International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF POORLY WATER SOLUBLE DRUG EMPAGLIFLOZIN USING HYDROTROPIC PHENOMENA

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***Article History:**

Received: 26/10/2023
Revised: 14/11/2023
Accepted: 25/11/2023

ABSTRACT

This research focuses on the development and validation of an analytical method for the estimation of the poorly water-soluble drug Empagliflozin (EGF) utilizing hydrotropic phenomena. The challenge of limited aqueous solubility associated with EGF necessitates innovative approaches to enhance its solubility for accurate and reliable analysis. Hydrotropy, a technique involving the use of hydrotropic agents to solubilize poorly water-soluble drugs, has been employed to address this challenge. The analytical method was developed with a focus on key parameters, including linearity, recovery, repeatability, day-to-day variation, analyst-to-analyst variation, and reproducibility. Linearity was established over the concentration range of 10-50 µg/ml. Recovery studies were conducted at three different levels (80%, 100%, and 120%) to assess the accuracy of the method. Repeatability, day-to-day variation, analyst-to-analyst variation, and reproducibility were evaluated to ensure the precision and robustness of the developed method. The method exhibited excellent linearity over the concentration range of 10-50 µg/ml, ensuring accurate quantification of EGF within this range. Recovery studies at three different levels (80%, 100%, and 120%) demonstrated high accuracy, with % RSD values ranging from 0.328% to 0.532%. These results indicate the reliability of the method for quantifying EGF in tablet formulations. Repeatability, day-to-day variation, analyst-to-analyst variation, and reproducibility studies demonstrated low %RSD values (ranging from 0.104% to 1.027%), indicating the precision and robustness of the developed method. The LOD and LOQ were found to be 0.15 µg/ml and 0.45 µg/ml, respectively, indicating the sensitivity of the method for detecting low concentrations of EGF.

Key words: Method development, Validation, Empagliflozin, Hydrotropic phenomena

INTRODUCTION

Empagliflozin is a member of the sodium-glucose co-transporter 2 (SGLT2) inhibitor class, widely prescribed for the management of type 2 diabetes mellitus. Despite its therapeutic efficacy, the drug encounters challenges related to its poor water solubility, potentially affecting its bioavailability. The solubility issue poses a hurdle in the development of analytical methods crucial for pharmacokinetic studies, quality control, and formulation development (Neumiller, 2014). Empagliflozin chemically,(1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran-3-yl-oxy) benzyl]-benzene is an orally administered selective sodium glucose

cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type 2 diabetes by blocking the reabsorption of glucose in the kidneys and promoting excretion of excess glucose in the urine. In patients with type 2 diabetes and hyperglycaemia a higher amount of glucose is filtered and reabsorbed. Empagliflozin improves glycaemic control in patients with type 2 diabetes by reducing renal glucose reabsorption. The amount of glucose removed by the kidney through this glucuretic mechanism is dependent on blood glucose concentration and GFR. Inhibition of SGLT2 in patients with type 2 diabetes and hyperglycaemia leads to excess glucose excretion in the urine (White, 2015; Tripathi, 2013).

In response to this challenge, the application of hydrotropic phenomena emerges as a promising strategy to enhance the solubility of poorly water-soluble drugs like empagliflozin. Hydrotropic agents, organic compounds with hydrotropic properties, have been increasingly explored in pharmaceutical research for their ability to improve the aqueous solubility of poorly water-soluble drugs (Agrawal *et al.,* 2004). This phenomenon involves the creation of micellar structures, facilitated by hydrotropes, leading to enhanced drug solubilization in aqueous media. Hydrotropy represents a versatile and effective approach to overcome solubility challenges, making analytical methods development for drugs like empagliflozin more feasible. The purpose of research to develop and validate specific and accurate UV spectrophotometric method of Empagliflozin by using different hydrotropic solubilizing agents.

MATERIALS & METHODS

Solubility enhancement

The synergistic effects observed in some combinations, such as the ammonium acetate and sodium citrate combination; suggest that certain mixtures of solvents can yield remarkably improved solubility. This information could be particularly useful in the development of pharmaceutical formulations and drug delivery systems, aiming to enhance the therapeutic effectiveness of empagliflozin. Empagliflozin were scanned in various hydrotropic agent in the spectrum mode over the UV range (200-400) and 2M Ammonium Acetate: 2M Sod. Citrate (1:1) was found to be most appropriate (Sundari *et al.,* 2012).

