International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page:www.pharmaresearchlibrary.com/ijcps

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 Empagliflozin and Linagliptin in Pharmaceutical Dosage Forms by RP-HPLC Method

Dr. K. Nageswara Rao[1*], Raghava Doonaboyina[2], S. Rajesh[3]

[1]*Professor and Head, Dept. of Pharmaceutical Analysis, K.G.R.L College of Pharmacy, KGRL Road, Bhimavaram, West Godavari, Andhra Pradesh, India.*
[2]*Associate Professor and Head, Dept. of Pharmaceutical Chemistry, K.G.R.L College of Pharmacy, KGRL Road, Bhimavaram, West Godavari, Andhra Pradesh, India.*
[3]*K.G.R.L College of Pharmacy, KGRL Road, Bhimavaram, West Godavari, Andhra Pradesh, India.*

A B S T R A C T

A new method was established for simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Empagliflozin and Linagliptin by using Agilent C1 85µm(4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer ph 4.0 : ACN (30:70%v/v), detection wave length was 254nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector996, Empower-softwareversion-2. The retention times were found to be 3.503 mins and 2.577 mins. The % purity of Empagliflozin and Linagliptin was found to be 100.3% and 101.1% respectively. The system suitability parameters for Empagliflozin and Linagliptin such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and 1.2, 2936.0 the resolution was found to be 9.4. The analytical method was validated according to ICH guidelines (ICH,Q2(R1)). The linearity study for Empagliflozin and Linagliptin was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient(r2)was found to be 0.999 and 0.999, %mean recovery was found to be 102.5% and 101.0%, % RSD for repeatability was 0.6 and 0.5, %RSD for intermediate precision was 0.7 and 0.6 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.1 and 3.02, and LOQ value was 10.1 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Empagliflozin and Linagliptin in API and Pharmaceutical dosage form.

Keywords: AgilentC18, Empagliflozin and Linagliptin, RP-HPLC

A R T I C L E I N F O

Corresponding Author
Dr. K.Nageswara Rao
1Professor and Head, Dept. of Pharmaceutical Analysis, K.G.R.L College of Pharmacy, KGRL Road, Bhimavaram, West Godavari, Andhra Pradesh, India.
MS-ID: IJCPS3741

PAPER-QRCODE

A R T I C L E H I S T O R Y: Received 31 August 2018, **Accepted** 19 September 2018, **Available Online** 27 November 2018

Citation: Dr. K.Nageswara Rao, *et al. Analytical Method Development and Validation for the Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Dosage Forms By RP-HPLC Method. Int. J. Chem, Pharm, Sci.*, 2018, 6(11): 313-318.

C O N T E N T S

1. Introduction

Empagliflozin (trade name Jardiance) is a drug of the gliflozin class, approved for the treatment of type 2 diabetes in adults in 2014. It was developed by Boehringer Ingelheim and Eli Lilly and Company. Empagliflozin is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), and causes sugar in the blood to be excreted by the kidneys and eliminated in urine.

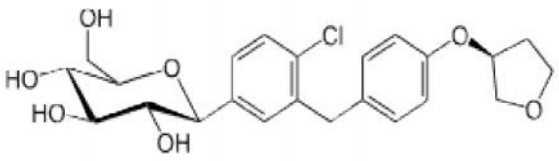

Fig 1: Structure of Empagliflzin

Linagliptin (INN, previously known as BI-1356, marketed under trade names Tradjenta (U.S.) and Trajenta (worldwide)) is a dipeptidyl peptidase-4 inhibitor developed by Boehringer Ingelheim for treatment of diabetes mellitus type 2.Once-daily linagliptin was approved by the U.S. Food and Drug Administration (FDA) on 2 May 2011 for treatment of type 2 diabetes. It is being marketed by Boehringer Ingelheim and Lilly.

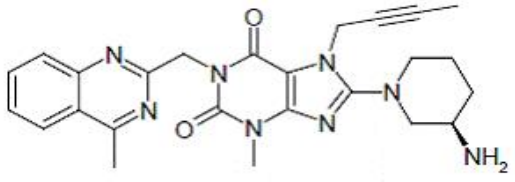

Fig 2: Structure of Linagliptin

2. Materials and Methods

Instrumentation

HPLC-auto sampler –UV detector, Separation module2695, UV.detector2487, Empower-software version-2, Waters, U.V double beam spectrometer, UV 3000+, U.V win soft ware, Lab India.

Chemicals

Water, Methanol, Acetonitrile, Potassium dihydrogen, Empagliflozin and Linagliptin.

