Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN :2277 -4572)

Research Article

METHOD DEVELOPMENT, VALIDATION BY SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN AND LINAGLIPTIN BY RP-HPLC METHOD

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DOI: 10.7897/2277-4572.091160

Received on: 12/06/19 Revised on: 30/09/19 Accepted on: 14/11/19

ABSTRACT

A very simple, accurate, precise, robust, rugged and stability indicating method with gradient elution was developed 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 C_{18} (Equisil BDS) column (4.6 × 250 mm) 5µ, flow rate was 0.7 ml/min, mobile phase ratio was (40:60 v/v) methanol: water, detection wavelength was 224 nm and 294 nm. The instrument used was SHIMADZU-HPLC system, Pump -HPD 20A, Detector - UV detector, Software - UV Probe. 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 20 µg-40 µg, 60 µg, 80 µg, 100 µg and 10 µg, 20 µg, 30 µg, 40 µg, 50 µg correlation coefficient (r2) was found to be 0.988 and 0.991, % recovery was found to be 99.1% and 99.6.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Keywords: Empagliflozin, Linagliptin, Method development, Validation, RP-HPLC, C_{18} (Equisil BDS) column.

INTRODUCTION

Empagliflozin, chemically designated as (2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy] phenyl} methyl) phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol (show in Figure 1). Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 Diabetes. Various analytical methods have been reported for the estimation of Empagliflozin as alone as well as in combination with other drugs. They include Spectrophotometric methods and HPLC[1,2].

Linagliptin chemically, 8-[(3R)-3-Aminopiperidin-yl]-7-(but-2yn-1-yl)-3-methyl-1- [(4- methyl quinazolin-2-yl) methyl]-3, 7-dihydro-1H-purine-2, 6- dione (show in Figure 2).

Linagliptin is an oral drug that reduces blood sugar levels in patients with type 2 diabetes. It is a competitive and reversible dipeptidyl peptidase-4 (DPP-4) enzyme inhibitor that slows the breakdown of insulinotropic hormone glucagon like peptide (GLP-1) for better glycemic control in diabetes patients. Literature shows the HPLC method to determine Linagliptin[3,4].

A novel anti-diabetic fixed-dose combination of Empagliflozin and Linagliptin is a new option for adults with Type-2 Diabetes. The combination of Empagliflozin/Linagliptin may provide the treatment option for patients intolerant to Metformin and/or have marked hyperglycemia, without the side effects of weight gain or increased risk of hypoglycemia. Literature review showed that few methods were described for the determination of Empagliflozin and Linagliptin in pharmaceutical preparation including spectrophotometry and chromatographic methods. The objective of the present study is to determine an RP-HPLC method for simultaneous estimation of Empagliflozin and Linagliptin and its comparison with the earlier reported method[5].

MATERIAL AND METHODS

Instrumentation

The liquid chromatography consisted of a WATERS® HPLC SYSTEM Model SHIMADZU-HPLC system High-Performance Liquid Chromatography. For the RP-HPLC system, a C18 (Equisil BDS) (250 mm x 4.6 mm, 05 µm) column was used. The system was equipped with a UV detector, with an automated sample injector a 10 µl injection loop, HPD 20A pump. The output signal was monitored and integrated using UV Probe and (PCI INSTRUMENT) 6.5 L (H) Ultra sonicator is use.

Reagents and Chemicals Used

All reagents and chemicals used were of AR grade and HPLC grade like methanol, Water and tablet of Glyxambi Manufactured by Boehringer Ingelheim pharma, Germany this tablet contains Empagliflozin (10 mg), Linagliptin (05 mg) and Excipients (q.s.)

Solubility of Drugs (show in Table 1)

Preparation of Standard Stock Solution (Stock- I) [EMP and LIN]

An accurately weighed quantity of Empagliflozin and Linagliptin (EMP and LIN) tablet were transferred into 50.0 ML volumetric flask. About 5.0 ml of methanol (HPLC Grade) was added to the volumetric flask and sonicated to dissolve the drug. The solution was cooled to the room temperature and made up to the mark with methanol (HPLC Grade) which gives the final concentrations of 200 µg/ml (stock solution) Empagliflozin and 100 µgm/ml Linagliptin.

Preparation of Working Standard Stock Solution (Stock -II) [EMP and LIN]

From the freshly prepared standard stock solution take 1.0 ml stock solution and make up the volume up to the mark with mobile phase to get 20 µg/ml Empagliflozin and 10 µg/ml Linagliptin Simultaneously add 2 ml to get-40 µg/ml, 3 ml for 60 µgm/ml, 4 ml for 80 µgm/ml, last dilution 5 ml from stock solution and make vol. with mobile phase to get 100 µg/ml Empagliflozin and 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml Linagliptin respectively.

Selection of mobile phase

Each mobile phase was vacuum degassed and filtered through 0.45 µ membrane filter. The mobile phase was allowed to equilibrate until steady baseline was obtained. The standard solution containing Empagliflozin and Linagliptin was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing Methanol and Water was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Empagliflozin and Linagliptin.

