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Development and Validation of RP- HPLC method for Estimation of Hydrochlorothiazide and Irbesartan in Pharmaceutical Preparation

T. M. Kalyankar*, S. J. Wadher, S. S. Pekamwar and N.G. Doiphode

School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India - 431606.

***Corres. author: dr.kalyankartm@gmail.com**
Contact No: - +91-8149026046

Abstract: A simple, precise, accurate, rapid and economical analytical method was developed and validated for simultaneous estimation of hydrochlorothiazide and irbesartan by RP-HPLC in Pharmaceutical Preparation. Analysis was performed on a C18 (250 mm x 4.6 mm, 5 µm) column with methanol: 0.05 M potassium dihydrogen ortho phosphate buffer pH 2.5 (60:40 v/v) as mobile phase, a flow rate 0.8 ml/min and column temperature 40ºC. Quantitation was achieved with UV detection at 226 nm. Both the drugs were well resolved on the stationary phase and the retention times were found to be 3.21 min for hydrochlorothiazide and 10.19 min for irbesartan. The calibration curves were linear in the concentration range of 5-30 µg/ml for hydrochlorothiazide and 60-360 µg/ml for irbesartan. Intra-day and inter-day relative standard deviations for both the components were < 2.0%. The Percentage recovery for hydrochlorothiazide and irbesartan are ranged between 99.72–100.38 and 100.00–100.44 respectively. The method proposed proved to be specific, rapid and accurate for the quality control of both drugs in pharmaceutical preparation.
Keywords: Hydrochlorothiazide, High performance liquid chromatography, Irbesartan, validation.

Introduction:

Fixed-dose combination of antihypertensive drugs can simplify dosing regimens, improve compliance, improve hypertension control, decrease dose-dependent side effects and reduce cost as the first-line treatment of hypertension[1]. These potential advantages make it recommendable for the combination antihypertensive therapy to be used as initial treatment, particularly in patients with target-organ damage or more severe initial hypertension [2, 3].

Chemically Hydrochlorothiazide (HYD) is 6-chloro- 1,1-dioxo- 3,4-dihydro- 2H- 1,2,4-benzothiazine-7-sulphonamides, is a thiazide diuretic that increases sodium and chloride excretion by distal convoluted tubule. Chemically Irbesartan (IRB) is 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 Angiotensin receptor blockers [9].

Literature survey reveals the availability of several methods for determination of irbesartan and hydrochlorothiazide includes spectrophotometry, liquid chromatography, thin-layer chromatography, as alone

or in combination with other drugs [10-16]. This paper is in continuation with our work where we studied Reversed-Phase Liquid Chromatographic method for simultaneous determination of multicomponent drugs [17-22]. The proposed method is validated according to ICH guidelines [23,24]. Present study emphasizes on the determination of hydrochlorothiazide and irbesartan in their combined dosage form by high performance liquid chromatography.

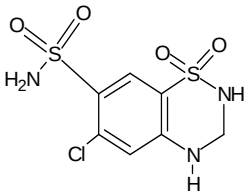

Figure 1: Structure of Hydrochlorothiazide

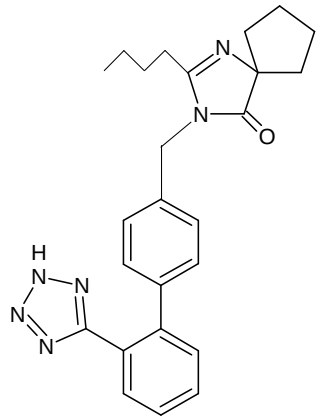

Figure 2: Structure of Irbesartan

Experimental Section:

Instrumentation

The LC system used for work was Perkin Elmer Quaternary pump Series 200 with auto sampler injector; and an Intelligence PDA detector connected to the Total Chrome Navigator version 6.3 software. For controlling the instrumentation as well as processing the data generated.

Material and reagents

HYD and IRB were obtained as gift sample from Ranbaxy Laboratories Limited (Gurgaon Haryana, India) respectively. Acetonitrile (HPLC grade), potassium dihydrogen orthophosphate (HPLC grade), methanol (HPLC grade), orthophosphoric acid (AR grade) were obtained from Rankem Pvt. Ltd. Delhi, India. The 0.45 µm membrane filter was used throughout the experiment. The tablets of HYD in combination with IRB (Irovrl-H) were purchased from Local market. Double distilled water was used throughout the experiment. Other chemicals used in the experiment either of analytical or HPLC grade.

Chromatographic conditions

The isocratic mobile phase consists of methanol: 0.05 M potassium dihydrogen orthophosphate buffer pH 2.5 in the ratio of 60:40 (v/v) with a constant flow rate of 0.8 mL/min. A Hypersil ODS C_{18} column (250mm × 4.6mm, 5 µ) was used as the stationary phase. HYD and IRB have different λmax but considering the chromatographic parameter, sensitivity, and selectivity of the method for these drugs, 226 nm was selected as the detection wavelength for PDA detector. The injection volume for sample was 10 µl.

