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ANALYSIS

RP-HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN, PIOGLITAZONE, AND METFORMIN IN BULK AND TABLET DOSAGE FORMS

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Abstract: Empagliflozin, pioglitazone, and metformin are antidiabetic drugs used alone or together to treat diabetes. An economical, simple, precise, selective, and stability-indicating RP-HPLC method has been established and validated to evaluate these drugs in bulk and tablet dosage forms. ICH guidelines were followed, where the separation of the drugs using a mobile phase prepared by mixing orthophosphoric acid buffer and acetonitrile (30 : 70 v/v) adjusted to pH 2.7 was followed. An ACE C18 – (250 mm x 4.6 mm), 5 µm column at a flow rate of 0.5 mL/min at 25°C, and detection monitored at 230 nm were used. The R2 was not < 0.9998 in the range of 20-250 ppm. For stability study, drugs were studied using variant stress conditions such as base, acid, neutral, oxidation, and thermal degradation. Results were validated for the limit of detection, the limit of quantification, precision, accuracy, and linearity. The method also proved robust concerning variations in pH of the mobile phase, detector-wavelength, temperature, and mobile phase composition. The retention time of empagliflozin, metformin, and pioglitazone was 3.2 min, 2 min, and 2.6 min, respectively, with a runtime of 7 min. Detector linearity was obtained at 10–100 ppm, with the correlation coefficient for empagliflozin, pioglitazone, and metformin being 0.9994, 0.9993, and 0.9998, respectively. The low relative standard deviation, i.e., <2%, validated results, and high recovery% affirm the suitability of this method for being employed for the routine analysis of bulk and tablets containing these drugs in pharmaceutical formulation.

Keywords: empagliflozin, mioglitazone, metformin, HPLC, method development, tablet, pharmaceutical dosage form

Empagliflozin

Empagliflozin (Figure 1) is one of the sodium-glucose cotransporter 2 (SGLT2) inhibitors, which is a relatively new class of oral medications used for type 2 diabetes mellitus (T2DM). Empagliflozin has a specific non-insulin-dependent mechanism of action that leads to an increase in glucose excretion and lowering blood glucose level, which has the advantage of no hypoglycemia effects due to the non-insulin-dependent character (1). Also, empagliflozin is very slightly soluble in water (pH 1-7.4) (2).

Pioglitazone

Pioglitazone (Figure 1) is one of the thiazolidinediones (TZDs) or glitazones, which is believed to have a role in increasing insulin-sensitizing that is being used in the treatment of type 2 diabetes mellitus (T2DM) (3). Pioglitazone is recommended in patients besides sulfonylurea or metformin, especially when there is limited glycemic control by controlling diet or exercise (4). In water, pioglitazone has a solubility of 46.85 mg/L at 25°C (5).

Metformin

Metformin (Figure 1) belongs to the biguanides group of drugs (6). Metformin does not lead to an increase in plasma insulin concentration and, as a result, does not cause severe hypoglycemia (7-9). Metformin powder is highly soluble in water at room temperature (10, 11).

Empagliflozin, pioglitazone, and metformin combinations

According to the Am erican Diabetes Association/European Association for the Study of Diabetes (ADA/EASD) guidelines, if the patient does

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not endure the metformin, pioglitazone is an alternative or a second-line therapy (12). Research encourages treatment with empagliflozin combined with other oral antidiabetics for reducing HbA1c, blood pressure, and weight of the body with excellent tolerability and safety profile (13).

When combined with metformin or pioglitazone, empagliflozin reduced body weight and HbA1c compared to placebo. Also, it resulted in a relevant clinical decrease in Fasting plasma glucose (FPG), diastolic, and systolic blood pressure compared to placebo (14).

MATERIALS AND METHOD

Materials and reagents

We used the following: orthophosphoric acid: AR grade, acetonitrile: HPLC grade, water: Mille-Q grade, methanol: HPLC grade. These chemicals were a generous gift from RAM Pharma.

The following tablets for empagliflozin, pioglitazone, and metformin were kindly donated by RAM Pharmaceuticals: Empagliflozin (Jardiance) 25 mg – Boehringer Ingelheim, batch number 906303. Pioglitazone HCL (Actos) 30 mg – Hikma Pharma, batch number 956397. Metformin HCL (Glucophage®) 1000 mg – Merk Pharma, batch number 98076.

