Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 28 May 2026
Revised 22 June 2026
Accepted 17 July 2026

Natural Resources for Human Health 2026, Vol. 6 (9s): 1070–1083

KEYWORDS:
C18 column, Rat Plasma, Freeze thaw Stability, Precision and Accuracy, Selectivity, Internal standard

Simple and Rapid HPLC Method for the Quantification of Irbesartan in Rat Biological Matrices Development and Bioanalytical Validation

Sunita Patnaik[1,2], Pratap Kumar Patra[2], Gurudutta Pattnaik[2], Himansu Bhusan Samal[2] Krishna Chandra Panda[1], Ch. Niranjan Patra[1]

[1]Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India.
[2]School of Pharmacy and Health Sciences, Centurion University of Technology and Management, Bhubaneswar, Odisha, India
Corresponding Author Sunita Patnaik
Email : sunita.patnaik01@gmail.com
Pratap Patra 0000-0002-1074-5749
Gurutta Pattnaik 0000-0002-3532-721X
Himansu Bhusan Sasmal 0000-0002-0803-6847
Ch. Niranjan Patra 0000-0002-5022-8746
Krishna Chandra Panda 0000-0001-7057-7559

ABSTRACT:

Objective: Irbesartan is one of the expansively utilized angiotensin II receptor blockers for treatment of hypertension and diabetic nephropathy. Accurate pharmacokinetic evaluation of irbesartan requires sensitive and trustworthy analytical methods. This study successfully established and thoroughly validated a novel and quick Ultra-Fast Liquid Chromatography (UFLC) bioanalytical method for the measurement of Irbesartan in rat plasma as a pure bulk medication.

Methods: Using an isocratic mobile phase consisting of methanol and PBS (pH 3.5) in an 80:20 (v/v) ratio, the chromatographic separation was successfully accomplished on a C18 column (4.6 × 250 mm, 5 µm) at room temperature.

Results: UV detection was tracked at 233 nm, and the flow rate was 0.75 mL/min. In the absence of endogenous plasma interference, a distinct, sharp analyte peak was seen at a retention time of 6.4minutes. Over a broad concentration range of 100–7000 ng/mL, the technique demonstrated high linearity. Additionally, good extraction recovery (mean ~95.5%), outstanding accuracy, intra-day and inter-day precision, and high stability under various storage conditions and bioanalytical study were attained through stringent validation.

Conclusion: In conclusion, a simple, rapid, and sensitive isocratic UFLC method was effectively developed and fully validated for the quantification of Irbesartan in rat plasma. The method exhibited high selectivity, excellent recovery (95.5%), robust stability under varied storage conditions, and low detection limits across a wide dynamic range (100–7000 ng/mL). Meeting all regulatory validation measures, this analytical approach aids as a reliable, cost-effective, and high throughput platform for routine preclinical

pharmacokinetic, bioavailability, and bioequivalence evaluations of Irbesartan. Finally, the method works well for standard bioanalytical applications.

INTRODUCTION

In order to accurately quantify therapeutic agents in complex biological matrices like plasma, serum, or tissue homogenates, a reliable, sensitive, and highly selective bioanalytical method must be developed and validated for pharmacokinetic (PK) and bioavailability (BA) studies[1]. A specially designed bioanalytical technique usually utilizing High-Performance Liquid Chromatography (HPLC) or Liquid Chromatography provides the sensitivity, linearity, accuracy, and precision required to capture true physiological concentration-time profiles because drugs frequently circulate at trace concentrations (nano or picogram levels) alongside an abundance of endogenous proteins, lipids, and potential metabolites[2].

Irbesartan is an angiotensin II receptor blocker that is primarily used to treat diabetic nephropathy and hypertension[3]. It has a broad plasma protein binding of about 96%[4]. Its terminal elimination half-life ($t_{1/2}$) is between 11 and 15 hours[5]. Irbesartan has demonstrated promise in lowering inflammation and oxidative stress in the central nervous system[6]. Irbesartan has neuroprotective qualities, including presumed involvement in antibacterial and antioxidant activities, repairing injured neural systems, controlling the vascular system, and boosting synaptic plasticity. It is categorized as a BCS Class II medication because it is nearly insoluble in aqueous medium (less than 1 mg/mL), which frequently acts as the rate-limiting step for its absorption and therapeutic efficacy[7].With a log P of 6, irbesartan is soluble in acetone and ethylacetate but nearly insoluble in water[8]. The chemical structure of Irbesartan is given in (Fig.1).

