International Journal of Research in Pharmacy and Life Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijrpls

Research Article **Open Access**

A New Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Empagliflozine and Metformin in its Pure and Pharmaceutical Dosage Forms

MD. Sadiq Ali[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

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of metformin and empagliflozin was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of metformin and empagliflozin were found to be from 100-500 µg/ml of metformin and 1-5µg/ml of empagliflozin. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of metformin and empagliflozin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Metformin, Empagliflozin, RP-HPLC

A R T I C L E I N F O

CONTENTS

Article History: Received 25 July 2016, **Accepted** 29 August 2016, **Available Online** 24 November 2016

***Corresponding Author**
Dr. Gampa Vijaya Kumar
Professor and Head, Dept. of Pharmacy,
KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.
Manuscript ID: IJRPLS3205

PAPER-QR CODE

Citation: Gampa Vijaya Kumar. A New Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Empagliflozine and Metformin in its Pure and Pharmaceutical Dosage Forms. *Int. J. Res. Pharm, L. Sci.*, 2016, 4(2): 86-93.

1. Introduction

Metformin, *N*, *N* - dimethyl imido di carbonimidic diamide (Figure 1), is a biguanide hypoglycemic drug that is regarded as the main drug in mixed therapies of oral hypoglycemics. Literature survey reveals that some methods have been reported for determination of MET in mixtures including LC/MS/MS and HPLC [1-2].

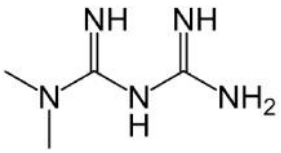

Figure 1: Structure of Metformin

Empagliflozin, (2S, 3R, 4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol, is used for Treating type 2 diabetes in certain patients. It is used along with diet and exercise. Empagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor [2]. It works by decreasing the amount of sugar the body absorbs and increasing the amount of sugar that leaves the body in the urine [3-5]. Literature survey reveals that only one spectrophotometric method [8] and one chromatographic method was reported for the determination of VDG in the presence of its synthetic intermediate [9].

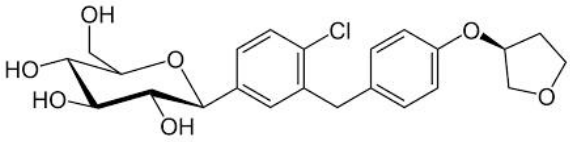

Figure 2: Structure of Empagliflozin

Due to the lack of reported HPLC methods describing determination of the mixtures under investigation, it was deemed useful to develop simple, sensitive and selective HPLC method that could be useful for the simultaneous determination of Metformin and Empagliflozin. The proposed method was designed to be suitable for the quality assessment of these mixtures in a tablet dosage form.

2. Materials and Methods

Table 1: Chemicals used

S.No	Chemical	Brand
1	Metformin	Mylon
2	Empagliflozin	Cipla
3	KH_2PO_4	Finer chemical LTD
4	Water and Methanol	Lichrosolv (Merck)
5	Acetonitrile for HPLC	Molychem
6	Ortho phosphoric Acid	Merck

Table 2: Solubility profile

Solvents	Metformin	Empagliflozin
Methanol	Soluble	Soluble
ACN	Soluble	Soluble
Water	Partially soluble	Insoluble
Chloroform	Soluble	Partially soluble

Instrumentation:

The HPLC system was an LC Waters (Waters, Milford, MA, USA) consisting of quaternary gradient system (600 Controller), in line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). Chromatographic separation assay was performed with a Water's C-18 analytical column (150 mm × 4.6 mm inner diameter, 5 µm particle size, Waters, Dublin, Ireland) maintained at 45 °C. The mobile phase was pumped at a flow rate of 1 mL min^{-1}.

Preparation of Buffer and Mobile Phase:

Preparation of Phosphate buffer:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Metformin and Empagliflozin10mg of working standard into a 10Ml & 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Metformin and Empagliflozin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3 ml of Metformine and Empagliflozin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Metformin and Empagliflozin peaks and calculate the %Assay by using the formulae.

