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Method Development and Validation For the Simultaneous Estimation of Irbesartan and Hydrochlorothiazide in Tablet Formulation by HPTLC

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ABSTRACT

High performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous investigation of Irbesartan and Hydrochlorothiazide in tablet formulation. Chromatographic separation was performed on aluminium plates precoated with silica gel 60F254, with methanol: ethyl acetate (7: 3 % v/v) as mobile phase. Detection was performed densitometrically at 254 nm. The Rf values of Irbesartan and Hydrochlorothiazide were 0.24 ± 0.10and 0.42 ± 0.06 , respectively. Linearity was found to be in the concentration range of 150-900 ng/spot for Irbesartan and 25- 150 ng/spot for Hydrochlorothiazide, accuracy (100.05 % for Irbesartan and 100.26 % for Hydrochlorothiazide) and specificity, in accordance with ICH guidelines. The method can be used for routine analysis of Irbesartan and Hydrochlorothiazide in tablet formulation.

Key words: HPTLC, Irbesartan, Hydrochlorothiazide.

INTRODUCTION

Irbesartan (IRBS) and Hydrochlorothiazide (HCTZ) is an combination therapy used for the treatment of hypertension. Irbesartan (IRBS) chemically known as 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl] phenyl} methyl) -1, 3-diazaspiro [4.4] non-1-en-4-one which is a angiotensin II receptor antagonists. Its chemical formula is $C_{25}H_{28}N_6O$.

Hydrochlorothiazide (HCTZ) is a diuretic of the benzodiazepine group which is chemically known as 6-chloro-3,4-dihydro-2H-1,2,4- benzothiadiazine-7-sulphonamide 1,1-dioxide. Its chemical formula is $C_7H_8ClN_3O_4S_2$.[1]. The chemical structures of Irbesartan and Hydrochlorothiazide are represented in Fig.1A and Fig. 1B.

Literature Survey represents that various analytical methods are provided for the determination of IRBS and HCTZ in combination such as spectrophotometry [2-5], Micro emulsion[6], HPLC [7-12], UPLC[13], Spectrofluormetric[14], Capillary electrophoresis[15], LC-MS[16] and HPTLC[17-19]. To the best of our knowledge, very few analytical methods have been reported for the determination of IRBS and HCTZ in combination by HPTLC.So, an attempt was made to develop an HPTLC method with an improved Rf value than the already reported method for the selected formulation.

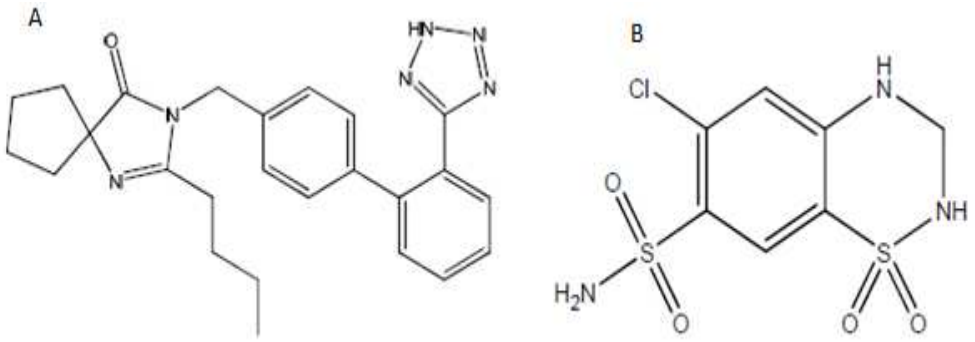

Fig.1: Chemical structure of Irbesartan (A) and Hydrochlorothiazide (B)

MATERIALS AND METHODS

Reagents and Chemicals

Irbesartan and Hydrochlorothiazide was a gift sample from Sun Pharmaceuticals Ltd, India. Pharmaceutical formulation of Irovel-H tablet containing 150 mg of IRBS and 12.5 mg of HCTZ was purchased from the local market. All chemicals and reagents used were of AR grade and were purchased from Merck, Mumbai, India**.**

Instrumentation

The HPTLC system (Camag,Switzerland) comprising of a Linomat IV semi-automatic spotting device, a glass twin-trough chamber (20×10 cm), a TLC Scanner-III, a data station by using win CATS software and an HPTLC syringe (100 µL capacity, Hamilton Company) was employed in the chromatographic studies. Linear ascending development was carried out in a twin trough glass chamber (20 cm x 10 cm).

