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

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). This impurity is classified as a probable human carcinogen and is believed to have been introduced into the finished products as a result of the manufacturing process of the drug substance. Subsequently, an additional nitrosamine, N-nitrosodiethylamine (NDEA), has also been detected in some valsartan products. OTR has been asked to develop a gas chromatography-tandem mass spectrometry (GC-MS/MS) method utilizing liquid injection.

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

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Drug Substance Limit of Quantitation (LOQ), ppm</th>
<th>Drug Product Limit of Quantitation (LOQ), ppm</th>
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</thead>
<tbody>
<tr>
<td>N-nitrosodimethylamine (NDMA)</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>N-nitrosodiethylamine (NDEA)</td>
<td>0.03</td>
<td>0.04</td>
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</tbody>
</table>
NDMA and NDEA Impurity Assay in Valsartan Drug Substance and Drug Product by Liquid Injection GC-MS/MS

Instrument and Equipment
Gas Chromatograph with Liquid Autosampler and a Triple Quadrupole Mass Selective Detector
Class A Glassware
Centrifuge
VF-WAXms GC Column: 30m x 0.25mm, 1.00µm
Vortex Mixer
15mL Disposable Glass Centrifuge Tubes
0.45µm Nylon filters
5mL Syringes

Reagents
Methylene Chloride
N-nitrosodimethylamine (NDMA): 100µg/mL in MeOH
N-nitrosodiethylamine (NDEA): 1mg/mL in MeCl₂
N-nitrosodimethylamine-C13-d6 labeled (NDMA:C13-d6): 1mg/mL in MeCl₂

Standard Preparation

Internal Standard Solution (IS)
To a 500mL of methylene chloride, transfer 25µL of NDMA:C13-d6 standard utilizing a 100 µL gas-tight syringe. Mix well. (~50ng/mL IS)

NDMA/NDEA 1µg/mL Standard Stock
Utilizing a 100 µL gas-tight syringe, transfer 200µL of NDMA stock standard to a 20 mL volumetric flask containing approximately 18mL of IS. Add 20µL of NDEA std via a 100µL gas-tight syringe. Dilute to volume with IS and mix well.

NDMA/NDEA 100ng/mL Standard (Std 1)
1:10 dilution of Standard Stock with IS utilizing class A glassware.

NDMA/NDEA 10ng/mL Standard (Std 2)
1:10 dilution of Std 1 with IS utilizing class A glassware.

NDMA/NDEA 5ng/mL Standard (Std 3)
5:10 dilution of Std 2 with IS utilizing class A glassware.

NDMA/NDEA 2.5ng/mL Standard (Std 4)
5:10 dilution of Std 3 with IS utilizing class A glassware.
NDMA/NDEA 50ng/mL Standard (Std 5)
5:10 dilution of Std 1 with IS utilizing class A glassware.

NDMA/NDEA 25ng/mL Standard (Std 6)
5:10 dilution of Std 5 with IS utilizing class A glassware.

NDMA/NDEA 80ng/mL Standard (Std 7)
2:25 dilution of Standard Stock with IS utilizing class A glassware.

Sample Preparation for Drug Substance
Accurately weigh approximately 0.5g of drug substance into a disposable 15 mL glass centrifuge tube. Add 5mL of IS via volumetric pipet. Cap tube. Vortex sample for 1 min and then place in the centrifuge. Spin at 4000 rpm for 2.5 min. Using a disposable pipet, transfer approximately 2mL of the bottom MeCl₂ layer to a 5mL syringe fitted with a 0.45µm Nylon filter. Filter 1mL of sample into a 2mL HPLC vial and cap.

Sample Preparation for Drug Product
Using a pill cutter, quarter one tablet and place the pieces into a disposable 15 mL glass centrifuge tube. Add 5mL of IS via volumetric pipet. Cap tube. Vortex sample for 1 min or until the tablet is dispersed, and then place in the centrifuge. Spin at 4000 rpm for 2.5 min. Using a disposable pipet, transfer approximately 2 mL of the MeCl₂ layer to a 5mL syringe fitted with a 0.45µm Nylon filter. Filter approximately 0.5mL of sample into a 2mL HPLC vial and cap. A 100µL glass vial insert can be utilized if needle depth into the sample is a concern.

