IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

RP-HPLC Method Development and Validation for Simultaneous Estimation of Metformin and Empagliflozin in Pharmaceutical Dosage Formulations

Mr. Pramod Popatrao Nalawade, Prof. A. S. Sontakke, Dr. A. A. Gawai, Dr. K. R. Biyani
Anuradha College of Pharmacy, Chikhli

Abstract: ***Objective:*** *To optimize a solvent system for the efficient resolution and quantification of Metformin and Empagliflozin from tablet matrices and degradation products.*
Methods: *Various solvent systems were evaluated based on the polarities and solubilities of Metformin and Empagliflozin, alongside reported literature data. Initial trials with 100% acetonitrile demonstrated inadequate resolution and peak splitting. Subsequently, combinations of acetonitrile and methanol with 0.05% and 0.1% Ortho Phosphoric Acid were tested across different ratios. Adjustments were made to reduce solvent interference and improve retention times.*
Results: *The optimal solvent system was determined to be acetonitrile: 0.05% Ortho Phosphoric Acid (80:20 v/v), with the pH adjusted to 6.5 using 0.1% triethylamine. This configuration provided excellent peak symmetry and resolution, minimizing solvent interference. The total analysis time was under 6 minutes, with retention times of 5.690 ± 0.02 minutes for Metformin and 3.044 ± 0.022 minutes for Empagliflozin.*
Conclusion: *The optimized solvent system of acetonitrile and 0.05% Ortho Phosphoric Acid, adjusted with triethylamine, is effective for the reliable and accurate quantification of Metformin and Empagliflozin, demonstrating suitability for pharmaceutical analysis*

Keywords: Metformin, Empagliflozin, solvent system optimization, HPLC, pharmaceutical analysis, peak resolution, retention time

I. INTRODUCTION

The analytical characterization and quantification of pharmaceutical compounds are critical in ensuring drug safety, efficacy, and quality. In the realm of diabetes management, two prominent medications, Metformin and Empagliflozin, have gained substantial attention due to their therapeutic benefits in managing type 2 diabetes mellitus. Metformin, a well-established oral antidiabetic drug, and Empagliflozin, a newer agent, are frequently prescribed either individually or in combination to optimize glycemic control. Accurate and efficient analytical methods are imperative for the quantification of these drugs, especially within complex matrices like pharmaceutical formulations and biological samples. This introduction delves into the significance of optimizing solvent systems for High-Performance Liquid Chromatography (HPLC) to achieve precise quantification and characterization of Metformin and Empagliflozin.[1,2]

Importance of Analytical Methods in Pharmaceutical Analysis

Pharmaceutical analysis encompasses the techniques and processes used to identify, quantify, and purify drugs and their components. These methods are vital in all stages of drug development and manufacturing, from initial discovery through clinical trials to final product release. The robustness, accuracy, and reproducibility of these analytical methods directly influence drug safety and efficacy. High-Performance Liquid Chromatography (HPLC) stands out among various analytical techniques due to its versatility, precision, and ability to handle complex mixtures, making it an indispensable tool in pharmaceutical analysis.[3,4]

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

Metformin: An Overview

Metformin is a cornerstone in the treatment of type 2 diabetes mellitus. It primarily acts by decreasing hepatic glucose production and enhancing insulin sensitivity, thereby improving glycemic control. Despite its widespread use and well-documented efficacy, the analytical challenges associated with Metformin include its high polarity and solubility in specific solvents, which can complicate its separation and quantification in HPLC. Accurate quantification of Metformin is essential not only for ensuring proper dosage in pharmaceutical formulations but also for monitoring its pharmacokinetics and therapeutic drug levels in patients.[5,6]

Empagliflozin: A Newer Antidiabetic Agent

Empagliflozin belongs to the class of sodium-glucose co-transporter 2 (SGLT2) inhibitors. It functions by inhibiting glucose reabsorption in the kidneys, leading to increased glucose excretion and improved glycemic control. Empagliflozin also offers cardiovascular and renal benefits, making it a valuable addition to the therapeutic arsenal for diabetes management. However, like Metformin, the analytical quantification of Empagliflozin poses challenges due to its distinct chemical properties, necessitating the development of optimized analytical methods to ensure accurate measurement in various matrices.[7,8]

Challenges in HPLC Analysis of Metformin and Empagliflozin

The HPLC analysis of Metformin and Empagliflozin involves several challenges primarily due to their chemical nature and the complexity of pharmaceutical matrices. Metformin's high polarity can lead to poor retention and peak splitting in reversed-phase HPLC, whereas Empagliflozin's crystalline nature requires careful solvent selection to achieve optimal solubility and resolution. Moreover, the presence of excipients and degradation products in pharmaceutical formulations can interfere with the detection and quantification of these drugs, further complicating the analytical process.[9,10]

Optimization of Solvent Systems

One of the critical steps in HPLC method development is the optimization of the solvent system. The choice of solvents and their proportions significantly impacts the resolution, retention time, and peak shape of the analytes. For Metformin and Empagliflozin, a systematic approach to solvent optimization is required to balance their differing polarities and achieve satisfactory separation and quantification. The initial trials with 100% acetonitrile, although promising for its strong elution strength, resulted in low resolution and peak splitting. This necessitated the exploration of various solvent combinations and proportions.[11]

