Combined Headspace N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), and N-Nitrosodiisopropylamine (NDIPA) 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 nitrosamine impurities. These impurities: (N-nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-diisopropylnitrosoamine (NDIPA), and N-ethyl-N-isopropylnitrosoamine (NEIPA) are classified as probable human carcinogens and are believed to have been introduced into the finished products because of the manufacturing process. OTR has been asked to develop a gas chromatography-mass spectrometry (GC/MS) headspace method to comprehensively detect the presence of NDMA, NDEA, NDIPA, and NEIPA in angiotensin II receptor blockers (ARBs).

Conclusions

The combined OTR GC/MS headspace method was developed successfully for the simultaneous evaluation of four nitrosamine impurities in ARB drug substance and drug product. The specific sensitivity details of the validated method for each of the four nitrosamine impurities are reported below. The method was developed and validated on valsartan drug substance and drug product.

Impurity	Drug Substance LOQ, ppm	Drug Substance LOD, ppm	Drug Product LOQ, ppm	Drug Product LOD, ppm
NDMA	0.05	0.01	0.05	0.01
NDEA	0.05	0.01	0.05	0.01
NEIPA	0.05	0.025	0.05	0.025
NDIPA	0.05	0.025	0.05	0.025

NDMA, NDEA, NEIPA, and NDIPA Impurity Assay in Valsartan Drug Substance and Drug Product by Headspace GC/MS

Instrument and Equipment

Gas Chromatography System with a Quadrupole Mass Spectrometry Detector and Headspace Auto-sampler
DB-Wax GC Column, 30 m x 0.25 mm, 0.5 µm, or equivalent
Analytical Balance
Wrist Action Mechanical Shaker
Vortex Mixer
Tablet Cutter
20 mL Headspace Vials
HS vial caps with Teflon/Silicone septa

Solvent

Dimethyl sulfoxide (DMSO), > 99.5%

Standard Stock Solutions

Stock 1: N-nitrosodimethylamine (NDMA): 1 mg/mL in MeOH
Stock 2: N-nitrosodiethylamine (NDEA): 1 mg/mL in MeOH
Stock 3: N-nitrosoethylisopropylamine (NEIPA): 1 mg/mL in MeOH
Stock 4: N-nitrosodiisopropylamine (NDIPA): 1 mg/mL in MeOH
N-nitrosodimethylamine-d6 labeled (NDMA d6): 1 mg/mL in MeOH
N-nitrosodiethylamine-d4 labeled (NDEA d4): 1 mg/mL in MeOH

Standards Preparation

Internal Standard Solution (NDMA d6 & NDEA d4**):**

To 100 mL volumetric flask containing approximately 90 mL DMSO, transfer 1 mL each of NDMA-d6 and NDEA-d4 standard stock solution (1 mg/mL) utilizing a 1000 µL pipettor. Make up the volume to 100 mL with DMSO and mix well to get 10 µg/mL concentration.

Nitrosamine Calibration Mix Standards (NDMA, NDEA, NEIPA, & NDIPA):

Refer to the table 1 below for a suggested calibration standards preparation scheme.

Table 1. Nitrosamine Calibration Standard Mix Solution Preparation Scheme:

CAL Level	Nitrosamine STD Mix Conc. (µg/mL)	ID of Spike Pool	Spiking Solution Conc. (µg/mL)	Spike Vol. (mL)	Blank DMSO Vol. (mL)	Total Vol. (mL)
1	100	Stock (1-4)	1000	1.0 mL from each stock	6.0	10
2	20.0	CAL 1	100	1.0	4.0	5.0
3	10.0	CAL 1	100	1.0	9.0	10.0

4	5.0	CAL 1	100	1.0	19.0	20.0
5	1.0	CAL 4	5.0	1.0	4.0	5.0
6	0.50	CAL 4	5.0	1.0	9.0	10.0
7	0.25	CAL 4	5.0	1.0	19.0	20.0
8	0.15	CAL 6	0.5	3.0	7.0	10.0
9	0.05	CAL 6	0.5	1.0	9.0	10.0

Working Standard Preparations for Headspace GC/MS (0.05 – 100 µg):
Transfer a 1.0 mL aliquot volume of the calibration standard solutions (table 1) and 0.5ml internal standard mix (10 µg/mL) into separate 20 mL headspace vials containing 3.5 mL of DMSO solvent. Immediately cap and crimp the headspace vials. Refer to the table 2 below for the working standard preparation scheme.

