INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR ESTIMATION OF PANTOPRAZOLE IN INJECTION

Patel Satish A*
Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Mehsana, Gujarat, India

Article Received on: 04/06/11 Revised on: 09/07/11 Approved for publication: 11/08/11

*Satish A. Patel, Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India.
Email: satishpatel_77@yahoo.com

ABSTRACT
A simple and sensitive high performance thin layer chromatography (HPTLC) method has been developed for the quantitative estimation of pantoprazole in its single component injection formulation (40 mg). Pantoprazole chromatographed on silica gel 60 F_{254} TLC plate using toluene: ethyl acetate: methanol: acetic acid (7:2:1:0.1 v/v/v/v) as mobile phase. Pantoprazole showed Rf value 0.40 $\pm$ 0.005 and scanned at 290 nm using a camag TLC scanner 3. The method was validated in terms of linearity (50 – 800 ng/spot), precision (intra-day variation, 1.28 to 2.40% and inter-day variation, 2.40 to 3.62%), accuracy (98.16 to 100.5%) and specificity. The limit of detection and limit of quantification for pantoprazole were found to be 8.45 ng/spot and 25.60 ng/spot, respectively. The developed method was successfully used for the assay of pantoprazole injection formulation. The method was found to be simple, sensitive, specific, accurate and precise and can be used for the routine quality control testing of pantoprazole in injection dosage form.
KEYWORDS: Pantoprazole, HPTLC, Validation, Injection

INTRODUCTION

Pantoprazole sodium (PNT) is chemically, sodium 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H – benzimida-zole[1]. Pantoprazole is a proton pump inhibitor drug that inhibits gastric acid secretion. Pantoprazole is used for short-term treatment of erosion and ulceration of the esophagus caused by gastro-esophageal reflux disease (GORD)[2]. Pantoprazole is a new H^+ K^+ ATPase inhibitor similar in potency and efficiency to omeprazole but more acid stable and less active at higher pH. It is the only proton pump inhibitor available for i.v. administration and is used to treat bleeding peptic ulcer and for prophylaxis of acute stress ulcers in particular. Pantoprazole is used in the treatment of peptic ulcer, NSAID-associated ulceration and Zollinger-Ellison syndrome[2]. Pantoprazole sodium is official in IP and USP. IP[3] and USP[4] describe HPLC methods for its estimation. The literature survey reveals spectrophotometry[5-11], HPLC[12-16], HPTLC[17] and LC/MS[18] methods for the determination of PNT in pharmaceutical dosage forms as well as in biological fluids. The literature survey does not reveal any spectrophotometric and chromatographic methods for the determination of PNT in injection dosage form. The present manuscript describes simple, sensitive, accurate, precise and specific HPTLC method for the estimation of PNT in injection.

MATERIALS & METHODS

Apparatus

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamp, Camag winCATS software, Hamilton syringe (100 µl), Sartorius CP224S analytical balance (Germany), Ultrasonic bath (Frontline FS-4, Mumbai, India) were used in the study.

Reagents and Materials

Pharmaceutical grade of PNT was kindly supplied as a gift sample from Astron Research Ltd, Ahmedabad, Gujarat, India. Silica Gel 60 F_{254} TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. The pharmaceutical injection formulation containing 40 mg of PNT was procured from the local pharmacy. Methanol (AR grade, Finar Chemicals Ltd, Ahmedabad, India) and toluene, ethyl acetate, acetic acid (AR grade, S.D. Fine Chemicals Ltd, Mumbai, India) were used for mobile phase preparation and as solvents.

Preparation of standard stock and sample solution

A standard solution of PNT (100 µg/ml) was prepared by accurately weighing PNT (10 mg) and transferred in 100 ml volumetric flask, dissolved in and diluted up to mark with methanol. The quantity of the injection solution equivalent to 40 mg of PNT was transferred to 100 ml volumetric flask and the volume was adjusted up to mark with methanol. The solution (2.5 ml) was further diluted to 10 ml to have concentration of PNT equivalent to 100 µg/ml.

