IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
July 2024 Vol.:30, Issue:7

Development and Validation of Analytical Method for the Simultaneous Estimation of Metformin and Empagliflozin by QbD Approach

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Khan Hajera*, More Urmila M, Nagargoje Ajay S, Kadam Rangnath S

Department Of Quality Assurance. SSS Indira college of pharmacy vishnupuri Nanded 431606. India.

Submitted: 20 June 2024
Accepted: 27 June 2024
Published: 30 July 2024

HUMAN JOURNALS
ijppr.humanjournals.com

Keywords: Quality by design, Metformin, Empagliflozin, UV Spectrophotometric method, Simultaneous equation method.

ABSTRACT

This study reports the QbD Approach in Formulation Design and Evaluation of Metformin & Empagliflozin. The concept of quality by design (QbD) has lately gained importance by application of design of experiments approach (DoE). QbD describes a pharmaceutical development approach especially in formulation design & development and manufacturing processes for the purpose of continue and enhance the product quality. There are several elements of QbD are there such as Quality Target Product Profile (QTPP), Critical Quality Attributes (CQAS), Quality Risk (Assessment) Management (QRM), Design Space etc. The current work is aimed in the application of quality by design (QbD) concept especially Target Product Profile in the formulation development of Metformin & Empagliflozin. UV spectrophotometric method was developed and validated for quantitative determination of Metformin & Empagliflozin. Different analytical validation parameter such as linearity, precision, accuracy, limit of detection, limit of quantification were determined according to ICH Q2[R1] guidelines. The proposed method is highly sensitive, precise and accurate and hence successfully applied for the estimating assay of tablet formulation.

INTRODUCTION

Metformin chemically n, n-dimethyl imido dicarbonimidic diamide (1) is available in the market in commercial forms under several brands including Glucophage. The drug is used as first line therapy in type II diabetes, due to its efficacy and safety in controlling hemoglobin A1c, reducing weight and decreasing cardiovascular mortality rate among people affected by the disease (2). The molecular structure of Metformin is shown in figure.no.1.

Figure.1: Structure of Metformin.

Empagliflozin chemical designation is (2S,3R,4R,5S,6R)-2[4-chloro-3-[[4-[(3S)-oxolan-3yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane3,4,5-triol. Empagliflozin is an inhibitor of SGL2, which is the major transporter involved in the reabsorption of glucose in the kidneys.(3,4)

Empagliflozin selectively inhibited SGLT2 (IC50 = 1.3 nm), resulting in decrease renal glucose re-absorption, and thereby increasing urinary glucose excretion (UGE) and lowered plasma glucose (PG) level in patients with type 2 diabetes.

The molecular structure of Empagliflozin is shown in figure.no.2.

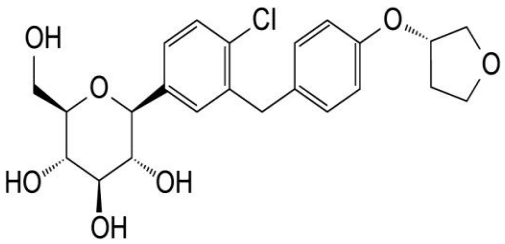

Figure.2: Structure of Empagliflozin

Literature survey revealed that many analytical method such as Spectrophotometric, HPLC, HPTLC, which have been reported for estimation of Metformin(5-6) & Empagliflozin(7,8).

Few stability indicating UV & HPLC(9-16) methods were also reported. The reported conventional spectrophotometric methods were monotonous & time consuming process of varying one factor at a time require large number of experimental run & always yields narrow vigorous method which has high risk of failure during handover/real time usage. The Analytical Quality by Design(AQbD) which is one of the alternative which dwindled the experimental timeand the cost of the drug analysis. In recent times, pharmaceutical company adopting QbD in analytics for trouble free completion with FDA & ICH guidelines. If an AQbD approach has been implemented in development stage flexibility of analytical method is granted without needof revalidation or regulatory review(17-20).

MATRIALS AND METHOD:

Instrument: Ultraviolet spectrophotometer: Double beam UV visible spectrophotometer (UV-1800, Shimadzu, Japan) software- UV probe (Version-2.35) Band width-2nm & Wavelength accuracy of ± 0.3nm Curette pair of 10mm matched Quartz cell. The other instrument used suchas Hot air oven, digital weighing balance.

Reagent & Material:

Standard gift sample of Metformin & Empagliflozin were provided by Swapnroop Pharmaceutical, Sambhajinagar.

