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Development of a Stability-Indicating Rp-Hplc Method for the Simultaneous Estimation of Metformin, Teneligliptin, And Dapagliflozin in Bulk and Combined Dosage Form

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KEYWORDS

RP-HPLC, Stability-indicating method, Metformin, Teneligliptin, Dapagliflozin.

ABSTRACT:

Introduction: The increasing worldwide prevalence of Type 2 Diabetes Mellitus makes it necessary to provide trustworthy analytical techniques for the simultaneous estimation and stability evaluation of formulations containing combinations of antidiabetic drugs.

Objectives: The objective of this study is to develop and validate a quick, sensitive and stability-indicating Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Metformin, Teneligliptin and Dapagliflozin in bulk and tablet dosage forms

Methods: Chromatographic separation was achieved using a C18 column with a mobile phase consisting of acetonitrile and 0.1% orthophosphoric acid in a 40:60 ratio. The detection wavelength was set at 220 nm. System suitability parameters including theoretical plates, tailing factor and resolution were evaluated. The method was validated for accuracy, precision and robustness. Forced degradation studies were conducted under acidic, basic, oxidative, photolytic and thermal stress conditions to assess the stability-indicating capability of the method.

Results: The developed method showed excellent linearity within the specified concentration ranges, with correlation coefficients greater than 0.999. System suitability parameters met the acceptable criteria. Validation results confirmed that the method is accurate, precise and robust. Forced degradation studies demonstrated that degradation products did not interfere with the analyte peaks, confirming the stability-indicating nature of the method.

Conclusions: The developed RP-HPLC method provides an efficient and reliable tool for routine quality control and stability analysis of combination antidiabetic formulations. It ensures safe therapeutic efficacy, regulatory compliance and formulation integrity, making it suitable for pharmaceutical industries and research laboratories involved in multi-drug therapy quality assessment due to its high reproducibility, sensitivity and selectivity.

1. Introduction

Metformin hydrochloride, scientifically referred to as 1-carbamimidamido-N,N-dimethyl methanimidamide, has a molecular formula of C4H11N5 and a molecular weight of 129.167 g·mol−1. It is classified within the biguanide category of oral hypoglycaemic agents. The primary mechanism through which it operates is by lowering blood glucose levels, achieved by decreasing hepatic glucose production via the inhibition of gluconeogenesis, with significant effects on the liver and skeletal muscles.(1) The activation of adenosine monophosphate-activated protein kinase (AMPK) is essential for improving insulin sensitivity and promoting peripheral glucose uptake. In the liver, the activation of AMPK inhibits critical enzymes involved in gluconeogenesis, resulting in a reduction of glucose output. (2) In both muscle and adipose tissues, metformin enhances glucose uptake by facilitating the translocation of glucose transporter type 4 (GLUT-4) to the cell membrane, while also reducing the absorption of glucose in the intestines. Unlike other antidiabetic drugs, metformin does not induce insulin secretion, which helps

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to lower the risk of hypoglycaemia. Furthermore, it has a beneficial effect on lipid profiles by decreasing triglycerides and LDL cholesterol, thereby further aiding in the management of type II diabetes. [3]

