DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR THE ESTIMATION OF METFORMIN, VILDAGLIPTIN, AND DAPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Abstract:

A simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of **metformin**, **vildagliptin**, and **dapagliflozin** in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a mobile phase consisting of **acetonitrile: phosphate buffer (pH 5.5): methanol (50:30:20 v/v)**, at a flow rate of **1.0 mL/min**, with detection at **220 nm**. The method showed linearity in the concentration ranges of **500–700 µg/mL** for metformin, **200–280 µg/mL** for vildagliptin, and **10–14 µg/mL** for dapagliflozin, with correlation coefficients (r^2) greater than **0.999** for all drugs. The **limits of detection (LOD)** were 5.85, 2.55, and 1.83 µg/mL, and the **limits of quantitation (LOQ)** were 17.74, 7.73, and 5.55 µg/mL for metformin, vildagliptin, and dapagliflozin, respectively. The analysis of the marketed formulation **Vysov DM** showed percentage purities of **100.03%, 100.08%, and 99.60%** for metformin, vildagliptin, and dapagliflozin, respectively. The **%RSD** values for precision and accuracy were below **2%**, confirming the method's reliability. Recovery studies indicated percentage recoveries of **99.67%, 99.65%, and 101.75%** for metformin, vildagliptin, and dapagliflozin, respectively, demonstrating accuracy and lack of interference from excipients. Ruggedness and robustness studies showed the method's stability against small variations in flow rate and mobile phase composition. Forced degradation studies revealed less than **20% degradation** under all stress conditions, confirming the method's stability-indicating nature. Thus, the developed RP-HPLC method is suitable for routine analysis and quality control of metformin, vildagliptin, and dapagliflozin in bulk and pharmaceutical dosage forms.

Keywords: RP-HPLC, Metformin, Vildagliptin, and Dapagliflozin

1. Introduction:

Metformin hydrochloride, chemically known as 1-carbamimidamido-N,N-dimethyl methanimidamide with the molecular formula C4H11N5 and a molecular weight of 129.167 $g \cdot mol^{-1}$,belongs to the biguanide class of oral hypoglycemic agents. Its primary mechanism of action involves lowering blood glucose levels by reducing hepatic glucose production through the inhibition of gluconeogenesis, primarily affecting the liver and skeletal muscles. [(1)] The activation of adenosine monophosphate-activated protein kinase (AMPK) plays a crucial role in enhancing insulin sensitivity and facilitating peripheral glucose uptake. In the liver, AMPK activation suppresses key enzymes responsible for gluconeogenesis, leading to decreased glucose output.[(2)] In muscle and adipose tissues, metformin promotes glucose uptake by increasing the translocation of glucose transporter type 4 (GLUT-4) to the cell membrane, while also diminishing intestinal glucose absorption. Unlike other antidiabetic medications, metformin does not stimulate insulin secretion, thereby reducing the risk of hypoglycemia. Additionally, it positively influences lipid profiles by lowering triglycerides and LDL cholesterol, further supporting its role in managing type II diabetes. [(3)]

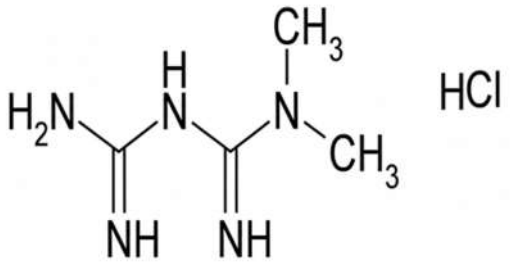


Fig.no.1. Metformin hydrochloride

Vildagliptin, representedby the chemical structure as 3-hydroxy-1-amino adamantine, has a molecular formula of C17H25N3O2 and a molecular weight of 303.399 g/mol. This oral antidiabetic medication is classified as a dipeptidyl peptidase-4 (DPP-4) inhibitor. Its primary function is to inhibit the DPP-4 enzyme, which plays a crucial role in the rapid breakdown of incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). By blocking DPP-4 activity, Vildagliptin effectively raises the levels and extends the action of these incretins, leading to enhanced glucose-dependent insulin secretion from pancreatic β-cells and reduced glucagon release from α-cells, thereby improving glycemic control.[(4)]

