Research Article

ABSORBANCE RATIO METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN HYDROBROMIDE HYDRATE (TENE) AND METFORMIN HYDROCHLORIDE (MET) IN TABLET DOSAGE FORM

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ABSTRACT

A precise, accurate UV spectroscopic method was developed and validated for simultaneous estimation of Teneligliptin hydrobromide hydrate (TENE) and Metformin hydrochloride (MET HCl) in pharmaceutical dosage form. Wavelength selected for estimation were 233 nm and 249.20 nm in absorbance ratio method for TENE and MET HCl. Linearity of developed method was found to be 6-16μg/mL for TENE and MET HCl as the r^2 value was found to be near 1. Accuracy was found to be 98.33% - 101.11% in absorbance ratio method for TENE and MET HCl. The Method validated as per ICH guidelines.

Key Words: Simultaneous estimation, Teneligliptin hydrobromide hydrate and Metformin hydrochloride, UV Spectroscopic method, Validation.

INTRODUCTION

Diabetes is a group of metabolic disease in which a person suffers with high blood glucose (blood sugar), either because of low insulin production or because of body's cells inability to produce insulin properly, or both. A combination of Teneligliptin hydrobromide hydrate and Metformin hydrochloride producing synergistic effect in diabetes[1,5,6]. Teneligliptin hydrobromide hydrate is a Dipeptidyl peptidase 4 (DPP- 4) inhibitor. It is highly potent, competitive, and long lasting DPP-4 inhibitor[2]. Metformin hydrochloride is an antihyperglycemic agent which lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization[3].
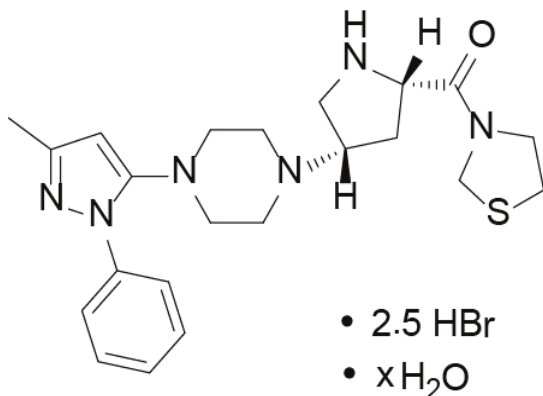

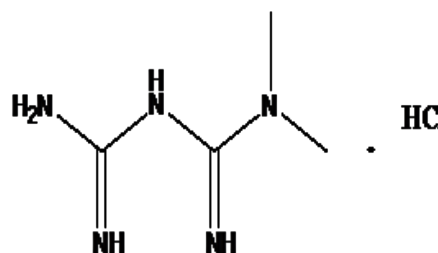**Figure 1: Structure of Teneligliptin hydrobromide hydrate**

Figure 2: Structure of Metformin hydrochloride

MATERIALS AND METHODS

Teneligliptin hydrobromide hydrate was kindly gifted by Glenmark Pharmaceuticals Ltd., Mumbai, Metformin Hydrochloride procured from Norris Medicines Ltd., Ankleshwar, Methanol and Acetic acid were procured from Fischer scientific, Mumbai. Potasium dihydrogen phosphate was procured from Merck, Mumbai

METHOD DEVELOPMENT
Selection of solvent

MET HCl and TENE were soluble in methanol and water. Hence water was selected for method development of both the drug as it was found that both the drugs were soluble in water[7].

Selection of wavelength

Standard drug solution of MET HCl and TENE were scanned separately in the range of 200-400 nm. Data was obtained by overlay spectra of MET and TENE. Data was obtained as 249.10nm maxima wavelength of MET HCl and 233nm maxima wavelength of TENE for simultaneous estimation method.

Preparation of solutions
Preparation of stock solution for Metformin hydrochloride

An accurately weighed quantity of MET HCl (10 mg) was transferred in to 100ml volumetric flask, 10-15ml of distilled water was added and sonicated for 5 min and diluted up to the mark 100ml with distilled water.

Preparation of stock solution for Teneligliptin hydrobromide hydrate

An accurately weighed quantity of TENE (10 mg) was transferred in to 100ml volumetric flask, 10-15ml of distilled water was added and sonicated for 5 min and diluted up to the mark 100ml with distilled water.

