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R E S E A E C H A R T I C L E

RP-HPLC Method Development and Validation for Simultaneous Estimation of Metformin and Empagliflozin in Bulk and Pharmaceutical Dosage Forms

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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: Methanol, Phosphate buffer, Inertsil C_{18} column, Metformin and Empagliflozin

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Metformin, marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome (PCOS). Limited evidence suggests metformin may prevent the cardiovascular disease and cancer complications of diabetes. It is not associated with weight gain. It is taken by mouth. Metformin is generally well tolerated. Common side effects include diarrhea, nausea and abdominal pain. It has a low risk of causing low blood sugar. High blood lactic acid level is a concern if the medication is prescribed inappropriately and in overly large doses. It should not be used in those with significant liver disease or kidney problems. While no clear harm comes from use during pregnancy, insulin is generally preferred for gestational diabetes. Metformin is in the biguanide class. It works by decreasing glucose production by the liver and increasing the insulin sensitivity of body tissues.

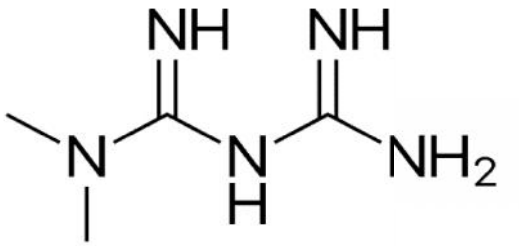

Fig 1: Structure of Metformin

Empagliflozin (trade name Jardiance) is a drug of the gliflozin class, approved for the treatment of type 2 diabetes in adults in 2014. It was developed by Boehringer Ingelheim and Eli Lilly and Company.Empagliflozin is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), and causes sugar in the blood to be excreted by the kidneys and eliminated in urine.

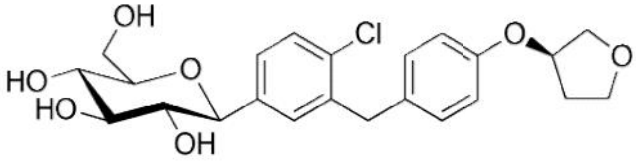

Fig 2: Structure of Empagliflozin

2. Materials and Methods

Instrumentation

HPLC Shimadzu Waters 996 LC 20 Software. UV/VIS spectrophotometer LABINDIA UV 3000+pH meter Adwa – AD 1020 Weighing machine.

Chemicals

Metformin and EmpagliflozinKH_2PO_4,Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Chromatographic Conditions:

Column	**:**Inertsil C18 column (4.6×150mm)5µ
Mobile phase ratio	**:**Phosphate buffer pH 3.0: Methanol (30:70%v/v)
Detection wavelength	**:**260 nm
Flow rate	**:** 0.8 ml/min
Injection volume	**:**10µl
Column temperature	**:** Ambient
Auto sampler temperature	**:** Ambient

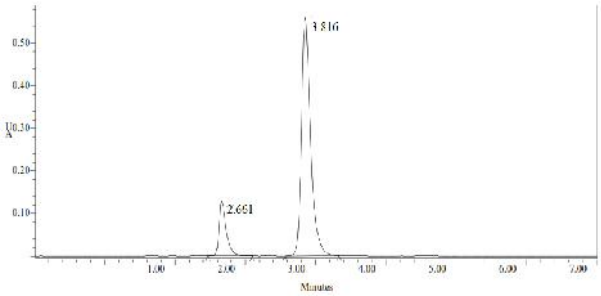

Fig 3: Optimized Chromatogram for Metformin and Empagliflozin

Observation: From the above chromatogram it was observed that the Metformin and Empagliflozin peaks are good it is final method.

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.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10mg 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.

Assay

Assay preparation of the Metformin and Empagliflozin standard and sample solution

Sample solution preparation:

1mg of Metformin and 10 mg Empagliflozin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

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

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

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

Method Validation

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.

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.

Accuracy:

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.

Linearity:

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.

Limit of Detection:

Limit of Detection: (For Metformin): 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.

Limit of Detection (For Empagliflozin): 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.

Limit of Quantification:

Limit Of Quantification (for Metformin):

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.

Limit of Quantification (for Empagliflozin):

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.

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.

3. Results and Discussions

Linearity:

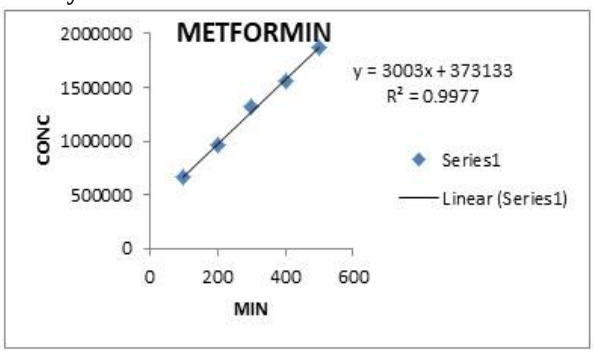

Fig 4: Calibration graph for Metformin

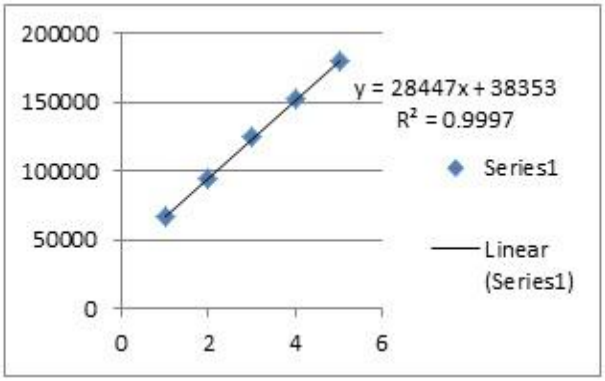

Fig 5: Calibration graph for Empagliflozin

Robustness:

