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Research Article

RP-HPLC Method for the Simultaneous Estimation of Irbesartan and Hydrochlorthiazide in Pharmaceutical Dosage Form

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ABSTRACT: A simple, precise, accurate reverse phase high performance liquid chromatographic method has been developed and validated for the simultaneous estimation of Irbesartan and hydrochlorothiazide in combined dosage forms. The mobile phase used was a mixture of sodium acetate buffer: acetonitrile (45:55). The elution was carried out at 260 nm. The method was validated in terms of suitability, precisicion, accuracy, linearity, ruggedness and robustness. The method can be successfully used to determine the drug content of marketed formulation.
KEY WORDS: Irbesartan, Hydrochlorothiazide, RP-HPLC, development, validation, simultaneous estimation

INTRODUCTION

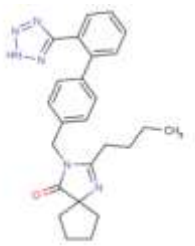
Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs.[1] It involves separating, identifying, and determining the relative amounts of components in a sample matrix. The RP-HPLC is a method of choice for assay that involves sophisticated equipment.

The aim was to develop a simple, rapid, specific and sensitive RP-HPLC method for the determination of Irbesartan and Hydrochlorthiazide in pharmaceutical dosage form (tablets). The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. The survey of literature reveals that few analytical methods are available for the drugs like Irbesartan and Hydrochlorthiazide, but the methods for simultaneous estimation of these drugs were still emerging.

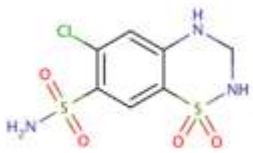
Irbesartan (2-butyl-3-[[4-[2-(2h-tetrazol-5-yl)phenyl]methyl]-1,3-diazaspiro[4.4]non-1-ene 4-one) is an angiotensin II receptor antagonist use and mainly for the treatment of hypertension. Hydrochlorothiazide (6-chloro-1,1-dioxo-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine-7-sulfonamide) is a thiazide diuretic which is used for the treatment of high blood pressure and management of edema.

These two drugs are used in the treatment of hypertension alone or on combination.[1]
Because of their synergestic potential as anti-hypertensive, both drugs are combined in a single dosage form and are available in market. [2]

Literature survey reveals that some methods have already been developed for the estimation of these drugs like HPTLC[1,3-4], HPLC[5-7], UV-Spectrophotometric[8], Spectrofluorometric[9], voltammetric[10], capillary zone electrophoretic[11], LC-MS[12-14] either for individual estimation or the simultaneous estimation of the drugs etc.



Irbesartan



Hydrochlorothiazide

EXPERIMENTAL WORK

Instruments, Reagents and Materials

A binary gradient schimadzu HPLC with spinochrome CFR software with UV-Visible detector (SPD-20A), pump (LC-10AT) and (LC-10ATVP) was used for the study. HPLC injecting syringe (25ug) HAMILTON and Hypersil pack BDS c18 RP column were used. Irbesartan and

hydrochlorothiazide pure samples were procured as gift samples. Acetonitrile HPLC grade and methanol HPLC grade were purchased from E.Merck (Mumbai, India), sodium acetate and o-phosphoric acid were obtained from SD fine chemical Ltd (Ahmedabad, India) and were of analytical grade. Water of HPLC grade was used.

Chromatographic condition of method

Hypesil BDS RP-18, 150 x 4.6 mm, 5µ column was used at ambient temperature. 8.2 g of sodium Acetate was weighed and 1000ml of Milli-Q water was added to it. The mobile phase was considered buffer: acetonitrile. pH was adjusted to 3.0 with ortho phosphoric acid and was filtered through 0.45µm nylon membrane filter and was degassed. Flow rate was maintained at 1ml/min. The elution was observed at 260 nm. Some trials were carried out with respect to change in the ratio of constituents of the mobile phase like 50: 60, 50:50, 45:55 (buffer: actonitrile). Injection volume and runtime were 20 µl and 10 mins respectively. In the ratio 45:55 retention time for hydrochlorothiazide and irbesartan were observed to be 2.98 and 4.83 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 standard stock solution

