Arabian Journal of Chemistry (2016) 9, S114–S119

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

A modified high-performance liquid chromatographic method for the analysis of pantoprazole sodium in pharmaceutical dosage forms using lansoprazole as internal standard

Safwan Ashour *, **Soulafa Omar**

Analytical Biochemistry Laboratory, Department of Chemistry, Faculty of Science, University of Aleppo, Aleppo, Syria

Received 2 January 2011; accepted 14 February 2011
Available online 19 February 2011

KEYWORDS

Pantoprazole sodium;
Liquid chromatography;
Pharmaceutical dosage form

Abstract A Simple and rapid reversed-phase high-performance liquid chromatographic method for the direct determination of pantoprazole in pharmaceutical dosage forms was developed and validated. Lansoprazole was used as internal standard. The chromatographic separation of pantoprazole and lansoprazole was achieved on a Nucleodur C_8 column (250 × 4.6 mm i.d., 5 μm particle size) using the photodiode array detector at 280 nm. The optimized mobile phase was consisted of a mixture of 0.1 M ammonium acetate solution and methanol (42:58, v/v), pumped at a flow rate 1.0 mL min^{-1}. The retention times for pantoprazole and lansoprazole were 8.10 and 11.15 min, respectively. Linearity range was 3.06–1243.0 μg mL^{-1} with limit of detection value of 0.78 μg mL^{-1}. The precision of the method was demonstrated using intra- and inter-day assay RSD% values which were less than 2.07%, while the recovery was 99.07–103.95%. No interference from any components of pharmaceutical dosage forms or degradation products was observed. According to the validation results, the proposed method was found to be specific, accurate, precise and could be applied to the quantitative analysis of pantoprazole in tablets.

1. Introduction

Pantoprazole, 5-difluoromethoxybenzimidazole-2-yl 3,4-dimethoxy-2-pyridylmethyl sulfoxide (CAS, 102625-70-7; MW, 383.4) is an irreversible proton pump (H^+/K^+-ATPase) inhibitor (PPI) that decreases acid secretion from gastric parietal cells (Cheer et al., 2003). It is also effective in Zollinger–Ellison syndrome and in preventing ulcer rebleeding. Thus pantoprazole is a valuable alternate to other PPIs in the treatment of acid-related disorders. The drug is officially listed in Martindale the extra pharmacopoeia (2005). A literature survey

* Corresponding author. Tel.: +963 933 604016.
E-mail address: profashour2010@myway.com (S. Ashour).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.02.010

reveals that spectrophometric (Wahbi et al., 2002; Karljikovic-Rajic et al., 2003; Salama et al., 2003; Moustafa, 2000; Syed and Syeda, 2008), kinetic spectrophotometric (Rahman and Kashif, 2005; Rahman et al., 2006), capillary zone electrophoresis (Eberle et al., 1997; Tivesten et al., 1999) and voltammetric (Radi, 2003; Chung et al., 2003; Erk, 2003) methods for the determination of pantoprazole in drug formulations have been described. In the literature only a few high-performance liquid chromatographic methods for the determination of pantoprazole in pharmaceutical formulations have been reported (Mansour and Sorour, 2001; Tanaka et al., 1995). An increasing number of publications are appearing describing the development of methods for pantoprazole determination in biological samples (Tanaka and Yamazaki, 1996; Ramakrishna et al., 2005; Storms and Stewart, 2002; Cass et al., 2002).

The objective of this work was to develop an analytical LC procedure, which would serve as reliable and rapid method for the determination of pantoprazole in pharmaceutical preparations. This manuscript describes the development and subsequent validation of isocratic reversed-phase HPLC method using C_8 column as stationary phase for the above determination. In the proposed LC method, pantoprazole and lansoprazole (internal standard) were well separated and eluted before 12 min run time. The precision of the described method for assay of pantoprazole has been checked in terms of *F*-test using a reported method as reference.

