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DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF TENELIGLIPTIN HYDROBROMIDE HYDRATE AND METFORMIN HYDROCHLORIDE IN COMBINED DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, reproducible and economical Dual Wavelength UV Spectrophotometric Method for estimation of Teneligliptin Hydro bromide hydrate and Metformin Hydrochloride in tablet dosage form has been developed.

Dual wavelength method is based on selection of wavelengths, where one drug shows equal absorbance (or difference between absorbance is zero) and other drug shows some response. The wavelengths selected for determination of Teneligliptin were 236 nm and 230.5 nm. Whereas, wavelengths selected for determination of Metformin were 238 nm and 249 nm. Methanol was taken as solvent.

Regression analysis of Beer's plots showed good correlation in concentration range of 5 - 25 µg/ml for Teneligliptin and 2-10 µg/ml for Metformin. Recovery studies for Teneligliptin and Metformin were performed and the percentage recovery for both the drugs was obtained in the range of 98.54-101.55% confirming the accuracy of the proposed method. The precision (interday, intraday and repeatability) of method was found within limits. The proposed method was successfully applied to determination of these drugs in commercial tablets.

KEYWORDS: Teneligliptin Hydro bromide hydrate, Metformin Hydrochloride, Dual wavelength method, Method validation.

INTRODUCTION:

Teneligliptin Hydro bromide hydrate (TNL) is a novel inhibitor of enzymatic activity of dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors have recently emerged as a new class of anti-diabetics that show favorable results in improving glycemic control with a minimal risk of hypoglycemia and weight gain. DPP-4 inhibitors stimulate glucose dependent insulin secretion and reduces glucagon level (1). Teneligliptin, is chemically known as a 3- {((2S,4S)-4-(4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl)pyrrolidin-2-yl carbonyl}thia zolidine hemi penta hydro bromide hydrate (2).

Metformin hydrochloride (MET) is chemically N, N dimethyl imido di carbonimidic diamide hydrochloride (1, 1- dimethyl biguanide hydrochloride) which acts by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity.

From literature survey it was found that, two UV spectrophotometric and HPLC method have been reported for estimation of TNL and MET in their combined dosage form (3, 4, 5).

The UV spectrophotometric method is often preferred over other more sensitive methods in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use. The aim of the present work is to develop simple, sensitive and reproducible UV Spectrophotometric method for estimation of TNL and MET in their combined tablet dosage form and hence, an economical method was developed and validated according to the ICH guidelines (6).

MATERIAL AND METHODS

Instruments

Absorbance measurements were made on Double beam UV-Visible spectrophotometer (Shimadzu UV-1700) having two matched quartz cells with 1 cm light path. Electronic analytical balance (Shimadzu AUW-220D) for weighing and Sonicator (Trans-O-Sonic) were used.

Chemicals and reagents

All chemicals were of analytical reagent grade. Teneligliptin Hydrobromide Hydrate was procured from Chemipharma Pvt. Ltd. Mumbai, India and Metformin Hydrochloride was gift sample from Dolffin Pharmaceuticals, Surat, India.

Procedure

Preparation of stock solution (1000µg/mL)

Accurately weighed 100 mg of Teneligliptin Hydro bromide Hydrate and Metformin were transferred to separate 100-ml volumetric flask. It was dissolved in about 70 ml of methanol by sonication for 5 min. The volume was adjusted to 100 ml with methanol to get concentration of 1000 µg/ml.

Preparation of working standard solution (100µg/mL)

From the above stock solution 10 ml each of TNL and MET was taken, transferred to separate 100ml volumetric flasks and the volume was made up to 100 ml with methanol.

Calibration Curve

For calibration curve, from the working standard solutions, appropriate dilutions in the range of 5-25 µg/ml and 2-10 µg/ml were prepared and analyzed for TNL and MET respectively.