Variation in Flow:

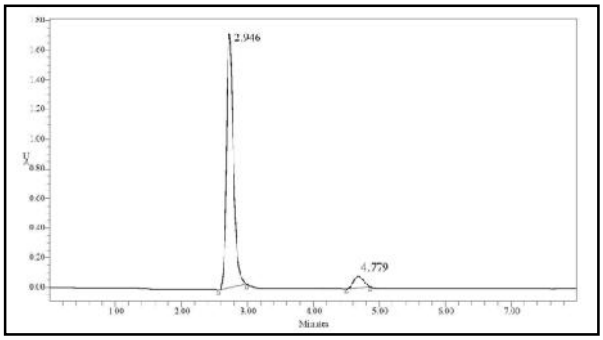

Fig 6: Chromatogram showing more flow rate 1.2ml/min

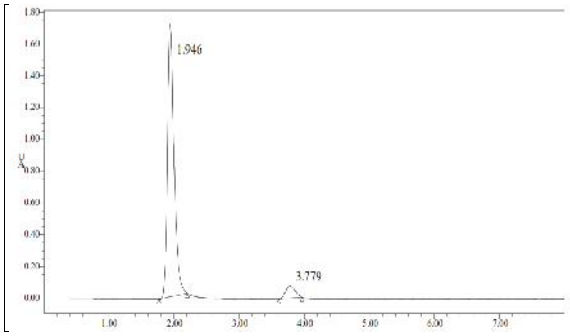

Fig 7: Chromatogram snowing more flow of 1.0ml/min

Variation in Mobile phase:

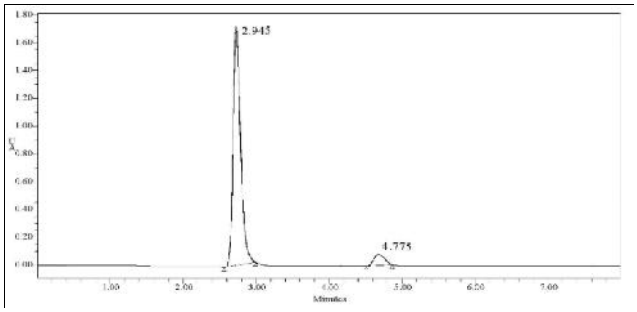

Fig 8: Chromatogram showing less organic composition

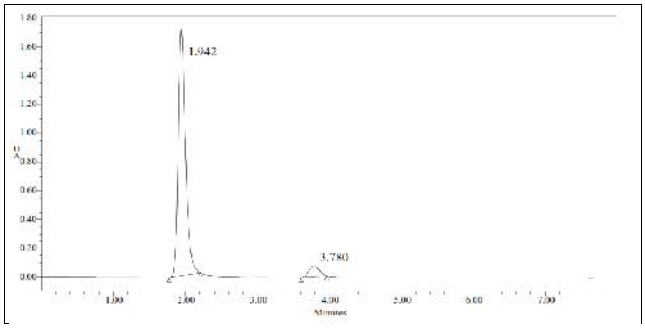

Fig 9: Chromatogram showing more organic composition

Table No 1: Results of system suitability parameters for Metformin and Empagliflozin

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Metformin**	2.5	124505	213642		1.2	4673.4
2	**Empagliflozin**	3.9	1308495	154566	6 0	1.3	6090.3

Table No 2: Results of method precession for Metformin and Empagliflozin

Injection	Area	
	Metformin	**Empagliflozin**
Injection-1	1302729	123149
Injection-2	1302947	123766
Injection-3	1303236	124271
Injection-4	1303977	124691
Injection-5	1309759	124956
Average	1304529.8	124162.7
Standard Deviation	2961.1	725.6
%RSD	0.2	0.6

Table No 3: Results of intermediate precession for Metformin and Empagliflozin

Injection	Area	
	Metformin	**Empagliflozin**
Injection-1	1300148	122487
Injection-2	1304520	122626
Injection-3	1305937	122632
Injection-4	1306476	122702
Injection-5	130871	122962
Average	1305070.2	122681.8
Standard Deviation	3061.8	174.8
%RSD	0.2	0.1

Table No 4: 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%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table No 5: Accuracy (recovery) data for Empagliflozin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table No 6: Area of different concentration of Metformin

S.No.	Linearity Level	Concentration	Area

1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table No 7: Area of different concentration of Empagliflozin

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Table No 8: Analytical performance parameters of Metformin and Empagliflozin

Parameters	Metformin	Empagliflozin
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table No 9: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Metformin	52	152	2.9
Empagliflozin	52	156	3

Table No 10: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Metformin	52	522	10.03
Empagliflozin	52	524	10.1

Table No 11: Flow Rate (ml/min) data for Metformin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table No 12: Flow rate (ml/min) data for Empagliflozin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table No 13: Change in Organic Composition in the Mobile Phase for Metformin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table No 14: Change in Organic Composition in the Mobile Phase for Empagliflozin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2

2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

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.

5. References

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