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.

Impurity	**Drug Substance Limit of Quantitation (LOQ), ppm**	**Drug Product Limit of Quantitation (LOQ), ppm**
N-nitrosodimethylamine (NDMA)	0.05	0.08
N-nitrosodiethylamine (NDEA)	0.03	0.04
Impurity	**Drug Substance Limit of Detection (LOD), ppm**	**Drug Product Limit of Detection (LOD), ppm**
N-nitrosodimethylamine (NDMA)	0.010	0.015
N-nitrosodiethylamine (NDEA)		

Note: LOQ's determined utilizing the ICH signal to noise approach. S/N = 10
LOD's determined based on the ICH's statistical formula: LOD = [3.3σ ÷ S]
where σ is the standard deviation of y-intercepts for the regression line and S is the slope of the regression line.

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 $MeCl_2$
N-nitrosodimethylamine-C13-d6 labeled (NDMA:C13-d6): 1mg/mL in $MeCl_2$

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 1min and then place in the centrifuge. Spin at 4000 rpm for 2.5 min. Using a disposable pipet, transfer approximately 2mL of the bottom $MeCl_2$ 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 $MeCl_2$ 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.

Gas Chromatograph (GC) Conditions	
Inlet Temperature Transferline Temperature	250°C 250°C
Injection Type	Pulsed Splitless: 12.285psi until 0.5min
Injection Volume	2µL
Flowrate	1mL/min
Oven Program	40°C for 0.5min→200°C at 20°C/min→250°C at 60°C/min and hold for 3min
Runtime	12.33min
Mass Spectrometer (QQQ) Conditions	
EI Source Temperature	250°C
Quad 1 Temperature	150°C
Quad 2 Temperature	150°C
Helium Quench Gas	4mL/min
Nitrogen Collision Gas	1.5mL/min
Electron Energy	-30eV
Solvent Delay	6.5min
QQQ Stop time	8.5min
NDMA MRM Start Time	4.00min
NDEA MRM Start Time	7.80min

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 ≥ 0.998.
The S/N ratio of the 5 ng/mL linearity standard should be ≥ 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:

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

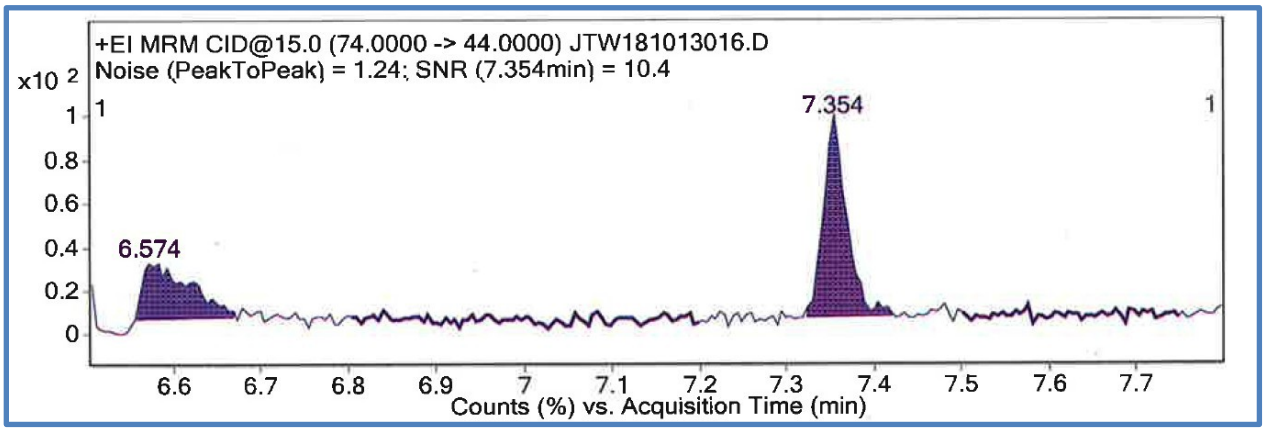
where: y = NDMA or NDEA to IS response factor
b = intercept of the linear curve
m = slope of the linear curve
EV = Extraction Volume = 5 mL
wt. = Valsartan API weight (g)

Report any NDMA peak ≥ 0.3 ppm and any NDEA peak ≥ 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.

