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METHOD DEVELOPMENT AND VALIDATION OF IRBESARTAN AND HYDROCHLORTHIAZIDE BY RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic method had been developed for simultaneous estimation of Irbesartan (IRBE) and Hydrochlorothiazide (HCTZ) in bulk and Pharmaceutical dosage form. A Phenomex Luna C-18 column having I'd of 150×4.6 mm and 5µm particle size was used. The method was carried out in gradient program using mobile phase, 0.02M Potassium dehydrogenate orthophosphate: acetonitrile (60:40 v/v) adjusted to pH-3.4 using dilute ortho phosphoric acid. Flow rate was adjusted to 1.0ml/min and effluents were monitored at 224nm. The retention time obtained for Irbesartan and HCTZ was 2.59 & 8.13min respectively. The calibration curves were linear in the concentration range of 100-300µg/ml for Irbesartan and 50-150µg/ml for HCTZ. The developed method was validated in accordance to ICH guidelines.

KEYWORDS: Irbesartan, Hydrochlorothiazide, Acetonitrile, Buffer, RP-HPLC.

INTRODUCTION

Irbesartan (IRB) is chemically 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 is an orally active specific angiotensin II, AT1 receptor antagonist, and clinically effective drug in the treatment of Hypertension. It is slightly soluble in alcohol and methylene chloride and practically insoluble in water. Due to its hydrophobic nature (octane/water partition coefficient 10.1 at pH of 7.4) IRB shows low dissolution profile in gastrointestinal fluid resulting poor absorption, distribution & consequently poor target organ delivery. Improvement of aqueous solubility in such cases

shall lead to improved therapeutic efficacy of the drug. Hydrochlorothiazide (HCTZ), or 6-chloro- 3, 4- dihydro-2H-1, 2, 4-benzothiadiazine-7-sulpho namide-1, 1-dioxide, is a widely used thiazide diuretic. It increases urinary excretion of sodium and water by inhibiting sodium re absorption in the renal tubules. It is indicated for the treatment of edema, control of essential hypertension and management of diabetes insipidus. Usually in combination with other antihypertensive agents with different mechanisms of action. This is not only because blood pressure control is often inadequate using mono therapy but also because combination therapy can simplify dosing regimens, improve compliance, decrease sideeffects and reduce cost. Several methods have been studied for simultaneous determination of IRB and HCTZ, but there are limited reports on method for combination. So the aim of our study is to develop simple, fast, accurate and specific reversed phase high performance liquid chromatographic method for simultaneous determination of Irbesartan and hydrochlorothiazide in bulk drugs and Pharmaceutical Dosage form.

MATERIALS AND METHOD

Chemicals and solvents

*Pure samples of IRB and HCTZ were obtained respectively from Spectrum pharma research solutions, Hyderabad, India. www.pharmascholars.com.*commercial pharmaceutical preparation Availed containing 150mg and 12.5mg IRB and HCTZ respectively (Marketed by Piramal Health care Pvt. Ltd) were procured from local pharmacy. Acetonitrile, Methanol and water used are of HPLC grade.

Instrumentation: The chromatographic separations were performed using HPLC-Waters alliance (Model-2695) consisting of an inbuilt auto sampler, a column oven and 2996 PDA detector. The data was acquired through Empower-2-software. The column used was Phenomex, Luna C18 (150×4.6mm I'd, 5µm particle size). Meltronicssonicator was used for enhancing dissolution of the compounds. Dig sun pH meter was used for adjusting the pH of buffer solution. All weighing was done on sarotorious balance (model AE-160).

Chromatographic conditions: A Phenomex Luna C-18 column having I'd of 150×4.6 mm and 5µm particle size was used .at ambient temperature. 1.36 g of Potassium dehydrogenate orthophosphate was weighed and 1000ml of Milli-Q water was added to it. The mobile phase was considered buffer: acetonitrile. PH was adjusted to 3.4 with Ortho phosphoric acid and was filtered through 0.45µm PVDF membrane filter disc and was degassed. Flow rate was maintained at 1ml/min. The elution was observed at 224nm. Some trials were carried out with

respect to change in the ratio of constituents of the mobile phase like 50: 60,50:50, 60:40 (buffer: actonitrile). Injection volume and run time were 20 µl l and 10 mins respectively. In the ratio 60:40 retention time for hydrochlorothiazide and Irbesartan were observed to be 2.59 and 8.13 min respectively. The two peaks were well resolved with good peak shape and symmetry was obtained. Hence this method was finalized for the simultaneous estimation of Irbesartan and Hydrochlorothiazide.

Preparation of buffer solution: Accurately weighed 1.36 gm of Potassium Dehydrogenate orthophosphate (0.02M) was transferred into 1000ml volumetric flask. Add about 900ml of Milli-Q water and Sonicate to dissolve, then make up to 1000ml and then pH was adjusted to 3.4 using dilute Ortho phosphoric acid.

Preparation of mobile phase: The prepared phosphate buffer solution and acetonitrile was used as mobile phase in ratio 60:40.

