DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND TENELIGLIPTIN HYDROBROIDE COMBINED DOSAGE FORM

T. M. Kalyankar[1]*, Sandeep Sasatte[1], M. R. Bodhankar[1] and Anitha K[2].
[1]School of Pharmacy Swami Ramanand Teerth Marathawada University,Nanded (431 606), MS India
[2]Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu-515003, Andhra Pradesh, India

Abstract : Simple, precise and reproducible UV spectrophotometric methods, simultaneous Equation analysis method, have been developed and validated for the simultaneous estimation of Teneligliptin Hydrobromide and Metformin Hydrochloride used as anti dibetic drugs available in Tablet dosage form. In UV spectrophotometric method, the solutions of Teneligliptin HBr and Metformin HCl were prepared in water. The methods are based on the measurement of absorbance of Teneligliptin Hydrobromide and Metformin Hydrochloride at 243 nm and 233 nm respectively. These methods obeyed Beer's law in the concentration range of 10-60 µg /ml for Teneligliptin HBr and 2-12 µg /ml for Metformin HCl. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed methods. These methods were successfully applied to the determination of these drugs in pharmaceutical dosage forms.

Keywords: Teneligliptin Hydrobromide and Metformin Hydrochloride *UV Spectrophotometry.*

I. INTRODUCTION

Teneligliptin hydrobromide hydrate is a dipeptidyl peptidase 4(DPP4) inhibitor is highly effective in lowering blood glucose levels. Teneligliptine hydrobromide hydrate is chemically described as {(2S,4S)-4-[4-(3-methyl-1phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3-thiazolidin-3-yl) methanone hemipentahydrobromide hydrate is a dipeptidyl peptidase inhibitor. It is highly potent, competitive, and long-lasting DPP-4 inhibitor that improves postprandial hyperglycemia and dyslipidemia. It is effectively used to treat type 2 diabetes mellitus.
Metformin Hydrochloride is 1, 1-dimethyl biguanide hydrochloride; Metformin Hydrochloride improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis.
Literature survey reveals that very few UV, HPLC, HPTLC methods in single form and in combination with other drugs have been reported for the estimation of Teneligliptin hydrobromide hydrate and Metformin Hydrochloride in pure and tablet dosage forms.

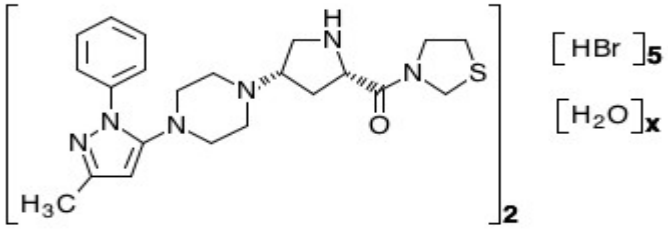

Figure 1: Molecular structure of Teniligliptin hydrobromide hydrate

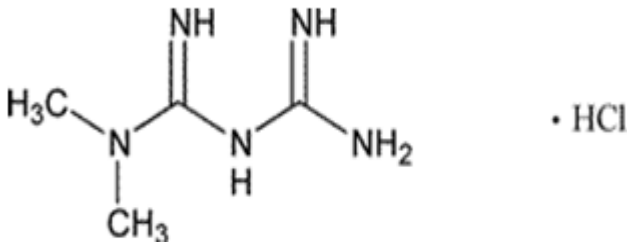

Figure 2: Molecular structure of Metformin Hydrochloride

II. MATERIAL AND METHODS

Chemical and Materials

The TEN and MET pure drugs were procured as a gift sample from Alkem Laboratory Mumbai and Getz Pharma Research, Mumbai India and the marketed formulation Tendia M (Eris Lifesciences Pvt.Ltd.) tablet (TEN 20mg and MET 500mg) was purchased from local market. All chemicals and solvents of AR grade were purchased from MERCK Ltd, Mumbai, India.

