How to Cite:
Laxman, B., Yojana, K., Chaitali, K., Shivam, K., Sagar, M., & Godge, R. K. (2022). A Rapid and Sensitive stability indicating Rp-HPLC method development for the quantitative analysis of empagliflozin & linagliptin in bulk & synthetic mixture. *International Journal of Health Sciences*, *6*(S3), 5526–5538. https://doi.org/10.53730/ijhs.v6nS3.7166

A Rapid and Sensitive stability indicating Rp-HPLC method development for the quantitative analysis of empagliflozin & linagliptin in bulk & synthetic mixture

Bulbule Laxman
Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Tq, Rahata, Dist. Ahmednagar, Maharashtra. (413736)

Kunjir Yojana
Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Tq, Rahata, Dist. Ahmednagar, Maharashtra. (413736)

Kolhe Chaitali
Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Tq, Rahata, Dist. Ahmednagar, Maharashtra. (413736)

Kote Shivam
Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Tq, Rahata, Dist. Ahmednagar, Maharashtra. (413736)

Magar Sagar
Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Tq, Rahata, Dist. Ahmednagar, Maharashtra. (413736)

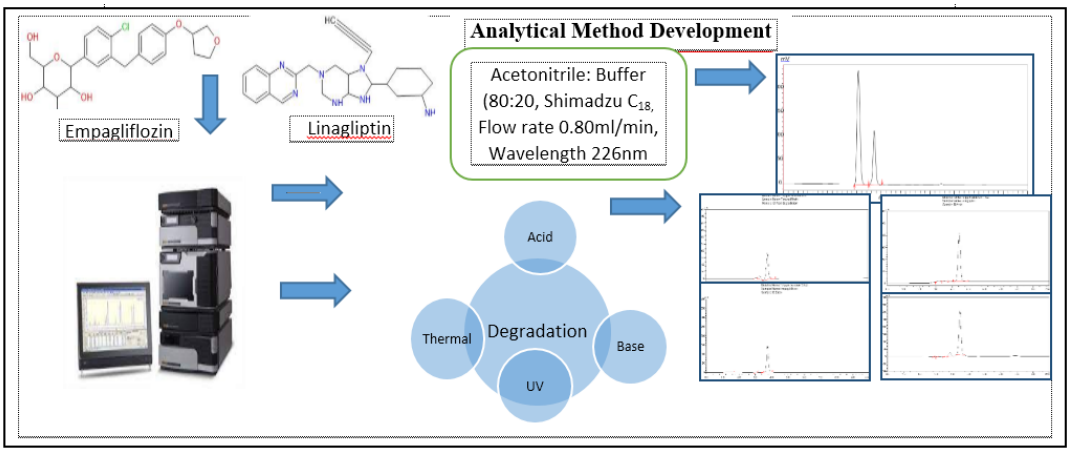
Dr. Rahul Keshav Godge
Assistant Professor, Pharmaceutical Chemistry Department, Pravara Rural College of Pharmacy Pravaranagar, Tal- Rahata, Dist- Ahmednagar, Maharashtra, India
*Corresponding author email: rahul.godge@pravara.in

***Abstract*---** An isocratic HPLC method was developed using, Shimadzu C18 column (250 mm × 4.6 mm, 5 µm) with an isocratic binary mobile phase consisting of Acetonitrile: Buffer in a ratio (80:20 v/v) pH 3.0 adjusted with Orthophosphoric acid and flow rate monitored at 0.80 ml/min. The UV detector was used for simultaneous analysis of two drugs at a common wavelength of 226 nm and each injection volume was 20 µl. The retention time for Empagliflozin and Linagliptin was found to be 3.714 min and 3.064 min, respectively. Empagliflozin and

Linagliptin produce degradation products in acidic, alkaline, thermal, and UV stress. The result of the assay of Empagliflozin and Linagliptin shows that the degradation product does not interfere with the analytical procedure quantitively when these drugs are analyzed.

Keywords---rapid, sensitive stability, empagliflozin, linagliptin, synthetic mixture.

Graphical Abstract



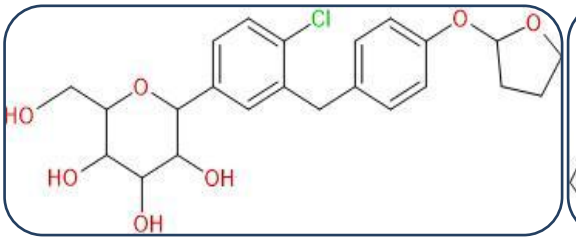
Introduction

Empagliflozin is hypoglycemic agent used to treat high blood sugar level caused by Type2 diabetes mellitus. Empagliflozin (EMP) acts as a sodium-glucose co-transporter-2 (SGLT-2) inhibitor to improve glycemic control in adult patients with type 2 diabetes. SGLT-2 co-transporters reabsorb glucose from the glomerular filtrate in the kidney and the glucuretic action resulting from the inhibition of SGLT-2 reduces renal absorption and lowers the renal threshold for glucose, therefore increasing glucose excretion which reduces hyperglycaemia and also helps in blood pressure reduction[1].

