Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Testing and Research
Division of Pharmaceutical Analysis

U.S. FOOD & DRUG ADMINISTRATION

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# Combined N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS-Headspace

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**Background:**

Valsartan products are used to treat high blood pressure and congestive heart failure. On July 13, 2018, FDA announced a recall of Valsartan tablets because of the potential for certain products to contain an impurity, N-Nitrosodimethylamine (NDMA). A second impurity was subsequently reported, N-Nitrosodiethylamine (NDEA). NDMA and NDEA are classified as probable human carcinogens and were believed to have been introduced into the finished products because of the manufacturing processes used to make the drug substance. OTR has developed a gas chromatography-mass spectrometry (GC/MS) headspace method to detect the presence of NDMA and NDEA in valsartan drug substance.

**Conclusions:**

The combined method has been validated to simultaneously quantify NDMA and NDEA.

| Impurity | LOD (ppm) | LOQ (ppm) |
|---|---|---|
| N-Nitrosodimethylamine (NDMA) | 0.005 | 0.10 |
| N-Nitrosodiethylamine (NDEA) | 0.02 | 0.05 |

**Equipment/Instrument:**
Gas Chromatography System with a Quadrupole Mass Spectrometry Detector and Headspace Auto-sampler
DB-1701 GC Column, 30 m x 0.25 mm, 1.00 µm, or equivalent
Analytical Balance
Vortex Mixer
20 mL Headspace Vials
HS vial caps with Teflon/Silicone septa

**N-Nitrosodimethylamine (NDMA) Reference Standard:**
Use commercially available NDMA standard solution in methanol. Alternatively, prepare a 1000 µg/mL standard solution in NMP from a NDMA reference standard. Correct for purity.

**N-Nitrosodiethylamine (NDEA) Reference Standard:**
Use commercially available NDEA standard solution in methanol. Alternatively, prepare a 1000 µg/mL standard solution in NMP from a NDEA reference standard. Correct for purity.

**N-Nitrosodimethylamine (NDMA) & N-Nitrosodiethylamine (NDEA) Mixed Stock Standard**
Prepare a 100 µg/mL mixed stock standard solution of NDMA and NDEA. Dilute to volume with diluent.

**Diluent:** 1-Methyl-2-pyrrolidinone (NMP); ≥ 99.0 %, GC grade

**Standard Solution Preparations (0.025 – 10 µg/mL):**
Transfer the appropriate aliquot volume of the designated mixed standard solution into separate volumetric flasks and dilute to volume with diluent. Refer to the table below for a suggested standard solution preparation scheme.

**Standard Solution Preparation Scheme:**

| Standard | Aliquot Vol. (mL) | NDMA/NDEA Std. Solution (µg/mL) | Total Vol. (mL) | NDMA/NDEA Conc. (µg/mL) |
|---|---|---|---|---|
| 1 | 1.0 | 100 µg/mL | 10.0 | 10.0 |
| 2 | 1.0 | 100 µg/mL | 20.0 | 5.0 |
| 3 | 1.0 | 5.0 µg/mL | 10.0 | 0.5 |
| 4 | 1.0 | 0.5 µg/mL | 5.0 | 0.100 |
| 5 | 1.0 | 0.5 µg/mL | 10.0 | 0.050 |
| 6 | 1.0 | 0.5 µg/mL | 20.0 | 0.025 |

**Working Standard Preparations (0.025 – 100 µg):**
Transfer a 1.0 mL aliquot volume of the standard solutions into separate 20 mL headspace vials containing 4.0 mL of NMP. Immediately cap and crimp the headspace vials. Refer to the table below for the working standard preparation scheme.

