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A review on method development and validation of stability indicating RP HPLC Method for metformin and Empagliflozin

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Abstract

Metformin HCl and empagliflozin are oral antidiabetic medications that help control blood sugar levels. A simple, precise, rapid, accurate, sensitive, specific, and stable RP-HPLC method was developed and validated for the simultaneous determination of Metformin and Empagliflozin in drug bulk form and drug dosage. This new RP-HPLC method is superior to formal reversed-phase HPLC with reduced solvent usage, retention time, resolution, and cost. Upper region separation was performed on a Waters HPLC system equipped with a PDA detector and autosampler. Both Metformin and Empagliflozin undergo stress conditions including acid, alkali, oxidative, thermal, and photodegradation, but significant degradation was observed in acid studies. The newly developed RP HPLC chromatographic method was validated for system compatibility, linearity, robustness, accuracy, and precision.

Keywords: RP-HPLC; Metformin; Empagliflozin; Stability-indicating method; Tablet dosage form

1. Introduction

Diabetes is a chronic metabolic disease characterized by high blood glucose levels. The most common form is type 2 diabetes, which usually occurs in adults when the body becomes resistant to insulin or does not produce enough insulin [1, 2]. Metformin HCl and empagliflozin are antacids that help control blood sugar levels. Metformin HCl, a drug of the biguanide class with the chemical 1, 1 dimethyl imido dicarbonimide diamide hydrochloride, works by reducing the production of glucose by the liver and increasing the sensitivity of body tissues to insulin [3].

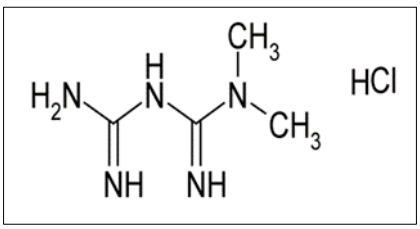

Figure 1 Chemical Structure of Metformin

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Empagliflozin is a drug of the gliflozin class with chemically 1-chloro-4-[b-Dglucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran-3-yl-oxy) benzyl, it is an inhibitor of the sodium-glucose cotransporter-2, it helps to stop sodium-glucose transport protein that has been filtered out of the blood by the kidney being reabsorbed back into the blood [4].

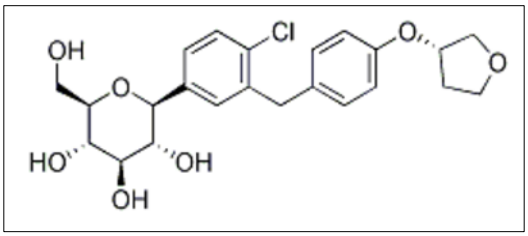

Figure 2 Chemical Structure of empagliflozin

A review of the literature shows that there are several methods for the separate determination of metformin HCl and empagliflozin in tablet dosage form by UV spectrophotometer [5-7], RP-HPLC [8-15], UPLC [16,17], and some analytical instruments. LC-MS/MS [18]. Therefore, an effort was made to develop a new stability-validated RP-HPLC method for the simultaneous estimation of metformin hydrochloride and empagliflozin in tablet dosage form according to the guidelines of the International Conference on Harmonization (ICH).

HPLC is a powerful analytical tool for the development of stable, fast methods. The importance of the HPLC method is to develop a simple and fast stability-promoting method. For reference, we chose HPLC to evaluate metformin hydrochloride and empagliflozin at therapeutic doses [19]. The currently proposed method can separate metformin hydrochloride and empagliflozin in one minute without affecting the peak shape and resolution and is useful for content homogeneity, elimination verification, and purification of the above drugs. The power of the method by adapting random variables to optimal conditions. QbD performs single-factor data reliability instead of multi-factor transformation, an optimized method useful for robustness and stability checks under all conditions. [20].

2. Material and methods

API gift samples of metformin hydrochloride and empagliflozin were provided by Spectrum Pharma Research Solutions, Hyderabad. HPLC grade Acetonitrile, water, and other chemicals from Rankem, Hyderabad. Waters HPLC 2695 SYSTEM is an integrated Autosampler with Quad Pump, Photo Diode Array Detector, and Empower 2 Software. Spectrophotometer T60, 2 mm and 10 mm broadband with quartz cells compatible with UV win 6 were used to measure the absorbance of Metformin and Empagliflozin solutions.