System Suitability: [4,5]

Tailing factor for the peaks due to Metformin and Empagliflozin in Standard solution

Should not be more than 2.0

Theoretical plates for the Metformin, Empagliflozin peaks in Standard solution should not be less than 2000.

Calculation: (For metformin)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:
AT = average area counts of sample preparation.
As= average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = label claim of Metformin mg/ml.

Calculation: (For Empagliflozin)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt.}}{\text{Label Claim}} \times 100$$

Where:
AT = average area counts of sample preparation.
As= average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = label claim of empagliflozinmg/ml.

Method Validation Summary:

Precision:

Preparation of stock solution: Accurately weigh and transfer 25 mg of Metformin and Empagliflozin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3 ml of Metformin & Empagliflozin of the above stock solution into a 10ml volumetric flask and dilute 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 day by using different make column of same dimensions.

Preparation of stock solution:

Accurately weigh and transfer 25 mg of Metformin and 10mg of Empagliflozin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml of Metformin & Empagliflozin of the above stock solution into a 10ml volumetric flask and dilute 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%.

Accuracy:

Preparation of Standard stock solution:

Accurately weigh and transfer 10 mg of Metformin andEmpagliflozin10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 5mg of Metformin and 5.3mg of Empagliflozin working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock Solution: Further pipette 3 ml of Metformine& 0.3 ml of Empagliflozin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration): Accurately weigh and transfer 10 mg of Metformin and 10 mg of Empagliflozin working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock Solution: Further pipette 3 ml of Metformin& 0.3 ml of Empagliflozin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 14.4mg of Metformin and 14.5mg of Empagliflozin working standards into a 10mL and 100ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3 ml of Metformin& 0.3 ml of Empagliflozin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Metformin & Empagliflozin and calculate the individual recovery and mean recovery values.

Acceptance Criteria:

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

Linearity:

Preparation of stock solution:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Metformin, Empagliflozin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (100ppm ofMetformin&1ppm of Empagliflozin): 1ml and 0.1 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (200ppm of Metformin&2ppm of Empagliflozin): 2ml and 0.2 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level–III (300ppm of Metformin & 3ppm of Empagliflozin): 3ml and 0.3 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level –IV (400ppm of Metformin&4ppm of Empagliflozin): 4ml and 0.4 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (500ppm of Metformin&5ppm of Empagliflozin): 5ml and 0.5 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

Limit of Detection [9]:

Limit of Detection: (For Metformin):

Preparation of 300µg/ml solution:

Accurately weigh and transfer 10 mg of Metformin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.12µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.4mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 152/52 = 2.9

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of Detection: (For Empagliflozin)

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Empagliflozin working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.015µg/ml solution): Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 156/52 = 3.0

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of Quantification:

Limit of Quantification (for Metformin HCL)

Preparation of 300µg/ml solution: Accurately weigh and transfer 10 mg of Metformin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.42µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Pipette 1.4 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

S/N = 522/52 = 10.03

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution.

Limit of Quantification: (for Empagliflozin)

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Empagliflozin working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.05µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 1.7mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

S/N = 524/52 = 10.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.8 ml/min to 1.2ml/min. Standard solution 300ppm of Metformin&3ppm of Empagliflozin was prepared and analysed using the varied flow rates along with method flow rate. 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 ±10%.

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

b). The Organic composition in the Mobile phase was varied from 50% to 50%. Standard solution 300 µg/ml of Setraline & 3µg/ml of Empagliflozin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

