



SPECTROPHOTOMETRIC DETERMINATION OF IRBESARTAN BY NQS REAGENT IN BULK AND TABLET DOSAGE FORM

Gousia Tazeen, K.Manju Sri, P.Lekhana, Y.Padmavathi, N.Raghavendra Babu*

G.Pulla Reddy College of Pharmacy, Mehdiapatnam, Hyderabad, Telangana-500028, India.

Abstract : A unique, simple, sensitive, and efficient approach has been developed for the measurement of Irbesartan in bulk and pharmaceutical formulations using the 1,2-naphthoquinone-4-sulphonic acid (NQS) reagent. The aim of this analytical validation method is to verify its effectiveness by laboratory testing, proving that the technique meets the necessary minimum standards for laboratory use. This approach was created using the chromogenic reagent NQS in a basic buffer at pH 12, producing a reddish-brown chromogen with an absorption maximum at 467 nm. This method can be efficiently employed to quantify drug content in pharmaceutical formulations. The analysis results have been statistically validated. A coefficient of determination (r^2) of 0.998 demonstrated compliance with Beer-Lambert's law within the concentration range of 100 to 150 $\mu\text{g/mL}$. The limit of quantification and limit of detection were established at 21.16 $\mu\text{g/mL}$ and 64.14 $\mu\text{g/mL}$, respectively.

IndexTerms - Irbesartan, NQS reagent, Pharmaceutical formulation, Chromogenic reagent.

I.INTRODUCTION

Irbesartan is employed either alone or in conjunction with other drugs to regulate hypertension. It was also employed to address renal disease associated with diabetes in persons with type 2 diabetes (a condition marked by the body's inadequate utilisation of insulin, resulting in an inability to control blood glucose levels) and hypertension [1]. Irbesartan is classified as an angiotensin II receptor antagonist. It operates by obstructing the actions of particular natural compounds that constrict blood vessels, so promoting smoother blood circulation and improving the heart's pumping efficacy[2].

Irbesartan with chemical name of 2-Butyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]-1,3-diazaspiro [4.4]non-1-en-4-one having molecular formula of $\text{C}_{25}\text{H}_{28}\text{N}_6\text{O}$ with molecular weight of 428.5gm [3].

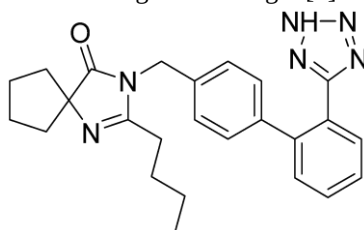


Figure.1. Chemical structure of Irbesartan

The literature survey outlines numerous approaches for the quantitative analysis of Irbesartan. The methodologies utilised for the analysis of Irbesartan include liquid chromatography-mass spectrometry [4-6], high-performance liquid chromatography [7-9], and high-high-performance liquid chromatography-tandem mass spectrometry [10]. Irbesartan has also been documented in combination with other drugs [11-15]. These methods need lengthy and arduous pre-treatment of the materials, as well as stringent cleanup procedures prior to analysis. No formal monograph for Irbesartan exists in any pharmacopoeia, and the quantification of Irbesartan in bulk and pharmaceutical formulations has not been documented.

A literature analysis has revealed a restricted number of spectrophotometric methods for quantifying Irbesartan in bulk pharmaceuticals and pharmaceutical formulations. Only a limited number of UV-Visible spectroscopic techniques have been described, and UV offers various advantages, including non-destructiveness, speedy results, convenience of use, and cost-effectiveness compared to alternative methods. We intended to establish a visible spectroscopic technique. 1,2-naphthoquinone-4-sulphonic sulphonate (NQS) has been employed as a chromogenic reagent for the spectrophotometric quantification of several medicinal amines [16-18]. Nevertheless, the interaction between NQS and Irbesartan has yet to be explored. This work evaluates NQS as a chromogenic reagent for developing a simple and rapid spectrophotometric method to determine Irbesartan in pharmaceutical dosage forms.

