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New RP-HPLC Method for the Estimation of Saxagliptin and Dapagliflozinin Bulk and Pharmaceutical Dosage Formulations

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ABSTRACT
A simple and Precise reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the separation and quantification ofSaxagliptin and Dapagliflozinin tablet dosage form and validated. Chromatographic separation of the two drugs was performed on a Phenomenex Luna C18 (4.6×250mm, 5µm) particle size.The mobile phase used was a mixture of Acetonitrile: Phosphate Buffer(45:55 v/v)adjust the pH 4.6 with diluted orthophosphoric acid. Detection was performed at 245nm and sharp peaks were obtained for Saxagliptin and Dapagliflozinretention times of 2.1±0.01 min and 3.5±0.01 min respectively. The calibration curve was linear in the concentration range 6-14 µg/ml for Saxagliptin 12-28 µg/ml for Dapagliflozin; the correlation coefficients were 0.9999 and 0.9999, respectively. The optimized method showed good performance in terms of specificity, linearity, detection and quantitation limits, precision and accuracy in accordance with the International Conference on Harmonization (ICH) Q2 (R1) guidelines. This assay was demonstrated to be applicable for routine quantitation of Saxagliptin and Dapagliflozinin tablet dosage form.

Key Words: Saxagliptin ,Dapagliflozin RP-HPLC and ICH Guidelines.

I. INTRODUCTION

Saxagliptin(SGT) Figure.1is used along with diet and exercise to lower blood sugar levels in patients with type 2 diabetes (condition in which blood sugar is too high because the body does not produce or use insulin normally). Saxagliptin is in a class of medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. It works by increasing the amount of insulin produced by the body after meals when blood sugar is high. It is used as monotherapy or in combination with other drugs.[1-3]

Dapagliflozin (DGZ)[4-9] is used along with diet and exercise, and sometimes with other medications, to lower blood sugar levels in adults with type 2 diabetes (condition in which blood sugar is too high because the body does not produce or use insulin normally). It is also used to reduce the risk of needing to be hospitalized for heart failure in adults who have type 2 diabetes along with heart and blood vessel disease or who have multiple risk factors for developing heart and blood vessel disease. Dapagliflozin is also used in adults with heart failure to reduce the risk of needing to be hospitalized and death due to heart and blood vessel disease. It is also used to reduce the risk of worsening kidney disease, the need to be hospitalized for heart failure, and the risk of death due to heart disease in adults with kidney disease. Dapagliflozin is in a class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors. It lowers blood sugar by causing the kidneys to get rid of more glucose in the urine.

Saxagliptin and Dapagliflozin combination (QTERN®) is used together with proper diet and exercise to treat type 2 diabetes. Saxagliptin helps to control blood sugar levels by making the pancreas gland release more insulin. It also signals the liver to stop producing sugar when there is too much sugar in the blood. Dapagliflozin works in the kidneys to prevent absorption of glucose (blood sugar). This helps lower the blood sugar level.

HPLC methods are available with the combination of above-cited drugs 17-21, with lower linearity range and or having longer retention timesHPLC methods are available with the combination of above-cited drugs [10-21], with lower linearity range and or having longer retention times. Therefore, an attempt was made to develop a novel, simple, accurate and precise method for the simultaneous estimation of Saxagliptin and Dapagliflozin in combined

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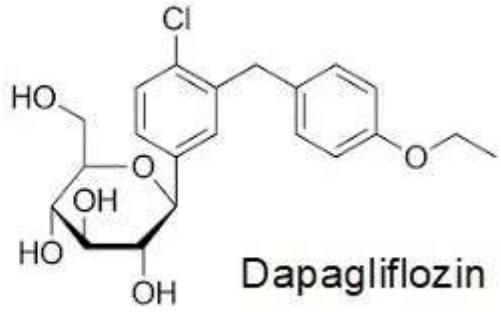
pharmaceutical dosage form. This manuscript describes the development and validation of RP-HPLC method for simultaneous estimation of these drugs as per ICH guidelines.[22-23]

Figure1.Structure of Saxagliptin and Dapagliflozin

Saxagliptin



Dapagliflozin

II. EXPERIMENTATION:

2.1 Equipment:

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

2.2. Reagents and chemicals:

Acetonitrile, Methanol and water of HPLC grade were procured from Lichrosolv (Merck) Ind Ltd. Working standard of Saxagliptin and Dapagliflozinwere provided by Sura labs Hydrabad.. Tri ethyl amine and orthophosphoricacid were A.R grade from Merck chemicals Mumbai, India. Tablets Qtern®were purchased from Indian market, containing a 10mg of Dapagliflozinand 5 mg of Saxagliptin per tablet.

