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Research Article

SIMULTANEOUS ESTIMATION OF METFORMIN AND TENELIGLIPTIN IN PHARMACEUTICAL FORMULATION BY USING UV SPECTROSCOPY

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ABSTRACT

Three new UV spectrophotometric methods namely simultaneous equation, absorbance ratio and dual wavelength methods were developed and validated for simultaneous estimation of Metformin and Teneligliptin in bulk drug and tablet formulation which were simple, rapid, sensitive, precise and accurate. In simultaneous equation method, absorbance was measured at 233nm for Metformin and 241nm for Teneligliptin. In absorbance ratio method, absorbance was measured at 244nm for Metformin and 233nm for Teneligliptin. In dual wavelength method, two wavelengths were selected for each drug, the absorbances was measured at 225 and 251nm for Metformin and 240 and 221nm for Teneligliptin. Developed methods were validated according to ICH guidelines including parameters viz., specificity, linearity and range, precision, accuracy, limit of detection and limit of quantification. All the three methods showed linear response in the concentration range of 2-10µg/mL for Metformin and 0.5-2.5µg/mL for Teneligliptin with a low correlation coefficient. Results of method validation parameters follows ICH guideline acceptable limits. Methods were found to be simple, rapid, sensitive, economical and hence can be useful for simultaneous estimation of Metformin and Teneligliptin in pure drug and commercial tablet formulation for routine quality control analysis.

INTRODUCTION

Metformin (MET) (Figure 1) is an oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes mellitus[1]. Chemically it is 1-carbamimidamido-N, N-dimethylmethanimidamide. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.[2-3]

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Teneligliptin (TEN) (Figure 2) is a pharmaceutical drug for the treatment of type 2 diabetes mellitus[4]. It belongs to the class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors. Chemically it is [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl) piperazin-1-yl]pyrrolidin-2-yl]-(1,3-thiazolidin-3-yl) methanone. Teneligliptin inhibits the enzyme dipeptidyl peptidase-4 (DPP4) which degrades incretin, a hormone adjusting blood glucose control[5,6].

Literature review revealed that few analytical methods have been reported for the simultaneous determination of Metformin and Teneligliptin in combined pharmaceutical dosage forms using spectrophotometry [7-12]. Hence the objective of the present work is to develop a new, simple, sensitive, specific, precise and accurate UV Spectrophotometric

method for the simultaneous determination of Metformin and Teneligliptin in bulk drug and in pharmaceutical formulations and validated the method as per ICH guidelines [13].

MATERIALS AND METHODS

Materials: The reference samples of Metformin and Teneligliptin were procured from Yarrow Chemicals, Mumbai. The commercial formulations (TENLIFIN M tablets containing 500mg of Metformin and 20mg of Teneligliptin) were purchased from the local market. 0.1N NaOH (AR grade) was purchased from E.Merck (India) Ltd., Mumbai and was used as diluent. Fresh purified distilled water was used throughout the experiment.

Instrumentation: Shimadzu UV1800 Double Beam UV-Visible Spectrophotometer was used for spectral studies. Shimadzu BL220H Digital Weighing Balance was used for weighing the materials.

Selection of Solvent: The solubility of Metformin and Teneligliptin was carried out in a variety of polar and non-polar solvents as per Indian Pharmacopoeia standards. Based on the solubility of the compounds finally 0.1N NaOH was selected as common solvent for both the drugs due to its positive results.

Preparation of Standard Stock Solution: The stock solutions were prepared by dissolving each of 100mg of Metformin and Teneligliptin in 100mL volumetric flask with 70mL of 0.1N NaOH. The solutions were sonicated for 15min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the 0.1N NaOH to get a stock concentration of Metformin and Teneligliptin. Further pipette 1mL of the above stock solution into a 10mL volumetric flask and the volume was made up to the mark with the 0.1N NaOH (100 µg/mL).

Preparation of Sample Solution: Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 50 mg of Metformin and 2mg of Teneligliptin was transferred into a 50mL clean dry volumetric flask containing 30 mL of 0.1N NaOH. The solution was sonicated for 15 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the 0.1N NaOH to get a stock concentration of Metformin and Teneligliptin. Further pipette 1 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the 0.1N NaOH. Later pipette out 1 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the 0.1N NaOH.