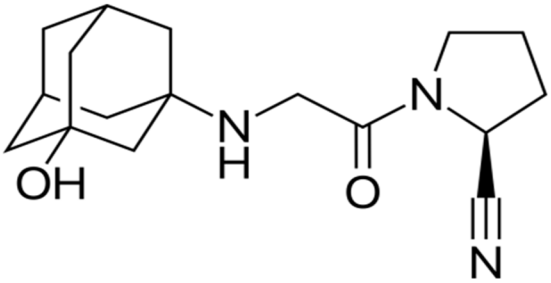


Fig.no.2. Structure of Vildagliptin

Dapagliflozin is characterized by its C-glycosyl structure, which includes beta-D-glucose, and has a molecular formula of C21H25ClO6, with a molecular weight of 408.873 g/mol. This medication functions as a selective inhibitor of the sodium-glucose co-transporter 2 (SGLT2) and is primarily utilized in the treatment of type 2 diabetes mellitus. Its main action involves decreasing the reabsorption of glucose in the proximal convoluted tubules of the kidneys. By blocking SGLT2, Dapagliflozin facilitates the excretion of glucose through urine, thereby lowering blood glucose levels. This process operates independently of insulin secretion or sensitivity, making it beneficial for patients who experience insulin resistance or have impaired β-cell function.[5]

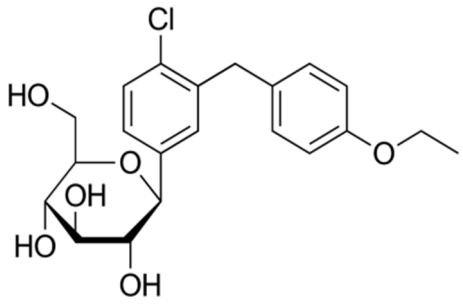


Fig.no.3. Structure of Dapagliflozin

2. Materials and Methods: [6-30]

Pharmaceutical-grade samples of Metformin, Vildagliptin, and Dapagliflozin were sourced from Madras Pharmaceuticals in Chennai. In addition, analytical-grade reagents such as acetonitrile, potassium dihydrogen orthophosphoric acid, and HPLC-grade water and methanol were procured from S.D. Fine Chemicals Pvt. Ltd. and Sigma-Aldrich Chemicals Pvt. Ltd. in India.

2.1.Instruments:

The current study utilized a variety of instruments, including the Shimadzu AUX-220 Digital Balance, a Sonicator ultrasonic cleaner (model 2200 MH), a centrifuge apparatus, Shimadzu LC, an ELICO pH Meter (Model LI – 120), and a melting point apparatus. SHIMADZU HPLC instrument, which includes pump specifications such as a five-line degassing unit for mobile phase and rinse solution, a flow rate range from 0.0001 to 10 ml/min, and a maximum pressure of 44 MPa. The auto sampler features a needle-in-flow path injection system with a volume range of 0.1 to 100 µl and a reproducibility of RSD <0.20% for volumes between 5.0 to 2000 µl. Additionally, the oven specifications allow for a capacity of six columns at a maximum of 10 cm, with a temperature setting range from room temperature to 90°C. The UV detector operates within a wavelength range of 190 to 700 nm, ensuring precise measurements under specified conditions.

2.2.Chromatographic method:

The choice of analytical method is determined by several factors, including the sample's characteristics, molecular weight, pKa value, and stability. This study specifically targeted polar drugs, which necessitated the use of reversed phase or ion exchange chromatography. For the initial separation, ultra-high-performance liquid chromatography (UHPLC) was employed due to its high efficiency. A C18 column was utilized as the stationary phase, while the mobile phase consisted of a blend of solvents, including acetonitrile, methanol, water, potassium dihydrogen orthophosphate, and orthophosphoric acid.

2.3.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.

