ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

A simple and sensitive RP-UPLC method for the determination of 2-(4', 4'-Dibromomethylphenyl) benzonitrile impurity content in Irbesartan drug substance

T.Kaleemullah[1*], Mansur Ahmed [1], Hemant Kumar Sharma[2]

[1]Post Graduate and Research Department of Chemistry, Islamiah College, Vaniyambadi, 635752, India

[2]Aurobindo Pharma Ltd Research Centre, 71 &72 Indrakaran Village, Sanga Reddy Mandal, Medak Dist, India

Received:
16th Oct 2012
Received in revised form:
29th Oct 2012
Accepted:
5th Nov 2012
Available online:
15th Nov 2012

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

This paper describes the development and validation of a simple reverse phase ultra performance liquid chromatography (RP-UPLC) method for the quantitative determination of 2-(4',4'-Dibromomethylphenyl)benzonitrile impurity content at low level in Irbesartan drug substance. Separation was achieved with 100mm x 2.1mm, 1.7µm particle size, ODS column. The mobile was a gradient prepared by simple phosphoric acid buffer, and Acetonitirle at a flow rate of 0.1 mL min^{-1}, UV detection was performed at 205nm. The method was validated to confirm selectivity, precision, linearity and accuracy parameters as per ICH guideline. Because of its speed and accuracy the detection limit and quantification limit are found to be 0.41 µg ml^{-1} and 1.46 µg ml^{-1} respectively. Repeatability is good, with a relative standard deviation of 2.1% to 3.2%. The drug substance was subjected to stability studies as well as stress condition of hydrolysis, oxidation, photolytic, thermal and humidity degradation using optimized method condition to enhance low level of detection within the minimum acquisition time indicating the accuracy of the optimized RP-UPLC method. This simple, efficient methodology can be used for quality control bulk manufacturing as well as routine analysis.

KEYWORDS: RP-UPLC, 2-(4',4'-Dibromomethylphenyl)benzonitrile, Irbesartan, validation.

1. INTRODUCTION

Irbesartan, is an non-peptide compound belongs to the family of Angiotensin II receptor antagonist [1-3]. Generally used for the treatment of high blood pressure (hypertension), kidney damage due to diabetes (diabetic nephropathy) and congestive heart failure [4-9]. The Chemical name of Irbesartan (IRB) is 2-Butyl-3-[[*p*-(0-1*H*-tetrzol-5-yl)phenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one. The molecular formula is $C_{25}H_{28}N_6O$ and the molecular weight is 428.53. N-Bromosuccimide (NBS) is used as brominating agent in the synthetic process of IRB [10]. In this process, NBS is used to convert 2-(4'-Methylphenyl)benzonitrile in to 2-(4'-Bromomethylphenyl)benzonitrile (or) Bromo impurity, which is the key material for the synthesis of IRB. The excess bromine residue, if present leads to its byproduct 2-(4',4'-Dibromomethylphenyl)benzonitrile (or) Dibromo impurity as depicted in Fig 1, with encircling of IRB active moiety.

NBS is an oxidizing and brominating agent, used in the radical substitution like allylic bromination as well as convenient source of cationic bromine [11, 12]. The bromination of NBS for substrate such as alcohol and amines, followed by elimination of Hydrogen bromide in the presence of a base, leads to the product of net oxidation in which no bromine has been incorporated as well as in Wohl-Ziegler reaction [13]. Bromine is considered as toxic substance according to OSHA 29 CFR 1910.1200 and its toxicity destroys thyroid and metabolism [14-15]. The residual organic impurities can come through the manufacturing process of the drug substance; the criteria for their acceptance are based on pharmaceutical studies or known safety data [16]. In view of this, monitoring of Dibromo impurity in IRB drug

*Corresponding author: T.Kaleemullah | Post Graduate and Research Department of Chemistry, Islamiah College, Vaniyambadi, 635752, Vellore Dist, India |E-mail: kaleemullaht@gmail.com | Tel.: +91-4174-235206| Fax: +91-4174-235876

substance is essential for preserving the desired quality of active substance. There are so many of the analytical procedures have been reported in the literature for the estimation of IRB [17-18] as well as NBS [19]. Hence, no method is available for determination of the residual Dibromo impurity. So, analytical liquid chromatographic technique UPLC has been chosen, due to advanced technology of chromatographic principles to run separations under minimal time with excellent sensitivity and resolution with higher pump operating pressure with low dimension columns. Therefore the developed and optimized simple RP-UPLC methodology to determine the content of Dibromo impurity in Irbesartan drug substance shows better separation of the peak with sufficiently at low level of detection. To the best of our knowledge no report has been published on the analysis of Dibromo impurity in IRB drug substance in literature.

