

Development and validation of a dissolution test for empagliflozin in film-coated tablets

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The present study proposes a validated dissolution method for empagliflozin (EMPA) in film coated tablets. A gradual *in vitro* dissolution profile for this formulation was obtained using 900 mL of hydrochloric acid 0.01 M at 37 °C ±0,5 °C as dissolution medium and USP apparatus 2 (paddle) at 50 rpm. The dissolved percentage of EMPA was quantified by ultraviolet spectrophotometric method to obtain cost technique and produce little residual solvents. Validation parameter for dissolution methodology such as the specificity, linearity, accuracy and precision were evaluated according to the international guidelines, giving results within the acceptable range. The method is linear in the range of 1 - 40 µg/mL, precise, with RSD value less than 2.62%, accurate (mean recovery 106.97%) and robust. Therefore, since no official method has been described, the proposed dissolution conditions represent a relevant contribution to evaluate the dissolution profile of coated tablet containing 25 mg of EMPA.

Keywords: Empagliflozin; Dissolution; Validation.

Article received at 11/03/2021 and accepted at 22/06/2021.

<https://doi.org/10.22456/2527-2616.112077>

Introduction

In vitro dissolution testing for oral dosage forms such as capsules and tablets are used to evaluate the batch quality of the drug, guide the process of new formulations and ensure quality after certain changes in the product (formulation, place of fabrication and scale-up). During the development of the method, it is important to evaluate drug characteristics such as solubility, permeability and pharmacokinetic data (1,2).

Empagliflozin (EMPA) is a potent and selective sodium glucose co-transporter 2 inhibitor (SGLT2). The SGLT2 inhibitors are used to management of glycemic control in type 2 diabetes mellitus. Approved by FDA in 2014, EMPA has an independent insulin action mechanism. (3,4). EMPA chemical designation is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol. The chemical structure is presented in Figure 1.

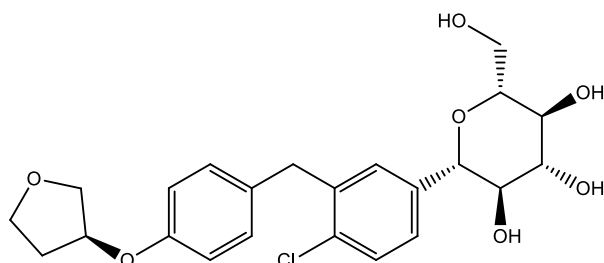


Figure 1. Chemical structure of empagliflozin.

Analytical methods were developed and validated for determination of EMPA in pharmaceutical formulation. Ayoub (2016) developed spectrophotometric and

chemometric method determination of EMPA and metformin. The wavelength used was 225 nm. (5). Padmaja and Veerabhadram develop RP-HPLC method for determination of EMPA with detection at 260 nm using PDA detector. (6) Ayoub and Mowaka using mass detector HPLC for EMPA determination. (7). Manoel J. *et al.* (2020) determine empagliflozin in the presence of its organic impurities using optimization by Quality by Design (8). However, there is no available method for the evaluation of dissolution profile of EMPA.

According to the biopharmaceutical classification system (BCS) EMPA is a class III drug, which presents a high solubility in and low permeability (water solubility = 0.111 mg/mL and log P = 1.79) (9,10). The FDA has described conditions used for the dissolution test. In analysis of EMPA tablets, FDA used these conditions: apparatus II (Paddle), 75 rpm, 0.05M Phosphate Buffer, pH 6.8, 900 mL and collection times of 5, 10, 15, 20 and 30 min. The FDA does not suggest analytical techniques for drug quantification and the dissolution method is not present in any pharmacopoeia. In this study, the HPLC method was used for the analysis of solubility of EMPA. Subsequently, the UV method was proposed to analyze the results as it is a simpler method and produces less solvent for disposal.

In this context, the purpose of this study was to develop and validate a dissolution method for EMPA tablets using UV-Vis spectrophotometry.

Material and methods

Chemical and reagents

EMPA (purity 99.3%) reference substance was purchase by Carbosynth (Berkshire, UK). Jardiance® (Boehringer

Ingelheim, Germany). Tablets for oral administration, containing 25mg of EMPA were obtained from local pharmacies and used within their shelf-life period. The excipients contained in the dosage form (lactose monohydrate, microcrystalline cellulose, hydroxypropylcellulose, croscarmellose sodium, colloidal anhydrous silica, magnesium stearate, hypromellose, titanium dioxide, talc, macrogol and iron oxide yellow) were all of pharmaceutical grades and acquired from different suppliers for placebo preparation.