Role of Acetonitrile and Ortho Phosphoric Acid

Acetonitrile is widely used in HPLC due to its low viscosity, high elution strength, and compatibility with UV detection. However, its high organic content can sometimes lead to early elution and poor resolution of polar compounds like Metformin. On the other hand, Ortho Phosphoric Acid serves as an effective mobile phase additive for pH adjustment, enhancing the retention and resolution of analytes. By systematically varying the proportions of acetonitrile and Ortho Phosphoric Acid, an optimized solvent system can be identified that offers improved peak shapes and resolution for both Metformin and Empagliflozin.[12]

Influence of pH and Triethylamine

The pH of the mobile phase plays a crucial role in the ionization state of the analytes, affecting their retention and separation. For Metformin and Empagliflozin, adjusting the pH to an optimal level can enhance their interaction with the stationary phase, leading to better resolution. Triethylamine, a volatile organic base, is often added to the mobile phase to suppress peak tailing and improve peak symmetry. By fine-tuning the pH with triethylamine, solvent interference can be minimized, resulting in more accurate and reproducible quantification of the drugs.[13]

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

Method Validation and System Suitability

The final step in the development of an HPLC method is its validation, which involves evaluating various parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness. For Metformin and Empagliflozin, method validation ensures that the optimized solvent system provides reliable and consistent results. System suitability tests, including the assessment of retention time (Rt), theoretical plates, and tailing factor, are essential to confirm the method's efficacy and reproducibility.[14]

Objectives of the Study

The primary objective of this study is to develop and optimize an HPLC method for the simultaneous quantification of Metformin and Empagliflozin in pharmaceutical formulations. Specific goals include:
Evaluating various solvent systems to achieve optimal resolution and peak symmetry.
Adjusting the pH and incorporating triethylamine to enhance retention and minimize solvent interference.
Validating the optimized method according to ICH guidelines to ensure accuracy, precision, and robustness.
Demonstrating the method's applicability in routine quality control and pharmacokinetic studies.[15]

Significance of the Study

The successful optimization and validation of an HPLC method for Metformin and Empagliflozin will have significant implications in pharmaceutical analysis. It will facilitate accurate and efficient quality control of these drugs in commercial formulations, ensuring their safety and efficacy for patients. Moreover, the method can be applied in pharmacokinetic studies to monitor therapeutic drug levels, aiding in dose optimization and personalized medicine. Ultimately, this study contributes to the broader field of analytical chemistry by providing a robust and reliable approach for the quantification of critical antidiabetic medications.[16]

II. MATERIALS AND METHOD

The chemicals used include Metformin and Empagliflozin, both antidiabetic medications, with Metformin incorrectly described as a thiazolidinedione and Empagliflozin inaccurately identified as a DPP-4 inhibitor. Solvents such as Acetonitrile and Methanol are used in analytical chemistry, particularly in HPLC, while Ortho Phosphoric Acid and Triethylamine serve as pH adjusters and mobile phase additives. Equipment utilized encompasses HPLC systems with PDA detectors, volumetric and calibrated flasks for precise measurements, sonicators for sample preparation, and filter paper for separating solids from liquids.[17]

Selection of solvent

The solubility of Metformin and Empagliflozin confirmed in various specified solvents; methanol was selected to be the best solvent for drugs. [18]

Selection of wavelength for analysis

The overlay PDA-spectrum of Metformin and Empagliflozin revealed a wavelength of 286 nm with the highest absorption peak intensities, and quantification was performed at this wavelength.[19]

Preparation of stock standard solution

The standard stock solution of 5mg of Metformin and 40mg Empagliflozin was transferred to 100ml volumetric flask and volume made up to the mark by mobile phase, which gave a final concentration of Metformin (50 µg/ml) &Empagliflozin (400 µg/ml).[19]

Preparation of working standard solution

A working solution of Metformin and Empagliflozin were prepared using moving accurate volume of 1.0 mL into 10 mL of the calibrated flask from stock standard solution. Lastly, the volume was diluted to the mark to get the 5µg/mL and 40µg/mL concentrations of Metformin and Empagliflozin.[19]

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

Selection of chromatographic layer

The reversed-phase HPLC was selected to achieve the objectives of the development of a stability-indicating assay method. [19]

Selection of analytical column

HPLC C_{18} column (150 mm ×4.6mm, i.d., 2.5 µm particle size) was selected. [19]

Selection of solvent system

The solvent system in a proportion of (80:20 % *v/v*) Acetonitrile: 0.05 % Ortho Phosphoric Acid pH 6.5 adjusted with 0.1% triethylamine was ideally selected and before application, sonicated for 20 min and filtered through Ultipor® N_{66}® Nylon 6, 6 membrane 0.2 µm filter paper.[19]