**Working Standard Preparation Scheme:**

| Working Standard | NDMA/NDEA Std. Solution (µg/mL) | Aliquot Vol. (mL) | NMP Vol. (mL) | Total Vol. (mL) | NDMA/NDEA Amount (µg) |
|---|---|---|---|---|---|
| 1 | 0. 025 | 1.0 | 4.0 | 5.0 | 0. 025 |
| 2 | 0.050 | 1.0 | 4.0 | 5.0 | 0.050 |
| 3 | 0.100 | 1.0 | 4.0 | 5.0 | 0.100 |
| 4 | 0.5 | 1.0 | 4.0 | 5.0 | 0.5 |
| 5 | 5.0 | 1.0 | 4.0 | 5.0 | 5.0 |
| 6 | 10.0 | 1.0 | 4.0 | 5.0 | 10.0 |
| 7 | 100 | 1.0 | 4.0 | 5.0 | 100 |

**Blank Preparation**
Transfer 5 mL of NMP into a 20 mL headspace vial. Cap and crimp the vial. Prepare as many as needed.

**Sample Preparation**
**Drug Substance**
Accurately weigh 500 mg of Valsartan drug substance into a 20 mL headspace vial. Add 5 mL of NMP to the vial and immediately cap and crimp the vial. Mix the sample solution using a vortex mixer. Drug substance weight could be increased or decreased, depending on the amount of NDMA/NDEA impurity in the drug substance. Vortex the sample for at least a minute or until the sample is dispersed

**Drug Product**
Using a pill cutter, cut the tablet(s) at least in half and accurately weigh into a 20 mL headspace vial. Target an API weight of at least 300 mg. The total weight of the tablet(s) should be around 1 g. Add 5 mL of NMP to the vial and immediately cap and crimp the vial. Shake the vial using a mechanical wrist action shaker and/or vortex mixer until the tablet is dispersed.

**GC/MS-HS Parameters:**
***Note: The method was optimized using an Agilent 7890B GC System with an Agilent 5977A MSD and an Agilent 7697A Headspace Auto-sampler.***

| **GC/MS - HS Parameters** | |
|---|---|
| Instrument: | Agilent 7890B GC with Agilent 5977A MSD and Agilent 7697A HS Auto-sampler |
| Column: | DB-1701, 30 m x 0.25 mm, 1.00 µm (PN: 122-0733), or equivalent |
| Inlet Temperature: | 220 °C |
| Column Flow: | 1 mL/min |
| Split Ratio | 5:1 |
| Oven Program: | 40 °C for 0.5 min.; 20 °C/min to 160 °C, hold for 0 min; 10 °C/min to 240 °C, hold for 2 min. |
| GC Run Time | 16.5 min. |
| GC Cycle Time: | 25 min. |
| **HS Auto-sampler Parameters** | |
| Oven Temperature: | 130 °C |
| Loop Temperature: | 180 °C |
| Transfer Line Temperature: | 185 °C |
| Vial Equilibration Time: | 15 min |

| | |
|---|---|
| Injection Time: | 1.0 min |
| Vial Size: | 20 mL |
| Vial Shaking: | Level 5 (71 shakes/min) |
| Fill Pressure: | 15 psi |
| Loop Size: | 1 mL |
| **MS Parameters** | |
| MS Source Temperature: | 230 °C |
| Quad Temperature: | 150 °C |
| Acquisition Type: | SIM |
| Gain Factor | 1 |
| Solvent Delay: | 3.0 min. |
| SIM Ion | m/z 74.0; m/z 102 |
| Dwell Time: | 150 ms |

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**System Suitability:**
The correlation coefficient (R) of the linear calibration curves should be ≥ 0.995.