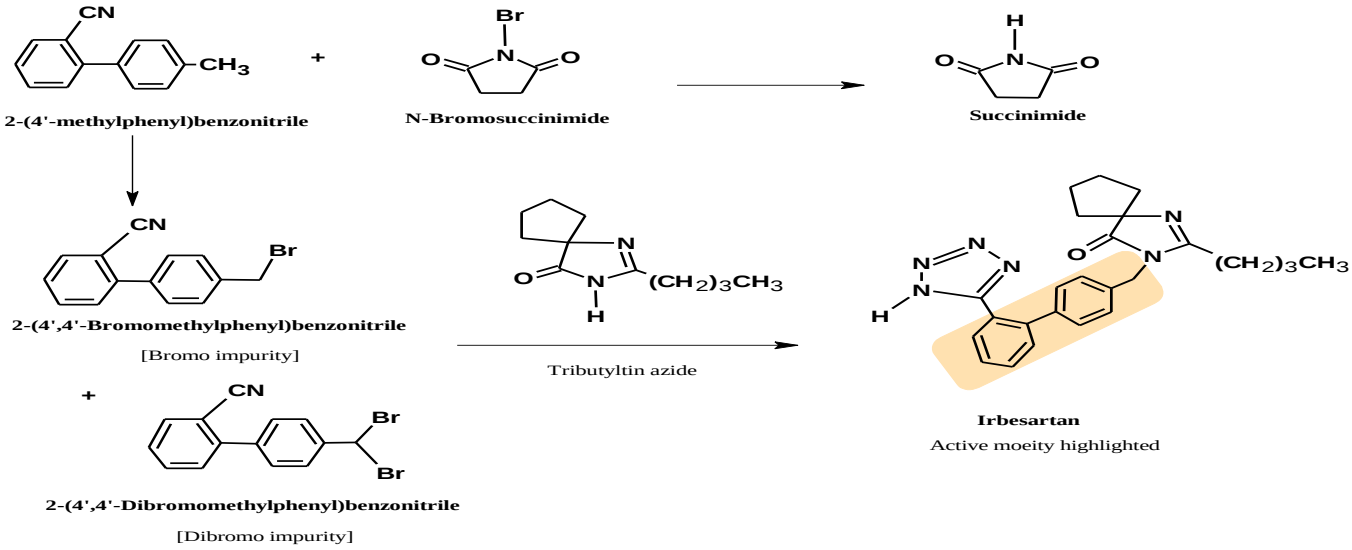

Fig.1. The Dibromo impurity and encircled active moiety in the chemical structure of IRB

2. MATERIALS AND METHODS

2.1. Chemicals, Reagents, and Samples

The standard, sample of IRB drug substance and known related substances of IRB, such as Impurity-I, Impurity-II, Impurity-III, Impurity-IV, USP related compound A and 2-(4',4'-Dibromomethylphenyl)Benzonitrile were obtained from Aurobindo Pharma Ltd., Hyderabad. Analytical reagent AR grade *ortho*-phosphoric acid**,** HPLC grade methanol and acetonitrile were purchased from E.Merck India. Highly purified water obtained from Millipore purification system. The photo degradation study was carried out in a photostability chamber, Model: PSC062.AHA.C (Sanyo Gallenkamp PLC, Leics, UK). Thermal studies carried out in a thermal oven, Model: NW-CON- 51 (Newtronics, Mumbai, India) and humidity studies were carried out in humidity chamber (containing saturated aqueous solution of potassium nitrate by creating ~85% relative humidity at 25°C).