Chromatographic conditions

The experiment was performed on an aluminium packed silica gel 60 F254 TLC plates, (20 cm × 10 cm, layer thickness 0.2 mm) prewashed with methanol and mobile phase comprising of methanol: ethyl acetate (7:3 % v/v). The solvent used for development was run up to 80 mm in Camag chamber formerly saturated by means of solvent mixture for 20 min.

Samples were spotted at a distance of 8 mm from lower edge the distance between the two bands was 7 mm. The developing solvent was run up to 80 mm and the development was performed at 25 ± 2°C. The average development time was 15 minutes. After development, the plate was air dried and scanned densitometrically at 254 nm by means of TLC scanner 3.

Preparation of Stock Standard Solution

Stock standard solutions of IRBS and HCTZ were prepared by dissolving 150 mg of IRBS and 12.5 mg of HCTZ in 100 mL methanol. The working standard solution was prepared by means of dilution of 1 mL of every stock solution to 10 mL with methanol.

The drugs were stable in methanol solution with no noteworthy diminish in their concentrations were observed after 12 h.

Preparation of Calibration Curves:

Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 µl of standard solutions of irbesartan and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 µL of hydrochlorothiazide were applied on the TLC plate (150 µg/ml of IRBS and 12.5 µg/ml of HCTZ).TLC plates were dried in air, developed and estimated photometrically.The standard calibration curve was computed using regression analysis by means of Microsoft excel. The results direct out that calibration plots were found to be linear over the concentration range of 150-900 ng/spot for IRBS and 25-150 ng/spot for HCTZ. Calibration curve was constructed by plotting peak area of IRBS and HCTZ against their relevant concentration.

Preparation of Sample solution

Powder from twenty tablets (Irovel-H containing 150 mg of IRBS and 12.5 mg of HCTZ for each tablet, manufactured by Sun Pharmaceuticals Ltd.) were weighed, their average weight was estimated and crushed to fine powder. An amount of powder equivalent to one tablet was transferred into a 100 mL volumetric flask containing 70 mL of methanol and mixed well. The solution was ultrasonicated for 30 min and then diluted to 100 mL with methanol. The solution was filtered by means of Whatman filter paper No.41. Further 1 mL of the filtrate is diluted

to 10 mL with methanol. The amount of each drug present in the sample was determined by comparing peak areas with that of the standard.

Validation of the proposed methods
HPTLC method was validated in conformity with ICH guidelines. The subsequent parameters were validated.

Specificity
The specificity of the method was ascertained by analysis of drug standards and samples. The identities of the peak for IRBS and HCTZ were established by comparing the Rf with those of standards.

Linearity
Standard stock solution of the drug was diluted to prepare linearity standard solutions containing IRBS in the concentration range of 150–900 ng/spot and 25–150 ng/spot for HCTZ, respectively. Every measurement was repeated six times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding concentration of the drug. Standard deviation, slope, intercept and coefficient of correlation (r^2) of the curves were calculated to determine linearity of the method.

Limit of detection and limit of quantitation
The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively, where σ is the standard deviation of the peak area and s is the slope of the ensuing calibration graph.

Precision
Repeatability of measurement of peak area was carried out by frequent scan of the similar spot (150 ng/ spot each of IRBS and 50 ng/spot of HCTZ) six times without altering the plate position. The % RSD for peak area was calculated. Repeatability of the sample application is based on six-time application of the combined standard solution. The % RSD for peak area was measured. Variations of results within the same day (intra-day precision) and among days (inter-day precision) are referred as reproducibility.

The intra-day precision (% RSD) was determined by analyzing standard solution of IRBS and HCTZ for 3 times on the same day. The inter-day precision (% RSD) was determined by analyzing standard solution of IRBS and HCTZ for 5 days.