Instruments:
Double beam UV- visible spectrophotometer (Shimadzu, Model UV-1800) having two matched quartz cells with 1cm light path and loaded with UV probe software. Electronic Analytical balance (Anamed). Ultrasonicator (HMG India).

Selection of Solvent System: The selection of solvent was made after assessing the solubility of both the drugs in different solvents like distilled water, methanol, 0.1N HCl etc. Both the drug Teneligliptine Hydrobromide and Metformin HCl are freely soluble in distilled water. Therefore water was sonicated for 20 min and made it soluble. According to their solubility distilled water (100%) is selected as a solvent system.

Preparation of standard stock solution (100µg/ml):
Accurately weighed 10mg of TEN and MET were transferred into two separate 100ml volumetric flask. Add sufficient amount of diluents (Distilled water) for 20min. and volume was made up to 100ml with diluent.

Preparation of working standard solution (10µg/ml):
From the above stock solution 1ml of TEN and 1ml of MET were taken, transferred to separate 10ml volumetric flasks and then volume was made up to 10ml with diluents.

Selection of analytical wavelength:
Working standard solution (10µg/ml) of TEN and 10µg/ml MET was scanned in the UV-region i.e. 400 to 200nm. In UV – Spectrophotometric method wavelengths 243nm and 233nm were selected for determination of simultaneous equation of TEN and MET respectively**.** The absorption spectra obtained for TEN is shown in fig.3 and absorption spectra obtained for MET is shown in fig.4. The overlain spectrum of both the drugs is shown in fig.5.

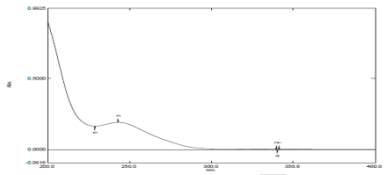

Figure 3: Absorption spectrum of TEN

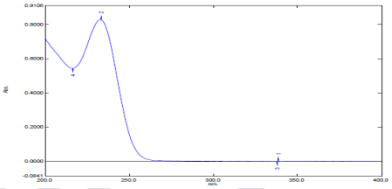

Figure 4: Absorption spectrum of MET

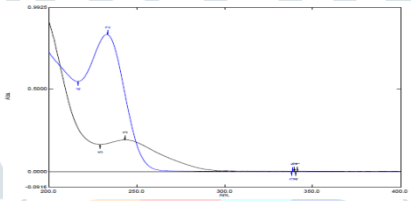

Figure 5: Overlay spectrum of TEN and MET

Selection of analytical concentration range and linearity study:
The standard stock solution of TEN and MET were diluted with distilled water to get series of concentration ranging from 10-60 µg/ml for TEN and 2-12 µg/ml for MET. Absorbance of these solutions was measured at 243nm for TEN and 233nm for MET in 1cm cell using solvent blank. Plot of absorbance Vs concentration were found to be linear and is depicted in Fig.6 and 7.

Determination of absorptivity coefficients at analytical wavelengths:
The absorptivity coefficients for the two drugs were determined at both the selected wavelength. The value obtained as a mean of six independent determinations were used for forming the simultaneous equations. The simultaneous equation formed were

$A_1=3.36\times C_1+4.9\times C_2$----------at 243nm
(For TEN)

$A_2=2\times C_1+82\times C_2$---------- ----------at 233nm
(For MET)

Where A_1 and A_2 are the absorbance of sample solution at 243nm and 233nm respectively, C_1 and C_2 are the concentration of TEN and MET respectively (gm/lit) in the sample solution. By solving the two simultaneous equations, the concentration of TEN (C_1) and MET (C_2) in sample solutions can be obtained.

