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A Novel Validated High Performance Thin Layer Chromatographic Method for the Simultaneous Quantification of Metformin Hydrochloride and Empagliflozin in Fixed-Dose Combination

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ABSTRACT

A precise and accurate method was developed for the simultaneous determination of Metformin hydrochloride and Empagliflozin in a fixed-dose combination using a CAMAG TLC system equipped with a CAMAG Linomat 5 applicator. Chromatographic separations were performed on silica gel plates as the stationary phase, employing a mobile phase composed of Butanol: Glacial acetic acid: Triethanolamine in a ratio of 6:3.5:0.5 v/v/v. The obtained Rf values were 0.608 for Metformin Hydrochloride and 0.717 for Empagliflozin. This method underwent validation for linearity, accuracy, and precision, with linear ranges established as 40 - 200 µg/band for Metformin Hydrochloride and 1 - 5 µg/band for Empagliflozin.

1 INTRODUCTION

Elevated blood glucose, often known as blood sugar, is a defining feature of diabetes, a chronic metabolic disease that eventually causes major harm to the heart, blood vessels, eyes, kidneys, and nerves. Insufficient or resistant insulin synthesis in the body is the primary cause of the most common kind of diabetes, type 2, which often affects adults. Over the last thirty years, type 2 diabetes has become far more common in all nations, regardless of economic status.[1] Dietary and exercise recommendations are typically the first steps in managing and treating diabetes.[2] Anti-diabetic medicines are a diverse class of medications with different pharmacological and molecular profiles.[3] Biguanides, or Metformin, are first-line conventional treatments for type 2 diabetes. Inhibitors of the sodium glucose cotransporter-2 (SGLT2), which is in charge of the kidney's reabsorption of glucose, are a relatively recent class of antidiabetic drugs. Glucosuria is brought on by inhibition of this transporter, which also lowers hyperglycemia in diabetes patients.[2]

Metformin, an anti-hyperglycemic agent, works by reducing hepatic glucose production, indicating that its primary site of action is likely in the liver.[4] Chemically, Metformin is known as 1-carbamimidamido-N,N dimethylmethanimidamide. To enhance solubility, Metformin is commonly formulated as Metformin Hydrochloride salt.[5]

Empagliflozin chemically known as 1-chloro-4-(β-D-glucopyranos-1-yl)-2-(4-(tetrahydrofuran-3-yloxybenzyl)benzene), is a competitive inhibitor of sodium-glucose co-transporter-2 (SGLT2) that can be taken orally and has anti-hyperglycemic properties. The way it works is that it blocks SGLT-2, which is mostly found in the kidneys' proximal tubules. Due to this inhibition, there is a decrease in glucose renal reabsorption and an increase in glucose excretion in the urine.[6]

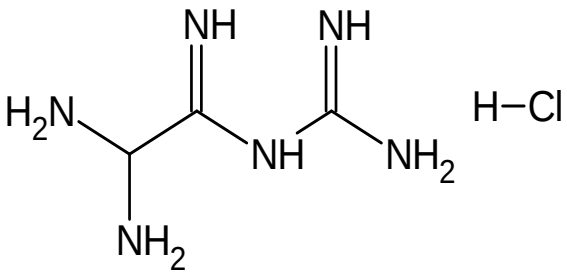

Fig 1 : structure of Metformin HCl

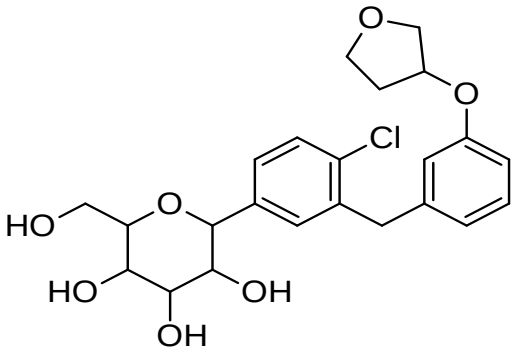

Fig 2structure of Empagliflozin

Better blood sugar management is achieved when Metformin Hydrochloride and Empagliflozin are combined because their modes of action complement one another. When either Metformin or Empagliflozin alone is insufficient to maintain optimal glycemic control, this combination treatment is frequently given.[7]