Preparation of standard stock solutions

IRBE (10mg) and HCTZ (5mg) were accurately weighed and transferred into 10ml volumetric flask, and dissolved in methanol. The volume was made up to the mark with methanol. From resulting stock solution 1ml was pipette out and was further diluted to 10ml with methanol to get the concentration of 150µg/ml IRBE and 12.5µg/ml HCTZ, which was used for calibration purpose of both the drugs.

Preparation of sample solution

Twenty tablets, each containing 150mg of IRBE and 12.5mg of HCTZ were accurately weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 150mg of IRBE and 12.5mg of HCTZ was accurately weighed and transferred into 10ml volumetric flask. 6ml of methanol was added to it and shaken until the drug gets dissolved. The volume was made up to the mark with methanol. The solution was sonicated for 10min, filtered through the 0.45µm PVDF membrane filter disc. This solution was further diluted with methanol to get the same concentration as that of final standard solution.

Method Validation: The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision, Specificity, Linearity, Accuracy, Limit of detection and Limit of quantification.

Linearity: Aliquots of 0.3, 0.75, 1.0, 1.5, 1.8 and 2.25 ml were taken from stock solution of concentration 1000µg/ml IRBE and 500µg/ml HCTZ, and then diluted up to mark with methanol. Such that the final concentrations were in the range 30-225µg/ml for IRBE and 2.5-18.75µg/ml for HCTZ. Volume of 10µl of each sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. The observations and calibration curve were shown in Table 1 and Fig. 2, 3.

Estimation of Irbesartan and Hydrochlorothiazide

Accurately weighed powder equivalent to 150mg of IRBE and 12.5mg of HCTZ was transferred into 10ml volumetric flask and made up to the mark with diluent methanol to obtain solution of IRBE (10mg/ml) and HCTZ (0.5mg/ml). From this each solution 1.5ml and 0.25ml was transferred to 10ml volumetric flask and made up to the mark with diluents methanol to obtain solution of IRBE (150µg/ml) and HCTZ (12.5µg/ml). The results were shown in Table-2. The chromatograms were shown in Fig-4, 5.

Accuracy

Accuracy of the method was done by recovery study. Sample solutions were prepared by Ramesh, et al. Int J Pharm 2013; 3(3): 521-526 **ISSN 2249-1848** www.pharmascholars.com 523spiking at about 50%, 100%, and 150% of specification limit to placebo and analyzed by the proposed HPLC method. Results are shown in Table-4.

Specificity

The specificity of the method was performed by injecting blank solution (without any sample) and then a drug solution of 10µl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both IRBE and HCTZ from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be Specific. ***Limit of detection (LOD) and Limit of quantification (LOQ):*** The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The linearity for IRBE and HCTZ was performed from 30-225µg/ml and 2.5-18.75µg/ml respectively. ***System precision:*** Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Standard solution of IRBE (150µg/ml) and HCTZ (12.5µg/ml) were prepared as per test method and injected for 3 times. Results are shown in Table-4. ***Method precision:*** Three samples were prepared and analyzed as per the test method

on same day and three different days and calculated the % RSD for assay of five preparations. Results were shown in Table- 5. ***Robustness:*** Robustness studies were carried out by variations in flow rate, mobile phase compositions and temperature. It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak interest. The degree of reproducibility of the results proven that the method is robust.

System suitability: The system suitability was determined by making six replicate injections from freshly prepared standard solutions. The observed RSD values were well within usually accepted limits (≤2%). Theoretical plates, tailing factor, resolution between IRBE and HCTZ were determined. The results are all within acceptable limits summarized in Table-6.

RESULTS AND DISCUSSION

The nature of sample, its molecular weight and solubility decides the proper selection of stationary phase. The drugs IRBE and HCTZ were preferably analyzed by reverse phase chromatography and accordingly C18 column was selected. The elution of the compounds from column was influenced by polar mobile phase. The ratio of phosphate buffer to Acetonitrile was optimized to (60:40) to give well resolved and good symmetrical peaks with short run time. The retention time of IRBE and HCTZ were found to be 2.59 & 8.13 min respectively. The calibration curve was linear over the concentration range of 30-225µg/ml (IRBE) and 2.5-18.75µg/ml (HCTZ). The linearity of the method was statistically confirmed. RSD values for accuracy and precission studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters were given in table-5. The analytical recovery at five different concentrations of IRBE and HCTZ was determined and the recovery results were in the range of 100-300µg/ml. Therefore proposed validated method was successfully applied to determine IRBE and HCTZ in Bulk and Pharmaceutical dosage form.

Table-1: Linearity.

S. No	Conc. IRBE in µg/ml	IRBE area	Conc. HCTZ in µg/ml	HCTZ area
1	30	1264305	2.40	340712
2	75	3085859	6.24	812164
3	105	4340564	8.69	1131180
4	150	6172409	12.3	1583355
5	180	7282931	15.2	1863839
6	225	9195918	18.60	2332427

Table. 2: IRBE and HCTZ assay.