Instrumentation

The ACE C18– (250 mm x 4.6 mm) made the chromatographic separation and a 5 µm column. The lot number was 942. The analysis was carried out on an HPLC (FINNIGAN SURVEYOR) machine (Thermo Electron Corporation, San Jose, CA, USA), equipped with the detector (UV-VIS plus Detector), the pump (solvent delivery systems pump) (LC Pump plus), and also the autosampler (Autosampler Plus).

Chromatographic analysis

Preparation of mobile phase

The buffer was prepared, and the adjusted pH for the buffer was 2.7 using orthophosphoric acid. The buffer was filtered by a 0.45 µm membrane filter and was also degassed by sonication. A 600 mL of acetonitrile was mixed with 400 mL of buffer solution within a ratio of 40 : 60.

Preparation of buffer

The buffer solution was prepared by adding orthophosphoric acid to 1 L of HPLC- grade water, and the pH was adjusted to 2.7 with orthophosphoric acid.

Preparation of stock and working solutions

Stock solutions of empagliflozin, pioglitazone, and metformin were prepared by weighing and transferring 100 mg (0.1 g of each active ingredient) into a volumetric flask and diluted up to 100 mL with the mobile phase (15).

Preparation of working standard solution:

A 10 mL of both pioglitazone and metformin stock solution was pipetted out to a 100 mL volumetric flask containing 10 mg empagliflozin. It was diluted up to the volume and mixed thoroughly, as detailed in Table 1.

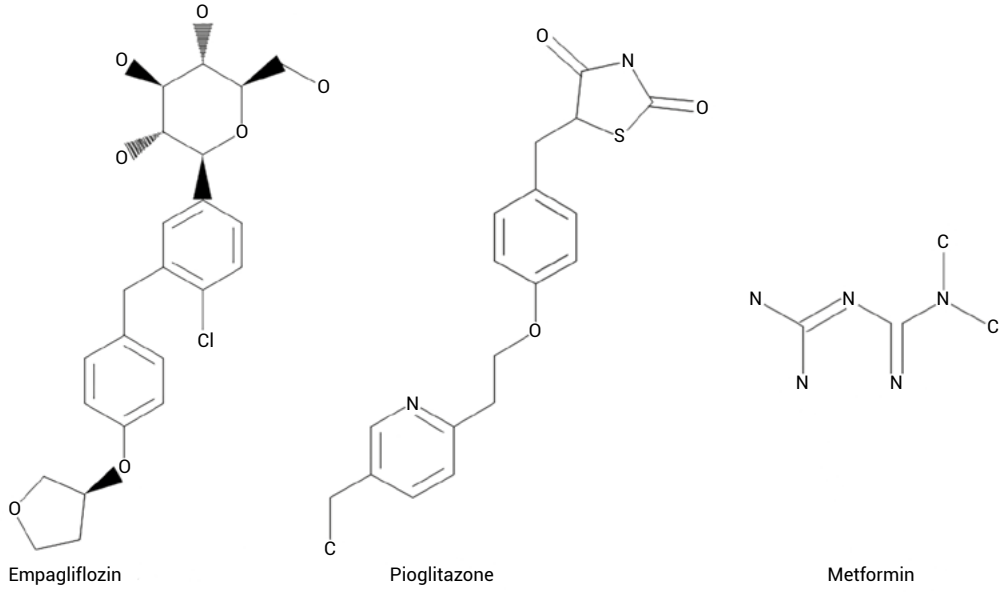

Figure 1. Chemical structures of empagliflozin, pioglitazone, and metformin

Table 1. Working solution preparation.

#	Stock solution $_{(40\ ppm\ MET + 100\ ppm\ PIO + 100\ ppm\ EMP)}$	Diluent (Mobile phase)
1	1 mL	9 mL
2	3 mL	7 mL
3	5 mL	5 mL
4	7 mL	3 mL
5	10 mL	0 mL

Preparation of standard stock solution

A 100 mg of each of the drugs separately was weighed and transferred into a 100 mL clean volumetric flask (1000 ug/mL = 1000 ppm). 50 mL of the mobile phase was added to metformin and empagliflozin separately, and 50 mL of acetonitrile was added to pioglitazone as a diluent. Then each flask was shaken separately to dissolve. It was then diluted up to the mark with the mobile phase and mixed thoroughly (16).

Preparation of sample stock solution

A physical mixture equal to 100 mg of empagliflozin, 100 mg of pioglitazone, and 100 mg of metformin hydrochloride was transferred into a 100 mL volumetric flask. 50 mL diluent was added and then sonicated for 30 minutes with intermitted shaking. It was then cooled down to room temperature and filtered to another 100 mL volumetric flask. Dilution was made up to the mark with the diluent and mixed thoroughly.

