Research Paper

Development and Validation of a HPTLC Method for the Simultaneous Estimation of Irbesartan and Hydrochlorothiazide in Tablet Dosage Form

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A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of irbesartan and hydrochlorothiazide in combined dosage forms. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of acetonitrile: chloroform: glacial acetic acid (7:3:0.1 v/v/v). The detection of spots was carried out at 260 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 100 to 700 ng/spot for irbesartan and 100 to 350 ng/spot for hydrochlorothiazide. The limit of detection and the limit of quantification for the irbesartan were found to be 30 and 100 ng/spot respectively and for hydrochlorothiazide 25 and 100 ng/spot respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

Irbesartan is a non-peptide compound, chemically described as a 2-butyl-3-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one[1] and chemically hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide,-1,1-dioxide. Irbesartan is rapidly and well absorbed. Irbesartan median peak plasma concentrations generally occurs 1.5-2 h after oral administration of irbesartan capsules and tablets. The addition of hydrochlorothiazide to irbesartan was more effective than each agent lowering blood pressure in patients with hypertension. Literature survey reveals that few HPTLC and HPLC methods are reported for the estimation of irbesartan in biological samples such as urine and plasma[2,3] and formulations[4]. So far no HPTLC method has been reported for the estimation of irbesartan and hydrochlorothiazide in combined dosage forms. In the present investigation an attempt has been made to develop accurate and precise HPTLC method for the simultaneous estimation of irbesartan and hydrochlorothiazide in combined dosage forms.

MATERIALS AND METHODS

Irbesartan and hydrochlorothiazide standard were procured as a gift sample from Sun Pharmaceuticals Ltd., Baroda. Silica gel $60F_{254}$ TLC plates (10x10 cm, layer thickness 0.2 mm, E. Merck, Mumbai) were used as a stationary phase. All chemicals and reagents used were of analytical grade. Tablets containing irbesartan (150 mg) and hydrochlorothiazide (12.5 mg) were procured from local market (Irovel-H, Sun Pharmaceutical and Xarb-H, Nicholas Piramal India Ltd). A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner 3, Camag WinCATS software, Camag Twin-trough chamber (10×10 cm) and ultrasonicator were used during study.

Preparation of standard and sample solutions:

Irbesartan and hydrochlorothiazide (25 mg) each were individually weighed accurately, dissolved and diluted with methanol to obtain the final concentration of 100 µg/ml of each drug. Twenty tablets (each containing 150 mg irbesartan and 12.5 mg hydrochlorothiazide) were weighed accurately and ground to fine powder. The powdered equivalent to 25 mg of irbesartan and 2.083 mg of hydrochlorothiazide were transferred to volumetric flask and dissolved in 5 ml of methanol. The solution was sonicated for 15 min. The extracts were filtered through Whatmann filter paper No. 41 and residue was washed thoroughly with methanol. The extracts and washing were

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pooled and transferred to a 250 ml volumetric flask and volume was made up to 250 ml with methanol to get 100 µg/ml of irbesartan and 8.33 µg/ml of hydrochlorothiazide.

Chromatographic conditions:

The experiment was performed on silica gel $60F_{254}$ aluminum sheets (10 × 10 cm) as stationary phase, using mobile phase comprised of acetonitrile: chloroform: glacial acetic acid (7:3:0.1 v/v/v). TLC plates were prewashed with methanol and activated in an oven at 50^0 for 5 min prior to chromatography. The sample solutions were applied on TLC plate as 6 mm bands at 4 mm interval under a stream of nitrogen gas. Ascending development to distance of 72 mm was performed in saturated (10 × 10 cm) Camag twin trough chamber for 30 min at room temperature. The developed TLC plated was air dried and then scanned between 200 and 400 nm using Camag TLC scanner 3 using Win CATS software. Both components show reasonably good response at 260 nm keeping the slit dimension of 5 × 0.45 mm. The overlain spectra for irbesartan and hydrochlorothiazide are shown in fig 1. Four microlitres of standard solutions of irbesartan and hydrochlorothiazide were spotted and developed.

Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 µl of standard solution of irbesartan and 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 µl of hydrochlorothiazide were applied on the TLC plate (100 µg/ml of drug). TLC plate was air dried, developed and analyzed photometrically as described earlier. The standard calibration curve was computed using regression analysis with Microsoft excel.