2.4.Procedure for preparation of solution:

2.4.1. Preparation of buffer:

To prepare a 0.05M KH2PO4 buffer, begin by precisely weighing 6.8 grams of potassium dihydrogen orthophosphate and placing it into a 1000 ml beaker. Next, add 900 ml of HPLC-grade water and sonicate the mixture for 20 minutes to ensure thorough dissolution. After sonication, bring the total volume up to the 1000 ml mark with additional HPLC-grade water and filter the solution through a 0.45 µ filter. Finally, adjust the pH to 5.5 by adding 1 ml of 0.1% OPA to the filtered solution.

2.4.2. 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.

2.4.3. Diluent preparation:

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

2.4.4. Preparation of standard stock solution

Accurate measurements were taken to weigh 500 mg of metformin, 100 mg of vildagliptin, 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, 1000 µg/ml for vildagliptin, and 100 µg/ml for Dapagliflozin.

2.4.5. 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.

2.4.6. Preparation of Calibration Graph

The aliquots of stock solution of metformin, Vildagliptin and Dapaglifozin (5.0- 70 ml of 5000µg/ml for metformin, 1000 µg/ml for vildagliptin 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.

2.4.7. 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.

2.4.8. 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.

2.4.9. Recovery studies

Recovery study was performed by standard addition method. The recovery experiment was done by adding known concentration of metformin, vildagliptin and Dapaglifozinworking standard to the pre- analyzed formulations. 50% pre-analyzed 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.

2.4.10. 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.

2.4.11. 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.Selection of wavelength:

The optimal wavelengths for the simultaneous analysis of Metformin HCL, vildagliptin, and Dapagliflozin were identified through their absorbance characteristics. While the peak absorbance occurred at 232 nm, the wavelengths of 223 nm and 210.0 nm were chosen for further examination due to their strong absorbance and enhanced sensitivity for detecting these pharmaceuticals. This selection makes them particularly suitable for spectrophotometric measurements.**(Table no. 1 &Fig. no. 3)**

A simple, precise and accurate RP-HPLC method was developed for the simultaneous estimation of metformin, vildagliptin and dapagliflozin in bulk and pharmaceutical dosage form. The mobile phase consists of Acetonitrile: phosphate buffer(pH 5.5): methanol in the ratio of 50:30: 20%v/v was selected for the analysis from the spectral characteristics, 220 nm was selected as wavelength for the analysis. Flow rate was 1.0 ml /min. various aliquots of stock solutions were prepared by diluting the stock solution with mobile phase to obtain concentration from 500-700 µg/ml for metformin, 200-280 µg/ml for vildagliptin and 10-14 µg/ml dapagliflozin.

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 500-700 µg/ml for metformin, 200-280 µg/ml for vildagliptin and 10-14 µg/ml dapagliflozin respectively.

Metformin was eluted at 1.454 min, Vildagliptin was eluted at 3.991 min and Dapagliflozin was eluted at 13.617min with good resolution **(Trial Chromatogram.03).** Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated. Out of 3 trails was selected for further studies because when compared to other trails 3th trails was found less in retention time due to the ratio or organic solvent in mobile phase. The method was found, based on the system suitability parameters like resolution, tailing factor and theoretical plates.**(Fig. no. 4)**

The tablet formulation (Vysov Dm tablet formulation containing 500 mg of Metformin, 100 mg of Vildagliptin and 10 mg of Dapagliflozin) was selected for analysis. The percentage label claim present in tablet formulation was found to be 100.03, 100.08 and 99.60 % for metformin, vildagliptin and dapagliflozin respectively.The % RSD values were found to be 0.0172, 1.1942 and 0.9046 for metformin, vildagliptin and dapagliflozin respectively. The reports were shown in **(Table no. 2& Fig. no. 5 &7).**

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

The specificity study demonstrated that the analytical method accurately quantified Metformin, Vildagliptin, and Dapagliflozin without interference from excipients or other components of the formulation. The percentage recoveries for Metformin, Vildagliptin, and Dapagliflozin were 100.03%, 100.08%, and 99.60%, respectively, with %RSD values well below 2%, indicating excellent precision, accuracy, and reproducibility of the method for all three drugs. The report was shown in table **(Table no. 4).**

