Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-155584
Volume-2025, Issue-1, pp-76-85
https://bfszu.journals.ekb.eg/journal
Research Paper DOI: **10.21608/bfszu.2025.294373.1396**

==========

Simultaneous quantification of Empagliflozin and Metformin in pharmaceutical dose form using HPLC technique

Alaa S. Amin *1, Soha F. Mohamed2 and Mourad M. Abo-Taleb2

1 Department of Chemistry, Faculty of Science, Benha University, Benha 13511, Egypt
2 Department of Chemistry, Faculty of Science, Zagazig University, Zagazig 44519, Egypt
2 Department of Chemistry, Faculty of Science, Zagazig University, Zagazig 44519, Egypt

***Corresponding Author**: E-mail: asamin2005@hotmail.com

ABSTRACT **:** ***A reversed-phase high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous quantification of empagliflozin and metformin in their combined dosage form. The analysis was performed on an Agilent 1200 system equipped with a diode array detector (DAD) using a Hypersil BDS C18 column (4.6 mm x 15 cm, 5 µm). The mobile phase consisted of an isocratic 70:30 (v/v) mixture of phosphate buffer (prepared by dissolving 6.8 g potassium dihydrogen orthophosphate in 1 L water and filtered with a 0.45 µm nylon filter) and acetonitrile. The flow rate was set to 1.5 mL/min, and the injection volume for both empagliflozin and metformin was 10 µL. Detection was performed at 255 nm using the DAD. The method demonstrated linearity over concentration ranges of 3.125-9.38 µg/mL for empagliflozin (retention time: 6.4 min) and 250-750 µg/mL for metformin (retention time: 2.6 min) with correlation coefficients exceeding 0.999. The relative standard deviation (RSD) was less than 2%. These results demonstrate that the method is rapid, accurate, precise, and sensitive, making it suitable for routine quality control of tablets containing both drugs in pharmaceutical settings.***

KEYWORDS: *Method validation Empagliflozin, Metformin, HPLC.*

Date of Submission: 01-06-2024 Date of acceptance: 02-02-2025

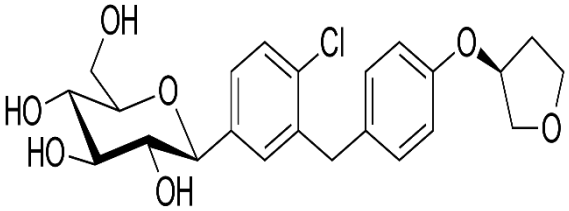
I. INTRODUCTION

Empagliflozin, a sodium-glucose cotransporter-2 (SGLT-2) inhibitor (molecular name: (2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy]phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol, Figure 1A), is used in combination with diet and exercise to improve glycemic control in adults with type 2 diabetes. SGLT-2 cotransporters are responsible for reabsorbing glucose from the kidney's glomerular filtrate. Inhibiting SGLT-2 with empagliflozin results in a diuretic effect, reducing renal glucose reabsorption and lowering the renal glucose threshold, leading to increased glucose excretion [1, 2]. This mechanism helps to lower hyperglycemia and can contribute to weight loss and blood pressure reduction.

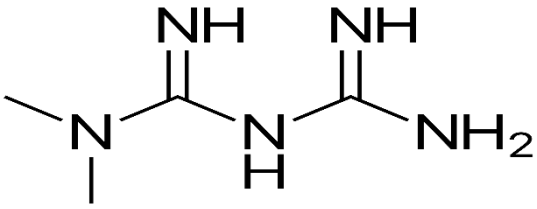
Metformin (molecular name: 1-carbamimidamido-N,N-dimethylmethanimidamide, Figure 1B), on the other hand, works by reducing gluconeogenesis in the liver while increasing glucose uptake by muscle and fat cells [3, 4]. Metformin hydrochloride is the preferred treatment for obese patients with type 2 diabetes (NIDDM) and can also lead to modest weight loss.

While some analytical methods exist for analyzing these medications individually, such as UV spectrophotometry [5], ultra-pressure liquid chromatography (UPLC) [6], and HPLC [7-12], a review of the literature revealed limited published methods for their simultaneous analysis in combination. This newly developed and validated HPLC method addresses this gap by offering a reliable and efficient approach for the quality control of tablets containing both empagliflozin and metformin. The validation process adheres to the International Council for Harmonisation (ICH) guidelines [13].

