Determination of Irbesartan Using Stability Indicating Reverse Phase Liquid Chromatographic and UV Spectrophotometric Method

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ABSTRACT

Objectives: The present research work involve the development of a novel UV-Spectrophotometric and stability-indicating RP-HPLC methods for the irbesartan in bulk as well as tablet dosage form. **Methods:** The RP-HPLC method is performed on the Phenomenex C_{18} column (150 x 4.6 mm, 5 µm) particle size, using 0.1% Formic acid buffer (pH 3.2): Acetonitrile (60:40 v/v) as the mobile phase at a flow rate of 1.0 mL/min at the 220 nm detection wavelength. For the UV method the 0.1% formic acid was used as solvent. Validation study was performed for both the methods as per ICH guidelines. **Results:** A linear range of 1.5-120 µg/mL for HPLC and 1.75-110.5 µg/mL for UV method was obtained with a correlation coefficient of 0.998 for UV and 0.999 for HPLC method. The retention time of irbesartan was found 2.226 min using optimised condition. In force degradation study the acidic and alkaline conditions decomposes the irbesartan more in compared to other stressed conditions. In UV method the measurement of the absorbance of Irbesartan at λ_{max} equals to 220 nm. The developed UV and HPLC methods was found suitable to assay the marketed tablet dosage form with the percentage purity values of 95.78% and 96.68%. All the other validation parameters were found within the limit that has been conducted as per the ICH guidelines. **Conclusion:** The developed methods were found novel, reliable and easy for the estimation of irbesartan in bulk and tablet dosage form.

Key words: HPLC, UV, Irbesartan, Method development, ICH Guidelines.

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INTRODUCTION

Irbesartan is 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. Irbesartan is an imidazole derivative with a bipenyl-tetrazole side chain shown in Figure 1. It does not require biotransformation to exert its pharmacological action. The molecule has a high affinity for the AT1 receptor in human vascular smooth muscle cells, inducing *in vitro* a rightward shift of the angiotensin II concentration-response curve and a depression of the maximal response to angiotensin II characteristic of insurmountable blockade of AT1 receptors.[1] Angiotensin II subtype 1 receptor (AT1R) antagonists are as effective or more effective in treating these conditions compared with ACE inhibitors.[2] Used as mono therapy or in association with hydrochlorothiazide, irbesartan is an effective antihypertensive drug in a variety of mild-to-moderate hypertensive populations, including patients with diabetes, obesity, renal insufficiency and cardiovascular disease. In comparative trials, irbesartan is at least as effective and sometimes superior to comparator agents of the major antihypertensive classes.[3] Extensive review of literature on irbesartan estimation reveals that few spectrophotometric methods.[4,5] One HPTLC method,[6] simultaneous estimation of irbesartan with other combination[7,8] has been available. Only two RP-HPLC methods were found for the estimation of irbesartan alone. In one method,[9] the retention time of irbesartan was found 11.93 min, which is consider too long in method optimization with a linearity range of 10-200 µg/mL. In another method,[10] the determination of irbesartan was reported along with other related impurities, where they reported 5.8 min as a retention time for

irbesartan. Keeping on view of the reported articles and their reported values with disadvantages, this can be state that there is a need to have a clear, easy, reliable validated UV and HPLC method for the estimation of irbesartan alone, which should successively utilize for the determination of irbesartan in marked formulation also. Therefore in this present research work, effort has been taken to minimize all the reported disadvantages and to develop a fast, easy, reliable stability indicating.[11] UV and HPLC method for the determination of irbesartan and to validate[12] as per the ICH Q2B guidelines.

MATERIALS AND METHODS

Chemicals

The required HPLC grade solvents eg. Methanol, acetonitrile, collected from the Merck, Mumbai. HPLC grade water was procured from Millipore. All other reagents were of AR grade (RANKEM) purchased from avantor performance materials India limited, Maharashtra. All active pharmaceutical ingredients (API's) of Irbesartan as reference standards were procured from spectrum labs, Hyderabad, India. Marketed formulation (Irovel tablet, Sun Pharma) was purchased from local market of Visakhapatnam, India.