An extensive review of the literature revealed that methodologies for the examination of Empagliflozin and Metformin HCl in bulk or in its commercial dose form included High Performance Liquid Chromatography (HPLC),[8-12] High Performance Thin Layer Chromatography (HPTLC),[4,13] Spectrophotometry,[14-17] and more. It was proposed to create a reliable HPTLC method using more eco-friendly and cheaper solvents because HPTLC is widely used in regular analysis. The current study outlines a high-performance thin-layer chromatographic method for the quantitative measurement of Metformin HCl and Empagliflozin that is straightforward, accurate, fast, selective, and reasonably priced. Validation of the suggested approach complies with ICH Q2 (R1) standards.[18]

2.0 MATERIALS AND METHOD

2.1 Instrument

The HPTLC apparatus is comprised of a 100 µL applicator syringe and a CAMAG Linomat V sample applicator (Hamilton, Bonadauz, Switzerland). Aluminum TLC plates measuring 10 cm×10 cm and precoated with silica gel 60-F_{254} were used for chromatography. The generated chromatogram was densitometrically scanned using the CAMAG TLC scanner 4.

2.2 Reagents and chemicals

Empagliflozin (EMPA) and Metformin HCl (MFN), were purchased from Synokem Pharmaceuticals, Ltd. Jardiance Met, the commercial formulation, was purchased from a local vendor. Merck Life Sciences Pvt, Ltd. provided Butanol, Glacial acetic acid, and Triethanolamine analytical quality.

2.3 Preparation of stock solution of standard drug mixture

Stock standard solution of MFN and EMPA mixture were prepared by dissolving 400 mg of MFN and 10 mg of EMPA in 10 mL of 99.9% absolute ethanol to yield concentrations of 40000 µg/mL and 1000 µg/mL, of MFN and EMPA respectively.

2.4 Preparation of sample solution

To ascertain the amount of each medication in the formulation, the commercial brand Jardiance Met tablets, which contain 500 mg of MFN and 12.5 mg of EMPA, were utilized. Twenty tablets were precisely weighed and finely powdered for this purpose. After precisely weighing an amount of powder equal to 400 mg of MFN and 10 mg of EMPA, it was transferred to a 10 mL stoppered flask and extracted using 5 mL of 99.9% absolute ethanol for 20 minutes at first. Next, ethanol was added to bring the volume up to 10 mL. After that, the mixture was centrifuged for 10 minutes at 800 rpm. For additional examination, the supernatant was gathered. The final mixture contained 1000 µg/mL of EMPA and 40000 µg/mL of MFN.

2.5 Development of solvent system

The polarity of the analyte (MFN and EMPA) and the stationary phase's adsorption characteristics determine which mobile phase is best. The choice of an appropriate solvent system is significantly influenced by the solubility of the medicines. Trial and error methods were used to select the solvent system.

2.6 Optimization of solvent system

Various solvents were tested based on their polarity, and a mixture comprising Butanol, Glacial acetic acid, and Triethanolamine in the ratio of 6:3:5:0.5 (v/v/v) yielded a chromatogram with superior resolution.

2.7 Chromatographic condition

Using silica gel 60 F_{254} aluminum sheets (10 X 10 cm) as the stationary phase and a mobile phase made up of Butanol, Glacial acetic acid, and Triethanolamine (6:3.5:.0.5 v/v/v), the experiment was carried out. Using an automated sample applicator, the CAMAG Linomat V, the solutions were placed to the TLC plate in the shape of bands with a width of 6 mm while being sprayed with nitrogen gas. In a 10 cm×10 cm CAMAG twin trough glass chamber that was saturated with the mobile phase for 15 minutes, ascending development to 70 mm was carried out. The produced TLC plate was allowed to air dry before being scanned using the CAMAG TLC scanner 4 with VISIONCATS software at a wavelength of 200 to 400 nm. At 254 nm, both components respond fairly well while maintaining a 5 x 0.45 slit dimension.

2.8 Preparation of calibration curve

Precisely measure and transfer 400 mg of MFN RS and 10 mg of EMPA RS into a 10 mL standard flask. It was dissolved in an adequate amount of 99.9% absolute ethanol, and the volume was adjusted using ethanol. MFN 40000µg/mL and EMPA 1000µg/mL were the concentrations in the solution.

From this, five distinct bands were spot-tested using 1µL, 2µL, 3µL, 4µL, and 5µL to determine the concentration of 40, 80, 120, 160, and 200 µg/band of MFN and 1, 2, 3, 4, and 5 µg/band of EMPA.

