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

Application News

Trace Level Quantitation of 6 Nitrosamines in Metformin API by Dynamic Headspace GC-MS/MS

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User Benefits

- Dynamic headspace equipped with GC-MS/MS was used for trace level quantitation of Nitrosamines in Metformin API
- GCMS-TQ8050 NX system equipped with HS-20 easily meets the criteria as per the regulatory guidelines on Nitrosamines
- Compared to static headspace, dynamic headspace has advantage in trace level detection of Nitrosamines

■ Introduction

Overview : Regulatory bodies related to pharmaceutical industry had extensively investigated the presence of genotoxic impurities, called Nitrosamines (NSA), in many drugs. Metformin (Figure 1) 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 and hence it is imperative to make Metformin drug available with safe levels of NSA.

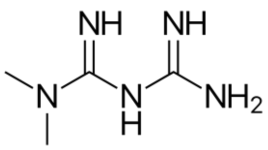

Figure 1: Structure of Metformin

NSA and their Limits : NSA are organic compounds of the chemical structure R2N−N=O, where R is usually an alkyl group. These are common chemicals found in water and foods including cured or grilled meats, dairy products and vegetables. Foods and drugs which are metabolized in human body, are also able to generate NSA. Thus, everyone is exposed to some level of NSA. These impurities may increase the risk of cancer if the exposure is above acceptable levels for a longer period. Regulatory counterparts around the world, has set internationally-recognized acceptable daily intake limits for NSA. If drugs contain levels of NSA above the acceptable daily intake limit, regulatory body recommends their recall by the manufacturer.
NSA can make their way into drug substance/product from varied sources. The sources of NSA can be related to the drug manufacturing process or its chemical structure or even the conditions in which they are stored or packaged.

Toxicity/Regulations/Method : The control strategy described in the USFDA industry guidance on NSA can be employed for Metformin Active Pharmaceutical Ingredient (API) & Finished Dosage Form (FDF) as well. These limits are applicable only if the API or FDF having Maximum Daily Dose (MDD) of 880 mg/day contains a single NSA, and lowest of which is 30 ppb. If more than one NSA is identified, the limit for 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 any NSA with Limit of Quantitation (LOQ) for total NSA below 30 ppb. Developing a method for determining total NSA < 30 ppb in API & FDF creates challenges in pharmaceutical industry.

Following are the Acceptable Intake (AI) limits for NSA in drug substance/drug product with MDD of 880 mg/day (Table 1).

Table 1: 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

There are several regulatory methodologies available, one such is USP General Chapter <1469> procedure-2 which makes use of static headspace.
For more details on static headspace GC-MS/MS analysis of Metformin API & FDF, please refer following
Application News: 06-SAIP-085-GC-026-EN

However, the results obtained here using dynamic headspace GC-MS/MS proved to be equally precise, accurate & even more sensitive as compared to static headspace GC-MS/MS.
This application note aims to provide a part validated analysis method using Shimadzu GCMS-TQ8050 NX with HS-20 dynamic headspace (Figure 2) for trace level quantitation for following NSA.

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

Summary of validation parameters is shown in Table 2.

Table 2: Summary of validation parameters

Parameters	Conc. in ppb (as such)	Conc. In ppb (w.r.t. sample)
System Precision	0.10	1.0
Precision a LOQ	0.05/0.1	0.5/1.0
Linearity	0.05 to 0.4	0.5 to 4.0
Accuracy	0.05 to 0.4	0.5 to 4.0

w.r.t. sample = with respect to sample (concentration 10% w/v)

Figure 2: GCMS-TQ™8050 NX with HS-20

■ Experimental

A mixture of NDMA, NDEA, NEIPA, NDIPA, NDPA and NDBA standards (1 ppm) 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.

The optimized MRM method was used for part method validation (As per ICH guidelines).

■ Method

The MRM transitions of 6 NSA standards are given in Table 3 and analytical conditions are in Table 4.

