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A NOVEL VALIDATED STABILITY INDICATING HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF IRBESARTAN AND HYDROCHLOROTHIAZIDE IN ACTIVE PHARMACEUTICAL INGREDIENTS

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Abstract

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 aluminum plate Pre coated with silica gel 60 F_{254}(make: Merk) and the mobile phase comprising ethyl acetate: isopropyl alcohol: toluene: ammonia 25% (4:4:2:0.2 v/v/v/v). Irbesartan and hydrochlorothiazide were well resolved with R_f 0.05 ± 0.078 and 0.39 ± 0.047 respectively. The wavelength selected for the quantization was 259 nm. Inherent stability of these drugs was studied by exposing both drugs to various stress conditions as per ICH guidelines viz. Neutral degradation, acidic degradation, alkaline degradation, oxidative degradation, thermal degradation and photolytic degradation. Both the drugs showed degradation under all the conditions but they are within the limit. The degraded products of irbesartan and hydrochlorothiazide were well resolved from the individual bulk drug response. 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.

Keywords: HPTLC, Hydrochlorothiazide, Irbesartan, Stability-indicating, Stress degradation.

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Introduction

Irbesartan[1-2] (IRB) (Angiotensin II Blocker) is chemically 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. Hydrochlorothiazide[1-2] (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 numbers of UV[6-11], RP-HPLC[12-25], HPTLC[25-28] and LC-MS[29] methods for individual irbesartan and hydrochlorothiazide drug or irbesartan and hydrochlorothiazide in combination. 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[3] and to develop a validated stability-indicating assay method[4-5] for this combination.

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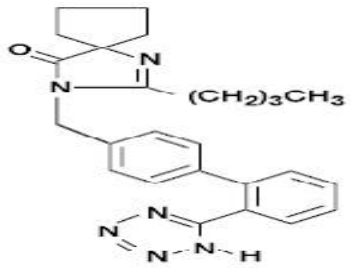
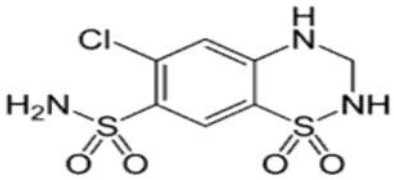


(a) Irbesartan



(b) Hydrochlorothiazide

Materials and methods

Materials

Irbesartan and hydrochlorothiazide were provided as gift sample by Ranbaxy Laboratories Limited, Gurgaon, India. Drugs were used without any further purification. All other reagents used for experimentation was of analytical reagent (AR) grade. Chemical used for this experiment were ethyl acetate, toluene, methanol and hydrochloric acid were purchased from M/s. SD Fine Chemicals, Gujarat, isopropyl alcohol, ammonia, glacial acetic acid, sodium hydroxide and hydrogen peroxide (H_2O_2) were purchased from M/s. CDH, New Delhi.

Instrumentation

Chromatographic separation of drugs was performed on Merck TLC aluminum plate Pre coated with silica gel 60 F_{254} (10 cm x 10 cm with 250 mm layer thickness) from E.Merck, Germany. The samples were applied onto the plates as a band with 8 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.1.4.3, SNR 1502W010). Electronic weighing balance 0.0001 g (Make Axis) was used for weighing purpose. A air dryer, silencio 1000, manufactured by Braun. All the glassware used for the experiment was, manufactured by Borosil, India.

Method Employed

HPTLC technique for the estimation of irbesartan and hydrochlorothiazide.

Selection of solvent

Ideal properties of solvents employed for HPTLC are;

- Drug should be soluble in solvent used,
- Drug should show stability in solvent used,
- Solvent should be volatile,
- It should be easily available,
- It should not show any kind of reactions with drugs to be analyzed.

Accordingly, methanol was selected as the solvent for further studies.

