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Analytical Method Development and Validation of RP-HPLC Method For Estimation of Empagliflozin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT
In this study, researchers aimed to develop and validate a reproducible and precise Reverse Phase High-Performance Liquid Chromatography technique for accurately estimating a chemical related to empagliflozin. The primary objective was to establish a method suitable for quality control of empagliflozin batches and its impurities. To achieve this, effective chromatographic separation has done by using two mobile phases: The mixture of 0.1 % Trifluoroacetic acid in Water and Methanol with flow rate 1.2 ml/min. The chromatographic separation was done by using a Phenomenex C-18, 250 mm X 4.6 mm, 5 µm. The results indicated successful chromatographic separation and accurate quantification. The proposed method demonstrated its efficacy for quality monitoring of bulk samples containing Empagliflozin, ensuring the reliability and consistency necessary for routine quality control purposes in the pharmaceutical industry

Keywords: Empagliflozin, RP HPLC, Anti Diabetic, Trifluoroacetic acid, Methanol

1. INTRODUCTION

Empagliflozin is a sodium glucose co transporter 2 (SGLT-2) inhibitor. That help lower blood sugar in people with type 2 diabetes by preventing the kidney from reabsorbing glucose back into the bloodstream, causing it to be excreted in urine. SGLT2 inhibitors lower blood sugar independently of insulin, making them a useful option for individuals who may not respond well to insulin based therapies.[1,2]

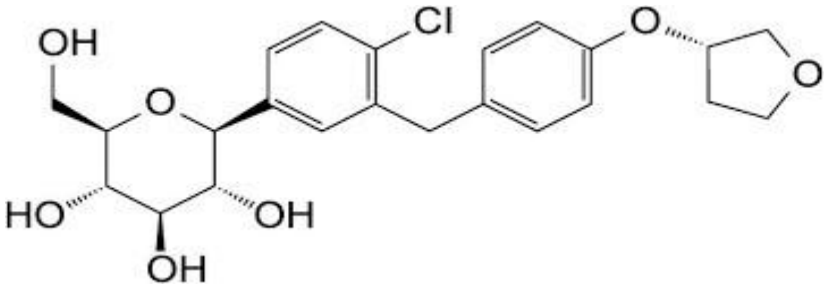

Fig 1: Structure of Empagliflozin

Table 1: General profile of Empagliflozin

Category	Anti- diabetes, type 2
Chemical Name	(2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3 yloxy] phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol
Molecular Formula	C23H27ClO7
Molecular Weight	450.91 g/mol
Description	White to off white powder.
Solubility	It is Slightly soluble in Acetonitrile and ethanol. Sparingly soluble in methanol, Very slightly soluble in water.
pKa	12.57
Melting point	152-157°C

Mechanism of Action:

The kidneys re absorb glucose through SGLT2, a key transporter in the kidneys, which accounts for 90% of total glucose reabsorption. Inhibiting this co-transport leads to increased glucosuria and decreased blood glucose levels. Empagliflozin, a potent inhibitor of renal SGLT2 transporters, lowers blood glucose levels by increasing glucosuria. It also appears to prevent heart failure, possibly through inhibition of Na+/H+ exchangers, blood pressure reduction, cardiac fibrosis prevention, and reduced pro-inflammatory adipokines.

Materials and Methods: [3]

We performed High-performance liquid chromatography (HPLC) using a Jasco instrument equipped with a manual sampler, a PDA detector, and ChromNAV CFR Chromatography Software (version 2.0, BS 4600S). A C18 column (5 µm, 250 mm × 4.6 mm) was used for the separation.

Empagliflozin were supplied by Vidisha analytical. Tablets containing Oboravo 25 mg tablet (Empagliflozin 25 mg) procured from local market which is manufactured by cipla Ltd.

Chromatographic Condition:

Table 2: Chromatographic Condition

Column	Inersil ODS 3V, 150 x 4.6 mm, 5µ
Mobile Phase	0.1% Trifluoroacetic acid (TFAA) in Water and Methanol (85:15) v/v
Flow Rate	1.2 mL/min
Injection Volume	20 µL
Wavelength	225 nm

Column Temp	35ºC
Sample Temp	10ºC
Run Time	7.0 minutes

Preparation of Solution: [4,5,6]

Preparation of Mobile phase:

Prepare mixture of 0.1% Trifluoroacetic acid (TFAA) in Water and Methanol in the ratio of 85:15 v/v respectively, mix well. Filter through 0.45µ nylon membrane disc filter. Sonicated for 15 min to degas the mobile phase.

