International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

A Validated RP- HPLC Method for the Simultaneous Estimation of Empagliflozin and Linagliptin in its Bulk and Pharmaceutical Dosage Forms

M. Jayalaxmi[1], Dr. T. Rajesh[1], Dr. Gampa Vijaya Kumar*[2]

[1]KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.
[2]Professor and Head, Department of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, 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 C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer (KH_2PO_4 and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 254 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Empagliflozin and Linagliptin was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 1.2 and 2.0, % RSD for intermediate precision was 1.1 and 1.1 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Keywords: Empagliflozin, Linagliptin, RP-HPLC, Agilent C18 column

A R T I C L E I N F O

CONTENTS

Article History: Received 29 September 2016, **Accepted** 31 October 2016, **Available Online** 27 December 2016

***Corresponding Author**
Dr. Gampa Vijaya Kumar
Professor and Head, Dept. of Pharmacy, KGR Institute of Technology and Management, Rangareddy, Telangana,
Manuscript ID: IJCPS3203

PAPER-QR CODE

Citation: Gampa Vijaya Kumar, *et al.* A Validated RP- HPLC Method for the Simultaneous Estimation of Empagliflozin and Linagliptin in its Bulk and Pharmaceutical Dosage Forms. *Int. J. Chem, Pharm, Sci.*, 2016, 4(12): 634-640.

Copyright© 2016 Gampa Vijaya Kumar, *et al.* This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [3]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [4]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [4].

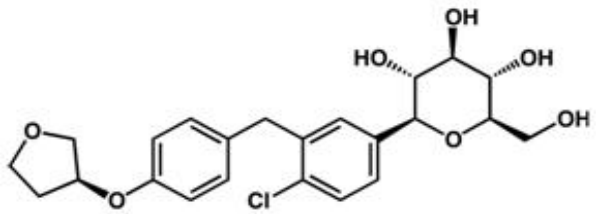

Figure 1: Empagliflozin

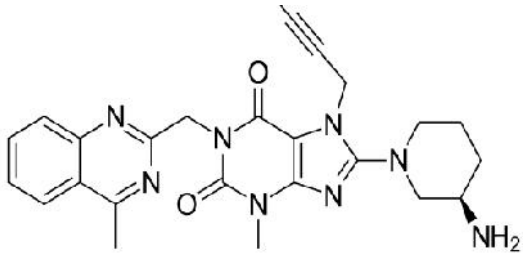

Figure 2: Linagliptin

2. Materials and Methods

Table 1: Chemicals required

Chemicals	Manufacturer Name
Water	Merck
Methanol	Merck
Acetonitrile	Merck
Ortho phosphoric acid	Merck
KH_2PO_4	Merck
K_2HPO_4	Merck
0. 22µ Nylon filter	Advanced lab
0.45µ filter paper	Millipore
Tancodep-2	Torrent pharmaceuticals
Empagliflozin and Linagliptin	In – House

Table 2: Solubility Profile

Solubility	Empagliflozin	Linagliptin
Water	Sliglty soluble	Insoluble
Methanol	soluble	Soluble
Acetonitrile	soluble	Soluble
Chloroform	In soluble	Soluble

Preparation of phosphate buffer

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation:

Mobile phase was used as the diluent.

Preparation of the individual Empagliflozin standard preparation: 10 mg of Empagliflozin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.2 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 20µg/ml.

Preparation of the individual Linagliptin standard preparation: 10 mg of Linagliptin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.4 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 40 µg/ml.

Preparation of the Empagliflozin and Linagliptin standard and sample solution

Sample solution preparation:

An equivalent tablet power such that 10 mg of Empagliflozin and 20 mg Linagliptin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Empagliflozin and 20 mg Linagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure:

10µL of the blank, standard and sample were injected into t he chromatographic system and areas for the Empagliflozin and Linagliptin the peaks were used for calculating the % assay by using the formulae.

System suitability

- Tailing factor for the peaks due to Empagliflozin and Linagliptin in standard solution should not be more than 1.5.
- Theoretical plates for the Empagliflozin and Linagliptin peaks in standard solution should not be less than 2000.

Analytical Method Validation

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.

Linearity

Preparation of stock solution

10 mg of Empagliflozin and 20 mg of Linagliptin working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (10ppm of Empagliflozin and 20 ppm of Linagliptin)

0.1 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – II (20ppm of Empagliflozin and 40ppm of Linagliptin)

0.2 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – III (30ppm of Empagliflozin and 60ppm of Linagliptin)

0.3 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – IV (40 ppm of Empagliflozin and 80ppm of Linagliptin)

0.4 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – V (50 ppm of Empagliflozin and 100 ppm of Linagliptin)

2.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Procedure

Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

Acceptance criteria

Correlation coefficient should be not less than 0.999.