Chromatographic conditions:

Column	:AgilentC18 5µm (4.6*250mm)
Mobile phase ratio	:Phosphate buffer ph 4.0: ACN (70:30%v/v)
Detection wavelength	: 254 nm
Flow rate	: 1ml/min
Injection volume	:10µl
Temperature	:Ambient

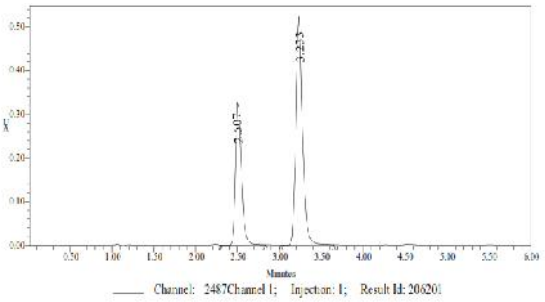

Fig 3: Optimized Chromatogram

Observation:

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Preparation of the individual Empagliflozin standard preparation:

10mg of Empagliflozin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Linagliptin standard preparation:

10mg of Linagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of Sample Solution :(Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Linagliptin and Empagliflozin (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

Method Validation

Accuracy:

Accurately weighed 10 mg of Linagliptin and 10mg of Empagliflozin working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks. About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark

with the same solvent. (Stock solution) Further 0.3ml and 0.3ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluents.

Precision:

Repeatability: Accurately 10 mg of Linagliptin and 10mg of Empagliflozin working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made 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 days by using different make column of same dimensions.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

LOD:

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

LOQ:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

Linearity:

Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Linagliptin and Empagliflozin (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Empagliflozin and Linagliptin respectively.

Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method. The flow rate was varied at 0.8ml/min to 1.2 ml/min. Standard solution 3ppm of Empagliflozin and 300 ppm of Linagliptin was prepared and analyzed using the varied flow rates along with method flow rate.

System suitability:

10 mg of Empagliflozin and 10 mg of Linagliptin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 20ml of diluant and sonicated to dissolve it completely and make volume up to the mark with the same solvent.

3. Results and Discussions

Wavelength Detection: The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Empagliflozin and Linagliptin was obtained and the isobestic point of Empagliflozin and Linagliptin showed absorbance's maxima at 254 nm.

Linearity: The resultant areas of linearity peaks are plotted against Concentration. It was shown in fig 4 and 5.

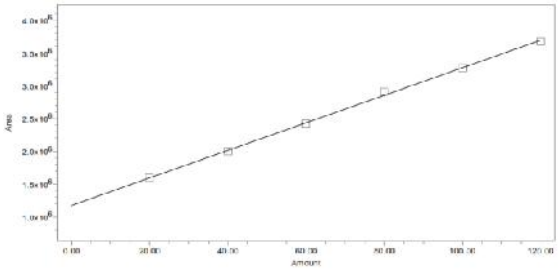

Fig 4: Calibration curve of Linagliptin

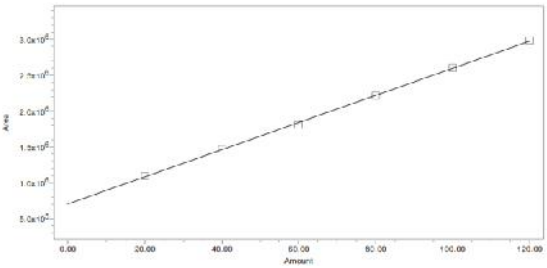

Fig 5: Calibration curve of Empagliflozin

Robustness: Flow Rate: The robustness was performed for the flow rate variations from 0.8 ml/min to 1.2ml/min. Standard solution 60 µg/ml of Empagliflozin & 60 µg/ml of Linagliptin was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

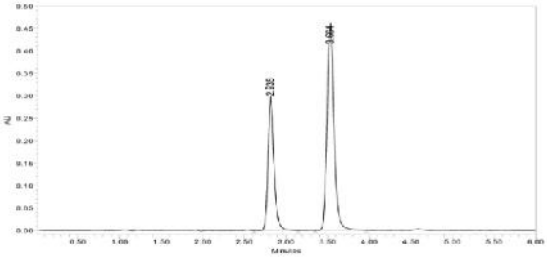

Fig 6: Chromatogram for Robustness Less flow

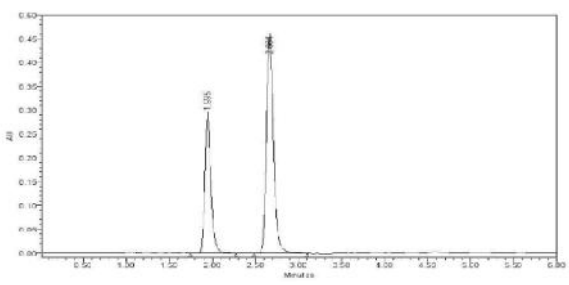

Fig 7: Chromatogram for Robustness More flow

Mobile phase:

