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Bio-analytical Method Development and Validation of Tenatoprazole using High Performance Liquid Chromatographic with UV Detection

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Abstract: A rapid and selective method for the extraction and reverse phase chromatographic estimation of Tenatoprazole from the human plasma is reported. Plasma samples were precipitated using acetonitrile and chromatographed using HiQ sil C18, (4.6 µm x 250 mm) column. Detection was accomplished using UV-VIS detector at 307 nm. The run time was 6.1 ± 0.21 minutes and the assay was liner from 0.1 to 5 µg/ml. The precision of the assay calculated as %RSD for intra-day and inter-day variability was less than 10% at concentration studied. Tenatoprazole was stable in plasma at -20 °C when stored for 30 days. The proposed method can be used for estimation of Tenatoprazole from human plasma sample for bioequivalence studies.
Keywords: Tenatoprazole, HPLC-UV, Plasma.

INTRODUCTION

Tenatoprazole (Fig 1) is imidazopyridine derivative (5-Methoxy-2- [(4-methoxy-3, 5-dimethyl-2-pyridyl-methyl) sulfinyl] -1H-imidazo [4,5-b] pyridine) a new proton pump inhibitor developed by Mitsubishi Pharma Corporation (Japan)[1], The literature survey shows very limited methods are published. Domagala et al[2] reported estimation of Teanatoprazole and its metabolite from urine and plasma using HPLC/MS/MS. Liu P et al[3] reported estimation of tenatoprazole in dog plasma by liquid-liquid extraction using HPLC UV method using 10 mm phosphate buffer (pH4.7)-acetonitrile (70:30, v/v).

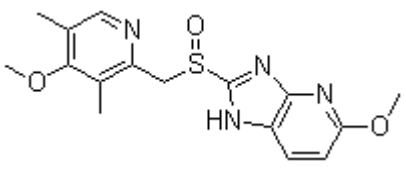


Figure 1:**Chemical structure of Tenatoprazole**

The internal standard used was pentoprazole. The resolution was achieved at 7.1 and 12.3 min, respectively. Guan J et al[4] reported enantioselective pharmacokinetics studied in Wistar rats. The method reported is by one-step extraction using hexane-dichloromethane-isopropanol (20:10:1, v/v/v) as extract solvent. Nirogi R et al[5] reported HPLC method with UV detection in rat plasma using single-step liquid-liquid extraction, the analyte and internal standard. All the method reported in the literature is based on liquid-liquid extraction. As sample preparation is most laborious step in the analysis of huge pharmacokinetic studies, an attempt was made to developed sample preparation by protein precipitation. The method is very simple meeting all the validation requirements and can be used in large scale plasma analysis of bioequivalence samples.

EXPERIMENTAL

Material and Methods

Tenatoprazole working standard, with certificate of analysis (COA) was obtained from Macleods Pharmaceuticals Ltd. Acetonitrile (HPLC grade) purchased from Qualigens Fine Chemicals, Mumbai and potassium dihydrogen phosphate, ortho phosphoric acid (all analytical reagent grade) were purchased from S.D Fine Chem. Ltd., Mumbai.

Preparation of Standard Solutions

Stock solution of tenatoprazole (1mg/ml) was freshly prepared in methanol (HPLC grade) before assay. Stock solution was further diluted with the methanol to get solutions with 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 µg/ml of tenatoprazole. Fresh frozen human plasma used in the method development was obtained from the National Plasma Fractionation Center, K.E.M. Hospital Mumbai, and was stored at -20°C until required.

Preparation of Quality Control Standards

Lowest quality control standards (LQC), median quality control standards (MQC) and highest quality control standards (HQC) were prepared by spiking drug free plasma with tenatoprazole to give solution containing 0.1, 2 and 5 µg/ml respectively. They were stored at –20 °C till analysed.

