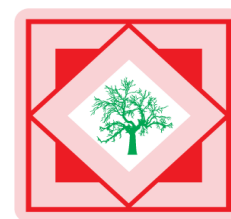




## Pelagia Research Library

Der Pharmacia Sinica, 2010, 1 (3): 69-78



Der Pharmacia Sinica

ISSN: 0976-8688  
CODEN (USA): PSHIBD

### Application of UV-Spectrophotometry and RP-HPLC for Simultaneous Determination of Rabprazole and Domperidone in Pharmaceutical Dosage Form

Arunadevi S. Birajdar\*, Subramania N. Meyyanathan, Bojraj Suresh

Department of Pharmaceutical Analysis, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu, India

#### ABSTRACT

Two simple, accurate and sensitive analytical methods have been developed for the simultaneous determination of Rabeprazole and Domperidone in pharmaceutical solid dosage form. The first method involves UV-spectrophotometric determination of both drugs in combination, using *Q*-analysis method. It involves absorbance measurement at 291.6 nm ( $\lambda_{max}$  of Rabeprazole) and 240 nm isobestic point in solvent (0.1N NaOH), Sodium hydroxide. The method obeys Beer's law over linearity range for Rabeprazole 2 – 12  $\mu\text{g mL}^{-1}$  and for Domperidone 3-18  $\mu\text{g mL}^{-1}$  respectively. The second method involves HPLC separation of Rabeprazole and Domperidone drugs in reverse phase mode using Phenomenx  $C_{18}$  column. Linearity was obtained in the concentration range of 0.5-5.0  $\mu\text{g mL}^{-1}$  for Rabeprazole and 0.75-7.5  $\mu\text{g mL}^{-1}$  Domperidone. The detection wavelength was 220 nm. Pantaprazole was used as internal standard in 10  $\mu\text{g mL}^{-1}$  concentration. Relative standard deviation (R.S.D) for both methods was found <2.0%. The proposed methods were successively applied to multicomponent formulation containing both the drugs. The methods were validated according to ICH guidelines.

**Keywords:** Rabeprazole; Domperidone; Pantaprazole; UV- spectrophotometry; RP-HPLC.

#### INTRODUCTION

Development of the simple and reproducible analytical methods for estimation of multicomponent drugs is very important part of quality control and for social awareness which is established in present work. Now day's new multicomponent formulations in market increasing with alarm rate which have better synergetic effect it is very essential that two or more number of drugs should be estimated simultaneously. Rabeprazole in combination with Domperidone used as antacid and  $H_1$  blocker in ulcer conditions as sustained release capsules.

Rabeprazole chemically 2-({[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl] methyl} sulfinyl)-1H-benzimidazole sodium. It belongs to a class of proton-pump inhibitors. It suppresses gastric acid secretion by specifically inhibiting the H<sup>+</sup>/K<sup>+</sup>- ATPase enzyme system at the secretory surface of the gastric parietal cell. Clinically, rabeprazole is used to heal, relieve symptoms and prevent a relapse of acid-peptic diseases, such as duodenal, gastric and oesophageal ulceration [1]. Literature survey revealed that various reports on stability in aqueous media [2], Dissolution test method [3], analytical methods such as HPLC [4], UV [5], enantiomeric determination [6], Estimation of photo degradation products by UV and HPLC [7,8] have been reported for individual estimation of Rabeprazole from its formulations.

Domperidone chemically (5-chloro-1-{1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl]-4-piperidiny]benzimidazol-2-one) is a dopamine antagonist with antiemetic properties. It stimulates gastro-intestinal motility and is used as an antiemetic for the short term treatment of nausea and vomiting of various aetiologies, including that associated with cancer therapy and with levodopa or bromocriptine therapy for parkinsonism [1]. The chemical structure of Rabeprazole, Domperidone and Pantaprazole (IS) is as shown in (Fig.1). There are very few methods reported for individual estimation of domperidone in pharmaceutical dosage form, which includes UV and HPLC [9,10]. The RP-HPLC methods for combination with other drugs [11-13].