2.1. Chemicals and Reagents

Potassium dihydrogen phosphate, acetonitrile, HPLC grade, HPLC grade water, Trimethylamine is used. Performance standards for metformin and empagliflozin. Trijardy XR tablets containing 1000 mg metformin and 10 mg Empagliflozin were purchased.

2.2. Preparation of standard solution

The exact weight is 125 mg of metformin HCl (API) and 12.5 mg of empagliflozin (API), separately filled into a 25 ml volumetric flask and dried with 100 ml. Add 3/4 of the mixture to both of these flasks, sonicate for 10 minutes, and finally label with diluent. The resulting concentrations were 5000 µg/ml metformin and 125 µg/ml empagliflozin.

2.3. Preparation of 0.1% Orth phosphoric Acid

Accurately pipette 1.0 mL of OPA into a clean & dried 1000 mL volumetric flask, add 100 mL of milli-Q water, stir well, and finally makeup up the mark with milli-Q water.

2.4. Preparation of Mobile Phase

It consisting a mixture of 0.1%OPA and Acetonitrile at a ratio of 50:50 v/v. Preparation of Diluent: It is a mixture of Acetonitrile and milli-Q water at a ratio of 50:50 v/v.

2.5. Preparation of Standard Working Solutions (100% solution)

Pipette 1 ml from each stock solution and transferred into a clean and dried 10 ml volumetric flask and finally makeup to the mark with diluent. The resultant concentrations are 500 µg/ml of metformin and 12.5 µg/ml of empagliflozin.

2.6. Preparation of Sample Stock Solutions

10 tablets are randomly selected and weighed and the average weight of each tablet is calculated, all tablets were grounded into fine powder. The weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, add 50 ml diluent, sonicated for 25 minutes and finally make up to the mark with diluent. All the content was passed through 0.45 µm filter paper. The resultant concentration was 5000 µg/ml of metformin and 125 µg/ml of empagliflozin.

2.7. Preparation of Sample Working Solution (100% solution)

Pipette 1 ml of filtered sample stock solution; transfer it into a 10 ml volumetric flask and make up to the mark with diluent. The resultant concentrations were 500 µg/ml of metformin and 12.5 µg/ml of empagliflozin.

2.8. Optimized Chromatographic Method

The separation of Metformin Hydrochloride and Empagliflozin was achieved on a Kromosil C18 column (250x4.6 mm; 5.6 µ) and eluting with a mobile phase consisting of a 50:50 v/v mixture of Acetonitrile and Buffer [0.1% orthophosphoric Acid (pH 2.8)] at a flow rate of 1.0 mL/min. The analytes were monitored at 260 nm. The injection volume was 10 µl. The total run time for the elution of the compound was 6 min.

- **Column:** Kromasil C18; 50 x 4.6 mm; 5
- **Column temperature:** 30°C
- **Flow rate:** 1 mL/min Lµ
- **Injection volume:** 10
- **Detector wavelength:** 260 nm
- **Run time:** 6 min

2.9. Instrumentation

Chromatographic separation was achieved in a UPLC water purification system equipped with a built-in autosampler and PDA detector. The processing of the selected components is carried out using Empower 2 software. A hot air oven is used for the thermal degradation of the sample, and a UV cutting machine and a model 23400 UV camera equipped with a UV fluorescent lamp with a wavelength between 200 and 300 nm are selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshconiv), digital pH meter (Adwa - AD 1020), and UV-Visible spectrophotometer (Labindia UV 3000) were used in the study. [22].

3. Results & Discussion

3.1. Method validation [23]

The US Pharmacopeia (USP) and US Food and Drug Administration (FDA) both refer to ICH guidelines. The most widely applied validation characteristics are accuracy, precision, specificity, linearity, range, robustness, the limit of detection, the limit of quantification, the limit of detection, and the limit quantification.

3.2. System Suitability

Table 1 System suitability parameters

Drug	Retention time	Area	USP plat count	USP tailing
Metformin HCl	2.192	9877896	4354	1.33
Empagliflozin	3.200	954988	7073	1.48

It is the checking of a system to ensure system performance before or during the analysis of the unknown. It tests are an integral part of the chromatographic method and are used to verify that the resolution & reproducibility of the system are adequate for the analysis to be performed. In this, plate count (N), tailing factor (T), resolution (Rs), and

reproducibility (%RSD) are determined from the replicate injection of the standard. The acceptable limit of %RSD is less than 2%. [Table 1].

3.3. Specificity

The method can accurately measure the analyte response in the presence of all potential sample components. In this study, the method was evaluated by injecting 10 µl of blank sample and placebo into HPLC. [Fig No. 3, 4, 5 & 6].