Chromatographic conditions

The chromatographic estimations were performed using following condition; stationary phase, precoated Silica Gel 60 F_{254} aluminum sheets (10 × 10 cm) (pre-washed with methanol and dried in air); mobile phase, toluene: ethyl acetate: methanol: acetic acid (7:2:1:0.1 v/v/v/v); chamber saturation time, 30 min; temperature, 25 ± 2°, migration distance, 80 mm; wavelength of detection, 290 nm; slit dimensions, 5 × 0.45 mm; scanning speed, 10 mm/s. Following spotting parameter were used - band width, 5 mm; distance from the plate edge, 10 mm; space between two bands, 5 mm and spraying rate, 1 µl/s.

Chromatographic separation

Six microlitres of standard solution of PNT (100 µg/ml) was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 80 mm at constant temperature using mixture of toluene: ethyl acetate: methanol: acetic acid (7:2:1:0.1 v/v/v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 290 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using winCATS software incorporating the track optimization option.

Validation of the proposed method

The proposed method is validated according to the International Conference on Harmonization (ICH) guidelines[19].

Linearity (Calibration curve)

Aliquots of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 µl of standard PNT solution (100 µg/ml) were spotted on precoated TLC plate using semiautomatic spotter under nitrogen stream. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of PNT by the standard addition method. Known amounts of standard solutions of PNT was added at 50, 100 and 150 % level to prequantified sample solutions of PNT (300 ng/spot). The amount of PNT was estimated by applying obtained values to the regression line equation.

Method Precision (% Repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of PNT (600 ng/spot) without changing the parameters of the proposed method.

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentration of standard solution of PNT (100, 300 and 500 ng/spot) for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines[19].

$$LOD = 3.3 \times \sigma$$

$$LOQ = 10 \times \sigma / S$$

Where σ Standard deviation of the response

S = Slope of calibration curve

Analysis of PANTO in injection

Three microlitres of sample solution from formulation was applied separately on TLC plate, developed and scanned as described in chromatographic separation. The amount of pantoprazole present in the sample solution was determined by fitting area values of peak corresponding to pantoprazole into the equation of line representing calibration curve of pantoprazole.

RESULTS & DISCUSSION

PNT is soluble in methanol; therefore methanol was selected as solvent. Several mobile phases were tried to accomplish good separation of PNT. Using the mobile phase toluene: ethyl acetate: methanol: glacial acetic acid (7:2:1:0.1 v/v/v/v) and 10 × 10 cm silica gel 60F_{254} aluminum-backed plates, good separation was attained with retardation factor (R_f) values of 0.40 ± 0.005 for PNT (Figure 1 and 2). A wavelength of 290 nm was used for quantification of the drug. Even saturation of TLC chamber with mobile phase for 30 min assured better reproducibility and better resolution.

Linearity range for PNT was found in the concentration range of 50 to 800 ng/spot, with a correlation coefficient of 0.9967. The average linear regression equation was represented as Y = 8.1160X + 651.28, where X = concentration of PNT in ng/spot and Y = peak area. The limit of detection and limit of quantification for

pantoprazole were found to be 8.45 ng/spot and 25.60 ng/spot, respectively indicate sensitivity of the method.

The intra-day precision (%RSD) was calculated for standard PNT solutions (100, 300 and 500 ng/spot) for 3 times on the same day. The inter-day precision (%RSD) was calculated for standard PNT solutions (100, 300 and 500 ng/spot) for 3 times over a period of one week. The intra-day and inter-day variation (%RSD) were found to be in the range of 1.28 – 2.40 and 2.40 -3.62, respectively. These values indicate that the method is precise.

Precision of the instrument was checked by repeated scanning of the same spot (600 ng/spot) of PNT six times without changing position of the plate and %RSD for measurement of peak area was found to be 2.40. The %RSD for measurement of peak area ensures proper functioning of HPTLC system indicates repeatability of the proposed method. Different validation parameters for the proposed HPTLC method for determining PNT content are summarized in Table 1.

Accuracy of the method was evaluated by calculating recovery of PNT by standard addition method at 3 different levels of the calibration curve (n = 5). The % recovery was found to be 98.16 to 100.5 ensuring that the method is accurate (Table 2).