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of drug in 80 mL mixed hydrotropic solution containing 2M Ammonium Acetate: 2M Sod. Citrate (1:1) and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to 100ml with mixed hydrotropic agent to get a concentration of 1000 µg/ml (Stock-A) for drug.

Preparation *of Sub Stock Solution (Stock-B)*

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of Empagliflozin and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with 2M Ammonium Acetate: 2M Sod. Citrate (1:1) that gave concentration of 100µg/ml (Stock-B) (Jain *et al.,* 2010).

Preparation of Working Standard Solution

Aliquots of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately

in 10 ml volumetric flask and volume was made up to 10 ml with 2M Ammonium Acetate: 2M Sod. Citrate (1:1). This gave the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml respectively for 2M Ammonium Acetate: 2M Sod. Citrate (1:1) (Das and Dhua, 2014).

Selection of wavelength for linearity

Solution of 10 µg/ml Empagliflozin were prepared separately the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of Empagliflozin was observed at 270 nm. Empagliflozin showed linearity in the concentration range of 10-50 µg/ml Calibration curve was plotted, absorbance versus concentration.

Validation of developed method

Linearity

Linearity of drug was established by response ratios of drug. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio (ICH Guidelines, 2005).

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of Empagliflozin to preanalysed tablet powder.

The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels (Mishra *et al.,* 2014).

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in tables 1.

Day-to-Day Variation

A day-to-day variation study evaluates how consistent or variable the measurements are when the same samples are measured on different days under similar conditions. The low standard deviation (S.D.) value of 0.127 suggests that the measurements of Empagliflozin are relatively close to the mean value of 99.262% across different days. This indicates good consistency in the measurements.

The % R.S.D. value of 0.128% reinforces the conclusion that the measurements are consistent and that the day-to-day variation is relatively small compared to the mean value. This is a positive indication of the precision of your method. The low % R.S.D. value suggests that the Empagliflozin measurements are stable and not subject to significant variations from day to day.

Analyst to analyst variation

An analyst-to-analyst variation study evaluates how consistent or variable the measurements are when different analysts perform the same measurements on the same samples.

Reproducibility

The higher standard deviation (S.D.) value of 0.966 suggests that there is relatively more variability in the measurements compared to

the mean value of 97.871%. This indicates a degree of variability across different conditions or analysts. The % R.S.D. value of 1.027% reflects the relative measure of reproducibility. While it's higher compared to the previous studies you've presented, it's still relatively low.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of tablet sample

Twenty marketed tablets of EGF were weighed and ground to a fine powder; amount equal to 10mg of EGF was taken in 10 ml volumetric flask. Then 8 ml of 2M Ammonium Acetate: 2M Sod. Citrate (1:1) solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of drug in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained. The procedure was repeated for five times.

RESULTS AND DISCUSSION

Table 1: Analysis of tablet formulation of EGF

Parameters		Values	%RSD
Linearity		10-50(µg/ml)	
Recovery study	80%	99.07±0.527	0.532
	100%	99.07±0.326	0.328
	120%	99.31±0.326	0.492
Repeatability		99.11±0.157	0.159
Day-to-Day Variation		99.26±0.127	0.128
Analyst-to-Analyst Variation		99.23±0.103	0.104
Reproducibility		97.87±0.966	1.027
LOD (µg/ml)		0.15	
LOQ (µg/ml)		0.45	

Table 2: Analysis of Tablet Formulation of EGF

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
EGF	10	9.83	98.30	0.223	0.245

CONCLUSION

The developed method for the estimation of Empagliflozin using hydrotropic phenomena has shown promising results in terms of accuracy, precision, linearity, and robustness. By enhancing the solubility of the poorly water-soluble drug, the method addresses a significant challenge in pharmaceutical analysis. The validated method provides a reliable and efficient means of quantifying Empagliflozin in tablet formulations or other matrices, ensuring the quality control and regulatory compliance of pharmaceutical products containing the drug. Additionally, the utilization of hydrotropic phenomena offers an innovative approach to enhance the solubility of poorly water-soluble drugs, which could have broader implications for drug development and analytical methods in the pharmaceutical industry. In conclusion, this study demonstrates the successful development and validation of a method for the estimation of Empagliflozin using hydrotropic phenomena. The method's accuracy, precision, and robustness make it a valuable tool for pharmaceutical analysis, with potential applications beyond Empagliflozin to address solubility challenges for other poorly water-soluble drugs.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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