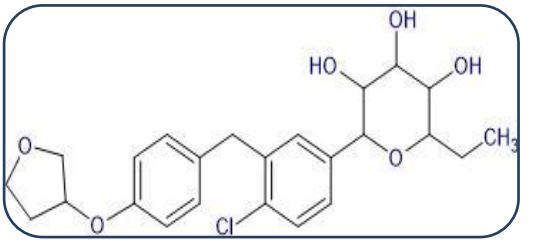
By orally administration, Empagliflozin inhibits SGLT2 within the kidneys, thereby suppressing the reabsorption process of glucose within the PT, due to the inhibition of SGLT2 urinary excretion of glucose is increased, and that leads to a decrease in the plasma glucose level. Inhibition of SGLT2 within the kidneys conjointly suppresses the renal reabsorption of 1, 5-anhydroglucitol (1,5AG). Empagliflozin is available in market under the brand name Jardiance, is a formulation used along with diet and regular exercise to treat Type2 diabetes mellitus[1]. FDA Approves Empagliflozin to treat Type 2 diabetes by 01-Aug-2014. It was found to be Empagliflozin has reduced the risk of cardiovascular death in adult with type2 diabetes so by 02-Dec-2016 FDA approved the use Empagliflozin in cardiac disease. EGN is available in combination of with Metformin in type2 diabetes treatment. Chemically EMP is 1-chloro-4-(glucopyranos-1-yl)-2-(4-

(tetrahydrofuran-3-yloxy) benzyl) benzene and having empirical formula is $C_{23}H_{27}ClO_7$ with molecular weight 450.91 g/mol[2].

Linagliptin (LNG) has competitive, reversible DPP-4 inhibitory action responsible for DPP-4 breakdown reduction of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). From beta cells of the pancreas, GLP-1 and GIP stimulate the release of insulin during inhibiting the release of glucagon from pancreatic beta cells. Together, these effects reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose. By prolonging the impact of GLP-1, Linagliptin increases insulin level and lowers glucose, thereby rising glycemic management in patients with type2 diabetes mellitus. [3] FDA approved, by 02-May-2011, the Linagliptin to be used along with diet and exercise to lower blood sugar in adult with Type 2 diabetes. By 17-Aug-2012, FDA approves updated prescribing information for Tradjenta tab for add-on therapy to insulin adults with type 2 diabetes. Linagliptin is marketed in 05 mg tablet under the brand name of Tradjenta. It is available in fixed dose combination with metformin under the name Jentadueto and with Empagliflozin marketed under the name Glyxambi[4].



Structure: Linagliptin



Structure: Empagliflozin

Materials and Methods

Chemicals

Ansalytical HPLC grade solvents were used in all experiments, including Acetonitrile, Water, Ortho phosphoric acid, Monobasic Potassium phosphate, Methanol, 1N HCl, 1N NaOH. Linagliptin and Empagliflozin were supplied by Shodh Advantech. Lab Aurangabad, Maharashtra.

Equipments

Analytical weighing balance make ESSAE, VIBRA+, and Capa: 220.0 g to 0.0001 g, Sonicator, pH METER: Make-LABINDIA, PHAN, MLH Stirrer with Hotplate Make- Remi, High Performance Liquid Chromatography Make: SHIMADZU, Water bath make Osworld, UV Chamber

Table 1
Chromatographic methods reported for the determination of EMP and LNG

Sr.	Mobile Phase	Column	Flow Rate	Wavelength	Reference
1.	Buffer: acetonitrile	Discovery C_{18}	1ml/min	210nm	[8]

	68:32 v/v	Column			
2.	Buffer: Acetonitrile 45:50 v/v	ODS C_{18} Column	1ml/min,	245nm	[9]
3.	phosphate buffer: methanol 70:30 v/v	C_{18} column (250mm × 4.6mm, 5µm)	1ml/min	240 nm	[10]
4.	Orthophosphoric acid: acetonitrile 60:40 v/v	BDS C_{18} Column	1 ml/min	230 nm	[11]
5.	Phosphate buffer: Acetonitrile 60:40 v/v	Inertsil ODS – 3V	1ml/min	226nm	[12]
6.	Acetonitrile: phosphate buffer 35:65 v/v	Water's X-Bridge C_{18} column	1 ml/min	230nm	[13]
7.	Phosphate buffer: Acetonitrile 80:20 v/v	Shimadzu C_{18}	0.70 ml/ Min	252 nm	Proposed Work

Chromatographic system
Preparation of Mobile Phase

By taking premix Acetonitrile: Buffer (80:20 v/v) pH 3.0 adjusted with ortho phosphoric acid taken as a mobile phase. For degassing the mobile phase prior to use, the reservoir was sonicated for 20 min and mixtures of solvent were used as mobile phase

Buffer preparation

Dissolved 6.8 g of monobasic Potassium phosphate in 500 ml of HPLC grade water. Adjusted pH of this solution to 3.0 + 0.05 with ortho phosphoric acid. Sonicated for 5 minutes and filtered through 0.45 µ filter paper.

