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Determination of Canagliflozin, Dapagliflozin, and Empagliflozin in Pharmaceutical Solid Dosage Forms by the HPLC Method

Israa Al-Ani[1], Marwah Thamer Abbas Al Saadi[2], Noor T. Alsaadi[2], Rima H. Al Omari[3], Mohammed F. Hamad[4], Wael Abu Dayyih[5]*, Laura G. Vicas[6], Riad Awad[7]

([1] Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, 19328, Jordan

[2] College of Pharmacy, Al-Muthanna University, Samawah, Iraq
[3] Pharmacological and Diagnostic Research Centre (PDRC), Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, 19328, Jordan

[4] Department of Basic Medical Sciences, Faculty of Medicine, Al-Balqa Applied University, Al-Salt, 19117, Jordan

[5] Faculty of Pharmacy, Mutah University, Al Karak, 61710, Jordan

[6] Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Oradea, Oradea, 410028, Romania

[7] Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, 11196, Jordan)

* Corresponding author: wabudayyih@mutah.edu.jo

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Abstract: This study aimed to develop a simple, sensitive, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of canagliflozin, dapagliflozin, and empagliflozin in their combined pharmaceutical dosage form or individually. HPLC separation was achieved utilizing a Hypersil-T C18 (150 mm x 4.6 mm, 5-µm particle size) analytical or equivalent column. A mixture of 1%

triethanolamine and 80% acetonitrile was used as the mobile phase at a flowrate of 1.2 ml/min. Detection was performed at 280 nm and ambient temperature. The retention times of canagliflozin, dapagliflozin, and empagliflozin were 1.854, 2.480, and 4.655 min, respectively. Linearity was obtained, ranging from 20 to 80 μg/ml for canagliflozin, dapagliflozin, and empagliflozin. The correlation coefficient for the method was greater than 0.999, indicating a strong linear relationship. The RP-HPLC method was thoroughly validated according to the ICH Q2 (R1) guidelines, and all validation parameters met the acceptance criteria. This validation demonstrated that the method's accuracy, precision, specificity, robustness, and limit of detection were within acceptable limits. Based on the successful validation and excellent method performance, this RP-HPLC method can be employed for the routine analysis of canagliflozin, dapagliflozin, and empagliflozin in raw materials or pharmaceutical dosage forms. Its simplicity, sensitivity, and accuracy make it a suitable choice for quality control and pharmaceutical research.

Keywords: canagliflozin; dapagliflozin; empagliflozin; high-performance liquid chromatography; validation; dosage form

HPLC法测定药物固体剂型中的卡格列净、达格列净和恩格列净

摘要：本研究旨在开发一种简单、灵敏且准确的反相高效液相色谱(RP-HPLC)方法，用于同时测定卡格列净、达格列净和恩帕格列净的联合药物剂型或单独剂型。采用海柏斯-T C18（150毫米x 4.6毫米，5微米粒径）分析柱或等效柱实现HPLC分离。以1%三乙醇胺和80%乙腈的混合物作为流动相，流速为1.2毫升/分钟。检测在280纳米和环境温度下进行。卡格列净、达格列净和恩帕格列净的保留时间分别为1.854、2.480和4.655分钟。卡格列净、达格列净和恩帕格列净的线性范围为20至80微克/毫升。该方法的相关系数大于0.999，表明具有很强的线性关系。根据ICH Q2 (R1)指南对RP-HPLC方法进行了全面验证，所有验证参数均符合验收标准。此验证表明该方法的准确度、精密度、特异性、耐用性和检测限均在可接受的范围内。基于成功的验证和出色的方法性能，此RP-HPLC方法可用于原材料或药物剂型中卡格列净、达格列净和恩帕格列净的常规分析。其简便性、灵敏度和准确性使其成为质量控制和药物研究的合适选择。

关键词：卡格列净；达格列净；恩格列净；高效液相色谱法；验证；剂型

1. Introduction

In recent years, the development of effective antidiabetic drugs has played a crucial role in managing diabetes mellitus (DM) and improving the quality of life of patients. Among the innovative therapeutic options available for the management of Type II DM, sodium-glucose co-transporter-2 (SGLT-2) inhibitors have gained significant attention because of their ability to lower blood glucose levels by effectively diminishing the reabsorption of glucose filtered by the kidneys and preventing its return to the bloodstream [1]. Remarkably, their insulin-independent mechanism of action allows them to be effective when other drugs that rely solely on stimulating insulin release, such as sulfonylureas and meglitinides, or partially rely on it, such as gliptins and GLP-1 analogs, are less effective [2]. SGLT-2 inhibitors exhibit 82% plasma protein binding, 36.8% partitioning into red blood cells, 78% bioavailability, and a half-life ranging from 5.6 to 13.1 hours when administered orally [3]. Canagliflozin, dapagliflozin, and empagliflozin are notable members of this class and have demonstrated remarkable efficacy in the treatment of type 2 diabetes [4].

