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Research Article

RP-HPLC and UV-Spectrophotometric Methods Development and Validation for Simultaneous Estimation of Teneligliptin and Metformin in Fixed Dose Combination.

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ABSTRACT

The reliable, economical, sensitive and reproducible RP-HPLC and UV- Spectrophotometric methods were developed and validated for the simultaneous estimation of Teneligliptin (TEN) and Metformin (MET) in combined dosage form. In the RP-HPLC method the mobile phase used was 50mM potassium dihydrogen orthophosphate (KH_2PO_4) buffer: Methanol (40:60) at P^H 3.0 and flow rate was 1.0 ml per min. The method was scanned at λmax 250 nm for both the drugs. The linearity range for MET and TEN was found to be 200 - 600 µg/ml and 1 - 30 µg/ml with regression correlation coefficient (R^2) 0.9996 and 0.9991 respectively. The retention time for Metformin and Teneligliptin was found to be 10.3 min and 21.56 min respectively. The UV-Spectrophotometric simultaneous equation and Absorption Ratio methods were developed and validated in which the λmax for Metformin and Teneligliptin was found to be 232 nm and 243 nm, the Isoabsorbtive point was found to be 249 nm. The linearity for both the drugs was found to be 5-30 µg/ml and regression coefficient equation was 0.9896 and 0.9988 for MET and TEN respectively. The developed methods of RP-HPLC and UV-Spectrophotometry were validated as per ICH guidelines.

KEYWORDS

Teneligliptin (TEN), Metformin (MET), Correlation equation; RP-HPLC, UV-Spectrophotometry, ICH Guidelines.

1. INTRODUCTION

Teneligliptin is a long-acting, orally bioavailable, pyrrolidine-based inhibitor of dipeptidyl peptidase 4 (DPP-4), with hypoglycemic activity. Teneligliptin may also reduce plasma triglyceride levels through a sustained increase in GLP-1 levels.[1] (Fig. 1)

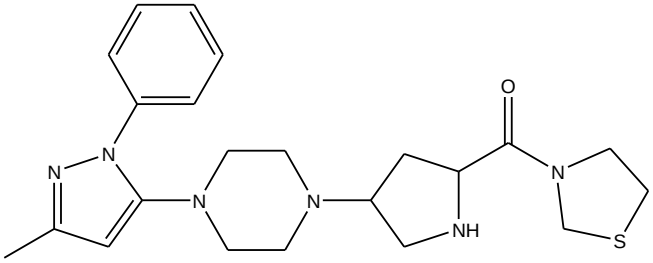

Fig. 1. Chemical structures of Teneligliptin (TEN*)*

Metformin hydrochloride is a biguanide hypoglycemic agent used in the treatment of noninsulin-dependent diabetes mellitus not responding to dietary modification. Metformin improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose, [2] (Fig. 2)

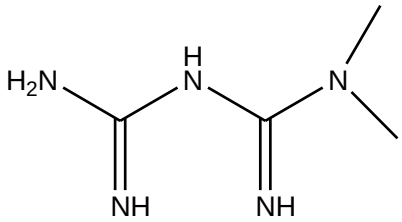

Fig. 2. Chemical structures of Metformin hydrochloride (MET).

The literature survey revealed that the Metformin individually and in combination with other drugs like Saxagliptin[3], Pioglitazone[4], Vildagliptin[5] and Glimeperide[6] is estimated. Similarly Teneligliptin individually is also estimated by using RP-HPLC and UV-Spectrophotometry. There are very few methods available for estimation of Teneligliptin and Metformin[7] in combined dosage forms; hence there is a need to develop the new, economical, sensitive and reliable method for estimation of Teneligliptin and Metformin in combined dosage forms.

2. MATERIALS AND METHODS

2.1. UV-Spectrophotometric Method

The UV method was performed on Schimadzu double beam spectrophotometer (Model: UV-1800) with 2 nm spectral bandwidth using 10 mm matched quartz cuvette. Data acquisition was done by using UV-Probe software version 2.42. The absorption spectra of reference and test solution were carried out over the range of 200–400 nm.

2.1.1. Determination of wavelength of maximum absorbance (λ_{max}) of TEN and MET

Wavelength of maximum absorption was determined by scanning 10 µg/ml solution of TEN and MET using UV–Visible double beam spectrophotometer by scanning from 200 to 400 nm using

Distilled Water as blank. The λmax Found for Metformin and Teneligliptin were 232nm and 243 nm, the Isoabsorbtive Point was found to be 249 nm (Fig. 5).

