SHIMADZU
Excellence in Science

Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ8050 NX, HS-20 NX

Ultra-Sensitive Dynamic Headspace GC-MS/MS Method for Trace Level Quantitation of Nitrosamines in Deferiprone API

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

User Benefits

- Dynamic headspace achieves high sensitivity for trace level estimation at desired quantitation levels.
- Compared to static headspace, dynamic headspace with multi-injection count option provides flexibility during method development.

■ Introduction

Overview : Regulatory bodies related to pharmaceutical industry have extensively investigated the presence of genotoxic impurities, called Nitrosamines (NSA), in many drugs. Deferiprone (Fig. 1) is an iron chelator used to treat patients with transfusional iron overload caused by thalassemia syndromes. Hence it is imperative to make Deferiprone drug available with safe levels of NSA.

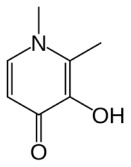

Fig. 1 Structure of Deferiprone

NSA and their Limits : NSA are organic compounds of the chemical structure $R_2N–N=O$, 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, have 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 Deferiprone Active Pharmaceutical Ingredient (API) and Finished Dosage Form (FDF) as well. These limits are applicable only if the API or FDF having maximum daily dose 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.
Furthermore, Deferiprone is used to treat the thalassemia syndromes which takes quite longer time to cure. Maximum daily intake is of 99 mg/kg/day. Considering worst case scenario, calculated maximum daily dose will be 9000 mg/day when weight of the patient as 90 kg. Hence, it is imperative to determine above mentioned NSA with Limit of Quantitation (LOQ) not exceeding 0.1 ppb. Developing method for determining total NSA at such low level in API & finish drug product creates challenges in pharmaceutical industry.

Following are the Acceptable Intake (AI) limits for NSA in drug substance/drug product with Maximum Daily Dose (MDD) of 9000 mg/day (Table 1).

Table 1 AI limits for NSA

Comp.	AI limit (ng/day)	Limit in ppm for MDD 9000 mg/day
NDMA	96.0	0.011
NDEA	26.5	0.003
NEIPA	26.5	0.003
NDIPA	26.5	0.003
NDPA	26.5	0.003
NDBA	26.5	0.003

There are several regulatory methodologies available, one such is USP General Chapter <1469> procedure-2 which makes use of static headspace.
However, the results obtained here using dynamic headspace GC-MS/MS proved to be equally precise, accurate and 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-TQ™8050 NX with HS-20 NX dynamic headspace (Fig. 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)	
	NDMA	5NSA*	NDMA	5NSA*
System Precision	0.36	0.1	11.0	3.0
Precision at LOQ	0.1	0.03	3.3	0.9
Linearity	0.1 to 0.54	0.03 to 0.15	3.0 to 16.5	0.9 to 4.5
Accuracy	0.1 to 0.54	0.03 to 0.15	3.0 to 16.5	0.9 to 4.5

w.r.t. sample = with respect to sample (concentration 3.3% w/v)
5NSA*= NDEA, NEIPA, NDIPA, NDPA & NDBA

Fig. 2 GCMS-TQ™8050 NX with HS-20 NX

■ 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	15
NDEA	102.00>85.10	5	102.00>44.10	11
NEIPA	116.00>99.10	5	116.00>44.10	11
NDIPA	130.00>88.10	5	130.15>42.10	11
NDPA	130.10>113.10	5	130.10>43.10	13
NDBA	116.00>99.10	5	158.00>99.10	9

Table 4 Analytical conditions

Note: Trap should be properly conditioned before starting the analysis. For conditioning procedure, please contact Shimadzu representative.

