



TITLE-SPECTRO SCOPIC DETERMINATION OF AZIDE CONTENT IN IRBISARTAN DRUG SUBSTANCE

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ABSTRACT:

Objective:

The synthesis of drugs often involves various reagents and intermediates, and azides are one such class of compounds that are commonly used in organic synthesis due to their ability to undergo nucleophilic displacement reactions. However, azides can also be potentially genotoxic, posing a risk to drug safety. Therefore, it is important to monitor and quantify azide levels in drug substances to ensure they remain within acceptable limits.

Irbesartan (also known as irbesartan) is a drug used to treat high blood pressure and diabetic nephropathy. The synthesis of irbesartan involve azide-containing intermediates, which necessitates the development of a method for detecting and quantifying any residual azides in the final drug substance. A UV method was developed to identify azide content in Irbesartan.

Methods:

This analytical method, developed for the estimation of azide by adding of ferric ammonium sulfate, gives a characteristic color at neutral pH showing an absorbance maximum of 458nm.

Results and conclusion:

Linearity studies indicated that estimation of azide between 6µg/g – 42µg/g was found to be linear with regression equation of $y=0.004x+0.034(R^2 0.998)$. This method was developed was validated for accuracy, precision, Linearity, Limit of detection (LOD), Limit of quantification (LOQ) studies. The relative standard deviation (% RSD) intraday and inter-day precisions are 0.86 and 0.75 respectively. In accuracy studies, recovery was found in the range of 98-101%. The Limit of detection (LOD) and Limit of quantification (LOQ) were found to be 1.96µg/g and 5.93µg/g respectively. The above analytical parameters indicated that the developed UV spectrophotometric method of azide content in was developed for the Irbesartan drug substance. This method is simple, Accurate & Reproducible, less expensive method.

Azide compounds have the potential to generate genotoxic impurities because of their ability to decompose and produce reactive nitrogen species. These species can damage cellular components, increasing the risk of genotoxic effects.

KEYWORDS:

Sodium azide, Irbesartan (IRB), Spectroscopic estimation of azide, Ultra violet spectrophotometric method of azide.

INTRODUCTION:

Irbesartan(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) is an angiotensin II receptor antagonist primarily used to treat hypertension[1]. Like other medications in this class, Irbesartan is indicated for managing high blood pressure. Additionally, it may help delay the progression of diabetic nephropathy and is also recommended for reducing renal disease progression in patients with type 2 diabetes[2]. Irbesartan (fig.1) is related to the vasoconstrictor peptide class of drugs called angiotensin receptor blockers (ARBs). By Using this relaxing blood vessels so that blood can flow more easily[3]. Azide (Fig.2) is the anion with the formula N_3^- . It is the conjugate base of hydrazoic acid (HN_3). Azide ion is a linear anion that is isoelectronic with CO_2 and N_2O . Per valence bond theory, azide can be described by several resonance structures, an important one being $N^-=N^+=N^-$. Azide is also a functional group in organic chemistry, RN_3 [4]. The principal source of the azide moiety is sodium azide. Nitrous oxide (N_2O) with sodium amide ($NaNH_2$) in liquid ammonia produce Sodium azide is produced. The overall stoichiometry is given by [5]. Some organic and other covalent azides are classified as highly explosive and toxic. Organic azides engage in useful organic reactions. The terminal nitrogen is mildly nucleophilic. Azides readily release diatomic nitrogen, a characteristic that is utilized in various reactions, including the Staudinger ligation, the Curtius rearrangement, and the synthesis of γ -imino- β -enamino esters.[6-7]. For the preparation of Irbesartan by reacting 1-[(2'-carboxamidobiphenyl-4-yl)methyl]-2-n-butyl-4-spirocyclopentane-2-imidazolin-5-one(XV) with trialkyl tin chloride selected from tributyl tin chloride and alkyl azide selected from sodium azide, potassium azide in a solvent selected from aromatic hydrocarbons selected from benzene, toluene, xylenes, most preferably o-xylene[8]. The Literature survey reveals many analytical methods for the determination of azide including Liquid chromatography tandem mass chromatography (LC MS MS)[9-10] and

Ionic Chromatography[11-12], High performance liquid chromatography (HPLC) [13] and Gas chromatography GC [14-15]. Therefore, an attempt was made to develop and validated[16] a simple spectrophotometric method for the determination of azide[17-19] levels of the present drug in Active pharmaceutical ingredient(API) and formulations i.e. Tablets [20].