As pharmaceutical industries strive to meet the increasing demand for SGLT-2 inhibitors, a sensitive and simple analytical method for their quantification is imperative. High-performance liquid chromatography (HPLC) has long been established as a powerful technique for drug analysis, providing a balance between simplicity, sensitivity, and accuracy [5]. The novelty of this study lies in the criteria for the choice of research.

In this study, we present a robust RP-HPLC method for the simultaneous estimation of canagliflozin,

dapagliflozin, and empagliflozin in their combined pharmaceutical dosage forms or as individual components. The method employs a Hypersil-T C18 analytical column or its equivalent, which has been widely recognized for its excellent separation capabilities.

2. Experiment

2.1. Instrument

Peak areas were measured using a Hitachi Chromaster HPLC system (Hitachi Group, Germany). This HPLC system is equipped with a 5410 UV detector, 5260 auto samplers, 5310 column oven, and 5160 quaternary pumps. The column used to achieve the separation was Symmetry® C18-(250 cm×4.6 mm, 5 µm, average particle size) (Waters Corp., Ireland). Chromatographic data analysis was performed using Clarity VA Chromatography System Version 8.1 [6].

2.2. Reagent and Chemicals

The chemicals and materials used were of HPLC-pharmaceutical grade, and distilled water was used throughout the investigation. Canagliflozin, dapagliflozin, and empagliflozin were gifted by Hikma (Jordan). Acetonitrile (HPLC grade) and Nylon Filter membranes (diameter = 47 mm, pore size = 0.45 µm) were obtained from Merck (Darmstadt, Germany), and trimethylamine (TMA) was purchased from Thermo Fisher Scientific (United Kingdom).

2.3. Chromatographic Conditions

A stationary phase consisting of an Inertsil C8 column (25 cm x 4.6 mm, 5 µm, GL Science, Japan) was utilized at ambient temperature for chromatographic analysis. Isocratic elution was conducted using a mobile phase with multi-gradient composition. Prior to use, the mobile phase was filtered through a 0.45-µm nylon disc filter (Millipore) and subjected to sonication for 20 min.

The chromatographic flow rate was set at 1.2 ml/min, and 20 µl of the sample was injected. The UV detector was operated at a wavelength of 280 nm, and separation was achieved using a Hypersil-T C18 column (3.9 x 150 nm) maintained at ambient temperature.

2.4. Preparation of Standard Solution

2.4.1. Preparation of Canagliflozin Standard Solution

Accurately weighed 5-mg canagliflozin was transferred to a 10-ml volumetric flask, dissolved, and diluted to the mark with mobile phase to obtain a final concentration of 500-µg/ml canagliflozin. Subsequently, the solution was filtered through a 0.20-µm membrane filter paper and sonicated for 5 min. Following this, 1 ml was transferred to a 10-ml volumetric flask and diluted to the mark with mobile phase to obtain a final concentration of 50-µg/ml canagliflozin.

2.4.2. Preparation of Dapagliflozin Standard Solution

Dapagliflozin (5 mg) was accurately weighed and transferred to a 10-mL volumetric flask, dissolved, and diluted to the mark with mobile phase to obtain a final concentration of 500-µg/mL dapagliflozin. Subsequently, the solution was filtered through a 0.20-µm membrane filter paper and sonicated for 5 min. One milliliter of this solution was then transferred to a 10-mL volumetric flask and diluted to the mark with mobile phase to obtain a final concentration of 50-µg/mL dapagliflozin.

2.4.3. Preparation of Empagliflozin Standard Solution

Accurately weighed empagliflozin (5 mg) was transferred to a 10-mL volumetric flask, dissolved, and diluted to the mark with mobile phase to obtain a final concentration of 500-µg/mL empagliflozin. Subsequently, the solution was filtered through a 0.20-µm membrane filter paper and sonicated for 5 min. A 1-mL aliquot was then transferred to a 10-mL volumetric flask and diluted to the mark with mobile phase to obtain a final concentration of empagliflozin of 50 µg/mL.

2.4.4. Preparation of Standard Mixture Solution of Dapagliflozin, Canagliflozin, and Empagliflozin (1:1:1)

Accurately weighed 5-mg samples of dapagliflozin, canagliflozin, and empagliflozin were transferred to a 10-ml volumetric flask, dissolved, and diluted to the mark with mobile phase to obtain a final concentration of 500-µg/ml dapagliflozin. Subsequently, the solution was filtered through a 0.20-µm membrane filter paper and sonicated for 5 min. Following this, 1 ml of the solution was transferred to a 10-ml volumetric flask and diluted to the mark with mobile phase to obtain final concentrations of dapagliflozin, canagliflozin, and empagliflozin (50:50:50 µg/ml).

