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Determination of Irbesartan in Pharmaceutical Preparations by HPLC

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ABSTRACT

In this study, a high-performance liquid chromatography (HPLC) method was developed to analyze irbesartan in both pure and pharmaceutical formulations. The mobile phase consisted of an acetonitrile-1.0 mM potassium dihydrogen phosphate solution (30:70, v/v), adjusted to pH 3.0 with phosphoric acid. The analysis was performed using an Ace C_{18} column, and a UV detector was employed to monitor the eluent at 220 nm. With a flow rate of 1.0 mL min^{-1}, the analysis was completed in under 6 minutes. The calibration curve was linear across the concentration range of 0.10-5.0 µg mL^{-1}. The accuracy of the method (relative error) for irbesartan was better than 2.67%, and the intra- and inter-day precision values were below 3.23%. The mean recovery of irbesartan in pharmaceutical formulations was 101.4%. The limits of detection and quantitation were found to be 0.03 and 0.10 µg mL^{-1}, respectively. Furthermore, the method proved to be effective for quantifying the drug and confirming the consistency of the formulation content in commercial irbesartan dosage forms.

Keywords: Irbesartan, HPLC, Validation, Drug analysis.

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Introduction

Cardiovascular diseases account for 17.9 million deaths annually, representing approximately 30% of all deaths worldwide [1]. Hypertension is responsible for at least 45% of heart disease-related deaths. Hypertension is a major public health issue due to its widespread prevalence worldwide and the increased risk of mortality when it coexists with other diseases. Antihypertensive medications are used not only to lower blood pressure but also to mitigate the adverse effects associated with hypertension. A variety of drug classes are employed to manage hypertension, including angiotensin-converting enzyme inhibitors (ACE-I), beta-blockers, calcium channel blockers, thiazide diuretics, and angiotensin receptor blockers (ARB). In cases where monotherapy with a single class of antihypertensive drugs is insufficient, most guidelines recommend combining a thiazide diuretic with an ARB. Combination therapy, compared to monotherapy, allows for the use of lower doses of each drug, which enhances treatment effectiveness and reduces side effects. Therefore, combining medications for hypertension treatment provides a more efficient approach with fewer adverse effects than using a single drug. Various medications have been used to treat hypertension, including beta-blockers, diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers [2].

Irbesartan belongs to the class of angiotensin II receptor antagonists. By preventing the constriction of blood vessels, irbesartan helps to lower blood pressure and enhance blood flow. It is commonly used to treat hypertension, or high blood pressure, and can be administered either alone or in combination with other antihypertensive drugs. Irbesartan is commonly used to treat hypertension and other cardiovascular diseases. However, like any medication, irbesartan can cause some side effects. In some individuals using irbesartan, particularly those with kidney problems, heart conditions, or other serious health issues, more severe side effects may occur. Chemically, irbesartan is identified as 2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one, a non-peptide molecule. Its structural formula is shown in Figure 1, and its empirical formula is $C_{25}H_{28}N_6O$. Irbesartan has a molecular weight of 428.5 g mol^{-1} and is typically found as a crystalline powder that ranges in color from white to off-white [3].

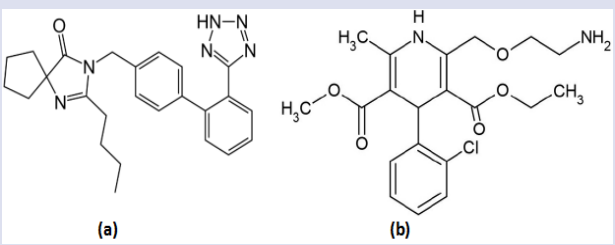

Figure 1. Chemical structures of irbesartan (a) and amlodipine (IS) (b)

Several analytical techniques have been reported for the detection of irbesartan, including UV-spectrophotometry [4-10], spectrofluorimetry [11], capillary electrophoresis [12], LC-MS [13-16] and HPLC

[17-22]. However, each of these methods presents certain challenges. Spectroscopic methods tend to have low sensitivity, while chromatographic techniques often require complex derivatization or lengthy extraction procedures, and are generally time-consuming and costly. Therefore, simpler, faster, and more affordable methods that still offer high sensitivity could provide a beneficial alternative.

