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Validated HPTLC method for simultaneous estimation of Irbesartan and Hydrochlorthiazide in a tablet dosage form

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ABSTRACT

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: ethyl acetate (8:2 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 600 ng/spot for irbesartan and 50 to 250 ng/spot for hydrochlorothiazide. The limit of detection and the limit of quantification for the irbesartan were found to be 22.19 and 73.98 ng/spot respectively and for hydrochlorothiazide 12.42 and 41.394 ng/spot respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

Keywords : Irbesartan, Hydrochlorthiazide, HPTLC.

INTRODUCTION

Irbesartan is a angiotensin II receptor antagonists, irbesartan is indicated for the treatment of hypertension, 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 and Hydrochlorothiazide (HCTZ), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a diuretic of the benzothiadiazine class. The two drugs (Fig. 1) are employed in antihypertensive pharmaceutical formulations, alone or in association. Irbesartan median peak plasma concentrations generally occurs 1.5-2 hr 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 one HPTLC methods was reported for the estimation of irbesartan and hydrochlorothiazide in combined tablet dosage forms. In the present investigation an attempt has been made to develop a new, accurate and precise HPTLC method for the simultaneous

estimation of irbesartan and hydrochlorothiazide in combined tablet dosage form and validation of the new developed method.

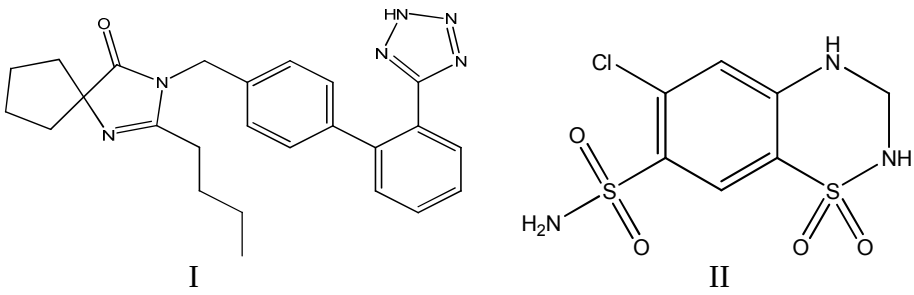

Fig. 1. Chemical structures of irbesartan (I) and hydrochlorothiazide (II).

MATERIALS AND METHODS

Instrument Used

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.

Materials

Irbesartan and hydrochlorothiazide standards were supplied by the institution. Silica gel 60F $_{254}$ TLC plates (10x10 cm, layer thickness 0.2 mm, E. Merck, Mumbai) was 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, manufactured by Sun Pharmaceutical).

Preparation of standard and sample solutions

Irbesartan and hydrochlorothiazide (10 mg) each were individually weighed accurately, dissolved and diluted with methanol to obtain the final concentration of 1 mg/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 powder equivalent to 10 mg of irbesartan and 0.83 mg of hydrochlorothiazide were transferred to 10 mL volumetric flask and dissolved in 5 mL of methanol. The extracts were filtered through Whatmann filter paper No. 41 and residue was washed thoroughly with methanol. The filtrate was kept in 10 mL volumetric flask and the volume was made up to 10 mL using methanol to get 1 mg/mL of Irbesartan and 83.3 µg/mL of hydrochlorothiazide. 1 mL of this solution is again diluted to 10 mL using 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:ethyl acetate (8:2 v/v). TLC plates were prewashed with methanol and activated in an oven at 50^{0}C for 5 min prior to chromatography. The sample solutions were applied on TLC plate as 5 mm bands at 5 mm interval under a stream of nitrogen gas. Ascending development to distance of 75 mm was performed in saturated (10 × 10 cm) Camag twin trough chamber 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 macro 5 × 0.45 mm**.** Ten micro-litre of standard solutions of irbesartan and hydrochlorothiazide were spotted and developed.