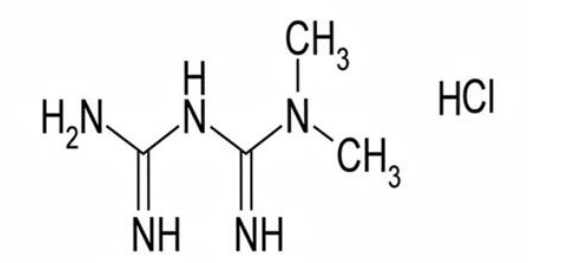

Fig.no.1. Structure of Metformin hydrochloride

Teneligliptin is an oral antidiabetic medication belonging to the dipeptidyl peptidase-4 (DPP-4) inhibitor class, primarily used for the management of type 2 diabetes mellitus. Its chemical formula is $C_{22}H_{30}N_6OS$, and its molecular weight is approximately 426.58 g/mol. The IUPAC name of teneligliptin is (2S, 4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-ylmethanone. Mechanistically, teneligliptin works by selectively inhibiting the enzyme DPP-4, which is responsible for the degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). By preventing incretin breakdown, it enhances glucose-dependent insulin secretion from pancreatic β-cells and suppresses glucagon release from α-cells, thereby reducing blood glucose levels without significantly increasing the risk of hypoglycaemia. Clinically, teneligliptin is used as monotherapy or in combination with other antidiabetic agents (such as metformin, sulfonylureas, or insulin) to improve glycaemic control in adults with type 2 diabetes mellitus. [4]

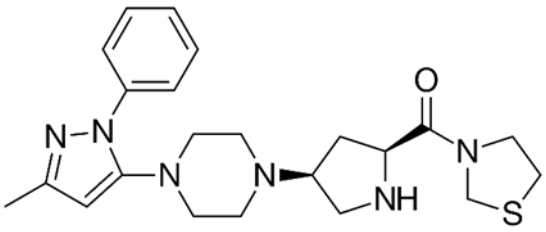

Fig.no.2. Structure of Teneligliptin

Dapagliflozin is an oral anti-hyperglycaemic agent used in the management of type 2 diabetes mellitus and belongs to the sodium–glucose cotransporter-2 (SGLT2) inhibitor class. Its chemical formula is $C_{21}H_{25}Cl\ O_6$, with a molecular weight of approximately 408.88 g/mol. The IUPAC name of Dapagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol. It acts by selectively inhibiting the SGLT2 protein in the proximal renal tubules of the kidneys, which is responsible for the reabsorption of filtered glucose back into the bloodstream. By blocking SGLT2, Dapagliflozin reduces renal glucose reabsorption, increases urinary glucose excretion, and consequently lowers plasma glucose levels in an insulin-independent manner. Clinically, Dapagliflozin is used to improve glycemic control in adults with type 2 diabetes mellitus and has additional therapeutic benefits in reducing the risk of hospitalization for heart failure and slowing the progression of chronic kidney disease in appropriate patients.[5]

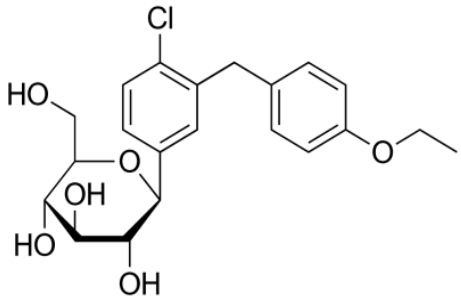

Fig.no.3. Structure of Dapagliflozin

2. Materials and Methods:

2.1. Drug Sample:

Metformin, teneligliptin, and Dapagliflozin were generously gifted by Madras Pharmaceuticals, Chennai. The marketed formulation used in the study was ZitaDM tablets manufactured by Glen mark Pharmaceuticals Ltd, containing 500 mg of metformin, 20 mg of teneligliptin, and 10 mg of Dapagliflozin. The tablet formulation was procured from a local pharmacy.

2.2. Instruments:

The present study employed various analytical instruments, including a Shimadzu AUX-220 Digital Balance, a sonicator ultrasonic cleaner (Model 2200 MH), a centrifuge apparatus, a Shimadzu LC system, an ELICO pH Meter LI-120, and a melting point apparatus. The Shimadzu HPLC system was equipped with a five-line degassing unit for the mobile phase and rinse solution, providing a flow rate range of 0.0001–10

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mL/min and a maximum pressure of 44 MPa (0.0001–5 mL/min). The auto-sampler featured a needle-in-flow path injection system with an injection volume range of 0.1–100 µL and reproducibility of RSD < 0.20% for volumes between 5.0 and 2000 µL. The column oven accommodated up to six columns (maximum 10 cm length) with a temperature range from ambient to 90°C. The UV detector operated within a wavelength range of 190–700 nm for accurate detection. The system was fitted with a 12 µL flow cell (10 mm, TC, 12 MP), with optional high-speed (8 µL, 10 mm, TC) and semi-micro (2.5 µL, 5 mm, TC) flow cells.