Drug	Label claim mg/tab	Amount found mg/tab	Label claim (%)	S.D*	% R.S.D
IRBE	150	149.90	99.90	0.189754	0.191106
HCTZ	12.5	12.49	99.93	0.329008	0.329008

Table. 3: Accuracy.

% linearity level	No. of times	% recovery	%mean recovery ± S.D	% RSD
50	1	99.95233	99.83± 0.1799	0.160652
	2	100.1493		
100	3	99.83102	99.24± 0.4652	0.17445
	1	99.55773		
	2	99.24518		
150	3	99.53192	99.93 ± 0.8668	0.36473
	1	100.0227		

Table. 4: System precession.

Injections	Areas(irbe)	Areas(hctz)
1	8033210	1123752
2	7955877	1127202
3	7992667	1130876
4	7964985	1126952
5	7930643	1122460
6	7999087	1129145
AVG	7979412	1126731
S.D	36325	3174.9
%R.S.D	0.5	0.5

Table-5: Method precession.

Drug	% Assay	Mean	S.D	% R.S.D
IRBE	99.40999 99.13919 99.14774 99.42698 99.54269 99.08979	99.29273	0.189754	0.191106
HCTZ	99.58895 100.0809 100.1466 99.98753 99.35761 100.1263	99.88131	0.329008	0.329399

Table. 6: characteristics of HPLC method.

Drug	Parameters defined	Obtained value
Irbesartan	Linearity range (µg/ml)	30-225µg/ml
	Slope	40682
	Intercept	31492
	Regression coefficient(r2)	0.999
	LOD (µg/ml)	2.5
	LOQ (µg/ml)	7.74
	Tailing factor	1.23
	Plate count	37319
Hydrochlorothiazide	Linearity range (µg/ml)	2.5-18.75 µg/ml
	Slope	12356
	Intercept	26595
	Regression coefficient(r2)	0.999
	LOD (µg/ml)	1.53
	LOQ (µg/ml)	4.64
	Tailing factor	0.99
	Plate count	4897

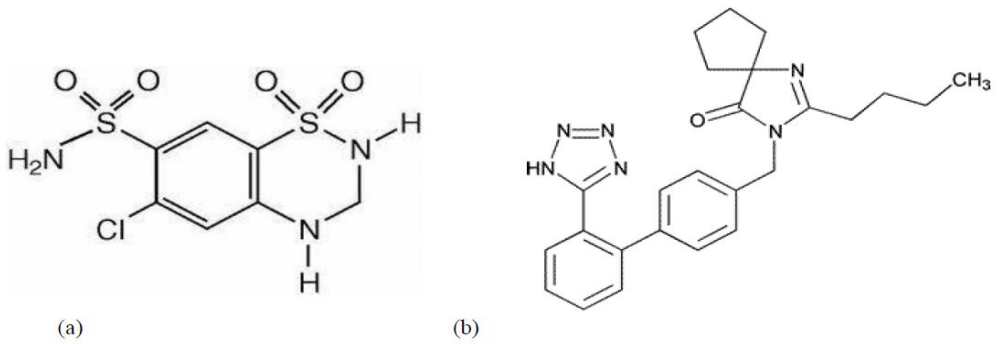

Fig. 1. Structures of a) Hydrochlorothiazide b) Irbesartan.

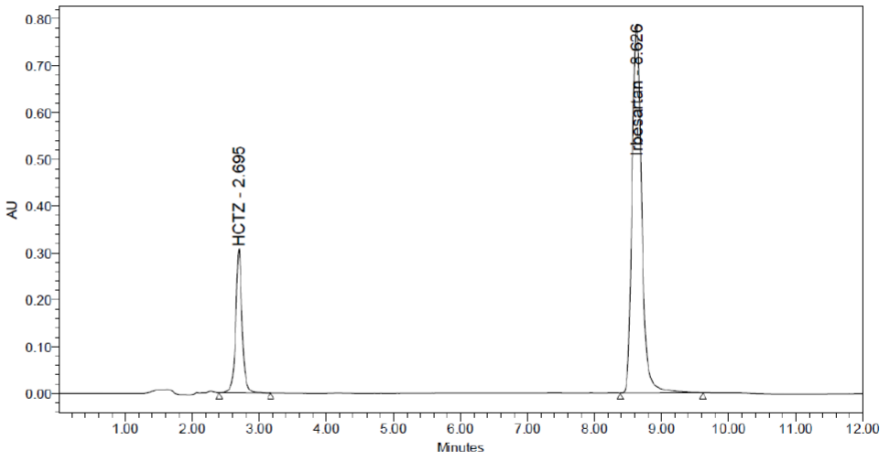

Fig. 2: chromatogram showing retention time of IRBE and HCTZ.

CONCLUSION

The developed method is accurate, simple, rapid and selective for the simultaneous estimation of IRBE and HCTZ in Bulk and pharmaceutical dosage form. The sample preparation is simple, the analysis time is short and the elution is by gradient method. The retention time of IRBE and HCTZ were found to be 2.59 & 8.13 min respectively. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. Hence the proposed method can be conveniently adopted for the routine quality control analysis in the combined formulation.

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