RESEARCH ARTICLE

UPLC Estimation of an Antidiabetic Drug by Method Development and Validation Followed by Stability Indicating Studies

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ABSTRACT

The analytical method development and validation of a common antidiabetic drug, Canaglifozin, by taking the aid of 'Ultra High-Performance Liquid Chromatography', along with stability indicating method. The canaglifozin drug was eluted from the optimized mobile phase in 70:30 ratio of water and methanol, at 1 ml/ min flow rate, under separation with C_{18} column of 100 x 2mm size with 1.8 diameters at isocratic mode. The detection was carried at 286 nm wavelength, resulting in 3.80 retention time, as run time being 10 min. The detection limit and quantification limit, was noticed at '0.0039 and 0.0119 µg/ml' correspondingly. The percentage RSD and recovery percentage was under ICH guidelines only. The stress degradation studies under acid, basic, neutral, and thermal conditions were carried on.

Keywords: Canaglifozin, antidiabetic drug, Stress degradation, Analytical method development.

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INTRODUCTION

Canagliflozin is essentially used to medicate diabetes mellitus of type 2. This is by acting as receptor inhibitor in co-transporting sodium glucose. Further plays beneficial role on kidney and heart organs such as patients suffering from renal failure, impairment of renal organ and so on.[1-3]

Chromatography has become one of the essential sciences that is paving ways in different areas in different techniques.[4,5] One such crucial technique in many disciplines is high-performance liquid chromatography (HPLC). Currently days, ultra performance liquid chromatography (UPLC) as an advanced version of HPLC have good attention. They also have excellent role in pharmaceutical fields.[6-9] This is because of their availability, good sensitivity, low sample requirement which was therefore chosen in the present work.[10-14]

Ther are reports on the work of analytical method development of Canagliflozin and other related drugs by using the RP-HPLC method,[15,16] UV method, HPTLC method, LC-MS/MS[17,18] method with different optimizing conditions and validation results.[19-22] So, the present work deals about the development and validation of the UPLC analytical method for an antidiabetic drug, Canagliflozin as in with ICH Q2 guidelines.

MATERIALS AND INSTRUMENTS

Pure Drug Samples

The drug samples of canagliflozin were received as gift samples from Intas Pharmaceuticals Pvt Ltd respectively.

Chemicals and Solvents

The required chemicals and solvents are detailed in Table 1.

Instruments Used

The instruments needed for the current work is presented in Table 2.

MATERIALS AND METHODS

UPLC System Specifications

Instrument - Ultra Performance Liquid Chromatography
Injector - Rheodyne (20µl loop)
Software - LC solutions
Detector - PDA

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Table 1: Chemicals and solvents

S. No.	*Chemicals/Solvents*	*Manufacturer*
1	HPLC Grade Acetonitrile	Lab Fine Chem Industries Ltd., Mumbai, India
2	HPLC Grade Methanol	Lab Fine Chem Industries Ltd., Mumbai, India
3	Ammonium Acetate	Gaurav Scientific and Chemicals, Mumbai, India
4	Hydrochloric acid	Lab Fine Chem Industries Ltd., Mumbai, India
5	Sodium Hydroxide	Lab Fine Chem Industries Ltd., Mumbai, India
6	Hydrogen peroxide	V.L. Products, Mumbai, India

Table 2: Details of instruments

S. No	*Instruments*	*Model*
1	UV Spectrophotometer	Shimadzu UV1800S
2	Ultra performance Liquid Chromatography	Agilent UHPLC-MS, E6125B
3	Liquid Chromatography Mass Spectrometry	Agilent 1290 Infinity II

Initial Chromatographic Conditions

Choosing a chromatographic technique for the separation

Reverse-phase chromatographic technique was chosen as the drug is polar.

Selection of wavelength

The selectivity of HPLC method with the PDA detector is dependent on suitable wavelength. A precise wavelength must be chosen for the medications to have a distinct and distinct response. 279 nm was chosen as the study wavelength from the canagliflozin drug's UV spectra.

Selection of mobile phase

The HPLC method's PDA detector selectivity is based on the wavelength of the PDA detector. For the drug to have a specific response, a precise wavelength must be chosen. A wavelength of 286 nm was carefully chosen from the UV spectra of the Canagliflozin drug because it was the most relevant. The trial results are given in Table 3.

After sufficient trials an optimized method have been chosen as in Table 4 that is giving good resolution, and sharp peaks in chromatogram as in Figure 1.