2.2. Ultra performance Liquid Chromatography (UPLC)

An, UPLC system equipped with acquity binary solvent manager, sample manager and PDA detector with Empower-2 data handling system [Waters Corporation, MILFORD, MA 017 57, USA] was used for chromatographic separations. The mobile phase-A consists of 0.01%v/v *ortho*-phosphoric acid in purified water and mobile phase-B was acetonitrile. The analysis was carried out on Acquity UPLC BEH C18 (Waters Corporation Ltd.,) 100 mm long, 2.1 mm i.d., 1.7 µm particle diameter column, thermo stated at 30°C. Mobile phase was passed through the column at a flow rate of 0.1 mL min^{-1} and pump was in gradient mode and the program was as follows: Time (min) / A (v/v) : B (v/v); $T_{0.01}$ / 40:60, T_{20}/ 25:75, $T_{20.5}$/ 40:60, T_{25} / 40:60. The injection volume was 8 µL. The acquisition time for the standard and sample was 20 min and the detection was set at 205 nm. The mixture of methanol and water in the ratio of 80:20 v/v was used as diluent. The retention time of the Dibromo peak was found to be at about 9.2 minutes as shown in the Fig 2. Relative standard deviation for the peak areas of six replicate injections of standard peak is not more than 5.0%.

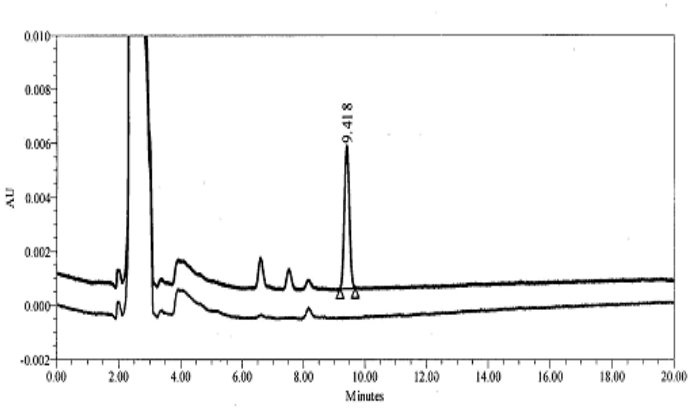

Fig.2. Overlay chromatogram in ascending order for Diluent and standard

2.3. Standard and Sample Solutions

Preparation of Standard Solution

Accurately weigh and transfer about 10mg Dibromo reference standard into a 50 mL clean, dry volumetric flask, add 35ml of methanol and sonicate to dissolve, make up to volume with water. Dilute 5 mL of this solution to 50mL with diluent. Dilute 5mL of this solution to 100mL with diluent. Further, dilute 5mL of this solution to 50mL with diluent. Filter through 0.22µ porous membrane.

Sample Solution

Accurately weigh and transfer about 100 mg of sample into a 10 mL clean, dry volumetric flask, add 8mL of methanol and sonicate to dissolve. Make up to volume with water. Filter through 0.22µ porous membrane.

3. RESULTS AND DISCUSSION

3.1. Development and optimization of chromatographic method

The scope of the chromatography method was to separate Dibromo impurity from IRB and its five related impurities. Method development was initiated with IRB drug substance and Dibromo impurity solubility studies, based on the experiments mixture of water and methanol in the ratio of 80:20 v/v was chosen as diluent. The impurities of the IRB were co-eluted with Dibromo impurity using different makes of stationary phases like C_8 and C_{18} as well as with different composition of mobile phases and organic modifiers. Hence, 0.1%v/v Ortho-phosphoric acid was chosen as buffer solution to exclude the precipitation of aqueous salt buffers with combination of higher organic modifier ratios. During the evaluation of various column chemistries, C_{18} has shown better resolution. In the optimized chromatographic condition, the Dibromo impurity was well separated and the retention time was found to be about 9.4 min with good asymmetry. Therefore the proposed RP-UPLC method was found to be specific for Dibromo impurity determination in IRB has been validated to evaluate the performance characteristics of the analytical method.