RESULTS AND DISCUSSION

Validation of the method

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. The results of the validation paramerters are shown in **table 1.**

Table 1. Validation of the parameters

Parameters	Values	
	Irbesartan	**Hydrochlorthiazide**
Linearity Range (ng/spot)	100 - 600	50 – 250
Correlation Co-efficient (r)	0.998	0.996
Regression Co-efficient (y=mx+c)	7.045	12.35
Slope (m)	6.9	12.03
Intercept (c)	62.82	58.2
Limit of Detection (LOD)	22.19 (ng/spot)	12.42 (ng/spot)
Limit of Quantification (LOQ)	73.98 (ng/spot)	41.394 (ng/spot)
Relative S.D	52.122	51.122

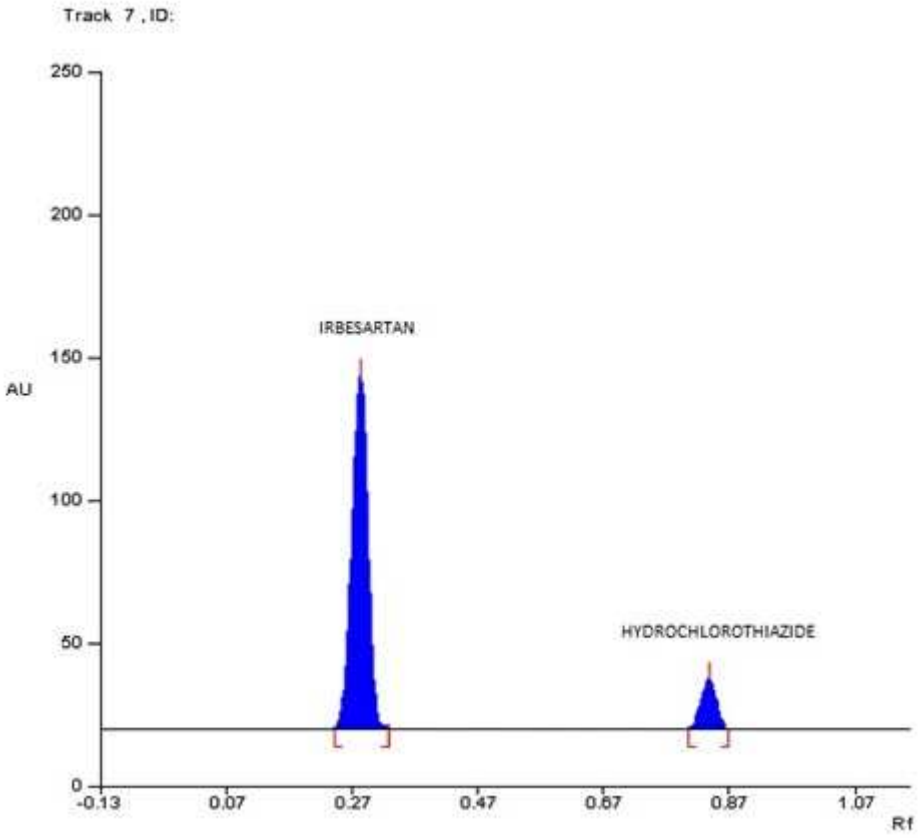

Fig.3: HPTLC Chromatogram for the assay of irbesartan and hydrochlorothiazide in a tablet dosage form

Repeatability test

Five replicate application to the HPTLC plate were performed for the standard solution containing 600 ng of Irbesartan and 250 ng of Hydrochlorothiazide. The results obtained by repeating the estimation procedure five times were as shown in **table 2**.

Table 2. System repeatability Parameters

Parameters	Irbesartan	Hydrochlorothiazide
Amount applied (ng)	600	250
Peak Area *	4224	3041
Standard Deviation	7.024	3.606
Relative Standard Deviation	0.166	0.119

