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SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF IRBESARTAN AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

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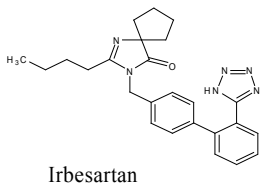
ABSTRACT
A simple, rapid, accurate and precise method is developed for the quantitative simultaneous determination of Irbesartan and Hydrochlorothiazide in combined pharmaceutical dosage form. Separation is achieved with an Ace5- $C_{18,}$(250 X 4.6)mm,5µ analytical column using buffer-acetonitrile (65:35, v/v) of pH 5.5, adjusted with acetic acid as the mobile phase. The buffer used in mobile phase contains 50Mm ammonium acetate in double distilled water. The instrumental setting is flow rate of 1 mL/ min, column temperature at 30°C, and detector wavelength of 260 nm. The internal standard method is used for the quantitation of the ingredients of this combination. Methylparaben is used as an internal standard. The method is validated and shown to Hydrochlorothiazide are 0.9998 and 0.9999, respectively. The relative standard deviations for six replicate measurements in two sets of each drug in tablets are always less than 2%. be linear for Irbesartan and Hydrochlorothiazide. The correlation coefficient for Irbesartan and Hydrochlorothiazide are 0.9998 and 0.9999 respectively. The relative standard deviation for six replicate measurements in two sets of each drugs in tablets are always less than 2%.

Key words: Irbesartan and Hydrochlorothiazide, RP-HPLC, Ace5-$C_{18,}$(250X 4.6)mm, 5µColumn, Validation.

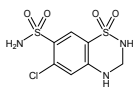
INTRODUCTION

Irbesartan(fig-I) is chemically (2-Butyl-3[[2'-(1H-tetrazole-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one) angiogenesis II type 1(All_1)-receptor antagonist. Hydrochlorothiazide (fig-I) is chemically (6-Chloro-3, 4-dihydro-2H-1,2,4-benzothiadiazine-7sulfonamide 1,1-dioxide) diuretics[1]. A combination of 150 mg of Irbesartan and 12.5mg of Hydrochlorothiazide is available commercially as tablets. An Irbesartan and Hydrochlorothiazide combination is used to lower blood pressure. Irbesartan controls high blood pressure (hypertension) by relaxing blood vessels. Hydrochlorothiazide is a diuretic. Irbesartan plus Hydrochlorothiazide is greater blood pressure lowering action than Irbesartan alone[2, 3].

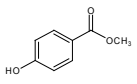
Figure 1. The structure of Irbesartan, Hydrochlorothiazide and Methylparaben IS



Irbesartan



Hydrochlorothiazide



Methylparaben IS

The literature survey reveals that several methods were reported for the individual estimation of Irbesartan and Hydrochlorothiazide. The methods for Irbesartan[4-8] and Hydrochlorothiazide[9-16] were reported for the estimation of these drugs in tablets and plasma. However, there is only one method was reported for the simultaneous determination of the Irbesartan and Hydrochlorothiazide in combined pharmaceutical-dosage form[4].

In the reported method internal standard was not used, it would require double time for analysis, as compared with the method would not be rapid, less expensive, or economical, whereas the developed method would save analysis time and also economy.

In the present research paper, attempts have been made to develop a method for the simultaneous estimation of the ingredients of this combination. An internal standards method was used for the quantization of Irbesartan and Hydrochlorothiazide. Methylparaben was used as an internal standard. A good separation of the analytes of this combination was achieved by using a mobile phase containing ammonium acetate. The proposed method is rapid, less expensive, and dissolution of the tablets containing Irbesartan and Hydrochlorothiazide.

The method[4-8] is only high-performance liquid chromatography (HPLC) and spectrophtometric methods reported in the literature for the estimation of Irbesartan in pharmaceutical preparations and plasma. The reported method[9-15] is for the estimation of Hydrochlorothiazide in combination with other drugs in plasma, serum and is in tablets by high-performance liquid chromatography (HPLC). The proposed method is rapid, accurate, and precise and is successfully applied for the simultaneous determination of Irbesartan and Hydrochlorothiazide in combined -dosage form (tablets) available in the commercial market. .

MATERIALS AND REAGENTS

Irbesartan and Hydrochlorothiazide standards were obtained from Glen mark pharmaceutical Ltd. (Mumbai, India), ammonium acetate; acetic acid and acetonitrile (HPLC grade) were obtained from Qualigens Fine Chemicals (Mumbai, India). Methyl paraben was obtained from Merck Laboratories Ltd., (Mumbai, India). The 0.45-Pump nylon filter was obtained from Advanced Micro devices Pvt. Ltd., (Ambala Cantt, India). The (Iroval-H) tablets (Ipca pharmaceutical,

Mumbai, India) of the combination of Irbesartan and Hydrochlorothiazide were purchased commercially. Double-distilled water was used throughout the experiment. Other chemicals used were have analytical or HPLC grade.