After analyzing the mixture, 5 mL of sample stock solution from each of the drugs separately was pipetted out to a 100 mL volumetric flask and diluted up to the mark with diluent and mixed thoroughly.

Wavelength selection

UV-VIS scan applied to each solution of empagliflozin, pioglitazone, and metformin was within the range of 200-400 nm (Figure 2). Maximum absorbance of 220-270 nm was obtained for all

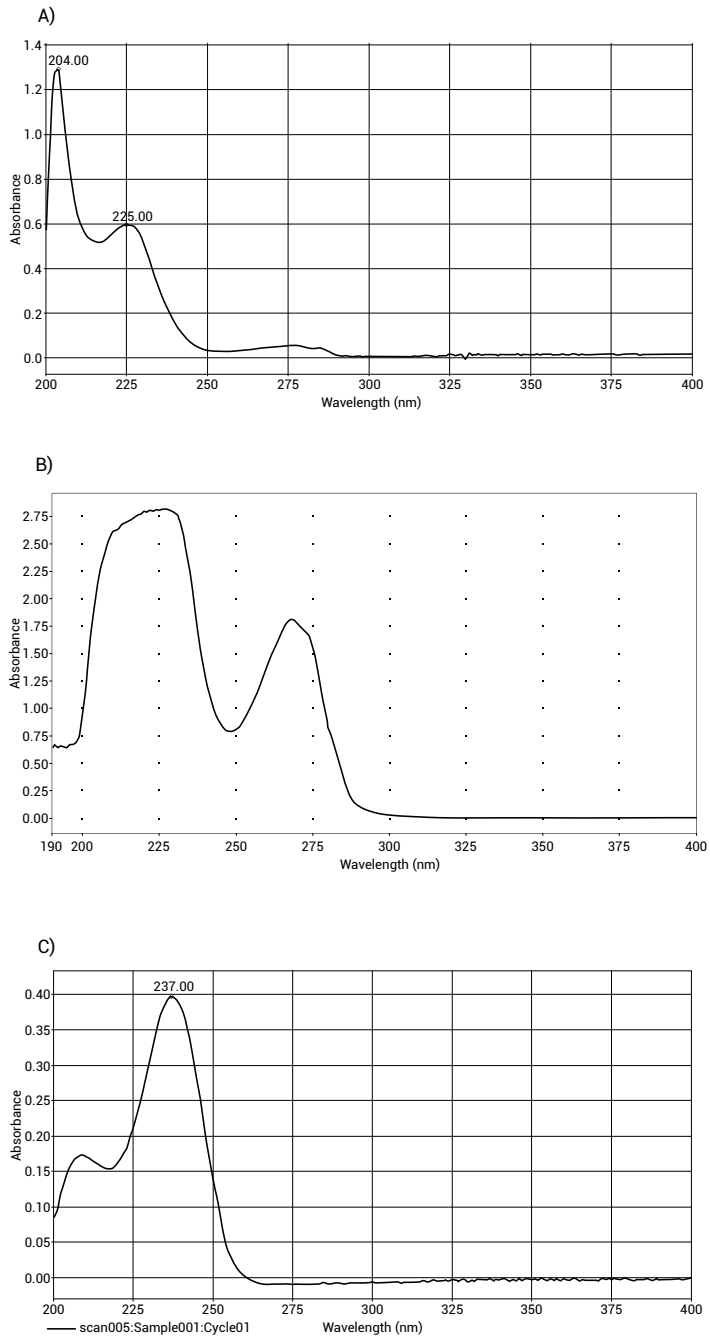

Figure 2. The absorbance profile of A) empagliflozin B) pioglitazone and C) metformin in the range of (200–400 nm).

three drugs. A wavelength of 230 nm was selected for analysis.

Method development

The study's main goal was to develop a better method for empagliflozin, pioglitazone, and metformin. The retention time was short, and the peaks were relatively symmetric.

The ion pair, pH, column, and composition of the mobile phase and the different effects of the chromatographic conditions on the separation of empagliflozin, pioglitazone, and metformin have been studied the determination of the drugs (17-19).

Chromatographic conditions

A Column: ACE C18 – (250 mm x 4.6 mm), 5 µm was used as the stationary phase, and the diluent: was used as a mobile phase: 30 : 70 ratios (buffer: acetonitrile), with a flow rate of 0.5 mL per min, and a column temperature of 25°C, detector wavelength was at 230 nm, injection volume of; 10 µL, in a run time of 7.0 min, the retention time for metformin was determined to be 2 min which shows a good peak with good symmetry, and it was 2.6 min, and 3.2 min for pioglitazone and empagliflozin respectively, according to a similar developed method of analysis (20).

