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# SPECTROFLUORIMETRIC ESTIMATION OF EMPAGLIFLOZIN IN THE PRESENCE OF ITS OXIDATIVE DEGRADATION PRODUCT

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**ABSTRACT**
A Spectrofluorimetric method has been developed and validated for the selective quantitative determination of empagliflozin in the presence of its oxidative degradation product. In this method the fluorescence of empagliflozin in methanol at 305 nm was measured after excitation at 234 nm. The proposed method was validated according to ICH guidelines and show high sensitivity, accuracy and precision. Furthermore this method was successfully applied to the analysis of empagliflozin in pharmaceutical dosage form without interference from additives and the results were statistically compared to a reported method and found no significant difference.

**KEYWORDS:** Empagliflozin; spectroflurimetry.

## 1. INTRODUCTION

Empagliflozin (IUPAC name: 1,5-anhydro-1-(4-chloro -3-tetrahydrofuran-3-yloxy)D-Glucitol) (Figure 1) is an anti-diabetic agent, a sodium-glucose co-transporter inhibitor, Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and there by excreting glucosevia the urine act as a anti -diabetic agent for treatment of type-2 diabetes.[1] Few methods have been reported for the estimation of empagliflozin either alone or in other combinations. These methods include spectrophotometry[2-6], UPLC with UV detection[7,8] and liquid chromatography/mass spectroscopy.[9]

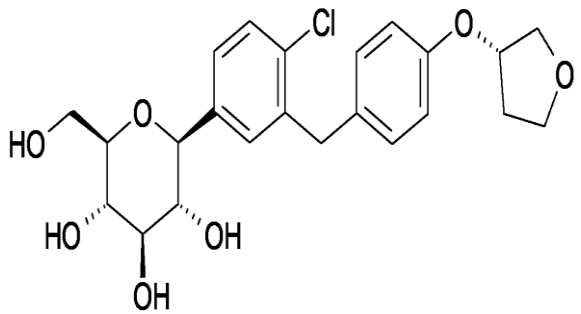


**Fig. 1: Chemical structure of empagliflozin.**

To the best of our knowledge *no* Spectrofluorimetric methods have been reported for determination of empagliflozin.

Hence, the aim of this work was to develop a new Spectrofluorimetric method which has the advantages of being more sensitive than the reported methods. The developed method also consider to be economic, time saving and operated by simple procedure which make it superior to HPLC or other tedious methods. Also the developed method allow the determination of empagliflozin in its pure form and pharmaceutical preparation without any interference.

## 2. Experimental

### 2.1. Instruments

- Jasco FP-6200 *Spectrofluorometer* (Japan), equipped with 150 Watt Xenon lamp, holographic gratting excitation and emission monochromators for all measurements. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. All measurements were carried out at medium sensitivity.
- *pH meter* 3510 (Jenway, U.S.A).

### 2.2. Materials and Reagents

- Empagliflozin (certified to contain 99.25%) was kindly supplied by Al Andalous for Pharmaceutical Industries, Obour city, Egypt.
- Pharmaceutical Preparation: "Jardiance 10 mg tablets batch no. 051, manufactured by Boehringer Ingelheim Pharmaceutical Company.
- Hydrogen peroxide 30% aqueous solution.
- Acetonitrile, chloroform, ethanol and methanol all of HPLC grades (Sigma-Aldrich, USA).

- Sodium hydroxide and hydrochloric acid; analytical grade, (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
- Monobasic potassium phosphate, potassium chloride, boric acid, glacial acetic acid and sodium acetate trihydrate; Analytical grade, (El-Nasr Company, Egypt).
- Deionized double distilled water.
- Buffers of different pH values prepared as prescribed in US pharmacopeia.[10]

1. Acetate buffer pH 4 to 6.
2. Phosphate buffer pH range from 6 to 8.
3. Borate buffer pH range from 8 to 10.

### 2.3. Standard solutions

Aliquots of standard drug solution (1µg/ml) containing (100–1000 ng) of intact empagliflozin were transferred into a series of 10 ml volumetric flasks. The solutions were diluted with methanol to 10 ml and mixed well. The fluorescence intensity was measured at 305 nm after excitation at 234 nm.

