Stability Indicating LC Method for Simultaneous Determination of Irbesartan and Hydrochlorothiazide in Pharmaceutical Preparations

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Abstract

A simple and precise stability-indicating liquid chromatography method is developed and validated for the quantitative simultaneous estimation of irbesartan (IRB) and hydrochlorothiazide (HCTZ) in combined pharmaceutical dosage form. A chromatographic separation of the two drugs was achieved with an Ace5 C_{18} 25-cm analytical column using buffer–acetonitrile (70:30 v/v). The buffer used in mobile phase contains 50 mM ammonium acetate pH adjusted 5.5 with acetic acid. The instrumental settings are flow rate of 1.5 mL/min, column temperature at 30°C, and detector wavelength of 235 nm using a photodiode array detector. IRB, HCTZ, and their combination drug products were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. Peak homogeneity data of IRB and HCTZ is obtained using photodiode array detector. In the stressed sample chromatograms, it demonstrated the specificity of the assay method for their estimation in presence of degradation products. The described method shows excellent linearity over a range of 10–200 µg/mL for IRB and 5–100 µg/mL for HCTZ. Methylparaben was used as internal standard. The correlation coefficient for IRB and HCTZ are 0.998 and 0.999. The mean recovery values for IRB and HCTZ ranged from 100.45% to 101.25%. The limit of detection for IRB and HCTZ were 0.019 and 0.023 µg/mL, respectively, and the limit of quantification were 0.053 and 0.070 µg/mL, respectively. The proposed method was suitable for quantitative determination and stability study of IRB and HCTZ in pharmaceutical preparations and also can be used in the quality control of bulk manufacturing and pharmaceutical dosage forms.

Introduction

Irbesartan (IRB), anonpeptide molecule (Figure 1) is chemically (2-Butyl-3 [[2'-(1H-tetrazole-5-yl)[1,1'-biphenyl]-4-yl] methyl]-1,3-diazaspiro [4.4] non-1-en-4-one), and hydrochlorothiazide (HCTZ) is chemically (6-Chloro-3, 4-dihydro-^{2}H-1, 2,4-benzothiadiazine-7sulfonamide 1,1-dioxide) diuretic. IRB is an angiotensin II Type 1(All_1)-receptor antagonist that is highly selective for Type 1 angiotensin II receptor. Angiotensin II is principle pressor agent of the rennin angiotensin system with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorbtion of sodium (1). Thiazide affects the renal tubular mechanisms of electrolytes reabsorbtion, directly increasing excretion of sodium salt and chloride in approximately equivalent amounts. The combination is useful in the treatment of mild-to-moderate hypertension, well-tolerated with a lower incidence of cough than ACE inhibitors. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin II, a vasoconstrictor substance. An IRB and HCTZ combination is used to lower blood pressure. IRB controls high blood pressure (hypertension) by relaxing blood vessels. IRB plus HCTZ is greater blood pressure lowering action than IRB alone (2).

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The assay of drug product in stability test sample needs to be determined using stability-indicating method, as recommended by the international Conference on Harmonization (ICH) (3). Although stability-

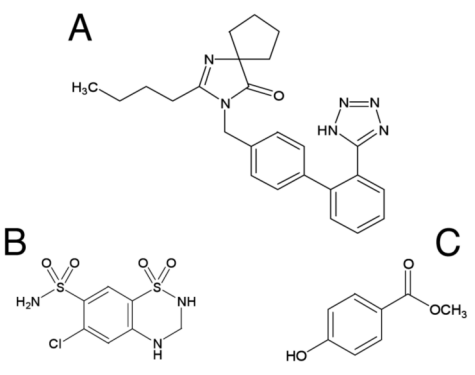

Figure 1. The structure of IRB (A), HCTZ (B), and methylparaben (C).

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indicating methods have been reported for assay of various drugs in drug products containing only one active drug substance, only few stability-indicating methods are reported for assay of combination drug products containing two or more active drug substances (4–6). The objective of this work was to develop a simple analytical liquid chromatography procedure, which would serve as stability indicating assay method for combination drug products of IRB and HCTZ.

