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A novel, sensitive and rapid method developed for simultaneous quantification of two potential genotoxic impurities in Irbseartan by UPLC

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ABSTRACT

A new, sensitive and rapid UPLC method developed and validated for the determination of two potential genotoxic impurities namely 4-Bromomethyl-2'-cyanobiphenyl and 4-Dibromomethyl-biphenyl-2-carbonitrile at trace levels in Irbesartan by applying the concept of threshold of toxicological concern (TTC), a limit of 5.0 ppm each were calculated based on the maximum daily dose of the drug substance. The proposed method is specific, linear, accurate and precise. The calibration curves show good linearity over the concentration range of 0.01–0.075 µg/mL for genotoxic impurities in Irbesartan. The correlation coefficient obtained is >0.999 in each case. Method has very low limit of detection (LOD) and quantification (LOQ). LOD and LOQ of all genotoxic impurities are as low as 0.0025 µg/mL and 0.01 µg/mL respectively. Method has accuracy with recovery in the range of 90.0–104.0% for all the genotoxic impurities. This method is a good quality control tool for quantitation of all the genotoxic impurities at very low levels in Irbesartan.

KEYWORDS: Genotoxic impurities, UPLC, Irbesartan, method validation.

INTRODUCTION

Irbesartan is an angiotensin II receptor antagonist used mainly for the treatment of hypertension. Irbesartan was developed by Sanofi Research It is jointly marketed by sanofi-aventis and Bristol-Myers Squibb under the trade names Aprovel, Karvea, and Avapro.[1-2]

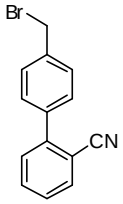
Starting materials, intermediates and by-products are often found as impurities in drug substances. Some of these known impurities are potential mutagens or carcinogens, but can be difficult or impossible to eliminate completely from the synthetic scheme. In Irbesartan, two potential structural alerts for genotoxicity were found due to the starting material used in the manufacturing process.

Based on the current regulatory guidances for genotoxic impurities, analytical methods should be developed to meet the required limit of 1.5µg/day daily intake of individual impurity.[3-6]

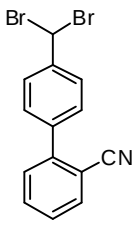
MATERIALS AND METHODS

Materials and reagents

Samples of IRB and its genotoxic impurities (Fig.1) were received from Pharma manufacturing company, Hyderabad, India. HPLC grade Acetonitrile, Potassium dihydrogen phosphate (KH2PO4) and orthophosphoric acid were purchased from Merck, Schuchardt OHG, Germany. Water was purified by a milli-Q-water purification system (Millipore, Bedford, MA, USA) used for preparation of all the solutions.



(a) Bromomethyl-2'-cyanobiphenyl (Impurity-1)



(b) 4'-Dibromomethyl-biphenyl-2-carbonitrile (Impurity-2)

Fig.1 Structures of Potential genotoxic impurities of Irbesartan

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Equipment

The HPLC system used for method development and method validation was a Waters 2695 binary solvent delivery pump, an auto sampler and a 2996 photo diode array detector.The output signal was monitored and processed using Empower2 software(Waters) on Pentium computer (Digital Equipment Co).

Chromatographic conditions

The chromatographic separation was achieved using Waters Acquity BEH Symmetry C-18, column 100 mm x 2.1 mm, 1.7 µm. Solvent A was 2mM KH_2PO_4 buffer and pH adjusted to 2.5 with dilute orthophosphoric acidand Solvent B was acetonitrile: water in the ratio of 90:10 (v/v). The gradient programme : T (min) / % B: 0.01/40, 2/40, 10/60, 15/60, 15.1/40 and 20/40 with flow rate of 0.2 mL/min was employed. The injection volume was 10.0 µL. The column temperature was maintained at 27°C and the chromatogram was monitored at the wavelength of 210 nm. A mixture of Acetonitrile and water in the proportion of 50:50 (v/v); respectively used as a diluent.

Preparation of standard and sample solutions

Standard solution preparation

A stock solution of impurity blend (mixture of Imp-1 and Imp-2) 10 µg/mL was prepared in diluent. An appropriate dilution was made from the stock to get the standard solution of 0.05 µg/mL of the two genotoxic impurities.