HPLC is a commonly used technique for analyzing drugs in pharmaceutical formulations and biological fluids, owing to its advantages like ease of use and affordability. Only two RP-HPLC methods have been found for estimating irbesartan alone. In one method [23], the retention time of irbesartan was found to be 11.9 minutes, which is considered too long for method optimization, with a linearity range of 10-200 µg mL^{-1}. In another method [24], the determination of irbesartan was conducted alongside other related impurities, with a reported retention time of 5.8 minutes for irbesartan. Based on the reported articles and their associated drawbacks, it can be concluded that there is a need for a clear, simple, reliable, and validated UV and HPLC method for estimating irbesartan alone, which could also be effectively used for the determination of irbesartan in marketed formulations.

Developing a novel technique for determining the concentration of irbesartan in pharmaceutical solutions or biological fluids is essential. To meet this need, the objective of the study is to propose an HPLC method with UV detection for the quantification of irbesartan in pharmaceutical products. In compliance with the International Conference on Harmonization (ICH) guidelines, the developed method was validated based on criteria such as linearity, stability, precision, accuracy, and recovery. This method offers a short six-minute run time and a simple mobile phase composition, allowing for the efficient analysis of a large number of samples.

Materials and Methods

Chemicals

Irbesartan, amlodipine besylate, internal standard (IS), methanol, acetonitrile and potassium dihydrogen phosphate were provided Sigma-Aldrich (St. Louis, MO, USA). The irbesartan-containing Karvea tablets were provided by the pharmacy in Erzurum, Türkiye. Every chemical was of the analytical purity.

HPLC System and Chromatographic Conditions

The method development and validation studies were conducted using Agilent HPLC equipment from the 1260 series. A UV detector (G71144A), auto injector (G7129A), and quaternary pump (G7111A) were included in this chromatographic system's equipment. The Ace C_{18} (250×4.60 mm ID, 5µm) analytical column was used for the separations, which were carried out at 25 °C. Phosphoric acid was used to modify the acetonitrile-1.0 mM potassium dihydrogen phosphate solution (30:70, v/v, pH 3.0) for the mobile phase. Mobile phase flow rate and UV detection of method were 1.0 mL min^{-1} and 220 nm, respectively.

Preparation of Standard and QC Solutions

The stock solutions of the irbesartan and IS were prepared in methanol at a concentration of 50 µg mL^{-1} and then stored at -20 °C. Methanol was used to dilute the stock solution to prepare irbesartan standard solutions in the range of 0.10-5.0 µg mL^{-1}. Additionally, quality control (QC) samples were prepared at concentrations of 0.75, 3.0, and 4.5 µg mL^{-1}, along with a 5.0 µg mL^{-1} IS solution.

Procedure for Pharmaceutical Preparations

Using the mass of the Karvea tablets, the average tablet mass was computed. Afterward, they were subjected to homogenization, fine grinding, and the careful weighing of a portion of the powder. The necessary amount of methanol was then poured to them in a 100 mL brown measuring flask in order to dilute the powder. After sonicating the mixture for a minimum of fifteen minutes to facilitate dissolution, it was filtered using a Whatman No. 42 paper. After an appropriate volume of filtrate was taken, it was further diluted with methanol to ensure that the final solution's irbesartan concentration fell within the working range.

Data Analysis

With the use of a computer program, SPSS 15.0 was used for the statistical analyses. The irbesartan standard line and calculations were made using regression analyses. The results' mean and standard deviation were given.

Results and Discussion

Development and Optimization of the Method

In recent years, the importance of the HPLC method for drug analysis in routine quality control has garnered significant attention. An appropriate technique for determining irbesartan in pharmaceutical dosage forms was proposed. For this purpose, the Ace C_{18} column (250×4.60 mm ID, 5µm) was selected. The chromatographic conditions were optimized to ensure the experiment's successful performance. The procedure utilized a mobile phase consisting of acetonitrile and 1.0 mM potassium dihydrogen phosphate solution (30:70, v/v, pH 3.0).