Chromatographic Conditions

An HPLC method was developed and validated at Bombay College of Pharmacy for analysis of Tenatoprazole in plasma samples with UV detection. The chromatographic system consisted of a model PU 980 Intelligent HPLC Pump and Variable Wavelength programmable UV-VIS detector (JASCO UV 975). Separation was obtained on reverse phase column HiQ sil C18, 4.6 µm x 250 mm. The injection was through 20 µl loop (Model 7125, Manual injector). The mobile phase was filtered through a nylon membrane filter (0.4520 µm, PALL) and degassed by an ultrasonic apparatus. The estimation wavelength was set at 307nm. Data processing was handled by Jasco-Borwin™ (Version 1.50 Build 15) and Chromatography Software was Hercule 2000 chromatography interface Star 800 interface module Interface (Version 2.0), Japan.

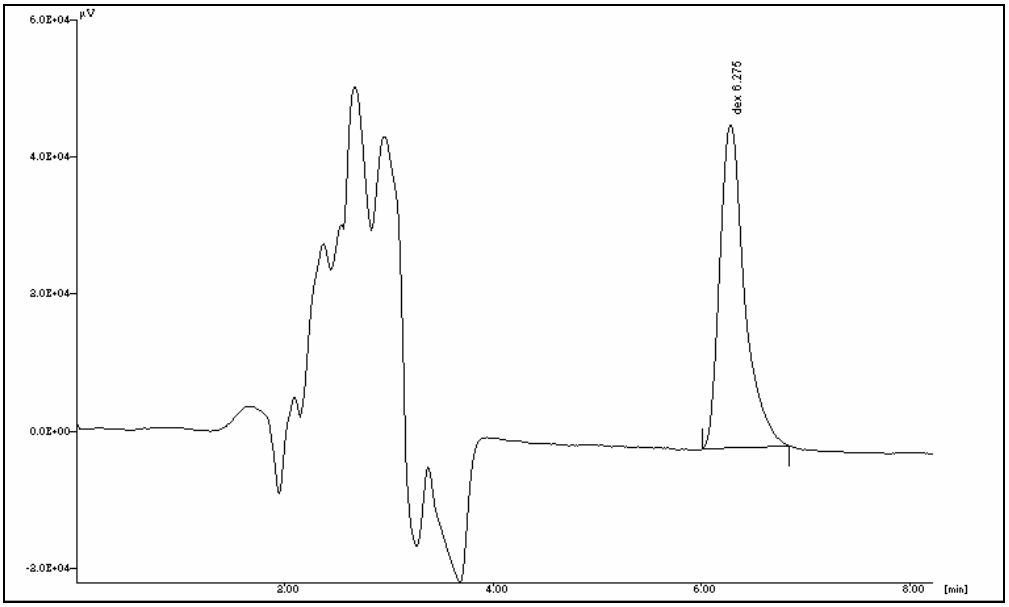
Sample Preparation for HPLC injection

To 1ml drug free plasma 1ml of acetonitrile was added and vortexed for 30 sec. The solution was centrifuged at 9000 rpm (micro centrifuge). 20 µl of supernatant was injected on the column.

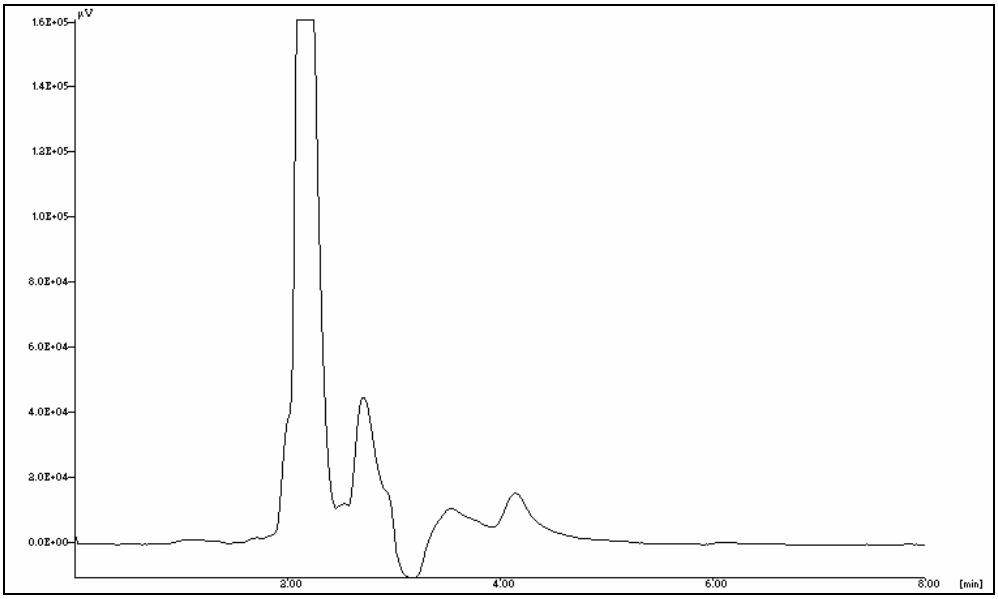
VALIDATION[6]