Solution preparation

Precisely weighed 100 mg of Linagliptin and Empagliflozin and transferred into two different 100 ml volumetric flasks containing mobile phase and the volume was made up to the mark with same which gives the stock solution having concentrations 1000 µg/ml. Pipetted out 1.0 ml from the above stock solutions, transferred into two different volumetric flasks of 100 ml and made up the volume with mobile phase to acquire the concentration of working standard stock solution 10 µg/ml of both drug samples.

Combination

Pipetted out 1.0 ml from the above stock solutions, transferred into 100ml volumetric flask and made up the volume with mobile phase to acquire the concentration of working standard stock solution 10 µg/ml of both drug samples.

Preparation of standard stock solution

Precisely weighed 100 mg of Linagliptin and Empagliflozin and transferred into two different 100 ml volumetric flasks containing mobile phase and the volume was made up to the mark with same which gives the stock solution having concentrations 1000 µg/ml. Pipetted out 1.0 ml from the above stock solutions, transferred into volumetric flask of 100 ml and made up the volume with mobile phase to acquire the concentration of working standard stock solution 10 µg/ml of both drug samples.

Table 2
Chromatographic Condition

Mobile Phase	Acetonitrile : Buffer 3 pH (80 : 20 v/v)
Column	4.6 mm x 2.5 cm 5 µm Shimadzu- C18 (4.6 mm x 2.5 cm 5 µm)
Flow rate	0.70 ml/min
Injection volume	20µl
Wavelength	252 nm
Run time	8 min
HPLC Make	Shimadzu

Method Development

The proposed method was designed by taking various trials and by optimizing the chromatographic conditions by going through various trial by changing the mobile phase compositions, altering columns to attain sharp peak and to obtain resolution in retention time as per ICH guidelines for ENP & LNG. As organic modifier acetonitrile was used in various concentration in the mobile phase. While developing the method, initially various concentrations of acetonitrile and prepared buffer solution were used as a mobile phase for chromatographic separation, exhibited peak asymmetry. After few trials asymmetric analyte peak with run time 08 min was achieved successfully by employing acetonitrile and buffer of 3 pH in ratio of 80:20 V/V at flow rate 0.70 ml/min along with C_{18} Shimadzu column as stationary phase. The solution of standard drug sample and mobile phase is injected volume was 20µl. The eluents were monitored at a wavelength of 252 nm.

System suitability

System suitability parameters plays major role in development of analytical method that ensures the optimum performance of the developed method. The analytical parameters such as retention time, % area, asymmetry (A), theoretical

plates (N) were scanned by injecting 4 times of standard Empagliflozin and Linagliptin solution at concentration 10 µg/ml.

Degradation studies

The forced degradation study is considered a vital analytical aspect of the drug development program for drug molecules. Forced degradation, commonly known as stress testing, is carried out to demonstrate as specificity to develop a stability-indicating analytical method, using high- performance liquid chromatography (HPLC), i.e., a single analytic method that is capable of separating the degraded peaks from the drug substance/drug product peak[14]. As per International Council on Harmonization (ICH) guidelines (Q1A), stability studies need to be performed to propose the shelf life of new drug substances and/or drug products. Shelf life studies are part of various regulatory submissions to the FDA. Generally, three kinds of stability studies need to be performed in order to propose the shelf life of a drug substance and/or drug product: accelerated stability (ACC), intermediate stability (INS), and controlled room temperature (CRT) stability[15].

Table 3
Experimental Conditions for Forced Degradation Studies

Degradation Type	Conc. Of Reagent	Conditions applied	Time	Remarks
Acid	1N HCl	80°c	4h	Observe physical appearance of samples after degradation
Alkali(Base)	1N NaOH	80°c	4h	
Thermal(Dry Heat)	NA	150°c	4h	
UV	Expose under UV Light at 254 nm Wavelength	Ambient temperature	24h	

There are a few molecules that do not degrade or show very little degradation under any harsh conditions and they are therefore considered rock stable molecules. This kind of molecule will not generate any additional impurities peaks during a stability study.

Selection and Procedures of Forced Degradation Condition

As per ICH guidelines and common industry practice, forced degradation is usually performed in different stress conditions, i.e., acid, alkali, peroxide, thermal, and UV, along with a control sample. There are no industrial guidelines about how much degradation should be achieved; however as per current industrial practices, 5 to 30 percent degradation should be achieved in any one of the applied stress conditions. The aim of the degradation to be achieved through stress testing is to mimic the control room temperature stability conditions. In cases where higher or lower degradations are observed, the conditions or concentrations of the reagent should be optimized.

Stability Indicating Parameters
Acid Degradation

10 mg each of Empagliflozin and Linagliptin were taken in separate beakers and 10 mL of 1 N HCl was added into the individual beaker and kept on water bath for hydrolysis for 4h at 80°C. After 4h of hydrolysis the samples were suitably diluted and analyzed using HPLC. The degraded products are separated efficiently.

