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Gas Chromatograph Mass Spectrometer GCMS-TQ™8040 NX, HS-20

Application News

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Quantitation of 5 Nitrosamines in Metformin API and Formulation by HS-GCMS/MS

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User Benefits

- A HS-GCMS/MS method for the determination of 5 Nitrosamines in Metformin API & formulation
- The GCMS-TQ8040 NX system easily meets the criteria as per the USFDA guidance on Nitrosamines

■ Introduction

Overview : The United States Food and Drug Administration (USFDA) has extensively investigated the presence of genotoxic impurities, called Nitrosamines (NSA) in many Active Pharmaceutical Ingredients (API) & Finished Dosage Formulations (FDF). Several drug products including Angiotensin II Receptor Blockers (ARB) and Ranitidine, have been found to contain small amounts of NSA such as N-nitrosodimethylamine (NDMA). There has been an ongoing investigation to further check the presence of NSA in other drug products as well, such as Metformin (Figure 1). Metformin is a prescription drug used to control high blood sugar in patients with type 2 diabetes. Patients should continue taking Metformin to keep their diabetes under control hence, it is imperative to make Metformin drug available with safe levels of NSA.

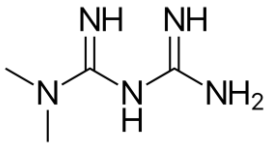

Figure 1: Structure of Metformin

NSA and their Limits : NSA are a common contaminant found in water, foods, dairy products and vegetables. Exposure at very trace levels from natural products may not cause harm to human life. However, may increase the risk of cancer if exposure to them is above acceptable levels. Hence, person taking a drug containing NSA at-or-below the acceptable daily intake limit is not expected to have an increased risk of cancer. USFDA recommends the following Acceptable Intake (AI) limits for drug products with Maximum Daily Dose (MDD) of 880 mg/day (Table 1).

Table 1: Acceptable Intake (AI) limits for NSA

Comp.	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

Control Strategy & Methodology : The control strategy described in the USFDA industry guidance on NSA can be employed for Metformin API & FDF as well.

These limits are applicable only if the API or FDF with MDD of 880 mg/day containing a single NSA impurity, and lowest of which is 30 ppb. Even if more than 1 NSA impurity is identified in API or FDF, the total NSA determined as listed in Table 1 should not be more than 26.5 ng/day or 30 ppb. Hence, it is imperative to determine below mentioned NSA with Limit of Quantitation (LOQ) for total NSA below 30 ppb. Developing method for determining total NSA < 30 ppb in API & FDF creates challenges in pharmaceuticals.
Out of several testing methods available, only few can detect NSA listed in Table 1 using GCMS or HS-GCMS/MS, hence, this application note aims to provide a part-validated method for quantitation of following 5 NSA in Metformin API/FDF with combined LOQ of 16.5 ppb using Shimadzu GCMS-TQ8040 NX with HS-20 (Figure 2).

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

■ 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 as described in Table 2 was generated in segments and used for analysis. For quantitation, five-point calibration curves ranging from 1.0 to 20.0 ppb for all 5 NSA were prepared in Dimethyl Sulfoxide (DMSO) and plotted after analyses using the conditions described in Table 3. The Limit of Detection (LOD) & LOQ for all 5 NSA were found to be 0.3 & 1.0 ppb, respectively. The S/N & % RSD at LOQ are shown in Table 4. (All concentrations mentioned above are as such)

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

Figure 2: GCMS-TQ™8040 NX with HS-20

■ Method

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

Table 2: MRM transitions of NSA

MRM Transitions				
Comp.	MRM-1	CE-1	MRM-2	CE-2
NDMA	74.00>44.10	5	74.00>42.10	14
NDMA d6	80.00>50.00	5	Not Applicable	
NDEA	102.00>85.10	5	102.00>56.10	14
NEIPA	116.00>99.10	5	116.00>42.10	27
NDIPA	130.00>88.10	5	130.00>42.10	14
NDBA	116.00>99.10	5	158.00>99.00	10