The selectivity of the method was checked for interference from plasma. The standard curve consisting of seven points ranging from 0.1 to 5 µg/ml was developed. Quality control samples i.e. LQC (0.1µg/ml), MQC (2 µg/ml) and HQC (5 µg/ml) were used to determine the intra and inter-day precision and accuracy of the assay. Peak area ratios of tenatoprazole was fit to linear equation (y= 324.71 X + 16487) and drug concentration in control samples along with the same day standard curve samples were calculated using this equation. For all the curves the correlation coefficients ($r2$) were never lower than 0.9904.

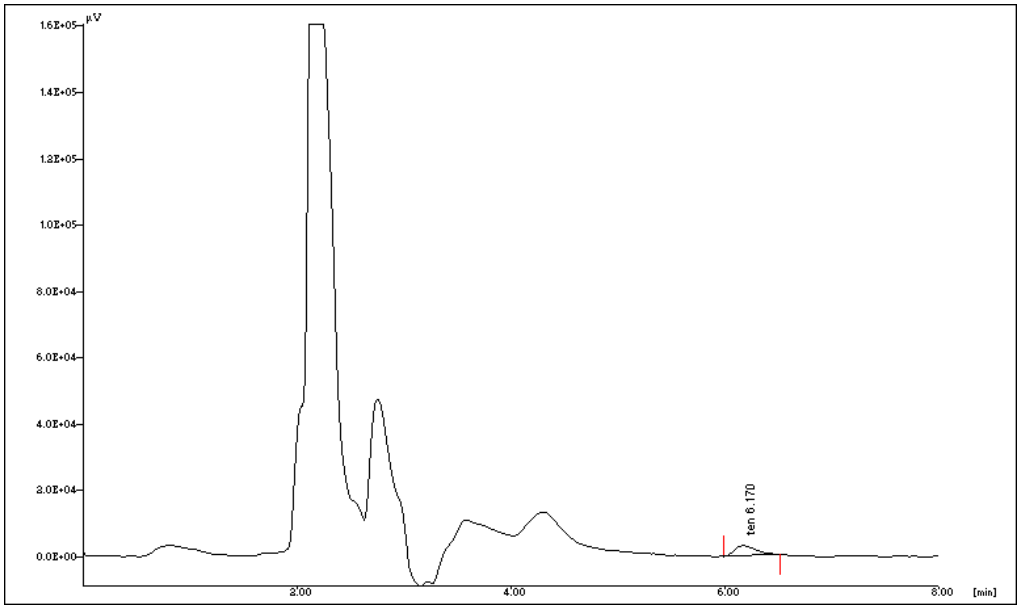
Fig. 2.