Method validation

The validation of the method was accomplished on different days. Each day, six calibration levels were prepared. The validation parameters did not surpass the limits set by the ICH Guidance (21).

System precision in terms of sample test preparation

It is essential to develop a precise method. A homogenous sample solution of empagliflozin, pioglitazone, and metformin was prepared by weighing and dissolving them in a 50 mL solution where the mobile phase was used as a solvent. It was then injected repeatedly (10 injections). The method was validated in terms of inter-day and intra-day precision (11).

Method precision in terms of test sample preparation

Six sample solution was prepared for the same homogenous sample solution prepared earlier and injected triply for every sample to calculate their assay% and RSD%.

Intermediate precision in terms of test sample preparation

The same six-sample preparation of method precision is injected triple times for every sample, but assay% and RSD% were calculated with different analysts and times.

Linearity for test sample preparation

Five standard sample concentrations (10%, 30%, 50%, 70%, and 100%) of empagliflozin, pioglitazone, and metformin were prepared to evaluate linearity. Triple injections analysis of each sample was done, and a linear analysis was done on average peak areas versus the concentration of level studied. A graph was plotted for the concentration of the corresponding drug versus the area. From the formers, the regression coefficient and regression equation were given (22).

Accuracy of sample test preparation

Three samples at three different concentration levels (70%, 100%, 130%) were prepared by dissolving them in a mobile phase solution and then diluting it in 50 mL mobile phase as in sample solution preparation. At every concentration level, it was injected in a triplicate and compared to the standard sample solution in the same way. The accuracy is presented as the percentage of the analyte recovery, and by measuring peak areas, the recovery was calculated.

Stability of analytical solution used in test preparation

The stability of the standard solution was evaluated at a room temperature of 25ºC. It was freshly prepared after 24 hours. According to (ICH) guidelines, the results attained were compared with a new standard solution of 100% (21, 23).

Robustness of test preparation

The robustness study was carried out to evaluate the power of influence of slight but deliberate variation in the chromatographic conditions. The robustness was checked by making two slight alterations. After every change, the sample solution was injected, and system suitability parameters were noticed (24, 25).

– Robustness regarding wavelength (+5 nm and -5 nm)

As mentioned earlier, the sample solution was also prepared, and the change was only in the wavelength. The wavelength detected earlier was 230 nm which changed to the UV detection reading respectively to 225 nm and 235 nm and triple injections.

– Robustness regarding pH change (+10% and -10%)

The sample solution was also prepared similarly; the change was only in the pH, adjusted by the orthophosphoric acid. Starting with a buffer solution of pH 2.7 and adjusting the final pH to 2.43 and 2.97, respectively.

– Robustness regarding temperature (+3 and -3)

The same preparation method was used to prepare the sample solutions at 25°C, as mentioned

Table 2. The best chromatographic conditions for simultaneous measurement of empagliflozin, pioglitazone, and metformin.

Column	Column: ACE C18 – (250 mm x 4.6 mm), 5 µm
Solvent system (mobile phase)	30 : 70 (Buffer: acetonitrile) adjusted to pH 2.7
Detection	Wavelength: 230 nm
Injection volume	10 µL
Flow rate	0.5 mL per min
Oven temperature	25°C
Runtime	7 mins.
Retention time Empagliflozin Pioglitazone Metformin	 3.2 min 2.6 min 2 min

earlier. The temperature was changed to 28°C and 22°C within the triple injections.

– Robustness regarding organic modified composition (+10% and -10%)

This test depended on changes in the organic phase. 10% acetonitrile was added to prepare the mobile phase (67 : 33). Other mobile phase solutions were produced by increasing the buffer solution (73 : 27) injected within the same sample solution, buffer concentration, and pH value.

Selectivity of test preparation

International and local drugs from the market were used for this test. The drugs were dissolved in a mobile phase solution as a solvent and then injected into the system (test formulation). The raw material was dissolved in the mobile phase with placebo content to inject the sample solution into the system (reference formula) (26).

Recovery (Accuracy)

By adding three known concentrations of the drug to the standard solution, accuracy was evaluated, and the spiked solutions were then analyzed under advanced conditions. Spiked samples were prepared by pre-analyzed sample solutions dissolved with the pure drug at three different concentration levels, each in triplicate, assuring 100% recovery. The recovery extent of analytes must be precise, reproducible, and consistent (27).