Selection of detection wavelength

The standard solution of Irbesartan and hydrochlorothiazide were scanned separately over wavelength of 200-400 nm by using UV-Visible spectrophotometer, irbesartan and hydrochlorothiazide shows the maximum absorbance at 205 nm and 271 nm respectively. From the overlay spectrum it shows two isobestic points at 215 nm and 259 nm. So the wavelength 259 nm was selected, at which, hydrochlorothiazide shows high absorbance compared to irbesartan. This could be used to compensate for relatively low concentration of hydrochlorothiazide compared to irbesartan in the marketed formulation. In tablet dosage form irbesartan and hydrochlorothiazide were found in the ratio of 150:12.5. Hence, the selected wavelength was convenient to obtain good response peaks for both the drugs.

Preparation of standard stock solution

A quantity of working standards equivalent to 150 mg of Irbesartan and 12.5 mg of hydrochlorothiazide were weighed and transferred in to a 100 mL clean dry volumetric flask, add about 10 mL of methanol to dissolve and make volume up to the mark with methanol. Further pipette out 1 mL from the stock solution transfer to a 10 mL clean dry volumetric flask and dilute up to the mark with methanol to yield concentration of irbesartan (150 µg/mL or 150 ng/µL) and hydrochlorothiazide (12.5 µg/mL or 12.5 ng/µL).

From the standard solution 8 µL was applied on TLC plate pre-coated with silica gel $60F_{254}$ as a band of length 8 mm at a distance of 10 mm from both x-axis and y-axis. It was developed in development chamber using optimized mobile phase consisting ethyl acetate: isopropyl alcohol: toluene: ammonia 25% (4:4:2:0.2 v/v/v/v). The plate was developed up to 90 mm, dried in air and scanned at 259 nm.

Preparation of sample solution

Contents of twenty tablets were accurately weighed and average weight per tablet was determined. An accurately weighed quantity of the pulverized powder equivalent to 150 mg of irbesartan and 12.5 mg of hydrochlorothiazide was weighed and transferred to a 100 mL volumetric flask, dissolved in 50 mL of methanol and sonicated for 30 min. The solution was filtered through whatmann filter paper No.41 and residue was washed with methanol. The solution was diluted up to the mark with methanol. Further pipette out 1 mL from the stock solution transfer to a 10 mL clean dry volumetric flask and dilute up to the mark with methanol to yield concentration of irbesartan (150 µg/mL or 150 ng/µL) and hydrochlorothiazide (12.5 µg/mL or 12.5 ng/µL). From this standard solution 8 µL was applied on TLC plate pre-coated with silica gel $60F_{254}$ as a band of length 8 mm 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 ethyl acetate: isopropyl alcohol: toluene: ammonia 25% (4:4:2:0.2 v/v/v/v). The plate was developed up to 90 mm, dried in air and scanned at 259 nm.

Selection of optimized mobile phase

A solvent system that would give dense compact spots, good separation from each other and separation from solvent front and application position was to be selected. Initially different solvent systems were tried (Table 1), Where bands closer to the solvent front and poor separation were observed. Finally, ethyl acetate: isopropyl alcohol: toluene: ammonia 25% (4:4:2:0.2 v/v/v/v), gave good sharp and symmetrical peaks.

Table No. 01: Different Solvent System Tried

Trial	Mobile phase	Observation
1	Methanol: Ether (5: 5v/v)	No spot observed
2	Acetone: Methanol (5: 5v/v)	No spot observed
3	Ethyl acetate: Methanol (5:5v/v)	One spots were observed
4	Toluene: Methanol (5:5v/v)	One spots were observed
5	Ethyl acetate: Toluene: Methanol (4:4:2 v/v/v)	Two spots were observed with poor separation
6	Ethyl acetate: Isopropyl alcohol: Toluene (4:4:2 v/v/v)	Two spots were observed with tailing but the distance between the spot is good
7	Ethyl acetate: Isopropyl alcohol: Toluene: 25% Ammonia (4:4:2:0.2 v/v/v/v)	Two spots were observed the separation and the distance between the spot is good

Among these systems, ethyl acetate: isopropyl alcohol: toluene: ammonia 25% (4:4:2:0.2 v/v/v/v), was selected because in this system good, compact, dense spots were obtained with good resolution between analyte, good separation from solvent front and sample application positions.

