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Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8050 NX, AOC-20i Plus

Quantitation of 5 NSA in Metformin API as per proposed USP General Chapter <1469> Procedure-4 by GC-MS/MS

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 GC-MS/MS method for the determination of 5 Nitrosamines in Metformin API
- The GCMS-TQ 8050 NX system easily meets the criteria as per the USP guidance on Nitrosamines

■ Introduction

Overview : The presence of Nitrosamine (NSA) as impurity has been detected recently in several drug substances and drug products. In 2018, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) were detected in some Valsartan Active Pharmaceutical Ingredients (API) and Finished Dosage Form (FDF). This led to an ongoing investigation to check presence of NSA in pharmaceutical substances and products. One such drug is Metformin; it is used to control high blood sugar observed in patients suffering from type 2 diabetes.

What are NSA? : NSA are organic compounds of the chemical structure $R_2N-N=O$, where R is usually an alkyl group. These compounds are listed as Class 1 mutagens in ICH M7: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit potential carcinogenic risk. As a result of the significant toxicity associated with these impurities, it is recommended to take steps to control and limit their presence in pharmaceutical materials.

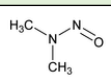
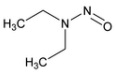
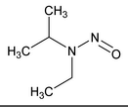
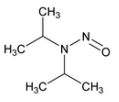
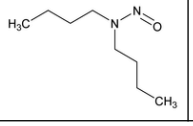
USP General Chapter <1469> : To protect patients from the adverse effects of NSA as impurities in drug products, Unites States Pharmacopeia (USP) has proposed a new general chapter <1469>.

This chapter is aligned with current scientific and regulatory approaches developed to ensure the appropriate control of NSA in pharmaceuticals. The proposed chapter comprises of 4 procedures for NSA determination in API and FDF. However, this application note aims to replicate the procedure-4 for quantitation of following 5 NSA (Table 1) in Metformin API using Shimadzu GCMS-TQ 8050 NX system with AOC-20i Plus autosampler (Figure 1).

1) N-nitrosodimethylamine (NDMA)
2) N-nitrosodiethylamine (NDEA)
3) N-nitrosoethylisopropylamine (NEIPA)
4) N-nitrosodiisopropylamine (NDIPA)
5) N-nitrosodibutylamine (NDBA)

Figure 1: GCMS-TQ™8050 NX system with AOC-20i Plus autosampler

Table 1: Commonly found NSA as contaminants in various API and FDF

Abbr.*	CAS	Structure	Chemical Formula	Molecular Weight
NDMA	62-75-9		$C_2H_6N_2O$	74.08
NDEA	55-18-5		$C_4H_{10}N_2O$	102.14
NEIPA	16339-04-1		$C_5H_{12}N_2O$	116.16
NDIPA	601-77-4		$C_6H_{14}N_2O$	130.19
NDBA	924-16-3		$C_8H_{18}N_2O$	158.25

* = Abbreviation

■ Experimental

A mixture of NDMA, NDEA, NEIPA, NDIPA and NDBA standards was analyzed using scan mode for identification. Steps such as precursor ion selection and MRM optimization at different Collision Energies (CE) were performed and method with optimum MRM and their CE in segments was generated and used for analysis. Optimized MRM and CE are very important for trace level determination and may vary for different instrument make. For quantitation, 8-point calibration curve ranging from 0.25 to 15.0 ppb for all 5 compounds as mentioned in the USP chapter were prepared in Dichloromethane (DCM) spiked with 5.0 ppb NDMA C13 d6 (Internal standard). The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for all 5 NSA were found to be 0.1 ppb and 0.25 ppb respectively.

(All concentrations mentioned above are as such)

Figures 3 to 7 depict the calibration curve, overlay of all linearity standards and chromatograms of LOQ solution for NDMA, NDEA, NEIPA, NDIPA and NDBA, respectively.

■ Method

The MRM transitions of 5 NSA and 1 internal standard are given in Table 2 and analytical conditions in Table 3.