## 3. Procedures

### 3.1. Linearity and construction of calibration graph

Aliquots of standard drug solution (1µg/ml) containing (100–1000 ng) of intact empagliflozin were transferred into a series of 10 ml volumetric flasks and 2 ml of phosphate buffer pH 6 was added. The solutions were diluted with methanol to 10 ml and mixed well. The emission of these solutions was measured at 305 nm after excitation at 234 nm and then plotted versus the final empagliflozin concentrations in ng $ml^{-1}$ to get the calibration graph. Alternatively, the regression equation was derived.

### 3.2. Assay of empagliflozin in synthetic mixture

Aliquots from empagliflozin working standard solution (1 µg $ml^{-1}$) ranging from (100 ng - 900 ng) with aliquots of oxidative degradation product working solution (1 µg $ml^{-1}$) ranging from (900 ng -100 ng) were transferred to a series of 10-ml volumetric flasks containing 2 ml of phosphate buffer pH 6 and the volume was completed to the mark with methanol. The emission of these solutions was measured at 305 nm after excitation at 234 nm. empagliflozin concentrations were calculated from the corresponding regression equation.

### 3.3. Application of the method to pharmaceutical formulation

Ten tablets were weighed and finely powdered after removeing the film coated by scratching and washing with methanol, Appropriate weight of powder equivalent to 1 mg empagliflozin was accurately transferred to 100-ml volumetric flask and the volume was made up to 75 ml with methanol. The solution was shaken vigorously for 15 min then sonicated for 30 min. The volume was completed to 100 ml with solvent then filtered through Whatman filter paper no. 41.

Necessary dilutions of the filtrate were made with methanol to obtain different concentration of empagliflozin samples as stated under linearity. The content of the tablets was determined from the corresponding regression equation.

## 4. RESULTS AND DISCUSSION

### 4.1. Spectral characteristics

Empagliflozin exhibits a native fluorescence in methanol and its emission can be measured at 305 nm ($\lambda_{em}$) after excitation at 234 nm ($\lambda_{ex}$), while oxidative degradation product has no fluorescence. The emission and excitation spectra of Empagliflozin in methanol are shown in

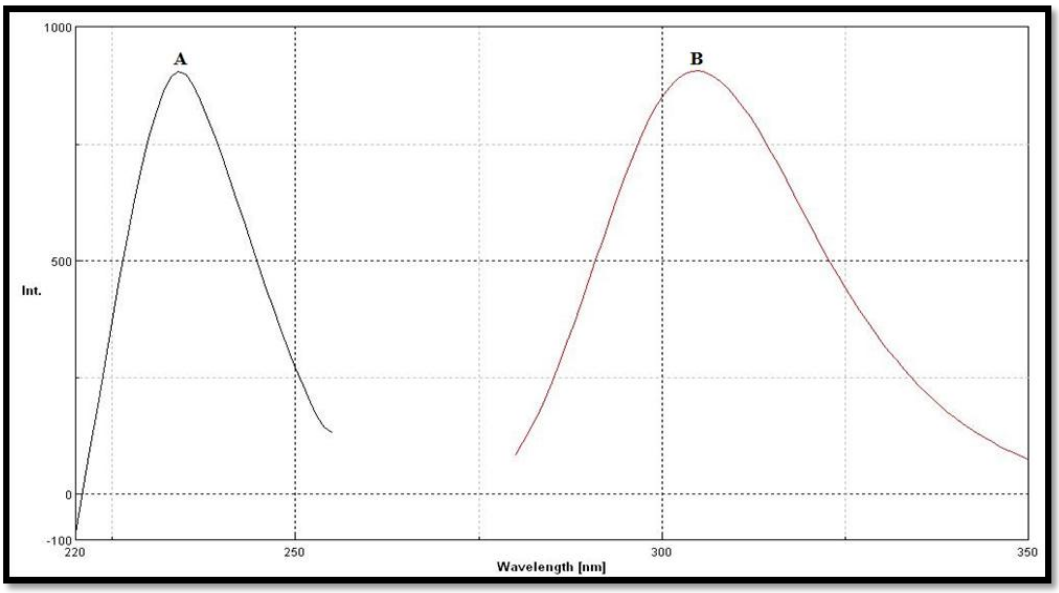


**Figure (2): (A) Excitation at 230 nm and (B) Emission at 305 nm spectra of empagliflozine (900 ng/ml) in methanol using 2 mL of phosphate buffer pH 6.**

### 4.2. Method optimization

#### 4.2.1 Effect of diluting solvents

The general procedure for the method was repeated using a fixed amount of empagliflozine (900 ng) and different diluting solvents, the results as shown in **figure (3)** prove that; methanol is the best diluting solvent.