System suitability testing demonstrated that all parameters for Metformin, Vildagliptin, and Dapagliflozin met the established acceptance criteria. The retention times, tailing factors, theoretical plate counts, and resolution values confirmed good peak symmetry, high column efficiency, and excellent analyte separation. These results indicate that the chromatographic system was stable, precise, and reliable for the simultaneous estimation of the three drugs. The system suitability results were within the limit. Hence the method was robust.The report was shown in table **(Table no. 5).**

An accuracy study was performed for Metformin, Vildagliptin, and Dapagliflozin at 50%, 100%, and 150% concentration levels using the standard addition method. Metformin showed percentage recoveries ranging from 98.93% to 100.05%, with a mean recovery of **99.67%** and %RSD of **0.642**, indicating high accuracy and precision. Vildagliptin exhibited recoveries between 99.29% and 100.16%, with a mean recovery of **99.65%** and %RSD of **0.4559**, reflecting excellent accuracy and repeatability. Dapagliflozin demonstrated slightly higher recoveries

ranging from 100.83% to 102.50%, with a mean of **101.75%** and %RSD of **0.8311**, confirming the method's reliability. Overall, the results confirm the analytical method is accurate, precise, and suitable for routine use.The report was shown in table **(Table no. 6).**

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 0.2257, 0.4668 and 0.2569 for metformin, vildagliptin and dapagliflozin respectively. The results of the analysis are shown in **(Table no. 7).**

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 1.2278, 1.1742 and 1.2054 for metformin, vildagliptin and dapagliflozin. The percentage RSD values for analyst II were 1.4426, 1.1149 and 1.1629 % metformin, vildagliptin and dapagliflozin respectively. The values are shown in **(Table no. 8).**

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 in **(Table no. 9).**

According to the suggested stability study methodology, both drugs demonstrated any discernible degradation under stress. For all stress condition, the percentage degradation was found to be less than 20%. Forced degradation studies can help with formulation, development, manufacturing, and packaging by identifying the degradation pathways and products of APIs that may occur during storage. So it can be concluded that the developed stability indicating HPLC method can be successfully employed for the routine analysis of metformin, vildagliptin and dapagliflozin in bulk and pharmaceutical dosage forms.

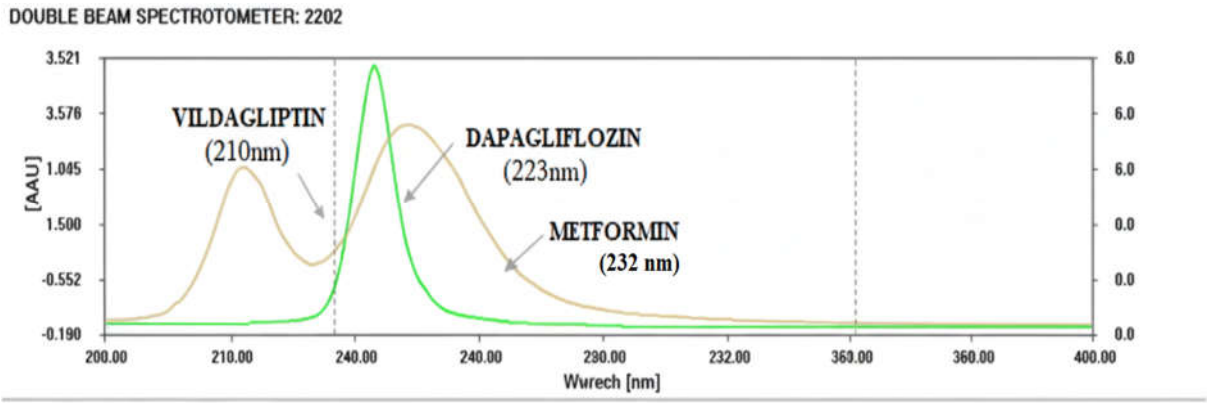
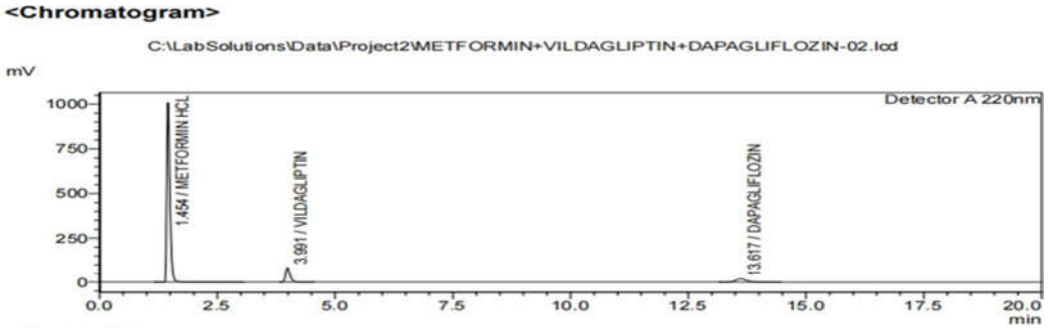