II. MATERIALS AND METHODS

Equipment

The UV-Visible spectrophotometer (Shimadzu-1800), interfaced with a computer equipped with Shimadzu UV Probe 2.0 software, has been utilised for every spectrophotometric measurement. The Shimadzu BL-220H electronic balance was utilised for sample weighing. The PCI Analytics 6.5-liter 2000H Ultrasonic bath sonicator is utilised for sonication purposes.

Reagents and solutions

All the reagents and chemicals used are of analytical grade and procured from SD fine chemicals Pvt Ltd, Mumbai. Irbesartan was obtained as gift sample from Aizant drug research solutions Pvt. Ltd, Hyderabad. Irovel 300mg was obtained from the local Pharmacy.

Preparation of 0.5% Naphthoquinone-4-sulphonic acid sodium (NQS) reagent

Dissolve 50mg of NQS in 10ml of double-distilled water. Protect it from direct light.

Preparation of 0.2M Potassium chloride

Within a 100mL volumetric flask, dissolve 0.746 g of KCl. Use distilled water to make up to the volume and sonicate.

Preparation of 0.2M Sodium hydroxide

Dissolve 400mg of NaOH in 100mL of distilled water. Sonicate and make up to the volume with distilled water.

Preparation Basic buffer (pH 12)

Transfer 25ml of 0.2M potassium chloride into a 100ml beaker. Add 66ml of 0.2M sodium hydroxide. Mix the solution and adjust the pH up to 12.

Preparation of Stock solution

After being weighed, ten milligrams of pure Irbesartan were moved to a 10-milliliter volumetric flask and dissolved in ethanol. To reach a concentration of 1000 µg/mL, the flask was shaken, sonicated, and filled with 10 mL of ethanol.

Analytical method development

To reach final concentrations between 100 and 150 µg/mL, fresh aliquots of Irbesartan, ranging in volumes from 1 to 1.5 ml from a stock solution of 1000 µg/ml, have been placed into a series of 10 ml volumetric flasks. In each flask, incorporate 0.5mL of 0.5% NQS reagent and allow it to stand for 5 minutes. Next, add 1mL of basic buffer at pH 12 and allow it to stand for an additional 10 minutes, until the colour develops to a reddish-brown. Add distilled water to reach the desired volume. Using the reagent blank, the absorbance of the generated chromogen was measured at 467 nm. The absorbance versus concentration graphs are plotted as shown in figures 2,3 and 4.