3.2. Method Validation

The proposed RP-UPLC method was validated as per the ICH guideline[20] individually in terms of specificity, forced degradation studies (stability indicating nature), limit of detection, limit of quantification, linearity, accuracy, precision (system precision, method precision and intermediate precision or ruggedness) and stability of sample solution.

3.3. Selectivity

Specificity is the ability of the method to determine the individual analyte in presence of other related substances of drug substance. For specificity determination, diluents, all the related substances of IRB and Dibromo impurity solutions were prepared individually and injected into UPLC as per methodology to confirm the retention times. After that solutions of IRB drug substance spiked with all related substances of IRB including Dibromo impurity (all spiked sample) were prepared and injected into UPLC as per methodology to confirm any co-elution with analyte peaks from respective diluents as well as with any of related substances peaks. The peak homogeneity was verified using Waters Empower software and found to be pure as summarized in Table 1.

Parameters	Components	Result	
System suitability [a]	Retention Time (R_T)	9.0 to 9.4 minutes	
	Peak Tailing	1.3	
	Plate counts	5341	
Selectivity [b]	**Solution**	**Purity angle**	**Purity Threshold**
	Standard	7.215	7.551
	Control sample	2.335	2.741
	Spiked sample	5.867	6.868

Table 1: System suitability and selectivity experimental summary data

[a] Average experimental observation,

[b] Empower software criteria for peak purity: Purity angle should be less than purity threshold

An overlay chromatogram of IRB drug substance, spiked sample with standard and all impurities spiked sample chromatogram is shown in Fig

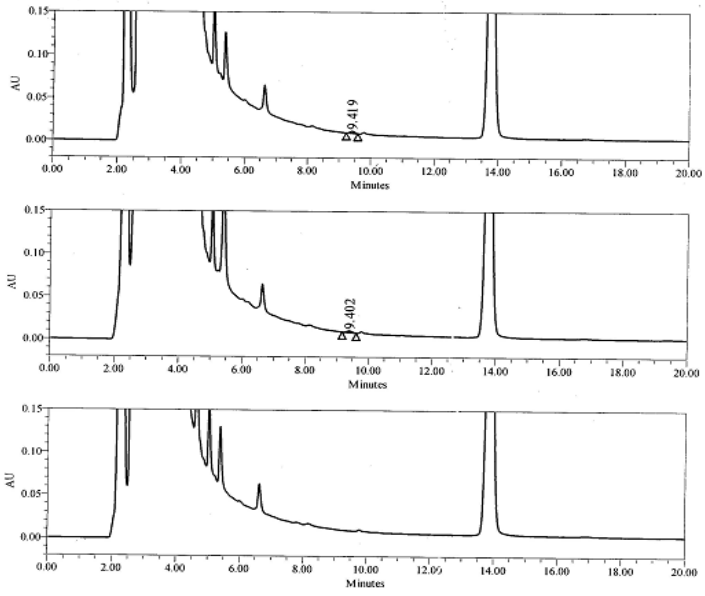

Fig.3. Overlay chromatogram in ascending order for IRB sample, spiked sample with standard and spiked sample along with all impurities

The stability indicating nature of the method was further evaluated by performing the forced degradation studies. To elucidate the inherent stability characteristics of the active substance stress testing is to be carried out to identify degradation products as per International Conference on Harmonization [20]. The integral part of stress study includes susceptibility to oxidation, hydrolytic, photolytic, humidity and thermal studies. In this study, Irbesartan drug substances were subjected to the following stress conditions:

- Thermal stress: The drug substances were subjected to dry heat at 105°C for 120 hrs.
- Stress study under photolytic condition (as per ICH) [20]: A sample was exposed to photolytic degradation (white fluorescent light (10K Lux/120 hrs followed by UV light 200 watt-hours/m^2.
- Stress study under humidity condition: A sample was exposed to degrade under 80% RH at 25°C for 120hrs.
- Stress study under an oxidative condition: A sample solution was mixed with a 30% H_2O_2 solution and exposed to 85°C for 60 min.
- Stress study under hydrolytic condition: A sample solution was mixed with purified water and kept aside for 12h.

