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RP-HPLC Method For Simultanious Estimation Of Metformin And Invokana In Pharmaceutical Dosage Form

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ABSTRACT

The high-performance liquid chromatographic method of reverse-phase (RP-HPLC) that was developed and validated was simple, precise, potent, and accurate in estimating Metformin and Invokana (Canagliflozin) in combined pharmaceutic dosage forms. The chromatographic separation was performed on Symmetry C18 column with size 4.6 X 150 mm, with 5 mum particle size with the mobile phase consisting methanol and phosphate buffer (PH 3.0) with 70:30 v/v ratio at flow rate of 0.8 mL/min and at 240 nm. Linearity of the method was outstanding in these concentration ranges, i.e., 50-250 g/mL of Metformin and 5-25 g/mL of Invokana, with correlation coefficients (R^2) being 0.999 of each analyte. Metformin, Invokana was retained at the time of 2.403 min and 3.907 min, respectively. Precision studies indicated that Metformin showed percentage recoveries of 99.18 to 99.91 percent and Invokana of 99.38 to 99.53 percent that were above the ICH acceptance criteria. Accuracy was determined by the fact that the %RSD was less than 2teen both repeatability and intermediate precision. The procedure was resistant to small changes in the flow rate and composition of the mobile phase. Limits of detection (LOD) and quantification (LOQ) were found 2.17 g/ml and 6.60 g/ml in the case of Metformin, and 0.0372 g/ml and 0.112 g/ml in the case of Invokana respectively. The validated procedure is definite, sensitive and reliable, and can be used as a normal quality control analysis of Metformin and Invokana even in mixture pharmaceutical composition.

INTRODUCTION

Due to the rising rate of type 2 diabetes mellitus (T2DM) readily observed in several countries, there has been a burgeoning need of effective combination therapies that combine multiple mechanism of glycemic control[1]. Metformin is a biguanide type of antidiabetic compound, which is the first line pharmacotherapy of T2DM because of its capacity to lower hepatic development of glucose and enhance the insulin response of the periphery[2]. Enhancement of urinary glucose excretion by targeting selective sodium-glucose cotransporter-2 (SGLT-2) inhibitor, Invokana

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(Canagliflozin) is largely used in combination with Metformin to have improved glycemic control on patients who do not adequately respond to monotherapy. Combinations of Metformin and Invokana in fixed dose combinations of pharmaceutical formulations have the synergistic value in therapeutic advantages, good patient compliance and clinical outcomes[3]. As the clinical application of this combination is growing, it is important that they have established reliable, simple and validated analytical procedures to estimate them simultaneously in bulk drug and pharmaceutical dose forms wherein quality and compliance are consistent and assured. HPLC (high-performance liquid chromatography) is still one of the most reproducible and sensitive methods of pharmaceutical analysis[4]. Although isolated HPLC methods have been obtained in both Metformin and Invokana, scanty literature is available on validated methods of estimating the same in combination dosage forms in a combined dosage form. Moreover, an affordable, strong and stability-pointing RP-HPLC procedure that can be used in quality control is required[5]. This is why it was the aim of the current research to design and establish a straightforward, as well as accurate, precise, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method of solving the problem of simultaneous determination of Metformin and Invokana in combined pharmaceutical dosage forms[6]. The methodology developed was validated and optimized based-on International Council for Harmonisation (ICH) Q2 (R1) measures that include parameters specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ)[7].

MATERIALS AND METHODS:

Chemicals and Reagents

The standards of working solutions of metformin and Invokana (canagliflozin) were obtained off the shelf (Torrent Pharmaceuticals). Tableted formulations produced containing both active pharmaceutical ingredients (APIs) were purchased to be analyzed. HPLC grade methanol, acetonitrile and water was bought in Merck (India). Orthophosphoric acid, potassium dihydrogen phosphate (KH2PO4) and disodium hydrogen phosphate (K 2 HPO 4) were obtained too as analytical reagent (AR) grade reagents supplied by Merck. They used nylon membrane filters (0.22 m), and Millipore filter papers (0.45 m) as tools of filtration.

