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Research Article

**METHOD DEVELOPMENT AND VALIDATION OF FIVE  
NITROSAMINES IMPURITIES CONTENT IN TELMISARTAN  
DRUG SUBSTANCE BY GC-MS/MS**

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Corresponding Author: Harendra.singh3333@gmail.com and [r.kumar31284@gmail.com](mailto:r.kumar31284@gmail.com)**Article Received:** April 2022**Accepted:** June 2022**Published:** July 2022**Abstract**

A proficient GC-MS/MSS method was developed for determination of N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine, N-Nitrosodibutylamine impurities in Telmisartan Drug Substance using column Rtx-5 Amine (30m length X 0.32mm diameter) 1.5µm film thickness, Part No.: 12369, Make: Restek. Helium is used as Carrier gas at with Linear velocity of 44.3 cm/sec. The proposed method was validated for System suitability, Specificity, Linearity, LOD and LOQ, Method precision, Intermediate precision and Recovery. All the parameters were found within the acceptable limits. Linearity in the range of LOQ to 150% for each impurity. The established methodology was commercially useful, specific, accurate, precise and suitable for the analysis of Nitrosamines impurities in Telmisartan drug substance

**Keywords:** Gas chromatography with mass spectrometry (GC-MS/MS), Telmisartan drug substance, Nitrosamine impurities, ICH guideline and Method Validation.

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**INTRODUCTION:**

Telmisartan is used in the treatment of Hypertension (high blood pressure), prevention of heart attack and stroke and Heart failure. Telmisartan is an angiotensin receptor blocker (ARB). It relaxes blood vessels by blocking the action of a chemical that usually makes blood vessels tighter. This lowers the blood pressure, allowing the blood to flow more smoothly to different organs and the heart to pump more efficiently.

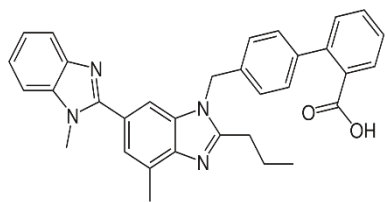
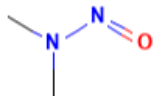
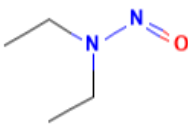
Nitrosamine impurities are unwanted chemicals, have no therapeutic value and are potentially harmful to the body. Nitrosamine substances are those which impact genetic material by means of mutations. Mutations can be chromosomal breaks, rearrangements, covalent binding or insertion into DNA during replication. The focus of this study is on reactive substances they have a potential to directly cause DNA damage when present low levels leading to mutations and there for potentially causing cancer. Because of this, it is important to identify Nitrosamine substances followed by monitoring and control at very low levels to ensure safety to the public health.

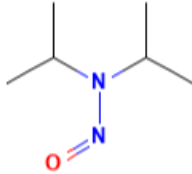
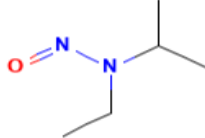
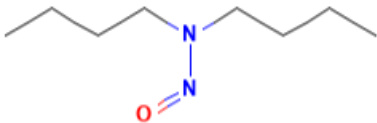
There are many sources of Nitrosamine impurities in pharmaceuticals active ingredient and drug products i.e. starting materials, byproducts, reagents, intermediates, degradation products, solvents or

unreacted products that get carried over into the final product. In addition, some of cases the Active pharmaceutical ingredient itself can decompose to form Nitrosamine impurities or they can form in the drug product by reaction between excipients and Active pharmaceutical ingredient or oxidation of drug products. The employment of these compounds within the synthetic process is logical as these substances are reactive building blocks that come together to form complex drug substances.

Impurity guidelines have been developed by international Conference on Harmonization (ICH). ICH Q3A regulates impurities in new drug substances with thresholds for reporting, identifying, and qualifying impurities. ICH Q3B is the equivalent guideline for impurities in new drugs. ICH Q3C controls residual solvent, and is the first time the ICH applied substance specific limits. Depending on their potential risk to human health. ICH Q3D is currently published and will include elements and limits for heavy metal impurities. Currently released ICH guidelines for impurity limits are not suitable for most Nitrosamine impurities. The Nitrosamine compounds considered unsafe at any level. The limit for Nitrosamine impurities with an understood toxicity can be calculated based upon the know PDE.