This combined approach provides valuable context for the method development. It highlights the rationale behind analyzing these drugs together and emphasizes the novelty of the proposed method for this specific application.



(A)



(B)

Fig. 1 Chemical structures of Empagliflozin (A) and Metformin (B)

II. MATERIALS AND METHODS

Chemicals and reagents

The analysis utilized empagliflozin and metformin obtained from Hetero Drugs (Hyderabad, India). Potassium dihydrogen orthophosphate was sourced from EL Nasr Pharmaceuticals Chemicals. Water for chromatography was of HPLC grade from Merck. HPLC-grade acetonitrile was obtained from Romil. A 0.45 µm nylon filter (ChromTech) was used for mobile phase filtration.

Equipment and chromatographic conditions

The separation and quantification of empagliflozin and metformin were achieved using a Hypersil BDS C18 column (4.6 mm x 15 cm, 5 µm particle size) on an Agilent 1200 HPLC system equipped with a diode array detector (DAD). An isocratic mobile phase was employed, consisting of a 70:30 (v/v) mixture of phosphate buffer (prepared by dissolving 6.8 g potassium dihydrogen orthophosphate in 1 L water) and acetonitrile. The mobile phase flowed through the system at a rate of 1.5 mL/min at room temperature. Samples (10 µL injection volume) were monitored at a detection wavelength of 255 nm with a maximum run time of 8.0 minutes. Table 1 summarizes the optimized chromatographic conditions for this analysis.

Table 1 Optimized chromatographic conditions

Parameters	**Conditions**
Detection wavelength (nm)	255nm
Injection volume (µL)	10
Stationary phase	Hypersil BDS C18, 150 x 4.6-mm, 5 µm
Column temperature (^{0}C)	Ambient (25 ^{0}C)
Flow rate (mL min^{-1})	1.5
Mobile phase	Phosphate Buffer : Acetonitrile (70:30 v/v)
Run time (min)	8.0

Standard Solution Preparation

Empagliflozin: Accurately weigh 62.5 mg of empagliflozin standard into a 200 mL volumetric flask. Add 100 mL of 50% methanol and sonicate for 5 minutes. Cool the solution and dilute to volume with 50% methanol to obtain stock solution (S1).

Metformin Hydrochloride: Accurately weigh 125 mg of metformin hydrochloride standard into a 50 mL volumetric flask. Add 25 mL of 50% methanol and sonicate for 5 minutes. Cool the solution and dilute to volume with 50% methanol to obtain stock solution (S2).

Working Solution: Combine 1 mL of S1 and 10 mL of S2 in a 50 mL volumetric flask. Dilute to volume with the mobile phase to prepare the working solution for analysis.

Linearity

The linearity of the analytical method was evaluated to ensure its ability to produce concentration-dependent responses within a defined range. Linearity was assessed using a single-point calibration approach, where the working solution was diluted to create a minimum of five distinct concentrations (50%, 80%, 100%, 120%, and 150% of the target concentration). Each concentration level was injected in triplicate to account for potential variability. The linearity of the response was determined by the correlation coefficient (R) calculated from the peak area responses. An acceptable R value, typically exceeding 0.99, indicates a strong linear relationship between the concentration of the analyte and the detector response. This demonstrates that the method can be reliably used to quantify empagliflozin and metformin within the tested concentration range.

Accuracy

The accuracy of the method was assessed by spiking a standard solution of empagliflozin and metformin hydrochloride into Empagliflozin/Metformin combination tablets (F.C. Tablets). This approach involves adding a known amount of the standard solution to the sample matrix (crushed tablets) at a concentration level determined to be within the desired working range. The spiked samples were then analyzed alongside unspiked samples (control) to determine the recovery of the analytes. Measurements were performed at this target concentration and at reasonable concentration levels above and below it to assess accuracy across a relevant range. The recovery of empagliflozin and metformin, expressed as a percentage of the spiked amount, was calculated to evaluate the accuracy of the analytical method.

Specificity

The selectivity and specificity of the method were evaluated to ensure that the target analytes (empagliflozin and metformin) could be accurately measured in the presence of potential interferences from the sample matrix (tablet excipients) or other drugs.