Instrumentation and Chromatographic Conditions

A Waters HPLC system (Model 2695), able to operate in chromatographic functions with its autosampler, quaternary pump, and UV detector (Model 2487) was used to run the chromatographic analysis (managed with the help of Empower Software Version- 2). The separation condition was accomplished on a Symmetry c18 column (4.6 150 mm, particle size 5m). The mobile phase consisted of 70:30(v/v) methanol and 0.05 M phosphate buffer (pH 3 0 adjusted with orth phosphoric acid.). The rate was set at 0.8 mL/min with detection of 240 nm. The volume of injection was 20 µL and all chromatographic runs were kept at an ambient room temperature[8].

Preparation of Solutions:

The phosphate buffer solution (pH 3.0) was prepared by dissolving 2.95 g of KH 2 PO 4 and 5.45 g of K 2 HPO 4 in HPLC-grade of water, 1000 mL in pH with orthophosphoric acid, son-purified and disc-filtered using a 0.22 m membrane filter. A new mobile phase was prepared by adding methanol and phosphate buffer in the described proportion to it and defoamed using an ultrasonic bath and then filtered. Metformin and Invokana drug solutions of different strengths were prepared by accurately weighing the drug in individual flask (10 mL volumetric flask) and after adding the diluent (prepared mobile phase) dissolved using the sonicate until drugs are fully dissolved. The same diluent was used to make the volumes up to the mark. Additional working dilute solutions were made by diluting with the diluent as necessary in order to be able to come up with the required assay and validation study concentrations. In sample preparation, tablet powder that is equivalent to 10 [micro]g of Metformin and 1 micro g of Invokana was weighed and delivered into 10 mL volumetric flask then dissolved in 2 mL of diluent and sonicated and diluted to a 10 mL mark. A proper aliquot of this stock was used on further dilution with the view of obtaining the test concentration[9,10].

Method Development and Optimization:

Method development involved systematic trials by varying mobile phase compositions, column types, detection wavelengths, and flow rates to optimize chromatographic separation. Multiple trials were performed using different stationary phases including Symmetry C18, Zodiacsil C18, and Hypersil RPC8, and varying mobile phase ratios comprising methanol, acetonitrile, and phosphate buffer at different pH values. The final optimized chromatographic conditions provided satisfactory resolution, peak shape, and system suitability, suitable for simultaneous estimation of Metformin and Invokana in combined dosage forms[11].

Analytical Method Validation:

This established procedure was confirmed with respect to ICH Q2(R1) specificity, linearity, accuracy, precision, robustness, limit of detection

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(LOD), limit of quantitation (LOQ) and system suitability.3

Specificity was also assessed at injecting blank, standard and sample to prove that the excipients and diluent did not interfere with the retention of time of both analytes[12].

Linearity was determined by elaborating calibration against the concentration ranges of 50 250 ug/mL of Metformin and 5 25 ug/mL of Invokana. In this, five various levels of concentrations were prepared and analyzed and calibration curves were developed by plotting peak area against concentration[13].

Accuracy had been predetermined through recovery experiments conducted at three concentration levels, 50%, 100 and 150 percent of the desired assay concentration. Pre-analyzed samples were pre-spiked with known quantities of the standard solutions and percentage recovery was computed[14].

Precision has been measured with regard to repeatability (intra-day), intermediate precision (inter-day). To determine repeatability, several injections of standard solutions were made by duplicate under the same experimental conditions; and intermediate precision was determined under different days and with different columns of the same specifications[15].

Robustness was studied by them by making small and controlled alterations of chromatographic conditions such as changes in flow rate and composition of the organic solvents of the mobile phase. The impact of these change in system suitability parameters was investigated[16].

LOD and LOQ The values were calculated using standard deviation of response and the slope of the calibration curve as calculated according to the formulae as mentioned in ICH guidelines.

System Suitability was established prior to validation by evaluating parameters such as theoretical plates, peak tailing factor, and retention times for both analytes, ensuring consistent chromatographic performance and reproducibility[17].