2.3. Chromatographic method:

The selection of the analytical method was based on several factors, including the sample characteristics, molecular weight, pKa value, and stability of the analytes. As the present study focused on polar drugs, reversed-phase or ion-exchange chromatography was considered appropriate. For the initial separation, ultra-high-performance liquid chromatography (UHPLC) was employed due to its superior resolution and efficiency. A C18 column was used as the stationary phase, and the mobile phase comprised a mixture of acetonitrile, methanol, water, potassium dihydrogen orthophosphate, and orthophosphoric acid.

2.4. **Optimized method parameters:**

The chromatographic analysis was conducted using an Agilent Technologies 1220 Infinity Series UHPLC system. Separation was achieved with a SUPELCO C18 analytical column measuring 250 mm × 46 mm and featuring a particle size of 5 µ. To maintain consistent performance and retention, the column oven temperature was set at 40°C. Detection occurred at a wavelength of 220 nm utilizing a UV-Visible detector. The mobile phase comprised a blend of acetonitrile, methanol, and phosphate buffer (pH 5.5) in a volume ratio of 50:30:20. The system operated in isocratic mode with a flow rate of 0.5 ml/min, and each run involved an injection volume of 20 µl, culminating in a total analysis time of 5 minutes.

2.5. Procedure for preparation of solution:

Preparation of buffer:

To prepare 0.05 M KH_2PO_4 buffer, accurately weigh 6.8 g of potassium dihydrogen orthophosphate and transfer it into a 1000 mL beaker. Add approximately 900 mL of HPLC-grade water and sonicate the solution for 20 minutes to ensure complete dissolution. After sonication, make up the volume to 1000 mL with HPLC-grade water and filter the solution through a 0.45 µm membrane filter. Finally, adjust the pH to 5.5 by adding 1 mL of 0.1% orthophosphoric acid (OPA) to the filtered solution.

Mobile phase preparation

About 500 ml of acetonitrile, 300 ml of phosphate buffer (0.05M) (pH 5.5 adjusted with 1M (NaOH) and 200ml of methanol were mixed and degassed in ultrasonic water bath for 5 min. Then it was filtered through 0.45 µ pore filter under vacuum and transferred into a 1000 ml volumetric flask.

Diluent preparation:

The selection of the diluent was based on the solubility characteristics of the drugs, with methanol being utilized as the diluent in the mobile phase.

Preparation of standard stock solution

Accurate measurements were taken to weigh 500 mg of metformin, 20 mg of teneligliptin, and 10 mg of Dapagliflozin, which were then individually transferred into a 100 ml volumetric flask. Each compound was dissolved using a minimal amount of mobile phase, and the total volume was adjusted to the mark with additional mobile phase. The resulting concentrations of the solutions were 5000 µg/ml for metformin, 200 µg/ml for teneligliptin, and 100 µg/ml for Dapagliflozin.

System suitability studies

The system suitability studies conceded as per ICH guidelines and USP. The parameters like peak area, resolution and retention time were calculated.

Preparation of Calibration Graph:

The aliquots of stock solution of metformin, teneligliptin and Dapagliflozin (5.0- 70 ml of 5000µg/ml for metformin, 200 µg/ml for teneligliptin and 100µg/ml for (da p a g l i f l o z i n) were transferred individually in to five 5 0 ml volumetric flasks and made up to mark with mobile phase. From this solution 20µl were injected and the chromatogram were recorded at 220 nm. The above concentration range was found to be linear and obeys beer's law. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

LOD and LOQ:

The linearity study was carried out for three times. The LOD and LOQ were calculated based up on the calibration curve method. The LOD and LOQ were calculated using average of slope and intercept.