Preparation of working standard solution for Metformin hydrochloride

From standard stock solution (100µg/ml) of MET HCl (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were taken and transferred in 10ml volumetric flask and make up the volume with distilled water, which gives (6, 8, 10, 12, 14, 16) µg/ml. Further absorbance of above prepared solutions was measured.

Preparation of working standard solution for Teneligliptin hydrobromide hydrate

From standard stock solution (100µg/ml) of TENE (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were taken and transferred in 10ml volumetric flask and make up the volume with distilled water, which gives (6, 8, 10, 12, 14, 16) µg/ml. Further absorbance of above prepared solutions was measured.

Method Validation
Linearity and Range

The linearity response was determined by analyzing 6 concentrations in the range of 6-16µg/ml for MET HCl and 6-16µg/ml for TENE. Accurately measured standard stock solutions of each MET HCl and TENE (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were transferred in to 10ml volumetric flask and make up the volume with distilled water to obtain concentration 6:6, 8:8, 10:10, 12:12, 14:14, 16:16µg/ml of MET HCl and TENE respectively. Absorbance of each solution was measured at 249.10nm and 233nm.
Range in term which calibration curve constructed by plotting absorbance vs. concentration[7,8].

Precision

Repeatability

Repeatability of MET HCl and TENE checked by repeated measurement of absorbance of solution (n=6) of 10µg/ml (MET HCl) and 10µg/ml (TENE) measured and %RSD was calculated.

Acceptance criteria: - %RSD should be less than 2.

Intraday precision

Three replicates of three concentration of MET HCl (8, 10, 12 µg/ml) and TENE (8, 10, 12 µg/ml) total nine determinations were analyzed at same day within short time interval and absorbance were measured and % RSD was calculated.

Acceptance criteria: - %RSD should be less than 2.

Intermediate Precision

Interday Precision

Three replicates of three concentration of MET HCl (8, 10, 12 µg/ml) and TENE (8, 10, 12 µg/ml) total nine determinations were analyzed at three consecutive days and absorbance were measured and % RSD was calculated.

Acceptance criteria: - %RSD should be less than 2.

Different instrument

Three concentration of MET HCl (8, 10, 12µg/ml) and TENE (8, 10, 12 µg/ml) total nine determinations were analyzed in two different instruments (UV-1800, UV-1700) on a same day and absorbance were measured and % RSD was calculated.

Acceptance criteria: - %RSD should be less than 2.

Robustness

Three different concentration of MET HCl (8, 10, 12 µg/ml) and TENE (8, 10, 12 µg/ml) were prepared and analyzed by different wavelength. The solution of MET HCl were analyzed at 232.5nm, 233nm, 233.5nm and TENE (8, 10, 12 µg/ml) were analyzed at 242.5nm, 243nm, 243.5nm. Absorbance at each wavelength were measured and % RSD was calculated.

Acceptance criteria: - %RSD should be less than 2.

Limit of Detection

The LOD was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

$$LOD = 3.3(SD/Slope)$$

Where, SD = Standard deviation of the Y- intercepts of the 5 calibration curves
Slope= Mean slope of the 5 calibration curves.

Limit of Quantification

The LOD was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

$$LOQ = 10 (SD/Slope)$$

Where, SD= Standard deviation of the Y- intercepts of the 5 calibration curves
Slope= Mean slope of the 5 calibration curves

Accuracy

The accuracy of the method was determined by calculating % recovery of Metformin hydrochloride and Teneligliptin hydrobromide hydrate by standard addition method. Known amount of standard solutions of MET HCl and TENE corresponding to 50, 100 and 150 % of target concentration were spiked with pre analyzed sample solution. The amounts of MET HCl and TENE were estimated by applying obtained values to regression equation of calibration curve.

Analysis of drug in marketed formulation

The response of sample solution was measured at 233nm and 243nm. The amount of MET HCl and TENE present in sample solution were calculated[9].

Preparation of sample solution:

Take 20 tablets, average weight was calculated and powdered. Weight equivalent to 25mg of Metformin hydrochloride and 1mg of Teneligliptin hydrobromide hydrate transferred in 100 ml volumetric flask. 20ml of distilled water was added and sonicated for 15min. The volume was adjusted with distilled water up to the mark. The solution then filtered through Whatman filter paper. To get final concentration 250µg/ml of Metformin hydrochloride and 10µg/ml of Teneligliptin. From this stock solution pipette out 2.5ml and transferred in 10ml volumetric flask and make up volume up to the mark with distilled water to get concentration 2.5 µg/ml of TENE and 62.5 µg/ml of MET HCl[4, 9].