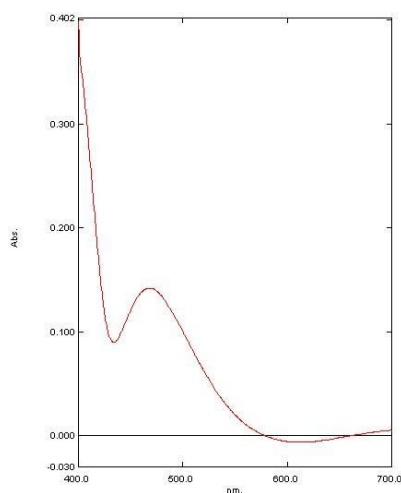


Figure.2. Absorption spectrum of Irbesartan with NQS reagent

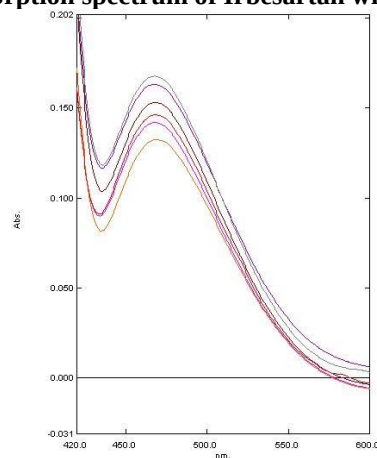


Figure.3. Overlay spectrum of Irbesartan with NQS reagent

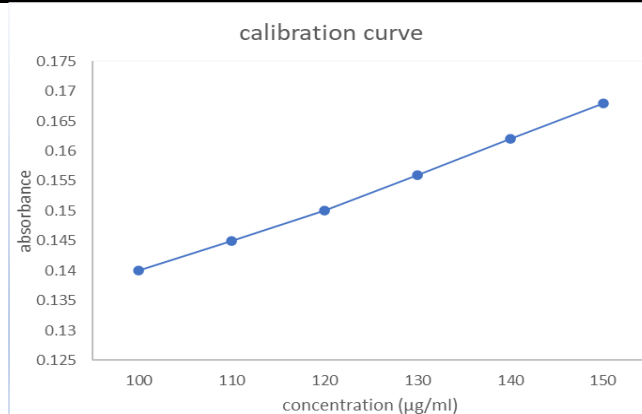
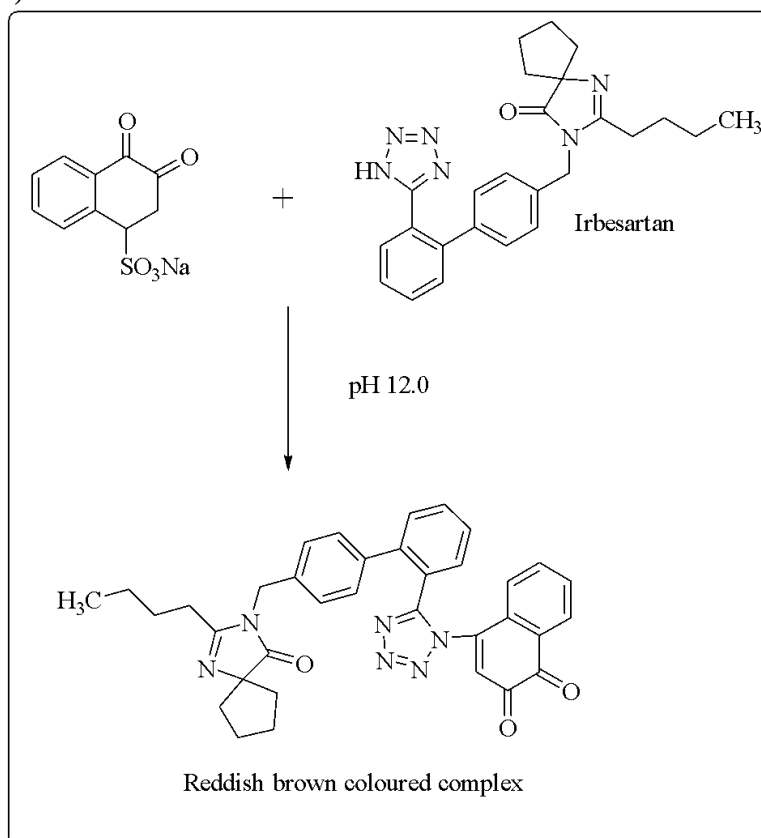


Figure.4. Calibration curve of Irbesartan with NQS reagent

III.RESULTS AND DISCUSSION

In this study, NQS undergoes reaction in the presence of basic buffer, leading to the development of reddish-brown complex that has been measured at 467nm. Here basically NQS reagent will react with the secondary amino group of Irbesartan to form the colored compound.(Scheme-1).



Scheme 1. Reaction of NQS reagent with Irbesartan

The optimisation of spectrophotometric conditions aimed to address the many objectives of technique development. Analytical conditions were refined by a series of exploratory tests.

The ideal circumstances for the reaction were meticulously examined. Optimal absorption at 467nm was achieved instantly with the use of buffer with pH and 0.5% NQS at room temperature, with the product maintaining stability for 60 minutes.

Stability of Chromogen

The interaction between Irbesartan and NQS concluded within 15 minutes. The produced reddish-brown colour was determined to be stable over an extended period, exhibiting no alteration in colour intensity over time. This enabled the approach to be applied for intra-day analyses.