Alkali (Base) Degradation

10 mg each of Empagliflozin and Linagliptin were taken in separate beakers and 10 mL of 1 N NaOH was added into the individual beaker and kept on water bath for hydrolysis for 4h at 80°C. After 4h of hydrolysis the samples were suitably diluted and analyzed using HPLC. The degraded products are separated efficiently.

UV Degradation

For UV degradation 50mg of Empagliflozin and Linagliptin were exposed to ultraviolet radiation for 24hr in UV chamber at 254nm. No change in appearance observed in both samples. After 24hr the samples were suitably diluted and analyzed. The degraded products are separated efficiently.

Thermal Degradation

For thermal studies, the samples were kept in drying oven at 150°C for 4 h. After exposure, the samples were cooled, suitability diluted and analyzed. No change in appearance observed in both samples. The degraded products are separated efficiently.

Results and Discussion

Specificity/selectivity

The specificity of the method was determined by injecting the standard and synthetic mixture through the developed method, it was found to be no co-eluting peak was observed. A sharp and symmetric peak shape illustrate the specificity or selectivity of the method. The resolution in the peak is according to ICH guidelines and retention time for Empagliflozin and Linagliptin was found to be 3.714 min and 3.064 min respectively. The chromatograms of the Empagliflozin and Linagliptin 10 ppm solution are in fig. 1 and 2.

