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Application News

NO. GC-23-ADI-079

GCMS-TQ8050 NX, HS-20

Quantitation of 7 Nitrosamines in Active Pharmaceutical Ingredient by HSGCMS/MS as per proposed USP General Chapter <1469>

Introduction

Overview : The Drug Regulatory Authorities first noticed the presence of the nitrosamine impurity (NSA), N-Nitrosodimethylamine (NDMA) in products containing valsartan in July 2018. Valsartan is an Angiotensin II Receptor Blocker (ARB) and belongs to a family of analogue compounds commonly referred to as the Sartans. Further, few other nitrosamines were subsequently detected in other drug substances belonging to the Sartan family & other Active Pharmaceutical Ingredients (API's) & Finished Pharmaceutical Products (FPP), including: N-Nitrosodiethylamine (NDEA), Nitrosodiisopropylamine (NDIPA), Nitrosoethylisopropylamine (NEIPA), N Nitrosodibutylamine (NDBA), N-Nitrosodi-n-propylamine (NDPA) & N-Nitroso-N'-methylpiperazin (NMPrZ).

What are Nitrosamines? : Nitrosamines refer to any molecule containing the nitroso functional group. Although they are also present in some foods and drinking water supplies, their presence in drugs is considered unacceptable.

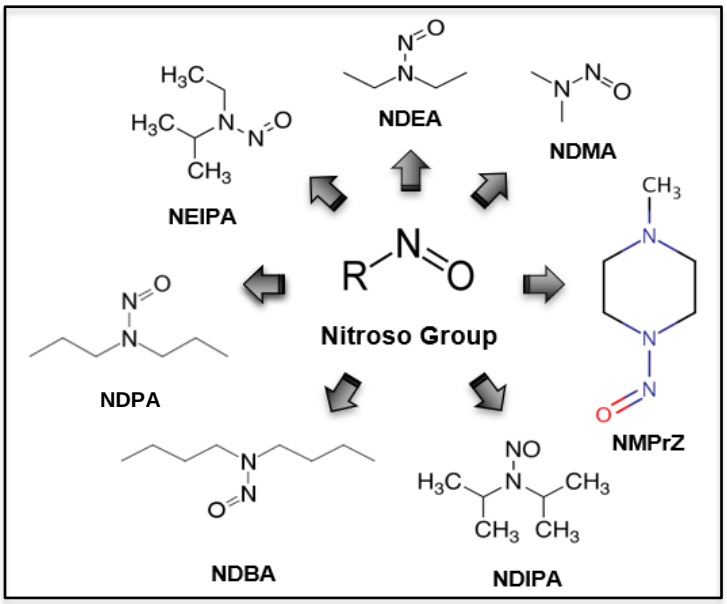

Figure 1: Structure of Nitroso group, NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA & NMPrZ.

Occurrence : Formation of nitrosamines is possible in the presence of secondary, tertiary, or quaternary amines and nitrite salts under acidic reaction conditions. Under these conditions, nitrite salts may form nitrous acid, which can react with an amine to form a nitrosamine. Apart from these there are other routes such as, vendor-sourced starting materials and raw materials, Recovered Solvents, Catalysts, and Reagents, cross contamination from common manufacturing facility, Quenching Process using Nitrous acid & packing/storage may result in Nitrosamine formation or contamination.

Toxicity/ Regulation/ Methods: NDMA and NDEA belong to the so-called "cohort of concern", which is a group of highly potent mutagenic carcinogens that have been classified as probably human carcinogens (PGI). Hence, United state Food & Drug Administration (USFDA) recommends the following acceptable intake (AI's) limits for NDMA, NDEA, NMBA, NMPA, NIPEA, and NDIPA (Table 1). These limits are applicable only if a drug product contains a single nitrosamine, and lowest of which is 0.03 ppm for drug substances (DS) with Maximum daily dose (MDD) of 880 mg/day. If more than one nitrosamine impurity is identified in the same DS the limit for total nitrosamines listed in table 1 is still not more than 26.5 ng/day or 0.03 ppm. Hence, it is imperative to detect above mentioned NSA's with Limit of Quantitation (LOQ) as low as possible to be sure that not just single nitrosamine impurity is below 0.03 ppm, but also total nitrosamine impurities are below 0.03 ppm.

Table 1. AI Limits for Nitrosamines

Nitrosamine	AI Limit (ng/day)	Limit in ppm for MDD 880 mg/day
NDMA	96.0	0.109
NDEA	26.5	0.030
NMBA	96.0	0.109
NMPA	26.5	0.030
NIPEA	26.5	0.030
NDIPA	26.5	0.030

The low levels at which the nitrosamine impurities occur creates challenges for testing in pharmaceuticals & to assist that the USFDA has published several test methods that may be considered when determining nitrosamines in the pharmaceutical products, also recently, the United states Pharmacopeia (USP) declared the proposed General Chapter <1469> for Nitrosamines in Sartans.

