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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN PHARMACEUTICAL DOSAGE FORMS.

Hanisha Thota *, G. Soundarya

Department of Pharmacy, Sri Sivani College of Pharmacy,
Affiliated to JNTUK, Srikakulam- 532402, Andhra Pradesh, INDIA.

***Corresponding Author E-mail :** hanishapharma96**@gmail.com**

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ABSTRACT

A simple, Accurate, precise technique was developed for the simultaneous estimation of Dapagliflozin and Saxagliptin in pharmaceutical dosage form. Chromatogram was run through Discovery- C_{18} column (1250 x 4.6mm, 5µ) column. Mobile phase containing Water: Methanol Buffer taken in the proportions 50:50v/v was pumped through column at flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimised wavelength selected was 222nm. Retention time of Dapagliflozin and Saxagliptin were observed to be 2.201min and 2.925min. %RSD of the Dapagliflozin and Saxagliptin were and observed to be 0.7 and 0.7 respectively. %Recovery was obtained as 99.55% for Dapagliflozin and 99.46% for Saxagliptin respectively. LOD, LOQ values obtained from regression equations of Dapagliflozin and Saxagliptin were 0.04, 0.13 and 0.01, 0.02 respectively. Regression equation of Dapagliflozin is y = 14132x + 2340, and y = 14870x + 421 of Saxagliptin. Retention times were decreased and that run time was decreased, so the technique developed was simple and conservative that can be embraced in regular quality control test in industries.

INTRODUCTION

Dapagliflozin is a sodium-glucose co-transporter 2 inhibitor indicated for managing diabetes mellitus type 2. When combined with diet and exercise in adults, dapagliflozin helps to improve glycemic control by inhibiting glucose resorption in the proximal tubule of the nephron and causing glycosuria. Saxagliptin (rINN) is an orally active hypoglycemic (anti-diabetic drug) of the new di-peptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Dapagliflozin and Saxagliptin is a combination medicine used together with diet and exercise to improve blood sugar control in adults with type 2 diabetes mellitus. Dapagliflozin and saxagliptin is also used to lower the risk of death from heart attack, stroke, or heart Failure in adults with type 2 diabetes who also have heart disease. [1-2]

MATERIALS AND METHOD:

Preparation of buffer: 0.1% OPA Buffer: 1ml of Conc. Ortho Phosphoric acid was diluted to 1000ml with water.

0.01N KH2PO4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Diluent: Based upon the solubility of the drugs, diluents were selected. Acetonitrile and water are taken in the ratio of 50:50 v/v.

Stock solution:

Preparation of Standard stock solutions: 5 mg dapagliflozin, 2.5 mg saxagliptin and 50 ml of volumetric flasks were precisely weighed separately. In these two vials, 3/4 of the diluents were applied and 10 minutes sonicated. Flasks have been made of diluents and labelled as regular 1and 2 stock solution. Dapagliflozin (100µg/ml) and Saxagliptin (50µg/ml).

Preparation of Sample stock solutions: Weighed 20 tablets and measured the average weight of each tablet, then transfer to a 100 ml volumetric fiber the weight of 1 tablet, added 5 ml of diluents and sonicated for 25 minutes, applied diluents and filtered HPLC filters to the amount.

Working solution:

Preparation of Standard working solutions: Every 1ml solution in inventory has been pipetted and taken in a 10ml volumetric flask and diluted. (10µg / ml Saxagliptin Dapagliflozin and 5µg / ml)

Preparation of Sample working solutions (100% solution): A 1ml sample stock solution filtered into a volumetric flask of ten ml has been transferred and diluted.

Procedure :

Sample solutions (10µL) in duplicates were injected and the peak responses were measured. % assay were calculated Dapagliflozin and Saxagliptin. [3]

RESULTS AND DISCUSSION:

Method validation: Specificity, linearity, range, Accuracy, precision, Repeatability, Intermediate precision, limit of detection, limit of Quantification, Robustness.

SPECIFICITY: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. The specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.

