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# A REVIEW ON DETECTION AND EVALUATION OF IMPURITIES IN REGADENOSON MONOHYDRATE DRUG MATERIAL BY RP-HPLC – A STABILITY STUDY

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***Abstract:*** The gradient HPLC technique was developed in order to identify and estimate seven related substances in Regadenoson drug material. Also known as organic impurities, they come from what are known as By-products of the synthesis of drug substance. In the present work the optimized method chromatogram was run through the column ACE 3 C18- PFP, 150×4.6mm 3.0µm or equivalent and the diluent used was Dimethyl Sulfoxide, whereas buffer was 1.0 ml of methane Sulfonic acid in 1000ml of water. The flow rate used was 0.8ml/min and the detector wavelength were at 272nm. The temperature was maintained at 30ºC and injection volume was 5µl. The total run time was 55 minutes and the retention time for Regadenoson was found to be 17.1 min. The correlation coefficient obtained was 0.9995, while the LOD and LOQ values were found to be in the range of 3.3 to 4.8 and 10.7 to 14.1 respectively. %RSD was found to be well within the limits.

***Keywords:*** Regadenoson, Methane Sulfonic Acid, Dimethyl Sulfoxide, Related Substances, RSD, LOD, LOQ.

**INTRODUCTION:**

Regadenoson is an $A_2A$ receptor agonist which is a coronary vasodilator and is mainly described as adenosine - 2, [4(methylamino)carbonyl)1H-pyrazol-1-yl-monohydrate with an empirical formula of $C_{15}H_{18}N_8O_5$ and molecular weight of 408.37 [(1)] It is specifically known by the brand name Lexiscan. [(2)] Each 1 ml in the 5 ml prefilled vial contains 0.084 mg of Regadenoson. It is approved to be used as a parenteral dosage form. [(3)] Its dosage is 0.4mg rapid IV injection into a periphery vein using a 22 gauge or needle (Table 1). The ultimate goal of the treatment is to achieve coronary vasodilation so that the blood flow will be normal. [(4-9)] According to literature review we found out that there are few methods developed for the estimation of related substances by HPLC. The reported methods carried out were either for 1 or 2 impurities and none mentioned the organic impurities that were produced as by product during the synthesis. [(10-15)]

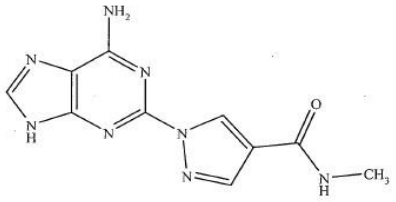


**Fig 1: Structure of Regadenoson Monohydrate**

**Table 1: Physiochemical Properties**

| | |
|---|---|
| **IUPAC name** | Adenosine,2-[4-[(methylamino)carbonyl]-1Hpyrazol-1-yl]-monohydrate. |
| **Molecular weight** | 408.38 |
| **Category** | Coronary vasodilator |
| **Water solubility** | 4.85mg/ml |
| **Molecular formula** | $C_{15}H_{18}N_8O_5.H_2O$. |
| **Route of administration** | Parenteral |
| **Melting point** | 194-196ºC |

**DOSAGE AND ADMINISTRATION:**

Intravenous solution - 0.4mg/5ml.

**PHARMACOLOGICAL STRESS TESTING**:

Indicated for radionuclide Myocardial Perfusion Imaging (MPI) in patients unable to undergo adequate exercise.

**PHARMACOKINETICS AND PHARMACODYNAMIC PROPERTIES:**

**Table 2: Pharmacokinetics and Pharmacodynamic Properties**

| | |
|---|---|
| **Protein binding** | Not available |
| **Elimination half life** | 2-4 minutes |
| **Excretion** | Renal clearance is 450ml/min |
| **Metabolism** | Cytochrome P 450 enzyme |
| **Route of administration** | Parenteral |
| **Toxicity** | Greater than 5% side effects |
| **Interaction** | Caffeine |
| **Food interaction** | Avoid caffeine for at least 12 hours |

**MECHANISM OF ACTION:**

Regadenoson is selectively a low affinity $A_2A$ receptor agonist that mimics the effects of adenosine in causing coronary vasodilation and increasing myocardial blood flow. It is a very weak agonist of the $A_1$ adenosine receptor.

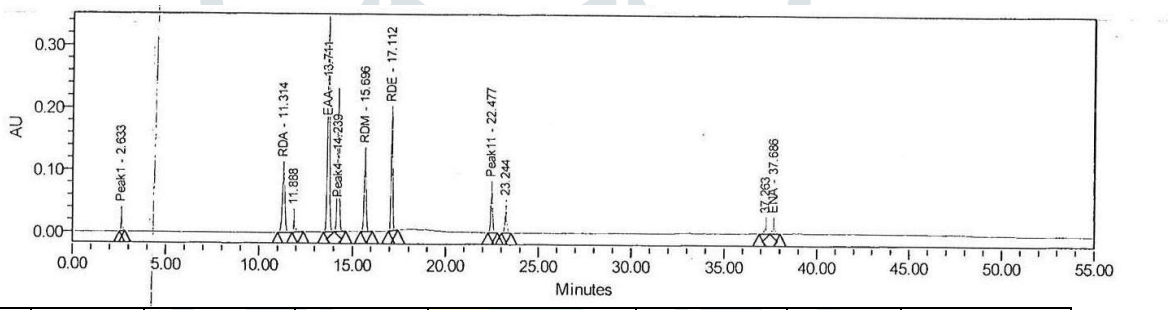
**ANALYSIS:**

A RP-HPLC method was chosen for the method development and validation of Regadenoson and its related substances. Validation was done according to the ICH guidelines. After so many trials, the Regadenoson was eluted at 17.1 minutes with all its impurities at the run time of 55 mins and this was selected as the optimized method and the acceptance criteria was met.