1.1.2. Preparation of stock solutions and test solutions (TEN, MET)

The stock solutions (1000 µg/ml) of TEN, MET were prepared by adding accurately weighed 100 mg of TEN and MET drugs in 100 ml of Distilled Water, then sonicated for 10 min and diluted up to 100 ml. Further pipette out 10 ml of this solution in 100 ml volumetric flask and make up the volume with Distilled Water to give the concentration 100 µg/ml. Series of test solutions were prepared in the concentration range of 05-30 µg/ml for Metformin and Teneligliptin by diluting appropriate volume of the stock solution with Distilled Water. The dilutions were first vortexed and then used for further analysis.

1.1.3. Preparation of calibration curve

The calibration curve was prepared by scanning test samples ranging from 1–30 µg/ml at 232 nm and 243 nm for TEN and for MET. The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, intra-day and inter-day measurements, accuracy and precision were determined for both. Mean of n = 6 determinations was plotted as the standard curve. The Calibration Curves are shown in Fig. 3 and Fig. 4 below for Metformin and Teneligliptin respectively.

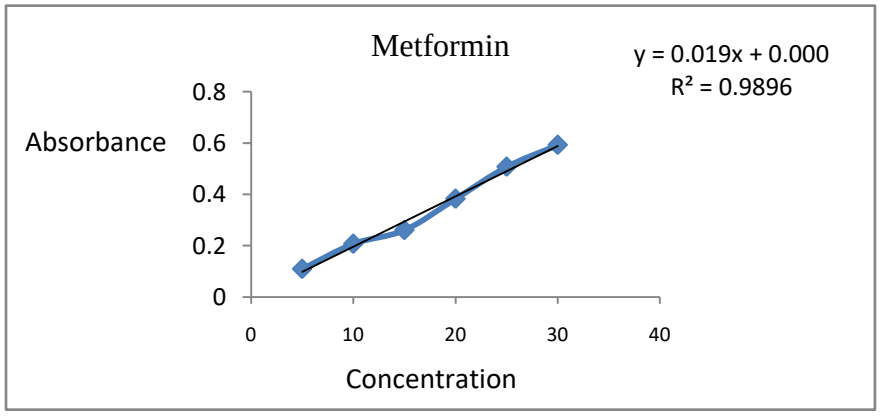

Fig. 3. Calibration Curve for Metformin.

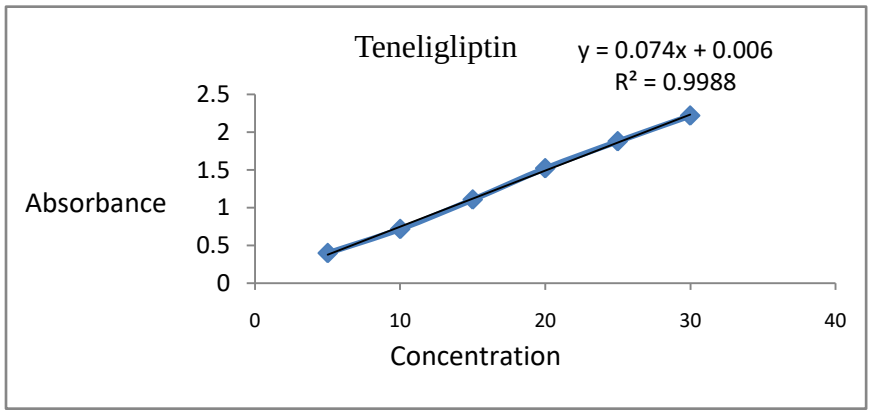

Fig. 4. Calibration Curve for Teneligliptin.

