SHIMADZU
Excellence in Science

Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8050 NX, HS-20

Quantitation of 7 Nitrosamines in API by HSGC-MS/MS as per proposed USP General Chapter <1469>

Nitish Suryawanshi, Dheeraj Handique, Prashant Hase, Sanket Chiplunkar, Durvesh Sawant, Aseem Wagle, Rahul Dwivedi, Jitendra Kelkar and Pratap Rasam
Shimadzu Analytical (India) Pvt. Ltd.,

User Benefits

- A HSGC-MS/MS method for the determination of 7 nitrosamines in Losartan API as per the proposed USP general chapter <1469>
- The GCMS-TQ8050 NX system easily meets the criteria as per the proposed USP general chapter <1469> procedure-2.

■ 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.

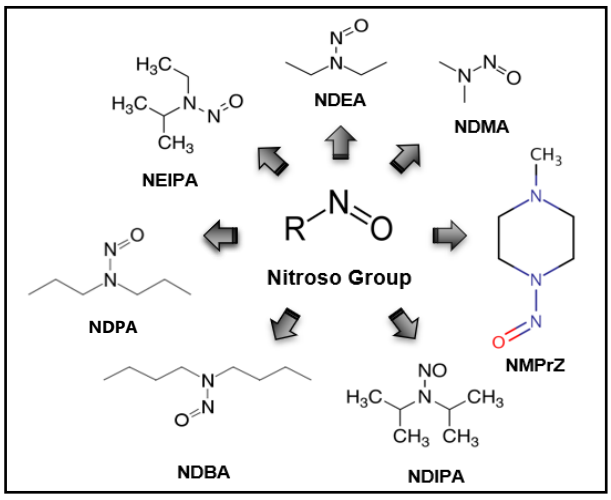

Figure 1: Structure of Nitroso group, NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA & NMPrZ

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, NEIPA, 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
NMBA	96.0	0.109
NDEA	26.5	0.030
NEIPA	26.5	0.030
NMPA	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.

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>.

Figure 2: GCMS-TQ™8050 NX with HS-20 system

■ Method

The MRM transitions of 7 nitrosamines standards & 1 internal standard are given in table 2 and analytical conditions in table 3.

Table 2: MRM transitions of nitrosamines

MRM Transitions				
Nitrosamine Impurity	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

Table 3: Analytical conditions

GCMS System	: GCMS-TQ8050 NX with HS-20		
Column	: SH-Stabilwax 30-meter, 0.32 mm I.D., 1.0 µm d_f		
Injection Mode	: Split		
Flow Control Mode	: Column Flow		
Carrier Gas	: Helium		
Column Flow	: 1.8 mL/min		
Diluent	: Methanol-Acetonitrile		
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
	MS Parameters		
Ionization Mode	: Electron Ionization (EI)		
Ion Source Temp.	: 250 ºC		
CID Gas	: Argon		
	HS Parameters		
Oven Temp.	: 110 ºC		
Pressurizing Gas Pressure	: 20 Psi		
Shaking Level	: off		
Equilibrating Time	: 10.0 Min		
Load Time	: 2.0 Min		
Injection Time	: 1.0 Min		
Cycle Time	: 45.00 Min		

■ 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.

N-nitrosodimethylamine

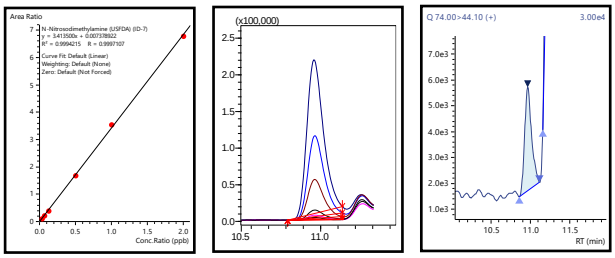

Figure 3: Calibration Curve, Overlay of Linearity Standards & LOQ Solution for NDMA

N-nitrosodiethylamine

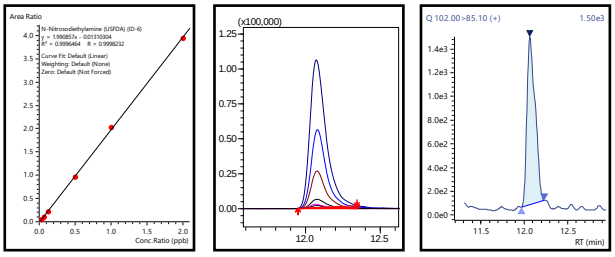

Figure 4: Calibration Curve, Overlay of Linearity Standards & LOQ Solution for NDEA

