EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 10
117-125
Year: 2024

A COMPREHENSIVE AND SYSTEMATIC REVIEW ON POTENT ANTIDIABETIC DRUG: EMPAGLIFLOZINE

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Article Received on 12/08/2024 **Article Revised on 02/09/2024** **Article Accepted on 22/09/2024**

ABSTRACT
The study involves quantitative analysis of 2 different brands (samples) of EMPAGLIFLOZIN (25 mg) tablets used in Vadodara (India), using and High-Performance Liquid Chromatographic methods, The mobile phase was prepared by mixing Methanol and Phosphate buffer (pH-5.0) in ratio 50:50%v/v. The prepared mobile phase was sonicated and filtered through 0.45µm membrane filter. There is no any official method available so as per EU criteria this method is validated as well as performed. Instrument- SHIMADZU HPLC -LC-2030, Software-Lab Solutions used for performing the analysis. at flow rate of 1mL/min and detection wavelength of 225nm. There is no official standard of Empagliflozin is available so here 25 mg of API takes as a standard, in the analysis for sample- 1 RT found 4.413 for 2nd sample RT time found- 4.290, both the samples found suitable for market as well as complies the %label claim.

KEYWORDS: Empagliflozin, Api, Tablets, Marketed Formulation, Hplc.

1. INTRODUCTION[1-2]

1.1 EMPAGLIFLOZIN

- Type 2 diabetes mellitus (T2DM) has become a global pandemic. The age-standardized prevalence of diabetes in adults has increased in most countries since 1980 and along with population growth and ageing, this has led to a near quadrupling of the number of adults with diabetes worldwide.
- Empagliflozin is an antidiabetic agent used in adult patients with type 2 diabetes mellitus. It was FDA-approved in 2014. Empagliflozin can be used as a single agent or as a combination agent with other antidiabetic products.

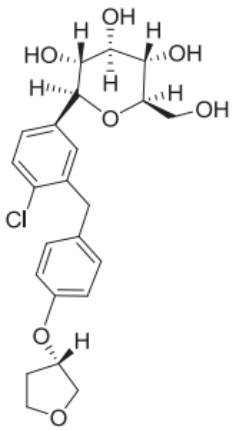

Fig 1: Chemical Structure of Empagliflozin.

- Empagliflozin Mainly work on SGLT2-inhibitos.
- SGLT2-inhibitos function not only as antidiabetics for normalizing blood glucose, but also have diuretic and hypotensive actions and resolve salt-sensitive hypertension, which plays an important role in diabetes. Therefore, SGLT2-inhibitos strongly prevent heart and renal failure.
- The mechanism by which empagliflozin reduces BP

has yet to be fully elucidated but may be related to improved glucose control, weight loss, volume contraction due to osmotic diuresis, and improved arterial stiffness.

2. DRUG PROFILE OF EMPAGLIFLOZIN[3]

Table 1: Drug Profile of Empagliflozin.

Sr No	Name	Empagliflozin
1)	IUPAC	(2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy] phenyl} methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol
2)	Class	SGLT2
3)	Category	Anti-Diabetic
4)	CAS No.	864070-44-0
5)	Molecular Formula	$C_{23}H_{27}ClO_7$
6)	Molecular Weight	450.91 g/mol
7)	Appearance	white to yellowish powder
8)	physical state	Solid
9)	Solubility	soluble in water, slightly soluble in acetonitrile and ethanol, sparingly soluble in methanol and practically insoluble in toluene
10)	pKa	PKa (Strongest Acidic) 12.57 pKa (Strongest Basic) -3
11)	Melting Point	194.0°C
12)	Partition coefficient (log P)	1.79

3. LITRATURE REVIEW

Table 2: UV method of Empagliflozin.