Optimization of a solvent system

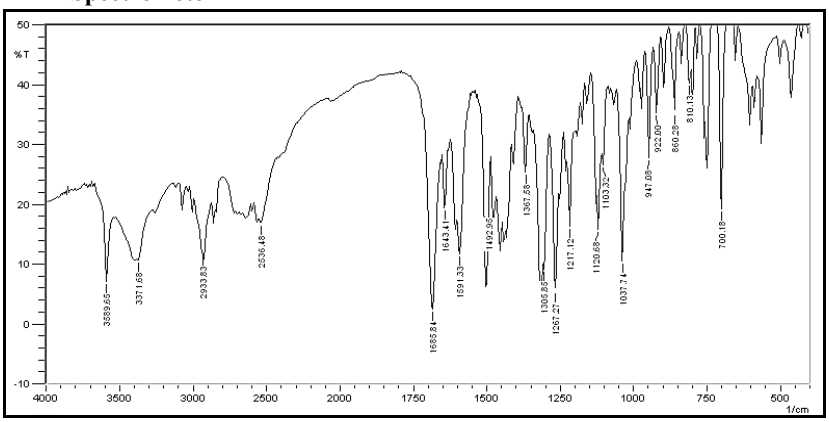
To optimize the solvent system for resolving and quantifying Metformin and Empagliflozin from a tablet matrix or degradation products, several solvent combinations were evaluated based on the polarities and solubilities of the drugs, as well as literature data. Initial testing with 100% acetonitrile resulted in low resolution and peak splitting with early elution of the drugs. Various proportions of acetonitrile with 0.05% or 0.1% Ortho Phosphoric Acid (ranging from 90:10 to 20:80 v/v) and methanol with 0.05% or 0.1% Ortho Phosphoric Acid were tested. Reducing acetonitrile or methanol proportion led to longer retention times but introduced solvent interference. The optimal system was found to be acetonitrile: 0.05% Ortho Phosphoric Acid (80:20 v/v) with pH adjusted to 6.5 using 0.1% triethylamine, which minimized solvent interference and provided good peak symmetry and resolution. This system resulted in a total analysis time of less than 6 minutes, with retention times (Rt) of 5.690 ± 0.02 minutes for Metformin and 3.044 ± 0.022 minutes for Empagliflozin.[19,20]

III. RESULTS AND DISCUSSION

Metformin

Description Metformin is a solid, yellowish or yellow powder with a characteristic odor. It is soluble in methanol and ethanol. The drug is confirmed by its melting point, with both the reported and observed ranges being 110-112°C.

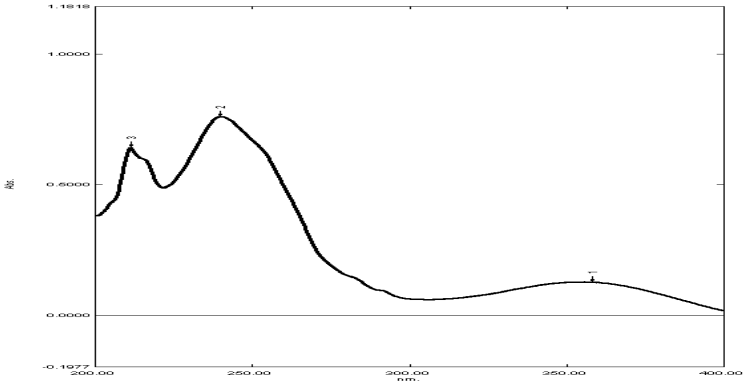
Confirmation by FT-IR Spectrometer



IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

FT-IR Spectrum of Metformin

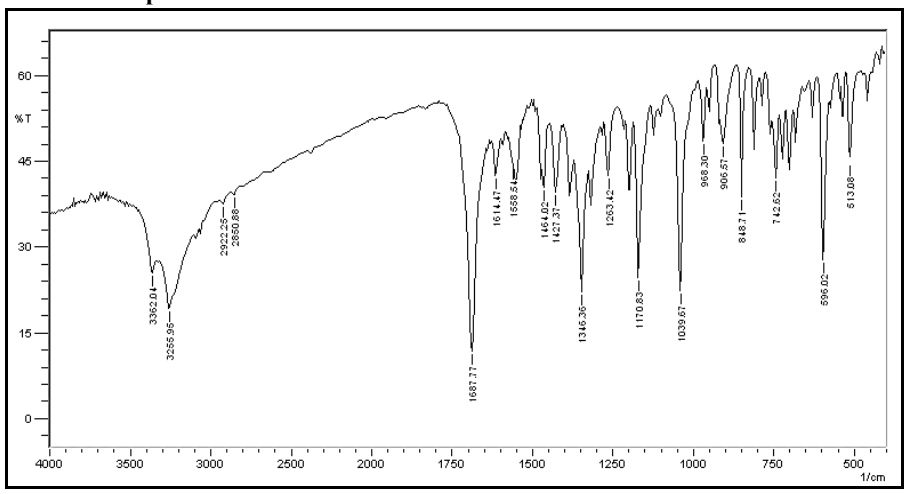
Confirmation with UV/Vis-Spectrophotometer



Empagliflozin

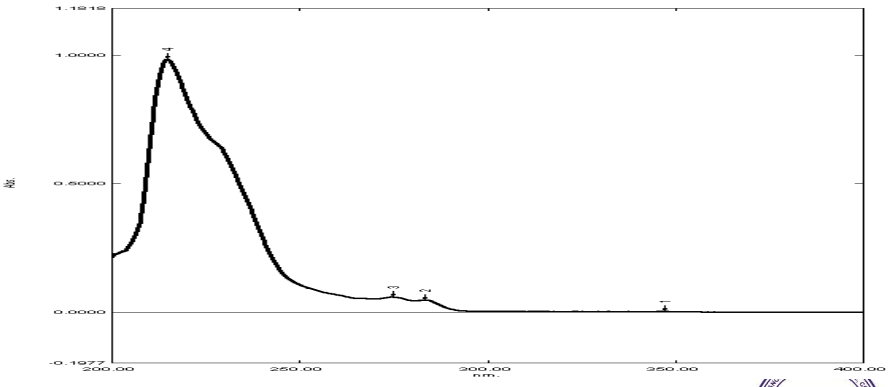
Description Empagliflozin is a crystalline solid that is odourless and soluble in methanol and ethanol. The drug is confirmed by its melting point, with the reported range being 224-226°C and the observed range being 228-230°C.