The retention time was observed to be 5.85 minutes. The total run time for the assay was approximately ten minutes. The mobile phase was chosen after conducting several experiments with different solvent mixtures. In selecting the mobile phase, factors such as peak properties (symmetry, tailing), run time, cost, and ease of preparation were carefully considered. A representative chromatogram obtained by applying the proposed method to the analysis of a standard irbesartan sample is shown in Figure 2. The retention time observations enabled rapid determination of the drug.

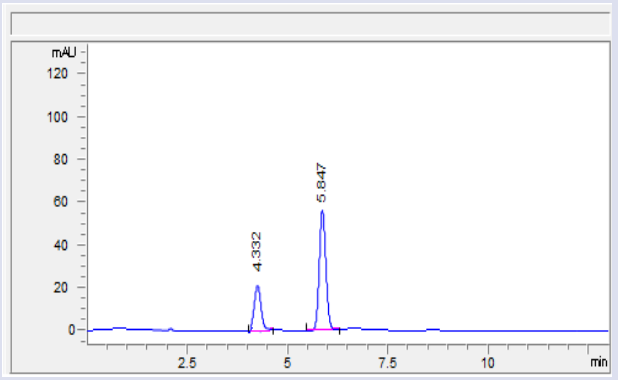

Figure 2. The chromatograms of irbesartan (5.0 μg mL^{-1}, Rt:5.847 min) and amplodipin (IS) (5.0 μg mL^{-1}, Rt:4.332 min)

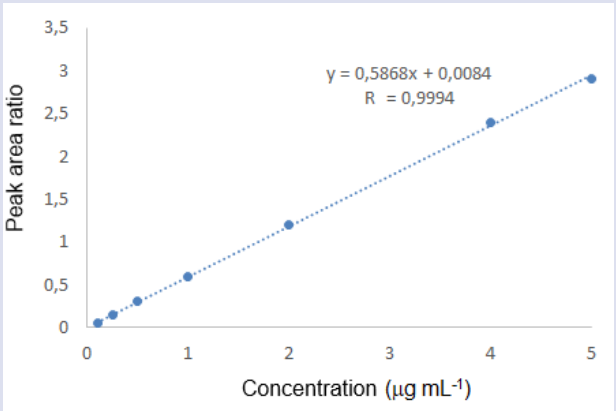

Figure 3. The standard curve of irbesartan (0.10, 0.25, 0.5, 1.0, 2.0, 4.0 and 5.0 μg mL^{-1})

Validation of the Method

The objective of method validation is to demonstrate that the method is suitable for its intended purpose, as outlined in the ICH guidelines. The method was validated for linearity, accuracy, precision, limits of detection and quantitation, recovery, stability, selectivity and system suitability [25-27].

Linearity

An analytical method is considered linear if it produces test results that are directly proportional to the analyte concentration in the sample within a specified range, either directly or through a well-defined mathematical transformation. Initially, this can be assessed visually by analyzing a plot of signal versus analyte concentration. If the relationship appears linear, the test results should be confirmed using appropriate statistical methods (e.g., by calculating a regression line using the least squares method). In some cases, a mathematical adjustment of the test results may be required to achieve linearity between the analyte's response and its concentration.

To mathematically assess the degree of linearity, the information derived from the regression line itself can be valuable. It is essential to report the slope of the regression line, the residual sum of squares, the y-intercept, and the correlation coefficient. Analysis was done on standard solutions containing 5.0 μg mL^{-1} of IS and 0.10-5.0 μg mL^{-1} of irbesartan. The standard curve (Figure 3) was created by plotting the concentration of irbesartan on the X-axis and the peak area ratio of irbesartan and IS on the Y-axis.

Using the least squares regression approach to construct the linear regression analysis, the linearity was assessed. The regression equation was computed from the calibration graphs (Table 1).

Table 1. Linearity of of irbesartan (n=3)

Range (μg mL^{-1})	Linear regression	R^2
0.10-5.0	y=0.5868x+0.0084	0.9988

Accuracy and Precision

Accuracy is described as how closely test results produced by the procedure resemble the actual value. By using known, added amounts of analyte in the experiment, it is frequently stated as the percent recovery. Accuracy measures the precision of the analytical process. The true values were used to calculate the variances of the obtained results, which were subsequently reported as percentage accuracy.