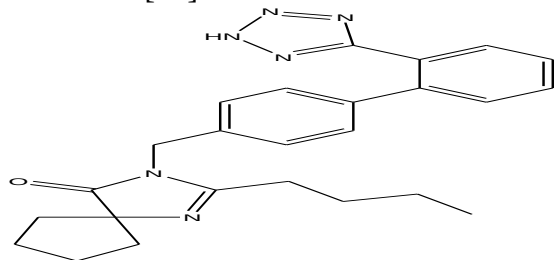


Fig. 1: Structure formulae of Irbesartan

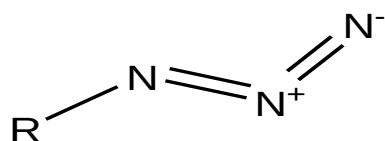


Fig. 2: Structure formulae Azide

MATERIALS AND METHODS

MATERIALS

- (1). 1M Hydrochloric acid solution
- (2). Sodium azide
- (3). Water
- (4). 1M Sodium hydroxide solution
- (5). Ferric ammonium sulfate solution about 2.0 M solution in water
- (6). 2% w/v Sodium sulfate solution in water

Materials procured from Sigma Aldrich and Avra,

INSTRUMENTATION:

- (1) Ultraviolet Visible Spectrophotometer (Suggested system Shimadzu, model No. UV-1601) with 1cm cell.

METHODS:

Preparation of solutions:**Identification test for azide:**

Dissolve 2.1 µg/ml of standard solution in water, add 5ml of potassium hydroxide solution, keep 30 min aside at room temperature shake 5 minutes intervals, add 25ml of sodium sulfate solution, neutralize the solution with hydrochloric acid solution. Add 25 ml of Ferric ammonium sulfate solution, make up to 100ml with sodium sulfate solution. Scan the solution between 600nm-400nm and measure absorbance maxima at 458nm.

Preparation of sample solution:

Transfer about 1g of sample into 100ml of volumetric flask, add 10ml Potassium hydroxide solution, keep 30 min aside at room temperature shake 5min intervals, add 25 ml of sodium sulfate solution, neutralize the solution with hydrochloric acid, add 25ml of ferric ammonium sulfate solution, make up to 100 ml with sodium sulfate solution, filter with 0.45µ filter paper.

Preparation of blank solution:

Prepare blank solution into a 100ml volumetric flask transfer 5ml of sodium hydroxide solution, add 25ml of sodium sulfate solution keep 30 min aside at room temperature, neutralize the solution with hydrochloric acid solution. Add 25 ml of Ferric ammonium sulfate solution, make up to 100ml with sodium sulfate solution.

Procedure: Scan the standard solution and sample solution with blank correction between 600nm-400nm and measure absorbance at 458nm values.

Preparation of calibration curve:

Aliquots of standard solutions from 0.006µg/ml – 0.042 µg/ml transfer into series of 100ml volumetric flasks, add 5ml of 0.1M Potassium hydroxide solution, add 25ml sodium sulfate and neutralize the solution with hydrochloric acid solution, add 25 ml of Ferric ammonium sulfate solution and make up to 100ml with sodium sulfate solution measure the absorbance at 458nm against the blank and calibration curve was plotted.

Method validation:

The developed method was validated for parameters like Specificity, Linearity, Precision, Accuracy, Limit of detection (LOD) and Limit of quantification (LOQ). The data for which presented in sample the low values of %RSD, value indicate that all the methods are precise and accurate.

Specificity:

In the specificity experiment all potential impurities spiked to the analyte. The developed method is used for the determination of azide in the presence of potential impurities.

Recovery (or) accuracy:

The sample spiked with a known amount of sodium azide (at LOQ, 100%, 150% levels) of analyte concentration levels each concentration level is prepared for three

times and measured the absorbance is at 458nm. The percentages of the recoveries are calculated.

Solution stability

In the solution stability experiment prepare the sodium azide solution in a volumetric flask tightly closed; keep it at room temperature. Measure the absorbance at 10 min intervals.