Fig.no. 3. Wavelength of Metformin, Vildagliptin&Dapagliflozin



<Peak Table>

Detector A 220nm

Peak#	Ret. Time	Area	Area%	Name	Peak Start	Peak End
1	1.454	4762676	84.52	METFORMIN HCL	1.175	3.042
2	3.991	551981	9.80	VILDAGLIPTIN	3.833	4.525
3	13.617	320220	5.68	DAPAGLIFLOZIN	13.167	14.442
Total		5634877	100.00			

Theoretical Plates/meter(USP)	Tailing Factor	Resolution(USP)
12293	1.492	--
52320	1.359	16.079
96742	1.241	30.444

Fig.no. 4.Optimized Chromatogram

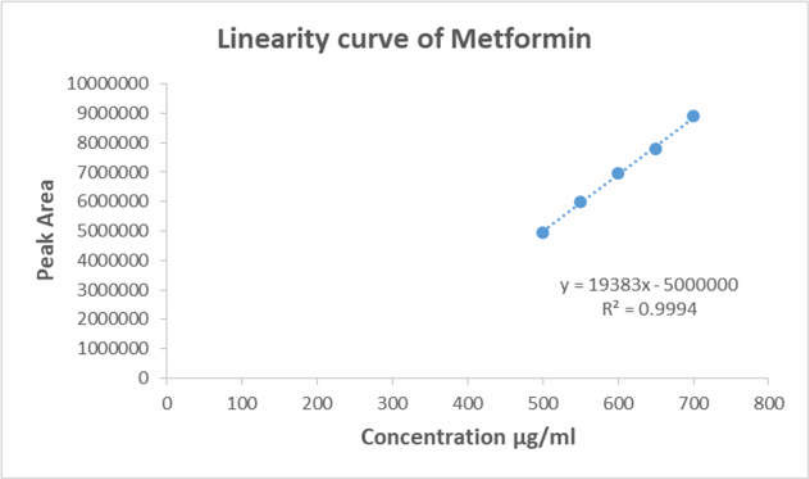


Fig.no. 5. Linearity of Metformin

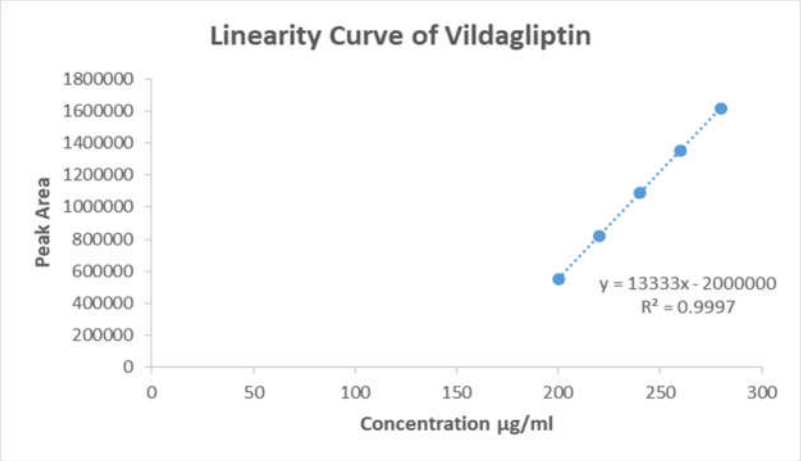


Fig.no. 6. Linearity of Vildagliptin

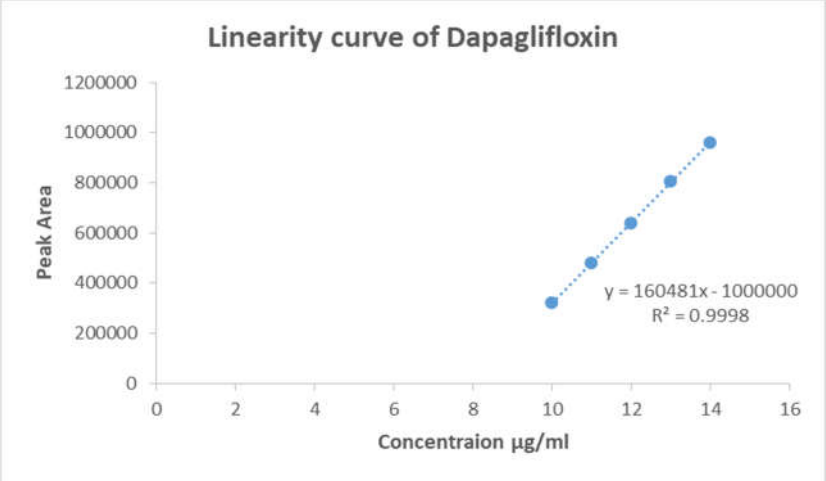


Fig.no. 7. Linearity of Dapagliptin

Table: 1. Wavelength of Metformin, Vildagliptin&Dapagliflozin

S.no	Wave Lengthʎ (nm)	Absorbance Range
1.	232 nm	1.1256
2.	223 nm	0.9850
3.	210 nm	0.8970

Table: 2. Linearity parameters

Parameters	Metformin	Vildagliptin	Dapagliflozin
Beers Law limit (µg/ml)	500-700	200-280	10-14
Correlationcoefficient (r^2)	0.9994	0.9997	0.9998
Regression equation	y=19383X-5000000	y=13333X-	y=16048X-

