Spectrophotometric analysis of empagliflozin tablets as SGLT2 inhibitors in pharmaceutical samples

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Abstract

Azo dyes account for 70% of dye chemistry, and their importance may grow in the future. Empagliflozin is a sodium-glucose co-transporter-2 (SGLT2) inhibitor. SGLT2 transporters are primarily responsible for glucose reabsorption in the kidney. In 2014, empagliflozin was approved for medical use in the United States and the European Union. With over 4 million prescriptions in 2019, it was the 146th most commonly prescribed medication in the United States in 2019. The spectrophotometric determination of empagliflozin is described using coupling agents such as 3-chloro-4-nitroaniline or sulfanilamide. These methods are straightforward and are based on the reaction of empagliflozin with diazotized products of 3-chloro-4-nitroaniline or sulfanilamide to produce colored azo dyes with absorption maxima at 470 and 480 nm. Empagliflozin was linear from 1.2 to 26.6 µg ml⁻¹ or 0.8 to 20.4 µg ml⁻¹ when combined with diazotized 3-chloro-4-nitroaniline or sulfanilamide, respectively. Empagliflozin’s molar absorptivity and Sandell’s sensitivity to 3-chloro-4-nitroaniline or sulfanilamide azo dyes were 3.179 × 10⁴ and 4.367 × 10⁴ mol⁻¹ cm⁻¹ or 3.67 × 10⁴ mol⁻¹ cm⁻¹ and 1.149 × 10⁻² µg cm⁻² or 8.368 × 10⁻² µg cm⁻², respectively. The formed colored azo dyes are stable for more than 12 hours. The optimal reaction conditions and other analytical parameters are assessed. Foreign organic compound interference has been studied. The method has been successfully used to determine empagliflozin in pharmaceutical samples.

Introduction

Azo dyes constitute 70% of dye chemistry, and their relative significance may increase in the future (Alsoghier et al., 2021; Benkhaya et al., 2020; Chen et al., 2021; Gester et al., 2020; Ben Mohamed-Smati et al., 2021; Omar et al., 2021; Prashantha et al., 2021; Rashidnejad et al., 2021; Selvaraj et al., 2021; Srinivasan and Sadasivam, 2021; Sweidan et al., 2018; Weldegebriel, 2020).

Empagliflozin (Fig. 1) is a competitive inhibitor of sodium-glucose co-transporter-2 that is orally active and has an antihyperglycemic effect (Hailat et al., 2022). It is approved for treating adults with type 2 diabetes in the EU, USA, and Japan, among other parts of the world (Frampton, 2018). This mechanism is independent of β-cell function; thus, these agents effectively treat type 2 diabetes mellitus at any disease stage (Levine, 2016; Mula-Abed and Aughsteen, 2005). Many methods have been adopted to determine empagliflozin (Ahmad et al., 2021). The liquid chromatography-mass spectrometry method was developed, optimized, and validated for simultaneous quantification of empagliflozin and metformin in human plasma using empagliflozin D4 and metformin D6 as an internal standard (Wattamwar et al., 2020). An Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) method was developed...

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to determine empagliflozin and metformin using a bridged ethylene hybrid C18 column (Ayoub and Mowaka, 2017). Another univariate spectrophotometric method and multivariate chemometric approach were developed and compared to determine empagliflozin simultaneously and metformin manipulating their zero-order absorption spectra with application to their pharmaceutical preparation (Mabrouk et al., 2019). 4-Nitroaniline forms molecular adducts with 4-aminobenzoic acid. It reacts with nitrite ion in a hydrochloric acid medium to form 4-nitrophenyldiazonium chloride, which couples with naphth-1-ol in an alkaline medium to give a purple azo dye. (Figure 2) Photocatalytic degradation of 4-nitroaniline in the presence of TiO$_2$ suspensions in a batch and continuous annular reactor has been studied (Abed-Elmageed et al., 2020; Ayoub et al., 2021; Baveja et al., 1981; Marchewka et al., 2011; Wu et al., 2012). Sulfanilamide is an organic sulfur compound similar to $p$-aminobenzoic acid (PABA) with antibacterial properties. Sulfanilamide competes with PABA for the bacterial enzyme dihydropteroate synthase, thereby preventing the incorporation of PABA into dihydrofolic acid, the immediate precursor of folic acid (Dionisio et al., 2018; United States Pharmacopeial Convention, 2007).

