September 2010 ISSN : 0974-7419 Volume 9 Issue 3

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 9(3) 2010 [320-325]

Development and validation of a HPLC method for separation and simultaneous determination of process-related substances of Irbesartan in bulk drugs

I.Ugandar Reddy[1,2]*, K.Hima Bindu[1], A.Madhuri[1], A.Rammohan Rao[1], Jaydeep Lilakar[1], P.Nageswara Rao[2], V.Ranga Reddy[1]

[1]Dr.Reddy's Laboratories Ltd., Active Pharmaceutical Ingredients, IPDO, Bachupally, Hyderabad - 500 072, A.P., (INDIA)

[2]Department of Chemisty, National Institute of Technology, Warangal - 506 004, A.P., (INDIA)

E-mail : ugandharri@drreddys.com; ugandar_reddy@yahoo.co.in

Received: 27th March, 2010 ; Accepted: 6th April, 2010

ABSTRACT

A simple, inexpensive and rapid reversed-phase high-performance liquid chromatographic method has been developed for the separation and simultaneous determination of related substances of Irbesartan, an anti-hypertensive drug, in bulk drugs. Degradation studies were performed on the bulk drug by heating to 105°C, exposure to UV light at an energy of 200 Watt hours/m^2 and to Visible light at an illumination of not less than 1.2 million lux hours, acid (0.5 N Hydrochloric acid), base (0.05 N sodium hydroxide), aqueous hydrolysis and oxidation with 6.0% v/v hydrogen peroxide. Considerable degradation was observed under acid, base and oxidation conditions. Good resolution between the peaks corresponding to impurities produced during synthesis, degradation products and the analyte was achieved on a Symmetry shield RP 18 LC column using a mobile phase consisting of a mixture of aqueous potassium dihydrogen phosphate and acetonitrile. Validation of the method was carried out as per ICH requirements.

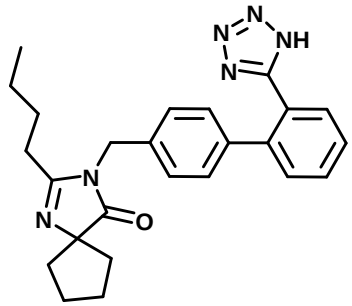
KEYWORDS

Column liquid chromatography;
Degradation studies;
Irbesartan;
Method validation.

INTRODUCTION

Irbesartan, 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl] phenyl} methyl)-1,3-diazaspiro [4.4] non-1-en-4-one (Figure 1), is an angiotensin II receptor (AT_1 subtype) antagonist mainly for the treatment of hypertension. As shown in seven placebo controlled clinical trials, Irbesartan provides clinically significant dose related reductions in blood pressure in patients with mild-to-moderate hypertension. Once daily dosing provides full 24 h blood pressure control with blood pressure reductions equivalent to those of twice daily dosing, and long-term control with monotherapy in a high percentage of patients. The antihypertensive effect of Irbesartan is comparable to or exceeds that of leading antihypertensive agents[1]. The empirical formula for Irbesartan is $C_{25}H_{28}N_6O$, and the molecular weight is 428.53.

The International Conference on Harmonization (ICH) guidelines[2] require stress testing of drug substances, which can help identify the likely degradation products, can be useful in establishing degradation pathways and in validating the stability-indicating power of the analytical procedures used. Moreover, a validated



2-Butyl-3-[2′-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1,3-diaza-spiro[4.4]non-1-en-4-one

Figure 1 : Structure and chemical name of Irbesartan

analytical method must be applied in stability studies[3].

Several methods have been described in the literature for the determination of Irbesartan in biological fluids. The techniques used include High-performance liquid chromatography (HPLC) with fluorescence detector[4,5]. UV[6,7], Diode-array[8]. LC-MS method was described for determining the levels of 4-methyl-2-cyano biphenyl and 4-bromomethyl-2-cyano biphenyl, which are key starting materials of Irbesartan[9]. HPLC method was mentioned in United states pharmacopeia[10] and European pharmacopoeia[11] for the estimation of one impurity. While studying the synthetic process we observed A, B, C, D, E, F, G and H as process-related impurities in the crude samples. Thus, there is a great need for development of analytical methods for separation and determination of related substances for the evaluation of quality of Irbesartan. It was simple method for the quantitative determination of process related impurities and degradents. The current work deals with the accelerated degradation of the drug substance under stress conditions like hydrolysis, oxidation, heating and UV light. The work also includes the validation of the stability-indicating method developed. This method can be used for quality control during manufacture and for assessment of the stability of bulk samples of Irbesartan.

