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RESEARCH ARTICLE

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REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND TENELIGLIPTIN IN TABLET FORMULATION

Abstract:
A simple, specific, precise and accurate RP-HPLC method has been developed for simultaneous estimation of Metformin (MET) and Teneligliptin (TEN) in tablet formulation. In the RP-HPLC method separation was achieved by HiQ silC-18 HS column (250 mm× 4.6 mm), with mobile phase containing Methanol: Buffer (Ammonium acetate) (70:30 v/v) and the Buffer was adjusted to pH 4 by Glacial acetic acid for. The flow rate was 1.2 mL/min and effluent was monitored at 249 nm. The retention time of MET and TEN were 2.29 min and 4.30 min respectively. The linearity for MET and TEN were in the range of 5-25 µg/mL and 5-25 µg/mL respectively. The recoveries of MET and TEN were found within the limits. The proposed method was validated as per ICH guidelines by means of different parameters likes Linearity, Precision, Accuracy, Limit of detection, Limit of quantitation, Range, Selectivity, Robustness Ruggedness, Solution stability and successfully applied to the estimation of MET and TEN in tablet dosage form.
Keywords: *Metformin, Teneligliptin, RP-HPLC, Analytical method, Validation.*

1. Introduction:

Metformin (MET) is chemically 1, 1-Dimethylbiguanide (Figure 1) with molecular formula and molecular weight $C_4H_{11}N_5$, HCl and 129.16 g/Mol respectively. It is a white to off-white crystalline compound. It is official in IP. MET is an anti-diabetic agent and used to treat type 2 diabetes mellitus in a dose ranges from 500-1000 mg. MET is recommended alone or in combination with other newer/existed antidiabetic drugs. MET is acted by decreasing the hepatic glucose production and intestinal absorption of glucose, it improves insulin sensitivity by increasing peripheral glucose uptake and utilization [1].

Teneligliptin (TEN) is chemically [(2S, 4S) -4-[4-(5-methyl-2-phenylpyrazol-3-yl) piperazin-1yl] pyrrolidin-2-yl] -(1,3-thiazolidin-3-yl) methanone;pentahydrobromide (Figure 2) With molecular formula and molecular weight $C_{22}H_{30}N_6OS$ and 426.58 g/Mol respectively. It is a white to off-white crystalline powder. TEN is a novel anti-diabetic agent and used to treat type 2 diabetes mellitus in a dose of 20 mg. TEN is suggested single drug or in combination with MET [2]. The TEN belongs to DPP-4 inhibitor antidiabetic drug, the anti-diabetic mechanism is to increase in creatine levels (GLP-1 and GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels.

The MET and with another drug in combination can be estimated by the reported HPLC chromatographic method, UV spectrophotometric method, HPTLC, SCF-TMS and UPLC methods [3-14]. The TEN can be determined by reported UV spectrophotometric method, HPLC, LC-MS/MS and UPLC methods. Literature survey also reveals that Metformin is official in I.P, B.P and Teneligliptin in I.P [15-19].

The fixed dose combination of Metformin (500 mg) and Teneligliptin (20 mg) in Glytrin Met or Zita Met Plus tablet is used for treatment of Type 2 diabetes mellitus generally in high blood glucose level. The literature survey reveals that there are assorted methods are available for estimation of Metformin and Teneligliptinin in the single formulation, but, no precise method has been reported for their simultaneous estimation in combined tablet dosage form. The current manuscript

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describes the analytical method development and validation of estimation of Metformin and Teneligliptin in Pharmaceutical dosage form using RP-HPLC. The proposed method is optimized and validated as per ICH guidelines [20, 21].

2. Materials and methods:

Standard and chemical reagents:

The standard drug Metformin and Teneligliptin were obtained as a gift sample from Ajanta Pharma Ltd., Paithan, Dist-Aurangabad, Maharashtra, India. Deionised distilled water (DIW) used was obtained from Loba Chemie Mumbai, India. HPLC grade methanol Merck Ltd., India, HPLC-grade acetonitrile, Merck Ltd., India. Buffering agent's Ammonium acetate, was procured from Fisher scientific, Mumbai. India. HPLC grade Glacial acetic acid was obtained from SD fine, Mumbai. India.

Chromatographic conditions:

Liquid chromatography was performed on JASCO Isocratic HPLC system model LC-NET II/ADC (JASCO Corporation, Japan). The system built with UV-2070 as UV-VIS detector and HiQ sil C18HS (4.6 × 250 mm, 5µm) column with a 20 µL manual sample injector. The HPLC system was equipped with Chrom-NAV software for data processing.

