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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND VILDAGLIPTIN BY USING RP-HPLC

Rasapelly Ramesh Kumar[1] Beluri Gayatri[2] Boddu Aishwarya[2] Chalasani Shivasree[2] Dyavanapally Thriveni[2]

[1],Department of Pharmaceutical Chemistry, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Gandimaisamma (M), Hyderabad, 500043, Medchal Dist, Telangana-500055.

[2],B. Pharmacy, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Gandimaisamma (M), Hyderabad, 500043, Medchal Dist, Telangana-500055.

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ABSTRACT:

This study presents a novel approach for the concurrent quantification of Dapagliflozin and Vildagliptin in tablet form. Chromatogram was analysed using a Discovery C18 column (4.6 x 150mm, 5µm). A mobile phase consisting of Acetonitrile and Na2hpo4 prepared in a 70:30 ratio was passed down the column at a flow rate of 0.8 ml/min. The solution employed in this technique is a phosphate buffer, and the pH is modified to 5.2 by the addition of 0.1% Formic acid. The temperature was kept constant at 30°C. The selected optimised wavelength was 220 nm. The observed retention times for Dapagliflozin and Vildagliptin were 2.307 minutes and 2.865 minutes, respectively. The relative standard deviation (RSD) of Dapagliflozin and Vildagliptin were determined to be 0.9 and 0.7 correspondingly. The observed recovery rates for Dapagliflozin and Vildagliptin were 100.41% and 99.94% respectively. The limit of detection (LOD) and limit of quantification (LOQ) values derived from the regression equations of Dapagliflozin and Vildagliptin were 0.02, 0.07, and 0.24, 0.72 correspondingly. The regression equation for Dapagliflozin is provided as y = 42540x + 1488.4. Furthermore, the equation y = 38485x + 2186.5 of Vildagliptin. By reducing retention times and run time, the new approach proved to be easy and cost-effective for use in routine quality control tests in industries.

Keyword: Dapagliflozin, Vildagliptin, RP-HPLC, Validation.

Address for Correspondence: Rasapelly Ramesh Kumar**,** Department of Pharmaceutical Chemistry, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Gandimaisamma (M), Hyderabad, 500043, Medchal Dist, Telangana-500055.; Phone Number: 8897802723 , **E-Mail:** rameshkumarrasapelly@gmail.com.

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INTRODUCTION

Type 2 diabetes is a medical disorder resulting from impaired regulation and utilisation of sugar as an energy source by the body. This sugar is also referred to as glucose. This chronic process leads to excessive blood sugar levels. Prolonged elevation of blood glucose levels can ultimately result in dysfunctions of the circulatory, neurological, and immunological systems.

Primarily, type 2 diabetes is characterised by two challenges. The pancreas lacks sufficient production of insulin, a hormone responsible for controlling the transportation of sugar into cells. And cells exhibit decreased responsiveness to insulin and uptake of glucose.

While formerly referred to as adult-onset diabetes, both type 1 and type 2 diabetes can manifest in both childhood and maturity. Prevalence of type 2 is higher in elderly individuals. However, the rise in the prevalence of childhood obesity has resulted in an escalation of type 2 diabetes incidence among adults of younger age.[1]

Type 2 Diabetes is a persistent endocrine disorder marked by increased levels of glucose in the bloodstream, accompanied with micro and macrovascular deficits [2,3,4]. Therefore, treatment with a combination of oral hypoglycemic drugs with distinct modes of action is often preferred for enhancing glycaemic control, as opposed to monotherapy. [5,6].

