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Original Article

Development and Validation of HPLC Method for Determination of Trace Level Potential Genotoxic Hydroperoxide Impurity in Canagliflozin

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ABSTRACT

Highly rapid, sensitive and selective High-performance liquid chromatography (HPLC) method was developed and validated for the determination of potential genotoxic hydroperoxide impurity in canagliflozin drug substances at trace level. HPLC column selected for the separation was Kromasil C18 (250 mm x 4.6 mm, 5 µm) kept at 25°C. The gradient elution mode was selected using mobile phase-A a 20Mm Potassium dihydrogen phosphate solution having 0.05% v/v orthophosphoric acid in water and mobile phase B as acetonitrile. The column flow rate was set to0.5 mL/min and a run time of 25 minutes. The UV detector was selected for the detection with 341 nm wavelength while injection volume was optimized to 50µL. The developed method was validated according to ICH guideline and found to be linear in the range of 0.83 ppm to 6.24 ppm for hydroperoxide impurity with a regression coefficient 0.9982. Limit of detection and limit of quantitation was found to be 0.32 ppm and 0.97 ppm, respectively. Recovery for this impurity was found between 90.58% and 114.16%. The method was found to be specific, selective, precise, and robust. The developed method can successfully be applied for the determination of hydroperoxide impurity in canagliflozin up to very low trace level concentration.

Keywords: Canagliflozin, Genotoxic, Hydroperoxide impurity, development and validation.

1. INTRODUCTION

Canagliflozin hemihydrate is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor drug used in the treatment of Type-2 diabetes [1, 2]. A potential genotoxic process and degradation hydroperoxide impurity chemically known as (2R,3R,4S,5S,6R)-2-(3-((5-(4-fluorophenyl)thiophene-2-yl)methyl)-4-methyl phenyl)-2-hydroperoxy-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol with molecular formula $C_{24}H_{25}FO_7S$ may be present in canagliflozin. Many of literature searches reveal that

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hydroperoxides impurities are genotoxic in nature [3, 4]. Structure alert by Quantitative structure-activity relationship (QSAR) software confirms that it is genotoxic. The reactivity of the hydroperoxide groups makes them particularly useful as alkylating reagents which could cause DNA damage involving genetic mutations. Oxidation is a common degradation pathway in drug substance and drug product where hydroperoxide impurities may be formed [5, 6]. Various guidelines and pharmacopoeia raise the concern to limit the potential genotoxic impurities (PGIs) in active pharmaceutical ingredients (APIs) to safety level, which is the threshold of toxicological concern (TTC).

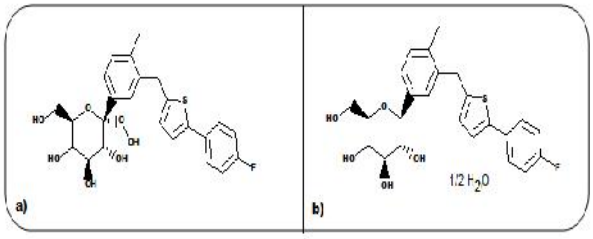

Fig 1: Structure of a) Hydroperoxide impurity and b) Canagliflozin Hemihydrate

Hence, in order to meet the regulatory requirements, it is essential to develop a very highly sensitive analytical method that can determine hydroperoxide impurity in canagliflozin at trace level. The threshold of toxicological concern (TTC) value of 1.5 µg/day intake of genotoxic impurity is permitted as per the regulatory guideline.

The concentration limit in ppm of genotoxic impurity in a drug substance is the ratio of TTC in µg/day intake and daily dose in g/day. Since 300 mg of canagliflozin is administered per day [7, 8]; therefore, the permissible limit for genotoxic impurities comes out to be 5 ppm/day. Hence the limit set for hydroperoxide impurities in canagliflozin is 5.0 ppm.

2. EXPERIMENTAL

2.1 Material

Canagliflozin bulk drug sample and hydroperoxide impurity were provided by Chemical research and development department (CRD) of Indoco Research Centre, Navi Mumbai. HPLC gradient grade acetonitrile was purchased from the J.T Baker. Potassium dihydrogen phosphate and orthophosphoric acid were purchased from Merck Chemicals (India), while water used for preparations of the solution was from Milli-Q.