2.3Optimized chromatographic Condition:

A Phenomenex Luna C18 (4.6×250mm, 5µm) particle sizecolumn was used as the stationary phase.The mobile phase used was a mixture Acetonitrile: Phosphate Buffer(47:53 % V/V, pH adjusted to 4.6 usingdiluted orthophosphoric acid. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1 ml/min. Detection was performed at 245nm and sharp peaks were obtained for Saxagliptin and Dapagliflozinretention times of 2.1±0.01 min and.3.5±0.01 min respectively. The injection volumes of samples and standard were 10µl.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDADetector 996 model.
Temperature : 35ºC
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.
pH : 4.6
Mobile phase : Acetonitrile: Phosphate Buffer(45:55 v/v)
Flow rate : 1ml/min
Wavelength : 245 nm
Injection volume : 10 µl
Run time : 7 min

Standard preparation:

2.4 Preparation of Buffer and Mobile Phase:

Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer (pH-4.6):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase:

Accurately measured 450 ml (45%) of Acetonitrile, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultra sonicater

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for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

2.5 Preparation of standard solution:

10 mg of Dapagliflozin and 10 mg of Saxagliptin were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) .The working standard solutions were prepared and further diluted in mobile phase to and Saxagliptin and Dapagliflozincontain a mixture of in over the linearity ranges from 6-14 µg/ml and 12-28 µg/ml.

2.6 Analysis of Tablet formulation:

Twenty tablets Qtern®v were weighed and finely powdered. A quantity of powder equivalent to10 mg of Dapagliflozin and 5mg of Saxagliptinwas weighed and transferred to a 10 ml volumetric standard flask and added 10 ml of mobile phase. The sample was kept in an ultrasonic bath for 20 min and further diluted using mobile phase to get 1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin. Then it is filtered through 0.22µ membrane filter paper. Then this primary sample solution were further diluted to get the concentration of 20 µg/mlDapagliflozin10 µg/ml of Saxagliptinsample solutions.

20µl of this solution was injected in to HPLC system and chromatograms were recorded. Concentrations of Dapagliflozin and Saxagliptinin the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table1.

Table1: Table for Assay

Drug Name	Amount present mg	Amount found* (mg/tab)	% label claim*
Dapagliflozin	10	9.98	99.98
Saxagliptin	5	4.99	99.99

III. RESULTS AND DISCUSSION:

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in Pharmaceutical industries. The chromatograms sample and standard solution of Saxagliptin and Dapagliflozin were shown in (Figure.1) and (Figure.2). There was clear resolution between Saxagliptin and Dapagliflozin with retention time of 2.120and 3.536 minutes respectively.

Figure 1: Typical Chromatogram of standard solution of Saxagliptin and Dapagliflozin

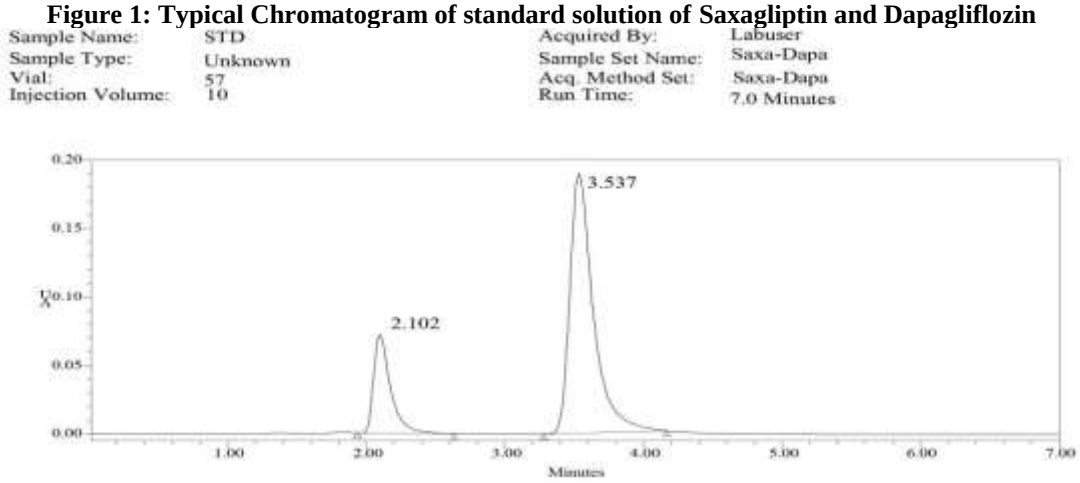

Figure 2: Typical Chromatogram of sample solution of Saxagliptin and Dapagliflozin