Sr No.	Title	Description	Ref
1.	Spectrophotometric Method Development and Validation of Empagliflozin in Active Pharmaceutical Ingredient and Tablet Dosage Form	**Solvent-** Methanol **λmax** – 238.5 nm **Linearity-** 2-10 µg/ml	[4]
2.	Development and Validation of Simple UV- Spectrophotometric Method for the Determination of Empagliflozin	**Solvent-** water: methanol (9.0:1.0 %v/v/v) **λmax -** 224 nm **Linearity-** 1-3 µg/ml	[5]
3	Novel UV and Visible Spectrophotometric methods for the analysis of Empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulation	**Solvent-** Double distilled water **λmax -** 247 nm **Linearity-** 5-30 µg/ml	[6]
4	Method development and validation for Estimation of Empagliflozin by UV spectrophotometry in human plasma	**Solvent-** methanol: acetonitrile (50:50%v/v) **λmax -** 223 nm **Linearity-** 6-14 µg/ml	[7]
5	Novel UV Spectrophotometer Methods for Quantitative Estimation of Empagliflozin (EMPA) and Linagliptin (Lina) Using Mixed Hydrotropy Solubilization	**Solvent-** 2M ammonium acetate: 2M sodium citrate and (50:50% W/W) **λmax -** 240 nm **Linearity-** 10-50 µg/ml	[8]
6	Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs and combined dosage form	**Solvent-** Methanol **λmax –** EMP-234nm MET- 272 nm **Linearity-** **EMP**- 5-25µg/ml **MET**- 2-12µg/ml	[9]
7	Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs	**Solvent-** Methanol **λmax –** **EMP**-224 nm **MET**-230 nm **Linearity** **EMP**- 1-3 µg/ml **MET**- 10-50 µg/ml	[10]

8	Development and validation of UV spectrophotometric method for simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and pharmaceutical dosage form	**Solvent-** Methanol **λmax –** **EMP**-276 nm **LIN**-293 nm **Linearity** **EMP**- 5-80 µg/ml **LIN**- 5-80 µg/ml	[11]
9	Development And Validation Of Q-Absorbance Ratio UV Spectrophotometric Method for The Simultaneous Estimation of Metformin and Empagliflozin in Bulk And Pharmaceutical Dosage Form	**Solvent-** Methanol **λmax –** **EMP**-258 nm **MET**- 235nm **Linearity** **EMP**- 10-50µg/ml **MET**- 3-11µg/ml	[12]

Table 3: HPLC method of Empagliflozin.

Sr no.	Title	Description	Ref
1	Empagliflozin: HPLC based analytical method development and application to pharmaceutical raw material and dosage form	**Mobile Phase-** 0.1% trifluoroacetic acid solution and acetonitrile (70:30 % v/v) **Stationary phase-** Hypersil GOLD C18 column (250 × 4.6 mm, 5 µm pore size). **λmax** – 237 nm **Flow Rate: -** 0.8 mL min **Retention time** –5.824 min **Linearity**– 0.025-30 µg/ml	[13]
2	Stability-indicating HPLC-DAD method for the determination of empagliflozin	**Mobile Phase-** Methanol/ acetonitrile/0.1%OPA (75:20:5%v/v/v). **Stationary phase-** Hypersil GOLD C18 column (250 × 4.6 mm, 5 µm pore size). **λmax** – 222 nm **Flow Rate: -** 1.0 mL min **Retention time** –2.54 min **Linearity**– 10-50 µg/ml	[14]
3	Development and Validation of Stability Indicating RP-HPLC Method for Empagliflozin	**Mobile Phase-** Methanol: Water (70:30% v/v) **Stationary phase-** C18 column (25 cm × 4.6 mm,5µm) **λmax** – 224 nm **Flow Rate: -** 1.0 mL min **Retention time** –2.54 min **Linearity**– 2-14 µg/mL	[15]
4	RP-HPLC Method for Quantification of Empagliflozin in Pharmaceutical Formulation	**Mobile Phase-** Methanol: Water (70:30% v/v) **Stationary phase-** e C18G (250 x 4.6 mm i.d.,5µ) **λmax** – 224 nm **Flow Rate: -** 1.0 mL min **Retention time** –6.2 min **Linearity**– 10-90 µg/mL	[16]
5	HPLC Method Development for the Estimation of Empagliflozin in Bulk and Pharmaceutical Formulation	**Mobile Phase-** Water: ACN (55:45 %v/v) **Stationary phase-** e C18G (250 x 4.6 mm) **λmax** – 225 nm **Flow Rate: -** 1.0 mL min **Retention time** –3 min **Linearity**– 2- 12 µg/mL	[17]
6	Development of an HPLC-UV Method to Assay Empagliflozin Tablets and Identification of the Major Photoproduct by Quadrupole Time-of-Flight Mass Spectrometry	**Mobile Phase-**methanol, acetonitrile and purified water (60:5:35 % v/v) **Stationary phase** - C18 column (250 × 4.6 mm i.d., particle size 5 µm) **λmax** – 225 nm **Flow Rate: -** 1 ml/min	[18]