Preparation of Diluent:

Prepare mixture of water and Methanol in the ratio of 10:90 v/v respectively, mix well.

Preparation of Blank:

Use diluent as blank.

Preparation of Standard solution:

Weighed and transferred accurately about 25 mg of Empagliflozin working standard into

50 mL clean and dry volumetric flask. Added about 30 mL of diluent, sonicate to about 15 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 5.0 mL of this stock solution to 50 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane nylon filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Empagliflozin standard solution: 50 ppm)

Preparation of Sample Solution:

Take average weight of 20 tablet. Crush the 10 tablet into mortal pestle into fine powder. Weighed and transferred crush powder equivalent to 50 mg of Empagliflozin in to 100 mL clean and dry volumetric flask. Added about 80 mL of diluent, sonicate for 30 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Further diluted above stock solution 5.0 mL of this sample stock solution to 50 mL volumetric flask make up with Diluent and mixed well. Filter the sample solution through 0.45µ membrane nylon filter. Discard first 4.0 mL of filtrate and then collected the sample. [4,5,6]

(Concentration of Sample Solution: 50 ppm)

2. CHARACTERISATION OF DRUG SUBSTANCE.

Selection of Solvent

Table 3: Drug Solubility Summary

Sr. No.	Name of Solvent	Observation	Conclusion
1	Water	Drug Particles seen after sonication	Drug was slightly soluble in water.
2	Methanol	No Drug Particles seen after sonication	Drug was found soluble in methanol.

Final Conclusion: Water: Methanol (10:90% v/v) will be used as a diluent for preparing stock solution.

Analytical Method Validation of UV Spectroscopic Method: [7]

System Suitability: System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

Specificity: Inject Blank (Diluent), standard soluton, placebo solution and sample solution.

Linearity: Linearity was evaluated in the range of 30% to 100% of the working concentration level. As the working concentration level of Empagliflozin.

Accuracy: Accuracy was evaluated three levels 30%, 50% and 100% of the working concentration level for Empagliflozin.

5) Precision:

I. Method Precision:

Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system.

II. Intermediate Precision:

Six independent sample preparation were prepared on different day and by different analyst and injected on the HPLC.

6)Robustness: This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

3. RESULTS AND DISCUSSION

A simple, precise and economic RP-HPLC method was developed and validated for estimation of Empagliflozin in bulk and tablet. The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, specificity and robustness.

Reverse Phase High Performance Liquid Chromatography Method Development and Optimization

Chromatographic Conditions:

Column	Agilent PLRP-S 100Aº (250 x 4.6mm) 10µ
Mobile Phase	Water: Acetonitrile (70:30)
Flow Rate	1 mL/min
Injection Volume	50 µL
Wavelength	225 nm
Column Temp.	25°C
Auto sampler Temp.	25°C

Table 4: Chromatographic Conditions

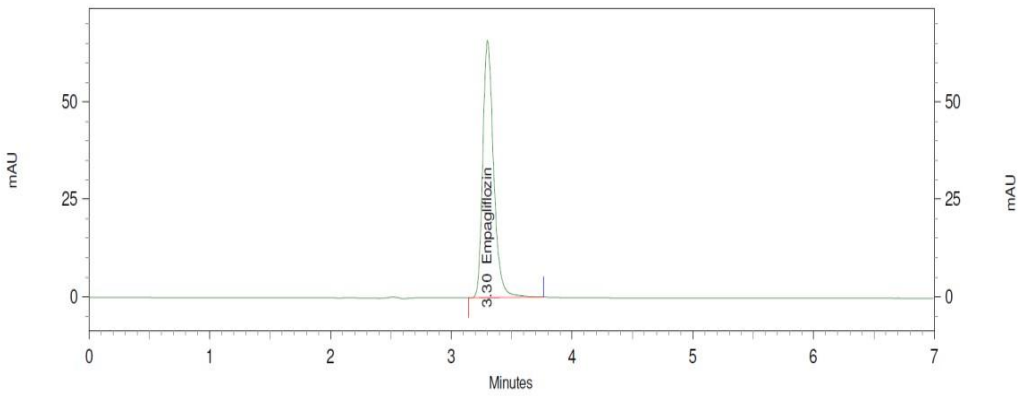

Fig. 9.10 Typical Chromatogram of Empagliflozin

Analytical Method Validation of RP-HPLC

1. System Sutability: System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

Tailing Factor	1.30
Theoretical plates	7763
Injection No.	Area
1	7362019
2	7365294
3	7361859
4	7349554
5	7360253
Mean	7359796
%RSD	0.1

