ORIGINAL ARTICLE

Development of a New Validated Stability Indicating RP-HPLC Method for the Determination of Irbesartan and Hydrochlorothiazide

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Abstract

Introduction: Irbesartan (IRB) is an angiotensin receptor blocker used mainly for the treatment of hypertension. Hydrochlorothiazide (HCTZ) is a first-line diuretic drug of thiazide class which acts by lowering the blood pressure initially by increasing sodium and water excretion. A simple, precise, accurate, and robust stability-indicating liquid chromatographic method (gradient mode) was developed and validated for the simultaneous determination of IRB and HCTZ in pharmaceutical dosage forms (Tablets). **Materials and Methods:** Chromatographic separation was achieved on an X Bridge shield RP C-18 (150 mm × 3.0 mm, 3.5 µm) column of Waters high-performance liquid chromatography with Empower2 software and photodiode array detector, maintained at 45°C using 0.1% v/v formic acid:acetonitrile (40:60, v/v), with flow rate 0.8 ml/min (ultraviolet detection at 235 nm). **Results and Discussion:** HCTZ and IRB obey Beer–Lambert's law over the concentration range of 2–600 µg/ml and 1.2–560 µg/ml, respectively, with regression equations y = 10669x −2468 (HCTZ) (R^2 = 0.9999) and y = 18222x + 18180 (IRB) (R^2 = 0.9997). The limit of quantification (LOQ) and limit of detection (LOD) for IRB were found to be 1.056 µg/ml and 0.349 µg/ml, respectively, whereas the LOQ and LOD for HCTZ were found to be 1.980 µg/ml and 0.654 µg/ml, respectively. IRB and HCTZ were subjected to stress conditions of acidic, alkaline, and oxidation degradations. The forced degradation studies were performed using HCl, NaOH, and H_2O_2. **Conclusions:** HCTZ and IRB are slightly sensitive toward alkaline conditions in comparison to other degradations. The method was validated as per ICH guidelines.

Key words: Hydrochlorothiazide, irbesartan, reversed-phase high-performance liquid chromatography, stability indicating, validation

INTRODUCTION

Chemically, irbesartan (IRB) is 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.[1] Angiotensin II receptor antagonists represent a relatively new pharmacological class which acts mainly by selective blockade of AT1 receptors and reduces the effects of angiotensin II.[2] They may be used alone or in combination with other antihypertensive or diuretic agents. IRB [Figure 1a] is an angiotensin receptor blocker used mainly for the treatment of hypertension. Hydrochlorothiazide (HCTZ) [Figure 1b] is chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7 sulfonamide which is a first-line diuretic drug of the thiazide class.[3] It acts by lowering blood pressure initially by increasing sodium and water excretion.

Literature survey revealed that there are a number of high-performance thin-layer chromatography (HPTLC)[4-7] spectrophotometric[8] and spectrofluorometric,[9,10] voltammetric,[11] capillary zone electrophoretic,[12] HPLC,[13-15] and liquid chromatography–mass spectrometry[16-17] methods

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for the determination of irbesartan and HCTZ individually or in combination. In the present study, a simple, robust, precise, and accurate RP-HPLC method (gradient mode) has been developed for the simultaneous determination of HCTZ and IRB and validated as per ICH guidelines.[18] The forced degradation studies were also conducted as per ICH guidelines.[19]

MATERIALS AND METHODS

Instrumentation and chromatographic conditions

Liquid chromatographic separation was achieved using an X Bridge shield RP C-18 (150 mm × 3.0 mm, 3.5 µm) column of Waters HPLC with Empower2 software and photodiode array detector, maintained at 45°C. Gradient mode elution was performed using acetonitrile (ACN) and 0.1% v/v formic acid. The overall run time was 20 min, and the flow rate of the mobile phase was 0.8 mL/min. The wavelength of the PDA detector was set at 235 nm. 5 µl of sample was injected into the HPLC system.

Chemicals and reagents

IRB standard (purity 99.0%) and HCTZ standard (purity 99.0%) are obtained from SUN Pharma (India). ACN (HPLC grade), formic acid, HCl, NaOH, and hydrogen peroxide (30% w/w) solution were purchased from Merck (India), and other chemicals and solvents used were of analytical grade. Water (HPLC grade) was obtained from Milli-Q RO system. The combination of IRB and HCTZ is available as tablets commercially with brand names IROVEL-H® and XARD-H® (IRB 150 mg and HCTZ 12.5 mg).