Table 3: 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
NDEA	102.00>85.10	5	102.00>56.10	14
NEIPA	116.00>99.10	5	71.00>56.10	5
NDIPA	130.00>88.10	5	130.10>42.20	14
NDPA	130.10>113.10	6	130.10>43.20	18
NDBA	116.00>99.10	5	158.00>99.00	7

Table 4: Analytical conditions

GCMS System	: GCMS-TQ8050 NX with HS-20 (Dynamic)		
Column	: WAX ms 30 m, 0.25 mm I.D., 1.0 µm d_f		
Injection Mode	: Split		
Flow Control Mode	: Column Flow		
Carrier Gas	: Helium		
Column Flow	: 4.0 mL/min		
Linear Velocity	: 71.9 cm/sec		
Split Ratio	: 10:1		
Purge Flow	: 5 mL/min		
Total Flow	: 49 mL/min		
Temp. Program	Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
	-	35.00	2.00
	5	105.00	1.17
	10	200.00	0.00
	30	240.00	12.00
Diluent	: Water (MS Grade)		
	MS Parameters		
Ionization Mode	: Electron Ionization (EI)		
Ion Source Temp.	: 240 °C		
Interface Temp.	: 240 °C		
CID Gas	: Argon		
	HS Parameters		
Oven Temp.	: 110 °C		
Sample Line Temp.	: 170 °C		
Transfer Line Temp.	: 190 °C		
Trap Cooling Temp.	: -15 °C		
Trap Desorb Temp.	: 260 °C		
Trap Equilib. Temp.	: -15 °C		
Shaking Level	: 5		
Multi Inj. Count	: 1		
Pressurizing Gas Pressure	: 192.0 kPa		
Dry Purge Gas Pressure	: 0.0 kPa		
Equilibrating Time	: 20.00 min		
Pressurizing Time	: 1.00 min		
Pressure Equilib. Time	: 0.10 min		
Load Time	: 0.50 min		
Load Equilib. Time	: 0.10 min		
Dry Purge Time	: 0.00 min		
Injection Time	: 25.00 min		
Needle Flush Time	: 25.00 min		
GC Cycle Time	: 55.00 min		

■ Linearity Solutions

Standard solutions for linearity were prepared in headspace vial as mentioned in Table 5.

Table 5: Linearity standard solution preparations

Linearity Levels	Linearity stock Conc. in (ppb)	Volume of standard stock (µL)	Volume of water (µL)	As such sample Conc. in (ppb)
Level - 1	5	10	990	0.05
Level - 2	10	10	990	0.1
Level - 3	20	10	990	0.2
Level - 4	30	10	990	0.3
Level - 5	40	10	990	0.4

■ Sample Analysis

Weigh 100 mg (± 10%) of Metformin API and add 300 mg (± 10%) of Na_2CO_3 in a 20 mL headspace vial. Add 1000 uL of water, crimp the vial with cap septa tightly and inject.

■ Spiked Recovery Test

Weigh 100 mg (± 10%) of Metformin API and add 300 mg (± 10%) of Na_2CO_3 in a headspace vial. Further add 1000 uL of respective linearity solution, crimp the vial with cap septa tightly and inject.

Figure 3 to 8 depicts the calibration curve, overlay of linearity standards, LOQ level chromatograms for NDMA, NDEA, NEIPA, NDIPA, NDPA & NDBA respectively.

NDMA

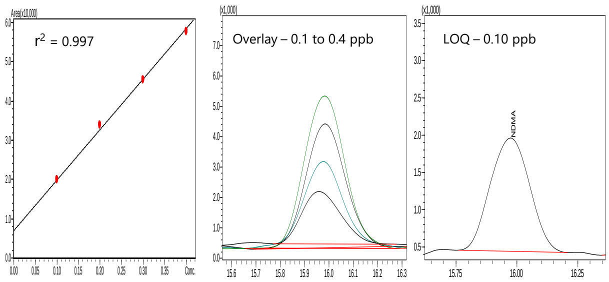

Figure 3: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NDMA

NDEA

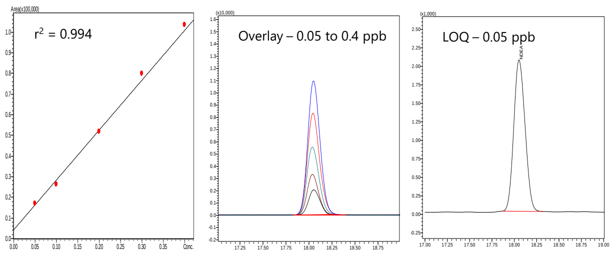

Figure 4: Calibration curve, overlay of linearity standards & chromatograph of LOQ solution for NDEA