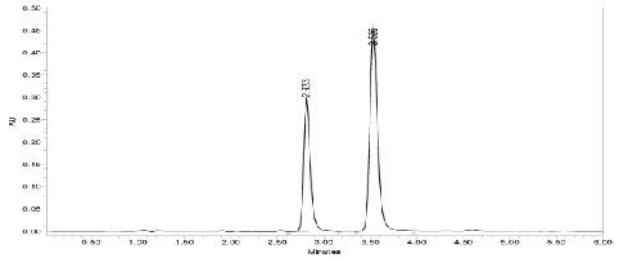

Fig 8: Chromatogram for Robustness less organic

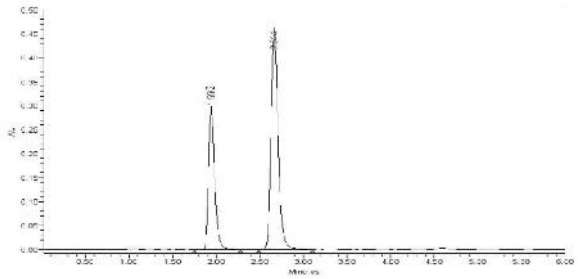

Fig 9: Chromatogram for Robustness more organic

Table No 1: Accuracy results of Empagliflozin

% concentration (at specification level)	Area	Amount added(m)	Amount found(m)	% Recovery	Mean Recovery
50%	1426646	5	4.9	101.8%	102.5%
100%	2551005	10	9.98	99.9%	
150%	2139845	15	15.0	100.0%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table No 2: Accuracy results of Linagliptin

% concentration (at specification level)	Area	Amount added(m)	Amount found(m)	% Recovery	Mean Recovery
50%	975578	5	5.0	101.3%	101.0%
100%	1718370	10	9.96	99.6%	
150%	1465857	15	14.9	99.3%	

Acceptance criteria: The % recovery for each level should be between 98.0 to 102.0 %

Table No 3: Repeatability results of Linagliptin and Empagliflozin

Name : Empagliflozin

	Name	RT	Area	Height (µV)	USP Plate Count	USP Tailing
1	Empag	2.506	1553631	316525	6346.5	1.3
2	Empag	2.516	1508002	296974	6197.1	1.2
3	Empag	2.519	1545624	307327	6184.0	1.3
4	Empag	2.531	1542374	302327	6176.0	1.2
5	Empag	2.544	1561368	302525	6382.1	1.3
Mean			1542200			
Std. Dev.			20490.0			
% RSD			1.33			

Name : Linagliptin

	Name	RT	Area	Height (µV)	USP Plate Count	USP Tailing
1	Linag	3.230	2790868	497608	7950.1	1.2
2	Linag	3.239	2661482	468477	8046.5	1.2
3	Linag	3.246	2706096	474632	8054.1	1.2
4	Linag	3.257	2703419	473234	8171.8	1.2
5	Linag	3.271	2695932	474830	8068.3	1.2
Mean			2711560			
Std. Dev.			47796.3			
% RSD			1.76			

Table No 4: Intermediate precision/Ruggedness Results for Empagliflozin

	Peak name	RT	Area
1	Empagliflozin	2.506	1763951
2	Empagliflozin	2.516	1794350
3	Empagliflozin	2.519	1792044
4	Empagliflozin	2.5531	1792044
5	Empagliflozin	2.544	1783951
Mean			1786782
Std.dev			10795.03
%RSD			0.60416

Table No 5: Intermediate precision/Ruggedness Results for Linigaleptin

	Peak name	RT	Area
1	Linagliptin	3.230	2575632
2	Linagliptin	3.230	2570930
3	Linagliptin	3.246	2613729
4	Linagliptin	3.227	2613729

5	Linagliptin	3.271	2575632
Mean			2586764
Std.dev			19163.75
%RSD			0.740839

Table No 6: Linearity Results Linagliptin

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Table No 7: Linearity Results Empagliflozin

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Table No 8: System suitability results for Linagliptin (Flow rate)

S.No	Flow Rate(ml/min)	Systemsuitabilityresults	
		USP Plate count	USP Tailing
1	0.8	3483	1.26
2	1.0	2936	1.3
3	1.2	2832	1.1

* Results for actual flow (1.0 ml/min) have been considered from Assay standard.

