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A VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF IRBESARTAN AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORMS

Md. Abdul Sattar*, M. Siva Sree lakshmi, M. Bhanu Sudha,

B. Laxmi Prasanna, G. Sravali, B. Rama Valkali

Department of Pharmaceutical Analysis, KJR College of Pharmacy, Rajahmundry, Andhra Pradesh, India.

ABSTRACT

A simple reverse phase RP-HPLC methodology was employed for simultaneous determination of Irbesartan and Hydrochlorothiazide in a pharmaceutical dosage form. The experiment employed a Hypersil BDS RP-c18 (250 x 4.6 mm), 5µ column manufactured by Thermo, operating in isocratic mode. The mobile phases consisted of pH 3.0 sodium acetate buffer and acetonitrile in a ratio of 45:55. The flow rate utilized in the experiment was 1.0 mL per minute, and the effluent was observed and measured at a wavelength of 260 nanometers. The respective retention times for Irbesartan and Hydrochlorothiazide were 4.83 and 2.99 minutes. The method was verified at concentrations 10-1000 ng/mL in accordance with ICH guidelines for the three analytes. The results indicate that the method is accurate, with average accuracies ranging from 98-102% for irbesartan and 97-101% for hydrochlorothiazide, as well as precise.

Keywords: Hypersil BDS C18 column, Irbesartan and hydrochlorothiazide, Chromatography.

INTRODUCTION

Annually, cardiovascular disease results in the death of 17.9 million individuals, accounting for almost 30% of all worldwide fatalities. Hypertension accounts for a minimum of 45% of fatalities attributed to cardiovascular disease[1]. Several pharmaceuticals, including angiotensin converting enzyme inhibitors, angiotensin-receptor blockers, beta-blockers, diuretics, and calcium channel blockers, have been utilized for the treatment of hypertension[2]. The combination of irbesartan (IRB) and hydrochlorothiazide (HCTZ) is utilized for the management of hypertension.

Irbesartan is a selective antagonist of AT-II Type 1 receptor; which is mainly used for the triage of hypertension[3]. Its physiological effects encompass vasoconstriction, stimulation of aldosterone synthesis and release, cardiac stimulation, and renal sodium reabsorption. The thiazide group's prototype, HCTZ impacts the renal tubular mechanisms of electrolyte reabsorption. Specifically, it increases the excretion of sodium, salt and chloride in roughly equal amounts. The combination of medications has demonstrated utility in managing hypertension of mild-to-moderate severity, exhibiting favourable tolerability with a reduced occurrence of cough when compared to ACE inhibitors. The exquisite mechanism of action is designed to impede the absorption of sodium and chloride at the beginning of the distal convoluted tubule[3,4]. IRB and HCTZ were simultaneously determined using spectrophotometric, spectrofluorometric and high-performance liquid chromatography (HPLC) techniques in both bulk and tablet dose form. The literature reveals a limited number of HPLC techniques have been documented for the concurrent

determination of IRB and HCTZ in tablet formulations. The objective of the current study was to establish and verify a highly sensitive HPLC technique in accordance with ICH guidelines, which could be utilised for the concurrent determination of IRB and HCTZ.

MATERIALS AND METHODS

The chromatographic separation was conducted using a Shimadzu HPLC system equipped with Spin Chrome CFR software, a UV-Visible detector (SPD-20A), and pumps (LC-10AT and LC-10ATVP). The separation was performed on a Hypersil-C18 BDS column.

REAGENTS AND MATERIALS

Sample was purchased from Spectrum laboratories, Hyderabad. All chemical substances were of analytical reagent grade and solvents were of HPLC grade.

HPLC instrument and chromatographic conditions

Chromatographic separation was conducted using a Shimadzu HPLC system equipped with Spin Chrome CFR software, a UV-Visible detector (SPD-20A), and pumps (LC-10AT and LC-10ATVP). The separation was carried out on a Hypersil-C18 BDS column. The mobile phase was prepared by combining Sodium acetate buffer and Acetonitrile in a volumetric ratio of 45:55. The isocratic elution process was conducted at room temperature. The flow rate was at 1.0 mL/minute, while the injection volume was recorded as 20 µl. The UV detection wavelength was configured to 260 nm.

