

## Development and validation of Analytical Method for Simultaneous Estimation of Empagliflozin and Linagliptin in Tablet Dosage Form.

### ABSTRACT:

This study outlines the establishment and evaluation of a straightforward, economical, as well as dependable UV-based analytical method for the assessment of Empagliflozin as well as Linagliptin both pure compounds and tablet dosage forms. Ethanol served as the solvent, with the maximum absorbance wavelengths identified at 222.80 nm for Empagliflozin and 294 nm for Linagliptin. The method underwent thorough validation as per ICH guidelines confirming its suitability for routine pharmaceutical analysis.

A linear response was observed within ranging between 2 to 16 µg/mL, showing high R-value ( $R^2 = 0.998$ ), which confirmed a consistent relationship between concentration and absorbance. Precision was evaluated through intraday and interday analyses at 10, 20, and 30 µg/mL, along the percent RSD values falling within permissible range, demonstrating the analytical precision and reliability. Recovery studies at low, medium, and high concentrations (50%, 100%, and 150%) demonstrated the accuracy of the method, with recovery rates consistently falling between 98% and 102%, highlighting the method's reliability.

Sensitivity studies provided LOD of Empagliflozin and Linagliptin 2.108 µg/ml and 1.517 µg/ml. And a limit of quantification (LOQ) of Empagliflozin as well as Linagliptin 0.528 µg/ml and 0.424 µg/ml respectively. Robustness testing, conducted by applying intentional, minor changes to experimental conditions such as solvent composition and detection wavelength, showed minimal influence on the results, confirming the method's stability.

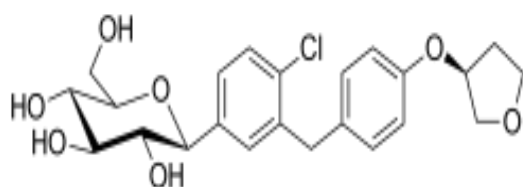
In conclusion, the developed UV-spectrophotometric method demonstrates excellent accuracy, precision, sensitivity, robustness, and cost-efficiency, making it highly suitable for routine quantitative analysis of Empagliflozin and Linagliptin in both volume as well as marketed dosage forms.

**KEYWORDS:** Empagliflozin, UV-Spectrophotometry, ICH Guidelines, Validation, Pharmaceutical Analysis.

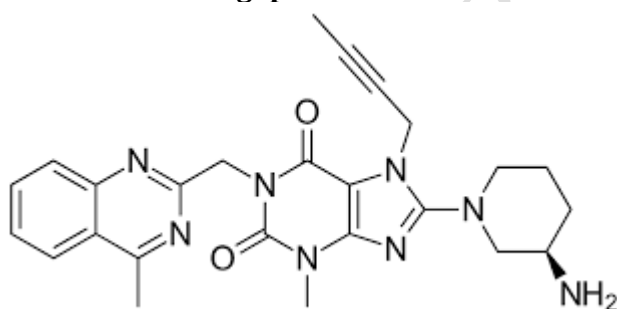
## INTRODUCTION :

Empagliflozin is targeted inhibitor of SGLT2 (sodium-glucose cotransporter 2), frequently prescribed for managing Type 2 diabetes mellitus.<sup>17</sup> It enhances glycemic control by increasing urinary glucose excretion, positioning it as an important agent in antidiabetic therapy. Accurate and reproducible quantification of Empagliflozin in both volume drug substances as well as finished medical formulations is crucial to secure product quality, therapeutic performance and conformity to industry regulations. Among various techniques, UV-spectrophotometry remains a widely accepted method due to its simplicity, cost-effectiveness, and suitability for routine quality control. While advanced techniques such as HPLC and LC-MS offer high sensitivity, UV spectrophotometry is often preferred for preliminary method development, especially in settings with limited resources.<sup>2,3,4,5</sup> Linagliptin is a reversible DPP-4 inhibitor that increases incretin concentrations like GLP-1 and GIP<sup>3,5</sup>, boosting insulin production as well as reducing glucagon release to improve glycemic control. Collectively, these actions lead to reduced hepatic glycogen breakdown and improved insulin response to blood glucose levels.<sup>5</sup> This study aims to devise and assess a UV-spectrophotometric method for estimating Empagliflozin as well as Linagliptin in bulk and tablet forms, as per ICH Q2(R1)<sup>13,15</sup> protocols.