Table 2. Working Standard Preparation Scheme for Headspace GC/MS:

Working Standard	Nitrosamine STD Mix Conc. (µg/mL)	Aliquot Vol. (mL)	IStd (10 µg/mL) Vol. (mL)	DMSO Vol. (mL)	Total Vol. (mL)	Nitrosamine Mix Amount (µg)
1	100	1.0	0.5	3.5	5	100
2	20.0	1.0	0.5	3.5	5	20.0
3	10.0	1.0	0.5	3.5	5	10.0
4	5.0	1.0	0.5	3.5	5	5.0
5	1.0	1.0	0.5	3.5	5	1.0
6	0.5	1.0	0.5	3.5	5	0.5
7	0.25	1.0	0.5	3.5	5	0.25
8	0.15	1.0	0.5	3.5	5	0.15
9	0.05	1.0	0.5	3.5	5	0.05

Sample Preparation for API
Accurately weigh 500 mg of Valsartan drug substance into a 20 mL headspace vial. Add 4.5 mL of DMSO and 0.5 mL of IStd mix solution 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 nitrosamine impurities in the drug substance.

Sample Preparation for Drug Product
Accurately weigh Valsartan drug product equivalent to 320 mg Valsartan into a 20 mL headspace vial. Add 4.5 mL of DMSO and 0.5 mL of IStd mix solution to the vial and immediately cap and crimp the vial. Mix the sample solution using a vortex mixer. Drug product weight could be increased or decreased, depending on the amount of nitrosamine impurities in the drug substance.

Note: The method was validated 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-WAX, 30 m x 0.25 mm, 0.5 µm (PN: 122-7033), or equivalent
Inlet Temperature:	220 °C
Column Flow:	1 mL/min
Split Ratio	5:1
Oven Program:	70 °C for 4 min.; 20 °C/min to 240 °C, Hold for 3.5 min.
GC Run Time	16 min.
GC Cycle Time:	24 min.
HS Auto-sampler Parameters	
Oven Temperature:	120 °C
Loop Temperature:	125 °C
Transfer Line Temperature:	130 °C
Vial Equilibration Time:	15 min
Injection Time:	1.0 min
Vial Size:	20 mL
Vial Shaking:	Level 9 (250 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	5
Solvent Delay:	6.0 min.
Group 1 (NDMA & NDMA-d6)	
Group Start Time : 6min	
Number of Ions : 4 (NDMA: 74.0 Dwell 60, 42.1 Dwell 60), (NDMA-d6: 80.1 Dwell 60, 46.1 Dwell 60)	
Group 2 (NDEA & NDEA-d4)	
Group Start Time : 7min	
Number of Ions : 4 (NDEA: 102.1 Dwell 60, 57.0 Dwell 60), (NDEA-d4: 106.1 Dwell 60, 61.1 Dwell 60)	
Group 3 (NDIPA & NEIPA)	
Group Start Time : 7.52min	
Number of Ions : 4 (NDIPA: 130.0 Dwell 60, 43.0 Dwell 60), (NEIPA: 116.0 Dwell 60, 56.0 Dwell 60)	

System Suitability:
System suitability test was conducted on each day during the method validation. Analyte retention time and total area under the curve (AUC) were tested for compliance with USP limits and determined acceptable. The average retention time for NDMA was 6.5 min, %RSD 0.03, NDEA was 7.3, %RSD 0.03, NDIPA was 7.9, %RSD 0.0 and NEIPA was 7.6 min, %RSD 0.04.

Linearity
The correlation coefficient (R) of the linear calibration curves should be ≥ 0.995.
The S/N ratio of the 0.05 µg working standard should be ≥ 10.

Calculations:
Plot the NDMA, NDEA, NDIPA, and NEIPA peak areas ratio against the standard concentration (µg) from 0.05 – 100 µg. Determine the intercepts, slopes and correlation coefficients of the linear curves. Calculate the NDMA, NDEA, NDIPA, and NEIPA impurity (ppm) using the formula below:

$$\text{NDMA, NDEA, NDIPA or NEIPA (ppm)} = [(y - b) / m] \div \text{wt.}$$

where: y = NDMA, NDEA, NDIPA or NEIPA peak area ratio
b = intercept of the linear curve
m = slope of the linear curve
wt. = Valsartan API weight (g)

