INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Analytical Method Development And Validation For Simultaneous Estimation Of Antidibetic Drugs In Bulk And Marketed Formulation

Kartik A. Bhatkar*[1], V. M. Waghulkar[2], M. P. Jadhao[3], M. D. Game[4], S. G. Jawarkar[5]

[1]*Vidya Bharti College of Pharmacy, Sant Gadge Baba Amravati University, Maharashtra, India, Student of Post-Graduation in Pharmacy, Quality Assurance Department.*
[2-5]*Vidya Bharti College of Pharmacy, Sant Gadge Baba Amravati University, Maharashtra, India, Faculty Pharmacy Department.*

ARTICLE INFO

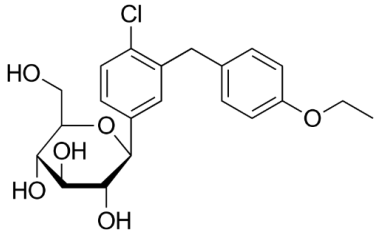
Received: 11 April 2024
Accepted: 15 April 2024
Published: 13 May 2024
Keywords:
HPLC, Method Validation, Linagliptin, dapagliflozin, ICH, UV, System suitability.
DOI:
10.5281/zenodo.11183408

ABSTRACT

A new, simple, selective, accurate, rapid and precise High-Performance Liquid Chromatographic technique of Dapagliflozin and Linagliptin was established as per ICH Guidelines. HPLC was performed on a Hypersil BDS Column C18, 5 µm particle size, 245 cm × 4.6 mm with phosphate buffer and acetonitrile, Methanol in the ratio of 15: 15: 70 v/v as a mobile phase and a flow rate of 1.0 ml min-1. UV detection was performed at 284 nm. The retention time of Dapagliflozin was found to be 3.961 minutes, and Linagliptin was found to be 3.451. Validation of the developed method was done as per USP and ICH guidelines. Method validation revealed that the method is rapid, accurate, precise, reliable, and reproducible. The high recovery and low coefficients of variation confirm the effectiveness of process in the dosage form. The validated method was successfully used for quantitative analysis of commercial available tablets.

INTRODUCTION

Dapagliflozin is a drug of the gliflozin class and it can be used to treat type-2 diabetes.(1-4) Dapagliflozin inhibits subtype 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 cause blood glucose to be eliminated through the urine (5-7).

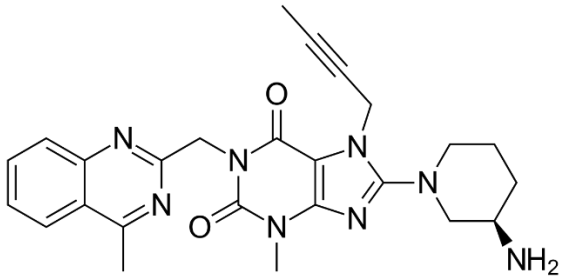


Drug structure - Dapagliflozin

***Corresponding Author:** Kartik Bhatkar
Address: *Vidya Bharti College of Pharmacy, Sant Gadge Baba Amravati University, Maharashtra, India, Student of Post-Graduation in Pharmacy, Quality Assurance Department.*
Email ✉: bhatkarkartik@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Dapagliflozin is chemically (2S,3R,4R,5S,6R)-2-(4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol. The molecular formula is C12H25CIO6. The molecular weight is 408.86g/mol. Dapagliflozin is soluble in organic solvent such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. Soluble in Methanol and Dichloromethane. Linagliptin is an oral drug that reduce blood sugar level in patients with type 2 diabetes.(9)Linagliptin is a member of a class of drug that inhibit the enzyme, dipeptidyl peptidase-4 (DPP-4). Following a meal, Incretin hormone such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) reduce are released from the intestine, and their levels increase in the blood. GLP-1 and GIP reduce blood glucose by increasing the production and release of insulin from the pancreas. GLP-1 also reduce blood glucose by reducing the secretion by the pancreas of the hormone, glucagon, a hormone that increase the production of glucose by the liver and raises the blood level of glucose. The net effect of increased released of GLP-1 and GIP is to reduce blood glucose level. Linagliptin inhibits the enzyme, DPP-4 that destroys GLP-1 and GIP and thereby increase the level and activity of both hormone. As a result, levels of GLP-1 and GIP in the blood remain higher, and blood glucose level fall(10).