Estimation of Irbesartan and Hydrochlorothiazide in Marketed Formulation

The proposed method was applied for the determination of irbesartan and hydro-chlorothiazide in pharmaceutical formulation of irbesartan and hydrochlorothiazide tablets. 8 µL of standard and sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed (Table 2 and 3).

Fixed chromatographic conditions	
Mobile phase	Ethyl acetate: Isopropyl alcohol: Toluene: 25% Ammonia (4:4:2:0.2 V/V/V/V)
Diluent	Methanol
Stationary phase	TLC aluminum plate Pre coated with silica gel 60 F_{254}(make: Merk)
Developing chamber	Twin trough chamber
Chamber saturation	30 minutes with filter paper (make: Whatmann-quantitative filter paper)
Separation technique	Ascending chromatography development to a distance of 90% from line of application at room temperature
Migration time	15 minutes
Detection wavelength	UV 259 nm using densitometer
Sample application volume	8µL / spot using linomate injector
Concentration	1200 and 100 ng/spot of irbesartan, and hydrochlorothiazide respectively
Densitometric method	Absorbance
Detector	UV-D_2×W
Scanner	Camag TLC scanner with 20mm/s scan speed
Slit dimensions	6×0.45mm
Lamp source	Deuterium lamp
Soft ware	Win CATS, V.1.4.3, SNR 1502W010.
Band length	8mm under stream of nitrogen gas using linomate injector

Table No. 02: Analyses of Irbesartan in Marketed Formulation

S.No	Standard area	Sample area	Label Claim (mg)	Amount found (mg)	% Assay
1	4917.8	4899.6	150 mg	149.09	99.39
2	4886.2	4845.4		148.40	98.93
3	4910.8	4938.1		150.48	100.32
4	4784.5	4914.7		149.18	99.41
5	4778.5	4889.5		150.62	100.38
6	4816.3	4873.6		151.43	100.95
				Average	**99.89**
				SD	**0.7682**
				%RSD	**0.7690**
				SE	**0.3136**
				CI (Confidence Interval 99%)	**98.62 – 101.15**

Table No. 03: Analyses of Hydrochlorothiazide in Marketed Formulation

S.No	Standard area	Sample area	Label Claim (mg)	Amount found (mg)	% Assay
1	1532.1	1542.8	12.5 mg	12.27	98.16
2	1527.0	1548.3		12.35	98.86
3	1514.1	1539.9		12.39	99.16
4	1505.8	1558.5		12.61	100.91
5	1530.9	1552.4		12.35	98.87
6	1531.2	1543.7		12.28	98.29
				Average	**99.04**
				SD	**0.9909**
				%RSD	**1.0005**
				SE	**0.4045**
				CI (Confidence Interval 99%)	**97.55 – 100.52**

Stress degradation studies

Control Sample

A quantity tablet powder equivalent to 150 mg of irbesartan and 12.5 mg hydrochlorothiazide were accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of methanol. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with methanol. Further pipette 1 mL of the above stock solution and transferred to 10 mL volumetric flask and made up to 10 mL with methanol. From the above resulting solution 8 µL of sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed

Neutral Degradation Studies

A quantity tablet powder equivalent to 150 mg of irbesartan and 12.5 mg hydrochlorothiazide were accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of methanol. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with methanol. Further pipette 1 mL of the above stock solution and transferred to 10 mL volumetric flask and made up to 10 mL with methanol and the solution was refluxed in water bath for 30 minutes at 80^0C. From the above resulting solution 8 µL of sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed

Acid Degradation Studies

A quantity tablet powder equivalent to 150 mg of irbesartan and 12.5 mg hydrochlorothiazide were accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of methanol. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with methanol. Further pipette 1 mL of the above stock solution and transferred to 10 mL volumetric flask to that add 1 mL of 1 N hydrochloric acid and the volume was made up to 10 mL with methanol then it was refluxed in water bath for 30 minutes at 80^0C. From the above resulting solution 8 µL of sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed

Alkaline Degradation Studies

A quantity tablet powder equivalent to 150 mg of irbesartan and 12.5 mg hydrochlorothiazide were accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of methanol. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with methanol. Further pipette 1 mL of the above stock solution and transferred to 10 mL volumetric flask to that add 1 mL of 1 N sodium hydroxide and the volume was made up to 10 mL with methanol then it was refluxed in water bath for 30 minutes at 80^0C. From the above resulting solution 8 µL of sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed

Oxidative Degradation Studies

A quantity tablet powder equivalent to 150 mg of irbesartan and 12.5 mg hydrochlorothiazide were accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of methanol. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with methanol. Further pipette 1 mL of the above stock solution and transferred to 10 mL volumetric flask to that add 1 mL of 3 % hydrogen peroxide (H_2O_2) and the volume was made up to 10 mL with methanol then it was refluxed in water bath for 30 minutes at 80^0C. From the above resulting solution 8 µL of sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed

Thermal Degradation Studies

A quantity tablet powder equivalent to 150 mg of irbesartan and 12.5 mg hydrochlorothiazide were accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of methanol. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with methanol. Further pipette 1 mL of the above stock solution and transferred to 10 mL volumetric flask and the volume was made up to 10 mL with methanol and the solution was placed in oven at 80^0C for 48 hours. From the above resulting solution 8 µL of sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed

Photolytic Degradation Studies

A quantity tablet powder equivalent to 150 mg of irbesartan and 12.5 mg hydrochlorothiazide were accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of methanol. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with methanol. Further pipette 1 mL of the above stock solution and transferred to 10 mL volumetric flask and the volume was made up to 10 mL with methanol and the solution was exposed to UV light by keeping the volumetric flask in UV chamber for 7 days. From the above resulting solution 8 µL of sample solution containing 1200 ng/spot of irbesartan and 100 ng/spot of hydrochlorothiazide were injected and from the peak area of irbesartan and hydrochlorothiazide, the amount of drug present in sample was computed

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 (300-1800 ng / spot) and hydrochlorothiazide (25-150 ng / spot) 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 interday and intraday 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 Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of irbesartan and hydrochlorothiazide was determined by using standard deviation of the response and slope approach as defined in 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 slop of the calibration curve.

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 ethyl acetate: isopropyl alcohol: toluene: ammonia 25% 4:4:2:0.2 v/v/v/v. The chamber was saturated with the mobile phase at room temperature. Developed mobile phase resulted in resolution for two drugs with R_f 0.05 ± 0.043 and 0.35 ± 0.057 for irbesartan and hydrochlorothiazide respectively. The representative chromatogram is given in Figure 1.

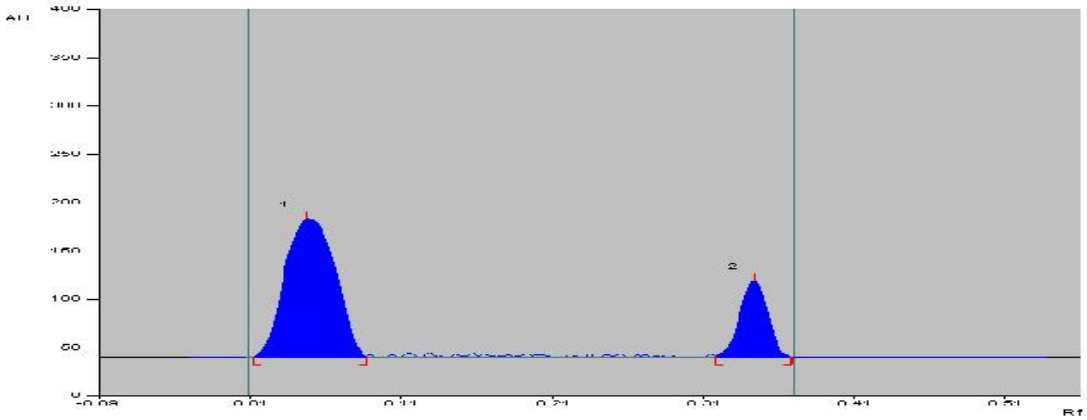


Fig. No. 01: Typical Densitogram of Irbesartan and Hydrochlorothiazide Standards with Rf of 0.05 and 0.35, respectively

Validation of the developed stability-indicating method

Linearity

The response for the drugs was found to be linear in the concentration range 300-1800 ng / spot of irbesartan and 25 - 150 ng / spot of hydro-. chlorothiazide with correlation coefficient of 0.9979 and 0.9986 respectively. The calibration graph was plotted against concentration Vs peak area Figure 2 and 3. The 3 dimensional chromatogram for the same is shown in the Fig. 4.