Table 1: Linearity study data of TEN

Sr. No.	Conc. (µg/ml)	Abs. at 243
1	10	0.199
2	20	0.386
3	30	0.581
4	40	0.784
5	50	0.986
6	60	1.168

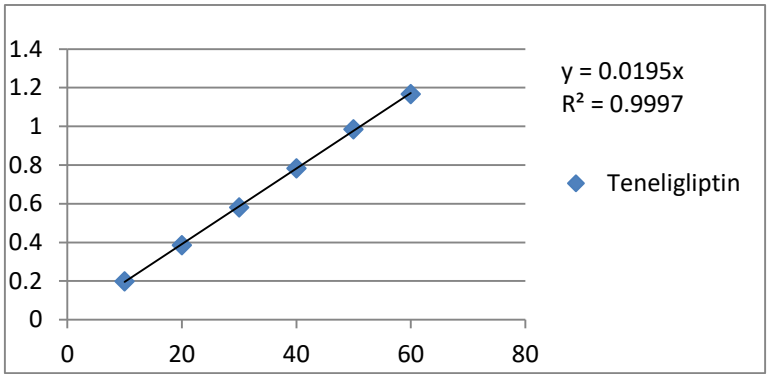

Figure 6: Calibration curve of TEN

Table 2: Linearity study data of MET

Sr. No.	Conc. (µg/ml)	Abs. at 233
1	2	0.187
2	4	0.361
3	6	0.546
4	8	0.731
5	10	0.920
6	12	1.064

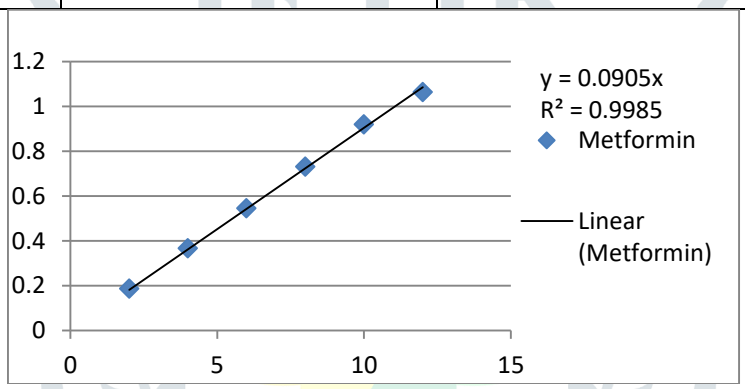

Figure 7: Calibration curve of MET

Analysis of marketed formulation:

Accurately weighed 20 tablets of marketed formulation TENDIA M and average weight were found to be 1020mg. Then these tablets were crushed to fine powder and from this 1020 mg of powder weighed containing equivalent weight of 20 mg of TEN and 500 mg of MET. It has been transferred into 100ml of volumetric flask and volume was made up to the mark with distilled water and this mixture was sonicated for about 20 min. After sonication, it was filtered through Whatmann filter paper no. 41. The solution was further diluted with methanol to get a final concentration of 1µg/ml of TEN and 25µg/ml of MET. The solution was scanned in UV-range (i.e., 200-400nm). The concentrations of the two drugs in the sample solutions were determined. The analysis procedure was repeated six times with tablet formulation. The analysis of tablet formulation study is shown in table no.3

Table 3: Analysis of tablet formulation

Sr. No.	Label claim (mg/tab)		Amount found (mg/tab)		% of Label claim	
	TEN	MET	TEN	MET	TEN	MET
1	20	500	19.98	499.3	99.90	99.86
2	20	500	20.18	497.7	100.94	99.54
3	20	500	20.19	498.8	100.96	99.76
4	20	500	20.18	503.95	100.9	101.79
5	20	500	19.79	507.1	98.95	101.42
6	20	500	19.99	504.05	99.98	100.81
			Avg*		100.27	100.53
			SD		0.8106	0.9466
			% RSD		0.81	0.94

*Indicates average of six determination

METHOD VALIDATION

The proposed method has been extensively validated according to ICH guidelines.

Linearity:

The linearity study was performed by preparing standard solution of 10-60µg/ml for TEN and 2-12µg/ml for MET. The calibration graph was plotted for each concentration versus absorbance often and MET separately.