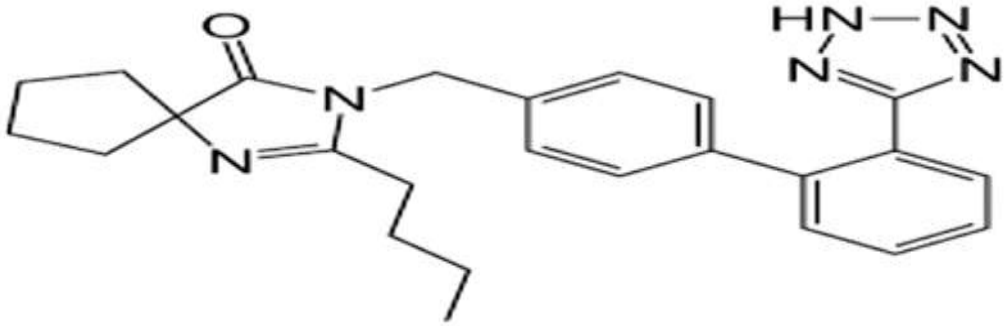

Figure 1: Irbesartan's Chemical Structure

An overview of the documented analytical techniques for irbesartan estimate is provided. Mohamed A. Amin et al. devised a technique for measuring irbesartan and hydrochlorothiazide simultaneously in pharmaceutical formulations. A Shimadzu (Prominence LC 20 UFLC XR) connected to a photodiode array (PDA) detector was used to perform the chromatographic separation utilizing an ACE column and C18/CN (100 x 4.6 mm, 5 µm) as the stationary phase. The isocratic mobile phase consisted of 0.1% phosphoric acid 85% and methanol in a 55:45 v/v ratio at a flow rate of 1.5 ml min-1. A highly sensitive and precise LC-MS method for quantifying irbesartan in human plasma was developed[9]. Chromatographic separation was performed on a Luna® HST C18 column (50 mm x 3 mm, 2.5 µm) using a mobile phase of 0.1% aqueous formic acid and acetonitrile (33:67, v/v) at a flow rate of 0.2 mL/min and a total runtime of 4.0 minutes. Irbesartan was discovered using tandem mass spectrometry with positive ionization mode and multiple reaction monitoring (MRM)[10]. The medications and tolbutamide using a gradient mode with a run time of 5 minutes and a flow rate of 1 ml/min using a YMC triart C18 column (50 mm × 4.6 mm i.d., 3 µm). It was found that a straightforward, precise UFLC method for estimating irbesartan in rat plasma in bulk[11].

MATERIALS AND METHODS

Materials

Irbesartan was given as a free gift sample by Medico Remedies Ltd. in Mumbai. E. Merck Private Limited in Mumbai supplied methanol and heparin. The remaining solvents were all analytical (AR) grade. A Shimadzu UFLC with PDA detector was used for this investigation.

Methods

A stock solution of 1000 µg/ml was created by dissolving 10 mg of Irbesartan in 10 milliliters of methanol. Additionally, samples in the concentration range of 500 ng/ml to 6000 ng/ml were obtained by diluting various quantities of stock solution with mobile phase (methanol: PBS pH3.5) (80:20 v/v). 200 µl of rat plasma was mixed with 100 µl aliquots of various solutions, and the mixture was vortexed for two minutes. One liter of acetonitrile, an extracting solvent, was then added, vortexed for two minutes, and centrifuged for five minutes at 1000 rpm. Following centrifugation, 500 µl of the supernatant was carefully moved and dried in a water bath kept at 60ºC.After reconstituting the dried residue with one milliliter of mobile phase, it was vortexed for two minutes.

Chromatography

A straightforward, quick, and precise reverse phase ultrafast liquid chromatographic method was used to analyze irbesartan. Chromatography was performed using a Photo Diode Array Detector (PDA) on a 250 mm 4.6 mm i.d., C18 column with an 80:20 (v/v) methanol: PBS pH 3.5 mobile phase at a flow rate of 0.75 mL/min[10,11].The UV wavelength was fixed at 233 nm .

Method Validation

Linearity

The linearity and range of the calibration curve were tested using seven calibration standards with various of irbesartan (500, 1000, 2000, 3000, 4000, 5000, 6000, 40000, and 7000 ng/mL). The investigation was undertaken in duplicate to validate the reproducibility of the data. Linear regression analysis was used to determine the test sample concentrations[12,13].

Selectivity

The selectivity of the proposed technique was tested by comparing chromatograms of blank plasma from individual rats with those of the corresponding standard plasma sample (Irbesartan spiked)[13].

Precision and Accuracy

The recovery study was carried out using spiking analysis. Three concentration levels 500, 1000, and 2000 ng/ml were used to assess the test method's accuracy[14]. Throughout the experiment, duplicate formulations were made at each concentration level. Six duplicates of quality control samples at three concentrations were evaluated to gauge the intraday precision and accuracy of the proposed method. Similarly, six replicate quality control samples at three different concentrations over three days were analyzed to evaluate interday precision and accuracy. The percentage relative error (%RE) was used to gauge the method's accuracy, while the percentage relative standard deviation (%RSD) was used to gauge its precision.

Lower limits for both detection and quantitation

The lowest concentration of an analyte that can be detected is known as the limit of detection, whereas the lowest concentration that can be precisely and accurately measured using the bioanalytical method is known as the limit of quantitation. The following formulas were used to calculate the previously specified restrictions.

$$Limit\ of\ Detection\ (LOD) = 3.3\ [\frac{SD}{S}]$$

$$Quntititation\ Limit\ (LOQ) =\ 10\ (\frac{SD}{S})$$

where S is the calibration curves slope and SD is the standard deviation.

Toughness

A homogeneous sample under purposefully variable operational conditions, including using various analysts and testing on different days and times The relative standard deviation (RSD) of a crucial chromatographic response, like peak area, confirms the robustness of the technique[15].

To assess the method's robustness, the effects of minor variations in chromatographic settings, such as the time and date of analysis, were investigated.

Recovery (extraction efficiency)

To test the extraction efficacy of the projected method, a set of samples (n=6) at each QC level were prepared by spiking drug into plasma samples and further processed (pre-extraction). Similarly, a second batch of plasma samples was processed first and spiked after extraction at each QC level. The extraction recovery for each analyte was estimated using the ratios of the peak areas of the pre-extraction and post-extraction samples. The percentage recovery for internal standard Losartan potassium was calculated at the MQC (1000 ng/ml). The slope and standard deviation of the calibration curve were used to determine the limits of quantitation (LOQ) and limits of detection (LOD) for the developed method.

Stability analysis

Three different types of stability studies were used to assess the stability of irbesartan in rat plasma, as detailed below[16].

Short-term Stability: The initial concentration of Irbesartan was determined by analyzing six samples at two concentration levels, 500 ng/ml and 2000 ng/ml. Six further samples were stored at 25°C for six hours before the concentration of irbesartan was measured. At last, the % stability was calculated.

Long-term stability: Irbesartan concentrations in six samples at two concentration levels (500 and 2000 ng/ml) were measured both at the beginning and after 14 days of storage at -20°C ± 5°C.