Table 3: Analytical conditions

GCMS System	: GCMS-TQ8040 NX with HS-20
Column	: SH-Stabilwax DA 60 m, 0.25 mm I.D., 0.25 µm d_f
Injection Mode	: Splitless
Flow Control Mode	: Linear Velocity
Linear Velocity	: 44.2 cm/sec
Carrier Gas	: Helium
Diluent	: DMSO
Temp. Program	Ramp Rate (°C/min) / Temp. (°C) / Hold Time (min): - / 40.00 / 0.00; 20 / 230.00 / 10.50
	MS Parameters
Ionization Mode	: Electron Ionization (EI)
Interface Temp.	: 230 °C
Ion Source Temp.	: 200 °C
CID Gas	: Argon
CID Gas Pressure	: 200 kPa
	HS Parameters
Oven Temp.	: 135 °C
Sample Line Temp.	: 150 °C
Transfer Line Temp.	: 180 °C
Shaking Level	: 3
Pressurizing Gas Pressure	: 105 kPa
Equilibrating Time	: 10.00 min
Load Time	: 0.50 min
Injection Time	: 1.00 min
Cycle Time	: 30.00 min

■ Sample Analysis (FDF/API)

1. For FDF sample, accurately weigh powdered FDF corresponding to 300 mg of API into a 20 mL headspace vial (P/N: 226-84520-01)
2. For API sample, accurately weigh 300 mg of API into a 20 mL headspace vial
3. Add 1.0 mL of DMSO to above headspace vial
4. Further, add 0.5 mL of 10 ppb* NDMA d6 internal standard prepared in deionized water
5. Crimp the vial tightly with cap-septa (P/N: 226-84525-11) and inject

■ Spiked Recovery Test (FDF/API)

1. For FDF sample, accurately weigh powdered FDF corresponding to 300 mg of API into a 20 mL headspace vial
2. For API sample, accurately weigh 300 mg of API into a 20 mL headspace vial
3. Add 1.0 mL of LOQ solution (1.0 ppb*) prepared in DMSO to above headspace vial
4. Further, add 0.5 mL of 10 ppb* NDMA d6 internal standard prepared in deionized water
5. Crimp the vial tightly with cap-septa and inject

* = Concentration as such

N-nitrosodimethylamine

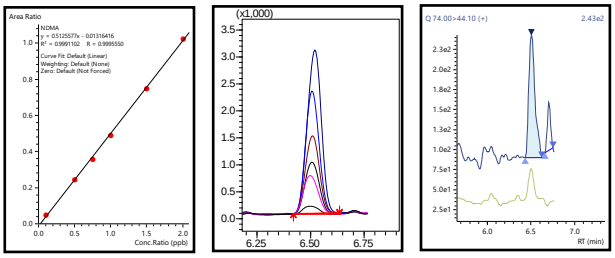

Figure 3: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NDMA

N-nitrosodiethylamine

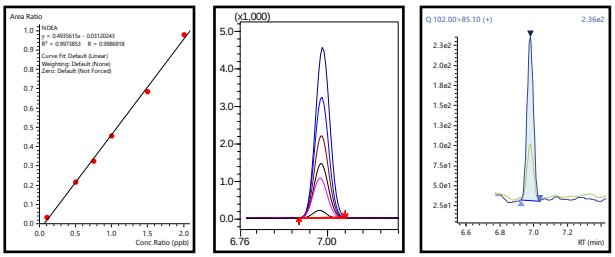

Figure 4: Calibration curve, overlay of linearity standards & chromatogram of LOQ Solution for NDEA