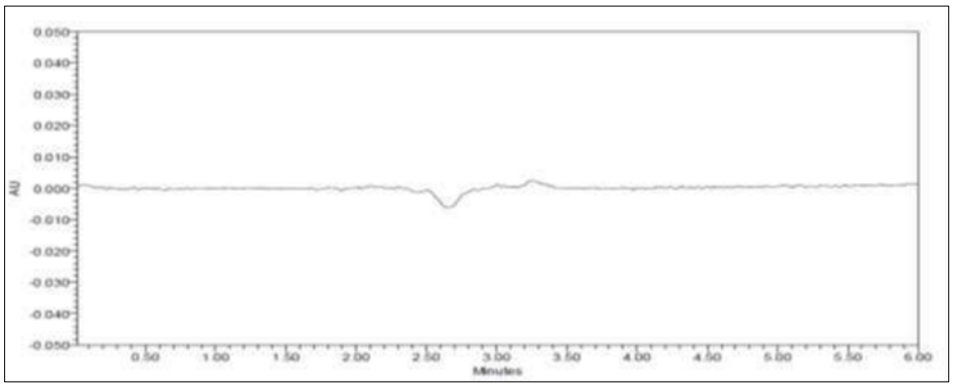

Figure 3 Typical chromatogram of blank

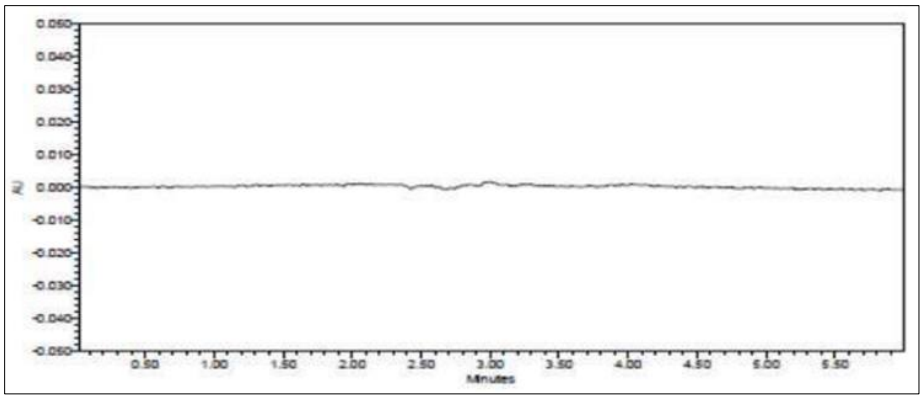

Figure 4 Typical Chromatogram of Placebo

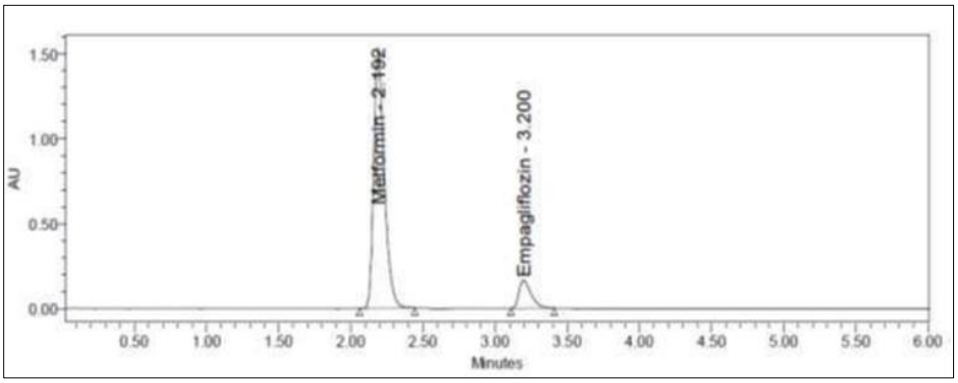

Figure 5 Standard chromatogram of metformin HCL and Empagliflozin

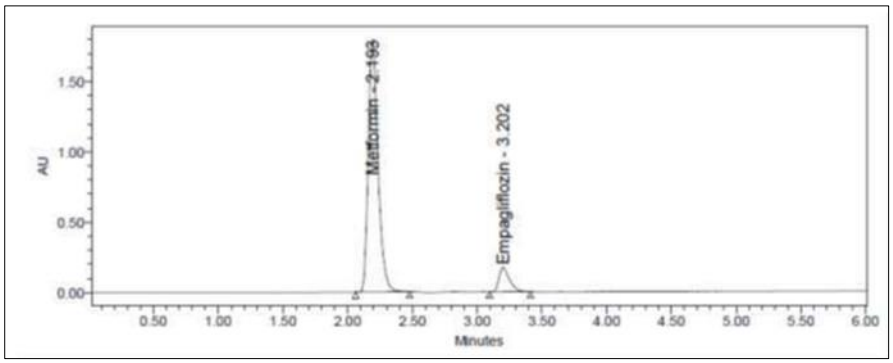

Figure 6 Typical chromatogram of a sample (Tablet Dosage Form)