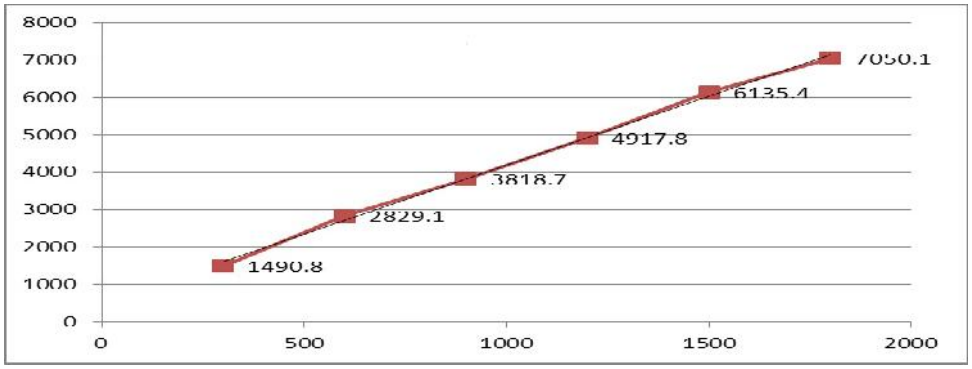


Fig. No. 02: Linearity Plot of Irbesartan

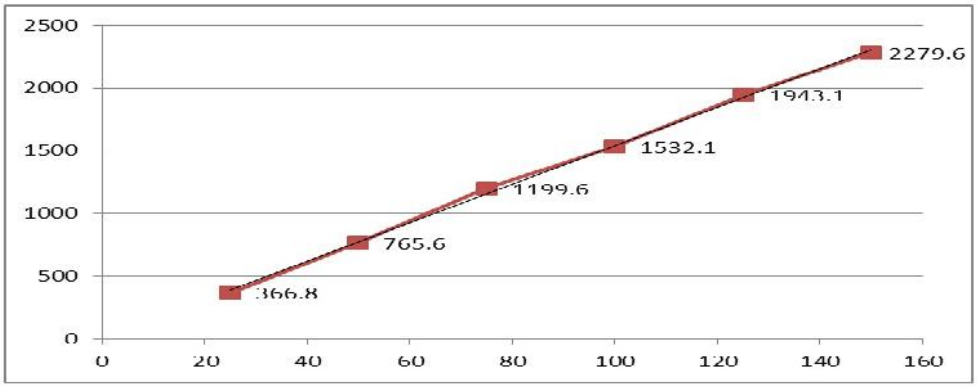


Fig. No. 03: Linearity Plot of Hydrochlorothiazide

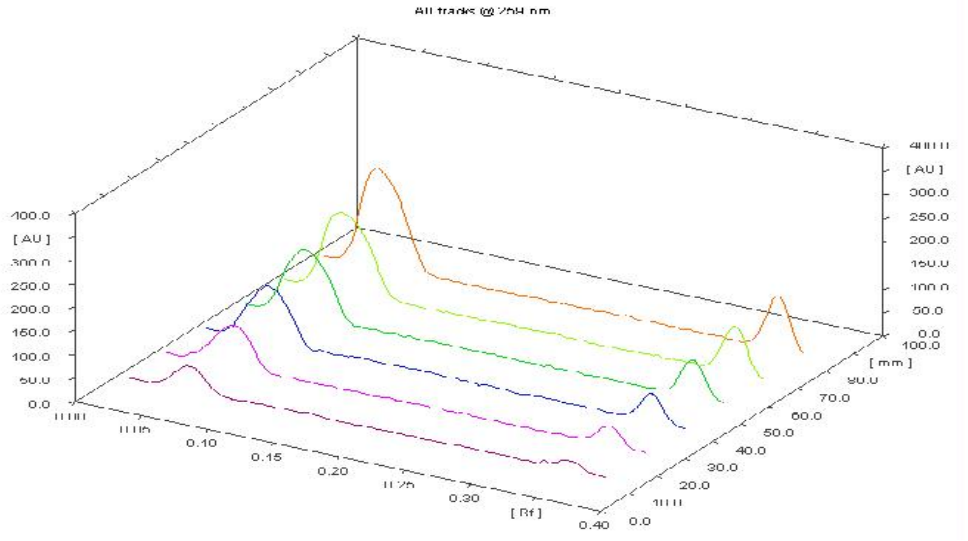


Fig. No. 04: Linearity 3Dimentional Chromatogram

Precision

The RSD value for intra-day and inter-day precision study was found to be within the limit, thus confirming precision of the method (Table: 4 and 5).

Table No. 04: Intra-day Precision Data of Irbesartan and Hydrochlorothiazide

S.No	Parameter	Irbesartan				
		Concentration (ng / spot)	Peak area*	% Amount found*	SD	%RSD
1	0 Hours		4890.23	99.43		
2	3 Hours	1200	4872.36	99.07	**0.5473**	**0.5524**
3	6 Hours		4882.1	92.27		
S.No	**Parameter**	**Hydrochlorothiazide**				
		Concentration (ng / spot)	**Peak area***	**% Amount found***	**SD**	**%RSD**
1	0 Hours		1530.11	99.86		
2	3 Hours	100	1518.91	99.13	**0.7742**	**0.7809**
3	6 Hours		1537.33	100.33		

*Mean of six determinations

Table No. 05: Inter-day Precision Data of Irbesartan and Hydrochlorothiazide

S.No	Parameter	Irbesartan				
		Concentration (ng / spot)	Peak area*	% Amount found*	SD	%RSD
1	**Day - I**		4853.46	98.68		
2	**Day - II**	1200	4861.93	98.79	**0.3944**	**0.3992**
3	**Day - III**		4911.0	99.85		
S.No	**Parameter**	**Hydrochlorothiazide**				
1	**Day - I**		1537.35	99.68		
2	**Day - II**	100	1527.16	99.68	**0.8067**	**0.8092**
3	**Day - III**		1527.23	99.67		

*Mean of six determinations

Accuracy