Analytical procedure repeatedly to several samplings of a homogenous sample is known as its precision. The standard deviation or relative standard deviation of a set of measurements is typically used to express the precision of an analytical procedure. A measure of precision could be the analytical method's repeatability or degree of reproducibility under typical operating conditions.

The assay method's accuracy for both intra- and inter-day variations was determined by evaluating the QC samples six times. Table 2 shows the accuracy and precision values for the QC samples intra and inter-day runs. Between 2.34% and 3.23% ranged the precision, and between 1.27% and 2.67% the accuracy.

Table 2. Precision and accuracy of irbesartan

Added (μg mL^{-1})	Found ± SD	Intra-day Accuracy (% relative error)	Precision RSD%	Found ± SD	Inter-day Accuracy (% relative error)	Precision RSD%
0.75	0.73 ± 0.019	-2.67	2.60	0.74 ± 0.022	-1.33	2.97
3.0	3.06 ± 0.08	2.00	2.61	2.98 ± 0.07	-0.67	2.34
4.50	4.57 ± 0.134	1.27	2.93	4.61 ± 0.149	2.44	3.23

LOD and LOQ

Irbesartan's LOD and LOQ values were ascertained by evaluating various irbesartan solutions and calculating the signal-to-noise ratio for every analyte. The concentration providing a signal-to-noise ratio of roughly 3:1 is the LOD, while the concentration providing a signal-to-noise ratio of roughly 10:1 with an RSD of less than 10% with triplicate analysis is the LOQ. The HPLC technique was determined LOD and LOQ values of 0.03 and 0.10 μg mL^{-1}, respectively.

Recovery

Recovery values were obtained by spiking different amounts of pure drug into tablet samples that had already been pre-analyzed, within the analytical concentration range of the proposed method. Using the described procedure, the added doses of each drug were determined. The results of the recovery experiments were considered satisfactory and are presented in Table 3.

Table 3. Recovery of irbesartan in pharmaceutical preparation (n=6)

Pharmaceutical preparation	Added (μg mL^{-1})	Found ± SD	Recovery (%)	RSD [a] (%)
Karvea tablet (1.0 μg mL^{-1})	0.5	0.51 ± 0.017	102.0	3.33
	2.5	2.53± 0.081	101.2	3.20
	3.5	3.54 ± 0.119	101.1	3.36

Stability

Based on stability experiments, the samples remained stable for 72 hours at ambient temperature, as well as at 4°C and -20°C under refrigeration. The stability study results presented in Table 4 demonstrated that no significant degradation was observed.

Table 4. Stability of irbesartan in solutions

Added (μg mL^{-1})	+25 °C stability (Recovery % ± RSD)		+4 °C stability (Recovery % ± RSD)		- 20 °C stability (Recovery % ± RSD)	
	24 h	72 h	24 h	72 h	24 h	72 h
0.50	102.1 ± 1.44	98.7 ± 3.17	99.7 ± 3.04	101.1 ± 2.94	98.3 ± 2.49	100.3 ± 2.09
2.5	101.4 ± 3.08	99.3 ± 2.71	99.4 ± 2.46	99.4 ± 3.07	101.4 ± 2.08	99.7 ± 2.65
4.5	99.4 ± 3.01	99.6 ± 2.97	101.2 ± 2.48	99.7 ± 3.19	100.4 ± 2.63	99.4 ± 3.09

Selectivity

In this study, the potential interferences of common excipients and additives were investigated. Control samples were prepared and analyzed. At the concentrations typically found in dosage forms, no evidence of interference from these substances was observed. The excipient used in this formulation is one of the most commonly employed by the pharmaceutical industry. The selectivity of the method was evaluated by checking for any interference from common tablet ingredients such as talc, lactose, sodium chloride, titanium dioxide, and magnesium stearate. These substances did not cause any harmful impact on the proposed method. Based on the analysis results, the procedure can be regarded as selective.