**Calculations:**
Plot the NDMA and NDEA peak areas against the standard concentrations (µg). Plot two calibration curves – one from 0.025 – 0.5 µg and the other from 0.025 – 100 µg. Determine the intercepts, slopes and correlation coefficients of the linear curves. NDMA and NDEA peaks ≤ the 0.5 µg working standard peak area should be quantitated using the 0.025 – 0.5 µg calibration curve. NDMA and NDEA peaks > the 0.5 µg working standard peak area should be quantitated using the 0.025 – 100 µg calibration curve. Calculate the NDMA and NDEA impurities (ppm) using the formula below:

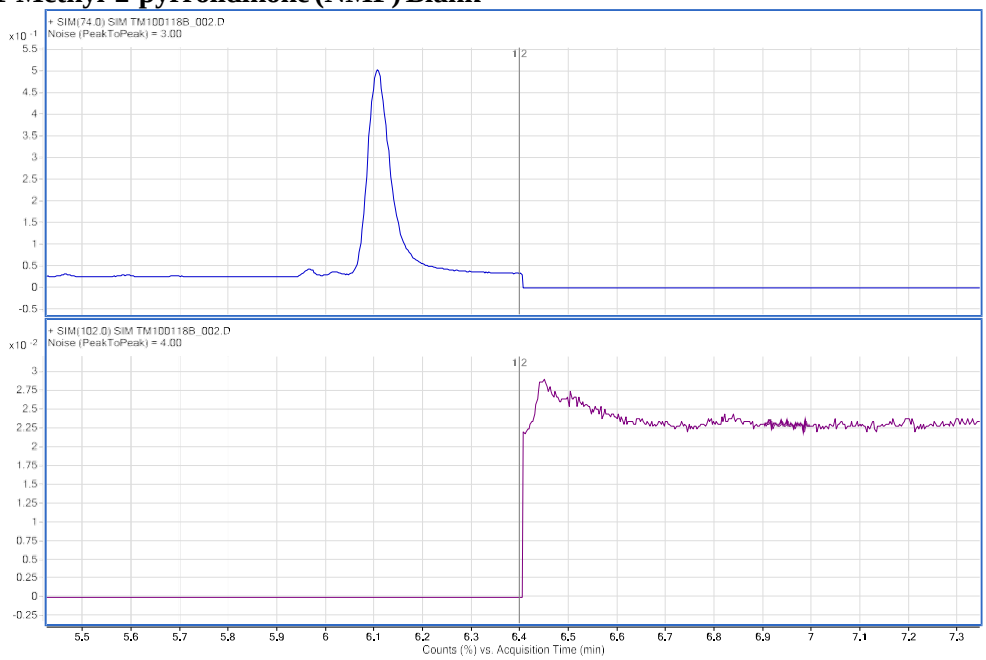
NDMA or NDEA (ppm) = [ (y – b) / m] ÷ wt.
where: y = NDMA or NDEA peak area
b = intercept of the linear curve
m = slope of the linear curve
wt. = Valsartan API weight (g)

Report NDMA/NDEA peaks > LOQ (NDMA LOQ = 0.10 ppm; NDEA LOQ = 0.05 ppm)

