# REVIEW OF SPECTROSCOPIC INVESTIGATION ON FUNDAMENTAL MODES OF DFT STUDIES ISRADIPINE

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**Abstract**

The main scope of the project work is evaluation of isradipine tablets it comprises of, Determination of physical properties. like appearance, solubility, freezing point , loss on drying, sulphated ash and heavy metals. Identification of Isradipine using infrared spectroscopy. Estimation of specified and unspecified impurities using high performance liquid chromatography. FTIR study was performed for analyzing and assigning the modes of vibrations and to spot the presence functional groups. H1-NMR and C13-NMR spectral studies revealed its structural identification

## INTRODUCTION

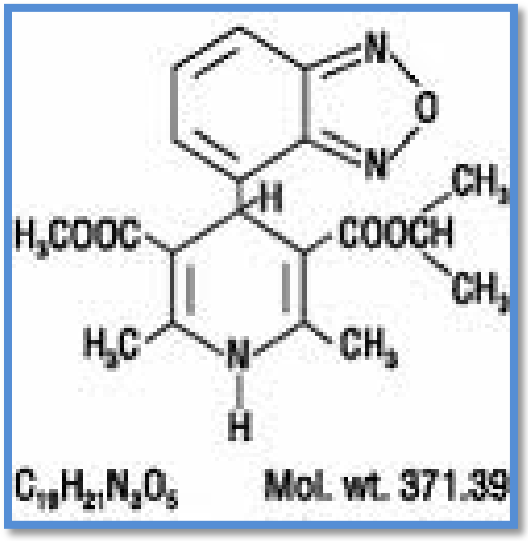
### Introduction

Isradipine is during a group of medicine called calcium channel blocker. It works by releasing the muscles of our heart and blood vessels. Isradipine is employed to treat high vital sign (hypertensive). it's some times given with other vital sign medications. Isradipine is light sensation, low actinic glassware should be used for all solution preparation be done under low actinic lighting. Isradipine may be a dihydropyridine calcium channel blocker. It binds to calcium channels with high affinity and specificity inhibits calcium flux into cardiac and smooth muscle. the consequences observed in mechanistic experiments in vitro and studied in intact animals and man is compatible with this mechanism of action and is typical of the category . Other drugs will affect isradipine cimetidine (Tagamet, tagamet HB) fentanyl (Duragesic, lonsys, actiq) or

rifampin

(Rifadin, Rimactane, Rifater). These could also be other drugs which will interact with isradipine. it'll be use hypertension. Isradipine is indicated within the management of hypertension. it's going to be used alone or concurrently with thiazide-type diuretics. Isradipine decreases peripheral resistance like other calcium blockers. Dynacirc (Isradipine) may occasionally produce symptomatic hypotension. How over symptoms like syncope and severe dizziness have rarely been reported in hypertensive patients administered isradipine, particularly at the initial recommended doses. Although acute hemodynamic studies in patients with congestive coronary failure have shown that isradipine reduced after load without impairing myocardial contractivity, caution should be exercised when using the drug in congestive coronary failure patients particularly n combination with a beta-blocker. Nitro glycerin: Dynacirc (Isradipine) has been safely Co-administered with nitroglycerin.

**Isradipine structure:**




Molecular formula C19 H21 N3 O

Molecular weight 371.39

Isradipine contains not less than 98.0 percent and not more than 102.0 percent of C19H21N3O5 Calculated on the dried basis.

**(A) Description:** Yellow fine crystalline powder.

**(B) Identification:**

**Infrared Spectroscopy** **:** The IR spectrum of sample should be concordant with the Spectrum of Isradipine reference standard.

**(C) Meting Range** **:** 168ºC

**(D) Loss on Drying** **:** 0.19% ( NMT =0.20% w/w )

**(E) Residue on Ignition**

**(Sulphated ash)** **:** 0.06% (NMT =0.10% w/w)

**(F) Heavy Metals** **:** ( NMT = 0.002% w/w )

**(G)Chromatographic purity** **:** **(By HPLC)**

**Experimental Methods**

The 1H & 13C NMR spectra were recorded on a BRUKER 400 MHz NMR spectrometer using DMSO as solvent. the space temperature Fourier transform infrared spectra of 2M5NA were recorded within the range 400-4000 cm-1 at a resolution of ±5 cm-1 employing a BRUKER spectrophotometer equipped with a LiTaO3 detector, a KBr beam splitter, a He-Ne laser source and a boxcar atomization used for 250 averaged interferograms collected for both the sample and therefore the background. High performance liquid chromatography. an appropriate HPLC instrument equipped with UV detector. Column using Themoquest Hypersil ODS 150 X 4.6mm, 5µm (or) equivalent. The quantum chemical computations of this heterodimer were employed with density functional theory (DFT) method by using Gaussian 09 [11] program package with basis set of 6-311++G (d,p). The SCXRD crystallographic information file (CIF) file of isradipine was used as an input. The optimized structure of this crystal was visualized using Chemcraft v1.8 software [13]. the very best occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), Molecular electrostatic potential (MEP) and hyperpolarizability of the isolated molecule were calculated from an equivalent basis set. the entire charge