The validation of method carried out as per ICH guidelines. This proposed method is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies [14-15].

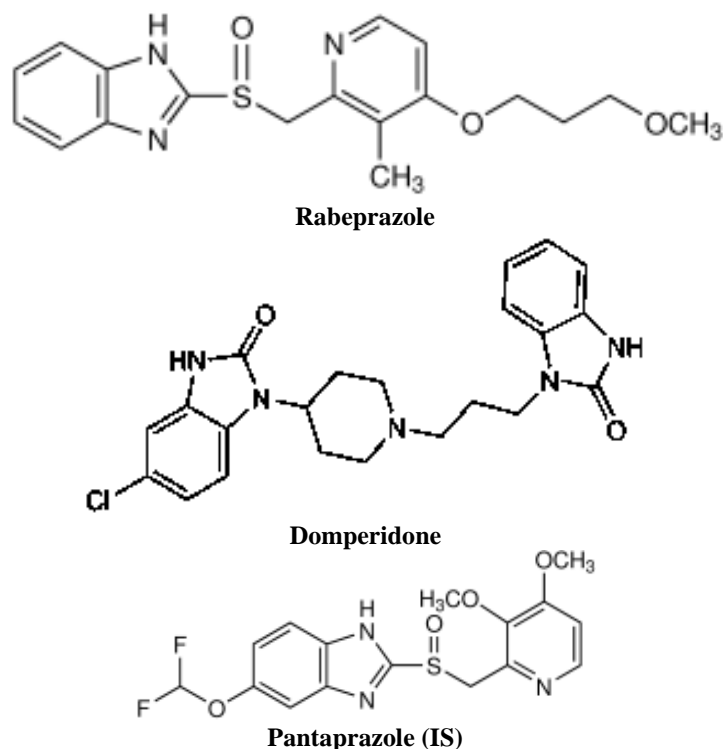


Fig 1. Chemical structure of Rabeprazole, Domperidone, Pantaprazole (IS).

## MATERIALS AND METHODS

### 2.1. Materials

Pharmaceutical grade Rabeprazole and Domperidone were pursued as a gift sample by Shreechem Pharmaceuticals Pvt Ltd. New Mumbai, India. Pantaprazole (IS) procured from Apex drugs and intermediates, Medka (A.P.) All chemicals and solvents of HPLC grade and were purchased from Qualigens fine Chemicals, Mumbai, India. Water HPLC grade was obtained from a Milli-QRO water purification system.

### 2.2. UV- spectrophotometry

Uv- spectrophotometer 1700 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells was used.

### 2.3. Preparation of standard stocks solution

Standard stock solutions of  $100 \mu\text{g mL}^{-1}$  were prepared by dissolving 10 mg of each in 100mL of in (0.1N NaoH), Sodium hydroxide . From these stock solutions, working standard solutions having concentration  $10 \mu\text{g mL}^{-1}$  each were prepared by appropriate dilutions. They were scanned in the wavelength range of 400–200 nm and the overlain spectrum of rabeprazole and domperidone was obtained (Fig 2). Two wavelengths 291.6 nm ( $\lambda_{\text{max}}$  of Rabeprazole) and 240 nm isobestic point for both the drugs were selected for estimation by Q-analysis. The calibration curves were found to be linear in the concentration range for Rabeprazole 2 – 12  $\mu\text{g mL}^{-1}$  and for Domperidone 3-18  $\mu\text{g mL}^{-1}$  as shown in (Fig 3).  $a_1$  and  $a_2$  were absorptivities  $\epsilon$  values of Rabeprazole and Domperidone were calculated at fixed concentration.