		Retention time – 7.65 min **Linearity** – 5-120 µg/ml	
7	Development and validation of a novel stability-indicating RP-HPLC Method for the determination of empagliflozin in bulk and Pharmaceutical dosage form	**Mobile Phase**-methanol and acetonitrile water (50:50 % v/v) **Stationary phase** - Intersil C18 (150mmx4.6mm, 5 µm) **λmax** – 265 nm **Flow Rate: -** 1 ml/min **Retention time** – 2.18 min **Linearity** – 50-150 µg/ml	[19]
8	Novel Simplified Analytical Method for Stress Degradation Study of Empagliflozin an Oral Anti-diabetic Agent by RP-HPLC Method	**Mobile Phase-** Acetate buffer: Acetonitrile in a ratio of (60:40% v/v) **Stationary phase** - ORBAxC18 (250 x 4.6mm, 5µm particle size) **λmax** – 232 nm **Flow Rate: -** 1 ml/min **Retention time** – 2.57 min **Linearity** – 10-120 µg/ml	[20]
9	Stability Indicating Simultaneous Estimation of Metformin and Empagliflozin in Pharmaceutical Tablet Dosage form by RP-HPLC	**Mobile Phase-** Buffer: Acetonitrile taken in the ratio (45:55% v/v,) **Stationary phase-** Kromasil C18 column (250mm×4.6mm, 5µm particle size) **λmax** – 226 nm **Flow Rate: -** 1.1 ml/min **Retention time** – MET- 2.182 min EMP-2.908 min **Linearity** – MET-125-750 µg/ml EMP- 3.12-18.75 µg/ml	[21]
10	A novel HPLC method for the simultaneous determination of empagliflozin and Dapagliflozin: Development, validation, robustness testing and greenness assessment	**Mobile Phase-** Water and ACN (30:70%v/v) **Stationary phase-** Kromasil C18 column (250mm×4.6mm, 5µm particle size) **λmax** – 230 nm **Flow Rate: -** 1.2 ml/min **Retention time** – DAP- 3.25 min EMP-1.75 min **Linearity** – 2.5-7.5 µg/ml	[22]
12	RP-HPLC method development and validation for the studies of sodium-glucose co-transporter 2 (sglt2) and dipeptidyl peptidase 4 (dpp-4) inhibitors empagliflozin and linagliptin in pharmaceutical dosage form.	**Mobile Phase-** ammonium acetate buffer (0.770 g per 1000 mL) as well as acetonitrile in a (65:35%v/v) **Stationary phase-** ODS column with measurements of 250 mm in length and 4.6 mm in diameter, and a 5 µm particle size **λmax** – 210 nm **Flow Rate: -** 1 ml/min **Retention time** – LIN- 4.056 min EMP-2.052 min **Linearity** – 4.0–24.0 µg/mL	[23]
11	Analytical quality by design based on design space in reversed-phase-high performance liquid chromatography analysis for simultaneous estimation of metformin, linagliptin and empagliflozin.	**Mobile Phase-** 0.043 M potassium dihydrogen orthophosphate buffer premixed with 0.05%v/v TEA (buffer pH 3.79 adjusted using orthophosphoric acid): methanol (34.4:65.6, %v/v/v) **Stationary phase-** Thermo Hypersil octa decyl silane (250 mm × 4.6 mm, 5 µm **λmax** – 225 nm	[24]

		Flow Rate: - 1.0 ml/min **Retention time** – MET- 2.787 min EMP-5.177 min LIN-3.883 min **Linearity** – MET-0.1-600 µg/ml EMP & LIN – 0.05-50 µg/ml	
11	Rp-Hplc Method Development for Simultaneous Estimation Of Empagliflozin, Pioglitazone, And Metformin in Bulk And Tablet Dosage Forms	**Mobile Phase-** orthophosphoric acid buffer and acetonitrile (30: 70 % v/v) **Stationary phase-** ACE C18 – (250 mm x 4.6 mm),5 µm **λmax** – 230 nm **Flow Rate: -** 0.5 ml/min **Retention time** – MET- 2.0 min EMP-3.2 min PIO-2.6 min **Linearity** – PIO-10-100 µg/ml MET -4-40 µg/ml EMP – 10-100 µg/ml	[25]
12	Development and Validation of a Stability-Indicating HPLC Method for Empagliflozin and Linagliptin in Tablet Dosage Form.	**Mobile Phase-** 0.1% orthophosphoric acid and acetonitrile (60:40 % v/v) **Stationary phase-** C-18 column [BDS 250 mm × 4.6 mm, 5 µm]5 µm **λmax** – 230 nm **Flow Rate: -** 1 ml/min **Retention time** – LIN- 4.056 min EMP-2.052 min **Linearity** – LIN-12.5-75 µg/ml EMP –25-150 µg/ml	[26]

