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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN DRUGS BY USING RP-HPLC METHOD

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ABSTRACT

In the research analysis a rapid, accurate and reliable High Performance Liquid Chromatography (HPLC) method was developed and validated by selecting chromatographic parameters for simultaneous estimation of Dapagliflozin and Saxagliptin in tablet dosage form. The HPLC method was developed using reverse phase Xterra C 18, column (4.6*150mm, 5µ) with mobile phases containing 0.1% OPA: Methanol: Acetonitrile (30: 60: 10). The flow rate was 1.0 ml / min with PDA detection at λ max 225 nm and the amount of injection was set at 20µl with 15 min run time. This method has been validated by the use of different validation parameters such as accuracy, precision, linearity and robustness. Such findings showed that the system could find practical use in its tablet dosage forms as a quality assurance tool for evaluating the drug in pharmaceutical industries.

INTRODUCTION

Dapagliflozin (DGZ) is an inhibitor of sodium-glucose co-transporter 2 (SGLT2), used as an alternative to diet and exercise to enhance glycemic regulation in adults with type 2 diabetes milletus. DGZ is a solid powder soluble substance present in ethanol, DMS, DMF, and methanol. DGZ is 91 percent protein bound. The binding of proteins is not altered in patients with renal or hepatic impairment. The DGZ metabolism is primarily mediated in humans and by UGT1A9. DGZ is extensively metabolized mainly to create 3-O-glucuronide dapagliflozin, which is an inactive metabolite. Saxagliptin (SXG), the latest class of drug inhibitor dipeptidyl peptidase-4 (DPP-4), is a potential oral hypoglycaemic product (anti-diabetic product). SXG inhibits the activity of DPP-4 enzymes over a 24-hour cycle.

SXG also decreased glucagon concentrations and increased glucose-dependent insulin secretion from beta-cells in the pancreas. The saxagliptin metabolism is mainly regulated by the enzyme cytochrome P450 3A4/5. The ingested dose of SXG would be subjected to hepatic metabolism by 50 percent. The main SXG metabolite is 5-hydroxy saxagliptin which is also an inhibitor of DPP-4. SXG is removed by both the hepatic and renal pathways .

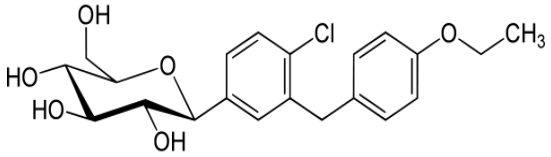

Fig.1 (a) – structure of Dapagliflozin

Fig.1 (b) – structure of Saxagliptin

Optimized Chromatographic Conditions:

The instrument used : Waters HPLC with auto sampler and UV detector.

Temperature : Ambient (25°C)

Mode of separation : Isocratic mode

Column : Xterra C 18, column (4.6*150mm, 5µ)

Buffer : 0.1% OPA

Mobile phase : 0.1% OPA: Methanol: Acetonitrile (30: 60: 10)

Flow rate : 1 ml /min

Wavelength : 225 nm

Inj. volume : 20 µl

Run time : 15 min

Standard Solution Preparation:

Precisely gauge and move 10 mg of Saxagliptin and 20 mg of Dapagliflozin working norm into a 100 ml clean dry volumetric carafe include around 7 mL of Diluent and sonicate to disintegrate it totally and make volume sufficiently with a similar dissolvable. (Stock arrangement) Further pipette 3.0 ml of the above stock arrangements into a 10ml volumetric flagon and weaken sufficiently with diluent.

Test Solution Preparation:

Precisely gauge and move comparable to 10 mg of Saxagliptin and 20 mg of Dapagliflozin test into a 100 ml clean dry volumetric flagon include around 7 mL of Diluent and sonicate to disintegrate it totally and make volume sufficiently with a similar dissolvable. (Stock arrangement) Further pipette 3 ml of the above stock arrangements into a 10ml volumetric flagon and weaken sufficiently with diluent.

Linearity:

Arrangement of stock arrangement:

Precisely gauge and move 10 mg of Saxagliptin and 20 mg of Dapagliflozin working norm into a 100 ml clean dry volumetric carafe include around 7 mL of Diluent and sonicate to disintegrate it totally and make volume sufficiently with a similar dissolvable. (Stock arrangement)

