A validated stability indicating HPTLC method for simultaneous estimation of irbesartan and hydrochlorothiazide

Abstract

Introduction: Irbesartan, a diazaspiro angiotensin II blocker, is marketed in combination with Hydrochlorothiazide, which is a diuretic acting on distal convoluted tubule; for synergistic anti-hypertensive action. The present study deals with development and validation of a stability indicating HPTLC method for simultaneous estimation of Irbesartan and Hydrochlorothiazide using TLC plates precoated with Silica gel 60F254 and the mobile phase comprising Acetonitrile: Chloroform in the ratio of 5:6 v/v. Irbesartan and Hydrochlorothiazide were well resolved with Rf 0.27 ± 0.03 and 0.45 ± 0.03, respectively. Wavelength selected for the quantization was 270 nm. Inherent stability of these drugs was studied by exposing both drugs to various stress conditions as per ICH guidelines viz. Dry heat, oxidative, photolysis (UV and cool white fluorescent light) and hydrolytic conditions under different pH values. **Results:** Both the drugs were not degraded under dry heat and photolytic conditions, but showed degradation under hydrolytic condition. The degraded products of Irbesartan and hydrochlorothiazide were well resolved from the individual bulk drug response. **Conclusion:** The developed method is found to be simple, specific, precise and stability indicating. The specificity of the method was confirmed by peak purity profile of the resolved peaks.

Key words: HPTLC, hydrochlorothiazide, irbesartan, stability-indicating, stress degradation

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INTRODUCTION

Irbesartan[1] (IRB) (Angiotensin II Blocker) is chemically 2-butyl-3-[[2′-(1*H*-tetrazol-5-yl) [1, 1′-biphenyl]-4-yl] methyl1, 3-diazaspiro [4, 4] non-1-en-4one. Hydrochlorothiazide[2] (HCTH) (Site 3 Diuretic) is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide,-1, 1-dioxide. Irbesartan and Hydrochlorothiazide are available in the market as combined dosage form for the treatment of hypertension. Literature survey revealed that there are number of HPTLC[3-9] and HPLC[10-12] methods for individual drug (IRB and HCTH) or IRB/HCTH in combination with other drug. There is stability indicating method[13-14] reported for individual drug Irbesartan and hydrochlorothiazide separately. But to the best of our knowledge, there is no stability-indicating method reported for this combination. The aim of the present study accordingly was to establish inherent stability of Irbesartan and Hydrochlorothiazide through stress studies under a variety of ICH recommended test conditions[15] and to develop a validated stability - indicating assay method for this combination.

MATERIALS AND METHODS

Irbesartan was provided as a gift sample by CIPLA Ltd. and Hydrochlorothiazide was provided as a gift sample by Torrent pharmaceutical Ltd. Drugs were used without any further purification. All other reagent used for experimentation was of analytical reagent (AR) grade. Chemicals used for this experiment were

Acetonitrile, Methanol, Chloroform, NaOH, HCl, and H_2O_2. These chemicals were purchased from M/s. Thomas Baker.

Instrumentation

Chromatographic separation of drugs was performed on Merck TLC plates pre-coated with silica gel 60 F254 (10 cm ×10 cm with 250 mm layer thickness) from E. Merck, Germany. The samples were applied onto the plates as a band with 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using Camag TLC scanner 3 and operated by winCATS software (V 1.4.2, CAMAG). Electronic balance (Make SHIMDZU Model AY-120) was used for weighing purpose.

Selection of detection wavelength

The wavelength was selected at 270 nm, at which, hydrochlorothiazide shows high absorbance compared to Irbesatan. This could be used to compensate for relatively low concentration of Hydrochlorothiazide compared to Irbesartan the marketed formulation. In tablet dosage form irbesartan and hydrochlorothiazide were found in the ratio of 150:12. Hence, the selected wavelength was convenient to obtain good response peaks for both the drugs.