spread is described by MEP. HOMO-LUMO energy gap affirms the chemical softness of the fabric . of these parameters were visualized using Gaussview software

**Disregard limit:**

Disregard any peak observed in blank and any secondary peak **Result and discussions**

INFRARED SPECTROSCOPY:

| Region in cm and intensity | Stretching |
|---|---|
| **3350** | N-H str |
| **3000-3100** | c≡c-H str |
| **3080** | C-H (aromatic) |
| **1710** | C=O str |
| **1680-1650** | N=O str |
| **2960-2850** | C-H str |
| **1020-1070** | C=C – O –C |
| **1500-1570** | Ar – NO2 |
| **1620-1535** | -N=0 |
| **~3500** | N-H str (1$^{o}$amine, amide) |
| **~3400** | N-H str (1$^{o}$amine, amides) H-bond |

Compare the spectrum with the reference spectrum. The sample spectrum exhibits maxima only at the utmost wave length that of reference spectrum. After analysis remove the pellet holder from the compartment discard the pellet.

**IR ABSORPTION SPECTRUM:**

**Sample Preparation:**

Isradipine indentification of infrared spectroscopy. The IR range is identified peaks in 1702 are **thanks to** c=o stretching (Ketone). From the position of absorption bands shows a band at 3348.76 in N-H stretching, 290 in C-H stretching, 1275.41 is (N=O) nitro compounds, 3080 is C-H stretching is aromatic ring and 1055.17 is C=C-O-C stretching. Absorption position of 602, 622.08, 670.11, and 696.84 is C-H def. There absorption at 2991.84 is C-H stretching.

| **Region in $cm^{-1}$ and intensity** | **Absorption** |
|---|---|
| **1702** | C=O str (Ketone) |
| **3348.76** | N-H str |
| **2940** | C-H str |
| **1275.41** | -N=0 str |
| **3080** | C-H str (Aromatic ring) |
| **1055.17** | C=C-O-C |
| **670.11, 696.84** | C-H def |
| **2991.84** | C-H str in $CH_3$ |

**Melting Point:**

Ensure that the sample is within the sort of fine powder, if not crush the sample to a fine powder. Fill sample during a meeting point capillary , to approximately 2mm height top it and insert it into the sample hole provides on the highest of the instrument. Observe through view glass the beginning of meeting. The lower display show the initial temperature press ‘stop’ at the top of the meeting. Now upper display shows the ultimate temperature write the initial and final temperatures. Stop the heater. Carefully remove the capillary from the part and permit the oil bath to chill right down to temperature.

**LOSS ON DRYING:**

Accurately weighted a clean previously dried(for half-hour ) and cooled LOD bottle(W1) Mix and weigh accurately about 1gm of sample with the LOD bottle(W2).Place the loaded LOD bottle within the drying chamber. Dry the sample at the temperature 105degree c for 4 hours. Take the LOD bottle and keep it inside the desiccators. Allow it to achieve the space temperature and weigh(W3).Continue the drying until constant weight is obtained. Until two consecutive weighing don't differ by quite 0.50mg per gram of substance taken, the second weighing following a further hour of drying.

**Calculation:**

$$\text{Loss on drying (\%W/W)} = \frac{Loss\ of\ weig\,ht\ (W2-W3)}{weig\,ht\ of\ sample\ (W2-W1)} \times 100$$

**Where,**

W1- Empty weight of the LOD Bottle with lid.

W2- Weight of the LOD bottle with lid and sample before drying.

W3- Weight of the LOD bottle with lid and sample after drying.

**RESIDUAL IGNITION (sulphated Ash)**

Weigh accurately about 1gm of the sample into a previously cleaned, ignited, and cooled and weighed a platinum/silica crucible. Moisten the sample with vitriol , and keep the crucible within the electric bunsen and ignite gently until the sample get charged continue ignition till no fumes are evolved. Transfer the crucible into muffle furnace.