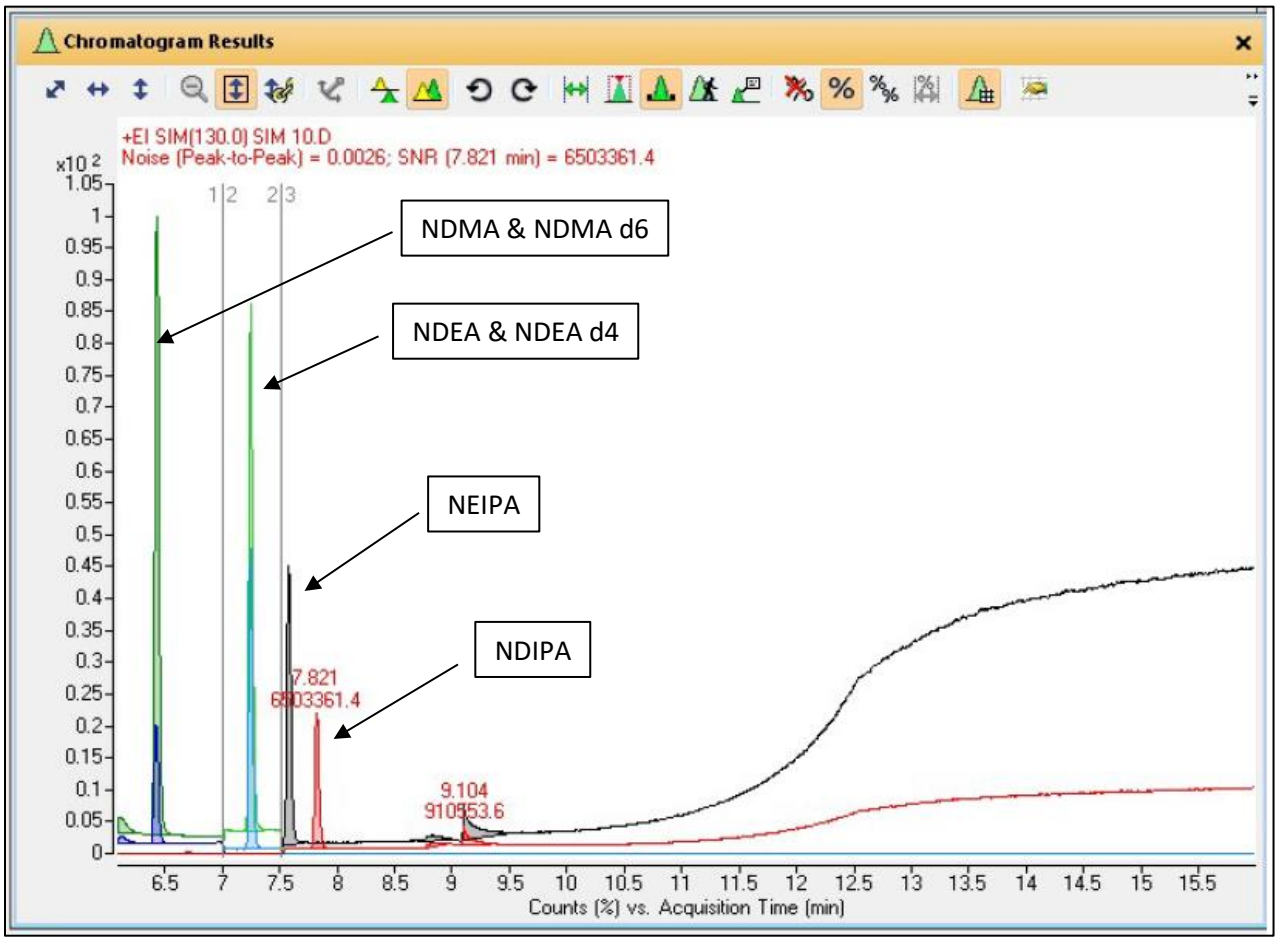
Report any NDMA, NDEA, NDIPA, NEIPA peak ≥ 0.05 ppm (LOQ)

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 a known amount of standard at different concentration levels into replicate samples (n = 3) of Valsartan drug substance. The spiked sample level with recoveries of 80 – 120% and a % RSD of ≤ 10 was designated as the method LOQ.

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

Example Chromatograms:

0.25 µg NDMA, NDEA, NDIPA, NEIPA Working Standard and IStd NDMA d6 and NDEA d4



500mg Valsartan Drug Substance and spiked 0.25 µg Working Standard and IStd NDMA d6 and NDEA d4