2.5. Validation of the Developed Method

The developed method was validated according to the ICH Guidelines for validation of analytical procedure Q2(R1) [7].

2.5.1. Specificity

Defining the ability to accurately and specifically measure the analyte of interest without interference from the blank: A solution containing 50-µg/ml dapagliflozin, 50-µg/ml canagliflozin, and 50-µg/ml empagliflozin was prepared. The prepared solutions were analyzed using the proposed method. The mobile

phase was utilized as a blank and analyzed according to the proposed method. Interferences from the blank that could affect the accurate and specific measurement of the analyte of interest were evaluated.

2.5.2. Linearity

A standard mixture containing 25-, 40-, 50-, 60-, and 75-µg/ml dapagliflozin, canagliflozin, and empagliflozin was prepared from the standard mixture solution as described above. The prepared solutions were analyzed using the proposed method. Five replicate analyses were conducted. The mean area, its standard deviation, and percent relative standard deviation of the peak area were calculated. Subsequently, the mean AUC was plotted against concentration to obtain the calibration curve. Regression equations and correlation coefficients were computed from the calibration curves.

2.5.3. Accuracy and Recovery Studies

Accuracy was calculated by adding standard drugs to pre-analyzed samples at three different concentration levels and computing the percentage recoveries. Accuracy was assessed using nine determinations over three concentration levels covering the specified range (e.g., 3 concentrations and three replicates for each of the total analytical procedures).

The prepared solutions were analyzed according to the proposed method, and the percentage recoveries were calculated from the absorbance ratio. The mean percentage recovery, along with its standard deviation and percent relative standard deviation, was calculated at each level.

2.5.4. Precision

The precision of the method was computed using two means: repeatability and intermediate precision.

Repeatability: system precision and method precision.

System precision: A solution containing a mixture of 50-µg/ml dapagliflozin, 50-µg/ml canagliflozin, and 50-µg/ml empagliflozin (100% test concentration) was prepared from their respective working mixture solutions. The prepared solution was analyzed five times using the proposed method. The mean peak area and its standard deviation were calculated for the drugs.

Method precision: Six replicate solutions containing mixtures of 50-µg/ml dapagliflozin, 50-µg/ml canagliflozin, and 50-µg/ml empagliflozin (100% test concentration) were prepared from their respective working mixture solutions. The prepared solution was analyzed according to the proposed method, and the mean peak area, its standard deviation, and relative standard deviation were computed for the drugs.

2.6. Ruggedness: Replication on Different Days

Six replicate solutions containing a mixture of 50-µg/ml dapagliflozin, 50-µg/ml canagliflozin, and 50-µg/ml empagliflozin (100% test concentration) were implemented on the first day; on the second day, five replicates of freshly prepared dapagliflozin, canagliflozin, and empagliflozin were analyzed. The same analyst performed both tests.

2.7. Robustness

A solution containing a mixture of 50-µg/ml dapagliflozin, 50-µg/ml canagliflozin, and 50-µg/ml empagliflozin (100% test concentration) was prepared from their respective working mixture solutions prepared as described above, and the prepared solution was analyzed according to the proposed method with a small but deliberate change in chromatographic conditions.

The mean peak area with standard deviation and % relative standard deviation was computed at each level.

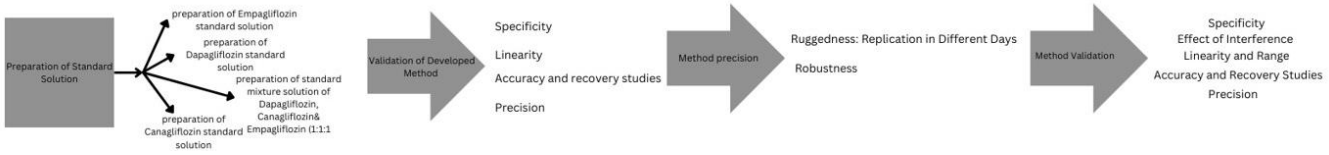

Figure 1. Change from column to column (The authors' elaboration)

3. Results and Discussion

3.1. Method Development

The HPLC method was accurate and precise for simultaneous determination of canagliflozin, dapagliflozin, and empagliflozin. Good resolution of both components was obtained with triethanol amine buffer: acetonitrile at a ratio of 97:3 v/v for 3 min and 85:15 v/v from 3 to 8 min with a flow rate of 1.2 ml/min was optimum. UV detection was performed at 280 nm. Canagliflozin, dapagliflozin, and empagliflozin were quantified at a wavelength of 280 nm. This wavelength was empirically determined to be the optimal choice for analysis. The average retention times for canagliflozin, dapagliflozin, and empagliflozin were found to be 1.854, 2.480, and 4.688 min, respectively, as shown in the chromatograms. The system suitability parameters for the chromatogram area were evaluated to assess the performance of the analytical system.

