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R E S E A E C H A R T I C L E

Analytical Method Development and Validation for the Simultaneous Estimation of Saxagliptin and Dapagliflozin in Combined Dosage Form by Using RP-HPLC

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A B S T R A C T

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Saxagliptin and Dapagliflozin was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Saxagliptin and Dapagliflozin were found to be from 100-500 µg/ml of Saxagliptin and 1-5µg/ml of Dapagliflozin . Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Saxagliptin and Dapagliflozin . LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Inertsil $C_{18,}$Saxagliptin and Dapagliflozin , RP-HPLC

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Saxagliptin, sold under the brand name Onglyza, is an oral hypoglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. Early development was solely by Bristol-Myers Squibb; in 2007 AstraZeneca joined with Bristol-Myers Squibb to co-develop the final compound and collaborate on the marketing of the drug.

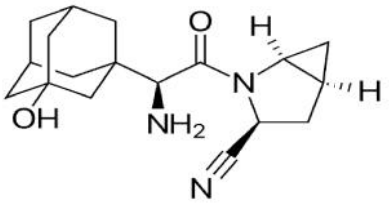

Fig 1: Structure of Saxagliptin

Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2) which are responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine. In clinical trials, dapagliflozin lowered HbA1c by 0.6 versus placebo percentage points when added to metformin.

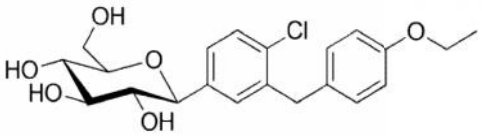

Fig 2: Structure of Dapagliflozin

2. Materials and Methods

Instrumentation: HPLC Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. UV/VIS spectrophotometer LABINDIA, UV 3000+pH meter, weighing machine.

Chemicals: Saxagliptin and Dapagliflozin, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Chromatographic Conditions:

Table No 1: Chromatographic Conditions

Parameter	Description
Flow rate	1.0 ml/min
Column	KromosilC18 column (4.6×150mm)5µ
Mobile phase ratio	Phosphate buffer: Methanol PH 4.5(20:80 v/v)
Detection wavelength	254 nm
Buffer	Potassium dihydrogen ortho phosphate PH 4.5 adjusted with Orthophosphoric acid
Injection volume	20µl
Column temperature	Ambient
Type of elution	Isocratic
Run time	10 min

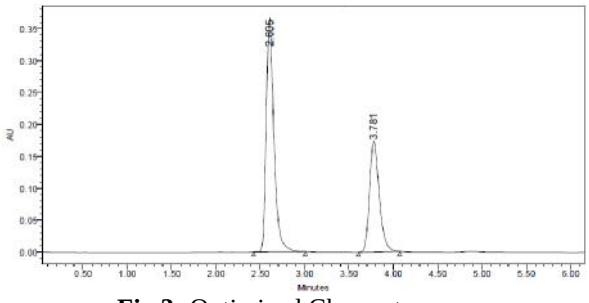

Fig 3: Optimized Chromatogram

Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Saxagliptin and Dapagliflozin 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Saxagliptin and Dapagliflozin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3 ml of Saxagliptin e and Dapagliflozin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

Precision: Accurately weigh and transfer 25 mg of Saxagliptin and Dapagliflozin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Accuracy: Accurately weigh and transfer 10 mg of Saxagliptin and Dapagliflozin 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Linearity: Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Saxagliptin and Dapagliflozin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection:

Accurately weigh and transfer 10 mg of Saxagliptin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it

completely and make volume up to the mark with the same solvent.