Selectivity: The chromatographic separation was assessed by injecting a placebo blank, a standard solution, and a sample spiked with the standard solution. The resolution between the peaks of empagliflozin and metformin, as well as any potential interfering peaks from the placebo or sample matrix, was determined to be at least 2. This indicates that the method can selectively isolate and quantify the target analytes.

Specificity: Peak purity of empagliflozin and metformin was confirmed using the photodiode array detector (PDA). The PDA allows for the acquisition of UV spectra at different points across the chromatographic peak. By comparing the spectra at the peak apex with those at the leading and trailing edges, any potential co-eluting components can be identified. This ensures that the measured peak area corresponds solely to the target analyte, demonstrating the method's specificity.

System suitability

The system suitability of the HPLC method was evaluated by injecting six replicate aliquots of the standard solution at 100% of the test concentration. This ensures the consistent performance of the chromatographic system throughout the analysis. The key parameters assessed were:

Tailing factor: This parameter reflects the symmetry of the chromatographic peak. Ideally, a tailing factor close to 1 indicates a symmetrical peak. The system suitability data, summarized in Table 2, demonstrates acceptable tailing factors for both empagliflozin (6.37) and metformin (2.66).

Retention time: This parameter refers to the time it takes for a specific analyte to elute from the column. Consistent retention times ensure reliable peak identification and quantification. Table 2 shows the average retention times for empagliflozin (2.10 min) and metformin (0.88 min), indicating good separation between the two analytes.

These results confirm that the HPLC system is suitable for the intended analysis, allowing for the simultaneous measurement of empagliflozin and metformin in sample drugs with distinct and well-defined chromatographic peaks. The complete system suitability data is presented in Table 2.

Table 2 System suitability parameters for Empagliflozin and Metformin

S. No.	Parameters	Empagliflozin	Metformin
1	Retention time	2.10	0.88
2	Tailing factor	6.37	2.66

Ruggedness

Ruggedness is a measure of the analytical method's ability to produce consistent results under slight variations in operational conditions. This ensures the method's reliability in routine use. The following factors were evaluated to assess ruggedness:

- **Day-to-Day**: The intra-day precision was evaluated by analyzing five replicates of the same sample (100% of the test concentration) on the same day. This assesses the method's consistency within a single day of analysis. On a separate day, a second set of five replicates was prepared from the same sample and analyzed by the same analyst. This evaluates the method's reproducibility across different sample preparations on different days.
- **Analyst-to-Analyst**: The inter-analyst precision was assessed by having two different analysts analyze five replicates of the same sample preparation. This evaluates the method's ability to produce consistent results regardless of the analyst performing the analysis.
- **Column-to-Column**: The impact of column variation was assessed by performing the analysis on two different HPLC columns with the same packing material and length but different batch numbers. This evaluates the method's robustness to slight variations in column performance.

Robustness

Robustness refers to the analytical method's capacity to remain unaffected by slight but deliberate variations in its operating parameters. This ensures the method's reliability under real-world laboratory conditions, where minor deviations from the ideal protocol might occur. In the context of HPLC, such variations could include:

Sonication duration: Deviations from the prescribed sonication time during sample preparation.

Aliquot stability: Potential degradation of the analyte solution over time after preparation.

By evaluating the method's response to these controlled variations, its robustness can be assessed. This demonstrates the method's ability to produce consistent results even when minor deviations from the standard operating procedure occur.

Limit of detection (LOD) and limit of quantitation (LOQ)

The signal-to-noise (S/N) ratio approach was employed to determine the LOD and LOQ for empagliflozin and metformin in this HPLC method. A series of standard solutions were prepared at different concentration levels to investigate these limits. Each solution was analyzed according to the established protocol, and the S/N ratio was determined for multiple injections at each concentration. The mean S/N ratio for each concentration level was then calculated.

Limit of Quantification (LOQ): The LOQ is defined as the lowest concentration level at which the analyte can be measured with acceptable precision and accuracy. In this study, the LOQ was established as the concentration that yields an S/N ratio of 10:1. This ensures that the analyte can be consistently detected and quantified with a high degree of confidence.

Limit of Detection (LOD): The LOD is defined as the lowest concentration level at which the presence of the analyte can be reliably distinguished from background noise. In this experiment, the LOD was determined as the concentration that produces an S/N ratio of 3:1. This indicates that the analyte can be confidently detected above the background level.

