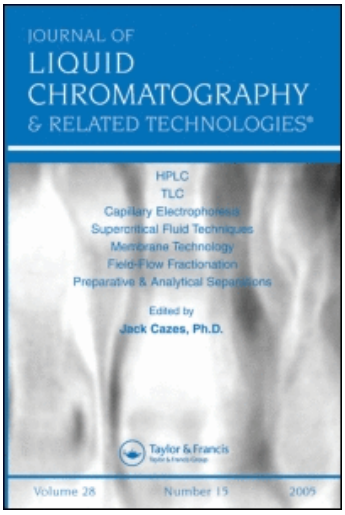

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Development and Validation of a RP-HPLC Method to Quantify Omeprazole in Delayed Release Tablets

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Abstract: An analytical method using a sensitive high performance liquid chromatographic technique was developed to quantify omeprazole in delayed release tablets. The analysis was carried out using a RP-C_{18} column with UV-Vis detection at 280 nm. The mobile phase was diluted with phosphate buffer (pH 7.4) and acetonitrile (70:30 v/v) at a flow-rate of 1.5 mL·min^{-1}. The parameters used in the validation process were: linearity, range, quantification limit, accuracy, specificity, and precision. The retention time of omeprazole was about 5 min with symmetrical peaks. The linearity in the range of 10.0–30.0 μg/mL presented a correlation coefficient of 0.9995. The excipients in the formulation did not interfere with the analysis and the recovery was quantitative. Results were satisfactory and the method proved to be adequate for quality control of omeprazole delayed release tablets.

Keywords: Omeprazole, Delayed release tablets, HPLC, Validation

INTRODUCTION

Omeprazole (Figure 1), chemically known as 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-pyridinyl) methyl]sulfinyl] -1H-benzimidazole, is the most significant of the substituted benzimidazole sulfoxides and belongs to the proton

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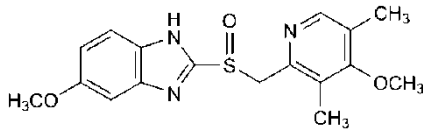

Figure 1. Structure of omeprazole.

pump inhibitors class. Its mechanism of action is selective by an irreversible inhibition of the hydrogen-potassium adenosine triphosphatase enzyme (ATPase H+/K+) of the parietal cells, leading to a reduction of the gastric acid secretion.[1–3]

Omeprazole may be considered a prodrug because it must be converted into an active form (sulfonamide), and only then is it able to react with the ATPase H+/K+ enzyme in the parietal cells of stomach. It is used in the treatment of acid peptic disorders such as reflux esophagitis, duodenal ulcer, and the Zollinger-Ellison syndrome.[4–6]

Pharmaceutical dosage forms containing omeprazole presents a great challenge, due to the drug's rapid decomposition at acid pHs, heat, moisture, organic solvents, and to some degree of light.[7–9]

Today, omeprazole is commercially available in capsules and powder for injection. Various analytical methods have been found in the literature, such as high performance liquid chromatography (HPLC) with UV or coulometric[7,10–14]derivative or UV-Vis spectroscopy[15–19] and polarography,[20] for the determination of omeprazole and its metabolites in bulk material, as well as pharmaceuticals formulations.

The official HPLC assay is described in the United States Pharmacopoeia USP 26, European Pharmacopoeia 4th edition and British Pharmacopoeia 1999, for the determination of omeprazole and its quantification within bulk material, but does not include individual monographs of pharmaceutical dosage forms. Furthermore, HPLC assays for determination of omeprazole in direct compressed tablets have not been reported in the scientific literature.

In fact, the liquid chromatographic method is the most suitable technique that can be used to quantify omeprazole in tablets, because there is no interference between the drug and its degradation products. For quality control, the development of a simple, rapid, and safe method is highly desirable.

Since our research involves the development and evaluation of a delayed release tablet containing omeprazole, the objective of the present paper was to develop and validate a simple, accurate, and stability indicating HPLC method of analysis.