Validation of RP-HPLC Method

System suitability studies

Table 5. presents the findings of a study conducted on system appropriateness factors such as - 'Resolution (Rs), tailing factor, retention duration, and number of theoretical plates (N)'.

Table 3: Mobile phase trials

Mobile phase composition	*Comment*
Water : Acetonitrile (75:25)	Tailing, Broad peaks
5 mM K_2HPo_4 : Acetonitrile pH5 (70:30)	Fronting, Tailing, Split Peaks
5mM K_2HPo_4 : Acetonitrile pH4.5 (70:30)	Tailing, Split Peaks
5mM K_2HPo_4 : Acetonitrile pH4 1 mL/min (70:30)	Tailing, Broad Peaks
Water: methanol (70:30)	Good Symmetric Peaks

Table 4: Optimized Chromatographic conditions

Parameter	*Condition*
Column	C_{18} column (100 x 2 mm, 1.8µ)
Flow rate	1ml/minute
Detector Wavelength	286nm
Injection volume	20µl
Column Temperature	50^0C
Auto sample Temperature	2^0C
Elution mode	Isocratic
Run time	10 minutes
Diluent	Water: methanol (70:30)

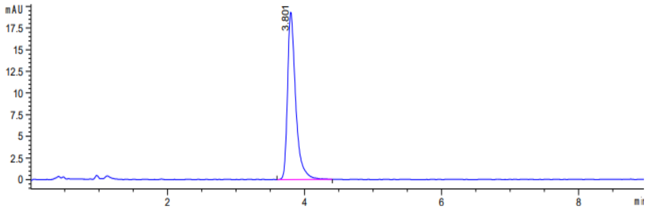

Figure 1: Optimized chromatogram of canagliflozin

Table 5: System suitability studies of Canagliflozin

Drug	*N*	*Rt*	*Tailing factor*
Canagliflozin	3711	3.801	1.5

Linearity and range

Based on the calibration curve, a 'linearity range at 10 to 50 µg/mL was established using least squares linear regression. A correlation coefficient of 0.999 was established between canagliflozin's slope and intercept. The linearity for five range of concentration from 10 to 50 ppm has been performed whose chromatograms are shown from Figures 6 – 10 while values at Table 6 and linearity proving graph at Figure 11.

Precision

Precision of method was demonstrated by -

- *Intraday precision*

Analysis of standard drug solutions in the linearity range at five different concentrations on the same day yielded intraday precision by calculating percent RSD, Table 7

- *Inter day precision*

By evaluating reference drug solutions at five concentrations in the linearity range for five days over the course of a week and computing the percent RSD shown in Table 8

Table 6: Linearity of Canagliflozin

Concentration (µg/ml)	*Mean peak area (A.U.)*
10	175.75
20	369.081
30	533.878
40	709.048
50	870.461
Linear regression equation (y = mx + c)	Y=17.311x+12.494
Slope(m)	17.311
Intercept(c)	12.494
Correlation coefficient (R^2)	0.999

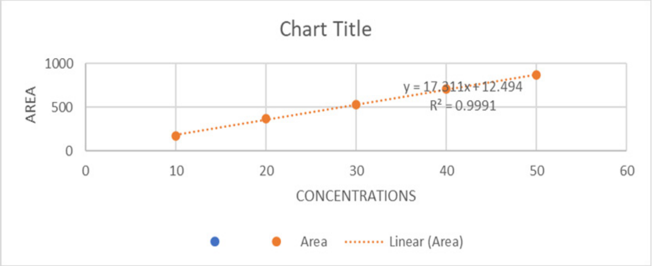

Figure 11: Calibration curve of canagliflozin

Table 7: Intraday precision of canagliflozin

Drug	*Conc (µg/ml)*	*Peak area*	*Average*	*%RSD*
Canagliflozin	10	175.75	175.806	0.028
	20	369.081	369.024	0.036
	30	533.878	533.878	1.567
	40	709.048	709.048	1.179
	50	870.461	870.466	1.258

Table 8: Inter-day precision of canagliflozin

Drug	*Conc (µg/ml)*	*Peak area*	*Average*	*%RSD*
Canagliflozin	10	175.752	175.766	0.0118
	20	369.081	369.064	0.0616
	30	533.87	533.854	0.026
	40	709.04	709.06	0.022
	50	870.461	870.46	0.023

Table 9: LoD and LoQ of Canagliflozin

Drug	*LoD*	*LoQ*
Canagliflozin	0.0039	0.0119

Limit of Detection (LoD) and Limit of Quantification (LoQ)

The LoD and LoQ of Canagliflozin was noticed at ‘0.0039 and 0.0119 µg/mL, respectively. It can be shown in Table 9

Robustness

It is possible to assess an analytical procedure’s robustness by observing how well it holds up under typical conditions, even when tiny but deliberate changes are made to the technique parameters. Changes in the composition of the buffer in the mobile phase and flow frequency were used to test the method’s robustness. Each condition’s RSD percentage was computed. Although several procedure parameters were deliberately varied, the findings derived from these changes were remarkably consistent.