Preparation of solution

Standard stock solution of Irbesartan and Hydrochlorothiazide were prepared by dissolving 25mg of each drug in methanol to obtain 25 ml stock solution (1,000 mcg/ml) and further diluted to get final concentration 100 mcg/ml. This sample was applied on TLC plate pre-coated with silica gel $60F_{254}$ as a band of length 4mm at a distance of 10 mm from both x-axis and y-axis. It was developed in development chamber using optimized mobile phase consisting of Acetonitrile: Chloroform in the ratio of 5:6 v/v. The plate was developed up to 90 mm, dried in air and scanned at 270 nm.

Stress degradation studies

Degradation under acid catalyzed hydrolytic condition

To 5 ml of working standard solutions of Irbesartan and Hydrochlorothiazide separately, each of conc. 1000 mcg/ml, 5 ml of 5 N HCl was added. The solutions were diluted to 50 ml with methanol and refluxed at 60°C for 1 hour. Appropriate volume of resultant solution was applied on TLC plate 100 ng per spot) and densitograms were developed.

Degradation under alkali catalyzed hydrolytic condition

To 1 ml of working standard solutions of Irbesartan and Hydrochlorothiazide separately, each of conc. 1,000 mcg/ml, 0.3 ml of 5 N NaOH was added. The solutions were diluted to 10 ml with methanol. Appropriate volume of resultant solution was applied on TLC plate (100 ng per spot) and densitograms were developed.

Degradation under neutral hydrolytic condition

To 5 ml of working standard solutions of Irbesartan and Hydrochlorothiazide separately, each of conc. 1000 mcg/ml, 5 ml of water was added. The solutions were diluted to 50 ml with methanol and refluxed at 60°C for 1 hour. Appropriate volume of resultant solution was applied on TLC plate (100 ng per spot) and densitograms were developed.

Degradation under oxidative condition

To 5 ml of working standard solutions of Irbesartan and Hydrochlorothiazide separately, each of conc. 1000 mcg/ml, 5 ml of 30% H_2O_2 was added. The solutions were diluted to 50 ml with methanol. This study was monitored with and without reflux. Appropriate volume of resultant solution (100 ng per spot) was applied on TLC plate and densitograms were developed.

Degradation under dry heat condition

Effect of dry on stability of these drugs was studied by keeping drug samples in oven (80°C) for a period of 8 hours. Samples were withdrawn at appropriate time and subjected to HPTLC analysis after suitable dilution with methanol.

Photo-degradation studies

Photolytic degradation studies were carried out by exposure of drugs to UV light up to illumination of 200 watt hours/square meter and subsequently cool fluorescent light to achieve an illumination 1.2 million Lux.Hr.

Method validation

Upon the study of samples exposed to stress degradation studies as mentioned above, it was established that the products of degradation do not interfere with the peak response for both Irbesartan and Hydrochlorothiazide. This optimized HPTLC

method was then validated for the parameters listed below as per ICH guidelines.

Linearity
Different concentrations of Irbesartan (200 ng to 1000 ng/ band) and Hydrochlorothiazide (200 to 600 ng/ band) were applied on TLC plate and densitograms were developed. The data of peak area v/s drug concentration were treated by linear least-square regression analysis

Precision
Interday and Intraday precision were evaluated by analyzing sample preparations obtained from homogenous sample, six times and % RSD value obtained was calculated to determine any intra-day and interday variation.

Accuracy
To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Mean percentage recovery was determined.

Limit of detection and limit of quantitation
The limit of detection (LOD) and limit of quantitation (LOQ) were obtained by calculating using the standard formula as per the ICH guidelines,

$$LOD = \frac{3.3\,\sigma}{S} \qquad LOQ = \frac{10\,\sigma}{S}$$

Where σ is Standard deviation of the response and S is slope of the calibration curve.