The concentration of two drugs in the mixture were calculated using equations as below,

For Rabeprazole

$$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{A}{a_1} \qquad Q_0 = \frac{\text{Absorbance of sample at 291.6 nm}}{\text{Absorbance of sample at 275 nm}}$$

For Domperidone

$$C_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{A}{a_2} \qquad Q_1 = \frac{\text{Absorbance of Rabeprazole at 291.6 nm}}{\text{Absorbance of Rabeprazole at 240 nm}}$$

$$Q_2 = \frac{\text{Absorbance of Domperidone at 291.6 nm}}{\text{Absorbance of Domperidone at 240 nm}}$$

In equation A was absorbance of sample at is absorptive point and  $a_1$  and  $a_2$  were absorptivities values of Rabeprazole and Domperidone respectively at iso absorptivity point.

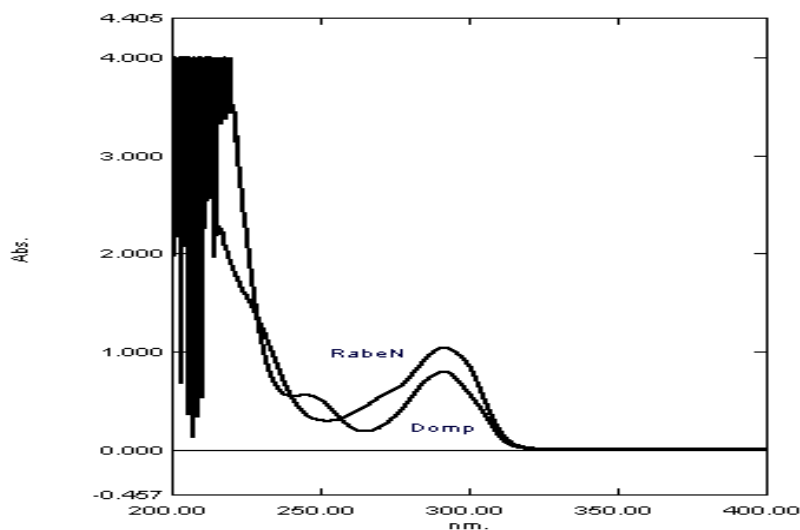


Fig 2. Overlain Spectrum of Rabeprazole and Domperidone in 0.1N NaOH (each  $10 \mu\text{g}\cdot\text{mL}^{-1}$ ) taken on UV – Vis spectrophotometer (SHIMADZU 1700)

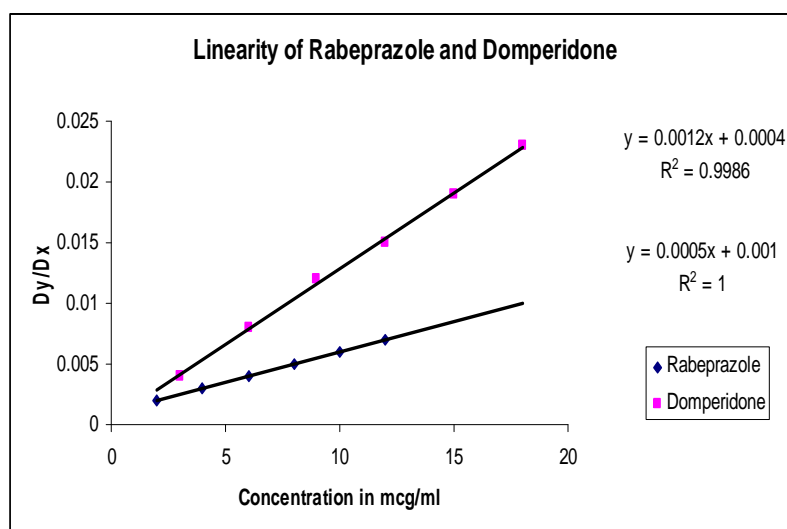


Fig 3. Linearity curve for Rabeprazole and Domperidone by spectrophotometry

## 2.4. HPLC method

### 2.4.1 Instrument

LC system used consisted of pump model (Waters 1515 isocratic solvent delivery system) with universal loop injector (Rheodyne 7725 i) of injection capacity  $20 \mu\text{L}$ . Detector consists Waters 2487 dual wavelength absorbance detector. The column used was phenomenex  $\text{C}_{18}$  (25 cm X 4.6 mm i.d.  $5 \mu\text{m}$  particle size) at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously.