IRB (12,000 µg/ml) and HCTZ (250 µg/ml) stock solutions were prepared by accurately weighing 1200 mg of IRB and 25 mg of HCTZ in a 100 ml volumetric flask with mobile phase. Working standard solutions were prepared on daily basis from the stock solutions by dilution with mobile phase, and the solutions were filtered through 0.45 µm membrane filter before injection. 1 mL of formic acid (HPLC grade) was diluted with water in a 1000 ml volumetric flask (0.1% v/v) and sonicated to dissolve completely and filtered through 0.45 µm filter.

Method development and optimization

For selection of column, a spiked sample of IRB was prepared with HCTZ and injected HPLC system with different columns. The required system suitability criterion was obtained using X Bridge Shield RP-18 (150 × 3.0mm), 3.5 µm column.

Method validation

Linearity

A series of solutions were prepared from by diluting the stock solutions of HCTZ (2–600 µg/ml) and IRB (1.2–560 µg/ml) with mobile phase. 5 µl of mixture of these solutions was injected into the HPLC system, and the peak area of each of the drug was noted from the chromatogram. A graph was drawn by taking the concentration of the drug solution on the x-axis and the corresponding peak area values on the y-axis.

Precision

The intraday precision of the assay method was evaluated by carrying out six independent assays of test samples of IRB and HCTZ (IRB 165 µg/ml and HCTZ 500 µg/ml) against a qualified reference standard and the % relative standard deviation (RSD) was calculated.

The interday precision study was performed on different days (n = 3) (IRB 165 µg/ml and HCTZ 500 µg/ml) and the % RSD was calculated.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100, and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of HCTZ and IRB, respectively, in the drug product and the % RSD was calculated.

Robustness

The robustness of the assay method was established by introducing small deliberate changes in the HPLC conditions which included flow rate (0.72 and 0.88 ml/min), percentage of ACN in the mobile phase (absolute ±2% composition), and column oven temperature (±5°C). Robustness of the method was studied using five replicates of IRB (6000 µg/ml) and HCTZ (500 µg/ml).

Analysis of commercial formulations

Twenty tablets of two different brands containing Irbesartan and Hydrochlorothiazide were procured from the local medical store and powder equivalent to 150 mg Irbesartan and 12.5 mg Hydrochlorothiazide was accurately weighed

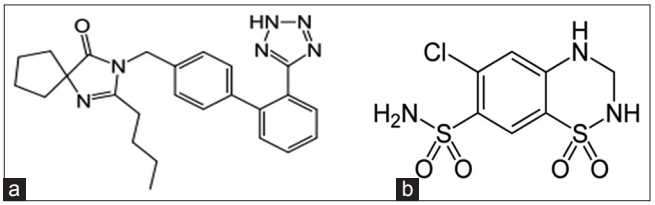

Figure 1: Chemical structure of (a) irbesartan and (b) hydrochlorothiazide

and transferred to a 50 ml volumetric flask. The contents were thoroughly sonicated with acetonitrile and filtered through 0.45 µm filter. The extracted solution was further diluted with mobile phase as per the requirement and 5 µl of each brand was injected into the HPLC system and the peak areas of the two drugs were noted from the chromatogram obtained and the percentage recovery was calculated from the linear regression equation.

Forced degradation studies

Forced degradation studies were intended to ensure the effective separation of IRB and HCTZ and their degradation peaks of formulation ingredients at the retention time of IRB and HCTZ , respectively. Forced degradation studies were performed with 560 µg/ml of IRB and 60 µg/ml of HCTZ .

Acidic degradation

The combined formulation of IRB and HCTZ was treated with 1 N HCl and refluxed for 2 h in thermostat maintained at 80°C. The stressed sample was cooled, neutralized, and diluted with mobile phase as per the requirement and filtered through 0.45 µm filter. 5 µL of this solution was injected into the HPLC system, and the peak area of the chromatograms was noted, and the corresponding peak purity plots were also recorded.

Alkaline degradation

The combined formulation of IRB and HCTZ was treated with 0.1 N NaOH and refluxed for 2 h in thermostat maintained at 80°C. The stressed sample was cooled, neutralized, and diluted with mobile phase as per the requirement and filtered through 0.45 µm filter. 5 µL of this solution was injected into the HPLC system, and the peak area of the chromatograms was noted, and the corresponding peak purity plots were also recorded.

Oxidative degradation

The combined formulation of IRB and HCTZ was treated with 1% H_2O_2 and refluxed for 2 h in thermostat maintained at 80°C. The stressed sample was cooled and diluted with mobile phase as per the requirement and filtered through 0.45 µm filter. 5 µL of this solution was injected into the HPLC system, and the peak area of the chromatograms was noted, and the corresponding peak purity plots were also recorded.