Reference solutions

The stock solutions of the drug were prepared by accurately weighing 10 mg of EMPA reference substance and dilution to volume with acetonitrile:water (50:50 v/v) to obtain the concentration of 1 mg/mL. This solution was further diluted with HCl 0.01M and other dissolution media employed.

Instruments

The HPLC system consisted of a Shimadzu 20 A, equipped with CBM-20A controller, LC-20AT pump, SIL-20 AC autosampler, CTO-20AC oven and PDA SPD-M20A detector. The software used for control and data acquisition was the LC-Solution from Shimadzu.

Spectral and absorbance measurements were performed with an UV-Vis Shimadzu model UV 1800 using 10 mm quartz cells and detection at 230 nm.

The dissolution analyses were performed with a Vankel® VK 7010 (Agilent Technology Group, USA) multi-bath (n=8) dissolution station, with VK 8000 auto-sampling station, VK bidirectional peristaltic pump and a VK 750D digitally controlled heater/circulator.

Chromatography conditions

A method using HPLC with UV detection at 230 nm was previously developed and validates in our laboratory by a wide range of parameters including specificity, linearity, precision, accuracy and robustness. Chromatography separations was achieved on a Phenyl column (4.6 mm x 250 mm i.d., particle size 5 µm). The mobile phase was composed of acetonitrile and water (72:28, v/v). The mobile flow rate was 0.84 mL/min with isocratic elution and was filtered before use through a 0.45 µm membrane filter. The injection volume was 20 µL and the temperature was maintained at 30 °C (8).

Sink conditions evaluation

EMPA sink conditions were checked in different media: purified water, 0.01 M hydrochloric acid, 0.05 M acetate buffer (pH 4.5), 0.05 M phosphate buffer (pH 6.8 ad 7.5). Vessels containing 15 mL of each medium were pre-heated to 37 ± 0.5°C before adding EMPA (10 mg). This relation mass by volume is 24 times major the concentration to be obtained by EMPA tablet dose and usual dissolution medium volume (900 mL). The preparations were

maintained in the bath for 30 min and visual solubilization was observed. Aliquots of these solutions were transferred to 10.0 mL volumetric flasks, diluted with mobile phase at final theoretical concentration of 40 µg/mL. These solutions were filtered through a 0.45 µm membrane filter and injection into the HPLC. Standard solution of EMPA was prepared in different media for comparison with sample solutions.

Dissolution test conditions

Tablets containing 25 mg of EMPA were evaluated using 900 mL (37 ± 0.5°C) of hydrochloric acid 0.01 M as dissolution medium using USP apparatus (paddle) at a rotation 75 rpm. The sample was performed by withdrawing 10 mL of aliquots with immediate filtration through 10 µm filters connected to the cannulas of the equipment. The dissolution samples were analyzed by the UV method at predetermined intervals (5, 10, 15, 20, 30, 40, 50 and 60 min), without dilution due to the low concentration of the drugs in the pharmaceutical formulation. The cumulative percentage of drug release was plotted against time in order to obtain the release profile. A correction approach was included in the calculations to account for the drug removed from the sampling.

Validation of dissolution procedure

Analytical method validation is the process of demonstrating that an analytical procedure is suitable for its intended purpose. To demonstrate that the UV method developed is applicable to the dissolution assessment, validation was performed according to official guidelines (1,11). The parameters evaluated were: specificity, linearity, precision, accuracy and robustness (11,12).

To prepare the product sample for validation procedure, tablets containing 25 mg of EMPA were weight and crushed to fine powder. An amount of 207.57 mg (equivalent to average weight of tablets) was transferred into the vessels containing 900 mL of media. After the collection times, samples were analyzed by the spectrophotometric method.

Results and discussion

Sink conditions evaluation

Solubility data were used as the basis for the selection of a dissolution medium for EMPA. Drug solubility was evaluated at 37 °C in different media and expressed as percentage of drug dissolved. Sink condition is the volume of medium at least three times that required in order to form a saturated solution of drug substance (1,13). To evaluate this condition, 6 different media were used, and the samples were analyzed by HPLC.

In the evaluation of the sink condition, tests were carried out with the medium of hydrochloric acid 0.01 M and 0.1 M, monobasic potassium phosphate buffer 0.05 M pH 6.8 and pH 7.4, sodium acetate buffer 0.05 M pH 4.5 and

water. In this protocol, conducted as little EMPA bulk was available, the test showed that EMPA presented sink conditions at all the tested media (900 mL), as quantity/volume 24 times major than dose/volume medium was completely dissolved at 37 °C. The percentage of dissolved drug varied from 100.20 a 104.33%, that is, all 10 mg was dissolved in 15 mL of each medium employed. The traditional shake flask method would require a lot of bulk drug, unworkable in this study.

