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Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Dapagliflozin And Saxagliptin In Bulk As Per Ich

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ABSTRACT

In the present work, a rapid, specific, accurate and precise reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage form. Successful chromatographic separation of Dapagliflozin (DGN) and Saxagliptin (SGN) was carried out with altima C18 column (150 ×4.6 mm, 5 µm) with mobile phase consisted of a mixture of Acetonitrile and Potassium dihyrogen phosphate buffer in the ratio of 35:65 v/v and the pH of the buffer was adjusted to 5.5, delivered at a flow rate of 1.0 ml/min. The eluents are monitored by PDA detector and peaks values were measured at 225 nm. The retention times for DGN and SGN were 2.953 min and 2.209 min respectively. The present analytical method was validated according to ICH guidelines (ICH, Q2 R1). The linearity study of DGN and SGN was found in the concentration range of 10-50 µg/ml for both and coefficient of variance was 0.997 and 0.998 respectively. % recovery was found to be 100.19% and 99.81 % for DGN and Saxagliptin respectively. LOD was 1.10 µg/ml and 1.18 µg/ml and LOQ was 3.01 µg/ml and 3.12 µg/ml for DGN and SGN respectively. It inferred that the developed method was successfully applied for the simultaneous estimation of DGN and SGN in bulk and could be used for the routine analysis of the studied drugs in quality control laboratories.

Keywords: *Dapagliflozin, Saxagliptin and RP-HPLC.*

1. INTRODUCTION

Saxagliptin[1] is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile and molecular formula C18H25N3O2 and mass 315.41 g/mol. It is White solid powder and soluble in Soluble in PEG-400, acetone, acetonitrile, ethanol, isopropyl alcohol, methanol; sparingly soluble in water and slightly soluble in ethyl acetate and half-life is 2.5-3.1 hours. It is a highly potent, reversible, competitive dipeptidyl peptidase-4 inhibitor indicated for the treatment of patients with type 2 diabetes.

Dapagliflozin[2] is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl) methyl]phenyl]-6(hydroxymethyl)oxane-3,4,5-triol and molecular formula C21H25ClO6 and mass 408.9 g/mol. It belongs to the class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors. It is White or off white crystalline solid and soluble in organic solvents like ethanol,

DMSO, Dimethyl formamide and sparingly soluble in aqueous buffers (0.173 mg/mL) and half-life is 12.9 h. It is used along with diet and exercise, and sometimes with other medications, to lower blood sugar levels in patients with type 2 diabetes.

Literature survey revealed a variety of analytical methods viz. HPLC, LC-MS and, GC has been reported for estimation of DGN and SGN individually or in combination with other drugs. The reported methods are Spectrophotometric [3-5], HPLC [6-8], LC-MS [9-10] and GC [11] method are reported for the simultaneous estimation of DAP and SAX in combined pharmaceutical formulation. The objective of the present study is to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage form. Validate the method according to ICH guidelines[12].

2. MATERIALS AND METHODS

The analytical method was performed by using the HPLC system Shimadzu (SPD-AT20) equipped with auto sampler, UV and PhotoDiode Array (PDA) detector, Rheodyne injector with 20 µl loop volume, analytical balance (Model AX200), pH analyser (Chemiline CL 180 based pH meter) and Toshcon Ultra Sonicator.

Instrumentation

Tokyo, Japan's Shimadzu SPD- AT20 HPLC system was used for the HPLC investigations. A photodiode array detector and a separation module were part of the system, and an autosampler was used to conduct the experiments in isocratic mode. LC solution software was used to gather and process the data. The eluents were measured at 225 nm, and the separation was carried out using altima C18 column (150 ×4.6 mm, 5 µm) analytical column.

Preparation of mobile phase

Mobile phase was prepared by mixing Acetonitrile and Potassium dihyrogen phosphate buffer in the ratio of 35:65 and the pH of the buffer was adjusted to 5.5 and was filtered through 0.45µ membrane.

Preparation of standard stock solution

Through the use of a digital microbalance, 100 mg of DGN and 10 mg of SGN were weighed into a volumetric flask of 10 millilitres. After adding few millilitres of diluent, it was sonicated to dissolve it. After that, the solution was diluted to volume with the diluent, and lastly, it was diluted to a final volume by adding more diluent.