RESULTS AND DISCUSSION

A simple, precise, accurate, and robust stability-indicating liquid chromatographic method (gradient mode) was developed and validated for the simultaneous determination of IRB and HCTZ in pharmaceutical dosage forms (Tablets). Chromatographic separation was achieved on an X Bridge shield RP C-18 (150 mm × 3.0 mm, 3.5 µm) column of Waters HPLC with Empower2 software and photodiode array detector, maintained at 45 ºC using 0.1% v/v formic acid:ACN (40:60, v/v), with flow rate 0.8 ml/min with ultraviolet detection at 235 nm.

The typical chromatograms of blank, HCTZ , and IRB were shown in Figure 2a and 2b where the HCTZ was eluted at 2.874 min and IRB at 9.439 min. The corresponding peak purity plots were shown in Figure 2c and 2d.

Beer–Lambert's law was obeyed over the concentration range of 2–600 µg/ml and 1.2–560 µg/ml [Table 1] with regression equations y = 10669x-2468 (R^2 = 0.9999) and y = 18222x + 18180 (R^2 = 0.9997) for HCTZ and IRB, respectively [Figure 3]. The limit of quantification (LOQ) and limit of detection (LOD) for IRB were found to be 1.056 µg/ml and 0.349 µg/ml, respectively, whereas the LOQ and LOD for HCTZ were found to be 1.980 µg/ml and 0.654 µg/ml, respectively.

The method is more precise as the % RSD was found to be 0.16–0.32 and 0.19–0.32 for intraday and interday precision studies, respectively, for HCTZ [Table 2] and the % RSD was found to be 0.14–0.26 and 0.19–0.32 for intraday and interday precision studies, respectively, for IRB [Table 3] (RSD <2). The % RSD in accuracy studies was found to be 0.65–1.09 (RSD <2) with percentage recovery 99.07–100.11 for HCTZ [Table 4] and 0.02–0.40 (RSD <2) with percentage recovery 98.87–99.33 for IRB [Table 5], respectively. The method is more robust as the % RSD was found to be 0.15–0.78 and 0.13–0.78 for HCTZ and IRB, respectively [Table 6]. The proposed method was applied for the determination of HCTZ and IRB tablets, and the percentage recovery was found to be 99.40–99.44 and 98.64–100.08, respectively.

The system suitability tests were performed to ensure that the complete testing system was suitable for the intended application. The tailing factor was (1.19 for HCTZ and 0.94 for IRB) <1.5–2 or <2, and the theoretical plates were >2000 for both HCTZ and IRB.

Forced degradation studies

Acidic degradation

The chromatogram shows peaks at 2.872 min and 9.345 min indicating the elution of HCTZ and IRB, respectively. On heating with 1 N HCl for 2 h, HCTZ (2.24%) and IRB (1.60%) have undergone decomposition very slightly, and therefore, it is concluded that HCTZ and IRB highly stable. The purity angle (0.198) was less than the purity threshold (1.016) indicating that IRB indicating that there is no interference. Similarly, the purity angle (0.019) was less than the purity threshold (1.017) for HCTZ.

Alkaline degradation

The chromatogram shows peaks at 2.867 min and 9.352 min indicating the elution of HCTZ and IRB,

Table 1: Linearity of IRB and HCTZ

HCTZ		IRB	
Conc. (µg/ml)	***Mean peak area**	**Conc. (µg/ml)**	***Mean peak area**
2.000	19856	1.202	21698
20.004	226981	2.403	44989
40.008	423754	12.017	221012
60.012	646789	24.034	436849
120.024	1280496	72.101	1300648
200.040	2134896	120.168	2193461
275.055	2908564	165.231	3069987
350.070	3709784	240.336	4476849
400.080	4269872	300.420	5593652
500.100	5304698	375.525	6794565
550.34	5838775	500.45	9222758
600.120	6469873	560.00	10098993

*Mean of three replicates HCTZ: Hydrochlorothiazide, IRB: Irbesartan

Table 2: Precision studies of HCTZ

HCTZ			
Intraday		**Interday**	
***Mean peak area±SD**	**RSD (%)**	***Mean peak area±SD**	**RSD (%)**
5316987±8507.179	0.16	5312649±12219.093	0.23
5309974±15398.925	0.29	5308746±13802.740	0.26
5326587±17045.078	0.32	5316452±15417.711	0.29
5301689±13784.391	0.26	5306996±16982.387	0.32
5369481±11812.858	0.22	5301698±11133.566	0.21
5298745±13776.737	0.26	5298746±10067.617	0.19

*Mean of three replicates. HCTZ: Hydrochlorothiazide, RSD: Relative standard deviation, SD: Standard deviation