*****Results for actual Mobile phase composition (55:45Methanol: Buffer (ph-2.8) has been considered from Accuracy stand

3. Results and Discussion

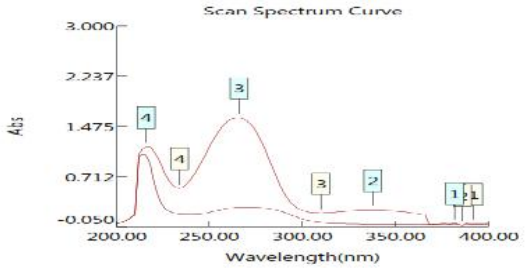

Figure 3: Spectrum showing wavelength of Metformin

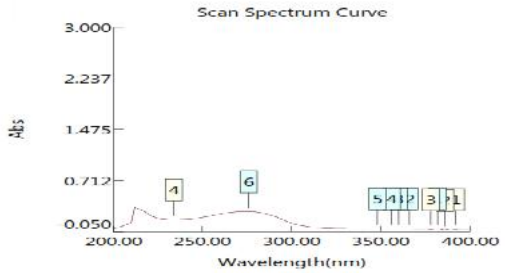

Figure 4: Spectrum showing wavelength of Empagliflozin

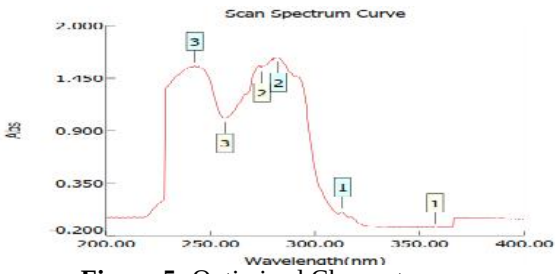

Figure 5: Optimized Chromatogram

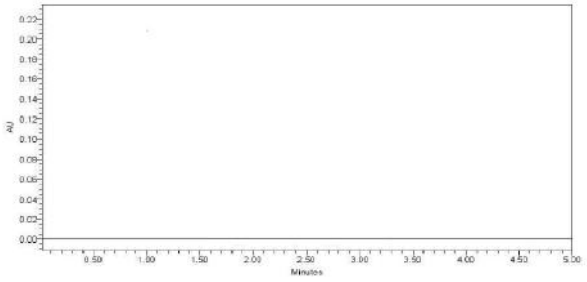

Figure 6: Chromatogram for blank

Observation: From the above chromatogram it was observed that there are no interferences.

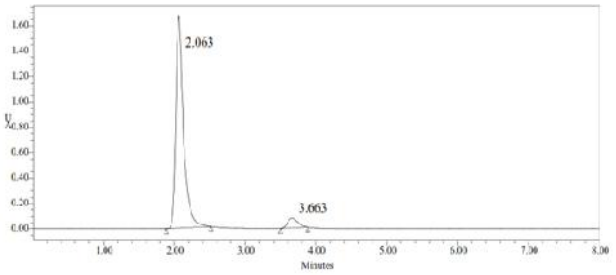

Figure 7: Chromatogram for Metformin and Empagliflozin sample Preparation

Observation: From the above chromatogram it was observed that the Metformin and Empagliflozin peaks are well separated. Retention time of Metformin – 2.063 min Retention time of Empagliflozin - 3.663 min.

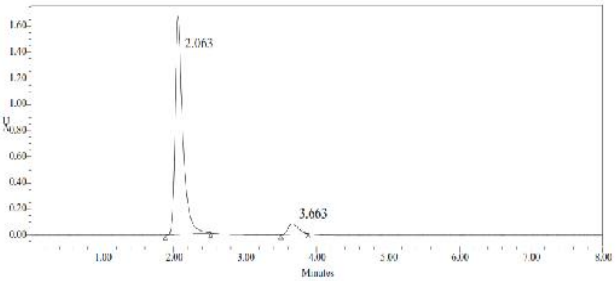

Figure 8: Chromatogram for Metformin and Empagliflozin Standard Preparation

Observation: From the above chromatogram it was observed that the Metformin and Empagliflozin peaks are well separated Retention time of Metformin–2.063 min, Retention time of Empagliflozin - 3.663 min.

