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Validated RP-HPLC Method Development for the Simultaneous Estimation of Irbesartan and Hydrochlorothiazide in Combined Dosage Form

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Abstract
A simple, sensitive, reproducible, accurate and precise RP-HPLC method was developed for simultaneous estimation of Irbesartan and Hydrochlorothiazide in tablet dosage form. The chromatographic separation was achieved on Quails 5 BDS C18 column (250 x 4.6mm, particle size 5µ) in low pressure gradient mode with mobile phase Acetonitrile: water (pH adjusted to 3.3 with orthophosphoric acid)in the ratio (42:58 v/v). The flow rate and injection volume were 1.1ml/min and 10 µL respectively and monitored on a PDA detector at 254nm. The developed method was found to be linear for Irbesartan and hydrochlorothiazide in the range of 12-84µg/ml and 1-7µg/ml with correlation coefficient (r^2) 0.9997 and 0.9992 respectively. The proposed method was validated as per ICH Q2B guidelines. Assay of the marketed formulation was found to be 98.26% and 98.47% for Irbesartan and hydrochlorothiazide respectively. In accuracy study the percentage recovery at three different levels was found to be in the range of 98.80% to 101.78%.

Keywords: *Irbesartan, Hydrochlorothiazide, Telmisartan, Internal standard method, RP-HPLC validation*

INTRODUCTION

Irbesartan (IRBN), 2-Butyl-3-[*p*-(*o*-1H-tetrazol-5-ylphenyl) benzyl]-1, 3-diazaspiro [4.4] non-1-en-4-onean antihypertensive agent[1,2] and Hydrochlorothiazide (HCZ), 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide a diuretic, are frequently available as combined dosage form [2]. The drugs IRBNshown in Fig.1 and HCZ shown in Fig.2 are majorly used in many antihypertensive pharmaceutical formulations as individual drugs and also in combination[3]. Literature survey reveals that few analytical methods such as capillary zone electrophoretic[5], HPTLC[6,7], UV spectrophotometric[8,9], LC-MS[10-12] and HPLC[13-17] are available for estimation of IRBN and HCZ as

individual drugs and also in combination with other drugs. Also, HPLC internal standard (IS) method is available for IRBN and HCZ in combination[18]. This reported method describes the use of mixture of three component mobile phase and column temperature maintained at 50°C. So, there is a scope to develop a HPLC method with simple chromatographic conditions. Therefore, present research work aims to develop simple, precise, accurate, sensitive and specific HPLC Internal Standard method for the estimation of IRBN and HCZ in pharmaceutical formulation.

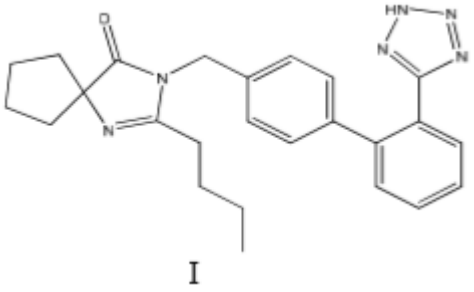

Figure 1: Structure of Irbesartan

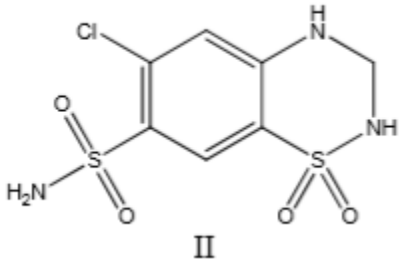

Figure 2: Structure of Hydrochlorothiazide

MATERIALS AND METHODS

Chemicals

IRBN and HCZ were obtained as gift samples from Glenmark Pharmaceuticals Ltd. Mumbai, India. Telmisartan (used as internal standard) was obtained from Vasudha Chemicals Pvt Ltd. Navi Mumbai, India.All HPLC grade solvents [acetonitrile (ACN), water and methanol and other chemicals like Orthophosphoric acid (OPA)] of Merck Life Science were procured from Local vendor. Tablet formulation IROVEL-H,Sun Pharma Laboratories with labelled claim for IRBN and HCZ as 150mg and 12.5 mg was purchased from the local pharmacy.