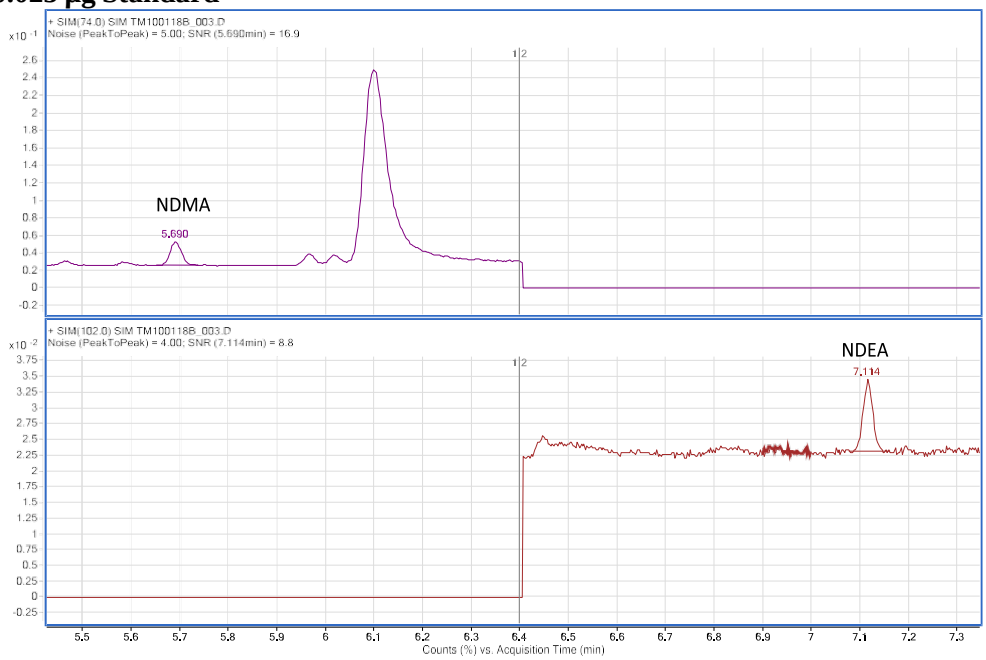
**Limit of Quantitation / Limit of Detection:**
Limit of detection (LOD) was determined by preparing standards of known concentrations and calculating the signal to noise ratio. The lowest standard concentration with a S/N of ≥ 3 was designated as the method LOD. Limit of Quantitation (LOQ) was determined by spiking known amounts of standards at different levels into replicate samples (n = 3) of Valsartan drug substance. Spiked sample level with recoveries of 80 – 120% and a % RSD of ≤ 10 was designated as the method LOQ.

## Example Chromatograms:

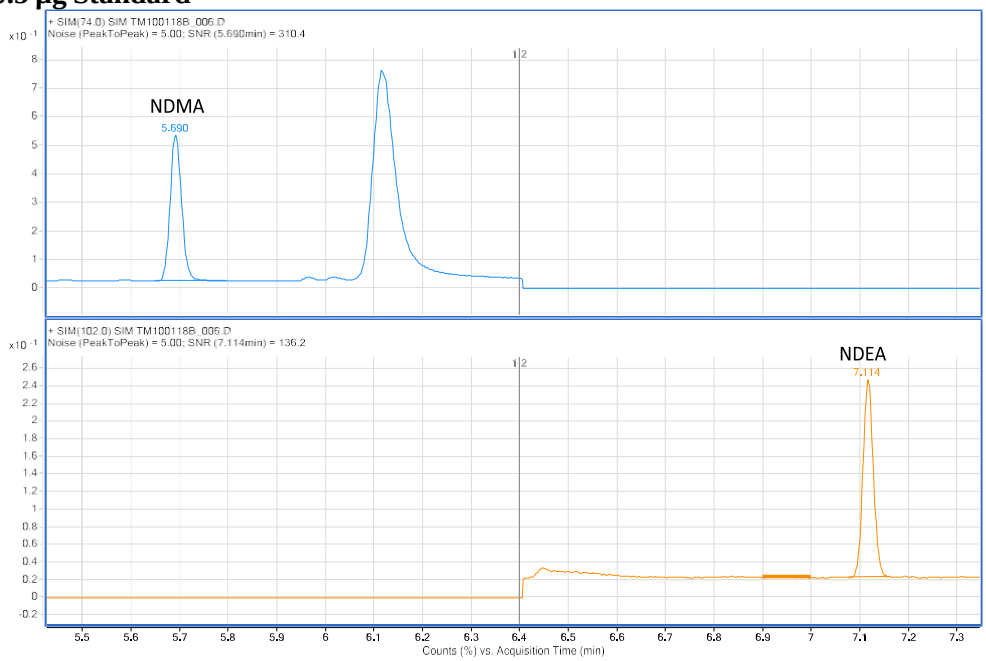
### 1-Methyl-2-pyrrolidinone (NMP) Blank




### 0.025 µg Standard



## 0.5 µg Standard




## Valsartan Drug Substance