Dissolution method optimization

Several conditions were evaluated in the selection of a dissolution method for analysis of EMPA tablets. The dissolution method should be discriminative, accurate, transferable and robust, adequate of detecting in the product any changes made in the manufacturing process. According to USP, the adjustments of the parameters evaluated in the dissolution of the drug must be made based on the solubility, pH and pka of the drug. The test should be carried out under moderate conditions, paddle for example 50 or 75 rpm, to generate a dissolution profile. Depending on the drug dissolution time, the need for collection intervals of 5 or 10 min is assessed (13).

In order to simulate the conditions of the gastrointestinal tract (gastric and intestinal fluid) and to follow FDA recommendations, at least three dissolution media should be tested. The choice of the dissolution medium was based on the physical characteristics of the drug and its solubility. The pH of the medium was evaluated with the intention of simulated the body compartments. The media constituted by acetate buffer pH 4.5 and phosphate pH 7.4 were not selected for the dissolution tests because in this work we prioritize the study with media that simulated gastric fluid (pH between 1.0 - 3.5) and intestinal fluid (pH 6.8 is recommended). According to the literature, the EMPA pka is equal to 13.23 ± 0.70 , therefore is in its unionized form in all the media employed (10).

Following the analysis, 0.1 M and 0.01 M hydrochloric acid, potassium phosphate pH 6.8 media were selected for the dissolution test. Preliminary tests were performed using apparatus 2 (paddle) at 50 rpm, with 900 mL of medium volume (37 ± 0.5 °C). The results (Fig. 2) demonstrated that in 15 min approximately 80% of the drug had already been dissolved. These conditions are in accordance with the recommendations for quality control of immediate release tablets (1).

The drug EMPA is classified as class 3 by BCS, that is, it has high solubility and low permeability. This characteristic was confirmed by observing the rapid solubility of the drug in the evaluated media. In the four media tested, EMPA dissolved the tablets close to 80% in just 15 min. Correlating with the behavior in vivo, these data suggest that the complete dissolution of EMPA probably occurs in the stomach. It is preferable that drugs with low permeability have rapid dissolution. Due to this characteristic, the drug quickly solubilizes in the medium and, thus, it will have more time in contact with the gastrointestinal epithelium to permeate slowly (14).

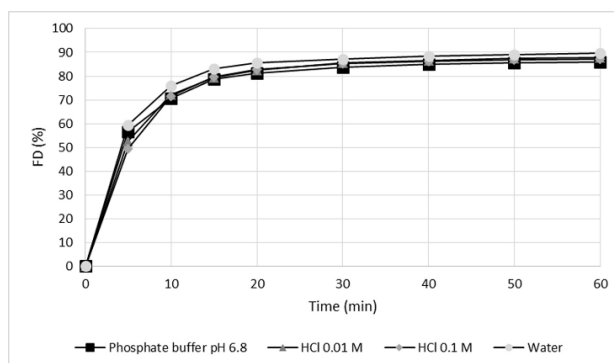


Figure 2. Dissolution profiles of empagliflozin from coated tablets using phosphate buffer pH 6.8, 0.01 M HCl, 0.1 M HCl and water; 50 rpm; 900 mL of media and apparatus 2 (paddle).

In this first preliminary test, it was observed that the dissolution plateau remained at 90% of the dose in all evaluated media. Because of this, a new dissolution test was performed with a rotation of 75 rpm, instead of 50 rpm. The HCL 0.1 M, HCL 0.01 M and potassium phosphate 0.05 M media were used again. The medium containing only water was not evaluated as it is not considered an ideal dissolution medium. There are reasons such as pH variability and surface tension, can vary depending on the source of water and may change during the dissolution tests itself, due to the influence of the active as inactive ingredients (1,13). The test was carried out under the conditions: apparatus 2 (paddle) at 75 rpm, with 900 mL of medium volume (37 ± 0.5 °C) for 100 min. The results of the EMPA dissolution profile are shown in Figure 3.