Simultaneous equation method (Method-I)

Detection of wavelength: Working standard solutions having 10µg/mL of Metformin and Teneligliptin in 0.1N NaOH were prepared and the solutions were scanned between 200-400 nm by using 0.1N NaOH as blank. The overlain spectrum was observed and suitable wavelengths for analysis were found to be 233 nm for Metformin and 241nm for Teneligliptin.

Preparation of calibration curve: Prepare linearity solutions of 2-10µg/mL for Metformin and 0.5-2.5µg/mL for Teneligliptin from standard stock solution using 0.1N NaOH respectively. The corresponding absorbances were measured at 233nm (λ_1) and 241nm (λ_2). Plot calibration curve by taking the concentration and absorbance values calibration curve was plotted taking concentration on x-axis and absorbance on y-axis which showed a straight line and the calibration curves of Metformin and Teneligliptin was shown in Fig. 3 & Fig. 4. Linearity results for Metformin and Teneligliptin were shown in Table 1 & 2.

The quantification analysis was of Metformin and Teneligliptin in binary mixture was performed by using equations 1 and equations 2 where Cx and Cy are the concentrations of Metformin and Teneligliptin respectively and ax1 and ax2 are the absorptivites of Metformin at λ_1 and λ_2 and ay1 and ay2 are the absorptivities of Teneligliptin at λ_1 and λ_2.

C x = (A1 aY2- A2 Ay1)/(aX1 aY2- aX2 aY1)…… Eq. 1

Cy =(aX1 A2- aX2 A1)/(aX1 aY2-aX2 ay1)……Eq. 2

Where, A1, A2 =Abs. of components

ax_1= Absorbitivity of Metformin at λ_1i.e. 233 nm

ax_2= Absorbitivity of Metformin at λ_2 i.e. 241 nm

ay_1= Absorbitivity of Teneligliptin at λ1

ay_2= Absorbitivity of Teneligliptin at λ2

Graphical absorbance ratio Q-Analysis method (Method-II)

In absorption ratio method, absorbances of both the drugs were calculated at two selected wavelengths; among which λ1 is the wavelength of isobestic point of both drugs and λ2 is the λmax of either drug among both drugs. From the overlain spectra, the absorbance was measured at 244 nm for Metformin and 233 nm for Teneligliptin were obtained. Linearity results for Metformin and Teneligliptin were shown in Table 3 & 4.

The concentration of the individual components was calculated by using the following equations;

Cx=Qm-Qy/Qx-Qy)×A1/ax1…… Eq. 3

Cy=Qm-Qy/Qy-Qx)×A1/ax1……Eq. 4

Where Qm = A2 /A1,

A1 is absorbance of sample at iso absorptive point, A2 is absorbance of sample at λmax of one of the two components,

$Qx = ax_2 / ax_1$, $Qy = ay_2 / ay_1$,

ax_1 and ax_2 represent absorptivities of Metformin at λ_1 and λ_2 and ay_1 and ay_2 denote absorptivities of Teneligliptin at λ1 and λ2 respectively; Cx and Cy be the concentration of Metformin and Teneligliptin respectively.

Dual Wavelength Method (Method-III)

In this method two wavelengths were selected for each drug in a way so that the difference in absorption is zero for one drug at a time. As per spectrum, the absorption of MET was same at 225 and 251nm so that these wavelengths were selected for estimation of TEN and same as in 240 and 221 nm, absorption of TEN were same hence these two wavelengths were selected for estimation of MET. All the mixed standards were scanned at these selected wavelengths and a calibration curve was plotted between absorbance difference and the respective concentrations. The value of coefficient of correlation was 0.9946 and 0.9964 for MET and TEN respectively. The sample solutions were measured at selected wavelengths and the values of difference in absorbance were extrapolated on the working standard curve to get the concentration. The linearity curves for Metformin and Teneligliptin were shown in Fig. 5 & Fig. 6. Linearity results for Metformin and Teneligliptin were shown in Table 5 & 6.

