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Quantitative UV-Spectrophotometric Method for the Analysis of Teneligliptin HBr and Metformin HCl in Pharmaceutical Dosage form: Development and Validation

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ABSTRACT

This study developed a UV-spectrophotometric method for the simultaneous quantification of Metformin HCl and Teneligliptin HBr. Both active pharmaceutical ingredients were found to be soluble in 0.1N sulfuric acid, which was thus chosen as the solvent for analysis. The maximum absorption wavelengths for Metformin HCl and Teneligliptin HBr were identified at 220nm and 240nm, respectively. Standard stock solutions were prepared, and samples from commercially available tablets were accurately measured and dissolved for testing. Method validation included evaluations of linearity, precision (intra-day and inter-day), accuracy, robustness, as well as detection (LOD) and quantification limits (LOQ). The method exhibited strong precision and accuracy, with %RSD values less than 2%. Both LOD and LOQ demonstrated sufficient sensitivity, and the method proved effective for analyzing commercial formulations, achieving compliance levels of 99.20% for Metformin and 102.00% for Teneligliptin.

Keywords: Teneligliptin HBr, Metformin HCl, Ultraviolet (UV) spectroscopy method, ICH Q2 R1 Validation.

INTRODUCTION

Teneligliptin HBr (TEN) is an inhibitor of dipeptidyl peptidase. A biguanide antidiabetic, Metformin HCl (MET) is the medication of choice for individuals who are overweight and are being treated for type 2 diabetes mellitus orally. Possible mechanisms of action include inhibiting hepatic gluconeogenesis, increasing insulin sensitivity, delaying glucose absorption from the GIT, and increasing glucose uptake in cells. Multiple medications are necessary for diabetic people to effectively control their blood sugar. With MET, TEN exhibits efficient blood sugar regulation, Fig. 1 and 2 illustrates the chemical structure[1,2,3].

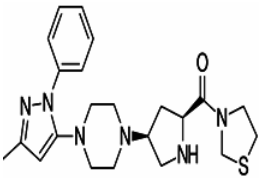

Fig. 1. Teneligliptin HBr

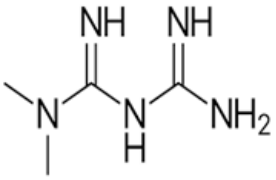

Fig. 2. Metformin HCl

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Materials, Chemical & Methods used

Teneligliptin was supplied as a gift sample by J.K. Print Pack (Pharma Division) Sara Industrial Estate Ltd. Dehradun. Tablets of 20 mg strength were purchased from the local pharmacy in Dehradun under the commercially available brand name Tenlimac (Macleods pharmaceutical Ltd.), tablets were used as pharmaceutical formulation for further analysis[4,5].

Development of a methodology

The solubility of Metformin HCl and Teneligliptin HBr was observed in 0.1N Sulphuric Acid. Therefore, it was chosen to create a technique for both drugs due to their solubility in 0.1 Sulphuric Acid[6].

Selection of wavelength

Metformin HCl and Teneligliptin HBr were individually analysed using a spectrophotometer within the wavelength range of 200 to 400 nanometres. For the simultaneous estimation approach[7,8], data were acquired as the 220nm and 240nm maximum wavelengths of Metformin HCl and Teneligliptin HBr respectively as depicted in Figure 3.