By employing the S/N ratio approach and establishing these limits, the sensitivity of the HPLC method for the analysis of empagliflozin and metformin was established. This ensures that the method can detect and quantify these drugs at relevant concentration levels in pharmaceutical samples.

III. RESULTS AND DISCUSSION

This study describes a novel and efficient HPLC method for the simultaneous determination of empagliflozin and metformin in their combined dosage form. The proposed method offers several advantages over existing techniques, including:

Reduced complexity: It requires fewer reagents and solvents, making it simpler and less time-consuming to implement.

Suitability for quality control: These characteristics make it a suitable approach for routine quality control applications in the pharmaceutical industry.

The method was thoroughly validated according to ICH guidelines [14] to ensure its reliability and performance. Validation parameters including linearity, specificity, accuracy, robustness, precision, limit of detection (LOD), and limit of quantification (LOQ) were all evaluated and demonstrated satisfactory results. As shown in Figure 2, the chromatogram confirms distinct resolution between empagliflozin (retention time: 6.4 min) and metformin (retention time: 2.6 min). This successful validation establishes the method's suitability for the accurate and reliable quantification of both drugs in pharmaceutical tablets.

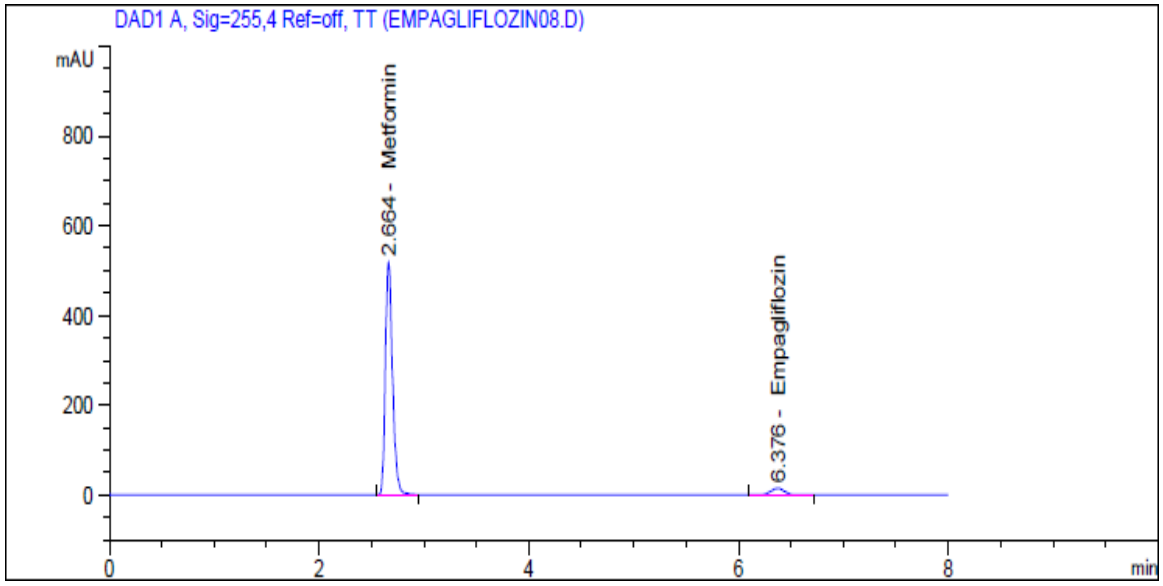


Fig 2. HPLC chromatogram for Empagliflozin and Metformin.

The linearity of the analytical method was evaluated to ensure its ability to produce concentration-dependent responses within a defined range. Linear calibration curves were obtained for both empagliflozin and metformin. The empagliflozin calibration range was from 3.125 to 9.38 µg mL-1 (concentrations used: 3.125, 5.0, 6.25, 7.5, and 9.38 µg mL-1), as shown in Figure 3. Similarly, the metformin calibration range was from 250 to 750 µg mL-1 (concentrations used: 250, 400, 500, 600, and 750 µg mL-1), as depicted in Figure 4 and tabulated in Table 4. Strong linear relationships were observed for both analytes, with correlation coefficients exceeding 0.99. This indicates that the method can be reliably used to quantify empagliflozin and metformin within the tested concentration ranges.