Chromatographic Conditions

A chromatographic system Systronic consisting of quaternary solvent delivery pump, a degasser, an auto- injector, column oven and photodiode array detector, 10A-VP series with IRIS 32 PLUS software. C18 (4.6 x 250) mm, 5 micron, Advance separation technology, US) column was used. The instrumental settings were a flow of 1 mL/min. The injection volume was 10 µL. The detection wavelength was 260nm for all three analytes. The peak purity was checked with the photodiode array detector from 10A-VP. The peak purity was checked with the photodiode array detector from 10A-VP.

Mobile Phase

The Mobile Phase consisted of buffer and acetonitrile in the ratio 65.35 (v/v). The pH of the mobile phase was adjusted to 5.5 with acetic acid. The buffer used in the mobile phase contained 50mM of ammonium acetate in double-distilled water. The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed.

Standard Stock Solutions

Standard solutions were prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluents used for the standards and sample preparation was prepared as follow, diluents A was composed of methanol and acetonitrile in the ratio 50:50 (v/v) and diluents B was composed of water and acetonitrile in the ratios of 65:35 (v/v).

Irbesartan

A 25-mg sample of Irbesartan (99.85%) was accurately weighed, transferred in a 25-mL volumetric flask, and dissolved with the diluents A.

Hydrochlorothiazide

A 12.5 - mg sample of Hydrochlorothiazide (99.77%) was accurately weighed, transferred in a 25-mL volumetric flask, and dissolved with diluents A.

Methylparaben

A 5-mg sample of Methylparaben was accurately weighed, transferred in a 25-ml volumetric flask, and dissolved with diluents A.

Mixed standard Solution

A mixed standard solution was prepared from these stock solutions by transferring 5 ml of Irbesartan standard solutions, 5 ml of Hydrochlorothiazide standard solution, and 5 ml of Methylparaben standard solution in a 50 ml volumetric flask and diluted with diluents B. This solution contained 100 µg/ml of Irbesartan, 50 µg /ml of Hydrochlorothiazide and, 20 µg /ml of Methylparaben.

Calibration Curve Solutions

The calibration curve solutions containing 25-150 µg/ml of Irbesartan, 10-75 µg/ml of Hydrochlorothiazide, and 20 µg/ml of Methylparaben in each calibration level were prepared.

Preparation of sample

Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 150 mg of Irbesartan and 12.5 mg of Hydrochlorothiazide was transferred in a 200ml volumetric flask. To this flask, 100 ml of diluents A was added, and the solution was sonicated for 10 min with intermittent shaking. An accurately measured volume of 10 ml acetonitrile was added to the flask and mixed well. Further sonication was performed for another 25 min with intermittent shaking. The solution was cooled to ambient temperature. An accurately measured volume of 20 ml methanol was added to the flask, and centrifuged at 10,000 rpm for 10 min. From the centrifuged solution, 5 ml of clear solution was transferred into a 50 ml volumetric flask, and 5 ml of internal standard solution was added into it and diluted to volume with diluent B.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Our objective of chromatographic method development was achieve peak tailing factor <2, retention time in between 5 to18 minutes, Along with resolution between Irbesartan, Hydrochlorothiazide and internal standard (Methylparaben) >2.

The chromatographic separation was achieved using end capped C18 (Ace5 $C_{18,}$ 25-cm) column. The chromatographic method was optimized by changing the composition of mobile phase and pH of the mobile phase.

From the development studies, it was determined that at 50ml ammonium acetate in water and acetonitrile in the ration of 65:35 (v/v) at pH 5.5, the analytes of this combination had adequate retentions and resolution and the chromatographic analysis time was less than 18 Min.

Validation of the method

Specificity

The specificity of the method was checked by a peak purity test of the sample preparation performed by a photodiode array detector. The peak purity for the peaks of Irbesartan, Hydrochlorothiazide and internal standard (Methylparaben) was observed to be 999, and 998 at wavelength 260 nm, which shows that the peaks of analyte were pure and also that formulation excipients were not interfering with the analyte peaks.

Calibration and linearity

An internal standard method was used for quantitative determinations. Linearity of the method was tested from 20% to 150% of the targeted level of the assay concentration (Irbesartan, 100 µg/ml and Hydrochlorothiazide 50 µg/ml) for both the analytes. Mixed standard solutions containing 20-150 µg/ml of Irbesartan, 10-75 µg/ml of Hydrochlorothiazide, and 20 µg/ml of Methylparaben in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area ratio against the concentration of the drugs. The equations of the calibration curves for Irbesartan and Hydrochlorothiazide obtained were y = 12999x – 1418.3 and y = 9694.5x + 2029.4, respectively. In the simultaneous determination, the calibration graphs were found to be linear in the aforementioned concentrations (the slopes and correlation coefficients are shown in Table –I).

Precision (repeatability)

The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The results of the precision study (Table I) indicate that the method is reliable (RSD% < 2).

