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DEVELOPMENT AND VALIDATION OF AN UV-SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND METFORMIN

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ABSTRACT

Teneligliptin Hydrobromide is a recently developed oral dipeptidyl peptidase 4 inhibitor indicated for the management of type 2 diabetes mellitus (T2DM) in adults along with diet and exercise. It has a potent and a long lasting antidiabetic action. Metformin is a biguanide which also is an antidiabetic and works by improving the peripheral sensitivity to insulin. A combination of these two drugs produced a synergistic effect and better control of blood sugar. Literature survey reveals some methods of simultaneous estimation of these two drugs in combination which include HPLC LC-MS/MS, RP-HPLC and very few UV methods using organic solvents. In this work an attempt was made to develop a green, ecofriendly, simple UV spectrophotometric method based on simultaneous equation method for the simultaneous estimation of the drugs using water as diluent. The wavelengths chosen were 243 nm as λmax of Teneligliptin and 233 nm as λmax of Metformin. The Beer Lambert's range for the two drugs was 5-50µg/mL, with correlation coefficient(r^2) of 0.999. The method was found to be precise as %RSD for Interday and intraday precision of the method was< 2%. The method was accurate as % recovery at 80%, 100% and 120% was within acceptance limits. The developed method was applied to the analysis of a marketed formulation of the two drugs and the % assay results were within 90-110%. Hence, the developed method can be successfully used as a quality control tool for the routine analysis of the drugs in bulk and in combined dosage form.

KEYWORDS: UV-visible spectrophotometer, Teneligliptin, Metformin.

INTRODUCTION

Diabetes is a chronic condition associated with abnormally high levels of sugar (glucose) in the blood. Insulin produced by the pancreas lowers blood glucose.[1] Absence or insufficient production of insulin, or an inability of the body to properly use insulin causes diabetes. The two types of diabetes are referred to as type I and type II. Type I is treated with insulin and type II with oral medications.[2,3]

Different categories of oral drugs are used in case of type II diabetes. DPP IV inhibitors or gliptins are available in combination with biguanides or sodium glucose co-transporter 2 inhibitors (SGLT2) inhibitors. Teneligliptin is a DPP-IV inhibitor which has a potent and a long lasting antidiabetic action Metformin is a biguanide which also is an antidiabetic and works by improving the peripheral sensitivity to insulin. It is widely used as an oral agent for the treatment of type 2 diabetes mellitus. Metformin helps in weight loss and has a record of efficacy and safety, with a lowest risk of hypoglycaemia.[4] Teneligliptin add-on to Metformin during the early course of treatment helps in delaying the exhaustion of pancreatic islet function.[5] Chemically Teneligliptin is 1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4-[(3S,5S)-5-(1,3-thiazolidine-3-carbonyl)pyrrolidin-3-yl]piperazine and Metformin is 1-carbamimidamido-N,N-dimethylmethanimidamide.[6,7]

A combination of these two drugs produced a synergistic effect and better control of blood sugar. Literature survey reveals LC-MS methods[8] and HPLC methods[9-15] and very few UV methods for simultaneous estimation of these two drugs using organic solvents.[16-19] In this work an attempt was made to develop a green, ecofriendly, simple UV spectrophotometric method based on simultaneous equation method for the estimation of the drugs using water as diluent.

MATERIALS AND METHODS

Instruments: UV-visible spectrophotometer Schimadu-1800, Sonicator- Ultrasonics, Weighing balance – CITIZEN- CY204.

Chemicals and reagents: Teneligliptin Hydrobromide and Metformin Hydrochloride were obtained as Gift samples from Zydus Cadila and Indocco Remedies Pvt Ltd, Goa respectively. Tablet dosage form was purchased from local market.

Trade name	Company	Dose
Tenglyn M	Zydus Cadilla	Metformin -500 mg, Teneligliptin- 20 mg.

Preparation of Standard: (1000 mcg/ml)

About 50 mg of Teneligliptin Hydrobromide and Metformin hydrochloride was accurately weighed and transferred into two separate 50 mL volumetric flasks and dissolved with sufficient volume of diluent. Volume was made up to 50 mL with diluent to get concentration of 1000 µg/mL. Solutions were scanned in the UV region 200 nm to 400 nm using diluent as blank. The λmax of the drugs were obtained.