Solvent used: HCl, Water.Marketed Formulations

Brand Name	**Component**	**Dose Quantity**	**Manufacturer**
Jardiance met	Empagliflozin & Metformin	12.5mg & 500mg	Boehringer Ingelheim
Synjardy	Empagliflozin & Metformin	12.5mg & 1000mg	Boehringer Ingelheim Limited

1. DOE (Design of Experiment) (21,22).

Methodology:-

- Selection of Independent& Dependent Variable
- Take absorbance of API's
- Put the data in Design Expert Software Version 13 in Actual Design
- Check Selected critical parameter for spectrophotometric analytical method
- Start Analysis of Dependent Variable
- Formation of Contour & on the basis of Contour 3D surface is form
- Validate the critical parameters

Independent Variables: -

Independent Variable	Low	High
Scan speed	1= Slow	3=High
Sampling interval	0.1	2.0

Dependent Variables: -

1	Absorbance of Metformin
2	Absorbance of Empagliflozin

METHODOLOGY

METHOD: SIMULTANEOUS EQUATION METHODEXPERIMANTAL

Preparation of stock solutions

The 100mg of pure drug Metformin & Empagliflozin were weighed accurately and transferred into 100 ml volumetric flask. Then drug was dissolved separately in 0.1N HCl solution to give stock solution of concentration 1 mg/ml (1000 µg/ml)of both drugs respectively.

Selection of analytical wavelengths: -

Appropriate dilutions were prepared for Metformin & Empagliflozin from the standard stock solution and scanned in the spectrum mode range from 400 nm to 200 nm. Metformin and Empagliflozin showed absorbance maxima at 203nm and at 223nm respectively.

Preparation of Working Solution:

Appropriate volume of 0.1ml of the Standard Stock solution of Metformin and Empagliflozin and was transferred into 10 ml volumetric flask, then diluted to mark with 0.1N HCl to give concentration of 10µg/ml for each drug. The coming solution was scanned in the UV range (200-400nm). In spectrum MET and EMP showed absorbance maxima at 203nm and at 223nm respectively.