### 2.4.2 Chromatographic conditions

The optimal composition of mobile phase was determined to be 30mM ammonium sulphate buffer (pH 5.5): acetonitrile (60:40 v/v). The flow rate was set to  $1 \text{ mL min}^{-1}$  and UV detection was carried out at 220 nm. Pantaprazole was used as an internal standard.

### 2.4.3 Preparation of standard solutions

Stock solutions were prepared by dissolving 10 mg of rabeprazole and domperidone in 100 mL volumetric flask separately with methanol. 10 mg of pantaprazole (internal standard) was taken in separate 100 mL volumetric flask and dissolved in methanol.

All solutions were stored at + 20 °C, these solutions were shown to be stable during the period of study. From the above stock solutions, dilutions were made for working standard the concentration range of rabeprazole 0.5–5.0  $\mu\text{g mL}^{-1}$  and of domperidone 0.75–7.5  $\mu\text{g mL}^{-1}$  respectively, and each solution contains 10  $\mu\text{g mL}^{-1}$  concentration of pantaprazole as an internal standard. Detection wavelength was 220 nm. A volume of 20  $\mu\text{L}$  of each working standard was injected into  $\text{C}_{18}$  column. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area ratios (response factor) of analyte to internal standard versus the corresponding drug concentration. This standard solution injected thrice and recorded chromatogram was shown as in (Fig 4).

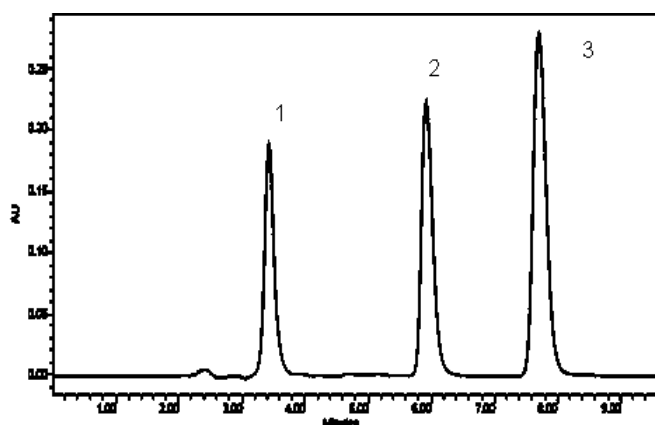


Fig 4. Chromatogram of Standard solution 1.Domperidone; (Rt 3.38), 2. Rabeprazole ; (Rt 5.82) 3. internal standard (Pantaprazole); (Rt 7.88) measured at 220 nm.

### 2.5. Analysis of Pharmaceutical Dosage Forms

To determine the content of rabeprazole and domperidone simultaneously in capsules (label claim: 20 mg Rabeprazole and 30 mg Domperidone); twenty capsules granules were weighed; their average weight determined and were finely powdered. The correct amount of powder equivalent to one capsule granule was dissolved in methanol by stirring and sonicating for 30 min. The excipients were separated by filtration. After filtration, an appropriate amount of internal standard was added and diluted up to mark with methanol. Further dilutions are made with mobile phase to get working standard solution containing 2  $\mu\text{g mL}^{-1}$  of rabeprazole, 3  $\mu\text{g mL}^{-1}$  of domperidone and 10  $\mu\text{g mL}^{-1}$ . This sample solution injected thrice and recorded chromatogram was shown as in (Fig 5). The amount of rabeprazole and domperidone were determined. The results are reported in Table 1.

