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DEVELOPMENT AND VALIDATION OF A RAPID RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN AND METFORMIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

Dhanush Ram Turkane[*1]

[1]Shri Shankaracharya Institute of Pharmaceutical Sciences and Research, Shri Shankaracharya Professional University, Junwani, Bhilai - 490020, Chhattisgarh, India.

***Corresponding Author: Dhanush Ram Turkane**

Shri Shankaracharya Institute of Pharmaceutical Sciences and Research, Shri Shankaracharya Professional University, Junwani, Bhilai - 490020, Chhattisgarh, India. **DOI:**

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ABSTRACT

A simple, rapid, precise, and robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Empagliflozin and Metformin Hydrochloride in pharmaceutical dosage forms. Chromatographic separation was achieved using a Hypersil ODS C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and 20 mM ammonium formate buffer (pH 3.5) in the ratio of 45:55 (v/v). The mobile phase was delivered at a flow rate of 1.0 mL/min, and detection was carried out at 230 nm using a diode array detector. The retention times of Empagliflozin and Metformin Hydrochloride were found to be 5.418 min and 2.851 min, respectively. The method exhibited excellent linearity over concentration ranges of 2.5–25 µg/mL for Empagliflozin and 12.5–125 µg/mL for Metformin Hydrochloride, with correlation coefficients of 0.9994 and 0.9997, respectively. Accuracy studies showed recoveries ranging from 99.62% to 100.54% for Empagliflozin and 99.85% to 100.42% for Metformin Hydrochloride. The method demonstrated satisfactory precision, ruggedness, robustness, and sensitivity, with all validation parameters meeting ICH guideline requirements. The developed method was successfully applied to the assay of marketed tablet formulations, yielding assay values within acceptable limits. The results indicate that the proposed RP-HPLC method is suitable for routine quality control and quantitative analysis of Empagliflozin and Metformin Hydrochloride in pharmaceutical dosage forms.

KEYWORDS: Empagliflozin; Metformin Hydrochloride; RP-HPLC; Method Validation; Simultaneous Estimation; Pharmaceutical Dosage Forms.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from defects in insulin secretion, insulin action, or both. Among its various forms, Type 2 diabetes mellitus (T2DM) is the most prevalent, affecting millions of individuals worldwide and posing a significant public health burden.[1] Effective management of T2DM often requires combination therapy involving drugs with complementary mechanisms of action to achieve optimal glycemic control. Empagliflozin and Metformin represent one such widely prescribed antidiabetic combination. Empagliflozin is a selective sodium-glucose co-transporter-2 (SGLT-2) inhibitor that lowers blood glucose levels by reducing renal glucose reabsorption and promoting urinary glucose excretion independent of insulin secretion.[2] Metformin, a member of the biguanide class, is considered the first-line pharmacological treatment for T2DM due to its ability to decrease hepatic glucose production, improve insulin sensitivity, and enhance peripheral glucose utilization.[3] The growing clinical use of fixed-dose combinations containing Empagliflozin and Metformin has created a need for reliable analytical methods capable of simultaneously quantifying both drugs in pharmaceutical formulations.[4] Reverse Phase High Performance Liquid

Chromatography (RP-HPLC) is widely recognized as a powerful analytical technique owing to its high sensitivity, precision, accuracy, and ability to separate complex mixtures within a short analysis time.[5] Therefore, the development of a robust RP-HPLC method for the simultaneous estimation of Empagliflozin and Metformin is essential for ensuring the quality, safety, and efficacy of pharmaceutical products containing these agents.[6] The present study aims to develop and validate a rapid RP-HPLC method for the simultaneous determination of Empagliflozin and Metformin in bulk drug substances and tablet dosage forms.[7] The validated method is intended to provide a reliable analytical tool for routine quality control analysis, stability studies, and formulation development in accordance with regulatory guidelines established for analytical method validation.