Metformin is almost often prescribed by endocrinologists as a therapeutic intervention for type II diabetes mellitus, except there's particular justifications for its avoidance [7] via inhibiting the mitochondrial respiratory chain of the liver, it stimulates the activation of AMP-activated protein kinase (AMPK), enhances insulin sensitivity (via influencing fat metabolism), and reduces cyclic adenosine monophosphate (cAMP) levels. Consequently, it decreases the activation of enzymes responsible for glucose synthesis and aids in the regulation of blood glucose levels. The compound dapagliflozin inhibits the function of sodium-glucose co-transporter 2 (SGLT2), therefore impeding the reabsorption of glucose that has undergone renal filtration. Consequently, enhanced excretion of glucose in the urine results in a reduction of blood glucose levels. The impact of pancreatic β cell activity or insulin sensitivity modification on the mechanism of action is negligible.[8] By forming a robust chemical connection with the enzyme's active site of dipeptidyl peptidase-4 (DPP-4), vildagliptin induces permanent inhibition of the enzyme. [9] Increased amounts of intact glucagon-like peptide-1 (GLP-1) are observed both postprandial and during fasting periods. Experimental studies have shown that it efficiently stimulates the secretion of insulin while inhibiting the secretion of glucagon in reaction to glucose levels.[10]

Background:

Dapagliflozin and Vildagliptin demonstrate promise as a drug combination of sodium-glucose cotransporter type 2 inhibitors (SGT2i) and dipeptidyl peptidase-4 inhibitors (DPP4i)[11]. SGLT2 inhibitors lower elevated blood glucose levels by enhancing the elimination of glucose in urine, while independent of insulin secretion or activity. DPP4 inhibitors, by impeding the breakdown of active incretin hormones, enhance glucose control and promote insulin secretion while decreasing glucagon liberation.

Dapagliflozin: Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor used to treat type 2 diabetes mellitus. When used in conjunction with diet and exercise in adults, dapagliflozin enhances glycaemic management by blocking the absorption of glucose in the proximal tubule of the nephron and inducing the excretion of glucose with urine. It is chemically known as (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl) oxane -3,4,5-triol.[12] Administered in conjunction with diet and exercise in adults, dapagliflozin improves glycaemic control by inhibiting the reabsorption of glucose in the proximal tubule of the nephron, leading to the elimination of glucose in the urine. It has been investigated as a monotherapy and as an adjunctive therapy with insulin or other oral hypoglycemic drugs.[13,14]

Vildagliptin: Vildagliptin (LAF237) is an orally administered antihyperglycemic medication that functions by specifically blocking the dipeptidyl peptidase-4 (DPP-4) enzyme. Its purpose is to control type II diabetes mellitus, a condition characterised by reduced GLP-1 production and insulinotropic effects. It is chemically known as (2S) -1-{2- [(3-hydroxyadamantan-1-yl)amino]acetyl} pyrrolidine-2-carbonitrile. [15]

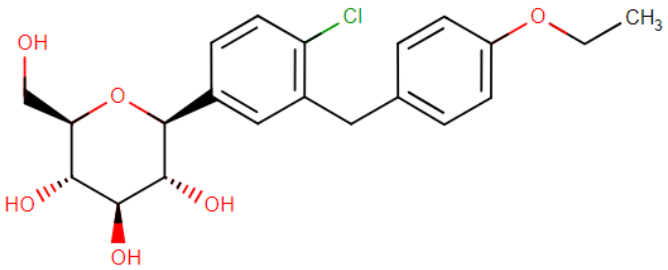

Figure No.1 structure of Dapagliflozin

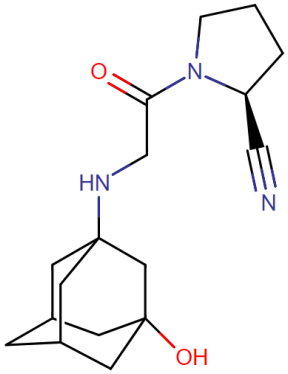

Figure No.2 structure of Vildagliptin

An extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Dapagliflozin, Vildagliptin, and their medicinal dose form using RP-HPLC.16-19 must be validated and developed as per ICH guidelines

Materials and Methods

Spectrum pharma Research Solution provide with Dapagliflozin and Vildagliptin pure drugs (API) gift samples and Combination Dapagliflozin and Vildagliptin tablets (Vildaily-DZ) received from local market. The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective:

The major aim of this work is to develop a precise, accurate, sensitive, specific, consistent, and efficient analytical technique for simultaneously measuring the amounts of Dapagliflozin and Vildagliptin in their pure state and tablet formulation.