Instrumentation and Chromatographic condition

The HPLC method was performed on a WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, auto sampler integrated with Empower 2 Software. The method was conducted using a reversed-phase
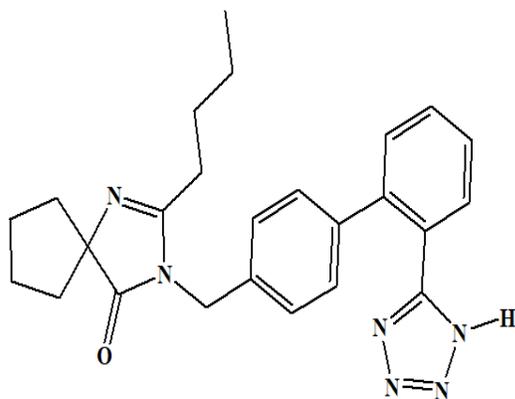
Figure 1: Chemical structure of Irbesartan.

technique. Irbesartan was eluted isocratically with a flow rate of 1.0 ml/min using a mobile phase consisting of 0.1% Formic acid buffer and Acetonitrile (60:40;v/v) at 220nm. Phenomenex C_{18} analytical column (150mm, 4.0 mm i.d., 5µm particle size) was used. The prepared mobile phase also used as a diluent UV method was performed on a UV-visible Spectrophotometer PG Instruments T60 at 220.0 nm using 2.0 cm quartz cells and UV win 6 software was used for all absorbance measurements.

High performance liquid chromatographic method development and validation

Preparation of standard and working solution for HPLC method

Accurately weighed 37.5mg of Irbesartan transferred 50ml and volumetric flasks; 5mL of acetonitrile was added to dissolve and sonicated for 10 min. Flasks were made up to the mark with diluents and labeled as standard stock solution (750µg/ml of irbesartan) from this solution working standard solution 100 µg/mL was prepared. The further diluted concentrations of 18.75, 37.5, 56.25, 75, 93.75 and 112.55µg/mL were made in 10 mL volumetric flask.

Preparation of sample for the assay of marketed formulations for HPLC method

10 tablets were weighed and calculate the average weight of each tablet then the tablet powder equivalent to 150 mg of irbesartan was transferred into a 100 mL. Volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipette out into a 100 ml volumetric flask and made up to the with diluents. This final diluted sample was injected into chromatographic system.

Accuracy

To study the accuracy of the proposed method, recovery studies were carried out by adding different amounts of pure drug of irbesartan to the pre-analysed formulation of concentration to achieve various levels (50%, 100% and 150%). All levels of the samples were injected in to chromatographic system and percentage recovery values were calculated.

Precision
Intraday and Inter day

To evaluate the intermediate precision which is also considered as ruggedness, was performed on different day. The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The percent relative standard deviation was calculated.

Repeatability

The precision of each method was ascertained separately from the peak areas and retention times obtained by actual determination of six replicates of a fixed amount of drug irbesartan. The percent relative standard deviation was calculated.

Specificity

The specificity of the optimised method was carried out by placebo interference test of the sample solution using 500 mg of placebo equivalent to one tablet dissolved in 100ml of mobile phase and the placebo solution was treated like a standard solution. The solution was injected to the chromatographic system to evaluate the possible interfering peaks.

System suitability

This study was evaluated to verify whether analytical system is working properly, by injecting the standard drugs of irbesartan six times. The RSD of the parameters like theoretical plates, peak area, retention time and asymmetric factor were calculated.

Linearity

The linearity justifies the ability of the developed method to obtain test results which are directly proportional to the concentration of analyte in the sample. For this study the solution was prepared in the range of 1.5-120 µg/ml and injected. Regression coefficient was calculated by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis.

LOD and LOQ

Limits of detection and quantification were calculated directly from the calibration curve using working standard solution. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively. Where σ is the standard deviation of intercept and S is the slope of the calibration plot.

Robustness

This was performed to investigate the effect of small deliberate changes in the chromatographic conditions such as change in Temperature (±2°C), flow rate (± 0.1ml/min) and organic phase content in mobile phase (±2%) were studied to determine the robustness of the developed.

Stability Studies
Acid degradation

10 mg of pure drug was transferred to a clean and dry round bottom flask. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water bath at 60°C for 4 hr. Allowed to cool to room temperature. The sample was then neutralized using dilute NaOH solution and final volume of the sample was made up to 100ml with mobile phase to prepare 40 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase (after optimizing the mobile phase compositions). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile.

Basic hydrolysis

10 mg of pure drug was accurately weighted and transferred to a clean and dry round bottom flask. 30 ml of 0.1N NaOH was added to it and it was refluxed in a water bath at 60°C for 4 hr. Allowed to cool at room temperature. The sample was than neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with mobile phase and finally diluted to 10 mL to prepare 10 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase after optimizing the mobile phase compositions. This experiment was repeated several

times using same concentration of NaOH such as 0.1N to observe its degradation profile is the degradation profile of irbesaran in 0.1N NaOH.

