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METHOD DEVELOPMENT AND VALIDATION OF OMEPRAZOLE BY USING UV SPECTROSCOPY

V. Vishwanath*, T. Ramya Krishna, D. Snidgha, Afzal Nazneen, Nerella Mounika
Department of Pharmaceutical Chemistry, Talla Padmavathi Pharmacy College, Orus, Kareemabad, Warangal, Telangana, India

ABSTRACT
An analytical methodology for quantification of omeprazole in presence of its metabolites is described. Full validation according to the FDA guidelines was performed, and the limit of quantification level of 15ng/ml was reached for omeprazole, with accuracy and precision levels within FDA requirements.
This method is suitable for the estimation of omeprazole in plasma samples. It was used for bioequivalence studies of two pharmaceutical dosage forms Omeran (Europharm) and Losec (Astra) and for estimation of the pharmacokinetic parameters of omeprazole in children and adults .
Key Words: Uv Spectroscopy, omeprazole

***Corresponding Author:**
V. Vishwanath,
Talla Padmavathi Pharmacy College,
Orus, Kareemabad,
Warangal,Telangana,India.

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INTRODUCTION
Analytic method development, validation, and transfer are key elements of any pharmaceutical development program. This technical brief will focus on development and validation activities as applied to drug products. Often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use"[1]. Method transfer is the formal process of assessing the suitability of methods in another laboratory. Each of these processes contributes to continual improvement of the methods and results in more efficient drug development.
Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance. According to the International Conference on Harmonization (ICH), the most common types of analytic procedures are: (i) identification tests, (ii) quantitative tests of the active moiety in samples of API or drug product or other selected component(s) in the drug product,(iii) quantitative tests for impurities' content, (iv) limits tests for the control of impurities.

EXPERIMENTATION MATERIALS AND METHODS
Reagents
Omeprazole (OPZ), hydroxyomeprazole (H-OPZ), omeprazole sulfone (S-OPZ) and phenacetine (PHE). Methanol, triethylamine, orthophosforic acid 85%, dichloromethane, diethyl ether. Reagent-grade water obtained from a Millipore Milli-Q system was used throughout the experiments. All other reagents were of analytical grade.
Preparation of solutions
Stock solutions of omeprazole, hydroxyomeprazole and omeprazole sulfone (0,1mg/ml) and the internal standard (I.S.) (0,1mg/ml) were prepared dissolving the drugs in methanol. Omeprazole, hydroxyomeprazole and omeprazole sulfone stock solution was further diluted to working solutions ranging from 25 ng/ml to

500 ng/ml. Phenacetine stock solution was further diluted to working solution at 100ng/ml. All solutions were stored at 4◦C and were stable for at least 2 months.

Sample preparation

Omeprazole, hydroxyomeprazole, omeprazole sulfone and the I.S. were extracted from human serum by liquid-liquid extraction. To 1ml serum samples there were added 80µl I.S working solution, 200µl NaOH 0.02M and 4.5ml of mixture of dichloromethane: diethyl ether (2:2.5 v/v). After mixing (30s on a vortex mixer) and centrifugation (5 min at 6000×g), the organic phase was removed and evaporated to dryness under a stream of nitrogen. 300µl mobile phase was added to the residue and after mixing for 10s (on a vortex mixer), 30 µl of this mixture was injected into the chromatograph.

UV-spectrophotometry

SHIMADZU double beam UV/Visible recording spectrophotometer (Model:1700) with 2 nm spectral bandwidth was employed for all spectrophotometric measurements using 10 mm matched quartz cell and Borosil glass wares were used for the study. Calibrated electronic single pan balances Sartorius CP 225 D, pH Meter, Enertech Fast Clean Ultrasonicator were also used during the analysis. UV-Spectrophotometric determination of two drugs was done using Vierordt's simultaneous equation method (Davidson et al., 2001).

Determination of λ_{max}

The standard solution of Omeprazole (10 µg/ml) were scanned in the wavelength region of 200–400 nm and the λ_{max} was found to be 269.4 nm (λ_{max} of Omeprazole). They were scanned in the wavelength range of 200–400 nm and the overlain spectrum was obtained.

Construction of calibration curve

Solutions containing 20-60 ppm of omeprazole were prepared. 20µl of each concentration were injected for 5 times and the mean area was calculated. A calibration plot was constructed between peak area and concentration. The regression equation obtained was y = 50417.3x + 2579.4 and the regression coefficient was found to be 1.0000. This equation was used later to estimate the amount of omeprazole in capsule dosage form.