**Fig 2: Chromatogram of Regadenoson and its impurities**

The validation parameters like Accuracy, Precision, Linearity, LOD, LOQ and Robustness were chosen to validate the method. Accuracy was found to be 102.8 - 111.1%, Precision was 0.24 - 1.18. Linearity was 0.9995, LOD and LOQ values were 3.3-4.8 and 10.7-14.1 respectively. And robustness was within the limit.

**Table 3: Peak result of Regadenoson and its impurities**




| S. No | RT | Area | Height | Name | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| **1** | 13.25 | 24815 | 2664 | Deribose Impurity | - | 1.10 | 46815.57 |
| **2** | 14.14 | 20099 | 2390 | Amine Impurity | 3.73 | 1.05 | 64751.70 |
| **3** | 17.81 | 115512089 | 1387404 | Regadenoson | 4.95 | 1.13 | 107013.88 |
| **4** | 19.05 | 24089 | 2852 | Acid impurity | 5.51 | 1.11 | 115645.36 |
| **5** | 19.71 | 13334 | 1680 | Dimethyl amine analogue | 2.99 | 1.09 | 139253.60 |
| **6** | 21.85 | 18575 | 2160 | Ethyl amine analogue | 9.59 | 1.07 | 148024.90 |
| **7** | 32.20 | 22103 | 2354 | EAC | 42.85 | 1.12 | 273319.02 |

**Table 4: Peak Results of Optimized Chromatogram**

| S. No | Parameter | Limit | Observation | Inference |
|---|---|---|---|---|
| **1** | Specificity | There should not be any interfering peaks in between | Within limit | Passed |
| **2** | System precision | Should not be more than 5.0 | System precision was 0.86 | Passed |
| **3** | Method Precision | Should not be more than 10.0 | 0.24 to 1.18 | Passed |
| **4** | Linearity | The correlation coefficient should NT 0.9995 | Correlation coefficient =0.9995 | Passed |
| **5** | Accuracy | The % recoveries should be between 85 to 115 | % Recoveries were 102.8 to 111.1 | Passed |
| **6** | Robustness | Should not be more than 10.0 | Were within the limit | Passed |
| **7** | LOD | Signal to noise ratio should be about 3:1 to 5:1 | LOD ranges are 3.3 to 4.8 | Passed |
| **8** | LOQ | Signal to noise ratio should be about 10.0 | LOQ ranges are 10.7 to 14.1 | Passed |

**STABILITY STUDIES:**

- The degradation study of Regadenoson indicates that the related compounds are stable upon exposure to Ambient, Thermal, Humidity, Photolytic, Dark and packaging conditions.
- No significant degradation was observed upon exposure to water.
- Significant degradation was observed upon exposure to oxidation condition and acid Hydrolysis.
- Rapid degradation was observed upon exposure to base hydrolysis.

It is concluded from the above observations that the HPLC method for the related substances in Regadenoson are stability indicative.

**Table 5: Solid state stability studies Results**

| **Major Impurities** | **Results in %** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Mother sample (As such)** | **Ambient Sample (25±2$^0$C)** | **Thermal Sample (Heated at 105±2$^0$C)** | **Humidity sample (At 90+ 5% RH)** | **Photolytic Sample** | **Dark Sample** | **Packing Sample** |
| | | | | | **(1.2 million LUX Hrs & 200 watt- hrs/Sq.mts)** | | |
| Deribose | ND | ND | ND | ND | ND | ND | ND |
| Acid | 0.02 | 0.02 | 0.03 | 0.03 | 0.02 | 0.03 | 0.03 |
| Dimethyl amine Analogue | ND | ND | ND | ND | ND | ND | ND |
| Ethyl amine Analogue | ND | ND | ND | ND | ND | ND | ND |
| EAS | ND | ND | ND | ND | ND | ND | ND |
| AUI | ND | ND | ND | ND | ND | ND | ND |
| **TI** | **0.02** | **0.2** | **0.03** | **0.03** | **0.02** | **0.03** | **0.03** |

*ND = Not detected. AUI = Any unspecified impurity, TI= Total impurities

**Table 6: Liquid state stability studies Results**

| **Major Impurities** | **Results in %** | | | |
|---|---|---|---|---|
| | **Water Hydrolysis (Heated at 80$^0$ C in $H_2O$ for 24 hrs)** | **Acid Hydrolysis (Heated at 80$^o$C in 1M HCL for 2 hrs)** | **Base Hydrolysis (Heated at 80$^0$C in 1M NaOH for 30 minutes)** | **Oxidation (Heated at 80$^0$C in 10% $H_2O_2$ for 24 hrs)** |
| Deribose | ND | 8.33 | 0.07 | 1.41 |
| Acid | 0.03 | 0.07 | 8.42 | 0.20 |
| Dimethyl amine Analogue | ND | ND | ND | ND |
| Ethyl amine Analogue | ND | ND | ND | 0.08 |
| EAC | ND | ND | ND | ND |
| AUI | **ND** | **0.04** | **0.48** | **0.37** |
| **TI** | **0.03** | **8.44** | **9.64** | **3.23** |

**CONCLUSION:**

The HPLC method was developed to detect and evaluate Deribose, Dimethyl Amine, Regadenoson acid, Ethyl Amine Analogue, EAC, EAS in Regadenoson drug material. The estimation of these impurities was found to be Specific, Precise, Accurate and Robust. The method was applied for successive batches of Regadenoson and these impurities were found to be below the specification limit (0.03%). Hence, these impurities are removed from the regular analysis of Regadenoson.

**CONFLICT OF INTEREST:**

The authors hereby confirm no Conflict of Interest.

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