The intra- and inter-day variation for determination of IRBS and HCTZ was carried out at three diverse concentration levels 150, 300 and 450 ng/spot for each of IRBS and 50,75 and 100 ng/spot of HCTZ.

Accuracy
To measure the accuracy of the developed method and to study the interference of formulation additives, recovery experiments were carried out by a standard addition method at 80, 100 and 120 % level. The experiment was conducted in triplicate. Percentage recovery and relative standard deviation were calculated.

RESULTS AND DISCUSSION

Method development
Various developing solvent systems composed of ethyl acetate,toluene, methanol, chloroform or a mixture thereof were tried for optimization of mobile phase used for HPTLC separation of IRBS and HCTZ. But the best resolution and symmetrical peak shapes were achieved by means of mobile phase system comprising of methanol: ethyl acetate (7: 3 % v/v). The Rf values were found to be 0.24 and 0.42 for IRBS and HCTZ, respectively.The summary of validation parameters is shown in Table 1.

Table 1. Summary of Validation parameters

Parameters	Irbesartan	Hydrochlorothiazide
Linearity Range (ng/spot)	150-900	25-150
Correlation coefficient (r^2)	0.999	0.999
Regression coefficient (y=mx+c)	Y= 16.45 x + 298.3	Y= 83.24 x + 116.1
Slope (m)	16.45	83.24
Intercept (c)	298.3	116.1
Limit of detection (ng/spot)	12.23	10.21
Limit of quantification (ng/spot)	37.08	30.96

Specificity

The chromatogram of formulation showed peaks at Rf values of 0.24 and 0.42 for IRBS and HCTZ respectively (Fig. 2), demonstrating that there is denial interference of the excipients present in the tablet formulation.

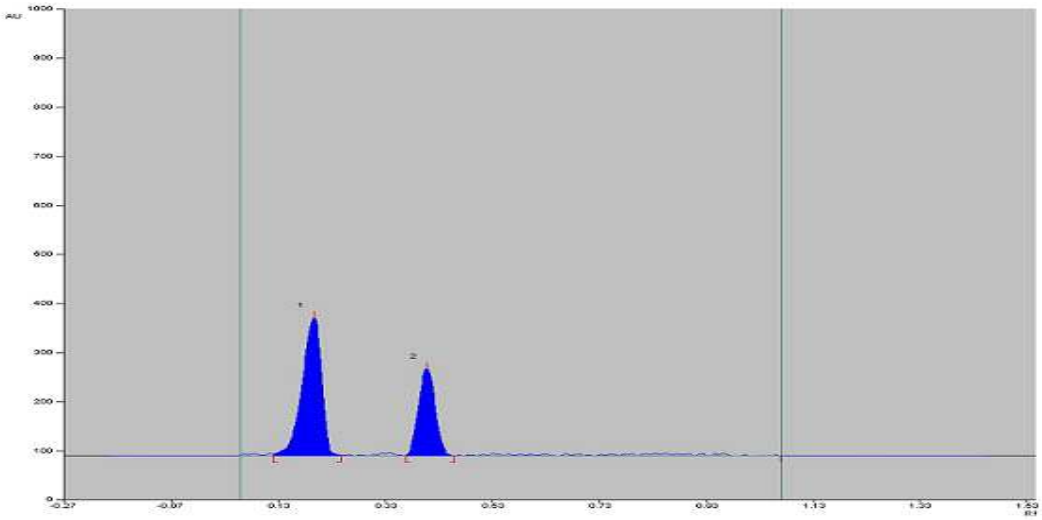

Fig.2:Typical Densitogram of Irbesartan and Hydrochlorothiazide

Linearity

Linear regression information for the calibration graph showed good linear relationships between response and concentration over the ranges 150-900 ng/spot for IRBS and 25-150 ng/spot for HCTZ, correspondingly. The linear regression equations were Y= 16.45 x + 298.3 (r^2= 0.999) for IRBS and Y= 83.24 x + 116.1 (r^2= 0.999). The plots obtained from linear regression study are given in Fig.3 for IRBS and Fig. 4 for HCTZ, respectively.

