Combined Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA) Impurity Assay by GC-MS/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. Subsequently, an additional nitrosamine, N-nitrosodiethylamine (NDEA), has also been detected in some valsartan products. N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA), and N-Nitrosomethyl-4-amino-butyric acid (NMBA) have also been flagged as potential nitrosamine impurities. OTR has been asked to develop a gas chromatography-tandem mass spectrometry (GC-MS/MS) method utilizing liquid injection to look for all these nitrosamine impurities.

Conclusions: The combined method has been validated to simultaneously quantify NDMA, NDEA, NEIPA, NDIPA, and NDBA in Valsartan API and verified for Valsartan drug products. It should be verified for other sartan API's and drug products.

Impurity	Drug Substance Limit of Quantitation (LOQ), ppm	Drug Product Limit of Quantitation (LOQ), ppm
N-nitrosodimethylamine (NDMA)	0.008	0.013
N-nitrosodiethylamine (NDEA)	0.005	0.008
N-nitrosoethylisopropylamine (NEIPA)	0.005	0.008
N-nitrosodiisopropylamine (NDIPA)	0.005	0.008
N-nitrosodibutylamine (NDBA)	0.025	0.040

Impurity	Drug Substance Limit of Detection (LOD), ppm	Drug Product Limit of Detection (LOD), ppm
N-nitrosodimethylamine (NDMA)	0.005	0.008
N-nitrosodiethylamine (NDEA)	0.001	0.002
N-nitrosoethylisopropylamine (NEIPA)	0.001	0.002
N-nitrosodiisopropylamine (NDIPA)	0.001	0.002
N-nitrosodibutylamine (NDBA)	0.010	0.016

- LOD chromatographically determined based on USP S/N = 3
- LOQ chromatographically determined based on USP S/N =10
- Drug substance LOD/LOQ calculations for this method were based on 500 mg of Valsartan API. Increasing the amount weighed out and extracted will lower the reported LOQ. Drug product LOD/LOQ calculations were based on one tablet containing 320 mg of Valsartan API.

NDMA, NDEA, NEIPA, NDIPA, and NDBA 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: 30 m x 0.25 mm, 1.00 µm
Single tapered splitless inlet liner (900µL) with deactivated glass wool
Vortex Mixer
15mL Disposable Glass Centrifuge Tubes
0.45 µm Nylon filters
5 mL Syringes

Reagents

Methylene Chloride
N-nitrosodimethylamine (NDMA): 1 mg/mL in MeOH
N-nitrosodiethylamine (NDEA): 1 mg/mL in MeOH
N-nitrosoethylisopropylamine (NEIPA): 1 mg/mL in MeOH
N-nitrosodiisopropylamine (NDIPA): 100 µg/mL in MeOH
N-nitrosodi-n-butylamine (NDBA): 1 mg/mL in MeOH
N-nitrosodimethylamine-C13-d6 labeled (NDMA:C13-d6): 1 mg/mL in $MeCl_2$

Standard Preparation

Internal Standard Solution (IS)

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

NDMA/NDEA/NEIPA/NDIPA/NDBA Stock Standard (1 µg/mL)

Utilizing a 100 µL gas-tight syringe, transfer 100 µL of NDMA, NDEA, NEIPA and NDBA reference standards (1 mg/mL) to a 100 mL volumetric flask containing approximately 90 mL of IS. Add 1 mL of NDIPA reference standard (100 µg/mL) via a volumetric pipet to the same volumetric flask. Dilute to volume with IS and mix well.

NDMA/NDEA/NEIPA/NDIPA/NDBA 100 ng/mL Standard (Std 1)

1:10 dilution of Stock Standard with IS utilizing class A glassware.

NDMA/NDEA/NEIPA/NDIPA/NDBA 80 ng/mL Standard (Std 2)

2:25 dilution of Stock Standard with IS utilizing class A glassware.

NDMA/NDEA/NEIPA/NDIPA/NDBA 40 ng/mL Standard (Std 3)

1:25 dilution of Stock Standard with IS utilizing class A glassware.

NDMA/NDEA/NEIPA/NDIPA/NDBA 20 ng/mL Standard (Std 4)

1:50 dilution of Stock Standard with IS utilizing class A glassware.