Recovery was made by making triplicates from each QC level of empagliflozin, pioglitazone, and metformin. Triplicates from each QC level were prepared in the diluent.

RESULTS AND DISCUSSION

System suitability, accuracy, robustness, linearity, the limit of quantification, limit of detection, and precision were ensured to be achieved according to the ICH guidelines as follows:

Method development

Abundant trials were made to establish a method for the three drugs with different absorbance, pH, column, mobile phase, and flow rate. The methods tested earlier showed some failures like asymmetrical peaks, unusual chromatograms, and overlapping for both drugs separately or accomplished by changing the mixture of solutions. The excellent ideal method for the three used drugs regarding the symmetry of peaks, their retention time, and a resolution was when they were analyzed by an HPLC system applied at (70 : 30) ACN: Buffer, with a pH adjusted to nearly 2.7.

Chromatographic conditions

Here the perfect representative chromatographic condition of the three drugs, empagliflozin, pioglitazone, and metformin, was occupied at the resolution and retention time for each drug. At pH 2.7, the mixture of the mobile phase administered the resolution. (Table 2 and Figure 3).

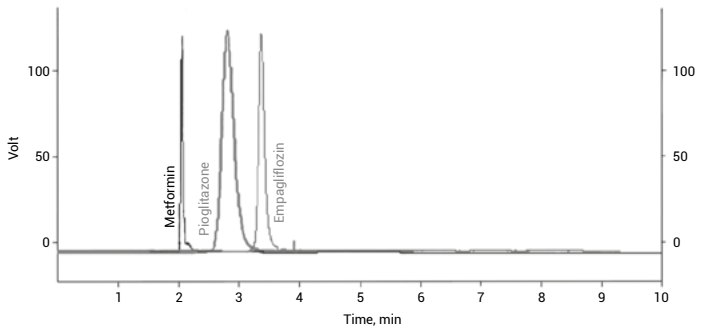

Figure 3. The overlay chromatogram of the three drugs: empagliflozin, pioglitazone, and metformin under the chromatographic conditions.

Table 3. System parameters for simultaneous measurement diluent-containing empagliflozin, pioglitazone, and metformin.

Parameters	Empagliflozin	Pioglitazone	Metformin
Average area of 10 injections	99.97	99.11	100.88
RSD%	1.19	0.65	1.37
Asymmetry	1.00	0.99	0.99
Theoretical plates	5245	2658	8547
Resolution	2.51	3.1	5.2
Initial retention time	2.01	2.40	3.1
Final retention time	2.23	2.52	3.4

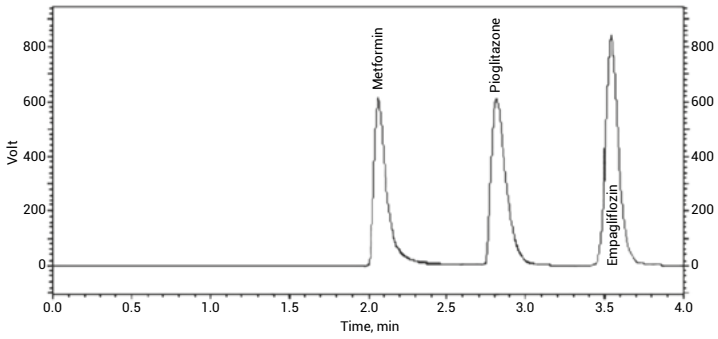

Figure 4. Chromatogram showing the precision of the method.

Identification

The essential objective of identification was to figure out if each of the drugs covered the previous chromatographic condition mentioned earlier. Studying the blank diluent demonstrated no peaks, implying no interference with the other drug peaks or any other alternative peaks found. The peaks of the drugs shown in Figure 3 show pleasant symmetry and resolution too.

Precision

System precision

The main objective of system precision was to determine the degree of agreement between each test result when the procedure was utilized regularly to multiple injections (10 injections) of the homogenous sample. More important was that the RSD% values were beneath 2%, suggesting suitable system suitability. Besides, the initial and the final retention time of each did not overrun or overlap, demonstrating fair resolution as presented in Table 3. These data displayed that the mean value of the assay% was within the range of 98-102%, and the relative standard deviation was lower than 2%. According to ICH guidelines, both of them were within the accepted range.

Method precision

The method's precision was achieved by analyzing the mixture of the three drugs six times. The RSD values were established below 2%, suggesting a precise method for samples in the diluent. Additionally, the recovered concentrations were established within the range of 98-102% for either sample, as shown in Figure 4.