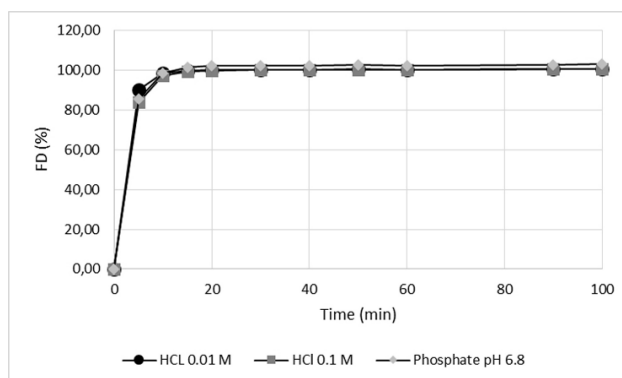


Figure 3. Dissolution profiles of empagliflozin from coated tablets using phosphate buffer pH 6.8, 0.01 M HCl, 0.1 M HCl and water; 75 rpm; 900 mL of media and apparatus 2 (paddle).

Figure 3 shows that the modification caused an increase in the dissolution of EMPA from the tablets. In just 5 min, the dissolution was greater than 80% in the three media evaluated. A test with a more gradual dissolution rate is more interesting for the evaluation of the quality control of the tablet. The use of surfactants was not evaluated at any time because the drug EMPA showed high solubility in the tested media.

The selected medium was HCl 0.01 M because, in addition to presenting satisfactory drug dissolution, it is easy to prepare for the testing routine in the quality control laboratory and corresponds to gastric pH but with low hydrogeonic concentration.

The spectra obtained in 0.01M hydrochloric acid demonstrate a maximum absorbance at 230 nm, so this wavelength was selected for the analysis of the dissolution test. The paddle apparatus was selected as it is the most used for the pharmaceutical form tablets. Regarding the speed of dissolution, 50 rpm was chosen, because even if in 60 min it did not dissolve 100% of the dose, the dissolution was more gradual in the analyzed time. The agitation at 75 rpm allowed the 100% dissolution percentage to be obtained, but in the first minutes the dissolved percentage has already exceeded 80%. Figure 4 shows the comparison of the dissolution profile of EMPA in HCL 0.01 M medium under agitation of 50 and 75 rpm, 900 mL of medium and 60 min analysis time.

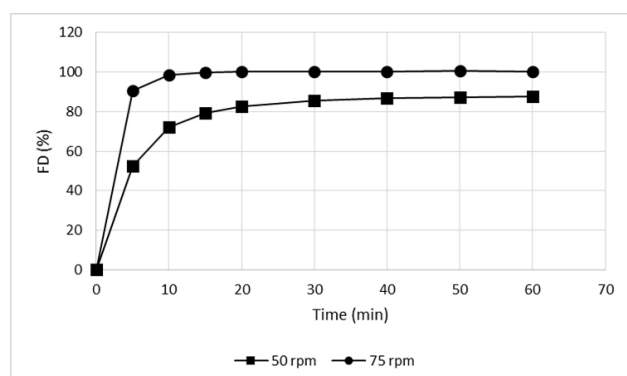


Figure 4. Dissolution profiles of empagliflozin from a coated tablets using 0.01 M HCl, 50 rpm e 75 rpm; 900 mL of media and apparatus 2 (paddle).

After selecting the dissolution conditions for EMPA tablets, the method was validated. Quantification was performed by UV-Vis spectrophotometry. The spectrophotometric method was selected due to the ease of sample preparation, low cost and to produce little residual solvents.

Validation of dissolution method

Specificity

The specificity of the method was evaluated to verify that the formulation excipients and the dissolution medium did not interfere in the analysis of EMPA. The specificity of the proposed method was established by preparing the placebo samples (mixture of all the tablet excipients in their usual concentration in pharmaceutical formulations without the active ingredient). An amount of mixture equivalent to an average weight of EMPA tablet was transferred to separate vessels (2) with 900 mL of the dissolution medium at 37 ± 0.5 °C and stirred for 60 min at 150 rpm using paddle. Aliquots were withdrawn and analyzed by UV-Vis spectrophotometry.

Figure 5 represents the spectrum obtained for the solutions of the tablets and the formulation excipients. The spectrum not demonstrated appreciable interference of pharmaceutical excipients in the wavelength 230 nm. The excipients interference was calculated as 0.97 % not

exceeding the limit of 2%, at the selected wavelength, compared to a standard solution of EMPA.