		2000000	1000000
Slope(m)	19383	13333	16048
Intercept(c)	5000000	2000000	1000000
LOD(µg/ml)	5.85	2.55	1.83
LOQ(µg/ml)	17.74	7.73	5.55

Table: 3. Stability of Metformin, Vildagliptin and Dapagliflozin

DegradationCondition	%Assay			%Degradation		
	MET	TEN	DAPA	MET	TEN	DAPA
0.1NHClAcidic/2hr	95.27	92.17	95.83	4.73	7.83	4.17
0.1NNaOH 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: 4. Specificity of Metformin, Vildagliptin and Dapagliflozin

Drug	Sample No	Labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average (%)	SD	% RSD
	1	500	500.26	100.05			
MET	2	500	500.15	100.03	100.03	0.0172	0.0172
	3	500	500.09	100.01			
	1	100	101.45	101.45			
VILDA	2	100	99.53	99.53	100.08	1.1941	1.1942
	3	100	99.26	99.26			
	1	10	10.05	100.5			
DAPA	2	10	9.87	98.7	99.60	0.9035	0.9046
	3	10	9.96	99.6			

Table: 5. System Stability of Metformin, Vildagliptin and Dapagliflozin

Parameters	Metformin	Vildagliptin	Dapagliflozin
Retention time (min)	1.454	3.991	13.661
Tailing factor	1.492	1.359	1.241
Peak Area	4762676	551981	320220
Theoretical plates (USP)	12293	52320	96742
Resolution (min)	0.00	16.079	30.444

