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RP- HPLC method for simultaneous determination of metformin and canagliflozin in pharmaceutical dosage form

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ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Canagliflozin and Metformin was done by RP-HPLC. The Phosphatebuffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (250mmx4.6mm,5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase.

Keywords: RP-HPLC, Metformin, Canagliflozin

INTRODUCTION

Canagliflozin is an inhibitor of subtype 2 sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of renal glucose reabsorption (SGLT1 being responsible for the remaining 10%). Blocking this transporter causes up to 119 grams of blood glucose per day to be eliminated through the urine,[13] corresponding to 476 kilocalories. Additional water is eliminated by osmotic diuresis, resulting in a lowering of blood pressure.

Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. When used alone, metformin does not cause hypoglycemia; however.

Stucture of Canagliflozin

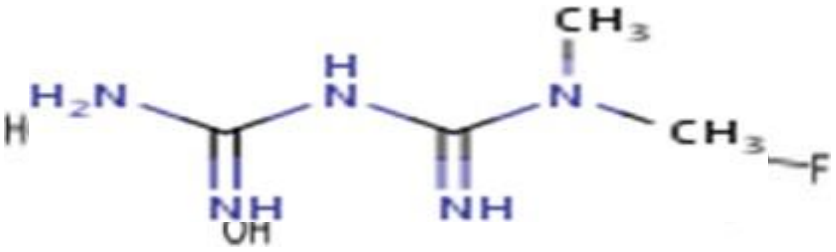


Fig 1: Structure of Metformin

METHODS AND MATERIALS

Instruments used Alliance, model No. BSA224SC LC+20AD HPLC, Software- UV WIN5, Solution, LABINDIA UV 3000+ UV/VIS spectrophotometer, Adwa – AD 102U pH meter.

Chemicals USED

SL.No	Chemical	Brand
1	CANAGLIFLOZIN	Mylon
2	METFORMIN	Cipla
3	KH_2PO_4	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

RESULT AND DISCUSSION

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30:70 v/v respectively.

Wave length selection

UV spectrum of 10 µg / ml Canagliflozin and metformin in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 254nm. At this wavelength both the drugs show good absorbance.

Optimization of Column

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil C18(250 x 4.6mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

Preparation of Phosphate buffer

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Canagliflozin and metformin 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

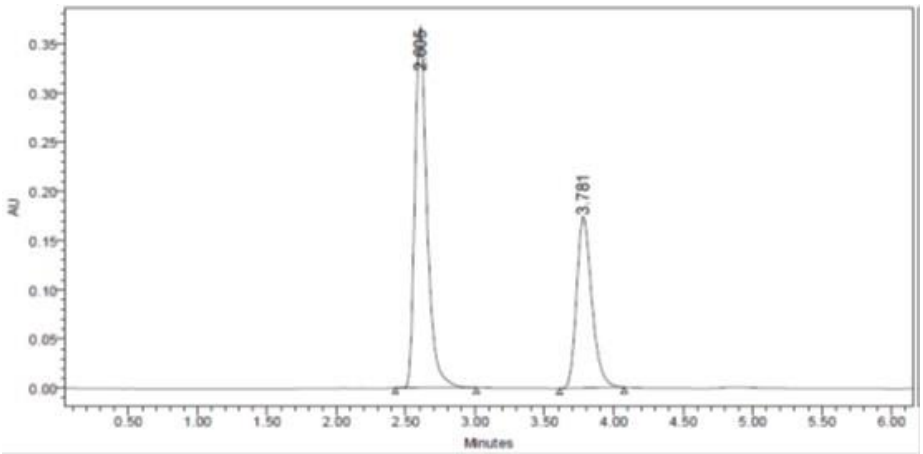
Sample Solution Preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Canagliflozin and metformin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Table 1: Optimized Chromatographic Conditions

Parameters	Description
Flow rate	1ml min^{-1}
Column	Inertsil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer:Methanol P^H 4.5(30:70 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 4.5 adjusted with Orthophosphoric acid
Detector	uv
Column temperature	Ambient

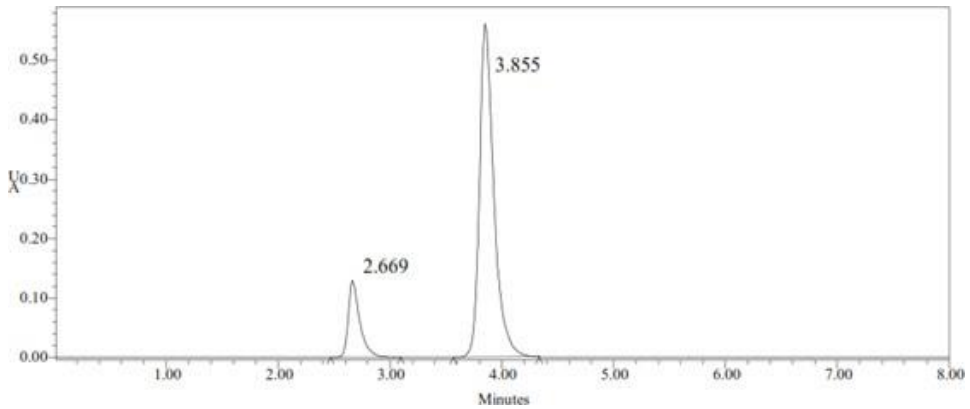
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min



The separation of two analytical peaks was good. The plate count also above 2000,tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Fig 2: Optimized Chromatographic ondition of canagliflozin and metformin

SYSTEM SUITABILITY



Tailing factor for the peaks due to Canagliflozin and metformin in Standard solutionShould not be more than 2.0. Theoretical plates for the Canagliflozin and metformin peaks in Standard solution should not be lessthan 2000.

Fig 3: Chromatogram for Canagliflozin and Metformin sample Preparation

Table 2: Results of system suitability parameters for Canagliflozin and Metformin

S.No	Name	Retentiontime (min)	Area (µVsec)	Height (µV)	USP resolution	USP tailing	USP platecount
1	Canagliflozin	2.5	124505	213642		1.2	4673.4
2	Metformin	3.9	1308495	154566	6 0	1.3	6090.3

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developedmethod were within the limit.

Precision

Precision of the method was carried out for standard solutions as described underexperimental work. The corresponding chromatograms and results are shown below.

Linearity

The linearity range was found to lie from 100µg/ml to 500µg/ml of Canagliflozin, 5µg/ml to25µg/ml 0f Metformin and chromatograms are shown below.

Table 3: Analytical performance parameters of Canagliflozin and Metformin

Parameters	Canagliflozin	Metformin
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearitywas established in the range of 10% to 50% of Canagliflozin and 5% to 25% of Metformin.

Robustness

The standard and samples of Canagliflozin and Metformin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 4: Change in Organic Composition in the Mobile Phase for Canagliflozin

S.No	Change in Organic Composition in theMobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 5: Change in Organic Composition in the Mobile Phase for Metformin

S.No	Change in OrganicComposition in theMobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	Actual	6090.3	1.2
3	10% more	6232.5	1.2

Percentage RSD should be below 2., The %RSD obtained for change of flow rate, variation in mobile phase was found to bebelow 1, which is within the acceptance criteria. Hence the method is robust

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Canagliflozin and Metformin was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (250mm x 4.6mm 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm.

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