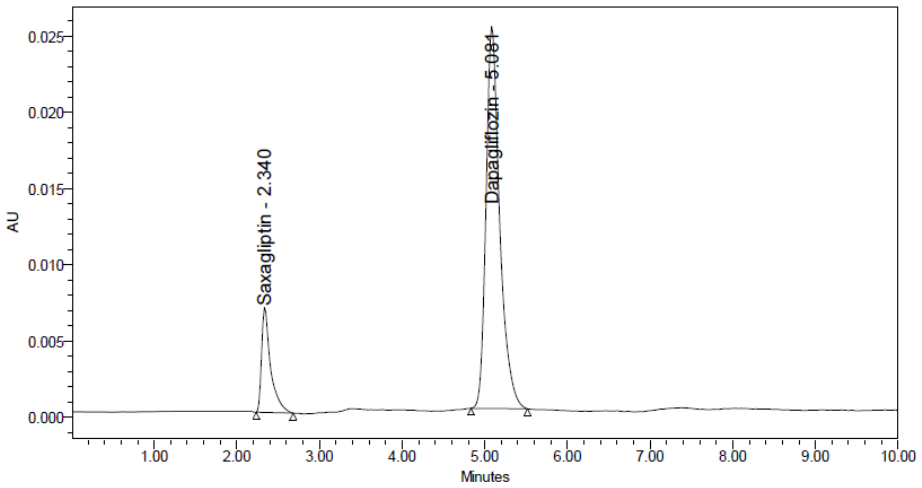

Fig.2 Optimised chromatogram

System Suitability:

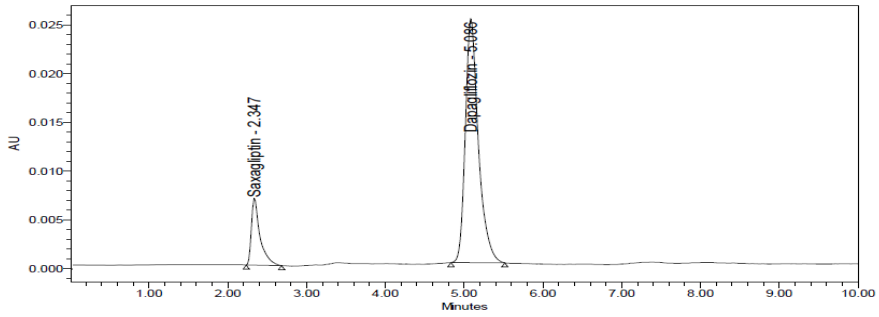

Fig.3 system suitability chromatogram

Table 1: Results of system suitability

S. No.	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Saxagliptin	2.347	56445	6857	--	1.41	2593.29
2	Dapagliflozin	5.086	320903	25250	11.53	1.18	4843.11

Linearity:

Table 2: Results of Linearity

S. No	Dapagliflozin		Saxagliptin	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	20	132359	10	17896
2	40	223105	20	37780
3	60	320315	30	56233
4	80	419173	40	74754
5	100	526461	50	93611

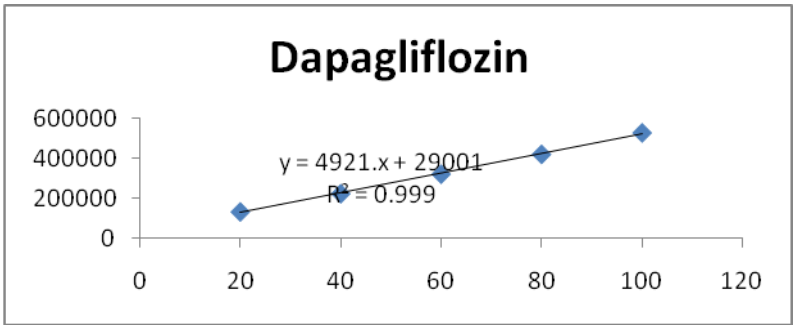

Fig. 4(a): Linearity curve for DGZ

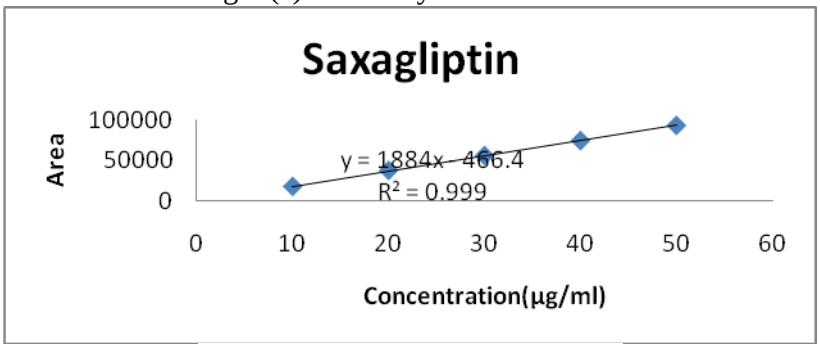

Fig. 4(b): Linearity curve for SXG

Precision:

Table 3(a): Results of Precision

Injection	Area for Dapagliflozin	Area for Saxagliptin
Inj-1	318752	56407
Inj-2	316862	56050
Inj-3	320903	56444
Inj-4	315150	56445
Inj-5	320979	56203
Inj-6	316258	56139
Average	318150.7	56281.3
Standard Deviation	2457.0	172.6
%RSD	0.8	0.3

Intermediate Precision (Ruggedness)

Table 3(b): Results of I.P.