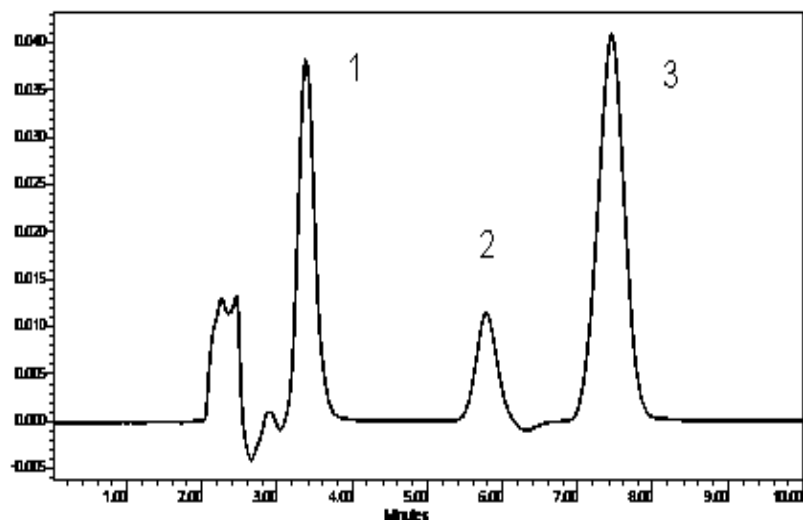


Fig 5. Chromatogram of Sample solution 1.Domperidone; (Rt 3.42), 2.Rabeprazole ; (Rt 5.89) 3.internal standard (Pantaprazole); (Rt 7.81)measured at 220 nm.

## 2.6. Recovery studies

To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 80, 100 and 120 % level. From the total amount of drug found, the percentage recovery was calculated. The results are reported in Table 1.

Table 1. Analysis data of tablet formulations

Parameter	UV-sepectrophotometry (Q-analysis)		HPLC	
	Rabeprazole	Domperidone	Rabeprazole	Domperidone
Label Claim	20	30	20	30
*Drug found	19.85	29.90	20.41	30.07
± S.D.	0.082	0.102	0.789	0.988
% Accuracy	99.50	99.56	102.05	100.90
*%Recovery ± S.D.	99.65±0.45	99.79±0.24	100.80±0.68	101.20±0.72

\*(n=6)

## RESULTS AND DISCUSSION

Both, UV spectrophotometric(Q-analysis) and HPLC methods were found to be reproducible, accurate, economic and rapid for routine simultaneous estimation of rabeprazole and

domperidone in tablet dosage forms. By UV spectrophotometric method, linearity was obtained in concentration range of Rabeprazole 2 – 12  $\mu\text{g mL}^{-1}$  and for Domperidone 3-18  $\mu\text{g mL}^{-1}$  with regression ( $R^2$ ) 1.0 and 0.999, intercept 0.0005 and 0.001 slope, intercept 0.0012 and 0.0004 slope for rabeprazole and domperidone respectively. Recovery was in the range of 99 – 101 %; the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method.

### 3.1. HPLC method development

By HPLC method chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate drugs and internal standard. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with 30 mM ammonium sulphate buffer pH 5.5: acetonitrile (60:40 v/v) with 1  $\text{mL}\cdot\text{min}^{-1}$  flow rate is quite robust. Pantaprazole was applied as an internal standard, neutralizing the error inherent in sample injection, eliminating random errors. A typical chromatogram for rabeprazole, domperidone and pantaprazole (internal standard) was shown in (Fig 4). The optimum wavelength for detection was 220 nm at which better detector response for drugs were obtained. The average retention times for rebeprazole and domperidone was found to be  $6.639 \pm 0.03$ ,  $9.010 \pm 0.03$  and  $11.822 \pm 0.02$  min, respectively.

### 3.2 Linearity and range

The calibration was linear for rabeprazole at concentration range of 0.5 – 5.0  $\mu\text{g mL}^{-1}$ , with regression 0.998, intercept – 0.0082 and slope 0.0642 and the calibration was linear for domperidone at concentration range of 0.75 – 7.5  $\mu\text{g mL}^{-1}$ , with regression 0.999, intercept +0.005 and slope 0.0649 respectively shown in (Fig 6). The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 100 – 102 %.