Preparation of working standard

The standard solutions were then diluted to get working standard solutions of the Teneligliptin and Metformin in the concentration range of 10-100 mcg/ml.

Analysis of Tablet Formulation

10 Tablets were accurately weighed and finely powdered with mortar and pestle. About 0.1 gm of the tablet powder containing about to 1.86 mg of Teneligliptin Hydrobromide 45.66 mg of Metformin hydrochloride and was weighed and transferred to 100 mL volumetric flask dissolved in water sonicated for 10 min and volume was made up to the mark. Solution was filtered using whattman filter paper (No.1). From above filtrate, 1 mL was pipetted out into a 10 mL volumetric flask and volume was made up to mark with diluent. The absorbance of the resulting solution was recorded at predetermined wavelengths The concentrations Cx and Cy were determined using the simultaneous equation method and the percent purity was calculated.

Validation parameters

1. Linearity

The solutions followed Beer Lambert's law in concentration range of 5- 50µg/mL and with correlation coefficient (r^2) = 0.999 and 0.999 for Teneligliptin Hydrobromide and Metformin hydrochloride respectively.

2. Precision

Intra-day Precision

From the mixed standard solution of drugs 2.5mL was withdrawn and transferred into 10 mL volumetric flasks and volume was made up to the mark with distilled water. Absorbance of

these solutions in triplicate were recorded against blank at predetermined wavelengths. The procedure was repeated three times in a day.

Inter-day precision

From the mixed standard solution of drugs 2.5mL was withdrawn and transferred into 10 mL volumetric flasks in triplicate and volume was made up to the mark with distilled water. Absorbance of these solutions were recorded against blank at predetermined wavelengths. This formed results of Day1. Similarly fresh solutions were prepared on Day 2 and Day 3 and absorbances were measured.

3. Accuracy (50%,100% and 150%.)

A quantity of 25 mg of placebo powder was weighed and transferred into 3 sets of volumetric flasks, each set in turn consisting of 3 volumetric flasks of 25mL. To the first set (50% level), second set (100% level) and to the third set (150% level). To this 2.0 2.5 and 3.0 ml of of mixed standard solution of drugs was added. About 17mL of distilled water was added to all the 3 sets and sonicated for about 5 minutes. Volume was made up to 25mL with the same diluent, and filtered through a Whatman filter paper. Fromthis about 2 ml was taken and diluted to 10 ml to give a conc of 16, 20 and 24 mcg/ml. Absorbance of these f was recorded at predetermined wavelengths.

Blank solution was prepared by weighing a 25mg of placebo powder and following the same procedure as above.

4. Limit of detection (LOD)

LOD was calculated using slope and standard deviation response of calibration curves of drugs at particular wavelength.

$DL=3.3\sigma/S$

5. Limit of quantitation (LOQ)

LOQ was calculated using slope and standard deviation response of calibration curves of drugs at particular wavelength.

$QL=10\sigma/S$

6. Robustness

Robustness of developed method was determined by.

Performing analysis by different analyst

Using a different UV spectrophotometer instrument,

By repeating the analysis on a different day

RESULTS AND DISCUSSION

Method development

Selection of solvent: The ideal solvent chosen for stock preparation was water based upon the solubility characteristics.

