



Stability indicating method and validation for the simultaneous estimation of metformin and empagliflozin by using RP-HPLC in a bulk and pharmaceutical dosage forms

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ABSTRACT

A simple and selective LC method is described for the determination of Metformin and empagliflozin in tablet dosage forms. Chromatographic separation was achieved on a C₁₈ column using mobile phase consisting of a mixture of 50 volumes of methanol and 50 volumes of phosphate buffer with detection of 255 nm. Linearity was observed in the range 60-140 µg /mL for Metformin ($R^2 = 0.999$) and 3-7µg /mL for empagliflozin ($R^2 = 0.999$) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Metformin and empagliflozin, Reversed phase HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books¹. Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis [1-3].

METHODOLOGY

Mobile phase [4-6]

A mixture of 50 volumes of Acetonitrile and 50 volumes of KH₂PO₄ buffer were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Preparation of standard stock solution of metformin [7]

10 mg of METFORMIN was weighed and transferred in to 100mL volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /mL of solution by diluting 1mL to 10mL with methanol.

Preparation of standard stock solution of empagliflozin [8]

10mg of EMPAGLIFLOZIN was weighed in to 100mL volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /mL of solution by diluting 1mL to 10mL with methanol.

RESULTS AND DISCUSSION

Metformin [9]

Soluble in methanol and in water, very slightly soluble in phosphate buffer.

Empagliflozin

Freely soluble in water, soluble in acetonitrile, sparingly soluble in methanol.

Wavelength determination

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately [10-13].

Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/mL solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1, 8.2 and 8.3 and the absorption curve shows characteristic absorption maxima at 240 nm for METFORMIN, 229 nm for EMPAGLIFLOZIN and 255nm for the combination [14-16].

Method development

Isobestic point of Metformin and empagliflozin

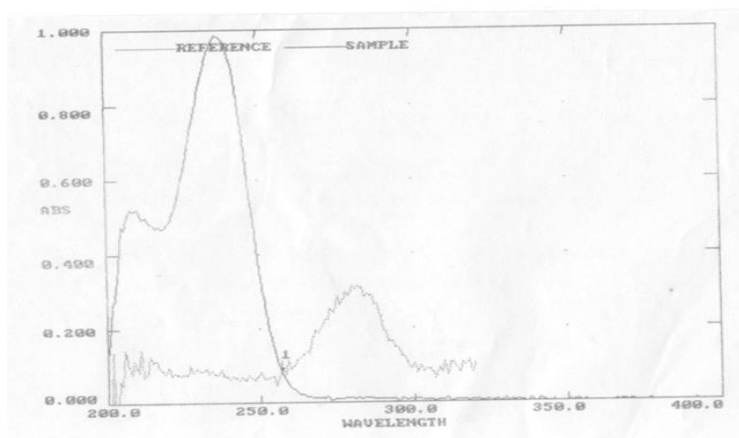


Figure 1: Isobestic point of Metformin and empagliflozin

Table 1: Optimized chromatographic conditions

Mobile phase	ACN: KH ₂ PO ₄
pH	4.0
Column	Inertsil ODS 3V column, C18(150x4.6 ID) 5µm
Flow rate	1.0 mL/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	255
Injection volume	20 µL
Run time	5 min
Retention time	About 2.463 min for metformin and 4.210 min for empagliflozin.

Preparation of mixed standard solution

Weigh accurately 100 mg of metformin and 5 mg of empagliflozin in 100 mL of volumetric flask and dissolve in 10mL of mobile phase and make up the volume with mobile phase. From above stock solution 1000 µg/mL of metformin and 50 µg/mL of empagliflozin is prepared by diluting 1mL to 10mL with mobile phase. This solution is used for recording chromatogram.

Tablet sample

Tablet stock solutions of empagliflozin and metformin (150 µg/mL) were prepared by dissolving weight equivalent to 5 mg of empagliflozin and 100 mg of metformin and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 50mL with mobile phase. Further dilutions are prepared in 5 replicates of 50µg/mL of

empagliflozin and 1000 µg/mL of metformin was made by adding 1 mL of stock solution to 10 mL of mobile phase.