**= mean of 5 values*

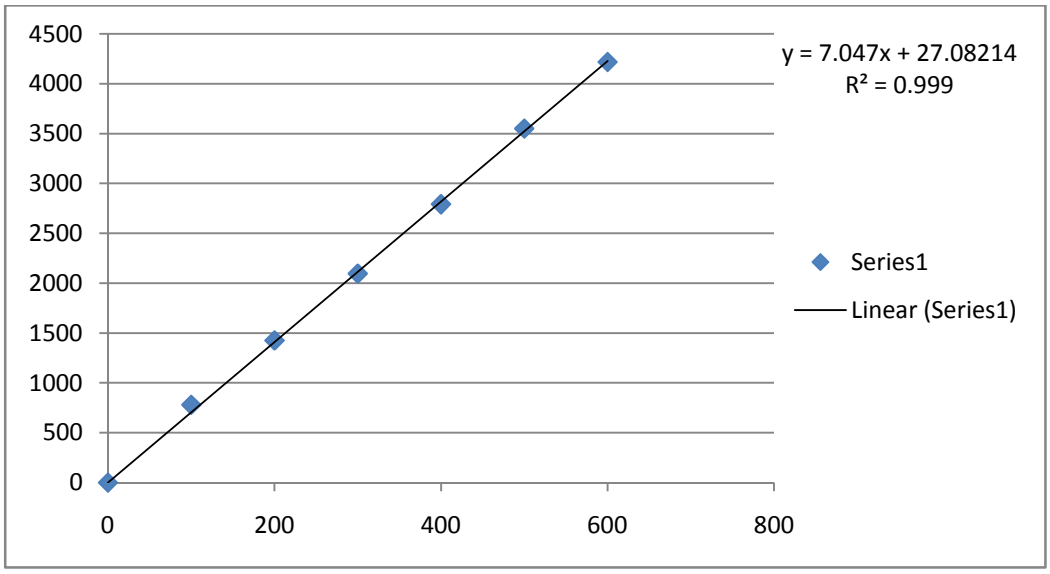

Fig. 4. Linearity Plot of Irbesartan (HPTLC)

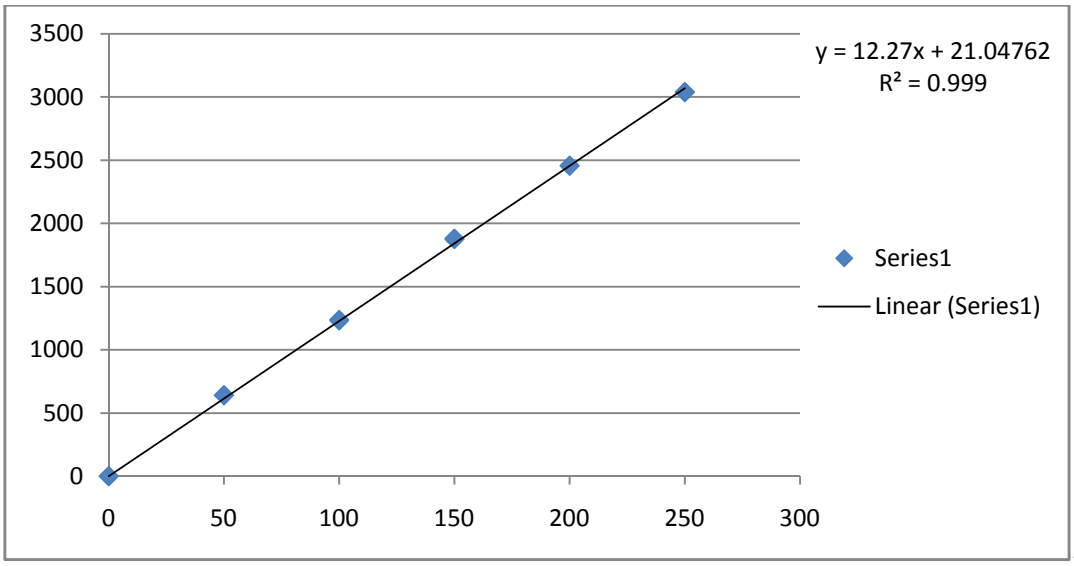

Fig. 5. Linearity Plot of Hydrochlorothiazide (HPTLC)