#### 4.2.2 Effect of pH and buffer

The general procedure for the method was repeated using a fixed amount of empagliflozine (900 ng) and different buffers with different pH values, the results as shown in **figure (4)** prove that; phosphate buffer pH 6 gives the best results.

#### 4.2.3 Effect of buffer volume

The general procedure for the method was repeated using a fixed amount of empagliflozine (900 ng) and different volumes of phosphate buffer pH 6, the results as shown in **figure (5)** prove that; 2 ml of phosphate buffer pH 6 gives the best results.

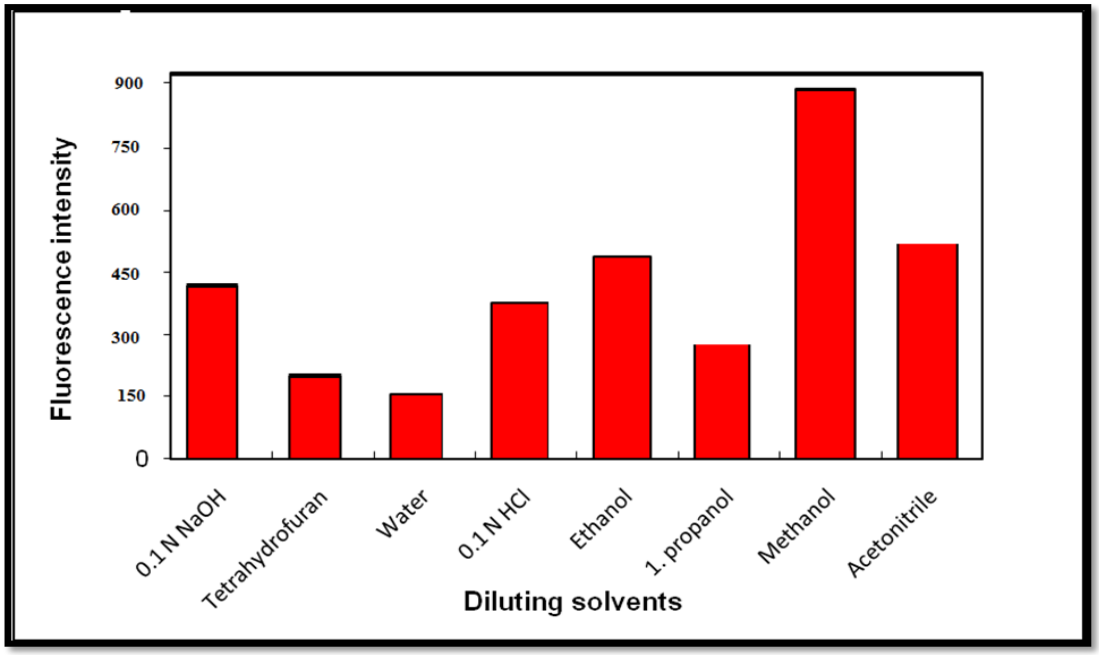