RESULTS AND DISCUSSION:

A number of method development experiments were carried out that varied mobile phase composition, wavelength of detection and stationary phase as well as flow rate. Opening runs of various columns and solvent system did not bring the desired resolution. The optimized conditions that were used in the final chromatographic system were a Symmetry C18 column (4.6 x 150 mm, 5 um), a mobile phase; methanol:phosphate buffer (pH 3.0) with ratio of 70:30 v:v, flow rate of 0.8 mL/ min and the UV detection of 240 nm. With these optimized conditions, Metformin and Invokana were eluted at 2.464 min and 3.746 min respectively(Figure 1).

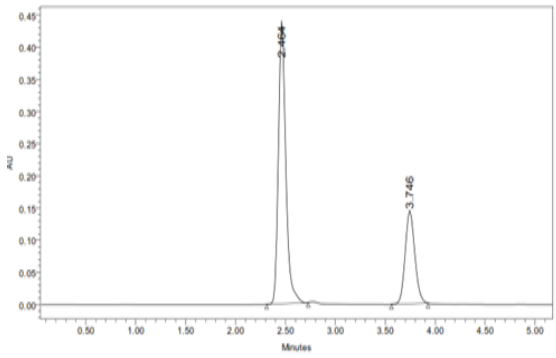

Figure 1. Chromatogram showing Optimized Method Injection

System Suitability:

The system suitability parameters were tested before the analysis to reveal proper performance of the system. Such parameters as theoretical plates, tailing factor, and resolution were of acceptable limits following the ICH guidelines (Table 1).

Table 1. System Suitability Results for Metformin and Invokana

Parameter	Metformin	Invokana
Retention Time (min)	2.403	3.907
Theoretical Plates	2294	4891
Tailing Factor	1.27	1.03
Resolution	—	8.67

Specificity

Specificity was verified through injection of blank, standard and sample solutions. The specificity of the method was found because no interference by excipients or diluent was observed at the retention times of Metformin and Invokana. This was graphically identified in the superimposed chromogram of null, standard and sample injection (Figure 2).

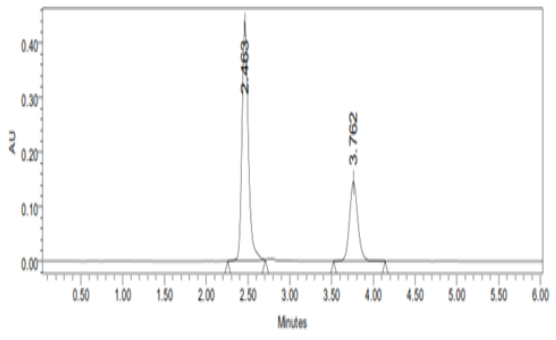

Figure 2. Overlay chromatogram confirming specificity.

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Linearity:
The evaluation of linearity was done on concentrations of Metformin and Invokana of 50-250 ug/mL and 5-25 ug/mL, respectively. Clearly displayed in Figures 3 and 4, the calibration curves were very linear (Correlation coefficients (R^2) = 0.999) and Tables 2 and 3 provide the details of linearity values of each antibiotic.

Table 2. Linearity for Metformin

Concentration (µg/mL)	Peak Area
50	800199
100	1589391
150	2264300
200	3071625
250	3894075
R²	**0.999**

Table 3. Linearity for Invokana

Concentration (µg/mL)	Peak Area
5	339009
10	689527
15	994963
20	1385006
25	1766425
R²	**0.999**

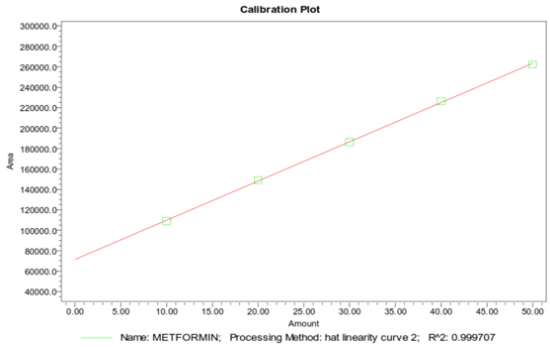

Figure 3. Linearity curve for Metformin.