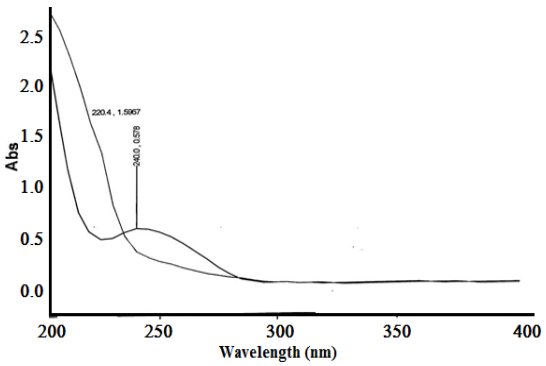

Fig. 3. Overlain UV spectra of Teneligliptin HBr and Metformin HCl

Standard Stock solution for Metformin HCl, Teneligliptin HBr

10 mg of Metformin HCl was precisely weighed and put into a 50 mL volumetric flask. Subsequently, 10-15 mL of 0.1 N sulfuric acid (H_2SO_4) was included, and the solution was subjected to sonication for 5 min to facilitate complete dissolution. Following sonication, the solution was diluted with 0.1 N sulfuric acid to a final volume of 50 mL, resulting in Stock A. In a similar manner, 10 mg of Teneligliptin HBr was measured and introduced into a separate 50 mL volumetric flask. Subsequently, 10-15 mL of 0.1 N H_2SO_4 was added, followed by sonication for 5 min, after which the solution was diluted to 50 mL, resulting in Stock B. From these stock solutions, 5 mL of Metformin (from Stock A) and 0.20 mL of Teneligliptin (from Stock B) were transferred into a 10 mL volumetric flask, and the final volume was adjusted with 0.1 N H_2SO_4, resulting in a solution of 100 µg/mL Metformin and 4.00 µg/mL Teneligliptin.[9,10]

Preparation of drug sample solution

Powder and calculate the average weight of 20 tablets. 100.00 mg of Metformin HCl and 4.00 mg of Teneligliptin HBr were added to a 100 mL volumetric flask. Added 20 mL of 0.1 N sulphuric acid and sonicated for 15 minutes. Using 0.1 N sulphuric acid as solvent, the volume was made up to the desired level. 1000 µg/mL of metformin hydrochloride solution, 40 µg/mL of teneligliptin solution were needed to create the final concentration. 5 mL of this stock solution taken in 50 mL flask, then add 0.1N Sulphuric acid to the mark until the volume is the desired 4.00 µg/mL Teneligliptin HBr and 100.00 µg/mL Metformin HCl concentration. After scanning in the UV region, absorbance (A1) and (A2) were measured at 240 and 220nm, respectively[11].

Method Validation

Linearity

A portion was taken out from a standard solution of Metformin HCl (100 µg/mL). (0.3, 0.6, 0.9, 1.2, and 1.5 mL) in a 10 mL volumetric flask. The remaining quantity was filled by 0.1 N sulphuric Acid, yielding (3, 6, 9, 12, 15) µg/mL. The additional solutions were prepared for Teneligliptin HBr (100 µg/mL) taking (1,2,3,4, and 5 mL) in a 10 mL flask. The remaining amount was filled with 0.1 N sulphuric Acid, yielding (10,20,30,40,50) µg/mL.

Precision and Accuracy

Repeatability (intraday and inter-day precision) was assessed for Metformin HCl and Teneligliptin HBr at a concentration of 12 µg/mL, utilising 0.1 N sulphuric acid as the solvent. Intraday and inter-day fluctuations demonstrated consistent outcomes, affirming accuracy. The accuracy was evaluated by augmenting pre-analysed test solutions with standard Metformin HCl and Teneligliptin HBr at 50%, 100%, and 150% concentrations, revealing dependable recovery results for both API.

Robustness

Robustness assesses an analytical method's capacity to produce consistent results

despite intentional alterations in experimental conditions. This study evaluated differences in wavelengths (219.5-220.5nm for Metformin and 239.5-240.5nm for Teneligliptin, both at 12 µg/mL), therefore verifying the method's dependability.

LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) for Metformin and Teneligliptin were established during the development of the UV technique. The LOD values indicated the minimum detectable concentration, but the LOQ denoted the minimum measurable concentration with sufficient precision and accuracy, hence assuring method sensitivity and validation reliability.

RESULT AND DISCUSSION

Linearity and Range

By examining five concentrations between 3-15 µg/mL for Metformin HCl and 10-50 µg/mL for Teneligliptin HBr at 220nm and 240nm, absorbance measurements were conducted for each solution as shown in Table 1.

Table 1: Data for Linearity study of Metformin and Teneligliptin by UV method

Sr. No	Concentration (µg/mL) Teneligliptin, Metformin	Concentration (µg/mL) Metformin HCl	Absorbance in 240nm Teneligliptin HBr	Absorbance in 240nm Metformin HCl	Absorbance in 220nm Teneligliptin HBr	Absorbance in 220nm Metformin HCl
1	10	3	0.2635	0.1310	0.2449	0.2080
2	20	6	0.4982	0.2287	0.4614	0.4454
3	30	9	0.7547	0.3310	0.6674	0.6773
4	40	12	1.0025	0.4417	0.8657	0.9297
5	50	15	1.2567	0.5517	1.1114	1.1328