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 10µg/ml-50µg/ml and 20µg/ml-100µg/ml of Empagliflozin and Linagliptin respectively.

Accuracy

Preparation of standard stock solution

10mg of Empagliflozin and 20mg of Linagliptin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)

5mg of Empagliflozin and 10 mg of Linagliptin working standard were accurately weighed and transferred into a 10 ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution).Further pipette out 10 ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 100% solution (with respect to target assay concentration)

10 mg of Empagliflozin and 20 mg of Linagliptin working standards were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 150% solution (with respect to target assay concentration)

15 mg of Empagliflozin and 25 mg of Linagliptin working standards into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for Empagliflozin and Linagliptin and calculate the individual % recovery and mean % recovery values.

Acceptance criteria

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

Precision

Repeatability

Preparation of stock solution

10 mg of Empagliflozin and 20 mg of Linagliptin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the

area of five replicate injections was found to be within the specified limits..

Acceptance criteria

The % RSD for the area of five standard injections results should not be more than 2.

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.

Preparation of stock solution

10 mg of Empagliflozin and 20mg of Linagliptin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The % RSD for the area of five sample injections results should not be more than 2%.

Limit of detection (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.

Formula: $\mathbf{LOD = 3.3\ X\ \frac{\sigma}{S}}$

Where

σ - Standard deviation (SD)

S - Slope

Limit of quantification

OQ'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.

Formula: $\mathbf{LOQ = 10\ X\ \frac{\sigma}{S}}$

Where

σ - Standard deviation

S - Slope

RobustnessAs 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 50 ppm of Empagliflozin and 100 ppm of Linagliptinwas prepared and analysed using the varied flow rates along with method flow rate.
- The organic composition in themobile phase was varied from 65% to75 %standard solution 50 µg/ml of Empagliflozin and 100 µg/ml ofLinagliptin were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

System suitability

10 mg of Empagliflozin and 20 mg of Linagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of Empagliflozin and Linagliptin from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

3. Results and Discussion

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Empagliflozin and Linagliptin by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Empagliflozin and Linagliptin in pharmaceutical dosage form.

Method Development

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.

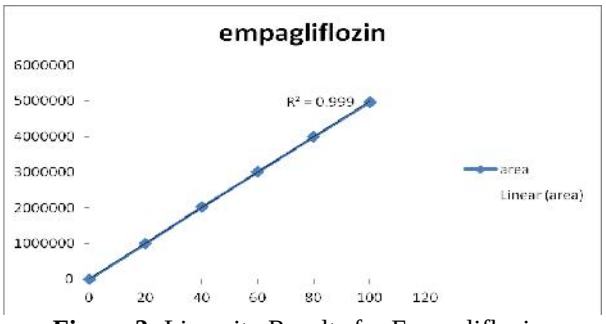

Figure 3: Linearity Results for Empagliflozin

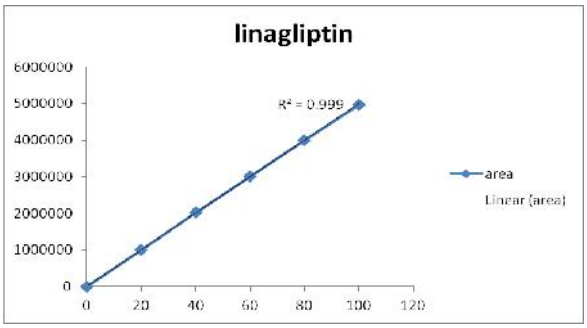

Figure 4: Linearity Results for Linagliptin

Accuracy:

The accuracy study was performed for 50%, 100% and 150 % for Empagliflozin, Linagliptin. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Precision

- Repeatability
- Intermediate Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision/Ruggedness

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Repeatability:

The precision study was performed for five injections of Empagliflozin and Linagliptin. Each standard injection was injected into chromatographic system. The area of each standard injection was used for the calculation of % RSD.