Than scanned in UV region and absorbance (A1) noted at 243 nm and absorbance (A2) at 233 nm. At this point quantify TENE concentration.

From above sample solution pipette out 0.5ml and transferred in 10 ml volumetric flask and make up volume up to mark with distilled water to get final concentration 0.5 µg/ml of TENE and 12.5 µg/ml of MET HCl.

Than scanned in UV region and absorbance noted (A1) at 243nm and absorbance (A2) at 233 nm and amount of MET HCl quantified.

RESULTS AND DISCUSSION

Method Validation
Selection of solvent

Water was selected for method development as both the drugs were found to be soluble in water.

Selection of wavelength

From the spectra of Metformin Hydrochloride, the wavelength maxima selected for estimation of Teneligliptin at 233 nm and isoabsorptive point was observed at 249.20 nm for absorption ratio method.
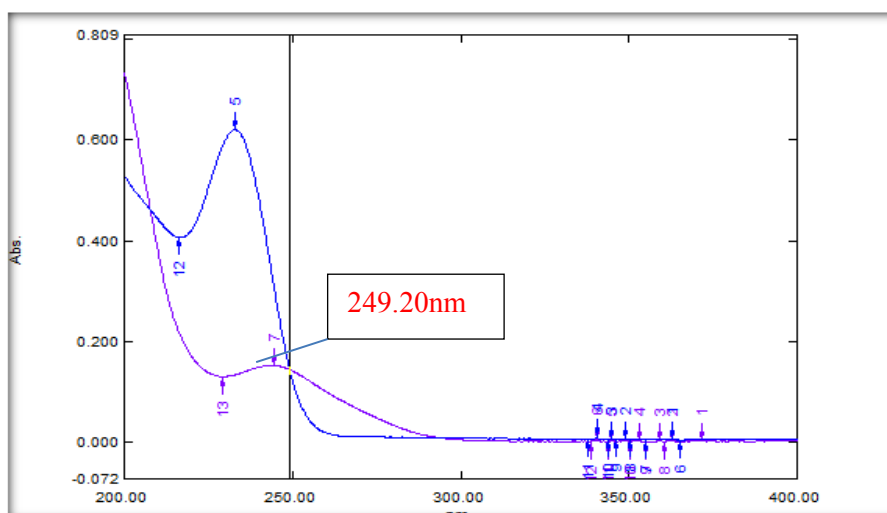

Figure 3: Overlay spectra of TENE and MET HCl (Absorbance ratio method)

Validation of Absorbance Ratio Method

Linearity and range
Linearity study was carried out for both the drugs at different concentration levels. Linearity of TENE and MET HCl was in the range of 6-16 µg/ml and 6-16 µg/ml respectively.
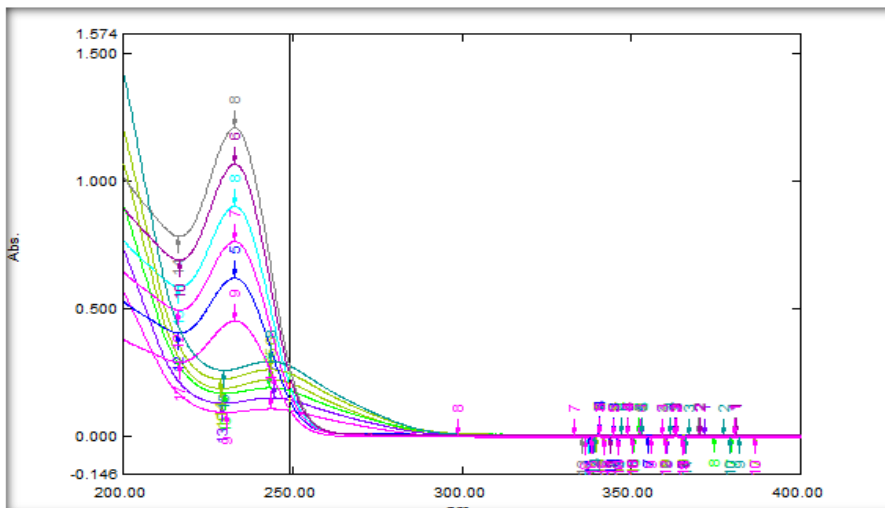
Figure 4: Overlay spectra of TENE and MET HCl