**Chemical Structure of N-Nitrosodimethylamine and Telmisartan:**

|   |   |
|---|---|
| <p><b>Telmisartan:</b><br/> <b>Chemical Name:</b> 2-(4-{{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-1-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl}benzoic acid<br/> <b>Molecular weight:</b> 514.6</p> |   |
| <p><b>N-Nitrosodimethylamine(NDMA):</b><br/> <b>Chemical Name:</b> N,N-dimethylnitrous amide<br/> <b>Molecular weight:</b> 74.04</p>  |  |
| <p><b>N-nitrosodiethylamine(NDEA):</b><br/> <b>Chemical Name:</b> N,N-diethylnitrous amide<br/> <b>Molecular weight:</b> 102.07</p>   |  |

|  |   |
|--|---|
| <p><b>N-Nitrosodiisopropylamine (NDIPA):</b><br/> <b>Chemical Name:</b> <i>N,N</i>-di(propan-2-yl)nitrous amide<br/> <b>Molecular weight:</b> 130.11</p>             |  |
| <p><b>N-Nitrosoisopropylethylamine (NIPEA):</b><br/> <b>Chemical Name:</b> <i>N</i>-ethyl-<i>N</i>-propan-2-ylnitrous amide<br/> <b>Molecular weight:</b> 116.09</p> |  |
| <p><b>N-Nitrosodibutylamine (NDBA):</b><br/> <b>Chemical Name:</b> <i>N,N</i>-dibutylnitrous amide<br/> <b>Molecular weight:</b> 158.14</p>                          |  |

### METHOD DEVELOPMENT:

#### Instrument, Chemicals and Reagents

##### Instrumentation

A Shimadzu Gas chromatograph with Auto sampler with Triple Quadrupole Mass spectrometer Detector (Shimadzu GC-2010 plus with TQ8050 MS), and Rtx-5 Amine (30m length X 0.32mm diameter) 1.5 $\mu$ m film thickness, Part No.: 12369, Make: Restek column was employed in the method. All the weighing in the experiments was done with Mettler toledo electronic

balance (Mettler Toledo / XSE 205) capable of measuring with an accuracy of 0.01 mg.

**Chemicals and Reagents:** Methanol (GCMS Grade, Make Merck), Dichloromethane (GCMS Grade, Make Merck), Acetone (GCMS Grade, Make Merck), Methane sulfonyl chloride (GCMS Grade, Make Merck), Anhydrous Sodium Sulphate (GCMS Grade, Make Merck), Sodium Hydroxide (AR Grade, Make Merck), Water (Milli-Q)

#### Chromatographic Conditions for GC:

|                      |   |
|----------------------|---|
| Column               | RTX-5 Amine, (30.0m x 0.32mm), 1.5 $\mu$ m, P. No. 12369, Make: Restek with Base deactivated guard column (5.0m x 0.32mm) Make: Restek, P. No: 10001. |
| Flow control Mode    | Constant Linear velocity  |
| Pressure             | 12.8 kPa  |
| Column Flow          | 1.49 mL/min   |
| Linear Velocity      | 44.3 cm/sec.  |
| Total Flow           | 11.9mL/min  |
| Injector Temperature | 200°C   |
| Injection mode       | Split less  |

|                       |  |                             |
|-----------------------|--|-----------------------------|
| Sampling time         | 2.0 minutes  |                             |
| Injection Volume      | 1.0 $\mu$ L  |                             |
| Carrier Gas           | Helium   |                             |
| Split Ratio           | 1:5  |                             |
| Purge Flow            | 3.0 mL/min   |                             |
| GC Equilibration time | 0.10 minutes   |                             |
| Split Ratio Program   | <b>Time (min)</b>  | <b>Split ratio</b>          |
|                       | 2.10   | 30                          |
| Oven Programing       | Initial 50.0°C, hold for 5.0min, Increase @ 20.0°C per min to 250.0°C, hold for 0 minute, Increase @ 30.0°C per min to 280.0°C, hold for 4.0 minutes |                             |
| Run time              | 20.0 Minutes   |                             |
| <b>Retention Time</b> | <b>Name</b>  | <b>Typical RT (minutes)</b> |
|                       | N-Nitrosodimethylamine   | 6.8                         |
|                       | N-Nitrosodiethylamine  | 9.7                         |
|                       | N-Nitrosoisopropylethylamine   | 10.5                        |
|                       | N-Nitrosodiisopropylamine  | 11.1                        |
|                       | N-Nitrosodibutylamine  | 13.3                        |