Excellent recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80%, 100% and 120% level) indicated the mean recovery between 99.32 % to 101.21 % and 98.19 % to 100.87 for irbesartan and hydrochlorothiazide respectively (Table: 6 and 7)

Limit of detection

The LOD as calculated by standard formula as given in ICH guidelines was found to be 7.8697 ng / spot and 0.3412 ng / spot for irbesartan and hydrochlorothiazide respectively (Table: 8)

Limit of quantitation

The LOQ as calculated by standard formula as given in ICH guidelines was found to be 23.8475 ng / spot and 1.0341ng / spot for irbesartan and hydrochlorothiazide respectively (Table: 8)

Robustness

The result of robustness it was observed that the %RSD were less than 2. This indicates that the developed method is robust (Table: 9 and 10)

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts (Table: 11)

Table No. 06: Accuracy Data of Irbesartan

Parameters	Amount present (ng/spot)	Amount added (ng/spot)	Peak area	Amount found (ng/spot)	Amount recovered (ng/spot)	% Amount recovered
			8860.7	2154.0	958.50	99.89
80 %	1200	960	8872.2	2156.80	961.30	100.18
			8843.5	2149.82	954.32	99.45
			9882.5	2402.32	1206.82	101.21
100%	1200	1200	9863.2	2397.70	1202.20	100.83
			9872.7	2400.01	1204.51	101.02
			10805.5	2626.77	1431.27	99.42
120%	1200	1440	10799.6	2625.34	1429.84	99.32
			10827.5	2632.12	1436.62	99.79
					Average	**100.12**
					SD	**0.7286**
					%RSD	**0.7277**
					SE	**0.2429**
				CI (Confidence Interval 99%)		**98.98 – 101.25**

