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RP-HPLC method for the determination of Tenatoprazole in pharmaceutical formulations

M. Sugumaran*, M. Poornima, M. Yogesh Kumar and Sree Ramarajasekhar

Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur, T.N.

ABSTRACT

A reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the estimation of Tenatoprazole in bulk drug and pharmaceutical dosage form. The quantification was carried out using C_{18} column and mobile phase consisting of 10mM phosphate buffer at p^H 2.4: acetonitrile (60:40 v/v), at flow rate of 1 mL/min. The separation was performed at ambient temperature. Eluents were monitored by UV detector set at 307 nm. The method was statistically validated for the linearity, precision, accuracy, LOD and LOQ. The linearity was found to be in the range of 1-6 µg/mL. The proposed method was found to be simple, precise, accurate, rapid and reproducible for the estimation of Tenatoprazole in bulk drug and tablets.

Key words: Tenatoprazole , RP-HPLC and UV-detector.

INTRODUCTION

Tenatoprazole (TPZ) is chemically, 3-methoxy-8- [(4-methoxy-3,5-dimethyl-pyridin-2 yl) methyl sulfinyl] 2,7,9-triazabicyclo [4.3.0] nona-2,4,8,10-tetraene. (Figure 1).It is a prodrug of the proton pump inhibitor (PPI) class, which is converted to the active sulfenamide or sulfenic acid by acid in the secretory canaliculus of the stimulated parietal cell of the stomach. This active species binds to luminally accessible cysteine of the gastric $H^+ K^+$ -ATP ase resulting in disulfide formation and acid secretion inhibition [1]. Literature review revealed that only HPLC methods are reported for the estimation of TPZ in rat and dog plasma and stability-indicating thin-layer chromatographic and chiral separation LC method for pharmaceutical dosage forms [2-5]. The objective of this study was to develop a simple, rapid, economic and sensitive RP-HPLC method for the analysis of TPZ in its tablet formulation using the most commonly employed RP-C_{18} column with UV-detection.

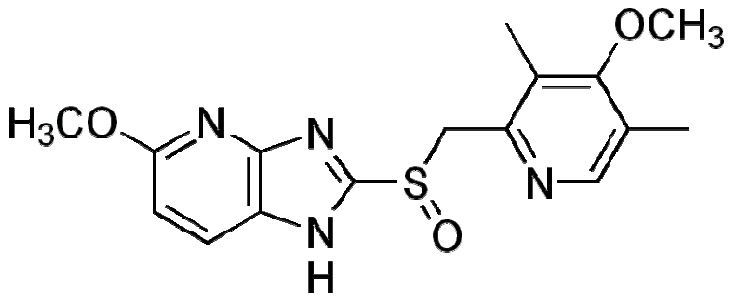


Figure 1 structural formula of tenatoprazole

MATERIALS AND METHODS

The pure drug sample of TPZ was obtained as gift sample from Reddy's Laboratory, Hyderabad. Allegro (enteric coated tablets) containing 40 mg of TPZ was purchased from SIDEM Pharmacy, U.K. Qualigens fine chemicals, Mumbai, supplied HPLC grade acetonitrile and water, sodium dihydrogen orthophosphate AR grade and Phosphoric acid AR grade. An isocratic high pressure liquid chromatograph (Schimadzu HPLC class VP series) with LC- 10 ATVP pump, variable wavelength programmable UV /Visible detector SPD-10 AVP system and operating software winchrome was used. The chromatography column used was a reverse phase phenomenax C_{18} column (250mm ×4.6 mm i.d, particle size 5µ). A mixture of 10mM phosphate buffer and acetonitrile (adjusted to pH 2.4 using ortho phosphoric acid) in the ratio of 60:40 v/v was used as mobile phase and was filtered before use through 0.45µ membrane filter. The flow rate of mobile phase was maintained at 1.0 ml/ min. Detection was carried out at 307nm at ambient temperature.

Preparation of 10 mM phosphate buffer pH 2.4

1.3609 g of potassium dihydrogen orthophosphate was dissolved in sufficient quantity of distilled water and made to produce 1000 mL and adjusted to pH 2.4 with ortho phosphoric acid [6].

Preparation of standard solution

25 mg of TPZ was dissolved in a minimum quantity of acetonitrile and the total volume was brought to 50 mL with acetonitrile, further dilutions were made with acetonitrile to get 10 µg/mL .

Preparation of calibration curve

Aliquots of standard solution of TPZ 1 to 6 mL was transferred into 10 mL volumetric flask and made up to the mark to get the concentration range from 1 to 6 µg/mL and the calibration curve was plotted between concentration and peak area.