Chromatographic Conditions:

Table No.1 chromatographic conditions

Mobile phase	Kh2Po4:Acetonitrile (70:30)
Flow rate	1 ml/min
Column	Discovery C18 (4.6 x 150mm, 5µm
Detector wave length	220nm
Column temperature	30°C
Injection volume	10µL
Run time	5.0 min
Buffer	0.01 N Na2HPO4 and 0.1% Formic Acid

Preparation of Standard stock solutions: Accurately weighed 5mg of Dapagliflozin, 50mg of Vildagliptin and transferred to 100ml volumetric flask. 3/4th of diluents was added to the flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution 1. (50µg/ml of Dapagliflozin and 500µg/ml of Vildagliptin)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (5µg/ml of Dapagliflozin and 50µg/ml of Vildagliptin)

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(100µg/ml of Dapagliflozin and 1000µg/ml of Vildagliptin)

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (5µg/ml of Dapagliflozin and 50µg/ml of Vildagliptin)

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Dapagliflozin(5ppm) and Vildagliptin(50ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The relative standard deviation (RSD) for the area of six standard injections should not exceed 2%. System suitability chromatogram was shown in figure 3 and values in table 1.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. The chromatogram were represented are shown in figure 4 and table 3.

Table No2: System suitability results

	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Vildagliptin	2.307	2135694	6207		1.4
2	Dapagliflozin	2.865	199228	9128	4.6	1.1

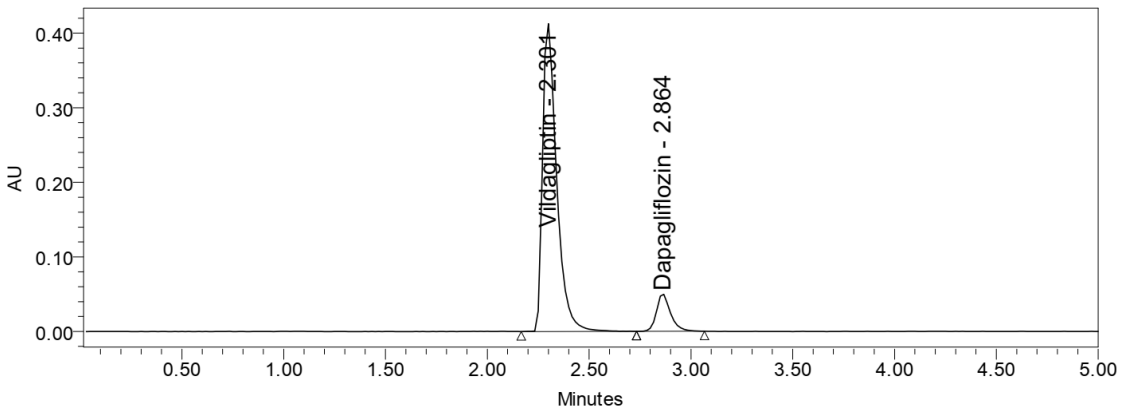

Figure 3: system suitability Chromatogram

Table No.3: Specificity data

Sample name	Retention time(mins)	Area
Vildagliptin	2.307	2135694
Dapagliflozin	2.865	199228