All compounds were eluted off the column with a mobile phase consisting of methanol: ammonium acetate buffer (70:30 v/v, PH 4 adjusted by acetic acid) at a flow rate of 1.2 mL/min in isocratic mode. The mobile phase was filtered through a 0.45 µm nylon filter and then ultrasonicated for 30 min. The injection volume was 20 µL and the eluent was detected at 249 nm, which was selected as wavelength for further analysis. The retention time of MET and TEN was around 2.29 and 4.30 min, respectively, and the total run was 10 min (Table 2).

The method was validated in accordance with the International Conference on Harmonization guidelines for validation of analytical procedures [20, 21].

Specificity and selectivity:

These parameters were determined by comparing the chromatograms of the MET and TEN standard, tablet and mobile phase as a solvent.

Linearity:

The linearity of an analytical procedure is its ability within a given range to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample [20, 21]. The linearity was tested by a linear least square method for MET and TEN in the concentration range value of 5-25 µg/mL.

Accuracy:

To check the degree of accuracy of the method, recovery studies were performed in triplicate by the standard addition method at 80%, 100% and 120%. Known amounts of standard MET and TEN were added to the pre-analyzed samples and were subjected to the proposed HPLC method.

Precision:

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The repeatability was calculated as the relative standard deviation with three replications and three different concentrations during the same day. Intermediate precision was studied by comparing the assays on three different days.

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Limit of Detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of detection can be calculated using the following equation as per ICH guidelines [20, 21].

$$LOD = 3.3 \times N/S$$

Where, N is the standard deviation of the peak area of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Limit of quantification can be calculated using the following equation as per ICH guidelines [20, 21].

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

Where, N is the standard deviation of the peak area of the drug and S is the slope of the corresponding calibration curve.

3. Experiment:

Preparation of buffer solution:

The buffer preparation was done by dissolving 1.925 gm of Ammonium acetate in 100 mL water, pH adjusted to 4 by using glacial acidic acid.

Preparation of mobile phase:

Firstly Buffer was prepared by using the 1.925 gm of Ammonium acetate in 100 mL water, pH adjusted to 4 by using Glacial acidic acid, then 20 min ultra-sonication of this buffer solution was done and Methanol: Buffer (70:30 v/v), the prepared mobile phase was degassed by ultra-sonication for about 20 min, lastly the mobile phase after degassing was filtered through 0.45µm membrane nylon filter.

Degassing of the mobile phase:

The mobile phase was prepared degassed by ultra-sonication for about 20 min, so as to avoid the disturbances caused by dissolved gases.

Filtration of mobile phase:

The mobile phase after degassing was filtered through 0.45µm membrane nylon filter to remove the smaller particles that may present in the mobile phase.

Preparation of standard stock solutions:

Metformin 10 mg and Teneligliptin 10 mg were accurately weighed on electronic balance and dissolved in 50 mL of mobile phase separately with shaking. Then the resulting solutions were sonicated and the volume was made up to 100 mL by addition of mobile phase to get the conc. 100 µg/mL. From the standard stock solution of drugs, appropriate dilutions were made with the mobile phase and the sample was filtered through 0.2 µm membrane nylon filter.

Loading of mobile phase:

Filtered and degassed mobile phase was loaded in the 500 mL reservoir. Priming was done in each freshly prepared mobile phase.

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Baseline stabilization:
The detector was turned on for an hour before the actual run in order to obtain the stable UV light. The mobile phase run was started at the desired flow rate and the run was continued until the stable baseline was obtained.

Loading of samples:
Well prepared and filtered sample of Metformin and Teneligliptin were loaded into the Rheodyne injector port using a 2 mL glass syringe and then the sample was injected.

Washing of the column:
Once the analysis of samples was finished, the column was first washed by flushing with the mobile phase for half an hour, afterwards with double distilled water and methanol in 1:1 proportion for another one hour.

Selection and Optimization of HPLC method:
After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity and accuracy.