3.2. Method Validation

3.2.1. Specificity

The method specificity was determined for canagliflozin, dapagliflozin, and empagliflozin samples and placebos of the chemical mixture. Canagliflozin, dapagliflozin, empagliflozin, and placebo spectra showed no interference for canagliflozin, dapagliflozin, and empagliflozin peaks. Therefore, the data obtained for canagliflozin, dapagliflozin, and empagliflozin were considered acceptable for method specificity, as can be seen from the respective chromatograms.

3.2.2. Effect of Interference

The utilization of interference materials, including saccharine sodium, lactose, microcrystalline cellulose, magnesium stearate, titanium dioxide, talc, sodium starch glycolate, and colloidal anhydrous silica, at a tenfold excess with a 5-mg/ml concentration of each drug demonstrated no interference with any of the substances.

3.2.3. Linearity and Range

Various concentrations of canagliflozin, dapagliflozin, and empagliflozin (20–80 μg/ml) were used to prepare standard solutions at 50–150% of the theoretical quantity of each drug. The objective was to establish good linearity, which was defined by achieving a correlation coefficient of not less than 0.999 when plotting the concentration against the peak area.

3.2.4. Accuracy and Recovery Studies

To assess the accuracy of the method, samples were prepared by spiking known quantities of canagliflozin, dapagliflozin, and empagliflozin standards into a placebo matrix containing all the excipients present in the product. The spiked samples were measured at three concentrations: 100%, 50%, and 150% of the target concentration. The accuracy of the method was evaluated by calculating the percentage recovery of each concentration, which represents the extent to which the measured values corresponded to the actual values of the spiked compounds.

3.2.5. Precision

System precision: Six replicates of the same solution from 50-μg/ml canagliflozin, 50-μg/ml dapagliflozin, and 50-μg/ml empagliflozin (RSD ≤ 1%).

Method precision: Six replicates of the same solution from 50-μg/ml canagliflozin, 50-μg/ml dapagliflozin, and 50-μg/ml empagliflozin (RSD ≤ 1%).

3.3. Ruggedness

Six replicates of the same solution from 50-μg/ml canagliflozin, 50-μg/ml dapagliflozin, and 50-μg/ml empagliflozin are implemented on the first day; on the second day, the method is rugged as the % (RSD not more than 3%).

3.4. Robustness

A modified column approach is employed to analyze six replicates of a single sample containing canagliflozin, dapagliflozin, and empagliflozin; the same analyst performs both tests.

The method is rugged as the %RSD is not more than 3%.

4. Synthetic Formulation Product Analysis

The synthetic formulation product contained 5-mg dapagliflozin, canagliflozin, and empagliflozin. A weight equivalent to 5 mg of the three drugs was transferred to a 10-ml volumetric flask. Mobile phase was added to volume, and the flask was sonicated for 5 min. The solution was then filtered through 0.20-um membrane filter paper. The filtrate solution was diluted with the mobile phase to obtain a final concentration of 50-μg/ml dapagliflozin, canagliflozin, and empagliflozin. The prepared solution was injected into the system under the chromatographic conditions described above. Chromatography was stopped after complete separation was achieved. The peak areas were recorded. The concentration of the synthetic formulation product was computed by setting the value of the peak areas in the respective standard regression equation obtained from the calibration curve. The analysis procedure was repeated thrice using the synthetic formulation product.

5. Applications

The proposed procedures, which are sensitive and accurate, can determine the substances studied in synthetic pharmaceutical formulations.

6. Conclusion

6.1. Main Findings of the Study

The investigation successfully developed and validated a stability-indicating RP-HPLC method for the simultaneous quantification of dapagliflozin, canagliflozin, and empagliflozin.

6.2. Comparison with Other Studies

The method was found to be more precise, sensitive, selective, robust, and linear over a concentration range of 25-80 μg/mL for all the three drugs. It was also free from interference from excipients used in the pharmaceutical formulations.

6.3. Implications of the Study

The developed method can be used for routine analysis in pharmaceutical factories to determine the three drugs individually, in a chemical mixture, or alone.

Declarations

Author Contributions

Conceptualization, I.A.-A.; methodology, M.T.A.A.S.; validation, M.F.H.; formal analysis, R.H.A.O.; investigation, N.T.A.; resources, R.A.; data curation, W.A.D.; writing—original draft preparation, all authors contributed equally; writing—review and editing, L.G.V.; supervision, I.A.-A.; project administration, M.T.A.A.S. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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