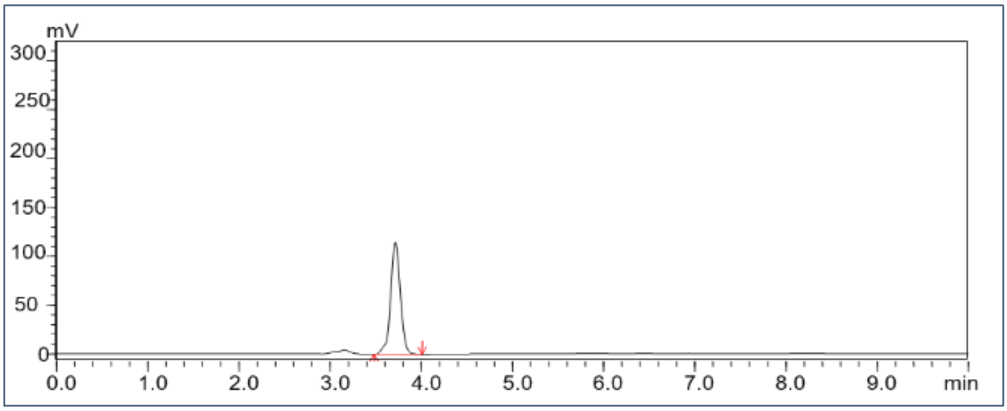


Fig 1. Chromatogram of Empagliflozin 10 ppm

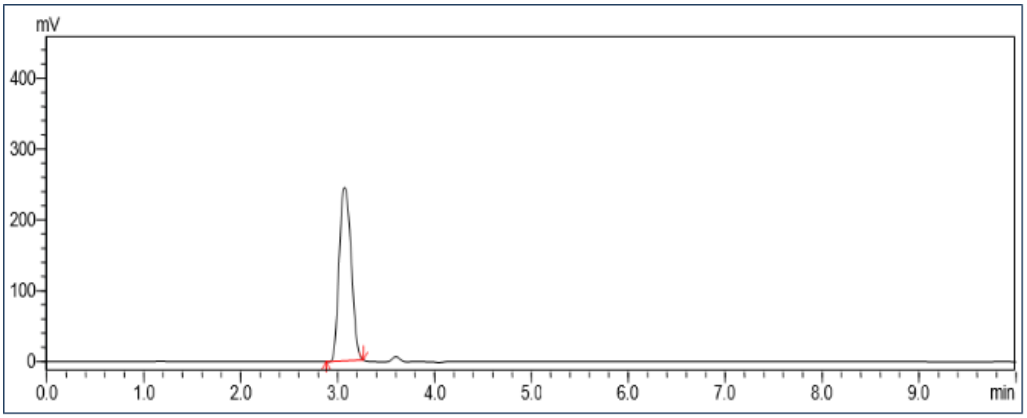


Fig 2. Chromatogram of Linagliptin 10 ppm

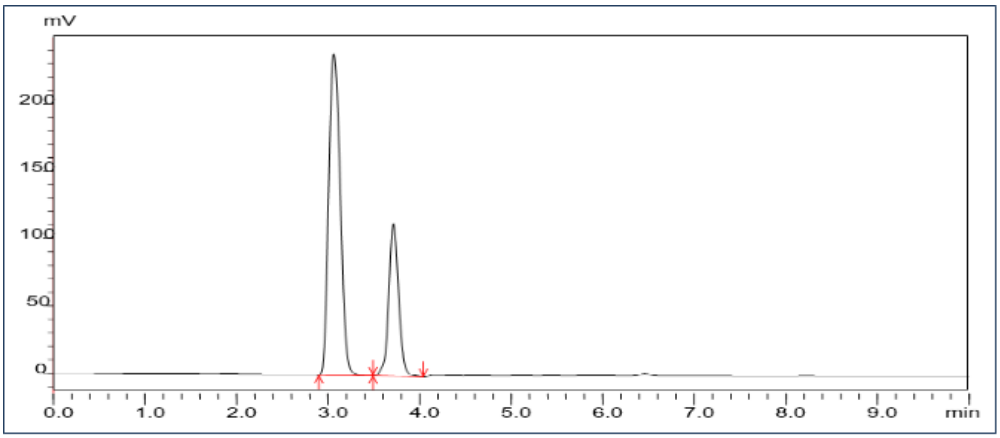


Fig 3. Chromatogram of Empagliflozin and Linagliptin 10 ppm in combination

Table 4
System Stability Parameters

Sr.	Parameters	Empagliflozin	Linagliptin
1.	Retention Time	3.714 min	3.064 min
2.	Area %	28.70%	71.29%
3.	Asymmetry factor (A)	1.026	1.168
4.	Theoretical Plates (N)	5418	2627
5.	Resolution	2.947	3.597

Results of Degradation studies

Empagliflozin and Linagliptin produces degradation products in acidic, alkaline, thermal and UV stress. The result of assay of Empagliflozin and Linagliptin shows that the degradation product does not interfere with the analytical procedure quantitively when these drugs are analyzed. The chromatograms of stability indicating study of Empagliflozin and Linagliptin are in fig 4 to 11. The planned analytical methodology is additionally helpful for the determination of Empagliflozin and Linagliptin in sample. The present method is stability indicating and able to separate the degraded products effectively and can be applied for the analysis of these drugs in pharmaceutical quality control.

Table 5
Results of Degradation studies

Empagliflozin			Linagliptin		
Stress conditions	Assay %	Degradation%	Stress conditions	Assay %	Degradation%
Acid degradation	86.988	13.012	Acid degradation	95.61	4.39
Base degradation	94.959	5.041	Base degradation	90.847	9.153
UV degradation	97.274	2.726	UV degradation	94.528	5.472
Thermal degradation	93.322	6.678	Thermal degradation	95.976	4.024