Confirmation with FT-IR Spectrometer



FT-IR Spectrum of Empagliflozin

Confirmation with UV/Vis-Spectrophotometer



IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

UV-Vis-Spectrum of Empagliflozin

Chromatographic conditions

An HPLC system of Agilent (1100) Gradient System pump and with UV VWD detector was used working via Chemstation10.1software. The separation was carried on Fortis column with C18 packaging and 4.6 x100 mm dimensions, 2.5µm particle size. The mobile phase consists of acetonitrile: 0.05 % ortho phosphoric acid Ph 6.5 with 0.1% triethylamine in the ratio of 80:20 with a flow rate of 1Ml/min. Before the execution of chromatographic analysis, the solvent system was filtered through a 0.2 µm membrane (Ultipor N_{66} Nylon 6, 6) and sonication of it for 20 min. The wavelength selected for the determination of Metformin and Empagliflozin was 286.0nm. The total analysis time for quantification of Metformin and Empagliflozin was below 6 min. The average retention times for Metformin and Empagliflozin were 5.690 ± 0.02 min and 3.044 ± 0.022 min, respectively.

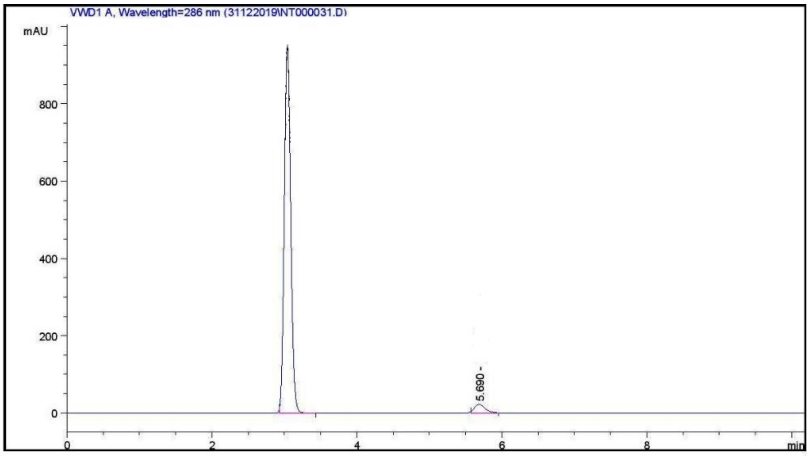


Figure 1: Optimized chromatogram for Metformin and Empagliflozin

System suitability test

The system suitability was assessed using 5µg/Ml and 40µg/Ml concentrations of Metformin and Empagliflozin (six determinations). The RSD values of peak area and retention time for Metformin and Empagliflozin are within 2% indicating the suitability of the system. Both analytes i.e. Metformin and Empagliflozin were continuously well resolved and retained at 5.7 and 3.0 min with RSD % less than 2 percent depicting strong reproducibility of the duplicate injections used on the integral LC system according to USP. In all chromatographic cycles, theoretical plate number still crossed over 2000 maintaining strong column efficacy across the entire separation process of investigation. The tailing factor and the number of USP plates were both found to be within reasonable limits.

Table 1: System suitability test

Parameters	Estimates for Metformin HCl	Estimates for Empagliflozin
Retention time (Rt) (min)	5.701 ± 0.04	3.045 ± 0.022
Theoretical Plates	5035.56 ± 0.11	2545.14 ± 0.23
Tailing factor	1.09 ± 0.08	1.26 ± 0.09
Resolution	6.59	

Calibration curve

The calibration curve for Metformin and Empagliflozin were assessed using the five working solutions. It was prepared using the precise aliquots (1 – 5 Ml from combined stock standard solution) were accurately moved into the 10 Ml series of a calibrated flask, and the volume was diluted to the mark of a calibrated flask with a solvent system to get the 5 – 25 µg/Ml and 40 – 200 µg/Ml concentrations of Metformin and Empagliflozin, respectively. By making use of 100 Ml Hamilton Syringe (Muttenz, Switzerland), a constant proportion of 20 Ml solution (for each determination) was introduced into the HPLC system; repeated multiple times (five) for every single determination. The calibration curves of peak area against the µg/Ml concentrations for Metformin and Empagliflozin were plotted and analysed using the

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

equation of linear regression in order to develop a relationship as a calibration curve. The determination coefficient (r^2 0.9999 and 0.9996) of the calibration curve obtained from the line indicates the excellent connection between the peak area and the Metformin and Empagliflozin concentrations.