Limit of Quantification: Accurately weigh and transfer 10 mg of Saxagliptin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection(For Dapagliflozin): Accurately weigh and transfer 10mg of Dapagliflozin working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification (for Dapagliflozin): Accurately weigh and transfer 10mg of Dapagliflozin working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

3. Results and Discussions

Linearity:

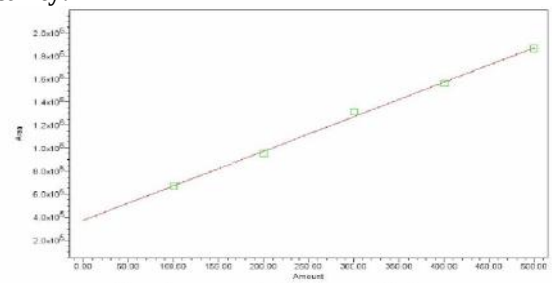

Fig 4: Calibration graph for Saxagliptin at 225 nm

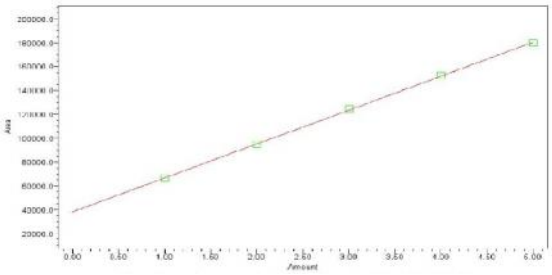

Fig 5: Calibration graph for Dapagliflozin at 225 nm

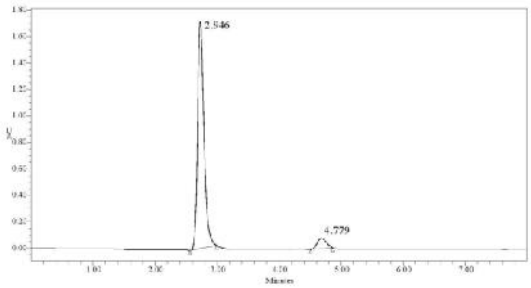

Fig 6: Chromatogram snowing less flow of 0.6ml/min

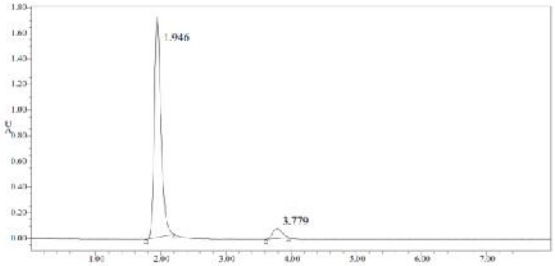

Fig 7: Chromatogram snowing more flow of 1.0ml/min

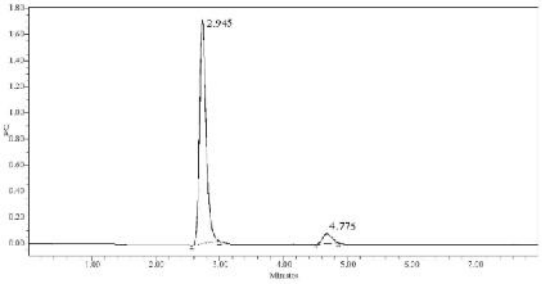

Fig 8: Chromatogram showing less organic composition

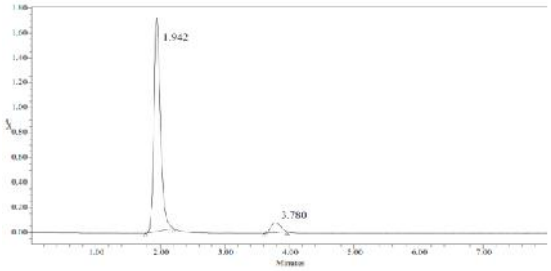

Fig 9: Chromatogram showing more organic compositio

Table No 2: Results of system suitability parameters for Saxagliptin and Dapagliflozin

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Saxagliptin**	2.5	124505	213642		1.2	4673.4
2	**Dapagliflozin**	3.9	1308495	154566	6 0	1.3	6090.3

Table No 3: Results of method precession for Saxagliptinand Dapagliflozin

Injection	Area	
	Saxagliptin	**Dapagliflozin**
Injection-1	1302729	123149
Injection-2	1302947	123766
Injection-3	1303236	124271
Injection-4	1303977	124691

Injection-5	1309759	124956
Average	1304529.8	124162.7
Standard Deviation	2961.1	725.6
%RSD	0.2	0.6