**Effect of acid, base concentration, and temperature used**

The effect of acid and base on the diazotization reaction of empagliflozin (2 µgml$^{-1}$) was studied by adding different acidic solutions (1 M) such as HCl, HNO$_3$, H$_2$SO$_4$, and CH$_3$COOH and basic solutions (1 M) such as KOH, NaOH, Na$_2$CO$_3$, and NH$_4$OH. It was observed that CH$_3$COOH gave low absorbance with low color stability. In contrast, HCl gave high absorbance with the highest color stability, whereas 1.0 ml of NaOH gave the maximum absorbance for the reaction of empagliflozin coupled with diazotized 3-chloro-4-nitroaniline or sulfanilamide. Therefore, 0.5 ml of 0.5 M HCl (Table 1) and 1.0 ml of 1 M NaOH solutions were preferred for the diazotization reaction of empagliflozin.

The effect of various acids such as HCl, HNO$_3$, H$_2$SO$_4$, and CH$_3$COOH (0.5 M) on the diazotization reaction was studied under the maximum absorbance by varying the volume of different acids between 0.25 and 1.0 ml while fixing all other parameters. It was found that 0.5 ml of HCl (0.5 M) gave the highest absorbance and was preferred for the diazotization reaction of empagliflozin (Table 2).

Room temperature (25°C ± 5°C) is recommended for these diazotization reactions because losses in color intensity and stability were observed at low or high temperature.

**Effect of nitrite concentration and coupling reagents**

The color is at maximum intensity when using 1 ml of a 0.1 M sodium nitrite solution using the current procedure with 2 µgml$^{-1}$ of empagliflozin and adding 1 ml of 0.02–0.16 M solutions of the nitrite in hydrochloric acid (0.5 M) to a series of nitrite solutions. A higher concentration did not build up the absorbance further, and at a lower concentration, no good results were obtained (Table 3).

The current procedure uses 3-chloro-4-nitroaniline or sulfanilamide as a coupling agent by taking 2 µgml$^{-1}$ of empagliflozin and adding 0.25–2.0 ml of 1% 3-chloro-4-nitroaniline or sulfanilamide to a string of nitrite solutions. The

**Table 1. Acid concentration on absorbance.**

| 0.5 ml HCl used (M) | Absorbance (A) |
|---------------------|----------------|
| 3-Chloro-4-nitroaniline | Sulfanilamide |
| 0.1 | 0.296 | 0.315 |
| 0.2 | 0.346 | 0.345 |
| 0.3 | 0.368 | 0.386 |
| 0.4 | 0.388 | 0.398 |
| 0.5 | 0.450 | 0.422 |
| 0.6 | 0.416 | 0.384 |

**Table 2. Different acid concentrations on absorbance.**

| 0.5 M acid concentration used | Absorbance (A)/ml of acid used |
|-----------------------------|-------------------------------|
| 0.25 ml | 0.5 ml | 0.75 ml | 1.0 ml |
| Acetic acid | 0.206 | 0.222 | 0.212 | 0.198 |
| Sulfuric acid | 0.246 | 0.322 | 0.306 | 0.294 |
| Nitric acid | 0.254 | 0.304 | 0.293 | 0.286 |
| Hydrochloric acid | 0.262 | 0.364 | 0.348 | 0.312 |
The firmest color was obtained with 1 ml of a 3-chloro-4-nitroaniline or sulfanilamide (1%) solution in 10.0 ml (Table 4).

**Effect of interference**

Some excipients generally present in the pharmaceutical preparations were examined by carrying out the determination of empagliflozin in the presence of different excipients such as glucose (1,200 µgml\(^{-1}\)), fructose (1,000 µgml\(^{-1}\)), lactose (800 µgml\(^{-1}\)), starch (600 µgml\(^{-1}\)), and urea (300 µgml\(^{-1}\)), which did not interfere.

**Analytical data**

A straight line is obtained in the graph by plotting absorbance beside the concentration of empagliflozin. (Figure 3) Beer’s law is obeyed in the range of 1.2–26.6 µgml\(^{-1}\) of empagliflozin with 3-chloro-4-nitroaniline or 0.8–20.4 µgml\(^{-1}\) of empagliflozin with sulfanilamide (Figure 3). The molar absorptivity of the colored azo dye of empagliflozin coupled with diazonium salt 3-chloro-4-nitroaniline or sulfanilamide was 3.179 × 10\(^4\) l mol\(^{-1}\)cm\(^{-1}\) or 4.367 × 10\(^4\) l mol\(^{-1}\)cm\(^{-1}\) (Figure 3). On the other hand, Sandell’s sensitivity to the colored system with nitrite-3-chloro-4-nitroaniline or nitrite-sulfanilamide was found to be 1.149 × 10\(^{-2}\) µgcm\(^{-2}\) or 8.368 × 10\(^{-3}\) µgcm\(^{-2}\), respectively.