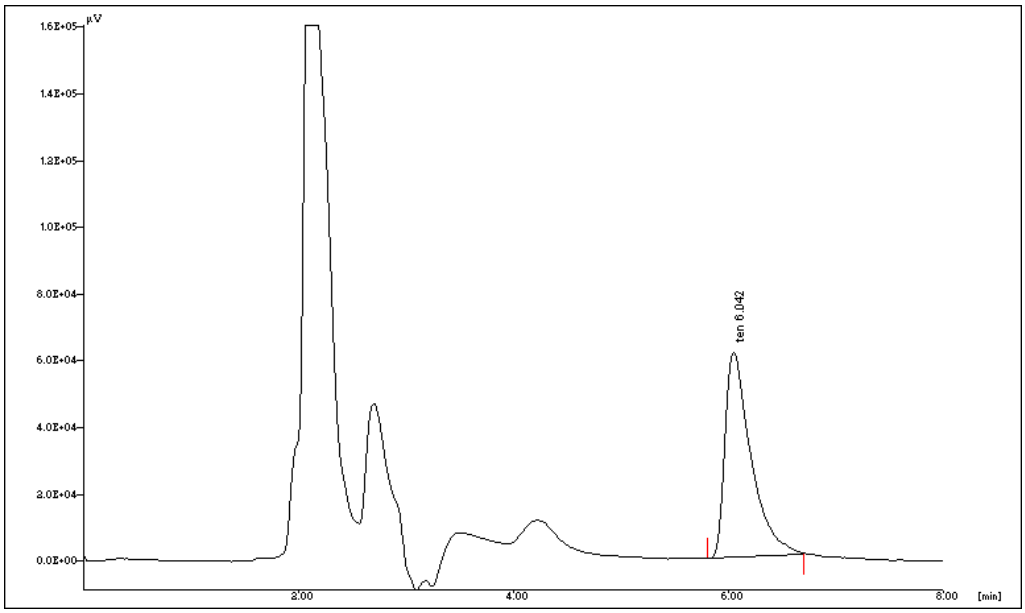
Chromatogram A



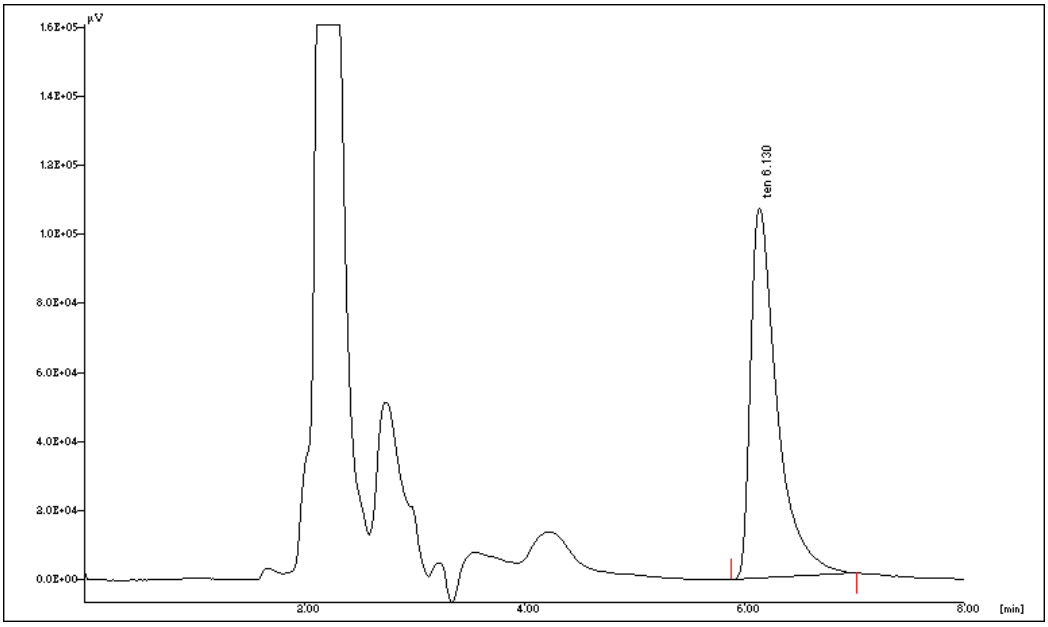
Chromatogram B



Chromatogram C



Chromatogram D



Chromatogram E

Fig.2. Representative Chromatograms: Chromatogram A: Calibrator standard chromatogram of tenatoprazole 1µg/ml (6.275 min), Chromatogram B: Selectivity chromatogram of blank human plasma, Chromatogram C, D, E: Linearity chromatogram of tenatoprazole 0.1 µg/ml (6.170 min), 3µg/ml (6.042 min) and 5 µg/ml (6.130 min) spiked in human plasma respectively