EXPERIMENTAL

Chemicals and reagents

Samples of Irbesartan and its impurities namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H (Figure 2) were received from the Process Research Department of Integrated Product Development Operations of Dr. Reddy's Laboratories, Hyderabad, India. LC grade acetonitrile, Potassium dihydrogen orthophosphate, and Ortho-phosphoric acid were purchased from Merck, Schuchardt, Germany. High purity water was prepared by using a Millipore Milli Q plus purification system (Bedford, MA, USA).

Instrumentation

The LC system was a Waters model 2996 equipped with a PDA (Waters Corporation, Milford, USA) for specificity. The output signal was monitored and processed using Empower software (Waters Corporation, Milford, USA) on a Pentium computer (Digital Equipment Co). Validation Performed on a Agilent 1100 series HPLC with quaternary pump. The output signal was monitored and processed with Chemstation software.

Chromatographic conditions

Chromatographic separation was achieved on a 5µm Symmetry shield RP18 column, (250 mm×4.6 mm), using a mobile phase with a buffer containing a mixture of 0.01 M aqueous Potassium dihydrogen orthophosphate . The mobile phase A was a mixture of buffer and acetonitrile (90:10, v/v) (Buffer pH adjusted to 2.6 using dilute ortho-phosphoric acid) and mobile phase B was a mixture of water and acetonitrile (20:80, v/v). The mobile phase was filtered and degassed through a 0.45µm nylon membrane filter. The mobile phase flow rate was 1.0mL min^{-1}. The LC gradient was time (min)/%B: 0/30, 5/30, 43/80, 53/80, 55/30, and 60/30. The column was maintained at 30°C and the effluent was monitored at 220nm. The injection volume was 10µL. Acetonitrile and mobile phase-A (1:1) was used as diluent during the preparation of the standard and test samples.

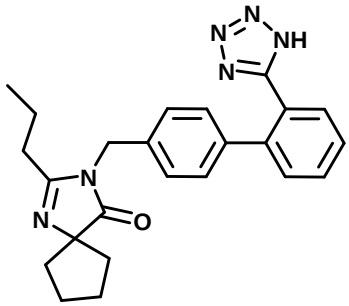
Preparation of standard solutions

A solution of Irbesartan (1000µg mL^{-1}) was prepared by dissolving an appropriate amount in the diluent. A stock solution of impurities at 100µg mL^{-1} was also prepared in diluent.

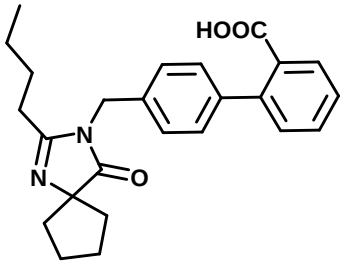
Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential im-

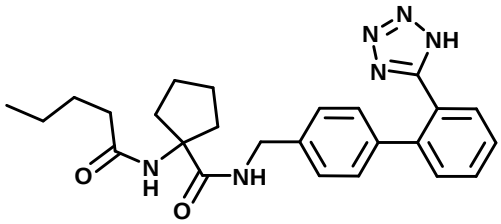
Full Paper



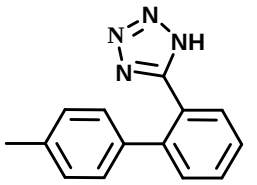
Imp-A : 2-propyl-3-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1,3-diaza-spiro[4.4]non-1-en-4-one



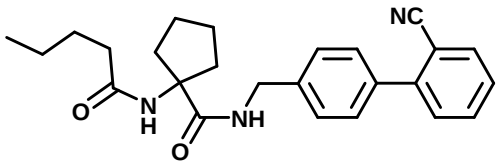
Imp-B : 4′-(2-butyl-4-oxo-1,3-diaza-spiro[4.4]non-1-en-3-ylmethyl)-biphenyl-2-carboxylic acid



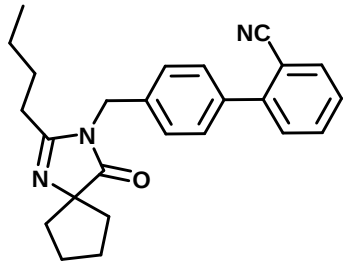
Imp-C : 1-pentanoylamino-cyclopentanecarboxylic acid [2′-(1H-tetrazol-5-yl)-biphenyl4-ylmethyl]-amide



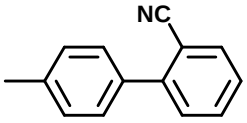
Imp-D : 5-(4′-methyl-biphenyl-2-yl)-1H-tetrazole



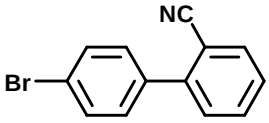
Imp-F : 1-pentanoylamino-cyclopentanecarboxylic acid (2′-cyano-biphenyl-4-ylmethyl)- amide