Intermediate precision

Intermediate precision was attained by running composite samples on two different days using different equipment. The six sample preparations were analyzed on the first day, and the data (RSD%, assay%) were collected. Then on the second day (different day and analyst with the same chromatographic conditions and concentration), the analysis was redone and repeated. The assay value attained was within the range of 99-102%, as shown in Tables 4-6 and Figure 5.

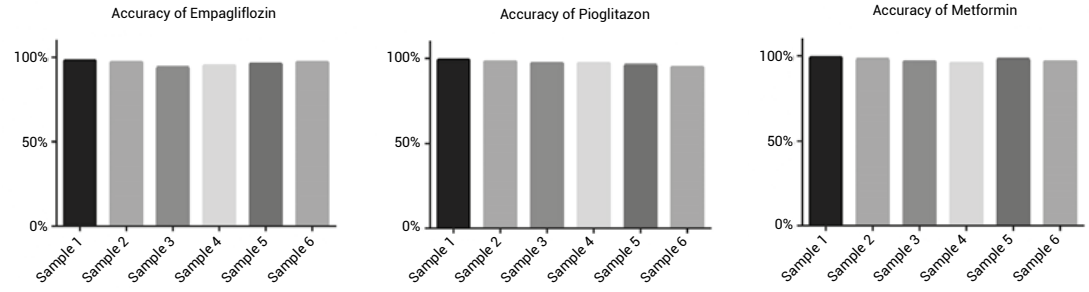

Figure 5. Accuracy of empagliflozin, pioglitazone, and metformin.

Table 4. Intermediate precision of the analytical method for a sample containing empagliflozin.

Sample No.	Conc.	Area	Conc. (Th.)	Accuracy	Avg.	RSD
Sample1	48.5	2150076	46.00	100.66	99.82	0.7
	49.1	2157549	45.00	98.99		
Sample2	48.8	2157549	45.00	100.01	100.15	
	48.5	2162395	45.00	100.28		
Sample3	48.1	2166837	43.00	101.35	100.38	
	49	2170159	44.00	99.41		
Sample4	50	2178141	43.00	99.59	99.51	
	49.3	2182820	43.10	99.44		
Sample5	48.4	2177129	43.50	99.23	98.68	
	48.9	2182149	44.00	98.12		
Sample6	48.9	2199924	48.50	99.80	100.44	
	48.2	2199581	48.00	101.07		

Table 5. Intermediate Precision of the analytical method for a sample containing pioglitazone.

Sample No.	Conc.	Area	Conc. (Th.)	Accuracy	Avg.	RSD
Sample1	40	1502535	42.42	102.63	102.03	1.2
	39	1444407	42.18	101.42		
Sample2	40	1461739	42.18	100.00	100.04	
	40.1	1466711	42.21	100.07		
Sample3	38.7	1412179	42.28	100.06	99.17	
	39.5	1416053	42.32	98.28		
Sample4	39	1387857	42.56	97.68	98.84	
	38.1	1388197	42.58	100.01		
Sample5	38.6	1398033	42.48	99.37	100.40	
	37.8	1397528	42.59	101.44		
Sample6	42.1	1587384	42.98	102.71	100.82	
	43.8	1590863	42.99	98.93		

Table 6. Intermediate Precision of the analytical method for a sample containing metformin.

Sample No.	Conc.	Area	Conc. (Th.)	Accuracy	Avg.	RSD
Sample1	38	1244283	36.83	96.93	98.16	1.4
	36.21	1215032	35.99	99.39		
Sample2	37	1225152	36.28	98.06	97.17	
	38	1235781	36.59	96.29		
Sample3	36.6	1188457	35.22	96.24	96.51	
	36.7	1198556	35.52	96.77		
Sample4	35.8	1179755	34.97	97.69	98.41	
	35.3	1180543	35.00	99.14		
Sample5	35.1	1186378	35.16	100.18	99.33	
	35.8	1189537	35.26	98.48		
Sample6	39.4	1343756	39.70	100.77	100.32	
	39.9	1348859	39.85	99.87		

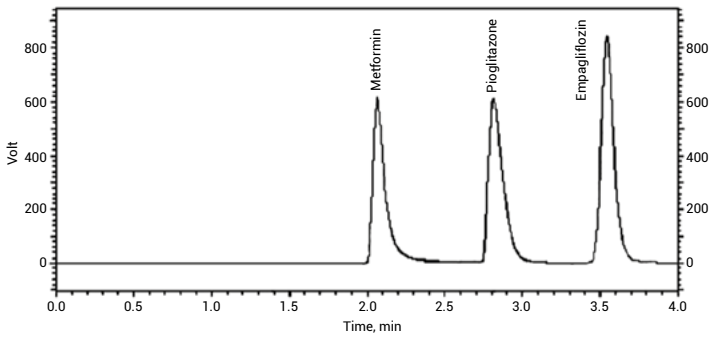

Figure 6. Chromatogram of intermediate precision.