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Precision

To evaluate the reproducibility of the method, six consecutive assays of the formulation were conducted using the same concentrations. Quantification of the drug content in the formulations was performed. The relative standard deviation (RSD) value was computed**.**

Recovery studies

Recovery study was performed by standard addition method. The recovery experiment was done by adding known concentration of metformin, teneligliptin and Dapagliflozin working standard to the pre- analysed formulations. 50% pre-analysed formulations solutions, known quantities of standard drug that is 50%, 100% and 150% of quantification concentration were added into series of 100 ml volumetric flasks, diluted with mobile phase and sonicated for 15minutes. After sonication the solution was made up to 100ml with mobile phase. The solution was filtered through Whatmann filter paper No.41, from each solution, 3.0ml, 6.0ml, and 9.0ml of clear filtrate was transferred into a series of 50 ml of volumetric flask and made up to the volume with water. The solution was injected and the chromatograms were recorded. The drug recovered was calculated using slope and intercept values from the calibration graph. The procedure was repeated for 3 times for each concentration.

Robustness

The robustness was studied by evaluating the effect of small but deliberate variation in the chromatographic conditions. The conditions studied were flow rate (± 0.2 ml/min) and composition of mobile phase (± 2 ml). For each condition, 20 µl solutions were injected into the chromatographic system and chromatograms were recorded. The system suitability parameters were checked.

Ruggedness

To assess the level of consistency in test findings obtained from the suggested analyze approach, the drug sample was analyzed using many analyzers.

3. Result and Discussion:

3.1. Reverse phase HPLC method:

In RP-HPLC, the retention of a compound is determined by its polarity, pka value, molecular weight, experimental conditions, mobile phase, column and temperature. The column typically octyl (C8) and octadecyl (C18) bonded phase is less polar than the water – organic phase, usually an almost or entirely mobile phase. Sample molecules partition between the polar mobile phase and nonpolar C8 and C18 stationary phase and more hydrophobic (non-polar) compounds are retained more strongly. Polar compounds are less strongly held and elute from the column first and vice versa. Usually the lower the polarity of the mobile phase, higher in its elution strength. RP-HPLC columns are efficient, stable and reproducible because of the solvents used. Generally gradient and isocratic elution techniques used for elution. Isocratic elution technique employed for resolution of compounds in the present study.

3.2. Method Validation:

3.2.1. Specificity:

Retention time of Metformin was eluted at 1.435 min and Retention time of Teneligliptin was eluted at 2.897 min and Retention time of Dapagliflozin was eluted at 6.475. We did not found and interfering peaks in blank at retention times of these drugs in this method. So this method was said to be specific. The results were shown in Table no: 1.

3.2.2. Linearity:

Preparation of Calibration Graph (Linearity):

The linearity of analytical method is the ability to elicit test results that are directly proportional to the analysed concentration in samples within a given range. Working stock solutions were prepared by diluting the stock solution with mobile phase to obtain concentration from 45-65 µg/ml for metformin, 18-26 µg/ml for teneligliptin and 9-13 µg/ml Dapagliflozin respectively. The solutions were injected and the chromatograms were recorded at 220 nm. It was found that the above concentration range was linear with the concentration range of 45-65 µg/ml for metformin, 18-26 µg/ml for teneligliptin and 9-13 µg/ml Dapagliflozin respectively. The correlation coefficient for the drugs was found to be above 0.999. The results were shown in table 2 and the calibration curve was shown in figure 5-7.