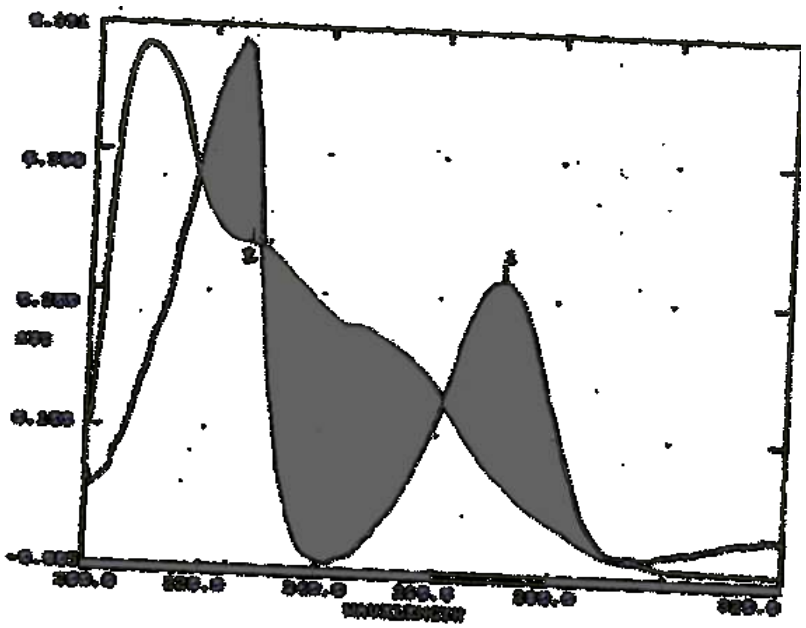
Accurately weighed about 75.0 mg of Irbesartan and 6.3mg of Hydrochlorothiazide working standard were transferred into a 25 ml clean dry volumetric flask, about 15 ml of methanol was added to it, sonicated for 5 minutes, and diluted to volume with methanol. 1ml from the standard stock solution was pipette out into a 25 ml clean dry volumetric flask, and diluted to the mark with 25 ml of diluent (mobile phase).

Sample preparation

Ten tablets weighed and powdered in a neat clean and dry motor and pestle .Weighed and transferred accurately about 0.2gm of the tablet powder into 25ml clean dry volumetric flask, added about 15ml of methanol, sonicated for 5 minutes, and diluted to volume with methanol . The solution was filtered through the Whatmann filter paper. From the filtrate 1ml of sample solution was pipetted out into a 25ml volumetric flask, the volume was made upto with diluent (mobile phase).

UV Spectras of Irbesartan and Hydrochlorothiazide

UV scan of the Irbesartan and hydrochlorothiazide was done individually and both were over layed upon each other to get the required wavelength .The wavelength of 260nm was found to be effective in determination of both the drugs at a time.



Evaluation of System Suitability

The column efficiency as determined from Irbesartan and Hydrochlorothiazide peaks is not less than 2000 USP plate count and the tailing factor for Irbesartan and Hydrochlorothiazide peaks is not more than 2.0. The relative standard deviation for the peak areas of the five replicate

injections is not more than 2.0%. 20µl of the blank, Standard (five injections) and sample solution were injected in duplicate into the liquid chromatograph and were recorded Peak areas were measured.

Fig: 7.1Typical chromatogram of Irbesartan and hydrochlorothiazide (blank)

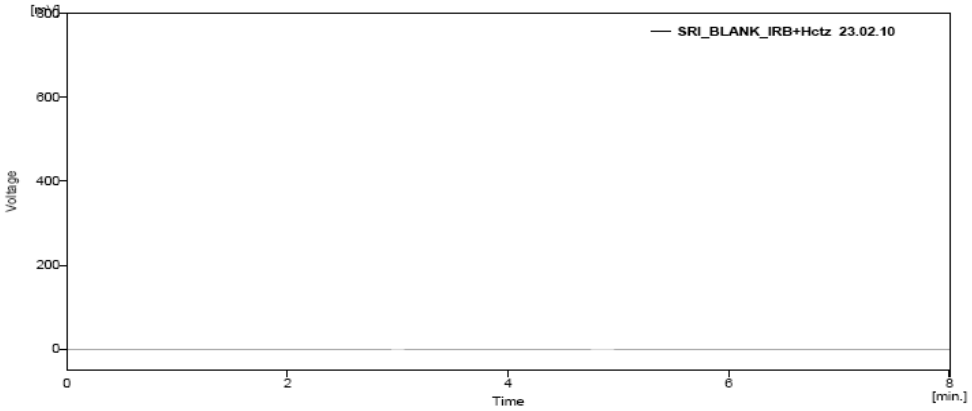


Fig: 7.2 Typical chromatogram of Irbesartan and hydrochlorothiazide (Standard)