Chromatographic conditions

High Performance Liquid Chromatography equipped with PDA detector.

For DGN and SGN (isocratic)

Column	:	Altima C18 column (150 ×4.6 mm, 5 µm) analytical column
Wavelength	:	225 nm
Injection Volume	:	20 µl
Column Temperature	:	Ambient
Flow rate	:	1.0 ml/min

At 2.293 minutes, the DGN peak was found to have an area of 785698, with a tailing factor of 1.15. As shown in Fig. 1 and Table 1, the SGN peak was seen at 2.209 min with a peak area of 160139, a tailing factor of 1.12, and a resolution of 2.25. This experiment was deemed optimal due to its positive outcomes and shorter retention duration. DGN has a retention time of around 2.293 minutes and SGN has a retention time of 2.209 minutes.

Table 1: System suitability parameters

S.No.	Name of the Peak	Retention Time (Mins)	Peak Area	Tailing Factor	Resolution	Plate Count
01	DGN	2.293	367852	1.15	---	8923
02	SGN	2.209	182567	1.12	2.25	5432

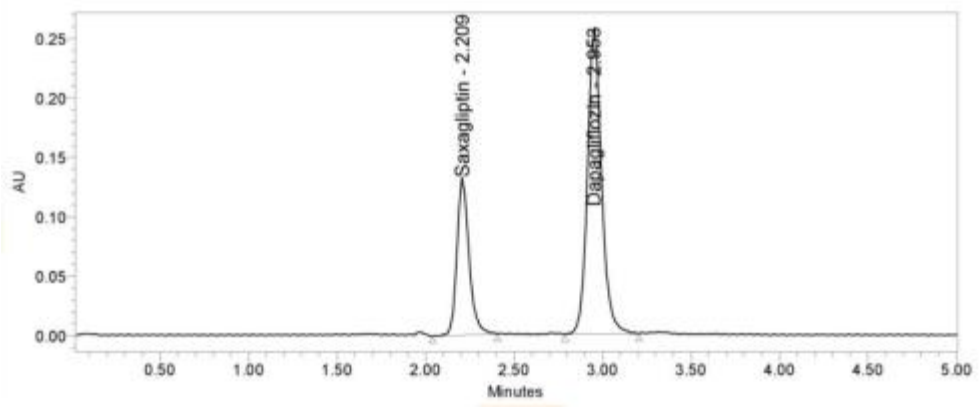

Fig.No. 01 : Typical Chromatogram of DGN and SGN

Preparation of sample solution

About 10 mg of sample was weighed into a 10 ml volumetric flask, and then 7 ml of diluent was added. The mixture was then sonicated to dissolve the material, and then diluted to volume with diluent. Further diluted to 10 ml with the diluent and filtered through 0.45µ Nylon syringe filter.

Procedure

Five injections of 20 µl each of active DGN and SGN standard solutions were performed. Chromatograms were obtained and peak responses were evaluated. The system's suitability was calculated by evaluating its parameters. The quantification of DGN and SGN in the sample was achieved by the analysis of the peak responses.

Method Validation

The present study examined many parameters to establish the validity of the HPLC methodology for quantifying DGN and SGN in accordance with the specified procedure, hence demonstrating its suitability for the intended use. The implementation of all validation criteria was done in compliance with the standards set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity and Range

The concentrations of DGN and SGN that showed a linear relationship with peak area were (10 - 50 µg/ml). Results are shown in (Fig.2 & 3), (Table 2 & 3), and the linearity of the calibration curve is confirmed by the high value of the correlation coefficient of the regression equation.