Table 4: Optical characteristics and other parameter

Parameters	TEN	MET
λ_{max}i.e. selected wavelength (nm)	243	233
Linearity range (µg/ml)	10-60	2-12
Slope (m)	0.019	0.090
Intercept (c)	0.011	0.014
Regression coefficient r^2	0.999	0.998
Limit of detection (ng band^{-1})	0.5964	0.7401
Limit of quantitation (ng band^{-1})	1.9740	2.4571

Precision

The precision of the method was evaluated by intraday and inter-day variation studies. In intraday studies, working solutions of standard and sample were analyzed thrice in a day and the percentage of relative standard deviation (% RSD) was calculated. In case of inter-day variation studies, the working solution of standard and sample were analyzed on three consecutive days and the percentage of relative standard deviation (% RSD) was calculated.

Table 5: Precision data of Tablet Formulation

Sr. No.	Interval of Time	Concentration (%w/w)		% found	
		TEN	MET	TEN	MET
I	Intra- day	1	25	0.998	24.86
II		1	25	0.999	24.89
III		1	25	0.981	24.96
I	Inter –day	1	25	1.019	24.87
II		1	25	0.998	24.89
III		1	25	1.008	24.99
		Mean		1.0005	24.91
		SD		0.0126	0.0471
		%RSD		1.26	0.19

Repeatability (Intra-assay precision)

To check the degree of repeatability of the method, suitable statistical evaluation was carried out. Six samples of the tablet formulation were analyzed for the repeatability study. The standard deviation and coefficient of variance was calculated.

Table 6: Result of repeatability study

Sr. No.	Concentration		Absorbance		% Amount Found	
	TEN	MET	TEN	MET	TEN	MET
1	1	25	0.0191	2.075	100	100.24
2	1	25	0.0192	2.073	100.5	100.14
3	1	25	0.0190	2.077	99.47	100.33
4	1	25	0.0192	2.074	100.5	100
5	1	25	0.0193	2.073	101.04	100.14
6	1	25	0.0189	2.072	98.95	100.09
			% Found*		100.07	100.15
			SD		0.7655	0.1154
			%RSD		0.7649	0.1152

Accuracy:

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80 %, 100 % and 120 %) as per ICH guidelines. The amount of TEN and MET were estimated by developed method. The accuracy data is shown in table7 and 8.

Table 7: Result of recovery study

Level of Recovery	Amount present (mg)		Added concentration(mg)		Amount Recovered(mg)		% Recovery	
	TEN	MET	TEN	MET	TEN	MET	TEN	MET
80%	20	500	18	400	37.44	884.25	98.53	98.25
	20	500	18	400	37.76	908.28	99.37	100.92
	20	500	18	400	38.28	891.9	100.76	99.1
100%	20	500	20	500	39.98	995.4	99.95	99.54
	20	500	20	500	39.96	994	99.9	99.4
	20	500	20	500	40.32	1008	100.8	100.8
120%	20	500	22	600	41.71	1100	99.33	100
	20	500	22	600	41.56	1095.6	98.96	99.6
	20	500	22	600	41.75	1104.4	98.42	99.42

Table 8: Statistical validation of recovery study data

Level of Recovery	% Mean Recovery *		SD*		% RSD*	
	TEN	MET	TEN	MET	TEN	MET
80%	99.55	99.42	1.262	1.3641	1.13	1.13
100%	100.21	99.91	0.5058	0.7711	0.50	0.77
120%	99.42	99.93	0.2438	0.5033	0.25	0.50

* Indicates average of three determinations

LOD AND LOQ:

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal to noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and calculated with use of the following equations:

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of response;S = the slope of the corresponding calibration curve. The result of LOD and LOQ are given in table 9.

Table 9: LOD& LOQ

Parameters	TEN	MET
LOD	0.5964	0.7401
LOQ	1.9740	2.4571

FORCE DEGRADATION STUDIES:

Stress degradation studies were performed to check the stability of the TEN and MET on different conditions. The stress conditions applied for degradation study involved acid, base, thermal, sunlight, oxidative and photolytic degradation.Standard stock solution of TEN10µg/ml and MET 10µg/ml was prepared in water. Summary of the forced degradation of TEN and MET are mentioned in Table 10.