Thermal degradation

Accurately weighed 10 mg of pure drug was transferred to a clean and dry round bottom flask. 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hr uninterruptedly. After the reflux was over, the drug became soluble and the mixture of drug and water was allowed to cool to room temperature. Final volume was made up to 100 ml and finally diluted to 10 mL to prepare 10 μg/ml solution. It was injected into the HPLC system against a blank of mobile phase.

Photolytic degradation

Approximately 10 mg of pure drug was taken in a clean and dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hr without interruption. Accurately weighed 1 mg of the UV exposed drug was transferred to a clean and dry 10 ml volumetric flask. First the UV exposed drug was dissolved in methanol and made up to the mark with mobile phase and finally diluted to 10 mL to prepare 10 μg/ml solution. This solution was injected into the HPLC system against a blank of mobile phase and chromatogram was obtained.

Oxidative hydrolysis (3% H_2O_2)

Accurately weighed 10 mg of pure drug was taken in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hr. Final volume was made up to 100 ml. using water and finally diluted to 10 mL to prepare 10 μg/ml solution. The above sample was injected into the HPLC system against a blank of mobile phase and chromatogram was obtained.

UV spectrophotometric method development and validation

Preparation of Standard and working solution for UV estimation method

Accurately weighed 7.5 mg of Irbesartan was dissolved in a little quantity of methanol and then made up to 10 ml to get a concentration of 750 μg/ml. From the standard stock solution, an aliquot of 1 ml was taken and made up to 10 ml with 0.1% Formic acid as blank to get a working standard stock solution of concentration 75 μg/ml. From the working standard stock solution, various diluted samples were prepare by withdrawing the aliquots of 0.2 ml, 0.4ml, 0.5ml, 0.8 ml, 1ml and 1.2 ml and made up to 10 ml with 0.1% Formic acid as blank to get working solutions of concentration 1.5, 3, 3.75, 6, 7.5 and 9 μg/ml, respectively.

Determination of absorption maxima ($λ_{max}$)

The working solutions were spectrum scanned in the wavelength range of 200-400 nm by using 0.1% Formic acid as blank. The wavelength at which the spectra showed the maximum absorbance was noted as $λ_{max}$.

Construction of calibration curve: Three series of working solutions were prepared and measured for absorbance in triplicate at 220 nm ($λ_{max}$) and a calibration curve was plotted by taking concentration on y-axis and average absorbance on x-axis.

Assay of Marketed dosage form

Accurately 10 tablets were weighed and the average weight of each tablet has been calculated then the tablet powder equivalent to 150 mg of irbesartan was transferred into a 100 mL volumetric flask, 25 mL of methanol was added and sonicated for 5 min, further the volume made up with 0.1% firmic acid and filtered. From the filtered solution 1ml was pipette

out into a 100 ml volumetric flask and the volume was made up to the mark with 0.1% formic acid.

Method Validation

The developed UV method for irbesartan was performed by optimizing the UV spectrophometric parameters eg. Solvent selection, $λ_{max}$ identification, linearity. The method was validated by studying the following validation parameters as per ICH guidelines for UV spectrophotometric method validation.

Linearity: The calibration curve was obtained with five concentrations of the standard solution, as 1.75—110.5 μg/ml for UV method. The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Recovery Studies

Recovery study was performed to access the accuracy of the developed method. A fixed amount of drug from dosage form was taken and pure standard drug at different concentrations within Beer's range was added the total concentration was found by the proposed method. The determination with each concentration was repeated three times and average percent recovery of the added standard was calculated.

Repeatability / Precision

The content of the drug in the working solution was determined using calibration curve 5 times in a day for intraday precision and on five different days for inters day precision. The average % RSD was calculated.

Limits of Detection and Quantification.

Calibration curve was prepared for 5 times at 220 nm and SD of the intercept was calculated for the drug. The LOD and LOQ are calculated.

Robustness

The Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal stage. Here the robustness of the method was determined by small changing the measurement wavelength and the drug content was determined.