Structure of Empagliflozin



Structure of Linagliptin



## MATERIALS AND METHODS:

### Materials

Empagliflozin API, Linagliptin API, commercial tablet formulation (Brand Name: Ajaduo 10mg and 5mg Tablet, Manufacturer: macleods Pharamceutics Ltd, Mumbai, India), and Ethanol (analytical grade) were used<sup>17,18</sup>.

### Technical Setup

A Shimadzu UV-1780 UV-Visible spectrophotometer, operated via UV Probe Software 2.35 and paired 1 cm quartz cuvettes was employed for the analysis<sup>2,3</sup>.

### Development of Standard Solution

Precisely 10 mg each of pure Empagliflozin and Linagliptin were individually transferred into specimen 10 mL volumetric flasks containing distilled water, mixtures were sonicated for 5 minutes as well as then adjusted to mass the mark using solvent mixture of Ethanol:Distilled Water (30:70)<sup>2,3,4</sup>. The resulting stock solutions had concentrations of 1 mg/mL (1000 µg/mL) for each drug.

### Determination of $\lambda_{\text{max}}$

Diluted solutions of Empagliflozin and Linagliptin were scanned from 200 to 400 nm.  $\lambda_{\text{max}}$  was observed at 294 nm for Empagliflozin and 222.80 nm for Linagliptin. An overlay spectrum confirms distinct absorbance peaks for both drugs.