**Figure (3): Effect of different diluting solvents on fluorescence intensity of empagliflozine (900 ng $mL^{-1}$).**

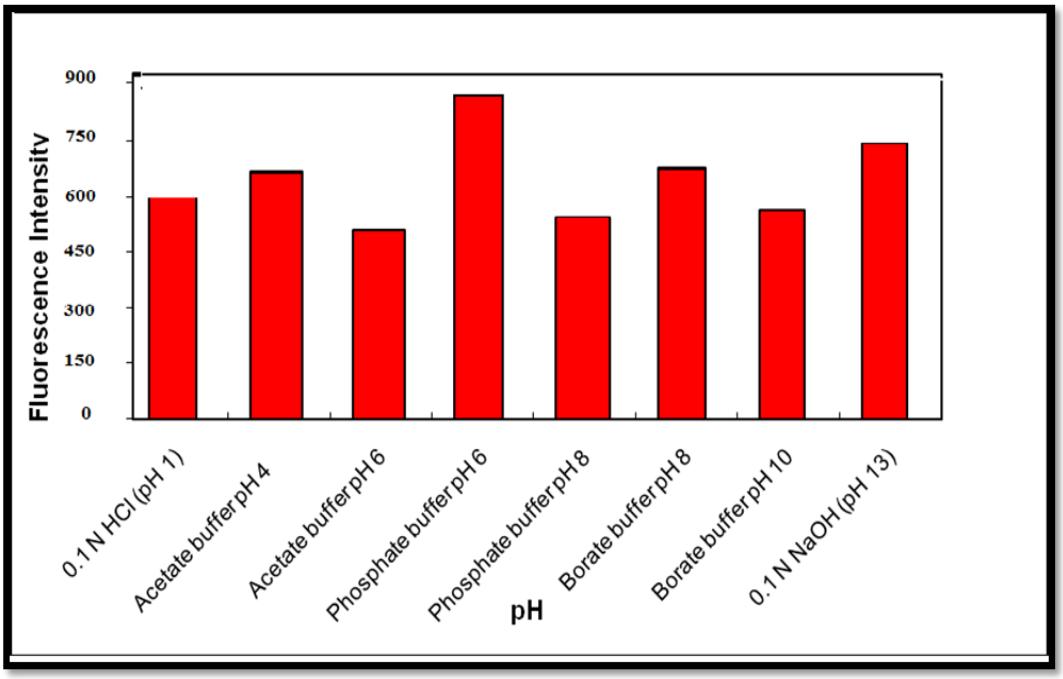


**Figure (4): Effect of different buffer systems (2 mL) on fluorescence intensity of empagliflozine (900 ng $mL^{-1}$).**

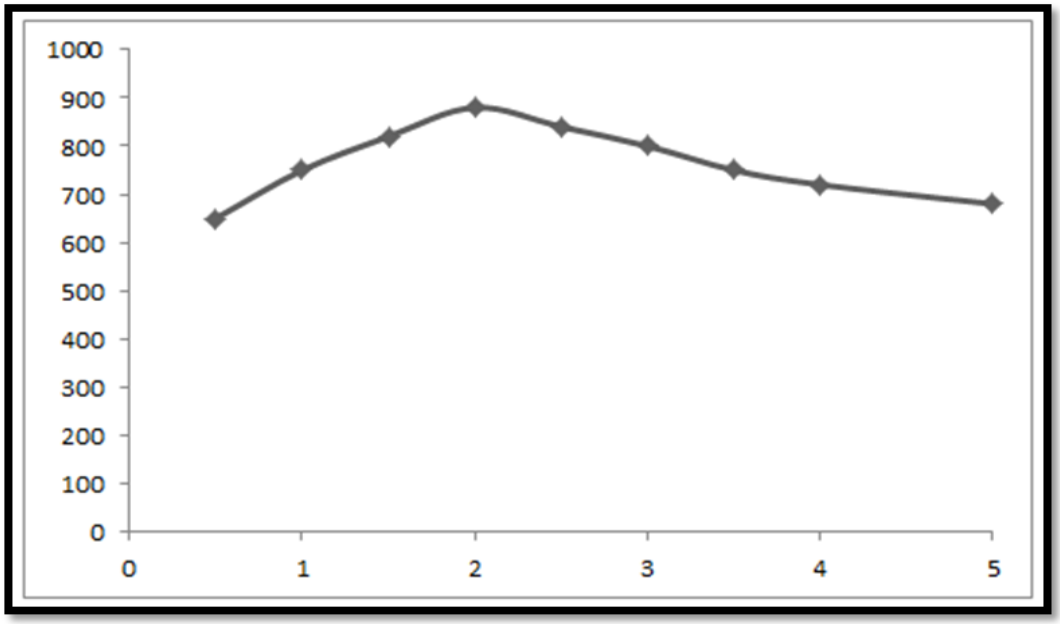


**Figure (5): Effect of volume of phosphate buffer pH 6 on fluorescence intensity of empagliflozine (900 ng $mL^{-1}$).**

#### 4.2.4. Effect of time

The general procedure for the method was repeated using a fixed amount of empagliflozine (900 ng) at different time intervals, the results as shown in **figure (6)** prove that; the florescence intensities were stable from zero time up to 60 min.