Table 4: HPTLC method of Empagliflozin.

Sr no.	Title	Description	Ref
1	Stability Indicating HPTLC Method for Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Formulation	**Mobile Phase** Methenol :Toulene: ethylacetate (2:4:4 % v/v/v) **Stationary phase**: Precoated silica plates coated with 0.2 mm lay- ers of silica gel 60 F254 **λmax** – 254 nm **Concentration range-** EMP - 0.2 -1.2 µg per band LIN-0.1-0.6 µg per band	[27]
2	Development and validation of high-performance thin layer Chromatographic method for estimation of metformin Hydrochloride and empagliflozin in combined tablet dosage Form using quality by design approach	**Mobile Phase- acetonitrile**: Toluene: 3% Ammonium Acetate in Methanol: Ethyl acetate: Ammonia (3: 5: 2: 0.4 % v/v/v/v) **Stationary Phase**: aluminium backed pre-coated with silica gel 60F254 **λmax**-230nm **Concentration range-** 500-2500 ng/band	[28]

Table 5: LC-MS method of Empagliflozin.

Sr no.	Title	Description	Ref
1	LC–MS/MS Determination of Empagliflozin and Metformin	**Stationary phase**- C18 column (50 mm × 2.1 mm, 1.7 µm) **Mobile Phase**: 0.1% aqueous formic acid: acetonitrile (75:25, % v/v) **Flow rate**: 0.2 mL / min **Detection:** Electrospray ionization by monitoring the transition pairs (precursor to prod- uct ion) of m/z 451.04–71.07 for empagliflozin and m/z 130.11–71.14 for metformin in the positive mode. The validation parameters were acceptable over concentration ranges of 5–1,000 ng mL−1 and 50–25,000 ng mL−1 for empagliflozin and metformin, respectively.	[29]

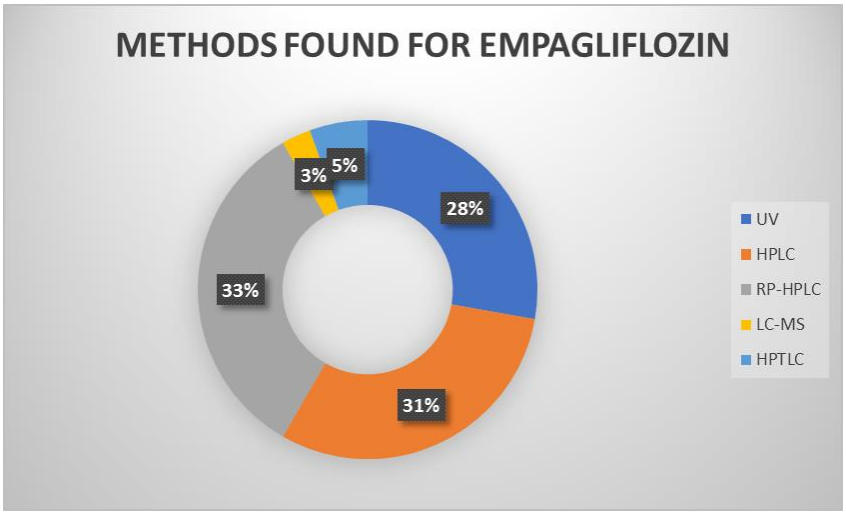

Fig-2: Methods found for Empagliflozin.