Method validation

Specificity of the method can be termed as the absence of any interference at retention times of samples. Specificity was performed by injecting blank, placebo, and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of the analyte[6,7].

Table 1: Solubility of Drugs

Solubility	Empagliflozin	Linagliptin
Water	Slightly soluble	Insoluble
Methanol	Soluble	Soluble
Acetonitrile	Soluble	Soluble
Chloroform	Insoluble	Soluble

Table 2: System Suitability data for Empagliflozin and Linagliptin

S. No.	Peak Name	Retention Time	Area
1	Empagliflozin	3.516	622842
2	Linagliptin	4.260	124384.5

Table 3: Linearity data for Empagliflozin and Linagliptin

Concentration range (µg/ml)		Empagliflozin		Linagliptin	
		RT	AREA	RT	AREA
20	10	3.526	146578.5	4.316	34596.5
40	20	3.593	272515.5	4.720	67081.5
60	30	3.621	355882.5	4.768	82694.5
80	40	3.515	465803.5	4.362	111875
100	50	3.520	543179.5	4.316	144360

Table 4: Result of Interday and Intraday Precision studies on RP-HPLC method for EMP and LIN

Concentration		Inter day		Intra day	
		Empagliflozin	Linagliptin	Empagliflozin	Linagliptin
20	10	197497	68902.5	189379	34888
40	20	278408	70038.5	270228	88247
60	30	383485	91858	389956	90469.5
Mean		286463.333	76933	283188	71201.5
Standard Deviation		93255.2965	12937.9	100915	31468
% RSD		1.442878	0.622929	1.393838	1.67484

Table 5: Accuracy data for Empagliflozin and Linagliptin

% concentration (at specific level)		Area	Amount added (mg)	Amount found (mg)	% recovery
EMP	80%	393457	20	7.92	99
	100%	503451	40	9.98	99.8
	120%	612348	60	11.82	98.5
LIN	80%	102954	10	3.987	99.675
	100%	131824	20	4.99	99.8
	120%	152967	30	5.97	99.5

Table 6: Robustness data for Empagliflozin and Linagliptin

Parameters	EMP		LIN	
	Retention time	Peak Area	Retention time	Peak Area
Flow rate-0.7 ml/min	3.652	265231	4.763	076228
Flow rate-1.0 ml/min	3.531	209853	4.642	67277
Wavelength 224 nm	3.782	722980	4.720	215206
Wavelength 294 nm	3.672	691529	4.725	199713

Table 7: LOD and LOQ data for Empagliflozin and Linagliptin

Parameter	EMP	LIN
LOD	1.052	3.190
LOQ	0.406	1.395

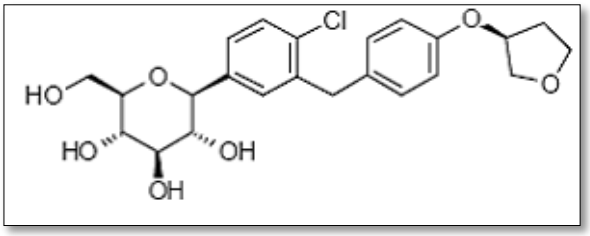

Figure 1: Structure of Empagliflozin

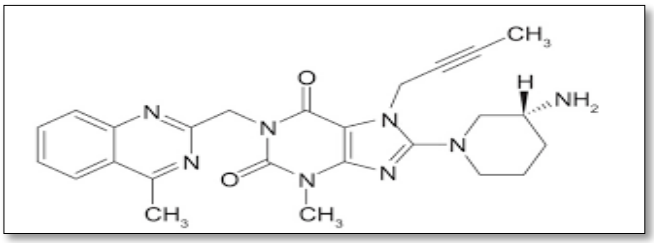

Figure 2: Structure of Linagliptin

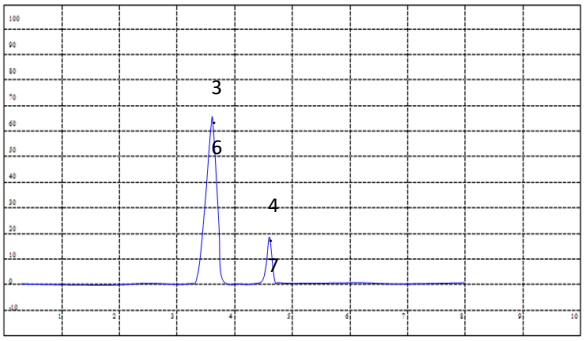

Figure 3: Chromatogram of Empagliflozin and Linagliptin

RESULT AND DISCUSSION

Linearity

A series of standard solutions in the range of 50-150 % of target concentrations were prepared. The plot of the average peak area versus the concentration is plotted and from this, the regression coefficient and the regression equation were generated[9]. The calibration data of Empagliflozin and Linagliptin show in Table 3.