Sample preparation:
Twenty tablets were taken, containing 500 mg Metformin and 20 mg of Teneligliptin. The tablets were crushed to fine powder and the amount of powder equivalent to 50 mg MET and 2 mg TEN was weighed accurately, and then transferred to 100 mL dried volumetric flask. Sufficient amount of mobile phase was added to dissolve the content and resulting solution was shaken for 20 min. The volume was made up to 100 mL with the mobile phase and then filtered through membrane filter and degassed in sonicator. From this solution appropriate dilutions of Metformin and Teneligliptin were made to get the final concentrations. After that sample was injected into the HPLC system to get chromatogram. The chromatogram obtained is shown in Figure 3 and the area obtained in each chromatogram of three replicates was correlated with regression equation and the amount found was calculated, which was within the limit results are recorded in Table 1 and optimal chromatographic conditions of tablet formulation tabulated in Table 2.

4. Results and Discussion:

Optimization of chromatographic conditions:
Chromatographic parameters comprising wavelength detection, mobile phase composition and proportions, pH and flow rate were prudently studied in order to identify the most appropriate chromatographic condition for analysis. The choice was based on the number of theoretical plates and best resolution in a reasonable time.

Selection of analytical wavelength:
By appropriate dilution of each standard stock solution in the mobile phase, various concentrations of MET and TEN were prepared separately. Each solution was scanned in between the range of 200-400 nm and their overlain spectrum was taken. The isobestic point was observed at 249 nm in the overlain spectra of MET and TEN. The wavelength selected for the HPLC analysis was 249 nm to which these two drugs showed significant absorbance and very good resolution.

Analytical method validation:
Linearity:
The linearity of the method was determined by constructing calibration curves. Standard solution of the MET and TEN of different concentration range (5-25 µg/mL) were used for this purpose. Each measurement was carried out in five replicates,

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which are presented in Table 3 and 4. The peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves (Figure 4 and 5) and correlation coefficients.

Accuracy:

To ensure the degree of accuracy of the method, recovery studies were carried out in triplicate by the standard addition method at 80%, 100% and 120%. Known amounts of standard MET and TEN were added to the pre-analyzed samples and were subjected to the proposed HPLC method. The solution was presented good recoveries and agreement with the standards of method validation [20, 21] as shown in Table 5 and 6.

Precision:

Repeatability of method was established by analyzing various replicates of standard Metformin and Teneligliptin solutions. All the solutions were analyzed three times, in order to record any intra-day and inter-day variation in the result. The data obtained for inter-day variations is shown in Table 7 and 8. The result obtained from intra-day variations is shown in Table 9 and 10.

Limit Detection (LOD):

The value for LOD was calculated from the following formula

$$LOD=3.3\sigma/S$$

Where, σ= Standard deviation of the response,

S= Slope of the calibration curve.

MET: 0.1424 µg/mL

TEN: 0.4944 µg/mL

Limit of Quantitation (LOQ):

The value for LOQ was calculated from the following formula

$$LOQ=10\sigma/S$$

Where, σ= Standard deviation of the response,

S= Slope of the calibration curve.

MET: 0.4315 µg/mL

TEN: 0.1498 µg/mL

Range:

The range of analysis for MET and TEN are as follows

Metformin : 5-25 µg/mL

Teneligliptin : 5-25 µg/mL

Selectivity:

After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity and accuracy. The optimized parameters were found to be suitable as well as there was no observation of any peak of the excipients or impurity other than the peak of MET and TEN during experimental work, hence the proposed method was selected for development, the results are shown in Figure 6.

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Ruggedness:

Different parameters like different laboratory condition, different source of reagents and solutions, as a result, there was no any significant change in the optimized parameters were observed.

Robustness:

The method must be robust enough to withstand slight changes and allow routine analysis of samples. Robustness of the method were determined by carrying out the analysis under conditions during which change in flow rate, change in the organic composition of the mobile phase, and change in pH were studied.

Variation of organic composition in the mobile phase, pH, and flow rate were seemed to have no significant impact on resolution, peak area, tailing factor, retention time and theoretical plate. The observations of robustness are shown in Table 11-13.

Solution stability:

Stability in solution was evaluated by the standard solution and the test preparation. The solution was stored at 25°C at ambient temperature without protection from light and tested after 12, 24, 36, and 48 hrs. The responses for the aged solution were evaluated by comparison with % assay of freshly prepared solutions. The stability study of the stored standard solution and test preparation was performed and solutions were found to be stable for up to 48 hrs. The assay values obtained after 48 hrs were statistically identical with the initial value without measurable loss which is shown in Table 14.