2. Experimental

2.1. Chromatographic system

Chromatographic analysis was performed on a modular HPLC system, Hitachi (Japan) consisted of binary pump (L-2130, flow rate range of 0.000–9.999 mL min^{-1}), auto sampler (L-2200, injection volume of 0.1–100 μL), column oven (L-2350, temperature range of 1–85 °C) and ultraviolet detector (L-2455, 190–850 nm) operated at wavelength of 280 nm and a quartz flow cell (10 mm path and 17 μL volume). Separation was achieved on a reversed-phase Nucleodur C8 column (250 × 4.6 mm, 5 μm particle size, Macherey-Nagel Germany). The mobile phase was a mixture of a 0.1 M ammonium acetate and methanol (42:58, v/v) and was filtered and degassed by ultrasonic agitation before use. The mobile phase was prepared weekly and was delivered at a flow rate of 1.0 mL min^{-1}. Data were monitored and processed using automation system software. Peak areas were integrated automatically by computer using the Ez-chrom Elite Hitachi software program. The injection volume was 10 μL. The system was operated at ambient temperature.

2.2. Chemicals

HPLC grade methanol and water were purchased from Merck (Darmstadt, Germany). Analytical reagent grade ammonium acetate from Merck (Darmstadt, Germany) and acetic acid glacial from SCP (England) were used to prepare the mobile phase.

2.3. Materials

Pantoprazole sodium ($C_{16}H_{35}F_2N_3NaO_4S$ = 426.52 g/mol, its purity was found to be 100.66% according to the compendial method) and the internal standard (lansoprazole USP, $C_{16}H_{14}F_3N_3O_2S$ = 369.37 g/mol, its purity was found to be 99.5%) were obtained from SL Drugs & Pharmaceuticals, India. Tablets containing pantoprazole sodium: Penta 40 mg (Alpha, Syria), Progast 40 mg (K.C. pharma, Syria) and Pantoprazol 40 mg (Amrit Medical Co., Syria).

2.4. Standard solutions

Standard solution of pantoprazole sodium (PPZ) was prepared by direct weighing of standard substance with subsequent dissolution in methanol. The concentration of the stock standard solution was 2.0 mg mL^{-1}. Stock standard solution of lansoprazole (LPZ) 1.0 mg mL^{-1} was prepared by dissolving appropriate amount of the compound in methanol. These solutions were stored in the dark at 2–8 °C and were found to be stable for one month at least. A series of working standard solutions of PPZ (3.06–1243.0 μg mL^{-1}) were prepared by diluting the stock standard solution with the methanol. In each sample 1 mL of LPZ was added. Standard solutions were found to be stable during the analysis time.

2.5. Calibration curve

To construct the calibration curve five replicates (10 μL) of each standard solution were injected immediately after preparation into the column and the peak area of the chromatograms was measured. Then, the mean peak area ratio of PPZ to that of the internal standard was plotted against the corresponding concentration of PPZ (3.06–1243.0 μg mL^{-1}) to obtain the calibration graph (Table 1).

2.6. Assay procedure for dosage forms

Twenty tablets containing PPZ were weighed and finely powdered. Five accurately weighed quantities of the powder equivalent to 50 mg of PPZ were transferred into 25 mL separated volumetric flasks. A 20 mL of methanol was then added to each flask and the mixture was sonicated for 10 min. Then, the volume of each mixture was adjusted to 25 mL with methanol. The sample solutions were filtered and a suitable concentration was prepared by diluting 0.5 mL of the filtrates with

Table 1 Calibration data for the estimation of pantoprazole by HPLC.