EXPERIMENTAL

Chemical and Reagents

The omeprazole reference standard (SQR FB 1030) with state purity of 100.1%, was obtained from the Brazilian Pharmacopoeia. The omeprazole

(sodium salt) raw material was provided by Eurofarma SA (São Paulo, Brazil). Ultrapure water was provided by a Milli-Q® purification system (Millipore, USA). Methanol, an acetonitrile of HPLC grade was purchased from Vetec®. Potassium phosphate and sodium hydroxide were purchased from Nuclear®. Other solvents and reagents used were analytical grade.

Production of Omeprazole Delayed Release Tablets

The tablets were obtained from direct compression, using a rotary tabletting machine, model URM-10 (USIROM, Brazil), fitted with 8 mm diameter standard concave tooling. The formulation was comprised of 20 mg of drug (Omeprazole sodium) and 160 mg of excipient, to a target weight of 180 mg.

The produced tablets were coated in a Rama Cota RD perforated pan, equipped with a Binks 460 spraying system gun and a Watson Marlon peristaltic pump. The tablets were coated with a sub-coating at a 6–8% level of Opadry® II (Colorcon) and coated with a Acryl-EZE® (Colorcon) enteric polymer system, to a range of 10–12% weight gain.

Instrumentation and Chromatography

The HPLC analysis was performed on a Shimadzu LC-10A system (Kyoto, Japan) equipped with a LC-10AD pump, SPD-10AV UV detector (set at 280 nm), SCL-10Avp controller unit, and the sample injection performed via a Rheodyne 7125 valve with a 20 μL loop. A RP C18A Merck column (150 mm × 4 mm i.d., 5 μm particle size) was employed with a mobile phase constituted by 0.05M phosphate buffer (pH 7.4) and acetonitrile (70:30 v/v) in an isocratic system, at a flow rate of 1.5 $mL \cdot min^{-1}$. The HPLC system was operated at 40 ± 1°C. The injection volume was 20 μL for all standards and samples. Data acquisition was performed using CLASS-VP software by the measurement of detected peak areas.

Standard and Sample Preparation

Standard Preparation

A stock standard solution of 200 μg/mL were prepared by dissolving a 20 mg of omeprazole reference standard (SQRFB 1030) in 5 mL of methanol and 50 mL of phosphate buffer (pH 11), transferred to 100 mL volumetric flask, and stirred in a ultrasonic bath for 10 minutes. The volume was completed with the same buffer.

Sample Preparation

Ten delayed release omeprazole tablets (20 mg/tablet) were finely powdered. An accurately weighed portion equivalent to the content of one tablet was quantitatively transferred to a 100 mL volumetric flask with 50 mL of phosphate buffer (pH 11), and stirred in an ultrasonic bath for 10 minutes. The volume was completed with the same buffer (200 μg/mL). A 5 mL volume of this solution was diluted with mobile phase in a 50 mL volumetric flask (20 μg/mL).

Sample Preparation for Content Uniformity

Ten delayed release omeprazole tablets were separately powdered, transferred to a 100 mL volumetric flask with 50 mL of phosphate buffer (pH 7.4), and stirred in an ultrasonic bath for 20 minutes. The volume was completed with the same buffer. The solution was filtered in a 0.45 μm nylon membrane, and 5 mL of the filtered solution was diluted with mobile phase in a 50 mL volumetric flask.

Method Validation

The method validation was based on the International Conference on Harmonization guidelines Q2(R1),[21] for validation of analytical procedures. The parameters used were required for the assay of a dosage form: linearity, range, quantification limit, accuracy, specificity, and precision.

Linearity and Range

The linearity response was assessed in the range of 10–30 μg/mL. Appropriate amounts of the stock solution were diluted with mobile phase, yielding concentrations of 10, 15, 20, 25, and 30 μg/mL. Triplicate injections of each were carried out. Peak area ratios of standard compounds were plotted against theoretical concentrations of standards. The linearity was expressed as a correlation coefficient by linear regression analysis.

Quantification Limit

The quantification limit (QL) was based on the standard deviation of the response and the slope of the constructed calibration curve. The QL may be expressed as:

$$QL = \left(\frac{10\sigma}{S}\right)$$

where σ is the standard deviation of the response and S is the slope of calibration curve.