NEIPA

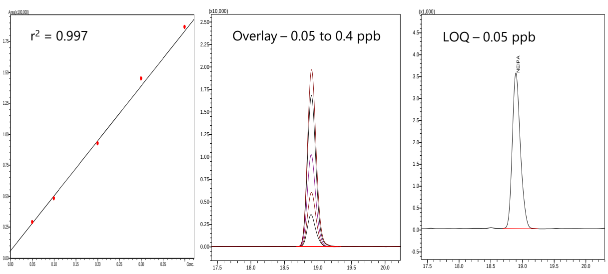

Figure 5: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NEIPA

NDIPA

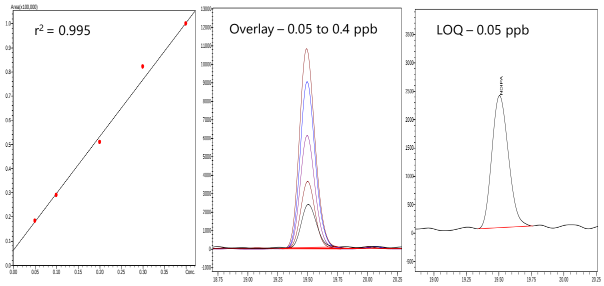

Figure 6: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NDIPA

NDPA

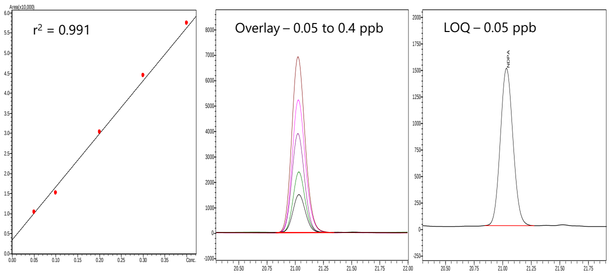

Figure 7: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NDPA

NDBA

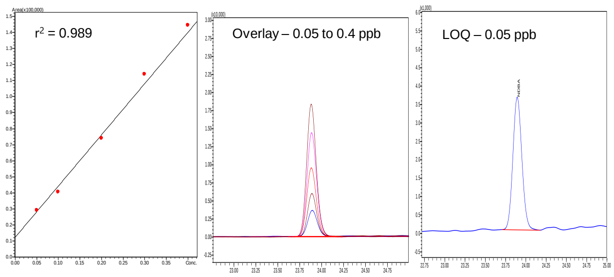

Figure 8: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for NDBA

■ Validation Parameters

System Precision :

Weigh 300 mg (±10%) of Na_2CO_3 in a headspace vial. Further, add 1000 uL of level-2 linearity solution, crimp the vial with cap septa tightly & inject (Table 6).

Table 6: Summary for system precision (n=6)

Comp.	Conc. in ppb (as such)	Conc. in ppb (w.r.t sample)	% RSD of area
NDMA	0.1	1.0	7.5
NDEA	0.1	1.0	6.6
NEIPA	0.1	1.0	11.8
NDIPA	0.1	1.0	7.1
NDPA	0.1	1.0	9.7
NDBA	0.1	1.0	6.2

RSD = Relative Standard Deviation

Precision at LOQ Level :

Weigh 300 mg (±10%) of Na_2CO_3 in a headspace vial. Further, add 1000 uL of level-1 linearity solution, crimp the vial with cap septa tightly & inject.
Summary for S/N and % RSD (area) at LOQ level standard solutions are shown in Table 7.

Table 7: Summary for LOQ system precision (n=6)

Comp.	Conc. in ppb (as Such)	Conc. in ppb (w.r.t sample)	% RSD of area	S/N^
NDMA*	0.10	1.0	7.5	20
NDEA	0.05	0.5	6.5	321
NEIPA	0.05	0.5	5.7	636
NDIPA	0.05	0.5	14.6	77
NDPA	0.05	0.5	10.6	230
NDBA	0.05	0.5	10.7	171

^ = Peak to peak
* = Data is taken from Table 6

Linearity :

Weigh 300 mg (±10%) of Na_2CO_3 in a 20 mL headspace vial. Further, add 1000 uL of respective linearity solution, crimp the vial with cap septa tightly & inject.

For quantitation, four-point calibration curve for NDMA (0.1, 0.2, 0.3 & 0.4 ppb) & five-point calibration curve for NDEA, NEIPA, NDIPA, NDPA & NDBA (0.05, 0.1, 0.2, 0.3 & 0.4 ppb) were plotted.

Summary of linearity standard solutions is shown in Table 8.

Table 8: Result summary for linearity (n=3)

Comp.	r^2	Conc. in ppb (as such)	Conc. in ppb (w.r.t. sample)
NDMA	0.997	0.1 to 0.4	1.0 to 4.0
NDEA	0.994	0.05 to 0.40	0.5 to 4.0
NEIPA	0.997		
NDIPA	0.995		
NDPA	0.991		
NDBA	0.989		

Accuracy :

For accuracy study, Metformin samples were diluted with 1000 uL of respective linearity solutions to get spiked concentration of 0.05, 0.1, 0.3 & 0.4 ppb (as such) in 20 mL headspace vial.