N-nitrosoethylisopropylamine

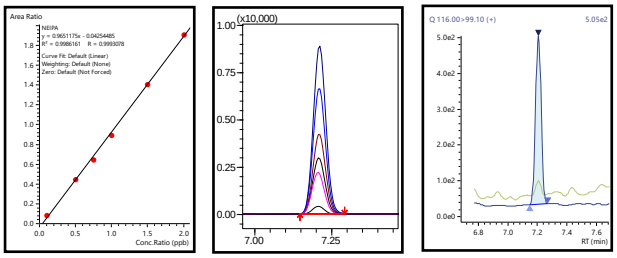

Figure 5: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NEIPA

N-nitrosodiisopropylamine

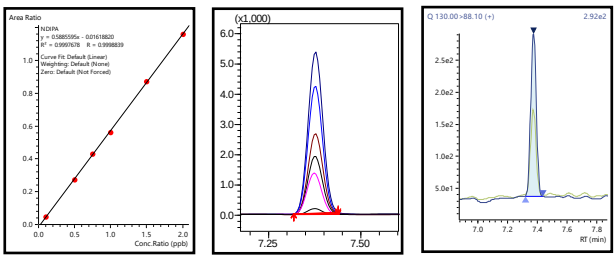

Figure 6: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NDIPA

N-nitrosodibutylamine

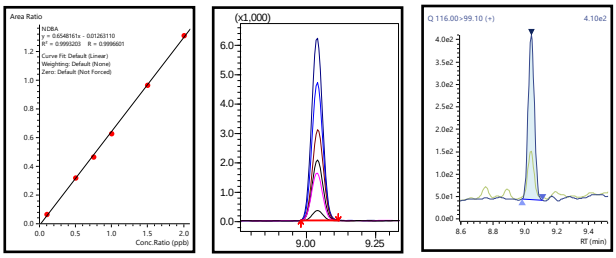

Figure 7: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NDBA

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

Table 4: Summary of Calibration curves

Comp.	Linearity range (ppb)	r^2	LOQ		
			Conc. (ppb)	% RSD (n=6)	S/N*
NDMA	3.3 to 66.7	0.999	3.3	4.9	51
NDEA		0.997		9.1	103
NEIPA		0.998		7.0	235
NDIPA		0.999		10.6	255
NDBA		0.999		4.4	366

* = Peak to Peak
Results expressed are relative to sample

The amount in sample, amount obtained, amount spiked & % recovery are shown in Table 5 & 6.

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

Metformin FDF				
Comp.	Amt. in sample (ppb)	Amt. spiked (ppb)	Amt. obtained (ppb)	% Recovery
NDMA	30.6	3.3	33.8	97
NDEA	Below LOQ	3.3	3.8	115
NEIPA	Below LOQ	3.3	3.4	103
NDIPA	Below LOQ	3.3	3.3	100
NDBA	Below LOQ	3.3	3.4	103

Table 6: The sample spiked study for Metformin API at LOQ level (Results expressed are relative to sample)

Metformin API				
Comp.	Amt. in sample (ppb)	Amt. spiked (ppb)	Amt. obtained (ppb)	% Recovery
NDMA	Below LOQ	3.3	3.6	109
NDEA	Below LOQ	3.3	3.0	91
NEIPA	Below LOQ	3.3	3.3	100
NDIPA	Below LOQ	3.3	3.0	91
NDBA	Below LOQ	3.3	3.5	106

■ Results

- Trace level quantitation of 5 NSA in Metformin API and FDF sample was successfully performed by using Shimadzu GCMS-TQ8040 NX with HS-20 headspace autosampler
- The correlation coefficient (r^2) was greater than 0.996 for all the 5 NSA (Table 4)
- The repeatability (% RSD for n=6) at LOQ level (3.3 ppb) was found to be less than 11% (Table 4)
- Accuracy study in terms of spiked recovery was performed at LOQ level (3.3 ppb), and results obtained were between 90 to 115 % (Table 5 & 6)

■ Conclusion

- Shimadzu HS-20 headspace autosampler provides high reproducibility ensuring reliable & accurate quantitation even at trace level.
- Shimadzu GCMS-TQ8040 NX equipped with a high sensitivity ion source, long-term ion stability and high-efficiency collision cell, provides sensitive, stable analyses over a long period of time. Also, since it incorporates the Nexis™ GC-2030, high-precision control over flowrate and temperature is assured, enabling the acquisition of highly reliable data.
- Thus, Shimadzu GCMS-TQ8040 NX with HS-20 is an excellent tool for determination of NSA in API and FDF at trace levels.

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