IJBPAS, February, 2022, 11(2): 708-714

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

METHOD DEVELOPMENT AND VALIDATION FOR IMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND IRBESARTAN BY RP-HPLC

A.PUSHPANJALI*, P. PRACHET, M. SIVA PRASAD AND RAMA RAO N

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Gunttur-522034, Andhra Pradesh, India

***Corresponding Author: A. Pushpanjali: E Mail: pushpanjalialla97@gmail.com; Tel.: +91 9133874100**

Received 20th March 2021; Revised 24th April 2021; Accepted 11th June 2021; Available online 1st Feb. 2022

https://doi.org/10.31032/IJBPAS/2022/11.2.5889

ABSTRACT

A simple precise, rapid and accurate reverse phase high performance liquid chromatography method has been developed and validated for the simultaneous estimation of hydrochlorothiazide and irbesartan. Shimadzu prominence i-series autosampler using Shimadzu column and separation was achieved with mobile phase acetonitrile: ammonium acetate (24:76 v/v) at flow rate 0.8 ml/min and wavelength is 290nm. Injection volume was 20 µl with 10min run time. The retention time for hydrochlorothiazide and Irbesartan is 7.488 and 2.746 min respectively. The linearity range for hydrochlorothiazide and irbesartan is 5-25 µg/ml and 6-30 µg/ml with correlation coefficient of 0.999. The %RSD for precision was found to be 0.93 and 0.92 for Hydrochlorothiazide and Irbesartan and % recovery for Hydrochlorothiazide and Irbesartan was 100.15 and 100.04 and method was robust. The developed method was validated according to ICH guidelines. Linearity, Accuracy, %RSD of precision, LOQ, LOD and robust values were found within the limits and the method was found to be satisfactory.

Keywords: Hydrochlorothiazide, Irbesartan, RP-HPLC

INTRODUCTION

Hydrochlorothiazide (HTZ) and irbesartan used in the treatment of hypertension. The drug of irbesartan (IRB), known as (2-butyl-3({4- [2- (2H-1,2,3,4-tetrazol-5yl)

phenyl] phenyl} methyl)1,3- diazaspirol [4,4] non-1-en-4-one), which is mainly used for the treatment of hypertension. IRB is an angiotensin II Type1 (All1)-receptor antagonist that is highly selective for Type 1 angiotensin II receptor. Hydrochlorothiazide is a chemical (6-chloro-3,4-dihydro-7-sulfamoyl-2H-1,2,4-benzothiadiazine 1,1-dioxide) diuretics **[1]**. IRB and HTZ are used together to lower blood pressure. IRB controls high blood pressureby relaxing the blood vessels. Thiazide affects the renal tubular mechanisms of electrolytes reabsorption, directly increases the excretion of sodium salt and chloride in approximately equivalent amounts. The combination is useful in the treatment of mild-tomoderate hypertension, well-tolerated with a lower incidence of cough than ACE (Angiotensin Converting Enzyme) inhibitors. HPLC method **[2-6]** was usually used for the determination of HTZ alone and in combination with other drugs. Spectrophotometer **[7]** and special methods combined with HPLC were used to determine HTZ in combination with other drugs in plasma and serum. HPLC was also used for the estimation of IRB in combination with other drugs in plasma and serum. However, there were several HPLC methods reported for simultaneous determination of HCTZ and IRB in combined dosage form but not stability indicating assay method **[8, 9]**. Only one method reported by Rane *et al* in 2010 **[6]** stated a stability indicating HPLC method for the determination of HTZ and IRB in combination with dosage forms. Unfortunately, this study suffered from some disadvantages as weak resolution between the two drugs and their degradation products in addition to long retention time, for instance, about 12 min of retention time, which set the method noneconomic and time consuming, so it is need to develop a stability indicating method to investigate the degradation behaviour of HTZ and IRB drugs. The aim of the present study was to develop an accurate, precise, specific, reproducible and stable indicating HPLC method for the estimation of HCTZ and IRB in the presence of their degradation products and get rid of the defects of the reported stability indicating method according to ICH guidelines. The HCTZ and IRB drugs were degraded by acid, alkali, oxide, dry heat and UV light treatment to check the stability and to develop stability indicating assay method.