Sample solution preparation

A Sample solution of 10 mg/mL was prepared for Irbesartan in diluent.

Analytical method validation

The developed chromatographic method was validated for sensitivity (limit of detection and limit of quantification), precision (repeatability and intermediate precision), linearity, accuracy, robustness and solution and mobile phase stability. [7-8]

Sensitivity

Sensitivity of the method was proven by establishing the limit of detection (LOD) and limit of quantitation (LOQ) for all the four impurities with signal-to-noise-ratios of 3:1 and 10:1, respectively. LOD and LOQ were determined by injecting a series of diluted solutions having known concentrations of

impurities. Precision of the method was also carried out at the LOQ level by injecting six individual preparations of the two impurities at LOQ concentration by calculating %RSD for the areas of each peak. The accuracy of the method was checked for all impurities at LOQ level by analyzing three replicate samples of IRB (10mg/mL)spiked with the two impurities at LOQ level and calculating the percentage recovery.

Precision

Precision was determined through repeatability (intra-day) and intermediate (inter-day) precision. Precision of the method was checked by injecting six individual preparations of the two impurities of IRB at 0.05 µg/mL level with respect to concentration of the drug substance. %RSD for area of each impurity was calculated.

Intermediate precision (ruggedness) of the method was evaluated by injecting six individual preparations on different days in the same laboratory.

Linearity

To establish the Linearity of the method, calibration solutions were prepared by diluting the impurity stock solution to the required concentrations at six different levels ranging from LOQ to 0.075 µg /mL of the two impurities of IRB. The linearity graph was drawn with concentration of linearity solution on x-axis and mean area counts on y-axis. The slope, y-intercept and correlation coefficient of the calibration curve were calculated.

Accuracy

To determine accuracy of the method, a recovery study was carried out by analyzing the drug substance spiked with impurities. Known amount of impurities were spiked to the drug substance IRB at different concentration levels of LOQ, 50%, 100% and 150% of the specification limit of the drug substance concentration (10 mg/ mL). Each concentration level was prepared in triplicate. Percentage recoveries for the two impurities in the drug substance IRB were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between the two impurities were evaluated.

To study the effect of flow rate on the resolution, it was changed by 0.1 units from 0.1mL/min to 0.3mL/min instead of 0.2 mL/min.. The effect of column temperature on resolution was studied at 32 °C and 22 °C instead of 27°C .In the all above varied conditions, the components of the mobile phase were held constant as per the method.

Solution stability and mobile phase stability

The solution stability of the two impurities was carried out by leaving spiked sample solution in tightly capped volumetric flask at room temperature for 48h.Content of the impurities were determined for every 12h interval up to the study performed. Mobile phase was also carried out for 48 h by injecting the freshly prepared sample solutions for every 12h interval. Content of the impurities were checked in test solutions. Mobile phase prepared was kept constant during the study period.

RESULTS AND DISCUSSION

Method Development and optimization of chromatographic conditions

The present method was developed by altering stationary and mobile phases sequentially and observing their influence on the resolutions of the two impurities. As a preliminary investigation, Acquity BEH Symmetry C18 (100mm*2.1mm×1.7µm) column was selected as appropriate stationary phase. Since, all the two impurities were neutral, the investigation is commenced with a water and acetonitrile taken in the ratio of 70:30 (v/v). All compounds were eluted, but the IRB peak shape was distorted leading to a broad peak and imp-1 was co eluted with IRB peak. It was planned to include acidic buffer in the mobile phase to get the better peak shape of IRB and to get the better resolution of the two impurities. In this condition, the IRB peak shape was improved but the interference of the impurities and the peaks due to IRB were observed. Various stationary phases like Acquity BEH Symmetry shield RP18, Acquity phenyl columns were tried to control the interference and different gradient programs of the mobile phase were studied. The method was finally optimized with Acquity BEH Symmetry C18 column with the mentioned chromatographic conditions and achieved the reasonable peak shape of IRB and adequate resolution between the two impurities.