Method Validation

Linearity: Linearity was performed by preparing standard solutions of Metformin and Teneligliptin at different concentration levels i.e., 2-12µg/mL for Metformin and 0.5-2.5µg/mL for Teneligliptin respectively. The absorbances were measured at 233 nm for Metformin and 241 nm for Teneligliptin. Each measurement was carried out in triplicate. Linearity was proven by regression analysis by the least square method. The straight line in the calibration curve obeyed linearity in the concentration range of 2-12µg/mL for Metformin and 05-2.5µg/mL Teneligliptin respectively. The correlation coefficient was found to be 0.9991 for Metformin and 0.9955 for Teneligliptin. Linearity results of comparative data of all three methods were furnished in Table 7.

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The intermediate precision of the method was confirmed by intra-day and inter-day analysis. The concentration used for the precision studies is 10µg/mL for Metformin and 2µg/mL for Teneligliptin was assumed as 100%.

Intra-day precision: To study the intra-day precision, the analysis of drugs was repeated for six times in the same day. Six replicate mixed standard solutions of Metformin and Teneligliptin was measured with the same concentration and the %RSD was calculated. The %RSD was found to be within the acceptable criteria of not more than 2.0. Results of intra-day precision were given in Table 8.

Inter-day precision: To study the inter-day precision, the analysis of drugs was repeated for six times on six consecutive days. Six replicate mixed standard solutions of Metformin and Teneligliptin was measured with the same concentration and the %RSD was calculated. The %RSD was found to be within the acceptable criteria of not more than 2.0. Results of inter-day precision were given in Table 9.

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed drug sample solution. Percent recovery was calculated by comparing the absorbance before and after the addition of the standard drug. The standard addition method was performed at three concentration levels in triplicate at 50%, 100% and 150%. All the dilutions should be prepared from 100µg/mL of standard and sample stock solutions.

The solutions were analyzed in triplicate at each level as per the proposed method. Each recovery was made three times and average value is considered. The percent recovery at each level was calculated. The accuracy results are presented in Table 10. Satisfactory recoveries ranging from 98.60% to 99.50% for Teneligliptin and 98.91% to 99.25% for Metformin respectively were obtained by the proposed method. This indicates that the proposed method was accurate.

Specificity: The wavelength was specific for Metformin and Teneligliptin according to its structure. A study conducted to establish specificity of the proposed method involved injecting blank and placebo using the spectrophotometric conditions defined for the proposed method. It was found that there is no interference due to excipients used in the tablet formulation and also found good correlation between the absorbances of standard and sample.

Robustness: The robustness study was performed by slight modification in method parameters (change in wavelength) of Metformin and Teneligliptin. Samples of Metformin and Teneligliptin were analyzed under these changed experimental conditions. It was observed that there were no marked changes in absorbances, which demonstrated that the developed method was robust in nature.

Limit of detection and Limit of quantitation: Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantitation (LOQ) is defined as the lowest concentration that can be quantified

reliably with a specified level of accuracy and precision. For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ of Metformin and Teneligliptin are given in Table 11.

Estimation of Metformin and Teneligliptin in tablet dosage forms: Commercial formulation of tablets was chosen for testing the suitability of the proposed method to estimate Metformin and Teneligliptin in tablet formulations. Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 100mg of Metformin and 4mg of Teneligliptin was transferred to a 100 mL volumetric flask containing 70mL of 0.1N NaOH. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of 0.1N NaOH. Further pipette 1mL of the above stock solution into a 10mL volumetric flask and the volume was made up to the mark with the 0.1N NaOH. Later pipette out 1mL of above stock solution into a 10mL volumetric flask and the volume was made up to mark with 0.1N NaOH.

The tablet sample solution was also subjected to analysis by simultaneous equation method. Absorbances of sample solutions were recorded at 233nm (λmax of MET) and 241nm (λmax of TEN) and concentration of two drugs in the sample were determined by using equations 5 and 6. The same tablet sample solutions were subjected to analysis by Q ratio method. The absorbance was measured at 244nm and 233nm for both drugs. The same tablet sample solutions were subjected to analysis by dual wavelength method. The difference in absorbance was measured at 225nm and 251nm for MET and 240nm and 221nm for TEN. The analysis procedure was repeated 6 times with tablet formulations. The assay results of tablet dosage forms of are Metformin and Teneligliptin furnished in Table 12.