Table 3: Showing %RSD results for Empagliflozin

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing	Injection
1	Empagliflozin	4.673	1197254	141033	2852.74	4.51	1.37	5
2	Empagliflozin	4.920	1171214	84462	2672.17	4.42	1.38	1
3	Empagliflozin	4.863	1207502	84306	2557.54	4.39	1.40	3
4	Empagliflozin	5.637	1212400	84282	2627.38	4.24	1.38	2
5	Empagliflozin	5.832	1203215	82319	2444.22	4.36	1.38	6
6	Empagliflozin	5.450	1184663	85374	2506.06	4.26	1.32	4
Mean			1196041.2					
Std. Dev.			15477.3					
% RSD			1.2					

Table 4: Showing %RSD results for Linagliptin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Linagliptin	3.174	1592260	146364	2973.61	1.49	1
2	Linagliptin	3.360	1600516	147364	2973.61	1.49	2
3	Linagliptin	3.323	1620416	144812	2873.05	1.52	3
4	Linagliptin	3.842	1605813	145422	2912.90	1.52	4
5	Linagliptin	4.002	1670034	136518	2654.63	1.50	5
6	Linagliptin	3.740	1571138	141267	2898.11	1.43	6
Mean			1610030				
Std. Dev.			33600.6				
% RSD			2.0				

Intermediate precision/Ruggedness

Table 5: Intermediate precision of Empagliflozin

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing	Injection
1	Empagliflozin	4.754	1637254	141033	2852.74	4.51	1.37	5
2	Empagliflozin	4.847	1621214	84462	2672.17	4.42	1.38	1
3	Empagliflozin	4.861	1657502	84306	2557.54	4.39	1.40	3
4	Empagliflozin	5.647	1602400	84282	2627.38	4.24	1.38	2
5	Empagliflozin	5.964	1643215	82319	2444.22	4.36	1.38	6
6	Empagliflozin	5.789	1634663	85374	2506.06		1.32	4
Mean			1632708					
Std. Dev.			18975.3					
% RSD			1.1					

The intermediate precision study was performed for five injections of Empagliflozin and Linagliptin. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

Table 6: Intermediate precision of Linagliptin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Linagliptin	3.054	1747384	146364	2973.61	1.49	1
2	Linagliptin	3.261	1720516	147364	2973.61	1.49	2
3	Linagliptin	3.532	1690416	144812	2873.05	1.52	3
4	Linagliptin	3.954	1705813	145422	2912.90	1.52	4
5	Linagliptin	4.125	1710034	136518	2654.63	1.50	5
6	Linagliptin	3.824	1721138	141267	2898.11	1.43	6
Mean			1715880				
Std. Dev.			19101.4				
% RSD			1.1				

Detection limit

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.

Formula: $\text{LOD} = 3.3 \text{ X } \frac{\sigma}{S}$

Where

σ - Standard deviation (SD)

S - Slope

The LOD was performed for Empagliflozin and Linagliptin was found to be 2.17and 0.0372 respectively.

Quantitation limit

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.

Formula: $\mathbf{LOQ = 10 \text{ X } \frac{\sigma}{S}}$

Where

σ - Standard deviation

S - Slope

The LOQ was performed for Empagliflozin and Linagliptin was found to be 6.60 and 0.112 respectively.

Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.0 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Empagliflozin and Linagliptin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

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 C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer ($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 254 nm. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Empagliflozin and Linagliptin was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 1.2and 2.0, % RSD for intermediate precision was 1.1 and 1.1 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Table 7: Linearity Results Linagliptin

S.No	Linearity level	Concentration	Area
1	I	20 ppm	2000516
2	II	40ppm	2420416
3	III	60ppm	2905813
4	IV	80ppm	3270034
5	V	100ppm	3671138
Correlation Coefficient			0.999

Table 8: Linearity Results for Empagliflozin

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	1097254
2	II	20 ppm	1471214
3	III	30 ppm	1807502
4	IV	40 ppm	2212400
5	V	50 ppm	2603215
Correlation Coefficient			0.999

Table 9: Showing accuracy results for Empagliflozin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1193706	5	4.96	99.91%	99.56%
100%	1601741	10	9.98	99.18%	
150%	2243270	15	15.02	99.60%	

Table 10: Showing accuracy results for Linagliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	0.5	0.99	99.53%	99.47%
100%	967998	1.0	1.05	99.38%	
150%	145437	1.5	1.495	99.52%	

Table 11: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Empagliflozin	371827.90	563365963	2.17
Linagliptin	5401.60	479884400	0.0372

Table 12: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Empagliflozin	371827.90	563365963	6.60
Linagliptin	5401.60	479884400	0.112

Table 13: Showing system suitability results for Empagliflozin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	**1**	**2294**	**1.27**
3	1.2	2146	1.26

Table 14: Showing system suitability results for Empagliflozin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	**1**	**4891**	**1.03**
3	1.2	4781	1.04

5. References

[1] Introduction to analytical method validation http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.

[2] Data elements required for assay validation, http://www.labcompliance.com/tutorial/methods/default.aspx.

[3] Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.

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

[5] 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.*

[6] 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)*

[7] 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)*

[8] 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)*

[9] 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)*

[10] 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.*

[11] 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*

[12] Praveen Kumar, G. Aruna, K. Rajasekar, P. Jayachandra Reddy. 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*