2.9 Analysis of marketed formulation

The tablet dosage form of MFN and EMPA was analysed by spotting 3 µL from the sample solution.

3.0 METHOD VALIDATION

In compliance with ICH criteria, the developed technique was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, and limit of quantitation. [18]

4.0 RESULTS AND DISCUSSION

4.1 Method Development

MFN and EMPA were found to be soluble in ethanol, therefore ethanol was chosen as the solvent. A solvent system comprising Butanol: Glacial acetic acid: Triethanolamine (6:3.5:0.5v/v/v) yielded dense and compact bands with appropriate Rf values. Consequently, this solvent system was selected as the mobile phase for quantifying MFN and EMPA in combined tablet dosage form. The HPTLC method established for this purpose was demonstrated to be simple, precise, and accurate, as evidenced by the Rf values listed in Table 1.

4.2 Determination of Rf value

Table 1: Rf values of the drugs

Drug	Rf values
Metformin Hydrochloride	0.608
Empagliflozin	0.717

4.3 Chromatogram of drugs with Rf value.

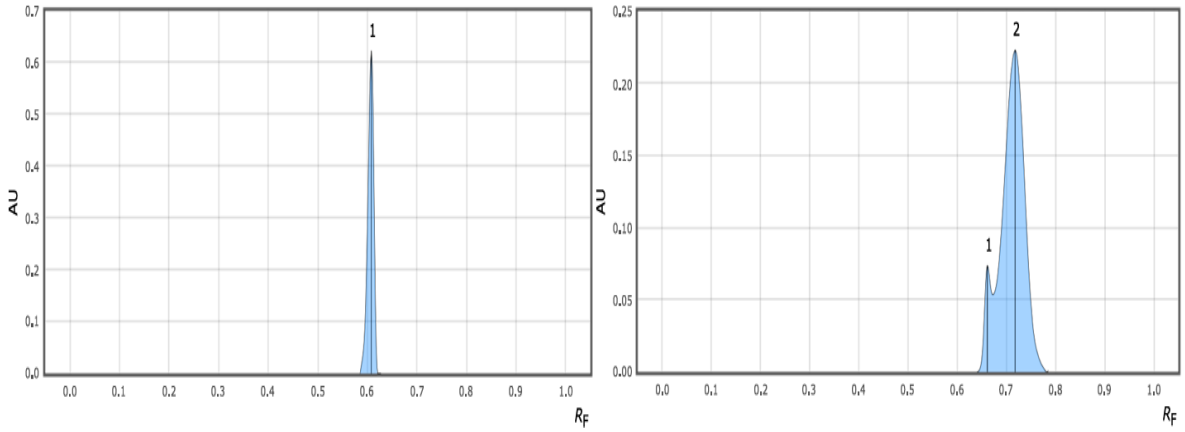

Fig 3a: chromatogram of MFN HCl with Rf value 0.608 Fig 3b: chromatogram of EMPA with Rf value 0.717

4.4 Chromatogram of Standard drug mixture and tablet mixture

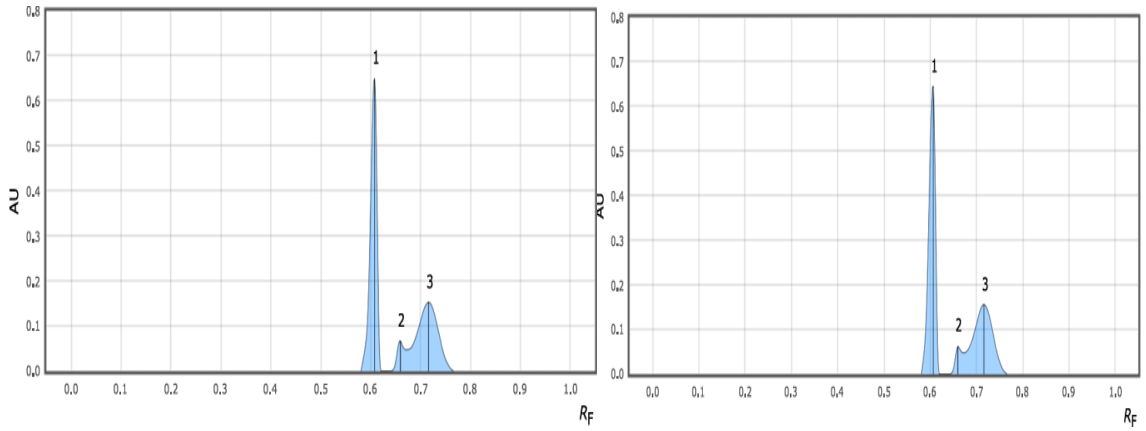

Fig 4a: standard drug mixture (3µL) Fig 4b: Tablet mixture (3µL)

The chromatograms of standard drug mixture and tablet mixture, with a concentration of 3 µg/band for Empagliflozin and 120 µg/band for Metformin hydrochloride, are shown in Figures 4a and 4b.