RESULT AND DISCUSSION

Method Development

Under the chromatographic conditions developed, tenatoprazole showed sharp peak. The retention time of the drug was found at 6.1 ± 0.21 minutes**.** Teanatoprazole is soluble in methanol and hence, standard solution was prepared in methanol. To develop suitable chromatographic condition, initially pure sample of tenatoprazole was used. Chromatograms of different concentrations of tenatoprazole were studied using HiQ sil C18, 4.6 µm x 250 mm at maximum absorption i.e., 307 nm.

Three different mobile phase compositions were studied during method development. Mobile phase A, B and C consisted of acetonitrile, phosphate buffer (0.01M) in 40:60 ratio at pH 3.0, acetonitrile, phosphate buffer (0.01M) in 40:60 ratio and acetonitrile, citric acid buffer (0.1M) in 20:80 ratio respectively. When chromatographed in mobile phase A, resulted poor resolution of drug, at pH 3.0 it was found that the rate of elution of drug was very slow and unsatisfactory in terms of peak shape and peak area. To make the peak shape sharp and increase the peak area citric acid was tried in mobile phase C, but when chromatographed tailing of the peak was observed and drug eluted at 10.12 min, which increases the analysis time. However chromatography with mobile phase-A, showed satisfactory result. The unadjusted pH of mobile phase was 5.0. At this pH and mobile phase ratio, good resolution of drug was obtained and retention time of drug was 6.1 min and total run time of the analysis was 7 min., which ultimately increase the speed of analysis and less solvents were consumed. The concentration of buffer to be used was also evaluated. A higher molar concentration of buffer than 0.01 M do not have significant effect on resolution pattern of the drug, thus we used the buffer concentration 0.01M, at this molar there was less salt deposition on the column and which ultimately increase the life span of column. Effect of flow rates on resolution pattern of drug was studied and found that 1ml /min flow rate was best as the retention time of the drug was 6.1 minutes.

The extraction of tenatoprazole was based on protein precipitation technique. Different protein precipitating agents such as methanol, acetonitrile, perchloric acid, and trifluroacetic acid were attempted for sample preparation. When recoveries with these agents were compared, acetonitrile was found to be best for denaturation of plasma proteins. The 1:1 ratio of plasma to acetonitrile was optimized for maximum denaturation. A less ratio of 0.8:1 resulted an increase LOQ level, but the plasma peaks become broader as all the proteins are not precipitated. Thus 1:1 ratio was optimized and used for complete denaturation. Precipitation by methanol resulted tailing and fronting of the peaks. Perchloric acid and trifluroacetic acetic

acid resulted very low analyte recovery and poor drug resolution. Since, the extraction of drug was done by denaturation of proteins, the internal standard were not required.

Specificity

Selectivity of the method described was investigated by screening six different batches of human plasma. Under the proposed assay condition tenatoprazole had retention time of 6.1 ± 0.2 min and rest of the peaks was due to plasma and do not interfere in the analysis. None of the drug free plasma samples studied in the assay yield endogenous interference at this retention time**.**

Linearity

The linearity of each calibration curve was determined by plotting the peak area ratio of tenatoprazole verses nominal concentration of tenatoprazole. For linearity study seven different concentration (0.1, 0.5, 1, 2, 3, 4, 5 µg/ml) was analysed. The peak area response was liner over the concentration range studied. Each experiment at all concentration was repeated three time on three separate days to obtain the calibration curve. The linear equation and coefficient of correlation 'r2' were found to be y= 324.71 X + 16487 and 0.9904 respectively. The limit of quantification and limit of detection were found to be 0.1 µg/ml and 0.05 µg/ml respectively.