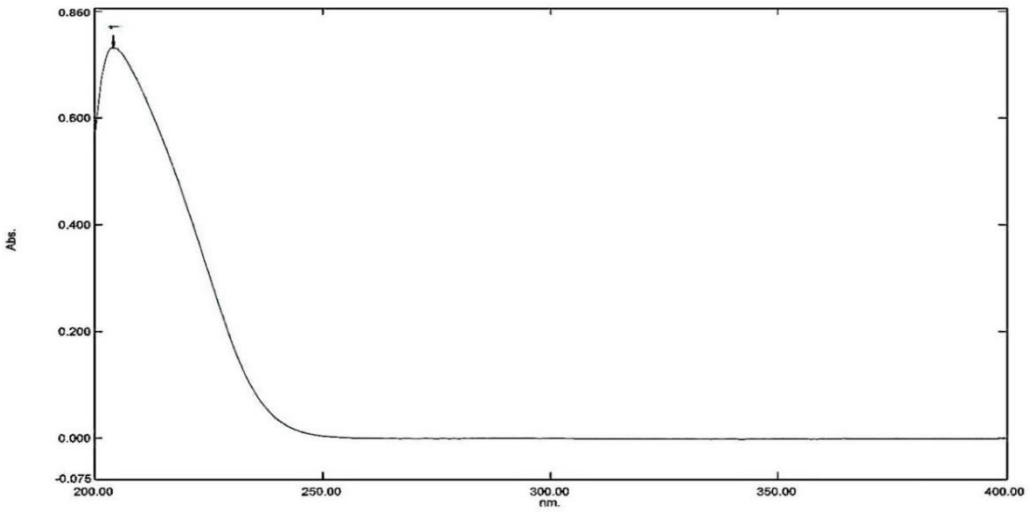

Figure.no.3: UV Spectrum of Metformin

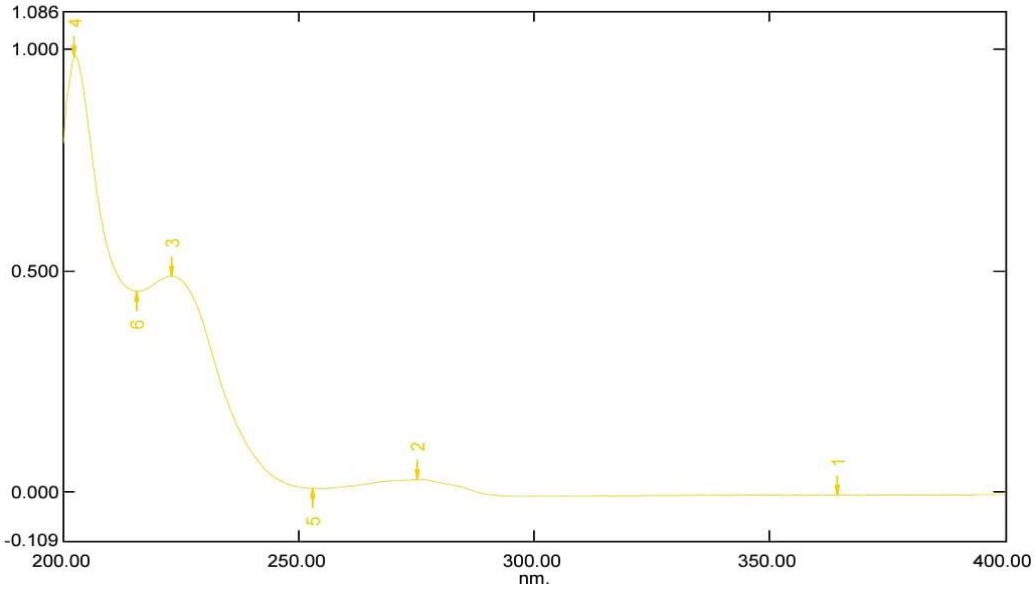

Figure.no.4: UV spectrum of Empagliflozin

Preparation of calibration curve

From the standard stock solution of MET and EMP appropriate aliquot was pipette out into 10 ml volumetric flask and dilution was made with 0.1 N HCl to create a concentration of 100 µg/ml again dilutions were made with 0.1 N HCl to obtain standard solutions of concentrations and 2 to 14µg/ml for MET and EMP respectively. Absorbance for these solutions were measured at 203 nm and 223nm respectively and diluting them in separate 10 ml volumetric flasks. A standard calibration curve of absorbance vs concentration was plotted. Both drugs follow Beer-Lamberts Law.

Table.1: Standard calibration Table of Metformin

Conc. (µg/ml)	**Absorbance at 203nm**
2µg/ml	0.152
4µg/ml	0.315
6µg/ml	0.470
8µg/ml	0.622
10µg/ml	0.801
12µg/ml	0.948
14µg/ml	1.109

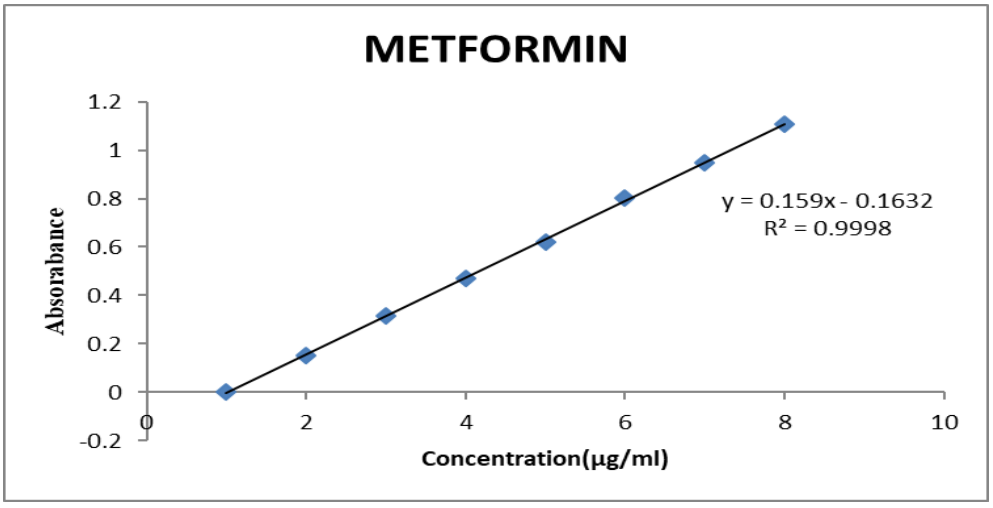

Figure.no.5: Calibration Curve of Metformin

Table.2: Standard calibration Table of Empagliflozin

Conc. (µg/ml)	Absorbance at 223 nm
2 µg/ml	0.152
4 µg/ml	0.261
6 µg/ml	0.370
8 µg/ml	0.479
10 µg/ml	0.588
12 µg/ml	0.697
14 µg/ml	0.806