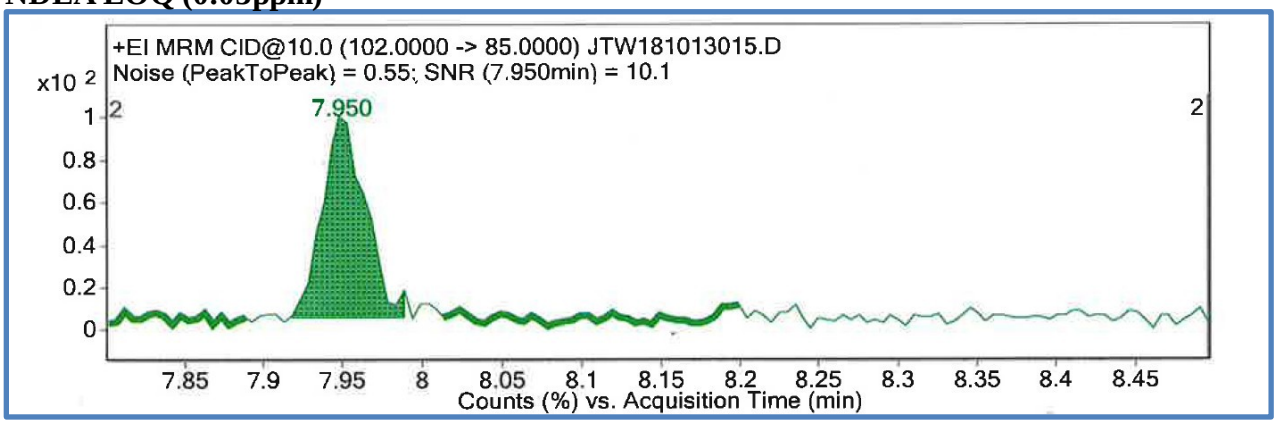
Example Chromatograms

NDMA LOQ (0.05ppm)



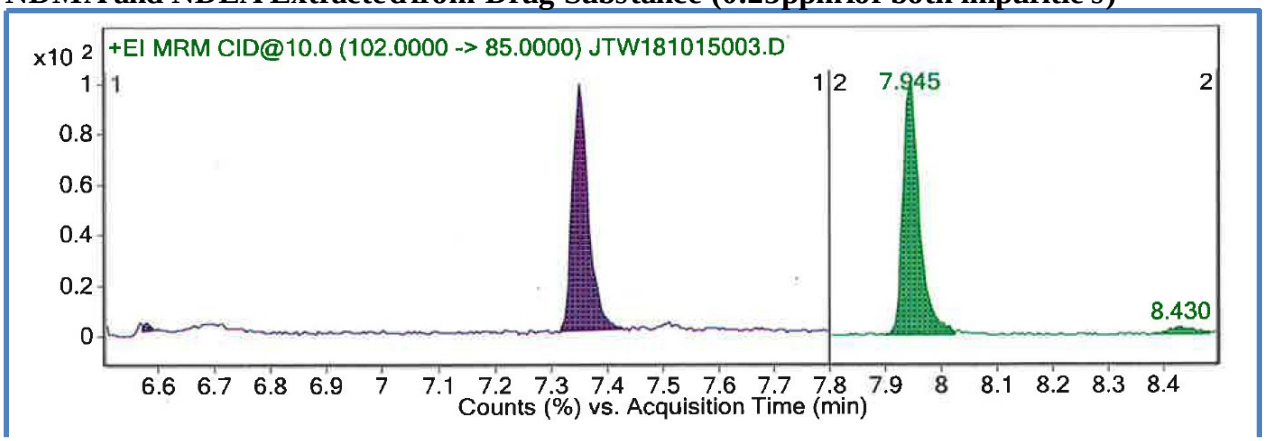
*The peak at 7.354min is NDMA.

NDEA LOQ (0.03ppm)



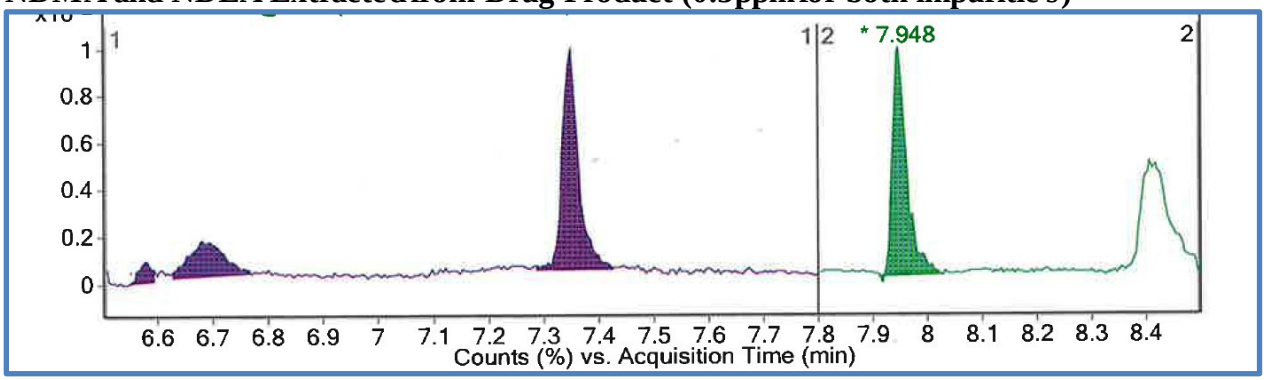
*The peak at 7.950min is NDEA.

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