MATERIALS AND METHODS

Materials

Empagliflozin and Metformin reference standards were generously supplied by AKUMS Drugs & Pharmaceuticals Ltd., Haridwar, Uttarakhand, India. Commercial tablet formulations containing Empagliflozin and Metformin were procured from the local pharmaceutical market in Moradabad, Uttar Pradesh, India. All solvents and reagents used in the study were of analytical or HPLC grade. HPLC-grade acetonitrile and water were obtained from Merck India, Mumbai, India, while ammonium formate was purchased from Rankem Laboratory Chemicals. The buffer solution was prepared using ammonium formate, and the pH was adjusted with formic acid to achieve the desired chromatographic conditions.

Chromatographic Conditions

Empagliflozin and Metformin were chromatographically separated using a Hypersil ODS C18 column (250 mm × 4.6 mm i.d., 5 µm particle size). The chromatographic analysis was performed on a Shimadzu HPLC system (Japan) equipped with two LC-20AD solvent delivery pumps, an SPD-M20A Diode Array Detector (DAD), a CBM-20A system controller, and LC Solution software for data acquisition and processing.[8] The mobile phase consisted of acetonitrile and 20 mM ammonium formate buffer (pH adjusted to 3.5 with formic acid) in the ratio of 40:60 (v/v). The mobile phase was filtered through a 0.45 µm membrane filter and degassed prior to use. The flow rate was maintained at 1.0 mL/min throughout the analysis. Detection was carried out using a Diode Array Detector at 230 nm, selected as the common wavelength providing adequate sensitivity for both analytes. The column was operated at ambient temperature, and the injection volume was fixed at 20 µL for all chromatographic runs.[9]

Mobile Phase Preparation

The mobile phase consisted of acetonitrile and 20 mM ammonium formate buffer (pH adjusted to 3.5 with formic acid) mixed in the ratio of 45:55 (v/v). The prepared mobile phase was filtered through a 0.45 µm nylon membrane filter to remove particulate matter and ensure system cleanliness. Subsequently, the mobile phase was degassed using an ultrasonic bath for 15 minutes to eliminate dissolved gases and prevent bubble formation during chromatographic analysis. The filtered and degassed mobile phase was then used for the separation and quantification of Empagliflozin and Metformin.[10]

Preparation of Buffer Solution

The buffer solution was prepared by dissolving 1.26 g of ammonium formate in 1000 mL of HPLC-grade water to obtain a 20 mM ammonium formate buffer. The pH of the solution was adjusted to 3.5 using formic acid. After pH adjustment, the buffer solution was filtered through a 0.45 µm membrane filter to remove any particulate impurities and subsequently degassed in an ultrasonic bath for approximately 15 minutes to eliminate dissolved gases. The prepared buffer was then used in the mobile phase preparation to ensure reproducible chromatographic performance and smooth operation of the HPLC system.[11]

Preparation of Standard Solution

A standard stock solution was prepared by accurately weighing 10 mg of Empagliflozin and 50 mg of Metformin hydrochloride and transferring them into a 100 mL volumetric flask. Approximately 80 mL of methanol was added, and the mixture was sonicated for 10 minutes to ensure complete dissolution of both drugs. The volume was then made up to the mark with methanol to obtain stock solutions containing 100 µg/mL of Empagliflozin and 500 µg/mL of Metformin hydrochloride. Working standard solutions were prepared by appropriate dilution of the stock solution with the mobile phase to obtain concentration ranges of 2.5–25 µg/mL for Empagliflozin and 12.5–125 µg/mL for Metformin hydrochloride, suitable for calibration and validation studies. Prior to chromatographic analysis, all prepared solutions were filtered through a 0.45 µm Millipore membrane filter to remove particulate matter and ensure sample clarity.[12,13]

Sample Solution Preparation

Twenty tablets containing Empagliflozin and Metformin hydrochloride were accurately weighed, and their average weight was determined. The tablets were then finely powdered using a mortar and pestle. An amount of powder equivalent to 10 mg of Empagliflozin and 50 mg of Metformin hydrochloride was accurately weighed and transferred into a 100 mL volumetric flask. Approximately 70 mL of mobile phase was added, and the mixture was sonicated for 15 minutes to ensure complete extraction and dissolution of the active pharmaceutical ingredients. After cooling to room temperature, the volume was made up to the mark with the mobile phase and mixed thoroughly. The resulting solution was filtered through a 0.45 µm membrane filter to remove insoluble excipients and particulate matter.