GCMS System	**: GCMS-TQ8050 NX with HS-20 NX (Dynamic)**
Column	: SH-PolarD 0.32 mm I.D. × 60 m, d.f.=0.5 µm. (P/N: 227-36276-01)
Injection Mode	: Split
Flow Control Mode	: Linear Velocity
Carrier Gas	: Helium
Linear Velocity	: 44 cm/sec
Split Ratio	: 5:1
Purge Flow	: 3 mL/min
Total Flow	: 20 mL/min
Temp. Program	: 70°C (0 min), 5°C/min to 140°C (0 min), 7°C/min to 210°C (0 min). 20°C/min to 250°C (9 min)
Diluent	: 0.5% NaOH in Water (MS Grade)
Ionization Mode	: Electron Ionization (EI)
Ion Source Temp.	: 250°C
Interface Temp.	: 235°C
CID Gas	: Argon
Oven Temp.	: 110°C
Sample Line Temp.	: 120°C
Transfer Line Temp.	: 130°C
Trap Cooling Temp.	: 80°C
Trap Desorb Temp.	: 280°C
Trap Equilib. Temp.	: 80°C
Shaking Level	: 5
Multi Inj. Count	: 3
Pressurizing Gas Pressure	: 160 kPa
Equilibrating Time	: 20.00 min.
Pressurizing Time	: 0.30 min.
Pressure Equilib. Time	: 0.10 min.
Load Time	: 0.30 min.
Load Equilib. Time	: 0.10 min.
Dry Purge Time	: 0.00 min.
Injection Time	: 10.00 min.
Needle Flush Time	: 10.00 min.
GC Cycle Time	: 45.00 min.
Detecor Voltage	: Adjust detector voltage to achieve intensity of 314 m/z as per tuning result

■ Linearity Solutions

Linearity Standard solutions of all NSA's were prepared in headspace vial as mentioned in Table 5.

Table 5 Linearity standard solution preparations

Linearity Levels	Volume taken from Linearity stock solution* (mL)	Final Volume of Linearity level (mL)	As such Conc. of NDMA in (ppb)	As such Conc. of rest of five (ppb)
Level - 1	0.30	10	0.11	0.030
Level - 2	0.50	10	0.18	0.050
Level - 3	0.75	10	0.27	0.074
Level - 4	1.00	10	0.36	0.099
Level - 5	1.50	10	0.54	0.149

**Linearity stock concentration for NDMA is 3.63 ppb and same for rest five impurities is 0.99ppb*

■ Sample Analysis

Weigh 33 mg (± 10%) of Deferiprone API and add 300 mg (± 10%) of Na_2CO_3 in a 20 mL headspace vial. Add 1 ml of diluent, crimp the vial with cap septa tightly and inject.

■ Spiked Recovery Test

Weigh 33 mg (± 10%) of Deferiprone API and add 300 mg (± 10%) of Na_2CO_3 in a 20 mL headspace vial. Further add 1 mL of respective linearity solution, crimp the vial with cap septa tightly and inject.

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

NDMA

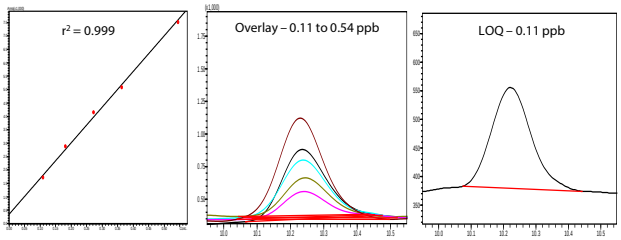

Fig. 3 Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDMA

NDEA

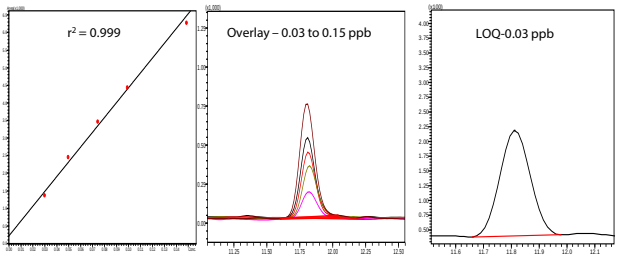

Fig. 4 Calibration curve, overlay of linearity standards and chromatograph of LOQ solution for NDEA