The required concentration of unstressed samples and each stressed sample were prepared and injected into UPLC using the analytical conditions. In unstressed samples, the result of Dibromo impurity content was found to be not detected and in each stressed sample, there was no peak observed at the retention time of Dibromo impurity. Due to insoluble nature of the sample in water the analysis of the sample has been conducted with filtrates. Hence, based on the above observation, the stressed conditions did not induce the degradation of API leading to Dibromo impurity. Therefore, this method is specific, selective and indicative nature. From the overall experiment, a system suitability rule has been established from the above experiment for the following parameters that, the column efficiency as determined from the Dibromo impurity peak is not less than 4000 USP plate count and the , USP peak tailing should not be more than 1.5.

3.4 Linearity:

By measuring area responses at different levels of Dibromo impurity over the range of 15% to 150% of analyte concentration the linearity data were validated. Required concentrations of solutions were prepared from stock solution for different level of 1.3µg mL^{-1}-12.8µg mL^{-1}, correlation co-efficient was found to be 0.99956. The statistical study was conducted with the peak area response with concentration of the Dibromo impurity using least square linear regression analysis plot [Area count in terms of Area count (AU) at Y-axis Vs Concentration (ppm) at X-axis]. The obtained result for statistical parameters slope, intercept, residual standard on deviation response and correlation co-efficient were shown in Table 2.

3.5. Sensitivity:

Sensitivity of method was determined by establishing the limit of detection (LOD) and limit of quantification (LOQ) values based on the residual standard deviation of a regression line and the slope. Dibromo impurity standard solution was injected into UPLC chromatograph from lower concentration (0.1µg mL^{-1}) to higher concentration (15µg mL^{-1}). A plot of peak area (AU) versus concentration (µg mL^{-1}) was drawn and LOD/LOQ values were predicted by STEYX (SD) and slope (S) method using the formula 3.3 x SD/S for LOD and 10 x SD/S for LOQ. The predicted concentration levels of LOD and LOQ solutions of Dibromo impurity was verified for precision by analyzing six replicates. The achieved précised values are tabulated in Table-2 and the chromatogram with these concentrations also shown in Fig 4.

Statistical parameters	Result
Calibration range (ppm)	1.3 – 12.8
Calibration Points	7
Correlation co-efficient (CC)	0.99956
Residual sum of square (r^2)	0.99911
Slope (S)	2909
STEYX (SD)	373
Intercept	409
Limit of Detection (ppm)	0.41
Limit of Quantification (ppm)	1.46
Precision of Limit of Detection (%RSD)	18.0
Precision of Limit of Quantification (%RSD)	2.2

Table 2: Summary of Linearity experimental data

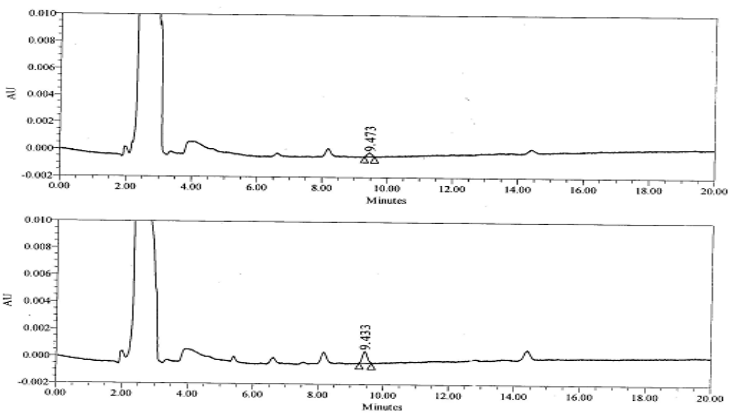

Fig.4. Chromatogram in ascending order for LOQ and LOD level concentration

3.5. Precision

The method was assessed by six replicate injections of Dibromo impurity standard solution (10µg mL^{-1}) into UPLC chromatographic system, and the percentage relative standard deviation of response for six replicate measurements was found to be 0.9% proves the repeatability of the system. Based on the TTC concept [21] the maximum daily dose for Irbesartan is 300mg, therefore the permitted daily exposure concentration was found to be 5ppm. Therefore the same concentration was considered in overall experiment. Reproducibility of the

method (Method precision) was demonstrated by preparing six replicate sample preparations by spiking known concentration (~5ppm) of Dibromo impurity in random selection of one batch of IRB drug substance. The samples were analyzed as per method, and the content of Dibromo impurity was determined. The degree of reproducibility is known as ruggedness, obtained by the analysis of the same sample concentration (which is used in the method precision) under a variety of conditions using different series of column, with different user on different day by using new standard also found to be within the acceptance criteria also proves that the method is rugged for the determination of Dibromo impurity under the experimental conditions. Achieved result along with statistical data tabulated in the Table 3.