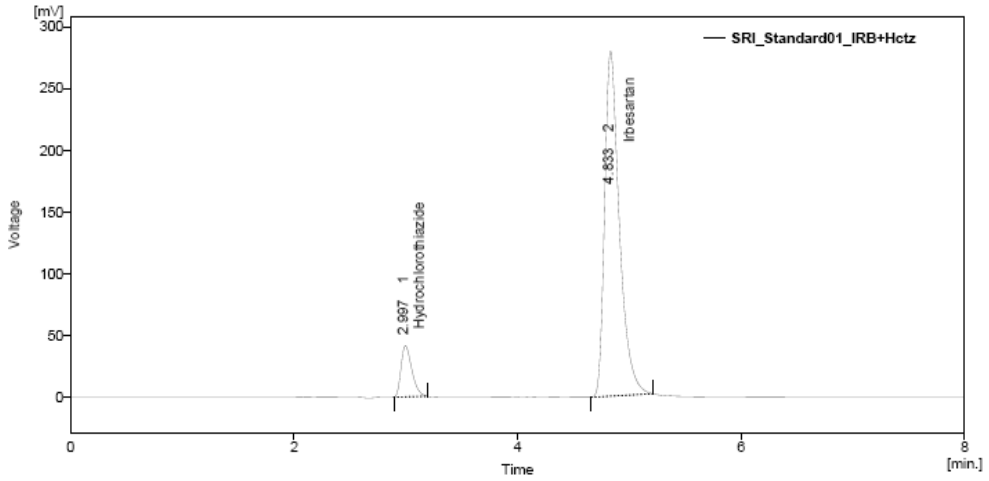
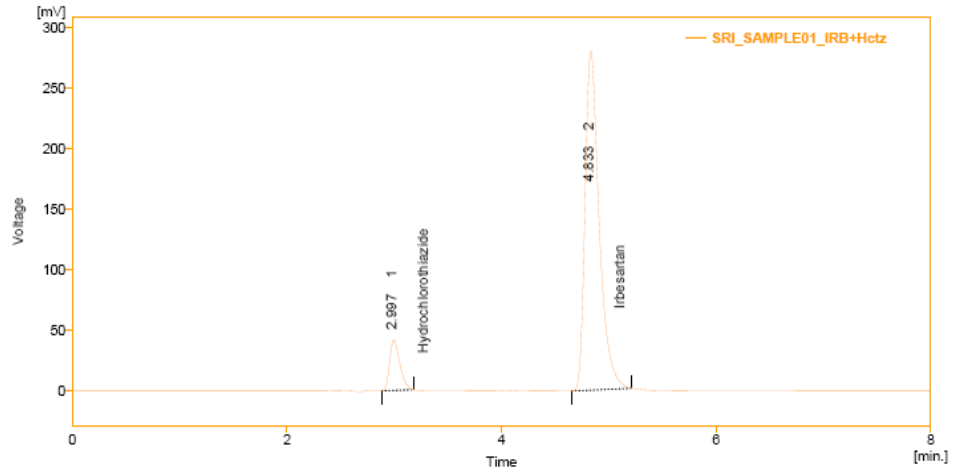


Fig: 7.3Typical chromatogram of Irbesartan and hydrochlorothiazide (sample)



Amount of each drug in each tablet was calculated by using the following formula

$$\text{Irbesartan (mg/tablet)} = \frac{A_I}{A_{SI}} \times \frac{D_S}{D_T} \times \frac{P}{100}$$

$$\text{Hydrochlorothiazide (mg/tablet)} = \frac{A_H}{A_{SH}} \times \frac{D_S}{D_T} \times \frac{P}{100}$$

Where,

$\mathbf{A_I}$ = Average area counts of injections for Irbesartan peak in the chromatogram of sample solution.

$\mathbf{A_{SI}}$ = Average area count of five replicate injections for Irbesartan peak in the chromatogram of standard solution.

$\mathbf{A_H}$ = Average area counts of injections for Hydrochlorothiazide peak in the chromatogram of sample solution.