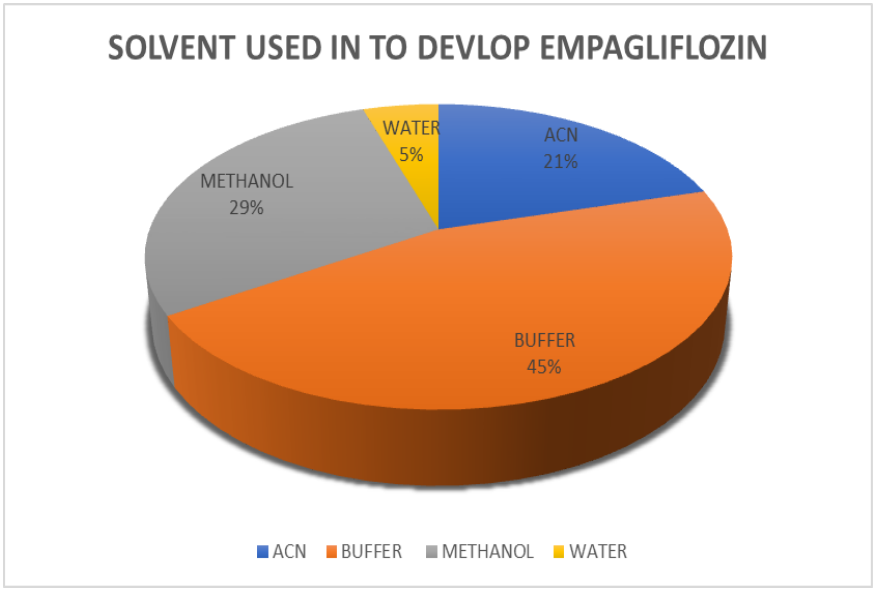

Fig-3: Solvent Used in To Develop Empagliflozin.

4. Quantification of Empagliflozin Tab. 25 mg by RP-HPLC method

- Although There are many methods available to perform Comparative study.
- But HPLC is known for its Accuracy and precision so this combative study done by using SIMADZU LC- 20 AD HPLC.

Instrument Details

- HPLC Shimadzu P series integrated HPLC was equipped with a quaternary gradient unit, a LC-20 AD solvent delivery unit, DGU20AR degassing unit, detector, a CTO10ASVP column oven, SPD-M40 PDA detector and a SIL-20AC programmable auto sampler controlled by LAB SOLUTION software. The Shimpack ODS C18 column 25 cm (4.6 mm x

250mm, 5 um) was used as a stationary phase. For filtration of solution, a Nylon-66 membrane filter was used.

- Two Different Sample of Empagliflozin Taken from Pharmaceutical shop both contain 25 mg of Empagliflozin. For authenticity of tablets bill was taken. (VADODARA).

5. Analysis Study

- Avg wait of different samples are given below.

Table 6: Showing the Average Weight of Tablets from Different Brands.

Samples	Weight (mg)
SAMPLE A	25.1
SAMPLE B	25.3

Preparation of mobile phase

The mobile phase was prepared by mixing Methanol and Phosphate Buffer) in ratio 50:50%v/v. The prepared mobile phase was sonicated and filtered through 0.45µm membrane filter.

Preparation of standard stock solution

- An accurately weighed 10.0mg of EMPA was transferred in a 10.0mL volumetric flask, dissolved in sufficient quantity of diluent to prepare a standard stock solution of 1000µg/mL of EMPA. The working standard solution of 50µg/mL was prepared by appropriate dilution of the stock solution with mobile phase.
- 100ml of the mobile phase was measured and added to each of the volumetric flask, and was put on to a sonicator for five minutes, for the drug molecules to dissolve.
- After sonicating for five minutes, the solutions were then filtered through a filter paper into clean beakers.
- 10ml of each filtrate was taken and put into different 100ml volumetric flask, and the mobile phase was added to make up the volume.
- From the above solutions, small portion of each was then put into different chromatographic sample vial, and the vials were put into the machine at different locations**.**
- The Peak area obtained for prepared concentration of different brands of Empagliflozin Shows below.

