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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF VILDAGLIPTIN HYDROCHLORIDE AND DAPAGLIFLOZIN BENZOATE BY RP-HPLC METHOD IN TABLET DOSAGE FORM

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ABSTRACT

The objective of this study was to develop a new simple, rapid, sensitive, and validated reversed phase-high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Vildagliptin Hydrochloride and Dapagliflozin Benzoate in tablet dosage form. By utilizing RP-HPLC, this method fills a gap in the existing literature. Optimizing various parameters of the chromatographic process was key to developing an effective method for separating and detecting drugs. International guidelines and regulatory requirements were followed for the validation of the method, including specificity, linearity, accuracy, Precision, robustness, and suitability for the system. For analysis of mixed solutions containing Vildagliptin Hydrochloride and Dapagliflozin benzoate, a Chromatographic separation was achieved on a Phenomenex C 18 column (150x4.6mm), 5µm, a mobile phase ratio consisting of Buffer: ACN: Methanol (30:5:65) at flow rate 1.2 ml/min, and total run time 8 min, The injection volume 20µl. The detection wavelength is 249nm. Approximately 2000

plates were found, indicating a successful chromatographic separation. With a tailing factor of less than 2 and a well-resolved peak, the peaks appear symmetrical and well-resolved. In order to ensure there were no interfering peaks, retention times for Vildagliptin Hydrochloride and Dapagliflozin benzoate were found to be 2.078 and 3.238 min. As for Vildagliptin Hydrochloride, the correlation coefficient (R^2) is 0.9993 and Dapagliflozin benzoate is 0.9996. This indicates a good linear relationship between drug concentrations and peak areas based on the high correlation coefficients. For Vildagliptin Hydrochloride and Dapagliflozin benzoate, the %RSD values were 0.09% and 1.42%, respectively, below the acceptable limit of 2%. As a result, the method is accurate and repeatable. In the study of Vildagliptin Hydrochloride, the mean percent recovery was 100.09%, while in the study of Dapagliflozin benzoate, the mean percent recovery 100.34%. Under varied conditions, both Vildagliptin Hydrochloride and Dapagliflozin benzoate showed %RSD values within the Acceptable range of 2%, demonstrating the robustness and reliability of the method. This Study concluded that valuable insight is provided into the use of validated RP-HPLC methods in this study, which contributes significantly to the evolution of pharmaceutical analytical techniques.

KEYWORDS: Vildagliptin Hydrochloride and Dapagliflozin benzoate, RP-HPLC, Method development, Method validation.

INTRODUCTION

Dapagliflozin (DAPA) **(Figure 1)** belongs to an emerging class of oral anti-diabetic medications known as Sodium Glucose Co-Transporter 2 (SLGT2) inhibitor. It is used to manage type 2 diabetes and improve glycemic control in adults when combined with diet and exercise. It is a sodium-glucose cotransporter 2 inhibitor that prevents the absorption of glucose in the kidney. Dapagliflozin is a first-generation SGLT inhibitor that selectively blocks SGLT2 over SGLT1, inhibiting glucose transport by roughly a hundred times.[1] Vildagliptin (VILDA) **(Figure 2)** is a biguanide antihyperglycemic agent used for treating noninsulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Vildagliptin is the only oral antihyperglycemic agent that is not associated with weight gain. Vildagliptin is the most common therapy for obese NIDDM patients due to its efficacy for weight loss. Vildagliptin does not generate hypoglycemia when used alone; Even so, it possesses the potential to enhance the hypoglycemic effects of

sulfonylureas and insulin.[2] Dapagliflozin and Vildagliptin are used to treat type 2 diabetic mellitus (T2DM). Particularly combined mechanism of action of dapagliflozin and Vildagliptin, as well as their high effectiveness and safety profiles, promote the use of this fixed-dose combination as a therapy option for T2DM patients. A literature review found few published RP-HPLC methods for determining Vildagliptin. A satisfactory study was conducted for the measurement of dapagliflozin by HPLC separately. This work successfully developed a speedy, precise, accurate, and cost-effective RP-HPLC method. The approach was verified and recovery studies were conducted following ICH criteria, employing several statistical parameters.[3]

MATERIALS AND METHODS

Chemicals and Reagents

Potassium dihydrogen ortho phosphate, Water and Methanol of HPLC grade were obtained from S.D. Fine Chemicals Pvt. Ltd., India, Sigma - Aldrich Chemicals Pvt. Ltd. Pharmaceutical grade Dapagliflozin and Vildagliptin were obtained from USV Formulations Pvt. Ltd., Pondicherry, India and Zydus Cadila Healthcare Pvt. Ltd., Gujarat, India respectively.