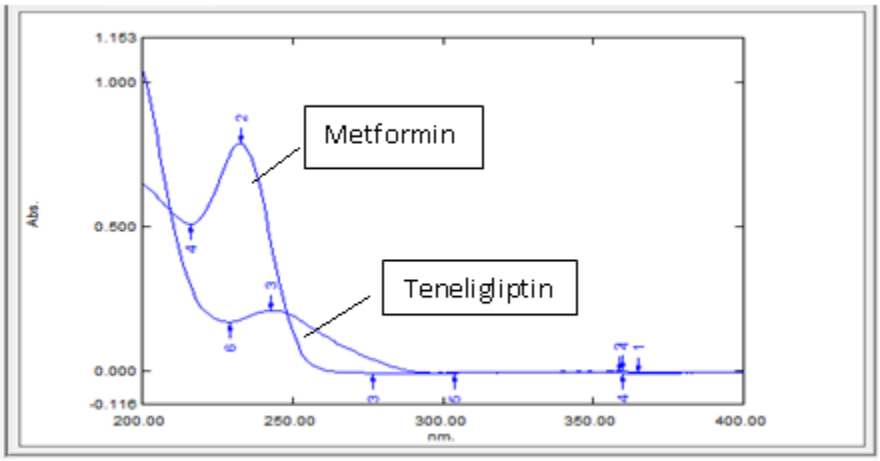

Fig. 5. Overlain spectra of Teneligliptin and Metformin.

2.1.4. Correlation equation method

Six standard solutions of each drug having concentration in the range of 05-30 µg/ml for Metformin and Teneligliptin were prepared in Distilled Water and absorbance at 232 nm and 243 nm was measured. Mixed standards containing TEN and MET were also prepared in the range of 05-30 µg/ml for Metformin and Teneligliptin. Using these data a regression correlation coefficient equation was established and was found to be 0.9896 and 0.9988 for MET and TEN respectively.

2.1.5. Method validation

2.1.5.1. Linearity

The methods were validated according to International Conference on Harmonization Q2B guidelines [8] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Calibration curves were generated with appropriate volumes of working standard solutions for both UV with the range of 5–30 µg/ml for both the drugs. The linearity was evaluated by the least square regression method using unweighted data. The (Fig. 3 and 4) above and the overlaid spectra is as above in the Fig. 5.

2.1.5.2. Precision and accuracy

Both precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range. The intermediate precision was studied by comparing the assays on 3 different days and the results documented as standard deviation and % R.S.D.

Accuracy was determined from nine determinations over three concentration levels covering the specified range. The results obtained for the same are shown in the Table 1, 2 and 3.

2.1.5.3. Specificity

The method specificity was assessed by comparing the scans obtained from the drug and the most commonly used excipient mixture with those obtained from blank.

2.1.5.4. LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated as, (Equation 1)

LOD ¼ 3:3r = S ---Equation 1
LOQ ¼ 10 r = S

Where r is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.

2.1.6. Analysis of marketed tablet formulation (ZITA-MET PLUS)

Twenty tablets of marketed formulation (ZITA-MET PLUS) were weighed and grounded to obtain fine powder. Accurately weighed powder sample equivalent to 10 mg of TEN and MET was dissolved in a 100 ml volumetric flask containing distilled water. The solution was kept for sonication for 20 min, filtered through whatmann filter paper No. 41. Aliquot of this solution was diluted to produce the concentration of 10 µg/ml for TEN and MET (n = 6). The absorbance of sample solutions at 232 nm and 243 nm was measured and the amount of drug present in the sample solution was calculated in the sample manner as that of pure mixed standard solution. The results of analysis and statistical validation for the marketed tablet formulation are reported in Table 3. The results of recovery studies conducted by the addition of different amounts of pure drugs at three different levels to a tablet solution were found to be satisfactory. (Table 2)

Table 1. Summary of the HPLC method and UV method validation.

Parameters	**HPLC method**		**UV-method**	
	TEN	**MET**	**TEN**	**MET**
Working λ_{max}	250	250	233 nm	243 nm
Beer's law limit	1-30 µg/ml	200 - 600 µg/ml	01–30 µg/ml	01 – 30 µg/ml
Regression coefficient (r_2)	0.9896	0.9988	0.998	0.989
Absorptivity*	NA	NA	749.87 17.016	42.32 199.28
Retention time (min)*	21.56	10.3	NA	NA
LOD*	0.11 µg/ml	2.97 µg/ml	0.291	0.070
LOQ*	0.34 µg/ml	9.02 µg/ml	0.883	0.212

Average of three determinations*

Table 2. Results of recovery study by UV methods.