The method was found to be specific for PNT. The specificity of the method was ascertained by analyzing standard drug and the samples. The spot for PNT in the sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. None of the formulation excipients were interferes in the quantification of PNT at this Rf value.

This method was applied to determine the content of PNT in market sample of single component PNT injection. The average percentage of PNT in market sample was found to be 99.62 ± 1.56 (n = 6). The results are in agreement with the labeled value of PNT in injection dosage form (Table 3). The results indicate that the proposed HPTLC method was found to be simple, sensitive, specific, precise and accurate for the estimation of PNT in injection formulations.

CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of PNT. The method can be routinty used for the analysis of the PNT in injection dosage form.

ACKNOWLEDGEMENT

The author is thankful to Astron Research Ltd, Ahmedabad, Gujarat, India for providing the gift sample of PNT and S. K. Patel College of Pharmaceutical Education and Research, Ganpat University for providing all the facilities to carry out the research work.

REFERENCES

1. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station: 2006. p. 1209.
2. Sean C Sweetman In., Martindale: The Complete Drug Reference, 35th ed, Pharmaceutical Press, London, UK, 2007, p. 1585.
3. Indian Pharmacopoeia, 6th ed, Vol. 3, New Delhi: Controller of Publication. Govt of India; 2010. p. 1856.
4. The United States Pharmacopoeia, USP 32, NF 27, Vol. 3, The United States Pharmacopoeial Convention, Inc., Rockville, M. D. 2009, p. 3200.
5. Karljikovic-Rajic K, Novovic D, Marinkovic V, Agbaba D. First-order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities. J Pharm Biomed Ana 2003; 32:1019-1027.
6. Wahbi Abdel-Aziz M, Abdel-Razak O, Gazy AA, Mahgoub H, Moneeb MS. Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. J Pharm Biomed Ana 2002;30(4):1133-1142.
7. Moustafa AAM. Spectrophotometric methods for the determination of lansoprazole and pantoprazole sodium sesquihydrate. J Pharm Biomed Anal 2000; 22:45-48.
8. Salama F, El-Abasawy N, Razeq SAA, Ismail M F, Fouad MM. spectrophotometric determination of omeprazole and pantoprazole sodium via chelates with iron, chromium, and cobalt, Bull. Fac Pharm 2003; 41:185-196.
9. Zenita Devi O, Basavaiah K, Vinay KB. Sensitive and selective spectrophotometricdetermination of pantoprazole sodium in pharmaceuticals using permanganate. Chem Ind Chem Eng 2010;16(1):97-102.
10. Basavaiah K, Anil kumar, UR, Kalsang T. Spectrophotometric determination of pantoprazole sodium in pharmaceuticals uising N-bromosuccinimide, methyl orange and indigo carmine as reagents. Iran J Chem Chem Eng 2009; 28(1):31-36.
11. Anil Kumar UR, Basavaiah K. Sensitive and validated spectrophotometric methods for the determination of pantoprazole sodium in pharmaceuticals using n-bromosuccinimide based on redox and complexation reactions. Bull Chem Soc 2008;22(1):135-141.
12. Ding G, Tang A, Wang J, Cong R, Bao J. Direct enantiomeric separation of pantoprazole sodium by high performance liquid chromatography on teicoplanin-bonded chiral stationary phase. Se Pu 2006;24(1):46-48.
13. Ding G, Cong R, Wang J. Direct enantiomeric separation of pantoprazole sodium by high performance liquid chromatography. Se Pu 2004;22(3):241-243.
14. Cass QB, Degani ALG, Cassiano NM, Pedrazolli J. Enantiomeric determination of pantoprazole in human plasma by multidimensional high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2002;766(1):153-60.
15. Zeinab AES, Afaf OM, Mohamed GEB, Mohamed FET. Reversed-phase high performance liquid chromatographic method for the determination of lansoprazole, omeprazole and

pantoprazole sodium sesquihydrate in presence of their acid-induced degradation products. Chem Pharm Bull 2006;54:814-818.
16. Patel BH, Suhagia BN, Patel MM, Patel JR. Determination of pantoprazole, rabeprazole, esomeprazole, domperidone and itopride in pharmaceutical products by reversed phase liquid chromatography using single mobile phase. Chromatogr 2007;65(11-12):743-748.
17. Danica A, Dragana N, Katarina KR, Valentina M. Densitometric determination of omeprazole, pantoprazole, and their impurities in pharmaceuticals. J Planner Chromatogr 2004;17(3):169-172.
18. Bhaskara BL, Anil Kumar UR, Basavaiah K. Sensitive liquid chromatography–tandem mass spectrometry method for the determination of pantoprazole sodium in human urine. Arabian J Chem 2011;4(2):163-168.
19. The International conference on harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology:2005