Table 2: MRM transitions of NSA

MRM Transitions				
Comp.	**MRM-1**	**CE**	**MRM-2**	**CE**
NDMA	74.00>44.10	6	74.00>42.10	15
NDMA C13 d6	82.00>48.00	20	Not Applicable	
NDEA	102.00>85.10	6	102.00>56.10	17
NEIPA	116.00>99.10	6	116.00>42.10	23
NDIPA	130.00>88.10	6	130.00>42.00	11
NDBA	116.00>99.10	6	158.15>141.10	5

Table 3: Analytical conditions

GCMS System	: GCMS-TQ 8050 NX with AOC-20i Plus :
Column	Wax MS 30 m, 0.25 mm I.D., 1.0 µm d_f
Injection Mode	: Splitless
Flow Control Mode	: Column Flow
Injector Port Temp.	: 250 °C
Carrier Gas	: Helium
Column Flow	: 1.0 mL/min
Injection Volume	: 2.0 µL
Diluent	: DCM*

Temp. Program

Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
-	40.00	0.50
20	200.00	0.00
60	230.00	4.00

Ionization Mode	: Electron Ionization (EI)
Interface Temp.	: 230 °C
Ion Source Temp.	: 230 °C
CID Gas	: Argon
CID Gas Pressure	: 200 kPa

* = Diluent is spiked with NDMA C13 d6; effective concentration 5 ppb

^ = The column used is wax (polar) and result in considerable baseline drift, this might increase as the column ages. Also, the column minimum bleed temperature is 250°C hence used 230°C. (Figure 2)

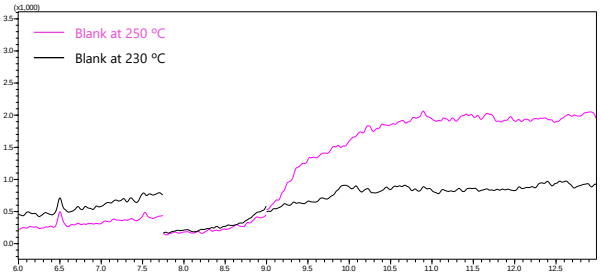


Figure 2: Comparison of blank injections analysed at 230 °C and 250 °C as maximum program temperature

■ Sample Preparation

1) Transfer 500 mg of the Metformin API into a disposable 15 mL centrifuge tube. Add 5 mL of the diluent.
2) Cap the tube. Vortex the sample for 1 min, and place in the centrifuge. Centrifuge the sample at 4000 rpm for 2.5 min.
3) Filter the extract using 0.45-µm nylon filter into GC autosampler vial and inject 2 µL on GC-MS/MS.

■ Spiked Sample Preparation

1) Transfer 500 mg of the Metformin API into a disposable 15 mL centrifuge tube. Add 5 mL of the LOQ solution. (0.25 ppb prepared in diluent)
2) Cap the tube. Vortex the sample for 1 min, and place in the centrifuge. Centrifuge the sample at 4000 rpm for 2.5 min.
3) Filter the extract using 0.45-µm nylon filter into a GC autosampler vial and inject 2 µL on GC-MS/MS.