Assay of omeprazole in capsule dosage form

Quantity of pellets (from a blend of 20 capsules) equivalent to 100 mg of omeprazole were weighed and transferred to a 250 ml Volumetric flask containing 150 ml of 0.1 N NaOH. The solution was sonicated for 15 min under controlled temperature not exceeding 30°C. The solution was made up to the Volume with 0.1 N NaOH. Centrifuge a portion of the sample at 3000 RPM for 15 minutes. The solution was diluted with the mobile phase to obtain a sample of 40µg/ml.

Validation of the proposed method

The precision, accuracy, linearity specificity, robustness and system suitability parameters were studied systematically to validate the proposed RP-HPLC method for the estimation of omeprazole. Solutions containing 30, 40, 50 ppm of omeprazole were subjected to intra-day and interday precision. The accuracy was of the method was determined by performing accuracy studies. Solutions ranging from 20-60 ppm were prepared and chromatograms were recorded by injecting the solutions. The mean area was calculated and a graph was constructed between concentration and peak area.

Specificity of the method was validated by forced degradation studies. The drug was subjected to acid degradation, base degradation, UV light stress, humidity stress. The method was found to be specific for the determination of omeprazole as no interference was observed from excipients and degradation products. The robustness of the method was studied by changing the organic phase proportion of mobile phase, flow rate, pH of the mobile phase.

RESULTS AND DISCUSSION

Validation of the method was performed according to latest FDA guidelines [5].

Selectivity and sensitivity

Six blank plasma obtained from six different sources were analysed according to the procedure previously described, in order to evaluate method specificity (Fig. 1a).

The limits of detection (LOD) and of quantification (LOQ) were determined by analysing six zero samples. HPLC-UV peak areas detected at the retention times of the analytes of interest were measured; the ratios with respect to chromatographic peak area of internal standard were calculated and calibration curves equations were used to determine 'virtual' mean analyte concentrations. LODs and LOQs were 9ng/ml and 15ng/ml, respectively

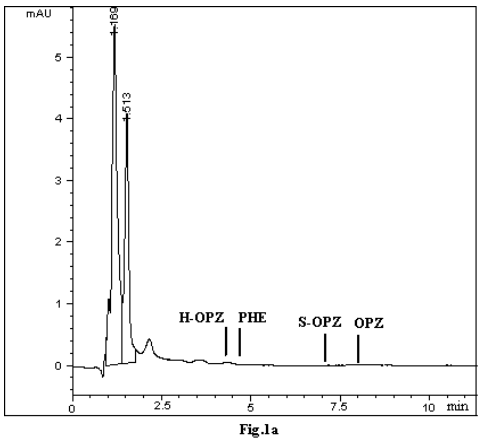

Figure 1a
Chromatogram of drug free plasma

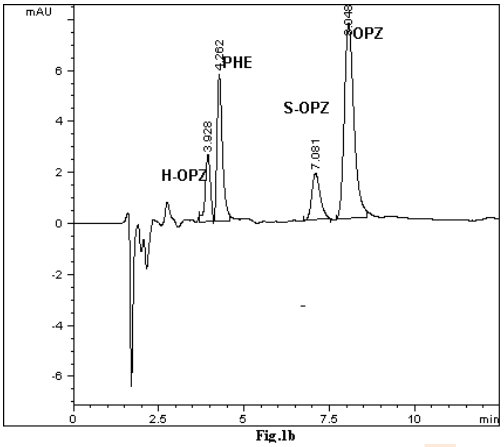

Figure 1b
Chromatogram of the plasma sample spiked with analytes

Linearity

Matrix-based calibration standards, in the range of 25-500ng/ml, were independently prepared and analysed in triplicate, in three different days.

Correlation coefficient of linear fit curve for omeprazole obtained from data was 0.999835 (Fig.2). Calibration standards concentrations were back calculated. Deviations from the nominal concentrations and CV values were from 0.33 to 7.82% and 0.30 to 7.12% for all analytes concentration levels.