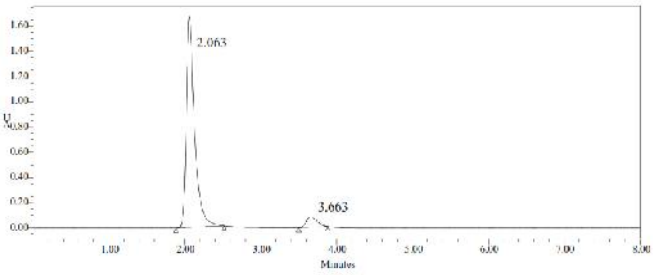

Figure 9: Chromatogram for system suitability

Table 3: Method precession for Metformin

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 4: Method precession for Empagliflozin

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table 5: Intermediate precision for Metformin

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 6: Intermediate precision for Empagliflozin

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Linearity: The linearity range was found to lie from 100µg/ml to 500µg/ml of Metformin, 5µg/ml to 25µg/ml 0f Empagliflozin and chromatograms are shown below.

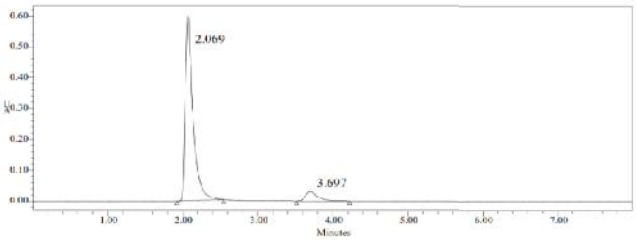

Figure 10: Chromatogram for linearity concentration-100µg/ml of Metformin& 5 µg/ml of Empagliflozin

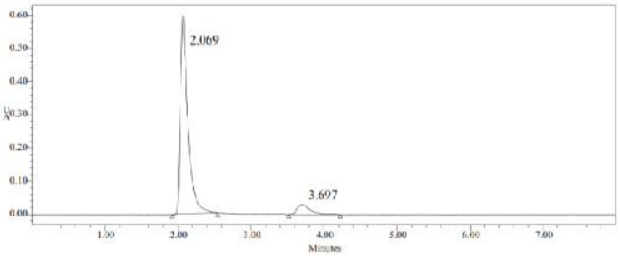

Figure 11: Chromatogram for linearity concentration-200µg/ml of Metformin & 10 µg/ml of Empagliflozin