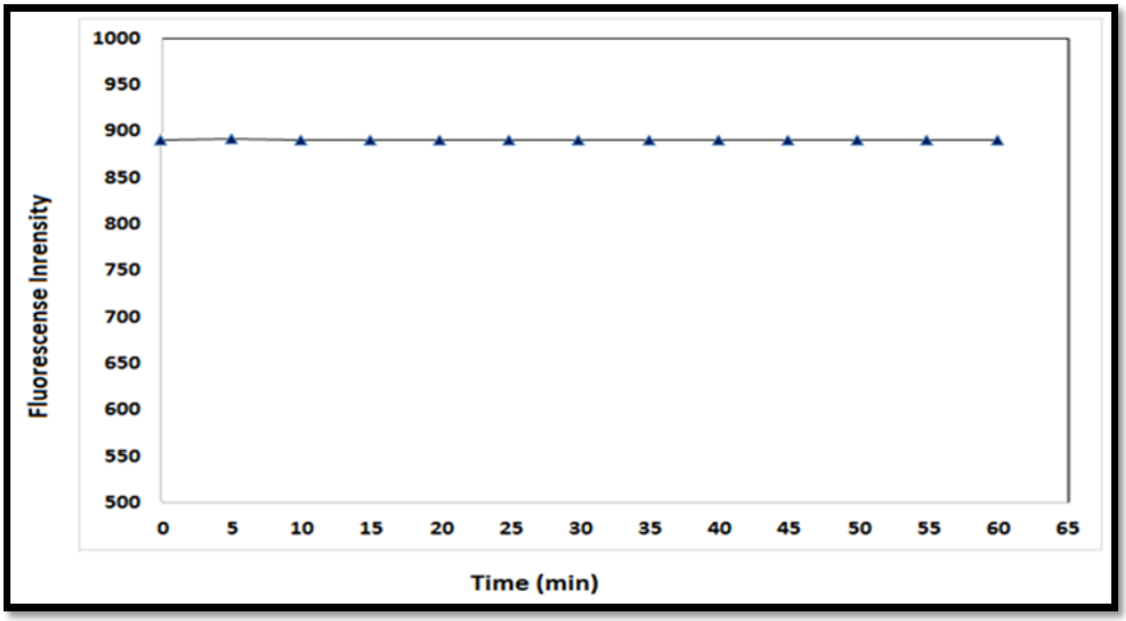


**Figure (6): Effect of time on fluorescence intensity of empagliflozine (900 ng $mL^{-1}$).**

### 4.3. Method validation

Method validation was performed according to the International Conference on Harmonization (ICH) guidelines.[11]

#### 4.3.1 Linearity and range

Linearity of the proposed method was evaluated and found to be in the concentration range of (100 - 1000 ng $ml^{-1}$). The regression plots was found to be linear over the mentioned range, the linear regression equation was:

**FI = 0.9838 C - 2.206 ($r^2$ = 0.9998).**

Where **FI** is the Fluorescence intensity, **C** is the drug concentration in ng $ml^{-1}$ and $\mathbf{r^2}$ is the squared correlation coefficient.

The high value of the correlation coefficient and the low intercept value indicate the excellent linearity of the proposed method. Linearity range, regression equation, intercept, slope and the squared correlation coefficient for the calibration data were presented in **table (1).**

#### 4.3.2 Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were from the following equations:

**LOD = 3.3 Sa / slope**

**LOQ = 10 Sa / slope**

Where **Sa** is the standard deviation of the intercept of regression line.

LOD and LOQ were found to be 15.26 and 46.25 ng $ml^{-1}$ respectively, as shown in **table (1).** The small values of LOD and LOQ indicate good sensitivity.