Freeze-thaw stability: Two samples with concentrations of 500 ng/ml and 2000 ng/ml were breezed (-70°c) then thawed to room temperature (25°C) and stored for another 24 hours. This finished one cycle. The test was repeated three more times in a similar fashion. The samples were examined and contrasted with newly made solutions following the fourth cycle.

For the bioanalytical study, 5 mL of rat blood was collected in EDTA and centrifuged at 10,000–15,000 RPM to isolate plasma, which was stored at -20 °C. During analysis, 200 µL of thawed plasma was spiked with 100 µL of drug solution, vortexed, and treated with 1 mL of acetonitrile for protein precipitation. After centrifugation (5,000–10,000 RPM), 500 µL of the supernatant was evaporated at 60 °C. The resulting residue was reconstituted in 1 mL of mobile phase and injected into the HPLC system for quantification.

RESULTS AND DISCUSSION

Linearity

Irbesartan concentration and the corresponding Area Under the Curve (AUC) at 233 nm shows a robust, equivalent connection using the reverse-phase UFLC method over a broad range of 100 to 7000 ng/ml. This vast dynamic range shows that the analytical method is extremely sensitive and able to precisely measure the drug's different concentrations. Additionally, the method's exceptional precision and analytical consistency are highlighted by the continuously low standard deviations seen at every concentration level (Table 1,2 and Fig.2).

Table 1. Chromatographic conditions of UFLC Method of Analysis

Parameters	**Chromatographic conditions**
Instrument	**Shimadzu HPLC Pump (LC-20AD)**
Column	5 µm C18 column
	(Dimensions 4.6 × 250 mm)
Detector	PDA Detector (SPD-20A)
Mobile phase	Methanol: PBS pH 3.5
Flow rate	0.75 ml/min
Detection wavelength	233 nm

Run time	10 min
Temperature	Room temperature (25°C)
Injection volume	20 µl
Retention time (Rt)	6.4min

Using the reverse-phase UFLC method, Irbesartan concentration and the corresponding Area Under the Curve (AUC) at 233 nm demonstrate a strong, proportional relationship over a wide range of 100 to 7000 ng/ml. This massive dynamic range demonstrates the analytical method's high sensitivity and ability to accurately quantify the drug's widely varying amounts. The steadily low standard deviations observed at all concentration levels further demonstrate the method's remarkable precision and analytical consistency (Table 1,2 and Fig. 2).

Table2. Calibration data for Irbesartan in Rat Plasma

Concentration (ng/ml)	AUC* (233nm)
100	470 ± 14
500	22326±77
1000	44275±289
2000	89138±315
3000	133526±263
4000	175121±401
5000	221684±329
6000	265736±346
7000	316236±412

*Mean ± SD (n=6)

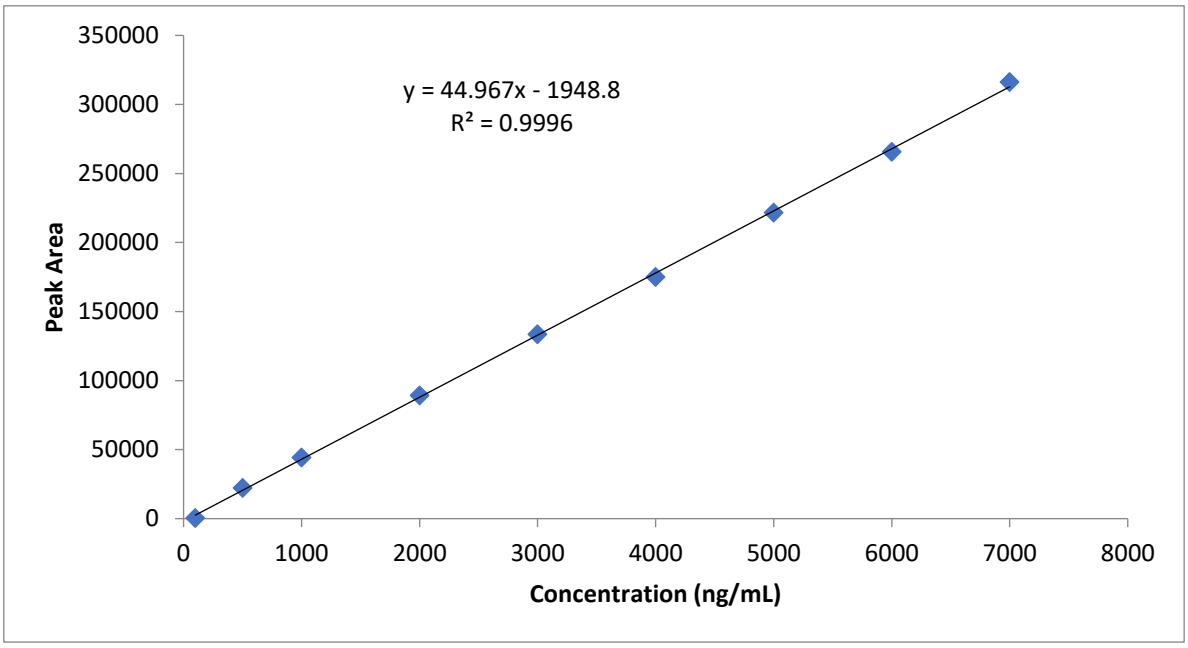

Figure 2: Calibration Curve for Irbesartan in Rat Plasma

Natural Resources for Human Health
eISSN: 2583-1194

Selectivity

Chromatogram 3A (blank) reflects the inherent, endogenous components of the plasma matrix, which elute largely early in the run (i.e., between 3.5 and 5.5 minutes). A noticeable, strong peak representing the eluted Irbesartan was shown in Chromatogram 3B during a retention duration of approximately 6.4 minutes. Chromatogram for internal standard 3C reflects retention time of 5.3 minutes. Chromatogram for internal standard sample spiked drug sample was represented in 3D (Fig.3).