$\mathbf{A_{SH}}$ = Average area count of five replicate injections for Hydrochlorothiazide peak in the chromatogram of standard solution.

$\mathbf{D_S}$ = Dilution factor of standard solution (weight÷dilution).

$\mathbf{D_T}$ = Dilution factor of sample solution.

P = Percentage purity of working standard used.

System Suitability

A Standard solution was prepared by using Irbesartan and Hydrochlorothiazide 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 % RSD from five replicate injections for Irbesartan and Hydrochlorothiazide retention times and peak areas.

Acceptance Criteria

The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%. The number of theoretical plates (N) for the Irbesartan and hydrochlorothiazide peaks is NLT 2000. The Tailing factor (T) for the Irbesartan and hydrochlorothiazide peaks is NMT 1.5.

Irbesartan Table

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.993	275.578	4363	1.1
2	2.997	275.115	4659	1.2
3	3.000	276.341	4382	1.4
4	2.997	277.838	4372	1.2
5	2.987	277.127	4607	1.2
Mean	2.9948	276.3998	4476.6	1.22
SD	0.00502	1.109816	144.1086	---
%RSD	0.1676	0.401525	---	---

Hydrochlorothiazide Table

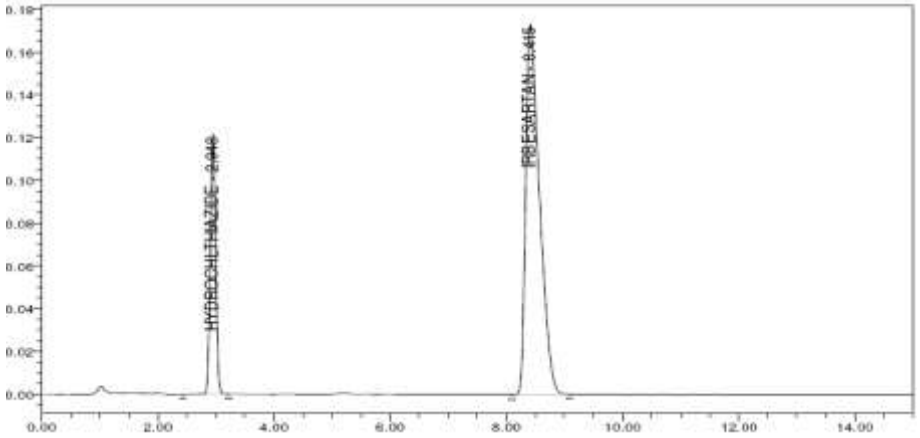
Injection	RT	Peak Area	USP Plate count	USP Tailing
1	4.83	2546.306	6920	1.3
2	4.833	2544.018	6603	1.2
3	4.833	2545.938	6603	1.2
4	4.833	2556.414	6603	1.2
5	4.837	2535.69	6939	1.3
Mean	4.8332	2545.673	6733.6	1.24
SD	0.00249	7.386462	178.9575	---
% RSD	0.0515	0.29016	---	---

Precision

a. System precision: Standard solution prepared of desired concentration and injected six times.

b. Method precision: Six replicate injection of the standard solution of the same concentration were prepared and injected six times one after the other.

The % relative standard deviation of individual Irbesartan and Hydrochlorothiazide from the six units should be not more than 2.0%.



System Precision

Table for Irbesartan

	Injection	Rt Irbesartan	Peak Areas of Irbesartan
Concentration 100%	1	4.82	508.545
	2	4.823	516.360
	3	4.823	518.154
	4	4.823	515.566
	5	4.823	513.083
Statistical **Analysis**	**Mean**	4.8224	514.3416
	SD	0.001342	3.717934
	% RSD	0.027821	0.722853

Table for hydrochlorothiazide

	Injection	Rt Hydrochlorothiazide	Peak areas of hydrochlorothiazide
Concentration 100%	1	2.993	279.81
	2	2.987	282.629
	3	2.99	281.937
	4	2.987	280.794
	5	2.987	279.006
Statistical **Analysis**	**Mean**	2.9888	280.8352
	SD	0.002683	1.485798
	% RSD	0.089778	0.529064