5. Conclusion:

The results shows that the RP-HPLC method presented here can be considered suitable for the analytical determination of MET and TEN in tablet dosage form. The proposed method is being linear in the concentration range used, high selectivity and specificity, high precision and adequate accuracy at the concentrations studied. The proposed method uses a simple mobile phase compared to the multi-component mobile phase in many reported methods. The separation and determination were achieved at an ambient temperature. Thus, it offers the advantages of low column back pressure, good peak shape, improved column efficiency, higher theoretical plates and consistent retention time. The developed method suggested non interference of formulation excipients in the estimation. Hence it can be easily and conveniently adopted for routine analysis.

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Table 1. Analysis of Tablet Formulation

Brand Name of Tablet Formulation	Drug	Label Claim	Peak area (µV/sec)	% of label claim determined	Mean %	SD	RSD
Glytrine	MET	500	82226	99.79%	99.63	0.6192	0.6197
met	PAN	20	43680	99.32%	99.37	0.9547	0.9568

n=3, Average of three replicates

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Table 2. Optimal chromatographic conditions of tablet formulation

Aspect	Description
Mobile phase	Methanol: Ammonium acetate buffer (70:30 v/v, PH 4)
HPLC Column	HiQ sil C18HS (4.6 × 250 mm, 5µm)
Flow rate	1.2 mL/min
Injection volume	20 µL
Retention time	for MET 2.29 min and for TEN 4.30 min
Runtime	10 min

Table 3. Linearity data for Metformin

Standard conc. →	**5 µg/mL**	**10 µg/mL**	**15 µg/mL**	**20 µg/mL**	**25 µg/mL**
Replicates ↓	**Peak area**				
1	50670	82246	135256	183244	224756
2	50659	82289	135256	183269	224790
3	51470	82379	135368	183356	224857
4	50745	82412	135247	183398	224849
5	50924	82567	155188	183456	224958
Mean	51472	82245	155872	183457	226890
±SD	95.15	595.21	397.60	315.38	98.94
RSD	0.0996	0.3783	0.0877	0.0866	0.0464

Table 4. Linearity data for Teneligliptin

Standard conc. →	**5 µg/mL**	**10 µg/mL**	**15 µg/mL**	**20 µg/mL**	**25 µg/mL**
Replicates ↓	**Peak area**				
1	29328	43501	66364	95008	106741
2	29378	43587	66399	95097	106795
3	29435	43643	66427	95134	106815
4	29497	43690	66487	95179	106870
5	29268	43489	66291	95213	106923
Mean	29451	43161	66317	95918	106494
±SD	58.25	87.92	99.25	941.18	602.41
RSD	0.4520	0.2070	0.2150	0.3020	0.0390

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Table 5. Recovery study of Metformin

Recovery level %	Metformin							
	Area (µV/ sec)	Amt. Taken (µg/ mL)	Amt. added (µg/ mL)	Total amount (µg/mL)	Amt. recovered (µg/mL)	% recovery	Average recovery% ± SD	RSD
80%	63973	5.0	4	9	8.95	99.48	99.99 ±0.621	0.134
	63998	5.0	4	9	8.96	99.52		
	64934	5.0	4	9	9.07	100.98		
100%	74798	5.0	5	10	9.99	99.97	100 ±0.521	0.090
	74821	5.0	5	10	10	100		
	74834	5.0	5	10	10	100.2		
120%	90641	5.0	6	11	10.81	99.58	99.01 ±0.0781	0.047
	90753	5.0	6	11	10.76	99.02		
	90821	5.0	6	11	10.68	98.43		

Table 6. Recovery study of Teneligliptin

Recovery level %	Teneligliptin							
	Area (µV/ sec)	Amt. taken (µg/mL)	Amt. added (µg/mL)	Total amount (µg/mL)	Amt. recovered (µg/mL)	% recovery	Average recovery% ± SD	RSD
80%	37195	5.0	4	9	8.96	99.60	99.73 ±0.251	0.031
	37256	5.0	4	9	8.98	99.76		
	37276	5.0	4	9	8.98	99.82		
100%	42316	5.0	5	10	9.98	99.85	100 ±0.462	0.077
	42397	5.0	5	10	10	100.04		
	42424	5.0	5	10	10.01	100.10		
120%	51000	5.0	6	11	10.96	99.73	100 ±0.0683	0.030
	51168	5.0	6	11	11	100		
	51234	5.0	6	11	11.02	100.19		

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Table 7. Inter-day variability of Metformin