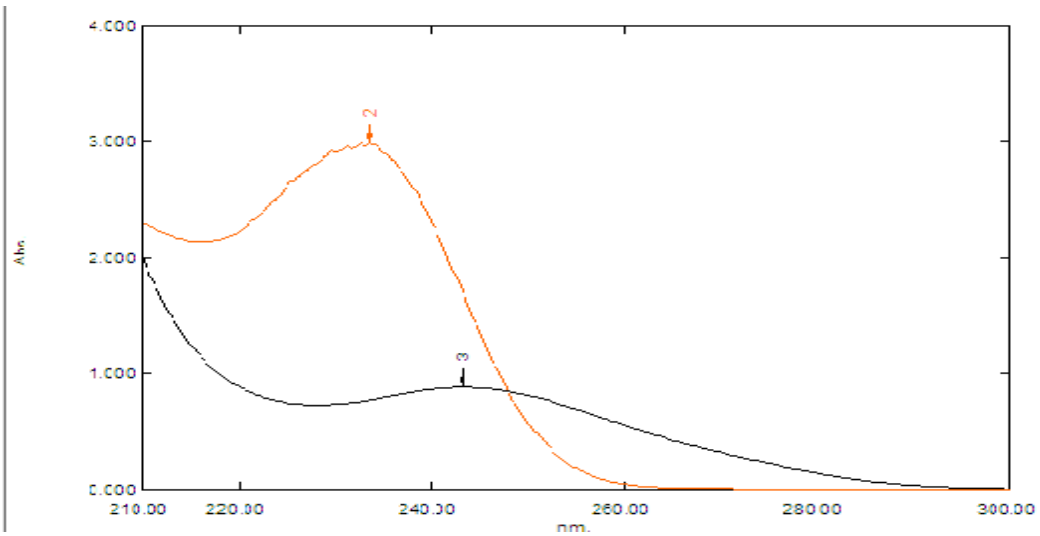
Selection of wavelengths for estimation of drugs

UV spectras of the standard drugs were obtained. Based upon the overlain spectra, the wavelengths at which both the drugs show maximum absorbance were chosen for analysis.

243 nm for Teneligliptin hydrobromide

233 nm for Metformin hydrochloride as absorbance of both the drugs was max at this wavelength.

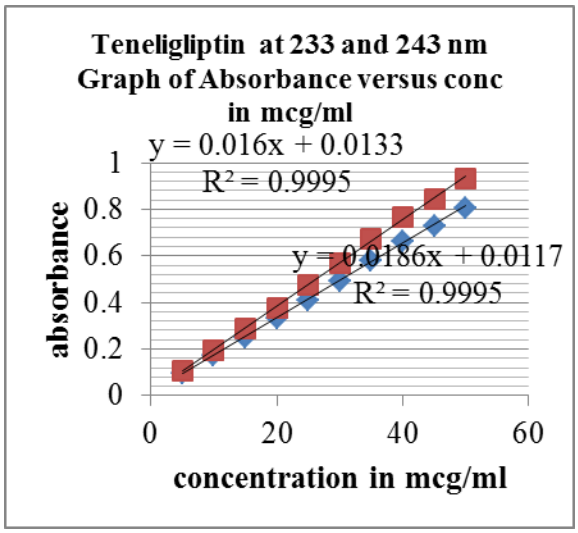
Overlay spectra of both the drugs.

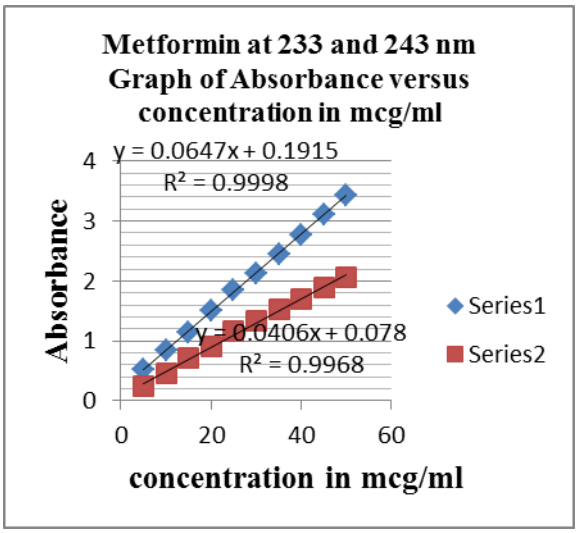


1. Assay

	Teneligliptin hydrobromide mg	Metformin Hydrochloride mg
Amount present in mg	1.860	45.660
Amount found in mg	1.900	45.240
% Assay	102.150	99.080

2. Linearity





Conc in mcg/ml	Teneligliptin 233 nm	Teneligliptin 243 nm
5	0.094	0.108
10	0.173	0.195
15	0.251	0.287
20	0.33	0.378
25	0.411	0.475
30	0.494	0.570
35	0.582	0.672
40	0.662	0.764
45	0.73	0.842
50	0.806	0.934