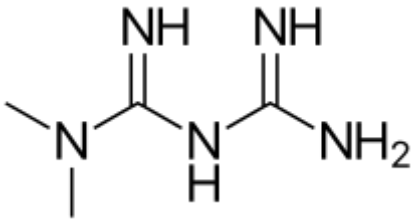

Fig. 1: Chemical structure of Metformin

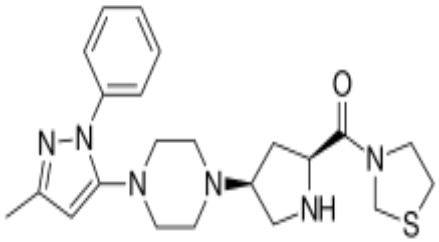

Fig. 2: Chemical structure of Teneligliptin

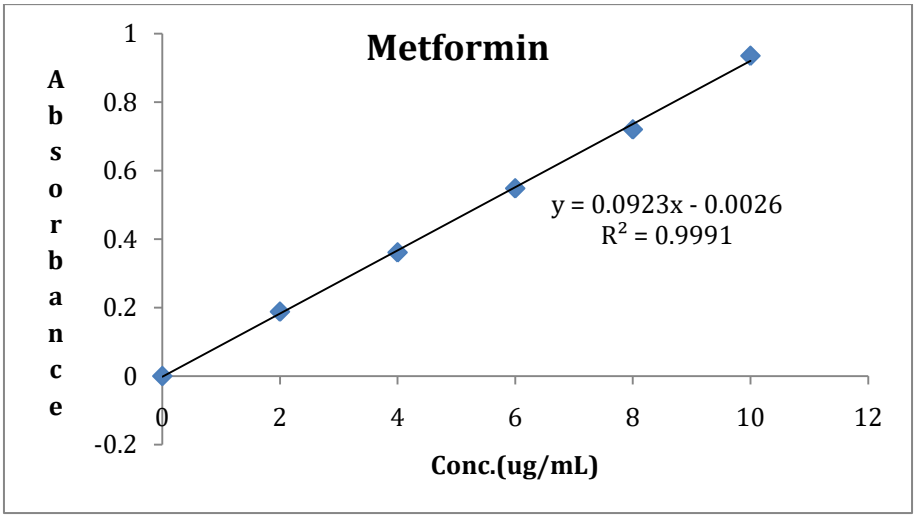

Fig. 3: Calibration curve of Metformin

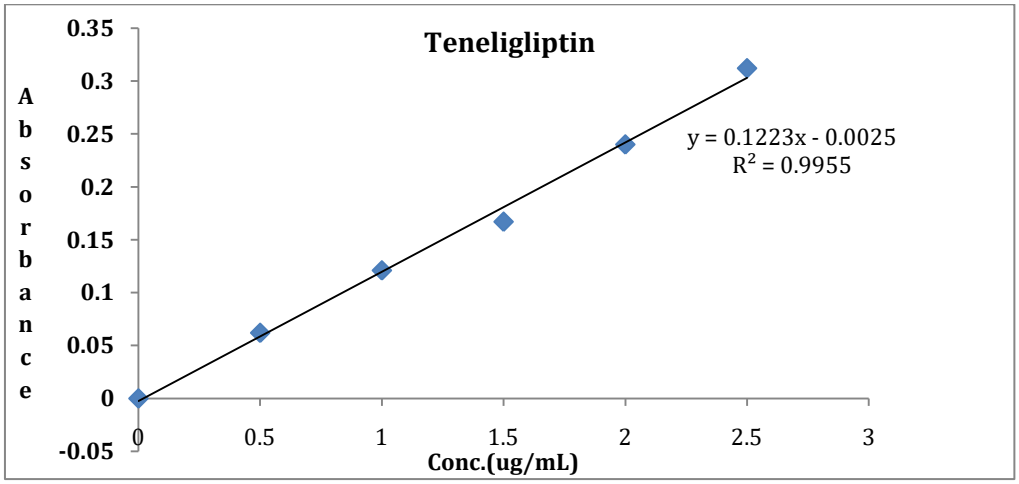

Fig. 4: Calibration curve of Teneligliptin

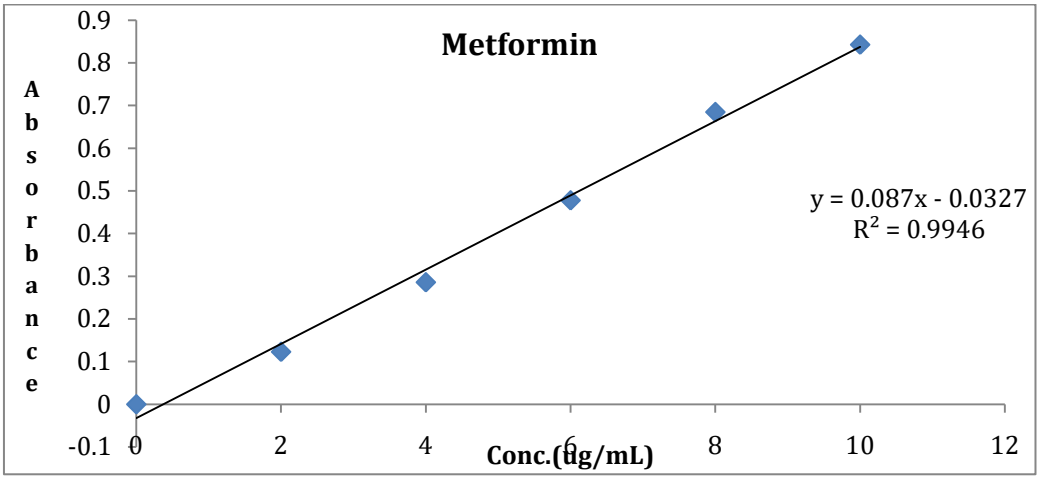

Fig. 5: Linearity curve of Metformin for dual wavelength method

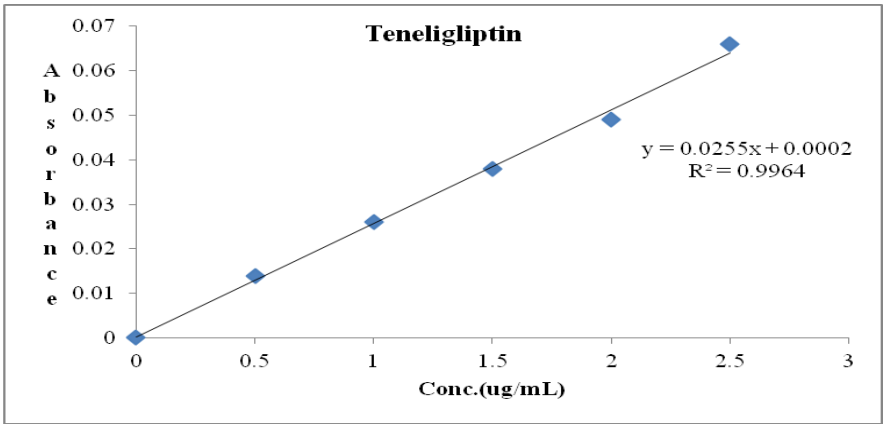

Fig. 6: Linearity curve of Teneligliptin for dual wavelength method

Table 1: Linearity results for Metformin

S. No.	Concentration (µg/mL)	Absorbance at λ1	Absorptivity at λ1 (ax1)	Absorbance at λ2	Absorptivity at λ1 (ax2)
1	0	0	0	0	0
2	2	0.188	0.090	0.127	0.064
3	4	0.361	0.091	0.244	0.061
4	6	0.548	0.090	0.383	0.064
5	8	0.720	0.094	0.497	0.062

6	10	0.935	0.094	0.607	0.061

Table 2: Linearity results for Teneligliptin

S. No.	Concentration (µg/mL)	Absorbance at λ1	Absorptivity at λ1 (ay1)	Absorbance at λ2	Absorptivity at λ1 (ay2)
1	0	0	0	0	0
2	0.5	0.062	0.124	0.039	0.078
3	1	0.121	0.121	0.075	0.075
4	1.5	0.167	0.111	0.101	0.067
5	2	0.240	0.120	0.142	0.071
6	2.5	0.312	0.1248	0.195	0.078

