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Development and Validation of RP - HPLC Method for the estimation of Tenatoprazole in Bulk and Tablet Dosage Form

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Abstract: A simple, precise, rapid, and reproducible RP -HPLC method was developed and validated for the determination of Tenatoprazole in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a PhenomenaxLunaC_{18} (ODS) column (150 X 4.6 mm i.d., particle size 5µ).The mobile phase consisted of Phosphate buffer at pH 2.5: Acetonitrile in the ratio 55: 45 v/v. An isocratic elution at a flow rate of 1 ml/ min at ambient temperature. The detection was carried out at 314nm using Shimadzu UV-Visible detector SpD-10AVP. The calibration curve was linear in the concentration range of 2–12µg/ ml (r2= 0.9999).The limit of detection and the limit of quantification were found to be 0.2515 µg/ml and 0.6623 µg/ml respectively. The amount of Tenatoprazole present in the formulation (Allegro) was found to be 99.95. The method was validated statistically using the SD, %RSD and SE and the values are found to be within the limits and the recovery studies were performed and the percentage recoveries was found to be 99.55± 0.7211 %. So, the proposed method was found to be simple, specific, linear, and rugged. Hence it can be used for applied for routine analysis of Tenatoprazole in the Pharmaceutical formulations.

Key words: Tenatoprazole**,** RP-HPLC, UV detection, Isocratic Elution, Development and validation of method, bulk drug and pharmaceutical formulation.

INTRODUCTION

Tenatoprazole (TPZ) a new drug and it is used as Anti Ulcer agent[1-3] which is chemically is 3-methoxy-8-[(4-methoxy-3,5-dimethyl-pyridin-2yl)methyl sulfinyl] 2,7,9-triazabicyclo [4.3.0] nona-2,4,8,10-tetraene(Fig.1).The review of literature revealed that no methods were reported for the estimation of Tenatoprazole in bulk and tablet dosage form. TPZ is not official in any pharmacopoeia.

The mechanism action[4-6] of Tenatoprazole is a prodrug of the proton pump inhibitor (PPI) class, which is converted to the active sulphonamide or sulfenic acid by acid in the secretary canaliculus of the stimulated parietal cell of the stomach. This active species binds to luminally accessible cysteines of the gastric H^+ K^+ -ATPase resulting in disulfide formation and acid secretion inhibition. Tenatoprazole binds at the catalytic subunit of the gastric acid pump with a

stoichiometry of 2.6 nmol mg^{-1} of the enzyme in vitro. In vivo, maximum binding of Tenatoprazole was 2.9 nmol mg^{-1} of the enzyme at 2 h after IV administration. The binding sites of tenatoprazole were in the TM5/6 region at Cys813 and Cys822 as shown by tryptic and thermolysin digestion of the ATPase labelled by Tenatoprazole. Decay of tenatoprazole binding on the gastric H^+K^+ -ATPase consisted of two components. One was relatively fast, with a half-life 3.9 h due to reversal of binding at cysteine 813, and the other was a plateau phase corresponding to ATPase turnover reflecting binding at cysteine 822 that also results in sustained inhibition in the presence of reducing agents in vitro.

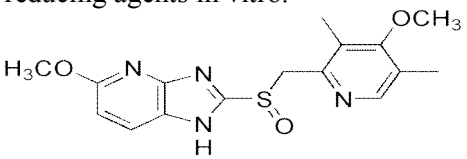

Fig.1: Chemical Structure of Tenatoprazole

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC[7-13] method with UV detection for the determination of TPZ in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 5 min) TPZ, good precision (R.S.D.less than 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH)[14-15] for the determination of TPZ in bulk and in tablet dosage form.

EXPERIMENTAL:

Chemicals and reagents

HPLC grade Acetonitrile and water was purchased from Loba fine Chemicals (Mumbai, India). Tenatoprazole standard sample was provided by Dr.Reddy's Laboratories (Hyderabad, India). Allegro (Enteric coated tablets) commercial formulation (SIDEM Pharmaceuticals (U.K) ® was procured from local market. The tablet dosage forms containing obtained was 40mg of TPZ for oral administration. The molecular weight is 346.40 for TPZ.