Optimization of Parameter

The ideal concentration and volume were determined based on their capacity to yield maximal absorbance. Various concentrations and volumes were tested for all chemicals by systematically altering one parameter at a time. The optimal concentration of NQS reagent was determined to be 0.5% w/v, with the basic buffer of pH 12.

Quantification

The constraints of Beer's law, the molar absorptivity, and the Sandell's sensitivity values were assessed and are presented in Table 1. Regression analysis of the Beer's law plots at their corresponding λ_{max} values demonstrated a strong association. Graphs depicting absorbance against concentration exhibited a zero intercept and are characterised by the regression equation, $y = bx + c$ (where y represents the absorbance of a 1 cm layer, b denotes the slope, c indicates the intercept, and x signifies the drug concentration in µg/ml) derived by the least-squares approach. The findings are encapsulated in Table 1

Table1. Optical characteristics and validation data of Irbesartan

Parameters	Conditions
λ_{max}	467nm
Beer's law range	100-150 $\mu\text{g/mL}$
Limit of detection(LOD) ($\mu\text{g/mL}$)	21.16 $\mu\text{g/mL}$
Limit of quantification(LOQ) ($\mu\text{g/mL}$)	64.14 $\mu\text{g/mL}$
Regression equation(y)	$y=0.0006x+0.0831$
Intercept	0.0831
Slope	0.0006x
Correlation coefficient (r ²)	0.998

$Y=mx+c$; c denotes the concentration of Irbesartan in microgram per millilitre, while 'y' signifies the absorbance determined at λ_{max} .

Analytical validation [19]

Limit of Detection and Limit of Quantification

The LOQ and LOD has been calculated as given below,

$$\text{LOD}=3.3\sigma/S$$

$$=21.16 \mu\text{g/mL}$$

$$\text{LOQ}=10 \sigma/S$$

$$=64.14 \mu\text{g/mL}$$

The sensitivity of the proposed method for measuring Irbesartan was found to have limits of detection (LOD) of 21.16 $\mu\text{g/mL}$ and quantification (LOQ) of 64.14 $\mu\text{g/mL}$.

Precision

Repeatability, intra-day precision, and inter-day precision studies have been determined in order to ascertain the precision.

Repeatability

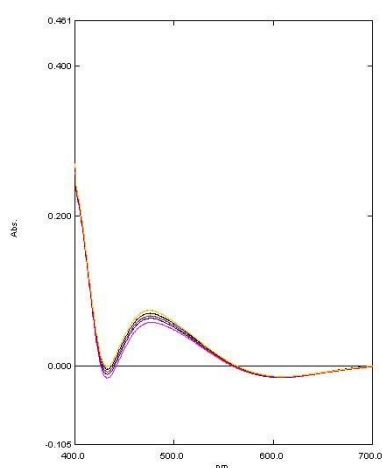
Repeatability results are derived from the preparation of six replicates of standard solutions of Irbesartan. The results are presented in Table 2 and Figure 5.

Table 2. Repeatability data of Irbesartan using NQS reagent

S.No	Concentration ($\mu\text{g/mL}$)	Absorbance	Mean $\pm$ standard* deviation	%RSD
1	130	0.069	0.068 $\pm$ 0.001211	1.78
2	130	0.068		
3	130	0.067		
4	130	0.070		
5	130	0.068		
6	130	0.070		

*Average of six replicates

The values of %RSD was obtained within the limits. Hence the method has found to be repeatable.