Instruments

The analysis was performed by using the Shimadzu digital electronics balance, pH meter - Elico Pvt. Limited, India, Jasco V-600 UV/ Vis- spectrophotometer, Shimadzu HPLC Prominence *i* LC – 2030 liquid chromatograph system with UV – VISIBLE detector and auto sampler injector. Chromatograms were recorded and integrated on PC installed with Lab solutions chromatographic software. Shimadzu liquid chromatograph equipped with LC – 10 AT VP pump, SPDM10A VP diode array detector and rheodyne 7725 *i* injected with a 20 µl loop. Chromatograms were recorded and integrated on PC installed with LC solutions chromatographic software.

REAGENTS AND SOLUTIONS

Preparation of Mobile Phase

a. Selection of mobile phase

Solvent type, solvent strength, strength of buffer and optimum pH were optimised to get the chromatographic conditions that gave best separation.

b. Selection of mobile phase ratio

Mobile phase system containing 10 mM potassium dihydrogen ortho phosphate and methanol was tested in different ratios like 30: 70, 35: 65 and 40: 60 v/v. A ratio of 35: 65% v/v gave good resolution and peak characteristics.

c. Selection of pH Different

pH's of 10 mM potassium dihydrogen orthophosphate such as 3, 3.5, 4, 4.5 and 5.4 were tried. Good peak characteristics was observed for pH 4 and hence selected for further studies.

Preparation of stock solution

`A stock solution of Dapagliflozin (2500 µg/ml) and Vildagliptin (100 µg/ml) was prepared in methanol.

Preparation of Sample solution

Ten tablets each containing 100 mg of Dapagliflozin and 10 mg of Vildagliptin were taken for the studies and the average weight was determined. Amount of powder equivalent to 125 mg of Dapagliflozin and 5 mg of Vildagliptin was taken and transferred to a 50 ml volumetric flask, and 25 ml methanol was added. The contents of the flask were shaken for 10 minutes, followed by dilution to volume with methanol to provide a solution containing 2500 µg/ml of Dapagliflozin and 100 µg/ml of Vildagliptin. This solution was filtered through a 0.45 mm membrane filter before injection.

Recording the chromatogram

A steady baseline was recorded with the fixed chromatographic conditions and 20 µg of standard drug solutions and sample solutions were injected and chromatograms were recorded. Calibration curve was plotted using the standard drug peak area versus concentration of standard solutions.

METHODS DEVELOPMENT[4-16]

The developed method was fully validated for the parameters as per ICH guidelines.

System suitability

A standard solution was prepared by using Dapagliflozin and Vildagliptin working standards as per test method. The system suitability parameters such as plate number (N), tailing factor (Tf), capacity factor (k`), resolution (Rs) were evaluated from standard chromatograms by calculating the % RSD from replicate injections for Dapagliflozin and Vildagliptin.

Linearity

Linearity is achieved through a series of three to five injections of five or more standards. Plot a graph of peak area (or heights) of the calibration standards on the Y-axis against the nominal standard concentration, and the linearity of the estimated curve is evaluated through the value of the co-relation coefficient (r2). Dapagliflozin was found to be linear in the concentration range of 25 – 250 µg/ml and Vildagliptin was found to be linear in the concentration range of 1 – 10 µg/ml.

Accuracy

Accuracy of the method was determined by inject the three replicate injections of concentrations range of 25 – 250 µg/ml were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the amount found and amount added for Dapagliflozin and Vildagliptin. Calculate the individual recovery and mean recovery values.

Precision

To determine the precision, intra-day, inter-day and Repeatability analysis was performed. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. Solutions corresponding to each concentration level were injected in duplicate. The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample.