LINEARITY: To establish the linearity of the method, serial dilutions were prepared to obtain the mixture of Dapagliflozin and Saxagliptin ranging from 2.5ml to 15ml and 1.25ml to 7.5ml, all the solutions were filtered through a 0.45µm Millipore filters. The final solution was injected in duplicate manner keeping the injection volume 10µl. Calibration curve was plotted between mean peak area and concentration. The correlation coefficient and slope were determined from the calibration curve. The linearity chats of Dapagliflozin and Saxagliptin was shown in figure no.5 and 6 . The correlation coefficient was found to be 0.999 for both drugs and hence the method was set to be linear. They were tabulated in table 1. [4]

ACCURACY: Accuracy was evaluated by standard addition method of three known concentration of the drug and the spiked solution were analysed. The recovery of the added drug was determined by calculating the pre-analysed drug concentration with concentration of spiked drug. The % recovery was calculated and the result was reported in table no. 2 & 3. [5]

PRECISION: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration of Dapagliflozin and Saxagliptin were injected at intermediate precision and repeatability. The %RSD was calculated and results were reported at table no. 4 and 5. [6]

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1.

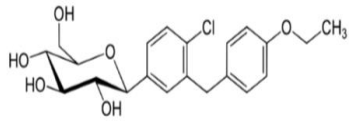

Fig-1: structure of Dapagliflozin

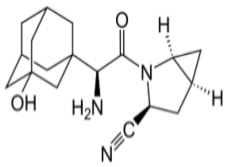

Fig-2: structure of Saxagliptin

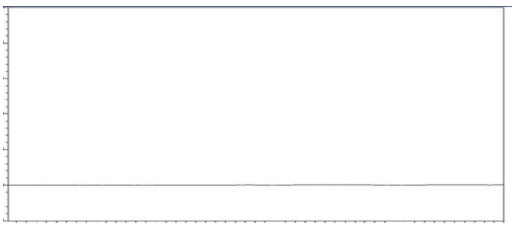

Fig-3: Chromatogram showing blank

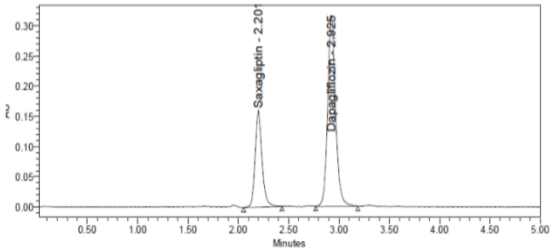

Fig-4: Chromatogram showing optimised condition

Table 1: Linearity results for Dapagliflozin and Saxagliptin

Dapagliflozin		Saxagliptin	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
2.5	39673	1.25	18538
5	74086	2.5	37271
7.5	107357	3.75	58267
10	145670	5	74379
12.5	177551	6.25	94074
15	213955	7.5	110756