Method	**Drug**	**% Recovery**	**Qty. present (µg/ml)**	**Qty. added (µg/ml)**	**Qty. found*** **(µg/ml)**	**Qty. Recovered*** **(µg/ml)**	**% Recovery***	**SD**	**% RSD**
UV method	TEN	50	10	05	15	14.97	99.80	0.68	04.54
		100	10	10	20	20.12	100.60	0.20	01.02

SEM		150	10	15	25	25.46	98.19	0.63	02.50
		50	10	05	15	14.56	97.06	0.47	03.24
	MET	100	10	10	20	20.17	100.85	0.67	03.33
		150	10	15	25	24.24	98.16	0.10	00.43
		50	10	05	15	14.14	94.30	0.16	01.19
	TEN	100	10	10	20	20.48	102.43	0.41	02.03
UV method ARM		150	10	15	25	25.19	100.77	0.60	02.41
		50	10	05	15	15.20	101.36	0.30	01.99
	MET	100	0	10	20	20.36	101.80	0.41	02.03
		150	0	15	25	25.46	101.85	0.60	02.40

*Average of 3 determinations.
SEM: - Simultaneous Equation Method
ARM: - Absorption Ratio Method

2.2. RP-HPLC method

2.2.1. Reagent and chemicals

Teneligliptin and Metformin (Ipca Laboratories Ltd, Mumbai) were received as gift sample. Marketed formulation (ZITA MET PLUS, Glenmark, India), containing 20 mg of TEN and 500 mg of MET was procured from local market. HPLC grade Methanol and purified grade potassium dihydrogen phosphate were purchased from Research Labs Ltd, India respectively. All other reagents employed were of high purity analytical grade. All weighing was done on a calibrated analytical balance. Calibrated glass wares were used throughout the work. Distilled water and double distilled water were used in the UV-Spectrophotometry and RP-HPLC method respectively.

2.2.2. Instrumentation

The HPLC method was performed on a system equipped with a universal loop injector (Rheodyne), PDA detector and HPLC pump. The column used was C18 (150 mm × 4.6 mm, 5.0 µ). The mobile phase used was 50mM potassium dihydrogen orthophosphate buffer: Methanol (40:60) at PH 3.0 by using orthophosphoric acid. Injection volume was 20µL. The flow rate was set to 1 ml/min and detection of both drugs was carried out at 250 nm by PDA detector.

2.2.3. Chromatographic Condition

The optimized composition of the mobile phase used was 50mm potassium dihydrogen orthophosphate buffer: Methanol (40:60) at PH 3.0 adjusted by using orthophosphoric acid. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min).Stock solution was prepared by dissolving TEN and MET (100 mg each) that were weighed accurately and separately transferred into 100 ml volumetric flasks. Both drugs were dissolved in 100 ml of mobile phase to prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with mobile phase. These standard stock solutions were observed to contain 1000 µg/ml of TEN and MET. Further 10 ml was pipette out in 100 ml volumetric flask and made up the volume up to the mark with mobile phase. Appropriate volume from this solution was further diluted to get appropriate concentration levels according to the requirement. From the above stock solutions, dilutions were made in the

concentration range of 200-600 µg/ml for Metformin and 1-30 µg/ml for Teneligliptin. A volume of 20 µL of each sample was injected into column.

2.2.4. Preparation of buffer

The buffer used was 50mm potassium dihydrogen orthophosphate buffer at PH 3.0 adjusted by using orthophosphoric acid. The pH was adjusted by orthophosphoric acid using pH meter (Equiptronics, India). The prepared buffer was passed through 0.22 µm membrane filter (Millipore, USA) and the same was used for mobile phase preparation.

2.2.5. Preparation of mobile phase

Mobile phase was prepared by mixing 50mm potassium dihydrogen orthophosphate buffer: Methanol (40:60) at PH 3.0 adjusted by using orthophosphoric acid. Mixture was shaken vigorously and sonicated for 30 min prior to use.

2.2.6. Preparation of stock solutions and test solutions (TEN, MET and binary mixture)

Aqueous solution (1000 µg/ml) of TEN, MET and its binary mixture was prepared by adding accurately weighed 100 mg of TEN and MET and binary mixture of both drugs in 100 ml of mobile phase then sonicated for 10 min and diluted up to 100 ml. Further pipetted out 10 ml of the solution in 100 ml volumetric flask and make up the volume with mobile phase to give concentration 100 µg/ml. Series of test solutions were prepared in the concentration range of 200-600 µg/ml for Metformin and 1-30 µg/ml for Teneligliptin by diluting appropriate volume of the stock solution (100 µg/ml) with mobile phase. The dilutions were first vortexed and then used for further analysis.