Parameters	Pantoprazole
Optimum concentration range (μg mL^{-1})	3.06–1243.0
Regression equation for the peak area of PPZ vs. concentration of PPZ in μg/mL, $A_{PPZ} = 0.762C_{PPZ} + 5.578$	
Correlation coefficient (r^2)	0.9999
Standard deviation of slope	0.0019
Standard deviation of intercept	0.7711
Regression equation for the ratio of peak area of PPZ to that of I.S. (LPZ) vs. concentration of PPZ in μg/mL, $R_{PPZ/LPZ} = 0.008C_{PPZ} + 0.060$	
Correlation coefficient (r^2)	0.9999
Standard deviation of slope	0.0018
Standard deviation of intercept	0.0033
Limit of quantification, LOQ (μg mL^{-1})	2.60
Limit of detection, LOD (μg mL^{-1})	0.78

methanol to 10 mL in volumetric flasks containing 1 mL of the internal standard LPZ. Finally, 10 μL of each diluted sample was injected into the column. Peak area ratios of PPZ to that of LPZ were then measured for the determination. PPZ concentrations in the samples were then calculated using peak data and standard curves.

2.7. Optimization procedure

On the basis of the optimization procedure the following factors were selected and tested in the experimental design: (A) volume percent of methanol (50–70%), (B) flow rate range (0.8–1.3 mL min^{-1}) and (C) pH of ammonium acetate solution (2.5–7.0). Factor levels are given in parenthesis. Experimental design indicates that the best conditions for separation of pantoprazole from internal standard are at mobile phase composition: (0.1 M) ammonium acetate and methanol (42:58, v/v).

2.8. Validation

The standard curve was a plot of the peak area ratios of PPZ–LPZ vs. the corresponding concentrations of PPZ in the standard curve samples. The linearity of the standard curve was evaluated using least-squares linear regression analysis. To determine recovery of RPZ at concentrations of 3.06, 10, 100, 200, 400, 800 and 1200 μg mL^{-1} and of LPZ at the concentration used in the assay (100 μg mL^{-1}) from bulk or formulations, an identical set of standards prepared in the methanol was analysed. Absolute recoveries at each concentration were measured by comparing the response of pre-treated standards with the response of standards which had not been subjected to sample pre-treatment. Intra- and inter-day coefficients of in variation of the assay were determined by the analysis of five samples at each concentration on the same day and of five samples at each concentration on three different days, respectively. The limit of quantification for this assay is defined as the lowest concentration of LPZ that can be detected.

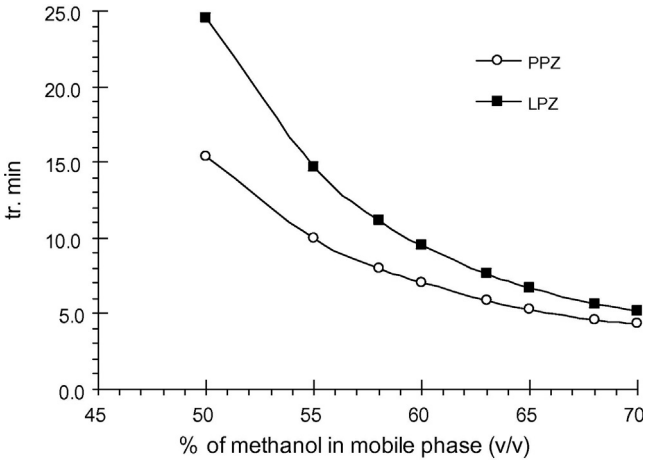

Figure 1 Plots of the retention time of PPZ and LPZ vs. methanol concentration in the mobile phase.

3. Results and discussion

3.1. Chromatography and selectivity

The goal of this study was to develop HPLC assay for the analysis of PPZ drug in pharmaceutical dosage form. Initial studies to develop HPLC assay involved the use of C_{18} and C_8 columns with various mobile phases containing acetonitrile- or methanol-aqueous ammonium acetate buffers. The C_8 column was chosen for further studies since it produced sharp and symmetrical peaks. The final selective HPLC mobile phase consisting of MeOH–NH_4OAC. The effect of composition of the mobile phase on the retention time of PPZ and the internal standard, LPZ, was investigated. Results of the effect of methanol in the mobile phase are presented in Fig. 1. An increase in the percentage of methanol decreases the retention of