Recovery

The mean extraction recoveries of tenatoprazole determined over the concentration of 0.1, 1 & 5 µg/ml were 87.21 ± 5.02 %, 93.06 ± 6.23 % and 95.01 ± 5.23 % respectively.

Precision

The intra-day precision for tenatoprazole was 0.08 ± 0.006, 2.1 ± 0.10, 4.8 ± 0.21 for the spiked concentration at 0.1, 2 and 5 µg/ ml and the percent coefficient of variation (%CV) was 6.9, 4.8 and 4.5 respectively. Inter day The intra-day precision for tenatoprazole was 0.09 ± 0.01, 2.3 ± 0.13, 5.1 ± 0.30 for the same spiked concentration and %CV was 11.9, 5.7 and 5.8 respectively.

Table 1 Precision of RP–HPLC method developed for the determination of tenatoprazole from human plasma

	Intra-day			Inter-day		
Spiked concentration µg/ ml	Mean Conc. (µg/ml) ± S.D (n=5)	Accuracy (%)	CV (%)	Mean Conc. (µg/ml) ± S.D (n=5)	Accuracy (%)	CV (%)
0.1	0.08 ± 0.006	83.8	6.9	0.09 ± 0.01	86.0	11.9
2	2.1 ± 0.10	107.7	4.8	2.3 ± 0.13	114.8	5.7
5	4.8 ± 0.21	96.6	4.5	5.1 ± 0.30	105.9	5.8

The table gives mean and standard deviation (±S.D.) of concentration found from the quality control samples, calculated from six samples at each of the concentrations mentioned (n = 6) on each day (intra-day) and six samples per day at each of the concentrations mentioned (n = 6) on different days (inter-day).The table also gives their coefficient of variation (%CV).

Table 2 Freez and thaw stability of tenatoprazole in human plasma

Spiked conc. (µg/ml)	Sample analysed immediately (0 hr) [mean ± S.D, µg/ml] (n=3)	CV (%)	Sample analysed at 24 hr after freeze thaw cycle [mean ± S.D, µg/ml] (n=3)	CV (%)
0.1	0.08 ± 0.006	7.5	0.09 ±0.016	12.7
2	2.30 ± 0.13	5.6	2.1 ±0.10	9.7
5	5.3 ± 0.34	6.4	5.1 ± 0.21	4.5

Table 3. Long-term stability of tenatoprazole in human plasma

Days of storage	LQC (0.1μg/ml) (n=3)	MQC (2 μg/ml) (n=3)	HQC (5 μg/ml) (n=3)
After 5 days	0.092	2.3	5.10
After 15 days	0.081	2.1	5.23
After 30 days	0.091	1.95	4.98
Mean	0.088	2.11	5.10
S.D.	0.006	0.17	0.12
% C.V.	6.91	8.29	2.45

The tables gives stability data of tenatoprazole at three different concentration i.e. lowest quality control (LQC), median quality control (MQC) , highest quality control (HQC), along with their standard deviation (± SD) and coefficient of variation (CV). Each sample was analysed in triplicate (n=3)

Stability study

Short-term and long-term stock solution stability study was evaluated, which proved no significant deviation from normal value when stored at 4 ◦C. The stability of tenatoprazole in plasma was determined by measuring concentration change in quality control samples over time. Stability was tested by subjecting the quality controls to three freeze-thaw cycles and compared with freshly prepared quality control samples. As shown in (Table 2 and 3), the mean concentration of tenatoprazole in quality control samples did not change significantly within the time period under the indicated storage conditions. Long-term stability studies results conclude that tenatoprazole is stable in plasma matrix at least for 30 days when stored at −20 ◦C.

System suitability

System suitability test was performed daily before the run of analytical batch to check detector response to the analyte. This method showed a good ruggedness, in fact little change in mobile phase ratio or normal laboratory condition of humidity, light, and air exposure temperature did not influence the retention time of tenatoprazole and internal standard.

CONCLUSION

An HPLC UV based method is developed and validated for quantification of tenatoprazole in human plasma. The sensitivity and simplicity of the method makes suitable for pharmacokinetic studies.

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