System Suitability

Prior to every validation, the chromatographic system underwent a system suitability test. Therefore, standard solutions containing 5.0 μg/mL of internal standard (IS) and 5.0 μg/mL of irbesartan were chosen. The efficiency, tailing factor, and area relative standard deviation were calculated for each of the five suitable injections. The average of the five suitable injections was used to quantify the check standard. The efficiency was ≥ 2967, the %RSD was ≤1.32%, and the tailing factor was ≤ 1.03 for all sample analysis.

Procedure for Pharmaceutical Preparations

The 300 mg irbesartan-containing Karvea tablet was carefully weighed and finely ground. The right amount of powder was dissolved in 50 milliliters of methanol. Next, a balloon flask was filled to the ultimate capacity of 100 mL. After the tablet solutions were appropriately diluted, a Whatman filter was employed to filter them in order to provide a final concentration that fell between the linearity limitations of the HPLC procedure (Figure 4). Irbesartan's drug concentration was determined using the calibration curve.

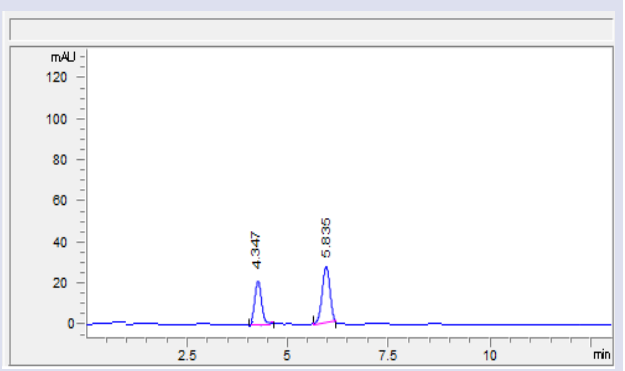

Figure 4. The chromatogram of Karvea tablet solution (2.50 μg mL^{-1})

Comparison of the Methods

Irbesartan is commonly prescribed to treat chronic renal failure, congestive heart failure, and hypertension. In this study, commercial formulations used in the pharmaceutical industry were analyzed using a fast and straightforward HPLC method. The popularity of the proposed approach lies in its simplicity and ease of implementation.

Recently, voltammetry has emerged as a promising new analytical technique for the electrochemical detection of drugs. Voltammetric methods are vital for pharmaceutical analysis due to their affordability, user-friendliness, and rapid analysis times [28, 29].

The results demonstrate the excellent reproducibility and reliability of the two techniques. To statistically compare the best outcomes, a t-test was performed. The calculated t-values did not exceed the theoretical values at a 95% confidence level (Table 5).

Table 5. Comparison of the proposed and reported methods of irbesartan

Parameters	Proposed method	Official method [30]	Reported method [8]
Mean (recovery %)	101.4	100.04	99.63
SD	0.621	-	-
% RSD	0.612	0.260	0.362
Variance	0.374	-	-
SE	0.253	-	-
t-test (2.228)[a]	0.897	-	-
F- test (5.1)[a]	3.74	-	-

SE: Standard error, (P > 0.05), [a]Theoretical values at P=0.05, Variance is a statistical measure that represents the spread or dispersion of a set of values.

As a result, the differences between the differential pulse polarography and square wave polarography techniques are minimal [28, 29]. The %RSD for the polarographic analysis of irbesartan tablets using the proposed methods was 1.09%. The recovery of standard additives further validated the accuracy of the methods applied to the irbesartan tablets. A mean recovery rate of 101.4% was achieved. The results from the drug analysis using the proposed techniques closely aligned with the stated values. The outcomes of the proposed methods were compared with those of the official [30] and reference methods [8]. The student t- and F-values, calculated at a 95% confidence level, indicated no significant differences in performance between the official or reference methods and the proposed techniques.

Conclusion

In this study, a quick and simple HPLC method has been developed and validated for the quantification of irbesartan. The chromatographic approach meets all the necessary criteria, such as accuracy, linearity, recovery, and precision, ensuring its reliability and practicality. With a run time of just 6 minutes, it allows for the efficient analysis of a large number of samples. As a result, this method can be used not only for routine testing of formulations and raw materials but also for analyzing samples in accelerated stability studies.

Conflicts of interest

The author states that did not has conflict of interests

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