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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CANAGLIFLOZIN IN DRUG SUBSTANCE BY RP-HPLC METHOD

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ABSTRACT

Analytical method was developed for the estimation of Canagliflozin drug substance by liquid chromatography. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V 150*4.6, 5um) at ambient temperature .the separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water: Methanol (10:90). The flow rate was 1.0 ml/ minute and ultra violet detector at 220nm. The average retention time for Canagliflozin found to be 2.4 min the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 100-300µg/ml for Canagliflozin.

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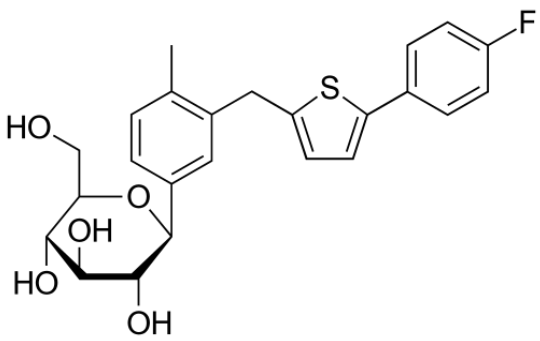
INTRODUCTION

Canagliflozin is an anti-diabetic drug used to improve glycemic control in people with type 2 diabetes. Canagliflozin is a drug of the gliflozin class or subtype 2 sodium-glucose transport (SGLT-2) inhibitors used for the treatment of type 2 diabetes. Secondary efficacy endpoints of higher reductions in weight and blood pressure (versus sitagliptin and glimiperide) were also observed in studies. Canagliflozin produces beneficial effects on HDL cholesterol whilst increasing LDL cholesterol to produce no change in total cholesterol.

Canagliflozin is chemically designated as (2S,3R,4R,5S,6R)-2-(3-{[5-(4-fluorophenyl)thiophen-2-yl]methyl}-4-methylphenyl)-6-(hydroxymethyl)oxane-3,4,5-triol.Its molecular formula is $C_{24}H_{25}FO_5S$ and its molecular weight is 444.516. Canagliflozin is a white-to-off white powder. It is freely soluble in methanol and practically insoluble in water.



Structure of Canagliflozin.

EXPERIMENTAL:

Equipments:

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Inertsil 5µ, 150 mm × 4.6 mm) as stationary phase ,Ultrasonic cleaner, Scaletech analytical balance ,Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials:

Pharmaceutically pure sample of Canagliflozin were obtained as gift samples from Fortune pharma training institute , sri sai nagar , KPHB and Hyderabad, India.

HPLC-grade Methanol was from qualigens reagents pvt ltd. Formic acid (AR grade) was from sd fine chem.

Chromatographic conditions

The sample separation was achieved on a C18 (5 µ, 15 cm X 4.6 mm i.d.) INERTSIL column, aided by mobile phase mixture of 0.1%v/v Formic acid in water : Methanol (10:90). The flow rate was 1.0 ml/ minute and ultra violet detector at 220nm , that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase:

Buffer Preparation:

Take accurately 1ml of formic acid in 1000mL of water

Mobile phase:

Then add 10 volumes of buffer and 90 volumes of Methanol mixed well and sonicated for 5 min.

Diluent preparation:

Water: Methanol 50:50 v\v

Preparation of standard stock solution:

A 50mg of pure Canagliflozin were weighed and transferred into 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 1000µg/ml. From the above solution 2ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 200µg/ml of Canagliflozin.

Preparation of sample solution:

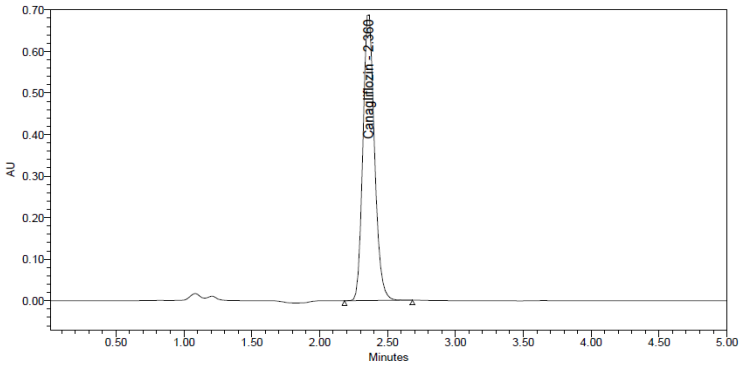
A 50mg of Canagliflozin sample were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 1000µg/ml. From the above solution 2ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 200µg/ml of Canagliflozin.