Specificity

The specificity was evaluated by analyzing a placebo tablet and a delayed release tablet without omeprazole. Triplicate injections of each were made.

Precision

The repeatability of the analytical method was evaluated by assaying six samples solutions of omeprazole 20 μg/mL, during the same day, under the same experimental conditions. Intermediate precision was evaluated by assaying solutions on 3 different days. Peak areas were determined and compared. Precision was expressed as percentage relative standard deviation (R.S.D).

Accuracy

The accuracy of the developed method was evaluated by a recovering test. An omeprazole sample solution 10 μg/mL was fortified with 3 known concentrations of reference standards, at 3 different levels lower, medium, and upper concentration (Table 1). The recovery of the added standard was determined in triplicate analysis and calculated by the formula:

$$R\% = \left(\frac{Fs - St}{Ss}\right) \times 100$$

in which R is the recovery, Fs is the fortified solution, Ss is the sample solution, and St is the standard solution.

RESULTS AND DISCUSSION

The reverse phase HPLC method was developed to provide an efficient and safe quality control determination of omeprazole in delayed release tablets. The chromatographic conditions were chosen as a function of the drug physical chemical parameters, such as the mobile phase, using a phosphate

Table 1. The recovery test performed

Fortified solution	Sample solution	Standard solution
15 μg/mL	10 μg/mL	5 μg/mL
20 μg/mL	10 μg/mL	10 μg/mL
25 μg/mL	10 μg/mL	15 μg/mL