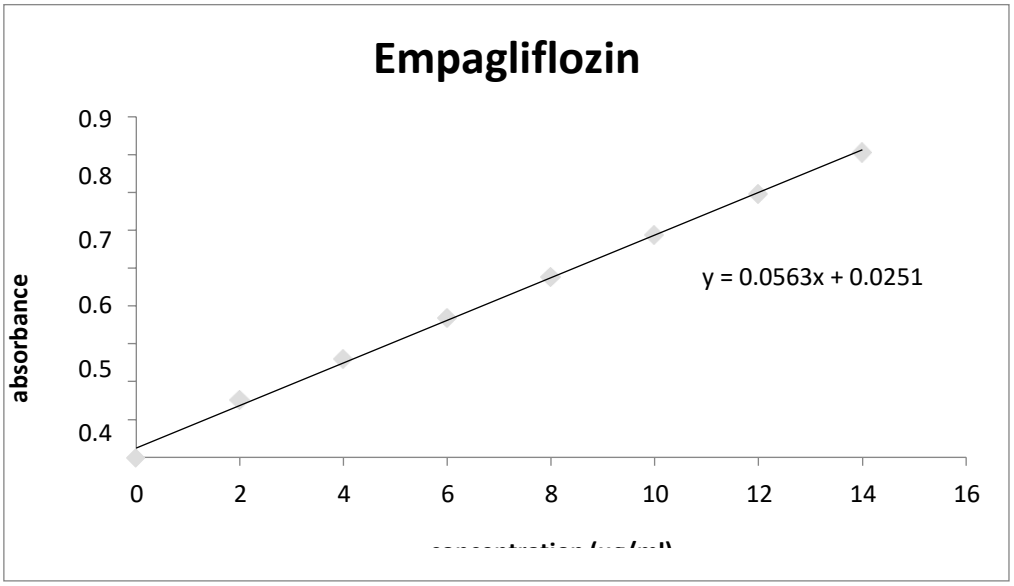

Figure.no.6: Calibration Curve of Empagliflozin

Table.3: Optical characteristics and Statistical data of the Regression equation in Simultaneous equation methods.

Parameters	Metformin	Empagliflozin
Max. wavelength	203	223
Beer's law limits(µg/ml)	2-14µg/ml	2-14µg/ml
Regression equation(Y*)	Y=0.159×-0.1632	Y=0.0563×+0.0251
Slope(b)	0.159	0.0563
Intercept(a)	0.1632	0.0251
Correlation coefficient (r2)	0.9998	0.9981

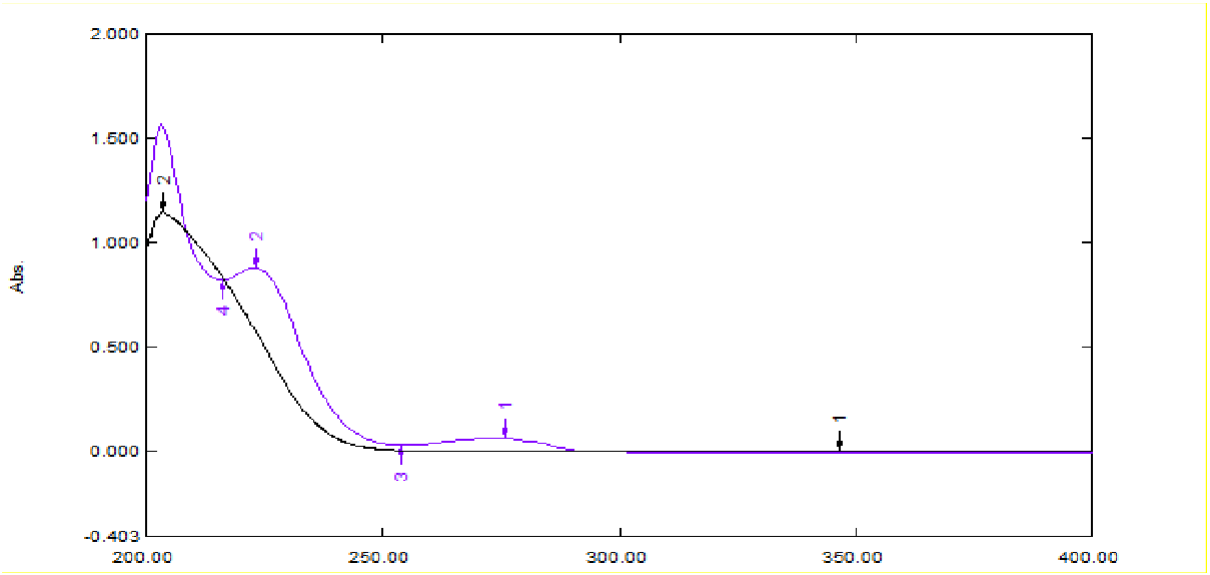

Figure.no.7: Overlay spectrum of Metformin and Empagliflozin Analysis of tablet formulation:

Twenty tablets of MET and EMP in combination were weighed; their average weight was determined and finally crushed to powder sample. from the triturate, tablet powder equivalent to 500 mg of MET and 12.5 mg of EMP was weighed and transferred to 100 ml volumetric flask and dissolve up to mark with 0.1 N HCl and the content was kept in ultrasonicator for 30 min. The solution was filtered through Whatman filter paper No.41.This tablet solution was further diluted to obtain 5O mcg/ml of MET and 1.25 mcg/ml of EMP. The mixed sample solution were analyzed to obtained spectra and absorbance value at 203 nm and 223 nm (λ_{max} of MET and EMP respectively) were noted. The concentration of MET and EMP were calculated from above equation and result shown below table 4.

Table.4: Analysis of Tablet Formulation.