RESULTS AND DISCUSSIONS:

Determination Of Working Wavelength (λ max):

10 mg of the Canagliflozin standard drug is taken in a 10 ml volumetric flask and dissolved in water:methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using methanol as blank. The λmax was found to be 220nm

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: Methanol (10:90). The flow rate was 1.0 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1.



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Canagliflozin	2.360	4089223	100.00	1.15	3754

Figure: 1 Chromatogram of Canagliflozin.

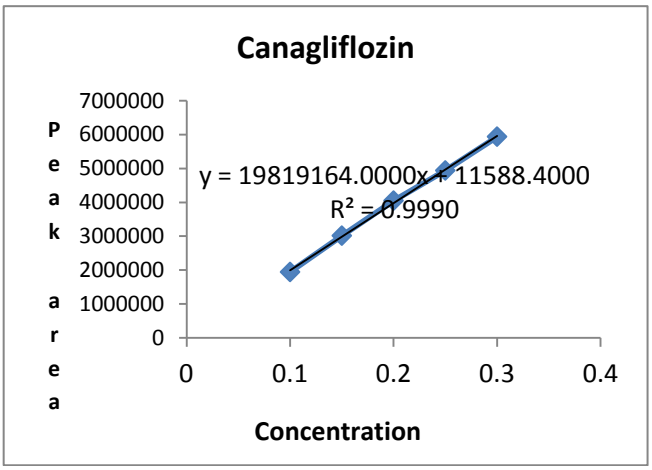
METHOD VALIDATION:

Linearity:

Linearity was studied by analyzing five standard solutions covering the range of 100-300 µg/ml of Canagliflozin. From the primary stock solution 1.0ml,1.5ml,2.0ml,2.5ml,3.0 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 100µg/mL , 150µg/mL ,200µg/mL ,250µg/mL and 300 µg/mL of Canagliflozin. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Table No: 1.

Level	Concentration (mg/mL)	Peak area
50%	0.1	1942537
75%	0.15	3014028
100%	0.20	4047677
125%	0.25	4937044
150%	0.30	5935820



FigureNo.2: Linearity (calibration) curve of Canagliflozin.

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

LOD = 3.3 δ/S ……. (3)
LOQ =10 δ/S …….. (4)

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Table no.2: LOD and LOQ values Calculated from calibration curve:

	mg
LOD	0.009
LOQ	0.029

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 100 µg/ml of CANAGLIFLOZIN without changing the parameter of the proposed chromatographic method.

Table.3: Summary of peak areas for method precision.

Sample No	Retention time	Peak area	% Assay
1	2.361	4092869	101.4
2	2.361	4076760	100.8
3	2.362	4081662	101.0
4	2.362	4086951	101.1
5	2.358	4097310	100.9
6	2.355	4132293	101.4
Mean	2.360	4094641	101.1
%RSD	0.12	0.49	0.25

Accuracy (recovery study):

The accuracy of the method was determined by calculating the recoveries of Canagliflozin by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Canagliflozin. The percentage recovery results obtained are listed in Table 4.

Table No.4: Recovery data.

LEVEL	S.No	%Recovery of Canagliflozin	Average
	1	99.2	
50	2	100.7	99.9%
	3	99.8	
	1	101.4	
100	2	101.0	101.1%
	3	99.1	
	1	101.1	
150	2	100.7	100.8%
	3	100.7	

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table no.5)

Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated i.e.,(limit <2%).
This indicates the method was rugged. The results were shown in Table no.6.

Table No.5: Results of Robustness study.

Parameter	Rt of Canagliflozin	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	2.613	3870	1.18
Increased flow rate (1.2ml/min)	2.149	3365	1.13
Wave Length 218nm	2.360	3754	1.15
222	2.358	3755	1.16

Table No.6: Results of Ruggedness.

		%Assay	%RSD
Analyst-1 Analyst-2	Canagliflozin	101.4 101.0	0.07%

Table No.7: Validation parameters of evaluated method:

S. No	Parameter	Limit	Value Obtained
1	Linearity concentrations Range (mg/mL) Correlation coefficient	NLT 0.990	0.1 to 0.3 mg\ml 0.9995
2	Method precision (Repeatability) (%RSD, n = 6)	98.0 to 102.0 %	100.8 to 101.4 %
3	ACCURACY(%Recovery)	98-102%	100.8 to 101.1%
4.	Robustness Flow Variation(0.8mL to 1.2 mL/min) Wavelength Variation (218nm to 222nm)	It should be meet System suitability criteria	Complies
5.	Ruggedness (Intermediate Precision) (%RSD analyst to analyst variation)	NMT2%	0.07%

*RSD = Relative standard deviation

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of CANAGLIFLOZIN was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

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