The literature survey reveals that several methods were reported for the individual estimation of IRB and HCTZ. The methods (7–11) for IRB in combination with other drugs in plasma and serum are done by high-performance liquid chromatography (HPLC), and methods (12–19) for the estimation of HCTZ in combination with other drugs in plasma and serum are done by HPLC. However, there is only one method reported for the simultaneous determination of the IRB and HCTZ in human plasma (20). None of the reported analytical procedures describe a method for simultaneous determination of the IRB and HCTZ in combined pharmaceutical dosage form in presence of their degradation products.

In the present study, attempts were made to develop a simple, precise, and accurate method for the simultaneous estimation of the ingredients of this combination in presence of their degradation products. This manuscript describes the development and validation of a stability-indicating isocratic reversed-phase HPLC method for simultaneous determination of IRB and HCTZ in the presence of their degradation products as per ICH guidelines.

Experimental

Chemicals and reagents

IRB and HCTZ standards were obtained from Sun Pharmaceutical Industries (Mumbai, India). Acetic acid and acetonitrile (HPLC-grade) were obtained from Merck Fine Chemicals (Mumbai, India). Ammonium acetate, sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were from Qualigens Fine Chemicals (Glaxo Ltd. Mumbai, India). The 0.45-µm pump nylon filter was obtained from Advanced Microdevices (Ambala Cantt, India). The (Iroval-H) tablets (Sun Pharmaceutical) of the combination of IRB and HCTZ were purchased commercially. Double-distilled water was used throughout the experiment. Other chemicals used were analytical- or HPLC-grade.

HPLC Instrumentation conditions

A Shimadzu HPLC system (Kyoto, Japan) consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven and photodiode array detector, and 10A-VP series with LC-10 software. Ace5 C_{18} (4.6 × 250 mm, i.d. column, 5 µm) (Advance Chromatography, Johns Island, SC), maintained at 30°C using column oven, eluted with mobile phase at the flow rate of 1.5 mL/min. The mobile phase consisted of buffer–acetonitrile (70:30 v/v). The buffer used in mobile phase contains 50 mM ammonium acetate pH adjusted 5.5 with acetic acid, filtered through 0.45-µm nylon filter, and degassed in ultrasonic bath prior to use. Measurements were made with injection volume 10 µL and UV detection at 235 nm. For analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 200–400 nm. The peak homogeneity was expressed in terms of peak purity and was obtained directly from the spectral analysis report obtained using the previously mentioned software.

Standard and sample solution preparations

Standard stock solutions

Standard solutions were prepared by dissolving the drug in the diluents and diluting them to the desired concentration. Diluent A was composed of methanol and acetonitrile in the ratio of 50:50, and diluent B was composed of water and acetonitrile in the ratio of 50:50 (v/v).

IRB

A 50 mg sample of IRB (99.87%) was accurately weighed, transferred into a 50-mL volumetric flask, and dissolved with diluent A.

HCTZ

A 25 mg sample of hydrochlorothiazide (99.56%) was accurately weighed, transferred into a 50-mL volumetric flask, and dissolved with diluent A.

Methylparaben

A 5-mg sample of methylparaben was accurately weighed, transferred in a 25-mL volumetric flask, and dissolved with diluent A.

Mixed standard solution

A mixed standard solution was prepared from these stock solutions by transferring 5 mL of IRB stock solution, 5 mL of HCTZ stock solution, and 5 mL of methylparaben stock solution in a 50-mL volumetric flask and diluted with diluent B. This solution contained 100 µg/mL of IRB, 50 µg/mL of HCTZ, and 20 µg/mL of methylparaben.

Calibration curve solutions

The calibration curve solutions contains 10–200 µg/mL of IRB, 5–100 µg/mL of HCTZ, and 20 µg/mL of methylparaben (IS).

Preparation of sample

Twenty tablets were weighed, their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to 150 mg of IRB and 12.5 mg of HCTZ was transferred in a conical flask. The drug was dissolved in acetonitrile. To ensure complete dissolution of drugs, it was sonicated for 5 min. The extract was filtered and residue was washed with acetonitrile. From the filtered solution, 5 mL of clear solution was transferred into a 50-mL volumetric flask, and 5 mL of internal standard solution was added into it and diluted to volume with diluents B.

Procedure for forced degradation study of drug substances

Forced degradation of each drug substances and the drug product were carried out under thermolytic, photolytic, acid/base hydrolytic, and oxidative stress conditions.