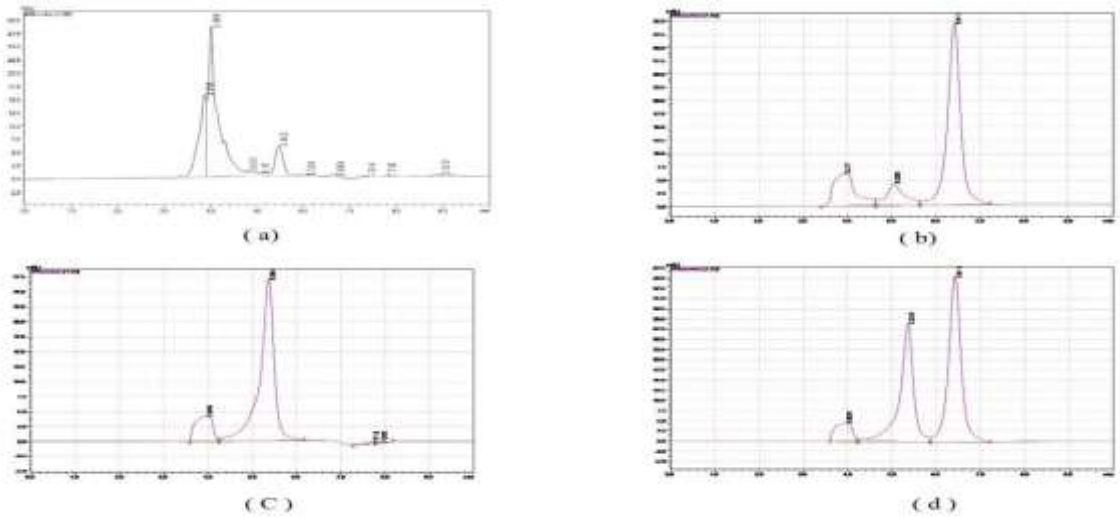

Figure 3 Chromatogram for blank Plasma (A) Chromatogram for Irbesartan (B) Chromatogram for Losartan (C) Chromatogram Losartan potassium sample spiked with drug Irbesartan (D)

The intrinsic, endogenous components of the plasma matrix are reflected in chromatogram 3A (blank), which elutes primarily early in the run (i.e., between 3.5 and 5.5 minutes). Chromatogram 3B (Irbesartan) displayed a prominent, strong peak that represented the eluted Irbesartan throughout a retention period of 6.4 minutes. Chromatogram for Losartan (C)as internal standard carries retention period of 5.3minutes. Chromatogram Losartan sample spiked with Irbesartan (D) (Fig. 3).

Inter-Day Accuracy and Precision

The range of the back-calculated accuracy is 98.97% to 100.77%. These findings, which fall well within the average analytical recovery range of 95%–105%, show strong technique robustness and quantitative accuracy. In the concentrations under study, the technique accuracy ranges from 1.02 to 1.22%. For the majority of analytical experiments, all results were well below the 2.0% acceptance threshold standard, demonstrating exceptional intra-day repeatability and extremely consistent instrument performance for six replicates[16,17]. (Table 3).

Table 3. Inter day Accuracy and Precision

Nominal Concentration (ng/mL)	Mean Peak Area (n = 6) DOCX	SD DOCX	Precision (CV %)	Accuracy (%)
500	22317	256	1.15%	98.97%
1000	44136	452	1.02%	100.77%
2000	86247	1052	1.22%	99.87%

The back-calculated accuracy falls between 98.97% and 100.77%. These results demonstrate virtuous procedure robustness and quantitative accuracy, falling well within the usual analytical recovery range of 95%–105%. The technique accuracy falls between 1.02 and 1.22% in the concentrations that are being studied. Excellent intra-day repeatability and very consistent instrument

performance for six replicates were demonstrated for most analytical studies, with all values falling far below the 2.0% acceptability threshold standard[17]. (Table 3).

Intraday Accuracy and precision

The data exhibits the intraday validation of an Ultra-Fast Liquid Chromatography (UFLC) analytical approach for the quantitative measurement of Irbesartan. All %CV values were significantly below the 2.0% criterion in the standard ICH guideline and high technique accuracy and repeatability was attained with several injection replicates.[14,18] Further the computed accuracy is well within the permitted pharmaceutical limit of 98.0% to 102.0% showing that the technology is capable of recovering the nominal concentrations of the medication. These statistical metrics collectively demonstrate the robustness of the procedure and prove it to be extremely suitable and dependable for routine pharmacokinetic analysis[18]. (Table 4).

Table 4. Intraday Accuracy and Precision

Working Day	Day 1			Day 2			Day 3		
Niminal Concentration of drug (ng/mL)	500	1000	2000	500	1000	2000	500	1000	2000
Measured Peak Area	22457	44101	89213	22415	44150	89150	22150	44050	89010
	22128	43972	89102	22280	44320	89420	22290	44210	89250
	22326	44236	89401	22350	43980	89300	22340	44180	89180
	22401	44198	89302	22490	44410	89050	22080	44350	89390
	22223	44721	89198	22210	44250	89500	22250	44090	89120
	22178	44203	89257	22380	44290	89280	22180	44260	89290
Mean (n =6)	22285.50	44238.50	89245.50	22354.17	44233.33	89283.33	22215.00	44190.00	89206.67
Standard Deviation	130.12	255.20	101.40	99.44	149.76	165.73	94.18	109.18	135.30
Precision (CV %)	0.58%	0.58%	0.11%	0.44%	0.34%	0.19%	0.42%	0.25%	0.15%
Accuracy (%)	100.70 %	99.49%	100.09 %	101.00 %	99.48%	100.13 %	100.38 %	99.38%	100.05 %

The data demonstrates the intraday validation of an analytical method for the quantitative detection of irbesartan using Ultra-Fast Liquid Chromatography (UFLC). High method accuracy and repeatability were achieved with many injection replicates, and all %CV values were much below the 2.0% requirement in the standard ICH guideline[14,18]. Additionally, the calculated accuracy is substantially within the allowed pharmaceutical limit of 98.0% to 102.0%, demonstrating that the technology can recover the medication's nominal concentrations. When taken as a whole, these statistical measures show how reliable and resilient the process is for routine pharmacokinetic analysis (Table4).