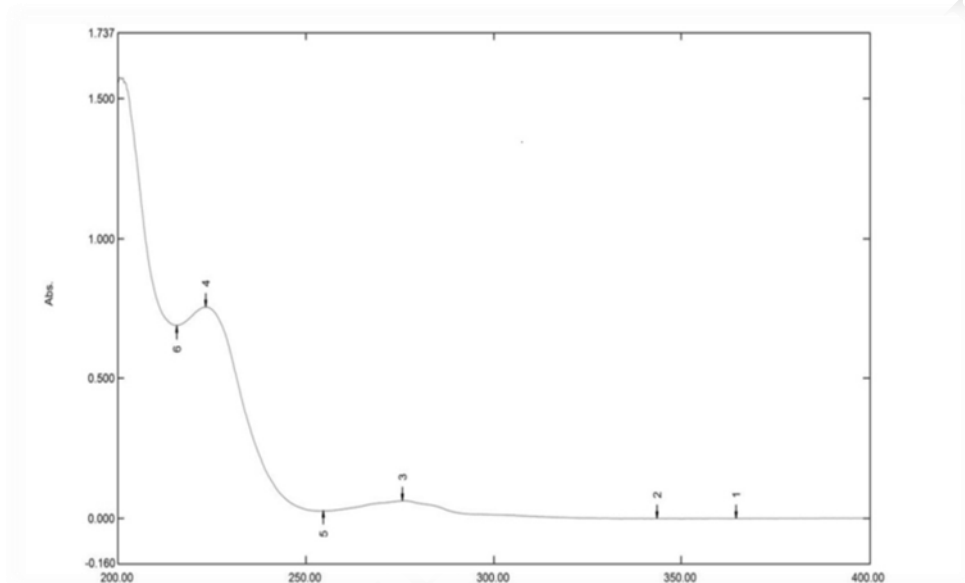


Fig 1: UV Spectrum of pure Empagliflozin

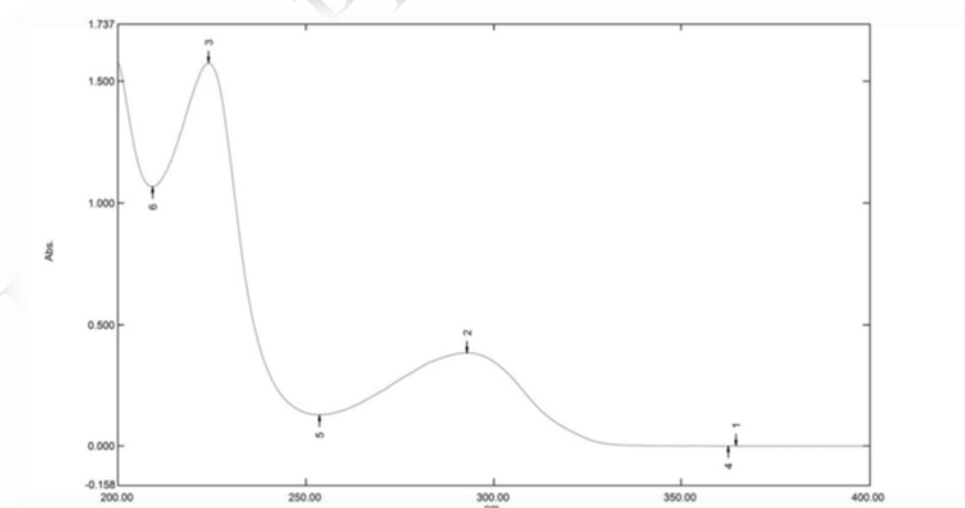
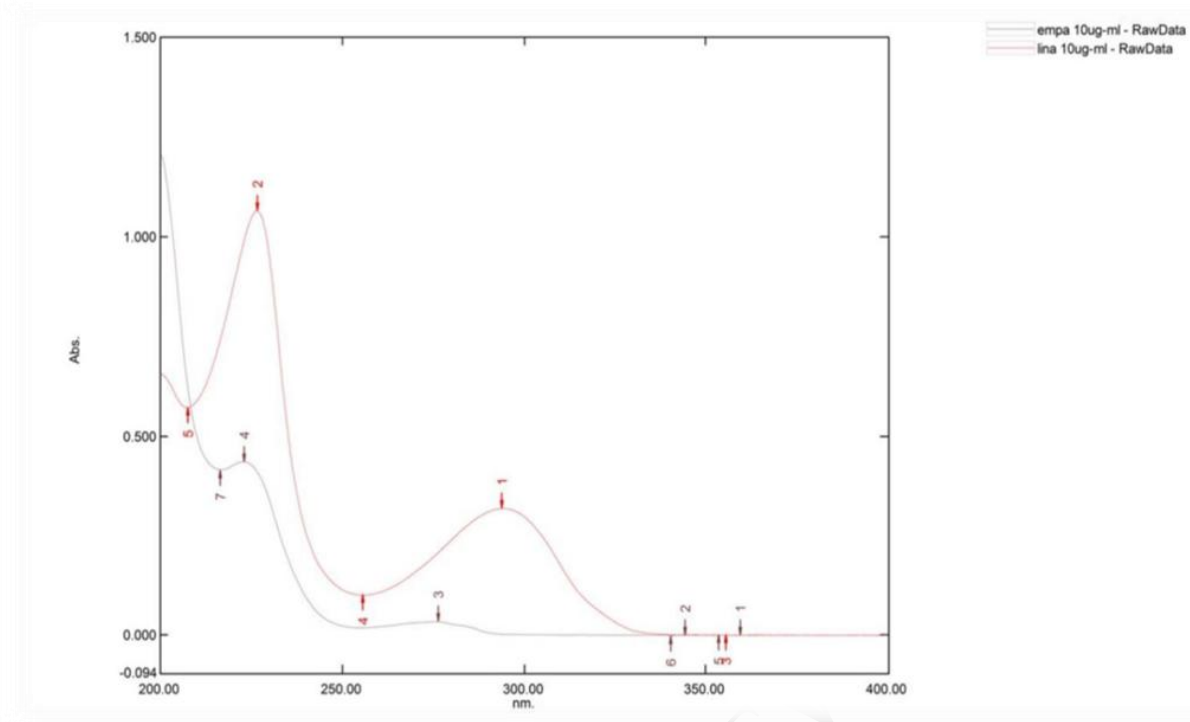