Photolytic degradation

The ICH guideline state the minimum desired exposure as 200 Wh/m^2 , which corresponds to a change in absorbance of 0.5 AU of quinine actinometer at 400 nm. This change was observed in 24 h of irradiation. A second photolytic stress test experiment with greater irradiation time was in 48 h.

Acid degradation

About 10 mg of IRB was accurately weighed and dissolved in 10 mL of diluent A, then 10 mL of 1 N HCl was added. The mixture was kept at room temperature for 48 h, then it was neutralized with 1 N NaOH to pH 7. Then the volume was made up to 50 mL with diluent B.

About 10 mg of HCTZ was accurately weighed and dissolved in 5 mL of diluent A, then 10 mL 1 N HCl was added, and the mixture was kept at room temperature for 48 h. The solution was neutralized with 1 N NaOH to pH 7 and volume made up to 100 mL with diluent B.

Alkali Degradation

About 10 mg of IRB was accurately weighed and dissolved in 10 mL of diluent A, then 10 mL of 1 N NaOH was added, and the mixture was kept at room temperature for 48 h. Then it was neutralized with 1 N HCl to pH 7, and the volume was made up to 50 mL with diluent B.

About 10 mg of HCTZ was accurately weighed and dissolved in 5 mL of diluent A. Then 10 mL 1 N NaOH was added, and the mixture was kept at room temperature for 48 h. The solution was neutralized with 1 N HCl to pH 7 and volume made up to 100 mL with diluent B.

Oxidative degradation

About 10 mg of IRB was accurately weighed and dissolved in 10 mL of diluent A. Then 10 mL of 30% H_2O_2 was added and the mixture kept at room temperature for 48 h. Then the volume was made up to 50 mL with diluent B.

About 10 mg of HCTZ was accurately weighed and dissolved in 5 mL of diluent A, then 10 mL of 30% H_2O_2 was added and the mixture kept at room temperature for 48 h. Then the volume was made up to 100 mL with diluent B.

Thermal degradation

About 50 mg of drug substance kept at 80°C for 48 h. Then the solution was prepared to achieve 100 µg/mL of IRB and 50 µg/mL of HCTZ.

UV degradations

About 50 mg of drug substance was exposed to UV short (254 nm) and UV long (366 nm) light for 48 h. Then, the solution was prepared to achieve 100 µg/mL of IRB and 50 µg/mL of HCTZ.

Procedure for forced degradation study of drug products

A forced degradation study of drug product in acidic, basic, and oxidative conditions were carried out using sonication and filtered solution (as described in "Preparation of sample") to achieve 100 µg/mL of IRB and 50 µg/mL of HCTZ.

For thermolytic and photolytic degradation, a quantity of powder equivalent to one tablet containing 50 mg of IRB and 12.5 mg of HCTZ was exposed. Then, the solutions were prepared as described in "Preparation of sample."

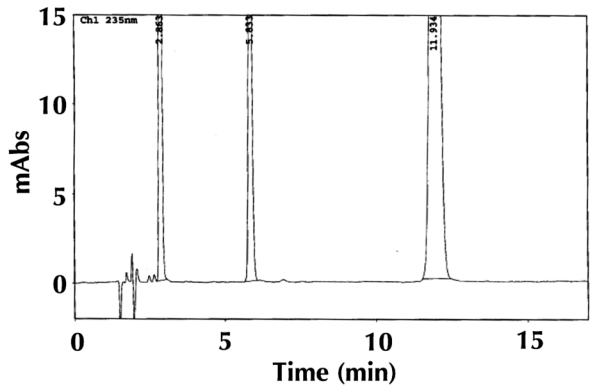

Figure 2. A typical chromatogram of the tablet solution (untreated): HCTZ (2.87 min.), methylparaben (5.8 min), and IRB (11.93 min.).

Results and Discussion

Optimization of chromatographic conditions

The primary target in developing this stability-indicating HPLC method is to achieve the resolution between the IRB, HCTZ and its degradation products. To develop a stability-indicating method different stationary phases (C_{18}, CN) and different mobile phases (containing buffers like phosphate, ammonium acetate, and ammonium formate) with different pH (pH 3–5) and using organic modifier (acetonitrile) were used.