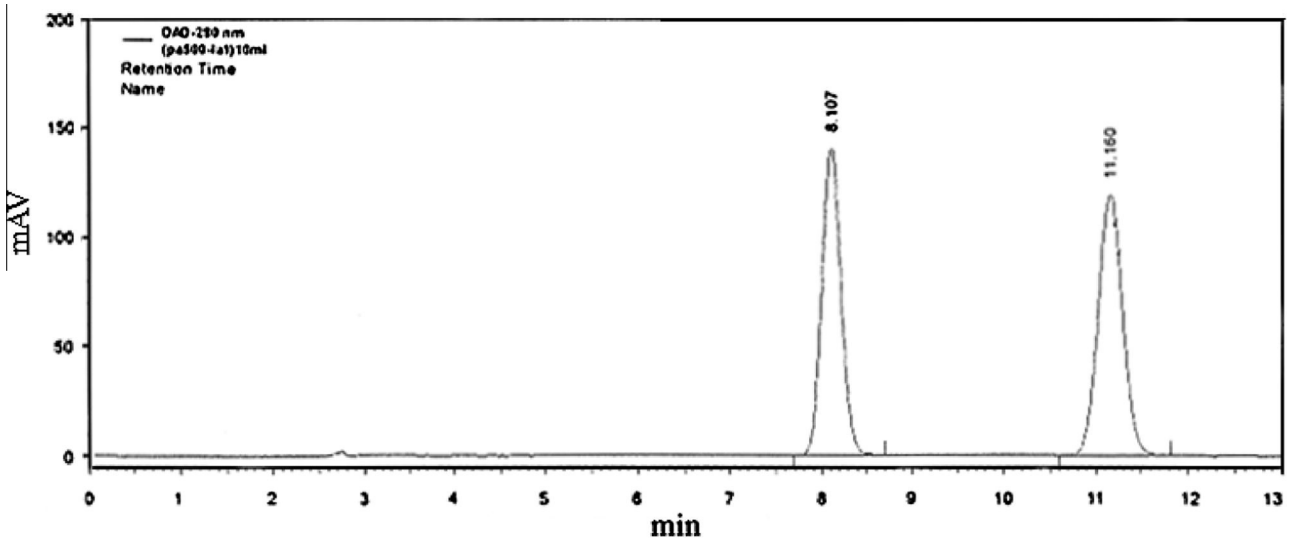

Figure 2 A typical chromatogram of a mixture of PPZ (500 μg mL^{-1}) and LPZ (100 μg mL^{-1}) at retention times 8.10 and 11.15 min, respectively. Chromatographic conditions: RP-HPLC on C8 column; mobile phase: 0.1 M ammonium acetate: methanol (42:58, v/v); flow rate 1.0 mL min^{-1} and detection at 280 nm.