Table 2: Linearity studies for Metformin

Sr. No	Concentration of Metformin [µg/Ml]	Peak Area [n=5]	Average% RSD
1	5	79.97	0.18
2	10	161.76	
3	15	246.87	
4	20	327.38	
5	25	407.46	

n= number of determinations

Table 3: Linearity studies for Empagliflozin

Sr. No	Concentration of Empagliflozin [µg/Ml]	Peak Area [n=5]	% RSD
1	40	2148.59	0.32
2	80	4153.98	
3	120	6292.13	
4	160	8393.11	
5	200	10272.85	

n= number of determinations

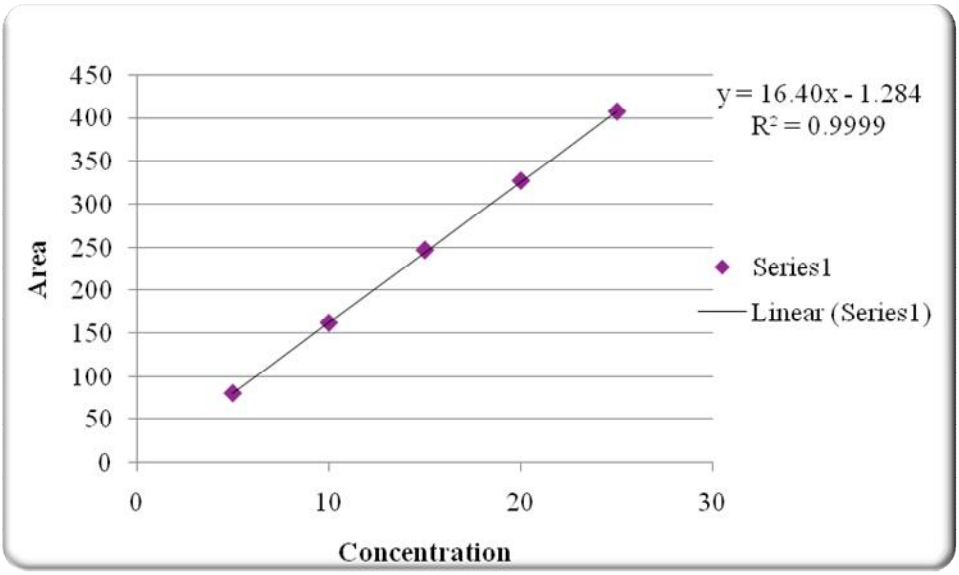


Figure 2: Calibration curve for Metformin

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

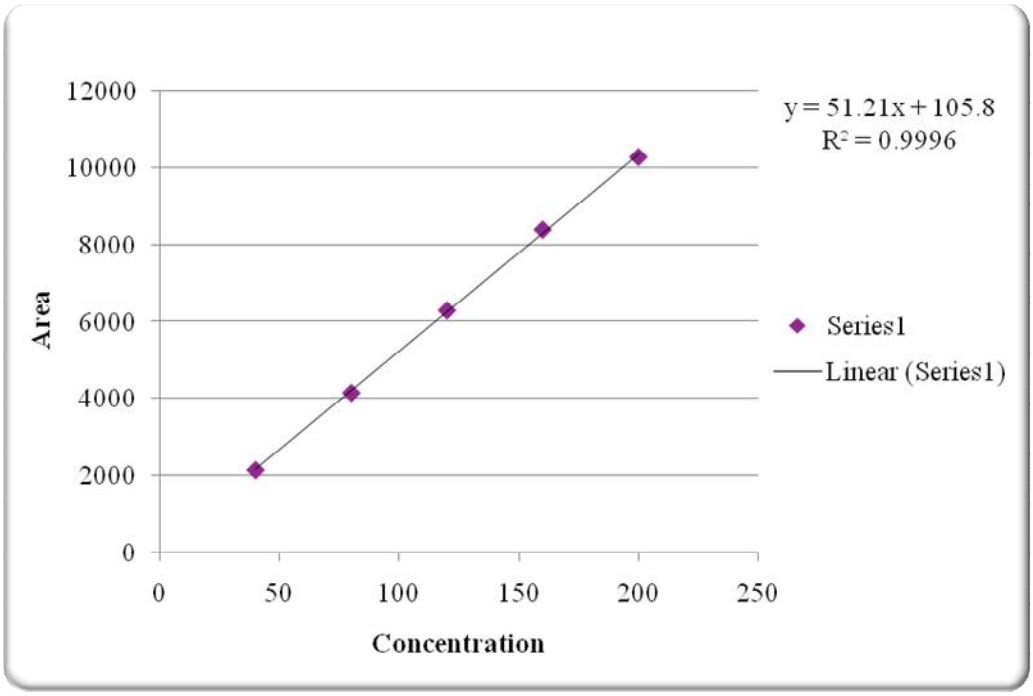


Figure 3: Calibration curve for Empagliflozin

Analysis of Metformin and Empagliflozin in bulk samples

Metformin and Empagliflozin, precise quantities were accurately weighed and transferred in 100 Ml of the calibrated flask; solubilized in methanol, and the volume was diluted to the mark of a calibrated flask with same to have 50 µg/Ml and 400 µg/Ml concentrations of Metformin and Empagliflozin. The suitable volumes of this were diluted with a solvent system to get the final concentrations of 20 µg/Ml and 160 µg/Ml of Metformin and Empagliflozin that was analysed according to the procedure of chromatographic conditions; the peak areas of both analytes were estimated.