Table 1: Data for linearity and range of TENE and MET HCl

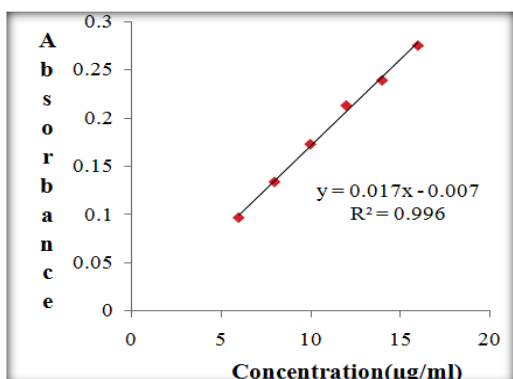| Sl.No.	Concentration (µg/ml)		At 233nm		At 249.20nm	
	TENE	MET HCl	TENE	MET HCl	TENE	MET HCl
1	6	6	0.097	0.453	0.103	0.104
2	8	8	0.134	0.620	0.144	0.144
3	10	10	0.173	0.763	0.180	0.179
4	12	12	0.213	0.900	0.211	0.210
5	14	14	0.239	1.066	0.247	0.246
6	16	16	0.275	1.207	0.276	0.276

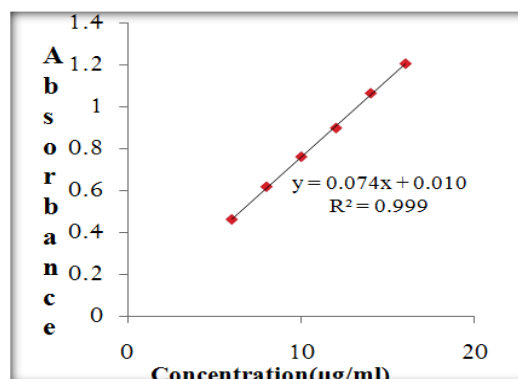**Figure 5: Calibration curve for TENE at 233 nm**

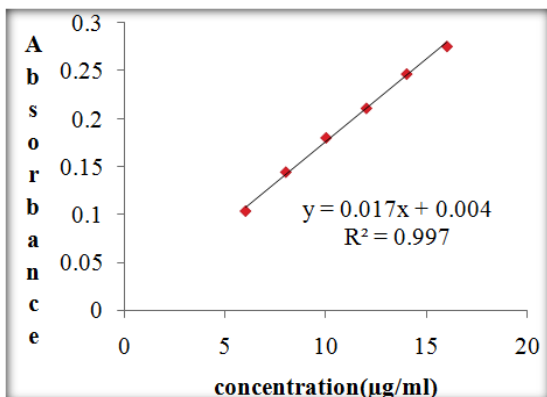**Figure 6: Calibration curve for MET HCl at 233 nm**

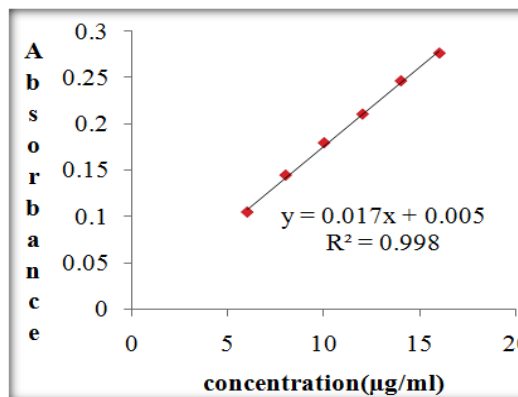**Figure 7: Calibration curve for TENE at 249.20 nm**

Figure 8: Calibration curve for MET HCl at 249.20 nm

Precision
Repeatability
The data for repeatability of absorbance measurement for TENE (10 μg/ml) and MET HCl (10 μg/ml) based on six measurements of same solution of TENE and MET HCl. The % RSD was found to be < 2.