Preparation of Standard stock solution

Accurately weigh and transfer about 75.0 mg of IRB and 6.3mg of HCTZ Working Standard into a 25 ml clean dry volumetric flask into a 25 ml clean dry volumetric flask. add about 15 ml of methanol, sonicate for 5 minutes, and dilute to volume with methanol.

mobile phase-a: Sodium acetate buffer and Acetonitrile were mixed in the ratio of 45:55 and sonicated to degas.

Standard stock preparation

Sample preparation

Weigh and powder about ten tablets in a neat clean and dry motor and pestle. Weigh and transfer accurately about 0.2gm of the tablet powder into 25ml clean dry volumetric flask, add about 15ml of methanol, sonic ate for 5 minutes, and dilute to volume with methanol. Filter the solution through the what Mann filter paper, from the filtrate pipette out 1ml of sample solution into a 25ml volumetric flask, make up the volume with diluent (mobile phase).

Buffer preparation

Weigh accurately 8.2 g of sodium Acetate and dissolve it in 1000ml of Milli-Q water. Adjust the pH to 3.0 with ortho phosphoric acid, filter through 0.45µm nylon membrane filter and degas.

RESULTS AND DISCUSSION

Method optimization

A simple reverse phase high-performance liquid chromatography method was developed for the determination of IRB and HCTZ in pharmaceutical dosage form using Hypersil-C18 BDS column. The developed RP-HPLC method was validated in terms of the following parameters according to the International Conference on Harmonization (ICH) guidelines[5]. The mobile phase consists of Sodium acetate buffer and Acetonitrile mixed in the ratio of 45:55. The flow rate is 1.0ml/min. The average retention times (RT) under the described conditions were 2.997 min for Irbesartan and 4.833 min for HCTZ (Table 1; Figure 3). Reverse-phase high-performance liquid chromatography method for simultaneous estimation of IRB and HCTZ accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantitation (LOQ), specificity, and system suitability studies were carried out.

System suitability

A Standard solution was prepared by using IRB and HCTZ working standards as per test method and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % relative standard deviation (RSD) from five replicate injections for IRB and HCTZRT and peak areas. The values were depicted Figure 3,4 and 5.

Precision

The precision of the drugs was determined by calculating the intra-day and inter-day variations. The present study aims to determine the percent RSD for the estimation of IRB and HCTZ. The coefficient of variation, expressed as the percentage %RSD, was provided in Table 2. It was observed that the replicate of five injections remained within the predetermined limits.

Intermediate precision/Ruggedness

To assess the intermediate precision, which is also referred to as ruggedness, precision was conducted on a separate day within the laboratory. The standard solution was injected for five times and the resulting area was measured using HPLC by two analysts. The RSD for the area of five replicate injections was determined to be within the specified limits. (Table 5).

Accuracy

The method's accuracy was assessed by using recovery experiments. The recovery experiments were conducted by incorporating predetermined quantities of the pharmaceutical substances into the placebo. The recovery experiment was conducted using three distinct concentrations, specifically 50%, 100%, and 150% of the label claim of the tablet. Chromatograms were subsequently recorded for each concentration. (Table 3)

Linearity

The linearity of the analysis was assessed by examining six standard solutions of IRB and HCTZ, across a concentration range of 20-160 µg/ml. The calibration curve was generated by constructing a graphical representation of the relationship between peak area and concentration. The equation for a straight line was determined. The linearity of the calibration plot was observed within the concentration range of 20 - 160 µg/ml for both IRB and HCTZ. The linearity of the detector for the drugs IRB and HCTZ was assessed by examining the relationship between the peak areas under the respective peaks and the concentrations of each drug. This analysis yielded correlation coefficients of 0.9989 and 0.9984 for IRB and HCTZ, respectively. (Figure 6,7).