Acid Hydrolysis

Accurately weighed 10mg of TEN and MET, transferred to two separate 100mL round bottom flasks, added 40mL distilled water and 10mL 0.1N HCl. These flasks were heated on water bath at 60°C for 4hr. Solutions were cooled and neutralized with 0.1N NaOH. And makeup volume up to 100mL, finally these solutions was diluted with distilled water to get 10µg/ml of TEN and 10µg/ml of MET and absorbance was measured at 243nm and 233nm for TEN and MET hydrochloride, respectively.

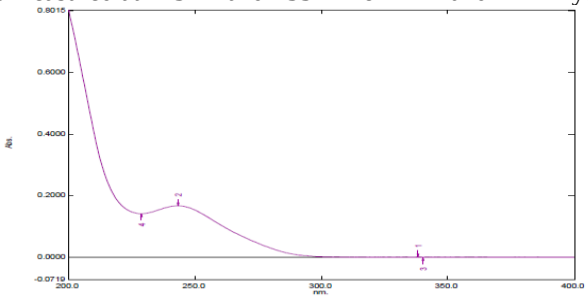

Figure 8: Acid degradation of TEN

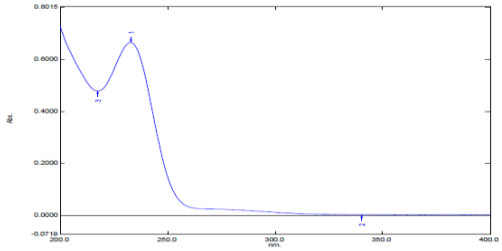

Figure 9: Acid degradation of MET

Base hydrolysis

Accurately weighed 10mg of TEN and MET, transferred to two separate 100mL round bottom flasks, added 40mL distilled water and 10mL 0.1N NaOH. These flasks were heated on water bath at 60°C for 4hr. Solutions were cooled and neutralized with 0.1N HCL. and makeup volume up to 100mL, finally these solutions was diluted with distilled water to get 10µg/ml of TEN and 10µg/ml of MET absorbance was measured at 243nm and 233nm for TEN and MET respectively.

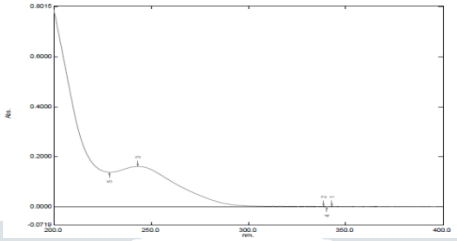

Figure 10: Base degradation of TEN

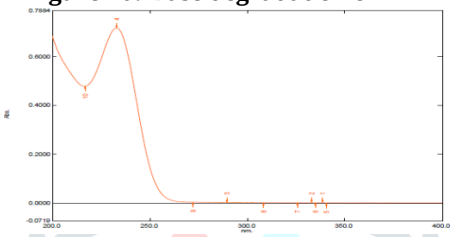

Figure 11: Base degradation of MET

Sunlight degradation

Sunlight degradation is performed by exposing the pure drugs TEN and MET to sunlight in open space for 24 hrs The samples after exposure to sunlight were diluted with distilled water to get TEN (10µg/mL) and MET (10µg/mL) and absorbance was measured at 243nm and 233nm for TEN and MET respectively. Finally absorbance of sample was compared with standard absorbance and percent degradation was calculated.