Conc. (µg/mL)	Peak area (µV/sec)			Mean area (µV/sec)	± SD	RSD
	Day 1	Day 2	Day 3			
5	51479	50745	50924	51049	382.71	0.7497
10	82246	82237	82245	82243	293.29	0.3224
15	13536	13524	13518	13526	91.76	0.0678

n=3, Average of three replicates

Table 8. Inter-day variability of Teneligliptin

Conc. (µg/mL)	Peak area (µV/sec)			Mean area (µV/sec)	± SD	RSD
	Day 1	Day 2	Day 3			
5	29435	29497	29268	29400	118.44	0.4028
10	43643	43690	43489	43607	105.14	0.2411
15	66427	66487	66291	66401	100.42	0.1512

n=3, Average of three replicates

Table 9. Intra-day variability of Metformin

Conc. (µg/mL)	Peak area (µV/ sec)			Mean area (µV/sec)	± SD	RSD
	Trial 1	Trial 2	Trial 3			
5	50670	50659	51479	50936	470.28	0.9232
10	82236	82237	82248	82238	254.10	0.2796
15	135125	135256	135368	135250	121.62	0.0899

n=3, Average of three replicates

Table 10. Intra-day variability of Teneligliptin

Conc. (µg/mL)	Peak area (µV/sec)			Mean area (µV/Sec)	± SD	RSD
	Trial 1	Trial 2	Trial 3			
5	29318	29378	104754	29380	53.53	0.1822
10	43501	43587	361633	43577	71.52	0.1641
15	66364	66399	548342	66396	31.56	0.0475

n=3, Average of three replicates

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Table 11. Robustness study of system suitability parameter: Change in flow rate (mL/min)

System suitability parameter	Drug	Change in flow rate (mL/min)			RSD		
		0.98	1.2	1.3	0.98	1.2	1.3
peak area (µV/sec)	MET	82238	82236	82269	0.1530	0.1560	0.1502
	TEN	43680	43690	43620	0.8122	0.8123	1.9083
Theoretical plates	MET	5613	7855	5614	0.5081	0.7323	0.9172
	TEN	3150	3326	3339	0.3268	0.3326	0.3339
Tailing factor	MET	1.730	1.741	1.752	0.7622	0.6678	0.5267
	TEN	1.243	1.232	1.234	0.6414	0.3989	0.3078
Retention Time (Min)	MET	2.281	2.291	2.293	0.8510	0.245	0.2754
	TEN	4.335	4.305	2.348	0.4060	0.3034	0.3017

n=3, Average of three replicates

Table 12. Robustness study of system suitability parameter: Change in O.Cof M.P Ratio

System suitability parameter	Drug	Change in O.C. of M.P. Ratio			RSD		
		75:25	70:30	65:35	75:25	70:30	65:35
peak area (µV/sec)	MET	82267	82236	82248	0.4400	0.2404	0.2380
	TEN	43688	43637	43611	0.4749	0.1073	0.2007
Theoretical plates	MET	5614	7855	5615	0.6066	0.5823	0.5432
	TEN	3151	3327	3340	0.3278	0.5308	0.5259
Tailing factor	MET	1.742	1.731	1.743	0.3673	0.9865	0.4547
	TEN	1.238	1.243	1.243	0.1449	0.9865	0.4538
Retention Time (Min)	MET	2.294	2.293	2.304	0.3080	0.2455	0.2407
	TEN	4.344	4.314	4.387	0.1150	0.3737	0.1088

n=3, Average of three replicates

Table 13. Robustness study of system suitability parameter: Change in PH

System suitability parameter	Drug	Change in PH			RSD		
		3.8	4.0	4.2	3.8	4.0	4.2
peak area (µV/sec)	MET	82248	82236	82278	0.1514	0.3070	0.7902
	TEN	43682	43637	43648	0.2000	0.1644	0.2208
Theoretical plates	MET	5613	7852	5659	0.274	1.269	0.0937
	TEN	3152	3369	3327	0.315	1.7196	1.2172
Tailing factor	MET	1.730	1.752	1.743	1.1449	1.7109	0.937
	TEN	1.232	1.244	1.243	0.3673	1.7161	0.1217
Retention Time (Min)	MET	2.287	2.296	2.297	0.0308	0.0311	1.8440
	TEN	4.339	4.315	4.378	0.1150	0.1056	0.1747

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n=3, Average of three replicates.