The detection limit \((D_L = 3.3 \sigma/S)\) and quantitation limit \((Q_L = 10 \sigma/S)\) of empagliflozin coupled with diazotized 3-chloro-4-nitroaniline or sulfanilamide were found to be 0.363 and 1.100 µgml\(^{-1}\) or 0.270 and 0.820 µgml\(^{-1}\) [where \(\sigma\) is standard deviation \((n = 5)\) and \(S\) is slope of the curve] and the correlation coefficient of empagliflozin with 3-chloro-4-nitroaniline or empagliflozin with sulfanilamide was 0.999 or 0.998. The better optical characteristics and statistical data were obtained under optimum conditions (Table 5).

**Applications**

This simple and uncomplicated method is beneficial for determining empagliflozin in different pharmaceutical samples. The results of the offered method are in good agreement with the acknowledged content. The relative standard deviation and percentage recoveries for all five samples ranged from 0.81% to 2.27% and 98.00% to 100.40% at 95% confidence. The additional ingredients present in pharmaceutical sample appearances did form, not hinder. The results (Table 6) are compared with the endorsed spectrophotometric method (Ayoub, 2016; Patil et al., 2017). These confirm no significant differences between

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**Table 3. Effect of NaNO\(_2\) on absorbance.**

| 1 ml of NaNO\(_2\) solution used (M) | Absorbance (A) | 1 ml of NaNO\(_2\) solution used (M) | Absorbance (A) |
|-------------------------------------|----------------|-------------------------------------|----------------|
| 0.02                                | 0.188          | 0.10                                | 0.353          |
| 0.04                                | 0.208          | 0.12                                | 0.326          |
| 0.06                                | 0.264          | 0.14                                | 0.315          |
| 0.08                                | 0.287          | 0.16                                | 0.314          |

Figure 3. Adherence to Beer’s law using empagliflozin coupled with diazotized 3-chloro-4-nitroaniline and sulfanilamide.
Table 4. Effect of 3-chloro-4-nitroaniline or sulfanilamide solution on absorbance.

| 1% 3-chloro-4-nitroaniline or sulfanilamide solution used (ml) | Absorbance (A) for 3-chloro-4-nitroaniline | Absorbance (A) for sulfanilamide |
|---------------------------------------------------------------|------------------------------------------|---------------------------------|
| 0.25                                                          | 0.298                                    | 0.258                           |
| 0.50                                                          | 0.304                                    | 0.264                           |
| 0.75                                                          | 0.321                                    | 0.303                           |
| 1.00                                                          | 0.344                                    | 0.312                           |
| 1.25                                                          | 0.331                                    | 0.306                           |
| 1.50                                                          | 0.328                                    | 0.300                           |
| 1.75                                                          | 0.325                                    | 0.287                           |
| 2.00                                                          | 0.326                                    | 0.281                           |

Table 5. Optical characteristics and statistical data.

| Parameter                              | Values obtained when 3-chloro-4-nitroaniline used | Values obtained when sulfanilamide used |
|----------------------------------------|---------------------------------------------------|----------------------------------------|
| Molar absorptivity                     | $3.179 \times 10^4$ l mol$^{-1}$ cm$^{-1}$          | $4.367 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ |
| Shandell’s sensitivity                 | $1.149 \times 10^{-2}$ µg cm$^{-2}$                | $8.367 \times 10^{-3}$ µg cm$^{-2}$      |
| Detection limit                        | $0.363$ µg ml$^{-1}$                               | $1.100$ µg ml$^{-1}$                    |
| Quantitation limit                     | $0.270$ µg ml$^{-1}$                               | $0.820$ µg ml$^{-1}$                    |
| Linearity range (µg ml$^{-1}$)         | $1.2$–$26.6$ µg ml$^{-1}$                         | $0.8$–$20.4$ µg ml$^{-1}$               |
| Regression equation                    | $y = 0.092x - 0.018$                               | $y = 0.121x - 0.039$                    |
| Calibration sensitivity                | 0.092                                              | 0.121                                 |
| Correlation coefficient ($R^2$)        | 0.999                                              | 0.998                                 |
| Color stability                        | 12 hours                                           | 12 hours                              |
| $\lambda_{\text{max}}$ (nm)            | 470                                                | 480                                   |

