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Original Article

Validated RP-HPLC Method for Simultaneous Estimation of Irbesartan and Hydrochlorothiazide in Tablet Dosage Form

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ABSTRACT

A simple and rapid reversed phase-high performance liquid chromatographic method was developed for simultaneous determination of Irbesartan and Hydrochlorthiazide in Tablet Dosage form. **Method**: The elution was done with a mobile phase of Methanol:0.05 % Orthophosphoric acid (90:10) on Intersil-BDS C_{18} column (250 × 4.6 mm, 5 µm particle size). The wavelength detector was set at 226 nm. **Results and discussion**: Retention times for Irbesartan and Hydrochlorthiazide were around 2.869 min, 3.942 min respectively. The reliability and analytical performance of the proposed HPLC procedure were statistically validated according to the respect of linearity, ranges, precision, accuracy, repeatability, reproducibility, detection and quantification limits. Linear ranges were established between 36-216 µg/mL for Irbesartan and 3-18 µg/mL for Hydrochlorthiazide. The LOD and LOQ for Irbesartan were found to be 0.65, 1.97 and for Hydrochlorthiazide were found to be 0.513, 1.556 respectively. The described High Performance Liquid Chromatography method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.

Keywords: Irbesartan, Hydrochlorthiazide , RP-HPLC, ICH guideline

1. INTRODUCTION

Irbesartan and hydrochlorothiazide combination is used in the treatment of hypertension. Irbesartan belongs to the category of Angiotensin -II receptor antagonist,[1] and chemically it is 2-butyl -3({4-[2-(2H-1, 2, 3, 4-tetrazol-5yl) phenyl] phenyl} methyl) 1, 3-diazaspirol [4, 4] non-1-en-4-one and it is used in the treatment of hypertension and Diabetic nephropathy.

Irbesartan, chemically 1-carbamimidamido-N, N dimethyl methanimidamide (Fig. 1) is a biguanide antihyperglycemic

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agent used for treating non insulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake.[1-2] Irbesartan is the only oral antihyperglycemic agent that is not associated with weight gain. Irbesartan may induce weight loss and is the drug of choice for obese NIDDM patients.[3] When used alone, Irbesartan does not cause hypoglycaemia; however, it may potentiate the hypoglycaemic effects of sulfonylurea and insulin. Its main side effects are dyspepsia, nausea and diarrhoea. Irbesartan decreases fasting plasma glucose, postprandial blood glucose and glycosolated hemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Irbesartan may also have a positive effect on lipid levels. [4-5] Hydrochlorthiazide is a new oral hypoglycaemic of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. This enzyme-inhibiting drug is to be used either alone or in combination with Irbesartan or a thiazolidinedione for control of type 2 diabetes mellitus. The drug works to competitively inhibit a protein/enzyme, dipeptidyl peptidase 4 (DPP-4), that results in an increased amount of active incretins (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin.[6-8] Several analytical methods based on UV, [9-12] HPLC, [13-14] and HPTLC [15] were reported for the determination of Irbesartan. Few analytical methods based on UV, [16-17] RP-HPLC, [18] were reported for the determination of Hydrochlorthiazide . Simultaneous determination of Irbesartan and Hydrochlorthiazide in bulk and tablet dosage form were reported by using spectrophotometric,[19] spectroflourometric[20] and HPLC[21] methods. However very few HPLC methods were reported for the simultaneous estimation of Irbesartan and Hydrochlorthiazide in tablet dosage form. The aim of present work was to develop and validate as per ICH guidelines, [22] a sensitive HPLC method that can be applied for simultaneous estimation of Irbesartan and Hydrochlorthiazide .

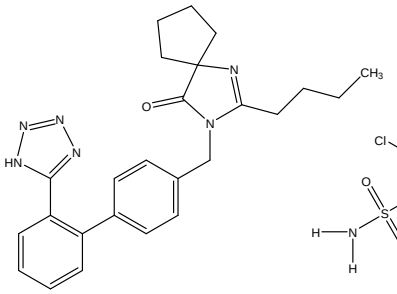

Fig.1 Irbesartan

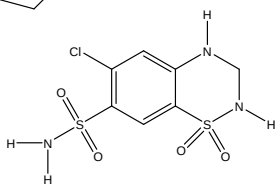

Fig.2 Hydrochlorthiazide

2. MATERIALS AND METHODS

Instruments

Chromatographic separation was performed using HPLC-WATERS Model NO.2690/5 equipped with PDA detector. A Intersil C18 column (250 × 4.6 mm, 5 µm particle size) was used.