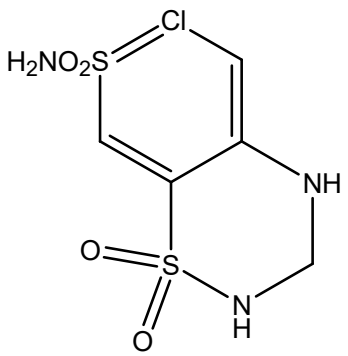

Figure 1: Structure of Hydrochlorothiazide

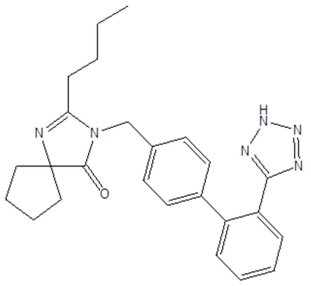

Figure 2: Structure of Irbesartan

MATERIALS AND METHODS

Materials: hydrochlorothiazide and irbesartan

Chemicals and instruments:

Instruments: The chromatographic separation was carried out by Shimadzu 2030C with photo diode array detector and separation is done by using shimadzu column at ambient temperature. Mobile phase consists of acetonitrile: ammonium acetate buffer (24:76 v/v) were filtered through 0.45µ membrane and mobile phase is purged for 2 minutes for the removal of air or gas bubbles. The flow rate is 0.8 ml/min and the column temperature was ambient. The injection volume was 20µl. The UV detector was set up at 290nm.

Preparation of stock solution: Weigh accurately 10mg of hydrochlorothiazide and irbesartan and transfer both into the volumetric flask separately. Hydro-chlorothiazide dissolve and make up in methanol. Irbesartan is dissolved and make up with 0.1N HCl.

Preparation of sample solution: 10 Tablets were weighed and their average weight were determined. They were crushed into fine powder, weigh the tablet powder equivalent to 10 mg of drug and transferred into volumetric flask and dissolved in methanol. The solution is filter through 0.45µ membrane filter which gives the concentration of 1000 µg/ml and this solution were used as a sample stock solution.

Method development:

Method optimization: A simple RP-HPLC was developed for estimation of hydrochlorothiazide and irbesartan in pharmaceutical formulation using shimadzu column. The mobile phase is acetonitrile: ammonium acetate buffer (24:76) the ratio of mobile phase chosen after several trials. The flow rate is 0.8 ml/min. The retention times is 7.488 min and 2.746 min for hydrochlorothiazide and irbesartan respectively.

System suitability: Prior to the validation of the proposed method, system suitability tests such as tailing factor, selectivity, resolution, tailing, and theoretical number of plates were calculated and reported. The retention factor values for IRBE and HCT were calculated as 2.08 for HCT and 3.35 for IRBE; the other system suitability test parameters are summarized in **Table 1**. Those results show that the proposed method for the determination of IRBE and HCT conformed to the ICH guidelines **[11]**.

Table 1: System suitability parameters

Parameters	Hydrochlorothiazide	Irbesartan	Acceptance criteria
Tailing	0.734	2.746	NMT 2.0
Plate count	2033	3456	NLT 2000
% RSD of peak area	0.54	0.55	NMT 2.0
Retention time	7.488	2.746	---

Specificity: The specificity of the method was demonstrated by injecting blank and standard preparations. Retention times from sample and standard were compared with each other.

Linearity: The linearity for hydrochlorothiazide and irbesartan was evaluated by relation between peak areas and concentration of each drug with a correlation coefficient of 0.999 for both drugs **(Table 2, Figure 3)**.

Pression: It is studied or evaluated by the repeatability studies which is determined by the injecting the same concentration of rug six times. The % RSD of two drugs for system precision is 0.75, 0.51 and for method precision is 0.93, 0.92 **(Table 3)**.

Accuracy: It is analyzed by conducting three different concentrations of the working standards. With the percentage of 50%, 100% and 150%inject each concentration three times into HPLC and calculate the average percentage recovery. The mean percentage recovery of Hydrochlorothiazide and Irbesartan is 100.15% and 100.04% **(Table 4)**.

Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ were calculate based on the ICH guidelines. Both LOD and LOQ is defined as the minimum concentration of signal-to-noise ratio of LOD= 3.3s/m and LOQ =10s/m using standard deviation of response (s) and the slope (m) of the calibration curve. LOD and LOQ values of hydrochlorothiazide and irbesartan is 0.5µg/ml, 1.5µg/ml and 0.6µg/ml, 1.8µg/ml.