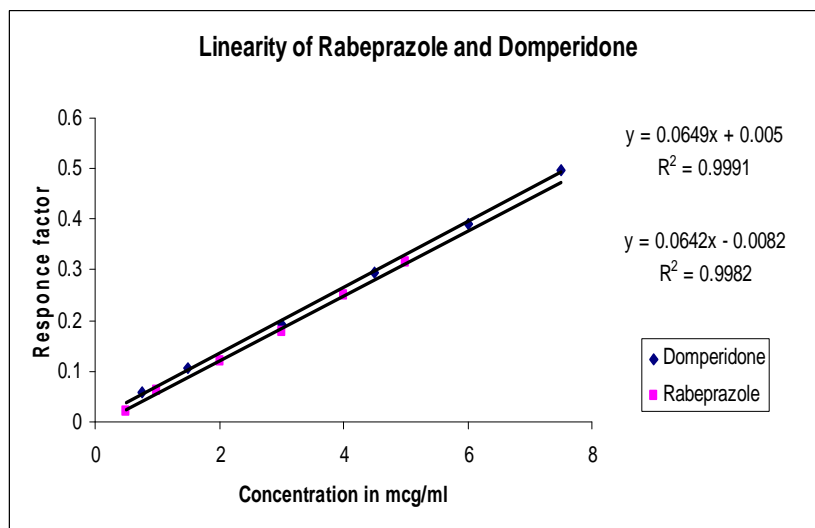


Fig 6. Linearity curve for Rabeprazole and Domperidone by HPLC

### 3.3 Precision and accuracy

Sample to sample precision and accuracy were evaluated using, three samples of three different concentrations, which were prepared and analyzed on same day. Day to day variability was

assessed using three concentrations analyzed on three different days, over a period of one week. These results show the accuracy and reproducibility of the assay.

System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1.

Thus, it was concluded that there was no significant difference on the assay, which was tested on an intra – day and inter – day basis. The % R.S.D. values reported in Table 2. shows that proposed methods provides acceptable intra –day and inter – day variation of rabeprazole and domperidone. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, using similar operational and environmental conditions; the % R.S.D. was found to be less than 2 %.

**Table 2. Validation parameters for Rabeprazole and Domperidone by HPLC**

Parameters	Rabeprazole	Domperidone
Linearity and range ( $\mu\text{g mL}^{-1}$ )	0.5- 5.0	0.75- 7.5
Correlation coefficient	0.998	0.999
Standard Deviation	0.112	0.160
Resolution Factor	3.80	2.55
Tailing Factor	1.0	0.99
Theoretical plates	6345	5432
Limit of Detection ( $\text{ng mL}^{-1}$ )	0.55	0.58
Limit of Quantification ( $\text{ng mL}^{-1}$ )	0.170	0.175
Precision (%R. S.D)		
Inter-day	1.0	0.98
Intra-day	0.87	0.69

The proposed methods are accurate, simple, rapid and selective for the simultaneous estimation of rabeprazole and domperidone in solid dosage form by internal standardization method. Hence, it can be conveniently adopted for the routine quality control analysis in the combination formulations. As the drug combination is available in market, hence, work is toward development of an analysis.

### 3.4 Comparison

According to shown in Table 3 the statistical comparison of the results was carried, there was no significant difference between uv-spectrophotometric and HPLC methods since the calculated *t*- and *F*- tests did not exceed the theoretical values at the 95% confidence level.



**Table 3. Statistical comparison of the results obtained by proposed methods**

Methods	F-test	t-Test
Q- Analysis method by UV and HPLC	0.04	0.43

n = 6; P = 0.05; t = 2.23; F = 5.05.

### CONCLUSION

The proposed one spectrophotometric Q-analysis method and one new HPLC method for simultaneous determination of rabeprazole and domperidone in multicomponent pharmaceutical dosage forms for both the drugs were developed and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by simultaneous equation estimation in spectrophotometric method and by RP-HPLC method. Both the methods were found to be simpler, accurate, economical and rapid and they can be applied for routine analysis in laboratories.

Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations, dissolution studies and also in future can be employed for bioequivalence studies for the same formulation.