Table 2 Recovery studies of metformin HCL and Empagliflozin

Drug	Level of spike solution	Amount present (mg/mL)	Amount added	Amount recovered	% Recovery	% RSD
Metformin HCL	50 %	500	250	249.82	99.93	0.73
	50 %	500	250	254.81	101.93	
	50 %	500	250	250.17	100.07	
	100 %	500	500	500.01	100	
	100 %	500	500	498.60	99.72	
	100 %	500	500	502.45	100.49	
	150 %	500	750	760.42	101.39	
	150 %	500	750	754.75	100.63	
	150 %	500	750	755.90	100.79	
Empagliflozin	50 %	12.5	6.25	6.20	99.29	0.75
	50 %	12.5	6.25	6.32	101.26	
	50 %	12.5	6.25	6.30	100.92	
	100 %	12.5	12.5	12.69	101.54	
	100 %	12.5	12.5	12.60	100.87	
	100 %	12.5	12.5	12.58	100.71	
	150 %	12.5	18.75	18.66	99.55	
	150 %	12.5	18.75	18.83	100.46	
	150 %	12.5	18.75	18.76	100.67	

3.4. Accuracy

The accuracy of this method is evaluated by the standard addition method. A certain number of standard data is added to the number of standard solutions at three different levels. Solutions were analyzed for mean recovery and %RSD. Studies were conducted for metformin and empagliflozin at three different concentrations of 50%, 100%, and 150%. 10 µL HPLC and % recovery and % RSD were injected. Shown in Table 2 [21].

3.5. Precision

Precision is the degree of agreement between individual test results when an analytical method is used several times to obtain several samples from the same sample. Precision is defined as reproducible precision and is studied for accuracy and intraday precision of the method with six injections of 10 µL and the peak area of duplicate injections [21].

3.6. Detection and Qualification limits

According to the Pharmacopoeial Guidelines (USP, 2011), the detection limit is defined as a concentration with a signal-to-noise ratio of 3:1, with a calculated ratio for the quantitative limit of 10:1. The detection value for metformin and empagliflozin are 0.01 and 0, respectively. .01 ppm. The limits of quantitation for metformin and empagliflozin were 0.04 and 0.02 ppm, respectively. (fig.no 7 & 8) [24].