Robustness

The robustness of the proposed method was determined by slight modifications to the experimental conditions, and the chromatographic resolution between HCTZ and IRB drugs was subsequently evaluated. The manipulation of analytical parameters at a minor level did not have a substantial impact on the RT, recoveries, resolution peak area, and peak height of the analytes. Hence, the methodology exhibited resilience towards slight variations in the flow rate. (Table 4)

Quantification limit

The LOD refers to the minimum concentration of the analyte that can be detected reliably, with a signal to noise ratio of 1:3. On the other hand, the LOQ represents the lowest concentration of the analyte that can be accurately and precisely quantified, with a signal to noise ratio of 1:10. The LOD for IRB was determined to be 2.3968396 µg/mL, while the LOD for HCTZ was found to be 1.75143 µg/mL. The LOQ for IRB was determined to be 7.2631503 µg/mL, while the LOQ for HCTZ was found to be 5.30736 µg/mL.

CONCLUSION

The analysis of drugs found in combined pharmaceutical dosage forms poses a significant challenge, prompting researchers to develop analytical methods for the relative substances of the drugs IRB and HCTZ present in these combined dosage forms. A simple reverse phase (HPLC) method was developed to concurrently determine the presence of Irbesartan and Hydrochlorothiazide in a pharmaceutical dosage form. The methods that have been proposed exhibit characteristics such as simplicity, selectivity, reproducibility, sensitivity, and accuracy, all of which are accompanied by a commendable level of precision. A Hypersil BDS RP-c18 (250 × 4.6 mm), 5µ column from Thermo in isocratic mode, with mobile phases pH 3.0 sodium Acetate buffer and acetonitrile was used. The column was operated in isocratic mode, utilising a mobile phase consisting of pH 3.0 sodium acetate buffer and acetonitrile. The flow rate utilised in the experiment was 1.0 mL/min, while the effluent was subjected to monitoring at a wavelength of 260 nm. The recorded RT for IRB and HCTZ were 4.83 and 2.99 minutes, respectively. As per ICH guide lines the method was validated over the range of 10–1000 µg/mL for the three analytes, and is accurate (average accuracies of three different concentrations ranged from 98 to 102% for IRB and 97 to 101% for HCTZ. The simplicity of the RP-HPLC method enables its utilisation in the quantitative determination of IRB and HCTZ in tablet formulations. The method was considered to be accurate, precise, linear, robust, and rugged as a result of its ability to effectively separate and resolve the chromatographic peaks. The suggested expeditious analytical methodology for antihypertensive medications is perceived as easily implementable and relevant for pharmaceutical companies that prioritise swift analysis. Moreover, this methodology can be utilised in research studies that involve real-life samples or in investigations pertaining to bioequivalence.