Specificity
The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peaks was ascertained by analyzing the spectrum at peak start, max position and at peak end. The peak purity was determined by winCATS software.

RESULTS AND DISCUSSION

Development of the optimum mobile phase
TLC procedure was optimized with a view to develop a stability-indicating assay method. The working standards of both the drugs were spotted on the TLC plates and developed in different solvent systems. Different mobile phases were tried to resolve Irbesartan and Hydrochlorothiazide. The optimum results were obtained with mobile phase consisting of Acetonitrile: Chloroform in the ratio of 5:6. The chamber was saturated with the mobile phase at room temperature. Developed mobile phase resulted in resolution for two drugs with R_f 0.27 ± 0.03 and 0.45 ± 0.03 for Irbesartan and Hydrochlorothiazide, respectively. The representative densitogram is given in Figure 1.

Validation of the developed stability-indicating method

Linearity
The response for the drugs was found to be linear in the concentration range 200-1000 ng/band for Irbesartan and 200-600 ng/band for Hydrochlorothiazide with correlation coefficient of 0.998 and 0.996, respectively. The linear regression equation obtained are y = 4.325(x) + 512.5 and y = 20.07(x) + 2,363 for Irbesartan and Hydrochlorothiazide, respectively.

Precision
The RSD value for intra-day precision study was found to be not more than 1.804 % and 1.8417% for Irbesartan and Hydrochlorothiazide, respectively and for interday precision was found to be not more than 1.8334% and 1.8765% for Irbesartan and Hydrochlorothiazide, respectively, thus confirming precision of the method.

Accuracy
Excellent recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80%, 100%, 120% level) indicated the mean recovery between 98% to 102% for both Irbesartan and Hydrochlorothiazide.

Limit of detection
The LOD as calculated by standard formula as given in ICH guidelines was found to be 30 ng/band and 66ng/band for Irbesartan and Hydrochlorothiazide, respectively.

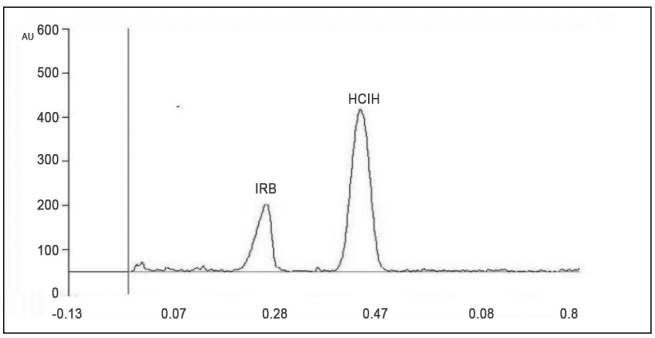

Figure 1: Representative Densitogram of Irbesartan and Hydrochlorothiazide with Rf 0.27 and 0.45, respectively

Limit of quantitation

The LOQ as calculated by standard formula as given in ICH guidelines was found to be 100 ng/band and 200 ng/band for Irbesartan and Hydrochlorothiazide, respectively.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s,m) 0.99981 and 0.99641 for IRB and HCTH, respectively indicating the non interference of any other peak of degradation product, impurity or matrix.

The validation summary is given in Table 1.

Degradation behavior

HPTLC studies on IRB and HCTH under different stress conditions suggested following degradation behavior

Hydrolytic studies

Acidic condition

Drugs, IRB and HCTH showed negligible degradation under acidic hydrolysis with reflux condition. No additional peaks were observed and drug peak area showed negligible decrease.