Stability studies:

The Stability of the analyses was confirmed by stability studies. The degradation studies was carried out by using 0.1N HCl, 0.1N NaOH, 0.1%H2O2 and photolytic. Based on the results the percentage degradation was found to be for 0.1N HCl – 4.73, 7.83 and 4.17%, 0.1N NaOH-3.87, 5.77 and 7.00%, 0.1% H2O2 – 7.47, 9.71 and 3.62 %, Photolytic degradation-5.85, 6.84 and 5.11% for metformin, tenegliptin and Dapagliflozin respectively. The degradation percentage

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was found to be for all stress conditions below 20% (ICH guidelines within the limit). Hence conclude that the analyses was stable under the above stress conditions. The report was shown in Table no: 3

System suitability:

System suitability test provides the added assurance that on a specific occasion the method is giving, accurate and precise results. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. Acceptance criteria for system suitability were asymmetry factor should not be more than 2.0, theoretical plates should not be less than 2000 and % RSD of peak area should not be more than 2.0. All variation parameters results were within the acceptance criteria mentioned above. The result of system suitability study of the developed method was shown in Table no: 4.

Analysis of market tablet:

The tablet formulation (ZitaDM containing 500 mg of, Metformin, 20 mg of Teneligliptin and 10 mg of Dapagliflozin) was selected for analysis. The percentage label claim present in tablet formulation was found to be 99.40, 99.83 and 101.43 % for metformin, teneligliptin and Dapagliflozin respectively. The reports were shown in Table no: 5.

Precision:

The precision of the method was confirmed by the analysis of formulation was repeated in six times. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.5609, 0.6465 and 0.5841 for metformin, teneligliptin and Dapagliflozin respectively. The results of the analysis are shown in Table no: 6.

Recovery studies:

Recovery studies confirmed the accuracy of the method. In the pre-analysed formulation, a known quantity of analysts raw materials were added at different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.15-100.52% for metformin, 99.68-101.81% for teneligliptin and 99.77 – 100.88 % for Dapagliflozin. The low % RSD value for analysts indicated that this percentage recovery revealed no interference due to the excipients used in the formulation. Therefore, the developed method was found to be accurate. The results were shown in the Table no: 7.

Ruggedness:

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. The percentage RSD value for analyst I found to be 0.8316, 0.8099 and 1.3267 for metformin, tenegliptin and Dapagliflozin. The percentage RSD values for analyst II were 0.4118, 0.9213 and 1.1586 % metformin, tenegliptin and Dapagliflozin respectively. The values are shown in Table no: 8.

Robustness:

The robustness study indicated that the factors selected remained unaffected by small variation of flow rate and the mobile phase composition. The system suitability results were within the limit. Hence the method was robust. The results were shown in table 12.

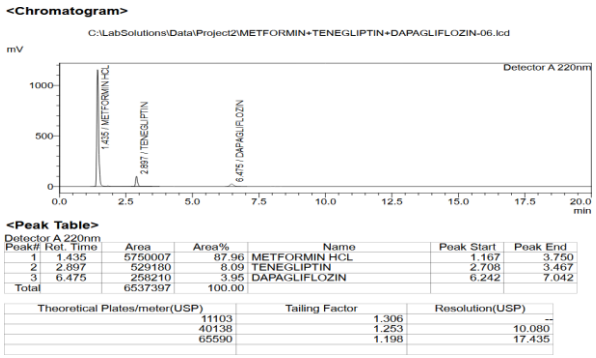

Fig. no. 4: Optimized Chromatogram.

Table no. 1: Specificity for Metformin, Teneligliptin and Dapagliflozin

Sample ID	Metformin		Teneligliptin		Dapagliflozin	
	RT	Area	RT	Area	RT	Area
BLANK	1.435	No peak observed	2.897	No Peak observed	6.475	No peak observed
STANDARD	1.435	5750108	2.897	529234	6.475	258360
SAMPLE	1.432	5746307	2.898	526367	6.464	258465

Table no. 2: Linearity parameters for Metformin, Teneligliptin and Dapagliflozin

S. no	Peak Area		
	Metformin	Teneligliptin	Dapagliflozin
1	3650032	266495	129161
2	4847946	398940	193549
3	5750007	529180	258210
4	647620	660290	323694
5	7092289	793010	387295
Mean	4397579	529583	258381.8
SD	**2445116**	**207822.7**	**102207.7**