Appropriate dilutions of the filtrate were prepared with the mobile phase to obtain concentrations within the calibration range for RP-HPLC analysis.[14]

Chromatographic Examination

Chromatographic analysis was performed using the prepared mobile phase under isocratic elution conditions. The mobile phase was delivered at a flow rate of 1.0 mL/min, and a 20 µL volume of each standard and sample solution was injected into the HPLC system. Detection of Empagliflozin and Metformin hydrochloride was carried out using a Diode Array Detector (DAD) at 230 nm. The chromatographic separation was achieved on a C18 column maintained at ambient temperature throughout the analysis. Data acquisition and processing were performed using the chromatographic software integrated with the HPLC system. Under the optimized conditions, well-resolved and symmetrical peaks for both analytes were obtained, enabling accurate quantitative determination in pharmaceutical dosage forms.[15]

RESULTS AND DISCUSSION

Method Creation and Optimization

The developed RP-HPLC method provided satisfactory separation of Empagliflozin and Metformin Hydrochloride with retention times of approximately 5.4 min and 2.8 min, respectively. Well-resolved and symmetrical peaks were obtained without interference from excipients. The chromatographic performance demonstrated good specificity and reproducibility, indicating the suitability of the method for simultaneous estimation of both drugs in pharmaceutical formulations (Figure 1).

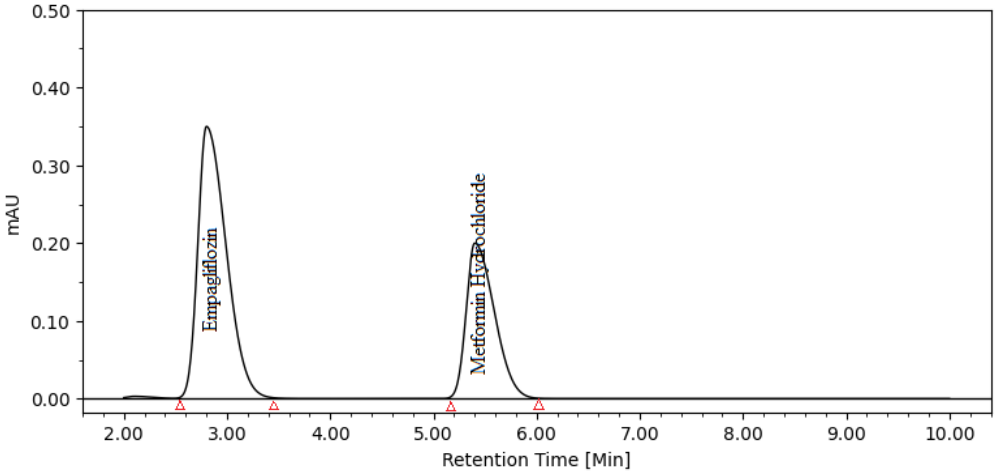

Figure 1: Chromatogram Showing Retention Peak.

System Appropriateness Parameters

The developed RP-HPLC method demonstrated satisfactory system suitability for the simultaneous estimation of Empagliflozin and Metformin Hydrochloride. The mean retention times of Empagliflozin and Metformin Hydrochloride were found to be 5.418 min and 2.851 min, respectively, indicating adequate separation of the two analytes. The %RSD values for peak area were 0.51% for Empagliflozin and 0.30% for Metformin Hydrochloride, confirming excellent precision of the chromatographic system. The mean USP tailing factors were 1.18 and 1.05, respectively, demonstrating good peak symmetry. Furthermore, the average theoretical plate counts of 6257 for Empagliflozin and 7135 for Metformin Hydrochloride indicated efficient column performance. All system suitability parameters were within the acceptable limits, confirming the reliability and reproducibility of the developed method for routine analysis (Table 1).

Table 1: System Suitability Parameters.