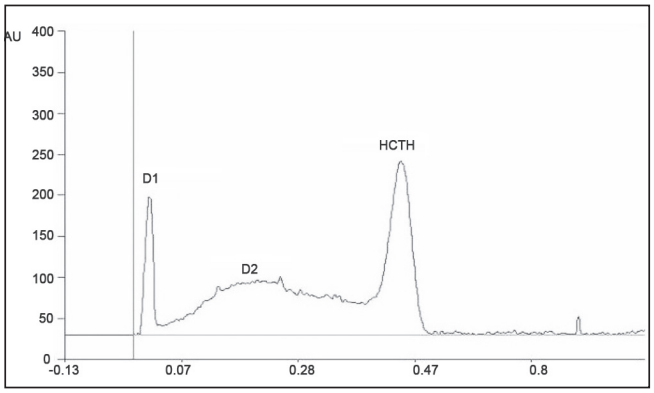

Figure 2: Representative densitogram showing degradation of irbesartan and hydrochlorothiazide under alkaline condition

Table 1: Summary of validation parameters

Validation parameter	Irbesartan	Hydrochlorothiazide
Linearity (r2)	y = 4.325 (x) + 512.5 0.998	y = 20.07 (x) + 2363 0.996
Beers law Range	200–1,000 ng/band	200–600 ng/band
Precision (% RSD)	NMT 2%	NMT 2%
Accuracy (mean recovery) (%)	98–102	98–102
LOD	30 ng/band	66 ng/band
LOQ	100 ng/band	200 ng/band
Specificity	Specific	Specific

Alkaline condition

IRB completely degraded under alkaline condition (0.3 ml of 5N NaOH) in short period of time. New peak was observed for product of IRB formed under these conditions at R_f 0.01. HCTH degraded to about 37.59% under alkaline condition (0.3ml of 5N NaOH) after keeping for four hours. One peak was observed for degradation product of HCTH along with HCTH drug peak at R_f 0.45 [Figure 2].

Neutral (water) condition

Drugs, IRB and HCTH showed negligible degradation under neutral hydrolysis with reflux condition. No additional peaks were observed and drug peak area remained almost constant.

Oxidative studies

Under reflux condition

IRB showed 9% degradation upon treatment with 30% H_2O_2 with reflux. Reduction in area of peak of IRB was observed but no other peak of degraded product was found. HCTH showed 66.17% degradation upon treatment with 30% H_2O_2 with reflux. Reduction in area of peak of HCTH was observed but no other peak of degraded product was found.

Without reflux condition

IRB showed 9% degradation under 30% H_2O_2 without reflux. Reduction in area of peak of IRB was seen but no other peak of degraded product was found. HCTH showed 42.7% degradation under 30% H_2O_2 without

Table 2: Summary of forced degradation study results

Conditions	Reflux time	Degradation	
		IRB	HCTH
Acid hydrolysis (5N HCl)	1 Hr.	Negligible	Negligible
Base hydrolysis (5N NaOH)	-	100%	37.59%
Neutral hydrolysis	1 Hr.	Negligible	Negligible
Oxidation (30% H2O2)	1 Hr.	9%	66.17%
Oxidation (30% H2O2)	-	9%	42.7%
Heat 800C	-	Negligible	Negligible
UV 200 watt hrs/sq m	-	Negligible	Negligible
Visible 1.2 Million Lux.Hr	-	Negligible	Negligible

reflux. Reduction in area of peak of HCTH was seen but no other peak of degraded product was found.

Thermal stress (dry heat) and photolytic studies

Under dry heat (80°C, 8 hours) and photolytic studies, no additional peaks were observed and drug peak area remained almost the same. This indicates stability of drugs upon exposure to dry heat, cool white fluorescent light and UV light for specified period. The forced degradation study results are summarized in Table 2

CONCLUSIONS

From the above study, we can conclude that the IRB and HCTH undergo degradation to different extent under different, above mentioned, stress conditions. In this study, the products formed after forced decomposition studies were resolved from the bulk drug response. From the peak purity profile studies, it was confirmed that the peak of the degradation product was not interfering with the response of drugs. It confirms that degradation product of drug can be separated from the drug by this method. The developed method is simple, accurate, precise, and specific. It is proposed for routine analysis of these drugs in the presence of degradation products in stability study.

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