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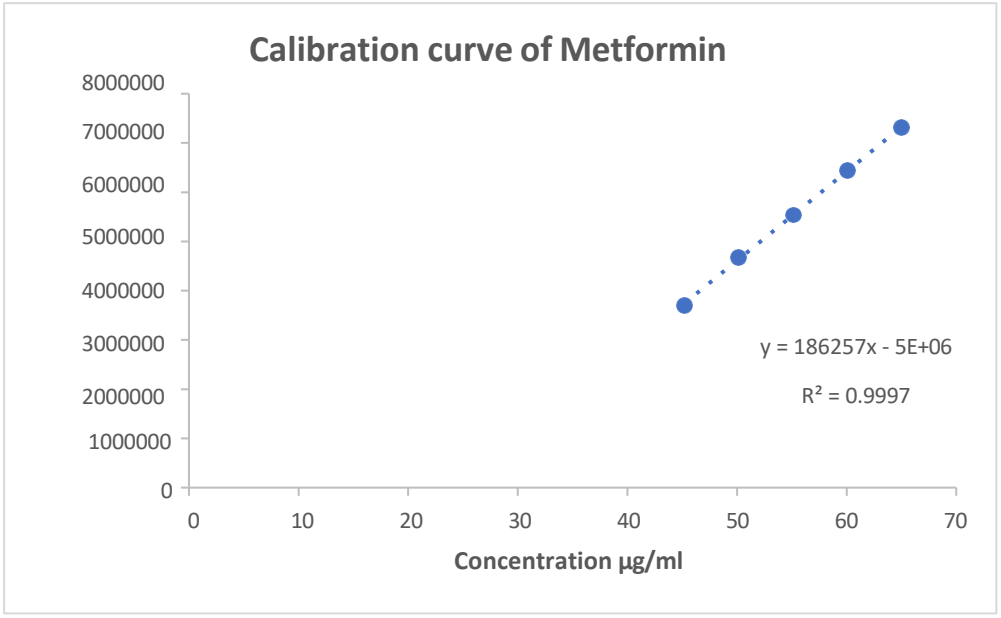