Table No 4: Results of Intermediate precision for Saxagliptinand Dapagliflozin

Injection	**Area**	
	Saxagliptin	**Dapagliflozin**
Injection-1	1300148	122487
Injection-2	1304520	122626
Injection-3	1305937	122632
Injection-4	1306476	122702
Injection-5	130871	122962
Average	1305070.2	122681.8
Standard Deviation	3061.8	174.8
%RSD	0.2	0.1

Table No 5: Accuracy (recovery) data for Saxagliptin

%Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table No 6: Accuracy (recovery) data for Dapagliflozin

%Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table No 7: Area of different concentration of Saxagliptin

S.No.	**Linearity Level**	**Concentration**	**Area**
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table No 8: Area of different concentration of Dapagliflozin

S.No	**Linearity Level**	**Concentration**	**Area**
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Table No 9: Analytical performance parameters of Saxagliptin and Dapagliflozin

Parameters	**Saxagliptin**	**Dapagliflozin**
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table No 10: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Saxagliptin	52	152	2.9
Dapagliflozin	52	156	3

Table No 11: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Saxagliptin	52	522	10.03
Dapagliflozin	52	524	10.1

Table No 12: Flow Rate (ml/min) data for Saxagliptin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table No 13: Flow rate (ml/min) data for Dapagliflozin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	7063.3	1.3
2	0.8	6090.3	1.2
3	1.0	6998.0	1.3

Table No 14: Change in Organic Composition in the Mobile Phase for Saxagliptin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table No 15: Change in Organic Composition in the Mobile Phase for Dapagliflozin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Saxagliptin and Dapagliflozin was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/v. InertsilC_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Saxagliptin and Dapagliflozin were found to be from 100-500 µg/ml of Saxagliptin and 1-5µg/ml of Dapagliflozin. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Saxagliptin and Dapagliflozin. LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

[1] Campbell DB, Lavielle R, Nathan C. The Mode Of Action And Clinical Pharmacology Of Gliclazide: A Review. Diab Res Clin Prac.1991; 14:S21-S36.

[2] Herman G, Bergman A, Liu F, Stevens C, Wang A, Zeng W, Chen L, Snyder K, Hilliard D, Tanen M, Tanaka W, Meehan A, Lasseter K, Dilzer S, Blum R, Wagner J. Pharmacokinetics and

Pharmacodynamic Effects Of The Oral DPP-4 Inhibitor Sitagliptin In Middle-Aged Obese Subjects. J.Clin.Pharmacol.2006; 46 (8): 876–886.

[3] Florentin T, Monica A .Specificity of An Analytical Hplc Assay Method Of Metformin Hydrochloride. Revue Roumaine De Chimie.2007; 52(6):603–609.

[4] Georgita C, Albu F, David V, Medvedovici A.Simultaneous Assay Of Metformin And Glibenclamide In Human Plasma Based On Extraction-Less Sample Preparation Procedure And LC /(APCI)MS. J.Chromatogr. B.2007; 854(1-2):211-218.

[5] Patil Sudarshan S. Et Al Development And Validation Of Analytical Method For Simultaneous Estimation Of Glibenclamide And Metformin Hcl In Bulk And Tablets Using UV – Visible Spectroscopy.

[6] Ramakrishna Nirogi, Vishwottam Kandikere, Koteshwara Mudigonda, Prashanth Komarneni, Raghupathi Al Eti, Rajeshkumar Boggavarapu. Sensitive Liquid Chromatography Tandem Mass Spectrometry Method For The Quantification Of Sitagliptin, A DPP-4 Inhibitor, In Human Plasma Using Liquid–Liquid Extraction. Biomed. Chromatogr. 2008; 22(2): 214–222.

[7] Shammi Goyal Et Al Development And Validation Of RP-HPLC Method For Estimation Of Glipizide In Bulk Drug And Pharmaceutical Formulation.

[8] Patil S.S., Bonde C. .Development And Validation Of Analytical Method For Simultaneous Estimation Of Glibenclamide And Metformin Hcl In Bulk And Tablets Using UV Visible Spectroscopy, Int. J. Chemtec. Res.2009; 1(4): 905-909.