Conc in mcg/ml	Metformin 233 nm	Metformin 243 nm
5	0.515	0.237
10	0.831	0.447
15	1.147	0.706
20	1.499	0.907
25	1.839	1.152
30	2.134	1.323
35	2.444	1.517
40	2.766	1.691
45	3.107	1.888
50	3.430	2.064

3. Summary of validation parameters

Parameters	Teneligliptin hydrobromide		Metformin hydrochloride	
	At 233 nm	At 243 nm	At 233 nm	At 243 nm
Linearity Range	5-50µg/ml	5-50µg/ml	5-50µg/ml	5-50µg/ml
Regression equation	y=0.016x+0.013	y= 0.018x + 0.011	y=0.064x+ 0.019	y=0.040x+ 0.078
Slope	0.0.016	0.018	0.0.064	0.040
Intercept	0.013	0.011	0.019	0.078
Correlation Coefficient (r^2)	0.999	0.999	0.999	0.999
LOD (mcg/ml)	---	1.306	0.755	---
LOQ (mcg/ml)	---	3.957	2.289	---
Accuracy (% recovery)				
50%	97.120		101.340	
100%	99.270		103.120	
150%	96.590		100.380	
Precision (%RSD)				
Interday $_{(n=6)}$	0.345	0.448	Interday $_{(n=6)}$	0.345
Intraday $_{(n=6)}$	0.578	0.673	Intraday $_{(n=6)}$	0.578

DISCUSSION

Choice of Diluent

Both the drugs i.e Teneligliptin Hydrobromide and Metformin hydrochloride were found to be soluble in water hence water was chosen as a diluent.

Choice of Wavelength

Solutions of Teneligliptin Hydrobromide and Metformin hydrochloride in the diluent when scanned in UV range 200-400 nm, showed λ max of the drugs at 243 nm and 233 nm respectively. Hence the the λ max of the two drugs i.e. Teneligliptin Hydrobromide and Metformin hydrochloride was chosen.

Results of Method Validation

Linearity: The solutions followed Beer Lambert's law in concentration range of 5- 50µg/mL and with correlation coefficient (r^2) = 0.999 and 0.999 for Teneligliptin Hydrobromide and Metformin hydrochloride respectively.

Precision: The % RSD for Teneligliptin Hydrobromide and Metformin hydrochloride at 243 nm and 233 nm was found to be less than 2% and hence the developed method was precise.

Accuracy: The % recovery at 50%, 100% and 150% for Teneligliptin hydrobromide was found to and for Metformin hydrochloride was 101.34, 103.12 and 100.38 respectively and it lies within the acceptable criteria of 90 – 110%. Hence the developed method was found to be accurate.

LOD & LOQ: The method was proved to be sensitive. LOD and LOQ for Teneligliptin hydrobromide was 1.306 and 3.957 mcg/ml respectively and for Metformin hydrochloride was 0.755 and 2.289 mcg respectively.

Robustness: Deliberate changes introduced (change in UV instrument, change of analyst, and change in the wavelength) did not affect the analysis. So UV method developed for Teneligliptin Hydrobromide and Metformin hydrochloride was found to be robust.

Assay of Marketed Formulation

The % assay for marketed formulation was found to be 102.15% and 99.08% for Teneligliptin Hydrobromide and Metformin hydrochloride 105% respectively which was within the acceptance criteria (90-110%).

CONCLUSION

- A novel and simple simultaneous equation method has been developed for simultaneous analysis of Teneligliptin Hydrobromide and Metformin hydrochloride in bulk and in tablet formulation.
- The two wavelengths, 243 nm and 233 nm (λ max of Teneligliptin Hydrobromide and of Metformin hydrochloride) were chosen for analysis. The developed UV spectroscopic method employed distilled water as a diluent.
- The method was found to be green, ecofriendly, accurate, precise and robust. Method validation was done as per ICH guidelines.
- The % assay Teneligliptin Hydrobromide and Metformin hydrochloride in tablet dosage form was found to be 97% and 103%, respectively, which met the acceptance criteria (90-110%).

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