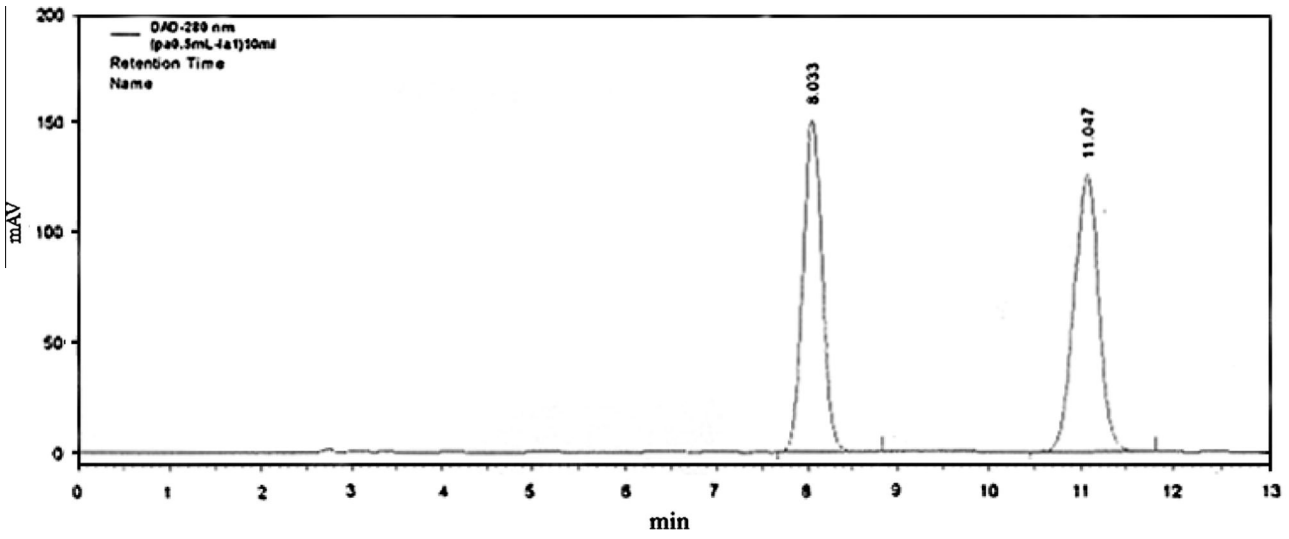

Figure 3 A typical chromatogram of a mixture of PPZ (500 μg mL^{-1}) and the internal standard, LPZ (100 μg mL^{-1}) in the mobile phase, prepared from PENTA 40 mg tablets. Chromatographic conditions: C8 column; mobile phase: 0.1 M ammonium acetate and methanol (42:58, v/v); flow rate 1.0 mL min^{-1} and detection at 280 nm.

compounds; PPZ and LPZ. Increasing methanol concentration to more than 70% RPZ peak is eluted with the solvent front, while at methanol concentration lower than 55% the elution of LPZ peak is seriously delayed. The optimum methanol concentration was found to be 58%.

The effect of pH in the chromatographic elution of both compounds was also investigated by changes in the pH values of the aqueous component of the mobile phase from 2.5 to 7.0. For all experimental concentration values, the drugs are eluted in order of PPZ and LPZ. A concentration value of 0.1 M NH_4OAC was chosen for the optimum separation of the compounds, as at this concentration the analyte peaks were well defined and resolved. The optimum wavelength for detection was at 280 nm, at which the best detector responses for all substances were obtained. The specificity of the HPLC method is illustrated in Fig. 2 where complete separation of the compounds was observed. PPZ was eluted at 8.10 min, while the internal standard LPZ was eluted at 11.15 min.

3.2. Linearity and limits of quantification and detection

Standard curve of PPZ was linear ($r^2 > 0.99$) over the concentration range 3.06–1243.0 μg mL^{-1}. Straight line for PPZ was obtained, when the area of the peaks were plotted vs. concentration (Table 1). Also, Linear relationship was obtained between the peak area ratio of PPZ to that of the internal

Table 2 Accuracy and precision of within and between run analysis for the determination of pantoprazole by HPLC.

Nominal concentration (μg mL^{-1})	Assayed concentration (μg mL^{-1})			
	Mean ± SD	RSD (%)	Recovery (%)	Relative error (%)
Intra-day (n = 14)				
3.06	3.07 ± 0.06	1.86	100.18	0.18
10.00	10.03 ± 0.14	1.41	100.31	0.31
100.00	103.95 ± 1.11	1.07	103.95	3.95
200.00	203.51 ± 1.95	0.96	101.76	1.76
400.00	402.10 ± 2.93	0.73	100.52	0.52
800.00	800.04 ± 4.64	0.58	100.01	0.01
1200.00	1202.65 ± 4.33	0.36	100.22	0.22
Inter-day (n = 14)				
3.06	3.10 ± 0.06	2.07	101.22	1.22
10.00	9.98 ± 0.16	1.56	99.78	−0.22
100.00	102.97 ± 1.07	1.04	102.97	2.97
200.00	198.14 ± 1.68	0.85	99.07	−0.93
400.00	402.54 ± 2.45	0.61	100.64	0.64
800.00	802.77 ± 4.01	0.50	101.31	1.13
1200.00	1194.33 ± 5.02	0.42	99.96	−0.04

Table 3 Determination of PPZ in pharmaceutical formulations by the proposed method and official method.