**GC-MS Programming**

|                        |                           |
|------------------------|---------------------------|
| Ion source temperature | 210.0°C                   |
| Interface temperature  | 230.0°C                   |
| Solvent cut time       | 5.50 min                  |
| Detector gain mode     | Relative to tuning result |
| Detector gain          | +0.4 kV                   |

**MS Conditions:**

| <b>MS/MS Table</b>                                |                                     |
|---|-------------------------------------|
| <b>N-Nitrosodimethylamine</b>                     |                                     |
| Ch1-m/z (Precursor>Product)<br>[Collision Energy] | 74.00>44.10 [6 V] {Quantifier Ion}  |
| Ch2-m/z (Precursor>Product)<br>[Collision Energy] | 74.00>42.10 [15 V] {Qualifier Ion}  |
| <b>N-Nitrosodiethylamine</b>                      |                                     |
| Ch1-m/z (Precursor>Product)<br>[Collision Energy] | 102.00>85.10 [6 V] {Quantifier Ion} |
| Ch2-m/z (Precursor>Product)<br>[Collision Energy] | 102.00>56.10 [15 V] {Qualifier Ion} |
| <b>N-Nitrosoisopropylethylamine</b>               |                                     |
| Ch1-m/z (Precursor>Product)<br>[Collision Energy] | 116.00>99.10 [6 V] {Quantifier Ion} |
| Ch2-m/z (Precursor>Product)<br>[Collision Energy] | 116.00>70.10 [15 V] {Qualifier Ion} |
| <b>N-Nitrosodiisopropylamine</b>                  |                                     |
| Ch1-m/z (Precursor>Product)<br>[Collision Energy] | 130.00>88.10 [6 V] {Quantifier Ion} |

|   |                                     |
|---|-------------------------------------|
| Ch2-m/z (Precursor>Product)<br>[Collision Energy] | 130.00>42.00 [10 V] {Qualifier Ion} |
| <b>N-Nitrosodibutylamine</b>                      |                                     |
| Ch1-m/z (Precursor>Product)<br>[Collision Energy] | 116.00>99.10 [6 V] {Quantifier Ion} |
| Ch2-m/z (Precursor>Product)<br>[Collision Energy] | 158.00>99.10 [10V] {Qualifier Ion}  |

**Preparation of blank, standard and sample solution:** The diluent used was homogeneous mixture of 4 gm sodium hydroxide/1 litre water. Nitrosamines impurities standard solution was prepared by using N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine reference standards to attain a concentration of about 0.0084 ppm of each impurity. For sample solution attain a concentration of about 2/mg/ml of sample.

**Acceptance criteria for System Suitability:**

**% RSD:** The %RSD for the area response of each impurity peak from initial six replicates of Standard Solution analyzed in the sequence should be not more than 25.

**Calculations and results:**

Calculate the impurity in the sample using the following formula (Result in ppm each nitrosamine impurity):

$$\text{Impurity(ppm)} = \frac{(AT-AB) \times WS \times DT \times P \times 106}{(AS-AB) \times DS \times WT \times 100}$$

AB = Average Peak area of respective impurity analysed in the chromatogram obtained from Blank, AT = Peak area counts of respective impurity analysed in the chromatogram of the sample solution, AS = Average peak area counts of respective impurity analysed in standard in the chromatogram of the

standard Solution, WS = Weight of respective impurity analyzed Standard, WT = Weight of sample solution, P = Purity/Potency of Impurity Standard used, DT = Dilution factor of sample solution, DS = Dilution factor of standard Solution.