Table 14. Solution stability of MET and TEN

Drug	% Assay Initial	After 12 hrs.	After 24 hrs.	After 36 hrs.	After 48 hrs.
MET	99.27%	99.22%	99.55%	98.68%	98.58%
TEN	99.69%	99.33%	99.67%	98.35%	98.07%

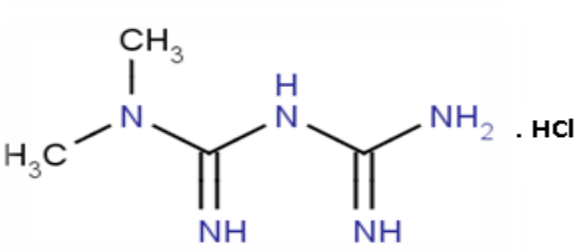


Figure 1. Chemical Structure of MET

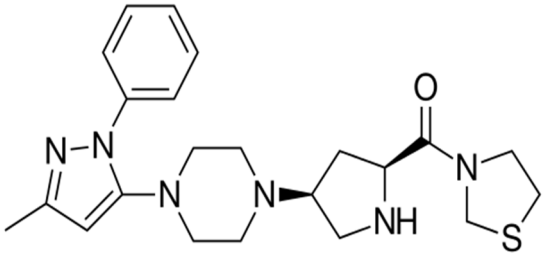
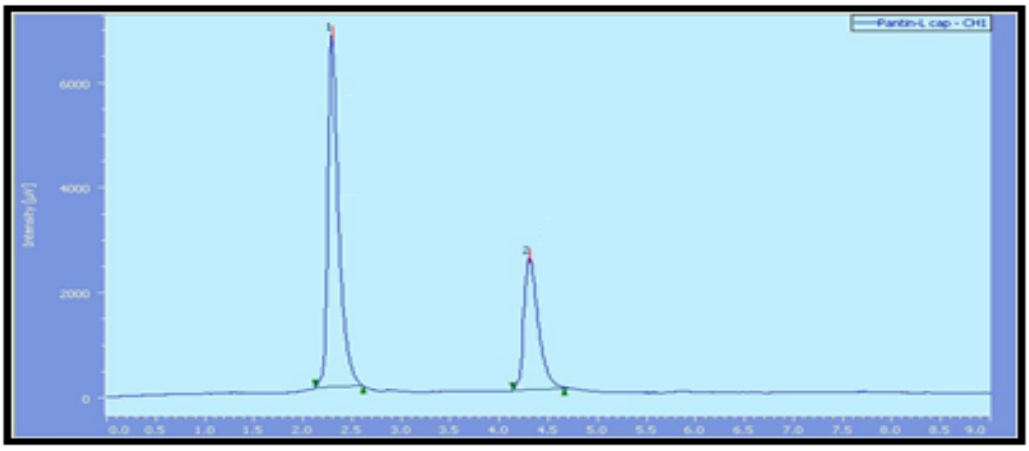


Figure 2. Chemical Structure of TEN



Drug	Retention time	Peak Area (µV/sec)	% area	Symmetric factor	NTP
MET	2.29	82237	100	1.74	6051
TEN	4.30	43628	100	1.23	7417