2.2 Instrumentation

Waters, Alliance 2695 series HPLC system (Milford) comprising a quaternary pump, an autosampler, a thermostatted column compartment, a solvent cabinet with degasser along with photodiode array (PDA) 2998 and ultraviolet (UV) 2487 detectors were used for separation and detection. Data acquisition and calculations were carried out using Waters Empower3 software (Milford). Sartorius (Germany) analytical balance was used for weighing the materials.

2.3 Methodology

2.3.1 Chromatographic condition

This novel method was developed using Kromasil C18 HPLC column having length 250 mm and an internal diameter of 4.6 mm, which is packed with 5 µm particle size. The separation was achieved by gradient elution mode (Table-1) by using Mobile phase-A and Mobile phase-B with a flow rate of 0.5 mL/min and injection volume of 50 µL. The column temperature was maintained at 25°C (± 2°C), and the peaks were monitored at wavelength 341 nm.

Table 1: Gradient elution

Time (min)	Mobile phase-A (%)	Mobile phase-B (%)
0	65	35
5	50	50
10	40	60
15	65	35
25	65	35

2.3.2 Preparation of Solutions

2.3.2.1 Mobile Phase-A

Transfer 2.72 g of potassium dihydrogen phosphate in a 1Litre bottle containing 1000 mL water and 0.5 mL of orthophosphoric acid, shake well and filter the solution through a 0.45 µm membrane filter and degas by sonication for 2 minutes.

2.3.2.2 Mobile Phase-B - Acetonitrile

2.3.2.3 Diluent

Prepared by mixing water and acetonitrile in the ratio of 90:10 (**v/v)** and degas by sonication for 2 mins.

2.3.2.4 Standard stock solution

Transfer 5.0 mg of hydroperoxide impurity standard into 100 mL volumetric flask, dissolve in 25 mL of diluent and make up to mark with the diluent.

2.3.2.5 Standard solution

Transfer 1.0 mL of the standard stock solution into 50 mL volumetric flask and make up to mark with diluent. Transfer 2.0 ml of the above solution into 25 mL volumetric flask and make up to mark with diluent.

2.3.2.6 Preparation of test solution

Transfer 400 mg canagliflozin sample into 25 mL volumetric flask, dissolve in about 10 mL of diluent and make up to mark with diluent. Sonicate the test solution for about 2 minutes and filter the solution through 0.45 µm syringe filter.

3. RESULT AND DISCUSSION

3.1 Analytical method validation

The analytical method validation work is conducted according to the International Conference on Harmonization (ICH) guidelines. The parameter with which analytical method is validated is Specificity, Limit of detection, Limit

of quantitation, Linearity, Accuracy, Precision, Robustness and Solution stability [9-12].

3.1.1 System suitability

To check the suitability of the system and quantification of hydroperoxide impurity in canagliflozin, six standard solutions were injected and calculated the percentage relative standard deviation for the hydroperoxide impurity peak, which was less than 10 and tailing factor was less than 2.0 throughout the validation.

3.1.2 Specificity

As specificity is the capability of the method to measure the analyte response in the presence of its potential impurities. Hydroperoxide impurity was spiked in a test sample at its limit level and analysed.

Canagliflozin and hydroperoxide impurity were well separated from each other in the spiked test sample (Figure-2, Table-2). There was no interference from peaks due to blank and test sample peaks. Peak purity of canagliflozin and hydroperoxide impurity were passing for spiked test sample solution (Table-3).

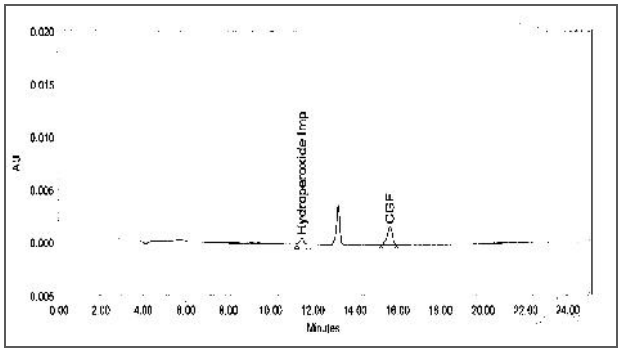

Fig 2: Canagliflozin spiked test sample chromatogram

Table 2: Retention and relative retention time

Component	Retention time (minutes)	Relative retention time
Canagliflozin	15.657	1.00
Hydroperoxide impurity	11.502	0.73

Table 3: Peak purity data

Peak name	Purity angle	Purity threshold	Peak purity
Canagliflozin (CGF)	0.433	0.462	Pass
Hydroperoxide impurity	10.153	13.687	Pass

3.1.3 Limit of detection and quantitation

A series of standard solutions of hydroperoxide impurity were prepared with a concentration ranging from 50% to 150% of target concentration (5 ppm w.r.t. sample). Limit of detection (LOD) and Limit of quantitation (LOQ) was calculated based on a residual standard deviation of the regression line and slope. Limit of detection obtained was 0.32 ppm and Limit of quantitation 0.97 ppm.