Our objective of the chromatographic method development was to achieve a peak tailing factor less than 2, and resolution between IRB, HCTZ, and degradation products greater than 1.5.

The chromatographic separation was achieved using Ace5 C_{18} column (250 × 4.6 mm i.d.). Resolution between IRB and HCTZ was observed on any C_{18} or CN column, but resolution between both drugs and degradation products was not completely achieved. Ace5 C_{18} column showed better perfor-

Table I. Results of Analysis of Forced Degradation Study Samples Using Proposed Method Indicating (%) Degradation and Peak Purity of IRB and HCTZ

	IRB			HCTZ		
Stress conditions/duration	Degradation (%)	Peak Purity*	Mass Balance Data†	Degradation (%)	Peak Purity*	Mass Balance Data†
Acidic/1N HCl/48 h	3.5	999.924	99.88	14.87	999.864	98.69
Basic/1N NaOH/48 h	12.75	999.508	98.95	7.12	999.827	99.12
Oxidative/30% H_2O_2/48 h	6.95	997.517	99.25	9.1	999.677	98.89
Thermal/80ºC/48 h	–	999.697	99.57	4.21	999.972	99.36
Photo/UV 254 nm/48 h	–	999.857	99.75	–	999.844	98.56
366 nm/48 h	–	999.816	99.59	–	999.998	99.15

* Peak purity values in the range of 990–1000 indicate a homogeneous peak.
† Mass balance (%assay + sum of all impurities + sum of all degradation products).

mance as compared to other columns as high carbon loading 15.5%, surface area 300 m^2/g.

From the development studies, 50 mM ammonium acetate with pH adjusted to 5.5 with mobile phase consisting of acetic acid and acetonitrile in the ratio of 70:30 (v/v) was used. The flow rate of mobile phase was 1.5 mL/min, UV 235 nm, and column temperature at 30°C. The analytes of this combination had proper peak shape, less tailing, and optimum resolution. In optimized conditions IRB, HCTZ, and their degradation products were well-separated. Typical retention times of HCTZ and IRB were about 2.86 and 11.93 min, respectively. Resolution between IRB and HCTZ founds to be more than 20.

Forced degradation study

Singh and Bakshi (21) in their article on stress testing suggested a target degradation of 20–80% for the establishing stability-indicating nature of the assay method as even intermediate degradation products should not interfere with any stage of drug analysis. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 20–80%, this could not be achieved in all degradation conditions even after exposure for prolonged duration. Table I indicates the extent of degradation of IRB and HCTZ under various stress conditions. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 990 indicates a homogeneous peak. The peak purity values for analyte peaks, IRB and HCTZ, were in the range of 999–1000 for degradation product, indicating homogeneous peaks and thus establishing the specificity of assay method. Figure 2 shows the chromatogram of IRB and HCTZ tablet solution. Figure 3 shows chromatogram of acid hydrolysis degradation. Figure 4 shows the chromatogram of base hydrolysis degradation. Figure 5 shows chromatograms of oxidative degradation, and Figure 6 shows chromatograms of heat degradation product (80°C). No other co-eluting peaks were found with the mean peaks suggested the specificity of the method for the simultaneous estimation of IRB and HCTZ in presence of degradation products.

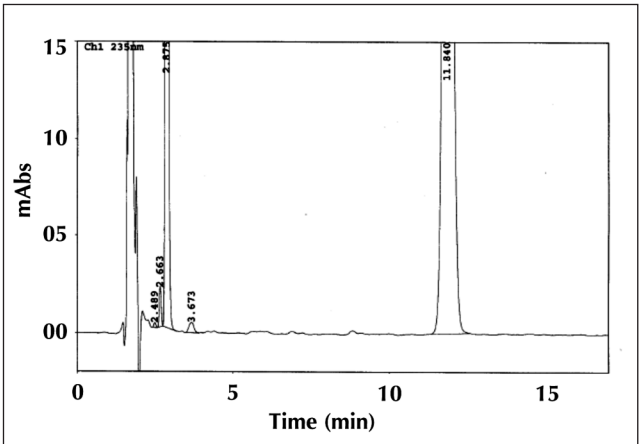

Figure 3. Chromatograms of acidic hydrolysis-degraded IRB, HCTZ, and tablet solution, respectively.