Table 10: Robustness studies of canagliflozin

Parameters	*Modifications*	*Robustness*	
		Plate count	*tailing factor*
Flow tate	0.8	3512	1.41
	Optimized	3711	1.50
	1.2	3704	1.41
Mobile phase composition	10% less	3711	1.41
	Optimized	3711	1.50
	10% more	3012	1.46
Wavelength	+5nm	3412	1.45
	Optimized	3711	1.50
	-5nm	3312	1.45
Column temperature	+5°C	3212	1.42
	Optimized	3711	1.50
	-5°C	3412	1.46

Ruggedness

The ruggedness of the procedure was determined by comparing the intra-day and inter-day Canagliflozin assay findings done by two analysts. The robustness of the procedure was demonstrated by the percent RSD for analysis in the same laboratory should not be beyond 2.

Accuracy

Through the use of recovery experiments, the accuracy of the drug’s recovery was established. The sample formulation was reanalysed using the suggested approach after it was mixed with a known quantity of standard pharmaceuticals. Both a 50 and a 100% sample were used in this study Table 11 displays the recovery results.

ANALYSIS OF FORMULATION

For formulation analysis, fixed chromatographic conditions were used.

Preparation of standard solutions

Dissolving 1 mg of canagliflozin in 10 mL of mobile phase yielded stock solutions. Transferred aliquots from the standard stock solution of canaglifozin were made into 10 mL volumetric flanks and a solution was created as needed to meet the linearity ranges.

Preparation of Sample Solutions

Measurement and powdering of individual Invokana pills yielded an average weight of canagliflozin. A 10-mL volumetric flask was required for the transfer of 100 mg of Canagliflozin. This can only be removed by using the mobile phase. It was necessary to use a 20-minute ultrasonication procedure to ensure that the medications were completely dissolved in the

Table 11: Accuracy studies of canagliflozin

Drug	*Levels (%)*	*Amount taken(µg/mL)*	*Amount added(µg/mL)*	*Amount recovered (µg/mL)*	*%recovered*
Canagliflozin	80	50	40	89.85	99.83
	100	50	50	100.35	100.35
	120	50	60	120.16	100.13

Table 12: Analysis of marketed formulation

Drugs	*Labeled amount*	*Amount found*	*%label claim*	*%RSD*
Canagliflozin	100	99.2	99.2%	0.5

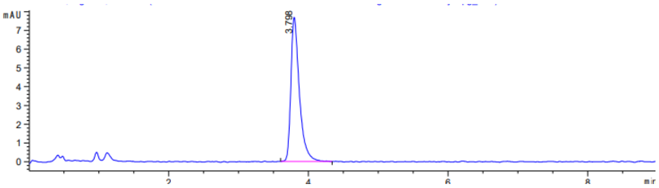

Figure 12: Formulation Chromatogram of Canagliflozin

volumetric flask. The HPLC was used to obtain a concentration within the linearity range by injecting the formulation solution in appropriate aliquots.

Recording of Chromatograms

Under the designated chromatographic conditions, standard drug solutions were injected, chromatograms were obtained, and a stable baseline was recorded. Canagliflozin was shown to have a retention period of 3.801 minutes. The injection of a sample solution taken from the preparation came next. Plotting of calibration curves was done using the peak areas of standard drugs vs the matching standard solution concentrations. The sample chromatograms' peak regions were compared, and the canagliflozin concentration was computed, as shown in Figure 6.2.8 and results are given in the Table 12.

Forced Degradation Study for Canagliflozin

Canagliflozin drug individual samples were taken for stress degradation analysis as in below table with their results. The primary and degraded samples of Canagliflozin were analyzed for the related compounds via UPLC as final method.

Degradation procedure

Forced degradation experiments of the medication in the presence of acid, alkali, H_2O_2, temperature, UV radiation, and HPLC grade water were used to establish the stability of the suggested technique.