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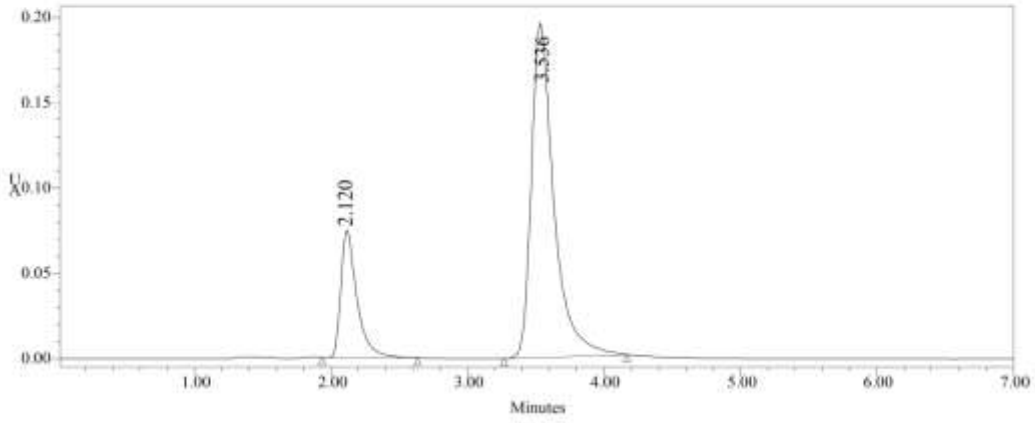
Sample Name: Assay Sample
Sample Type: Unknown
Vial: 56
Injection Volume: 10

Acquired By: Labuser
Sample Set Name: Saxa-Dapa
Acq. Method Set: Saxa-Dapa
Run Time: 7.0 Minutes



3.1. VALIDATION OF THE METHOD:

3.1.1. System suitability:

The column efficiency, resolution and peak symmetry were calculated for the standard solutions. Table.2. The RSD of system suitability factors was satisfactory that is less than 2% and resolution was satisfactory. The peaks obtained for Saxagliptin and Dapagliflozin were sharp and have clear base line separation.

Table 2: System suitability parameters for Saxagliptin and Dapagliflozin

Parameter	Saxagliptin ±RSD,%	Dapagliflozin ±RSD,%
Retention time,min	2.1±0.21	3.5±0.74
Tailing factor	0.43 ±0.15	1.10±0.81
Theoretical plates	9256.13±0.94	8156.21±0.45
Capacity factor	0.21±0.32	0.76±0.12
Resolution	9.23±0.55	

3.1.2. Linearity:

The response for the detector was determined to be linear over the range of 6-14 µg/ml for Saxagliptin (6,8,10,12,14,) and 12-24µg/ml (12,16,20,24,28,) for Dapagliflozin. Each of this concentration was injected in six times to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level.(Figure 3& Figure 4.) The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. The results show that an excellent correlation exits between response factor and concentration of drugs within the concentration range indicated above. (Table 3)

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Figure.3. Linearity graph for Saxagliptin