4.5 Drug content per tablet determined by the proposed method

Table 2: Drug content per tablet

Drug	Area wise (mg)	Area wise (%w/w)
Metformin Hydrochloride	478.60	95.72
Empagliflozin	11.87	94.96

Table 2 shows the drug content and % label claim determined per tablet of marketed formulation of Jardiance Met.

5.0 Validation of proposed method

5.1 Accuracy

By using the standard addition method to undertake recovery trials, the created method's accuracy was verified. In order to compute the drug concentrations, standard drug solution was added to the pre-analysed sample solution at three distinct levels: 80, 100, and 120%.

Table 3: Recovery Study –Statistical validation data of Metformin Hydrochloride and Empagliflozin

Level of % recovery	**Mean % recovery**		**Standard deviation (SD)**		**Relative standard deviation % RSD**	
	Area wise		**Area wise**		**Area wise**	
	MFN	**EMPA**	**MFN**	**EMPA**	**MFN**	**EMPA**
80	94.66	93.84	0.110	0.2512	0.116	0.2677
100	94.94	94.68	0.063	0.3161	0.066	0.3339
120	95.55	95.39	0.446	0.1919	0.467	0.2011

The findings of the percentage recovery study were deemed adequate for the recovery of MFN and EMPA at each of the three levels. For MFN and EMPA, the recovery rates were determined to be 94.66% to 95.55% and 93.84% to 95.39%, respectively.

5.2 Precision

Precision was determined at two levels: Repeatability and intermediate precision. The investigation on repeatability utilized a test concentration of 100%. A chromatogram was generated for this solution, revealing 3 µg/band of Empagliflozin and 120 µg/band of Metformin Hydrochloride. The peak region was scanned six times at 254 nm.

The intra-day precision was done by scanning the chromatogram of one concentrations of both drugs three time on three days. A standard mixture containing 1000 µg/mL of Empagliflozin and 40000 µg/mL of Metformin Hydrochloride was prepared. 3 µL of this solution was spotted, resulting in 3 µg/band of Empagliflozin and 120 µg/band of Metformin Hydrochloride, respectively. Chromatograms were developed and scanned at 254 nm. Peak areas for each band were measured three times over three days.

Table 4a: Repeatability study- statistical validation

Method	Drug	Mean	Standard deviation	RSD%
Area wise	**MFN**	99.70	0.6853	0.00687
	EMPA	94.28	0.8515	0.00903

Table 4b: intra- day precision statistical validation

Drug	Method	Mean	SD	RSD
MFN	**Area**	100.02	1.1706	0.011704
EMPA		94.08	0.9573	0.009573

Recurring studies of both standard and sample solutions yielded relative standard deviation (RSD) values less than 2%, demonstrating the method's good precision and repeatability.

5.3 Linearity and range

To assess the linear relationship across the range of analytical procedures, a linearity study was carried out. Linearity was determined by using five different concentrations of each drug. Chromatogram was developed and scanned at 254 nm and peak area were determined.