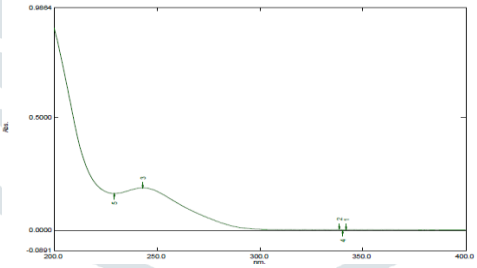

Figure 12: Sunlight degradation of TEN

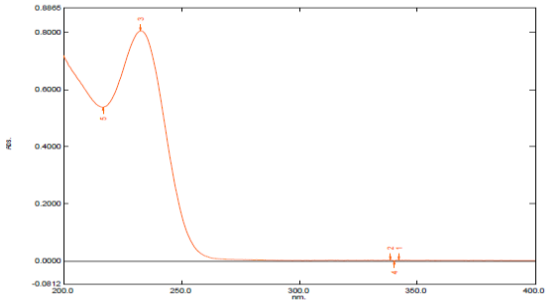

Figure 13:Sunlight degradation of MET

Oxidative degradation

Accurately weighed 10mg of TEN and MET hydrochloride, transferred to two separate 100mL round bottom flasks, added 40mL distilled water and 10mL 3% H_2O_2. These flasks were refluxed for 4hrs at 60°C for 4hr. Solutions were cooled and makeup volume up to 100mL with distilled water, finally these solutions were diluted with distilled water to get 10µg/ml of TEN and 10µg/ml of MET and absorbance was measured at 243nm and 233nm for TEN and MET respectively.

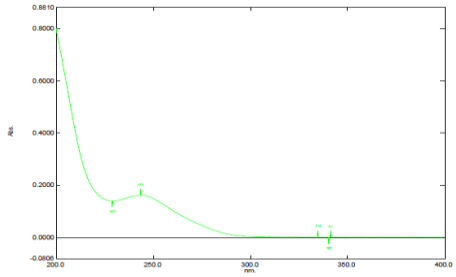

Figure 14: Oxidative degradation of TEN

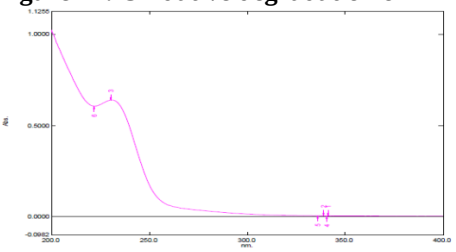

Figure 15: Oxidative degradation of MET

Photolytic degradation

Pure drugs were exposed to UV radiations for 4hrs. The samples after exposure to light were diluted with distilled water to get TEN (10µg/mL) and MET (10µg/mL) and absorbance was measured at 243nm and 233nm for TEN and MET respectively. Finally absorbance of sample was compared with standard absorbance and percent degradation was calculated.

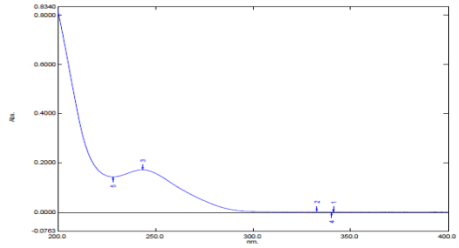

Figure 16: Photolytic degradation of TEN

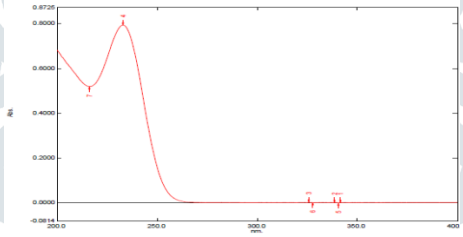

Figure 17: Photolytic degradation of MET

Thermal degradation

Thermal degradation was carried out by exposing pure drugs to dry heat at 60ºC for 4hrs.The samples after exposure to heat were diluted with distilled water to get TEN (10µg/mL) and MET (10µg/mL) and absorbance was measured at 243nm and 233nm for TEN and MET respectively. Finally absorbance of sample was compared with standard absorbance and percent degradation was calculated.