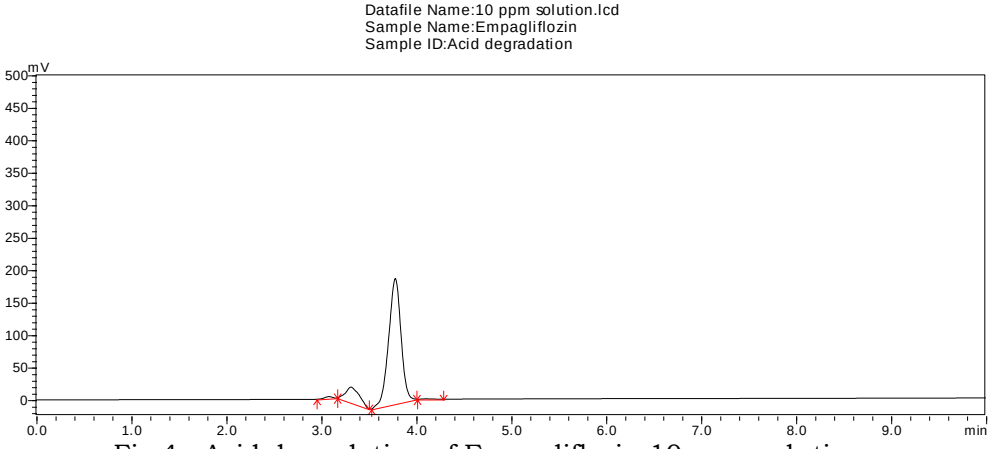


Fig 4. Acid degradation of Empagliflozin 10 ppm solution

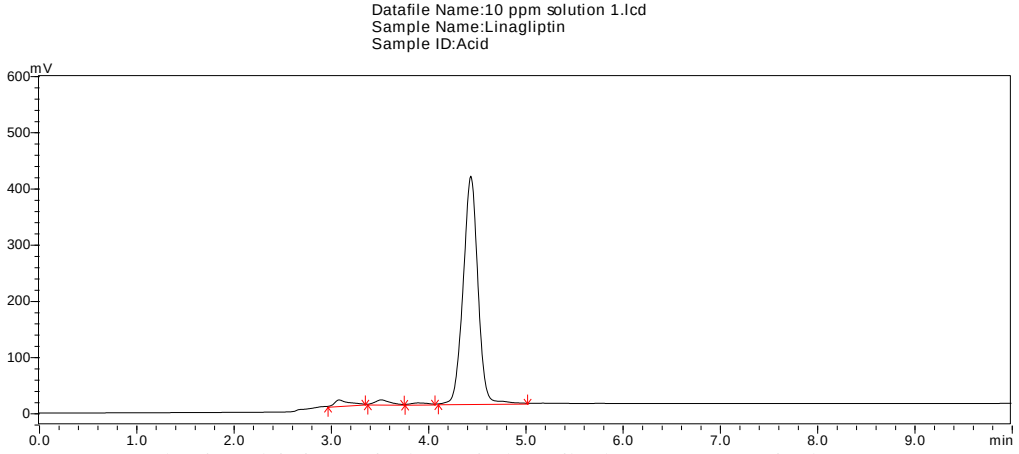


Fig 5. Acid degradation of Linagliptin 10 ppm solution

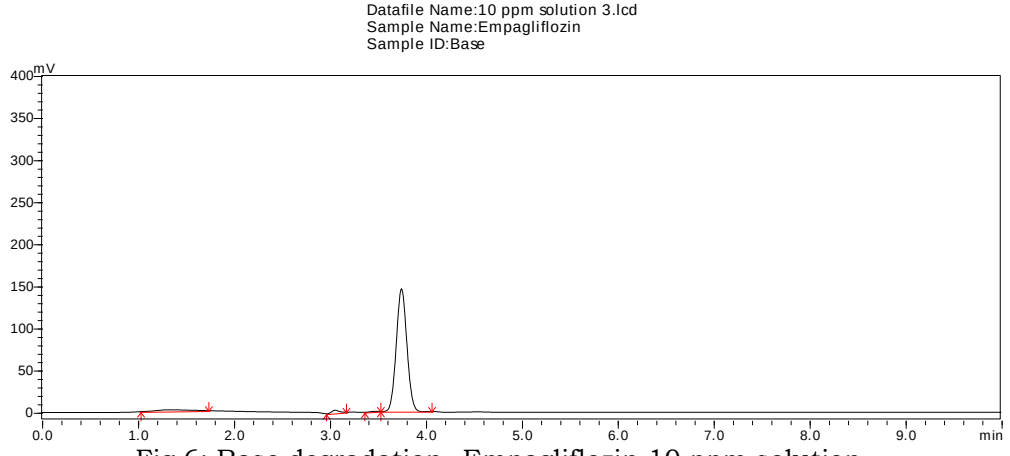


Fig 6: Base degradation- Empagliflozin 10 ppm solution

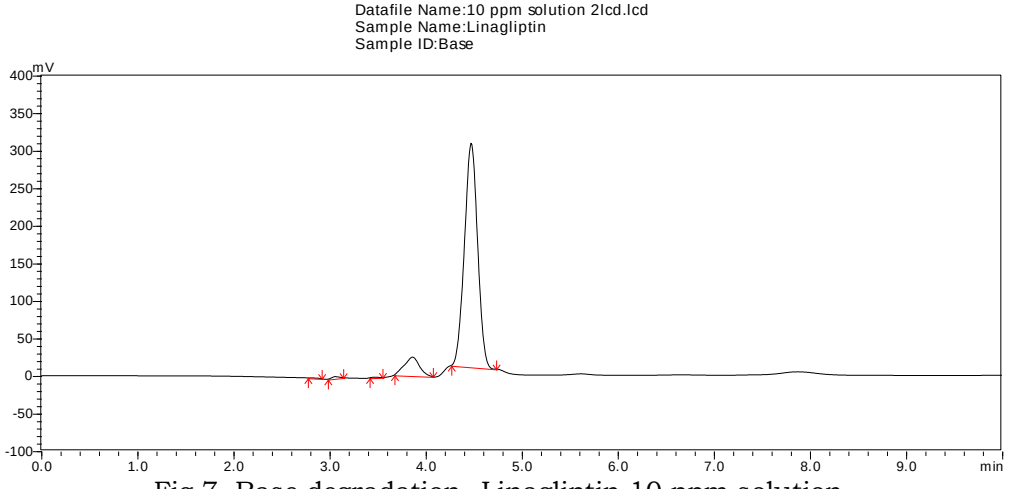


Fig 7. Base degradation- Linagliptin 10 ppm solution

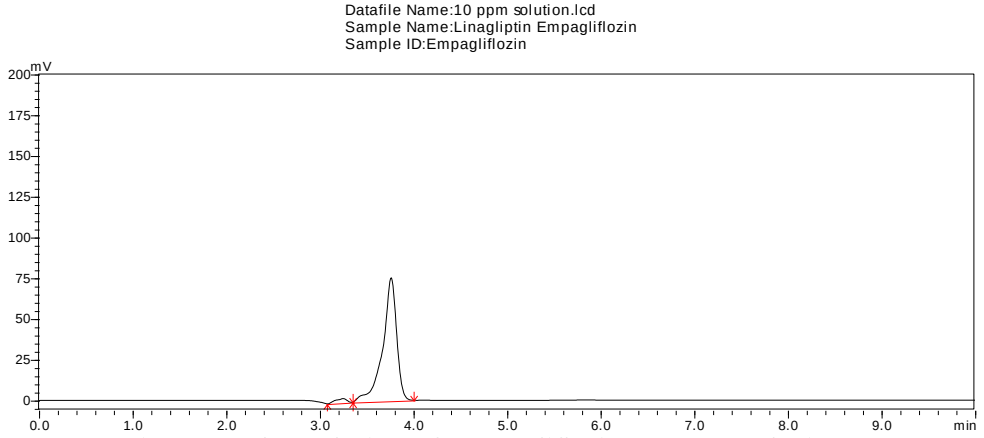


Fig 8. UV degradation of Empagliflozin 10 ppm solution

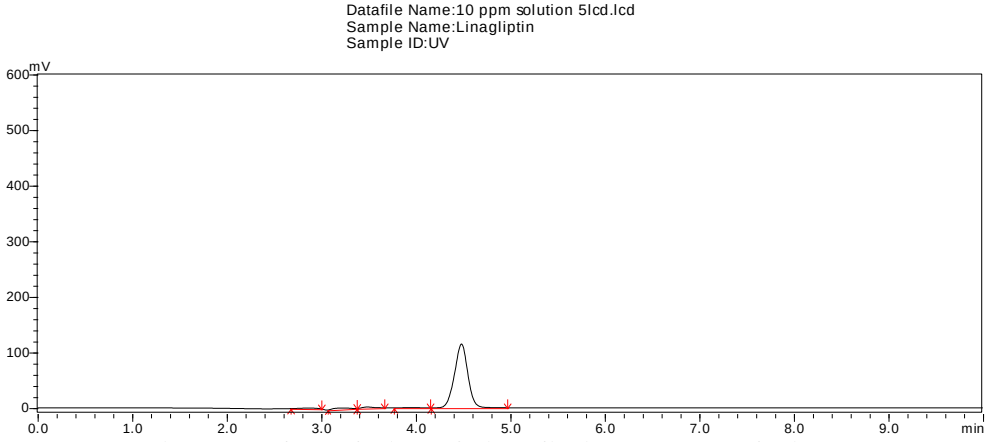


Fig 9. UV degradation of Linagliptin 10 ppm solution

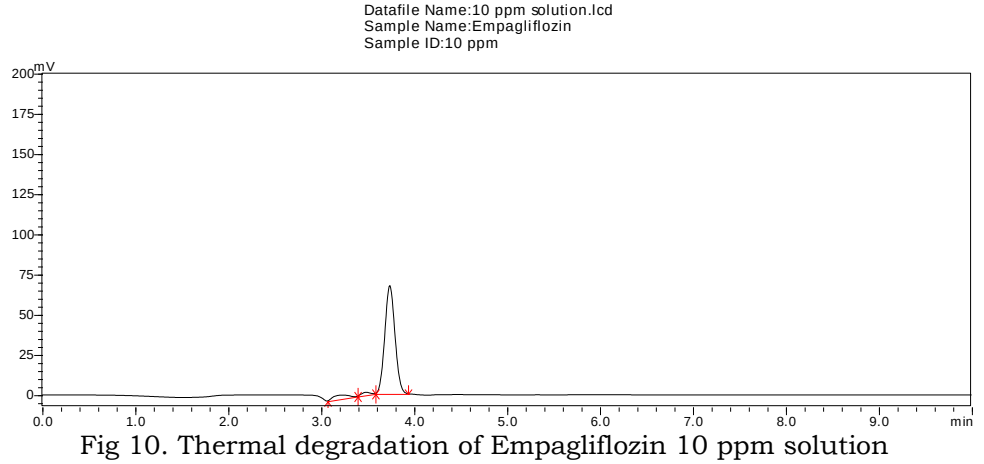


Fig 10. Thermal degradation of Empagliflozin 10 ppm solution

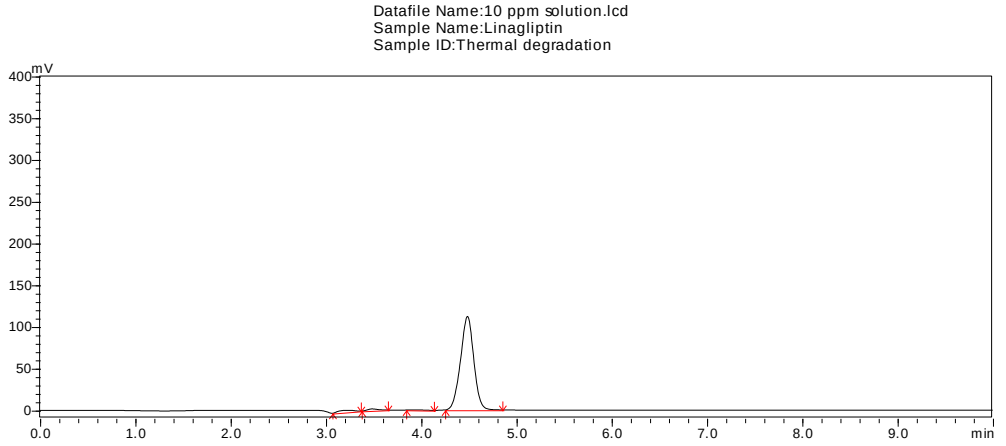


Fig 11. Thermal degradation of Linagliptin 10 ppm Linagliptin

Conclusion

A new, simple, sensitive, specific, precise, inexpensive, and accurate Reverse Phase-High Performance Liquid Chromatography (RP- HPLC) method has been developed for simultaneous estimation of Empagliflozin, and Linagliptin in bulk and synthetic mixture. As per the International Conference on Harmonisation (ICH) Q2 (R1) guideline, proposed RP-HPLC method development has been carried out. The proposed RP-HPLC method was repeatable and selective as per statistical analysis and it can be used for simultaneous estimation of Empagliflozin and Linagliptin in bulk and synthetic mixture. The proposed method can be applied for simultaneous estimation of two drugs in pharmaceutical formulation. Stress studies for stability of the drugs were performed as per the method developed for simultaneous estimation of Linagliptin and Empagliflozin by RP-HPLC method. For conducting stress studies referred ICH-guidelines Q1A (R2) on stability testing of drug-substances API and finish product. To understand the degradation