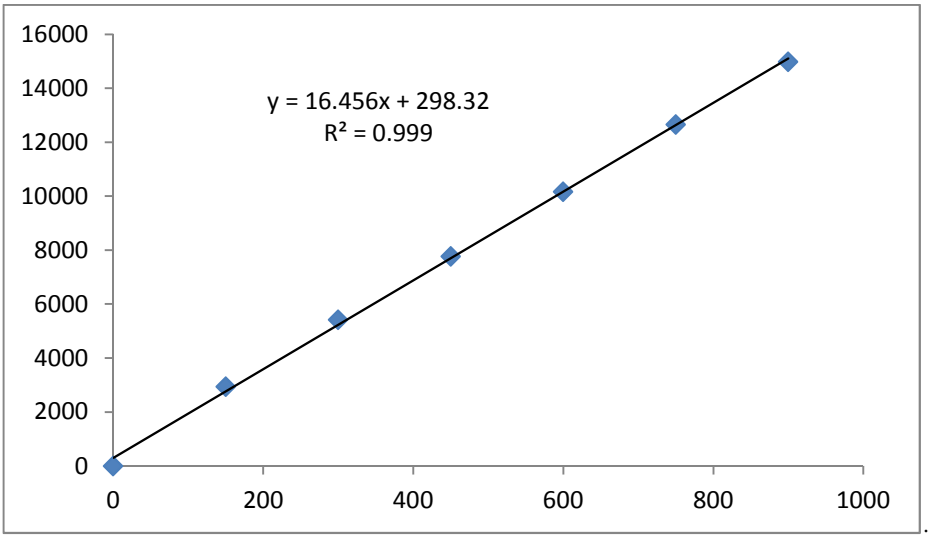

Fig.3: Linearity plot of Irbesartan

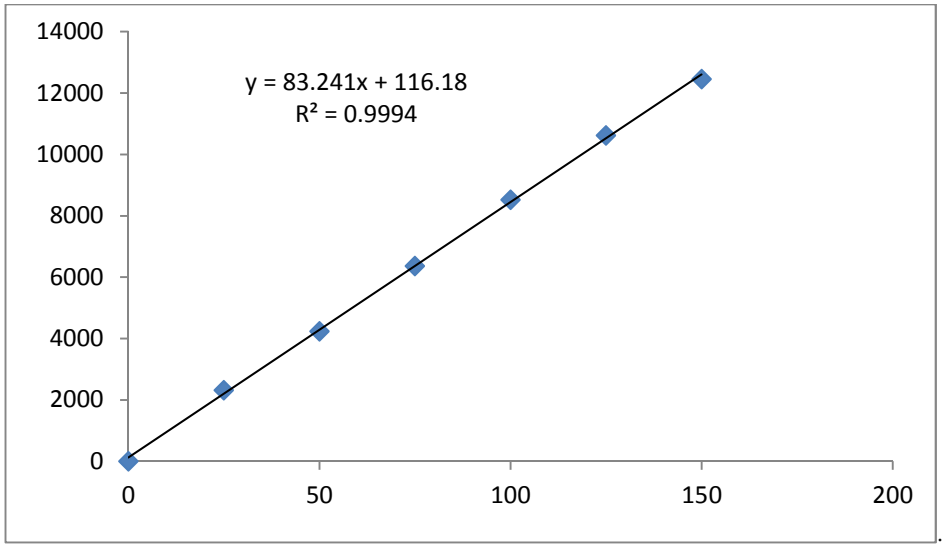

Fig.4: Linearity plot of Hydrochlorothiazide

Limits of Detection and Quantitation

The limits of detection and quantitation were found to be 12.23 ng/spot and 37.08 ng/spot respectively, for IRBS and 10.21 ng/spot and 30.96 ng/spot for HCTZ. This represents the developed method is sufficiently sensitive.

Precision

The precision of the method was expressed in terms of the relative standard deviation (% RSD) as shown in Fig.5. The results shown in Table 2 expose the high precision of the method.