Calculation

The amount of empagliflozin and metformin present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ assay} = \frac{at}{as} \times \frac{ws}{ds} \times \frac{dt}{wt} \times \frac{p}{100} \times \frac{aw}{lc} \times 100$$

Where,

as: average peak area due to standard preparation

at: peak area due to assay preparation

ws: weight of empagliflozin /metformin in mg

wt: weight of sample in assay preparation

dt: dilution of assay preparation

Table 2: Assay of metformin and Canagliflozin

Metformin	Empagliflozin			
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	3941.191	3933.444	378.411	372.761
Injection-2	3925.782	3930.759	367.951	371.408
Injection-3	3941.042	3936.783	375.523	370.373
Injection-4	3925.782	3920.484	550.591	541.451
Injection-5	3941.191	3945.931	384.450	378.411
Average Area	3937.618	3933.479	411.385	406.880
SD	0.824		0.752	
%RSD	2.0		0.9	
Assay(%purity)	99.89		100.9	

Observation

The amount of metformin and empagliflozin present in the taken dosage form was found to be 99.89% and 100.9% respectively.

VALIDATION

Specificity by direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 10mg of metformin and 10 mg of canagliflozin in 100 mL of volumetric flask and dissolve in 10mL of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/mL of metformin and canagliflozin is prepared by diluting 1mL to 10mL with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains canagliflozin–50mg, metformin -500 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of canagliflozin and metformin (µg/mL) were prepared by dissolving weight equivalent to 10 mg of

canagliflozin and 20 mg of metformin and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10mL with mobile phase. Further

dilutions are prepared in 5 replicates of 10µg/mL of canagliflozin and metformin was made by adding 1 mL of stock solution to 10 mL of mobile phase.

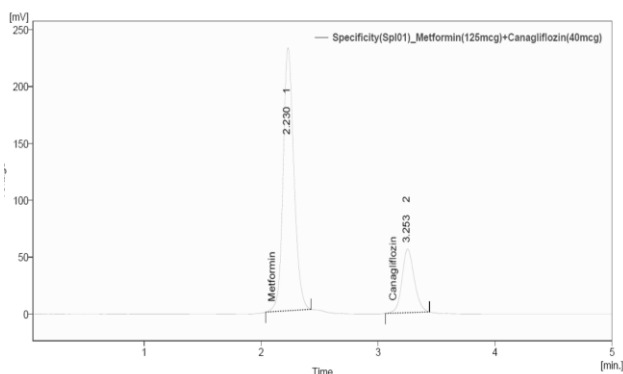


Figure 2: Chromatogram for specificity of metformin and Canagliflozin sample

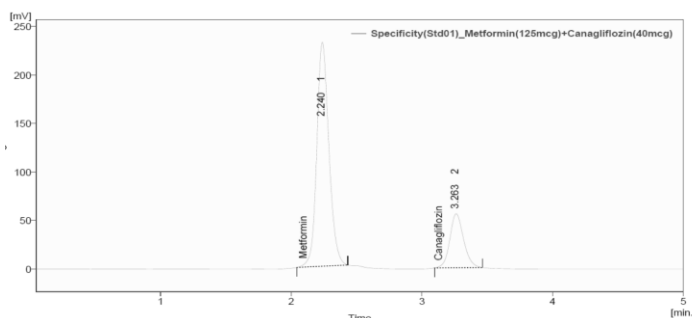


Figure 3: Chromatogram for Specificity of metformin and Canagliflozin standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the metformin and Canagliflozin peaks.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of metformin and canagliflozin (µg/mL) were prepared by dissolving 10 mg of metformin and canagliflozin dissolved in sufficient mobile phase and dilute to 100 mL with mobile phase.

Table 3: Linearity Preparations

Preparations	Volume from standard stock transferred in mL		Volume made up in mL (with mobile phase)	Concentration (µg /mL)	
				Metformin	Canagliflozin
Preparation 1	0.75	0.24	10	75	24
Preparation 2	1	0.32	10	100	32
Preparation 3	1.25	0.4	10	125	40
Preparation 4	1.50	0.48	10	150	48
Preparation 5	1.75	0.56	10	175	56

Table 4: Linearity of metformin

Concentration (µg/mL)	Area
75	927.341
100	1235.752
125	1477.14
150	1722.958
175	2041.082

Table 5: Linearity curve of canagliflozin

Concentration (µg/mL)	Area
24	281.784
32	372.204
40	438.317
48	520.564
56	627.846