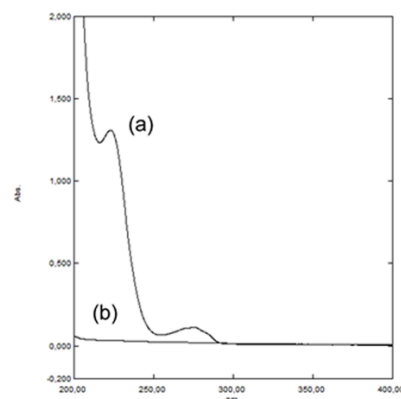


Figure 5. UV spectrum of empagliflozin reference standard at 28.0 µg/mL (a) and placebo (b) in 0.01 M hydrochloric acid.

Linearity

Appropriate amounts of EMPA stock solution (1000 µg/mL) prepared in acetonitrile:water (50:50 v/v) were diluted with 0.01 M hydrochloric acid to give concentrations of 1.0, 5.0, 10.0, 20.0, 30.0 and 40.0 µg/mL. This range includes the low concentrations found in the initial EMPA dissolution time points and exceeds the maximum dose that can be dissolved. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis and also by analysis of variance (ANOVA).

The representative linear equation for this drug in 0.01 M HCl at 230 nm was $y = 0,0366x + 0,0236$ and the coefficient of determination, R^2 was 0.9995. The variance analysis ($P = 0.05$) was performed to verify the good fitting of the method and the results showed that no deviation from linearity was revealed for EMPA ($F_{\text{calculated}} < F_{\text{critical}}$). According to ANOVA there is linear regression and there is no deviation from linearity.

Accuracy

The accuracy was determined by adding known amounts of powdered EMPA tablets pool corresponding to drug level of 25, 100 and 125% of the labeled amount in an average weight equivalent to a tablet. The dissolution test was run for 30 min using 900 mL of the medium and Apparatus 2 at 50 rpm. This approach was used because of the limited availability of the EMPA reference standard. The drug content in the tablets pool was 100.64% of the labeled dose.

The accuracy of the method was demonstrated by the recovery of the known amounts of EMPA from the tablet pool to the dissolution vessels. Three levels were evaluated, low, medium, and high, were corresponding a 6.25, 25.0 and 31.25 mg of EMPA, respectively. The results obtained are described in Table I. The percentage recoveries ranging demonstrate the satisfactory accuracy of the method (105.19 – 108.87%).

Table 1. Accuracy of UV method for determination of EMPA.

Level (%)	Added EMPA (mg)	Added tablets "pool" (mg)	Mean recovery \pm RSD (%)
25	6.25	51.89	108.87 \pm 1.19
100	25.0	207.57	105.19 \pm 1.74
125	31.25	259.46	106.87 \pm 3.60

Precision

Repeatability and intermediate precision were used to assess method precision. Repeatability was evaluated through the relative standard deviation (RSD) from adding known amounts of the powdered EMPA tablets pool, corresponding to a drug of 100% (labeled amount in an average weight equivalent to a tablet). The dissolution test was run for 30 min using 900 mL of medium at 37 ± 0.5 °C and Apparatus 2 at 50 rpm, over two days (n = 6). The assay evidenced a low RSD equal 2.38 and 3.07 for intra-day precision and 2.62 for inter-day precision. RSD values lower than 5% indicates the good precision of this method.

Robustness

The robustness was studied by analyzing small changes in the dissolution conditions, such as comparing the dissolution medium with and without using the ultrasonic bath, and change porosity filter of 70 μ m. Labeled amount in an average weight equivalent to a tablet was used for this parameter analysis. The dissolution test was evaluated for 30 min using 900 mL of medium at 37 ± 0.5 °C and Apparatus 2 at 50 rpm. Two vessels for each modification were used to evaluate the proposed changes.

None of the changes made affected the dissolution process. The average content with deaeration of the medium was 99.18% (\pm 0.69) and when changing the filter porosity to 70 μ m it was equal to 99.11% (\pm 0.92).

Conclusion

The *in vitro* dissolution evaluation is essential for providing process control and quality assurance of pharmaceutical products. The dissolution test developed and validated for EMPA was considered satisfactory. The dissolution test for EMPA tablets was developed using 900 mL of 0.01 M hydrochloric acid medium and paddle (USP apparatus 2) at 50 rpm of rotation, different from the conditions presented by the FDA. The percentage of drugs dissolved was determined by the ultraviolet spectrophotometric method. The proposed dissolution method was successfully validated according to the international requirements. The method has the advantages of being fast and simple. The method is suitable for its purpose and could be applied in routine quality control of EMPA in tablet formulation since there is no official monograph for this drug in the pharmacopoeias.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors wish to thank CNPQ (Scientific Project MCT/CNPq N° 014/2013).

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