Table 3: Precision studies of IRB

IRB			
Intraday		**Interday**	
***Mean peak area±SD**	**% RSD (%)**	***Mean peak area±SD**	**RSD (%)**
2986234±7764.208	0.26	2998746±7796.740	0.26
2976495±5655.341	0.19	2988759±7471.898	0.25
2968746±6828.116	0.23	3016549±7843.027	0.26
2988745±6575.239	0.22	2998745±8696.361	0.29
2994395±4192.153	0.14	2984687±5670.905	0.19
2976843±7739.792	0.26	2998745±9595.984	0.32

*Mean of three replicates. IRB: Irbesartan, RSD: Relative standard deviation, SD: Standard deviation

respectively. On heating with 0.1 N NaOH for 2 h, only 5.42% of IRB has undergone alkaline degradation and 5.91% of HCTZ was decomposed. The purity angle (0.226) for IRB was less than the purity threshold (1.017) indicating that no interference of degradants. Similarly, the purity angle (0.098) was less than the purity threshold (1.008) for HCTZ.

Oxidative degradation

The chromatogram shows peaks at 2.863 min and 9.352 min indicating the elution of HCT and IRB, respectively. On refluxation with hydrogen peroxide, about 1.75% of IRB and 1.56% of HCTZ have undergone oxidative degradation without degradants. The purity angle (0.319) was less than

Table 4: Accuracy studies of HCTZ

Drug added (µg/ml)	Drug recovered (µg/ml)	% Recovery	Statistical data
10.653	10.579	99.31	Mean=99.78
10.857	10.748	99.00	SD=1.09
10.662	10.771	101.02	% RSD=1.09
12.571	12.545	99.79	Mean=99.07
12.758	12.669	99.30	SD=0.85
12.912	12.671	98.13	% RSD=0.86
15.538	15.650	100.72	Mean=100.11
15.395	15.422	100.18	SD=0.65
15.558	15.470	99.43	% RSD=0.65

HCTZ: Hydrochlorothiazide, RSD: Relative standard deviation, SD: Standard deviation

Table 5: Accuracy studies of IRB

Drug added (µg/ml)	Drug recovered (µg/ml)	% Recovery	Statistical data
120.58	118.72	98.46	Mean=98.87
120.72	119.58	99.06	SD=0.36
121.22	120.13	99.10	% RSD=0.36
151.17	149.73	99.05	Mean=99.06
151.05	149.62	99.05	SD=0.02
150.68	149.31	99.09	% RSD=0.02
180.12	179.74	99.79	Mean=99.33
180.99	179.35	99.09	SD=0.40
181.09	179.49	99.12	% RSD=0.4

RSD: Relative standard deviation, SD: Standard deviation

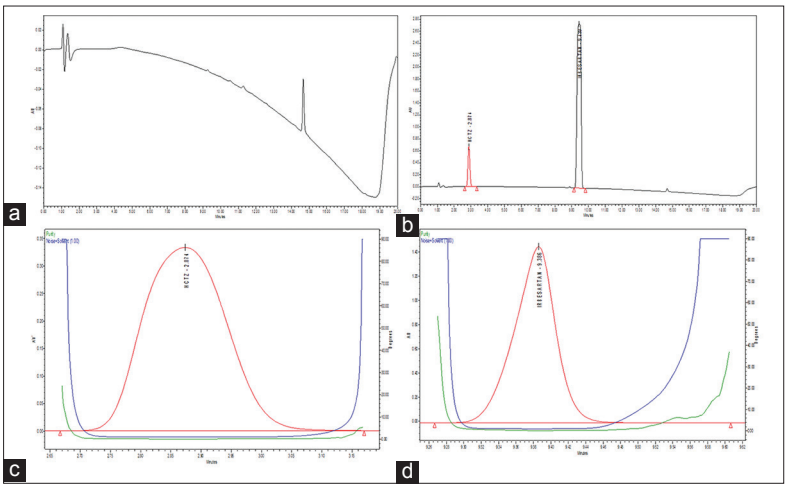

Figure 2: Typical chromatograms of (a) blank (b) hydrochlorothiazide (HCTZ) (500 µg/ml) and irbesartan (IRB) (6000 µg/ml) and peak purity plots of (c) HCTZ (d) IRB

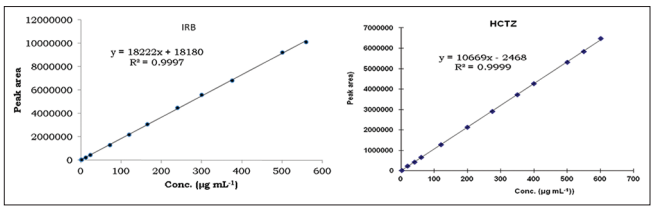

Figure 3: Calibration curves of and Irbesartan and Hydrochlorothiazide HCTZ

the purity threshold (1.016) indicating that IRB peak was well separated, and similarly, the purity angle (0.330) was less than the purity threshold (1.007) for HCTZ.