Limit of detection and Limit of quantification

The LOD and LOQ values were determined by injecting lower concentrations of the drugs. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be determined quantitatively with suitable precision and accuracy. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact Value. The determination of the limit of quantification is carried by minimum concentration at which the analyte can reliably be quantified is established.

Stability

The standard drug solutions were subjected to stability studies under room temperature and refrigerated conditions. Stability of solutions were analysed by looking for any changes in retention time, resolution, peak shape etc.

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters like Flow plus and minus, Mobile phase ratio plus and minus, pH plus and minus. Important parameters in the method were systemically varied and their effect on separation was measured as follows:-

- ± 0.1 units of flow rate,
- ± 2 units of mobile phase ratio
- ± 0.5 units of Ph

Specificity (forced degradation studies)

Sample degradation is also a technique for assessing specificity by deliberately degrading the sample and to look for the appearance of other peaks in the chromatogram. Here, the drugs were subjected to acid degradation (0.5 N HCl), base degradation (0.5 N NaOH), oxidative degradation (3% H2O2) and neutral conditions to achieve 10 to 20% degradation from the initial material.

RESULT AND DISCUSSION

The present study aimed to create a new RP-HPLC method for separating and quantifying Dapagliflozin and vildagilptin in bulk and pharmaceutical dosage forms. Various mobile phase compositions were tested. In RP-HPLC method, optimizations of different chromatographic parameters like selection of chromatographic method, detection wavelength, selection of mobile phase, mobile phase ratio, etc., were done. A wavelength of 247 nm was selected for the study. It was found that a system comprising of 10 mM potassium dihydrogen orthophosphate: methanol in the ratio of 35: 65%v/v which gave good resolution and peak characteristics. The column used was Hibar, C18 column 250mm X 4.0mm, particle size 5µm with flow rate of 1.0 ml/min with pH adjusted to 4.0 using UV detection at 247 nm.

1. System suitability

System suitability parameters like plate number (N), tailing factor (Tf), capacity factor (k`), resolution (Rs) and relative standard deviation of peak area for repetitive injections were studied and it was found that the values were within the limits.(Table. 1 & fig.3).

2. Linearity

From the linearity studies, specified concentration levels were determined. Dapagliflozin was found to be linear in the concentration range of 25 to 250 µg/ml. Vildagliptin was found to be linear in the concentration range of 1 to 10 µg/ml. It shows that the good correlation exist between the drug and response. The results are summarized in the (Table No.2&3, fig. 4 & 5).

3. Accuracy

Recovery studies were done for determining accuracy parameter. It was done by spiking known quantity of standard drug with the analysed sample formulation and the contents were reanalysed by the proposed method. Recovery studies were carried out at 80, 100 and 120% levels. The percentage recovery and its %RSD were calculated, (Table no.4 & 5).

4. Precision

a. Intraday precision

Intra-day precision was studied by carrying out the analysis of the standard drugs at two different concentrations in the linearity range of the drugs for three times on the same day and %RSD was calculated, (Table no.6).

b. Inter-day precision

Inter-day precision was studied by carrying out the analysis of the standarddrugs at two different concentrations in the linearity range of the drugs for three days over a period of one week and %RSD was calculated, (Table no.7)

c. Repeatability of injection

Standard drug solution was injected six times and its %RSD was calculated, (table no.8)

5. Limit of Detection and Limit of Quantification

The LOD values for Dapagliflozin and Vildagliptin were found to be 5 and 10 ng/ml, (Fig. 6 & 7) and their LOQ values were found to be 10 and 70 ng/ml respectively (Fig. 8 & 9).

6. Robustness

The analysis was performed in different conditions to fine the variability of test results. The conditions are checked for variation of results. Results are summarized in (Fig. 10)

7. Assay: (Recording the chromatogram)

Assay was performed with the above formulation. Average percentage purity for Dapagliflozin is 98.55 % and Vildagliptin is 96.66 % and % RSD was found 1.42 and 1.14. The results of formulation analysis are given in, Table.9

Table No.1: System Suitability of Proposed Method.