Table 2: Repeatability data of TENE and MET HCl

Repeatability		
Concentration (10:10μg/ml)	TENE at 233nm	MET HCl at 249.20nm
1	0.169	0.179
2	0.164	0.176
3	0.164	0.178
4	0.171	0.177
5	0.168	0.178
6	0.168	0.177
Mean	0.167	0.177
SD	0.0028	0.0010
%RSD	1.6761	0.5908

Intraday Precision

It was performed by taking three replicates of standard solution of TENE and MET HCl using that 3 concentration (8,10,12μg/ml) and (8, 10,12 μg/ml) werePrepared thus total nine determination were analyzed within the short period of time interval. The % RSD was found to be < 2.

Table 3: Intraday Precision Data for TENE and MET HCl

Sl. No.	Concentration (μg/ml)		Wave-Length (nm)	At 11am	At 2pm	At 5pm	Mean	SD	%RSD
	TENE	MET HCl							
1	8	8	233	0.134	0.136	0.134	0.134	0.0011	0.8574
			249.20	0.144	0.142	0.141	0.142	0.0015	1.0732
2	10	10	233	0.173	0.171	0.169	0.171	0.0020	1.1695
			249.20	0.179	0.182	0.183	0.181	0.0020	1.1479
3	12	12	233	0.213	0.210	0.213	0.212	0.0017	0.8170
			249.20	0.210	0.212	0.214	0.212	0.0020	0.9433

Intermediate Precision
Interday Precision

Three replicates of 3 concentrations of standard solution of TENE and MET HCl, total 9 determinations were analyzed at three consecutive day and absorbance were measured at 233 nm and 249.20 nm. % RSD was calculated.

Table 4: Interday Precision data for TENE and MET HCl

Sl. No.	Concentration (μg/ml)		Wave-Length (nm)	Day 1	Day 2	Day 3	Mean	SD	%RSD
	TENE	MET HCl							
1	8	8	233	0.134	0.136	0.137	0.135	0.0015	1.1259
			249.20	0.144	0.142	0.145	0.143	0.0015	1.0632
2	10	10	233	0.173	0.172	0.171	0.172	0.0010	0.5813
			249.20	0.179	0.181	0.183	0.181	0.0020	1.1049
3	12	12	233	0.213	0.214	0.215	0.214	0.0010	0.4672
			249.20	0.210	0.214	0.216	0.213	0.0030	1.4320

Different instrument

Three different concentrations of TENE and MET HCl were analyzed at UV 1800 and UV1700 and record the absorbance at 233 nm and 249.20 nm. %RSD was calculated.

Table 5: Different Instrument Data for TENE and MET HCl

Sl. No.	Concentration (μg/ml)		Wave-Length (nm)	UV-1800	UV-1700	Mean	SD	%RSD
	TENE	MET HCl						
1	8	8	233	0.124	0.122	0.123	0.0014	1.1497
			249.20	0.144	0.141	0.142	0.0021	1.4886
2	10	10	233	0.165	0.167	0.166	0.0014	0.8519
			249.20	0.179	0.176	0.177	0.0021	1.1951
3	12	12	233	0.192	0.190	0.191	0.0014	0.7404
			249.20	0.210	0.213	0.211	0.0020	1.002

Robustness

Robustness carried by changing wavelength ± 0.5 nm. %RSD for TENE and MET HCl was calculated.

Table 6: Different wavelength Data for TENE and MET HCl

Drug	Concentration (µg/ml)	232.5 Nm	233 nm	233.5 nm	Mean	SD	%RSD
TENE	8	0.133	0.134	0.135	0.213	0.0010	0.7462
	10	0.172	0.173	0.174	0.174	0.0010	0.5780
	12	0.212	0.213	0.214	0.213	0.0010	0.4694
MET HCl	Concentration (µg/ml)	248.7 Nm	249.20 nm	249.7 nm	Mean	SD	%RSD
	8	0.143	0.144	0.145	0.144	0.0010	0.6944
	10	0.177	0.179	0.180	0.178	0.0015	0.8549
	12	0.209	0.210	0.213	0.210	0.0020	0.9881

LOD and LOQ

Calibration curves were repeated for five and standard deviation of intercept was calculated, then LOD and LOQ were calculated as follows:

Table 7: LOD and LOQ Data for TENE and MET HCl

Parameters	TENE at 249.20nm	MET HCl at 233nm
SD of the Y-Intercepts of 5 Calibration curve	0.0004	0.0004
Mean slope of 5 calibration curve	0.0162	0.0724
LOD(µg/ml)	0.0203	0.0912
LOQ(µg/ml)	0.0617	0.2765

Accuracy

From marketed formulation at three level of standard addition accuracy of the method was confirmed by recovery study. % recovery of TENE and MET HCl were found between 98% to 102%.