NDMA/NDEA/NEIPA/NDIPA/NDBA 10 ng/mL Standard (Std 5):

1:10 dilution of Std 1 with IS utilizing class A glassware.

NDMA/NDEA/NEIPA/NDIPA/NDBA 5 ng/mL Standard (Std 6)

1:20 dilution of Std 1 with IS utilizing class A glassware.

NDMA/NDEA/NEIPA/NDIPA/NDBA 2.5 ng/mL Standard (Std 7)

5:10 dilution of Std 6 with IS utilizing class A glassware.

Sample Preparation for API

Accurately weigh approximately 0.5 g of API into a disposable 15 mL glass centrifuge tube. Add 5 mL 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 2 mL of the $MeCl_2$ layer to a 5 mL syringe fitted with a 0.45 µm Nylon filter. Filter 1 mL of sample into a 2 mL HPLC vial and cap.

Sample Preparation for Drug Product

Using a pill cutter, quarter one tablet (smaller tablets may be halved) and place the pieces into a disposable 15 mL glass centrifuge tube. Add 5 mL of IS via volumetric pipet. Cap tube. Vortex sample for at least 1 min or until the tablet is dispersed in the solution and then place in the

centrifuge. Spin at 4000 rpm for 2.5 min. Using a disposable pipet, transfer approximately 2 mL of the $MeCl_2$ layer to a 5 mL syringe fitted with a 0.45 µm Nylon filter. Filter approximately 0.5 mL of sample into a 2mL HPLC vial and cap. A 100 µL glass vial insert can be utilized if needle depth into the sample is a concern.

Gas Chromatograph (GC) Conditions	
Inlet Temperature	250 ºC
Transfer line Temperature	250 ºC
Injection Type	Pulsed Splitless: 12.285 psi until 0.5min
Injection Volume	2 µL
Flowrate	1 mL/min
Oven Program	40 ºC for 0.5 min→200 ºC at 20 ºC/min→250 ºC at 60 ºC/min and hold for 3 min
Runtime	12.33 min
Mass Spectrometer (QQQ) Conditions	
EI Source Temperature	250 ºC
Quad 1 Temperature	150 ºC
Quad 2 Temperature	150 ºC
Helium Quench Gas	4 mL/min
Nitrogen Collision Gas	1.5 mL/min
Electron Energy	-40 eV
Solvent Delay	6.50 min
NDMA:C13-d6 MRM Start Time	6.50 min
NDMA Start Time	6.50 min
NDEA Start Time	7.60 min
NEIPA MRM Start Time	8.03 min
NDIPA MRM Start Time	8.25 min
NDBA MRM Start Time	8.70 min
NDMA MRM 1 (Quantitation)	74 amu→44 amu (Dwell Time: 150 ms, CE=15 V
NDMA MRM 2	74 amu→42 amu (Dwell Time: 50 ms, CE=20 V)
NDEA MRM 1 (Quantitation)	102 amu→85 amu (Dwell Time: 150 ms, CE=10 V)
NDEA MRM 2	102 amu→56 amu (Dwell Time: 150 ms, CE=18 V)
NEIPA MRM 1 (Quantitation)	116 amu→99 amu (Dwell Time: 150 ms, CE=10 V
NEIPA MRM 2	71 amu→56 amu (Dwell Time: 150 ms, CE=10 V)
NDIPA MRM 1 (Quantitation)	130 amu→88 amu (Dwell Time: 150 ms, CE=10 V)

NDIPA MRM 2	130 amu→42 amu (Dwell Time: 150 ms, CE=10 V)
NDBA MRM 1 (Quantitation)	158 amu→99 amu (Dwell Time: 150 ms, CE=10 V)
NDBA MRM 2	84 amu→56 amu (Dwell Time: 150 ms, CE=22 V)
NDMA:C13-d6 MRM (Quantitation)	82 amu→48 amu (Dwell Time: 100 ms CE=20 V)
MS1 and MS2 Resolution	MS1: Unit MS2: Unit

System Suitability:
The coefficient of determination (R^2) of the linear calibration curve should be ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.
% RSD of six replicate injections of the 40 ng/mL standard should be ≤ 5

Calculations:
Plot the response factor of the nitrosamine 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 nitrosamine impurities (ppm) using the formula below:

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

where: y = Nitrosamine 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)

Example Chromatogram