Instruments

For present study High Performance Liquid Chromatography system of Shimadzu Corporation, Japan (Shimadzu LC20 AD) equipped with LC lab solution software with Qualisil 5 BDS C18 column as stationary phase with dimension as 250 x 4.6mm x 5µand PDA detector was used.

Preparation of standard stock solution

IRBN(100 mg) and HCZ (100 mg) were transferred into two separate 100ml volumetric flask containing 60ml methanol. The content was dissolved by sonication for 5 mins and volume was topped up with diluent.Further dilutions were carried out with the diluent to get the desired concentrations.

Preparation of Internal Standard solution

Telmisartan (100 mg) was accurately weighed and dissolved in 30ml methanol by sonication and diluted upto 100ml with the diluent. An aliquot was further appropriately diluted using diluent to get a concentration of 10µg/ml.

Preparation of Sample solution and Assay of marketed formulation

Tablet powder (403mg, equivalent to 150mg of IRBN and 12.5mg of HCZ) was dissolved in 60ml methanol by sonication and volume was topped up with diluent.Resulting solution was filtered using membrane filter (0.22µ), further diluted appropriately (36 µg/ml of IRBN and 3 µg/ml of HCZ) and analyzed by HPLC.

Chromatographic Conditions

During mobile phase optimization ACN: Water (pH adjusted to 3.3 with orthophosphoric acid) in the ratio of 42:58 was found to be satisfactory for IRBN and HCZ. The mobile phase was degassed and filtered through membrane filter (0.22µ) and delivered at 1.1ml/min inlow-pressure gradient modeat ambient temperature. The volume of sample injected was 10 µL and the run time was 10 mins. The detection was carried out at 254nm using PDA detector. Water:ACN (58:42) was used as a diluent in the study.

Method Development &Validation

Mobile phase consisting of ACN: Water (adjusted pH to 3.3 with OPA) in the ratio of (42:58) at 254 nm with aflow rate1.1 ml/min was used for the present method. The validation of present method was performed for system suitability, linearity and range, precision, accuracy, robustness, ruggedness quantitation limit (LQ) and detectionlimit (LD) [19].

System Suitability

The mixed standard solution(36 µg/ml of IRBN and 3 µg/ml ofHCZ and IS of concentration 10µg /ml)wasanalyzed in six replicates as per the method describe for sample.

Specificity

Standard solutions(36 µg/ml of IRBN and 3 µg/ml of HCZ) and sample solutions(36 µg/ml of IRBN and 3 µg/ml of HCZ)were analyzed intriplicates.

System and Method Precision

System precision was evaluated by analyzing the six replicates of standard solutions(36µg/ml ofIRBNand 3µg/ml ofHCZ) using chromatographic conditions mentioned earlier.The results were expressed in terms of percent relative standard deviation of peak area. For method precision the six replicates of sample solutions(36µg/ml of IRBN and 3µg/ml of HCZ)were analyzed. The percentage assay of IRBN and HCZwas calculated and results were expressed in terms of % RSD.

Intermediate Precision

Intra Day and inter day precision studies were carried out to determine the Intermediate precision at three different concentration level at regular interval of time in a day and on three different days in triplicates. The percentage assay of IRBN and HCZwas calculated and the results were expressed in terms of %RSD.

Linearity and Range

Working standard solutions of IRBN and HCZin the range of 12-84µg/ml and 1-7µg/mlrespectively were analyzed. The linearity of the responses was calculated by regression analysis of the concentration *vs* ratio of peak area.

Accuracy

Recovery experiments were performed by standard addition method at80%, 100%, and 120% level in triplicate. The percentage recoveries were calculated against respective levels.