Injection No.	Retention Time (min) Empagliflozin	Peak Area Empagliflozin	Retention Time (min) Metformin HCl	Peak Area Metformin HCl	USP Tailing Factor Empagliflozin	USP Plate Count Empagliflozin	USP Tailing Factor Metformin HCl	USP Plate Count Metformin HCl
1	5.412	485621	2.846	1754382	1.18	6245	1.05	7124
2	5.428	488154	2.859	1760125	1.17	6268	1.04	7142
3	5.401	482793	2.841	1749854	1.19	6215	1.06	7095
4	5.419	487062	2.853	1757643	1.18	6284	1.05	7160
5	5.437	489205	2.861	1763458	1.17	6298	1.04	7178
6	5.408	484356	2.848	1752065	1.18	6232	1.05	7110

Mean	5.418	486199	2.851	1754588	1.18	6257	1.05	7135
SD	0.013	2494.8	0.008	5258.6	–	–	–	–
%RSD	0.24	0.51	0.29	0.30	–	–	–	–

Accuracy and Recovery

The accuracy of the developed RP-HPLC method was evaluated through recovery studies at 80%, 100%, and 120% spiking levels for Empagliflozin and Metformin Hydrochloride. The percentage recoveries obtained for Empagliflozin ranged from 99.62% to 100.54%, while those for Metformin Hydrochloride ranged from 99.85% to 100.42%. The %RSD values at all concentration levels were less than 2%, indicating excellent accuracy and precision of the method. The recovery values were within the acceptable range of 98–102%, confirming that the developed method is reliable for the quantitative estimation of both drugs in pharmaceutical formulations (Table 2). The low %RSD values and recoveries close to 100% demonstrate the accuracy of the proposed RP-HPLC method and its suitability for routine quality control analysis of Empagliflozin and Metformin Hydrochloride tablets.

Table 2: Accuracy and Recovery Data (n = 6)

Drug	% Spiking Level	Drug in Tablet (µg)	Standard Drug Added (µg)	Total Drug (µg)	Total Drug Recovered (Mean ± SD) (µg)	%RSD	Recovery (%)
Empagliflozin	80%	5.0	4.0	9.0	9.05 ± 0.086	0.95	100.54
	100%	5.0	5.0	10.0	9.96 ± 0.121	1.21	99.62
	120%	5.0	6.0	11.0	11.03 ± 0.094	0.85	100.27
Metformin HCl	80%	25.0	20.0	45.0	45.19 ± 0.142	0.31	100.42
	100%	25.0	25.0	50.0	49.93 ± 0.165	0.33	99.86
	120%	25.0	30.0	55.0	54.92 ± 0.188	0.34	99.85

Precision

Empagliflozin (10 µg/mL) and Metformin Hydrochloride (50 µg/mL). The mean peak areas obtained were 486199 for Empagliflozin and 1754588 for Metformin Hydrochloride. The %RSD values for peak area were 0.51% and 0.30%, respectively, indicating excellent system precision. Similarly, the mean assay values were 100.12% for Empagliflozin and 100.08% for Metformin Hydrochloride, with corresponding %RSD values of 0.42% and 0.29% (Table 3). Since all %RSD values were below the acceptance limit of 2%, the method demonstrated satisfactory repeatability and precision for routine quantitative analysis.

Table 3: Precision Study Data.

Injection No.	Peak Area of Empagliflozin	Peak Area of Metformin HCl	% Assay of Empagliflozin	% Assay of Metformin HCl
1	485621	1754382	99.48	99.72
2	488154	1760125	100.26	99.95
3	482793	1749854	99.84	100.42
4	487062	1757643	100.58	100.15
5	489205	1763458	100.12	99.86
6	484356	1752065	100.44	100.38
Mean	**486199**	**1754588**	**100.12**	**100.08**
SD (±)	**2494.8**	**5258.6**	**0.42**	**0.29**
%RSD	**0.51**	**0.30**	**0.42**	**0.29**

Acceptance Criteria: *%RSD ≤ 2.0* ***Concentration:*** *Empagliflozin (10 µg/mL) and Metformin Hydrochloride (50 µg/mL).*

Intermediate Accuracy (Ruggedness)

The intermediate precision of the developed RP-HPLC method was evaluated through intraday and interday studies at three concentration levels of Empagliflozin and Metformin Hydrochloride. The intraday %RSD values ranged from 0.42% to 0.86%, while the interday %RSD values ranged from 0.78% to 1.24%. All values were well below the acceptance limit of 2%, indicating excellent reproducibility of the method under normal laboratory conditions. The results from Table 4 confirmed that the developed method is rugged and suitable for routine quality control analysis.