Table: 6. Accuracy of Metformin, Vildagliptin and Dapagliflozin

Drug	Percentage	Amount Present (µg/ml)	Amount Added (µg/ml)	AmountEstimated (µg/ml)	Amount recovered (µg/ml)	%Recovery	SD	%RSD
MET	50 100 150	600.0 600.0 600.0	300.0 600.0 900.0	900.0 1200.0 1800.0	300.1 600.3 890.4	100.03 100.05 98.93	0.639	0.6420
					Mean	99.67		
VILDA	50 100 150	240.0 240.0 240.0	120.0 240.0 360.0	360.0 480.0 600.0	119.15 240.40 358.26	99.29 100.16 99.51	0.453	0.4559
					Mean	99.65		
DAPA	50 100 150	12.0 12.0 12.0	6.0 12.0 18.0	18.0 24.0 36.0	6.15 12.23 18.15	102.50 101.91 100.83	0.845	0.8311
					Mean	101.75		

Table: 7.Precision of Metformin, Vildagliptin and Dapagliflozin

S.No	PeakArea		
	Metformin	Vildagliptin	Dapagliflozin
1	6947944	1086757	640054
2	6958536	1096439	643672
3	6938259	1084639	641945

Table: 8.Ruggedness of Metformin, Vildagliptin and Dapagliflozin

4	6928492	1083659	644631
5	6967944	1087249	643926
Mean	6948235	1087748.6	642845.6
SD	15683.96	5078.14	1651.78
%RSD	0.2257	0.4668	0.2569

S.no	Drug	Condition	Mean	±SD	%RSD
1	**Metformin**	Analyst 1	99.90	1.2277	1.2278
2		Analyst 2	100.44	1.4490	1.4426
3	**Vildagliptin**	Analyst 1	100.92	1.1850	1.1742
4		Analyst 2	100.61	1.1217	1.1149
5	**Dapagliflozin**	Analyst 1	100.42	1.2105	1.2054
6		Analyst 2	100.55	1.1693	1.1629

Table no. 9. Robustness of Metformin, Vildagliptin and Dapagliflozin

Peak Name	Parameter	Conditions	Theoretical plate	Tailing factor
Metformin	Mobilephase (Acetonitrile concentration) Flowrate	43ml	11087	1.28
		40ml	11103	1.30
		37ml	11156	1.31
		0.8ml/min	11046	1.25
		1.0ml/min	11105	1.30
		1.2ml/min	11134	1.36
Vildagliptin	Mobilephase (Acetonitrile concentration) Flowrate	43ml	40312	1.27
		40ml	40138	1.25
		37ml	40298	1.28
		0.8ml/min	40312	1.27
		1.0ml/min	40140	1.25
		1.2ml/min	40298	1.28
		43ml	65610	1.21

Dapagliflozin	Mobilephase (Acetonitrile concentration) Flowrate	40ml	65590	1.19
		37ml	65638	1.20
		0.8ml/min	65634	1.25
		1.0ml/min	65595	1.19
		1.2ml/min	65625	1.23

4. Conclusion:

A straightforward, precise, accurate, and robust RP-HPLC method for the simultaneous estimation of metformin, vildagliptin, and dapagliflozin has been successfully validated. This method exhibited excellent linearity, with correlation coefficients exceeding 0.999 across the tested concentration ranges. It also demonstrated high accuracy, as indicated by percentage purity and recovery studies nearing 100%. The precision was confirmed by low % RSD values, typically below 2%, reflecting both high repeatability and intermediate precision. Furthermore, the method showed ruggedness with minimal variability among different analysts and robustness, remaining stable despite minor changes in flow rate and mobile phase composition. Importantly, the method proved to be stability-indicating, effectively separating and quantifying the drugs even under stress conditions, with degradation percentages remaining below 20%. Consequently, this stability-indicating HPLC method is suitable for routine analysis of metformin, vildagliptin, and dapagliflozin in both bulk and pharmaceutical dosage forms.

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