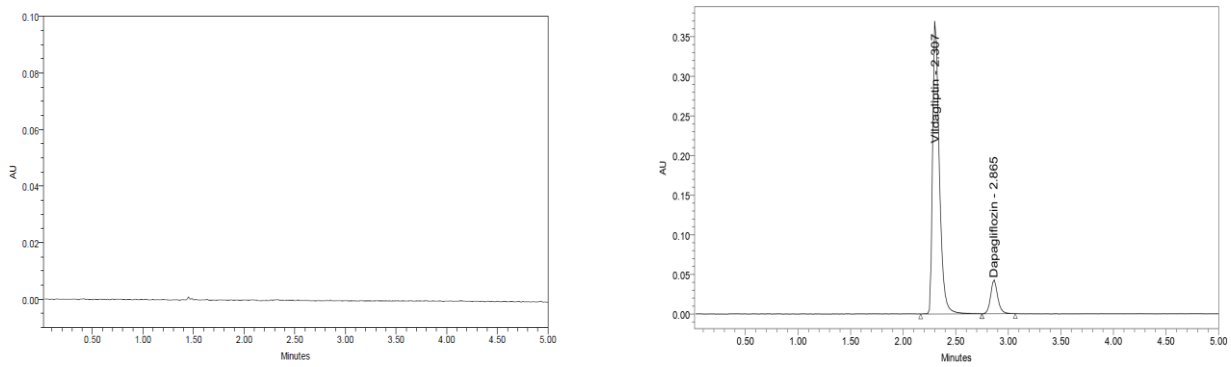

Figure 4: Specificity of Dapagliflozin and Vildagliptin

Linearity:
Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 5,6

Table No.4: Calibration data of Dapagliflozin and Vildagliptin

Dapagliflozin		Vildagliptin	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
1.25	53879	12.5	465341
2.5	110689	25	976032
3.75	162543	37.5	1453473
5	214053	50	1926125
6.25	264482	62.5	2439526
7.5	321439	75	2857076

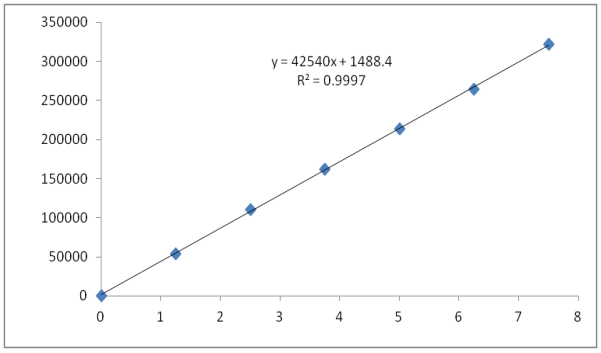

Figure No.5 Calibration curve of Dapagliflozin

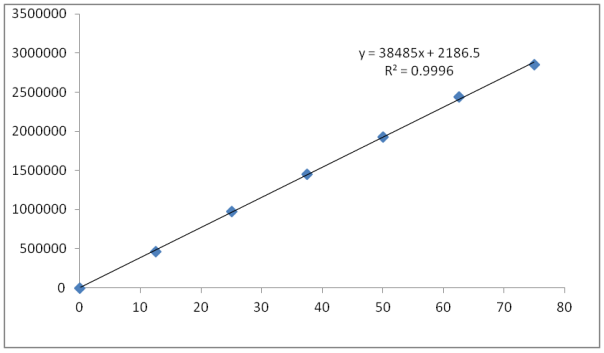

Figure No.6 Calibration curve of Vildagliptin

Table No.5: regression data

Parameter	Dapagliflozin	Vildagliptin
Conc range (µg/mL)	1.25-7.5µg/ml	12.5-75µg/ml
Regression Equation	y = 42540x + 1488.4	y = 38485x + 2186.5
Co-relation	0.999	0.999

Accuracy:
Recovery data shown in table 6

Table No.6: recovery data of Dapagliflozin and Vildagliptin

	Dapagliflozin			Vildagliptin		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	2.5	2.52	100.78	25	25.11	100.46
	2.5	2.62	104.97	25	24.76	99.06
	2.5	2.52	100.62	25	24.83	99.31
100%	5	5.01	100.29	50	50.10	100.20
	5	4.98	99.58	50	49.74	99.48
	5	4.97	99.43	50	50.00	99.99
150%	7.5	7.45	99.39	75	75.53	100.71
	7.5	7.43	99.08	75	75.53	100.71
	7.5	7.47	99.54	75	74.63	99.51
% Recovery	100.41			99.94		

System precision was performed and the data was shown in table 7

Table No.7: System precision of Dapagliflozin and Vildagliptin

S. No	Area of Dapagliflozin	Area of Vildagliptin
1.	215448	1925609
2.	216029	1956714
3.	213751	1959867
4.	215366	1940114
5.	211456	1953520
6.	216791	1942334
Mean	**214807**	**1946360**
S.D	**1923.4**	**12858.7**
%RSD	**0.9**	**0.7**

The % RSD for the peak areas of Dapagliflozin and Vildagliptin obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Dapagliflozin and Vildagliptin and shown in table 8.