Drug name	Number of theoretical plates (N)	Tailing Factor (Tf)	Capacity factor (k`)	Resolution (Rs)	Relative standard deviation of peak area (%RSD)
DAPAGLIFLOZIN	1652	1.3	0.657	4.746	0.09
VILDAGLIPTIN	1381	1.5			0.43

Table No. 2: Linearity (Linearity data of Dapagliflozin).

Concentration (µg/ml)	Peak Area
25	1354287
50	2611634
75	3796175
100	4972965
125	6188947
150	7408120
175	8428047
200	9700838
225	10771794
250	11866670
Slope	**46749.2**
Intercept	**281930**
Correlation coefficient	**0.9998**

Table No. 3: Linearity (Vildagliptin-Calibration data).

Concentration(µg/ml)	PeakArea
1	15169
2	29070
3	46856
4	62792
5	79957
6	98550
7	112943
8	131102
9	147915

10	157651
Slope	**16392.2**
Intercept	**-1954.47**
Correlation coefficient	**0.9991**

Table No. 4: Accuracy (Recovery studies for Dapagliflozin).

Level	%Recovery	%RSD*
80%	99.72	0.65
100%	101.25	0.38
120%	100.92	0.55

***RSD of 6 observations**

Table No. 5: Accuracy (Recovery studies for Vildagliptin).

Level	%Recovery	%RSD*
80%	99.99	0.19
100%	100.98	0.64
120%	101.06	0.64

***RSD of 6 observations**

Table No. 6: Precision (Intraday precision).

Concentration	Peak Area		%RSD	
	Dapagliflozin	Vildagliptin	Dapagliflozin	Vildagliptin
100(µg/ml) Dapagliflozin 4(µg/ml) Vildagliptin	5123139	60866	0.88	0.46
	5173112	61212		
	5214763	61427		
125(µg/ml) Dapagliflozin 5 (µg/ml) Vildagliptin	6299618	78644	0.98	0.29
	6279424	79109		
	6184767	78945		

Table No. 7: Precision (Interday precision).

Concentration	PeakArea		% RSD	
	Dapagliflozin	Vildagliptin	Dapagliflozin	Vildagliptin
100(µg/ml) Dapagliflozin 4(µg/ml) Vildagliptin	5172965	62792	0.65	0.19
	4967626	62553		
	5027176	62720		
125(µg/ml) Dapagliflozin 5(µg/ml) Vildagliptin	6188947	79957	0.71	1.15
	6203468	79833		
	6271502	78311		

Table No. 8: Precision (Repeatability of injection).

Concentration	Peak Area		% RSD	
	Dapagliflozin	Vildagliptin	Dapagliflozin	Vildagliptin
125 (µg/ml) Dapagliflozin 5 (µg/ml) Vildagliptin	6278397	79627	0.09	0.43
	6276164	79874		
	6279061	78968		
	6279365	79738		
	6266020	79736		
	6268003	79864		

Table No. 9: Assay (Analysis of formulation).

Drug	Amount of drug (mg/tablet)		%Label Claim	%RSD*
	Amount taken	Amount Found		
Jalra DP & Dapagliflozin 10mg/Vildagliptin100 mg)	20 mg	19.71 mg	98.55	1.42
	500 mg	483.33 mg	96.66	1.14

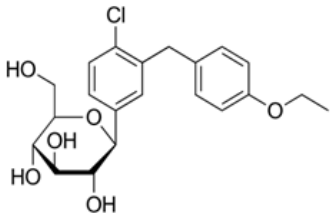

Fig. 1: Dapagliflozin.

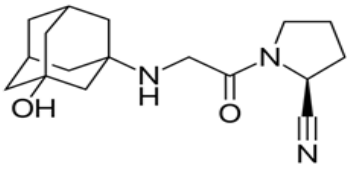

Fig. 2: Vildagliptin.