RESULTS

HPLC Method optimization

During the HPLC method development study, before to select the optimised chromatographic condition several of preliminary trials has been conducted with different combination of solvents, flow rate, various buffers, pH, temperature and columns in order to justify the retention time, peak shape, resolution and other chromatographic parameters. Various analytical columns were tested with U.V detection at 220 nm. Finally a selected mobile phase consisting of 0.1% formic acid buffer and acetonitrile (60:40;v/v) at 220nm. Phenomenex C_{18} analytical column (150mm, 4.0 mm i.d., 5μm particle size) was used at the flow rate of 1 mL/min, the chromatographic conditions were successfully separated at 2.22 min retention time for irbesartan with a better sensitivity and excellent peak shape, shown in Figure 2.

Method Validation by developed HPLC method

The subsequent validation study and force degradation study as per ICH guidelines were conducted by utilizing the optimised chromatographic condition. In the study of specificity no correspond peak was found at the retention time of the analyte. In the study of system suitability, parameters like tailing factor and theoretical plates were be taken into con-

sideration. The %RSD of theoretical plates, tailing factors and retention time were 0.56 %, 0.78%, 0.68 % and 0.52% respectively.

The assay of tablet "Avapro" (150 mg irbesartan) using developed HPLC method was conducted and the amount of drug was found 145.02 mg and % assay was 96.68%. The details was given in Table 1 and chromatogram was shown in Figure 3. The calibration curve showed linearity in the range of 1.5-120 μg/mL, for irbesartan (API) with correlation coefficient (r^2) of 0.999. The limit of detection (LOD) and limit of quantified (LOQ) were found to be 0.40 and 0.13 μg/ml respectively. The chromatograms of detection and quantitation were shown in Figure 5. In the study of intra and interday precision, the % RSD was found 0.72 and 0.91. The results of the validation parameters were included in Table 2. The average recovery of the irbesartan using HPLC method was 97.68 %, details of the results shown in Table 3. The method was found robust with the change in flow rates from ±0.1 mL/min, 0.1% formic acid buffer and acetonitrile ratios (62: 38, 58: 42 and), Detection wave-
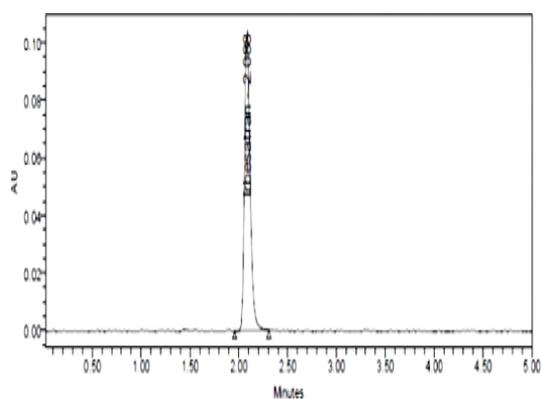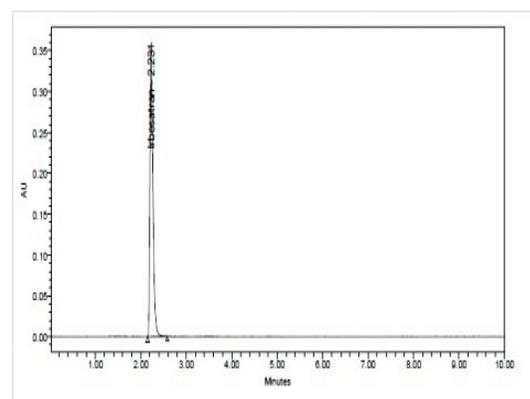

Figure 4: Optimised (A) and linear (B) UV spectrum of Irbesartan.

Figure 2: Optimised chromatogram of irbesartan.

Figure 3: Chromatogram of irbesartan tablet assay.
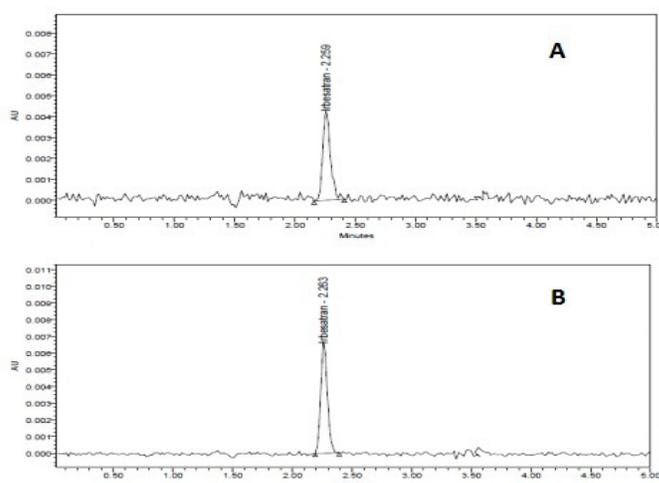
Figure 5: Limit of detection (A), Limit of quantitation (B) chromatograms of irbesartan.