Precision

Six replicate injections in the same concentration were analyzed on the same day for repeatability and the % RSD for Empagliflozin and Linagliptin found to be 0.23 and 0.24 respectively[10]. The % RSD for Empagliflozin and Linagliptin found to be within the acceptable limit of ≤ 2 and hence the method is reproducible, and the results are shown in Table 4.

Accuracy

To pre-analyzed sample solution, a definite concentration of standard drug (50%, 100% and 150% level) was added and recovery was studied[11]. The % Mean recovery for Empagliflozin and Linagliptin are 100.65 and 100.47 respectively and these results are within the acceptable limit of 98-102%. Results obtained are shown in Table 5.

Robustness

Study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by making two small changes[12,13].

Robustness of the method was studied by changing flow rate ± 0.02 mL/minutes and temperature ± 5ºC. After each change sample solution was injected and system suitability parameters were observed. The results were shown in Table 6.

LOD and LOQ

It is for Empagliflozin and Linagliptin were calculated as suggested by ICH guidelines using equations LOD = 3.3 σ/s and LOQ = 10 σ/s, respectively[14,15]. Where σ is the SD of the response and S is the slope of the calibration curve. Results were showed in Table 7.

CONCLUSION

The developed HPLC method was found to be linear over concentration range. Therefore, the developed HPLC method can be applied for routine quantitative and qualitative analysis of Empagliflozin and Linagliptin in pharmaceutical formulations like Tablet. The developed HPLC method was validated as per the ICH guidelines.

The HPLC method was developed using isocratic system with run time 10 min. The assay method in Indian Pharmacopoeia (2014) is based on gradient system with run time 10 min.

ACKNOWLEDGEMENT

The author would like to thank Ruhita Medicals, Amravati for providing the samples of tablet. We are highly grateful to Dr. K. K. Tapar sir Principal of Vidya Bharati College of Pharmacy Amaravati for providing the necessary facilities to carry out this research work.

REFERENCES

1. Kim ES, Deek ED. Empagliflozin/Linagliptin: A Review in Type 2 Diabetes. Drugs 2015; 75(13): 1547-1557.
2. Padmaja N and Veerabhadram G. Development and validation of a novel stability – indicating RP – HPLC method for the determination of Empagliflozin in bulk and pharmaceutical dosage and forms, International Journal of Pharmaceutical Sciences and Research 2016; 7(11): 4523-4530.
3. Badugu LR. A validated RP – HPLC method for the determination of Linagliptin. American Journal of Pharmatech Research 2012; 2(4): 462-470.
4. Patil SD, Amurutkar SV, Chatpalliwar VA, Upasani CD. Development and validation of RP- HPLC method for Empagliflozin and Metformin HCL. Journal of Innovations in Pharmaceutical and Biological Sciences 2017; 4(4): 185-189.
5. Sujatha K and Seshagirirao JVLN. A new RP – HPLC method for the estimation of Linagliptin in tablet dosage forms. Indo American Journal of Pharmaceutical Research 2013; 3(10): 8376-8381.
6. Godasu SK and Sreenivas SA. A new validated RP – HPLC Method for the determination of Metformin HCl and Empagliflozin in bulk and pharmaceutical dosage and forms. International Journal of Pharmaceutical Sciences and Research 2017; 8(5): 2223-2232.
7. Jyothirmai N, Anil Kumar M and Nagaraju B. Novel UV and Visible Spectrophotometric methods for the analysis of Empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulations. Journal de afrikana 2016; 3(1): 177-187.
8. Andrew L, DeFronzo RA, Patel S, *et.al*. Initial Combination of Empagliflozin and Linagliptin in Subjects with Type 2 Diabetes, Diabetes Care 2015; 38: 394–402.
9. Padmaja N, Veerabhadram G. Development and validation of analytical method for Simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy. Der Pharmacia Lettre 2015; 7(12): 306-312.
10. Srivani J. Development and validation of stability indicating HPTLC method for simultaneous determination of Linagliptin and Metformin, International Journal of Pharmacy and Pharmaceutical Sciences 2016; 8: 112-115.
11. Ayoub BM. UPLC simultaneous determination of Empagliflozin, Linagliptin and Metformin, RSC Advances 2015; 116(5): 95703-95709.
12. Madhusudhan P, Reddy R, Deanna N. RPHPLC Method development and Validation for Simultaneous determination of Linagliptin and Empagliflozin in Tablet Dosage Form. International Advanced Research Journal in Science, Engineering and Technology 2015; 2(2): 95-99.
13. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development, 2nd ed., New York, Wiley Inter Science; 1997. p. 1-56.
14. Willard HH, Merritt L, Dean JA, and Settle FA, Textbook of Instrumental Methods of Analysis, 7th ed., New Delhi, CBS Publishers and distributors; 1986. p. 625-650.
15. International Conference on Harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use, validation of analytical procedure: definitions and terminology, Geneva; 1996.

How to cite this article:

Abhijeet N. Raut *et al*. Method development, validation by simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC Method. J Pharm Sci Innov. 2020;9(1):1-4.
http://dx.doi.org/10.7897/2277-4572.091160

Source of support: Nil, Conflict of interest: None Declared