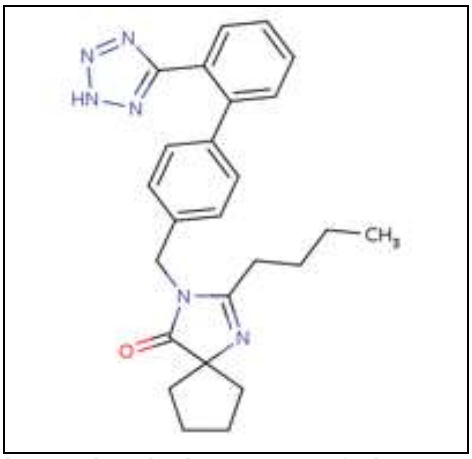

Fig. 1: Chemical structure of Irbesartan

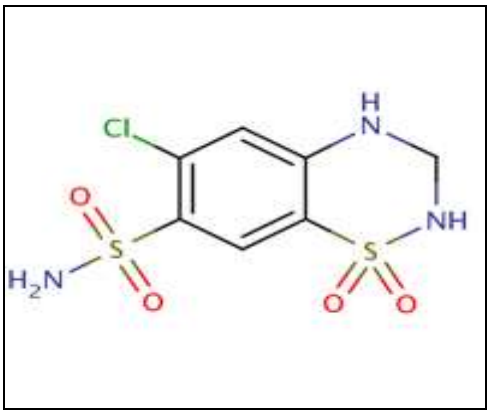

Fig. 2: Chemical structure of hydrochlorothiazide

Table 1: Optimised method

Parameters	Method
Stationary phase (Column)	Hypersil BDS RP-c18 (250 × 4.6 mm, 5µ)
Mobile Phase	Sodium acetate buffer and Acetonitrile were mixed in the ratio of 45:55
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (^{0}C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength	260 nm
Drug RT (min)	2.997 min for Irbesartan and 4.833 min for Hydrochlorothiazide.

Table 2: Results of precision studies

Precision IRB	Repeatability (Intra-day)	Reproducibility (Inter-day)
%Assay	100.25 ± 0.9	100.37 ± 0.898
%RSD	0.6857	0.7228
HCTZ		
%Assay	100.79± 0.25	99.99 ± 0.84
%RSD	0.2428	0.5290

Table 3: Accuracy (% recovery) data for IR and HCTZ

Drug	Spiked level (µg/ml)	Concentration(µg/ml)		
		Amount added	Amount Mean Recovered	% Recovery
IRB	80	20	20.51	98.69
	100	40	39.98	100.25
	120	60	61.53	99.94
HCTZ	80	20	21.79	99.99
	100	40	40.31	100.79
	120	60	60.80	99.61

Table 4: Results of Robustness

Robustness	Modification in Flow rates		
	0.8 ml/min	1.0 ml/min	1.2 ml/min
IRB			
Tailing Factor	1.1	1.2	1.1
% RSD	0.0189	0.01025	0.0552
HCTZ			
Tailing Factor	1.1	1.2	1.1
%RSD	0.0620	0.0456	0.02533

Table 5: Results of Ruggedness

Ruggedness	Analyst 1	Analyst 2
IRB		
Peak area	397.927	380.559
%RSD	0.00235	0.00236
HCTZ		
Peak Area	4.597	4.460
%RSD	0.00557	0.00552

