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DEVELOPMENT AND VALIDATION OF UV- SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN ETABONATE AND TENELIGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY SIMULTANEOUS EQUATION METHOD.

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ABSTRACT
A simple, precise and economical method for simultaneous estimation of Remogliflozin Etabonate and Teneligliptin in combined dosage form has been developed. The λmax of Remogliflozin Etabonate and Teneligliptin were found to be at 236 nm and 246 nm, respectively. The calibration plot was found to be linear between concentration range 5-25µg/ml for Teneligliptin and 0.5-2.5µg/ml for Remogliflozin Etabonate with their correlation coefficient values (r^2) 0.9993 and 0.9995. Limit of detection and quantification values were determined to be 0.0123µg/ml and 0.0373µg/ml for Remogliflozin Etabonate and 0.0289µg/ml and 0.0877µg/ml for Teneligliptin, respectively. Percentage recovery for assay was found to be 100.83% and 99.16% for Remogliflozin Etabonate and Teneligliptin, respectively. The method was found to be precise as %RSD was less than 2. The recovery results indicated that Remogliflozin Etabonate and Teneligliptin could be quantified by this procedure simultaneously in combined dosage form without the interference of common excipients. The simultaneous estimation method was simple, precise, accurate, reproducible and economical which can be efficiently and easily applied to pharmaceutical dosage form.

KEYWORDS: Teneligliptin, Remogliflozin Etabonate, Simultaneous Estimation, Spectrophotometric method.

INTRODUCTION
Teneligliptin hydrobromide hydrate (TEN) is {(2S,4S)-4-[4-(3-Methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3- thiazolidin-3-yl) methanone hemipentahydrobromide hydrate, an oral dipeptidyl peptidase inhibitor (DPP-4). DPP-4 inactivates incretin hormones (glucagon-like peptide-1; GLP-1 and glucose-dependent insulinotropic polypeptide; GIP) which are responsible for enhancing insulin secretion. It is indicated for the management of type 2 diabetes mellitus (T2DM).Remogliflozin etabonate IUPAC name Ethyl [(2R, 3S, 4S, 5R, 6S)-3,4,5 trihydroxy-6- {[5 methyl -1-(propan-2-yl) -4- {[4-(propan-2-yloxy) phenyl] methyl}-1H-pyrazol-3-yl] oxy} oxan-2-yl] methyl carbonate. It is Soluble in methanol. It is used for type-2 diabetes. Also use in case of non-alcoholic hepatitis. It isa Inhibitor of Sodium-Glucose Cotranspoter-2 which is responsible for glucose reabsorption in the kidney, blocking this transporter causes blood glucose to be eliminated from the urine, it is a selective SGLT-2.[1-7]

From literature survey it was found that, UV spectrophotometric method have been reported for estimation of Teneligliptin and Remogliflozin Etabonate in Single dosage form but no report was found in in their combined dosage form[8-11]. 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 Teneligliptin and Remogliflozin Etabonate in their combined dosage form and hence, an economical method was developed and validated according to the ICH guidelines.

SIMULTANEOUS EQUATION METHOD DEVELOPMENT
Working solutions of both drugs were scanned in the UV range 200– 400 nm. The overlay spectra of both drugs were recorded (Figure 1). From overlain spectra, wavelengths 236 nm (λmax of REMO) and 246 nm (λmax of TEN) were selected for analysis of both drugs using simultaneous equation method (λ1- 236 nm for REMO and λ2-246 nm for TEN). Consequently, it may

be possible to determine both drugs by the technique of from method or simultaneous equation method.

Five standard solutions having concentrations of 5, 10, 15, 20 and 25 μg/mL for REMO and 0.5, 1.0, 1.5, 2.0 and 2.5 μg/mL for TEN were prepared in Methanol and their corresponding absorbance was measured at 236 nm and 246 nm. The concentration of drugs x (REMO) and y (TEN) in sample solutions were determined by the SE method using the following formula.

$Cx = A2ay1 - A1ay2\ ax2ay1 - ax1ay2,$

$Cy = A1ax2 - A2ax1\ ax2ay1 - ax1ay2,$

where Cx and Cy are the concentration of REMO and TEN, $A1$ and $A2$ are the absorbance of sample solution at 236 nm and 246 nm, respectively, $ax1$ and $ax2$ are absorptivity of REMO at 236 nm and 246 nm, and $ay1$ and $ay2$ are absorptivity of TEN at 236 nm and 246 nm, respectively.

Determination of Absorptivity Value

The absorptivity value of REMO and TEN from each solution was calculated using following formula:

Absorptivity = $\frac{\text{Absorbance}}{\text{conc}}$(gm/100 ml).

Developed method was validated as per ICH guidelines.

MATERIALS AND METHOD

Apparatus: A double beam UV-Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2nm, 1cm quartz cell was used to measure absorbance of solutions.

Chemical and Reagents: All chemicals were of analytical reagent grade and solutions were prepared with methanol AR grade (FINAR). Active Pharmaceutical Ingredient (API) Remogliflozin Etabonate was purchased from Benzchem Enterprise, Vadodara, India and Teneligliptin was received as gift sample from Prayosha Healthcare Private Limited, Ankleshwar, India.