NEIPA

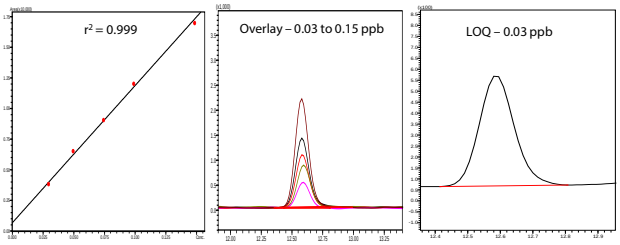

Fig. 5 Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NEIPA

NDIPA

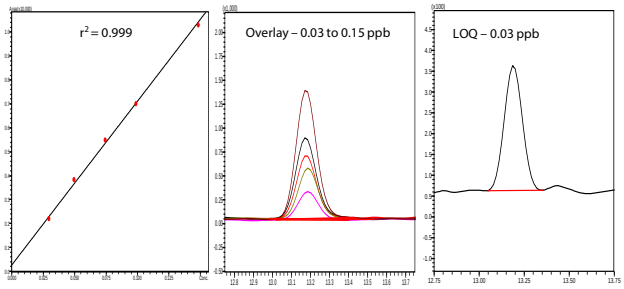

Fig. 6 Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDIPA

NDPA

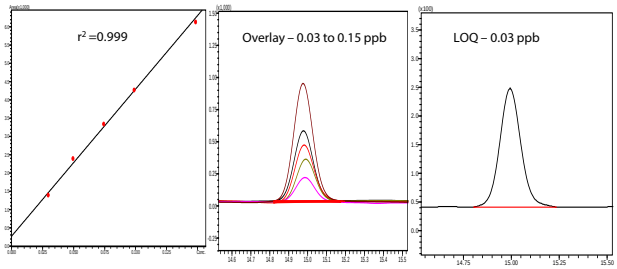

Fig. 7 Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDPA

NDBA

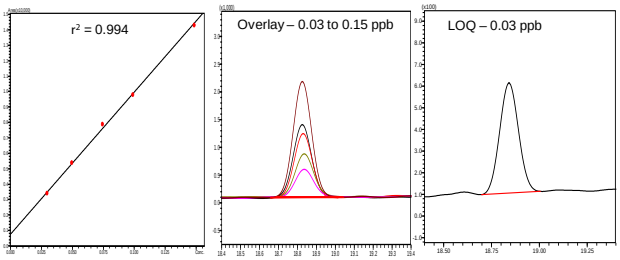

Fig. 8 Calibration curve, overlay of linearity standards and chromatogram of LOQ solution for NDBA

■ Validation Parameters

System Precision :

Weigh 300 mg (±10%) of Na_2CO_3 in a headspace vial. Further, add 1 mL of level-4 linearity solution, crimp the vial with cap septa tightly and 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	0.36	11.0	4.6
NDEA	0.099	3.0	2.6
NEIPA	0.099	3.0	3.4
NDIPA	0.099	3.0	3.0
NDPA	0.099	3.0	3.0
NDBA	0.099	3.0	3.0

Where RSD* = Relative Standard Deviation

Precision at LOQ Level :

Weigh 300 mg (±10%) of Na_2CO_3 in a headspace vial. Further, add 1 mL of level-1 linearity solution, crimp the vial with cap septa tightly and 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	0.11	3.30	8.9	21
NDEA	0.03	0.90	3.4	98
NEIPA	0.03	0.90	3.3	178
NDIPA	0.03	0.90	5.2	50
NDPA	0.03	0.90	4.8	10
NDBA	0.03	0.90	3.8	252

^ = Peak to peak

Linearity :

Weigh 300 mg (±10%) of Na_2CO_3 in a 20 mL headspace vial. Further, add 1 mL of respective linearity solution, crimp the vial with cap septa tightly and inject.

For quantitation, five-point calibration curve for all the analyte impurities were plotted. Their concentrations were as per table no.-5
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	0.999	0.11 to 0.54	3.3 to 16.5
NDEA	0.999	0.03 to 0.15	0.9 to 4.5
NEIPA	0.999		
NDIPA	0.999		
NDPA	0.999		
NDBA	0.994		

Accuracy :

For accuracy study, 33 mg of Deferiprone API sample was diluted with 1 mL of respective linearity solutions to get desired spike concentration in 20 mL headspace vial.

Accuracy study was performed for all the six impurities at four different concentration level including LOQ level. For Method precision, six replicates of Accuracy level-3 (100% spiked sample solution) were injected.
Results observed for the accuracy and method precision parameters was well within the criteria of 70% to 130%.

Accuracy study for level 1 to 4 for NDMA, NDEA, NEIPA, NDIPA, NDPA and NDBA is summarized in Table 9, 10, 11, and 12 respectively.