Table 5: System Suitability Test of Empagliflozin

Conclusion: The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

2 Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table

Solution	Specificity data		
	Retention time (min)	Purity Match	
Blank solution	NA	NA	
Placebo solution	NA	NA	
	3.30	Purity angle	Purity threshold

Standard solution		5.84	7.35
Sample solution	3.29	4.98	6.14

Table 6: Specificity (Identification and Interference)

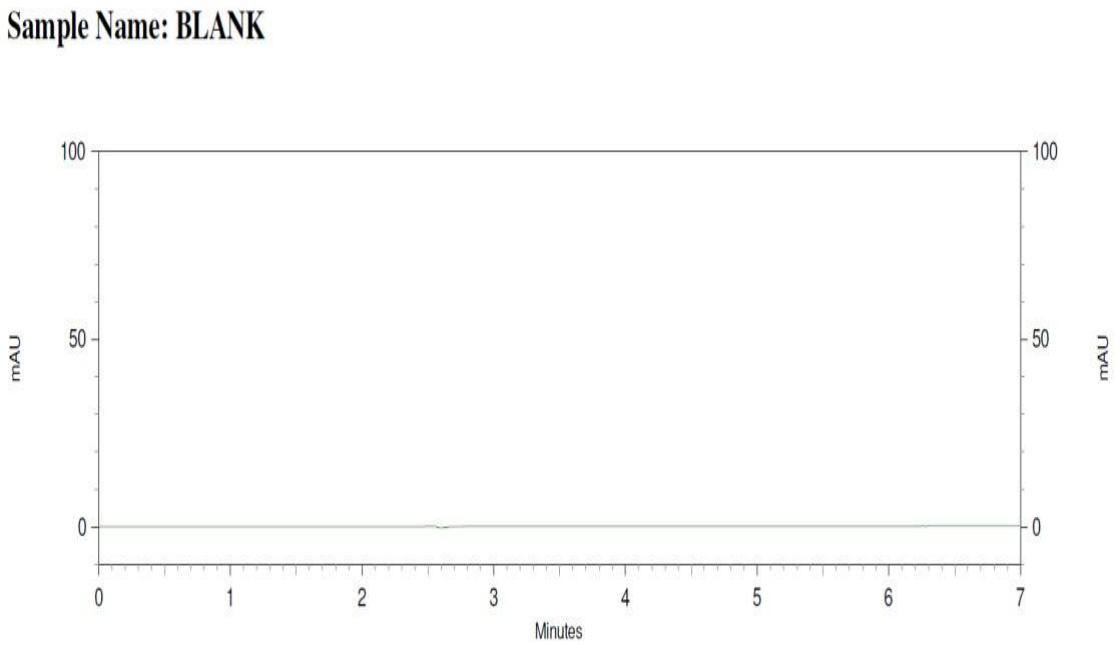

Fig 2: Chromatogram of Blank

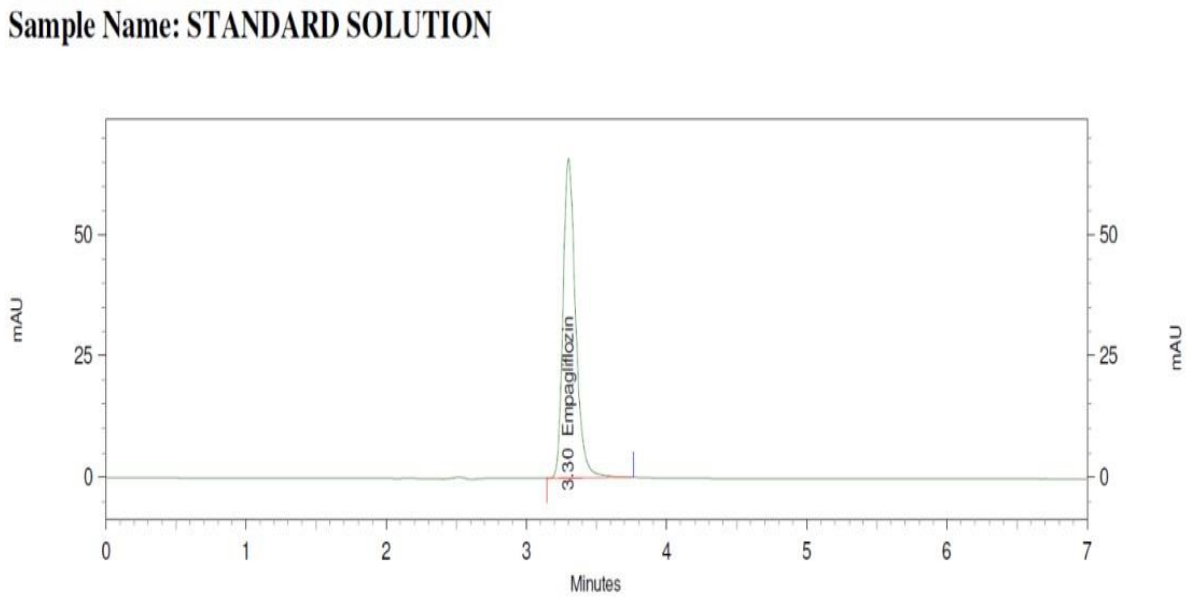

Fig 3: Chromatogram of Standard