However, high interference was observed at retention time of NDMA from Metformin API in presence of Na_2CO_3 hence, accuracy study was not carried out for NDMA.

For low level quantification of NDMA in Metformin, different salts such as Ammonium Sulfate/Sodium Chloride can be used instead of Na_2CO_3, keeping analytical method parameters same.

Accuracy study for NDEA, NEIPA, NDIPA, NDPA & NDBA is summarized in Table 9, 10, 11 & 12.

Table 9: Summary for recovery at 0.05 ppb (n=3)

Comp.	Amount spiked (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% Average Accuracy
NDEA	0.05 (as such)	BLOQ	0.043	86
NEIPA		BLOQ	0.053	106
NDIPA		BLOQ	0.053	106
NDPA		BLOQ	0.057	114
NDBA		BLOQ	0.053	106

BLOQ = Below Limit Of Quantitation

Table 10: Summary for recovery at 0.1 ppb (n=3)

Comp.	Amount spiked (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% Average Accuracy
NDEA	0.1 (as such)	BLOQ	0.101	101
NEIPA		BLOQ	0.120	120
NDIPA		BLOQ	0.127	127
NDPA		BLOQ	0.133	133
NDBA		BLOQ	0.098	98

Table 11: Summary for recovery at 0.3 ppb (n=3)

Comp.	Amount spiked (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% Average Accuracy
NDEA	0.3 (as such)	BLOQ	0.266	89
NEIPA		BLOQ	0.298	99
NDIPA		BLOQ	0.311	104
NDPA		BLOQ	0.316	105
NDBA		BLOQ	0.280	93

Table 12: Summary for recovery at 0.4 ppb (n=3)

Comp.	Amount spiked (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% Average Accuracy
NDEA	0.4 (as such)	BLOQ	0.401	100
NEIPA		BLOQ	0.448	112
NDIPA		BLOQ	0.493	123
NDPA		BLOQ	0.486	121
NDBA		BLOQ	0.417	104

Comparison between Dynamic & Static headspace :

Comparison study between dynamic & static headspace analysis is summarized in Table 13 & 14.

Table 13: Summary for dynamic headspace analysis

Dynamic Headspace (Trap)				
Comp.	LOQ (n=6)			
	Conc. in ppb (as such)	Conc. in ppb (w.r.t sample)	S/N	% RSD
NDMA	0.10	1.0	20	7.5
NDEA	0.05	0.5	321	6.5
NEIPA	0.05	0.5	636	5.7
NDIPA	0.05	0.5	76	14.6
NDBA	0.05	0.5	171	10.7

w.r.t. sample = with respect to sample (concentration 10% w/v)

Table 14: Summary for static headspace analysis

Static Headspace (Loop)				
Comp.	LOQ n=6)			
	Conc. in ppb (as such)	Conc. in ppb (w.r.t sample)	S/N	% RSD
NDMA	0.99	3.3	51	4.9
NDEA	0.99	3.3	103	9.1
NEIPA	0.99	3.3	235	7.0
NDIPA	0.99	3.3	255	10.6
NDBA	0.99	3.3	366	4.4

w.r.t. sample = with respect to sample (concentration 30% w/v)

For more details on static headspace GC-MS/MS analysis of Metformin API & FDF, please refer following
Application News: 06-SAIP-085-GC-026-EN

■ Results

- Trace level quantification of 5 NSA impurities in Metformin API was successfully performed by using Shimadzu GCMS-TQ8050 NX with HS-20 headspace sampler (Dynamic mode)
- Repeatability for all 6 NSA at LOQ level was found to be less than 15.0% (Table 7)
- The correlation coefficient (r^2) was greater than 0.988 for all NSA (Table 8)
- Accuracy study in terms of spiked recovery was carried out at 0.05, 0.1, 0.3 and 0.4 ppb levels that matched the acceptance criteria between 80 to 135 % (Table 9, 10, 11 & 12)

■ Conclusion

- Dynamic headspace mode, outperforms the current regulatory limits, delivering 10 to 20 times more sensitivity compared to static headspace mode for NSA analysis.
- Shimadzu GCMS-TQ8050 NX features a new highly efficient detector and superior noise reduction technology that enhance sensitivity and enables quantitation of NSA even at trace levels.

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