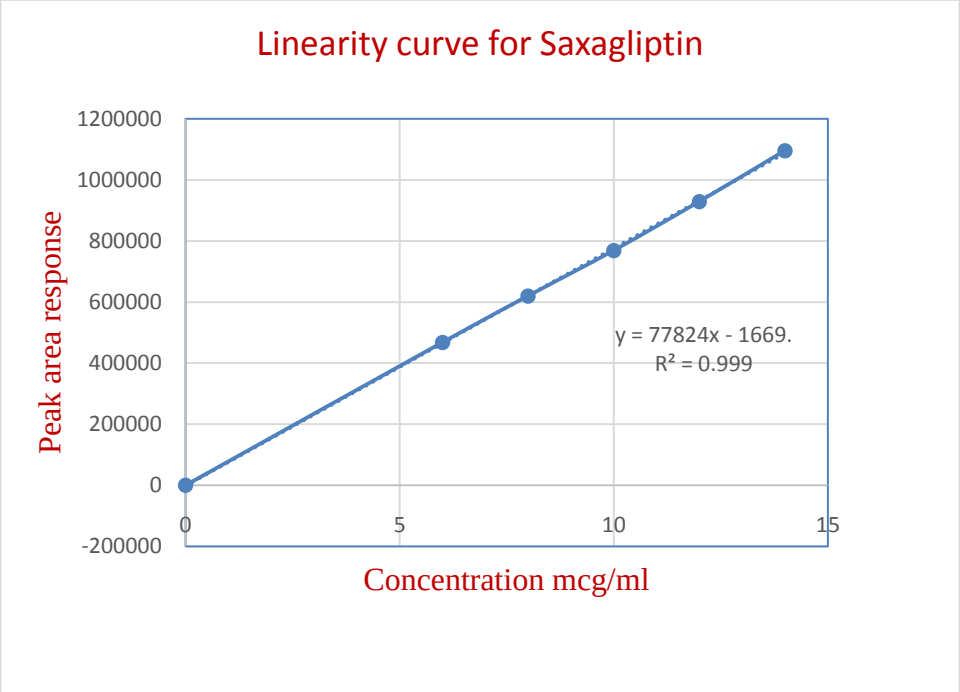

Figure 4.Calibration graph for Dapagliflozin

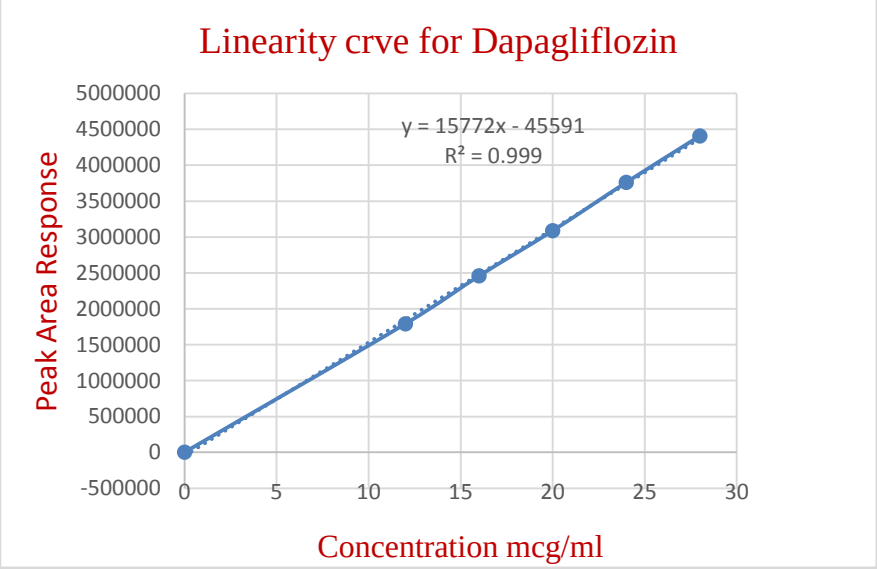

Table3: Summary of analytical method validation

S.No	Parameters	Acceptance criteria	Saxagliptin	Dapagliflozin.
1	Linearity	r^2=0.995 to1.0	0.9999	0.9999
2	Specificity	No interference with placebo	specific	specific
3	Accuracy(Recovery studies)	Recovery98.0-101.0%	99.94%	99.01%

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4	**Precision**			
	Intraday	RSD NMT 2.0%	0.224	0.421
	Interday	RSD NMT 2.0%	0.521	0.215
5	**Robustness**			
	Change inflow rate	NMT±1%	0.4%	0.2%
	Change in mobile phase ratio	NMT±1%	0.4%	0.2%
	Change in p^H	NMT±1%	0.2%	0.%
6	Limit of detection µg/ml	----------	0.6µg/ml	0.8µg/ml
	Limit of Quantification µg/ml	----------	1.8µg/ml	2.4µg/ml

3.1.3. Precision and Accuracy:

Recovery studies were carried out by applying the standard addition method. A known amount of standard Saxagliptin and Dapagliflozinc or responding to 50%, 100%, and 150% of the label claim was added to pre analyze sample of tablet dosage form separately. The recovery studies were carried out six times at each level of recovery. From the data obtained, recoveries of standard drugs were found to be accurate (Table.3). The %RSD of interday and intraday precision obtained was less than2%for both selected drugs. The intraday and inter day precision of Saxagliptin was 0.0.224 and 0.521 and Dapagliflozin was 0.421and 0.215 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

3.1.4 Specificity of the method:

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 10 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Saxagliptin and Dapagliflozinfrom any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

3.1.5LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and10∂/S, respectively asper ICH guidelines, where ∂ is the standard deviation of the response (y-intercept)and S is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD for Saxagliptin and Dapagliflozinwas found to be 0.6µg/ml and 0.8µg/ml, respectively. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10).The LOQ was 1.8µg/ml and 2.4µg/ml for Saxagliptin and Dapagliflozin respectively.(Table 3)

3.1.6 Ruggedness:

The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, and different days. The %RSD of below 2% indicated that the method was accurate with high precision.

3.1.7 **Robustness:**

Robustness is a measure of the performance of a method when small deliberate changes are made to the conditions of the method. The results of the robustness study are summarized in Table 3.

IV. CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of

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Saxagliptin and Dapagliflozinin bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. The solvent system used in this method was economical. High percentage recovery of drug shows the method is free from inference of excipients present in the formulation

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Saxagliptin and Dapagliflozinin bulk drug and in Pharmaceutical dosage forms.

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