Ignite it at 600+- 50 °c until the carbon is consumed. Allow the crucible to chill to temperature and weigh continue the ignition until constant weight is obtained. Until two consecutitive weighing don't differ by quite 0.50mg per gram of substance taken the second weighing following a further 15 to half-hour ignition period.

**Calculations:**

Residue on ignition (%W/W) = $\frac{weig\,ht\ of\ the\ residue\ (in\ g)}{weig\,ht\ of\ the\ sample\ (in\ g)} \times 100$

**HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OR HIGH PRESSURE LIQUID CHROATOGRAPHY HPLC**

RELATED SUBSTANCES BY HPLC CONDITIONS:

CHROMATOGRAPHIC PURITY [BY HPLC]

**CHROMATOGRAPHIC CONDITIONS:**

| | | |
|---|---|---|
| Wave Length | : | 230 nm |
| Flow Rate | | : 1.0 mL/min |
| Injection volume | : | 20µL |
| Date Acquistion Time | : | 45 min |
| Total Run time | | : 55min |

**MOBILE PHASE :**

**SOLUTION A :**

Dissolve about 1.54g of ammonium acetate (AR grade) in 1000mL of HPLC grade/Milli-Q water and filter through 0.45µ membrane filter.

**SOLUTION B:**

Degassed mixture of methanol: Acetonitrile (65:35v/v)

**GRADIENT PROFILE;**

| TIME MIN | % MOBILE PHASE A | %MOBILE PHASE B | FLOW(mL/MIN) |
|---|---|---|---|
| **0.01** | 90 | 10 | **1.0** |
| **35** | 20 | 80 | **1.0** |
| **45** | 20 | 80 | **1.0** |

| EQUILIBIRIUM TIME: | | | |
|---|---|---|---|
| **46** | **90** | **10** | **1.0** |
| **55** | **90** | **10** | **1.0** |

Flow rate and other mobile phase parameters may be adjusted to achieve system suitability requirements

## ISRADIPINE IMPURITIES SOLUTION PREPARATION: [RESOLUTION SOLUTION]

**SOLUTION A:**

Weigh accurately about 20mg of Isradipine USP reference standared.Internal reference standard in a 10mL volumetric flask dissolve in 0.5mL of methanol and make up to volume with methanol.

**SOLUTION B:**

Weigh accurately about 10mg of Isradipine related compound-A into a 10mL of volumetric flask and dissolve in 0.5mL of methanol and dilute to volume with methanol.

**System Suitability solution: (Solution-c)**

Pipette out 4mL of solution A and 0.4mL of solution B into a 10mL volumetric flask and dilute to volume with methanol.

**Run Sequence:**

Inject methanol (blank) and record the chromatogram.

(i) Inject resolution solution'c' record the chromatogram.

(ii) Inject standard preparation (solution E) in five replicates

(iii) Inject 50ppm IS4 standard solution record the chromatogram.

(iv) Inject the preparation (solution E) record the chromatogram

(v) Reject any peak arising in the test preparation, due to methanol(blank)

**System Suitability criteria: (Solution-C)**

- The resolution 'R" between Isradipine and Isradipine related compound-A in solution –c is not less than 1.5
- The relative standard deviation for the area of 5 injections of Isradipine standard preparation (solution –E) is not more than 2.0

observed in standard from the sample chromatogram.

**Standard preparation:**

**Soution-D:** Diluted 4mL of solution-A to 10mL with methanol.

**Solution-E:** Diluted 3mL of solution-D to 100mL with methanol.

**IS4 impurity stock solution:**

Separately weigh about 8 mg of IS4 impurity into a 100mL volumetric flask dissolve and make up to the volume with methanol. Further dilute 1mL of the above solution into 100mL volumetric flask and dilute and make up to the volume with methanol.

**Impurity Stock solution:**

Weigh accurately 10mg of each impurity ISO, IS1, IS2, IS3, IS5, IS6, IS7, IS8, IS9 and Isradipine into a 100mL volumetric flask dissolve and dilute with methanol.

**50ppm IS4 standard solution:**

Pipette out 5mL of IS4 impurity stock solution into 10mL volumetric flask dilute and make up to the volume with methanol.

**Test sample preparation:**

Weigh accurately about 200mg of Isradipine sample in a 25mL volumetric flask; dissolve in 5mL of methanol and make up to volume with methanol.

**SPIKE SMAPLE PREPARATION:**

Weigh accurately about 200mg of Isradipine sample in a 25mL volumetric flask and add each 6mL impurities stick solution and 12.5mL of IS4 impurity stock solution then dissolve and make up to volume with methanol.