LOD and LOQ

The Limit of Quantification (LOQ) is approximately 3.11 ng/ml while the Limit of Detection (LOD) is approximately 1.03 ng/ml. These data demonstrate a highly sensitive bioanalytical technique for identifying analyte trace quantities in intricate biological matrices. In intensive pharmacokinetic (PK) profiling, a LOQ of 3.11 ng/ml is especially useful[19]. (Table 5).

Table 5. Data of Recovery of Irbesartan

Peak areas of UFLC Chromatogram						
Pure Solutions in Mobile Phase				Extracted Plasma Samples		
(ng/mL)	500 ng/ml	1000 ng/ml	2000 ng/ml	500 ng/ml	1000 ng/ml	2000 ng/ml
	23350	46500	93200	22457	44101	89213
	23280	46380	93050	22128	43972	89102
	23410	46600	93300	22326	44236	89401
	23310	46410	93110	22401	44198	89302
	23300	46480	93180	22223	44721	89198
	23366	46444	93108	22178	44203	89257
Mean (n = 6)	23336.00	46469.00	93158.00	22285.50	44238.50	89245.50
SD	47.96	77.26	86.85	130.12	255.20	101.40
Percentage Recovery (%)				95.50%	95.20%	95.80%
Average Recovery				95.5		

By comparing the peak regions of the extracted samples with those of the unextracted mobile phase standards in six replicates, the absolute recovery of irbesartan from plasma was ascertained. The method's extraction effectiveness was shown to be highly consistent across all tested concentration levels, with mean percentage recoveries falling within a very tight range of 95.20-95.80%. Additionally, the precision for both extracted and unextracted samples showed extremely low coefficient of variation (%CV) values, all of which were significantly less than 1.0%. These findings demonstrate the robustness and high reproducibility of the sample preparation technology, which yields excellent analyte isolation with minimal matrix interference or processing loss.

The limit of detection (LOD) is roughly 1.03 ng/ml, and the limit of quantification (LOQ) is 3.11 ng/ml. These findings provide a very sensitive bioanalytical method for determining trace amounts of analytes in complex biological matrices. A LOQ of 3.11 ng/ml is particularly helpful in thorough pharmacokinetic (PK) profiling. Self-nanoemulsifying drug delivery systems (SNEDDS), nanostructured lipid carriers (NLCs), and novel polyherbal composite therapeutic agents are examples of advanced formulation strategies that often exhibit altered absorption kinetics and extended systemic circulation.[12,18] These sustained release nanocarriers' terminal elimination phase can be accurately measured at extended time intervals with a low LOQ (Table 5).

On the other hand, the percentage recovery for Losartan (88.53%) was relatively lower compared to that for Irbesartan. Such percentages might be due to the difference in partition co-efficient (log P) between the two drugs (Table 6).

Table 6. Data of Recovery of Losartan Potassium

	Pure solutions(ng/ml)	Extracted Plasma samples(ng/ml)
Concentration	1000	1000
Peak Area	46450	41045
	46374	41140
	46580	41110
	46390	41125
	46472	41200
	46433	41120
Mean(n=6)	46449	41123
Recovery (%)		88.53

The percentage recovery for Losartan Potassium (88.53%) was relatively lower compared to that for Irbesartan. Such percentages might be due to the difference in partition co-efficient.(log P) between the two drugs is mentioned in (Table 6).

Ruggedness was assessed by assessing a 500 ng/mL Irbesartan sample on two different days and at different times of the day. With the peak area exhibiting a slight shift and the assay percentage staying extremely consistent at 99.63% and 98.91%, respectively, the obtained data suggest no variance between the two operational sets[19].The method is sufficiently robust and dependable for routine quantitative analysis, as evidenced by its high degree of reproducibility under various temporal conditions (Table 7).

Table 7. Statistical validation of Ruggedness

Parameters	Set I	Set II
System	Shimadzu	Shimadzu
Sample	Irbesartan	Irbesartan
Solvent	Methanol : PBS pH 3.5 (80:20)	Methanol : PBS pH 3.5 (80:20)
Date	01.06.2026	02.06.26
Time	11.30 am	4 pm
Lab	Central Instrument room	Central Instrument room
Sample Concentration	500 ng/mL	500 ng/mL
Peak Area	22457	22304
Assay %	99.63	98.91

By evaluating a 500 ng/mL Irbesartan sample on two separate days and at different times of the day, the analytical method's robustness was evaluated[19]. The acquired data indicate little variation between the two operational sets, with the peak area showing a minor shift and the assay percentage being remarkably stable at 99.63% and 98.91%, respectively. The method's high degree of consistency under different temporal situations shows that it is sufficiently reliable and resilient for routine quantitative analysis (Table 7).

Robustness

The robustness of the analytical approach was assessed by evaluating a 500 ng/mL Irbesartan sample in triplicate at a wavelength of 233 nm[20]. The study produced peak areas of 22451, 22314, and 22173, resulting in a mean peak area of 22312.67 and a standard deviation of 139.01. The most notable finding was that the relative standard deviation (%RSD) was only 0.62%. Because this %RSD is extraordinarily low, it demonstrates that the analytical method is highly robust, reliable, and capable of sustaining constant performance despite any tiny procedure deviations (Table 8).

Table 8. Statistical Validation of Robustness

S. No	Wavelength	Concentration (ng/mL)	Peak area	Mean	SD	%RSD
1	233	500	22451			
2	233	500	22314	22312.67	139.01	0.62
3	233	500	22173			

By analysing a 500 ng/mL Irbesartan sample in triplicate at a wavelength of 233 nm, the analytical method's robustness was evaluated[20]. The investigation yielded peak areas of 22451, 22314, and 22173, with a standard deviation of 139.01 and a mean peak area of 22312.67. The relative standard deviation (%RSD) was only 0.62%, which was the most noteworthy discovery.