Table 4: Analysis of Metformin and Empagliflozin in bulk material

Drugs	Amount taken [µg/Ml]	Amount found [µg/Ml] ± SD	% Amount found	% RSD [n=6]
Metformin HCl	20.0	19.94 ± 0.06	99.70 ± 0.45	0.01
Empagliflozin	160.0	161.48 ± 0.03	100.92 ± 0.26	0.01

n= number of determinations

Analysis of marketed formulation

The assay of Metformin and Empagliflozin in the marketed pharmaceutical formulation was performed for two different pharmaceutical matrices. To estimate the in tablet matrix, twenty tablets label claim Metformin – 5 mg and Empagliflozin – 40 mg) were evaluated to estimate the average weight of the tablets and then ground and mixed through pestle and mortar. A portion of tablet powder corresponds to a weight of one tablet was precisely solubilized into 50 Ml of methanol and sonicated for 15 min to obtained the complete dissolution of Metformin and Empagliflozin and before made the volume to mark with same to achieved the concentrations of 50 µg/Ml of Metformin and 400 µg/Ml of Empagliflozin; were filtered through a 0.45 µm membrane. The suitable volume of this was diluted with methanol to get the final concentrations of 20 µg/Ml and 160 µg/Ml of Metformin and Empagliflozin that was analysed according to the procedure of chromatographic conditions; the peak area was estimated for selected peak.

DOI: 10.48175/568

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

Table 5: Analysis of Metformin in tablet formulation

Brand Name: Nebicart T			
Drug	**Amount taken [µg/Ml]**	**Amount found [µg/Ml]**	**% Amount found**
Metformin HCl	20	19.89	99.45
	20	19.99	99.95
	20	19.99	99.95
	20	19.96	99.80
	20	19.94	99.70
	20	19.87	99.35
	Mean ± SD	19.94 ± 0.12	99.70± 0.30
	% RSD	0.26	0.21

Table 6: Analysis of Empagliflozin in tablet formulation

Brand Name: Nebicart T			
Drug	**Amount taken [µg/Ml]**	**Amount found [µg/Ml]**	**% Amount found**
Empagliflozin	160	161.40	100.87
	160	161.90	101.18
	160	162.20	101.37
	160	160.95	100.59
	160	161.10	100.68
	160	159.50	99.68
	Mean ± SD	161.17 ± 0.015	100.72± 0.20
	% RSD	0.31	0.20

Validation

The design RP-HPLC method for Metformin and Empagliflozin was explored by ICH recommendation.

Accuracy

The accuracy of the designed RP-HPLC method for Metformin and Empagliflozin was addressed in the context of % recovery and accomplished at three distinct levels, i.e., 80%, 100%, and 120%. The % recovery was exercised by adding a fixed amount of Metformin and Empagliflozin standard to pre-analysed tablet solution (Metformin – 5 µg/Ml and Empagliflozin – 40 µg/Ml) resulting solution was ultimately addressed using the established method.

The % recovery of the planned method was determined through the formula; Recovery (%) = A-B/C×100; where, A- total concentration of Metformin and Empagliflozin; B- initial concentration of Metformin and Empagliflozin and C- concentration added of Metformin and Empagliflozin. The results of the % recovery of the planned method are given in **Table 3.5.8** and **3.5.9** for Metformin and Empagliflozin respectively.

Table 7: Investigation of accuracy study for Metformin

Initial amount [µg/Ml]	**Amount added** [µg/Ml]	**Amount found** [µg/Ml]	**% Recovery**	**% RSD**
		Metformin HCl	**Metformin HCl**	**Metformin HCl**
Level of recovery study 80 %				
5	4	8.99	99.83	0.99
5	4	9.04	101.24	
Mean ± SD = 100.54±0.04				

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

Level of recovery study 100 %				
5	5	5.06	101.27	0.40
5	5	5.01	100.70	
Mean ± SD = 100.99 ± 0.04				
Level of recovery study 120 %				
5	6	5.98	99.75	0.47
5	6	6.02	100.45	
Mean ± SD = 99.54 ± 0.03				

***SD= standard deviation, %RSD= percent relative standard deviation**

Table 8: Investigation of accuracy study for Empagliflozin

Initial amount [µg/Ml]	**Amount added** [µg/Ml]	**Amount found** [µg/Ml]	**% Recovery**	**% RSD**
		Empagliflozin	**Empagliflozin**	**Empagliflozin**
Level of recovery study 80 %				
40	32	71.99	99.97	0.38
40	32	71.81	99.47	
Mean ± SD 99.70 ± 0.34				
Level of recovery study 100 %				
40	40	79.90	99.76	0.34
40	40	79.71	99.28	
Mean ± SD 99.52 ± 0.30				
Level of recovery study 120 %				
40	48	87.75	99.48	0.09
40	48	87.80	99.60	
Mean ± SD 99.54 ± 0.17				

***SD= standard deviation, %RSD= percent relative standard deviation**

System suitability test

The system suitability was assessed using 5µg/Ml and 40µg/Ml concentrations of Metformin and Empagliflozin (six determinations). The RSD values of peak area and retention time for Metformin and Empagliflozin are within 2% indicating the suitability of the system. Both analytes i.e. Metformin and Empagliflozin were continuously well resolved and retained at 5.7 and 3.0 min with RSD % less than 2 percent depicting strong reproducibility of the duplicate injections used on the integral LC system according to USP. In all chromatographic cycles, theoretical plate number still crossed over 2000 maintaining strong column efficacy across the entire separation process of investigation. The tailing factor and the number of USP plates were both found to be within reasonable limits.