Table No. 07: Accuracy Data of Hydrochlorothiazide

Parameters	Amount present (ng/spot)	Amount added (ng/spot)	Peak area	Amount found (ng/spot)	Amount recovered (ng/spot)	% Amount recovered
			2744.2	180.34	79.65	100.08
80 %	100	80	2721.7	178.87	78.18	98.19
			2738.4	179.89	79.20	99.47
			3048.5	200.34	99.65	99.95
100%	100	100	3062.3	201.25	100.56	100.87
			3044.5	200.08	99.39	99.69
			3348.5	220.06	119.37	99.10
120%	100	120	3362.7	220.99	120.30	99.87
			3356.1	220.56	119.87	99.51
					Average	**99.63**
					SD	**0.7330**
					%RSD	**0.7357**
					SE	**0.2443**
				CI (Confidence Interval 99%)		**98.49 – 100.76**

Table No. 08: LOD and LOQ Data of Irbesartan and Hydrochlorothiazide

S.No	Irbesartan		Hydrochlorothiazide	
	Slope	**Y-Intercept**	**Slope**	**Y-Intercept**
1	3.6966	492.2	15.3523	9.12
2	3.6940	497.51	15.3491	6.9
3	3.7167	480.49	15.3538	8.4066
4	3.6970	499.49	15.3491	8
5	3.6991	493.13	15.3474	4.9
6	3.7275	478.21	15.3474	6
Average	**3.7051**	**490.1744**	**15.3498**	**7.2211**
SD		**8.835**		**1.5874**
	LOD (ng/spot)	**7.8697**	**LOD (ng/spot)**	**0.3412**
	LOQ (ng/spot)	**23.8475**	**LOQ (ng/spot)**	**1.0341**

Table No. 09: Robustness Data of Irbesartan

Parameters	R_f value*	Mean area*	% Amount found*	SD	%RSD
nm plus (261)	0.05	4928.6	99.81	**0.4259**	**0.4267**
nm minus (257)	0.05	4892.4	99.86	**0.2445**	**0.2449**
Chamber saturation (28 minutes)	0.06	4852.9	99.55	**0.5756**	**0.5782**
Chamber saturation (32 minutes)	0.05	4912.5	100.11	**0.1528**	**0.1526**
Migration time (13 minutes)	0.05	4946.3	99.95	**0.2171**	**0.2172**
Migration time (17 minutes)	0.05	4899.5	100.30	**0.3158**	**0.3149**

*Mean of six determinations

Table No. 10: Robustness Data of Hydrochlorothiazide

Parameters	R_f value*	Mean area*	% Amount found*	SD	%RSD
nm plus (261)	0.35	1548.6	100.84	**0.1472**	**0.1460**
nm minus (257)	0.35	1528.5	99.67	**0.3742**	**0.3755**
Chamber saturation (28 minutes)	0.35	1530.9	99.98	**0.2410**	**0.2410**
Chamber saturation (32 minutes)	0.37	1546.8	100.51	**0.2322**	**0.2310**
Migration time (13 minutes)	0.35	1525.4	99.94	**0.2855**	**0.2856**
Migration time (17 minutes)	0.37	1549.6	100.99	**0.1471**	**0.1456**

*Mean of six determinations

Table No. 11: Ruggedness Data of Irbesartan and Hydrochlorothiazide

S.No	Parameters	Irbesartan				
		Concentration (ng/spot)	**Peak area***	**% Amount found***	**SD**	**%RSD**
1	Different Analyst	1200	4969.0	99.25	**0.6695**	**0.6698**
S.No	**Parameters**	**Hydrochlorothiazide**				
		Concentration (ng/spot	**Peak area***	**% Amount found***	**SD**	**%RSD**
1	Different Analyst	100	1542.8	100.09	**0.3623**	**0.3619**

Table No. 12: Summary of linear regression and validation data

S.No	Parameters	Irbesartan	Hydrochlorothiazide
1	R_f value	0.05	0.35
2	Linearity range (ng / spot)	300-1800	25-150
3	Correlation Coefficient	0.9979	0.9986
4	Slop	3.7051	15.3498
5	Intercept	490.1744	7.2211
6	LOD (ng / spot)	7.8697	0.3412
7	LOQ (ng / spot)	23.8475	1.0341
8	Robustness (% RSD)	Within the limit	Within the limit
9	Ruggedness (% RSD)	0.6698	0.3619

Degradation behavior

HPTLC studies on irbesartan and hydro-chlorothiazide under different stress conditions suggested following degradation behavior.