Table 2: Summary of validation parameters.

Parameters	Irbesartan by UV	Irbesartan by HPLC
Linearity range (μg/ml)	1.75-110.5	1.5-120
Intraday Precision (% RSD)	0.12	0.72
Interday Precision (% RSD)	0.17	0.91
Regression coefficient	0.998	0.999
LOD μg/ml	0.27	0.04
LOQ μg/ml	0.82	0.13

Table 1: Assay of marketed dosage form by UV and HPLC method.

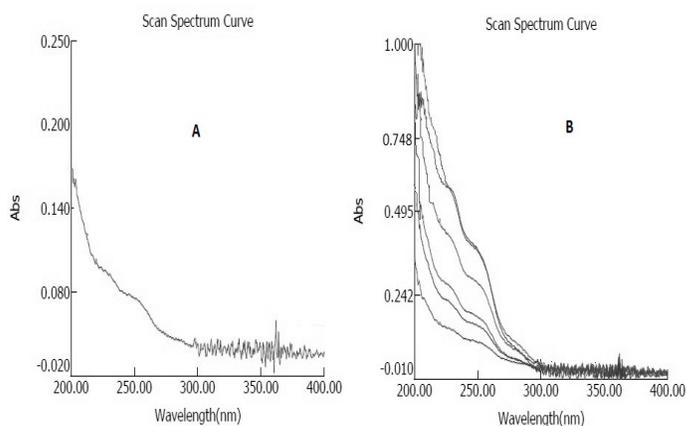| Formulation	Label Claim (mg)	*Amount found (mg)		% purity	
		UV method	HPLC method	UV method	HPLC method
AVAPRO (Irbesartan 150mg)	150 mg	143.67	145.02	95.78	96.68

* Average of Three experiments

length (222 nm, 224nm). The details of the study results are presented in Table 4. The stress degradation study were conducted in various stressed condition and % degrading was calculated. It was observed that in acidic and alkaline stressed conation the percentage degradation were 25.60 % and 26.26 %. The percentage degradation 17.38 % was found in peroxide induced stressed condition. The thermal and UV condition the % degradation were 15.95 % and 5.43 %. The details was given in Table 5 and degradation chromatograms were cited in Figure 6

Table 3: Recovery studies of irbesartan by UV and HPLC method.

Formulation	Label Claim in mg	Conc. of pure drug added in mg	* Amount of drug found in mg		% Recovery ± SD		Avg % ± SD	
			UV method	HPLC method	UV method	HPLC method	UV method	HPLC method
Etura	150	--	143.57	144.28	95.71±0.124	96.18±0.154	97.43 ±0.133	97.68 ±0.146
	--	100	245.03	246.65	98.01±0.154	98.66±0.173		
	--	200	343.96	342.56	98.27±0.262	97.87±0.109		
(Avapro 150 mg)	--	300	439.86.	441.08	97.74±0.106	98.01±0.146		

* Average of three determinations

Table 4: Robustness study.

Change in parameter	% RSD
Flow (1.1 ml/min)	0.94
Flow (0.9 ml/min)	0.89
Mobile phase ratio(+2)	0.78
Mobile phase ratio(-2)	0.81
Wavelength of Detection (218 nm)	0.93
Wavelength of detection (222 nm)	1.27

Table 5: Stress degradation study.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid Hydrolysis (0.1 M HCl)	25.60	0.167	0.369
2	Alkali Basic Hydrolysis (0.I M NaOH)	26.26	0.185	0.372
3	3 % Hydrogen peroxide	17.38	0.212	0.427
4	Thermal Degradation (60 ⁰C)	15.95	0.156	0.374
5	UV (254nm)	5.43	0.195	0.311

Figure 6: Chromatograms of force degradation study, Acidic (A), Alkaline (B), Peroxide (C), Thermal (D), Photo (E).

UV Optimization

To determine the suitable solvent system to obtain the linear UV spectrum of irbesartan various solvents, mixture of solvents and buffer systems at several volume ratios were tested. Finally methanol as a dissolving solvent and 0.1% formic acid was utilized to optimize the solvent system and also utilize for subsequent validation study. The optimised spectrum and overlay spectrum was shown in Figure 4. With this solvent system the linear UV spectrum were obtained at the λ_{max} 220nm as shown in optimised spectrum Figure. The above solvent system also used for the assay of marketed dosage form.