**VALIDATION OF GC-MS/MS METHOD**

**Specificity:**

The specificity is defined as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as residual, degradation product and matrix components. In HPLC method, it is assured/proved by complete separation of peak of analyte from other peaks that are of other impurities that might be present in sample or blank.

Inject the Blank and Standard solution, Check the interference at the retention time of all five analytes. There should not be any interference in blank and standard at the retention time of each impurity and each impurity shall be well separated from each other. If any peak is present at the retention time of analyte its response should not be more than 20% of the response at the quantification limit (LOQ).

There is not any interference at retention time of all five analyte (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine). Refer Table-I

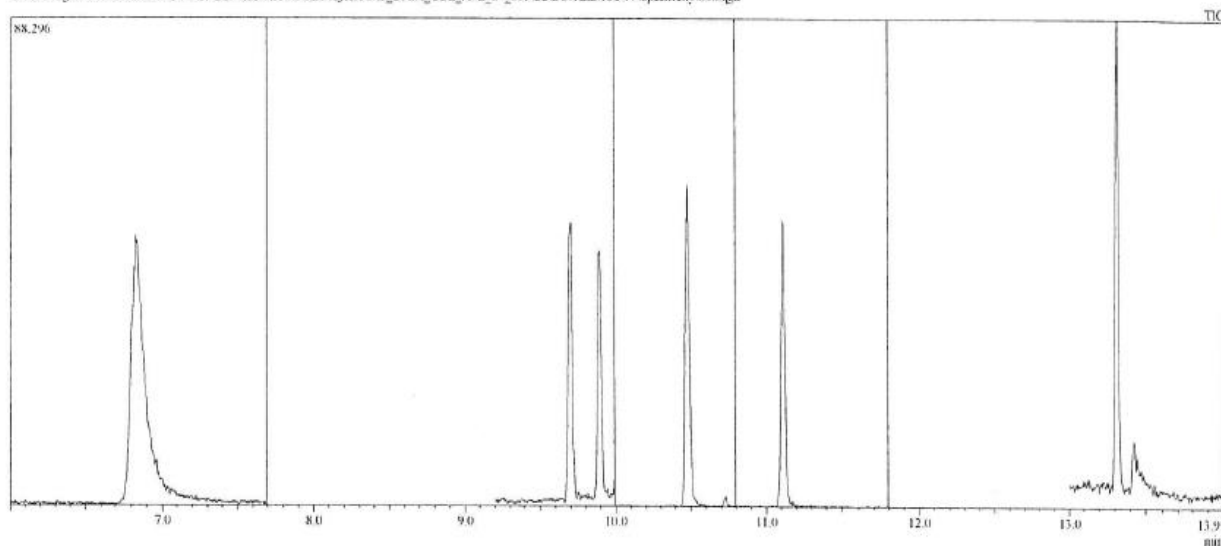
Table-I

| Name of Solvent              | Retention Time (Minutes) |          |
|------------------------------|--------------------------|----------|
|                              | Blank                    | standard |
| N-Nitrosodimethylamine       | ND                       | 6.8      |
| N-Nitrosodiethylamine        | ND                       | 9.7      |
| N-Nitrosoisopropylethylamine | ND                       | 10.5     |
| N-Nitrosodiisopropylamine    | ND                       | 11.1     |
| N-Nitrosodibutylamine        | ND                       | 13.3     |

No peak was observed in blank at the retention time of any impurity peak and all peaks are well separated from *each other*.

### Chromatograms of study

Chromatogram Standard Solution-1 C:\GCMS\Software\Data\Project1\MS\_DATA\_CBL\_AVL\_IN\_065\22\DS\TELM\AMV\Specificity\008.qst



Quantitative Result Table

| ID# | Name                         | Conc. | R. Time | m/z            | Area   | Height | S/N  |
|-----|------------------------------|-------|---------|----------------|--------|--------|------|
| 1   | N-Nitrosodimethylamine       | 0.000 | 6.838   | 74.00 > 44.10  | 235165 | 26091  | 590  |
| 2   | N-Nitrosodiethylamine        | 0.000 | 9.702   | 102.00 > 85.10 | 71146  | 13026  | 1183 |
| 3   | N-Nitrosoisopropylethylamine | 0.000 | 10.480  | 116.00 > 99.10 | 103856 | 19146  | 2584 |
| 4   | N-Nitrosodiisopropylamine    | 0.000 | 11.114  | 130.00 > 88.10 | 59157  | 11070  | 700  |
| 5   | N-Nitrosodibutylamine        | 0.000 | 13.315  | 116.00 > 99.10 | 94751  | 18277  | 708  |

#### Linearity:

A linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration of analyte. The linearity of method was determined using different concentration of N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine. Calibration curve found to be linear from LOQ to 150% of specification level.

