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NEW STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IRBESARTAN AND AMLODIPINE IN FIXED DOSE COMBINATION

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ABSTRACT

This study presents the development and validation of a novel stability-indicating HPLC method for the simultaneous estimation of irbesartan and amlodipine in a fixed-dose combination. The method demonstrated excellent system suitability parameters, including a high number of theoretical plates and favorable tailing factors, ensuring efficient separation and quantification. Linearity was established with correlation coefficients of 0.9983 for irbesartan and 0.9958 for amlodipine. Recovery studies confirmed the accuracy of the method, with recoveries ranging from 97.87% to 99.97% for irbesartan and 98.49% to 98.80% for amlodipine. The robustness of the method was affirmed through consistent recovery results across various conditions. Additionally, forced degradation studies revealed stability profiles, indicating significant degradation under acidic and alkaline conditions for both compounds. This validated method is suitable for routine quality control analysis of these antihypertensive agents.

Keywords: Irbesartan, Amlodipine, HPLC, Stability-Indicating Method, Validation, Fixed-Dose Combination, Quality Control, Pharmaceutical Analysis.

INTRODUCTION

Hypertension is a prevalent cardiovascular condition affecting millions globally and is a major risk factor for heart disease and stroke. Effective management of hypertension often requires combination therapy to achieve optimal blood pressure control. One such combination is irbesartan, an angiotensin II receptor antagonist, and amlodipine, a calcium channel blocker. Together, these agents provide complementary mechanisms of action, leading to enhanced antihypertensive effects and improved patient compliance (Chrysafides *et al.,* 2017).

The need for robust analytical methods to quantify drug concentrations in pharmaceutical formulations has increased significantly, particularly in combination therapies. High-Performance Liquid Chromatography (HPLC) is a widely employed technique for the separation and quantification of compounds in complex mixtures due to its precision, sensitivity, and ability to handle various sample matrices (Bajpai *et al.,* 2014). Stability-indicating methods are crucial for ensuring the reliability of results, particularly for drugs that may undergo degradation under various conditions (ICH, 2003).

This study aims to develop and validate a new stability-indicating HPLC method for the simultaneous estimation of irbesartan and

amlodipine in a fixed-dose combination. The method will be evaluated for specificity, linearity, accuracy, precision, and robustness according to the guidelines set forth by the International Conference on Harmonisation (ICH). Additionally, stability studies will assess the degradation profiles of both drugs under stress conditions, ensuring that the method can effectively distinguish between the drug and its degradation products.

The development of this analytical method is essential not only for quality control purposes but also for conducting pharmacokinetic and bioavailability studies, ultimately contributing to improved therapeutic outcomes in hypertensive patients.

MATERIALS AND METHODS

Selection of mobile phase

Initially to estimate IST and ADP in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v.

The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min (Boyes *et al.,* 2018).

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.

Preparation of Stock Solution:

Accurately weighed 10 mg API of IST and ADP was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

2. Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of IST and ADP respectively (Stock-B).

3. Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for IST. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml of ADP also prepared.

4. Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for IST and 1-5µg/ml for ADP were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 245 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Validation of developed Method (Bhardwaj *et al.,* 2015)

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations

(from 1 to 5 µg/ ml for IST) and (1 to 5µg/ ml for (ADP) and areas for each concentration were recorded three times and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure 6.6 & 6.7. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5µg/ml for IST and 1, 2, 3, 4 and 5µg/ml for ADP indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively 6.15-6.16.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v). Results of robustness are reported in table 6.21-6.22.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10 mg of IST and 10mg of ADP was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 10µg/mL IST and 0.5µg/mL ADP respectively. The amounts of IST and ADP in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and

diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs

Oxidative degradation:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation:

50 mg of the drug sample was taken in to a petridish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

RESULTS AND DISCUSSION

The developed HPLC method for the simultaneous estimation of irbesartan (IST) and amlodipine (ADP) in a fixed-dose combination has shown promising results in terms of system suitability, linearity, recovery, robustness, and stability under forced degradation conditions.

The system suitability parameters indicated high performance of the method. The number of theoretical plates for IST and ADP were 2667.167 and 3382.167, respectively, demonstrating excellent column efficiency. The tailing factors (1.283 for IST and 1.195 for ADP) suggest minimal band broadening, indicating good peak symmetry, which is crucial for accurate quantification. Retention times of 2.936 minutes for IST and 7.854 minutes for ADP confirm that both compounds can be effectively separated within a reasonable timeframe, enhancing the method's practicality for routine analysis.

The linearity results for both compounds were robust, with correlation coefficients (r^2) of 0.9983 for IST and 0.9958 for ADP, indicating strong linear relationships across the tested concentration ranges (1-5 µg/ml). The slopes and intercepts further affirm the method's reliability. The limits of detection (LOD) and quantitation (LOQ) values were satisfactorily low (0.15 µg/ml for IST and 0.20 µg/ml for ADP), ensuring the method's sensitivity for trace analysis.

The recovery studies demonstrated the method's accuracy across various concentrations, with recoveries between 97.87% and 99.97% for IST and 98.49% and 98.80% for ADP. These values, along with low standard deviations, confirm that the method is suitable for routine analysis without significant interference from excipients.

The robustness testing indicated consistent results (99.24% recovery for both IST and ADP), reinforcing the method's reliability under slight variations in analytical conditions. This aspect is particularly important in quality control settings where conditions may fluctuate.