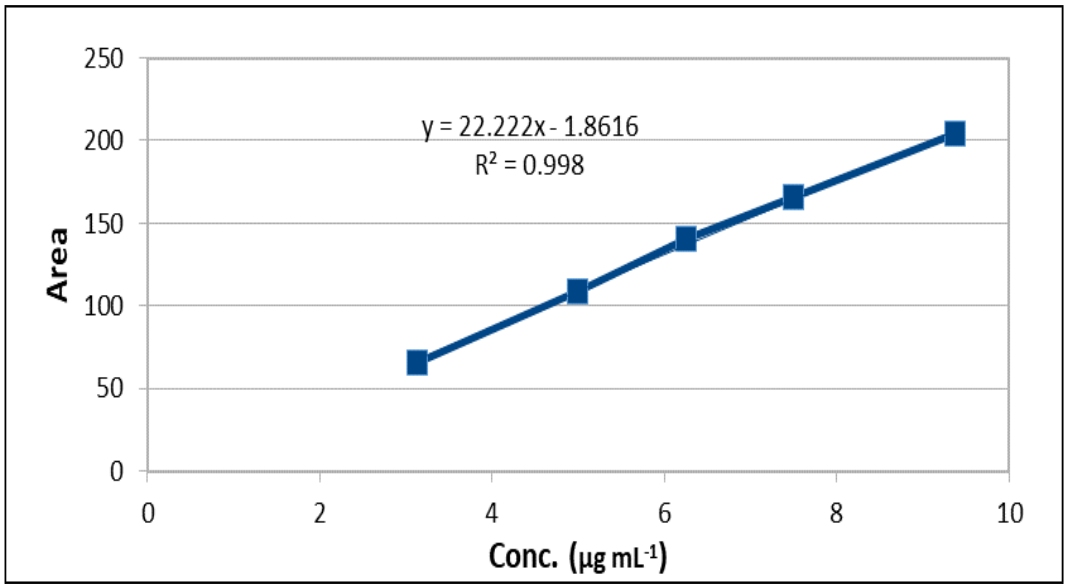


Fig. 3 Calibration curve of Empagliflozin.

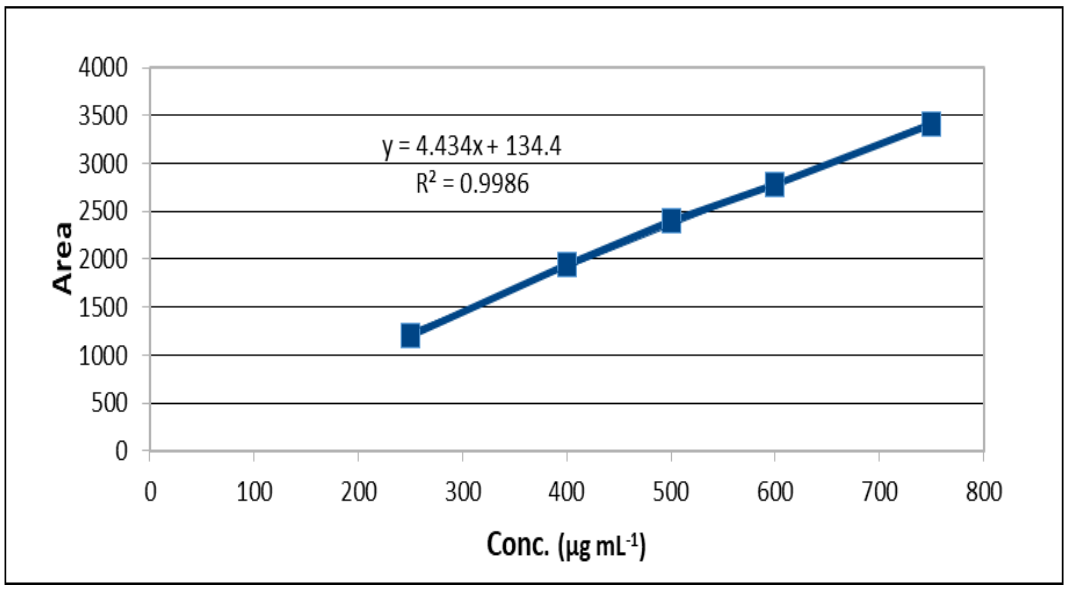


Fig. 4 Calibration curve of Metformin.