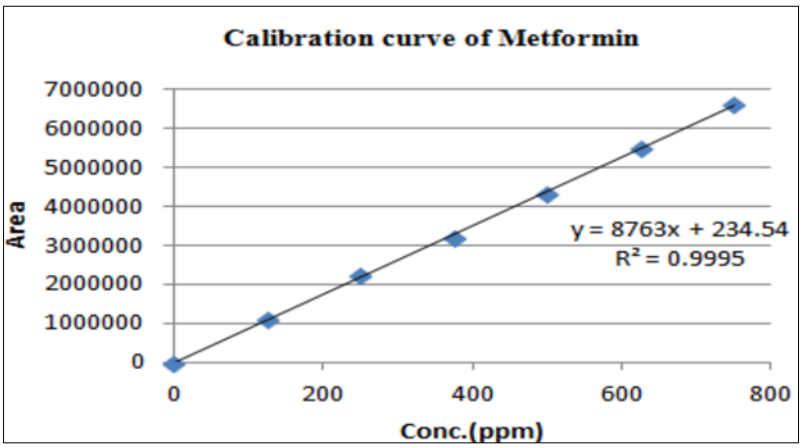

Figure 7 Linearity for Metformin

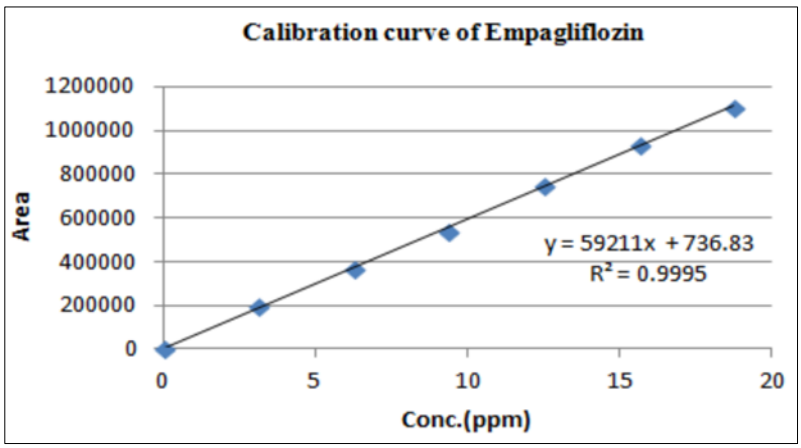

Figure 8 Linearity for Empagliflozin

3.7. Robustness

It is the capacity of a method to remain unaffected by small, deliberate variations in method parameters. It was indicated by changing the flow rate, mobile phase composition, and temperature.

3.8. Limit of Detection (LOD) & Limit of Quantification (LOQ)

LOD is the lowest concentration of an analyte in a sample that can be detected. LOQ is the lowest concentration of an analyte in a sample that can be quantized. The LOD and LOQ of metformin and empagliflozin were determined from a standard deviation of the response and the slope.

3.9. Assay Procedure

The assay was performed by the marked product (Synjardy XR 12.5 mg/500 mg of metformin and empagliflozin. The prepared sample and standard solution were injected into HPLC and peak areas were recorded. Finally, the percentage amount of the drug was calculated.

3.10. Degradation studies

Forced degradation is the degradation of new drug substances and drug products at conditions more than accelerated conditions. These studies show the chemical behavior of the molecules which in turn helps the development of formulation and packaging. Hence, in the present study forced degradation studies were established by subjecting the samples of Metformin and Empagliflozin standard solution to degradation in Oxidation, Acid, Alkaline, Dry heat, Photostability, and Neutral degradation.

3.10.1. Oxidation

Pipette 1ml of standard stock solution of Metformin and Empagliflozin into a volumetric flask separately, add 1ml of 20% hydrogen peroxide (H_2O_2), and these solutions were kept for 30 minutes at 600C. The resultant solutions were diluted to obtain 500 µg/ml and 12.5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3.10.2. Acid Degradation Studies

Pipette 1ml of stock solution of Metformin and Empagliflozin into a volumetric flask separately, add 1 ml of 2N Hydrochloric Acid, and Reflex for 30 minutes at 60 ℃. The resultant solutions were diluted to obtain 500 µg/ml and 12.5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3.10.3. Alkali Degradation Studies

Pipette 1ml of stock solution of metformin and Empagliflozin into a volumetric flask separately, add 1ml of 2N sodium hydroxide, and reflex for 30 minutes at 60 ℃. The resultant solutions were diluted to obtain 500 µg/ml and 12.5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3.10.4. Dry Heat Degradation Studies

The standard Drug solutions were placed into the oven at 105 ℃ for 6 hours. The resultant solutions were diluted to obtain 500 µg/ml and 12.5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3.10.5. Photo Stability Studies

The photochemical stability of the drug was also studied by exposing the stock solutions to UV light by keeping the beaker in a UV chamber for 7 days or 200-watt hours/m2 in a photostability chamber. The resultant solutions were diluted to obtain 500 µg/ml and 12.5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3.10.6. Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at 60 ℃. The resultant solutions were diluted to obtain 500 µg/ml and 12.5 µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

4. Discussion

The research currently reported in this review is the development of a new RP-HPLC method and validation of the simultaneous assay of metformin and empagliflozin. The developed method is followed by the choice of wavelength. The optimized wavelength is 226 nm. Different mobile phases and columns are used to obtain an optimized RP-HPLC method.

A new isocratic RP-UPLC-DAD method showing new stability was developed and validated for the determination of Metformin and Empagliflozin in bulk and tablet form. bulk dosage forms and tablets. for precision, specificity, LOQ, LOD, precision, linearity, and robustness.

5. Conclusion

This method is simple, precise, accurate, and reliable for the simultaneous estimation of metformin HCL and empagliflozin in combined doses and considers the stability of both drugs according to ICH guidelines. The %RSD of all results is less than 2-5%, indicating a high level. The proposed method showed good accuracy, linearity, precision, and determination of four drugs in laboratory-prepared mixtures with different drug doses, making them suitable for quality control.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing financial interest.

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