Table No.8: method Precision

S. No	Area of Dapagliflozin	Area of Vildagliptin
1.	213975	1934284
2.	214477	1963915
3.	216471	1959490
4.	215235	1960000
5.	214711	1960937
6.	213620	1949851
Mean	**214748**	**1954746**
S.D	**1015.0**	**11092.0**
%RSD	**0.5**	**0.6**

From the above results, the % RSD of method precision study was within the limit for Dapagliflozin and Vildagliptin.

Robustness: Robustness conditions like Flow minus (0.7ml/min), Flow plus (0.9ml/min), mobile phase minus (65B:35A), mobile phase plus (75B:25A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table No.9: Robustness data for Dapagliflozin and Vildagliptin.

Condition	%RSD of Dapagliflozin	%RSD of Vildagliptin
Flow rate (-) 0.7ml/min	1.3	0.7
Flow rate (+) 0.9ml/min	1.4	0.9
Mobile phase (-) 65B:35A	0.9	0.9
Mobile phase (+) 75B:25A	1.7	0.9
Temperature (-) 27°C	0.2	0.5
Temperature (+) 33°C	0.3	0.4

Force Degradation Studies: table 10 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 7, 8

Table No.10: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

Table No.11: degradation data

Type of degradation	Dapagliflozin			Vildagliptin		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	210668	97.68	2.32	1903613	97.61	2.39
Base	211117	97.89	2.11	1917442	98.32	1.68
Peroxide	202306	93.80	6.20	1823726	93.51	6.49
Thermal	211077	97.87	2.13	1905627	97.71	2.29
Uv	213186	98.85	1.15	1911428	98.01	1.99
Water	214932	99.66	0.34	1930942	99.01	0.99

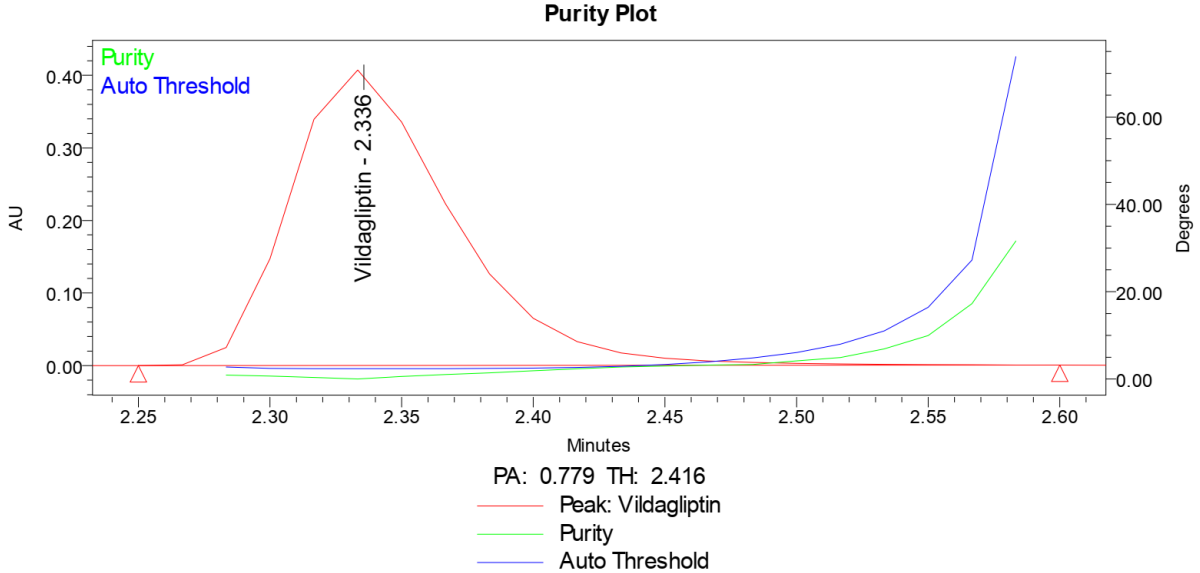

Figure No.7 Purity plots for Acid Condition for Vildagliptin.