The test method linearity was established from, six levels of concentration over the range LOQ to 150% of, ICH limit for each residual solvent impurity. A

linear correlation, and regression were determined among the concentrations, and peak area responses of each residual solvent. The correlation coefficient ( $r$ ) and regression coefficient ( $R^2$ ) values, for all five nitrosamine impurities found to be higher than, 0.990. The statistical characteristics like slope, y-intercept and, % y-intercept were interpreted and found within the acceptable limit for all five analyte (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine). The data tabulated in Table-II demonstrate the linearity of procedure.

Table-II

| Linearity Conc. level           | N-Nitrosodimethylamine |           | N-Nitrosodiethylamine |           | N-Nitrosoisopropylethylamine |           | N-Nitrosodiisopropylamine |           | N-Nitrosodibutylamine |           |
|---------------------------------|------------------------|-----------|-----------------------|-----------|------------------------------|-----------|---------------------------|-----------|-----------------------|-----------|
|                                 | Conc. (ppm)            | Mean area | Conc. (ppm)           | Mean area | Conc. (ppm)                  | Mean area | Conc. (ppm)               | Mean area | Conc. (ppm)           | Mean area |
| LOQ level                       | 0.33                   | 68688     | 0.09                  | 21410     | 0.09                         | 25996     | 0.08                      | 17886     | 0.08                  | 29825     |
| 50% level                       | 0.55                   | 112796    | 0.15                  | 34416     | 0.14                         | 43371     | 0.14                      | 30929     | 0.14                  | 44530     |
| 75% level                       | 0.82                   | 168478    | 0.23                  | 52078     | 0.22                         | 65093     | 0.2                       | 45812     | 0.2                   | 67532     |
| 100% level                      | 1.09                   | 218388    | 0.3                   | 68885     | 0.29                         | 87293     | 0.27                      | 61320     | 0.27                  | 86587     |
| 125% level                      | 1.37                   | 274823    | 0.38                  | 84299     | 0.36                         | 105499    | 0.34                      | 77277     | 0.34                  | 110513    |
| 150% level                      | 1.64                   | 331561    | 0.45                  | 105303    | 0.43                         | 127351    | 0.41                      | 91751     | 0.41                  | 134100    |
| Correlation coefficient         | 1.00                   |           | 1.00                  |           | 1.00                         |           | 1.00                      |           | 1.00                  |           |
| Squared Correlation coefficient | 0.99975                |           | 0.99725               |           | 0.9991                       |           | 0.99949                   |           | 0.9979                |           |
| Slope                           | 199386.6               |           | 228824.0754           |           | 294072.3241                  |           | 225686.1039               |           | 318436.7532           |           |
| Y-Intercept                     | 3048.66                |           | 45.41322              |           | 778.72402                    |           | -2.16493                  |           | 2423.01255            |           |
| Residual sum of square          | 11958731               |           | 13505980.5            |           | 6577226.572                  |           | 1981326.631               |           | 16427990.82           |           |

**Limit of detection (LOD):**

It is the smallest amount or concentration of an analyte that can be estimated. The detection limit is determined by the analysis of standard with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The limit of detection is determined by establishing the signal to noise ratio. Inject the blank and standard solutions at lower concentration and calculate the signal to noise ratio.

A signal-to-noise ratio between 3:1 estimating the detection limit.

The detection limit for N-Nitrosodimethylamine is found 0.12ppm and for remaining four nitrosamine impurities i.e. N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine is found 0.03 ppm w.r.t. Telmisartan sample concentration. For details, refer Table III.