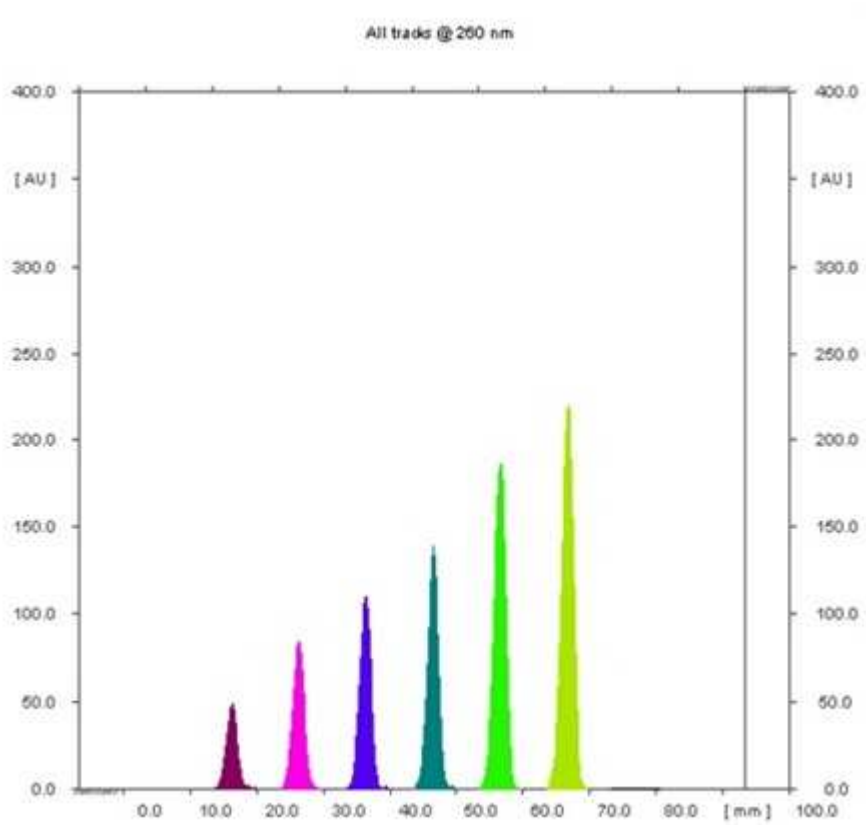

Fig.6 . Linearity chromatogram of Irbesartan

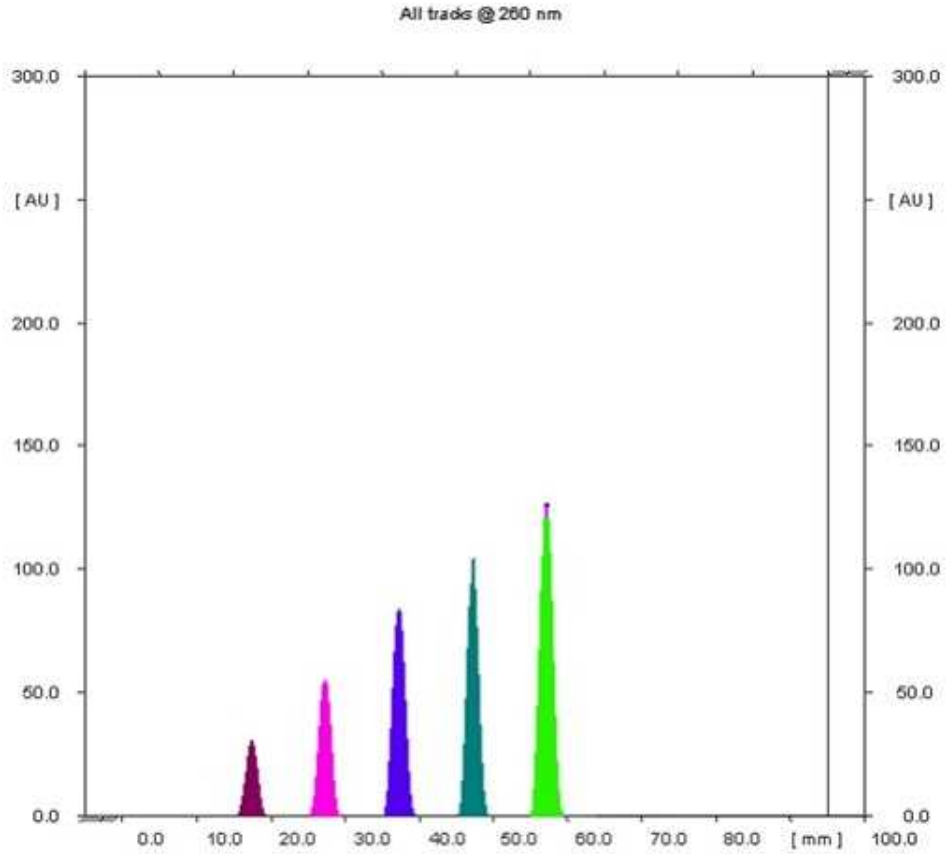

Fig.7 . Linearity chromatogram of Hydrochlorothiazide

Linearity Studies

To evaluate the linearity range of Irbesartan and Hydrocholothiazide Aliquots of 1, 2, 3, 4, 5, 6 µL of standard solution of irbesartan and 0.5, 1.0, 1.5, 2.0, 2.5 µL of hydrochlorothiazide were applied on the TLC plate (concentration of 100 µg/mL for both drugs). TLC plate was air dried, developed and analyzed photometrically as described earlier. The standard calibration curve was computed using regression analysis with Microsoft excel as shown in **fig 4** and **fig 5** for IRB and HCTZ respectively. The chromatogram of the linearity was shown in **fig 6** and **fig 7** for IRB and HCTZ respectively.