Figure 3. Chromatogram of Tablet solution of MET and TEN

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RESEARCH ARTICLE

Shantaram Gajanan Khanage et al, The Experiment, 2018, Vol 45(2), 2583-2597

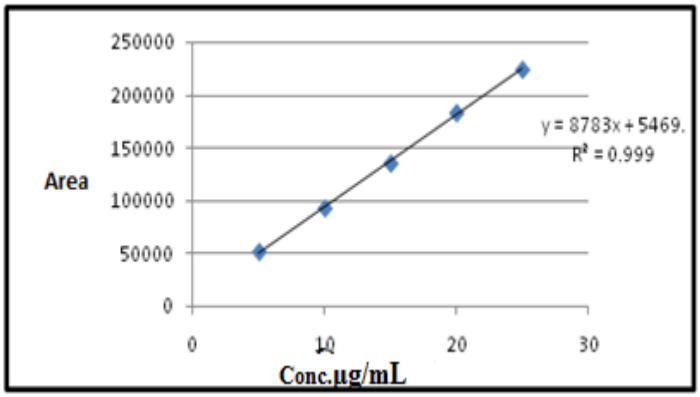

Figure 4. Calibration curve for MET

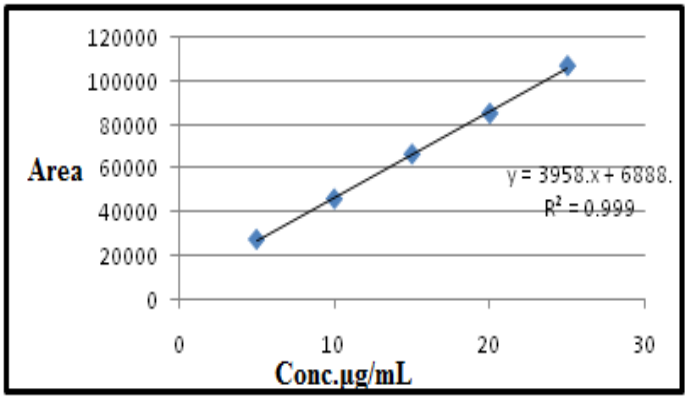

Figure 5. Calibration curve for TEN

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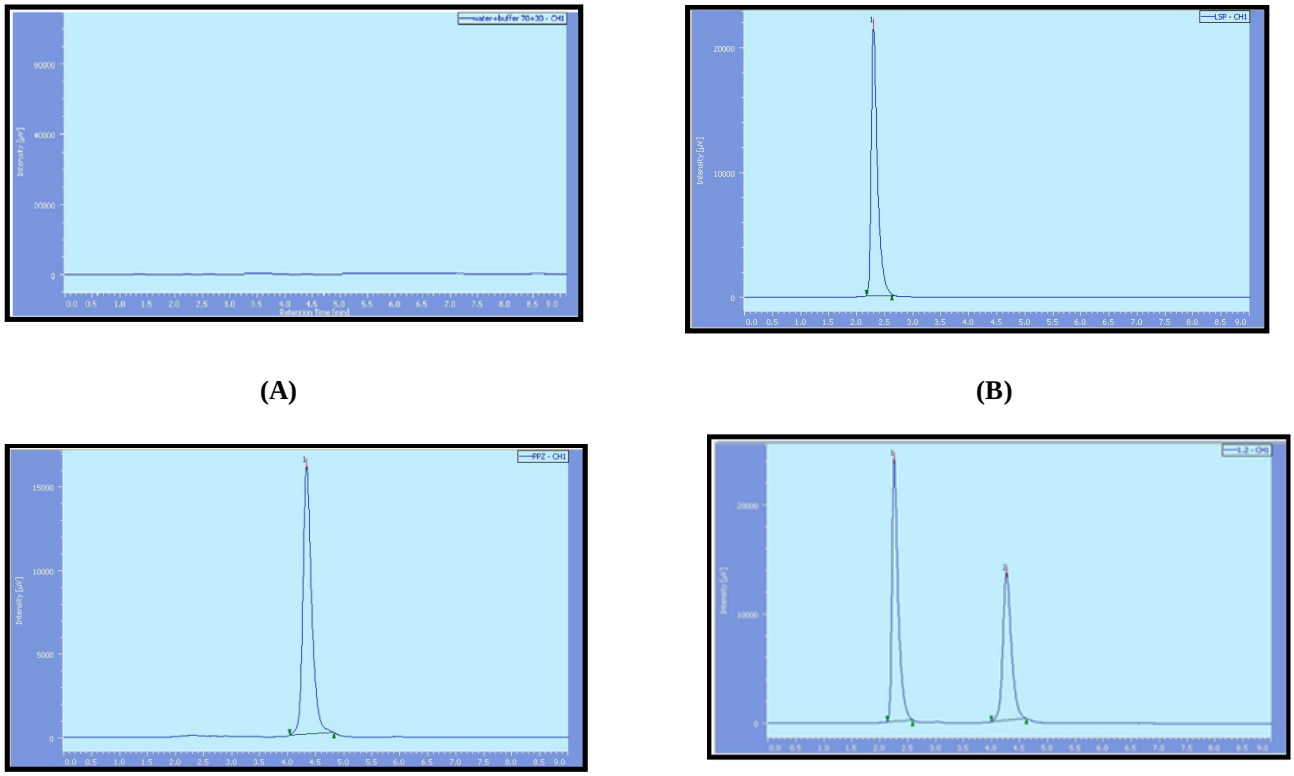

(A) **(B)**

(C) **(D)**

Figure 6. Method selectivity of MET and TEN analysis
(A: Blank, B: Standard solution of MET, C: Standard solution of TEN, D: Tablet solution)

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