N-nitrosoethylisopropylamine

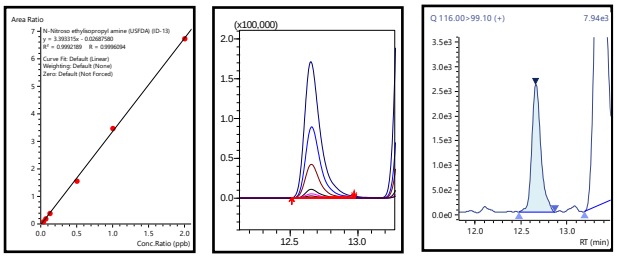

Figure 5: Calibration Curve, Overlay of Linearity Standards & LOQ Solution for NEIPA

N-Nitroso diisopropyl amine

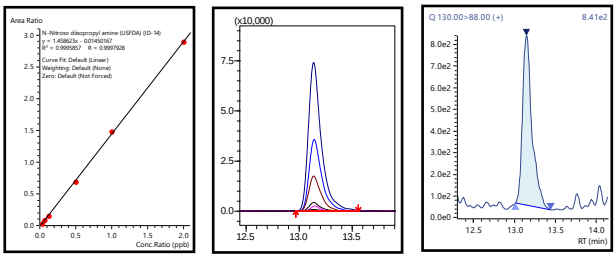

Figure 6: Calibration Curve, Overlay of Linearity Standards & LOQ Solution for NDIPA

N-Nitroso-di-n-propylamine

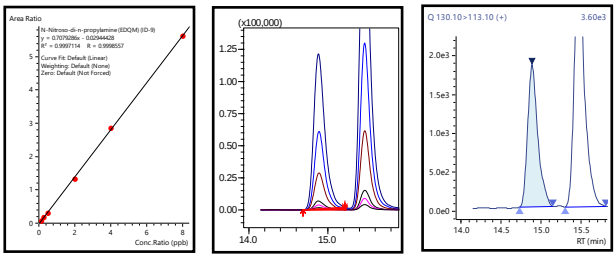

Figure 7: Calibration Curve, Overlay of Linearity Standards & LOQ Solution for NDPA

N-nitrosodibutylamine

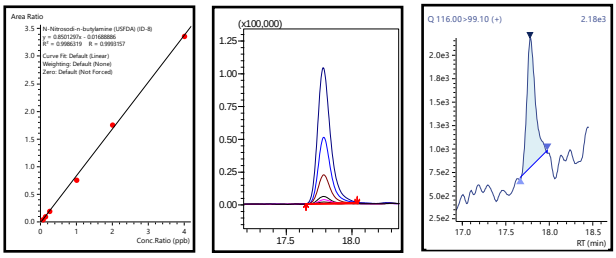

Figure 8: Calibration Curve, Overlay of Linearity Standards & Overlay of LOQ Solution for NDBA

N- Nitroso methyl piperazine

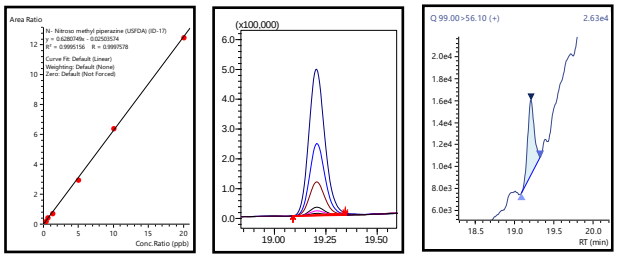

Figure 9: Calibration Curve, Overlay of Linearity Standards & LOQ Solution for NMPrZ

Table 4: Summary of Calibration Curves

Comp.	CC range (ppb)	r^2	LOQ		
			Conc. (ppb)	%RSD (n=6)	S/N*
NDMA	2.5 to 160	0.999	2.5	8.5	50
NDEA		0.999		12.6	472
NEIPA		0.999		6.6	651
NDIPA		0.999		9.5	399
NDPA	10 to 640	0.999	10	7.5	612
NDBA	5 to 320	0.999	5	9.2	58
NMPrZ	25 to 1600	0.999	25	14.6	28

* = Peak to peak

The range for calibration curves, LOQ established from S/N and % RSD at LOQ are shown in table 4. (Conc. expressed are relative to sample)

Table 5: The sample spiked study for Losartan API at LOQ level (Results expressed are relative to sample)

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

Table 6: 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

■ Results

- The 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)
- The Correlation coefficient (r^2) was greater than 0.999 for all the seven nitrosamines. (Table 4)
- The repeatability (n=6) at LOQ level was found to be less than 15% RSD. (Table 4)
- Recovery analysis was performed at LOQ level and it matched to the acceptance criteria between 70 to 130 %. (Table 5)

■ Conclusion

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.

06-SAIP-079-GC-024-EN First Edition: Apr. 2021

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

Shimadzu Analytical (India) Pvt.Ltd.
www.shimadzu.in