Instrumentation and analytical conditions:

The HPLC system (Shimadzu, Japan) consisted of a pump (LC-10 ATVP series pump) equipped with a Rheodyne model -7161 injection valve with a 20µl loop (Rheodyne Inc.,Cotati, CA, USA), an UV-visible detector (type SPD 10 AVP) set at 314 nm. TheAnalytical column,a Phenomenax $LunaC_{18}$(150mm×4.6mmi.d., 5µ particle size) was operated at ambient temperature (20 ± 1°c).Isocratic elution with Acetonitrile: Phosphate buffer (45:55% v/v pH2.5) was used at a flow rate of 1ml/ min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use (Soltec, Soluzioni tecnologiche, Luglio, Italy). The UV spectrum of TPZ for selecting the working wavelength of detection was taken using a Shimadzu UV-1700, UV -Visible spectrophotometer (Shimadzu, Kyoto, Japan).

Stock and working standard solutions:

Stock standard solution of 1000µg/ ml of TPZ was prepared freshly by accurately weighing 25mg of TPZ into 25ml volumetric flask. Dissolved and made up to the volume with Phosphate buffer (pH 2.5). The solution was diluted by pipetting 1ml into 25ml volumetric flask to obtain 40 µg/ ml solution.

The solution was further diluted with mobile phase in 10ml volumetric flask to obtain six working standards in the concentration range 2-12 µg/ ml of TPZ. All the solutions were prepared in triplicates. Before being subjected to analysis, all the working standard solutions were filtered through 13mm membrane syringe filter (Pore size 0.2 µm).

Before injecting solutions, the column was equilibrated for at least 60 min with the mobile phase flowing through the system. The calibration curve was plotted with the six concentrations of the 2- 12 µg/ ml working standard solutions. Chromatogram was recorded thrice for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of sample preparation:

The contents of twenty commercial tablets (labeled concentration 40 mg of TPZ) were weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 25 mg of TPZ was quantitatively transfer into a 25 ml volumetric flask with about 20 ml of phosphate buffer pH 2.5. The solution was sonicated for 10 min, brought to the volume with phosphate buffer, mixed well and filtered through 13mm membrane syringe filter (pore size 0.2 µm). 1 ml filtered test solution was transferred into 25 ml volumetric flask and made up to the volume with mobile phase (40 µg/ ml). 1.5 ml aliquot was transferred into a 10 ml volumetric flask. The

theoretical TPZ concentration after dilution was 6 µg/ ml (100% of TPZ). An aliquot of this solution was filtered through a 13mm membrane syringe filter (Pore size 0.2 µm) prior to the injection into the HPLC system. Peak area of Tenatoprazole was measured for the determinations.

Validation procedure:

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, short term stability and system suitability.

Standard plots were constructed with six concentrations in the range of 2 - 12 µg/ ml prepared in triplicates to test linearity. The peak area of TPZ was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared TPZ test solution in the same equipment at a concentration of 100% (6µg/ ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of TPZ was determined and precision was reported as % R.S.D. Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analyzing samples of TPZ at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of TPZ recovered in the samples.

Sample solution short term stability was tested at ambient temperature (20 ± 1°C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re injected after 24 and 48 hrs at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION:

Screening and optimization

Selection of the detection wavelength

The UV spectra of Tenatoprazole in 55:45 v/v mixtures of phosphate buffer and Acetonitrile in the region between 200 and 400 nm are shown in Fig 2. It shows that at 314 nm, TPZ have maximum absorbance. Hence λ max of Tenatoprazole in mobile phase was selected as an optimum detection wavelength for the quantification of TPZ.

Optimization of the chromatographic conditions:

Proper selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. The drug TPZ is non polar. Non polar compounds preferably analyzed by reverse phase columns. Among C_8 and C_{18}, C_{18} column was selected. Non polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of Phosphate buffer and Acetonitrle was selected as mobile phase and the effect of composition of mobile phase on the retention time of TPZ was thoroughly investigated. The concentration of acetonitrile (30-45%v/v) and water (40-55%v/v) were optimized to give symmetric peak with short run time (Fig 3).A short run time and the stability of peak asymmetry were observed in the ratio of 55:45 % v/v of phosphate buffer and acetonitrile. It was found to be the optimum mobile phase concentration.