Method validation by developed UV spectroscopy method

The optimised method was applied for the estimation of irbesartan in marketed dosage form "Avapro" (150 mg irbesartan). The obtained amount was 143.67 mg and the percentage purity was 95.78 %, shown in Table 1. The linearity study of the developed method was found linear in the range of 1.75 – 110.5μg/ml, with a regression coefficient (R^2) of 0.998, cited in Table 2. In accuracy study of the developed method the average percentage recovery of irbesartan was found 97.43 %, as shown in Table 3. The average % RSD for the intraday and interday precision study was found 0.12 and 0.17. In the study of limit of detection and limit of quantitation, limit was found 0.27μg/ml and 0.82 quantitation limit was found 0.389 μg/ml for irbesartan. The details of these values included in the summary of validation parameters as shown in Table 2.

DISCUSSION

The optimised chromatographic conditions, for the developed HPLC method of irbesartan was confirmed with a mobile phase of 0.1% formic acid buffer and acetonitrile in the ratio of 60:40(v/v) using WATERS HPLC 2695 SYSTEM equipped with phenomenex C_{18} column, quaternary pumps, photo diode array detector and auto sampler integrated with Empower 2 Software and the flow rate of 1 mL/min was found optimum. Irbesartan was eluted with a retention time of 2.22 min with a better sensitivity and excellent peak shape. For the developed UV method of irbesartan methanol as a dissolving solvent and 0.1% formic acid as bulk solvent was found suitable to optimised the UV spectrum at 220nm. For both the developed methods the assay of marketed dosage form was found suitable with satisfactory percentage of purity as shown in the results. In the precision study the percentage of RSD using both UV and HPLC methods were found less than 2, which gives a clear indication for the precision of the developed method. In linearity study of both UV and HPLC method, the regression coefficient values were 0.999 and 0.998, clearly indicates the linearity of the developed methods. The results of average percentage recovery 97.43 % for UV method and 97.68% for HPLC method which indicated the accuracy of the developed methods as the values are within the acceptance limit. LOD and LOQ study results
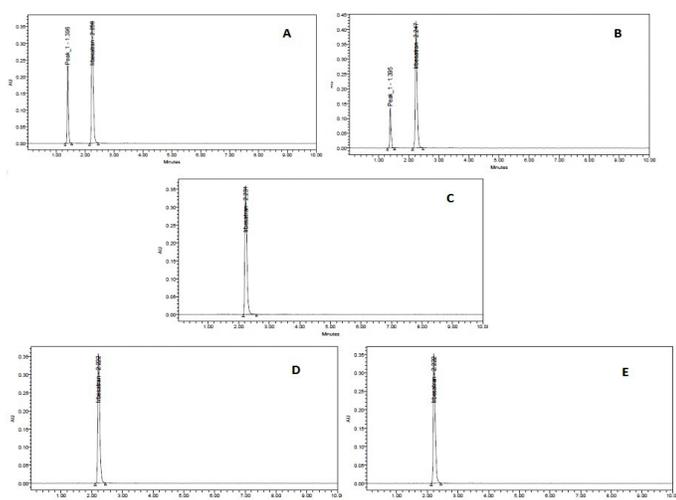
indicated that the both the developed methods was and easily quantifiable and found sensitive to determine irbesartan. The robustness study results of the HPLC method indicates that there is no significant changes in the result on small deliberate changes in detection wavelength, mobile phase ratio and flow rate of the mobile phase in chromatographic system, which indicated that the developed method was robust. In force degradation study there is a very narrow level degradation occurs in most of the decomposition conditions. In comparison to photolytic and thermal degradation, acidic and alkaline stressed conditions caused more decomposition of the irbesartan. The obtained result shows that the drug is resistant to the above degradation conditions, because in every stressed condition the percentage of degradations were found within the limit as per ICH guideline to claimed[13] the developed method stable.

CONCLUSION

The empirical evidences of the validation study results indicated that the developed RP-HPLC and UV spectrophotometric methods were highly accurate, precise and robust and are in good agreement with the labeled claim of the drug and obtained assay results. Therefore it can be concluded that, the present methods were highly suitable for the routine analysis of irbesartan in bulk and its tablet dosage form in pharmaceutical quality control laboratory.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ABBREVIATIONS

HPLC: High performance liquid chromatography; **ICH:** International conference on harmonization; **PDA:** Photo diode array; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **SD:** Standard deviation; **RSD:** Relative standard deviation.

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