Imp-E : 4′-(2-butyl-4-oxo-1,3-diaza-spiro[4.4]non-1-en-3-ylmethyl)-biphenyl-2-carbonitrile



Imp-G : 4′-methyl-biphenyl-2-carbonitrile



Imp-H : 4′-bromo-biphenyl-2-carbonitrile

Figure 2 : Structures and chemical names of impurities

purities. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21CFR Sect. 211 requires the development and validation of a stability-indicating assay method. In order to determine whether the determination of impurities and the assay method were adequate, Irbesartan API sample was submitted to forced degradation studies. The specificity of the developed LC method for Irbesartan was carried out in the presence of its impurities.

The current regulatory guidelines do not indicate detailed degradation conditions in stress testing. However, the conditions used were found to effect a degradation of preferably not less than 5% but not complete degradation. Degradation conditions employed were UV light (200 Watt hours/m^2), Visible light(1.2 million lux hours) , heating to 105°C, acid hydrolysis with 0.5 N HCl, base hydrolysis with 0.05 N NaOH, water hydrolysis and oxidative degradation using 6% H_2O_2. Peak purity testing was carried out on the stressed samples of Irbesartan by using the PDA detector.

Method validation

Precision

The precision of the determination of the impurities was checked by injecting six individual preparations of (1000µg mL^{-1}) Irbesartan spiked with 0.15% of Imp-A, Imp-B, Imp-C, Imp-D Imp-E, Imp-F, Imp-G and Imp-H and calculating the % RSD for the area of each impurity.

Limit of detection and limit of quantification

The LOD and LOQ for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H were estimated at a S/N of 3:1 and 10:1 respectively, by inject-

TABLE 1 : LOD, LOQ, regression, precision and accuracy data

Parameter	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Imp-G	Imp-H
LOQ (μgmL^{-1})	0.006	0.005	0.01	0.01	0.01	0.008	0.005	0.013
LOD (μgmL^{-1})	0.0020	0.002	0.003	0.0024	0.002	0.003	0.0016	0.0026
Regression equation (y)								
Slope (b)	0.7293	0.5288	0.5412	0.8367	0.6577	0.2922	1.2639	0.8978
Intercept (a)	-0.3089	-0.0406	-.3263	-0.9928	-0.9008	0.4824	0.2518	0.0074
Correlation coefficient	0.9999	0.9999	0.9999	0.9999	0.9969	0.9997	0.9998	0.9999
Y-intercept at 100% level	-0.42%	-0.08%	-0.61%	-1.21%	-1.39%	1.61%	0.20%	0.01%
R square value	0.9958	0.9988	0.999	0.9992	0.9997	0.9997	0.9984	0.9974
Precision(n = 6)								
(% RSD)#	5.44	4.99	5.22	2.93	7.90	4.58	4.43	3.96
Accuracy (n = 3)								
(% Recovery at LOQ)	105.3	105.1	107.8	98.3	105.3	91.6	102.2	99.1
(Recovery at 50%)	108.0	98.0	100.5	100.3	105.8	100.3	99.5	95.0
(Recovery at 100%)	99.2	99.7	98.9	100.2	100.6	100.5	100.5	98.7
(Recovery at 150%)	96.2	99.7	100.1	100.6	99.2	100.3	101.5	98.4

Linearity range is LOQ- 150 % with respect to 1.0mg/mL of Irbesartan for impurities, # six determinations using LOQ solution for impurities

ing a series of dilute solutions with known concentrations. Precision study was also carried at the LOQ level by injecting six individual preparations of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H, and calculated the %RSD for the areas.

Linearity

Linearity test solutions for the impurities were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration levels from LOQ to 150% with respect to the impurities specification level of 0.15% (i.e. LOQ, 0.025, 0.050, 0.075, 0.10, 0.125 and 0.150%). The calibration curves were drawn by plotting the peak areas of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H against the corresponding concentration.

Accuracy

Accuracy of the determination of the impurities was carried out in triplicate at 0.05, 0.075, 0.10 and 0.15% of the Irbesartan concentration (1000$\mu g\ mL^{-1}$).The percentages recoveries for the impurities were calculated.

Robustness

To determine robustness, experimental conditions were purposely altered and the resolution between the impurities and Irbesartan peak was evaluated.

To study the effect of flow rate on the resolution between impurities and Irbesartan peak, it was changed by 0.2 units from 0.8 to 1.2mL min^{-1}. The effect of column temperature on the resolution between the impurities and Irbesartan peak was studied at 25 and 35°C. In all the above conditions, the components of the mobile phase were held constant.