### Acknowledgements

The author's thank to Shreechem Pharmaceuticals Pvt Ltd. New Mumbai, India. for providing gift samples of Rabeprazole and Domperidone and Apex drugs and intermediates, Medka ( A.P.) for providing a gift sample of pantaprazole. The authors are thankful to Mr. Supe (Drug Inspector). The author's are grateful to "His Holiness Jagadguru Sri Sri Shivarathree Deshikendra Mahaswamigalavaru" of Sri Suttur Mutt, Mysore and AICTE (QIP) cell for providing facilities to carry out this work.

### REFERENCES

- [1] The Martindale 35th ed., The complete drug reference. (2006) published pharmaceutical press, lambeta high street, london SE1 75M, UK.
- [2] Shan Rena, Mi-Jin Park, Hongkee Sah and Beom-Jin Lee (2008) Effect of pharmaceutical excipients on aqueous stability of rabeprazole sodium. *International J. Pharmaceutics* 350: 197.
- [3] Cassia V, Garcia , Clesio S Paim, Martin Steppe, Elfrides E S and Schapoval (2006) Development and validation of a dissolution test for rabeprazole sodium in coated tablets. *J. Pharm. Biomed. Anal.*, 41: 833.
- [4] Ramakrishna NVS, Vishwottam KN, Wishu S, Koteswara M, Suresh S Kumar (2005) High-performance liquid chromatography method for the quantification of rabeprazole in human plasma using solid-phase extraction. *J. of Chromatography B*, 816: 209.
- [5] Shweta S Sabnis, Nilesh D Dhavale, Vijay Y Jadhav, Santosh V Gandhi (2008) Spectrophotometric simultaneous determination of Rabeprazole Sodium and Itopride Hydrochloride in capsule dosage form. *Spectrochimica Acta Part A* 69: 849.

- [6] Masatomo Miura , Hitoshi Tada , Shigeru Satoh, Tomonori Habuchi, Toshio Suzuki (2006) Determination of rabeprazole enantiomers and their metabolites by high-performance liquid chromatography with solid-phase extraction. *J. Pharm. Biomed. Anal.*, 41: 565.
- [7] C´assia V Garcia, Norma S Nudelmanb, Martin Steppe, Elfrides E S Schapoval (2008) Structural elucidation of rabeprazole sodium photodegradation Products. *J. Pharm. Biomed. Anal.* 46: 88.
- [8] Alaa El-Gindy, Fawzy El-Yazby, Moustafa M Maher (2003) Spectrophotometric and chromatographic determination of rabeprazole in presence of its degradation products. *J. Pharma. Biomed. Anal.* 31: 229.
- [9] Maria Kobylin´ska, Kamila Kobylin´ska (2000) High-performance liquid chromatographic analysis for the determination of domperidone in human plasma. *J. Chromatography B* 744:207.
- [10] Maurizio Cignitti, Marina Cotta Ramusino, Leandro Rufini (1995) UV spectroscopic study and conformational analysis of Domperidone. *J. Molecular Structure* 350: 43.
- [11] Mohammed Shahid Ali , Mohsin Ghori, Aamer Roshanali Khatri (2006) Stability indicating simultaneous determination of domperidone (DP),methylparaben (MP) and propylparaben by high performance liquid chromatography (HPLC). *J. Pharma. Biomed. Anal.* 41: 358.
- [12] Sivakumar T., Manavalan R., and Valliappan K (2007) Development and validation of a reversed-phase hplc method for simultaneous determination of domperidone and pantoprazole in pharmaceutical dosage forms. *Acta chromatographica*, no. 18:260
- [13] Sivakumar T., Manavalan R., Muralidharan, C. and Valliappan K (2007) Multi-criteria decision making approach and experimental design as chemometric tools to optimize HPLC separation of domperidone and pantoprazole. *J. Pharma. Biomed. Anal.* 43:1842.
- [14] ICH, Q2A Text on validation of analytical procedures (1994) International Conference on Harmonization.
- [15] ICH, Q3B Validation of analytical procedures (1996) Methodology, International Conference on Harmonization.