Fig 2: UV Spectrum of linagliptin

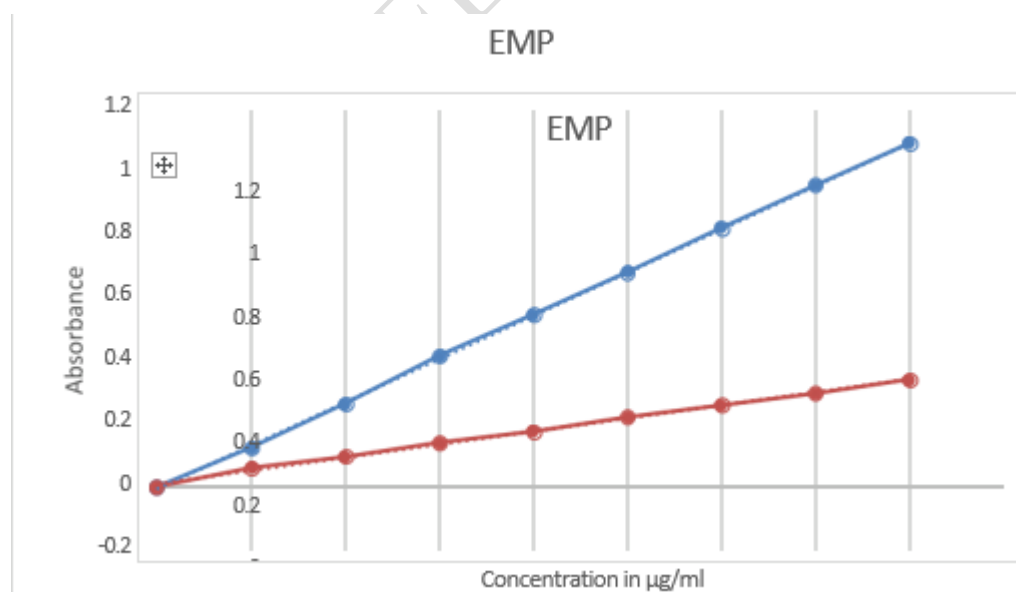


**Fig 3: Overlay Spectrum of EMP And LIN**

### Linearity

A series of dilutions (2–16  $\mu\text{g/mL}$ ) were prepared for analysis, with absorbance measured at both 294 nm as well as 222.80 nm for Linagliptin and Empagliflozin respectively. calibration curve was plotted<sup>2,3,4,17</sup>.(fig.4)

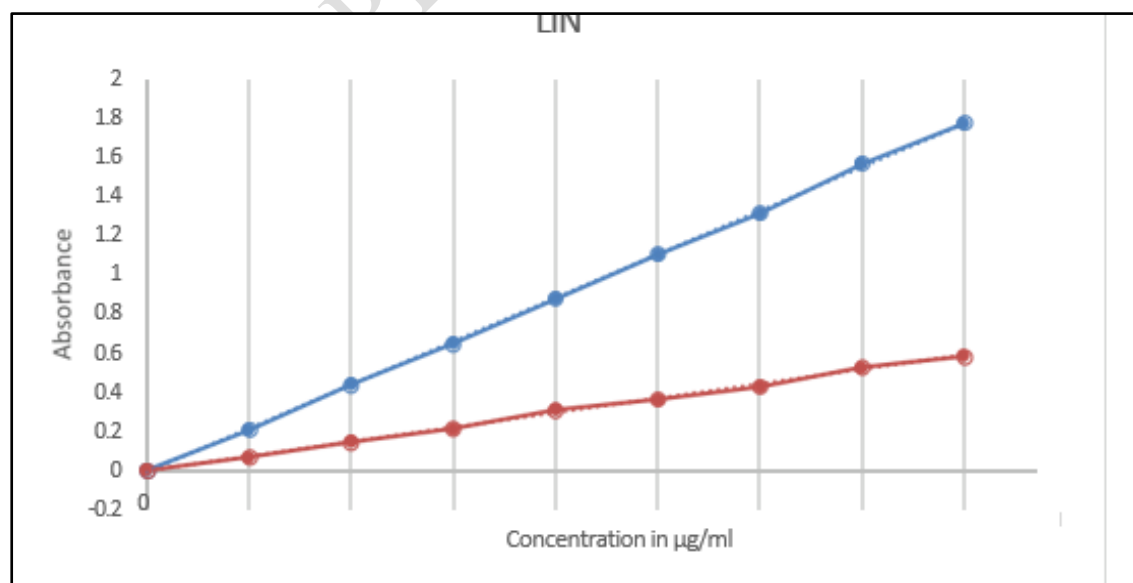
Table summarise optical characteristics of both drug.