Injection	Area for Dapagliflozin	Area for Saxagliptin
Inj-1	316450	56082
Inj-2	318607	56734
Inj-3	316347	56133
Inj-4	319509	56124
Inj-5	319175	56948
Inj-6	317693	56919
Average	317963.5	56490.0
Standard Deviation	1359.9	419.8
%RSD	0.4	0.7

Accuracy:

Accuracy (recovery) data for Dapagliflozin

Table 4(a): Results of accuracy for DGZ

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	161058.3	10	10.04	100.39	100.64
100%	323719.3	20	20.18	100.89	
150%	484374.0	30	30.19	100.64	

Accuracy (recovery) data for Saxagliptin

Table 4(b): Results of accuracy for SXG

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	28244.7	5	5.01	100.26	100.36
100%	56457.3	10	10.02	100.20	
150%	85035.3	15	15.09	100.61	

Limit Of Detection for Dapagliflozin And Saxagliptin

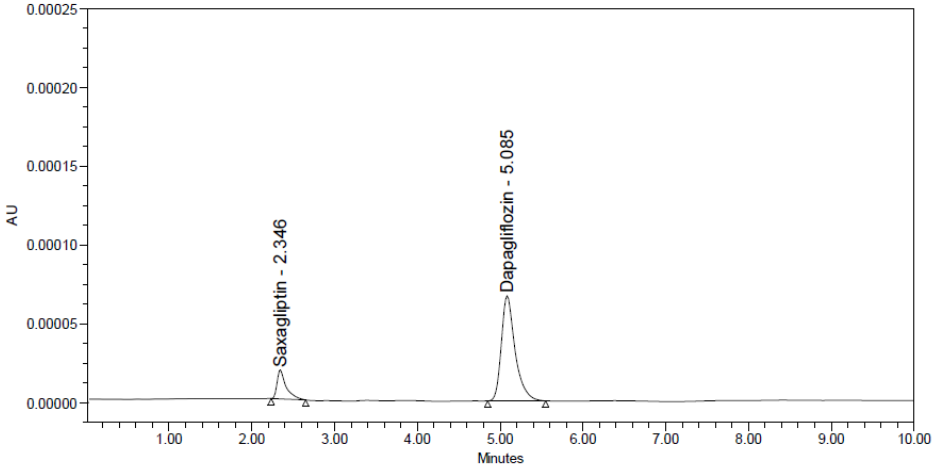

Fig. 5(a): Chromatogram for LOD of DGZ and SXG

Limit of Quantification For Dapagliflozin And Saxagliptin

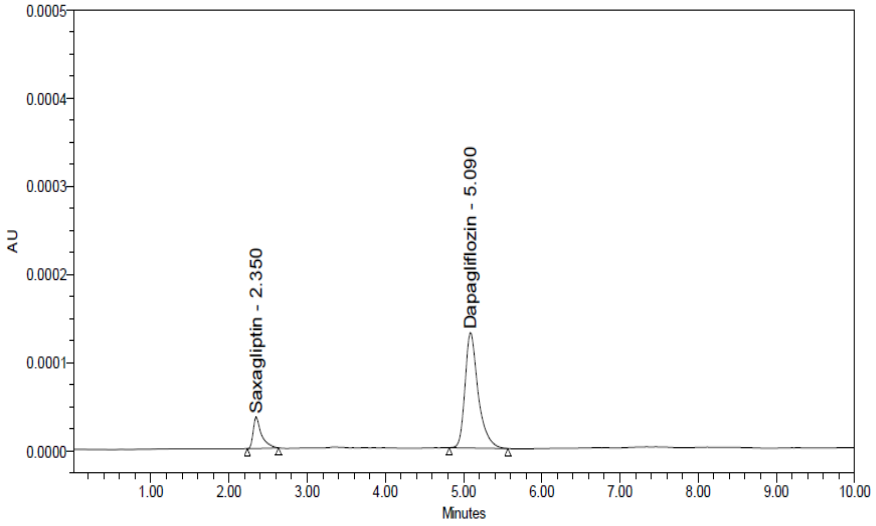

Fig. 5(b): Chromatogram for LOQ of DGZ and SXG

Degradation Studies:

Table 5: Results of Degradation studies

Sample Name	Dapagliflozin		Saxagliptin	
	Area	% Degraded	Area	% Degraded
Standard	320211.3	--	56232.7	--
Acid	295636	7.67	54275	3.48
Base	302783	5.44	52453	6.72
Peroxide	289767	9.51	53967	4.03
Thermal	316254	1.24	51867	7.76
Photo	286735	10.45	50162	10.80

CONCLUSION:

The RP-HPLC method was developed in pharmaceutical dosage form for the simultaneous estimation of drugs DGZ and SXG. By studying various media and conditions, the HPLC method was optimised. The Sample recoveries were in excellent accordance with their respective labelled claims in all formulations and the proposed method was validated in all

suitable parameters as per ICH guidelines. This method can be used in quality control testing process of anti-diabetic drugs (Dapagliflozin and Saxagliptin) combination in pharmaceutical dosage forms.

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