Method Precision

Table for Irbesartan

	Injection	**Rt Irbesartan**	**Peak Areas of Irbesartan**
Concentration 100%	1	4.823	503.122
	2	4.827	507.643
	3	4.827	506.702
	4	4.827	512.719
	5	4.823	513.217
Statistical **Analysis**	**Mean**	4.8254	508.5806
	SD	0.027979	4.26614
	% RSD	0.0203049	0.838833

Table for hydrochlorothiazide

	Injection	**Rt Hydrochlorothiazide**	**Peak Areas of Hydrochlorothiazide**
Concentration 100%	1	2.98	277.226
	2	2.98	278.376
	3	2.983	278.346
	4	2.99	276.749
	5	2.993	275.545
Statistical **Analysis**	**Mean**	2.983	276.267
	SD	2.984833	277.0848
	% RSD	0.005419	1.134046

Accuracy (Recovery)

A study of accuracy was conducted by preparing three different concentrations of the working standards of Irbesartan and Hydrochlorothiazide i.e. 80%, 100% and 120%.inject them into the HPLC and the obtained parameters are considered to be standard. Later inject each concentration three times and compare the parameters with that of the standard. The average % recovery of Irbesartan and Hydrochlorothiazide was calculated.Separately inject the blank, Irbesartan and Hydrochlorothiazide in to the chromatograph.

The mean % recovery of the Irbesartan and Hydrochlorothiazide at each level should be not less than 95.0% and not more than 105.0%

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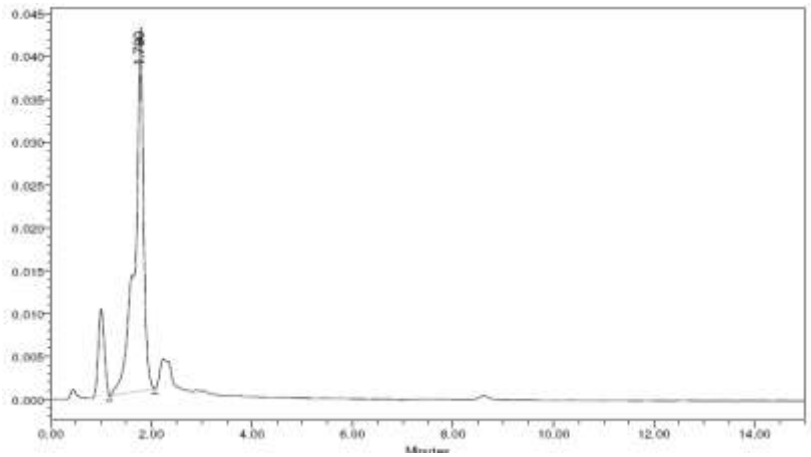


Table for irbesartan

Concentration of Irbesartan	**Peak area**	**Amount found**	**%recovery**		
standard 80mcg	370.12				
80mcg inj 1	365.414	78.9828	98.728	**MEAN**	98.69433
80mcg inj 2	367.578	79.45	99.313	**SD**	0.636168
80mcg inj 3	362.873	78.433	98.042	**%RSD**	0.6445
standard 100mcg	503.213				
100mcg inj 1	505.837	100.52	100.52	**MEAN**	100.2517
100mcg inj 2	501.921	99.74	99.74	**SD**	0.443293
100mcg inj 3	505.704	100.495	100.495	**%RSD**	0.4422
standard 120mcg	589.214				
120mcg inj 1	587.038	119.59	99.66	**MEAN**	99.94867
120mcg inj 2	589.958	120.15	100.126	**SD**	0.252161
120mcg inj 3	589.556	120.06	100.06	**%RSD**	0.25229

Table for hydrochlorothiazide

Concentration of Hydrochlorothiazide	**Peak Area**	**Amount found**	**%recovery**		
standard 80mcg	2065.11				
80mcg inj 1	209.898	81.47	101.83	**MEAN**	99.99467
80mcg inj 2	202.472	78.587	98.234	**SD**	1.799645
80mcg inj 3	205.957	79.94	99.92	**%RSD**	0.0179925
standard 100mcg	271.658				
100mcg inj 1	274.849	101.116	101.12	**MEAN**	100.79
100mcg inj 2	276.242	101.68	101.68	**SD**	1.091100
100mcg inj 3	270.499	99.57	99.57	**%RSD**	0.010825
standard 120mcg	323.929				
120mcg inj 1	320.682	118.797	98.99	**MEAN**	99.61
120mcg inj 2	323.64	119.89	99.91	**SD**	0.19226
120mcg inj 3	323.717	119.92	99.93	**%RSD**	0.31005