Drug structure - Linagliptin

Linagliptin may be taken with or without food. The recommended dose is 5 mg/day. The most common side effect of Linagliptin are stuffy or many nose and sore throat. Hypoglycemia may occur when Linagliptin is combined with insulin or a sulfonylurea-type drug. Allergic reaction and muscles pain also may occur, Pancreatitis also has been reported. Rifampin decrease the blood concentration of Linagliptin by stimulating break down of Linagliptin by CYPAS liver enzyme, other drugs that increase activity CYPAS may also reduce the blood concentration of Linagliptin. Very few method have been developed for the estimating of Linagliptin in pharmaceutical dosage forms by HPLC.(11) The aim of the present work was the Method development and validation.(6-13)

EXPERIMENTAL WORK

Material, Reagent and Pharmaceutical Product

Linagliptin 100%, Dapagliflozin 100% were purchased from, East Zone of Economic development Zone, Pingyuan Country, Dezhou City, Shandong Province, China. Linagliptin 5 mg and Dapagliflozin 10mg tablet (Linabite D) tablet were obtained from local pharmacy. Analytical reagent grade was obtained from Qualigens Thermo fisher scientific India Pvt. Ltd Mumbai.

Instrumentation

The HPLC system used for the method development and validation of Shimadzu and UV detector manual sampler. Data acquisition, recording and chromatographic integration was performed by lab solution. Analysis and separation has been done on an Hypersil BDS column C18 (250 mm × 4.6mm, 5µm) in an air lab condition. The mobile phase consist of Methanol, Acetonitrile and Sod. Acetate buffer adjust pH 3.6, the flow rate was set at 1ml/min in an isocratic mode and the injection volume was set at 20 µl for all sample.

Preparation of the buffer solution

Weight accurately 0.82 gm of Sod. Acetate and dissolve in 900 ml HPLC grade water, adjust pH to 3.6 with dilute acetic acid solution make up volume to 1000ml with HPLC grade water.

Preparation of standard stock solution (A and B)

Weight accurately 15 mg and transferred into 100 ml volumetric flask and 20 ml of mobile phase mixture was added to Linagliptin and sonicated for 10 min, the final volume was made up to 100 ml, using the mobile phase mixture (flask A). In a separate volumetric flask, the same procedure was followed to dissolve 30 mg Dapagliflozin (flask B).

Preparation of working solution

An pipette out 2 ml from flask A and 2 ml from flask B were transferred into 20 ml with help of diluent shake well, sonicate for 2 min, filter the solution through 0.2µm syringe filter and volume was made up with mobile phase to give working solution.

Preparation of pharmaceutical sample

20 tablet of Linabite D were weight and crushed. Linabite D is a combination of Linagliptin 5mg and Dapagliflozin 10mg. The powder was transferred in 500 volumetric flask and make up volume with distilled water with mobile phase mixture shake well for 5min, And pipette out into 20 ml in volumetric flask and mobile phase was added to the mark to produce final concentration is ready.

Method development and optimization

Due to the significant difference of Linagliptin and Dapagliflozin, several mobile phase and column were initially trialed in order to have both eluents on the same chromatogram. The suitability of the column and mobile phase used in the optimized method have been decided upon the basis of the selectivity, sensitivity as well as acceptable chromatogram parameter of the produced peak.

Selection of wavelength

Linagliptin has lambda max at 295 nm and Dapagliflozin has lambda max at 230 nm in methanol. An acceptable response was obtained upon detection of both drugs at 284 nm either individually or in combination.

Method validation

The optimized method for simultaneous determination of Linagliptin and Dapagliflozin has been validated as per ICH guideline, for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

System suitability

System suitability parameter with respect to tailing factor, repeatability, number of theoretical plate and resolution between Linagliptin and Dapagliflozin peak were assessed by injecting a blank mobile phase followed by 6 replicate of Linagliptin and Dapagliflozin.

Precision

System and method precision were assessed by injecting 6 independent combined sample of Linagliptin and Dapagliflozin on the same day under same operating conditions. Intermediate or inter-day precision was assessed by comparing the result of 4 independent on 2 different day.

Linearity

The standard stock solution of Linagliptin is diluted in the concentration range of (7.5-22.5µg/ml). Concentration range were prepared and plotted on Linagliptin calibration curve. The standard stock solution of Dapagliflozin is diluted in the concentration range of (15-45µg/ml),such concentration range were prepared and plotted on a Dapagliflozin calibration curve. Calibration curve were determined to ensure linearity of the analytical method.