This extremely low %RSD shows that the analytical approach is very robust, dependable, and able to maintain consistent performance even in the face of small process changes (Table 8).

Stability Study

Short-term stability analysis

The short-term bench-top stability of the analyte at two different quality control levels (500 and 2000 ng/mL) during routine sample handling and preparation was examined to determine the structural and chemical integrity of the analyte. This test ensures that, for six hours before extraction and instrumental analysis, the analyte is unaffected by ambient laboratory conditions. The mean measured concentration for the low-concentration quality control (LQC) samples marginally changed from an initial 499.89 ng/mL to 491.74 ng/mL after 6 h.

This amounts to a computed stability of 98.37%. Similarly, the high-concentration quality control (HQC) samples moved from an initial mean of 2000.58 ng/mL to 1974.77 ng/mL, indicating a stability of 98.71%. The total average stability across the examined con-centration levels was 98.54%. According to normal USFDA and ICH M10 regulatory criteria for bioanalytical technique validation, the accuracy of stability samples must re-main within ± 15% of the nominal concentration. The detected differences indicate less than a 2% deviation from the initial baseline across both QC levels, which is well within the acceptable regulatory criteria (Table 9).

Table 9. Short term Stability Study

	Low Concentration		**High Concentration**	
Time Period	**Initial**	**6 hours**	**Initial**	**6 hours**
Nominal Concentration (ng/mL)	500	500	2000	2000
Measured Concentration (ng/mL)	500.12	492.15	2002.36	1975.21
	499.03	490.85	2000.56	1973.85
	501.54	491.60	2000.14	1976.40
	498.54	493.05	1999.84	1972.15
	499.71	489.90	1999.74	1974.90
	500.41	492.89	2000.83	1976.11
Mean (n =6)	499.89	491.74	2000.58	1974.77
Stability (%)	**98.37%**		**98.71%**	
Average Stability (%)	**98.54%**			

This results in a 98.37% computed stability. The high-concentration quality control (HQC) samples also showed 98.71% stability, moving from an initial mean of 2000.58 ng/mL to 1974.77 ng/mL. Across all concentration levels under investigation, the overall average stability was a strong 98.54%. The accuracy of stability samples must remain within ± 15% of the nominal concentration in accordance with the USFDA and ICH M10 regulatory standards for bioanalytical technique validation. Less than a 2% departure from the initial baseline across both QC levels is indicated by the discovered discrepancies, which is well below the acceptable regulatory standards (Table 9).

Long Term Stability Study

After a storage period of 14 days, the low-concentration quality control (LQC) samples revealed a small mean reduction from the initial baseline of 501.25 ng/mL to 487.77 ng/mL, equal to a calculated long-term stability of 97.31%. The mean measured concentration changed from an initial 1998.50 ng/mL to 1955.33 ng/mL at the high-concentration quality control (HQC) level, resulting in a stability of 97.84%. 97.58% was determined to be the combined average stability for both concentration levels.

The accuracy of stability test samples must stay within ± 15% of the nominal or initial measured concentration in accordance with strict bioanalytical method validation requirements, such as those described by the USFDA and ICH M10. Over the course of the 30-day test period, the synthesized medicine had less than 3% degradation, according to the analytical data(Table 10).

Table 10. Long term stability

	Low Concentration		High Concentration	
Time Period	**Initial**	**14 days**	**Initial**	**14 days**
Nominal Concentration (ng/mL)	500	500	2000	2000
Measured Concentration (ng/mL)	502.10	488.50	2001.20	1957.10
	500.85	486.90	1996.80	1953.50
	501.60	488.10	1999.50	1956.80
	499.95	486.50	1997.40	1954.20
	502.40	489.00	2000.60	1958.00
	500.60	487.62	1995.50	1952.38
Mean (n =6)	501.25	487.77	1998.50	1955.33
Stability (%)	97.31		97.84	
Average Stability (%)	97.58			

The low-concentration quality control (LQC) samples showed a little mean decrease from the initial baseline of 501.25 ng/mL to 487.77 ng/mL after a 14day storage period, which corresponds to a computed long-term stability of 97.31%. The initial 1998 mean concentration was altered. 97.84% stability was achieved at the high-concentration quality control (HQC) level, ranging from 50 ng/mL to 1955.33 ng/mL. The combined average stability at both concentration levels was found to be 97.58%. Stringent bioanalytical method validation requirements, including those outlined by the USFDA and ICH M10, demand that the accuracy of stability test samples remain within ± 15% of the nominal or initial measured concentration. The analytical findings showed that the synthesised medication had less than 3% deterioration during the 14 day test period (Table 10)

Freeze Thaw Stability Study

The first mean measured concentration for the low-concentration quality control (LQC) samples was 502.50 ng/mL. After three cycles of freezing and thawing, the mean concentration slightly fell to 493.05 ng/mL, equivalent to a stability of 98.12%. For the high-concentration quality control (HQC) samples, the mean concentration moved from an initial baseline of 1995.00 ng/mL to 1953.50 ng/mL post-thaw, which yields a stability of 97.92%. 98.02% was the average stability over the two concentration levels that were assessed. The mean concentration of the stability samples must be within ± 15% of the nominal or first determined baseline, per standard bioanalytical technique validation criteria (e.g., USFDA and ICH M10). According to the analytical findings, the medication exhibited approximately 2% degradation during the three severe freeze-thaw cycles, easily satisfying the requirements for regulatory acceptance (Table 11).