Table 2: Linearity data of DGN

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	122888
3	20	246753
4	30	367852
5	40	490756
6	50	603089
Slope		**12044**
Intercept		**4946.1**
Regression		**0.9997**

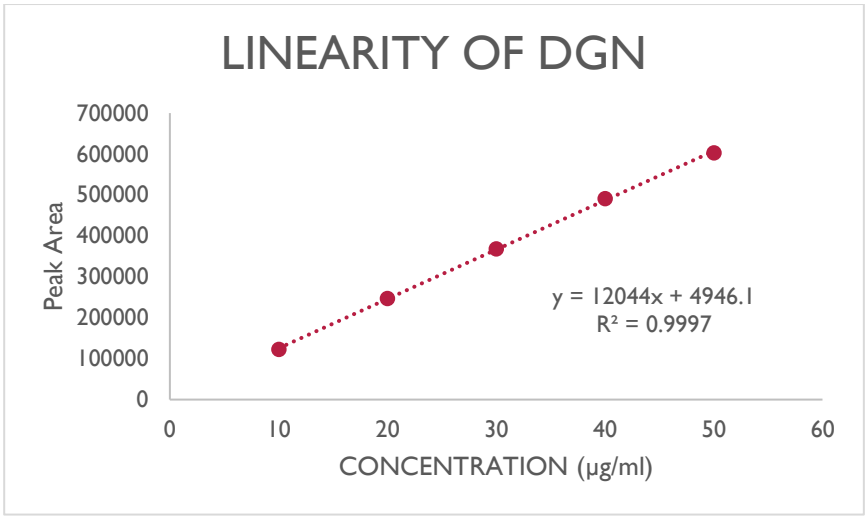

Fig.No. 02 : Linearity of DGN

Table 3: Linearity data of SGN

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	60877
3	20	121567
4	30	182567
5	40	247653
6	50	305674
Slope		**6156,8**
Intercept		**-1036.4**
Regression		**0.9998**

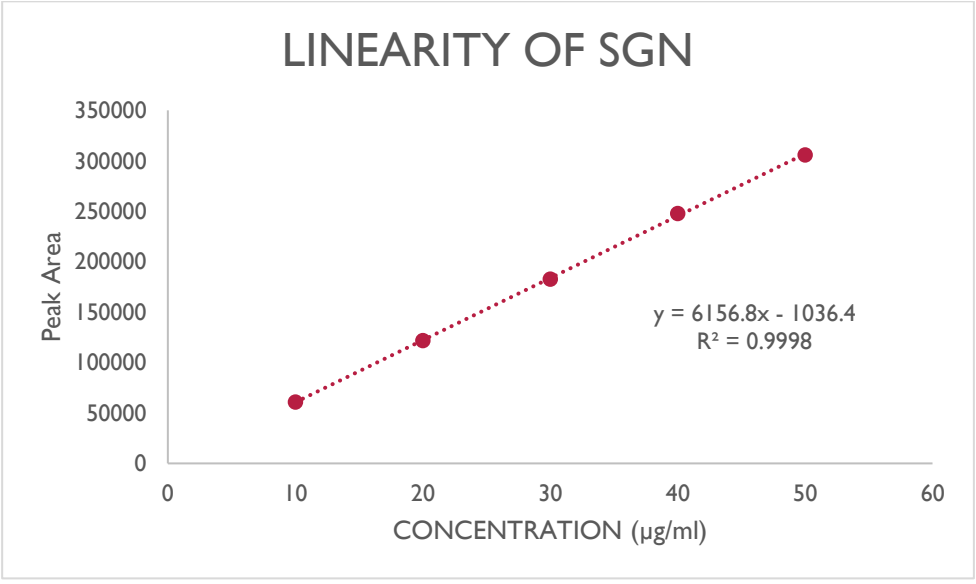

Fig.No. 03: Linearity of SGN

Accuracy and Precision:

Accuracy as recovery was examined by spiking previously analyzed test solution with extra Standard drug at three different concentration levels. With a relative standard deviation (RSD) of less than 2%, we observed that the suggested technique is accurate for the simultaneous estimate of both DGN and SGN , with a recovery of 99.97 % for DGN and 100.24% for SGN , respectively. The high reproducibility and low RSD values show that the Method is reliable. (table-4 & 5).