The assay% and RSD% values were achieved within the accepted range, which indicated a valid and accurate method. The chromatogram of intermediate precision for the three drugs: metformin, pioglitazone, and empagliflozin is shown in the following figure (Figure 6).

Linearity and range

Linearity was assessed by running a series of standard samples at different concentrations of the target compounds were processed. Later, after analyzing every preparation in duplicate, a linear regression analysis was achieved on the average peak areas versus the concentration of the levels studied. An excellent linear relationship ($R^2 = 0.9994$) was noticed between the concentrations of empagliflozin and the corresponding average area. The linear equation of empagliflozin = 46206x – 66208 was obtained. The calibration curve of empagliflozin was linear over the concentration range studied (10-100 ppm). The correlation coefficient was 0.9994, suggesting good linearity for empagliflozin, within the stated limit to detect the linearity validation method. Pioglitazone and metformin were analyzed in the same range.

An excellent linear relationship ($R^2 = 0.9993$) was noticed between the concentrations of pioglitazone and the corresponding average area. The calibration curve equation = 38767x – 88953 was obtained. The calibration curve of pioglitazone was linear over the concentration range (10-100 ppm).

Also, an excellent linear relationship ($R^2 = 0.9998$) was noticed between metformin concentrations and the corresponding average area. The calibration curve equation was absorbance = 86700x – 33112. The calibration curve of metformin was linear over the concentration range (4-40 ppm). The calibration curves affirmed linearity in the range of 20-250 ppm for all the three drugs dissolved in the diluent with $R^2 > 0.99$.

Recovery "Accuracy"

In order to estimate the accuracy, samples at three divergent concentration levels (70%, 100%, and 130%) were analyzed, and a triplicate injection was given in each level of concentration compared to the standard sample. The% of recovery equation was: % accuracy = (recovered amount / actual amount) X 100. According to ICH guidelines (21), the accepted recovery limits were within the percent range of 98%-102%.

Concentration levels were 35, 50, and 65 ppm for empagliflozin, 31.6, 45, 58.6 ppm for pioglitazone, 28, 40, and 52 ppm metformin. They were analyzed and calculated from a standard curve.

A direct relationship between the peak height and the concentration was declared from the noticed profiles of the three different concentrations.

There was a good, acceptable separation noticed from the peaks above, presenting the relationship in which a change in concentration levels would change the peak area (AUC); also, by increasing the concentration, the AUC would increase. However, the above three drugs, empagliflozin, pioglitazone, and metformin, showed a valid and acceptable test accuracy result.

Table 7. Stability for empagliflozin, pioglitazone, and metformin in the diluent solution.

Drug	Time and temperature	Assay%
Empagliflozin	Standard fresh sample	99.7
	24 hours at 25°C	99.4
Pioglitazone	Standard fresh sample	101.1
	24 hours at 25°C	101.0
Metformin	Standard fresh sample	99.8
	24 hours at 25°C	99.9

Stability of drugs in analytical solution

It is essential to notice the level at which the analyte solutions were stable. The stability of the solution was to be evaluated for 24 hours by storing the solution under a well-known concentration at a room temperature of 25°C and then compared to a new standard solution. A 100% concentration level was analyzed against the standard solution. The concentration for empagliflozin was 50 ppm, 45 ppm for pioglitazone, and 37 ppm of metformin. These were within the stated and acceptable limit of the range (98-102%) for fresh samples and in 24 hours.

The given results (Table 7) showed that the assay percentage under all tested conditions was mentioned as per the ICH guidelines. Results implied that empagliflozin, pioglitazone, and metformin were stable under the test conditions.

Robustness

The robustness test was applied to enhance the method by varying the procedure parameters and specific limits without changing the results. Robustness altered, along with the procedure tested. Generally, it was done by altering procedure parameters and observing its effect on the analyte analysis. Robustness was applied using solutions prepared in the same way as method or system precision. The number of replicates was usually three. It was figuring out according to system suitability parameters, or else the recovered amounts were compared to data generated using the original method. The trailing changes were separately done (Figure 7).