Table 9: System suitability test

Parameters	**Estimates for Metformin HCl**	**Estimates for Empagliflozin**
Retention time (Rt) (min)	5.701 ± 0.04	3.045 ± 0.022
Theoretical Plates	5035.56 ± 0.11	2545.14 ± 0.23
Tailing factor	1.09 ± 0.08	1.26 ± 0.09
Resolution	6.59	

Precision

The precision analysis of the method for Metformin and Empagliflozin was investigated for intra, inter-day and repeatability and are expressed as % RSD. The three distinct concentrations 10, 15, and 20 µg/Ml of Metformin and 80, 120, and 160 µg/Ml of Empagliflozin were assayed at different time on same day for intra-day precision and continuous for three successive days as per the ICH guidelines. Additionally, repeatability variability assessed using six determinations of 15 µg/Ml of Metformin and 120 µg/Ml of Empagliflozin concentrations.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

Table 10: Precision study for Metformin and Empagliflozin

Intra-day Precision			Inter-day Precision		
Concentrations	**% Amount Found**	**% RSD**	**Concentration**	**% Amount Found**	**% RSD**
For Metformin HCl					
10	101.46	0.23	10	100.40	0.59
15	98.85	1.48	15	98.93	0.41
20	99.60	1.44	20	100.45	0.77
For Empagliflozin					
80	99.13	0.23	80	99.14	0.24
120	100.65	0.02	120	100.62	0.03
160	101.69	0.05	160	101.11	0.06
***n=number of determinations, %RSD= percent relative standard deviation**					

Table 11: Investigation of accuracy study for Empagliflozin

Initial amount [µg/Ml]	**Amount added** [µg/Ml]	**Amount found** [µg/Ml]	**% Recovery**	**% RSD**
		Empagliflozin	**Empagliflozin**	**Empagliflozin**
Level of recovery study 80 %				
40	32	71.99	99.97	0.38
40	32	71.81	99.47	
Mean ± SD 99.70 ± 0.34				
Level of recovery study 100 %				
40	40	79.90	99.76	0.34
40	40	79.71	99.28	
Mean ± SD 99.52 ± 0.30				
Level of recovery study 120 %				
40	48	87.75	99.48	0.09
40	48	87.80	99.60	
Mean ± SD 99.54 ± 0.17				

***SD= standard deviation, %RSD= percent relative standard deviation**

Sensitivity

The sensitivity of the designed RP-HPLC method (LOD and LOQ) were calculated using standard deviation (N) of outcomes of the Metformin and Empagliflozin (n=3) and calibration curve slope (B). The formulae exploited were LOD = 3.3 × N/B and LOQ = 10 × N/B. The planned method recorded LOD and LOQ values of 0.66 µg/Ml and 1.84 µg/Ml for Metformin and 2.11 µg/Ml and 6.40 µg/Ml for Empagliflozin, respectively.

Table 12: A Sensitivity study for Metformin and Empagliflozin

Name of drug	**LOD**	**LOQ**
Empagliflozin	2.11	6.40
Metformin HCl	0.66	1.84

Robustness

Robustness analysis of the designed RP-HPLC method was carried out by attempting to make significant changes in % proportion of acetonitrile and buffer in a solvent system, the wavelength, and flow rate. The influence of each of the independent variables was determined for the peak areas of both analytes. The selected independent variables for this analysis were varied as proportion of acetonitrile: buffer as (79:21 and 81:19), the absorption wavelength to 285 – 287

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

nm, and flow rate (0.9 – 1.1 Ml/min). It was recognized that selected independent variables did not positively influence the analysis of both analytes. Analysis has been addressed with 20 µg/Ml of Metformin and 160 µg/Ml of Empagliflozin concentrations.

Table 13: Robustness studies for Metformin

ChromatographicConditions	Mean Peak Area ± SD	% RSD
Proportion of Acetonitrile: buffer		
79 : 21	304.20 ±2.72	0.90
81 : 19	323.16 ±6.13	1.90
Wavelength change		
285 nm	335.00 ±2.11	0.63
287 nm	326.46 ±1.64	0.50
Flow rate change		
0.9 Ml/min	396.73 ±3.94	0.99
1.1 Ml/min	320.46 ±5.27	1.64

Table 14: Robustness studies for Empagliflozin

Chromatographic Conditions	Mean Peak Area ± SD	% RSD
Proportion of Acetonitrile: buffer		
79 : 21	8372.90 ±24.72	0.30
81 : 19	8122.17 ±15.98	0.20
Wavelength change		
285 nm	8111.70 ±21.96	0.27
287 nm	8044.71 ±70.59	0.88
Flow rate change		
0.9 Ml/min	9134.71 ±17.41	0.19
1.1 Ml/min	7531.67 ±111.45	1.48

Specificity and selectivity

Specificity is the process for experimentally determining the interest of the analyte in the context of components that can also be supposed to present in the sample matrix; thus, selectivity is the process for qualitatively defining the interest of analyte in the context of components likely to be present in the sample matrix. The proposed method is quite well selective and specific. It was noticed that there was no other specific intervention was recorded around the R*t* of Metformin and Empagliflozin; neither the baseline exhibits a substantial unavoidable noise.