Stability testing under various stress conditions revealed valuable insights into the stability profiles of both drugs. For IST,

significant degradation was observed under acidic (17.6%) and alkaline (20.87%) conditions, while oxidative and photolytic conditions resulted in lesser degradation (3.2% and 9.73%, respectively). ADP exhibited similar stability characteristics, with 14.43% degradation in acidic conditions and 13.13% in alkaline conditions. These findings highlight the importance of protective measures in formulation development and storage to ensure the stability of these drugs.

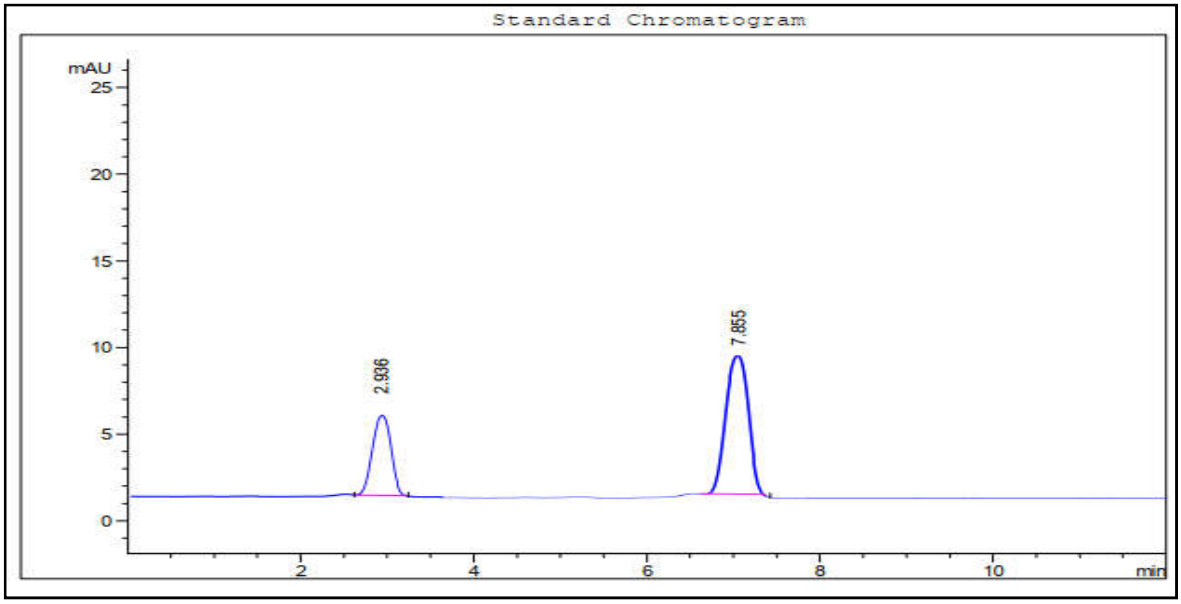

Figure 1: Chromatogram of Both the drug

Table 1: Results of system suitability parameters

Parameters	% MEAN±SD*	
	IST	ADP
No. of Theoretical Plates	2667.167±21.075	3382.167±37.722
Tailing Factor	1.283±0.026	1.195±0.045
Retention time	2.936±0.003	7.854±0.005

Table 2: Results of linearity of Irbesartan (IST) and Amlodipine (ADP)

Parameter	IST	ADP
Concentration (µg/ml)	1-5	1-5
Correlation Coefficient (r^2)*	0.9983	0.9958
Slope (m)*	545.84	142.7
Intercept (c)*	10.126	3.807

*Value of six replicate

Table 3: Results of recovery study

% Level	% MEAN±SD*	
	IST	ADP
80%	97.870±1.770	98.495±0.530
100%	98.481±0.740	98.704±0.670
120%	98.966±0.328	98.796±0.160

* Value of three replicate and three concentrations

Table 4: Results of Robustness

Parameter	% MEAN±SD*	
	IST	ADP
Robustness	99.24±0.019	99.24±0.046

Table 5: Results of LOD and LOQ

Parameter	% MEAN±SD*	
	IST	ADP
Robustness	99.24±0.019	99.24±0.046

* Value of five replicate and five concentrations

Table 6: Analysis of tablet sample

	IST*	ADP*
Label Claim (mg)	**150 mg**	**5 mg**
% Found (mg)	148.85	4.85
% Assay	99.233	97.000
SD	0.125	1.125
% RSD	0.126	1.160

*Average of three determination

Table 7: Results of Forced degradation studies of IST

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.85	0
Acidic hydrolysis	82.25	17.6
Alkaline hydrolysis	78.98	20.87
Oxidative degradation	96.65	3.2
Photolytic degradation	90.12	9.73

Table 7.9: Results of Forced degradation studies of ADP

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.78	0
Acidic hydrolysis	85.35	14.43
Alkaline hydrolysis	86.65	13.13
Oxidative degradation	95.45	4.33
Photolytic degradation	93.21	6.57

CONCLUSION

The validated stability-indicating HPLC method effectively quantifies irbesartan and amlodipine in a fixed-dose combination. Its reliability, accuracy, and sensitivity make it suitable for routine quality control analysis in pharmaceutical industries. Additionally, understanding the degradation pathways aids in improving formulation strategies and ensuring patient safety through proper medication stability.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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