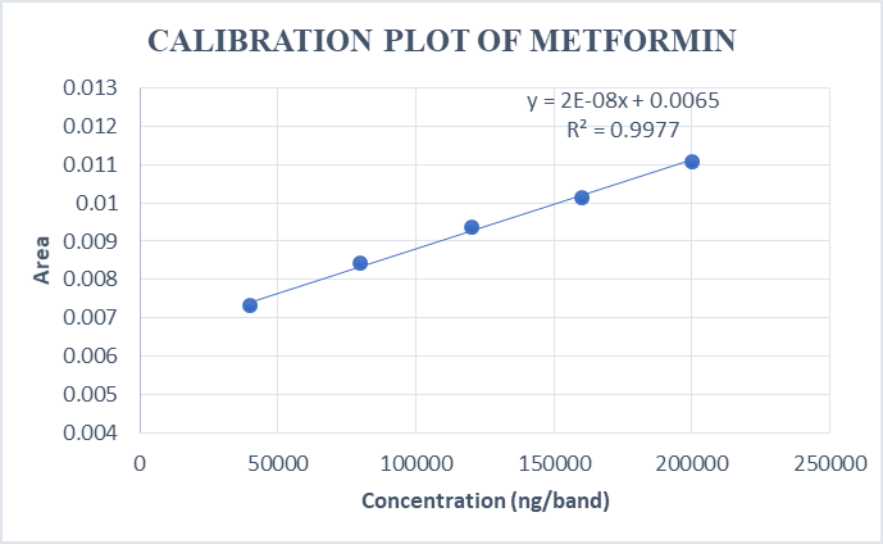

Figure 5a : calibration plot of Metformin Hydrochloride (concentration Vs peak area)

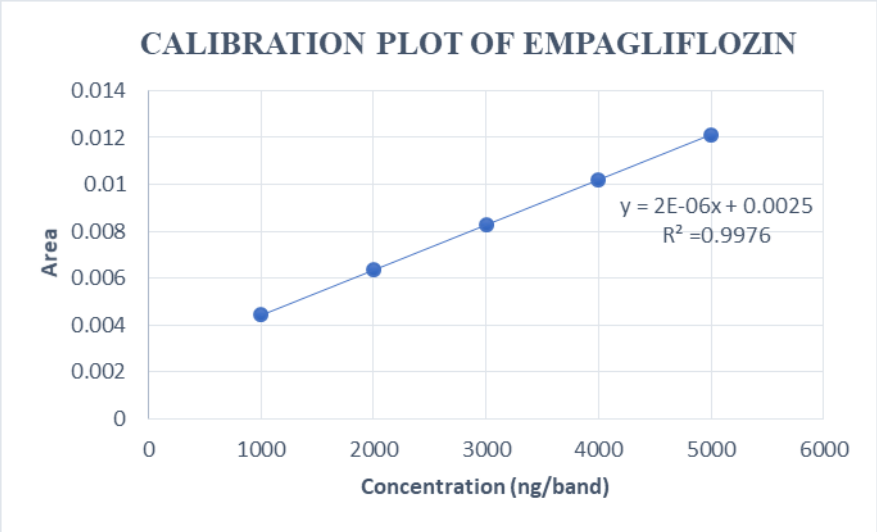

Figure 5b : calibration plot of Empagliflozin (concentration Vs peak area)

The calibration plots for MFN and EMPA, respectively, in the concentration ranges of 40 - 200 µg/band and 1 - 5 µg/band, are shown against area in Figures 5a and 5b. Within the previously mentioned concentration range, linear results were observed. For metformin hydrochloride, the correlation coefficient and linear regression equation were determined to be y = 2E-08x + 0.0065 and R^2 = 0.9964, respectively, and for empagliflozin,

y = 2E-06x + 0.0025 and R^2 = 0.9976.

Table 5: Linearity and Range

Method parameters	**Metformin Hydrochloride**	**Empagliflozin**
	Area wise	**Area wise**
Linearity range (µg/spot)	40000-200000	1000-5000
Slope	2E-08	2E-06
Intercept	0.0065	0.0025
R^2 value	0.9977	0.9976

5.4 LOD and LOQ

The LOD and LOQ were estimated from five calibration curves drawn for each drug in their respective linearity range and calculated by the equation.

LOD = 3.3* (σ/S)

Where, σ= Standard deviation of y- intercepts of regression lines S= Slope of Calibration curves

$$\mathbf{LOQ= 10* (\sigma/S)}$$

Where, σ = Standard deviation of y- intercepts of regression lines S= Slope of Calibration curve

Table 6: LOD & LOQ

Drug	Method	LOD (µg/band)	LOQ (µg/band)
MFN	**Area wise**	10063.95	30496.83
EMPA	**Area wise**	208.71	632.45

6.0 CONCLUSION

The established HPTLC method is durable, accurate, specific, and exact. The results of validation studies showed that the suggested approach can accurately estimate both metformin HCl and empagliflozin in bulk and in pharmaceutical formulations without the excipients interfering. Since the approach was created using affordable and readily available solvents for drug analysis, it might be regarded as economical. The ICH guidelines suggestions were followed in the validation of the methods. As a result, they are easily suited for regular quality evaluation of the tablet composition.

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