Procedure for determination of wavelength for measurement

Solutions of pure MET (8 µg/ ml) and TNL (20 µg/ ml) were scanned in the SPECTRUM basic mode. Using the cursor function, two wavelengths have been selected at which one analyte shows same absorbance and at this two wavelengths difference in absorbance is used for estimation of second analyte. The difference in absorbance between 236.00 nm and 230.5 nm (difference is zero for MET) were plotted against the concentration of TNL. Similarly difference in absorbance between 238.00 nm and 249.00 nm (difference is zero for TNL) were plotted against the concentration of MET. (figure. 1).

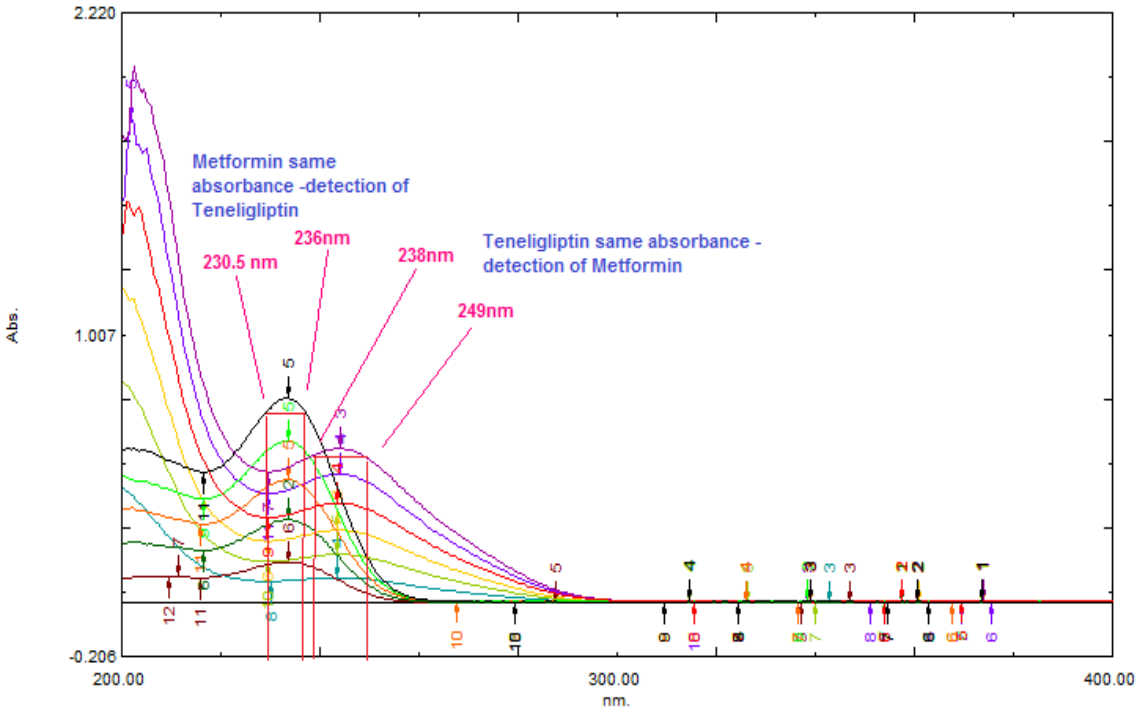

Figure 1: Overlain spectra of different concentrations of TNL (5-25 µg/ml) and MET (2-10 µg/ml)

METHOD VALIDATION

Method was validated accordance to ICH guidelines for linearity, range, precision, accuracy and ruggedness.

1. Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5-25 µg/ml and 2-10 µg/ml for TNL and MET respectively (n=3). The solutions were analyzed.

2. Precision

Precision of the methods was determined by performing method repeatability studies, interday variation and intraday variation. In repeatability study, standard solution of 10, 15, 20 µg/ml of TNL and 4, 6, 8 µg/ml of MET measured three times. In intraday variation the absorbance of standard solutions of 5-25 µg/ml TNL and 2-10 µg/ml of MET were measured three times in a day. In interday variation, the absorbance of standard solutions of 5-25 µg/ml TNL and 2-10 µg/ml of MET were measured on three consecutive days and analysed.

3. Accuracy and Recovery studies

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels according to ICH guidelines. A series of solutions of TNL and MET at 80%, 100%, and 120% of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the absorbance values at determined wavelengths. To a fixed concentration of the formulation, varying concentrations of pure drug solutions were added and percentage recoveries calculated.

4. Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous samples by different analysts using similar operational and environmental conditions.

Procedure for Assay of tablet dosage form

20 tablets were weighed and powdered. Powder equivalent to 10 mg of TNL and 250 mg of MET was weighed and transferred to a 100 ml of volumetric flask and dissolved in 70 ml methanol. This tablet solution was sonicated for 15 minutes, diluted up to mark with methanol and filtered through Whatman filter paper no.42. First few ml of filtrate were discarded (100 µg/ml of TNL and 2500 µg/ml of MET). 2.0 ml of the above solution was diluted to 10 ml with methanol. (20 µg/ml of TNL and 500 µg/ml of MET). Measurement of Teneligliptin was done from this solution. Further 1 ml of the above solution was diluted to 50 ml with methanol. (0.4 µg/ml of TNL and 10 µg/ml of MET). Measurement of Metformin was done from this solution. Each solution was analyzed using developed method. The concentration of each drug was calculated using equation of straight line.

RESULT AND DISCUSSION

The wavelengths selected for determination of Teneligliptin were 236 nm and 230.5 nm, where Metformin show equal absorbance. Wavelengths selected for determination of Metformin were 238 nm and 249 nm, where Teneligliptin show equal absorbance.

The response for the TNL was found to be linear in the concentration range 5-25 µg/ml and response for the MET was found to be linear in the concentration range 2-10 µg/ml. The low % RSD of intraday and interday indicate that the proposed method is precise. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table-1. The recovery experiment was performed by the standard addition method. The results (Table-2) obtained (n=3 for each level 80%, 100%, 120% level) indicated the % recovery in range of 99.07-101.38 and 98.54-101.55 for TNL and MET, respectively. These values of recovery experiment reveal that the proposed method is highly accurate. The proposed validated method was successfully applied for determination of TNL and MET in their tablet dosage form. The % assay of TNL and MET in tablet samples was calculated compared with label claim and recorded in Table-3. No interference of the excipients with the absorbance of analytes of interest observed; hence the proposed method is applicable for the routine analysis of TNL and MET in tablet dosage form

REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR TNL AND MET

Sr. No	Parameters	Result	
		Teneligliptin Hydro bromide Hydrate	Metformin Hydrochloride
1	Linearity and Range	5-25 µg/ml	2-10 µg/ml
2	Correlation coefficient	0.9929	0.9998
3	Straight line equation	y = 0.0012x - 0.0002	y = 0.0495x + 0.0022
4	Precision (% C.V.)		
	Repeatability (n=3)	2.43 -4.33	0.50-0.1.56
	Intraday precision (n=5)	1.84- 5.00	0.61-1.50
	Interday precision (n=5)	1.82 – 5.00	0.64-1.58
5	Accuracy (%recovery)	99.07-101.38	98.54-101.55
6	Ruggedness (%C.V) (n=2)	0.21 -0.86	0.13 - 1.08

TABLE 2: RECOVERY DATA OF TNL AND MET

Drug	Amount in sample (µg)	Amount of Std added (µg)	Total amount (µg)	Total amount of Std found (µg) Mean ± S.D (n=3)	% Recovery of Std (n=3)
TNL	10	8	18	8.11 ± 0.0833	101.38
	10	10	20	10.05 ± 0.1273	100.55
	10	12	22	11.89 ± 0.2926	99.07
MET	4	3	7	2.99 ± 0.0534	98.54
	4	4	8	4.03 ± 0.0562	100.92
	4	5	9	5.08 ± 0.0925	101.55

CONCLUSION

Based on the results which have been obtained from the analysis using proposed method, it can be concluded that the method has linear response in the range 5-25 µg /ml for TNL and 2-10 µg/ml for MET. The result of the analysis of marketed tablet dosage form by the proposed method is highly reproducible, reliable, as well as in agreement with label claim of the drugs. The additive present in the tablet dosage form did not interfere in the analysis. So that, the method can be used for the routine analysis of drugs in combination.

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