Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2014, 6 (3):277-280
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Bioanalytical method for measurement of rabeprazole in human plasma

Shankar S. and Suneetha V.

School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu

---

ABSTRACT

Bioanalytical method for the measurement of rabeprazole in human plasma was developed and validated. Rabeprazole was resolved on a reverse phase C18 column from the endogenous plasma components by isocratic elution of 30% 25mM phosphate buffer (pH 7) in acetonitrile at a flow rate of 1mL/min. Accuracy, precision and recovery of rabeprazole from plasma was determined. System suitability parameters such as theoritical plates, resolution, assymetry factor, LOQ and LOD are reported. The optimized method is simple, accurate, precise, robust and thereby could be used for the determination of bioeqvivalence of rabeprazole between drug products in human subjects.

Keywords: Rabeprazole, HPLC, Isocratic elution, Stability, Bioequivalence

---

INTRODUCTION

Rabeprazole,2-4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1H-benzimidazole is a benzimidazole proton-pump inhibitor. Rabeprazole is activated by conversion to sulphenamide in the acid environment. It is also reported that independent of pH, rabeprazole instantly converts into sulphenamide[1,2,15]. Therefore, it is very critical to establish a sensitive and robust analytical method for the measurement of rabeprazole in biological matrices. Several researchers have published methods for the quantification of rabeprazole using HPLC with UV detection [3], spectrophotometric [4,3,7] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods [5,6]. Several investigators have also reported the stability studies of rabeprazole under different conditions such as acidic, alkaline, oxidative and photolysis [4,8,9].

Though, more sensitive and selective LC-MS/MS methods are reported, a validated method addressing stability of rabeprazole and for application to bioequvialence studies by HPLC is necessary.

Therefore, we present in this report development and validation of a rugged and reliable HPLC-UV method to accurately measure rabeprazole in human plasma

MATERIALS AND METHODS

Reagents

Acetonitrile of HPLC grade, Ortho-Phosphoric acid and Disodium hydrogen orthophosphate, Triethylamine, Perchloric acid, and Trichloroacetic acid AR grade were purchased from Qualigens Fine Chemicals and S.D. Fine chemicals. Water HPLC grade obtained from Milli-Q RO system, Working Standards of Rabeprazole and Pantoprazole (as internal standard) were obtained as gift samples from the manufacturers.[10,12,15]

Instrumentation
Waters HPLC system with 1515 solvent delivery system (pump), Rheodyne 7725i injector with 100 µl loop, 2487 Dual wavelength absorbance detector. Shimadzu gradient HPLC system with LC-10 AT-VP solvent delivery system (pump), Rheodyne 7725i injector with 100 µl loop, SPD M-10AVP photo diode array detector, Perkin-Elmer FT-IR 1600 series, Sartorius single pan digital balance (R200D & 1702), Systronics - pH meter, µ pH system 361[10,11.13]

Selection of wavelength
An uv spectrum of 10 µg/ml Rabeprazole in a mixture of water and acetonitrile (1:1 V/V) was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength of 280 nm was selected. At this wavelength Rabeprazole showed good absorbance.[14]

Selection of chromatographic method
Proper selection of the method depends upon the nature of the sample (ionic / ionisable / neutral molecule), its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase or ion-pair or ion-exchange chromatography method may be used. The reversed phase HPLC was selected for the initial separations because of its simplicity and suitability.

During method development several factors such as effect of pH, effect of peak modifier, effect of nature of stationary phase, effect of solvent strength, effect of ratio of oraganic-to-aqueous mobile phase, effect of flow rate was studied and approprite analog internal standard was selected (pantoprazole). The optimized chromatographic conditions was sepeartion of rabeprazole from endogenous plasma components on a C18 colum (Waters Symmetry C18, 15 cm x 4.6mm, 5 µ particle size) by isocratic elution consisting of 30% 25mM phosphate buffer (pH 7) in acetonitrile at a flow rate of 1mL/min. Injection volume was 100 µL and rabeprazole was detected by UV detection at 280 nm.