**RELATIVE RETENTION TIME (RRT) TABLE:**

Retention time of Isradipine = About 30.0 minutes

| NAME OF THE IMPURITY | RELATIVE RETENTION TIME | RELATIVE RESPONSE FACTOR(RRF) |
|---|---|---|
| IS0 | 0.77 | 2.79 |
| IS1 | 0.81 | 1.86 |
| IS2 | 0.42 | 3.53 |
| IS3 | 0.38 | 3.14 |
| IS4 ISOMER 1* | 0.75 | 2.84 |
| IS4 ISOMER 2* | 0.82 | 2.84 |
| IS5 | 0.87 | 0.93 |
| IS6 | 1.11 | 1.07 |
| IS7 | 0.95 | 2.83 |
| IS8 | 0.99 | 0.80 |
| IS9 | 1.19 | 1.95 |
| Isradipine | 1.00 | |

IS4 impurity is observed as two isomeric peaks. Hence it is labeled as IS4 isomer-1 and IS4 isomer-2

**INDIVIDUAL KNOWN IMPURITY:**

$$\frac{\begin{array}{c}Area\ of\ known\ peak\ in\ the\ chromatogram\ of\ test\ (solution-F)\end{array}}{\begin{array}{c}Mean\ area\ of\ principal\ peak\ in\ the\ chromatogram\ or\ standard\ (solution-E)\end{array}} \times \frac{Weight\ of\ USP\ RS/IRS}{10} \times \frac{1}{10} \times \frac{3}{100} \times \frac{25}{Weight\ of\ sample} \times$$

$$RRF\ of\ USP \times Purity \frac{RS}{IRS}$$

Where RRF=Relative Response factor of the specific impurity

**Unknown Impurity:**

$$\frac{\begin{array}{c}\text{Area of unknown peak in the chromatogram of test (solution} - \text{F)}\end{array}}{\begin{array}{c}\text{Mean area of principal peak in the chromatogram or standard (solution} - \text{E)}\end{array}} \times \frac{\text{Weight of USP RS/IRS}}{10} \times \frac{1}{10} \times \frac{3}{100} \times \frac{25}{\text{Weight of sample}} \times \text{Purity of USP RS/IRS}$$

***Total Impurities:***

Sum of known impurities + sum of unknown impurities

**Known Impurities:**

| **Impurity:** | **Chemical name of impurity:** |
|---|---|
| IS0 | 4-Methyl Benzofurazan |
| IS1 | 4-Bromomethyl benzofurazan |
| IS2 | 4-Hydroxymethyl benzofurazan |

IS3 Benzofurazan-4-carboxaldehyde

IS4 2-Acetyl-3-(benzofurazan-4yl)acrylicacidmethyl ester.

IS5 4-(4-benzofurazanyl)-1,4-dihydro-2 , 6-dimethyl-3,5-pyridine dicarboxylic acid dimethyl ester

IS6 4-(4-Benzofurazanyl)-1, 4-dihydro-2, 6-dimethyl-3,5-prridine dicarboxylic acid di isopropyl ester

IS7 7-bromo-4-methyl benzofurazan

IS8 Isopropyl methyl-4-(4-benzofurazanyl)-2, 6-dimethyl-3, 5-pyridine dicarboxylate

IS9 Iso propyl methyl-4-(2, 1, 3-benzoxadiazol-4-yl)-2-[2-(2,1,3-benzoxadiazol-4-yl)ethenyl]-1,4-dihydro-6-methyl pyridine-3,5-dicarboxylate

**HEAVY METALS:**

(i) Reagents:

(a) Water : Distilled water (or) Milli-Q-Water

(b) Acetic acid (IN) : Dilute 6ml of Glacial Acetic acid to 100ml with water

(c) Ammonium Hydroxide (6N0): Dilute 44.5ml of 13.5ml Ammonia solution to 100ml with water.

(d) Hydrochloric acid (6N) : Dilute 51ml of concentrated HCl to 100ml with water

(ii) Preparation of Glycerin base TS:

To 200mg of glycerin, add water to bring the total weight to 235gm. Add 140ml if 1N NaOH and 50ml of water.

(iii) Preparation of Thioacetamide TS:

Dissolve 4gm of Thioacetamide is 100ml of water.