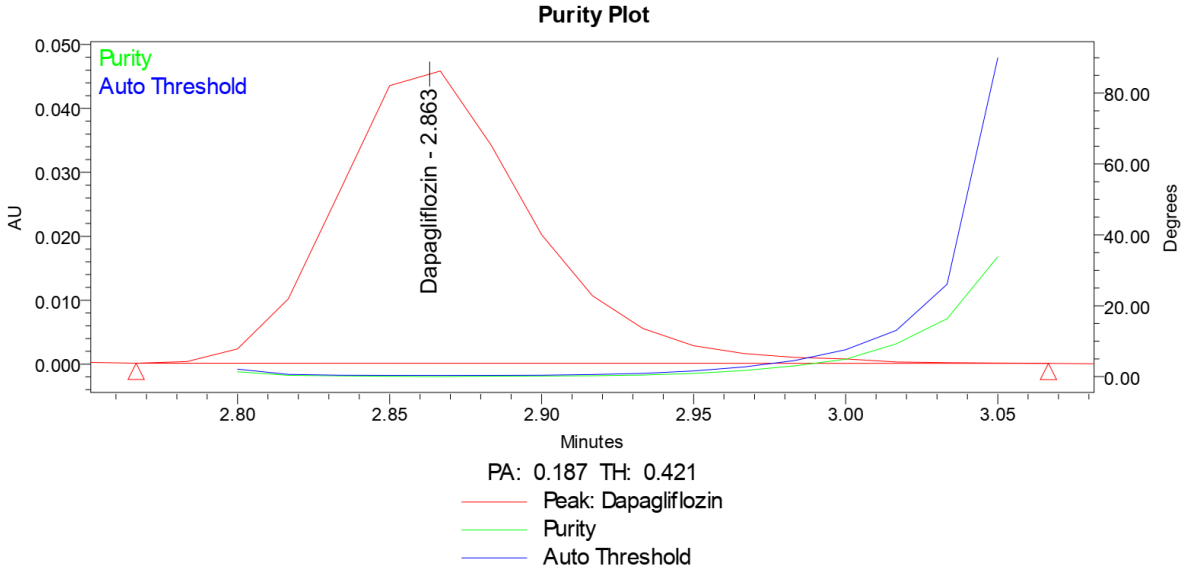

Figure No.8: **Purity plots for Acid Condition for Dapagliflozin.**

Assay: Vildaily, bearing the label claim Dapagliflozin 100mg, Vildagliptin 10mg. Assay was performed with the above formulation. Average % Assay for Dapagliflozin and Vildagliptin obtained was 100.00% and 100.02% respectively.

Table No.12: assay data

	Dapagliflozin			**Vildagliptin**		
S.no	Std Area	Sample area	% Assay	Std Area	Sample area	% Assay
1	1925609	1934284	99.18	1925609	1934284	99.18
2	1956714	1963915	100.70	1956714	1963915	100.70
3	1959867	1959490	100.47	1959867	1959490	100.47
4	1940114	1960000	100.50	1940114	1960000	100.50
5	1953520	1960937	100.55	1953520	1960937	100.55
6	1942334	1949851	99.98	1942334	1949851	99.98
Avg	**1946360**	**1954746**	**100.23**	**1946360**	**1954746**	**100.23**
Stdev	**12858.7**	**11092.0**	**0.569**	**12858.7**	**11092.0**	**0.569**
%RSD	**0.7**	**0.6**	**0.6**	**0.7**	**0.6**	**0.6**

Assay was calculated by the formula:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure No.9 formula

CONCLUSION

The results of this study will greatly aid in the quality control of generic pharmaceutical formulations containing Dapagliflozin and Vildagliptin. This may be ascribed to the study's straightforward sample preparation method, which utilises a small amount of mobile phase and requires only a brief analysis period. Following an investigation of two medications in a combination dose form, the results indicated a close approximation of

100% efficacy using the recently developed analytical method. The recovery studies produced consistent favourable results, indicating the lack of any influence from the excipient.