Selection of solvent: On the basis of solubility study Methanol was selected as the solvent for dissolving Remogliflozin Etabonate and Teneligliptin.

Preparation of Standard Stock Solutions of REMO and TEN (1000 µg/ml)

Remogliflozin Etabonate Stock Solution: An Accurately weighed 10 mg of Remogliflozin Etabonate was transferred to a 10ml volumetric flask and dissolved, diluted up to mark with methanol by sonication for 5 min. The volume was adjusted to 100 ml with methanol to obtain standard solution having concentration of REMO (1000 µg/ml).

Teneligliptin Stock Solution: An Accurately weighed 10 mg of Teneligliptin was transferred to a 10 ml volumetric flask and dissolved, diluted up to mark with methanol by sonication for 5 min. The volume was adjusted to 100 ml with methanol to obtain standard solution having concentration of Teneligliptin (1000 µg/ml).

Preparation of Working Standard Solutions: Working standard solutions of 10µg/ml were prepared by diluting 1ml each of above standard solution to 100ml with methanol and scanned it in the range 200nm-400nm to obtain the absorbance spectra.

RESULTS AND DISCUSSION

The analytical method was validated with respect to parameters such as linearity, precision, accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ).

Linearity: Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 5 – 25 µg/ml for Remogliflozin Etabonate and 0.5 – 2.5 µg/ml for Teneligliptin. Absorbances obtained were plotted against respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficient were found to be 0.9993 and 0.9995 for Remogliflozin Etabonate and Teneligliptin (Figure) respectively. The results are given in Table 1.

Precision: To check the reproducibility of the method, suitable statistical evaluation was carried out. The concentrations of two drugs were measured three times on the same day at intervals of 1 hour and on three different days for intra and inter day study respectively. The standard deviation and Relative standard deviation (RSD) were calculated. The results are given in Table 2.

Accuracy: Recovery studies were carried out by applying the method to drug sample to which known amount of standard Remogliflozin Etabonate and Teneligliptin corresponding to 99.11-100.83% and 99.16 -100.55% of label claim has been added. At each level of the amount six determinations were performed. The results are given in Table 3.

LOD and LOQ: The LOD of Remogliflozin Etabonate and Teneligliptin Was Found to be 0.0123µg/ml and 0.0289 µg/ml respectively and LOQ was found to be 0.0373µg/ml and 0.0877 µg/ml respectively. The results are given in Table 4.

Table 1: Linearity and Correlation Coefficient.

Parameters	REMO	TEN
Regression Equation	y = 0.5361x - 0.0436	y = 0.1148x + 0.0962
Linearity (µg/ml)	5-25	0.5-2.5
Correlation Coefficient (r^2)	0.9993	0.9995