Table 1- System suitability and precision result (acceptance limit RSD % < 2)

Sr.no	Retention time		Tailing factor		Theoretical plate	
	Linagliptin	**Dapagliflozin**	**Linagliptin**	**Dapagliflozin**	**Linagliptin**	**Dapagliflozin**
1.	3.183	4.017	1.05	1.09	11,755	14906
2.	3.183	4.017	1.03	1.10	11743	14505
3.	3.183	4.017	1.00	1.01	11791	12478
4.	3.167	4.100	1.14	0.98	7895	19450
5.	3.167	4.000	1.08	1.04	8054	12328
Mean	3.1766	4.030	1.06	1.04	11,755	14906
Standard Deviation	0.008	0.039	0.053	0.051	20.75	28.81

Accuracy study and recovery

Accuracy of the proposed method was confirmed was by placebo tablet with Linagliptin and Dapagliflozin separately at 3 different levels 80%, 100% and 120%. Determination of 3 levels have been recorded to obtain the mean and % RSD.

LOD and LOQ

LOD and LOQ for Linagliptin and Dapagliflozin were calculated from the linear regression equation based on standard deviation of the intercept and the slope using the formula.

LOD = 3.3Q/S and LOQ = 10Q/S

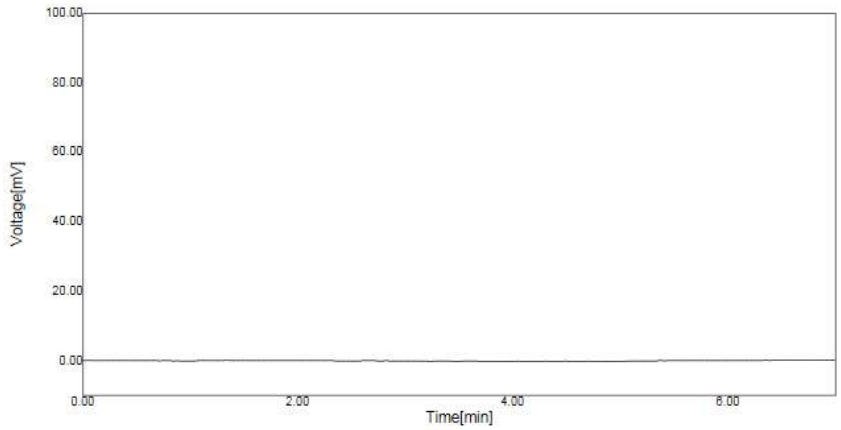
Where Q is the standard deviation of the intercept, S is slope of the calibration curve.

Robustness

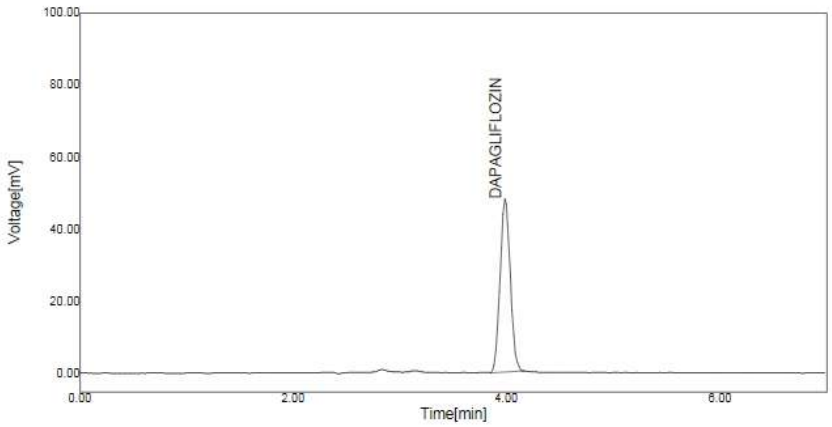
The robustness of an analytical method is a measure of its ability to remain unaffected by small, but deliberate change in method parameter and provide an indication of to remain normal during usage. Robustness tests examine the impact of operational parameter on the analysis result. The robustness parameter should be considered during the development phase. Robustness shows the reliability of an analytical procedure with respect to deliberate variation in method parameter.