Linearity, Limit of detection (LOD) & Limit of quantification (LOQ)

Aliquots solution of standard solutions from 0.006µg/ml – 42 µg/ml transfer into series of 100ml volumetric flasks, add 5ml of 0.1M Potassium hydroxide solution, add 25ml sodium sulfate and neutralize the solution with hydrochloric acid solution, add 25 ml of Ferric ammonium sulfate solution and make up to 100ml with sodium sulfate solution measure the absorbance at 458nm against the blank and calibration curve was plotted, determine LOD, LOQ.

Precision

Precision was determined by performing content of azide by spiking with the known concentration of sodium azide in Irbesartan drug substance under the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision) performed on different consecutive days by different analysts, at working concentration.

Intraday precision

In the precision experiment prepared six individual preparations of mg/ml of Irbesartan solution spiked with 0.021µg/ml of azide stock solution. Measure the absorbance at 458nm and calculate the % Relative standard deviation of impurity.

Intermediate Precision (Interday precision / Ruggedness)

Six consecutive recordings of absorbance at 478 nm of the azide from the same homogeneous mixture at working concentration on three consecutive days by three different analysts. Calculate the % Relative Standard Deviation of impurity.

Results and Discussions

The identification test proposed absorbance max at 458nm in the neutral solution.

Precision

System precision

Six replicate recordings of absorbance at 458 nm of standard solution at working concentration showed a % Relative Standard Deviation less than 1.0 concerning absorbance for the azide, which indicates the acceptable. System precision results are tabulated in (Table 1).

Table 1. System precision results of azide

Number of determinations	Absorbance
1	0.117
2	0.115
3	0.116
4	0.117
5	0.118
6	0.117
Mean	0.117
SD ^a	0.001
%RSD ^a	0.86

^a **Standard deviation,** ^a **Relative standard deviation**

Intraday and Inter day Precision were determined by performing content of azide by spiking with the known concentration of azide to the Irbesartan drug substance under the tests of (i) Repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intraday precision)

Six consecutive recordings of absorbance at 478 nm of the azide from the same homogeneous mixture at working concentration showed % RSD less than 1, which indicates that the developed method is precise by the test of consistency and hence can that the method gives consistently reproducible results (Table 2).

Table 2: Intraday precision results of Azide Content (21µg/g) spiked in Irbesartan drug substance

Number of determinations	Azide Content(µg/g)
1	0.114
2	0.116
3	0.116
4	0.114
5	0.115
6	0.116
Mean	0.115
SD	0.001
%RSD	0.85

Intermediate Precision (Inter day precision / Ruggedness)

Six consecutive recordings of absorbance at 478 nm of the azide from the same homogeneous mixture at the working concentration on three consecutive days by three different analysts, showed % Relative Standard Deviation (RSD) less than 1 within and between days, which indicates the method developed is inter day precise / rugged (Table 3).

Table 3: Interday precision results of azide (21 μ g/g) spiked to Irbisartan drug substance

S.no	azide content(μ g/g)		
	Day 1	Day2	Day 3
1	21.32	21.42	21.25
2	21.05	21.25	21.10
3	21.00	21.05	21.00
4	21.20	21.20	21.35
5	21.05	21.21	21.05
6	21.00	21.10	21.02
Mean	21.10	21.21	21.13
SD	0.13	0.13	0.14
%RSD	0.61	0.61	0.67

Linearity

The zero order Ultraviolet spectrum of azide content in the irbisartan drug substance showed an absorbance max at 458nm. The calibration plot was constructed between the absorbance and concentration. The calibration plot Fig.3 was found to be linear over the range of 6-42(μ g/g) with a correlation coefficient of 0.996. The limit of detection and limit of quantification are 1.96 (μ g/g) & 5.93(μ g/g) respectively.