Acid degradation

Among all, hydrolysis is frequent one for chemical process. Hydrolysis is a chemical process in which a chemical component is broken down by the addition of water to the reaction. Ionizable groups in molecules can be used to catalyze hydrolysis in acidic conditions. Drug substances are exposed to an acidic environment in order to produce 'primary degradants in a desired range of concentrations. The stability of the drug material specifies the type and concentration of acid used. For acid degradation to break down a, use hydrochloric acid (0.1 to 1 M). Co-solvents can be used to dissolve substances for stress testing that are insoluble in water. To choose a co-solvent, it is necessary to know the drug's structure. Normal stress testing trials begin at ambient temperature and progress to increased temperatures ranging from 50–70°C if no degradation is seen. Stress testing should not last for longer than seven days at the most. To prevent further breakdown, an acid or buffer is used to neutralize the degraded sample. Results shown in Table 13.

Alkaline degradation

For base hydrolysis, reagents such as sodium hydroxide or potassium hydroxide (0.1 M) are recommended. Co-solvents can be employed to dissolve the stress-testing chemicals in NaOH if they are insoluble in water. In order to choose a co-solvent, it is necessary to know the drug's structure. Normal stress testing trials begin at ambient temperature and progress to increased temperatures ranging from 50 to 70°C if no degradation is seen. Stress testing should not last for longer than seven days at the most. To prevent further degradation, the sample is neutralized with a base, or buffer shown in Table 14.

Oxidative degradation

Forcible degradation studies can also make use of additional oxidising agents. It is important to know the drug material before selecting an oxidizing agent, its concentration, and the conditions for use. It has been reported that significant

Table 13: Acid degradation of canagliflozin

S. No	*Optimized and degradation types*	*Retention time*	*Peak Area*	*%*
1	Canagliflozin Peak	4.256	$8.354e^3$	96.484
2	Impurity-A	0.453	74.552	0.861
3	Impurity-B	0.739	14.303	0.165
4	Impurity-C	1.402	10.416	0.120
5	Impurity-D	1.766	103.701	1.198
6	Impurity-E	2.522	93.170	1.076
7	Impurity-F	2.878	8.227	0.096

Table 14: Alkaline degradation of canagliflozin

S. No	*Optimized and Degradation types*	*Retention time*	*Peak area*	*%*
1	Canagliflozin Peak	4.304	$2.853e^3$	68.719
2	Impurity-A	0.343	40.236	0.969
3	Impurity-B	0.474	$1.243e^3$	29.944
4	Impurity-C	1.812	15.255	0.362

Table 15: Oxidative degradation of canagliflozin

S. No	*Optimized and degradation types*	*Retention time*	*Peak area*	*%*
1	Canagliflozin Peak	4.258	12.410	0.070
2	Impurity-A	0.470	1.708e4	95.739
3	Impurity-B	1.538	747.755	4.191

Table 16: Thermal degradation of canagliflozin

S. No	*Optimized and degradation types*	*Retention time*	*Peak area*	*%*
1	Canagliflozin Peak	4.274	$8.368e^3$	96.403
2	Impurity-A	0.453	76.794	0.898
3	Impurity-B	0.740	14.592	0.169
4	Impurity-C	1.404	10.600	0.141
5	Impurity-D	1.771	104.179	1.203
6	Impurity-E	2.530	93.280	1.086
7	Impurity-F	2.886	8.806	0.101

degradation products can be produced by hydrogen peroxide exposure for seven days at room temperature and neutral pH, or up to a maximum of 20% degradation. Electrons are transferred between anions and cations as a result of the oxidative decomposition of drugs. 'Electron transfer oxidation of amines, sulphides, and phenols produces N-oxides, hydroxylamine, sulfones, and sulfoxides. Oxidation of labile hydrogen-containing functional groups such as those in the benzylic carbon, the allylic, and the tertiary carbon positions with regard to the hetero atom can result in hydro peroxides, hydrogen or ketone formation. As shown in Table 15.

Thermal degradation

Under extreme challenging conditions both dry and wet heat has to experimented that described in ICH Q1A (accelerated testing). Both dry and moist heat has to be given to drug compounds whereas heat is for liquid drugs. For short span of time higher temperatures are provided shown nin Table 16.

CONCLUSION

The present work is simple, precise and accurate in the development and validation of canaflogzin by the UPLC method estimation. The resultant validation parameters of LoD, LoQ, % RSD, accuracy and precision recovery are as in ICH guidelines. The stress degradation has also been detailed along with impurities study.

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