Validation of the method[5]:

The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application.

Analysis of the marketed formulations:

Four microlitres of sample solutions of the marketed formulation was spotted on to the same plate followed by development scanning. The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded.

RESULTS AND DISCUSSION

The mobile phase consisting of acetonitrile: chloroform: glacial acetic acid (7:3:0.1 v/v/v) gave R_f values of 0.60±0.04 and 0.70±0.03 for irbesartan and hydrochlorothiazide, respectively (fig. 2). The linear regression data (n=5, Table 1) showed a good linear relationship over a concentration range of 100-700 ng/spot, with a correlation coefficient of 0.9961 and 100-350 ng/spot, with a correlation coefficient of 0.9914 for irbesartan and hydrochlorothiazide, respectively. The limit of detection and limit of quantification for irbesartan was found to be 30 and 100 ng/spot and for hydrochlorothiazide, 25 and 100 ng/spot, respectively.

The intra-day precision was determined by analyzing standard solutions in the concentration range of 200 ng/spot to 600 ng/spot for irbesartan and 150 ng/spot to 300 ng/spot for hydrochlorothiazide for 3 times on the same day while inter-day precision was determined by analyzing corresponding standards daily for 3 day over

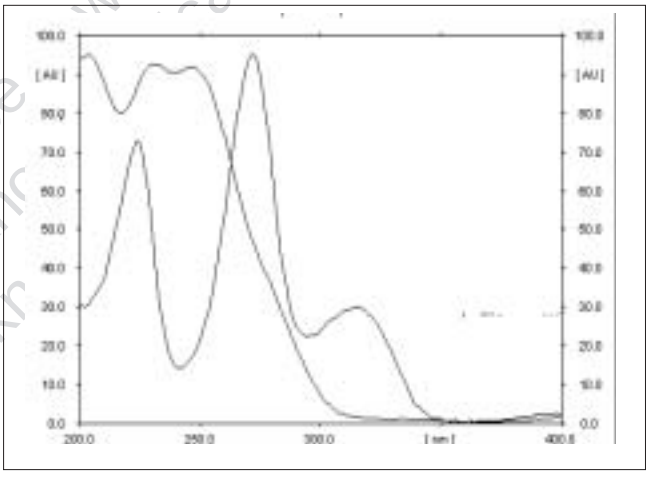

Fig. 1: Spectrum of irbesartan and hydrochlorothiazide.
1 and 2: represents spectrum of irbesartan and hydrochlorothiazide.

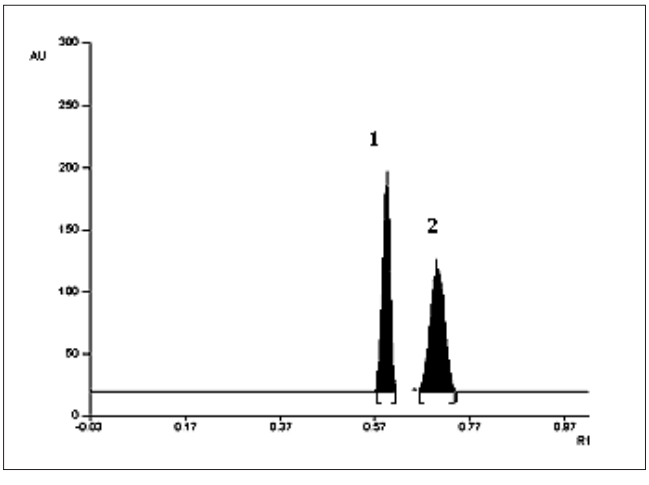

Fig. 2: A typical HPTLC Chromatogram of irbesartan and hydrochlorothiazide.
1 and 2: represents peak of irbesartan and hydrochlorothiazide.