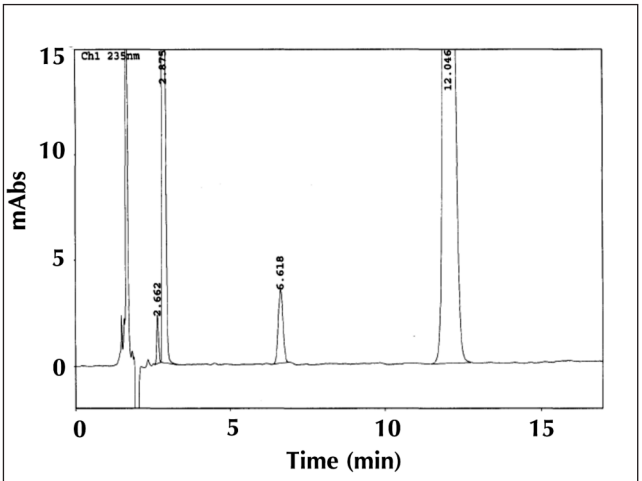

Figure 4. Chromatograms of base hydrolysis-degraded IRB, HCTZ, and tablet solution, respectively.

Method validation

Specificity

The peak purity values were carried out using photodiode array detector. The peak purity was more than 997 for drug substance and drug products at 235 nm, which shows that the peaks of analytes were pure and that formulation excipients and degradation were not interfering with analytes peaks.

The described method was validated with respective specificity, linearity, system suitability, accuracy, robustness, limit of detection (LOD), limit of quantitation (LOQ), and intermediate precision. Characteristics parameters for regression equation and system suitability are given in Table II.

Precision (Repeatability)

The precision of the method was studied by determining the concentration of each drug in the tablets six times. The area % RSD for IRB and HCTZ were 0.24 and 0.58. The results of the precision study indicate that the method is reliable (Table III).

The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts in different day in same laboratory. Results are shown in Table III.

Table II. Regression Characteristics and System Suitability Parameters of Proposed RP-HPLC method

Parameters	IRB	HCTZ
Retention time (min)	2.8	11.1
Tailing factor	1.2	1.1
Resolution	27.5	
Theoretical plates	6694	12823
Linearity range (µg/mL)	10–200	5–100
Limit of detection (LOD) (µg/mL)	0.019	0.023
Limit of quantitation (LOQ) (µg/mL)	0.053	0.070
Regression equation		
Slope (*m*)	1299.9	9694.5
Intercept (*c*)	–1418.3	2029.4
Correlation coefficient (*r*)	0.998	0.999
Method precision (RSD%) (*n* = 6)	0.98	1.15

Accuracy

The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels: 80%, 100%, and 120% of the label claim of the tablet (50 mg of IRB and 12.5 mg of HCTZ). Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The mean recovery values for IRB and HCTZ ranged from 101.25% to 100.97% and 100.52 to 100.78%, respectively, as shown in Table IV.

Table III. Result of Precision of Test Method*

Sample number	Analyst I Intra-day precision		Analyst 2 Inter-day precision	
	IRB	HCTZ	IRB	HCTZ
1	99.25	98.56	99.34	99.23
2	99.29	99.93	98.89	98.12
3	99.42	99.12	99.61	98.69
4	99.82	98.91	99.21	99.79
5	99.22	99.95	99.59	99.95
6	99.54	99.32	99.23	99.12
Mean	99.22	99.36	99.44	99.15
RSD	0.24	0.58	0.27	0.69

* Assay of IRB and HCTZ as % of Labeled Amount.

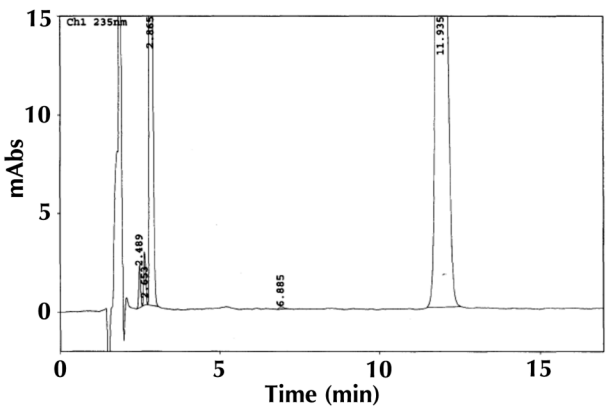

Figure 5. Chromatograms of oxidative-degraded IRB, HCTZ, and tablet solution, respectively.