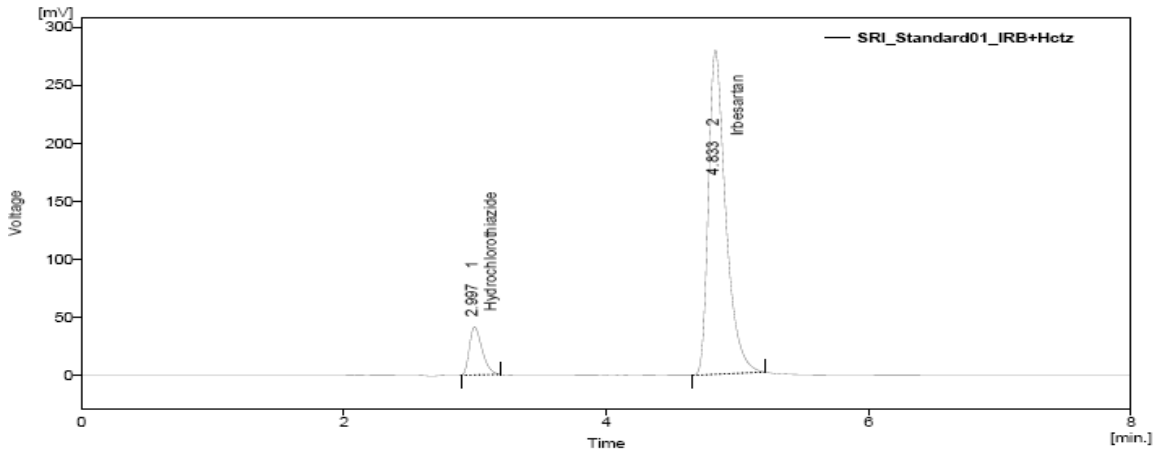

Fig. 3: Standard chromatogram for optimized condition

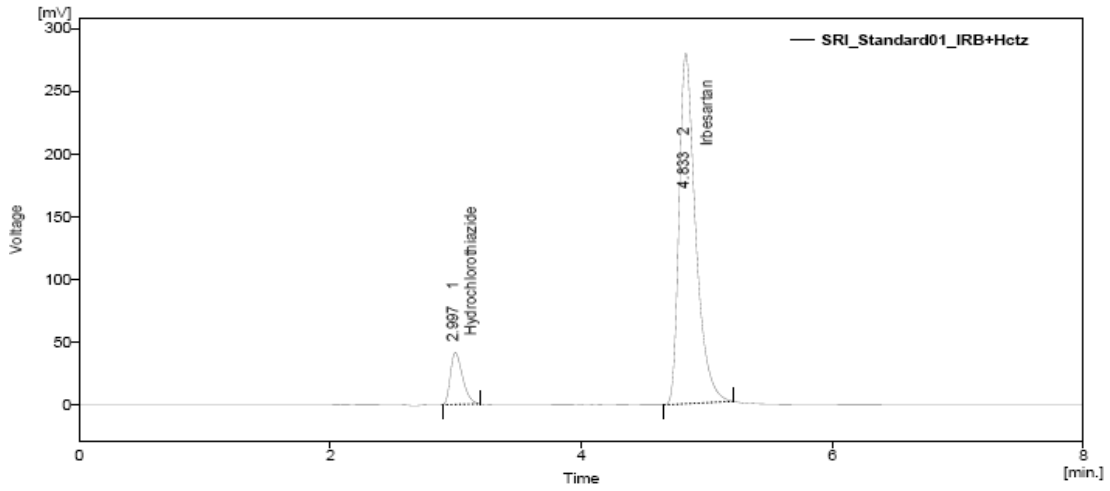

Fig. 4: Standard Chromatogram System Suitability

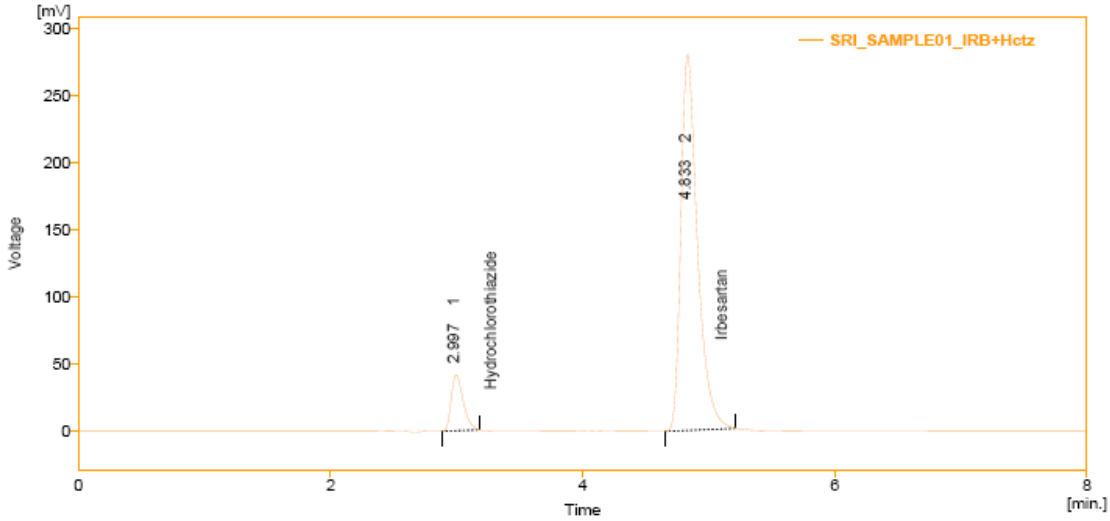

Fig. 5: Sample Chromatogram System Suitability