Linearity

Preparation of linearity stock solution

Transfer an accurately weighed quantity of about 4.5mg of hydrochlorothiazide and 12.5mg of Irbesartan into a 50ml volumetric flask. Add about 25ml of the diluent and sonic ate to dissolve. Make the volume up to the mark with the diluent. From the stock serial dilutions were made by taking 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6ml into 10ml volumetric flask and diluted with the diluent up to the mark. Inject these solutions into the HPLC system and record the area of analyte peaks. Plot a graph of concentration (in x-axis) vs. analyte peak area (in y-axis).evaluate the correlation coefficient between concentration and peak area on y-intercept of the correlation plot. Correlation Coefficient should be not less than 0.9990. % of y- Intercept should be ±2.0. % of RSD for level 1 and Level 6 should be not more than 2.0%.

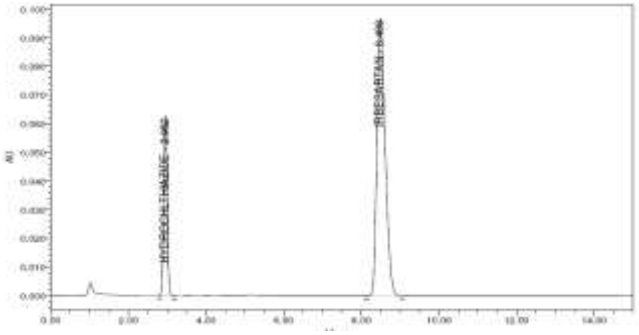
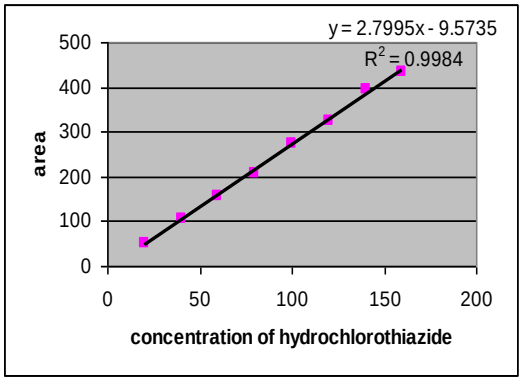


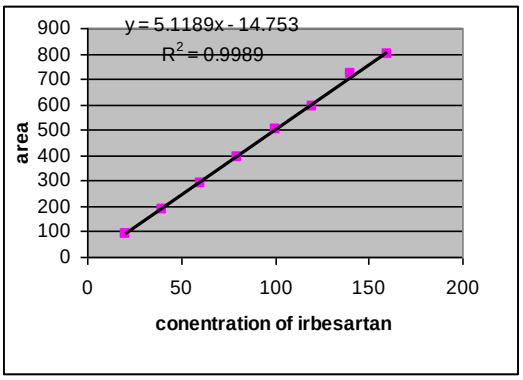
Table for irbesartan

Concentration of Irbesartan	Average area	Statistical Analysis	
20	89.766	Slope	5.1189
40	189.629	y-Intercept	- 14.753
60	290.797	Limit of detection	2.3968396
80	390.12	Limit of quantification	7.2631503
100	503.213	r^2 coefficient of determination	0.9989
120	589.214		
140	717.977	Corelation coefficient r	0.9994
160	796.858		

Table for hydrochlorothiazide

Concentration of Hydrochlorothiazide	Average area	Statistical Analysis	
20	48.979	Slope	2.7995
40	104.243	y-Intercept	- 9.5735
60	157.375	Limit of detection	1.75143
80	206.11	Limit of quantification	5.30736
100	271.658	r2 coefficient of determination	0.9984
120	323.929		
140	392.663	Corelation coefficient r	0.99919
160	434.103		





Ruggedness

System to system /Analyst to Analyst/column to Column variability:

System to system /Analyst to Analyst/column to Column variability study was conducted on different HPLC systems, different columns and different analysts under similar conditions at different times. Six samples were prepared and each were analysed as per test method.