**Fig 4: Calibration curve of Empagliflozin**

| Sr. No. | Conc. ( $\mu\text{g/ml}$ ) | EMP       |        |
|---------|----------------------------|-----------|--------|
|         |                            | 222.80 nm | 294 nm |
| 1       | 2 $\mu\text{g/ml}$         | 0.125     | 0.060  |
| 2       | 4 $\mu\text{g/ml}$         | 0.265     | 0.095  |
| 3       | 6 $\mu\text{g/ml}$         | 0.418     | 0.140  |
| 4       | 8 $\mu\text{g/ml}$         | 0.548     | 0.175  |
| 5       | 10 $\mu\text{g/ml}$        | 0.683     | 0.221  |
| 6       | 12 $\mu\text{g/ml}$        | 0.823     | 0.259  |
| 7       | 14 $\mu\text{g/ml}$        | 0.959     | 0.296  |
| 8       | 16 $\mu\text{g/ml}$        | 1.092     | 0.341  |

**Table 1: Standard calibration Table for Empagliflozin**



**Fig 5: Calibration curve of linagliptin**

116 **Table 2: Standard calibration Table for Linagliptin**

| Sr. No. | Conc.(µg/ml) | LIN      |        |
|---------|--------------|----------|--------|
|         |              | 222.80nm | 294 nm |
| 1       | 2 µg/ml      | 0.205    | 0.068  |
| 2       | 4 µg/ml      | 0.440    | 0.146  |
| 3       | 6 µg/ml      | 0.650    | 0.214  |
| 4       | 8 µg/ml      | 0.876    | 0.311  |
| 5       | 10 µg/ml     | 1.104    | 0.364  |
| 6       | 12 µg/ml     | 1.314    | 0.428  |
| 7       | 14 µg/ml     | 1.567    | 0.527  |
| 8       | 16 µg/ml     | 1.776    | 0.584  |

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118 **Absorptivity Determination at Selected Wavelengths(1%, 1cm) =**

119 **Absorbance/ Concentration (g/mol)**

120 Measured volumes of LIN and EMP stock solutions were transferred into separate 10 mL  
 121 standard flasks. The distilled water was used for dilution of the solutions to obtain final  
 122 levels of 10 µg/mL for LIN as well as both 10 µg/mL and 100 µg/mL for EMP.

123 Absorbance was recorded at 222.80 nm as well as 294 nm.

124 The corresponding absorptivity values for EMP and LIN were determined and recorded.

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128 **Table 3: Absorptivity values of Empagliflozin  $\lambda_1$  and  $\lambda_2$**

| Sr. No         | Absorbance of EMP |               |
|----------------|-------------------|---------------|
|                | 222.80 nm         | 294 nm        |
| 1.             | 0.551             | 0.0060        |
| 2.             | 0.543             | 0.0050        |
| 3.             | 0.483             | 0.0070        |
| <b>MEAN</b>    | <b>0.525</b>      | <b>0.0060</b> |
| <b>S.D.</b>    | <b>0.037</b>      | <b>0.001</b>  |
| <b>%R.S.D.</b> | <b>0.0037</b>     | <b>0.0001</b> |