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The proposed chapter is aligned with current scientific and regulatory approaches developed to ensure the appropriate control of nitrosamine impurities in drug substances and drug products. The objective of this standard is to provide a science-based approach for the control of nitrosamine impurities, eliminating or reducing their presence in drug products. This application note is based on procedure 2 of General Chapter <1469>.

Experimental:

Table 2: GCMS-TQ8050 NX with HS-20 Operating Conditions.

Instrument Details	**Shimadzu GCMS-TQ8050 NX with HS-20**		
GC Parameters			
Column Details	SH-Stabilwax, 30 m, 0.32 mm I.D., 1.0 µm df		
Injection Mode	Split		
Flow Control Mode	Column Flow		
Detector	Mass spectrometer		
Carrier Gas	Helium		
Column Flow	1.80 mL/min		
Linear Velocity	48.5 cm/sec		
Temp. Program	**Ramp Rate (ºC/min)**	**Temp. (ºC)**	**Hold Time (min)**
	-	45.00	3.00
	10	130.00	3.00
	15	190.00	0.00
	40	240.00	15.25
Diluent	Acetonitrile-Methanol		
MS Parameters			
Ion Source Temp.	250ºC		
Ionization Mode	EI		
Mode	MRM		

MRM Transitions				
	MRM-1	**CE-1**	**MRM-2**	**CE-2**
NDMA	74.00>44.10	6	74.00>42.10	21
NDMA d6	80.00>50.00	5	Not Applicable	
NDEA	102.00>85.10	6	102.00>56.10	15
NEIPA	116.00>99.10	5	71.00>56.10	5
NDIPA	130.00>88.00	6	130.10>42.20	12
NDPA	130.10>113.10	6	130.10>43.20	18
NDBA	116.00>99.10	5	158.00>99.00	10
NMPrZ	99.00>56.10	12	99.00>72.10	9

HS Parameters	
Oven Temp.	110ºC
Pressurizing Gas Pressure	20 psi
Shaking Level	Off
Load Time	2.0 Min
Injection Time	1.0 Min
GC Cycle Time	45.00 Min

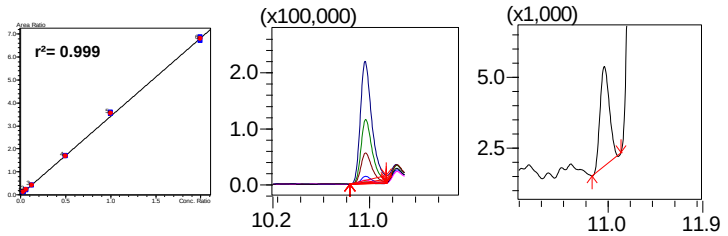
Linearity of the Calibration Curve:

Six-point calibration curves for all 7 NSA's were prepared in methanol and analyzed using the conditions described in Table 2. The range for calibration curves, LOQ established from S/N and % RSD at LOQ are shown in table 3. The figure 2 to 8 depicts the calibration curves, overlay of linearity standards & LOQ solution chromatograms for NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA & NMPrZ respectively.

Table 3: Standard summary. (Results expressed are relative to sample)

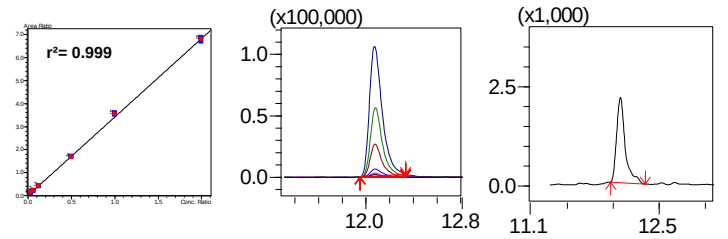
Comp.	**Calibration Range**	**LOQ**		
		Conc. (ppb)	**S/N ***	**% RSD**
NDMA	2.5 to 160 ppb	2.5	50	8.5
NDEA			472	12.6
NEIPA			651	6.6
NDIPA			399	9.5
NDPA	10 to 640 ppb	10	612	7.5
NDBA	5 to 320 ppb	5	58	9.2
NMPrZ	25 to 1600 ppb	25	28	14.6

* = Peak to Peak



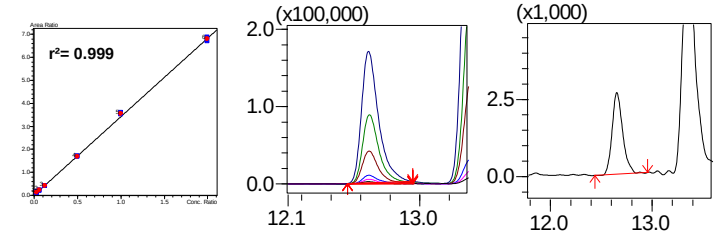
NDMA

Figure 2: Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDMA.