Hydrolytic studies

Acidic condition

Drugs, irbesartan and hydrochlorothiazide showed negligible degradation under acidic hydrolysis with reflux condition. New peaks were observed for product of irbesartan and hydrochlorothiazide and drug peak area remained almost constant. Irbesartan and hydrochlorothiazide showed 8.96 % and 5.13 % degradation upon treatment with 1N HCl respectively. The extra peaks were eluted at the R_f value of 0.05, 0.13, 0.15, 0.17 and 0.27.

Alkaline condition

Drugs, irbesartan and hydrochlorothiazide showed negligible degradation under alkaline hydrolysis with reflux condition. New peaks were observed for product of irbesartan and hydrochlorothiazide and drug peak area remained almost constant. Irbesartan and hydrochlorothiazide showed 9.39 % and 7.26 % degradation upon treatment with 1N NaOH respectively. The extra peaks were eluted at the R_f value of 0.05, 0.13, 0.18, 0.26 and 0.44.

Neutral condition

Drugs, irbesartan and hydrochlorothiazide showed negligible degradation under neutral hydrolysis with reflux condition. New peaks were observed for product of irbesartan and hydrochlorothiazide and drug peak area remained almost constant. Irbesartan and hydrochlorothiazide showed 0.48 % and 0.49 % degradation respectively. The extra peak was eluted at the R_f value of 0.33.

Oxidative condition

Drugs, irbesartan showed 9.55 % degradation and hydrochlorothiazide showed 9.07 % degradation upon treatment with 3 % H_2O_2 with reflux condition. New peaks were observed for product of irbesartan and hydrochlorothiazide and drug peak area remained almost constant. The extra peaks were eluted at the R_f value of 0.05, 0.13 and 0.18.

Thermal and photolytic condition

Under dry heat (80^0C for 48 hours) and photolytic studies, new additional peaks were observed for product of irbesartan and hydrochlorothiazide and drug peak area remained almost the same. This indicates stability of the drugs upon exposure to dry heat exposed and to UV light for specified period. The forced degradation study results are summarized in Table 13 and 14.

Table No. 13: Forced Degradation Study Data of Irbesartan

Parameters	Degradation time	Peak area*	% Degradation	% of Active drug present after degradation
Control sample	-	4962.3	-	-
Neutral degradation	30 minutes	4938.1	0.48	100.42
Acidic degradation	30 minutes	4521.9	8.96	91.94
Alkaline degradation	30 minutes	4501.6	9.39	91.51
Oxidative degradation	30 minutes	4492.8	9.55	91.37
Thermal degradation	48 hours	4796.4	3.31	97.59
Photolytic degradation	7 days	4813.2	3.04	97.86

*Mean of six determinations

Table No. 14: Forced Degradation Study Data of Hydrochlorothiazide

Parameters	Degradation time	Peak area*	% Degradation	% of Active drug present after degradation
Control sample	-	1526.8	-	-
Neutral degradation	30 minutes	1519.3	0.49	99.72
Acidic degradation	30 minutes	1448.5	5.13	95.07
Alkaline degradation	30 minutes	1416.2	7.26	92.95
Oxidative degradation	30 minutes	1388.6	9.07	91.14
Thermal degradation	48 hours	1470.4	3.69	96.51
Photolytic degradation	7 days	1511.9	2.61	97.59

*Mean of six determinations

Conclusions

From the above study, we can conclude that the irbesartan and hydrochlorothiazide 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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Disclosure of interest

The authors that they have no conflicts of interest concerning this article.

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