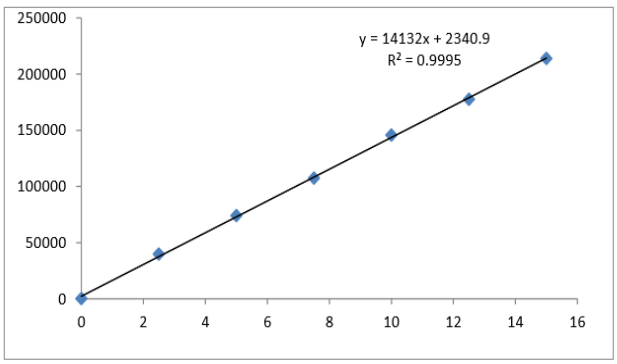

Fig-5: showing calibration curve of Dapagliflozin

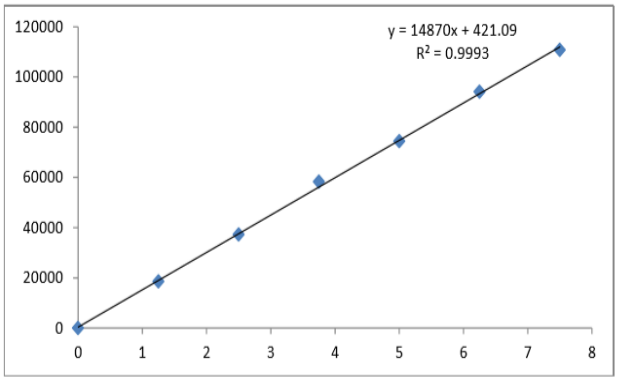

Fig-6: showing calibration curve of Saxagliptin

Table 2 : Accuracy data for Dapagliflozin

% Level	Amount Spiked (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.98	99.69	99.26%
	5	4.97	99.35	
	5	4.99	99.88	
100%	10	9.94	99.38	
	10	9.93	99.29	
	10	9.93	99.27	
150%	15	14.90	99.36	
	15	14.93	99.55	
	15	14.89	99.69	

Table 3 : Accuracy data for Saxagliptin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	2.5	2.47	98.99	99.46%
	2.5	2.48	99.37	
	2.5	2.50	99.87	
100%	5	4.96	99.23	
	5	4.97	99.50	
	5	4.99	99.77	
150%	7.5	7.42	98.95	
	7.5	7.48	99.76	
	7.5	7.48	99.67	

Table-4: Intermediate precision data for Dapagliflozin and Saxagliptin

S. No	Area of Dapagliflozin	Area of Saxagliptin
1.	145393	74018
2.	145648	74880
3.	147415	74418
4.	146112	74589
5.	147197	74784
6.	147630	73576
Mean	146566	74378
S.D	967.0	497.1
%RSD	0.7	0.7

Table-5: Repeatability results for Dapagliflozin and Saxagliptin

S. No	Area of Dapagliflozin	Area of Saxagliptin
1.	149122	73534
2.	149914	73524
3.	147114	73032
4.	147954	73634
5.	147829	73007
6.	148375	73371
Mean	148385	73350
S.D	999.1	269.8
%RSD	0.7	0.4

Table-6: LOD and LOQ data for Dapagliflozin and Saxagliptin

Molecule	LOD	LOQ
Dapagliflozin	0.04	0.13
Saxagliptin	0.01	0.02

Table-7: Robustness data for Dapagliflozin and Saxagliptin

S.No	Condition	%RSD of Dapagliflozin	%RSD of Saxagliptin
1	Flow rate (-) 0.9ml/min	1.2	1.4
2	Flow rate (+) 1.1ml/min	1.3	1.1
3	Mobile phase (-) 65B:35A	1.0	1.0
4	Mobile phase (+) 55B:45A	1.1	0.4
5	Temperature (-) 25°C	0.5	0.7
6	Temperature (+) 35°C	0.8	0.8

The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no. 6 . [7]

ROBUSTNESS: The small deliberate changes in method like flow rate was made but there were no recognized change in the result and are within the range as per ICH guide lines. Robustness condition like flow minus (0.9ml/min), flow plus (1.1ml/min), temperature ambient was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no.7 . [8]

CONCLUSION:

For the simultaneous estimation of Dapagliflozin and Saxagliptin in tablet form, a simple, reliable, precise method has been developed. Retention time was found to be 2.201min and 2.925min for Dapagliflozine and Saxagliptin. 0.7 aznd 0.7 respectively were and were found to be percent RSD of Dapagliflozin and Saxagliptin. Recovery for Dapagliflozin and Saxagliptin was obtained as a percentage of 99, 55% and 99, 46% respectively. LODs, LOQ values from Dapagliflozin and Saxagliptin regression equations were of respectively 0.04, 0.13 and 0.01, 0.02. Dapagliflozin regression equation is = 14132x + 2340 and y = 14870x + 421 Saxagliptin regression equation. Conservation times were reduced and the running time was reduced, which makes the process that could be used in industries in the daily quality assurance test simple and economical.

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