Table 9 Summary for recovery at Accuracy Level-1 (LOQ) (n=3)

Comp.	Amount spiked as such (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% Average Accuracy
NDMA	0.11	BLOQ	3.83	117
NDEA	0.03	BLOQ	0.89	97
NEIPA		BLOQ	0.82	91
NDIPA		BLOQ	0.82	92
NDPA		BLOQ	0.84	93
NDBA		BLOQ	1.14	127

BLOQ = Below Limit Of Quantitation

Table 10 Summary for recovery at Accuracy Level-2 (n=3)

Comp.	Amount spiked as such (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% Average Accuracy
NDMA	0.18	BLOQ	5.57	102
NDEA	0.05	BLOQ	1.33	87
NEIPA		BLOQ	1.27	85
NDIPA		BLOQ	1.30	88
NDPA		BLOQ	1.34	88
NDBA		BLOQ	1.60	107

BLOQ = Below Limit Of Quantitation

Table 11 Summary for recovery at Accuracy level-3 (n=6)

Comp.	Amount spiked as such (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% of Average Accuracy
NDMA	0.36	BLOQ	10.95	100
NDEA	0.10	BLOQ	2.67	87
NEIPA		BLOQ	2.58	86
NDIPA		BLOQ	2.69	90
NDPA		BLOQ	2.85	94
NDBA		BLOQ	2.92	97

BLOQ = Below Limit Of Quantitation

Table 12 Summary for recovery at Accuracy Level-4 (n=3)

Comp.	Amount spiked as such (ppb)	Amount in sample (ppb)	Amount obtained (ppb)	% of Average Accuracy
NDMA	0.54	BLOQ	12.14	93
NDEA	0.15	BLOQ	2.82	77
NEIPA		BLOQ	2.81	78
NDIPA		BLOQ	2.98	84
NDPA		BLOQ	3.08	85
NDBA		BLOQ	3.13	87

BLOQ = Below Limit Of Quantitation

Method precision was performed by injecting six replicates of 100% spiked sample solution. %RSD for content observed from those spiked sample solution was calculated. Refer summarized results in Table 13.

Table 13: Summary for Method Precision (n=6)

Comp.	Amount spiked as such (ppb)	Mean Amount obtained (ppb)	%RSD of content
NDMA	0.36	0.28	5.5
NDEA	0.10	0.08	7.3
NEIPA	0.10	0.07	7.0
NDIPA	0.10	0.08	6.2
NDPA	0.10	0.08	6.2
NDBA	0.10	0.09	11.1

■ Results

- Trace level quantification of 6 NSA impurities in Deferiprone API was successfully performed by using Shimadzu GCMS-TQ8050 NX with HS-20 NX headspace sampler (Dynamic mode).
- Precision for all 6 NSA at LOQ level was found to be less than 15.0% (Refer Table 7).
- The correlation coefficient (r^2) was greater than 0.99 for all NSA (Refer Table 8).
- Accuracy study in terms of spiked recovery was carried out at four accuracy level in which first level is LOQ level. LOQ was achieved easily at such a trace level which is difficult to achieve with static headspace technique. Further LOQ is confirmed by accuracy and precision. (Refer Table 9, 10, 11, 12 and 13 respectively).

■ Results (Cont.)

- Method precision in terms of repeatability was performed. %RSD for content observed from six replicate injections of spiked sample solution was well within the acceptance criteria of not more than 10% (Refer Table 13).

■ Conclusion

- Dynamic headspace mode, outperforms the current regulatory limits, delivering high sensitivity compared to static headspace mode for NSA analysis.
- Shimadzu GCMS-TQ8050 NX has features like a new highly efficient shielded detector and superior noise reduction technology enhances sensitivity and enables quantitation of NSA even at trace levels.

■ References

- ICH Q2 (R2): Validation of analytical procedures: Test and Methodologies
- ICH M7 (R1): Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk
- USP <1469> General chapter for Nitrosamine Impurities

06-SAIP-GC-036-EN First Edition: May 2023

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

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

› Please fill out the survey

Related Products Some products may be updated to newer models.

› GCMS-TQ™8050 NX

Triple Quadrupole Gas Chromatograph Mass Spectrome···

› HS-20 NX series

Headspace Sampler

Related Solutions

› Small Molecule Pharmaceutical

› Price Inquiry

› Product Inquiry

› Technical Service / Support Inquiry

› Other Inquiry