Table 4: Precision data of DGN and SGN

Injection Number	DGN				SGN			
	Retention Time	**Peak Area**	**Plate Count**	**Peak Symmetry**	**Retention Time**	**Peak Area**	**Plate Count**	**Peak Symmetry**
1	2.293	367852	8967	1.12	2.209	182567	5567	1.1
2	2.292	369852	9167	1.25	2.209	183567	5582	1.42
3	2.291	377852	8991	1.11	2.209	182887	5625	1.41
4	2.293	368752	8978	1.1	2.209	179453	5582	1.56
5	2.293	367222	8923	1.13	2.209	179999	5715	1.1
6	2.292	369852	8912	1.45	2.209	182834	5699	1.21
Average	**2.292**	**1403270**			**2.209**	**181885**		
Standard Deviation	**0.001**	**3879.90**			**0.000**	**1712.92**		
% RSD	**0.0356**	**0.28**			**0.00**	**0.94**		

Table 5: Accuracy data of DGN and SGN

Sample Preparation No.	DGN Assay (%)	SGN Assay (%)
1	100.11	99.78
2	100.34	99.83
3	101.34	99.89
4	100.23	99.98
5	99.99	99.25
6	99.13	100.11
Mean	**100.19**	**99.81**
SD	**0.7095**	**0.2967**
RSD (%)	**0.7081**	**0.2973**

Robustness:

The results of the robustness analysis are shown in Table no.6. Both components exhibited comparable tailing factors, elution orders, resolutions, relative standard deviations, and recoveries. The analysis revealed that the relative standard deviation (RSD) of the peak sites was much below 2.0%.

Table 6: Robustness data of DGN and SGN

Condition	DGN			SGN		
	% RSD	**Tailing Factor**	**% Recovery**	**% RSD**	**Tailing Factor**	**% Recovery**
1) Change in Flow rate						
Normal Condition (1.0 ml per minute)	0.23	1.11	100.11	0.12	1.10	99.21
Flow rate (0.8 ml per minute)	0.28	1.10	99.45	0.11	1.11	99/99
Flow rate (1.2 ml per minute)	0.29	1.22	99.98	0.23	1.35	99.98
2) Change in minor component in the mobile phase						
Normal Condition (acetonitrile and Phosphate buffer in a ratio of 35:65)	0.32	1.11	99.34	0.23	1.28	100.89
(acetonitrile and Phosphate buffer in a ratio of 45:55)	0.42	1.12	99.99	0.25	1.26	99.89
(acetonitrile and Phosphate buffer in a ratio of 25:75)	0.45	1.10	100.56	0.28	1.21	100.21
3) Change in Wave Length						
Normal: Wave Length 225 nm	0.21	1.15	99.89	0.25	1.23	99.87
Wave Length 220 nm	0.23	1.17	98.99	0.22	1.28	99.95
Wave Length 230 nm	0.28	1.19	98.89	0.29	1.21	100.93
4) Change in pH						
Normal: pH 5.5	0.21	1.22	99.89	0.34	1.24	100.12
pH 5.0	0.22	1.18	99.23	0.23	1.21	99.49
pH 6.0	0.32	1.10	98.56	0.21	1.29	99.99

Ruggudness:

DGN and SGN had respective mean peak areas of 785777 and 160142 with an RSD of 0.35 and 0.28%, respectively.

3. SUMMARY

A novel and validated RP-HPLC method has been created to evaluate DGN and SGN in bulk. Given the results of the literature review, which showed that there are few techniques for estimating DGN and SGN in large numbers, a straightforward, economical, and accurate solution to this problem is desperately needed. A combination of phosphate buffer, acetonitrile, (65:35) with a pH of 5.5 was injected onto an Altima C18 column (150 ×4.6 mm, 5 µm) analytical column to measure the concentrations of DGN and SGN . The injection volume was 20 µl, and the flow rate was set at 1.0 ml/min. The SGN peak eluted after 2.209 minutes, while the DGN peak had a retention time of 2.293 minutes.

Following improvement, the method was verified for linearity, sensitivity parameters, precision, accuracy, resilience, and system compatibility in accordance with ICH requirements. Every validation parameter produced results that fell within reasonable bounds. The tests' relative standard deviations (RSDs) were below two. The range of recoveries was 98% to 102%.

4. CONCLUSION

The suggested RP-HPLC technology provides a quick and easy method that is nevertheless straightforward, quick, accurate, precise, resilient, and economical. As a result, it is a preferred technique for determining SGN and DGN simultaneously. Every part of the implemented method was carefully checked to ensure it complied with ICH rules.

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