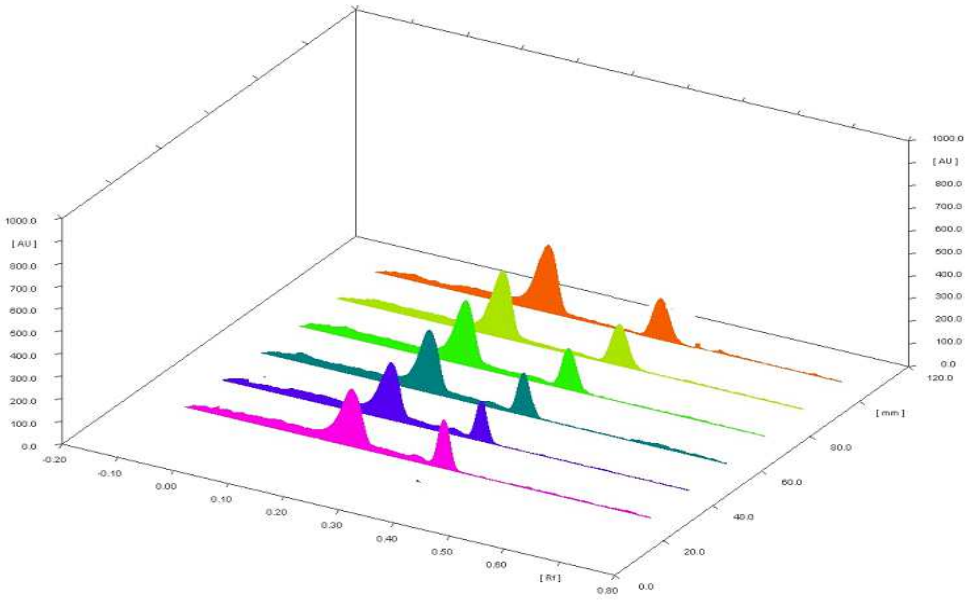

Fig.5: Precision Densitogram of IRBS and HCTZ

Table 2.Precision of the method

Concentration (ng/spot)	Intra day Presision			Inter day Precision		
	Measured Conc (ng/spot)	% RSD	% Content Found	Measured Conc (ng/spot)	% RSD	% Content Found
Irbesartan						
150	149.86	0.98	99.90	149.64	1.12	99.76
300	298.97	1.13	99.65	298.35	1.03	99.45
450	449.25	1.17	99.83	449.58	1.15	99.90
Hydrochlorothiazide						
50	49.78	1.12	99.56	49.83	1.08	99.60
75	74.83	1.18	99.77	74.85	1.14	99.80
100	99.65	1.20	99.65	99.89	1.09	99.89

Accuracy

The proposed HPTLC method when used for recovery studies for IRBS and HCTZ from pharmaceutical formulation following spiking with added standard drug afforded recovery between 99.56–100.35 % and average recoveries for IRBS and HCTZ from the marketed dosage form are listed in Table 3.

Table 3. Percentage Recovery

Drugs	Amount Added (ng)	Amount Recovered (ng)	% Recovery	Average Recovery	% RSD
Irbesartan	240	240.15	100.06	100.05	0.093
	300	300.34	100.12		
	360	359.92	99.97		
Hydrochlorothiazide	20	20.27	101.35	100.26	0.954
	25	24.89	99.56		
	30	29.96	99.87		

Analysis of marketed formulation

Investigational reports of the quantity of IRBS and HCTZ in tablet formulation, expressed as a percentage of label claims were in admirable compliance with the label claims thus representing that there is no interference from any of the excipients which are normally available in tablets. The average drug content was found to be 99.89 % for IRBS and 99.76 % for HCTZ.

CONCLUSION

The developed HPTLC method provides precise, accurate and reproducible quantitative analysis for the simultaneous determination of IRBS and HCTZ in Irovel-H tablets. The developed method was validated as per the ICH guidelines.

The robustness of the projected method were considered and establish to be robust at deliberate changes made in investigational conditions.

Statistical analysis designate that the proposed HPTLC method diminish the duration of the analysis and emerge to be equally appropriate for routine determination of IRBS and HCTZ simultaneously in tablet formulation in quality control laboratories.

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