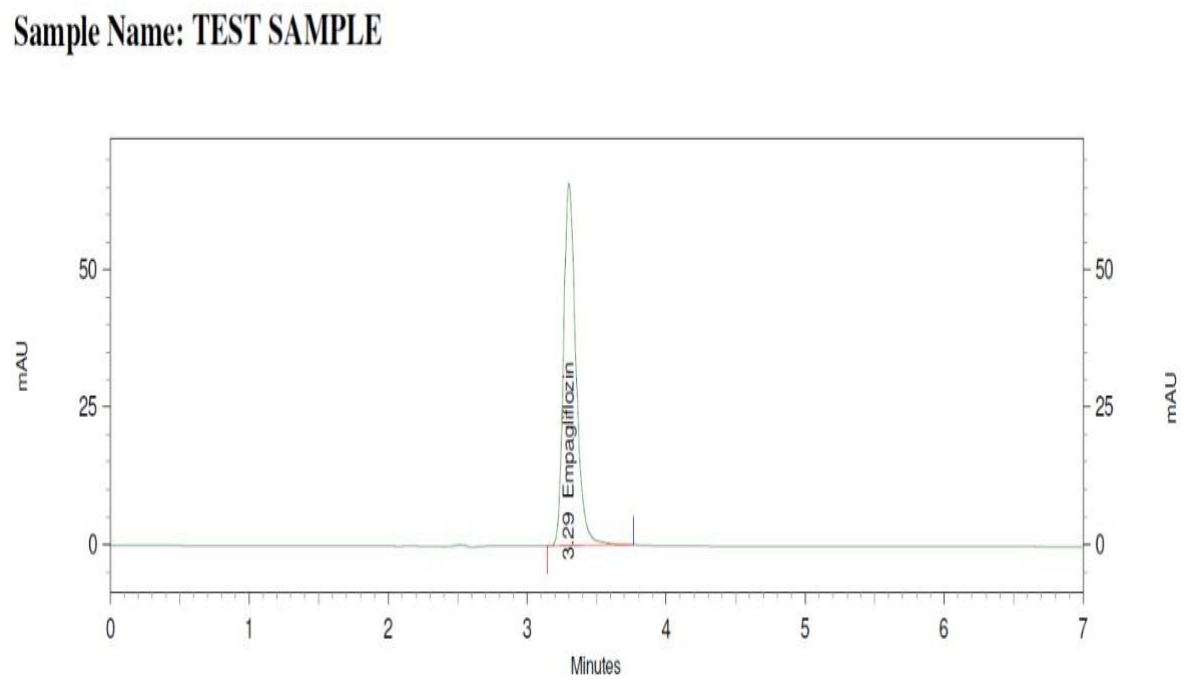

Fig 4: Chromatogram of Sample

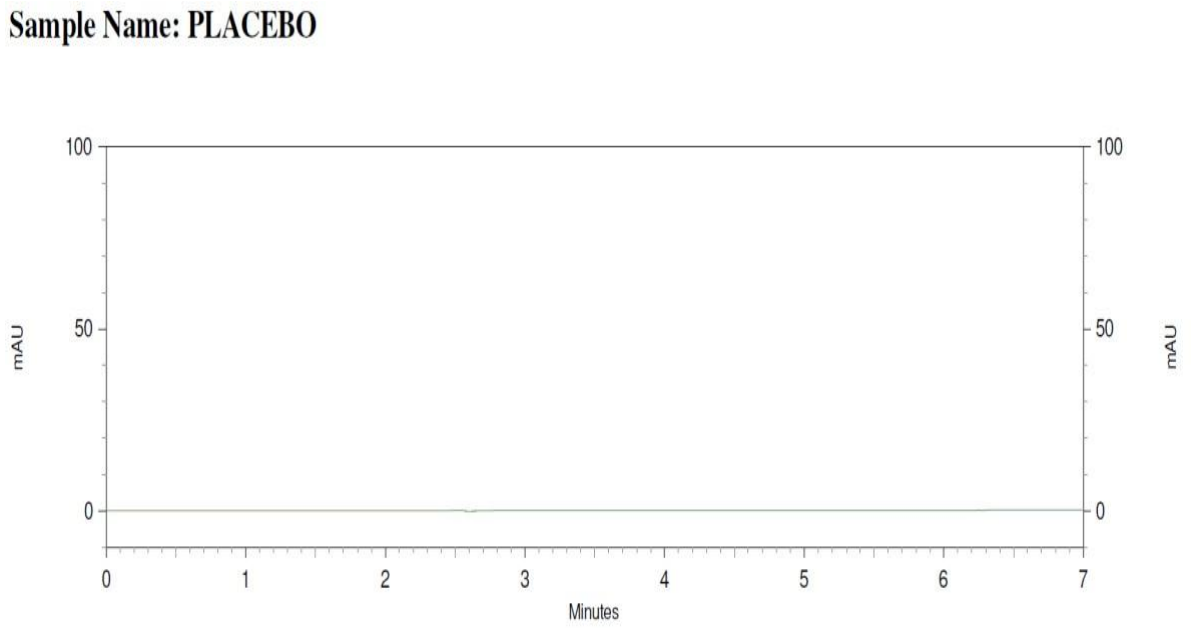

Fig 5: Chromatogram of Placebo

4. CONCLUSION:

The data demonstrates that retention time in standard and sample is same for Empagliflozin peak.

The data demonstrates that there is no interference in blank and placebo at the retention time of Empagliflozin peak. Peak Purity match in both chromatograms obtained from Standard and Sample solution.

3. **Linearity**:

Linearity was evaluated in the range of 50 % to 150 % of Empagliflozin for working concentration. The working concentration of Empagliflozin in solution is 50 µg/mL. The data summarized in Table.

Table 7: Linearity

Level	Conc (µg/mL)	Area	Mean
50%	10.0	3625961	3623579
		3634182	
		3610593	
75%	15.0	5579854	5582564
		5582641	
		5585196	
100%	20.0	7360529	7365436
		7364183	
		7371597	
125%	25.0	9236521	9239566
		9242519	
		9239658	
150%	30.0	11025964	11035028
		11032591	
		11046529	
Corr. Coeff			0.9993
Intercept			18032
Slope			146293
% Y-intercept			0.24

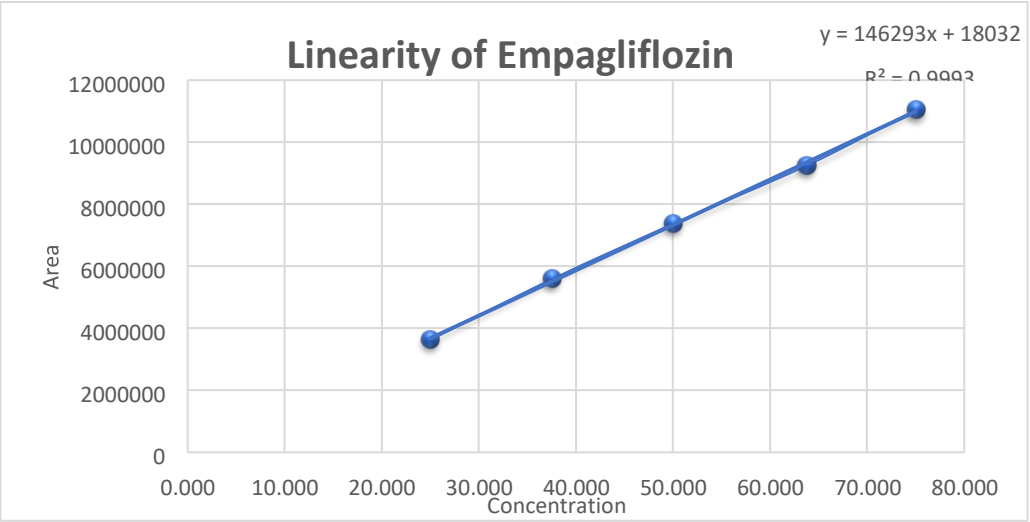

Fig 6: Linearity plot of Empagliflozin Conclusion:

The data shows that system suitability is fulfilled.

The data shows that the response is found to be linear.

Co-relation coefficient (r^2)was found 0.9998.

4. Accuracy (Recovery):

Evaluated accuracy from 50% to 150% of Empagliflozin tablet, working concentration level. Each level prepared in triplicates.

Table 8:% Recovery for Empagliflozin

Level (%)	Empagliflozin Added Conc (µg/mL)	Empagliflozin Recovered conc	Area	% Recovery	Mean % Recovery
50	25.24	25.07	3705851	98.35	99.35
	25.32	25.23	3765889	99.16	
	25.18	25.24	3766524	100.54	
100	50.24	50.08	7389525	99.21	99.68
	50.38	50.13	7405968	98.75	
	50.44	50.66	7602597	101.08	
150	75.10	74.96	11025635	99.54	100.12
	75.24	75.24	11128481	100.00	
	75.38	75.62	11269237	100.80	

Conclusion: The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%102.0% and individual recovery for 50% to 150% is in the range of 95.0% - 105.0%.

5) Precision:

1. Method Precision: Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system.

Table 9: Method precision

Sample	Area	% Assay
Sample 1	7253201	98.70
Sample 2	7196524	97.97
Sample 3	7226598	98.27
Sample 4	7165200	97.21
Sample 5	7236591	98.67
Sample 6	7192568	97.77
Mean		98.10
STD DEV		0.5741
% RSD		0.585

Conclusion:

The data shows that system suitability is fulfilled.

The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

2. Intermediate Precision: six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 10: Intermediate Precision

Sample	Area	% Assay
Sample 1	7250419	98.71
Sample 2	7194105	97.52
Sample 3	7300597	99.27
Sample 4	7214853	98.03
Sample 5	7156208	97.38
Sample 6	7236590	98.14
Mean		98.18

STD DEV	0.7155
% RSD	0.729

Table 11: Intermediate Precision pool Data

Parameter	Method Precision (Analyst-I)	Intermediate Precision (Analyst-II)
HPLC NO.	AD/HPLC-022	AD/HPLC-018
Column No.	HPLC-21	HPLC-08
Sample No.	%Assay	
1	98.70	98.71
2	97.97	97.52
3	98.27	99.27
4	97.21	98.03
5	98.67	97.38
6	97.77	98.14
Mean	98.10	98.18
Mean of Precision % Assay	98.14	
Absolute Mean difference % assay	0.6	

Conclusion:

The data shows that system suitability is fulfilled.

The data shows that % Assay is of six samples is not more than 2.0

The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

6) Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table 12: Robustness for Empagliflozin

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	7253201	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.3 ml/min	7145961	-1.5
	1.1 ml/min	7132594	-1.7
Change in wavelength (±2 nm)	227 nm	7128968	-1.7
	223 nm	7325029	1.0

Conclusion:

System suitability criteria were fulfilled.

The difference of % assay value in each modified condition is within acceptance criteria.

5. CONCLUSION:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Empagliflozin tablet formulation. HPLC Agilent 1260 Infinity II with Inertsil ODS-3V (150 mm X 4.6 mm), 5µm column and UV/PDA detector with Openlab EZ Chrome workstation Software was used for the study. The standard and sample solution of Empagliflozin were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

The mobile phase that was found to be most suitable was 0.1 % Trifluoroacetic acid water, and

Methanol the wavelength 225 nm were selected for the evaluation of the chromatogram of

Empagliflozin respectively. The selection of the wavelength was based on the λmax obtained by UV scanning of standard laboratory mixture in water: methanol. This system gave good resolution and optimum retention time with appropriate tailing factor (<2).

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by procedure described under Materials and methods. It gave accurate, reliable results and was extended for estimation of drugs in tablet formulation.

The results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their formulation.

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