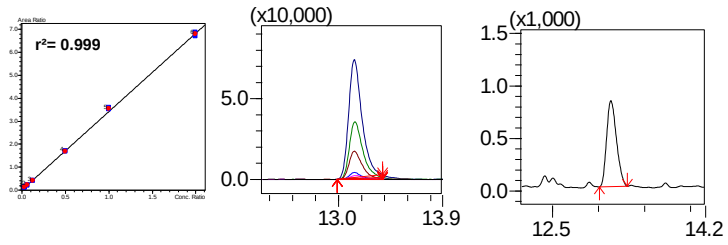
NDEA

Figure 3: Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDEA.



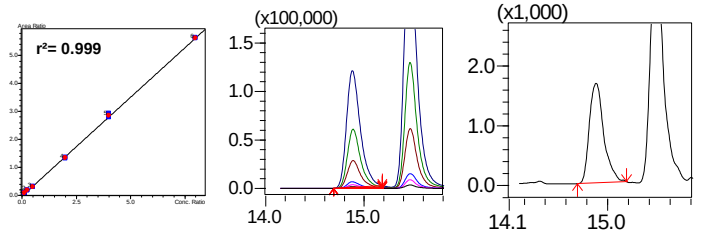
NEIPA

Figure 4: Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NEIPA.



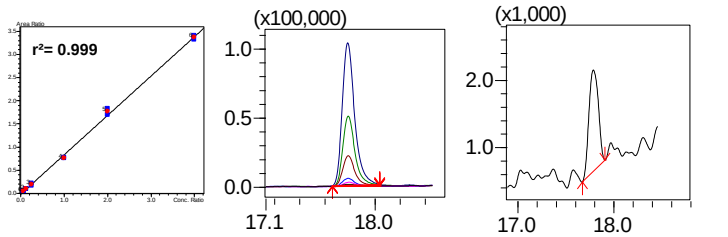
NDIPA

Figure 5: Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDIPA.



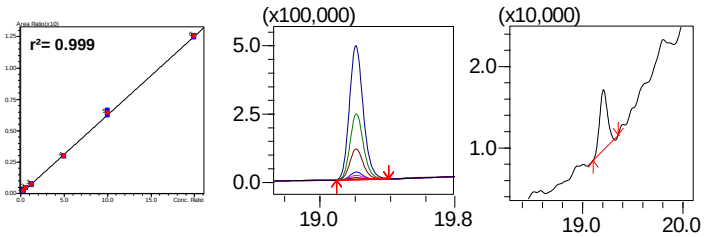
NDPA

Figure 6: Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDPA.



NDBA

Figure 7: Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDBA.



NMPrZ

Figure 8: Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NMPrZ.

Sample Analysis:

Weigh 200 ± 10 mg of Losartan API and 100 mg of imidazole in a headspace vial. Add 1.0 mL of 16.0 µg/L internal standard solution prepared in acetonitrile and 1.0 mL of methanol, crimp the vial tightly.

Spiked Recovery Test:

Weigh 200 ± 10 mg of Losartan API and 100 mg of imidazole in a headspace vial. Add 1.0 mL of 16.0 µg/L internal standard solution prepared in acetonitrile and 1.0 mL of LOQ solution, crimp the vial tightly.

Table 4: Shows results of the sample spiked study for Losartan API (Results expressed are relative to sample)

Losartan API				
Name	**Spiked Amt. (ppb)**	**Sample Amt. (ppb)**	**Found Amt. (ppb)**	**% Recovery**
NDMA	2.5	BLOQ	2.63	**105**
NDEA		BLOQ	2.24	**90**
NEIPA		BLOQ	2.44	**97**
NDIPA		BLOQ	3.19	**127**
NDPA	10.0	BLOQ	10.56	**106**
NDBA	5.0	BLOQ	5.54	**111**
NMPrZ	25.0	BLOQ	27.75	**111**

Note: Criteria for % Recovery as per USP <1469> is 70 to 130%.

BLOQ: Below Limit of Quantitation

Table 5: Shows LOQ comparison of USP <1469> Vs Shimadzu Application note.

Name	**USP <1469>**	**Shimadzu Application Note**
	LOQ (ppb)	**LOQ (ppb)**
NDMA	20.0	**2.5**
NDEA		
NEIPA		
NDIPA		
NDPA	Not Applicable	**10.0**
NDBA		**5.0**
NMPrZ		**25.0**

Conclusion:

- USP General Chapter is applicable to only 4 NSA's (NDMA, NDEA, NEIPA & NDIPA) whereas Shimadzu methodology can be used for quantitation of additional 3 NSA's. (NDPA, NDBA & NMPrZ)
- Shimadzu GCMS-TQ8050 NX with high sensitivity shielded detector offers outstanding noise elimination with excellent Sensitivity, Repeatability & Precision while outperforming the current regulatory limits by delivering 8 times more sensitivity.

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