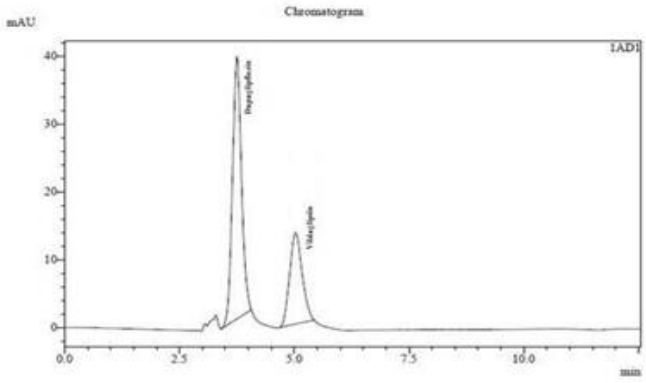

Fig. 3 System suitability chromatogram.

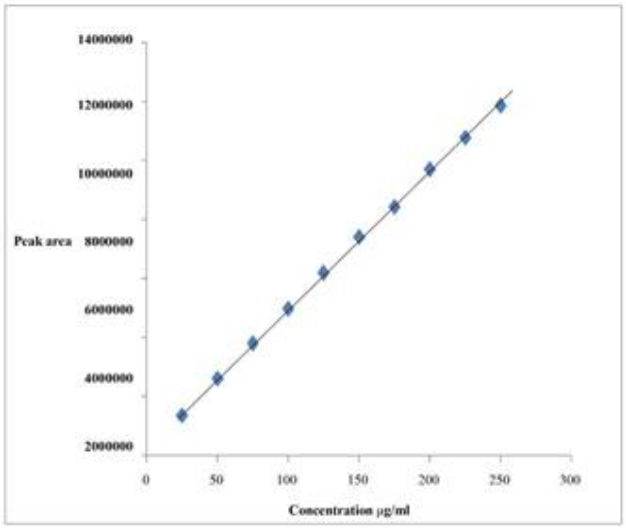

Fig. 4: Linearity data of Dapagliflozin.

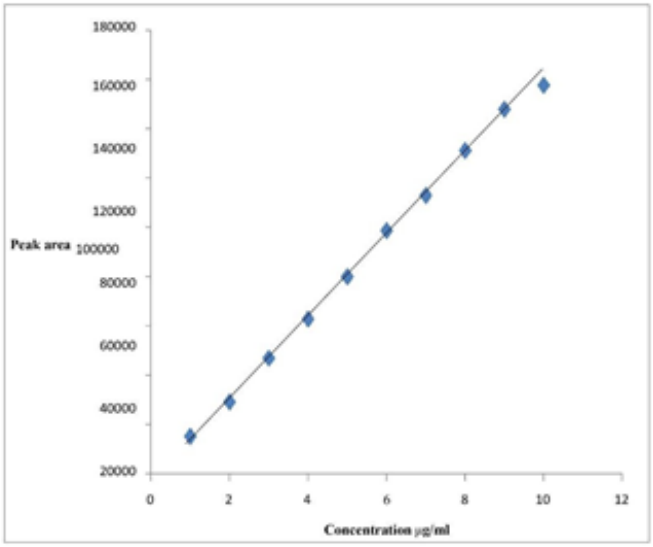

Fig. 5: Linearity data of Vildagliptin.

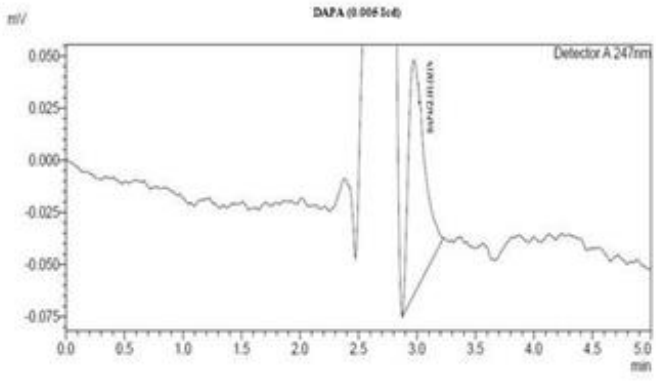

Fig. 6: LOD of Dapagliflozin.