Table 3 Statistical data of calibration curves of Empagliflozin

Working Conc. (%)	Working Conc. (µg/ml)	Observed peak areas		
		Mean	SD	RSD
50%	**3.125**	65.62	0.82	1.25
80%	**5.00**	109.19	0.81	0.74
100%	**6.25**	140.30	0.83	0.59
120%	**7.50**	166.02	0.80	0.48
150%	**9.38**	204.00	1.15	0.56
Slope	**22.222**			
Intercept	**-1.8616**			
R 2	**0.998**			

Table 4 Statistical data of calibration curves of Metformin

Working Conc. (%)	Working Conc. (µg/ml)	Observed peak areas		
		Mean	SD	RSD
50%	**250.00**	1204.00	14.92	1.24
80%	**400.00**	1950.17	14.85	0.76
100%	**500.00**	2405.06	4.92	0.20
120%	**600.00**	2773.49	5.46	0.20
150%	**750.00**	3405.01	39.93	1.17
Slope	**4.434**			
Intercept	**134.4**			
R 2	**0.999**			

Linearity is a critical parameter in analytical methods, ensuring the relationship between the detector response (peak area) and the analyte concentration is proportional within a defined range. To establish linearity for empagliflozin and metformin, calibration curves were constructed. Each concentration level was injected in triplicate to account for potential variability. Peak areas were then plotted against the corresponding concentrations. The resulting data were subjected to linear regression analysis, yielding the following linear regression equations.

Y Empagliflozin = 22.222x -1.8616, r2 = 0.998

Y Metformin = 4.434x +134.4, r2 = 0.999

In these equations, Y represents the peak area, x represents the concentration of the analyte (µg/mL), and r^2 represents the correlation coefficient. The high correlation coefficients ($r^2 > 0.99$) indicate strong linear relationships between the peak area and concentration for both empagliflozin and metformin within the tested range. This confirms the method's linearity and its capacity to produce reliable quantitative results across the employed concentration levels.

The signal-to-noise (S/N) ratio approach was employed to determine the LOD and LOQ for empagliflozin and metformin in this HPLC method. Serial dilutions of the standard solutions were prepared to create a range of concentrations for analysis. The LOD was established as the concentration level that yields an S/N ratio of 3:1. This indicates that the presence of the analyte can be reliably distinguished from background noise at this concentration. In this study, the LOD for empagliflozin and metformin were determined to be 0.352 µg/mL and 36.80 µg/mL, respectively.

The LOQ, on the other hand, is defined as the lowest concentration level at which the analyte can be measured with acceptable precision and accuracy. In this context, the LOQ was set at a concentration that produces an S/N ratio of 10:1. This ensures a high degree of confidence in both the detection and quantification of the analyte. The LOQ values for empagliflozin and metformin were found to be 1.055 µg/mL and 110.401 µg/mL, respectively.

These limits demonstrate the sensitivity of the HPLC method for analyzing empagliflozin and metformin at relevant concentrations in pharmaceutical samples.

The accuracy of the method was assessed by spiking a standard solution of empagliflozin and metformin hydrochloride into pre-analyzed Empagliflozin/Metformin combination tablets (F.C. Tablets). This approach involves adding a known amount of the standard solution to the sample matrix at three distinct concentration levels. The spiked samples were then analyzed alongside unspiked samples (control) to determine the recovery of the analytes. The percentage recovery, which is the amount of the analyte recovered from the sample compared to the amount spiked, was calculated for each concentration level.

According to the International Council for Harmonisation (ICH) guidelines [14], the acceptable range for percentage recovery in such studies is typically between 98% and 102%. As shown in Tables 5 and 6, the percentage recovery values for both empagliflozin and metformin fell within this recommended range. This confirms that the method produces accurate results for the quantification of these drugs in the target sample matrix.

Table 5 Accuracy and recovery results for determinations of Empagliflozin

Level (%)	Actual µg/ml	Theoretical µg/ml	Recovery(%) (n=3)
80	4.98	5.03	99.06
100	6.24	6.29	99.16
120	7.46	7.55	98.90
Average Recovery			**99.04**
SD			**0.130**
% RSD			**0.131**

Table 6 Accuracy and recovery results for determinations of Metformin

Level (%)	Actual µg/ml	Theoretical µg/ml	Recovery(%) (n=3)
80	400.35	402.46	99.47
100	501.72	503.08	99.73
120	602.25	603.69	99.76
Average Recovery			**99.65**
SD			**0.157**
% RSD			**0.157**

Specificity is a crucial parameter in analytical methods, ensuring that the target analytes (empagliflozin and metformin) can be accurately measured in the presence of potential interferences from the sample matrix (tablet excipients) or other drugs.