Table 3: Absorptivity values of Metformin for Q-ratio method

S. No.	Concentration (µg/mL)	Absorbance at λ1	Absorptivity at λ1 (ax1)	Absorbance at λ2	Absorptivity at λ1 (ax2)
1	0	0	0	0	0.094
2	2	0.152	0.076	0.188	0.090
3	4	0.315	0.078	0.361	0.091
4	6	0.486	0.081	0.548	0.090
5	8	0.624	0.078	0.720	0.093
6	10	0.781	0.078	0.935	0.094

Table 4: Absorptivity values of Teneligliptin for Q-ratio method

S. No.	Concentration (µg/mL)	Absorbance at λ1	Absorptivity at λ1 (ax1)	Absorbance at λ2	Absorptivity at λ1(ax2)
1	0	0	0	0	0
2	0.5	0.054	0.108	0.062	0.124
3	1	0.089	0.089	0.121	0.121
4	1.5	0.126	0.084	0.167	0.111
5	2	0.173	0.086	0.240	0.120
6	2.5	0.217	0.086	0.312	0.124

Table 5: Absorbance values of Metformin for dual wavelength method

S. No.	Concentration (µg/mL)	Absorbance at λ1	Absorbance at λ2	Result (λ1- λ2)
1	0	0	0	0
2	2	0.178	0.055	0.123
3	4	0.387	0.101	0.286
4	6	0.621	0.143	0.478
5	8	0.883	0.198	0.685
6	10	1.060	0.217	0.843

Table 6: Absorbance values of Teneligliptin for dual wavelength method

S. No.	Concentration (µg/mL)	Absorbance at λ1	Absorbance at λ2	Result (λ1- λ2)
1	0	0	0	0
2	0.5	0.099	0.085	0.014
3	1	0.128	0.102	0.026
4	1.5	0.168	0.13	0.038
5	2	0.199	0.15	0.049
6	2.5	0.287	0.221	0.066

Table 7: Linearity results comparative data

Methods	Method-I		Method-II		Method-III	
Parameters	**MET**	**TEN**	**MET**	**TEN**	**MET**	**TEN**
Linearity (µg/mL)	2-10	0.5-2.5	2-10	0.5-2.5	2-10	0.5-2.5
Slope	0.0923	0.1223	0.0785	0.0845	0.074	0.0255
Intercept	-0.0026	-0.0025	0.0007	0.0042	-0.0043	0.0002
Correlation coefficient	0.9991	0.9983	0.9993	0.9968	0.9942	0.9964

Table 8: Intra-day precision results

S. No.	Time (Hours)	Absorbance					
		Method-I		Method-II		Method-III	
		MET	**TEN**	**MET**	**TEN**	**MET**	**TEN**
1	0	0.935	0.312	0.835	0.695	0.719	0.052
2	3	0.932	0.325	0.834	0.693	0.723	0.053
3	6	0.919	0.316	0.835	0.696	0.725	0.054
4	9	0.924	0.32	0.839	0.698	0.724	0.053
5	12	0.921	0.322	0.838	0.699	0.726	0.053
6	15	0.926	0.314	0.832	0.697	0.721	0.054
Mean		0.926	0.318	0.836	0.696333	0.723	0.053
SD		0.006	0.005	0.003	0.00216	0.002	0.001
%RSD		0.674	1.570	0.310	0.310232	0.329	1.416

Table 9: Inter-day precision results

S. No.	Time (Hours)	Absorbance					
		Method-I		Method-II		Method-III	
		MET	**TEN**	**MET**	**TEN**	**MET**	**TEN**
1	1	0.935	0.312	0.835	0.695	0.719	0.055
2	2	0.928	0.314	0.833	0.696	0.722	0.056
3	3	0.931	0.318	0.836	0.697	0.721	0.055
4	4	0.933	0.319	0.839	0.698	0.724	0.054
5	5	0.935	0.322	0.837	0.7	0.723	0.056
6	6	0.922	0.322	0.831	0.697	0.722	0.055
Mean		0.931	0.318	0.835	0.697	0.722	0.055
SD		0.005	0.004	0.003	0.002	0.002	0.001
%RSD		0.538	1.296	0.342	0.247	0.239	1.365