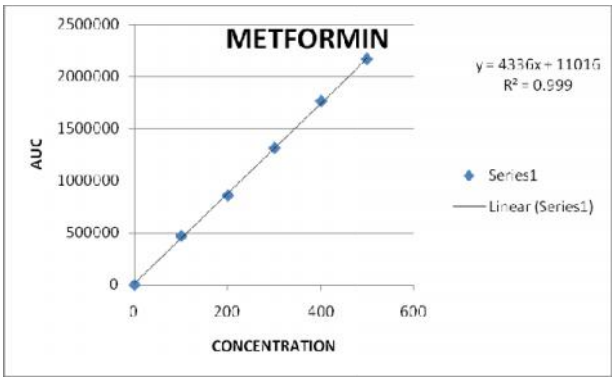

Figure 12: Calibration graph for Metformin at 225 nm

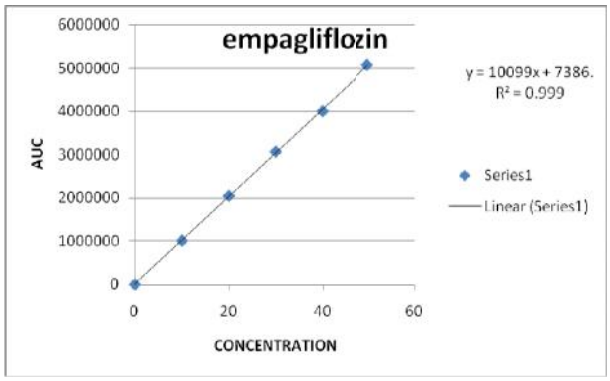

Figure 13: Calibration graph for empagliflozin at 225 nm

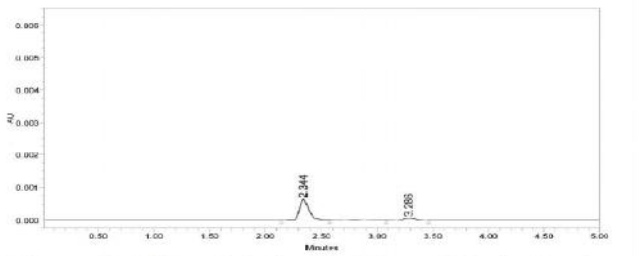

Figure 14: LOD of Metformin &Empagliflozin showing

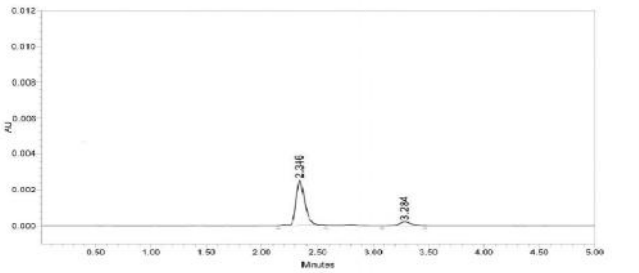

Figure 15: LOQ of Metformin &Empagliflozin showing

Limit of Detection (LOD)

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio, signal to noise ratio shall be 3 for LOD solution.

Limit of Quantification (LOQ)

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio, signal to noise ratio shall be 10 for LOQ solution

Table 7: Accuracy (recovery) data for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
50%	656658.9	5.1	5.010	100.2%	
50%	656654.2	4.9	5.10	101.2%	
100%	1304258	10.0	10.003	100.0%	
100%	1304256	10.2	10.3	100.4%	100.6%
100%	1304249	10.2	10.3	100.4%	
150%	1854608	14.4	14.2	98.780%	100.21%
150%	1854605	14.9	14.8	99.98%	
150%	1854609	15.1	14.9	100.12%	

Table 8: Accuracy (recovery) data for Empagliflozin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65850	5.3	5.04	100.8%	99.99%
50%	65842	5.1	5.01	100.5%	
50%	65684	5.0	5.02	101.2%	
100%	124353	10	10.10	100.01%	101.6%
100%	124712	10.2	10.0	100.05%	
100%	120424	10.5	10.4	101.4%	
150%	177940	14.9	14.75	98.68%	101.21%
150%	177950	15.1	14.9	99.99%	
150%	1854609	15.2	15.02	100.10%	

Table 9: Analytical performance parameters of Metformin and Empagliflozin

Parameters	Metformin	Empagliflozin
Slope (m)	4336	10099
Intercept (c)	7389	10099
Correlation coefficient (R^2)	0.999	0.999

4. Conclusion

The estimation of Metformin and empagliflozin was done by RP-HPLC. The Phosphate buffer was p^H3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil ODS C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Metformin and empagliflozin were found to be from 100-500 µg/ml of Metformin and 1-5µg/ml of empagliflozin. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Metformin and empagliflozin. LOD and LOQ were found to be within limit.

5. References

[1] 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. 2014, 2(3):1332-1341.

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

[3] 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, 5(1): 230.

[4] Ghazala Khan, Dinesh Sahu Agrawal Y P, Neetu Sabarwal, Avnish Jain, Gupta A K. Simultaneous Estimation of Metformin and Sitagliptin In Tablet Dosage Form. Asian J. Biochem. Pharma. Res. 2011, 1(2): 352-358.

[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, 5(2): 202-218.

[6] Ravi Pratap Pulla, Sastry B S, Rajendra Prasad Y, Appala Raju N. Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in Tablet Dosage Forms by RP-HPLC. Res. J.

Pharm. Tech. 2011, 4(4): 646-649.

[7] 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.

[8] Zeng W, Xu Y, Constanzer M, Woolf EJ. Determination of sitagliptininin human plasma using protein precipitation and tandem mass spectrometry. J. Chromatogr. B. 2010, 878(21): 1817-1823.

[9] WeiZeng, Donald Musson G, Alison Fisher L, Li Chen, Michael Schwartz S, Eric Woolf J, Amy Qiu Wang. Determination of sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectrometry. J. Pharm. Biomed. Anal. 2008, 46(3): 534-542.