S.No	Repeatability of Standard [c] (AU)	Repeatability of Sample [d] (ppm)	Reproducibility of Sample [d] (ppm)
1	59360	4.425	4.325
2	59235	4.325	4.318
3	59082	4.125	4.316
4	59287	4.425	4.128
5	58874	4.325	4.145
6	59170	4.125	4.261
Mean	59168	4.292	4.249
SD	173	0.14	0.09
RSD (%)	0.3	3.2	2.1
95%CI(±)	304	0.34	0.22
Overall statistical data (n=12)		Mean	4.270
		SD	0.11
		RSD (%)	2.7
		95%CI(±)	0.28

Table 3: Precision data statistically obtained for determination of Dibromo impurity

3.6. Stability of sample solution

The IRB drug substance were spiked with known concentration of Dibromo impurity (10µg mL^{-1}) with respect to sample concentration and the solution was stored at 25 ± 2°C temperature condition then injected into chromatographic system at different time intervals. The content of Dibromo impurity was determined at each interval, the sample solution was found to be stable over a period of 7 hours. The % difference between the peak area obtained at initial and different time interval was found to be 2.1. However, it is observed from the experimental condition the stability of the sample was found to be stable for at least 7 hour at room temperature (~25°C).

3.7. Accuracy

The recovery studies during the method was evaluated by preparing sample solution spiked with known amount of Dibromo impurity at different concentration levels in the range between 25%, 50%, 100% and 150% with respect target concentration with IRB sample. Each concentration of sample solution was prepared in triplicate and analyzed as per the method. The overall percent recoveries were found to be within the acceptable criteria**,** when calculated against the known added amount, and the results also shown in Table 4**,** indicating that the method is accurate.

Components	Level-I/ 20%	Level-II/ 50%	Level-III/ 100%	Level-IV/ 150%
Added (ppm) [e]	1.431	2.418	4.416	6.336
Found (ppm) [e]	1.432	2.380	4.392	6.273
Recovery (%)	100.0	98.5	99.5	99.1
%RSD [f]	2.6	2.9	2.6	0.6
Overall statistical data				
Mean Recovery (%)	**SD [g]**	**%RSD [g]**	**95%CI (±)[g]**	
99.3	2.2	2.2	2.3	

Table 4 Experimental summary for Accuracy analysis
[e] Calculated with respect to 5ppm, [f] Average of n=12 determination, [g] Overall experimental result

3.8. Robustness

To assess the robustness of the method, experimental conditions were deliberately altered for specified range for temperature (±5°C), flow of mobile phase (±10%) and wavelength (±5nm). The result obtained from the robustness indicated that, the experimental method parameters were tolerance limit with minor changes to optimize the method.

3.9. Stability Studies

To present stability studies on IRB drug substance for the determination of Dibromo impurity content, the analysis were conducted on samples from variable sources of temperature and humidity storage of accelerated (40°C/75%RH), long term (25°C/60%RH) and refrigerated (5°C±3°C) storage condition [22]. The results obtained from the above storage conditions are found to be not detected. Hence the formation of Dibromo in IRB drug substances resulting as process related impurity, in view of that the sample shows no degradation profile with respect to storage at different conditions of temperature and humidity. The experimental condition shows precise results with good repeatability on inter and intra day with other analyst and different chromatograph with different lot of the column shows the method is rugged for the determination of Dibromo impurity content.

4. CONCLUSION

The proposed stability-indicating ultra performance liquid chromatographic method results demonstrated that the method is specific, sensitive, linear, precise, rugged and accurate. The method provided the satisfactory validation data for the tested parameters as per the ICH guidelines.