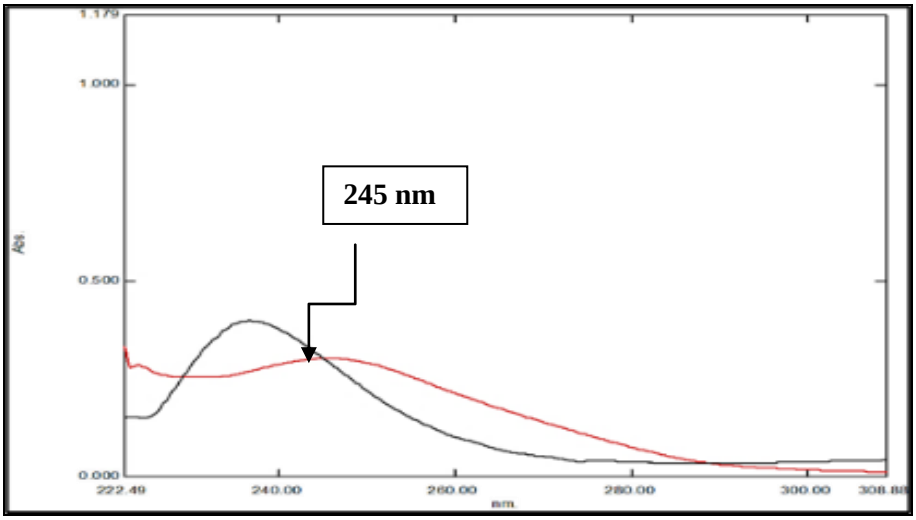

Fig. 1: Overlay Spectra of REMO & TEN

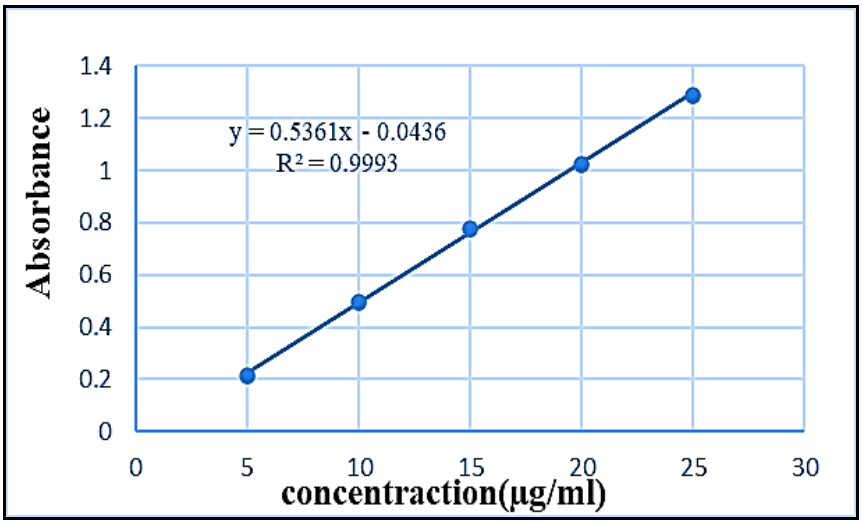

Fig. 2: Calibration curve of REMO at 236 nm.

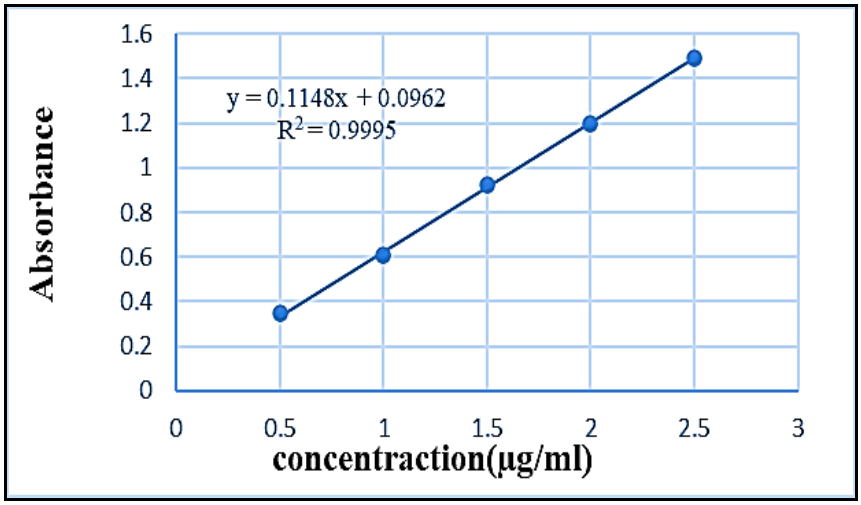

Fig. 3: Calibration Curve of TEN at 246 nm.

Table 2: Precision Studies for Remogliflozin Etabonate.

Parameter	Remogliflozin Etabonate			Teneligliptin		
	Conc.	Abs	%RSD	Conc.	Abs	%RSD
Intraday (n=3)	10	0.475 ± 0.001	0.321	1.0	0.199± 0.003	1.612
	15	0.778 ± 0.002	0.267	1.5	0.266±0.001	0.377
	20	0.993 ± 0.001	0.153	2.0	0.337± 0.001	0.466
Interday (n=3)	10	0.472 ±0.002	0.440	1.0	0.207±0.002	0.339
	15	0.773 ±0.001	0.197	1.5	0.261±0.002	0.795
	20	0.994 ±0.001	0.100	2.0	0.328±0.001	0.304
Repeatability (n=6)	15	0.776	0.23%	1.5	0.265	1.028%
		0.775			0.268	
		0.771			0.261	
		0.773			0.265	
		0.775			0.263	
		0.774			0.261	
		$\bar{y}$ =0.774 ± 0.001			$\bar{y}$ = 0.2638 ± 0.002	

Table 3: Accuracy.

Drug	Level of %Recovery	Amount added	Total Amount Recovered	% RSD	% Recovery
Remogliflozin Etabonate	80	4.5	12	0.164	100.83
	100	7.5	15	0.199	100.6
	120	10.5	18	0.167	99.11
Teneligliptin	80	0.45	1.2	0.892	99.16
	100	0.75	1.5	0.378	97.33
	120	1.05	1.8	0.870	100.55

Table 4: LOD and LOQ.

Validation Parameters	REMO	TEN
LOD (µg/ml)	0.0123	0.0289
LOQ (µg/ml)	0.0373	0.0877

Analysis of Marketed Formulation

Twenty tablets each containing 100mg of Remogliflozin Etabonate, 10mg of Teneligliptin were weighed, average weight was calculated and powdered. A quantity equivalent to 10mg of Remogliflozin Etabonate and 10mg of Teneligliptin was weighed and transferred in to 100ml volumetric flask. It is extracted with distilled water. The volumetric flask was sonicated for 2mins to affect the complete dissolution of the drugs and the solution was made up to the volume with distilled water and filtered. Suitable aliquots of formulation were prepared and scanned to obtain concentration of the two drugs in the linearity range. The concentration of each analyst was determined using the simultaneous equation.

CONCLUSION

The proposed UV Spectrophotometric method was found to be simple, precise, accurate and economical for the simultaneous estimation of Remogliflozin Etabonate and Teneligliptin in combined dosage forms. Hence, this method can be easily used for routine quality control analysis of Remogliflozin Etabonate and Teneligliptin in pure and its combined dosage form.

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