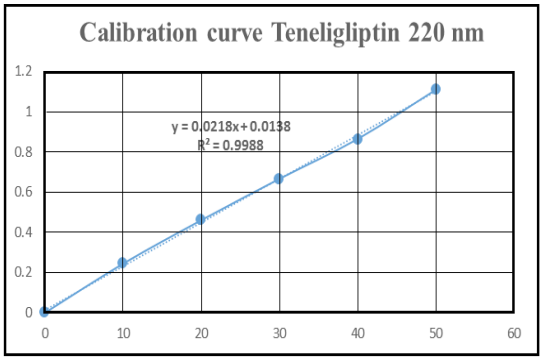

Fig. 4. Linearity Graph of Teneligliptin

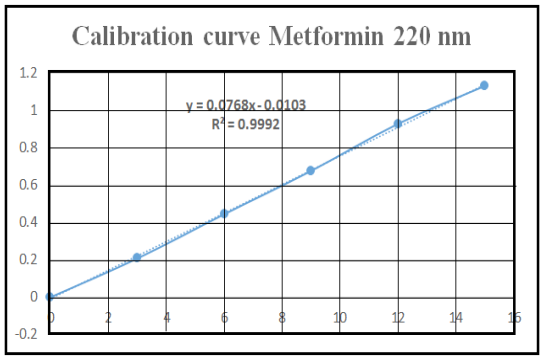

Fig. 5. Linearity Graph of Metformin

Precision

Repeatability

By repeatedly measuring the absorbance of solutions (n=6) containing 12 µg/mL of Metformin HCl, 12 µg/mL of Teneligliptin HBr, and then calculating %RSD, the repeatability of Metformin HCl, Teneligliptin HBr were examined as shown in Table 2. Approval requirements: The %RSD must be less than 2[12,13].

Table 2: Data for Repeatability study of Metformin and Teneligliptin by UV method

Concentration (12µg/mL)	Teneligliptin HBr at 240nm	Metformin HCl at 220nm
1	0.2966	0.2431
2	0.2946	0.2410
3	0.2958	0.2433
4	0.3027	0.2501
5	0.2942	0.2419
6	0.3050	0.2405
Mean	0.2981	0.2433
SD	0.0045	0.0035
%RSD	1.52	1.44

Intraday and Inter day precision

Twelve determinations totalling three duplicates of six different Metformin HCl (12 µg/mL) and Teneligliptin HBr (12 µg/mL) concentrations were examined on the same day and in a short interval time of 3 h, shown in Table 3 and the next day (Inter day) shown in Table 4. The absorbance was measured and the percent RSD was determined.

Table 3: Data for Intraday Precision study of Metformin and Teneligliptin by UV method

Drug sample	Concentration (µg/mL)	11AM Absorbance	2 PM Absorbance	5 PM Absorbance	Mean	S D	%RSD
Teneligliptin HBr	12	0.2978	0.2910	0.2897	0.2928	0.0043	1.48
Teneligliptin HBr	12	0.2878	0.2850	0.2901	0.2876	0.0025	0.88
Teneligliptin HBr	12	0.2977	0.2887	0.2913	0.2925	0.0046	1.58
Metformin HCl	12	0.8907	0.8887	0.8892	0.8895	0.0010	0.11
Metformin HCl	12	0.9011	0.9023	0.8950	0.8994	0.0039	0.45
Metformin HCl	12	0.8898	0.9002	0.8911	0.8903	0.0095	1.07

Table 4: Data for Inter day Precision study of Metformin and Teneligliptin by UV method

Drug sample	Concentration (µg/mL)	11 AM Absorbance	2 PM Absorbance	5 PM Absorbance	Mean	SD	%RSD
Teneligliptin HBr	12	0.2966	0.2895	0.2944	0.2935	0.0036	1.23
Teneligliptin HBr	12	0.3043	0.3108	0.3088	0.3079	0.0033	1.08
Teneligliptin HBr	12	0.2876	0.2815	0.2905	0.2865	0.0045	1.60
Metformin HCl	12	0.9087	0.8970	0.9101	0.9052	0.0071	0.79
Metformin HCl	12	0.9145	0.9120	0.9180	0.9148	0.0030	0.33
Metformin HCl	12	0.8955	0.9054	0.8920	0.8976	0.0069	0.77

Robustness

Three different concentrations of Metformin HCl and Teneligliptin HBr (12 µg/mL) were produced and examined using various wavelengths. Teneligliptin HBr (12 µg/mL) was assessed at 239.5, 240, and 240.5nm, whereas the Metformin HCl solution was examined at 219.5, 220, and 220.5nm, data shown in Table 5. Each wavelength's absorbance was measured, and the percent RSD was computed.