Scheme 1. Formation of colored azo dye.
Table 6. Determination of empagliflozin in different pharmaceutical samples using 3-chloro-4-nitroaniline or sulfanilamide as a coupling agent for three trade names of empagliflozin.

| Pharmaceutical samples | Sample taken (μg ml\(^{-1}\)) | Using 3-chloro-4-nitroaniline | Using sulfanilamide |
|------------------------|---------------------------------|-------------------------------|---------------------|
|                        | Sample found (μg ml\(^{-1}\)) ± SD ± RSD | Rec. (%)                     | Sample found ± (μg ml\(^{-1}\)) ± SD ± RSD | Rec. (%) |
| Jardiance 25 (25 mg/tab.), Boehringer Ingelheim International GmbH, Germany | 5.000 | 4.90 ± 0.08 ± 1.63 | 98.00 | 4.96 ± 0.06 ± 1.21 | 99.20 |
|                        | 10.000 | 9.91 ± 0.12 ± 1.21 | 99.10 | 10.00 ± 0.10 ± 1.00 | 100.0 |
|                       | 15.000 | 14.94 ± 0.18 ± 1.20 | 99.60 | 14.96 ± 0.28 ± 1.87 | 99.73 |
|                        | 20.000 | 19.92 ± 0.25 ± 1.25 | 99.60 | 19.96 ± 0.35 ± 1.75 | 99.80 |
| Empagliflozin tab. (25 mg/tab.), Cipla Ltd, India | 5.0 | 4.96 ± 0.06 ± 1.20 | 99.20 | 4.98 ± 0.10 ± 2.00 | 99.60 |
|                        | 10.0 | 9.94 ± 0.12 ± 1.21 | 99.40 | 9.94 ± 0.16 ± 1.61 | 99.40 |
|                        | 15.0 | 14.92 ± 0.20 ± 1.34 | 99.40 | 14.94 ± 0.24 ± 1.60 | 99.60 |
|                        | 20.0 | 19.91 ± 0.38 ± 1.91 | 99.50 | 19.92 ± 0.30 ± 1.50 | 99.60 |
| Emjard 25 (25 mg/tab.), Square Centre, Bangladesh | 5.0 | 4.92 ± 0.04 ± 0.81 | 98.40 | 5.02 ± 0.06 ± 1.19 | 100.40 |
|                        | 10.0 | 9.93 ± 0.14 ± 1.41 | 99.30 | 9.95 ± 0.18 ± 1.81 | 99.50 |
|                        | 15.0 | 14.95 ± 0.16 ± 1.07 | 99.70 | 14.91 ± 0.28 ± 1.87 | 99.40 |
|                        | 20.0 | 19.92 ± 0.36 ± 1.81 | 99.66 | 19.90 ± 0.42 ± 2.11 | 99.50 |

* Mean (n = 5) ± SD (standard deviation) ± RSD (relative standard deviation).
the offered and endorsed methods. The precision and accuracy were evaluated by replicate analysis of three different samples containing empagliflozin at different concentrations.

CONCLUSION

Sulfanilamide and 3-chloro-4-nitroaniline, the first spectrophotometric coupling agents used to determine empagliflozin, are inexpensive and equitably selective. Compared to other methods, this one is simple, quick, sensitive, and reproducible, has good precision and accuracy, and has high dye stability (12 hours).

As low relative standard deviation and percentage recovery values highlighted good accuracy and precision of the proposed methods, no tedious separation or solvent extraction procedures were required. There is no interference from excipients in results obtained using the proposed methods. The proposed method examined empagliflozin levels in pharmaceutical samples, which can be applied to more complex samples. For example, a blood sample to determine the blood level of empagliflozin helps in various pharmacokinetic and toxicological studies.

ACKNOWLEDGMENTS

The authors would like to thank those who helped finish this research, especially the Faculty of Pharmacy at Mutah University.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FUNDING

There is no funding to report.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

PUBLISHER’S NOTE

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How to cite this article:
Abu Dayyih W, Hailat M, Al Hujran T, Magharbeh M, Zakaraya Z, Al Tamimi L, Aburumman AM, Abumansour H, Awad R. Spectrophotometric analysis of empagliflozin tablets as SGLT2 inhibitors in pharmaceutical samples. J Appl Pharm Sci, 2022; 12(10):140–146.