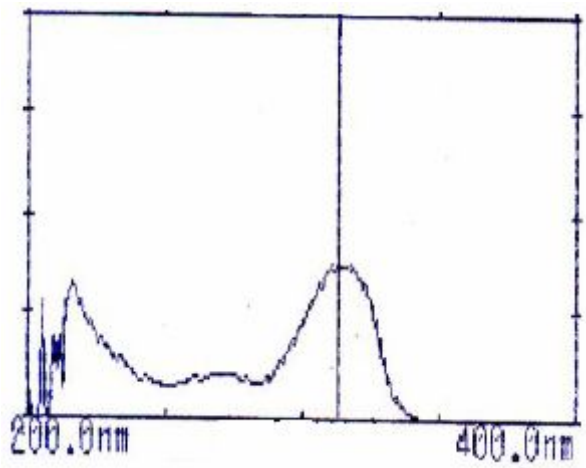

Fig.2. Absorption Spectrum of Tenatoprazole.

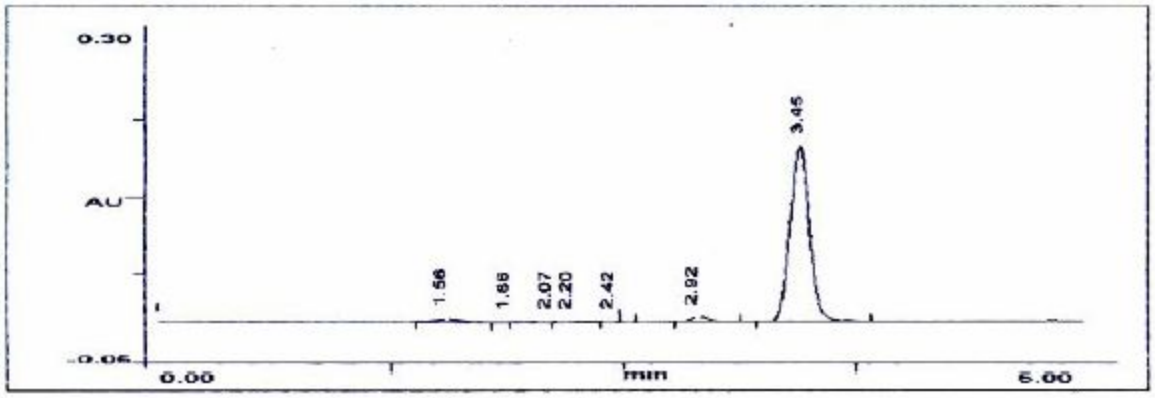

Fig.3: Typical chromatogram obtained from the analysis of Tenatoprazole standard solution. Retention time of Tenatoprazole was 3.45 min.

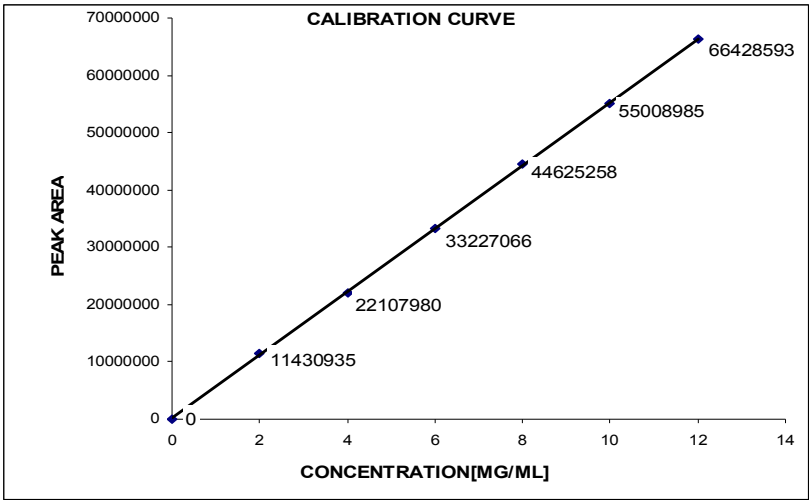

Fig.4: Calibration curve of Tenatoprazole.