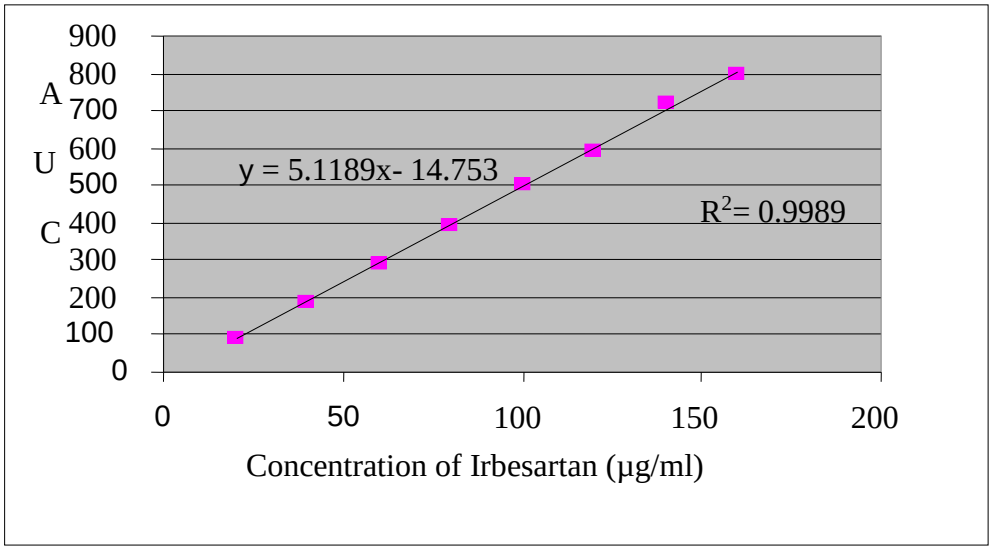

Fig. 6: Irbesartan Linearity Plot (Concentration vs Response)

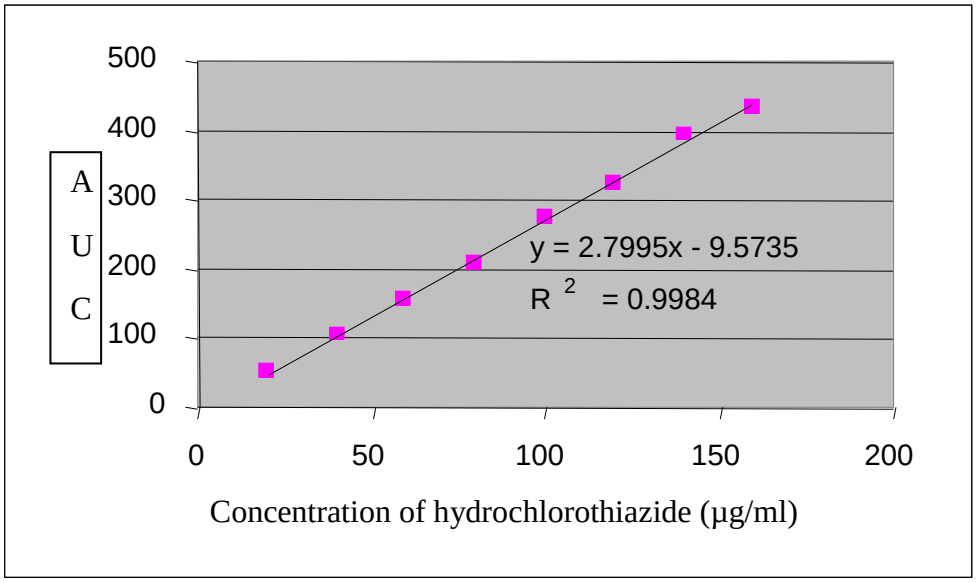

Fig. 7: Linearity Plot for Hydrochlorothiazide (Concentration vs Response)

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