Sr. No.	Label Claim mg/tab		Amount Found mg/tab		% of Label Claim	
	MET	EMP	MET	EMP	MET	EMP
1.	500	12.5	499.81	12.415	99.96	99.32
2.	500	12.5	499.85	12.417	99.97	99.34
3.	500	12.5	499.25	12.426	99.85	99.41
				MEAN	99.9266	99.3566
				SD	0.0665	0.0472
				%RSD	0.0665	0.0475

n=3 SD-Standard deviation, %RSD- Relative standard deviation

Actual Design in Design Expert:

Run	A)Scan speed	B)Sampling Interval	1)Response Absorbance of Metformin	2)Response absorbance of Empagliflozin
1	2	2	0.761	0.588
2	1	0.1	0.753	0.513
3	3	2	0.753	0.588
4	2	1	0.754	0.512
5	3	0.1	0.754	0.513
6	2	1	**0.754**	**0.512**
7	1	2	0.754	0.588
8	1	1	0.759	0.513
9	3	1	0.764	0.512
10	2	0.2	0.757	0.511
11	2	1	0.754	0.512

Normal Plot :

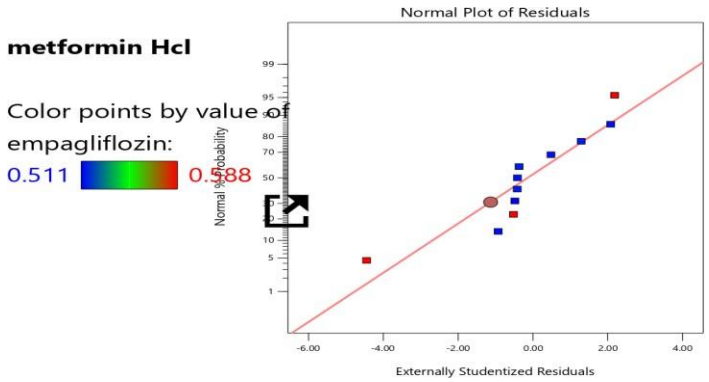

Figure.no.8: Normal Plot of Metformin

B) Counter Plate:

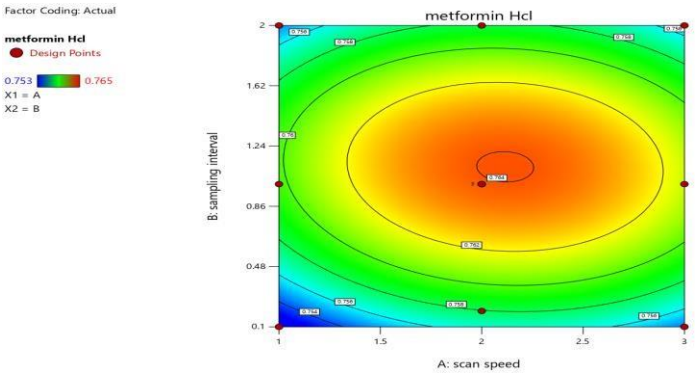

Figure.no.9: Counter Plate Graph of Metformin

C) 3D Surface of Metformin:

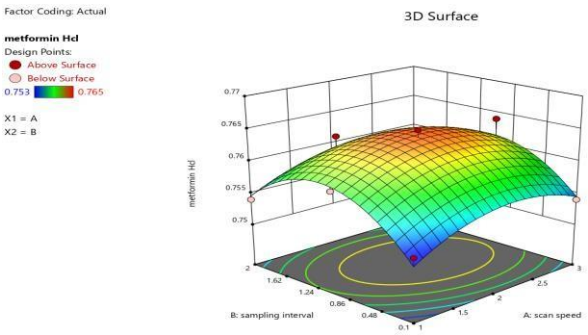

Figure.no.10: 3D Surface Graph of Metformin

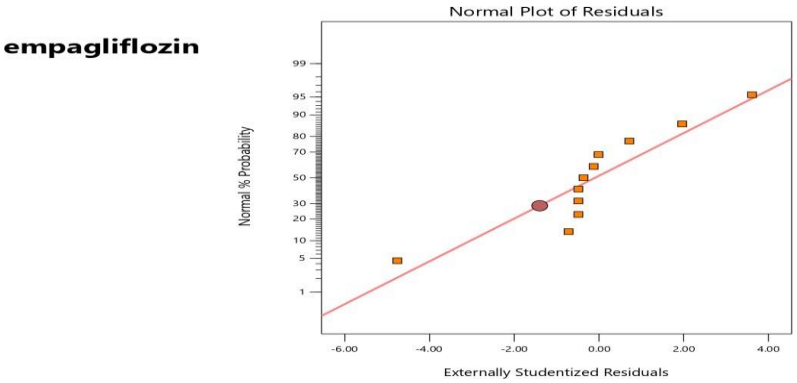

Figure.no.11: Normal Plot of Empagliflozin

B) Counter Plate

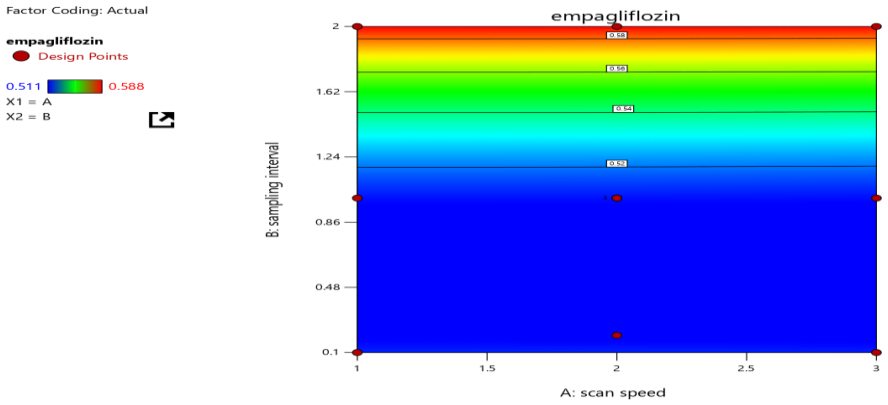

Figure.no.12: Counter Plate of Empagliflozin

C) 3D Surface of: Empagliflozin

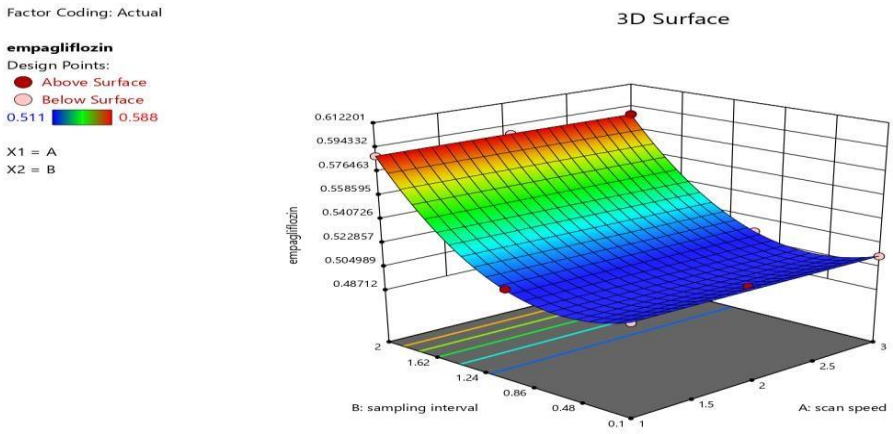

Figure.no.13: 3D Surface of Empagliflozin

RESULTS AND DISCUSSION:

Validation of Proposed method: -

a) Linearity:

MET & EMP was found to be linear in the concentration range of 2-14µg/ml at 203nm & 223nm.

b) Recovery studies:

The proposed method was validated following the guidelines set by the International Council for

Harmonization (ICH). We took pre-analyzed sample solutions with a concentration of 10 µg/ml for both MET and EMP. To these solutions, we added known amounts of standard solutions containing pure drugs, specifically 8, 10, and 12 µg/ml of MET, and 8, 10, and 12 µg/ml of EMP (from the standard stock solution).

We measured the total concentration of the resulting mixture using equations I and II. The results of these measurements are presented in Table.

$$\% \text{ Recovery} = A/B+C \times 100$$

Where,

A= Total amount of drug estimated

B= Amount of drug found on reanalyzed bases

C= Amount of pure drug added

Table.5: Results of Recovery Studies:-

Level of % recovery	**Amount of Standard added(µg/ml)**		**Amount recovered (µg/ml)**		**% Recovery**	
	MET	**EMP**	**MET**	**EMP**	**MET**	**EMP**
80	17.767	17.877	1.172	2.574	98.71	99.32
100	19.464	19.782	2.071	2.813	97.32	98.91
120	21.850	21.682	1.031	2.780	99.32	98.31
			Mean		98.45	98.84
			SD		1.025	0.5079
			% RSD		1.0411	0.5138

C) Precision: -

The precision of an analytical method refers to how closely the test results agree with each other.It is commonly expressed as either ± S.D. (standard deviation) or % RSD (relative standard deviation) of a series of measurements. In this case, we verified the precision of the method using stock solutions containing equal amounts of 10 µg/ml of MET and 10 µg/ml of EMP. To assess the consistency of the system, we repeated the assay three times for three dilutions of the same

concentration.

Each repetition was carried out every two hours on the same day (intraday precision). Additionally, we evaluated the inter-day precision by performing the assay on three separate sets of samples after 24 hours and 48 hours. You can find the results of these precision tests in Table.