The typical chromatograms [Figure 4] and the results of forced degradation studies were shown in Table 7. IRB and HCTZ are more resistant toward acidic and oxidation degradations in comparison to alkaline stressed conditions.

Table 6: Robustness study

Conditions	Para meter	System suitability					
		HCTZ			IRB		
		Tailing factor	Theoretical plates	%RSD	Tailing factor	Theoretical plates	%RSD
Flow rate (± 0.08, mL/min)	0.72	1.16	2662	0.62	0.94	46684	0.13
	0.88	1.04	2979	0.78	0.96	49875	0.52
ACN: formic acid (± 2%, v/v)	58:42	1.12	2699	0.19	1.01	50454	0.16
	62:38	1.07	2836	0.54	1.06	50987	0.36
Column oven temperature (± 5°C)	40°C	1.11	2864	0.15	0.98	49874	0.44
	50°C	1.09	2861	0.36	0.99	50469	0.78

*Mean of three replicates. HCTZ: Hydrochlorothiazide, RSD: Relative standard deviation, SD: Standard deviation, IRB: Irbesartan, ACN: Acetonitrile

Table 7: Forced degradation studies of HCTZ and IRB

Stress conditions	*Mean peak area		*Drug recovered (%)		*Drug decomposed (%)		Purity angle		Purity threshold	
	HCTZ	IRB	HCTZ	IRB	HCTZ	IRB	HCTZ	IRB	HCTZ	IRB
Untreated	610329	9980882	100	100	-	-	0.096	0.213	1.008	1.018
Acidic degradation	596664	9820831	97.76	98.40	2.24	1.60	0.019	0.198	1.017	1.016
Alkaline degradation	574272	9439774	94.09	94.58	5.91	5.42	0.098	0.226	1.008	1.017
Oxidative degradation	600789	9806219	98.44	98.25	1.56	1.75	0.330	0.319	1.007	1.016

*Mean of three replicates. HCTZ: Hydrochlorothiazide, RSD: Relative standard deviation, SD: Standard deviation, IRB: Irbesartan

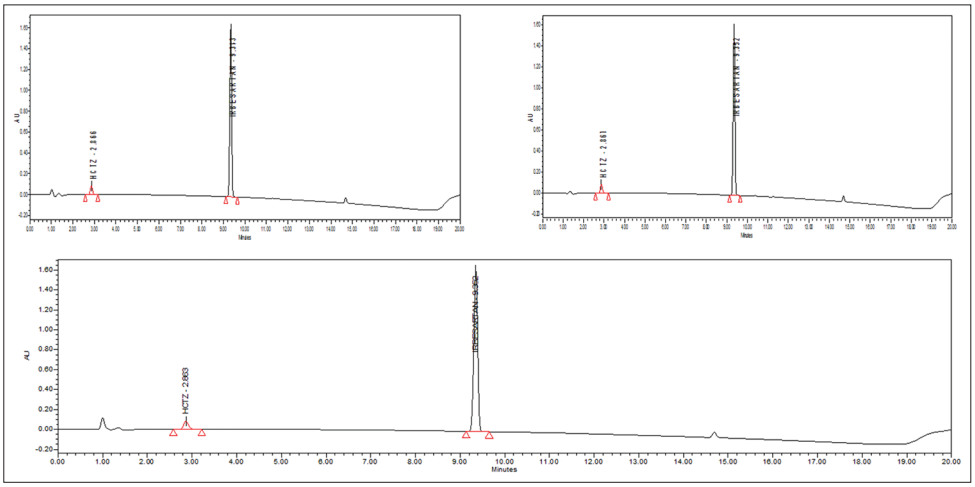

Figure 4: Typical chromatograms of hydrochlorothiazide (60 µg/ml) and IRB (560 µg/ml) during forced degradation studies, (a) Acidic degradation, (b) Alkaline degradation, (c) Oxidative degradation

CONCLUSION

The present developed stability-indicating RP-HPLC method was simple, specific, precise, accurate, and robust, and therefore, it can be applied for the pharmacokinetic studies as well as for the determination of HCTZ and IRB in pharmaceutical dosage forms. IRB and HCTZ are more resistant toward acidic and oxidation degradations in comparison to alkaline stressed conditions.

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