Table 4: Intraday and Interday Precision Data.

Concentration (µg/mL)	Intraday Precision (%RSD)	Interday Precision (%RSD)
Empagliflozin 8 & Metformin HCl 40	0.42	0.78
Empagliflozin 10 & Metformin HCl 50	0.56	1.02
Empagliflozin 12 & Metformin HCl 60	0.86	1.24

Linearity and Range

The linearity of the developed RP-HPLC method was evaluated over the concentration range of 2.5–25 µg/mL for Empagliflozin and 12.5–125 µg/mL for Metformin Hydrochloride. A strong linear relationship was observed between concentration and peak area for both analytes. The calibration curves exhibited correlation coefficients (R^2) of 0.9994 for Empagliflozin and 0.9997 for Metformin Hydrochloride, demonstrating excellent linearity over the studied concentration ranges. The results confirm that the proposed method is suitable for accurate quantitative analysis of both drugs across a wide concentration range. The corresponding calibration plots are shown in Figure 2.

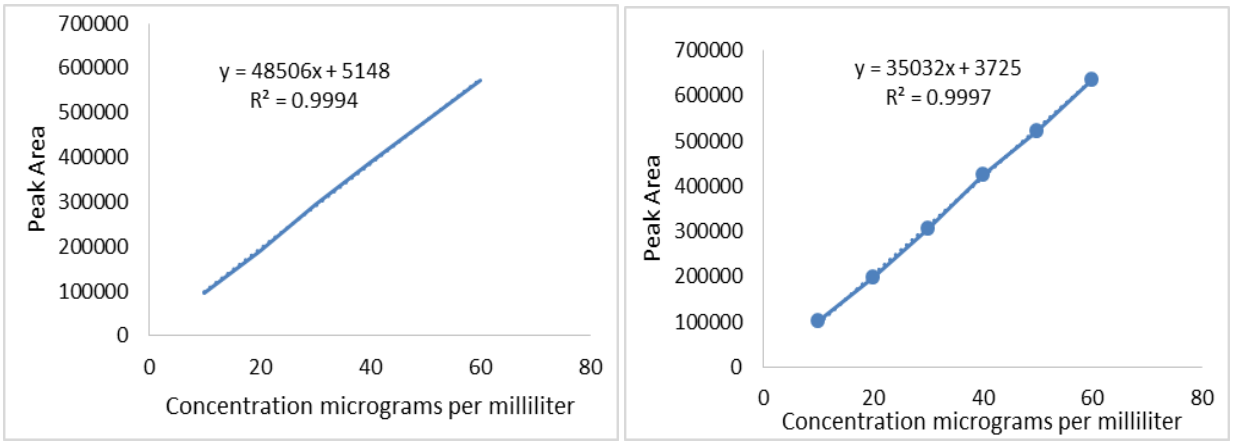

Figure 2: Calibration curve of Empagliflozin and Metformin Hydrochloride.

Robustness Data

The robustness of the developed RP-HPLC method was evaluated by introducing small deliberate variations in flow rate, mobile phase pH, detection wavelength, and mobile phase composition. The %RSD values for Empagliflozin and Metformin Hydrochloride were found to be below 2% under all modified conditions. The tailing factors remained within acceptable limits, and the percentage recoveries ranged from 99.09% to 101.42% for Empagliflozin and 98.95% to 101.21% for Metformin Hydrochloride. These results (Table 5) indicate that minor changes in chromatographic conditions did not significantly affect method performance, confirming the robustness and reliability of the proposed method for routine pharmaceutical analysis.