Validation of the Method

Precision

In the precision study, the % relative standard deviation (R.S.D.) for the content of two impurities were within 0.6. The %RSD obtained in the intermediate precision study for the content of two impurities are well within 0.8.The %RSD values are presented in Table-1. The typical chromatogram was presented in Fig.2.

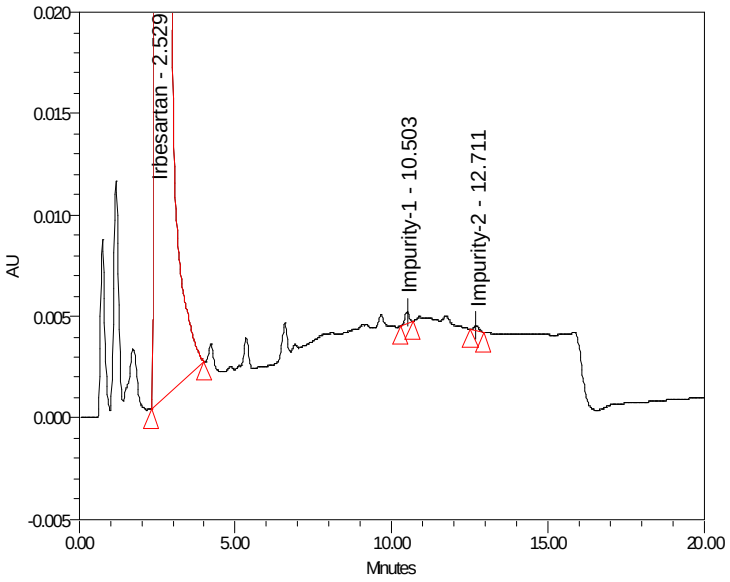

Fig.2 Typical LC chromatogram of Irbesartan spiked with 5.0ppm of two potential genotoxic impurities

Table-1. LOD,LOQ, Regression and Precision Data

Parameter	Impurity-1	Impurity-2
LOQ (µg/mL)	0.010	0.010
LOD (µg/mL)	0.003	0.003
Linearity		
Slope	105136.1	56954.0
Intercept	22.8	12.7
Correlation coefficient	0.9999	0.9999
%y-intercept at 100% level	0.57	0.58
r^2 value	0.999	0.999
Precision (%RSD)	0.64	0.59
Intermediate Precision (%RSD)	0.43	0.76
Precision at LOQ (%RSD)	1.64	3.71

Sensitivity

The determined LOD, LOQ, precision and accuracy at LOQ values for all the two impurities are reported in Table-1.

Accuracy

Recovery of the two impurities in IRB ranged from 90.0 to 104.0%. The %recovery values for impurities were presented in Table-2.

Table-2:Evaluation of Accuracy

Impurity Name	Spike level (%)	Added (µg/mL)	Recovered (µg/mL)	Recovery (%)
Impurity-1	LOQ	0.010	0.0090	90.0
	50	0.025	0.0245	98.0
	100	0.050	0.0520	104.0
	150	0.075	0.0760	101.3
Impurity-2	LOQ	0.010	0.0095	95.0
	50	0.025	0.0260	104.0
	100	0.050	0.0510	102.0
	150	0.075	0.0740	98.7

Linearity

Linearity calibration plot for the two impurities were obtained over the calibration ranges tested i.e., LOQ to 150% of the specification limit. The correlation coeffiecient obtained was greater than 0.999. The result shows that an excellent correlation existed between the peak area and the concentration of the two impurities. The regression data is presented in Table-1.

Robustness

In all the deliberate varied chromatographic conditions (flow rate and column temperature), all impurity peaks were adequately resolved and elution order of the impurities remained unchanged. The resolution between the two impurities were greater than 5.0.

Solution and Mobile phase stability

No significant changes in the content of the two impurities were observed during solution stability and mobile phase experiments. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and solutions in the mobile phase were stable for up to 48 h during determination of impurity content in IRB.

CONCLUSION

The proposed method describes a sensitive, simple, rapid and accurate validated UPLC method for estimation of two different potential genotoxic impurities in the drug substance.

This method shows adequate linearity, precision and accuracy with no sample matrix interference observed. This method can be further applied to control any of these two potential genotoxic impurities to other drug substances.

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