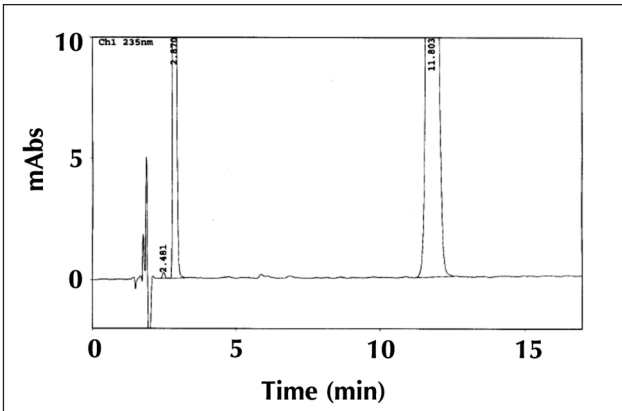

Figure 6. Chromatograms of heat (80°C)-degraded IRB, HCTZ, and tablet solution.

Linearity

Linearity of the method was tested from 10 to 200% of the targeted level of the assay concentration (100 µg/mL IRB and 50 µg/mL HCTZ) for both the analytes. Mixed standard solutions containing 10–200 µg/mL of IRB and 5–100 µg/mL of HCTZ. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area ratio against the concentration of the drugs. The equations of the calibration curves for IRB and HCTZ obtained were $y = 1299.9x - 1418.3$ and $y = 9694.5x + 2029.4$, respectively. Slopes and correlation coefficients are shown in Table II.

Robustness

The robustness of a method is the ability to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered, and chromatographic resolution between IRB and HCTZ were evaluated.

The flow rate of the mobile phase was 1.5 mL/min. To study the effect of flow rate on resolution of IRB and HCTZ, it was changed to 0.1 units from 1.5 to 1.6 mL/min and 1.4 mL/min. The effect of column temperature on resolution was studied at 28°C and 32°C instead of 30°C, while other mobile phase components were held constant. The resolution between IRB and HCTZ was not less then 1.5 in the robustness study. The robustness results are shown in Table V.

LOD and LOQ

The LOD and LOQ for IRB and HCTZ were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations (22). The LODs for IRB and HCTZ were 0.019 and 0.023 µg/mL, and the LOQs were 0.053 and 0.070 µg/mL, respectively, for 10 µL injection volume.

Table V. Results of Robustness Experiment

Parameter	Resolution between HTCZ and IRB
Flow rate (mL/min)	
1.4	28.5
1.5	27.1
1.6	25.3
Column temperature (°C)	
28	28.9
30	27.3
32	26.1

Table IV. Results of the Recovery Tests for the IRB and HCTZ

Level of addition (%)	Ingredient	Amount added (n = 3) (mg)	% Recovery*	% Average recovery†
80	IRB	120.0	101.25 (0.45)	101.15 (0.45)
	HCTZ	10.0	100.52 (0.37)	100.45 (0.34)
100	IRB	150.0	100.97 (0.24)	
	HCTZ	12.5	100.78 (0.55)	
120	IRB	180.0	101.23 (0.49)	
	HCTZ	15.0	100.87 (0.31)	

Assay IRB and HCTZ from its tablets dosage forms

The assay results of IRB and HCTZ in tablet dosage forms were comparable with the value of label claimed. The results presented in Table IV indicate the suitability of the method for routine analysis of IRB and HCTZ from their combination drug products.

Conclusion

The developed method is a stability-indicating while separating degradation products from mean peak. No co-eluting peaks with the main peaks and the method is specific for the estimation of IRB and HCTZ in the presence of degradation products. The method has linear response in stated range and is accurate and precise. The described method can be used as a stability-indicating method for assay of IRB and HCTZ in their combination drug product. The proposed method can be conveniently used by quality control departments to determine the assay of pharmaceutical preparations and also stability samples.

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