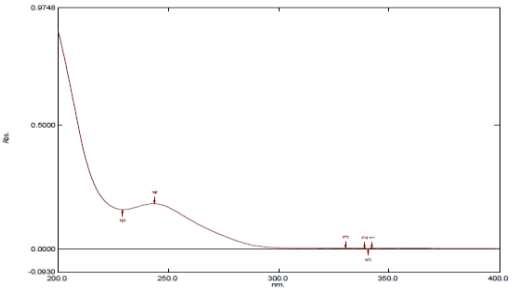

Figure 18: Thermal degradation of TEN

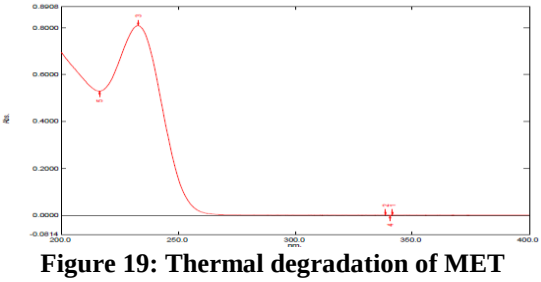

Figure 19: Thermal degradation of MET

Table 10: Data of forced degradation study

Sr. No.	Condition	% Degradation		% Assay	
		TEN	MET	TEN	MET
01.	Acid hydrolysis	12.78	19.76	87.22	80.24
02.	Base hydrolysis	15.50	13.45	84.50	86.55
03.	Oxidative degradation	17.81	22.69	82.19	77.31
04.	Photolytic degradation	3.57	4.23	95.77	96.43
05.	Thermal degradation	2.20	4.35	95.65	97.80
06.	Sunlight degradation	1.94	1.47	98.53	98.06

III. RESULT AND DISCUSSION

The validated stability indicating spectrophotometric method for estimation of degradation behaviour of TEN and MET in tablet formulation has been developed using water as solvent. TEN and MET show maximum absorbance at 243nm and 233nm, respectively. The simultaneous equation method TEN and MET follow Beer's law in the concentration range of 10-60µg/ml and 2-12µg/ml (r^2 = 0.999, 0.999).The optimized method showed mean recovery within acceptable limit for TEN and MET respectively. Results within the range indicate non interference with the excipients of formulation.

The mean percent label claim of tablet formulation was found to be 100.27 % for TEN and 100.53% for MET. The precision was calculated as repeatability, inter day and intraday variation and results was found to be within acceptable limits (i.e. RSD≤2). The LOD and LOQ values of TEN and MET were found to be 0.5964 and 0.7401 µg/ml and 1.9740 and 2.4571 µg/ml respectively. The forced degradation showed TEN and MET undergo degradation in acidic, basic, photolytic, thermal, and peroxide condition and the percentage degradation was found.

IV. SUMMARY AND CONCLUSION

Summary:

In the present investigation by UV analysis, the simultaneous equation method is employed for the assay of TEN and MET in Pharmaceutical formulation. The solvent selected for stock solution preparation was distilled water required concentrations were prepared by using the same. The λ_{max} for detection of TEN and MET were selected as 243nm and 233nm respectively. The calibration curve showed the concentration range10-60µg/ml TEN and 2-12µg/ml for MET. The correlation coefficient obtained with linear regression curve of 0.999 and 0.999 for TEN and MET respectively. Marketed formulation of Tendia M tablet (TEN 20mg and MET 500mg) tablet manufactured by Eris Lifesciences Pvt. Ltd.

For the precision study, the detector response from the standard and sample were used to calculate the amount of the drug in the tablet; percentage estimation was near 100 % and %RSD below 2 % indicate the method is precise.

The forced degradation of drug was carried out by exposing to acid hydrolysis, base hydrolysis and, oxidative degradation, photolytic, Sunlight and thermal condition both drug shows degradation.