TABLE 1: METHOD VALIDATION PARAMETERS OF PROPOSED METHOD

Parameters	Values	
	Irbesartan	Hydrochlorthiazide
Linearity range (ng/spot)	100-700	100-350
Correlation coefficient (r)	0.9961	0.9914
Regression equation (y=mx+c)	10.15	19.42
Slope (m)	268.35	-638.98
Intercept (c)	30 ng/spot	25 ng/spot
Limit of detection (LOD)	100 ng/spot	100 ng/spot
Limit of quantification (LOQ)	0.64	0.85
Precision (%CV)		
Repeatability of application (n=5)	0.90	0.95
Repeatability of measurement (n=5)		

TABLE 2: PRECISION DATA OF IRBESARTAN AND HYDROCHLOROTHIAZIDE

Drug	Concentration (µg/spot)	Intra-day precision % RSD	Inter-day precision % RSD
Irbesartan	0.2	100.28±0.79	100.23±1.18
150 (mg)	0.25	100.10±0.49	99.70±0.81
	0.3	101.04±0.08	101.76±0.15
Hydrochlorthiazide	0.2	100.30±0.24	99.71±0.73
12.5 (mg)	0.25	101.32±0.11	101.67±0.45
	0.3	100.17±0.07	101.37±0.15

RSD – Relative standard deviation.

TABLE 3: RECOVERY STUDIES OF IRBESARTAN AND HYDROCHLOROTHIAZIDE

Brand name	Label Claim mg/tablet	Amount added %	Total Amount added (mg)	Amount recovered* (mg)±SD	% Recovery ±SD
Irovel-H	Irbesartan	100	150	150.6±0.21	100.40±0.77
	150	200	300	300.6±0.79	100.20±0.19
		300	450	452.34±0.99	100.52±0.36
	Hydrochlorthiazide	50	6.25	6.37±0.04	99.38±0.74
	12.5	100	12.50	12.47±1.18	99.83±0.39
		150	18.75	18.70±0.99	100.15±0.10
Xarb-H	Irbesartan	100	150	152.73±1.31	101.82±1.01
	150	200	300	298.77±0.33	99.59±0.32
		300	450	448.47±1.63	99.66±0.48
	Hydrochlorthiazide	50	6.25	6.52±0.33	102.50±0.27
	12.5	100	12.50	12.39±0.05	99.14±0.22
		150	18.75	18.84±0.01	100.53±0.68

*Each value is a mean±standard deviation of three determinations. Irovel-H is a brand of Sun Pharmaceutical, Baroda and Xarb-H is a brand of Nicholas Piramal India Ltd., Mumbai.

TABLE 4: ANALYSIS OF IRBESARTAN AND HYDROCHLOROTHIAZIDE

Brandname	Label Claim mg/tablet	Amount found (mg)	% of drug found*	% RSD
Irovel-H	Irbesartan			
	150	151.62	101.08±1.06	1.04
	Hydrochlorthiazide			
Xarb-H	12.5	12.48	99.87±0.35	0.35
	Irbesartan			
	150	148.68	99.12±0.55	0.55
	Hydrochlorthiazide			
	12.5	12.45	99.37±0.80	0.80

*Each value is mean of three determinations.

a period of one week. The intra-day and inter-day coefficients of variation are given in Table 2. Repeatability of sample application was assessed by spotting 4 µl of drug solution 5 times on a TLC plate followed by development of plate and recording the peak area for 5 spots. The % RSD for peak area values of irbesartan and hydrochlorothiazide were calculated to be 0.64 and 0.85, respectively. Repeatability of measurement of peak area were determined by spotting 4 µl of irbesartan and hydrochlorothiazide solution on a

TLC plate and developing the plate. The separated spot was scanned five times without changing the position of the plate and % RSD for measurement of peak area of irbesartan and hydrochlorothiazide were found to be 0.90 and 0.95, respectively. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of irbesartan and hydrochlorothiazide.

Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels i.e. multiple level recovery studies. The recovery of the added standard was studied at three different levels viz 100%, 200% and 300% for irbesartan and 50%, 100% and 150% for hydrochlorothiazide, of the estimated amount of the drug. Sample stock solutions from tablet formulation of 100 µg/ml were prepared. Dilutions were made and recovery studies were performed. Percentage Recovery was found to be within the limits as listed in Table 3. The assay value for the marketed formulation was found to be within the limits as listed in Table 4. The low RSD value indicates precision and suitability of the method for routine analysis of irbesartan and hydrochlorothiazide in pharmaceutical dosage forms.

The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of irbesartan and hydrochlorothiazide in bulk drug and tablet formulations.

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