Table.6: Results of Precision Studies (Intra-day): -

Sr.No.	**Amount found in µg/ml**		**% Estimation**	
	MET	**EMP**	**MET**	**EMP**
Morning	0.792	0.0587	98.91	99.91
Afternoon	0.793	0.576	99.01	98.12
	Mean		99.01	99.15
	SD		0.1050	0.9250
	%RSD		0.1060	0.9329
Evening	0.794	0.584	99.12	99.42

Table.7: Results of Precision Studies (Inter-day): -

Sr. No.	**Amount found in µg/ml**		**% Estimation**	
	MET	**EMP**	**MET**	**EMP**
Day-1	0.790	0.587	98.71	99.91
Day-2	0.789	0.580	98.62	98.71
Day-3	0.793	0.584	99.12	99.42
	Mean		98.81	99.34
	SD		0.2665	0.6033
	%RSD		0.2696	0.6072

D) Ruggedness

To determine the ruggedness of the proposed method, aliquots (small portions) from a homogeneous sample were analyzed by two different analysts. Both analysts followed the same operational and environmental conditions during the analysis. The results of this analysis are reported in Table.

Table.8: Results of Different analyst studies

Sr.No.	Amount found in µg/ml		% Estimation	
	MET	**EMP**	**MET**	**EMP**
Analyst-1	0.792	0.587	98.91	99.91
Analyst-2	0.793	0.580	99.01	98.71
	Mean		98.96	99.31
	S D		0.0707	0.8485
	%RSD		0.0714	0.8543

E) Robustness

The term "robustness" refers to the ability of a method to remain accurate and precise even when certain factors, such as different solvents, vary. To assess the robustness of the method, we performed the assay three times using different solvents while using the same equipment. The results of the same are presented in Table.

Table.9: Results of Different solvent studies:-

Session	Absorbance At		% Estimation	
	MET	**EMP**	**MET**	**EMP**
0.2 N HCl	0.790	0.586	98.71	99.82
0.05 N HCl	0.792	0.572	98.91	97.42
	Mean		98.81	98.62
	S D		0.141	1.697
	%RSD		0.1426	1.7207

G) Limit of quantitation (LOQ)

Table.10: Results of LOD & LOQ

Sr. No.	Drug Name	LOD (µg/ml)	LOQ (µg/ml)
1	Metformin	0.6033	1.1991
2	Empagliflozin	0.4012	1.215

A new drug combination containing Metformin and Empagliflozin was selected for Analytical method development as no any method was reported for its simultaneous estimation. Pure sample of Metformin and Empagliflozin were procured as gift sample from industries.

Method:

UV-Spectrophotometric method for estimation of Metformin & Empagliflozin by Simultaneous Equation Method

Since both Metformin (MET) and Empagliflozin (EMP) are soluble in 0.1 N HCl solution, we conducted the estimation by dissolving the drugs in the same solvent and diluting them accordingly. We found that MET had an absorbance peak at 203.0 nm, while EMP had an absorbance peak at 223.0 nm. This means that when one drug absorbs at its respective λ_{max}, the other drug absorbs minimally. Both drugs exhibited linearity in the concentration range of 2-14 µg/ml. We observed that the absorbance of both drugs was additive at both wavelengths. To validate the method, we applied it to a tablet formulation and found that the percentage estimation of MET and EMP was 99.92% and 98.35%, respectively. The estimated number of drugs using the proposed method agreed well with the actual estimation.

To assess the accuracy of the method, we conducted recovery studies at different levels. The % recovery for MET and EMP was found to be 99.45% and 99.84%, respectively. The percentage relative standard deviation (% RSD) values were less than 2, indicating good accuracy of the method. We also evaluated the precision of the method through inter-day, intra-day, and repeatability analysis. The % RSD values were all less than 2, demonstrating the method's precision. Furthermore, there was no statistical difference observed between operators, suggesting that the developed method was rugged and reliable.

Table.11: Validation parameters of MET and EMP

Parameter	**Metformin**	**Empagliflozin**
Linearity Range	2-14 µg/ml	2-14 µg/ml
Regression Equation (y=mx+c)	Y=0.159×-0.1632	Y=0.0563×+0.0251
Recovery (%RSD)	1.0411	0.5138
Intra-day	1.060	0.9329
Inter-day	0.2696	0.6072
Robustness	0.1426	1.7207
Ruggedness	0.0714	0.8543
LOD	0.6033	0.4012
LOQ	1.1991	1.215

CONCLUSION:

Spectroscopic method was developed according to QbD approach as per ICH Q8 (R2) guidelines. QbD approach was carried out by varying various parameters. The critical parameters were determined by using principal component analysis as well as by observation. The estimated critical parameters in Zero order spectroscopic method were sample preparation tablet, **slit width:1.0, scan speed: medium, sampling interval:1.0.** The above method was validated according to ICH Q2 (R1) guidelines. Proposed method can be used for routine analysis of in tablet dosage form as they were found to be robust and specific.