Conclusion:

The method used is simple and rapid and does not involve the use of complex instrument, low value of Standard deviation showed that the method is precise and high percentage of recovery shows that the method is accurate. Force degradation study shows that both the drug shows lowest degradation in sunlight degradation. TEN shows degradation in oxidative and base condition and MET shows degradation in oxidative and alkali hydrolysis. Thus from the results it is concluded that above developed stability indicating UV method is suitable for estimation of TEN and MET in tablet formulation. The estimated method is sensitive, precise, accurate, specific and economic. Hence, the method can be used successfully for quality control and routine analysis of finished pharmaceutical dosage form.

REFERENCES

1. Beckett AH, Stenlake JB, Practical pharmaceutical chemistry. Part –II. 4th ed.; New Delhi: CBS Publishers and Distributors; p.157-67; 2002.
2. Anjeneyulu Y, Chandrasekhar K, Manikam V. A textbook of analytical chemistry. New Delhi: Parma Book Syndicate; p. 3-5; 2005.
3. Rao GA. Textbook of pharmaceutical analysis. 2nd ed.; New Delhi: Birla publications Pvt. Ltd.; Vol.2; p. 1-4, 18-22; 2006.
4. Settle F. Handbook of instrumental techniques of analytical chemistry. 1st ed.; New Jersey: Upper Saddle River; p. 19-21, 609-17; 2004.
5. Jeffery GH, Basset J, Mendham J, Denney RC. Vogel's textbook of quantitative analysis. 5th ed.; Longman Scientific and Technical; p. 10-11; 1999.
6. Skoog DA, West DM, Holler FJ. Analytical Chemistry: an Introduction. 6th ed., Saunders College Publishing House: pp. 3, 710, 714, 734, 567, 1996.
7. Sharma BK. Instrumental methods of chemical analysis.23rd ed., Meerut; Goel Publishing House: pp. 10, 2004.
8. Settle F. Handbook of instrumental techniques of analytical chemistry. 1st ed.; New Jersey: Upper Saddle River; p. 19-21, 609-17; 2004.
9. Pikering WF. Modern Analytical Chemistry. New York; Marcel Dekker Inc: pp. 165, 1971.
10. Sykes PA, Shiue HC, Walker JR, Bateman RC. Determination of myoglobin stability by visible spectroscopy. J. Chem. Ed. 76 (9), 1283–1284, 1999.
11. Talsky G. Derivative Spectrophotometry; VCH Publishers: New York, 1994.

12. Thomas M. Ultraviolet and visible spectroscopy. In Analytical Chemistry by Open learning; Ando, D.J., Ed.; John Wiley &Sons: New York, 1997.
13. Willard HH, Merrit Jr. LL, Dean JA, Settle Jr. FA. "Instrumental methods of analysis"; 7th edition, CBS Publishers and distributors, New Delhi; p. 170, 580-610, 1986.
14. Silverstein RM, Bassler GC, Morrill TC, Spectrometric Identification of Organic Compounds, 4th ed. (New York: Wiley, 1981), 166.
15. Pavia DL, Lampman GM, Kriz GS. Introduction to Organic Laboratory Techniques A Microscale Approach 4th Edition. Belmont, Ca. The Thomson Corporation; 13-101, 2007.
16. Sohan S. Chitlange, Diptee G. Rawat, Sneha Chandani, estimation of anti-diabetic teneligliptin hydrobromide hydrate by RP-HPLC and derivative spectroscopic method, Indo American Journal of Pharmaceutical Research, 2016, 6 (07),6144-6152
17. Vishnu C. Shinde, development and validation of UV spectrophotometric method and high performance thin layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation, Scholars Research Library Der Pharmacia Lettre, 2016, 8 (8), 291-301.
18. Chandana M., Prasad Rao M., Samrajyam B., Sireesha K.S.K.D., Naga premi V.V., developed analytical method development and validation of teniligliptin in phrmaceutical dosage form by RP-HPLC method, IJRDO-Journal of Health Sciences and Nursing, 2016, 1 (12) ,1-12.
19. Rathod E. A., developed HPLC and UV-spectrophotometric estimation of teneligliptin from tablet dosage form, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry, 2016, 4(3),148- 156.