Reagents and materials

All chemicals substances were of analytical reagent grade and solvents were of HPLC grade.

Chromatographic conditions

Chromatographic separation was performed on a Chromosil Intersil C18 column (250 × 4.6 mm, 5 µm particle size) . The mobile phase consisted of Methanol: 0.05 % Orthophosphoric acid in ratio 90: 10 were filtered before use through a 0.45 µ membrane and degassed for 10 min.. The flow rate was 1 mL/min and the column temperature was maintained at 27°C.The volume of injection was 20 µl. The UV detector was set up at 226 nm.

Preparation of standard stock solution

Accurately weigh and transfer 100 mg of Irbesartan and 10 mg of Hydrochlorthiazide working standards into a 100 ml clean dry volumetric flask, add about 10 ml of mobile phase and make volume up to the mark with the mobile phase. Further pipette out 1 ml from above stock solution into 10 ml volumetric flask and dilute up to the mark with mobile phase.

Preparation of sample solutions

Ten tablets were accurately weighed and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 10 mg of Irbesartan was weighed and transferred to 10 ml volumetric flask and dissolved in 5 ml Methanol and the content was kept in ultra-sonicator for 10 min. Finally the volume was made up to the mark with methanol. The solution was filtered through Whatmann filter paper No.41 which gave a concentration of 1000 µg/ml and this solution was used sample stock solution**.**

3. RESULTS AND DISCUSSION

Method optimisation

A simple reverse phase high-performance liquid chromatography method was developed for the determination of Irbesartan and Hydrochlorthiazide in pure form and in pharmaceutical formulations using Chromosil Intersil C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of Methanol: 0.05 % Orthophosphoric acid in ratio 90: 10. The mobile phase was chosen after several trials to reach the optimum stationary /mobile phase matching. The flow rate is 1.0ml/min. The average retention times under the conditions described were 2.869 min, 3.942 min for Irbesartan and Hydrochlorthiazide respectively (Table 1,Fig.3).

System Suitability

The resolution, capacity factor, theoretical plates/meter, Rt values and peak symmetry were calculated for the standard solutions. The values (Table 2) obtained demonstrated the suitability of the system for the analysis of the above drug combinations.

Linearity

The linearity of the method was determined by Preparing serial dilutions of minimum 6 concentration of standard stock solutions each in duplicate. Take the average area of each injection and plot the graph of average peak area versus actual concentration of each solution in µg/ml. Linearity ranges were found to be 36-216 µg/mL for Irbesartan and 3-18 µg/mL for Hydrochlorthiazide (Fig. 4,5).

Accuracy and precision

The accuracy of the method was determined by recovery experiments which were carried out and the percentage recovery and % relative standard deviation was calculated. From the data obtained, recoveries of standard drugs were found to be accurate (Table 3).The precision was carried out for System precision, Method precision and Intermediate precision (Table 4).

Specifity of the method.

In formulations, chromatograms with some additional peaks were observed which may be due to excipients present in the formulations. These peaks however did not interfere with the standard peaks .The results revealed that the peak is free from interferences, which shows that the HPLC method is specific.

Quantification Limit

The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (1:3) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (1:10). The LOD of Irbesartan and Hydrochlorthiazide are found to be 0.65µg/ml and 0.513µg/ml respectively. The LOQ of Irbesartan and Hydrochlorthiazide are found to be 1.97µg/ml 1.556µg/ml respectively.

Robustness

The robustness of the method was studied by changes in the method like alteration in flow rate (0.2 ml/min of set value i.e. 0.8 ml/min and 1.2 ml/min), detection are evaluated (Table 5,Fig 6,7).

Table 1: Optimised Method

Parameters	Method
Stationary phase (column)	Inertsil -BDS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : 0.05 % Orthophosphoric acid (90:10)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	226 nm
Drug RT (min)	2.869min for Irbesartan and 3.942for Hydrochlorthiazide