Robustness and Ruggedness

The robustness of the developed method was determined byaltering the pH from 3.3 to 3.1, 3.5 and flow rate from 1.1 ml/min to 1.0ml, 1.2ml. The ruggedness of method was determined by injecting the six replicates of preanalyzed sample as per the proposed method by different analyst.The results were represented as percentage RSD.

DetectionLimit (LD) and QuantitationLimit (LQ)

The LD and LQwere calculated using theslope of the linearity curve and standard deviations the peak area.

RESULTS AND DISCUSSION

Thetablet IROVEL-H was analyzed by using optimized chromatographic conditions (Mobile phase consisting of ACN: Water with pH adjusted to 3.3 with orthophosphoric acid in the ratio of 42:58 at 254 nm with a flow rate 1.1 ml/min) and Telmisartan as an IS to minimize the errors caused by variations in chromatographic conditions. The percentage assay was found to be 99.58% w/w for IRBN and 99.51% w/w for HCZ. The recorded chromatograms of standard and sample solution are shown in figure 3 and 4.The method was found to be suitable for the estimation of IRBN and HCZ in tablet dosage forms as reflected by the system suitability parameters which were found to be well within the acceptable limits (Table 1).Mobile phase and excipients in sample did not show any interference at the retention time of IRBN and HCZ which indicates that the method is specific for estimation of IRBN and HCZ. InSystem precision study the %RSD was found to be 0.82% and 0.75% for IRBN and HCZ respectively andin method precision study the %RSD was found to be 0.96% and 0.99% for IRBN and HCZ respectively which was found to be well within the acceptable limits (less than 2.0%).The intraday and interday precision studies were carried out by injecting the sample solutions at three levels at different time interval in a day and on three different days at three concentration levels.In intra-day precision study, the %RSD was found to be 0.87% and 0.85% for IRBN and HCZ respectively (Table 2). In interday precision study, the %RSD was found to be 0.78% and 0.94% for IRBN and HCZ respectively(Table3). The %RSD values in precision study, indicates that the method is precise for their estimation. Linearity of method was determined by analyzing the mixed standard solutions in the concentration range from 12µg/ml to 84µg/ml for IRBN and from 1µg/ml to 7µg/ml for HCZ (Table4) along with fixed concentration (10ppm) of Telmisartan (IS). The linearity curve of IRBN and HCZ is shown in figure 5-6 respectively and the R^2 value for IRBN and HCZ was found to be 0.9997 and 0.9992 respectively. Accuracy of the method was determined by recovery study at 80%, 100% and 120%. The results of recovery study are shown in Table 5. The mean percentage recovery was found to be in the range of 99.01% to 101.78% indicating that method is accurate for the determination of IRBN and HCZ. . No significant change was observed in system suitability and percentage assay even after slight variation in pH and flow rate of mobile phase which reflects the robust (Table 6). The % assay remains unaffected by change in analyst which indicates the ruggedness of the method(Table 7). The LD and LQfor IRBN were found to be 1.2ng/ml and 3.8ng/ml respectively. While the LD and LQfor HCZ was found to be 0.28ng/ml and 0.86ng/ml respectively. The

chromatographic conditions and results of validation parameters of the present work are summarized in Table 8.