pattern in both the drugs samples were prepared for individual drug and injected separately. The suitability of the method for detection of degradation products was confirmed as well as that the method is stability indicating (all peaks were sufficiently separated from the drug substance peak and show no sign of co-elution).

References

1. Bulbule Laxman, Godge Rahul et.al, Empagliflozin and Linagliptin an Analytical Review, Journal of The Maharaja Saiyajirao University of Baroda, Vol. 55 No. 2 (Science & Technology) 2021 page no.39-45.
2. Fitchett D, Inzucchi SE, Cannon CP, McGuire DK, Scirica BM, Johansen OE, Sambevski S, Kaspers S, Pfarr E, George JT, Zinman B, Empagliflozin Reduced Mortality and Hospitalization for Heart Failure Across the Spectrum of Cardiovascular Risk in the EMPA-REG OUTCOME Trial. Circulation. 2018 Dec 6;
3. Schwaiger E, Burghart L, Signorini L, Ristl R, Kopecky C, Tura A, Pacini G, Wrba T, Antlanger M, Schmaldienst S, Werzowa J, Säemann MD, Hecking M, Empagliflozin in post transplantation diabetes mellitus: A prospective, interventional pilot study on glucose metabolism, fluid volume and patient safety. American journal of

transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2018 Dec 26;
4. Liver Tox: Clinical and Research Information on Drug Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012- Linagliptin. [Updated 2018 Jan 3].
5. Patil SD, Chaure SK and Kshirsagar S: Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs. Asian Journal of Pharmaceutical Analysis 2017; 7(2): 117-23.
6. P. Madhusudhan M., Radhakrishna Reddy and N. Devanna, (02-Dec-0215) 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, Vol.02, 2015.
7. Bakshi A, Mounika A, Bhutada S and Raju MB: Simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC method. World Journal of Pharmacy and Pharmaceutical Sciences 2018; 7(8): 1062.