RESULT AND DISCUSSION

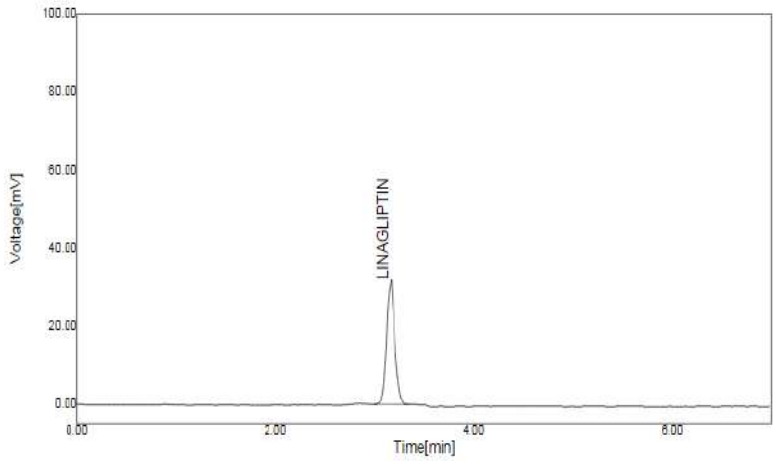
Fig no 1:- Chromatograms of placebo matrix, Dapagliflozin, Linagliptin and typical chromatogram of Dapagliflozin and Linagliptin mixture.



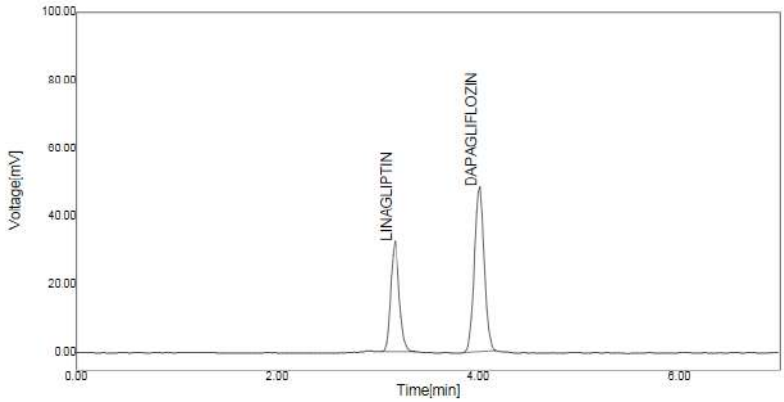
Blank Chromatograph



Typical Chromatograph of Dapagliflozin



Typical Chromatograph of Linagliptin



Typical chromatograph of Linagliptin and Dapagliflozin

At the first trial of method development of chromatogram result is not good, the result of first chromatogram is Tailing factor and theoretical plate were not found within acceptance criteria, peak shape for Linagliptin found but it s splitting. Second trial result is same condition of the first trial and the chromatogram was not found satisfactory. Third trial result change the column C18 column use TP, TF and resolution observe within acceptance criteria peak shape is good, but peak are eluting at longer time. Fourth trial result TP, TF and resolution criteria, peak shape are good but peaks are eluting too early about void volume due to concentration of mobile phase then change

the concentration of mobile phase change. Last trial use C18 column and mobile phase concentration are MeoH: ACN: Buffer is 70:15:15, and result are TF, TP and resolution criteria are so good and peak also.

Table -2 Recovery result for Linagliptin and Dapagliflozin (Acceptance limit recovery % = 98-102)

Dapagliflozin Recovery

Sample name	Theoretical (claimed) concentration µg/ml	The concentration found in µg/ml	Recovery mean	Statistical data
S1 80%	24.00	23.85	99.36	Mean= 100 Standard deviation = 0.6889 % RSD = 0.69
S2 80%	24.20	24.37	100.69	
S3 80%	24.19	24.19	100.35	
S4 100%	29.64	29.64	98.48	Mean= 99.59 Standard deviation = 0.9760 % RSD = 0.98
S5 100%	29.99	29.99	99.97	
S6 100%	30.20	30.20	100.32	
S7 120%	36.12	36.12	100.61	Mean= 101.04 Standard deviation = 0.3777 % RSD = 0.37
S8 120%	36.43	36.43	101.20	
S9 120%	36.57	36.57	101.31	

Linagliptin Recovery

Sample name	Theoretical (claimed) concentration µg/ml	The concentration found in µg/ml	Recovery mean	Statistical data
S1 80%	12.00	12.02	100.76	Mean= 99.59 Standard deviation = 1.167 % RSD = 1.17
S2 80%	12.10	12.17	100.56	
S3 80%	11.90	11.71	98.36	
S4 100%	14.80	14.63	98.88	Mean= 99.28 Standard deviation = 0.37 % RSD = 0.37
S5 100%	15.00	14.90	99.35	
S6 100%	15.10	15.04	99.62	
S7 120%	18.10	18.10	100.02	Mean= 100.92 Standard deviation = 0.79 % RSD = 0.79
S8 120%	18.20	18.41	101.17	
S9 120%	17.90	18.18	101.56	

System suitability

The obtained result of 4 replicate injection showed that the parameter tested were within the acceptable range, Linagliptin and Dapagliflozin were repeatedly retained and separated at 3 min and 4 min expressing excellent resolution both peak with % RSD of recorded retention time.