Table 11. Freeze Thaw Stability Data

	Low Concentration		High Concentration	
Time Period	**Initial**	**4 cycles**	**Initial**	**4 cycles**
Nominal Concentration (ng/mL)	500	500	2000	2000
Measured Concentration (ng/mL)	503.15	493.80	1997.20	1955.10

	501.90	492.20	1993.50	1951.80
	502.80	493.50	1996.80	1954.90
	501.40	492.80	1992.10	1952.30
	503.50	494.10	1998.00	1956.40
	502.25	491.90	1992.40	1950.50
Mean (n =6)	502.50	493.05	1995.00	1953.50
Stability (%)	98.12		97.92	
Average Stability (%)	98.02			

The low-concentration quality control (LQC) samples showed a little mean decrease from the initial baseline of 501.25 ng/mL to 487.77 ng/mL after a 14 day storage period, which corresponds to a computed long-term stability of 97.31%. The initial 1998 mean concentration was altered.97.84% stability was achieved at the high-concentration quality control (HQC) level, ranging from 50 ng/mL to 1955.33 ng/mL. The combined average stability at both concentration levels was found to be 97.58%. According to bioanalytical technique validation standards (e.g., USFDA and ICH M10), the stability samples' mean concentration must be within ± 15% of the nominal or first determined baseline. The analytical results showed that over the three severe freeze-thaw cycles, the drug showed around 2% degradation, readily meeting the conditions for regulatory acceptance (Table 10)

Bioanalytical Study Procedure for Rat Plasma Sample

For the bioanalytical study, 5 ml of rat blood was collected and treated with EDTA to prevent coagulation. The blood sample was centrifuged at 10,000–15,000 RPM for 10–15 minutes using a refrigerated centrifuge. After centrifugation, the resulting supernatant was carefully separated and stored in a deep freezer at -20 °C for future analysis. Simultaneously, a stock solution of the drug was prepared. Before analysis, the stored supernatant was thawed to room temperature. In a centrifuge tube, 200 µL of the thawed plasma was combined with 100 µL of the previously prepared drug solution and vortexed for 2 minutes to ensure thorough mixing. Subsequently, 1 ml of acetonitrile was added as the extracting solvent, followed by another 2minute vortexing. The mixture was centrifuged (Remi RM-12 CBL) at 5,000–10,000 RPM for 5–10 minutes to separate the components. After centrifugation, 500 µL of the clear supernatant was carefully transferred to a vial and evaporated to dryness in a water bath maintained at 60 °C. The dried residue was reconsti-tuted with 1 mL of a mobile phase followed by brief vortexing to ensure complete dissolution. The prepared sample was then ready for injection into a High performance liquid chromatography (HPLC) system for comprehensive analysis (Table 12).

Table 12. Bio Analytical Study of Rat Plasma in HPLC

Concentration (ng/ml)	AUC (233nm)
1000	44275±270
3000	66731±287
5000	244594±311
100000	606163±358
200000	1256101±410
400000	2737071±425
600000	3864810±451

*Mean ± SD (n=6)

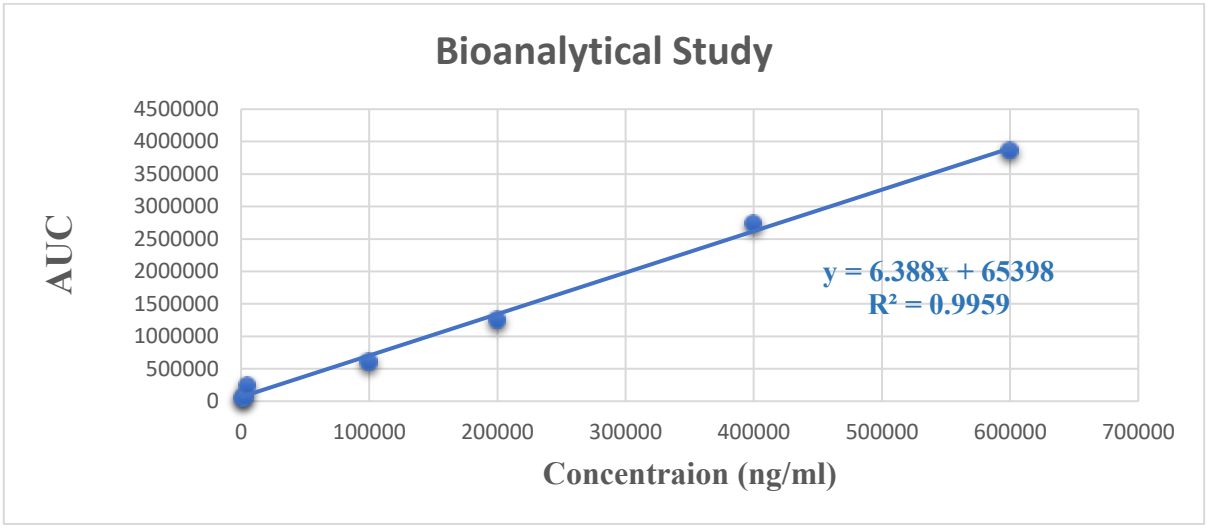

Figure 4: Calibration Curve of Bioanalytical study of Rat Plasma

CONCLUSION

In conclusion, a simple, sensitive, and robust Ultra-Fast Liquid Chromatography (UFLC) approach was successfully developed and completely validated for the quantitative quantification of Irbesartan in rat plasma. The test demonstrated negligible interference from endogenous plasma components and achieved outstanding selectivity with a distinct retention time of 6.4 minutes by using a C18 column and an isocratic mobile phase made up of methanol and PBS (pH 3.5) at an 80:20 (v/v) ratio. The suggested approach is very beneficial for trace-level pharmacokinetic profiling because it demonstrated excellent linearity throughout a wide dynamic range of 100 to 7000 ng/mL and reached exceptionally low limits of detection (1.03 ng/mL) and quantification (3.11 ng/mL). Crucial validation characteristics, including as accuracy, ruggedness, and intra-day and inter-day precision, easily satisfied strict regulatory acceptance requirements. This was reinforced by a very stable absolute recovery rate that averaged about 95.5%. Additionally, comprehensive stability evaluations demonstrated that the medication stays chemically intact under short-term, long-term (14 days), and stringent freeze-thaw conditions. Overall, this highly reproducible UFLC approach provides a viable analytical platform for conducting sophisticated bioavailability and pharmacokinetic studies of Irbesartan, accommodating the pure bulk drug.