Mobile phase

The mobile phase consisted of methanol: 0.05 M potassium dihydrogen orthophosphate buffer in the ratio 60:40 (v/v). The pH of the buffer was adjusted to 2.5 with orthophosphoric acid. The buffer used in the mobile phase consisted of 0.05 M potassium dihydrogen orthophosphate in double distilled water. The mobile phase was premixed and filtered through a 0.45-µm membrane filter and degassed.

Standard stock solution of Hydrochlorothiazide and Irbesartan (100 µg/ml)

10 mg of standard drugs separately hydrochlorothiazide and irbesartan dissolved in 100 ml of mobile phase and then volumes were made up to the mark with mobile phase to get 100 µg/ml of standard stock solutions and

sonicated for 10 minute. These stock solutions were filtered through 0.45 µm membrane filter paper. For the preparation of working standard, suitable aliquots of stock solutions were pipette out and volumes were made up to the mark with mobile phase.

Calibration curve solutions

From the mentioned stock solutions of HYD and IRB calibration curve solutions containing 5-30 µg/mL of HYD and 60-360 µg/mL of IRB in each calibration level were prepared.

Preparation of sample solutions

Twenty tablets were weighed and finely powdered. A quantity equivalent to one tablet containing 1.5 mg of HYD and 5 mg of IRB was transferred in a 50 mL volumetric flask and volume was made with mobile phase. The contents were sonicated for 20 min with mobile phase, and filtered through 0.45µm membrane filter.

Result and Discussion

Optimization of chromatographic conditions

The chromatographic method was optimized after performing different experiments to achieve the adequate retentions and resolution for the peaks of HYD and IRB. To set the adequate retentions and resolution, the effects of the mobile phase components, changes in ionic strength were studied, initially methanol and water in different ratios were tried. But HYD gave broad peak shape While IRB gave no peak, so water was replaced by potassium dihydrogen buffer (0.05 M), and mixture of methanol and potassium dihydrogen phosphate buffer in different ratios were tried. It was found that both peaks show broad peaks. Finally methanol: 0.05 M potassium dihydrogen orthophosphate buffer pH 2.5 adjusted with OPA in ratio of 60:40 v/v gave acceptable retention time (3.21 min for HYD and 10.19 min for IRB) and resolution for HYD and IRB was found to be 23.42 at the flow rate of 0.8 mL/min.

Validation of the method

The proposed method was validated by studying several parameters such as Specificity, linearity, precision, accuracy and limit of detection (LOD), limit of quantitation (LOQ).

Specificity

The specificity of the method was checked by a peak purity test of the sample preparation done by PDA detector. The peak purity for HYD and IRB was found to be 0.999. The result of the peak purity analysis shows that the peaks of the analytes were pure and also the formation excipients were not interfering with the analyte peaks.

Calibration and linearity

The standard solutions containing 5 - 30 µg/mL of HYD and 60-360 µg/mL of IRB in each linearity level were prepared. Linearity solutions were injected in triplicate. Calibration graphs were found to be linear for both the analytes in the mentioned concentrations. The coefficient of correlation was found to be 0.998 and 0.999 for HYD and IRB, respectively.

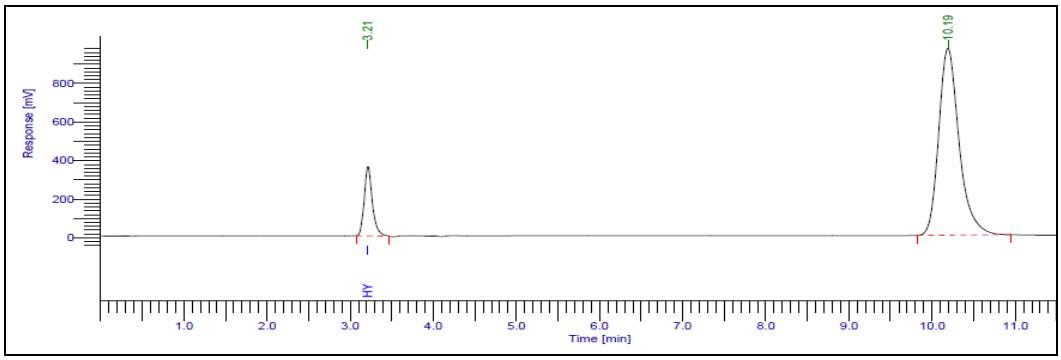

Figure No.1: Typical chromatogram of mixture

Table No.1: Calibration table for HYD

Sr.No.	Concentration (µg/ml)	Area (mV)
1.	5	450365
2.	10	849164
3.	15	1201144
4.	20	1550036
5.	25	1984434
6.	30	2353030

Figure No.3: Calibration curve of HYD.