129 **Table 4: Absorptivity values of Linagliptin  $\lambda_1$  and  $\lambda_2$**

| Sr. No         | Absorbance of LIN |                |
|----------------|-------------------|----------------|
|                | 222.80 nm         | 294 nm         |
| 1.             | 0.397             | 0.462          |
| 2.             | 0.411             | 0.467          |
| 3.             | 0.391             | 0.461          |
| <b>MEAN</b>    | <b>0.399</b>      | <b>0.463</b>   |
| <b>S.D.</b>    | <b>0.010</b>      | <b>0.0032</b>  |
| <b>%R.S.D.</b> | <b>0.0010</b>     | <b>0.00032</b> |

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133 **Table 5: Absorptivity values of Mixture  $\lambda_1$  and  $\lambda_2$**

| Sr. No         | Absorbance of Mixture at |               |
|----------------|--------------------------|---------------|
|                | 222.80 nm                | 294 nm        |
| 1.             | 0.932                    | 0.485         |
| 2.             | 0.912                    | 0.455         |
| 3.             | 0.899                    | 0.462         |
| <b>MEAN</b>    | <b>0.914</b>             | <b>0.467</b>  |
| <b>S.D.</b>    | <b>0.016</b>             | <b>0.015</b>  |
| <b>%R.S.D.</b> | <b>0.0016</b>            | <b>0.0015</b> |

136 **Validation of Proposed method**

137 **RECOVERY STUDY:**

138 The devised method was in accordance with ICH instructions<sup>13,14</sup>. To pre-analyzed solutions containing  
 139 10 µg/mL of EMP and 5 µg/mL of LIN, known amounts of standard drugs were added: 8, 10, and  
 140 12 µg/mL for EMP, and 4, 5, and 6 µg/mL for LIN. Total concentrations subjected to analysis to assess  
 141 recovery.

$$142 \quad \%RECOVERY = \frac{A * 100}{(B + C)}$$

144 Where,

145 A= Total drug estimation.

146 B= Amount of drug found on pre-analyzed bases.

147 C= Amount of pure drug added

148 **Table 6: Results of Recovery Studies of Empagliflozin**

149 **Table 7: Results of Recovery Studies of Linagliptin**

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| Sr.No | Amount Added (µg/ml)<br>EMP | Absorbance At<br>222.80nm | Amount<br>Recovered | %<br>Recovery |
|-------|-----------------------------|---------------------------|---------------------|---------------|
| 1     | 8                           | 1.485                     | 7.7                 | 99.41         |
| 2     | 10                          | 1.589                     | 9.6                 | 99.36         |
| 3     | 12                          | 1.685                     | 11.3                | 99.16         |
| Mean  |                             |                           |                     | 99.31         |
| SD    |                             |                           |                     | 0.100         |
| % RSD |                             |                           |                     | 0.0010        |

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153 **Table 6: Results of Recovery Studies of Empagliflozin**

| Sr.No | AmountAdded(µg/ml)<br>LIN | Absorbance At<br>268.60 nm | Amount<br>Recovered | %<br>Recovery |
|-------|---------------------------|----------------------------|---------------------|---------------|
| 1     | 4                         | 0.654                      | 3.8                 | 99.47         |
| 2     | 5                         | 0.699                      | 4.8                 | 99.08         |
| 3     | 6                         | 0.738                      | 5.5                 | 99.03         |
| Mean  |                           |                            |                     | 99.19         |
| SD    |                           |                            |                     | 0.042         |
| % RSD |                           |                            |                     | 0.42          |

**Table 7: Results of Recovery Studies of Linagliptin**

**A) Accuracy**

It denotes extent of agreement among the measured and true value. It was assessed through recovery studies performed at multiple concentration levels to validate the dependability of the method.