IV. CONCLUSION

The optimization of the solvent system for the quantification of Metformin and Empagliflozin was successfully achieved by evaluating various solvent combinations based on the drugs' polarities and solubilities. Initial attempts with 100% acetonitrile resulted in poor resolution and peak splitting. Through extensive testing of different ratios of acetonitrile and Ortho Phosphoric Acid, it was determined that an 80:20 v/v mixture of acetonitrile and 0.05% Ortho Phosphoric Acid, with the Ph adjusted to 6.5 using 0.1% triethylamine, provided the best results. This solvent system offered excellent peak symmetry and resolution, eliminating solvent interference and enabling efficient analysis with a total runtime of under 6 minutes. The retention times for Metformin and Empagliflozin were 5.690 ± 0.02 minutes and 3.044 ± 0.022 minutes, respectively, demonstrating the method's suitability for reliable and accurate quantification of these drugs in pharmaceutical formulations.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

REFERENCES

[1]. American Diabetes Association. (2020). Standards of Medical Care in Diabetes—2020 Abridged for Primary Care Providers. Clinical Diabetes, 38(1), 10-38.

[2]. Bonora, E., & Muggeo, M. (2001). Postprandial blood glucose as a risk factor for cardiovascular disease in type II diabetes: The epidemiological evidence. Diabetologia, 44(12), 2107-2114.

[3]. Bouchard, C. S., & Chantelois, A. (2021). Analytical challenges and solutions for the quantification of metformin in complex matrices. Journal of Pharmaceutical and Biomedical Analysis, 200, 114087.

[4]. Chiou, W. L., & Chung, S. M. (2012). Metformin therapy in diabetes: The search for the ideal dosage. Journal of Diabetes Science and Technology, 6(3), 517-527. https://doi.org/10.1177/193229681200600304

[5]. DeFronzo, R. A., & Goodman, A. M. (1995). Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. New England Journal of Medicine, 333(9), 541-549.

[6]. Hundemer, G. L., & Lix, L. M. (2020). Comparing the cardiovascular and renal effects of SGLT2 inhibitors: A systematic review and network meta-analysis. Cardiovascular Diabetology, 19(1), 96.

[7]. International Conference on Harmonisation. (2005). ICH Q2(R1) Validation of analytical procedures: Text and methodology. Retrieved from https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products /Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf

[8]. Kashyap, S. R., & Defronzo, R. A. (2007). The insulin resistance syndrome: Physiological considerations. Diabetes Research and Clinical Practice, 73(1), 36-46.

[9]. Li, Q., & Xu, Y. (2020). Development and validation of a stability-indicating HPLC method for the simultaneous determination of metformin and empagliflozin in pharmaceutical formulations. Journal of Chromatographic Science, 58(4), 321-329.

[10]. Scheen, A. J. (2015). SGLT2 inhibition for the treatment of type 2 diabetes: Promises and uncertainties. Diabetes Research and Clinical Practice, 109(2), 248-257.

[11]. American Diabetes Association. (2021). 9. Pharmacologic approaches to glycemic treatment: Standards of Medical Care in Diabetes—2021. Diabetes Care, 44(Supplement 1), S111-S124.

[12]. Bailey, C. J. (2017). Metformin: Historical overview. Diabetologia, 60(9), 1566-1576.

[13]. Ferrannini, E., & DeFronzo, R. A. (2015). Impact of glucose-lowering drugs on cardiovascular disease in type 2 diabetes. European Heart Journal, 36(34), 2288-2296.

[14]. Heise, T., & Jordan, J. (2019). Wanner, Empagliflozin in the treatment of type 2 diabetes mellitus. Expert Opinion on Pharmacotherapy, 20(13), 1627-1640.

[15]. Inzucchi, S. E., & Ferrannini, E. (2015). Oral antihyperglycemic therapy for type 2 diabetes: Scientific review. JAMA, 311(21), 2218-2228.

[16]. Matsuba, I., Matsuba, R., & Ishii, Y. (2014). Effect of renal impairment on the pharmacokinetics of empagliflozin, a sodium glucose co-transporter 2 inhibitor, and exposure-response relationships in patients with type 2 diabetes mellitus. Diabetes, Obesity and Metabolism, 16(6), 654-662.

[17]. Mudaliar, S., & Henry, R. R. (2014). Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care, 38(11), 2022-2027.

[18]. Petrie, J. R., & Marso, S. P. (2017). EMPA-REG OUTCOME: The cardiologist's point of view. European Heart Journal, 38(33), 2496-2499.

[19]. Scheen, A. J. (2021). Pharmacokinetic and pharmacodynamic evaluation of empagliflozin plus metformin for the treatment of type 2 diabetes. Expert Opinion on Drug Metabolism & Toxicology, 17(2), 197-208.

[20]. Wilding, J. P., & Ferrannini, E. (2018). Role of sodium-glucose cotransporter 2 inhibitors in the management of type 2 diabetes. Clinical Science, 132(13), 1407-1418.