Table 5: Robustness Study Data for Empagliflozin and Metformin Hydrochloride Under Various Analytical Conditions.

Parameter	Level	%RSD (Empagliflozin)	Tailing Factor (Empagliflozin)	% Recovery (Empagliflozin)	%RSD (Metformin HCl)	Tailing Factor (Metformin HCl)	% Recovery (Metformin HCl)
Flow Rate (mL/min)	0.8	0.72	1.12	99.41	0.21	1.04	99.89
	1.0	0.55	1.07	100.15	0.32	1.09	99.52
	1.2	0.89	1.31	99.09	0.48	1.13	100.39
pH	3.5	0.11	1.02	99.76	0.57	1.22	99.26
	4.0	0.71	1.41	99.63	0.51	1.16	98.95
Wavelength (nm)	228	0.66	1.20	99.27	0.85	1.10	99.14
	230	0.09	1.04	99.48	0.54	1.08	99.74
	232	0.57	1.33	100.76	0.92	1.21	99.42
Mobile Phase Ratio (ACN)	40:60	0.95	1.38	101.42	0.24	1.09	101.21
	45:55	0.49	1.05	99.31	0.83	1.07	100.74
	50:50	0.85	1.06	99.38	0.61	1.18	

Detection and Quantitation Limits

The sensitivity of the developed RP-HPLC method was evaluated by determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Empagliflozin and Metformin Hydrochloride. The LOD values were found to be 0.32 µg/mL for Empagliflozin and 1.75 µg/mL for Metformin Hydrochloride, while the corresponding LOQ values were 0.98 µg/mL and 5.30 µg/mL, respectively. The low LOD and LOQ values in Table 6 indicate the high sensitivity of the developed method and its suitability for the detection and quantification of both analytes at low concentration levels.

Table 6: LOD and LOQ Data.

Drug	LOD (µg/mL)	LOQ (µg/mL)
Empagliflozin	0.32	0.98
Metformin Hydrochloride	1.75	5.30

Assay of Marketed Formulations

The developed RP-HPLC method was successfully applied to the assay of marketed tablet formulations containing Empagliflozin and Metformin Hydrochloride. The chromatographic peaks were well resolved, and the retention times were found to be reproducible across all formulations. The percentage assay values ranged from 99.24% to 101.18% for Empagliflozin and 99.68% to 100.84% for Metformin Hydrochloride, indicating good agreement with the labeled claim. The results in Table 7 confirm the applicability of the proposed method for routine quality control analysis of combined tablet dosage forms.

Table 7: Assay Data for Marketed Formulations.

Marketed Formulation	Drug	Retention Time (min)	Peak Area	% Assay
SYNJARDY®	Empagliflozin 10 mg	5.41	485621	99.24
	Metformin HCl 500 mg	2.85	1754382	99.68
EMPA-MET	Empagliflozin 10 mg	5.43	489154	100.35
	Metformin HCl 500 mg	2.86	1762145	100.22
GLYXAMBI-MET	Empagliflozin 10 mg	5.39	492706	101.18
	Metformin HCl 500 mg	2.84	1770854	100.84

CONCLUSION

A rapid, accurate, precise, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Empagliflozin and Metformin Hydrochloride in bulk drugs and pharmaceutical dosage forms. The method provided satisfactory chromatographic separation with retention times of 5.418 min for Empagliflozin and 2.851 min for Metformin Hydrochloride. Validation studies demonstrated excellent linearity over the concentration ranges of 2.5–25 µg/mL and 12.5–125 µg/mL, respectively, with correlation coefficients greater than 0.999. The method exhibited acceptable accuracy, precision, ruggedness, robustness, and sensitivity, with all validation parameters complying with ICH guidelines. Assay results of marketed formulations showed drug contents within the acceptable range of 99–101% of the label claim. Therefore, the proposed RP-HPLC method can be reliably employed for routine quality control, assay determination, and analysis of pharmaceutical formulations containing Empagliflozin and Metformin Hydrochloride.

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Conflict of Interest

The authors announce that there is no disagreement of interest associated with this research work.

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