**Table 8 : Result of Accuracy results**

| Sr. No. | Amount Added (µg/ml) |     | % Recovery |        |
|---------|----------------------|-----|------------|--------|
|         | EMP                  | LIN | EMP        | LIN    |
| 1       | 8                    | 4   | 99.21      | 99.71  |
| 2       | 10                   | 5   | 99.89      | 99.69  |
| 3       | 12                   | 6   | 99.60      | 99.72  |
| Mean    |                      |     | 99.56      | 99.70  |
| SD      |                      |     | 0.2786     | 0.0124 |
| % RSD   |                      |     | 0.0027     | 0.0012 |

**B) Precision:**

Replicability of results is indicated by the standard deviation ( $\pm$  SD) or %RSD of repeated measurements. It was assessed using stock solutions in a 10:5 ratio containing EMP and LIN at concentrations of 10 and 5 µg/mL, respectively of LIN. Intraday reproducibility<sup>13,14</sup> was assessed by analyzing triplicate dilutions across three time points, spaced two hours apart. To assess interday precision, three sets of samples were analyzed at 24-hour and 48-hour intervals. The findings are presented below

**Table 9: Results of Precision Studies (Intra-day)**

| Session   | Absorbance at |       | % Estimation |       |
|-----------|---------------|-------|--------------|-------|
|           | 222.80        | 294   | LIN          | EMP   |
| Morning   | 0.924         | 0.468 | 99.96        | 99.71 |
| Afternoon | 0.918         | 0.465 | 99.30        | 99.07 |

|                |       |       |              |              |
|----------------|-------|-------|--------------|--------------|
| <b>Evening</b> | 0.911 | 0.462 | 98.46        | 98.43        |
| <b>MEAN</b>    |       |       | <b>99.24</b> | <b>99.07</b> |
| <b>S.D.</b>    |       |       | <b>0.75</b>  | <b>0.63</b>  |
| <b>% RSD</b>   |       |       | <b>0.075</b> | <b>0.064</b> |

**Table 10: Results of Precision Studies (Inter-day)**

| <b>Session</b> | <b>Absorbance at</b> |            | <b>% Estimation</b> |              |
|----------------|----------------------|------------|---------------------|--------------|
|                | <b>222.80</b>        | <b>294</b> | <b>EMP</b>          | <b>LIN</b>   |
| <b>Day 1</b>   | 1.208                | 0.561      | 99.41               | 99.69        |
| <b>Day 2</b>   | 1.202                | 0.556      | 99.17               | 98.66        |
| <b>Day3</b>    | 1.195                | 0.551      | 98.79               | 97.67        |
| <b>MEAN</b>    |                      |            | <b>99.13</b>        | <b>98.67</b> |
| <b>S.D.</b>    |                      |            | <b>0.31</b>         | <b>1.01</b>  |
| <b>% RSD</b>   |                      |            | <b>0.03</b>         | <b>0.010</b> |

### C) Ruggedness

The method's ruggedness was tested by having two analysts independently analyze identical sample portions under consistent laboratory conditions<sup>23,13,14</sup>. Outcomes are presented below.

**Table 11: Results of Different analyst study**

| <b>Session</b>   | <b>Absorbance at</b> |               | <b>% Estimation</b> |               |
|------------------|----------------------|---------------|---------------------|---------------|
|                  | <b>222.80nm</b>      | <b>294 nm</b> | <b>EMP</b>          | <b>LIN</b>    |
| <b>ANALYST 1</b> | 1.209                | 0.561         | 99.55               | 99.66         |
| <b>ANALYST 2</b> | 1.203                | 0.558         | 99.08               | 99.11         |
| <b>MEAN</b>      |                      |               | <b>99.31</b>        | <b>99.39</b>  |
| <b>S.D.</b>      |                      |               | <b>0.33</b>         | <b>0.38</b>   |
| <b>% RSD</b>     |                      |               | <b>0.0033</b>       | <b>0.0038</b> |

#### D) Robustness

| Session                                     | Absorbance at |        | % Estimation |        |
|---------------------------------------------|---------------|--------|--------------|--------|
|                                             | 222.80nm      | 294 nm | EMP          | LIN    |
| CH <sub>3</sub> OH + H <sub>2</sub> O (1:9) | 1.204         | 0.560  | 99.12        | 99.53  |
| 0.1M NaOH                                   | 1.198         | 0.554  | 98.86        | 98.30  |
| MEAN                                        |               |        | 98.99        | 98.92  |
| S.D.                                        |               |        | 0.18         | 0.87   |
| % RSD                                       |               |        | 0.0018       | 0.0088 |

varying solvents, while operational parameters and instrumentation remained unchanged. Outcomes are presented in below.