Table-III

| Residual Solvent                     | LOD Level (ppm) w.r.t SPL | Mean S/N ratio |
|--------------------------------------|---------------------------|----------------|
| N-Nitrosodimethylamine (10.0%)       | 0.12                      | 7              |
| N-Nitrosodiethylamine (10.0%)        | 0.03                      | 11             |
| N-Nitrosoisopropylethylamine (10.0%) |                           | 9              |
| N-Nitrosodiisopropylamine (10.0%)    |                           | 7              |
| N-Nitrosodibutylamine (10.0%)        |                           | 10             |

**Limit of quantitation (LOQ):**

The Quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.



The limit of quantification is determined by establishing the signal to noise ratio. Inject the blank sample and the spiked sample at LOQ level in six replicates and calculate signal to noise ratio and the % RSD at LOQ level.

A signal-to-noise ratio between 10:1 estimating the quantification limit.

The quantification limit for N-Nitrosodimethylamine is found 0.36ppm and for remaining four nitrosamine impurities i.e. N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine is found 0.09 ppm w.r.t. Telmisartan sample concentration. For details, refer Table IV.

Table-IV

| Residual Solvent                     | LOQ Level (ppm) w.r.t SPL | Mean S/N ratio | %RSD (six replicates) |
|--------------------------------------|---------------------------|----------------|-----------------------|
| N-Nitrosodimethylamine (30.0%)       | 0.36                      | 22             | 7.7                   |
| N-Nitrosodiethylamine (30.0%)        | 0.09                      | 19             | 6.3                   |
| N-Nitrosoisopropylethylamine (30.0%) |                           | 17             | 8                     |
| N-Nitrosodiisopropylamine (30.0%)    |                           | 17             | 6.3                   |
| N-Nitrosodibutylamine (30.0%)        |                           | 18             | 2.8                   |

#### Recovery:

Recovery means the percentage of the true concentration of a substance recovered during the analytical procedure.

Recovery assessed using a minimum of 6 determinations over a minimum of 3 concentration levels.

Acceptable limits for a recovery result during validation should be within the range of 80% - 120%.

The percentage of average recovery for all five analyte (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) in Telmisartan found >90% at LOQ, 100% and 150%. For details, refer Table V.

Table V

| Accuracy of N-Nitrosodimethylamine |           |                                  |                                      |              |                      |
|------------------------------------|-----------|----------------------------------|--------------------------------------|--------------|----------------------|
| Level (%)                          | Sample ID | Amount added (ppm w.r.t. Sample) | Amount recovered (ppm w.r.t. Sample) | Recovery (%) | Average Recovery (%) |
| LOQ                                | Sample-1  | 0.361                            | 0.3517                               | 97.42        | 94.3                 |
|                                    | Sample-2  |                                  | 0.3191                               | 88.39        |                      |
|                                    | Sample-3  |                                  | 0.3505                               | 97.09        |                      |
| 100                                | Sample-1  | 1.2035                           | 1.2126                               | 100.75       | 100.2                |
|                                    | Sample-2  |                                  | 1.2004                               | 99.74        |                      |
|                                    | Sample-3  |                                  | 1.2032                               | 99.97        |                      |
| 150                                | Sample-1  | 1.8053                           | 1.6531                               | 91.56        | 96.6                 |
|                                    | Sample-2  |                                  | 1.7522                               | 97.05        |                      |
|                                    | Sample-3  |                                  | 1.825                                | 101.09       |                      |



| N-Nitrosodiethylamine |           |                                  |                                      |              |                      |
|-----------------------|-----------|----------------------------------|--------------------------------------|--------------|----------------------|
| Level (%)             | Sample ID | Amount added (ppm w.r.t. Sample) | Amount recovered (ppm w.r.t. Sample) | Recovery (%) | Average Recovery (%) |
| LOQ                   | Sample-1  | 0.0891                           | 0.0863                               | 96.85        | 97.3                 |
|                       | Sample-2  |                                  | 0.0847                               | 95.06        |                      |
|                       | Sample-3  |                                  | 0.0891                               | 100          |                      |
| 100                   | Sample-1  | 0.2971                           | 0.3078                               | 103.6        | 102.3                |
|                       | Sample-2  |                                  | 0.294                                | 98.95        |                      |
|                       | Sample-3  |                                  | 0.3104                               | 104.47       |                      |
| 150                   | Sample-1  | 0.4457                           | 0.4235                               | 95.01        | 98                   |
|                       | Sample-2  |                                  | 0.4378                               | 98.22        |                      |
|                       | Sample-3  |                                  | 0.4494                               | 100.83       |                      |