(iv) Preparation Of Thioacetamide – Glycerin base TS:

Mix 0.2ml if thioacetamide Ts and 1ml of glycerin base TS and heat is a boiling water bath for 20 seconds, use the mixture immediately

**Lead Nitride Stock Solution:**

Dissolve 159.8mg of load nitride is 10ml water to which has been added 1ml of nitric acid, mix and dilute to 1000ml with water. If should be shared is glass containers free from soluble lead salts. The lead nitrate stock solution prepared can be used for a period of three months from the data of preparation.

**Standard Lead Solution:**

On the day of use dilute 10ml of lead nitride stock solution to 100ml with water. (Each ml of standard lead solution is equivalent to 10mg of lead). A comparison solution prepared on the basis of 100ml of standard lead solution per gm of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

**Preparation of pH 3.5 Acetated buffer:**

Dissolve 25gm of Ammonium Acetated in 25 ml water and add
38 ml of 6N HCl acid. Adjust the pH to 3.5, if necessary hydroxide, dilute to 100ml with water and mix.

**Standard Preparation:**

Pipette out 4ml of the standard lead solution is to a suitable test tube, and add 10ml of 6N HCL acids.

**Test preparation:**

Pipette out ml of the standard lead solution in to a suitable test tube, and add 10ml of 6N hydrochloric acid

**Test Preparation:**

Weigh 1gm of sample (from the formula 2.0/1000L,where 'L' is heavy metals limit in %) in suitable silica crucible, add sufficient quantity of sulphuric acid to web the substance. Ignite it carefully at a low temperature in electrical Bunsen burner until thoroughly charred. (The crucible may be loosely covered with suitable lid during the charring). Add to the carbonized mass 2ml of nitric acid and 5 drops of sulphuric acid and heat it cautiously until white fumes no longer are evolved. Ignite in the muffle furnace at 500$^{o}$-600$^{o}$c until the carbon is completely burned off (No longer than 2 hours). If carbon remains, allow to residue to cool, add a few drops of sulphuric acid, evaporate and ignite again. Cool, add 5ml of 6N HCL acid cover and digest on a stream both for 10 minutes. Cool and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5ml portion of 6N HCl acid, and transfer the rinsing to the test tube.

**Procedure:**

Adjust the answer in each of the tubes containing the quality preparation, the test preparation and therefore the monitor preparation with ammonia water , added cautiously and drop risen to a pH of 9, cool, and adjust with glacial ethanoic acid , added drop wise, to a pH of 8, then add 0.5ml in excess. employing a pH meter or short-range pH indicator paper as external indicator, check the pH and adjust if necessary, with in ethanoic acid or 6N ammonia water to a pH between 3.0 and 4.0, filter if necessary, washing with a couple of ml of water in to a 50ml color comparison tube, then add 1.2 ml of thio acetamide- Glycerin base Ts, dilute with water to 50ml, mix and permit to face for two minutes and consider down ward over a white surface. the color of the answer from the test preparation isn't darker than that of the answer from the quality preparation.

**MELTING POINT:**

Melting point of Irradipine = 168$^{o}$c

**Sulphated Ash: (Residue on Ignition)**

Temperature = 600$^{o}$c

Weight of Ash = 0.00056 g

**Calculation:**

Sulphated Ash = 0.06% (NMT 0.10% W/W)

**LOSS ON DRYING:**

Loss of Weight = 0.00193 g

LOD = 0.19% (NMT0.20% W/W)

**Chromatography Purity by HPLC:**

**Standard Preparation:**

| | | | |
|---|---|---|---|
| Retention time | | = | 30.4 |
| Run – 01 | Area | = | 1290.21 |
| Run -02 | Area | = | 1281.68 |
| Run – 03 | Area | = | 1285.10 |
| Run – 04 | Area | = | 1268.96 |
| Run – 05 | Area | = | 1291.01 |

Area of Isradipine = 1283.40

**RELATED SUBTANCES BY HPLC:**

Distance about 3.08001gm of ammonium acetate (AR grade) in 2000ml of HPLC grade/Milli-Q-water and filter through 0.45ml membranes filter.

**Solution B:**

Degassed mixture of methanol added to 1300ml and then added to 700ml of acetonitrile and diluted in.

HPLC grade Acetonitrile

Detector wave length : 230nm

Injection Volume : 20 ml

Flow rate : 1.0ml/min

Date Acquisition time: 45min

Total Run Time : 55 min

**Diluent: -** Methanol

**Resolution Solution:**

Weigh accurately about 19.95 mg in 10ml volumetric flask dissolved in 0.5ml methanol and make up to volume.

**Solution B:**

**Solution –B:** Weight of isradipine = 10.1 mg

10.1mg of Isradipine related compound-A 10ml of volumetric flask, and 0.5ml of methanol and dilute to volume with methanol.