Figure 4. Overlay spectrum of Irbesartan for determination of Repeatability (6 $\mu\text{g/mL}$)

Intraday and Interday Precision

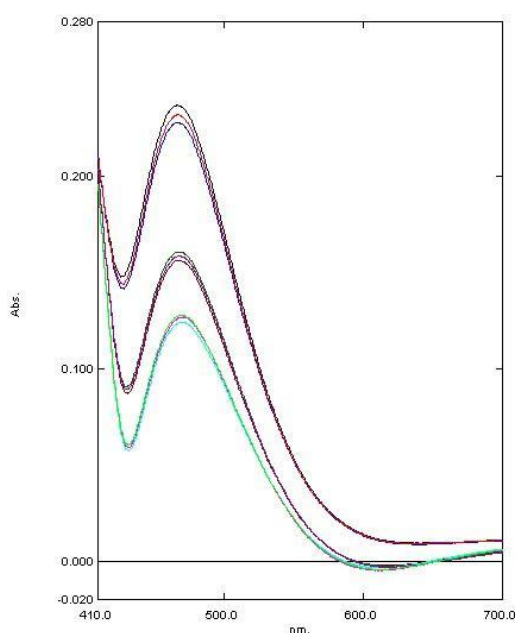
Three replicates of three distinct concentrations of Irbesartan were prepared utilising NQS reagent to assess inter-day and intra-day precision results. The results obtained are presented in the Table 3 and Figure 6.

Table 3. Intraday and Interday Precision data of Irbesartan with NQS reagent

S.No	Concentration (µg/mL)	Intraday-absorbance mean±standard* deviation	%RSD	Interday-absorbance mean±standard* deviation	%RSD
1	110	0.125±0.002	1.66	0.129±0.00152	1.17
2	130	0.158±0.002	1.26	0.163±0.003	1.84
3	150	0.232±0.004	1.94	0.225±0.003	1.33

* Average of three replicates

The developed method demonstrates precision, as the %RSD values are within the acceptance criteria, specifically less than 2%.

**Figure.5. Overlay spectrum of Intra-day Precision of Irbesartan at 467 nm****Accuracy**

The %RSD value for Irbesartan was calculated to evaluate the accuracy of the developed method; the results are shown in Table 4.

Table 4. Accuracy results of Irbesartan using NQS reagent

S.No	Concentration (µg/mL)	Absorbance Mean±standard deviation*	%RSD
1	104	0.053±0.00110	1.88
2	130	0.074±0.00115	1.57
3	156	0.124±0.00153	1.25

*Average of six replicates

The method developed demonstrates accuracy across three distinct levels (80%, 100%, 120%), with %RSD values consistently meeting the acceptance criteria of less than 2%.

The accuracy was assessed and validated through recovery trials at three distinct levels (80%, 100%, 120%) by combining a known quantity of pure medication with the drug formulation, and the percentage recovery has been determined. The results obtained are documented in Table 5.

Table 5. Accuracy results of Irbesartan dosage form using NQS reagent

Tablet used	Amount of sample added (µg/mL)	Amount of standard Added (µg/mL)	Total amount added (µg/mL)	Total amount recovered (µg/mL)	%Recovery
Irovel 300mg	104	130	234	230.72	98.6
	130	130	260	261.82	100.7
	156	130	286	285.14	99.7

*Average of six replicates

The results fall within the range of 98.6-100.7%, which is considered acceptable as it aligns with the acceptance level of 98-102%. The new approach has achieved %RSD and % recovery of the drug product within acceptable limits, confirming its accuracy.

Application of developed spectrophotometric method for the estimation of Irbesartan utilizing NQS reagent.

The established method was further utilised for the Irbesartan quantification in the commercial formulation Irovel 300mg, produced by Sun Pharma in East Sikkim, India, which contains 300mg of Irbesartan. The drug's % purity was estimated. The results obtained are given in Table 6.

Table 6. Assay results of Irbesartan using NQS reagent

Tablet used	Label claim (mg)	Amount found (mg)	%Purity
Irovel-300mg	300	273.6	91.2

*Average of six replicates

The results were determined to be within the permissible range of 90-110%.

IV.CONCLUSION

The developed method is straightforward, sensitive, and reliable, exhibiting commendable precision and accuracy. The proposed method is precise in estimating commercial formulations without the involvement of excipients and other additives. Consequently, the suggested method is applicable for the regular spectrophotometric quantification of Irbesartan in both pure samples and pharmaceutical formulations.

V. REFERENCES

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