Quantification of Tenatoprazole in formulation

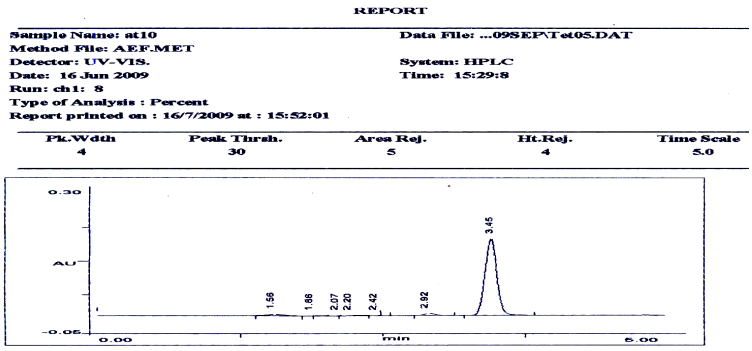
Twenty tablets containing 40mg of TPZ were accurately weighed and finely powdered. The powdered tablet equivalent to 25 mg of TPZ was weighed and transferred into a 50 ml volumetric flask, added sufficient quantity of acetonitrile and was sonicated for few minutes and made up to the mark with acetonitrile.The solution was filtered through Whatmann filter paper No.41.From this clear solution, further dilution was made with acetonitrile to produce 10

µg/ml solution. The peak area measurements were done by injecting the sample (3µg/mL) six times and the amount of TPZ was calculated from the respective calibration curve.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drug by RP-HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The melting point of TPZ (126º C) was recorded to check the identification of the drug.

The mobile phase containing phosphate buffer p^H 2.4 and acetonitrile in the proportion 60:40v/v was selected because it was found to give a peak for TPZ with minimal tailing. With the above mentioned composition of mobile phase, sharp peak was achieved with reasonable short run time of 10 min. The criteria employed for assessing the suitability of above said solvent system were cost, time required for analysis, solvent noise, preparatory steps involved in the use of same solvent system for the extraction of the drug from formulation excipient matrix for the estimation of drug content. A 10 µg/mL solution of TPZ was prepared and scanned in the UV region. From the spectra , 307 nm was selected as an analyzing wavelength. The absorbance at λ_{max} 307 nm was stable for up to 2 h and 30 min. The retention time for TPZ was found to be 3.54 min. A typical chromatogram of test solution is shown in Fig.2.



No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.56	619.43	0.90	0.75	619	0.00	0.00
2	1.86	5339.56	0.79	0.59	5339	0.00	0.00
3	2.07	666.81	0.64	0.28	666	0.00	0.00
4	2.20	414.05	1.71	2.41	414	0.00	0.00
5	2.42	4832.09	1.54	2.08	4832	0.00	0.00
6	2.92	4624.47	1.13	1.16	4624	0.00	0.00
7	3.45	5273.79	1.08	1.08	5273	0.00	0.00

Figure 2 Typical chromatogram of the sample solution

The peak shape was symmetrical and asymmetry factor was less than 2. When the concentrations of TPZ and its respective peak areas were subjected to regression analysis [7] by least square method, a good linear relationship (r=0.9998) was observed between the concentration of TPZ and the respective peak areas in the range of 1-6 µg/ mL. The regression of TPZ was found to be Y= 578169.2x-7876.82 where Y is the peak area and X is the concentration of TPZ. The regression equation was used to estimate the amount of TPZ either in tablet formulations or in validation study. The RP-HPLC method developed in the present study has been used to quantify TPZ in tablet dosage forms. TPZ tablets were analyzed as per procedure described above and the average drug content was found to be 100.3% of the labeled amount (Table 1). The limit of

detection (LOD) and limit of quantification (LOQ) were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y- intercepts of regression lines and slope of the calibration curves were used to calculate LOD and LOQ. The LOD and LOQ values are 0.2515 and 0.7623 µg/mL respectively.

Table 1 Quantification of TPZ formulation

Labeled claim (mg/tab)	Amount found(mg)	Percentage purity obtained	Mean ± S.D	% RSD
40	40.08	100.20	100.35±1.6431	1.6046
40	40.20	100.50		
40	40.08	100.20		
40	40.20	100.50		
40	40.08	100.20		
40	40.20	100.50		

RSD: Relative standard deviation

The proposed method was validated as per the standard analytical procedures [8]. Each sample was injected six times and the retention times were same. Accuracy of the method was calculated by recovery studies (n=3) at three levels. Standard drug solution containing drug in the range 100.0, 66.66 and 33.33% of nominal concentration was added to previously analyzed test solution. Amount of drug recovered at each level was calculated. The sample recovery in the formulation (Table 2) was in good agreement with the label claim.

Table 2 Recovery studies of TPZ formulation

Percentage	Amount present (µg/ml)	Amount Added (µg/ml)	Total Estimated* (µg/ml)	Amount recovered * (µg/ml)	% recovery	Mean ± S.D	% RSD
100.00	3.15	3	6.11	2.96	98.60	99.20± 0.7211	0.7269
66.66	3.12	2	5.12	2.00	100.00		
33.33	3.06	1	4.05	0.99	99.00		

** Mean of three observations*

Table 3 System suitability parameters of TPZ

Parameter	Value
Retention Time	3.45
Tailing factor	1.08
Capacity factor	1.54
Asymmetric factor	1.08
No of theoretical plates	5273

High percentage recovery and low % RSD showed that the method was free from interference of the commonly used excipients in the formulation. System suitability parameters of the proposed method for TPZ are given in Table 3. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

CONCLUSION

The procedure described here is simple, rapid, sensitive, selective and cost effective. It is evident from the results that the recommended procedure is well suited for assay and evaluation of drug, in dosage forms. It can be applied for direct determination of TPZ in drug control laboratories.

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