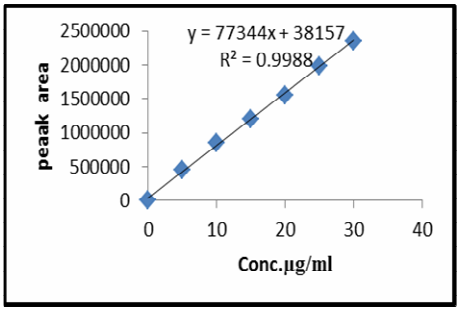

Table No. 2: Calibration table for IRB

Sr. No.	Concentration (µg/ml)	Area (mV)
1.	60	1308271
2.	120	1756383
3.	180	3918669
4.	240	42733229
5.	300	12840387
6.	360	17346293

Figure No.4: Calibration curve of IRB.

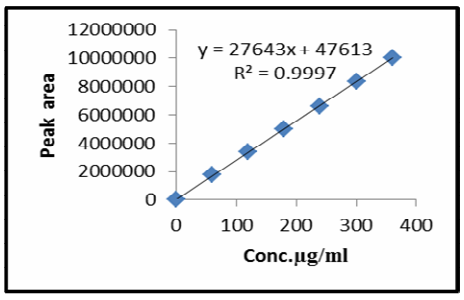

Table No.3: Linear regression data for calibration curve of HYD and IRB

Sr. No.	Linearity range	r^2	Slope	Intercept
HYD	5-30	0.998	77344	38157
IRB	60-360	0.999	27643	47613

Accuracy (recovery test)

The accuracy of the method was done by recovery study. The recovery experiments were performed by adding known amounts of the pure drug to the pre analyzed sample. The recovery was done at three levels: 80%, 100%, and 120% of the label claim. Three samples were prepared for each recovery level. The recovery values for HYD and IRB was 100.12 and 100.25 respectively.

Table No.4: Statistical validation of Recovery study.

Level of % recovery	% Mean recovery*		S.D.		% R.S.D.	
	HYD	IRB	HYD	IRB	HYD	IRB
80	99.72	100.44	0.4054	0.5727	0.4065	0.5701
100	100.26	100.33	0.1960	0.2613	0.1954	0.2604
120	100.38	100.00	0.3046	0.2594	0.3034	0.3034

*Average recovery = average of three levels, nine determinations

Precision (repeatability)

The precision of the method was studied by determining the concentrations of each ingredient in the tablet six times. In the precision study, percentage relative standard deviation of the HYD and IRB were found to be 1.3335 and 0.1198 respectively. The results of precision study indicate that the method is reproducible.

Intermediate precision

Intermediate precision of the method was done by analyzing the sample of six replicate on different days, by different chemists, using analytical column of different make and different HPLC systems. The percentage assay was calculated using the calibration curve. The assay results are shown in Table No.5.

Table No.5: Assay Results of Active Ingredients in Tablets

Set	Ingredients	Label claim (mg)	Found (mg) †	% Label claim ± %RSD
Precision	HYD	1.5	1.51	100.66 ± 1.024
	IRB	5	4.991	99.41 ± 0.768
Intermediate precision	HYD	1.5	1.49	99.33 ± 1.022
	IRB	5	4.951	99.53 ± 0.711

† Average of six analyses

Determination of the limits of detection and Quantitation

For determining the limits of detection (LOD) and quantitation (LOQ), the method based on the RSD of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curve was studied using samples in the range of the detection and quantitation limits. The LOD for HYD and IRB were 0.0425 and 0.0119 µg/mL, and the LOQ were 0.140 and 0.0395 µg/mL respectively.

System suitability

For system suitability studies, five replicate injections of mixed standard solutions were injected, and the parameters like RSD of peak area ratio, column efficiency, resolution, and tailing factor of the peaks were calculated. Results are reported in Table No.6.

Table No.6: System Suitability Parameters

Parameters	**HYD**	**IRB**
Retention time (min)	3.21	10.19
Resolution	23.42	
Theoretical Plates	4304.53	6466.69
Tailing factor	1.43	1.55

Analysis of Marketed Formulations.

The proposed method was successfully applied to the determination of HYD and IRB in their combined dosage form. The % recovery ± S.D. was found to be 99.93±0.294 and 100.21± 0.827, respectively, for HYD and IRB (Table 7) which were comparable with the corresponding labeled amounts.

Table No. 7: Assay results of tablet dosage form using proposed method.

Formulations	Labelled amount (mg)		% Recovery [c]	
	HYD	IRB	HYD	IRB
Irovrl-H	1.5	5	99.93±0.294	100.21±0.827

[c] mean value ± standard deviation of determinations; Tablet formulation Irovrl-H (Sun Pharmaceutical Pvt. Ltd. Himachal Pradesh) containing labeled amount of Hydrochlorothiazide 1.5 mg and Irbesartan 5 mg.

Conclusion

It can be concluded from present study that method can be used for the simultaneous determination of Hydrochlorothiazide and Irbesartan in the pharmaceutical preparation. The method is validated and shown to be accurate and precise. It can be used in the quality control departments for the analysis of Hydrochlorothiazide in combination with Irbesartan.

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