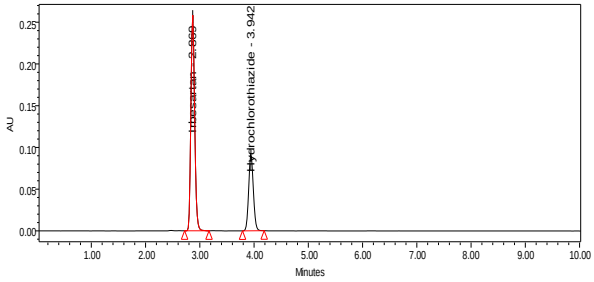

Fig 3: Optimised Chromatogram

Table 2: System suitability

Parameters	Irbesartan	Hydrochlorthiazide	Acceptance Criteria
USP Tailing	1.07	1.59	NMT 2.0
USP Plate count	9574	10768	NLT 2000
% RSD of peak Areas	0.01	0.04	NMT 2.0
Retention time	2.868	3.942	----

Table 3: Accuracy data

Accuracy level	Mean recovery of Irbesartan%	Mean recovery of Hydrochlorthiazide %	% RSD Irbesartan	% RSD Hydrochlorthiazide
Accuracy 50%	99.69	100.06	0.92	0.18
Accuracy 100%	99.83	100.04	0.41	0.09
Accuracy 150%	99.97	100.02	0.31	0.09

Table 4: Precision

Sl no	Analyte	System precision		Method precision		Intermediate precision	
		Peak area ±SD	% RSD	Peak area ±SD	% RSD	Peak area ±SD	% RSD
1	Irbesartan	1705542 ±739.0046	0.35	1702688 ±771.5483	0.38	1706333 ±2572.599	1.24
2	Hydrochlorthiazide	844318 ±8241.164	1.10	838004 ±4988.879	0.81	844608 ±8392.59	1.10

Table 5: Robustness

Parameters	% RSD	
	Irbesartan	Hydrochlorthiazide
0.8ml/min	0.11	0.06
1.2ml/min	0.35	0.09

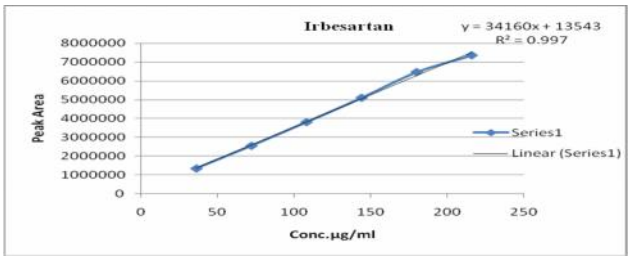

Fig 4: Linearity of Irbesartan

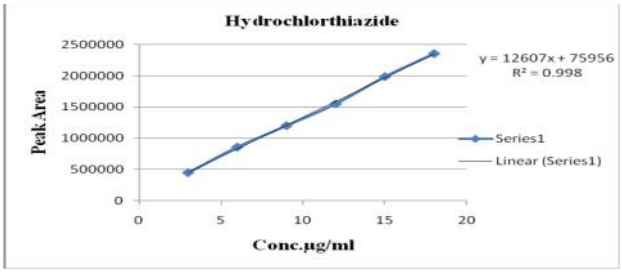

Fig 5: Linearity of Hydrochlorthiazide

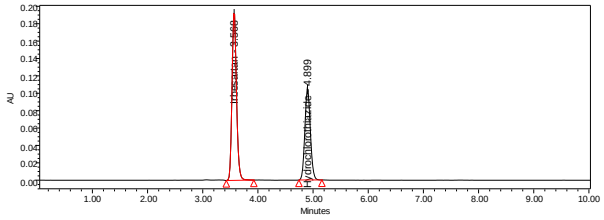

Fig 6: Flow rate for 0.8 ml/min

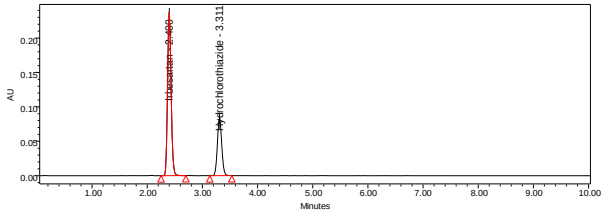

Fig 7: Flow rate for 1.2 ml/min

4. CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Irbesartan and Hydrochlorthiazide and from pure pharmaceutical formulations. The mobile phase is simple to prepare and the run time was less than 5min which consumes only less than 5ml of mobile Phase shows that the method was economical. The sample recoveries in all formulations were in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Irbesartan and Hydrochlorthiazide in combined dosage forms .The simplicity ensures that the RP-HPLC method can be applied for estimation of Irbesartan and Hydrochlorthiazide in tablet dosage forms. Since the good separation and resolution of the chromatographic peaks, the method was found to be accurate, precise, linear, robust and rugged.

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