To evaluate the method's specificity, the following approach was employed: A blank placebo tablet solution was injected to assess any background interferences at the detection wavelength (255 nm). A standard solution containing only empagliflozin and metformin was then injected to confirm the presence of well-defined peaks for each analyte. Finally, an actual sample solution prepared from Empagliflozin/Metformin tablets was injected to determine if any matrix components or degradation products co-eluted with the target analytes.

By comparing the chromatograms of these injections, the method's ability to separate and quantify empagliflozin and metformin in the presence of potential interferences was evaluated. Since the proposed method achieved good separation with no interfering peaks observed in the sample solution, it demonstrates adequate specificity for the quantification of the active ingredients in the tablet formulation

IV. Conclusion

This study describes a novel and efficient reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of empagliflozin and metformin in their combined dosage form. The proposed method offers significant advantages over existing techniques, including:

- Simplicity: Fewer reagents and solvents are required, making it less complex and faster to implement.
- Sensitivity: The method demonstrates excellent sensitivity, with low limits of detection and quantification for both analytes.
- Accuracy and Specificity: Validation according to ICH guidelines confirmed the method's accuracy, specificity, and ability to distinguish the target drugs from potential interferences.
- Robustness: The method was found to be robust to minor variations in operating parameters, ensuring reliable performance in routine use.

These features make the proposed HPLC method a valuable tool for quality control applications in the pharmaceutical industry. It provides a reliable and efficient approach for the simultaneous quantification of empagliflozin and metformin in combined tablet formulations.

V. References

1. National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 11949646, Empagliflozin.
2. J Endocrinol Metab. (2024). Pharmacological Treatment of Diabetes Mellitus: An Overview of New Sodium-Glucose Cotransporter 2 Inhibitors for the Treatment of Diabetes Mellitus, 14(3):89-102
3. Polianskyte-Prause, Z., Tolvanen, T. A., Lindfors, S., Dumont, V., Van, M., Wang, H., Dash, S. N., Berg, M., Naams, J. B., Hautala, L. C., Nisen, H., Mirtti, T., Groop, P. H., Wähälä, K., Tienari, J., & Lehtonen, S. (2019). Metformin increases glucose uptake and acts renoprotectively by reducing SHIP2 activity, 33(2), 2858–2869.
4. Hunter, R. W., Hughey, C. C., Lantier, L., Sundelin, E. I., Peggie, M., Zeqiraj, E., Sicheri, F., Jessen, N., Wasserman, D. H., & Sakamoto, K. (2018). Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase, 24(9), 1395–1406.
5. Lakshmi, K. K., Hejmady, S., Shridula, S., Alexander, A., Agrawal, M., Singhvi, G., & Dubey, S. K. (2021). Analytical quality by design for spectrophotometric method development. *Handbook of Analytical Quality by Design* (pp. 15-44). Academic Press.
6. Vankalapati, K. R., Alegete, P., & Boodida, S. (2021). Stability-indicating ultra-performance liquid chromatography method development and validation for simultaneous estimation of metformin, linagliptin, and empagliflozin in bulk and pharmaceutical dosage form. Biomedical chromatography: BMC, 35(4), e5019.
7. Akula, R., et al. (2013). A validated RP-HPLC method for the simultaneous estimation of metformin hydrochloride and Gliclazide.
8. Bhoomaiah, B., & Shree, R. (2014). Development and validation of an RP-HPLC method for the simultaneous determination of metformin and miglitol.
9. Prathap, S., et al. (2014). Development and validation of simultaneous estimation of vildagliptin and metformin hydrochloride by RP-HPLC.
10. Shyamala, K., et al. (2016). A validated stability-indicating RP-HPLC method for the determination of empagliflozin.
11. Padmaja, K., & Veerabhadram, G. (2015). Development and validation of an RP-HPLC method for empagliflozin estimation.
12. Ali, M., et al. (2014). Simultaneous determination of metformin and pioglitazone by HPLC.
13. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [ICH]. (2005). Validation of Analytical Procedures: Text and Methodology (ICH Q2(R1))