**Table 12: Results of Different solvent study**

#### LOD and LOQ

The LOD was derived from the equation:

$$\text{LOD} = 3.3 \times (\text{SD} / \text{Slope})$$

where *SD* is the standard deviation of the Y-intercepts from the calibration curves, and *Slope* is the average slope of those curves.

Based on this, the LOD was determined to be:

- **2.108 µg/mL** for Empagliflozin
- **1.517 µg/mL** for Linagliptin

The LOQ was estimated using the equation:

$$\text{LOQ} = 10 \times (\text{SD} / \text{Slope})$$

utilizing the same calibration data.

.LOQ for Empagliflozin 0.528µg/ml andLOQ for Linagliptin is 0.424µg/ml

**Table 13 : LOD & LOQ of EMP and LIN**

| Sr. No. | DRUG NAME | LOD (µg/ml) | LOQ (µg/ml) |
|---------|-----------|-------------|-------------|
| 1.      | LIN       | 1.517       | 0.424       |
| 2.      | EMP       | 2.108       | 0.528       |

**Table 14: Validation Parameters of LIN andEMP**

| Parameters                    | LIN                    | EMP                   |
|-------------------------------|------------------------|-----------------------|
| Working Wavelength(nm)        | 294nm                  | 222.80nm              |
| Linearity Range(µg/ml)        | 2-16 µg/ml             | 2-16 µg/ml            |
| Limit of Detection (µg/ml)    | 1.517µg/ml             | 2.108 µg/ml           |
| Limit of Quantitation (µg/ml) | 0.424 µg/ml            | 0.528 µg/ml           |
| Y= mx+c                       | Y=0.054x-0.003         | Y=0.202x-0.009        |
| Slope ± S.D.                  | 0.111                  | 0.068                 |
| Intercept ± S.D.              | 0.011                  | 0.0042                |
| Regression Coefficient ±S.D.  | R <sub>2</sub> = 0.999 | R <sub>2</sub> =0.999 |

## RESULT:

The results confirmed the suitability of methods for estimating EMP and LIN<sup>2,3,4,17,18,22</sup>. Linearity was established across the tested range with high regression coefficients. Accuracy studies showed excellent recovery, precision studies confirmed reproducibility. The low LOD and LOQ values indicate high method sensitivity. Ruggedness confirmed the method's consistency under altered conditions.

The technique was linear over 2–16 µg/ml with  $R^2 = 0.997$ . Intraday and interday precision (%RSD) was within acceptable limits. Accuracy (% recovery) ranged from 98–102%. LOD of Empagliflozin and Linagliptin were 2.108 µg/ml and 1.517 µg/ml, respectively. LOQ of Empagliflozin and Linagliptin 0.528 µg/ml and 0.424 µg/ml. Robustness studies showed no significant variation. The method is suitable for routine use.

## CONCLUSION:

The validated UV-spectrophotometric method enables accurate, precise, and sensitive joint assessment of Empagliflozin and Linagliptin in both volume as well as tablet formulations. Compliant with ICH Q2(R1)<sup>13,14</sup> instructions, the method demonstrated strong linearity, reproducibility, and robustness under varied conditions. Its simplicity, cost-effectiveness, and reliability make it highly suitable for routine quality control and pharmaceutical analysis.

## CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

## ACKNOWLEDGMENTS:

Gratitude is extended to JSPM's Rajarshi Shahu College of Pharmacy and Research, Pune, for their infrastructural and technical support during the course of this research.

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