ACKNOWLEDGEMENT

Authors are thankful to Principal and management SSS. Indira College of Pharmacy, Vishnupuri, Nanded. For providing required facilities for research work.

REFERENCES:

1. Maruthur NM, Tseng E, Hutfless S, Wilson LM, Suarez-Cuervo C, Berger Z, Bolen S. Diabetes medications as monotherapy or metformin-based combination therapy for type 2 diabetes: a systematic review and meta-analysis. Annals of internal medicine. 2016;164(11),:740-751.
2. Indian Pharmacopoeia, Government of India, ministry of health and family welfare, 2010:(3): (1854).
3. Mubeen G, Khalikha N. Spectrophotometric method for analysis of metformin hydrochloride. Indian J Pharm Sci. 2009;71(1):100-2.
4. Mousumi K, Choudhury PK. HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Indian J Pharm Sci. 2009;71(3):318-20.
5. Madhukar, Prince A, Vijay KR, Sanjeeva Y, Jagadeeshwar K, Raghu PD. Simple and sensitive analytical method development and validation of metformin hydrochloride by RP- HPLC. Int J Pharm Pharm Sci. 2011;3(3):11720. 10.
6. Himal PC, Panna T, Schepdael AV. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharm J. 2014;22(5):483-7.
7. Sharmila Donepudi validated HPLC-UV method for simultaneous estimation of Linagliptin and Empagliflozin in Human plasma, Ijap, Vol10, Issue 3, 2018, 56-61.
8. Anjali Bakshi, Simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC Method, 'world Journal of Pharmacy and Pharmaceutical Sciences, Volume 7, Issue 8, 1062- 1071.
9. Mowaka S, Mohamed D. Novel contribution to the simultaneous analysis of certain hypoglycemic drugs in the presence of their impurities and degradation products utilizing UPLCMS/MS. RSC Adv. 2015;5(74):60467-81. 16.
10. Nidhi K, Jayvadan P. Development and validation of Q-absorbance ratio UV- spectrophotometric method for simultaneous estimation of canagliflozin and metformin in bulk and combined dosage form. Indo Am J Pharm Res. 2018;8:447-55. 17.
11. Wafaa AZ, Shereen M, Moataz SH. Comparative liquid chromatographic study for concurrent determination of canagliflozin and metformin in combined tablets. J Anal Methods Chem. 2017;9197230. [cited on 2018 Oct 8]. Available from: https://doi.org/10.1155/2017/9197230.
12. Nareddy PR, Naga TC. RP-HPLC method development and validation for the simultaneous estimation of metformin and canagliflozin in tablet dosage form. Int J Pharm Sci. 2015;5(4):1155-99.

13. Uttam PP, Sunil KR. A Novel Validated RP-HPLC-DAD method for the simultaneous estimation of metformin hydrochloride and canagliflozin in bulk and pharmaceutical tablet dosage form with forced degradation studies. Orient J Chem. 2015;31(3):1489-507.
14. Deepak G, Patil RN, Mangesh H. A validated stability indicating RP-HPLC method for simultaneous determination of metformin and canagliflozin in pharmaceutical formulation. World J Pharm Pharm Sci. 2015;4:631-40.
15. Sonia DS, Krishna M, Sushmitha SG, Vasantharaju SG. Stability indicating assay method development and validation to simultaneously estimate metformin hydrochloride and canagliflozin by RP-HPLC. Curr Trends Biotechnol Pharm. 2016;10(4):334-42.
16. Vinutha K, Chowdary KPR, Prasad SVUM. Development and validation of a new RP- HPLC method for simultaneous estimation of metformin hydrochloride and canagliflozin and its comparison with the reported methods. World J Pharm Pharm Sci. 2017;6(3):696-713.
17. Parag D, Animesh M. Analytical Quality by Design (AQbD): A new horizon for robust analytics in pharmaceutical process and automation. Int J pharm. 2017;5:324-37.
18. Devesh AB, Smita IR. QbD approach to analytical RP-HPLC method development and itsvalidation. Int J Pharm Pharm Sci. 2011;3(1):179-87.
19. Mohini B, Sanju N. Analytical quality by design (AQbD): New paradigm for analytical method development. Int J Dev Res. 2015;5(2):3589-99.
20. Peraman R, Bhadraya K, Padmanabha RY, Surayaprakash RC, Lokesh T. Analytical Quality by Design approach in RP-HPLC method development for the assay of etofenamate in dosage forms. Indian J Pharm Sci. 2015;77(6):751-7
21. Mayang Kusuma Dewi, Reza Pratama, Mia Arifka, Amiss Yohana Chaerunisaa. 2022."Quality by Design: Approach to Analytical Method Validation" Sciences of Pharmacy 1, no. 1:38-46.
22.N.V.V.S.S Raman, Usen Reddy Bapatu. Analytical QbD Approach to Test method development and validation in Drug substance manufacturing. 2015.