Table 10: Results for recovery studies

% Level	Drug	Amount of drug added (µg/mL)	Method-I		Method-II		Method-III	
			% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
50	MET	2	100.05	±0.052	98.96	±0.063	99.98	±0.068
	TEN	1	98.95	±0.116	98.86	±0.166	100.15	±0.096
100	MET	4	99.95	±0.085	99.25	±0.078	99.56	±0.052
	TEN	2	100.26	±0.286	98.95	±0.195	98.93	±0.139
150	MET	6	99.98	±0.065	100.02	±0.064	100.28	±0.055

	TEN	3	101.15	±0.164	98.89	±0.159	101.19	±0.126

Table 11: LOD and LOQ data

Drug	Method-I		Method-II		Method -III	
	LOD (µg/mL)	LOQ (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
MET	0.033	0.099	0.164	0.498	0.103	0.093
TEN	0.031	0.095	0.035	0.108	0.012	0.283

Table 12: Assay results of Metformin and Teneligliptin formulations

Drug	Method-I	Method-II	Method-III
MET	99.25	100.25	100.52
	99.96	99.23	100.25
	100.55	98.99	99.62
	100.52	101.25	100.12
	99.85	99.36	99.32
	98.96	100.25	99.94
% Mean assay	99.85	99.89	99.962
% RSD	0.649	0.853	0.4356
TEN	100.28	100.32	101.23
	100.13	100.25	101.52
	99.95	99.36	100.25
	101.45	99.54	99.99
	100.23	98.95	100.35
	99.56	99.92	100.22
% Mean assay	100.27	99.72	100.593
% RSD	0.636	0.536	0.624

RESULTS AND DISCUSSION

The spectrophotometric study of Metformin and Teneligliptin were carried out in 0.1 M NaOH as it was found to be suitable solvent for both the drugs. Three different spectrophotometric methods were developed for simultaneous estimation of MET and TEN in pure and tablet dosage form.

Method-I: Spectral study showed that the λmax for MET at 233nm and that for TEN at 241nm. Thus these wavelengths were selected for the estimation of drugs by simultaneous equation method. Thus a simultaneous spectrophotometric method that involves solving simultaneous equations based on maximum absorptivity at 233nm in the range of 2-10µg/mL for MET and 241nm in the range of 0.5-2.5µg/mL for TEN respectively was developed.

Method-II: A reliable Q-ratio method was developed as the overlain spectra of both MET and TEN shows isobestic point at 244nm (λ_1). Metformin λmax i.e. 233nm (λ_1) was selected another wavelength for the simultaneous estimation of drugs by this method. Beer's law obeyed in the concentration range of 2-10 µg/mL for MET and 0.5-2.5 µg/mL for TEN respectively.

Method-III: A simple and rapid dual wavelength spectrophotometric method was developed for simultaneous determination of MET and TEN in combined formulation. From the overlain spectra four wavelengths are selected. The absorbance at 225 nm (λ_1) and 251nm (λ_2) wavelengths was found to be with same absorbance for TEN. These two wavelengths were selected to determine the concentration of MET from the mixture of MET and TEN. In the same way, the absorbance at 240nm (λ_3) and 221nm (λ_4) wavelengths was found to be with same absorbance for TEN. These two selected wavelengths were employed to determine the concentration of TEN from the mixture of MET and TEN. The suitable linear range for this method is 2-10µg/mL for MET and 0.5-2.5µg/mL for TEN.

All the three methods were validated as per ICH guidelines and the results are within the acceptance criteria.

CONCLUSION

Three different UV Spectrophotometric methods were proposed for simultaneous estimation of Metformin and Teneligliptin in pure and tablet dosage form using 0.1 N NaOH as solvent. All the

three methods were satisfies the Beer-Lambert's law in the range of 2-10µg/mL for Metformin and 0.5-2.5µg/mL for Teneligliptin with a low correlation coefficient. Recovery studies were performed by adding a known amount of standard drug to pre-analyzed sample and percentage recovery was found to be within the limits. % RSD for inter- and intra-day variations was found to be less than 2% disclosed the reproducibility of method. The proposed methods applied for the estimation of the drugs in marketed tablet formulation and very encouraging results were obtained. Hence the proposed methods are new, simple, economical and can be applied for the simultaneous estimation of Metformin and Teneligliptin in pharmaceutical formulation.

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