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REFERENCES

1. https://www.mayoclinic.org/diseases-conditions/type-2-diabetes/symptoms-causes/syc-20351193
2. Chatterjee, S.; Khunti, K.; Davies, M.J. Type 2 diabetes. Lancet 2017, 389, 2239–2251. [Google Scholar] [CrossRef]
3. Mouhayyar, C.; Riachy, R.; Khalil, A.B.; Eid, A.; Azar, S. SGLT2 inhibitors, GLP-1 agonists, and DPP-4 inhibitors in diabetes and microvascular complications: A review. Int. J. Endocrinol. 2020, 2020, 1762164. [Google Scholar] [CrossRef] [PubMed]
4. Cade, W.T. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys. Ther. 2008, 88, 1322–1335. [Google Scholar] [CrossRef] [PubMed] [Green Version]
5. Abdul-Ghani, M.A.; Puckett, C.; Triplitt, C.; Maggs, D.; Adams, J.; Cersosimo, E.A.; DeFronzo, R. Initial combination therapy with metformin, pioglitazone and exenatide is more effective than sequential add-on therapy in subjects with new-onset diabetes. Results from the Efficacy and Durability of Initial Combination Therapy for Type 2 Diabetes (EDICT). Diabetes Obes. Metab. 2015, 17, 268–275. [Google Scholar] [CrossRef] [Green Version]
6. American Diabetes Association. 9. Pharmacologic approaches to glycemic treatment: Standards of medical care in diabetes-2020. Diabetes Care. 2020, 43, S98–S110. [Google Scholar] [CrossRef] [Green Version]
7. Tan X et all.., Combination therapy for type 2 diabetes: dapagliflozin plus metformin. Expert Opin Pharmacother. 2016.
8. Guarino E, et all..,Combination therapy with metformin plus vildagliptin in type 2 diabetes mellitus. Expert Opin Pharmacother. 2012.
9. Kasahara M, et all.., Comparison of efficacy between dipeptidyl peptidase-4 inhibitor and sodium-glucose cotransporter 2 inhibitor on metabolic risk factors in Japanese patients with type 2 diabetes mellitus: Results from the CANTABILE study,Diabetes Res Clin Pract, 2021.
10. Scheen AJ. DPP-4 inhibitor plus SGLT-2 inhibitor as combination therapy for type 2 diabetes: from rationale to clinicalaspects. Expert Opin Drug Metab Toxicol. 2016.
11. Min SH et all., Combination of sodium-glucose cotransporter 2 inhibitor and dipeptidylpeptidase-4 inhibitor in type 2 diabetes: a systematic review with meta-analysis. Sci Rep. 2018 13th March 8
12. https://go.drugbank.com/drugs/DB06292
13. Komoroski B et all.., In vitro characterization and pharmacokinetics of dapagliflozin (BMS-512148), a potent sodium-glucose cotransporter type II inhibitor, in animals and humans, Drug Metab Dispos. 2010 Mar.
14. Sl Anderson, Dapagliflozin efficacy and safety: a perspective review. Ther Adv Drug Saf. 2014 Dec.
15. https://go.drugbank.com/drugs/DB04876
16. Bhavesh H. Patel, Nishit H. Teraiya, Simultaneous Estimation and Stability Indicating Method of Vildagliptin and Dapagliflozin by Rp-hplc in Pharmaceutical Dosage Form, (2024), Research Square.

17. Pintu Prajapati, Bageshree Rana, Veera Shakar Pulusu, Shailesh Shah, Simultaneous Chromatographic Estimation of Vildagliptin and Dapagliflozin Using Hybrid Principles of White Analytical Chemistry and Analytical Quality by Design, J AOAC Int (2024) Jan 4;107(1):212-222. doi: 10.1093/jaoacint/qsad108.
18. Vishva S. Patel, Pankaj Kapupara, Jitendra O. Bhangale, Stability Indicating Rp-Hplc Method for Simultaneous Estimation of Dapagliflozin And Vildagliptin In Pharmaceutical Dosage Form, (2023), EuroChemBull, ISSN 2063-5346.
19. P Priya, P Janki, P Bhumi, P Ankita, T Urvi, Stability Indicating Rp-Hplc Method Development and Validation For Simulaneous Estimation Of Dapagliflozin Propanediol Monohydrate And Vildagliptin In Tablet Dosage Form, (2023) IJCRT, Volume 11, Issue 3 March 2023, ISSN: 2320-2882.