Robustness: Robustness should be considered during development phase and also depends on the type of procedure under study. The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, %organic solvent strength and buffer concentrations, etc. to determine the robustness of the method experimental conditions were purposely altered, and chromatographic characters were evaluated. In this work we alter the ratio of mobile phase and flow rate of the mobile phase.

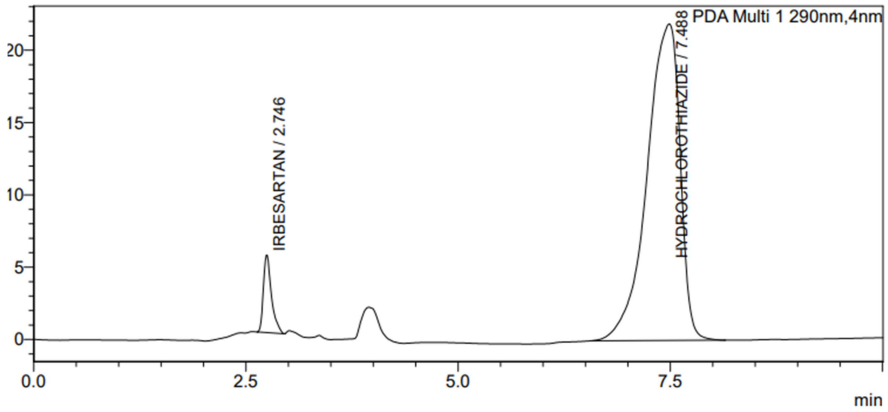

Figure 3: Standard chromatogram

Table 2: Linearity ranges for Hydrochlorothiazide and Irbesartan

S. No.	Hydrochlorothiazide		Irbesartan	
	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
1.	0.5	197129	6	118144
2.	1	394458	12	235897
3.	1.5	591088	18	353831
4.	2	778217	24	471778
5.	2.5	98248	32	599816
6.	Correlation coefficient	0.999	Correlation coefficient	0.9998