Accuracy, precision, limit of quantification (LLOQ) and recovery
Accuracy, intra and inter-day precision of the method was determined for rabeprazole according to FDA guidance for bioanalytical method validation [6]. Six replicates of human plasma samples spiked with rabeprazole were analysed to determine intra and inter-day precison at three different concentrations (25, 100 and 250 ng/ml. Accuracy was calculated as deviation of the mean from the nominal concentration. Intra and inter-day precision was expressed as the relative standard deviation of each calculated concentration. For the concentration to be accepted as LOQ, the percent deviation from the nominal concentration (accuracy) and the relative standard deviation has to be ±20% and less than 20%, respectively. Average recovery of each compound was determined by comparing AUC obtained after injection of the processed QC samples with those achieved by direct injection of the same amount of drug in aqueous solution at different concentrations (six samples for each concentration level).[15]

Stability of Rabeprazole in Plasma
The stability of the drug spiked human plasma samples at three levels (25, 100 and 250 ng/mL) were studied for three freeze thaw cycles. The mean concentrations of the stability samples were compared to the nominal concentrations. Similarly, short term (3 h), long term (4 weeks) and standard solution stability were evaluated. The stability of the internal standards was also performed[14].

RESULTS AND DISCUSSION

Selection of wavelength
The sensitivity of the HPLC method that uses UV detection depends upon the proper selection of wavelength. The standard solution was scanned in UV spectrophotometer and UV spectra were recorded. From the UV spectra the detection wavelength of 280 nm was selected.

Optimisation of chromatographic conditions
Chromatographic conditions were optimized taking into account the various goals of the method development and weighing each goal (resolutions, run time, sensitivity, peak symmetry, etc) accurately, so as to achieve a chromatographic method that can reliably measure rabeprazole in plasma samples.

System suitability
System suitability parameters such as column efficiency (theoretical plates), resolution factor and peak asymmetry factor of the optimised methods were found satisfactory (Table 1).

Linearity

Optimised method was linear within a specific concentration range for rabeprazole. The calibration curves were plotted between response factor and concentration of the standard solutions (Table 2). The linearity range for Rabeprazole was found to be 5, 10, 25, 50, 100 and 250ng/ml, respectively and their slope (k) and the intercept values (B) were 557.330 and 0.0699, respectively. The calibration curves were constructed on six different days over a period of two weeks to determine the variability of the slopes and intercepts. The results indicated that no significant interday variability of slopes and intercepts over the optimised concentration range.

Table 1: System Suitability Parameters

S.No.	Parameters	Rabeprazole	Pantoprazole (Internal standard)
1	Theoretical Plate number	19367	16238
2	Resolution factor	1.09	
3	Asymmetric factor	0.919	1.012
4	LOD (ng/ml)	1.00	1.00
5	LOQ (ng/ml)	4.00	4.00

Table 2: Linearity of rabeprazole in plasma

S.No	Rabeprazole Concentration (ng/ml)	Internal Standard Concentration (ng/ml)	Response factor
1	5	1000	0.0089
2	10	1000	0.0181
3	25	1000	0.0448
4	50	1000	0.0891
5	100	1000	0.1791
6	250	1000	0.4486

Table 3: Intra-day and Inter-day precision and accuracy

Day	Replicates	Obtained concentration (ng/mL)		
		Low Quality control (25 ng/mL)	Mid Quality Control (100 ng/mL)	High Quality control (250 ng/mL)
Day-1	1	24.537	101.869	251.519
	2	24.998	101.837	249.287
	3	23.904	100.779	251.591
	4	25.725	100.916	249.513
	5	24.135	100.065	249.104
	6	24.267	101.043	248.815
Mean		**24.594**	**101.085**	**249.972**
Stdev		**0.6692**	**0.6848**	**1.2479**
Intra day Precision (%CV)		**2.72**	**0.68**	**0.50**
Accuracy (% nominal)		**98.38**	**101.08**	**99.99**
Day-2	7	24.421	99.845	251.215
	8	23.954	100.021	250.965
	9	23.904	101.586	248.287
	10	27.914	102.427	246.871
	11	30.182	98.752	253.982
	12	30.961	102.125	250.754
Day-3	13	28.425	100.024	250.369
	14	26.453	100.059	250.034
	15	29.845	99.854	239.654
	16	25.124	98.327	248.365
	17	28.351	97.953	250.954
	18	30.021	101.652	251.682
Mean		**26.507**	**100.507**	**249.609**
Stdev		**2.5396**	**1.3065**	**2.9723**
Precision (%CV)		**9.58**	**1.30**	**1.19**
Accuracy (% nominal)		**106.03**	**100.51**	**99.84**

Accuracy, precision, and recovery

The results from the validation (precision and accuracy) of the method in human plasma are listed in Table 3. The method proved to be accurate and precise. Accuracy at three concentrations (25, 100 and 250 ng/mL) ranged from 98.38 to 106.03% for rabeprazole. The intra and inter-day precision ranged from 0.50 to 2.72% and 1.19 to 9.58%, respectively. The absolute recoveries for rabeprazole ranged from 96.67 to 98.45% (Table 4).