Product[a]	Pharmaceutical company (country of origin)	%Found[b] ± SD	
		Proposed method	Official method
Penta 40 mg	Alpha (Syria)	102.23 ± 1.08 t = 1.87 F = 1.12	99.44 ± 1.02 t = 1.92
Progast 40 mg	K.C. Pharma (Syria)	100.40 ± 0.78 t = 1.43 F = 1.48	100.70 ± 0.64 t = 1.75
Pantoprazol 40 mg	Amrit Medical Co. (Syria)	104.83 ± 0.37 t = 2.05 F = 1.42	103.17 ± 0.31 t = 2.25

[a] The dose is 40 mg expressed as pantoprazole sodium for all products.
[b] Five independent analyses. At 95% confidence level t-value is 2.776 and F-value is 6.26.

standard LPZ and the corresponding concentration of PPZ (3.06–1243.0 μg mL^{-1}), as shown by the equations presented in Table 1.

The minimum level at which the investigated compound can be reliably detected (limit of detection, LOD) and quantified (limit of quantitation, LOQ) were determined experimentally. LOD was expressed as the concentration of drug that generated a response to three times of the signal to-noise (S/N) ratio, and LOQ was 10 times of the S/N ratio. The LOD of PPZ attained as defined by IUPAC (Long and Winefordner, 1983), $LOD_{(k=3)} = k \times S_a/b$ (where b is the slope of the calibration curve and S_a is the standard deviation of the intercept), was found to be 0.78 μg mL^{-1}. The LOQ was also attained according to the IUPAC definition, $LOQ_{(k=10)} = k \times S_a/b$, and was found to be 2.60 μg mL^{-1}.

3.3. Accuracy and precision

The precision and accuracy of the method were evaluated by intra- (analysis of standard solutions of PPZ in replicates of five in the same day) and inter-day (analysis of standard solutions of PPZ in replicates of five on three different days from day 1 to 30 after preparation) assay variance (Table 2). The standard deviation, relative standard deviation, recovery and relative percentage error of different amounts tested were determined, as recorded in Table 2. The accuracy of the method is indicated by the excellent recovery (99.07–103.95%) and the precision is supported by the low standard deviation. Table 2 shows that the percent error of the method was always less than 3.95%; therefore, it was concluded that the procedure gives acceptable accuracy and precision for the analyte.

3.4. Application of the assay

The present method has been successfully applied for the determination of pantoprazole sodium in three different pantoprazole preparations. The resulted chromatogram has been shown in Fig. 3. Student's t-test was used for statistical analysis of the data and statistical significance was defined at the level of $P < 0.05$. The results obtained with the proposed method were compared with the official method (British Pharmacopœia, 2009) and have been shown in Table 3. Good agreement with results obtained by the official method was observed. The proposed method is simple, rapid, accurate, highly sensitive and suitable for the routine quality control without interference from the excipients and additives such as starch, glucose and lactose.

4. Conclusion

The proposed analytical method was simple, rapid, accurate, precise and inexpensive, because the lower solvent consumption along with the short analytical run time of 12 min, and hence can be used for the routine analysis of PPZ in bulk and pharmaceutical formulations. The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulations excipients in the estimation. Moreover, the present method is fast with respect to analysis time as compared to sophisticated chromatographic techniques. The method provided excellent specificity and linearity with a limit of quantification of 2.60 μg mL^{-1} and limit of detection of 0.78 μg mL^{-1}. The major advantage of this method is the wide range of linearity.

References

British Pharmacopœia, 2009. Her Majesty's Stationery Office Ltd., London, p. 4536.

Cass, Q.B., Cassiano, N.M., Degani, A.L.G., Pedrazolli, J.J.R., 2002. Enantiomeric determination of pantoprazole in human plasma by multidimensional high-performance liquid chromatography. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 766, 153.

Cheer, S.M., Prakash, A., Faulds, D., Lamb, H.M., 2003. Pantoprazole: an update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs 63, 101.