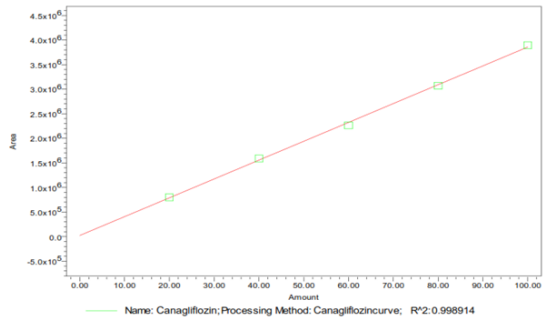

Figure 4. Linearity curve for Invokana.

Accuracy (Recovery Studies)
The accuracy was determined by recovery studies at three percentages that included 50, 100 and 150 percent. Percentage recovery was found between 99.18 to 99.91 and between 99.38 to 99.53 in Metformin and Invokana respectively which is indicative of the truth of the method followed as shown in Table 4.

Table 4. Accuracy (Recovery) Results for Metformin and Invokana

% Level	Metformin Recovery (%)	Invokana Recovery (%)
50%	99.91	99.53
100%	99.18	99.38
150%	99.60	99.52
Mean	**99.56**	**99.47**

Precision:
Repeatability (Intra-day Precision):
Repeatability (intra-day precision) was performed injecting six times the standard solution. The %RSD in Metformin and Invokana was determined as 0.3%, which revealed a high level of precision with respect to methods.

Intermediate Precision (Inter-day Precision):
Intermediate precision (inter-day precision) was determined through injection of replicate solutions and different columns at different days. The values of %RSD were 1.3 and 0.4 supporting the ruggedness of Metformin and Invokana respectively.

Limit of Detection (LOD) and Limit of Quantification (LOQ):
Calculation of LOD and LOQ was done by considering standard deviation of the response and the slope of the calibration curve using the following equation. Table 5 provides the results.

Table 5. LOD and LOQ for Metformin and Invokana

Drug	LOD (µg/mL)	LOQ (µg/mL)
Metformin	2.17	6.60
Invokana	0.0372	0.112

Robustness:
The reliability of the procedure was checked by changing the flow rate (0.6 mL/min, 0.8 mL/min and 1.0 mL/min) and composition of the organic phase (pm 5). All the parameters of system suitability such as plate count, tailing factor were within acceptable limits in all conditions. Table 6 presented the results.

Table 6. Effect of Flow Rate on System Suitability

Flow Rate (mL/min)	Metformin USP Plate Count	Tailing Factor	Invokana USP Plate Count	Tailing Factor
0.6	2590	1.39	5435	1.04
0.8	2294	1.27	4891	1.03
1.0	2146	1.26	4781	1.04

CONCLUSION:
The reverse-phase high-performance liquid chromatographic technique (RP-HPLC) appeared as a new, simple, and also robust technique used in estimating both Metformin and Invokana simultaneously in combined pharmaceutical dosage forms, which was found to be successfully developed and validated. The chromatographic

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separation was obtained with Symmetry C18 column (4.6 x 150 mm, 5 m), a mobile phase consisting of methanol and a phosphate buffer (pH 3.0) of 70:30 v/v mixtures, a flow rate of 0.8 mL/min on a detector at 240 nm. In these optimized conditions Metformin and Invokana were eluted at 2.403 min and 3.907 min respectively. The prepared method showed outstanding system capability but with theoretical plate 2294 and 4891, tailing factor 1.27 and 1.03, and resolution of 8.67. The procedure has been justified as per ICH Q 2 (R1). During the 50-250 and 5-25 mg/ml concentrations Linearity was observed in both Metformin and Invokana and the correlation coefficients (R 2) were observed to be 0.999 in both Metformin and Invokana. Accuracy data indicated that percentage recoveries of Metformin were 99.18-99.91 and percentage recovery of Invokana were 99.38-99.53 with respectively mean recovery of 99.56 and 99.47. The precision was shown by the %RSD of the two drugs 0.3 percent in repeatability, 1.3 percent in Metformin and 0.4 percent in Invokana in intermediate precision. Following the LOD of 2.17 ug/mL and 0.0372 ug/mL of Metformin and Invokana, respectively, the sensitivity of the method was identified. The method was found sensitive with LOQ value of 6.60 ug /mL and 0.112 ug/mL of Metformin and Invokana, respectively. Robustness trials established that slight intentional variations in flow rate and mobile phase composition did not produce any meaningful changes of system suitability parameters.