| N-Nitrosoisopropylethylamine |           |                                  |                                      |              |                      |
|------------------------------|-----------|----------------------------------|--------------------------------------|--------------|----------------------|
| Level (%)                    | Sample ID | Amount added (ppm w.r.t. Sample) | Amount recovered (ppm w.r.t. Sample) | Recovery (%) | Average Recovery (%) |
| LOQ                          | Sample-1  | 0.0883                           | 0.086                                | 97.39        | 97.5                 |
|                              | Sample-2  |                                  | 0.0862                               | 97.62        |                      |
|                              | Sample-3  |                                  | 0.086                                | 97.39        |                      |
| 100                          | Sample-1  | 0.2946                           | 0.3088                               | 104.82       | 101.8                |
|                              | Sample-2  |                                  | 0.2943                               | 99.89        |                      |
|                              | Sample-3  |                                  | 0.2967                               | 100.71       |                      |
| 150                          | Sample-1  | 0.4419                           | 0.4146                               | 93.82        | 96.8                 |
|                              | Sample-2  |                                  | 0.4317                               | 97.69        |                      |
|                              | Sample-3  |                                  | 0.4373                               | 98.95        |                      |

| N-Nitrosodiisopropylamine |           |                                  |                                      |              |                      |
|---------------------------|-----------|----------------------------------|--------------------------------------|--------------|----------------------|
| Level (%)                 | Sample ID | Amount added (ppm w.r.t. Sample) | Amount recovered (ppm w.r.t. Sample) | Recovery (%) | Average Recovery (%) |
| LOQ                       | Sample-1  | 0.0899                           | 0.0841                               | 93.54        | 96.1                 |
|                           | Sample-2  |                                  | 0.0832                               | 92.54        |                      |
|                           | Sample-3  |                                  | 0.0919                               | 102.22       |                      |
| 100                       | Sample-1  | 0.2999                           | 0.3021                               | 100.73       | 100.1                |
|                           | Sample-2  |                                  | 0.2963                               | 98.79        |                      |
|                           | Sample-3  |                                  | 0.3026                               | 100.9        |                      |
| 150                       | Sample-1  | 0.4498                           | 0.4237                               | 94.19        | 97                   |
|                           | Sample-2  |                                  | 0.4403                               | 97.88        |                      |
|                           | Sample-3  |                                  | 0.4444                               | 98.79        |                      |

| N-Nitrosodibutylamine |           |                                  |                                      |              |                      |
|-----------------------|-----------|----------------------------------|--------------------------------------|--------------|----------------------|
| Level (%)             | Sample ID | Amount added (ppm w.r.t. Sample) | Amount recovered (ppm w.r.t. Sample) | Recovery (%) | Average Recovery (%) |
| LOQ                   | Sample-1  | 0.0857                           | 0.083                                | 96.84        | 97.2                 |
|                       | Sample-2  |                                  | 0.0819                               | 95.56        |                      |
|                       | Sample-3  |                                  | 0.0851                               | 99.29        |                      |
| 100                   | Sample-1  | 0.2856                           | 0.2905                               | 101.71       | 100.7                |
|                       | Sample-2  |                                  | 0.2794                               | 97.82        |                      |
|                       | Sample-3  |                                  | 0.2932                               | 102.66       |                      |
| 150                   | Sample-1  | 0.4285                           | 0.4019                               | 93.79        | 96.6                 |
|                       | Sample-2  |                                  | 0.4187                               | 97.71        |                      |
|                       | Sample-3  |                                  | 0.4216                               | 98.38        |                      |

### Precision: (Method Precision)

The precision determined under equal conditions with same homogeneous spiked sample (six different sample preparation) as per recommended test method and % RSD of the results obtained shall be calculated.