<table>
<thead>
<tr>
<th><strong>Gas Chromatograph (GC) Conditions</strong></th>
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<tbody>
<tr>
<td>Inlet Temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Transferline Temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Injection Type</td>
<td>Pulsed Splitless: 12.285psi until 0.5min</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>2µL</td>
</tr>
<tr>
<td>Flowrate</td>
<td>1mL/min</td>
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<tr>
<td>Oven Program</td>
<td>40°C for 0.5min → 200°C at 20°C/min → 250°C at 60°C/min and hold for 3min</td>
</tr>
<tr>
<td>Runtime</td>
<td>12.33min</td>
</tr>
</tbody>
</table>

**Mass Spectrometer (QQQ) Conditions**

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>EI Source Temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Quad 1 Temperature</td>
<td>150°C</td>
</tr>
<tr>
<td>Quad 2 Temperature</td>
<td>150°C</td>
</tr>
<tr>
<td>Helium Quench Gas</td>
<td>4mL/min</td>
</tr>
<tr>
<td>Nitrogen Collision Gas</td>
<td>1.5mL/min</td>
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<tr>
<td>Electron Energy</td>
<td>-30eV</td>
</tr>
<tr>
<td>Solvent Delay</td>
<td>6.5min</td>
</tr>
<tr>
<td>QQQ Stop time</td>
<td>8.5min</td>
</tr>
<tr>
<td>NDMA MRM Start Time</td>
<td>4.00min</td>
</tr>
<tr>
<td>NDEA MRM Start Time</td>
<td>7.80min</td>
</tr>
</tbody>
</table>
NDMA MRM 1 (Quantitation) 74amu→44amu (Dwell Time: 150ms, CE=15V)
NDMA MRM 2 74amu→42amu (Dwell Time: 50ms, CE=20V)
NDEA MRM 1 (Quantitation) 102amu→85amu (Dwell Time: 150ms, CE=10V)
NDEA MRM 2 102amu→56amu (Dwell Time: 150ms, CE=18V)
NDMA:C13-d6 MRM (Quantitation) 82amu→48amu (Dwell Time: 100ms CE=20V)
NDMA MRM: MS1 and MS2 Resolution MS1: Unit MS2: Wide
NDEA MRM: MS1 and MS2 Resolution MS1 and MS2: Wide
NDMA:C13-d6 MRM: MS1 and MS2 Resolution MS1: Unit MS2: Wide

System Suitability:
The coefficient of determination ($R^2$) of the linear calibration curves should be $\geq 0.998$. The S/N ratio of the 5 ng/mL linearity standard should be $\geq 10$.

Calculations:
Plot the response factor of the NDMA and NDEA peak areas to the IS peak area against the standard concentration (ng/mL). Determine the intercepts, slopes and coefficients of determination for each linear curve. Calculate the NDMA and NDEA impurities (ppm) using the formula below:

$$(ppm) = \frac{[(y - b) / m] \times EV \times 1 \mu g/1000 ng}{wt.}$$

where: $y =$ NDMA or NDEA to IS response factor
$
b =$ intercept of the linear curve
$m =$ slope of the linear curve
$EV =$ Extraction Volume = 5 mL
$wt.$ = Valsartan API weight (g)

Report any NDMA peak $\geq 0.3$ ppm and any NDEA peak $\geq 0.08$ ppm

Note:
Drug substance LOQ calculations for this method were based on 500mg of Valsartan drug substance. Increasing this amount weighed out and extracted will lower the reported LOQ. Drug product LOQ calculations for this method were based on 320mg of Valsartan drug substance.
Example Chromatograms

NDMA LOQ (0.05ppm)

*The peak at 7.354min is NDMA.

NDEA LOQ (0.03ppm)

*The peak at 7.950min is NDEA.
NDMA and NDEA Extracted from Drug Substance (0.25ppm for both impurities)

NDMA elutes at 7.351min and NDEA at 7.945min.

NDMA and NDEA Extracted from Drug Product (0.3ppm for both impurities)

NDMA elutes at 7.352min and NDEA at 7.948min.