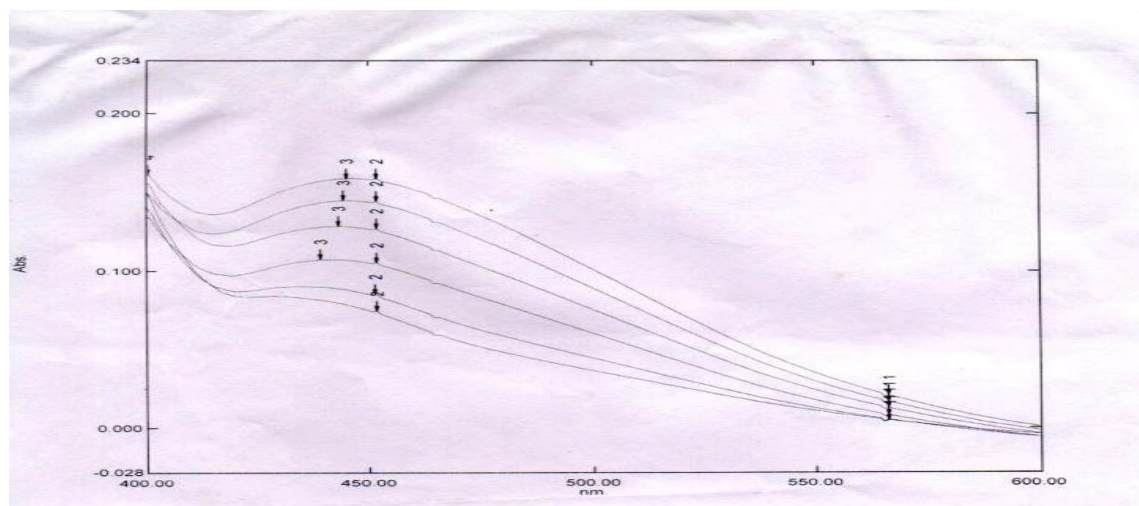


Fig.3 -linearity

Accuracy

Accuracy was determined by using the spiking experiments the recovery was determined by spiking of the sample at three different levels (Limit of detection to 150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in (Table 4). The accepted limits of recovery are 98% - 101% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 4: Recovery results from spiking of Irbisartan drug substance with azide

Accuracy Average of triplicate	Level-1	Level-2	Level-3
	Day 1	Day2	Day 3
Amount Added (µg/g)	5.93	21.00	32.00
Amount Found (µg/g)	5.85	20.70	31.50
Recovery(%)	98.65	98.57	98.44

Sensitivity

The sensitivity of measurement of azide content by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and limit of detection (LOD). The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 1.96 µg/g and 5.93 µg/g respectively. Optical characteristics results are summarized in (Table 5).

Regression analysis data summarizes of validation parameters for the proposed method.

Table-5: Regression analysis data

S.No	Parameters	Azide
1	Wavelength measurement	458nm
2	Beer's law limit (ug/g)	6-42
3	Regression equation ($Y = mx + c$)	$Y = 0.011x - 0.034$
4	Slope	0.011
5	Intercept	0.0034
6	Correlation Coefficient (R^2)	0.996
7	Precision(%RSD)	
	System precision(n=6)	0.86
	Intraday (n=6)	0.85
	Interday (n=6)	0.61
8	Accuracy (%Recovery) (For drug substance)	98.5
9	Limit of detection (µg/g)	1.96
10	Limit of quantification (µg/g)	5.93

Conclusion:

A cheap and a rapid Ultraviolet spectrophotometric method was developed and validated for the quantitative estimation of azide in Irbesartan drug substance as per International Council for Harmonisation (ICH) guidelines[21]. The developed method resulted in azide exhibiting linearity in the range 6µg/g to 42µg/g. The interday and intraday precision is exemplified by a relative standard deviation of 0.85 % and 0.61%. Percentage Mean recovery was found to be in the range of 98-101%, during accuracy studies. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.98 µg/g and 5.93 µg/g respectively. It can be concluded that the developed Ultraviolet (UV) spectrophotometric method is accurate, precise, linear, rugged, and robust, making it suitable for the routine analysis of azide content in Irbesartan drug substances.

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7. Reaction conditions:
a) Sodium azide (4 eq.), acetone, reflux for 18 hours, 92% chemical yield.
b) Isopropylamine, titanium tetrachloride, diethyl ether, reflux for 14 hours, 83% yield.
8. Azide 2 is formed through a nucleophilic aliphatic substitution reaction, where the azide anion displaces chlorine in 1. The ketone reacts with the amine to form an imine, which tautomerizes to the enamine in 4. In the subsequent rearrangement, nitrogen is expelled, and a proton is transferred to 6. The final step involves another tautomerization, leading to the formation of enamine 7 as a mixture of cis and trans isomers.
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