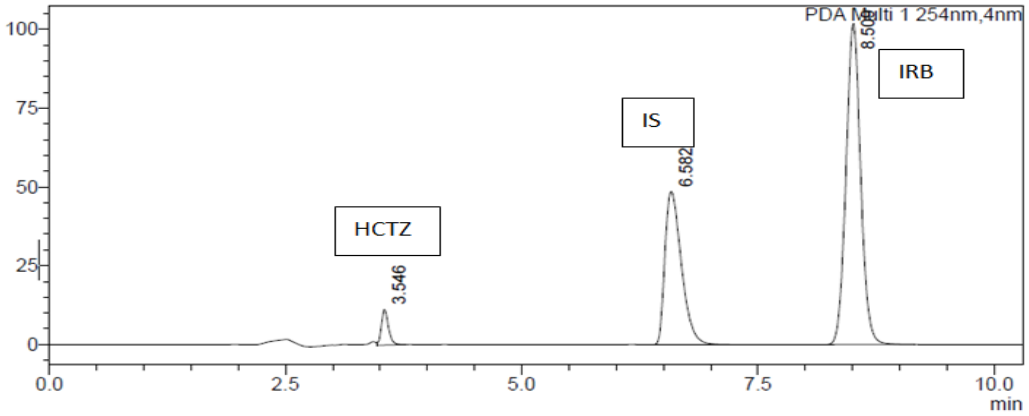

Figure3: Chromatogram of Standard IRBN and HCZ with IS

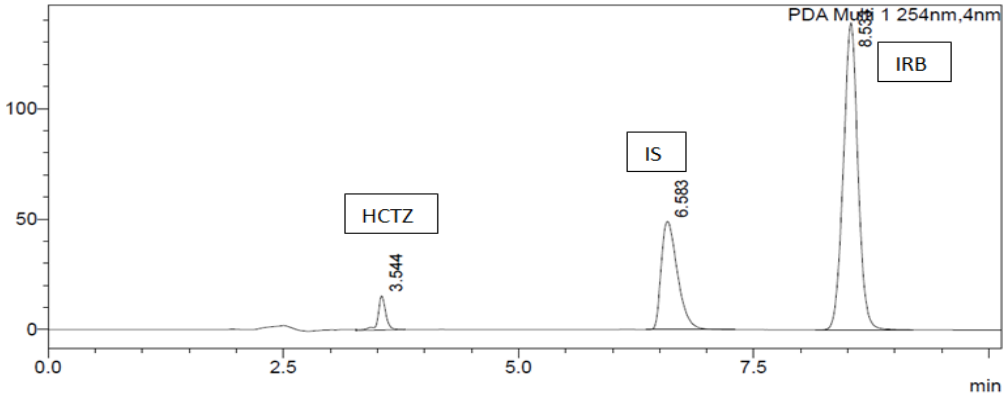

Figure3: Chromatogram of Sample

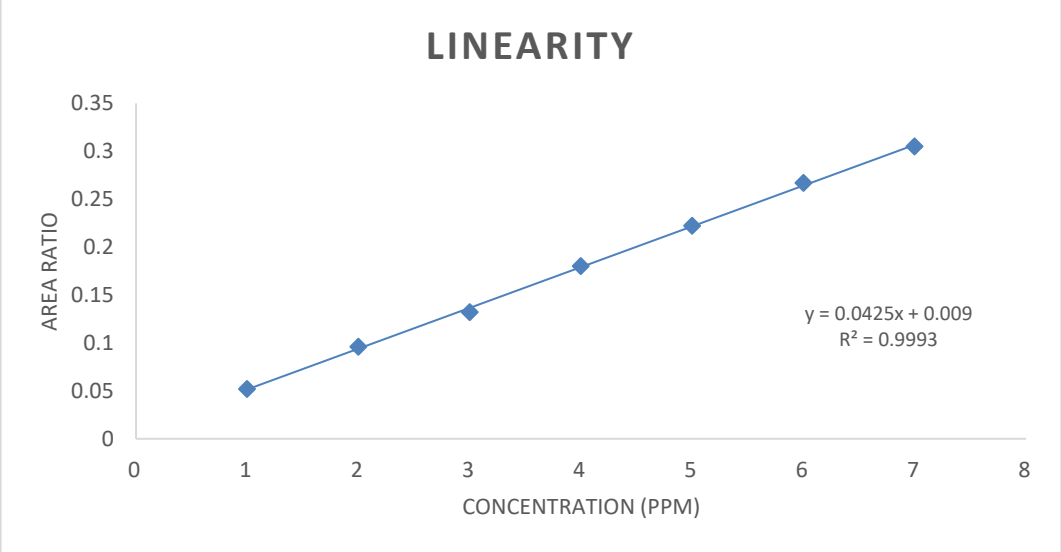

Figure 6: Linearity Curve of HCZ

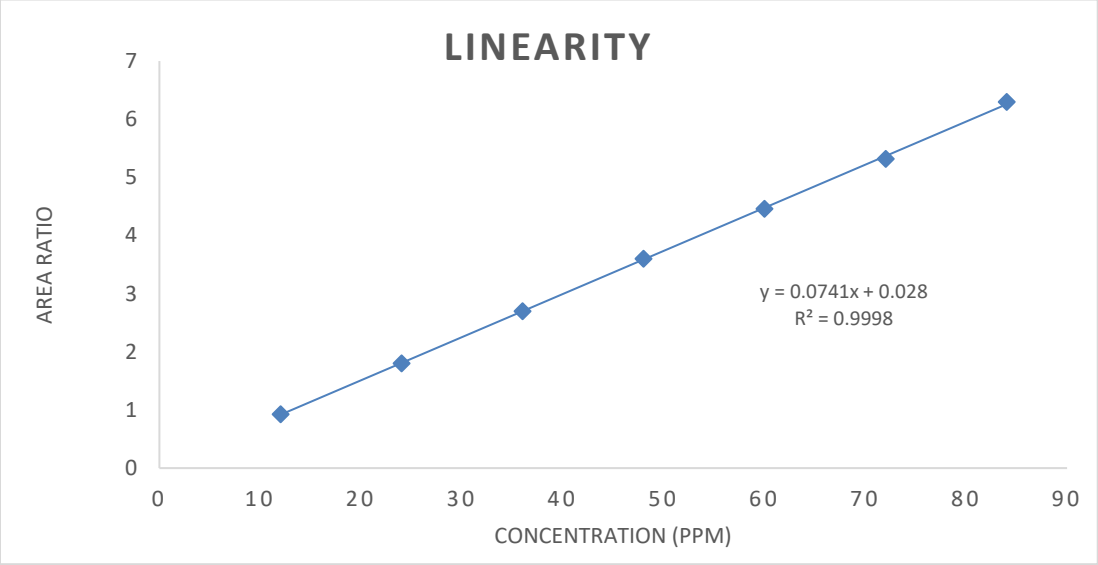

Figure 5: Linearity Curve of IRBN

Table 1: System suitability Parameters

Parameters	**USP Tailing**	**USP Plate Count**	**%RSD of Peak areas**	**Retention Time**
Irbesartan	1.058	13243	0.98	8.500
Hydrochlorothiazide	1.025	8704	6467	3.546
Telmisartan (IS)	1.559	6467	0.82	6.582
Acceptance Criteria	NMT 2.0	NLT 2000	NMT 2.0	--

Table 2: Intra Day PrecisionStudy of IRBN and HCZ

Sr. no.	**IRBN**			**HCZ**		
	Concentration (µg/ml)	**±SD**	**%RSD**	**Concentration (µg/ml)**	**SD**	**%RSD**
1	24	10078.53	0.93	2	416.85	0.71
2	36	11578.46	0.77	3	797.87	0.91
3	48	18417.33	0.91	4	1216.5	0.95

Table 3: Inter Day Precision Study of IRBN and HCZ

Sr. no.	**Day**	**IRBN**			**HCZ**		
		Concentration (µg/ml)	**SD**	**%RSD**	**Concentration (µg/ml)**	**SD**	**%RSD**
1	Day 1	24	9846.8	0.90	2	443.97	0.76
	Day 2						
	Day 3						

