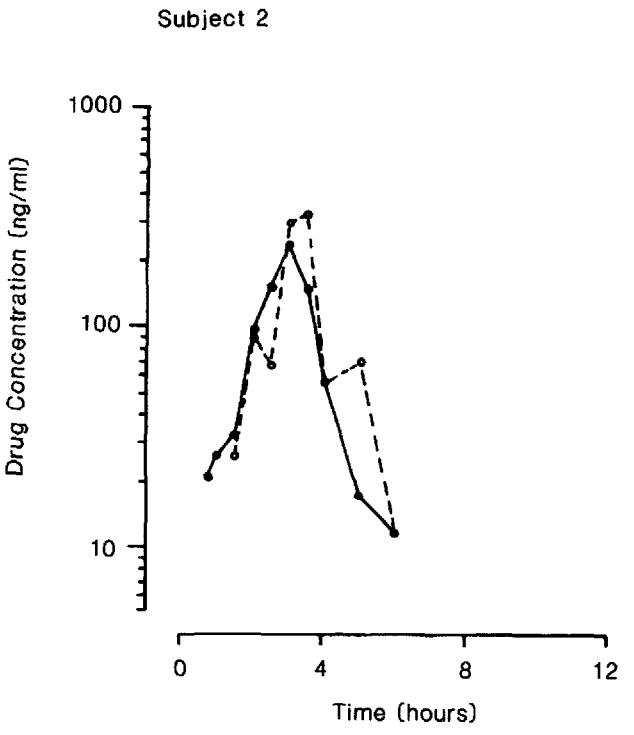



Fig. 5. Plasma levels of omeprazole (•) and omeprazole sulphone (○) following 40-mg oral dosage (subject 2).

TABLE II

OMEPRAZOLE AND OMEPRAZOLE SULPHONE PHARMACOKINETIC PARAMETERS CALCULATED FOR SUBJECTS 1 AND 2, AFTER 40-mg ORAL DOSAGE

| Pharmacokinetic parameters* | Subject 1 | Subject 2 |
|---|---|---|
| *Omeprazole* | | |
| $T_{lag}$ (h) | 1.8 | 1.7 |
| $C_{pk}$ (ng/ml) | 519.0 | 239.6 |
| $T_{pk}$ (h) | 3.5 | 3.0 |
| $k_a$ ($h^{-1}$) | 0.81 | 1.27 |
| $\beta$ ($h^{-1}$) | 1.05 | 1.06 |
| $t_{½\beta}$ (h) | 0.66 | 0.65 |
| $AUC_{0\to\infty}$ (ng h $ml^{-1}$) | 1024.3 | 429.4 |
| *Omeprazole sulphone* | | |
| $C_{pk}$ (ng/ml) | 400.6 | 317.7 |
| $T_{pk}$ (h) | 4.0 | 3.5 |
| $\beta$ ($h^{-1}$) | 0.42 | 1.08 |
| $t_{½\beta}$ (h) | 1.64 | 0.64 |

*$T_{lag}$ = lag time; $C_{pk}$ = peak plasma concentration; $T_{pk}$ = time to reach peak plasma concentration; $k_a$ = absorption rate constant; $\beta$ = elimination rate constant; $t_{½\beta}$ = elimination phase half-life; $AUC_{0\to\infty}$ = area under plasma concentration—time curve from time 0 to infinity.

prazole sulphide in excess of the minimum detectable concentration for this metabolite [in subsequent chronic studies this metabolite has been identified at low concentrations in human plasma (unpublished observations)]. The resultant pharmacokinetic parameters for omeprazole and omeprazole sulphone are summarised in Table II. After an initial lag, absorption of omeprazole was rapid with peak levels being reached at approximately 3.25 h. Thereafter, omeprazole plasma levels fell monoexponentially with a short apparent elimination half-life of 39 and 40 min. Plasma levels of omeprazole could be followed for 5—6 half-lives before they fell below the minimum detectable concentration of 5 ng/ml by 8 h post-dose.

Omeprazole undergoes rapid metabolism in part to omeprazole sulphone, the levels of which follow a similar pattern to that seen with omeprazole. This metabolite was eliminated with apparent half-lives of 98 and 38 min.

Neither omeprazole nor omeprazole sulphide were detected in any urine samples; 2.3% and 2.5% of the dose were recovered in urine as omeprazole sulphone.

In summary, the proposed method is sufficiently sensitive, selective and precise for the quantitation of omeprazole and its sulphone and sulphide metabolites in human plasma and urine.

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