2.2.7. Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 200-600 µg/ml for Metformin and 1-30 µg/ml for Teneligliptin solutions manually in triplicate to the HPLC system at detection wavelength of 250.0 nm. Mean of n = 6 determinations was plotted as the standard curve. The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both inter-day and intra-day measurements.

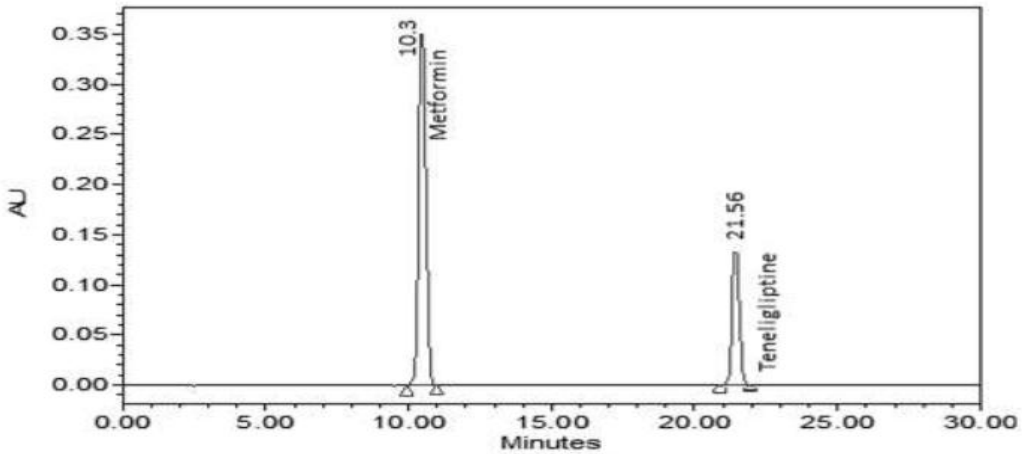

Fig. 6. Chromatogram of Combination of Teneligliptin and Metformin

Table 3. Assay of marketed formulation ZITA-MET PLUS by HPLC and UV methods.

Assay	Drug	Label claimed (mg/tab)	Content found (mg/tab)***	% Label claimed	SD*	% RSD**
HPLC method	TEN	20	20.18	100.94	0.083	0.326
	MET	500	499.55	99.91	0.816	0.819
UV method	TEN	20	19.86	99.34	0.446	2.4
	MET	500	500.90	100.18	0.425	2.236

*** Average of six determinations.

Table 4. Assay of marketed formulation ZITA-MET PLUS by HPLC.

	Level of recovery Study	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered	S.D. (n = 3)	% Recovery	% RSD
Metformin	50%	200	100	100.65	0.1823	0.1823	0.1053
	100%	200	200	196.86	1.3424	0.7804	0.7895
	150%	200	300	299.47	0.0251	0.0146	0.0146
Teneligliptin	50%	10	5	4.9718	0.1401	0.0814	0.0819
	100%	10	10	9.9676	0.3165	0.1840	0.1846
	150%	10	15	14.9835	0.3218	0.1871	0.1870

3. RESULTS AND DISCUSSION

RP-HPLC and UV-Spectrophotometric methods were developed for TEN and MET which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The chromatographic conditions were optimized in order to provide a good performance of the assay. The retention times (R_t) for TEN and MET was found to be 10.2 min and 21.56 min respectively. The chromatogram shows good resolution (Fig. 6). The regression with correlation coefficient (R^2) for MET and TEN was found to be 0.9896 and 0.9988 respectively with less than 2% RSD (Table 1). The developed HPLC method was accurate, precise, reproducible and very sensitive. All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines. (Table 1 and 2) The intra-day and inter-day precision at different concentration levels was found to be less than 2%. The percent recovery of both drugs in the commercial formulations by UV-Spectrophotometry and RP-HPLC was found to be in specified limit (Table 2 and 4 respectively). The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. Hence, the methods were suitably employed for assaying both the drugs in commercial marketed formulation (Table 3).

4. CONCLUSION

Simple, rapid, accurate and precise RP-HPLC as well as UV-Spectrophotometric methods have been developed and validated for the routine analysis of TEN and MET in tablet dosage forms without interference of each other. The results obtained for validation of these methods are satisfactory in the specified limit as per ICH guidelines hence these methods can also be conveniently adopted for estimation of TEN and MET in commercial formulation.

5. ACKNOWLEDGEMENT

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