Table No 9: System suitability results for Empagliflozin (Flow rate)

S.No	Flow Rate (ml/min)	Systemsuitabilityresults	
		USP Plate count	USP Tailing
1	0.8	6645	1.3
2	1.0	5824.4	1.3
3	1.2	6059.0	1.2

* Results for actual flow (1.0ml/min) have been considered from Assay standard

Table No 10: System suitability results for Linagliptin (Mobile phase)

S.No	Changein Organic Compositionin the Mobile Phase	Systemsuitabilityresults	
		USP Plate count	USP Tailing
1	10%Less	3254.5	1.1
2	Actual	3516	1.2
3	10%More	3215	1.2

Table No 11: System suitability results for Empagliflozin (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	6691	1.3
2	Actual	6532.1	1.2

3	10%More	6557	1.3

* Results for actual Mobile phase composition (55:45Water: Methanol) have been considered from Accuracy standard

4. Conclusion

A new method was established for simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Empagliflozin and Linagliptin by using Agilent C185 µm (4.6*250mm) column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer ph 4.0: ACN (30:70%v/v), detection wave length was 254nm. The instrument used was WATERS HPLC Auto Sampler, Separation module2695, PDA Detector996, Empower-softwareversion-2. The retention times were found to be 3.503mins and 2.577 mins. The % purity of Empagliflozin and Linagliptin was found to be 100.3% and 101.1% respectively. The system suitability parameters for Empagliflozin and Linagliptin such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and1.2, 2936. 0 the resolution was found to be 9.4. The analytical method was validated according to ICH guidelines (ICH, Q2(R1)). The linearity study for Empagliflozin and Linagliptin was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient (r2) was foundtobe 0.999 and 0.999, %mean recovery was found to be 102.5% and 101.0%, %RSD for repeatability was 0.6 and0.5, %RSD for intermediate precision was 0.7 and 0.6 respectively. The precision study was precise, robust and repeatable. LODvaluewas3.1and3.02, and LOQ value was 10.1 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Empagliflozin and Linagliptin in API and Pharmaceutical dosage form

5. References

[1] Lakshman Raju Badugu et al, A Validated RP-HPLC Method for the Determination of Linagliptin. Am. J. Pharm Tech Res. 2012; 2(4)).

[2] Dnyaneshwar Thakare et al, Development and Validation of spectrophotometric method for the determination of DPP-4 Inhibitor, Metformin and Linagliptin, in its pharmaceutical preparations World journal of pharmacy and pharmaceutical sciences volume 2,issue3,1332-1341.

[3] Kavitha. K. Y et al, Development and Validation of spectrophotometric method for the determination of DPP-4 InhibitorMetformin and Linagliptin, in its pharmaceutical preparations. Journal of Chemical and Pharmaceutical Research, 2013, 5(1):230-235).

[4] Geetha, G et al, Development and Validation of Stability Indicating UV Spectrophotometric Method for the Estimation of Metformin and Linagliptin in Bulk and Tablet Dosage Form. Journal of Chemical & Pharmaceutical Research;2013, Vol. 5 Issue 1, p230).

[5] Rutvik H Pandya et al,Spectroflourometric and Spectrophotometric Methods for the Determination of Linagliptin in Binary Mixture with Metformin and Ternary Mixture with Metformin and Linagliptin Alkaline Degradation Product. Pharmacophore 2014, Vol. 5 (2), 202-218).

[6] Janardhan Swamy et al, Validated RP-HPLC for simultaneous estimation of Linagliptin and Metformin hydrochloride in tablet dosage form. Int. J Pharm 2013; 3(3): 594-600).

[7] Chirag and Amrita Parle et al, Development and validation of UV spectrophotometric method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms. Der Pharma Chemica, 2014, 6(1):303-311.

[8] G. Satya Sri, S. Ashutosh Kumar, J. Saravanan, Manidipa Debnath, V. Greeshma, N. Sai Krishna. et al,A New Stability Indicating Rp-Hplc Method Development For Simultaneous Estimation Of Metformin And Alogliptin In Bulk As Well As In Pharmaceutical Formulation By Using Pda Detector. Indo American journal of pharmaceutical research.

[9] A. Praveen Kumar, G. Aruna, K. Rajasekar, P. Jayachandra Reddy et al, Analytical Method Development And Validation of Alogliptin And Metformin Hydrochloride Tablet Dosage Form By RP-HPLC Method. International Bulletin of Drug Research. 3(5): 58-68, 2013.

[10] Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, (2002), PP 1-7.