**Table 1: Spectral data for determination of empagliflozine by the proposed methods.**

| *Parameters* | *Spectroflurimetric method* |
|---|---|
| **Wavelength (nm)** | $\lambda_{em}$ (305) $\lambda_{ex}$ (234) |
| **Linearity range (ng $mL^{-1}$)** | 100-1000 |
| **LOD (ng $mL^{-1}$)** | 15.26 |
| **LOQ (ng $mL^{-1}$)** | 46.25 |
| **Slope ± SD**<br>**Intercept ± SD** | 0.9838 ± 0.013<br>-2.206 ± 0.020 |
| **Squared correlation coefficient ($r^2$)** | 0.9998 |

#### 4.3.3 Accuracy

Three replicate determinations of three different concentrations of empagliflozine in pure forms within linearity range were performed by the proposed method. Accuracy as percent recovery (%R) was calculated, the calculated values of %R confirms excellent accuracy as shown in **table (2).**

#### 4.3.4 Precision

Three replicate determinations of three different concentrations of empagliflozine in pure form within linearity range were performed in the same day (repeatability) and in three successive days (intermediate precision) using the proposed method. Small values of % RSD confirm the precision of the method as shown in **table (2).**

**Table 2: Accuracy and precision of the proposed method.**

| **parameters** | | **empagliflozine** |
|---|---|---|
| **Accuracy (%R)** | **Intraday** [a] | 100.96 |
| **precision (% RSD)** | **Repeatability** [b] | 1.026 |
| | **Intermediate precision** [c] | 1.217 |

[a] *Average of %R of three replicates determinations for three concentrations (200, 500, and 800* ng $mL^{-1}$*) of* empagliflozine.

[b] *Average of %RSD of three replicates determinations for three concentrations (200, 500, and 800* ng $mL^{-1}$*) of* empagliflozine *in the same day.*

[c] *Average of %RSD of three replicates determinations for three concentrations (200, 500, and 800* ng $mL^{-1}$*) of* empagliflozine *at three successive days.*

#### 4.3.1 Specificity

The specificity of the proposed procedure was assured by applying it to laboratory prepared mixtures of empagliflozine and its oxidative degradation product. The proposed procedure was adopted for the selective determination of empagliflozine in presence of oxidative degradation product. The percentage recovery ± % RSD was (98.05 ± 0.198), as shown in **table (3).**

**Table (3): Determination of empagliflozine in synthetic mixtures with oxidative degradation product by the proposed method.**

| **Empagliflozine (ng $mL^{-1}$)** | **Degradate (ng $mL^{-1}$)** | **Empagliflozine found (ng $mL^{-1}$)** | **Recovery % of empagliflozine** |
|---|---|---|---|
| 100 | 900 | 98.74 | 98.74 |
| 300 | 700 | 299.03 | 99.68 |
| 500 | 500 | 496.79 | 99.36 |
| 700 | 300 | 693.85 | 99.12 |
| 900 | 100 | 903.89 | 100.43 |
| ***Mean*** | | | 99.74 |
| ***%RSD*** | | | 0.642 |

## 5. Application to pharmaceutical formulation

The proposed procedure was applied for determination of empagliflozine in Jardiance® 10 mg tablets. Satisfactory results were obtained in good agreement with the label claim, indicating no interference. The obtained results were statistically compared to those obtained by the

reported method.[2] No significant differences were found by applying t-test and F-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as shown in **table (4).**

**Table (4): Statistical comparison between the results obtained by applying the proposed and reported methods for determination of empagliflozine in Jardiance® 10 mg tablets.**

| Parameter | Proposed method | Reported method [2] *** |
|---|---|---|
| **N*** | 5 | 5 |
| **Mean** | 100.23 | 99.35 |
| **variance** | 0.923 | 1.116 |
| **t**** | 1.209 (2.306) | —— |
| **F**** | 3.003 (6.388) | —— |

** No. of experimental.*

*** The values in the parenthesis are tabulated values of t and F at (p= 0.05).*

## CONCLUSION

In this study, a Spectrofluorimetric methods was developed and validated according to ICH guidelines. Selective determination of empagliflozine in presence of oxidative degradation product in their combined dosage form by using the proposed methods.

The developed method was found to be extremely simple, sensitive, accurate, precise and economic unlike HPLC procedure which is time consuming and expensive.

Finally; the developed methods can be applied for routine analysis of empagliflozine in its pure form and in tablets.

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