3.1.4 Linearity

Series of linearity solution of hydroperoxide impurity were prepared from LOQ to 150% of target concentration (5.0 ppm w.r.t. sample). Linearity curves were drawn by plotting the peak area of hydroperoxide impurity against its corresponding concentration of linearity solution. Regression coefficient and % y-intercept are reported (Figure-3). Regression coefficient observed was 0.9982 and % y-intercept 4.75. The method is found to be linear between 0.83 and 6.24 ppm range.

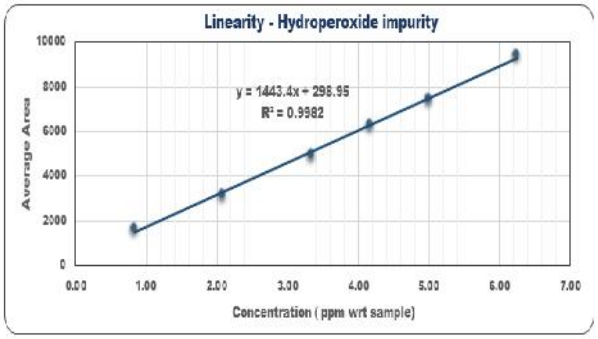

Fig 3: Linearity graph of hydroperoxide impurity

3.1.5 Precision

System precision was carried out by analysing six standard solutions of hydroperoxide impurity at a limit level concentration (5.0 ppm). The relative standard deviation for the peak area of hydroperoxide impurity was calculated and found to be 0.34 %. Precision at LOQ solution was prepared at a LOQ concentration of hydroperoxide impurity and injected six times.

The relative standard deviation for the peak area for hydroperoxide impurity obtained was 1.73%. For repeatability and intermediate precision; six solutions were prepared by spiking the hydroperoxide impurity in the test sample at a limit level concentration (5.0 ppm). Relative standard deviation observed in the spiked hydroperoxide impurity content in repeatability and the intermediate precision solution was 7.13 % and 4.91 %, and cumulative was 10.52 %.

3.1.6 Accuracy

The accuracy of the method was established by performing the recovery studies of hydroperoxide impurity, which was spiked at LOQ, 50%, 100% and 150% in the canagliflozin test sample in triplicate and analysed for its recovery. Recovery for hydroperoxide impurity obtained was between 90.58% and 114.16%.

3.1.7 Robustness

For robustness three deliberate changes were done with respect to flow rate, column oven temperature and buffer concentration. Each change consists of one lower set and one upper set (Table-4) except the column oven temperature. For each set, three preparations were made by spiking the hydroperoxide impurity in the test sample at the limit level concentration and analyzed. The relative standard deviation for spiked hydroperoxide impurity content observed was less than 10.0%. The cumulative relative standard deviation of

robustness and repeatability determination was less than 15% (Table-4).

Table 4: Robustness parameter changes

Sr.No.	Changes	RSD	Cumulative RSD
1	Mobile phase flow rate to 0.4 mL/min	0.89 %	9.45 %
2	Mobile phase flow rate to 0.6 mL/min	1.70 %	8.43 %
3	Column Oven Temperature to 30°C	5.74 %	12.37 %
4	Buffer concentration decreases by 10%	0.93 %	9.29 %
5	Buffer concentration increases by10%	1.38 %	10.65 %

3.1.8 Solution stability

Test solution stability was established by injecting the same test sample solution kept at room temperature after every six hours time interval for 24 hours. Hydroperoxide impurity content in test sample solution for all determination was calculated, and relative standard deviation for impurity content was found out to be less than 10.0%; thus solution stability was established up to 24 hours.

4. CONCLUSION

The reverse phase HPLC method is developed for the quantitative determination of hydroperoxide impurity of canagliflozin. This method is validated and found out to be linear, accurate, precise, robust and specific. Acceptable data for all method validation parameters tested and found out to be satisfactory. The developed method can suitably use by the quality control department to determine the genotoxic hydroperoxide impurity up to trace level in commercial and stability test samples of canagliflozin.

5. ACKNOWLEDGEMENT

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