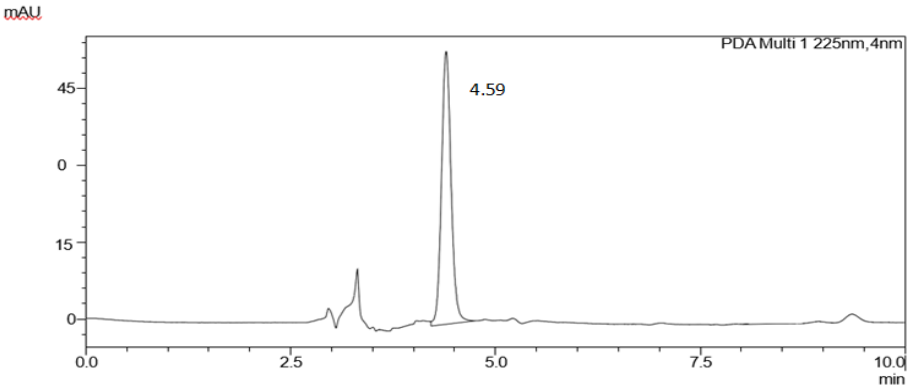

Fig 4: Standard Chromatogram of EMPA (API,25 mg)

Table 7: Peak Table for API.

Peak#	Ret. Time	Area	Height	Theoretical Plates/meter (USP)
1	4.59	691744	47822	46174
Total		691744	47822	46174

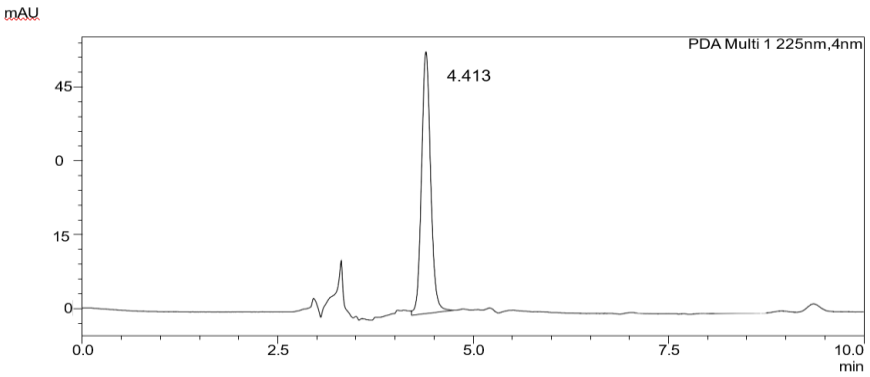

Fig-5: Chromatogram of Type -A Tablet of EMPA 25 mg.

Table 8: Peak Table for Sample A.

Peak#	Ret. Time	Area	Height	Theoretical Plates/meter (USP)
1	4.413	690464	47712	46147
Total		690464	47712	46147

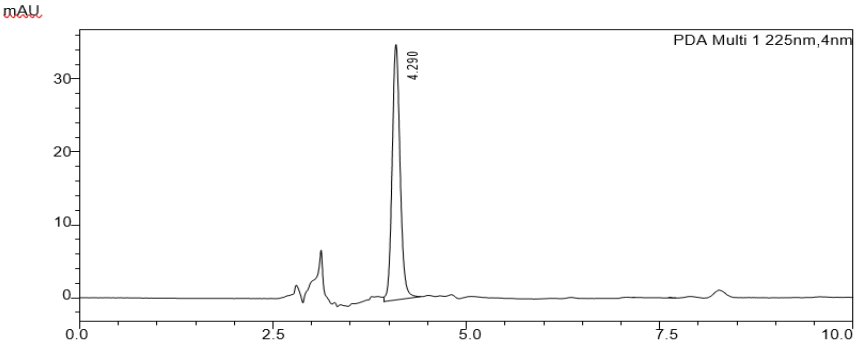

Fig- 6: Chromatogram of Type -B Tablet of EMPA 25 mg.

Table 9: Peak Table for Sample B.

Peak#	Ret. Time	Area	Height	Theoretical Plates/meter (USP)
1	4.290	625387	44417	50795
Total		625387	44417	50795

- Empagliflozin was found to be highly soluble in methanol and Phosphate Buffer. Using these solvents working standard solutions were prepared of desired concentration for RP-HPLC estimation of Empagliflozin. The mean percentage amounts of Empagliflozin estimated from tablet formulation using RP-HPLC method was found to be 96%
- .%Label claim is within the limit of the range provided by the EU guideline, the result of estimate of Empagliflozin given below

Table 10: RESULT AND DISCUSSION.

Sample solution	Concentration (mg/ml)	Peak Area	% Content	Content (mg)
A	0.014	690464	100.61	25.1
B	0.0.12	625387	97.96	24.2

6. CONCLUSION

It can thus conclude that all the brands A, B, are within limit as laid down by EU and HPLC method. Both brands of Empagliflozin successfully comply limit of EU. And suitable for market Usage.

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