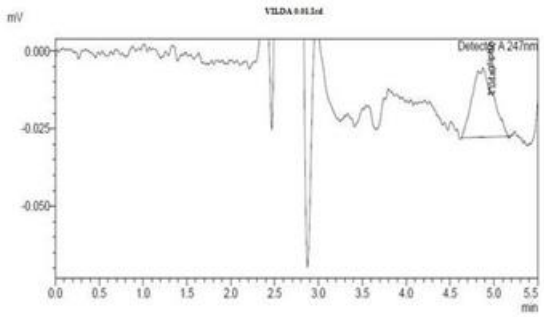

Fig. 7: LOD of Vildagliptin.

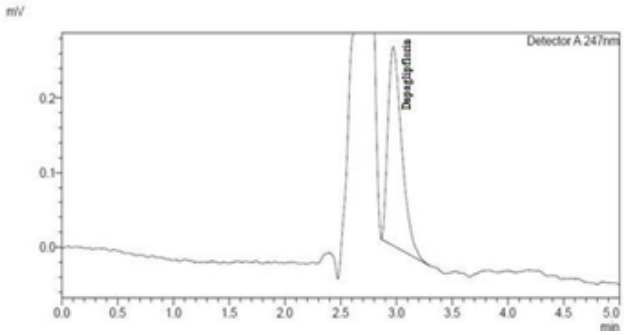

Fig. 8: LOQ of Dapagliflozin.

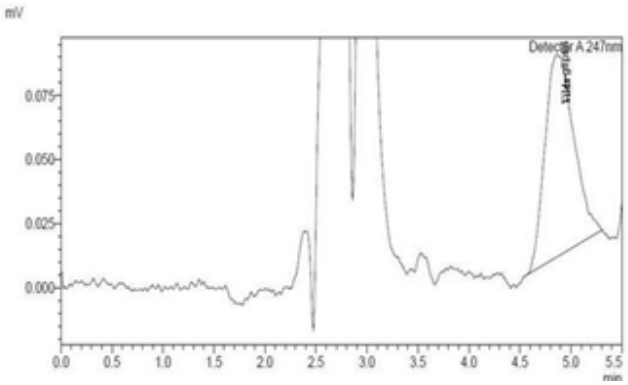

Fig. 9: LOQ of Vildagliptin.

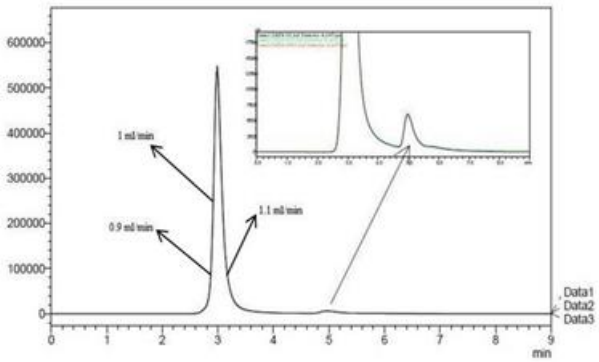

Fig. 10: Robustness (Effect of flow rate).

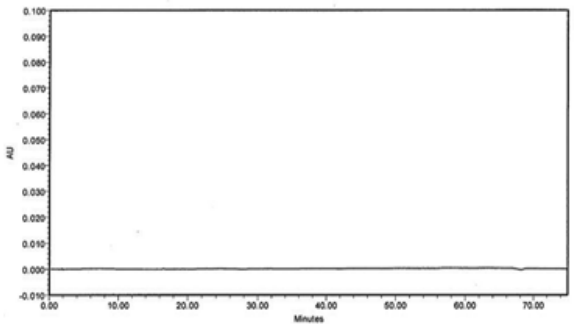

Fig. 11: Assay (Blank chromatogram).

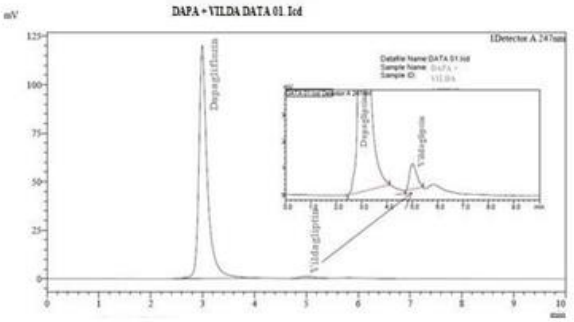

Fig. 12: Assay (Chromatogram of standard 1).