The relative standard deviation for Irbesartan and Hydrochlorothiazide were found to be below 2 % on the columns, systems and Analysts. Comparison of both the results obtained on two different HPLC systems, different column and different analysts shows that the assay test method is rugged for System to system /Analyst to Analyst/column to Column variability.

The % relative standard deviation of Irbesartan and Hydrochlorothiazide from the six sample preparations should be not more than 2.0%.

Robustness

Effect of variation in mobile phase composition

A study was conducted to determine the effect of variation in Organic phase composition in mobile phase. Standard solution prepared as per the test method was injected into the HPLC system using two mobile phases. The system suitability parameters were evaluated and found to be with in the limits for mobile phase having 95% and 110% of method highest organic phase. Irbesartan and hydrochlorothiazide blend solution at target concentration was chromatographed using mobile

phase having 95% and 110% of the method organic phase.

Irbesartan and hydrochlorothiazide were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having 100% of the organic phase.

From the study it was established that the allowable variation in mobile phase composition is 95% to 110% of the method highest organic phase of mobile phase.

The Tailing Factor of Irbesartan and Hydrochlorothiazide standards should be NMT 1.5 for Variation in Organic Phase.

Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.9ml/min and 1.1ml/min. The system suitability parameters were evaluated and found to be with in the limits for 0.9ml/min and 1.1ml/min flow.

Irbesartan and hydrochlorothiazide were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.

0ml/min. From the above study it was established that the allowable variation in flow rates is 0.9ml/min and 1.1ml/min.

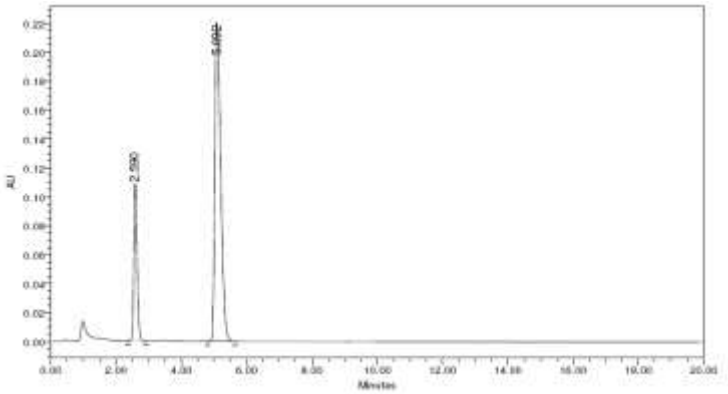
The Tailing Factor of Irbesartan and Hydrochlorothiazide standards should be NMT 2.0 for Variation in Flow.

Effect of variation of pH:

A study was conducted to determine the effect of variation in pH. Standard and sample solutions were prepared as per the test method and injected into the HPLC system using pH 2.8 and 3.2. The system suitability parameters were evaluated and found to be with in the limits for pH 2.8 and 3.2.

Irbesartan and hydrochlorothiazide were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having pH 3.0. From the above study it was established that the allowable variation in pH 2.8 and 3.2.

The Tailing Factor of Irbesartan and Hydrochlorothiazide standard should be NMT 1.5 for Variation in pH.



Parameters	Optimum range	Conditions in procedure	Remarks
Mobile phase composition (% of Acetonitrile)	10% variations in isocratic	isocratic	Beyond the optimum range of % of Acetonitrile, the resolution factor and relative retention and asymmetry factor were decreased
Flow rate ml/min	0.9-1.1	1.0	At lower flow rates the asymmetry factor was increased and at higher flow rates the relative retentions was decreased
Temperature	25-30^{o}C	Ambient	Beyond the optimum range peak shape and symmetry was lost
PH of mobile phase	2.8-3.2	3.0	Beyond the optimum range of pH of the mobile phase, better resolution was not found. When it is reduced or increased beyond optimum range asymmetry factor was increased.

DISCUSSION

In the study for system suitability %RSD for retention times and peak areas were found to be with in the limits. The precision study has shown that the test method is precised. The recovery results indicating that the test method has an acceptable level of accuracy. In linearity studies the correlation coefficient was found to be 0.99958. From the study it was established that the linearity of test method is from 20% to 160% of the target concentration. The ruggedness study reveals that the %RSD is within limits. Robustness study had shown that the tailing factor is within limits.

CONCLUSION

From the above study it can be concluded that the method developed for the simultaneous estimation of irbesartan and hydrochlorothiazide is well validated.

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