**System Suitability Preparation:**

Pipette out 4ml of solution –A and 0.4ml of solution –B is 10ml of volumetric flask and dilute to volume with methanol.

**Standard Preparation:**

**Solution-D:** Dilute 4ml of solution-A to 10ml with methanol.

**Solution-E:** Dilute 3ml of solution-D to 100ml with methanol.

**Is4 Imparity Stock Solution:** 8.1mg of IS4 impurity in 100ml volumetric flask, further dilute 1ml of the above solution in to 100ml volumetric flask, and dilute to volume with methanol.

**Impurities Stock Solution:**

10.2mg of ISO, 10.1 mg of IS1, 10.1 mg of IS2, 10.1 mg of IS3, 10.1 mg of IS5, 10.08 mg of IS6, 10.2 mg of IS6, 10.2 mg of IS7, 10.1 mg of IS8, and 10.2mg of IS9 and 10.1 mg of isradipine standard is 100ml of volumetric flask, dissolved and dilute to volume with methanol.

**Impurity Mixture Solution:**

Pipette out 2.4ml of impurities stock solution in to 10ml of volumetric flask and 5ml of IS4 impurity stock solution then dilute and make up to volume with methanol.

**50ppm IS4 Standard Solution:**

Pipette out 5 ml of IS4 imparity stock solution in to 10 ml volumetric flask, dilute and make up to volume with methanol.

**Test Sample Preparation:**

**Test Sample Preparation:**

B: no:PID/ISRA/001 Weight of isradipine = 0.19739 g

197.39 mg of dissolved in 25 ml volumetric flask and make up to volume with methanol.

**Spiked Sample Preparation:**

B: no: 197.39 mg of dissolved in 25 ml of volumetric flask and 6 ml impurities stock solution and 12.5 ml of impurities stock solution then dissolved and make up to volume with methanol.

**RETENTION TIME**

IS0 =0.75(RRT=0.77), IS1 =0.78(RRT=0.81), IS4 ISOMER1 =0.73(RRT=0.75), IS4 ISOMER2 =0.81(RRT=0.82), IS5 =0.86(RRT=0.87) , IS6 =1.12(RRT=1.11), IS7 =0.94(RRT=0.95), IS8 = 0.99(RRT=0.99), IS9 = 1.28(RRT=1.19), ISRADIPINE = 1.00(RRT=1.00)

**Individual Known impurity:**

Area of peak in the chromatogram of test

$$= \frac{SOLUTION-F}{\substack{MEAN\ AREA\ OF\ PRINCIPAL\ PEAK\ IN\ THE\ CHROMATOGRAM \\ OF\ STANDARD\ SOLITION-E}} \times \frac{\frac{WEIGHT\ OF\ STANDARD\ SOLUTION}{10}}{} \times \frac{\frac{SOLUTION\ D}{10}}{} \times \frac{\frac{SOLUTION\ E}{10}}{} \times \frac{TEST\ SOLUTION}{WEIGHT\ OF\ SOLUTION} \times RRF\ OF\ USP \times$$

$$PURITY \times \frac{RS}{IRS}$$

**IS0:** = 0.007%. **IS1:** = 0.002%, **ISIsomer1:** = 0.004%, **ISIsomer** = 0.001%, **IS5:**= = 0.092%, **IS6:** =0.223%, **IS7:** = 0.182%, **IS8:** = 0.001%, Unknown Impurities: = 0.065%, Total Unknown Impurities = 0.323%, Total Impurities: =0.677%

**Results Obtained During The Analysis:**

**Identified (known) Impurity:**

4-methyl Benzo Furazan (ISO) : 0.007% (NMT 0.30%)

4- BromomethylBenzo furazan (IS1) : 0.002% (NMT 0.03%)

2- Acetyl -3 – (Benzofurazan-4 yl) Acrylic acid methyl ester (IS4 Isomer1) :0.004 %( NMT 0.030%)

2- Acetyl – 3 – (Benzofurazan – 4yl) Acrylic acid methyl ester (IS4 Isomer 2) :0.001% (NMT 0.03%)

4- (4- Benzofurazanyl) -1, 4- Dihydro – 2, 6- dimethyl – 3, 5 – pyridine Dicarboxylic acid dimethyl ester (IS5) : 0.092 %( NMT0.030%)

4-(4-Benzofurazanyl)-1, 4-dihydro – 2, 6 – Dimethyl -3, 5-phyidine di iso propyl ester (IS6) : 0.223% (NMT 0.30%)