FUNDING

This research received no external funding.

CONTRIBUTION BY EACH AUTHOR:

Sunita Patnaik: Performed the practical research experimental work. Pratap Patra: Guided while executing research work. Gurudutta Pattnaik: Contributed in writing research work. Himansu Bhusan Sasmal: Contributed in language editing. Ch.Niranjan Patra: Reviewd Manuscript writing. Krishna Chandra Panda: Contributed in conducting experimental work.

CONFLICT OF INTEREST

All authors acknowledge that there are no conflicts of interest or financial interests linked to this research work.

REFERENCES

[1] Pai, P.-Y., et al., *Angiotensin II receptor blocker Irbesartan enhanced SIRT1 longevity signaling replaces the mitochondrial biogenetic survival pathway to attenuate hypertension-induced heart apoptosis.* Journal of Cardiovascular Development and Disease, 2022. **9**(8): p. 266.

[2] Vardanyan, R. and V. Hruby, *Synthesis of best-seller drugs.* 2016: Elsevier.

[3] Croom, K.F., et al., *Irbesartan: a review of its use in hypertension and in the management of diabetic nephropathy.* Drugs, 2004. **64**(9): p. 999-1028.

[4] Markham, A., C.M. Spencer, and B. Jarvis, *Irbesartan: an updated review of its use in cardiovascular disorders.* Drugs, 2000. **59**(5): p. 1187-1206.
[5] Marino, M.R., et al., *Pharmacokinetics and pharmacodynamics of irbesartan in healthy subjects.* The Journal of Clinical Pharmacology, 1998. **38**(3): p. 246-255.
[6] Gouveia, F., et al., *Protective effects of irbesartan against neurodegeneration in APP/PS1 mice: Unraveling its triple anti-apoptotic, anti-inflammatory and anti-oxidant action.* Biomedicine & Pharmacotherapy, 2025. **188**: p. 118167.
[7] Kaur, N., et al., *Understanding the oral absorption of irbesartan using biorelevant dissolution testing and PBPK modeling.* AAPS PharmSciTech, 2020. **21**(3): p. 102.
[8] Cho, C.-K., et al., *Physiologically based pharmacokinetic (PBPK) modeling to predict the pharmacokinetics of irbesartan in different CYP2C9 genotypes.* Archives of Pharmacal Research, 2023. **46**(11): p. 939-953.
[9] Elgawish, M.S., M.K. Soltan, and M.M. Sebaiy, *An LC–MS/MS spectrometry method for the simultaneous determination of rosuvastatin and irbesartan in rat plasma: Insight into pharmacokinetic and drug-drug interaction studies.* Journal of Pharmaceutical and Biomedical Analysis, 2019. **174**: p. 226-234.
[10] Pongnarin, P., et al., *Development and validation of a highly specific for quantification of irbesartan in human plasma and its application to a bioequivalence study.* Pharmaceutical Sciences Asia, 2025. **52**(3).
[11] Bhargavi, P.D., et al., *The simultaneous quantification of Sitagliptin and Irbesartan in rat plasma using the validated LC-MS/MS method is applied to a pharmacokinetic study.* Journal of Chromatography B, 2023. **1221**: p. 123677.
[12] Maruthi, R., et al., *Analytical method development and validation of teneligliptin by rp-uflc.* Research Journal of Pharmacy and Technology, 2020. **13**(9): p. 4035-4040.
[13] Qureshi, H.K., C. Veeresham, and C. Srinivas, *Analytical method development and validation of filgrastim by UV and RP-UFLC methods.* American Journal of Analytical Chemistry, 2021. **12**: p. 333-346.
[14] Sajjad, S., et al., *Validated analytical RP-UFLC method development and pharmacokinetic evaluation of Thiolated chitosan nanoparticles of Vonoprazan fumarate in rabbit.* Microchemical Journal, 2026: p. 118166.
[15] Patro, R., S. Mallick, and C.N. Patra, *Development and Validation of a UV Spectroscopic Method for the Estimation of Gepirone Hydrochloride in Bulk and Tablet Dosage Form in Dissolution Media.* Journal of Applied Spectroscopy, 2025: p. 1-7.
[16] Inamdar, A., et al., *A green UFLC approach for vancomycin therapeutic drug monitoring in human plasma: method development, validation, and clinical application in critically ill patients at a tertiary care hospital.* Results in Chemistry, 2026: p. 103354.
[17] Liu, J., et al., *Simultaneous UFLC–ESI–MS/MS determination of piperine and piperlonguminine in rat plasma after oral administration of alkaloids from Piper longum L.: Application to pharmacokinetic studies in rats.* Journal of Chromatography B, 2011. **879**(27): p. 2885-2890.
[18] Shao, Y., et al., *Simultaneous determination of eight bioactive components of Qishen Yiqi Dripping Pills in rat plasma using UFLC–MS/MS and its application to a pharmacokinetic study.* Biomedical Chromatography, 2017. **31**(8): p. e3941.
[19] Khanra, S., et al., *Development and Validation of an RP-UFLC Method for the Estimation of Oxaliplatin Drug for the Preparation of Oxaliplatin Nanoparticles.* Current Pharmaceutical Analysis, 2024. **20**(8): p. 863-873.
[20] Nalini, C.N. and N. Mathivanan, *A review on analytical methods of irbesartan and its combinations in pharmaceutical dosage forms.* Current Pharmaceutical Analysis, 2020. **16**(8): p. 1020-1029.