Table 8: % recovery data for TENE and MET HCl

Drug	% Level	Amt. of Sample taken (µg/ml)	Amt. of Standard Spiking (µg/ml)	Total Amt. (µg/ml)	Conc. Found (µg/ml)	% Recovery
TENE	I (50%)	6	3	9	9.02	100.2
		6	3	9	9.10	101.20
		6	3	9	8.99	99.88
TENE	II (100%)	6	6	12	11.80	98.33
		6	6	12	11.93	99.41
		6	6	12	12.01	100.8
TENE	III (150%)	6	9	15	14.87	99.13
		6	9	15	14.99	99.93
		6	9	15	14.89	99.26
MET HCl	I (50%)	6	3	9	8.90	98.88
		6	3	9	8.85	98.33
		6	3	9	9.05	100.55
MET HCl	II (100%)	6	6	12	12.02	100.20
		6	6	12	11.99	99.91
		6	6	12	11.85	98.75
MET HCl	III (150%)	6	9	15	14.86	99.06
		6	9	15	14.92	99.46
		6	9	15	15.03	100.2

Analysis of Marketed Formulation
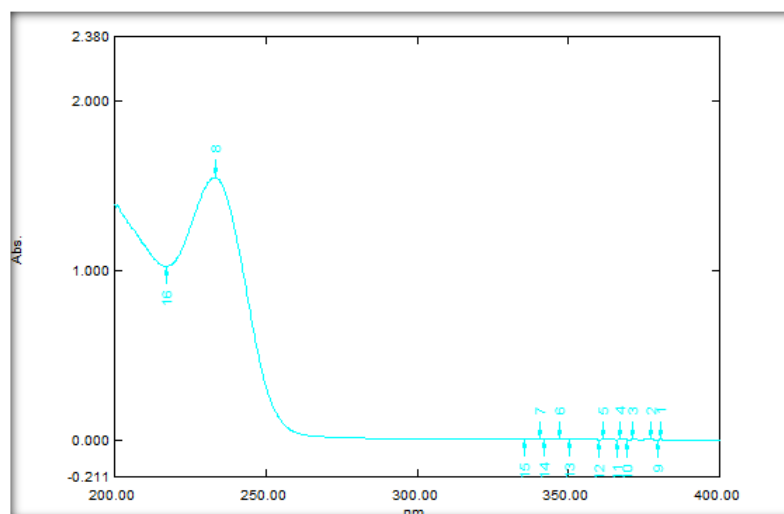
Figure 9: Spectra of TENIVA-M Tablet

Table 9: Analysis of TENIVA-M Tablet

Sample	Label Claim%		Amt. Found		%Assay	
	TENE (mg/tab)	MET HCl (mg/tab)	TENE (mg/tab)	MET HCl (mg/tab)	TENE	MET HCl
1	20	500	19.80	490	99	98
2	20	500	20.01	499.45	100.06	99.89
3	20	500	19.72	500.05	98.62	100.01
4	20	500	19.86	492.5	99.30	98.50
5	20	500	19.91	497.5	99.56	99.50
6	20	500	19.96	496.3	99.80	99.26
Average			**19.87**	**495.96**	**99.39**	**99.11**

DISCUSSION

Absorbance ratio method was developed and validated for Teneligliptin hydrobromide hydrate and Metformin hydrochloride. Linearity was found near to 1 for Teneligliptin hydrobromide hydrate and Metformin hydrochloride. For Intraday, Interday, Intermediate precision, Robustness, % RSD was found less than 2. % Recovery was found to be between range 98-102% for both the drugs. These results indicate that the method is accurate, precise and simple.

CONCLUSION

Both the drugs showed better solubility and stability in Methanol. Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed method is new, simple, accurate, sensitive, economic and precise and can be adopted for routine analysis and in tablet dosage form.

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