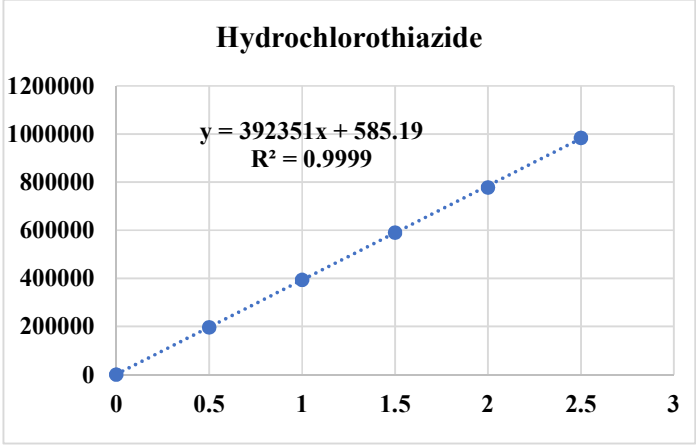

Figure 4: Calibration curve of Hydrochlorothiazide

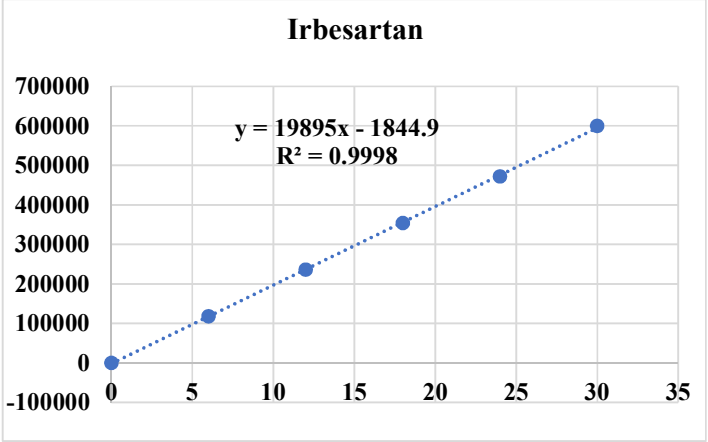

Figure 5: Calibration curve of Irbesartan

Table 3: Precision for Hydrochlorothiazide and Irbesartan

S. No.	System precision		Method precision	
	Peak area		Peak area	
	Hydrochlorothiazide	Irbesartan	Hydrochlorothiazide	Irbesartan
1.	609888	189512	598862	185632
2.	598692	190021	596213	189563
3.	598721	189632	608999	187461
4.	598762	187456	598421	189523
5.	599876	188979	598231	189952
6.	598625	189959	592139	189754
Avg	600751	189259.8	598811	188647.5
Std. dev	4501.943	959.4777	5544.663	1733.882
%RSD	0.75	0.51	0.93	0.92

Table 4: Accuracy for Hydrochlorothiazide and Irbesartan

% level	% recovery		Mean % recovery	
	Hydrochlorothiazide	Irbesartan	Hydrochlorothiazide	Irbesartan
50%	100.20	99.87	100.15	100.04
	100.11	99.83		
	100.40	99.76		
100%	100.34	99.79		
	100.04	99.89		
	100.51	99.98		
150%	99.86	100.53		
	99.92	100.36		
	100.03	100.40		

CONCLUSION

In the present work a new, simple, precise, accurate RP-HPLC method was developed and validated according to the ICH guidelines. RP-HPLC equipped with schimadzu c18 column with mobile phase of acetonitrile and ammonium acetate buffer (24:26)at flow rate 0.8ml/min and detected wavelength is 290nm, injection volume is 20µl. Retention time is 7.488 and 2.746 for Hydrochlorothiazide and Irbesartan and correlation coefficient for both drugs is 0.999. Mean %recovery is 100.15 and 100.04. In precision %RSD for both drugs is 0.93 and 0.92. Parameters such as theoretical plates, tailing factors, %RSD, % recovery was within the limit as per ICH Q2 guidelines. Hence this can be employed for routine analysis in laboratories and in pharmaceutical industries.

REFERENCES

[1] O' Neil M J, Smith A, Heckelman P E, Budavari, "The Merck Index: An encyclopedia of chemicals drugs, and biologicals: Diphenyl 13th ed. Merck, Whitehouse Station, NJ, 2001.

[2] Gao F, Zhang M, Cui X.J. Chem, "Simultaneous quantitation of hydrochlorothiazide and metoprolol in human plasma by liquid chromatography-tandem mass spectrometry" Journal of Pharmaceutical and Biomedical Analysis, 2010; Vol. 52, Issue 1, pg. 149-154.

[3] Li H, He J, Liu Q, "Simultaneous determination of

hydrochlorothiazide and reserpine in human urine by LC with a simple pre-treatment" Chromatographia 73 (1), 171-175, 2011

[4] Al-Momani I F, "Determination of hydrochlorothiazide and enalapril maleate int ablet formulations by RP-HPLC" Turkish Journal of Chemistry 2001, 25: 49-54.

[5] Sasa S I,Jala I M, Khalil H S. J. Liq. Chromatogr., 1998, 11: 1673-1696.

[6] Kirschbaum J, Perlman S, "Analysis of captopril and hydrochlorothiazide combination tablet formulations by liquid chromatography" Journal of pharmaceutical sciences 1984, 73(5): 686-697.

[7] Erturk S, Cetin S M, Atmaca S, "Simultaneous determination of moexipril hydrochloride and hydrochlorothiazide in tablets by derivative spectrophotometric and high-performance liquid chromatogram methods" 2003, 33(3): 505-511.

[8] Wankhede SB, Tajne M R, Guptha K R, Wadodkar S G, "RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form" 2007, 69: 298-300.

[9] Milind B. Ubaleetal: Simultaneous HPLC determination of Irbesartan and Hydrochlorothiazide, JPSI 1 (1) JAN-FEB 2012: 25-28.

[10] Rane V P, Patil K R, Sangashetti J N, Yeole R D, Shinde D B, "Stability indicating LC method for simultaneous determination of irbesartan and hydrochlorothiazide in pharmaceutical preparations" Journal of chromatographic science. 2010, 48: 595-600.

[11] ICH Guidelines T. Validation of Analytical Procedures, Methodology International Council on Harmonization. Brussels, Belgium; 1995.