Fig. no. 5: Calibration Curve for Metformin

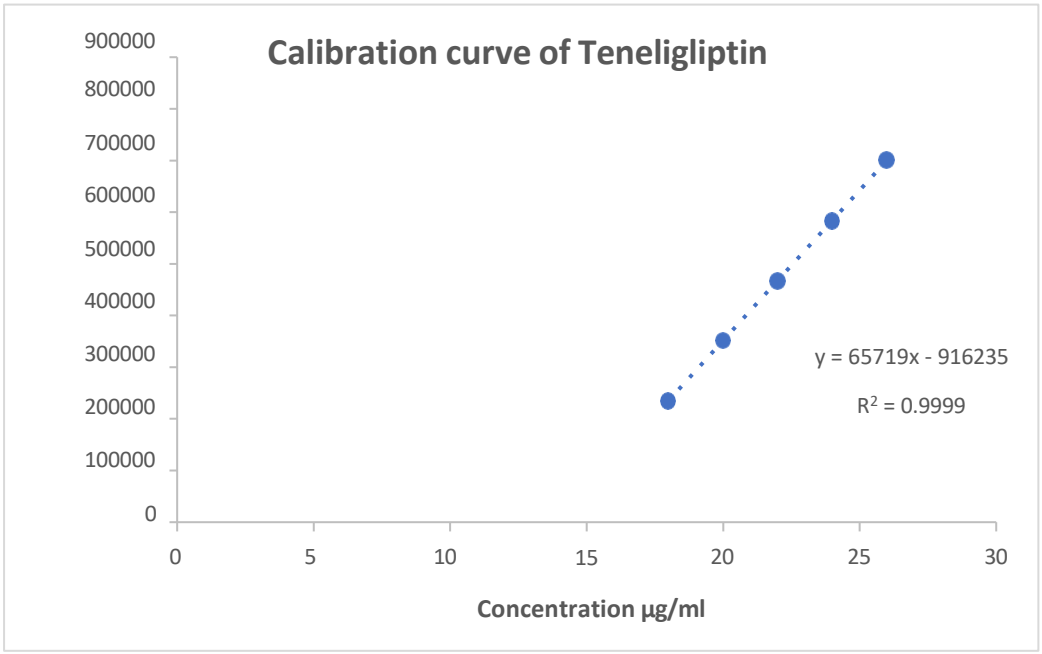

Fig. no. 6: Calibration Curve for Teneligliptin

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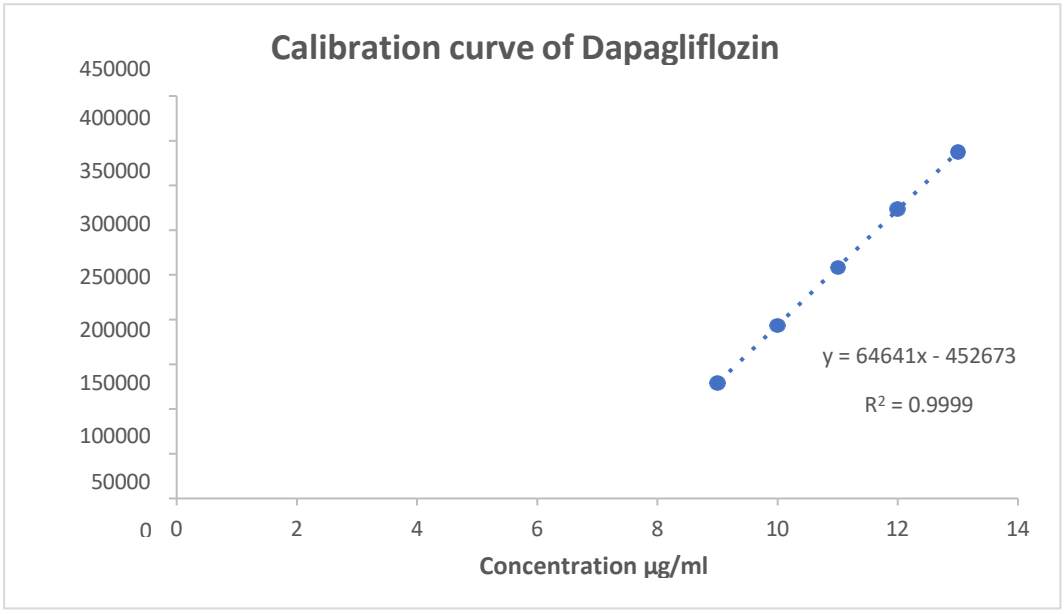

Fig. no. 7: Calibration Curve for Dapagliflozin

Table no. 3: Data for Stability studies

Degradation Condition	% Assay			% Degradation		
	MET	TEN	DAPA	MET	TEN	DAPA
0.1N HCl Acidic / 2hr	95.27	92.17	95.83	4.73	7.83	4.17
0.1N NaOH Basic / 2hr	96.13	94.23	93.00	3.87	5.77	7.00
1% H2O2 Peroxide/2hr	92.53	90.29	96.38	7.47	9.71	3.62
Photo/ UV light/ 24hr	94.15	93.16	94.89	5.85	6.84	5.11

Table no. 4: System suitability parameters of optimized method

Parameters	Metformin	Teneligliptin	Dapagliflozin
Retention time(min)	1.435	2.897	6.475
Tailing factor	1.306	1.253	1.198
Peak Area	5750007	529180	258210
Theoretical plates(USP)	11103	40138	65590

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Resolution(min)	0.00	10.080	17.435