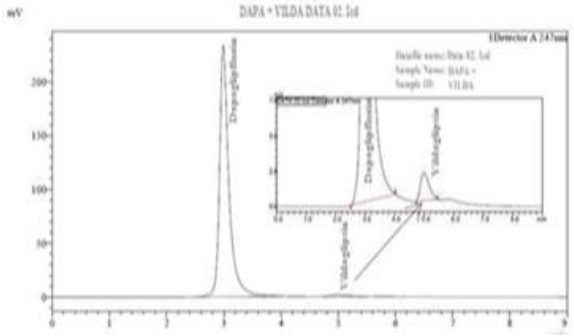

Fig. 13: Assay (Chromatogram of standard 2).

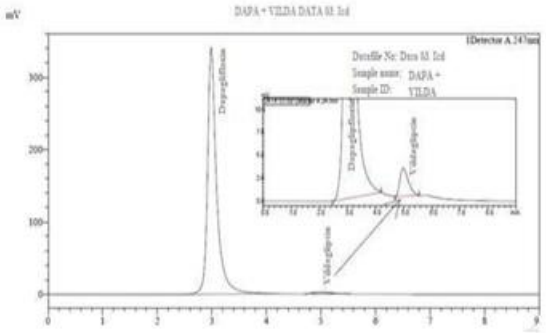

Fig. 14: Assay (Chromatogram of standard 3).

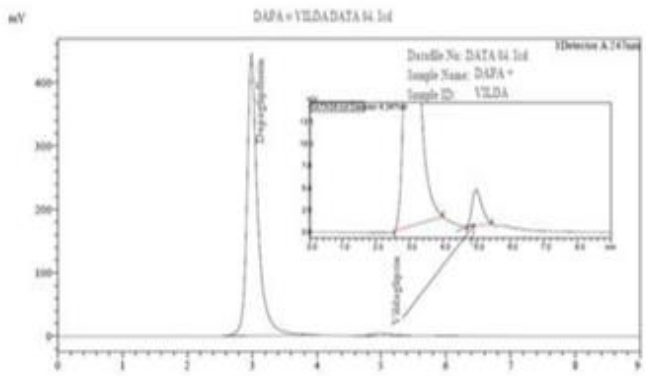

Fig. 15: Assay (Chromatogram of standard 4).

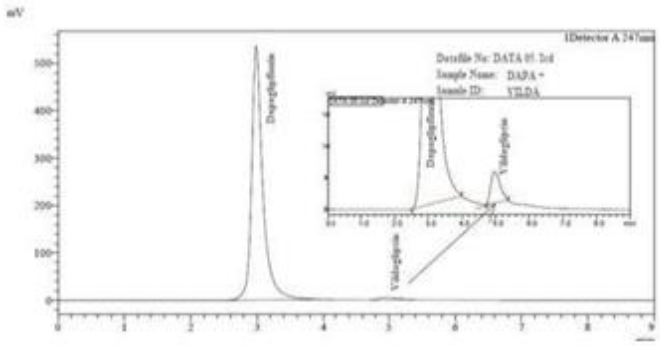

Fig. 16: Assay (Chromatogram of standard 5).

CONCLUSION

The present work aims to develop and validate a new simple RP-HPLC method for simultaneous estimation of Dapagliflozin and Vildagliptin in combined dosage form, as existing methods for determining these drugs are limited. The study developed a RP-HPLC method using a 247 nm wavelength, using a 10 mM potassium dihydrogen orthophosphate: methanol system. The Hibar C18 column was used for quantitative estimation, identifying individual peaks of Dapagliflozin and Vildagliptin. The RP-HPLC method, based on experimental data, offers advantages such as less time-consuming standard and sample preparation, no tedious extraction for formulation analysis, suitable for raw material analysis, dissolution studies, and content uniformity studies. RP-HPLC method was found to be simple, precise, specific and accurate. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, limit of detection, limit of quantitation, ruggedness, robustness.

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