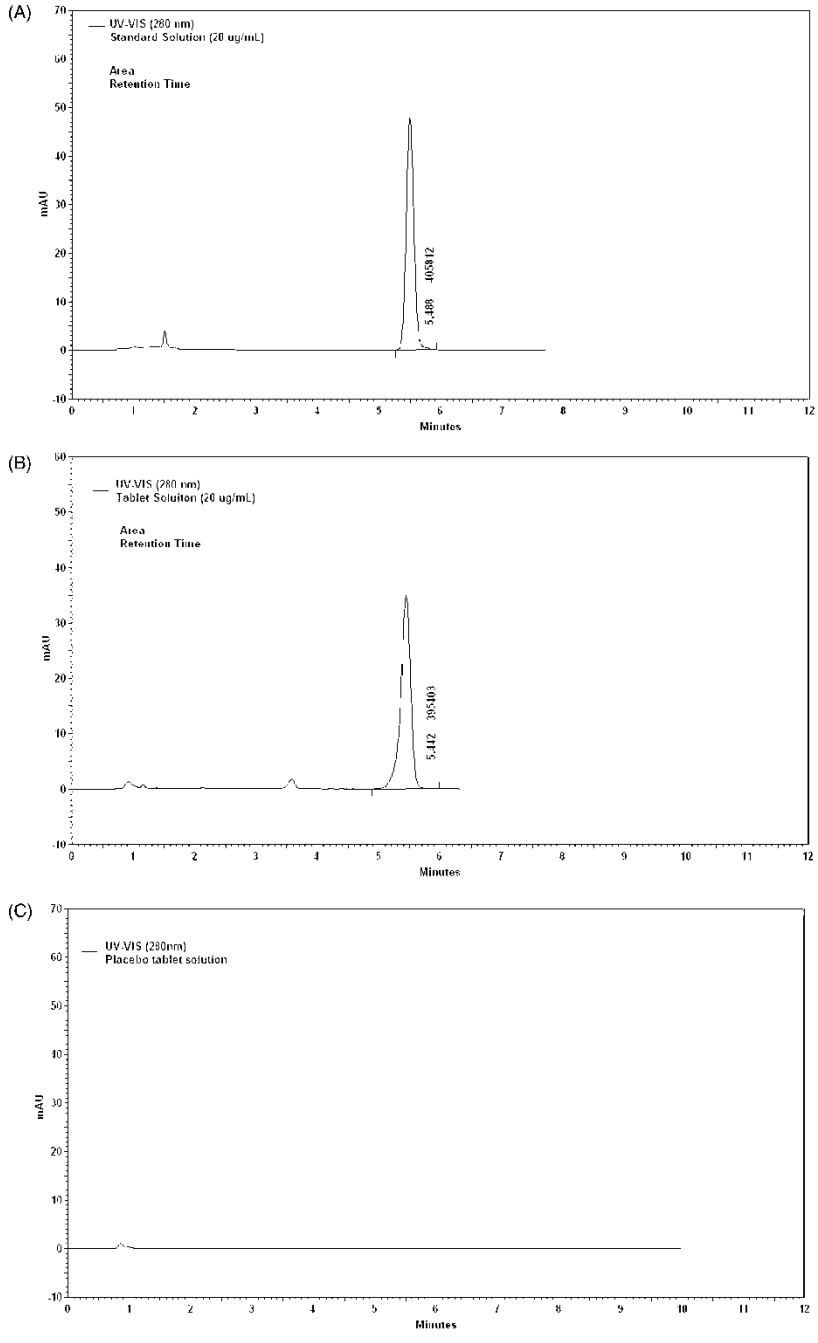

Figure 2. Typical chromatograms obtained under the experimental conditions. (A) Standard solution of 20 μg/mL, (B) Tablet solution of 20 μg/mL, and (C) Placebo solution.

Table 2. The linearity and quantification limit

Range of linearity (μg/mL)	Peak area (mean and R.S.D)[a]
10	212746.67 ± 0.296
15	318672.67 ± 1.441
20	432250.00 ± 1.172
25	554348.00 ± 0.431
30	660928.33 ± 0.026
Equation	y = 22641x + 17026
r^2	0.9995
QL	0.56 μg/mL

[a]Mean ± R.S.D (n = 3).

buffer of pH 7.4, ensuring the omeprazole stability. The column, flow rate and the portion of mobile phase were adjusted to obtain a rapid assay method with a reasonable retention time and a sharp peak.

Under the experimental conditions, a typical chromatogram of a standard and sample solution of omeprazole (Figure 2A and B) showed a single peak of the drug in around 5 min.

The peak area ratios of the standard analysis were plotted against its theoretical concentrations in the range of 10–30 μg/mL. The linearity was evaluated by construction of a calibration curve by least square linear regression. The method was linear, showing a correlation coefficient of 0.9995. All the results are shownin Table 2. The representative linear equation for was: y = 22641x + 17026. The quantification limit calculated was 0.56 μg/mL.

The specificity of the method was evaluated by analyzing a sample of a delayed release tablet without omeprazole (placebo). The chromatograms showed that the method is specific, and there is no interference or overlaps of the excipients with the omeprazole response at 280 nm detection wavelength. Any detection near to the retention time of the drug (Figure 2C) was not observed.

The repeatability and intermediate precision of the method was determined. The mean peak area and the relative standard deviation are reported in Table 3. The obtained values indicate good precision.

Table 3. Results from the precision

Sample solution (20 μg/mL)	Mean peak area ± RSD[a]
Day 1	413235.50 ± 0.56
Day 2	402887.67 ± 0.67
Day 3	401120.33 ± 0.28
Inter-day	405923.29 ± 1.44

[a]Mean ± R.S.D (n = 6).

Table 4. Results from the recovery test

Fortified solution	Mean recovery ± RSD[a]
15 μg/mL	101.3 ± 1.78
20 μg/mL	100.4 ± 2.30
25 μg/mL	101.0 ± 1.16

[a]Mean ± R.S.D (n = 3).

The accuracy was determined using the recovery test, by fortifying the samples with the omeprazole standard at 3 levels and assaying by the developed method. Percentage recovery was calculated from the differences between the peak areas obtained for fortified and sample solutions (Table 4).

The validation results indicate that the developed method can be used to quantify omeprazole from the produced delayed release tablet. The mean value obtained for the quantitative assay was 97.9 ± 0.35% and the mean value found for uniformity of content was 98.8 ± 3.56%.

CONCLUSIONS

A simple, rapid, and safe RP-HPLC method was developed and validated. The analytical procedure proved to be more adequate compared to some other LC methods that ensures the stability of the drug. Furthermore, the excipients did not interfere in the analysis, and the proposed method proved to be a suitable technique for quality control of omeprazole delayed release tablets, such as quantitative assay and content uniformity.

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