Table no. 5: Quantification of formulation data (ZitaDM)

Drug	Sample No	Labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average (%)	SD	% RSD
MET	1	500	500.19	100.03	99.40	0.2466	0.2469
	2	500	500.28	100.05			
	3	500	498.10	99.62			
TENE	1	20	20.22	101.10	99.83	1.2271	1.2291
	2	20	19.95	99.75			
	3	20	19.15	98.65			
DAPA	1	10	10.16	101.6	101.43	0.9606	0.9473
	2	10	10.04	100.4			
	3	10	10.23	102.3			

Table no. 6: Precision for Metformin, Teneligliptin and Dapagliflozin

S. No	Peak Area		
	Metformin	**Teneligliptin**	**Dapagliflozin**
1	5750007	529180	258210
2	5789012	520360	257012
3	5723119	527891	258910
4	5709871	526780	255430
5	5767990	526539	259010
Mean	5747999.8	526150	257714.4
SD	32243.20	3401.62	1505.44
%RSD	**0.5609**	**0.6465**	**0.5841**

Table no. 7: Recovery Analysis of formulation data

Drug	Percentage	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	SD	% RSD

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MET	50 100 150	55.0 55.0 55.0	27.50 55.0 82.50	82.50 110.0 137.50	27.55 55.29 82.63	100.18 100.52 100.15	0.205	0.2049
					Mean	100.28		
TEN	50 100 150	22 22 22	11.0 22.0 33.0	33.0 44.0 55.0	11.20 21.93 32.90	101.81 99.68 99.69	1.2268	1.2220
					Mean	100.81		
DAP	50 100 150	9 9 9	4.5 9.0 13.5	13.5 18.0 22.5	4.54 8.98 13.56	100.88 99.77 100.44	0.5589	0.5569
					Mean	**100.36**		

Table no. 8: Ruggedness study data- Different Analyst

S.no	Drug	Condition	Mean %	± SD	% RSD
1	**Metformin**	Analyst 1	100.18	0.8331	0.8316
2		Analyst 2	100.16	0.4125	0.4118
3	**Teneligliptin**	Analyst 1	101.84	0.8248	0.8099
4		Analyst 2	99.69	0.9185	0.9213
5	**Dapagliflozin**	Analyst 1	100.96	1.3394	1.3267
6		Analyst 2	99.81	1.1568	1.1586

Table no. 9: Robustness study data

Peak Name	Parameter	Conditions	Theoretical plate	Tailing factor
Metformin	Mobile phase (Acetonitrile concentration)	43ml	11087	1.28
		40ml	11103	1.30
		37ml	11156	1.31
	Flow rate	0.8ml/min	11046	1.25
		1.0ml/min	11105	1.30
		1.2ml/min	11134	1.36

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Teneligliptin	Mobile phase (Acetonitrile concentration)	43ml	40312	1.27
		40ml	40138	1.25
		37ml	40298	1.28
	Flow rate	0.8ml/min	40312	1.27
		1.0ml/min	40140	1.25
		1.2ml/min	40298	1.28
Dapagliflozin	Mobile phase (Acetonitrile concentration)	43ml	65610	1.21
		40ml	65590	1.19
		37ml	65638	1.20
	Flow rate	0.8ml/min	65634	1.25
		1.0ml/min	65595	1.19
		1.2ml/min	65625	1.23

4. Conclusion:

A simple, precise, accurate, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of metformin, teneligliptin, and dapagliflozin in bulk and tablet dosage form. The method showed excellent linearity ($r^2 > 0.999$), low LOD and LOQ values, high accuracy with recovery close to 100%, and %RSD values below 2%, indicating good precision and reproducibility. Robustness, ruggedness, and system suitability parameters were within acceptable limits, and forced degradation studies confirmed the stability-indicating nature of the method. Therefore, the developed RP-HPLC method can be effectively used for routine quality control analysis of these drugs in pharmaceutical formulations.

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