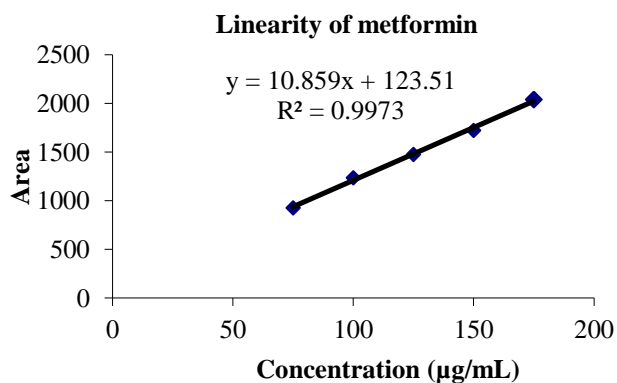


Figure 4: Linearity graph of metformin

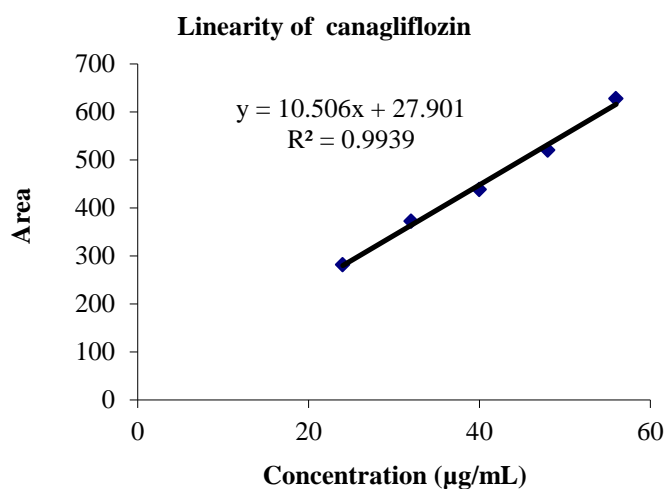


Figure 5: Linearity graph of canagliflozin

The relationship between the concentration of metformin and canagliflozin and area of metformin

and canagliflozin should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. area for standard preparations of metformin and canagliflozin is 0.999 and 0.996. The relationship between the concentration of metformin and canagliflozin and area of metformin and canagliflozin is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed

sample), the reference standards of the drugs were added at the level of 100%, 120%, 140%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 100%, 120%, 140%.

Acceptance criteria

The % recovery of metformin and canagliflozin should lie between 98% and 110%.

Table 6: Recovery results for metformin

Recovery level	Accuracy of metformin			Average % Recovery
	Amount taken(mcg/mL)	Area	%Recovery	
100	75	410.811	197.6411668	102.83
	75	411.371	197.9105828	
	75	411.224	197.8398611	
120	125	522.084	125.5873028	99.36
	125	527.341	126.8518741	
	125	529.651	127.4075446	
140	175	621.351	89.67958741	99.103
	175	621.351	89.67958741	
	175	615.264	88.80105072	

Table 7: Recovery results for canagliflozin

Recovery level	Accuracy canagliflozin			Average % Recovery
	Amount taken(mcg/mL)	Area	%Recovery	
100	24	410.811	197.6411668	102.83
	24	411.371	197.9105828	
	24	411.224	197.8398611	
120	40	522.084	125.5873028	99.36
	40	527.341	126.8518741	
	40	529.651	127.4075446	
140	56	621.351	89.67958741	99.103
	56	621.351	89.67958741	
	56	615.264	88.80105072	

PRECISION

Method precision

Prepared sample preparations of omeprazole and aspirin as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of assay preparations of omeprazole and aspirin should be not more than 2.0%.

Table 8: Method precision of metformin and Canagliflozin

Metformin			Canagliflozin		
S.No.	RT	Area	S.No.	RT	Area
1	3.145	978370.000	1	6.211	340457
2	3.165	962064.000	2	6.224	341907
3	3.151	967422.000	3	6.212	339323.000
4	3.148	955774.000	4	6.194	339473.000
5	3.126	951906.000	5	6.168	339074
6	3.116	962532.000	6	6.170	340503.000
AVG	3.1418	963011.333	AVG	6.197	340122.833
SD	0.0178	9297.067	SD	0.023	1058.443
%RSD	0.57	0.97	%RSD	0.38	0.31

Observation

Test results for canagliflozin and metformin are showing that the %RSD of assay results are within limits.

Robustness**Acceptance criteria**

The system suitability should pass as per the test method at variable conditions.

Table 9: Result of Robustness study

Parameter	Metformin		Canagliflozin	
	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor
Flow Rate				
0.8 mL/min	2.562	1.679	5.059	1.263
1.2 mL/min	2.148	1.678	4.235	1.264
Wavelength				
249 nm	2.566	1.687	5.052	1.262
253 nm	2.570	1.686	5.065	1.265

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Table 10: Results for Ruggedness

Metformin	%Assay	Canagliflozin	%Assay
Analyst 01	100.5	Analyst 01	98.9
Analyst 02	99.5	Analyst 02	100.6

Observation

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

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