Precision

The peak areas obtained following injecting 4 independent combined Linagliptin and Dapagliflozin sample were repeatable and precise over 2 consecutive days. The result of both intra-day and inter day determined ensure the high precision and repeatability express in % RSD and never exceed (acceptance limit <2).

Linearity

The analytical calibration curve constructed for both Linagliptin and Dapagliflozin were liner in the specific range indicated by the closeness of the correlation coefficient (R2= 0.9999).

Recovery

Accuracy of the proposal analytical method was evaluated by determining the added analyze in at 3 different levels (80%, 100%, 120%) and express in term of % recovery of Linagliptin and Dapagliflozin. At different levels proved the accuracy of the proposed method where,>99% of recovery from excipient. Result shown in Table 1.

LOD and LOQ

The calculated LOD and LOQ were 0.97 µg/ml, 1.61µg/ml for Dapagliflozin and 0.98µg.ml, 1.31µg.ml for Linagliptin.

Robustness

No significant changes detected upon applying small variation to the chromatographic conditions ensuring that the method is robust to small changes applied in terms of flow rate, pH of buffer or different mobile phase ratio.

CONCLUSION

The validated HPLC method has been developed for the determination of Linagliptin and Dapagliflozin in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 7 min allows the analysis of a large no. of sample in short period of time.

REFERENCES

1. Beckett AH, Stenlake JB (1988) Practical Pharmaceutical Chemistry: Part II. 4th edn. A&C Black 2: 275-298.
2. Quality Assurance (2003) Worth the effort. Inforum 7: 4.
3. Shethi PD (1997) Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd edn., pp: 51-56.
4. Kasture K, Shethi PD (2001) Hand book of Pharmaceutical Analysis. High Performance Liquid Chromatography 1: 11.
5. Validation of Analytical Procedures, Methodology (1996) ICH Harmonized Tripartite Guidelines.
6. Text on Validation of Analytical Procedures (1994) ICH Harmonized Tripartite Guidelines.
7. Ravi S (2015) A Text book of Pharmaceutical Analysis. 3rd edn. p: 22
8. FDA Approves Type 2 Diabetes Drug from Boehringer Ingelheim and Lilly". 3 May 2011.
9. Four Phase III Trials Confirm Benefits of BI's Oral, Once-Daily Type 2 Diabetes Therapy". Genetic Engineering & Biotechnology News. 28 June 2010.
10. H. Spreitzer (September 1, 2008). "Neue Wirkstoffe - BI-1356" (in German).Österreichische Apothekerzeitung(18/2008): 918
11. Wang Y, Serradell N, Rosa, E Castaner, R BI-1356.Drugs of the Future 2008; 33(6):473–477.
12. Lakshmi B, Reddy TV. A novel RP – HPLC Method for the Quantification of Linagliptin in formulations. J Atoms and Molecules 2012; 2(2):155–164.
13. Krause SO. Analytical method validation for biopharmaceuticals: a practical guide. Guide to Validation. Supp to BioPharm Int. 2005 Mar; 26–34.
14. Kanarek AD. Method validation guidelines. Guide to Bioanalytical Advances. Supp to BioPharm Int. 2005 Sep; 28–33.
15. US FDA. Guideline for industry: text on validation of analytical procedures: ICH Q2A. Rockville, MD: Mar 1995
16. Rodbard D. Statistical estimation of the minimal detectable concentration (sensitivity) for radio ligand assays. Analytic Biochem 1978; 90:1–12.
17. Hauck WW, Capen RC, Callahan JD, De Muth JE, Hsu H, Lansky D, et al. Assessing parallelism prior to determining relative potency. PDA J Pharma Sci Technol 2005; 59(2):127–37.
18. ICH Q5C: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (July 1996)
19. ICH Q6A: Specifications: Test Procedures and Acceptance Criteria for New Drug Substances.

HOW TO CITE: Kartik A. Bhatkar, V. M. Waghulkar, M. P. Jadhao, M. D. Game, S. G. Jawarkar, Analytical Method Development And Validation For Simultaneous Estimation Of Antidibetic Drugs In Bulk And Marketed Formulation , Int. J. of Pharm. Sci., 2024, Vol 2, Issue 5, 524-531. https://doi.org/10.5281/zenodo.11183408