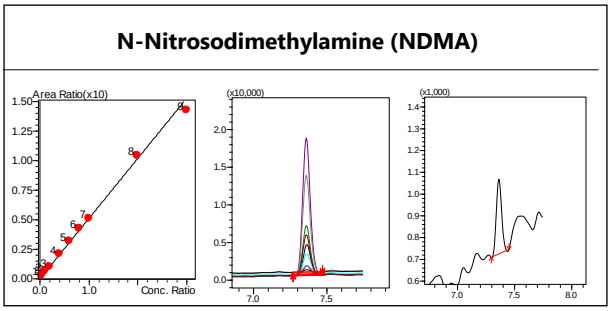


Figure 3: Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDMA

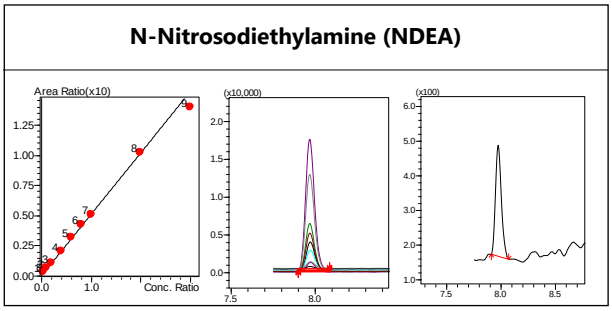


Figure 4: Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDEA

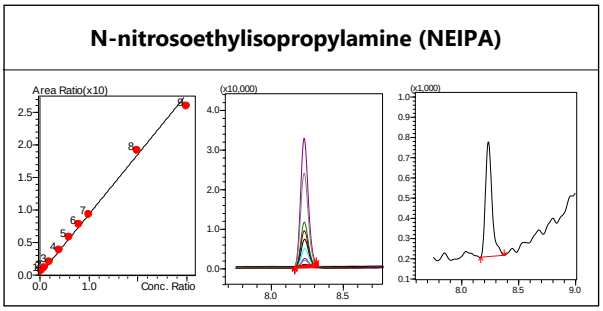


Figure 5: Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NEIPA

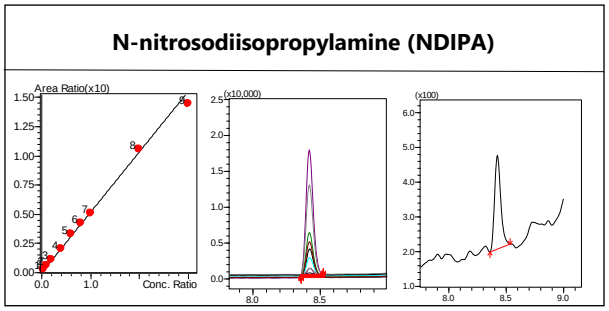


Figure 6: Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDIPA

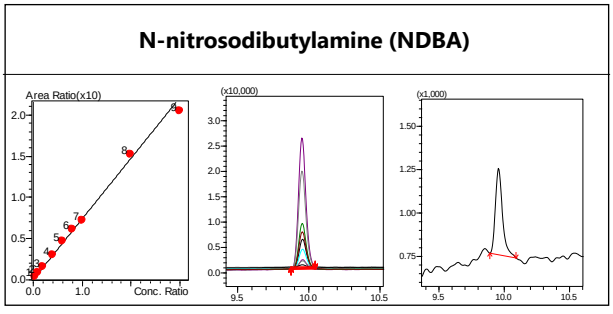


Figure 7: Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDBA

The range for calibration curve, LOQ established from S/N and %RSD at LOQ are shown in Table 4.

Table 4: Summary of calibration curve

Comp.	Linearity range (ppb)	r^2	LOQ		
			Conc. (ppb)	% RSD (n=6)	S/N*
NDMA	2.5 to 150.0	0.998	2.5	6.0	30.60
NDEA		0.995		6.9	118.00
NEIPA		0.998		8.3	47.75
NDIPA		0.998		8.8	104.67
NDBA		0.996		6.4	57.50

* = Peak to Peak
Concentrations expressed are relative to sample

The concentration in sample, concentration in spiked sample, concentration spiked and % recovery are shown in Table 5.

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

Metformin API				
Comp.	Conc. in sample (ppb)	Conc. in spiked sample (ppb)	Conc. spiked (ppb)	% Recovery
NDMA	BLOQ	2.75	2.5	110
NDEA	BLOQ	2.53	2.5	101
NEIPA	BLOQ	2.42	2.5	97
NDIPA	BLOQ	2.67	2.5	107
NDBA	2.81	4.59	2.5	71

LOQ levels mentioned in USP <1469> Procedure-4 in comparison to those obtained in this application news are shown in Table 6.

Table 6: LOQ comparison of USP <1469> Procedure-4 Vs Shimadzu Application news. (Results expressed are relative to sample)

Comp.	LOQ (ppb)	
	USP <1469> Procedure-4	Shimadzu Application News
NDMA	5.0	2.5
NDEA		
NEIPA		
NDIPA		
NDPA		

■ Results

- Trace level quantitation of 5 NSA in Metformin API sample was successfully performed by using Shimadzu GCMS-TQ 8050 NX with liquid autosampler AOC-20i Plus
- The determination coefficient (r^2) was greater than 0.99 for all the 5 NSA (Table 4)
- The repeatability (%RSD for n=6) at LOQ level was found to be less than 10% (Table 4)
- Accuracy study in terms of spiked recovery was performed at LOQ level (2.5 ppb), and results obtained were between 70 to 130 % (Table 5)

■ Conclusion

Shimadzu GCMS-TQ 8050 NX with high sensitivity shielded detector offers outstanding noise elimination with excellent sensitivity, repeatability nad precision while outperforming the current regulatory limits.

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