– Mobile phase composition (± 10%) acetonitrile volume.
– Temperature ± 3°C.
– The pH of the mobile phase (± 10%) unit of the specified value defines any effect of changes in the pH value.
– Detector wavelength (± 5 nm)

Robustness regarding wavelength

Minor variations of (±5) in wavelength have been made to the analytical method of procedure to evaluate the capacity of the method to stay unaffected by minor alterations. One analytical concentration was analyzed at every level versus the standard solution. The RSD% was less than 2%, suggesting that a minor change in wavelength did not disturb the assay detection parameters. It was observed that changes in a wavelength (±5) could lead to a minor variation in the AUCs of empagliflozin, pioglitazone, and metformin. However, RSD% values remain within the accepted, confirmed range (< 2%), implying that the current method was robust.

Robustness regarding pH

Likewise, minor changes in the pH of the diluent were done to evaluate if the change would affect the detection parameters of the drugs in this manner. By agreement, the RSD% and assay% were close to 100% and < 2%, respectively, which indicated that a minor pH change did not affect the detection parameters of empagliflozin pioglitazone or metformin. The results revealed no expressed or significant change in the peak areas; this indicated that the analytical method for empagliflozin, pioglitazone, and metformin was robust.

Robustness regarding the organic modified composition

To evaluate and measure the method's capacity to last without being affected by slight modifications, minor variations (+10% and -10% ACN) in the composition of the mobile phase have been done with the analytical method. The analytical concentration was analyzed against the standard solution at level 100%.

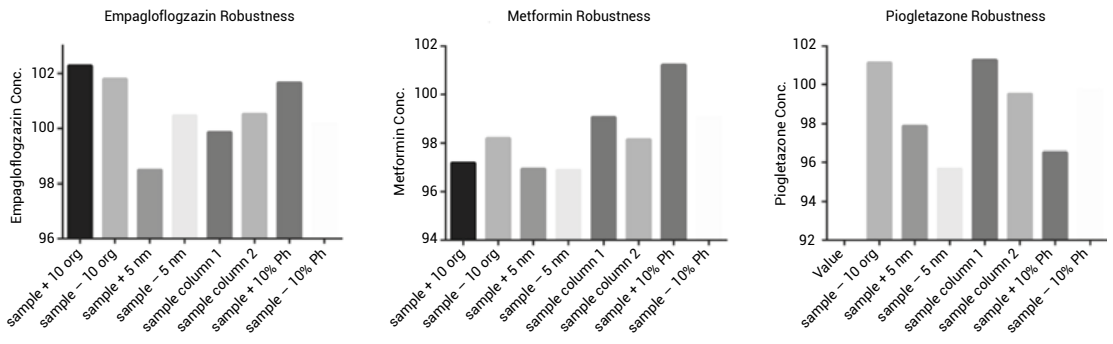

Figure 7. Robustness of empagliflozin, metformin, and pioglitazone.

Robustness regarding temperature

Changing the setting temperature of the instrument was also done on the method parameters. The results were attained by comparing the standard solution tested under ±3 Celsius change of 25°C. The RSD% values of lower or higher temperatures were less than 2%).

Selectivity

It was essential to study the method's selectivity to figure out the capability of the analytical procedure to precisely measure the existence of the placebo, active ingredients, or another ingredient. Following the parameters stated under the developed method, standard, solvent, placebo, and sample solutions were injected into the column. It was initiated that there is no interference between the analyte and both the placebo and solvent, which implies good selectivity of the method.

Placebo effect

A placebo solution was prepared with the same solvents used to prepare the sample solutions and standards performed during the analysis. The addition of water prepared a placebo solution, water: CAN: methanol, in a ratio of 1 : 1 : 1 and later analyzed in the analytical system. No peaks were identified, suggesting no interference between the active ingredients and the excipients.

CONCLUSION

The proposed HPLC method provided specific, simple, accurate, reproducible, and precise quantitative analysis for the simultaneous analysis of empagliflozin, pioglitazone, and metformin in pharmaceutical formulations. According to the ICH guidelines, the method was validated for accuracy, reproducibility, linearity, robustness, and precision. The designed method could be used for routine analysis and quality control assay of empagliflozin, pioglitazone, and metformin in pharmaceutical formulations. We recommend that future bioanalytical methods promote and take advantage of this method to estimate empagliflozin, pioglitazone, and metformin in the various biological methods, with slight or no modifications.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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