Validation of method:

Linearity

Six point's calibration graphs were constructed covering a concentration range 2-12 µg/ ml (Three independent determinations were performed at each concentration. Linear relationships between the of peak area signal of TPZ the corresponding drug concentration was observed as shown in Fig.4. The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.9999.The statistical analysis of calibration is shown in Table No.1.

Precision:

The validated method was applied for the assay of commercial tablets containing 40 mg of TPZ (Allegro). Sample was analyzed in for six times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, was found to be 99.95 ± 1.02 for Allegro showing that the content of TPZ in tablet formulations confirmed to the content requirements (95 - 105 %) of the label claim. Low values of standard deviation denoted very good repepitabilty of the measurement.

Thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day and on three consecutive days (n=3) indicated a R.S.D. of 0.0455 and 0.0395% respectively. Both values were far below to 2%, the limit percentage indicated a good method precision. The results of analysis were shown in Table No.2& 3.

Table No.1: Statistical analysis of calibration curves in the HPLC determination of Tenatoprazole (n=6)

Parameters	Values
λ_{max}(nm)	314 nm
Linearity range	2-12
Correlation coefficient (r)	0.9999
Regression equation (y=mx+c)	Y=382587.9X-16639.53
Slope(m)	382587.9
Intercept(c)	16639.53
LOD (µg/ml)	0.2515722
LOQ (µg/ml)	0.6623
Standard error of mean	15654.53

Table No.2: Intraday and interday precision of the method

Amount Found (Percentage Obtained)		% RSD	
Intraday*	**Interday***	**Intraday***	**Interday***
99.35	100.38	0.0455%	0.0395%

Table No.3: Repeatability of Tenatoprazole

S. No	Labelled claim (mg/tab)*	Amount found(mg)*	% purity obtained*	Average (%)	S.D.	% RSD	S.E
1.	40	39.96	99.96	99.95	1.02	1.656	0.623
2.	40	40.01	100.01				
3.	40	40.36	100.23				
4.	40	39.63	99.65				
5.	40	39.99	99.23				
6.	40	41.56	101.96				

*Mean of SIX observations

Accuracy:

The data for accuracy were expressed in terms of percentage recoveries of TPZ in the real samples. These results are summarized in Table 4**.** The mean recovery data of ESZ in real sample were within the range of 98.60 and 101.05 %. Mean % R.S.D. was 0.7269 %, satisfying the acceptance criteria for the study. It proved that there is no interference due to excipients used in tablet formulation .Hence the accuracy of the method was conformed.

Stability:

The stability of TPZ in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 1°C). The solutions were checked in triplicate after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that TPZ is stable in standard and sample solutions for at least 2 days at ambient temperature.

Table No. 4: Accuracy study for Tenatoprazole (n =9)

S. No	Percentage	Amount present (µg/ml)	Amount Added (µg/ml)	Total Estimated * (µg/ml)	Amount recovered* (µg/ml)	% recovery	Mean ± S.D	% RSD
1	100.00	6.15	6	12.11	5.97	98.60	99.55± 0.7211	0.7269
2	66.66	6.12	4	10.12	4.01	101.05		
3	33.33	6.06	2	8.06	1.99	99.00		

* Mean of three observations

Table 5: System suitability study of Tenatoprazole

S.NO	Parameters	Tenatoprazole
1.	Tailing factor	1.08
2.	Asymmetrical factor	1.08
3.	Theoretical plates	257379
4.	Capacity factor	1.50
5.	HETP	0.0266

System suitability:

The system suitability parameter like capacity factor, asymmetric factor, tailing factor, HETP and No. of theoretical plates also calculated. It was observed that all the values are within the limits (Table No.5).

The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of TPZ in tablet formulation.

CONCLUSION:

A validated isocratic HPLC - UV method has been developed for the determination of Tenatoprazole in tablet dosage form. The proposed method is simple, rapid, accurate, precise, and specific. Its chromatographic run time of 7 min allows the analysis of a large number of samples in a short period of time. There fore, it is suitable for the routine analysis of Tenatoprazole in pharmaceutical dosage form. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as GC-MS that is complicated, costly and time consuming rather than a simple HPLC-UV method. Hence the proposed method could be useful for the national quality control laboratories in developing countries.

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