Table I. Results of the Linearity study and Precision

Ingredient (n= 6)	Precision (%RSD)	Linearity (µg/ml)	Slopes* (n = 3)	Coefficients of correlations
Irbesartan	1.11	20-150	12999(0.0281)	0.9998
HCTZ	1.15	10-75	9694.5(0.0222)	0.9999

*Standard deviation shown in parentheses
HCTZ-Hydrochlorothiazide

Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known

amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120% of the label claim of the tablet (150mg of Irbesartan and 12.5 mg of Hydrochlorothiazide). Placebo equivalent to one tablet was transferred into a 200 ml volumetric flask, and the amounts of Irbesartan and of Hydrochlorothiazide at 80%, 100% and 120% of the label claim of the tablet were added to it. The recovery samples were prepared as per the procedure mentioned, and then 5 mL of each of the solutions were transferred into a 50 ml volumetric flask; 5 ml of internal standard solution was added to it and diluted to volume with diluent B. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Irbesartan and Hydrochlorothiazide ranged from 100.96% to 101.63% and 99.31-100.42%, respectively (Table II). The average recovery of three levels (nine determinations) for Irbesartan and Hydrochlorothiazide were 101.04% and 100.51%, respectively.

Table II. Results of the Recovery Tests for the Drugs

Level of addition (%)	Ingredients	Amounts added(n=3)(mg)	% Recovery	% Average recovery
80	Irbesartan	120.0	101.25 (0.45) 100.52	101.35(0.45) 100.22
	HCTZ	10.0	(0.37)	(0.34)
100	Irbesartan	150.0	100.97 (0.24) 100.78	100.75 (0.39) 100.55
	HCTZ	12.5	(0.55)	(0.56)
120	Irbesartan	180.0	101.23 (0.49) 100.87	100.97 (0.45) 101.32
	HCTZ	15.0	(0.31)	(0.33)

* RSD shown in parenthesis

Intermediate precision (reproducibility)

Intermediate precision of the method was determined by analyzing the samples six times on different days, by different Chemists, by using different analytical columns of the same make and different HPLC systems. The percentage assay was calculated using calibration curves. The assay results are shown in Table III.

Table III. Assay Results of Active Ingredients in Tablets

Set	Ingredient	Label value (mg)	Found (mg)*	% Label claim
Repeatability	Irbesartan	150.0	150.99	100.398
	HCTZ	12.5	12.48	99.79
Reproducibility	Irbesartan	150.0	151.45	100.69
	HCTZ	12.5	12.39	99.06

* Average of six analysis

Determination of limit of quantification and limit of detection (LOQ & LOD)

For determining the limit of detection (LOD) and limit of quantification (LOQ), the method based on the residual standard deviation of a regression line and slope was adopted[16]. To determine the LOD and LOQ, a specific calibration curve was constructed using samples containing the analytes in the range of LOD and LOQ. The LODs for Irbesartan and Hydrochlorothiazide were 0.018 and 0.023 µg/ml, and the LOQs were 0.053 and 0.070 µg/ml, respectively.

Solution stability

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 75 h for Irbesartan, Hydrochlorothiazide and the Methylparaben internal standard were 0.65 %, 0.34 %, and 0.39 %, respectively. The assay values were within ±2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature. Standard solutions were used, and the RSD of peak area ratio, column efficiency, resolution, and tailing factor of the peaks were calculated. The results are shown in Table IV.

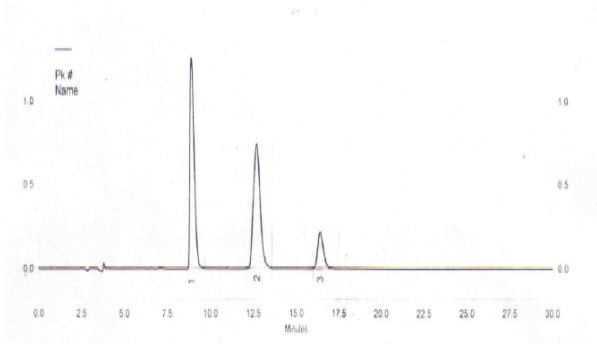
Table IV. System Suitability Parameters

Parameter	HCTZ	Methylparaben	Irbesartan
Theoretical plates (Per column length)	8409	14600	12315
Resolution	15.74	-	6.54
Tailing factor	1.21	1.15	1.1
% RSD	-	0.21	0.18

Determination of active ingredients in tablets

The contents of two drugs in tablets were determined by the proposed method using the calibration curve. The determinations were done in two sets, one for precision and the second for raggedness, and six samples were prepared for each set. The results are shown in Table III. The chromatogram of the tablet sample is shown in (Figure II).

Figure 2. A typical chromatogram of the tablet: and Irbesartan (8.9), HCTZ (12.5), Methylparaben (16.9).



CONCLUSION

This method can be used for the simultaneous determination of Irbesartan and Hydrochlorothiazide in the pharmaceutical-dosage form. The method is validated and shown to be accurate and precise. It can be used in the quality control departments for the assay and dissolution of tablets of the combined pharmaceutical-dosage forms containing Irbesartan and Hydrochlorothiazide.

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