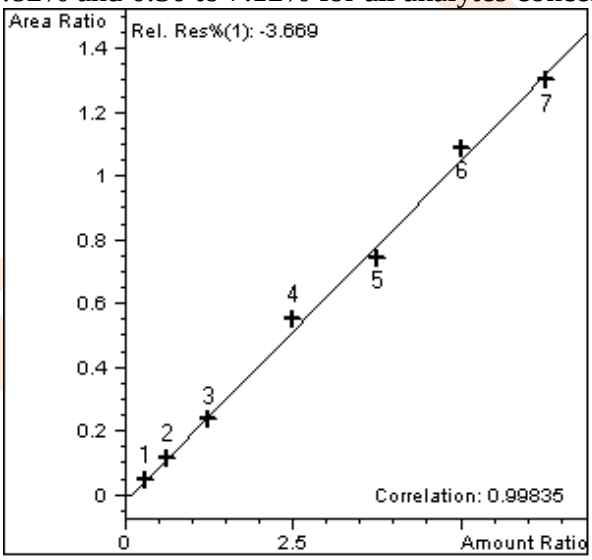

Figure 2 Calibration curve of omeprazole in plasma

The accuracy and precision of the analytical method were evaluated by analysing quality control samples at seven concentration levels (QCi). The obtained values are reported in Table II and Table III resulted within FDA requirements for all the investigated analytes. In particular, the Acc% (accuracy) and coefficient of variation CVs (coefficient of variation) values of QC7 samples were < 7.5% in all of the examined cases. At concentrations close to the LOQ levels Acc% is within 20% deviation.

Table II Recovery, accuracy and intra-day precision

Theoretical concentration (ng/ml)	Calculated concentration ng/ml ±SD	Precision CV %	Accuracy %	Recovery % ±SD
25	26.286 (1.73)	7.12	5.1	86.1(3.29)
50	51.543 (1.70)	3.18	3.1	94.7(2.91)
100	107.33 (0.33)	0.30	7.3	99.9(1.80)
200	196.75 (2.30)	1.18	-1.6	90.2(5.61)
300	297.57 (5.01)	1.69	-0.8	91.3(1.40)
400	384.47 (3.88)	1.02	-3.9	81.1(0.90)
500	518.50 (7.82)	1.49	3.7	89.8(1.87)

Table III Recovery, accuracy and inter-day precision

Theoretical concentration (ng/ml)	Calculated concentration ng/ml ±SD	Precision CV %	Accuracy %	Recovery % ±SD
25	23.959 (1.68)	7.12	-4.2	92.6(3.32)
50	54.536 (1.75)	3.18	9.1	98.6(2.99)
100	107.72 (0.38)	0.30	7.7	99.1(1.92)
200	193.61 (2.42)	1.18	-3.2	98.3(5.58)
300	301.16 (5.11)	1.69	0.4	92.4(1.25)
400	382.65 (3.98)	1.02	-4.3	82.2(1.12)
500	528.94 (7.91)	1.49	5.8	90.6(1.83)

Stability

Short-term stability after 6 and 24 h at room temperature was studied to verify if analytes degrade over the course of analyses. Short-term stability can be evaluated by analysing either working solutions or matrix-based samples added to working solutions and kept at room temperature before the extraction step. The working solutions were left at room temperature for at least 1h. The matrix-based samples added to working solutions were left at room temperature for 24h.

Long-term stability was studied in order to be sure that analytes present in plasma samples ("real" samples) do not degrade in the storage conditions before being analysed. Hence, long-term stability was studied on matrix-based samples stored at −20 ◦C for 3 months.

The stability of analytes (expressed as percentage) at room temperature and in the storage conditions was evaluated by comparing the analytical responses, respectively, of working solutions analysed after 6 and 24 h with respect to that of the same solutions immediately analysed, and of samples extracted after 3 months with respect to that of samples immediately extracted and analysed.

The obtained results, show that analytes in the working solutions and in matrix-based samples added to working solutions are stable at room temperature for 6h. Plasma samples can be stored at −20 ◦C for 3 months without a relevant loss of signal.

CONCLUSION

An analytical methodology for quantification of omeprazole in presence of its metabolites is described. Full validation according to the FDA guidelines was performed, and the limit of quantification level of 15ng/ml was reached for omeprazole, with accuracy and precision levels within FDA requirements.

This method is suitable for the estimation of omeprazole in plasma samples. It was used for bioequivalence studies of two pharmaceutical dosage forms Omeran (Europharm) and Losec (Astra) and for estimation of the pharmacokinetic parameters of omeprazole in children and adults .

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