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED HPTLC METHOD

Parameters	Results
Linearity range (ng/spot)	50 - 800
Slope	8.1160
Intercept	651.27
Correlation co-efficient (r^2)	0.9967
Precision (% RSD) Intra-day (n = 3) Inter-day (n = 3)	 1.28 – 2.40 2.40 – 3.62
Repeatability of peak area (% RSD) (n = 6)	2.40
Accuracy (%Recovery) (n = 5)	98.16 – 100.5
Limit of detection (LOD) (ng/spot)	8.45
Limit of quantification (LOQ) (ng/spot)	25.60
Specificity	Specific

n is number of determination and RSD is relative standard deviation.

TABLE 2: RECOVERY DATA FOR THE PROPOSED METHOD

Drug	Level	Amount of sample taken (ng/spot)	Amount of standard spiked (%)	Mean % Recovery ± S.D. (n = 5)
PNT	I	300	50 %	98.16 ± 1.08
	II	300	100 %	100.5 ± 1.84
	III	300	150 %	99.14 ± 1.65

n is number of determination and S.D. is standard deviation.

TABLE 3: ANALYSIS OF MARKETED INJECTION FORMULATION OF PNT BY PROPOSED HPTLC METHOD (n = 6)

Sample No.	Label claim (mg)	Amount found (mg)	% Label claim
1	40	40.70	101.85
2	40	39.97	99.93
3	40	39.29	98.24
4	40	40.30	99.11
5	40	39.64	102.83
6	40	39.10	97.75
Mean		39.83	99.62
S.D.		0.61	1.56

n is number of determination and S.D. is standard deviation.

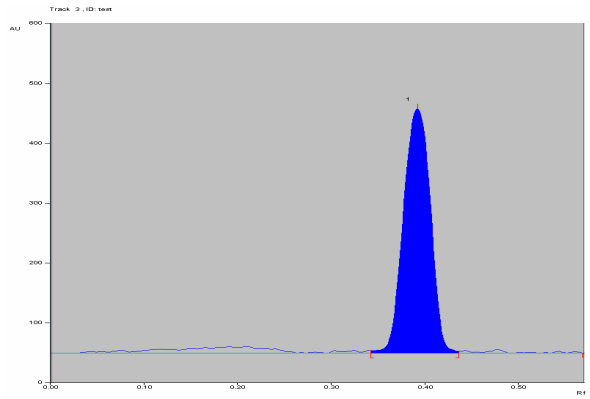

Figure 1: HPTLC chromatogram of PNT with corresponding Rf value at 290 nm

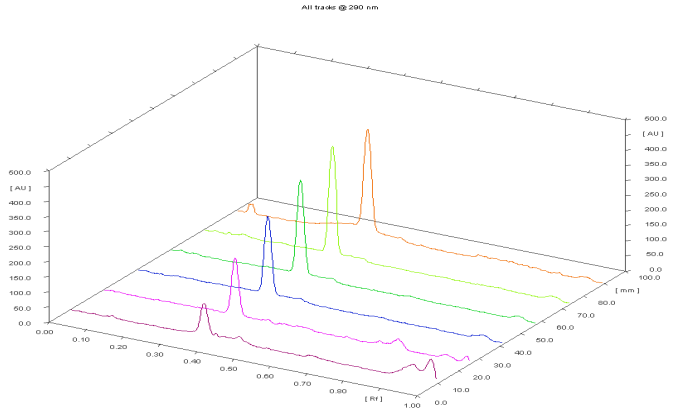

Figure 2: 3-D Chromatogram showing peaks of PNT in different concentrations at 290 nm

Source of support: Nil, Conflict of interest: None Declared