7- Bromo – 4 – methylbenzofurazan (IS7) : 0.182 %( NMT 0.30%)

Iso propyl methyl -4- (4 – Benzofurazanyl) -2, 6-dimethyl -3, 5-phyidine dicarboxylate (IS8) : 0.001% (NMT 0.30%

Iso propyl methyl -4- (2, 1, 3-benzoxadiazal -4-yl)-2-[2-(2,1,3-benzoxodiazol -4 – yl) ethenyl]-1,4-dihydro -6- methyl pyridine -3-5- dicarboxylate (IS9) :0.180% (NMT 0.30%)

Unidentified (unknown) impurity : 0.065% (NMT 1.00%)

Total Impurities : 0.677 % (NMT 1.00%)

**Proton NMR spectral analysis**

Nuclear magnetic resonance (NMR) spectral analysis is an important analytical technique used to determine the structures of organic compounds. Figure shows that the proton NMR spectra of the molecule NMNPB, exhibits a three singlet peaks were 3.77 & 2.26 ppm (S, 3H, CH3), 1.32 ppm (S, 3H, CH) due to the presence of methyl group. The secondary amine hydrogen (NH-) signal appeared at 8.91 ppm as a sharp singlet. Two doublet peaks were observed at 7.04 ppm (d, 2H, ArH),7.76 ppm (d, 2H, ArH), due to the presence of aromatic protons of phenyl ring.. Two triplet peaks were observed at 7.12 (t, 3H, ArH) & 7.53 ppm (t,3H, ArH) in aromatic ring.

**$^{13}$C NMR spectral analysis**

Fig.7. shows the $^{13}$C NMR spectra of Isradipine shows the carbon signals at 167.2 ppm in the downfield respectively due to the highly deshielded carbonyl carbon of the benzamide moiety. The peaks due to aromatic carbons appears at 21.7, 68.4, 120.4, 127.9, 131.1, 112.8, 148.4, 104.1, 150.2, 19.20 ppm in the upfield region is due to methyl carbon.

## CONCLUSION:

Isradipine is a crucial substance, which widely used as vital sign , Hypertension, coronary failure .Some important analytical parameters like pH freezing point , moisture content, loss on drying, sulphated ash, Heavy metals were administered to work out the purity of raw materials used for the assembly of isradipine. The purity of ultimate product Isradipine decided by the determination of loss on drying, sulphated ash, Heavy metals, High performance liquid chromatography. All the analytical parameters determined were correlated with the theoretical and standard values. Isradipine is identified by Infrared and freezing point . Some specified impurities such IS0, IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, are present in Isradipine and it's determine by using High performance liquid chromatography. The dominant peaks are the Infrared spectra of isradipine are correlated with observed value and theoretical value. The results are suitably interoperated.

The purity of the Isradipine tablet (99%) is confirmed by High performance liquid chromatography methods. From these methods it's concluded that the purity of the tablet is 96.8% .If impurities present within the tablet itwill be produce some side effects.

**GUASSIAN TABLE**

| Geometric parameters | From single crystal | From Gaussian 09 software |
|---|---|---|
| **Bond length (Å)** | | |
| C(1)-C(2) | 1.381(3) | 1.393 |
| C(1)-C(6) | 1.389(3) | 1.4 |
| C(1)-H(1) | 0.93 | 1.085 |
| C(2)-C(3) | 1.374(4) | 1.393 |
| C(2)-H(2) | 0.93 | 1.084 |
| C(3)-C(4) | 1.367(4) | 1.395 |
| C(3)-H(3) | 0.93 | 1.084 |
| C(4)-C(5) | 1.388(3) | 1.391 |
| C(4)-H(4) | 0.93 | 1.084 |
| C(5)-C(6) | 1.375(3) | 1.4 |
| C(5)-H(5) | 0.93 | 1.083 |
| C(6)-C(7) | 1.491(3) | 1.503 |
| C(7)-O(3) | 1.229(2) | 1.218 |
| C(7)-N(1) | 1.346(3) | 1.385 |
| C(8)-C(13) | 1.382(3) | 1.399 |
| C(8)-C(9) | 1.398(3) | 1.415 |
| C(8)-N(1) | 1.418(2) | 1.402 |
| C(9)-C(10) | 1.389(3) | 1.395 |
| C(9)-C(14) | 1.503(3) | 1.508 |
| C(10)-C(11) | 1.379(4) | 1.39 |
| C(10)- | 0.93 | 1.084 |
| C(11)-C(12) | 1.382(4) | 1.388 |
| C(11)- | 0.93 | 1.084 |
| C(12)-C(13) | 1.374(3) | 1.389 |
| C(12)-N(2) | 1.465(3) | 1.482 |
| C(13)- | 0.93 | 1.078 |
| C(14)- | 0.96 | 1.09 |
| C(14)- | 0.96 | 1.09 |
| C(14)- | 0.96 | 1.09 |
| N(1)-H(1A) | 0.84(3) | 1.007 |
| N(2)-O(1) | 1.202(3) | 1.226 |
| N(2)-O(2) | 1.203(3) | 1.223 |