Solution stability and mobile phase stability

The solution stability of Irbesartan and its impurities in the related substances method was carried out by leaving spiked sample solution in a tightly capped volumetric flask at room temperature for 48 h. Content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H were determined at 24 h intervals. Mobile phase stability was also carried out for 48 h by injecting freshly prepared sample solutions at 24 h intervals. Content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H were checked in test solutions. Mobile phase composition was kept constant during the study period.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Attempts were made by using different C_{18} and C_8

stationary phases[12]. The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. Effects of pH (2-7) and acetonitrile content were investigated using phosphate and acetate buffers. It was found that the retention time of Irbesartan shifts significantly at basic pH. The optimum conditions are given in 'Experimental'. Irbesartan, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H were well separated with a resolution of greater than 1.5.

Results of forced degradation studies

Degradation was not observed in Irbesartan bulk sample during stress conditions like photolytic and thermal degradation at 105°C. Slight degradation was observed in aqueous hydrolysis. Major degradation was observed in basic, acidic and oxidation conditions. Imp-C was the major degradent in basic degradation.

Peak purity test results confirmed that the Irbesartan peak was homogeneous and pure in all the analyzed stress samples.

Results of method validation studies

Precision

The RSD of area of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H were within 2.5%, confirming the good precision of the method.

Limit of detection and limit of quantification

The limits of detection (LOD) of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H were found to be in the range of 0.0016 to 0.003% (of analyte concentration 1000μg mL^{-1}) in each case for a 10μL sample size. The limits of quantification (LOQ) of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H were found to be in the range of 0.012 to 0.005%. The precision for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H at LOQ level was below 8.0% RSD.

Linearity

Linear calibration plots for the impurities were obtained over the calibration ranges tested, i.e. LOQ to 015% for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H. The correlation coefficients obtained were greater than 0.999. The results show that an excellent correlation between the peak area and concentration of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H.

Accuracy

The percentage recovery of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H in bulk drug samples ranged from 96.2 to 108.

Robustness

In all the deliberately varied chromatographic conditions (flow rate and column temperature), the resolution between the impurities and Irbesartan peak was more than 2.0. which reveals the adequate robustness of method.

Solution sand mobile phase stability

No significant change was observed in the content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and Imp-H during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirm that sample solutions and mobile phase used during assays were stable up to 48 h.

CONCLUSIONS

The simple RP-LC method developed for the quantitative determination of Irbesartan and its possible degradation products and impurities is precise, accurate and specific for the analysis of bulk material. The method was fully validated, showing satisfactory results for all the parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples.

ACKNOWLEDGMENTS

The authors wish to thank the management of Dr. Reddy's group for supporting this work. The authors wish to acknowledge the Process Research Group for providing the samples.

REFERENCES

[1] G.Hubert Pouleur; American Journal of Hypertension, **10(12)**, **Supplement(1)**, 318S-324S **(1997)**.

[2] ICH [Validation of Analytical Procedures: Methodology (Q2R1)]; International Conference on Harmonization, 'Food and Drug Administration', USA, November (1996) and November **(2005)**.

[3] ICH [Stability Testing of New Drug Substances and Products (Q1AR2)]; International Conference on Harmonization, 'Food and Drug Administration', USA, November (1996) and February **(2003)**.

[4] K.Ashok Shakya, M.Yusuf Al-Hiari, M.O.Omran Alhamami; Journal of Chromatography, B, **848(2)**, 245-250 **(2007)**.

[5] Shu-Ying Chang, B.Daisy Whigan, N.Nimish Vachharajani, Rajesh Patel; Journal of Chromatography, B, Biomedical Sciences and Applications, **702(1-2)**, 149-155 **(1997)**.

[6] Najma Sultana, M.Saeed Arayne, S.Shahid Ali, Shahnawaz Sajid; Chinese Journal of Chromatography, **26(5)**, 544-549 **(2008)**.

[7] Nevin Erk; Journal of Chromatography, B, **784(1)**, 195-201 **(2003)**.

[8] E.Caudron, S.Laurent, E.M.Billaud, P.Prognon; Journal of Chromatography, B, **801(2)**, 339-345 **(2004)**.

[9] G.V.Ram Reddy, A.Praveen Kumar, B.Venkateswara Reddy, J.Sreeramulu, J.H.Park; Analytical letters, **42(13)**, 2087-2095 **(2009)**.

[10] Irbesartan; USP32-NF27, 2686.

[11] Irbesartan; European Pharmacopoeia, 6.7, 5679-5680.

[12] L.R.Snyder, J.L.Glajch, J.J.Kirkland; Practical HPLC Method Development, 2nd Edn., Wiley Interscience, New York, **(1997)**.