The repeatability is established by estimating the six replicates of spiked sample and calculates the % RSD of the results obtained for each analyte.

The %RSD of results for the analysis of spiked sample should not be more than 25%.

The % RSD of six different sample preparation found <5% refer Table-VI.

Table-VI

| Preparation | Results (ppm)           |                        |                               |                            |                        |
|-------------|-------------------------|------------------------|-------------------------------|----------------------------|------------------------|
|             | N-Nitroso dimethylamine | N-Nitroso diethylamine | N-Nitroso Isopropylethylamine | N-Nitroso Diisopropylamine | N-Nitroso Dibutylamine |
| 1           | 1.2                     | 0.28                   | 0.29                          | 0.29                       | 0.27                   |
| 2           | 1.2                     | 0.29                   | 0.29                          | 0.28                       | 0.27                   |
| 3           | 1.21                    | 0.28                   | 0.29                          | 0.3                        | 0.28                   |
| 4           | 1.18                    | 0.29                   | 0.3                           | 0.3                        | 0.28                   |
| 5           | 1.27                    | 0.29                   | 0.29                          | 0.31                       | 0.27                   |
| 6           | 1.26                    | 0.31                   | 0.3                           | 0.31                       | 0.28                   |
| Mean        | 1.22                    | 0.29                   | 0.29                          | 0.29                       | 0.27                   |
| SD          | 0.036                   | 0.01                   | 0.005                         | 0.011                      | 0.005                  |
| %RSD        | 3                       | 3.4                    | 1.7                           | 3.8                        | 1.9                    |

### Precision: (Intermediate Precision)

Intermediate Precision means the susceptibility of an analytical method to changes in experimental conditions which can be expressed as different columns, different analyst and different days.

Intermediate Precision of the method is established by estimating the six replicates of spiked sample by different analysts, on different days and on different columns. Calculate the % RSD of the results obtained.

The %RSD of results for the analysis of spiked sample should not be more than 210%.

The % RSD of six different sample preparation found 5%, refer Table-VII.

Table-VII

| Preparation | Results (ppm)           |                        |                               |                            |                        |
|-------------|-------------------------|------------------------|-------------------------------|----------------------------|------------------------|
|             | N-Nitroso dimethylamine | N-Nitroso diethylamine | N-Nitroso Isopropylethylamine | N-Nitroso Diisopropylamine | N-Nitroso Dibutylamine |
| 1           | 1.06                    | 0.27                   | 0.27                          | 0.26                       | 0.25                   |
| 2           | 1.16                    | 0.27                   | 0.28                          | 0.27                       | 0.26                   |
| 3           | 1.11                    | 0.26                   | 0.27                          | 0.25                       | 0.25                   |
| 4           | 1.15                    | 0.29                   | 0.28                          | 0.27                       | 0.27                   |
| 5           | 1.11                    | 0.27                   | 0.28                          | 0.26                       | 0.27                   |
| 6           | 1.16                    | 0.28                   | 0.3                           | 0.28                       | 0.27                   |
| Mean        | 1.12                    | 0.27                   | 0.28                          | 0.26                       | 0.26                   |
| SD          | 0.039                   | 0.01                   | 0.01                          | 0.01                       | 0.009                  |
| %RSD        | 3.5                     | 3.7                    | 3.6                           | 3.8                        | 3.5                    |

### DISCUSSION:

A chromatographic method involves demonstrating specificity, which is the ability of the method to accurately measure the all five Nitrosamines impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) response in the presence of all potential sample components. The chromatographic and mass spectroscopy parameters were fixed and GC-MS/MS system was studied for suitability of Nitrosamine impurity analysis. The developed method was performed for linearity, precision, Accuracy, specificity, LOD and LOQ.

### CONCLUSION:

A simple and sensitive method for the determination all five Nitrosamines impurities (N-Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosodiisopropylamine, N-Nitrosoisopropylethylamine and N-Nitrosodibutylamine) in Telmisartan drug substance by using GC-MS/MS was developed, validated and applied for the analysis of Telmisartan drug substance samples. The method was validated to ensure the feasibility of the method for its application in routine analysis. The LOQs achieved through this method were lower than the genotoxic impurities limit.

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