| Geometric parameters | From single crystal | From Gaussian 09 software |
|---|---|---|
| **Bond angle** | | |
| C(2)-C(1)-C(6) | 120.2(2) | 120.3 |
| C(2)-C(1)-H(1) | 119.9 | 118.9 |
| C(6)-C(1)-H(1) | 119.9 | 120.7 |
| C(3)-C(2)-C(1) | 120.1(2) | 120.1 |
| C(3)-C(2)-H(2) | 120 | 120.2 |
| C(1)-C(2)-H(2) | 120 | 119.8 |
| C(4)-C(3)-C(2) | 120.0(2) | 119.9 |
| C(4)-C(3)-H(3) | 120 | 120.1 |
| C(2)-C(3)-H(3) | 120 | 120.1 |
| C(3)-C(4)-C(5) | 120.3(2) | 120.2 |
| C(3)-C(4)-H(4) | 119.9 | 121.1 |
| C(5)-C(4)-H(4) | 119.9 | 119.8 |
| C(6)-C(5)-C(4) | 120.2(2) | 120.3 |
| C(6)-C(5)-H(5) | 119.9 | 118.8 |
| C(4)-C(5)-H(5) | 119.9 | 119.2 |
| C(5)-C(6)-C(1) | 119.19(19) | 123.5 |
| C(5)-C(6)-C(7) | 118.71(18) | 117.2 |
| C(1)-C(6)-C(7) | 121.95(18) | 120.2 |
| O(3)-C(7)-N(1) | 122.52(18) | 123.5 |
| O(3)-C(7)-C(6) | 120.56(17) | 121.8 |
| N(1)-C(7)-C(6) | 116.89(16) | 114.7 |
| C(13)-C(8)-C(9) | 120.94(18) | 120.1 |
| C(13)-C(8)-N(1) | 120.41(18) | 122.1 |
| C(9)-C(8)-N(1) | 118.65(18) | 117.7 |
| C(10)-C(9)-C(8) | 118.1(2) | 118.7 |
| C(10)-C(9)-C(14) | 120.1(2) | 120 |
| C(8)-C(9)-C(14) | 121.7(2) | 121.2 |
| C(11)-C(10)-C(9) | 122.0(2) | 122 |
| C(11)-C(10)-H(10) | 119 | 119.2 |
| C(9)-C(10)-H(10) | 119 | 118.8 |
| C(10)-C(11)-C(12) | 117.8(2) | 117.7 |
| C(10)-C(11)-H(11) | 121.1 | 122 |
| C(12)-C(11)-H(11) | 121.1 | 120.3 |
| C(13)-C(12)-C(11) | 122.4(2) | 122.9 |
| C(13)-C(12)-N(2) | 118.5(2) | 118.4 |
| C(11)-C(12)-N(2) | 119.1(2) | 118.7 |
| C(12)-C(13)-C(8) | 118.7(2) | 118.6 |

| | | |
|---|---|---|
| C(12)-C(13)-H(13) | 120.6 | 120.4 |
| C(8)-C(13)-H(13) | 120.6 | 120.9 |
| C(9)-C(14)-H(14A) | 109.5 | 111.9 |
| C(9)-C(14)-H(14B) | 109.5 | 111.8 |
| H(14A-C14-H(14B) | 109.5 | 107.7 |
| C(9)-C(14)-H(14C) | 109.5 | 110.7 |
| H(14A)-C(14)- | 109.5 | 107.1 |
| H(14B)-C(14)- | 109.5 | 107.4 |
| C(7)-N(1)-C(8) | 124.50(16) | 128.7 |
| C(7)-N(1)-H(1A) | 117.0(16) | 115.4 |
| C(8)-N(1)-H(1A) | 118.3(16) | 115.5 |
| O(1)-N(2)-O(2) | 122.7(3) | 124.6 |
| O(1)-N(2)-C(12) | 118.7(3) | 117.9 |
| O(2)-N(2)-C(12) | 118.6(2) | 117.4 |

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