2	Day 1 Day 2 Day 3	36	14568.31	0.98	3	828.15	0.94
3	Day 1 Day 2 Day 3	48	16821.97	0.83	4	1186.2	0.92

Table 4: Linearity of IRBN and HCZ

Sr. No.	**IRBN**		**HCZ**	
	Concentration of IRB (ppm)	**Average Peak area ratio of IRB and IS**	**Concentration of HCTZ (ppm)**	**Average Peak area ratio of HCTZ and IS**
1	12	0.92	1	0.05
2	24	1.80	2	0.10
3	36	2.70	3	0.13
4	48	3.60	4	0.18
5	60	4.46	5	0.22
6	72	5.32	6	0.27
7	84	6.30	7	0.30
(Correlation coefficient) R^2	0.9997		0.9992	

Table 5: Recovery study for IRBN and HCZ

Recovery level	**IRBN**		**HCZ**	
	% Recovery	**Average % Recovery**	**% Recovery**	**Average % Recovery**
80% - 1	101.91	101.78	98.80	99.06
80% - 2	101.76		98.97	
80% - 3	101.68		99.41	
100% - 1	99.77	99.95	101.12	101.06
100% - 2	100.34		100.90	
100% - 3	99.75		101.16	
120% - 1	98.77	98.80	100.76	99.67
120% - 2	99.30		99.01	
120% - 3	98.33		99.24	

Table 6: Robustness Study

Parameter		**IRBN**			**HCZ**		
		Retention time (Rt)	**USP Tailing**	**% RSD of peak areas**	**Retention time (Rt)**	**USP Tailing**	**% RSD of peak areas**
Flow (mL/min)	1.0	8.491	1.023	0.84	3.514	1.012	0.94
	1.1	8.501	1.058	0.75	3.546	1.025	0.96
	1.2	8.483	1.045	0.83	3.521	1.044	0.91
pH	3.0	8.456	1.012	0.94	3.542	1.110	0.89
	3.2	8.510	1.066	0.98	3.584	1.036	0.92
	3.5	8.497	1.123	0.96	3.536	1.027	0.94

Table 7: Ruggedness Study

Sample No.	**% Assay of IRBN**	**% Assay of HCZ**
1	97.85	98.25
2	97.52	99.84
3	99.66	97.37
4	99.55	98.55
5	98.28	99.23
6	98.85	97.65
Mean	98.62	98.48
SD	0.89	0.94
% RSD	0.90	0.95

Table 8: **Summary of** Chromatographic Conditions and Results of Validation Parameters

Sr. No	**Parameters**	**IRBN**		**HCZ**	
		Average	**% RSD**	**Average**	**% RSD**
1.	Optimized Mobile Phase	Water (pH adjusted to 3.3 using OPA): acetonitrile (58:42 v/v)			
2.	Wavelength, flow rate, injection volume	254nm, 1.1ml/min, 10 µl			
3.	Retention time	8.500		3.546	
4.	Assay of marketed formulation	99.58%w/w		99.51%w/w	
5.	Method Precision	98.26%w/w	0.96	98.47%w/w	0.99
6.	Inter-day Precision	98.20%w/w	0.78	98.58%w/w	0.94
7.	Intra-day Precision	98.81%w/w	0.87	98.46%w/w	0.85

8.	Linearity	12-84µg/ml	0.9998 (R^2)	1-7µg/ml	0.9992 (R^2)
9.	Recovery	99.95%w/w	-	101.00%w/w	-
10.	Robustness	Robust		Robust	
11.	Ruggedness	98.62	0.90	98.48	0.95

CONCLUSION

In present research work separation of IRBN and HCZ was carried out in less than 10min run time by using simple chromatographic conditions at ambient temperature using two component mobile phase (ACN: Water pH 3.3 adjusted with OPA) in the ratio of 42:58 using Telmisartan as internal standard at 254nm. System suitability parameters showed that the method is appropriate and found to be satisfactory for the estimation of IRBN and HCZ in tablet dosage form. Theproposed method was validated as per ICH for specificity, linearity, precision, accuracy, robustness, ruggedness, LD and LQ and was found to be well within the acceptable limit. Hence, method can be used for the routine analysis of IRBN and HCZ in bulk and dosage form.

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