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CONFLICT OF INTEREST:

Nil.

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REFERENCES:

1. Nareddy PR, Chevela NT. RP-HPLC method development and validation for the simultaneous estimation of Metformin and Canagliflozin in tablet dosage form. *Int J Pharm Sci*. 2015;5(4):1155–1159.
2. Panigrahy UP, Reddy ASK. A novel validated RP-HPLC-DAD method for simultaneous estimation of Metformin hydrochloride and Canagliflozin in bulk and tablet dosage form with forced degradation. *Orient J Chem*. 2015;31(3):1489–1507.
3. Gandla KS, Lalitha R, Mounika C, Soumya B, Sudheer Kumar D. A validated RP-HPLC method for simultaneous determination of Metformin and Canagliflozin in pharmaceutical formulation. *Asian J Pharm Anal*. 2018;8(2):73–77.
4. Patil SD, Kshirsagar SJ. Development and validation of stability-indicating RP-HPLC method for Canagliflozin. *World J Pharm Pharm Sci*. 2015;4(12):631–640.
5. Patel FN, Pandey AK, Patel UR. Analytical method development and validation for determination of Canagliflozin and Metformin in API and synthetic mixture by RP-HPLC. *Discover Chem*. 2024;1:63.
6. Khan N, Ahmed A. Stability-indicating RP-HPLC method for simultaneous determination of Canagliflozin and Metformin in fixed-dose combination. *J Anal Sci Technol*. 2022;14:22.
7. Sharma G, Patil RN, Huidong M. Stability-indicating RP-HPLC method for simultaneous determination of Metformin and Canagliflozin in pharmaceutical formulation. *World J Pharm Pharm Sci*. 2015;4(12):631–640.
8. Ishpreet I, Sharad W, Harsharan PS. A novel validated RP-HPLC-DAD method for simultaneous estimation of Metformin hydrochloride and Canagliflozin in bulk and tablet dosage forms with forced degradation studies. *Orient J Chem*. 2015;31(3):1489–1507.
9. Patel D, Shah U, Patel J, Joshi H, Patel P. A stability-indicating RP-HPLC method validation for simultaneous estimation of Metformin HCl and Canagliflozin in pharmaceutical dosage form. *J Pharm Res Int*. 2021;33(56A):180–192.
10. Reddy MB, Bhutada S. Analytical method development of Metformin, Canagliflozin and Empagliflozin by RP-HPLC. *Asian J Pharm Anal*. 2020;10(3):95–100.
11. Silpa K, Sirisha K, Vivek Sagar P. RP-HPLC method for simultaneous determination of Metformin and Canagliflozin in dosage form. *Int J Novel Res Dev*. 2024;9(7):420–431.
12. Patel BA, Patel FN, Pandey AK, Patel UR. Analytical method development and validation for determination of Canagliflozin and Metformin in API and synthetic mixture by RP-HPLC. *Discover Chem*. 2024;1:63.
13. Khan N, Ahmed A. Stability-indicating RP-HPLC method for simultaneous determination of Canagliflozin and Metformin in fixed-dose combination. *J Anal Sci Technol*. 2022;14:22.
14. Devakumar KPR, Athi Narayanan P; Panigrahy UP, Reddy ASK. A novel validated RP-HPLC-DAD method for the simultaneous estimation of Metformin Hydrochloride and Canagliflozin in bulk and tablet dosage form with forced degradation studies. *Orient J Chem*. 2015;31(3):1489–1507.
15. Khan N, Ahmed A. Development and validation of stability-indicating RP-HPLC method for simultaneous estimation of Canagliflozin and Metformin in fixed-dose combination. *J Pharm Res Int*. 2021;33(56A):180–192.
16. Chowdary VKPR, Prasad SVUM. Development of a new stability indicating RP-HPLC method for simultaneous estimation of Metformin HCl and Canagliflozin and its validation as per ICH guidelines. *Int J Pharm Sci Res*. 2017;8(8):3427–3435.
17. Nachiket SD, Shinde GS, Shinde VB. Simultaneous estimation and validation of Canagliflozin and Metformin HCl in bulk and dosage form by RP-HPLC. *Res J Pharm Technol*. 2019;12(10):4953–4957.