Chung, E.W., Ho, E.N.M., Leung, D.K.K., Tang, F.P.W., Yiu, K.C.H., Wan, T.S.M., 2003. Determination of pantoprazole by adsorptive stripping voltammetry at carbon paste electrode. IL Farmaco 58, 535.

Eberle, D., Hummel, R.P., Kuhn, R., 1997. Chiral resolution of pantoprazole sodium and related sulfoxides by complex formation with bovine serum albumin in capillary electrophoresis. J. Chromatogr. A 759, 185.

Erk, N., 2003. Differential pulse anodic voltammetric determination of pantoprazole in pharmaceutical dosage forms and human plasma using glassy carbon electrode. Anal. Biochem. 323, 48.

Karljikovic-Rajic, K., Novovic, D., Marinkovic, V., Agbaba, D., 2003. First-order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities. J. Pharm. Biomed. Anal. 32, 1019.

Long, G.L., Winefordner, J.D., 1983. Limit of detection. A closer look at the IUPAC definition. Anal. Chem. 55, 712A.

Mansour, A.M., Sorour, O.M., 2001. High performance liquid chromatographic determination of pantoprazole in tablet dosage form. Chromatographia 53, S478.

Martindale the Extra Pharmacopoeia, 2005. 34th ed. Royal Pharmaceutical Society, London, p. 1283.

Moustafa, A.A.M., 2000. Spectrophotometric for the determination of lansoprazole and pantoprazole sodium sesquihydrate. J. Pharm. Biomed. Anal. 22, 45.

Radi, A., 2003. Square-wave adsorptive cathodic stripping voltammetry of pantoprazole. J. Pharm. Biomed. Anal. 33, 687.

Rahman, N., Kashif, M., 2005. Initial-rate method for the determination of pantoprazole in pharmaceutical formulations using 1-fluoro 2,4-dinitrobenzene. Pharmazie 60, 197.

Rahman, N., Bano, Z., Azmi, S.N.H., 2006. Kinetic spectrophotometric analysis of pantoprazole in commercial dosage forms. Anal. Sci. 22, 983.

Ramakrishna, N.V.S., Vishwottam, K.N., Wishu, S., Koteshwara, M., 2005. High-performance liquid chromatography method for the quantification of pantoprazole in human plasma. J. Chromatogr. B 822, 326.

Salama, F., El-Abasawy, N., Abdel-Razeq, S.A., Ismail, M.M.F., Fouad, M.M., 2003. Validation of the spectrophotometric determination of omeprazole and pantoprazole sodium via their metal chelates. J. Pharm. Biomed. Anal. 33, 411.

Storms, M.L., Stewart, J.T., 2002. Development of a reversed-phase liquid chromatographic method for the analysis of amoxicillin, metronidazole and pantoprazole in human plasma using solid-phase extraction. J. Liq. Chromatogr. Rel. Technol. 25, 2433.

Syed, A., Syeda, A., 2008. Spectrophotometric determination of certain benzimidazole proton pump inhibitors. Indian J. Pharm. Sci. 70, 507.

Tanaka, M., Yamazaki, H., 1996. Direct determination of pantoprazole enantiomers in human serum by reversed-phase high-performance liquid chromatography using a cellulose-based chiral stationary phase and column-switching system as a sample clean up procedure. Anal. Chem. 68, 1513.

Tanaka, M., Yamazaki, H., Hakushi, H., 1995. Direct HPLC separation of enantiomers of pantoprazole and other benzimidazole sulfoxides using cellulose-based chiral stationary phases in reversed-phase mode. Chirality 7, 612.

Tivesten, A., Folestad, S., Schonbacher, V., Svensson, K., 1999. Nonaqueous capillary electrophoresis for the analysis of labile pharmaceutical compounds. Chromatographia 49, S7.

Wahbi, A.M., Abdel-Razak, O., Gazy, A.A., Mahgoub, H., Moneeb, M.S., 2002. Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. J. Pharm. Biomed. Anal. 30, 1133.