Stability of Rabeprazole in Plasma

Stability of rabeprazole in plasma subjected for three freeze-thaw cycles, stored for 3 h at room temperature, 4 weeks at -20 °C was found stable. Stock solutions stored under different conditions were also found to be within 20% of the nominal concentration.

Table 4: Recovery of rabeprazole from human plasma

Level	Concentration of drug added (ng/ml)	Amount of drug recovered (ng/ml) in plasma sample	Percentage Recovery (%)
Level-I	25.00	24.82 ± 1.092	Mean : 96.670 % CV : 4.368 N : 6
Level-II	100.00	97.294 ± 4.072	Mean : 98.456 % CV: 4.072 N : 6
Level-III	250.00	246.538 ± 7.629	Mean : 97.619 CV : 3.051 N : 6

CONCLUSION

In conclusion, the developed method for the estimation of Rabeprazole in plasma by HPLC is accurate, precise, selective and linear and is therefore, can be employed for a comparative bioavailability/ bioequivlaence study to evaluate its applicability.

Acknowledgement

The author wants to express her gratitude to Dr G. Viswanathan, Founder and Chancellor VIT University for his constant support and encouragement Sri Sankar Viswanathan, Sri Sekar Viswanathan and Sri G.V Selvam ,Vice presidents, VIT university for their constant motivation and Raj Vuppu Temple university ,Singapore providing constant help throughout this research.

REFERENCES

[1] N.V.S. Ramakrishna, K. N. Vishwottam, S. Wishu, M. Koteshwara, S. Suresh Kumar*J. Chromatogr. B*, 816:1-2 (**2005**), p. 209-214
[2] P. Richardson, C.J. Hawkey, W.A. Stack,. *Drugs*, 56:3 (**1998**), p. 307-335
[3] H. Nakai, Y. Shimamura, T. Kanazawa, S. Yasuda, M. Kayano. *J. Chromatogr. B*, 660:1 (**1994**), p. 211-220
[4] El-Gindy, F. El-Yazby, M.M. Maherv, *J. Pharm. Biomed. Anal*., 31:2 (**2003**), p. 229-242
[5] Y. Zhang, X. Chen, Q. Gu, D., Zhong *Anal. Chim. Acta*, 54:2 (**2004**), p. 171-174
[6] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), **2001**.
[7] Suneetha V., Bishwambhar Mishra, Parul Kamat, Gopi chandT., Saranya C., Rani Anupama,, *Applied Mathematical Sciences.* **2013**, 7, 1563 – 1567.
[8] Suneetha Vuppu and Bishwambhar Mishra, *Der Pharmacia Lettre*, **2013**, 5, 100-106.
[9] Sanjeeb Kumar Mandal And Suneetha Vuppu, *Int J Pharm Bio Sci*. **2013**, 4,193 – 200.
[10] Siddharth Sharan, Naina Thangaraj, Bishwambhar Mishra, Suneetha V . *Int. J. Drug Dev. & Res*.,**2013**, 5, 138-144.
[11] Sonika Jha , Vineet Vaibhav and Suneetha V. *Int. J. Drug Dev. & Res.*,**2013**, 5, 362-367.
[12] Vaishnavi R and Suneetha V. *Der Pharmacia Lettre*, **2013**, 5, 71-75.
[13] K. Lohitesh, Alok Kumar Behera, Allen Abey Alexander and Suneetha V, *Der Pharmacia Lettre*, **2013**, 5, 163-169.
[14] Suneetha V, Bishwambhar M, Gopinath R.,Shrestha S R, Kartik G K.B., Pravesh C, Apoorvi C, Kalyani R, *Asian Journal of Microbiology Biotechnology and Environmental Sciences*,**2013**,14,405-412.
[15] Suneetha Vuppu, Bishwambhar Mishra and Ching Lee, *International Journal of Pharmaceutical Sciences and Research*,**2013**,3,4242-4246.
[16] Suneetha V and Raj V .*International Journal of Drug Development & Research*,**2012**, 4, 1-6.