Hence the proposed method may be conveniently used in bulk manufacturing laboratories.

5. ACKNOWLEDGEMENTS

The authors express their sincere thanks to Post Graduate and Research Department of Chemistry, Islamiah College (Autonomous), Vaniyambadi, Tamil Nadu, India, and APL Research centre-II, Hyderabad, India for their co-operation

.

6. REFERENCES

1. Birkenhager WH, Leeuw PW. Non-peptide angiotension type I receptor antagonist in the treatment of hypertension. J Hypertens.1999;177:876-81.
2. Burnier MW. Angiotensin II type I receptor blocker. Circulation. 2001;103(6):904-12.
3. Aronson JK. Irbesartan: Meyler's side effect of cardiovascular drugs, Angiotensin II receptor antagonists, General Information. Elsevier publication. 2009, 30-31.
4. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, and Pohl MA et.al. Collaborative Study Group: Renoprotective
5. effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type diabetes
6. N Engl J Med. 2001; 345(12): 851-60.
7. Massie BM, Carson PE, McMurray JJ and Komajda M, McKelvie R, Zile MR, Anderson S, Donovan M, Iverson E, Staiger C, Ptaszynska A, I-PRESERVE Investigators. Irbesartan in patients with heart failure and preserved ejection fraction. N Engl J Med. 2008; 359 (23):2456-63.
8. Lee HY, Kim CH, Acute oliguric renal failure associcate with angiotensin II receptor antagonist. Am J med. 2001; 111(2):162-3.
9. Anglada Ointadojc, Gallego Oureti P, Zapata Lopez H, Cayon Blanco H. Fracaso renal agudo asociado con [Acute renal failure associate with Irbesartan]. Med Clin (Barc). 1999; 113(9):358-9.
10. Markham A, Spencer CM, Jarvis B. Irbesartan: an updated review of its use in cardiovascular disorder. Drugs.2000; 595:1187-206.
11. Johnson CI, Pharmacology of Irbesartan. Expert Opinion on Investigational Drugs. 1999; 8(5):655-670.
12. Process for the preparation of Irbesartan, US patent no: US 8,106,216 B2., 2012.
13. Dauben Jr Hyp J, McCoy Layton L. N-Bromosuccinimide. I. Allylic Bromination, a General Survey of Reaction
14. Variables. J Am Chem Soc. 1959, 81 (18): 4863–4873.
15. http://www.organic-chemistry.org/chemicals/oxidations/n-bromosuccinimide-nbs.shtm
16. Carl Djerassi. Brominations with N-Bromosuccinimide and related compounds, The Wohl- Ziegler reaction. Chem Rev. 1948; 43 (2): 271–317.
17. http://preventdisease.com/news/10/030910_bromine_toxicity_thyroid.shtml.
18. Withers RMJ, and Lees FP. Journal of Hazardous material, 1986, 12(3):279-299.
19. International conference on harmonization Q3A (R2): Draft revised Guidance on impurities in new drug substances; 2006.
20. Basaviah K, Somashekar BC. Titrimetric and spectrophotometric determination of Metaprolol tartrate in pharmaceuticals using N-Bromosuccinimide. E Journal of chemistry. 2007;4(1): 117-127.
21. Ibrahim A Darwish, Samiha AH, Ashraf MM,Ahmed IH. N-Bromosuccinimide/system for spectrophotometric determination of H2-receptor antagonists in their dosage forms. Int J Res Pharm Sci. 2010; 1(2): 86-93.
22. Kaleemullah T, Mansur Ahmed, Hemant Kumar Sharma, Validation of RP-HPLC method for the quantification of N-Bromosuccinimide in Angiotensin II receptor antagonists in Pharmaceuticals. Der Pharma Chemica. 2011; 3(1): 372-380.
23. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use, ICH harmonized tripartite Guideline, Validation of analytical procedure: Text and methodology, Q2 (R1), step 4, 2005.
24. Kroes R, Kleiner J, Renwick A. Toxicological sciences. 2005; 86(2): 226.
25. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use, ICH harmonized tripartite Guideline, Stability testing of new drug substances and products Q1A (R2), 2003.

Conflict of Interest: None Declared