8. Sharamali Donepudi, Suneetha Achanta (10-Mar-2018) Validated HPLC-UV Method for Simultaneous Estimation of Linagliptin and Empagliflozin, International Journal of Applied Pharmaceutics, Vol.10, Issue 3, 2018.
9. P. Madhusudhan M., Radhakrishna Reddy and N. Devanna, (02-Dec-0215) 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, Vol.02, 2015.
10. Bakshi A, Mounika A, Bhutada S and Raju MB: Simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC method. World Journal of Pharmacy and Pharmaceutical Sciences 2018; 7(8): 1062.
11. Ashok K. Shakya, Wael Abu Dayyih, Ramadan I. Chandrabatla Varaprasad, Md. Asif and K. Ramakrishna, RP-HPLC method for simultaneous estimation Of Metformin and Linagliptin in tablet dosage form, (2015), Vol. 8, No.4, pg.no.426 - 432.
12. Godge R.K, Shinde G.S, Simultaneous estimation and validation of Dapagliflozin and saxagliptin in bulk drug and dosage form by HPLC, 2019, Vol. 11, Issue 1, 59-63.
13. Mallikarjun Rao N, GOWRI SANKAR D, RP-HPLC method for simultaneous and stability indicating study of Metformin and Linagliptin in pure and pharmaceutical dosage form (29-10-2014), Int. J Pharm Sci., Vol. 7, Issue 3, 191-197.
14. Monika Bakshi Saranjit Singh Development of validated stability-indicating assay methods-critical review, Journal of Pharmaceutical and Biomedical Analysis Volume 28, Issue 6, 15 June 2002, Pages 1011-1040.
15. Godge, R. K, Shinde, G S, Bhosale M.S, RP-HPLC Method for estimation of Alogliptin and Glibenclamide in Synthetic mixture, 2020, Vol. 13, Issue 2,555-589.
16. Mohamed A.Korany[a] Rim S.Haggag [a] Marwa A.A.Ragab[a] Osama A.Elmallah[b] A validated stability-indicating HPLC method for simultaneous determination of Silymarin and Curcumin in various dosage forms Volume 10, Supplement 2, May 2017, Pages S1711-S1725.