Estimation Method

The sample solution was spotted on the TLC plate with the help of Linomat V spotting system. The chromatographic plate was developed in a twin trough chamber containing the Mobile Phase. The Chromatograms were recorded and Rf values were determined for Irbesartan and Hydrochlorothiazide. The amount of drug present was calculated by comparing the peak area values of standard with that of sample as follows. The results were as shown in **table 3** and **table 4.** The chromatogram of the sample drug is as shown in **fig. 3.**

$$\textbf{Amount of drugs in a tablet} = \frac{\text{peak area of test}}{\text{Peak area of Standard}} \times \frac{\text{Standard dilution factor}}{\text{Sample dilution factor}} \times \text{Avg. Weight}$$

Table 3. Assay of combined tablet dosage forms

Drug	Sample No	Label Claim	Amount found	% of label claim
Irbesartan	1	150	150.68	100.39
	2	150	150.11	100.07
	3	150	149.42	99.61
	4	150	151.23	100.82
	5	150	148.91	99.27
Hydrochlorothiazide	1	12.5	12.62	100.96
	2	12.5	12.59	100.72
	3	12.5	12.43	99.44
	4	12.5	12.55	100.4
	5	12.5	12.34	98.72

Table 4. Statistical Validation

Drugs	Label Claim (mg/tablet)	Amount Found * (mg/tablet)	Standard Deviation	% RSD	Standard Error
Irbesartan	150	150.07	0.701	0.468	0.244
Hydrochlorothiazide	12.5	12.51	0.118	0.941	0.081

**= Mean of 5 values*

Table 5. Percentage Recovery

Drugs	Amount Added (ng)	Amount Recovered (ng)	% Recovery	% Average recovery	% RSD
Irbesartan	100	100.23	100.23	100.12	0.127
	300	300.41	100.14		
	500	499.89	99.98		
Hydrochlorothiazide	50	50.67	101.34	100.59	0.658
	150	150.55	100.34		
	250	250.23	100.09		

Recovery Studies

To ensure the reliability of the method, mixing a known quantity of standard drug with the pre-analyzed sample formulation carried out recovery studies and contents were analysed by the proposed method. The percentage recovery was found to be as shown in **table 5.**

The intra/inter day variations of the method were performed using five replicate injections of three different, which were prepared and analysed on the same and on three different days over a period of two weeks. The intra and inter day variation in the peak area ratio of the drug solution was calculated in the terms of percentage relative standard deviation and the results are shown in **table 6.**

Table 6: Intra-day and inter-day precision of Irbesartan and Hydrochlorothiazide standards

Drugs	Theoretical Amount (ng)	Intra-day concentration measured (ng)		Inter-day concentration measured (ng)	
		Mean (a)	RSD %	Mean (a)	RSD %
IRBESARTAN	100	100.22	0.054	100.95	0.32
	300	299.89	0.034	300.79	0.45
	500	500.85	0.047	501.11	0.25
HYDROCHLO-ROTHIAZIDE	50	50.92	0.097	50.87	0.55
	150	150.52	0.086	150.98	0.39
	250	250.97	0.107	250.45	0.71

A. Mean values represent five different standards for each concentration.

B. Inter-day reproducibility was determined from five different runs for each concentration over a week period.

CONCLUSION

The proposed HPTLC method has been evaluated over the linearity, precision, accuracy, stability, specificity and proved to be convenient and effective for the quality control of Irbesartan and hydrochlorothiazide in a tablet dosage form. The measured signal was shown to be precise, accurate, and linear over the concentration range tested with a correlation coefficient of 0.999 for both drugs and shows an acceptable limit of RSD. The good separation of the two drugs in the TLC plate shows that the mobile phase used is best for its separation. Thus the proposed method is rapid, selective, requires a simple sample preparation procedure, Moreover, the lower solvent consumption leads to a cost effective and represents a good procedure of of Irbesartan and hydrochlorothiazide in a tablet dosage form.

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