Table 5: Data for study of Robustness Metformin and Teneligliptin by UV method

Parameters	Metformin (12 µg/mL)			Teneligliptin (12 µg/mL)		
Wavelength(nm)	219.5	220.0	220.5	239.5	240.0	240.5
	0.9012	0.9102	0.9114	0.2995	0.3025	0.3110
	0.8955	0.9055	0.9089	0.3014	0.2978	0.3088
	0.9041	0.9112	0.9103	0.3042	0.2969	0.3078
Mean	0.9003	0.9090	0.9102	0.3017	0.2991	0.3091
SD	0.0043	0.0031	0.0012	0.0023	0.0030	0.0016
% RSD	0.48	0.35	0.14	0.78	1.01	0.53

Accuracy

The pre-analysed Test solution was spiked into standard Metformin HCl and Teneligliptin HBr solutions in known quantities equivalent to 50, 100, and 150% of the desired concentration. By utilising the acquired data in the regression equation of the calibration curve[14]. we were able to make estimations of the quantities of Metformin HCl and Teneligliptin HBr shown in Table 6.

Table 6: Data for Accuracy study of Metformin and Teneligliptin by UV method

Drug	%Level	Test(µg/mL)	Reference (µg/mL)	Total (µg/mL)	Conc.(µg/mL)	Recovery amount
Teneligliptin HBr	I (50%)	6	3	9	8.87	98.55
Teneligliptin HBr	II (100%)	6	6	12	11.85	98.75
Teneligliptin HBr	III (150%)	6	9	15	14.76	98.40
Metformin HCl	I (50%)	6	3	9	8.91	99.00
Metformin HCl	II (100%)	6	6	12	12.10	100.8
Metformin HCl	III (150%)	6	9	15	14.59	98.80

LOD and LOQ

For calculating LOD and LOQ, data from the linearity equation (Fig. 4 and 5) that is slope and standard deviation were used. For the present study computed LOQ values were determined to be 17.29 µg/mL, while the LOD values were 5.88 µg/mL. The sensitivity of the technique is indicated by the low values of LOD and LOQ[15].

Drug analysis in commercially available formulation

The assay findings for Metformin and Teneligliptin with the UV technique indicate a concentration of 99.20 µg/mL for Metformin, which is near the asserted 100 µg/mL, resulting in a 99.20% compliance with the claim. Teneligliptin was quantified at 4.20 µg/mL compared to the asserted 4.00 µg/mL, resulting in a 102.00% claim, as seen in Table 7.

Analysis of Marketed formulation

Table 7: Data for Assay study of Metformin and Teneligliptin by UV method

Drug	Concentration found (µg/mL)	Concentration Claimed (µg/mL)	%Claim
Metformin	99.20	100	99.20
Teneligliptin	4.20	4.00	102.00

DISCUSSION

The research assessed the linearity, precision, accuracy, and robustness of a UV spectrophotometric technique for the concurrent quantification of Metformin HCl and Teneligliptin HBr. Linearity was noted throughout the concentration ranges of 3-15 µg/mL for Metformin HCl and 10-50 µg/mL for Teneligliptin HBr, exhibiting robust correlation coefficients. The precision, both intraday and inter day, exhibited low %RSD values, hence validating the method's repeatability. Accuracy was evaluated using spiked samples, yielding recoveries close to 100%, therefore confirming the method's dependability. The robustness was assessed by varying wavelengths, and the %RSD stayed within acceptable thresholds, indicating the method's stability with minor fluctuations. Low limits of detection (LOD) and quantification (LOQ) values demonstrated great sensitivity, and the examination of commercial formulations yielded findings around the stated amounts, validating the method's suitability for routine analysis of these pharmaceuticals.

CONCLUSION

Both APIs were more soluble and stable with 0.1 N Sulphuric Acid. The recovery investigation demonstrated precisely any tiny variation in drug concentration in the solution, and low LOD and LOQ values indicated strong sensitivity. Both medicines had good wavelength regression values. Thus, the recommended method is creative, simple, exact, sensitive, affordable, and suitable for routine analysis of tablet dosage.

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Conflict of interests

The authors declare that they have no Conflict of Interest.

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