SHIMADZU

MP 205

Ultra-sensitive dynamic headspace GC-MS/MS method for trace level quantitation of Nitrosamines in Deferiprone API

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1. Introduction

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

Pharmaceutical drug product which contains Deferiprone, is supposed to consume for a longer time. Maximum daily intake is of 99 mg/kg/day. Considering worst case scenario, calculated Maximum Daily Dose (MDD) will be 9000 mg/day when weight of the patient as 90 kg. Hence, it is imperative to determine NSA with Limit of Quantitation (LOQ) < 0.1 ppb. Limits were calculated as per MDD of 9000 mg / day and reported in Table-1 with established LOQ's for each NSA impurity. Developing method for determining total NSA at such low level in Active Pharmaceutical Ingredient (API) & Finish Dosage Form (FDF) creates challenges in pharmaceutical industry.

This poster describes a validated dynamic headspace GC-MS/MS procedure for quantification of various NSAs like N-nitroso-dimethylamine (NDMA), N-nitroso-diethylamine (NDEA), N-nitroso-ethyl-isopropylamine (NEIPA), N-nitroso-diisopropylamine (NDIPA), N-nitroso-dipropylamine (NDPA), and N-nitroso-dibutylamine (NDBA) in Deferiprone Active Pharmaceutical Ingredient (API).

Table 1: Limits for Deferiprone and established LOQs

Impurity	AI limit (ng/day)	Limit in ppb for MDD 9000 mg/day	LOQ (ppb)
NDMA	96	11	3.3
NDEA	26.5	3	0.9
NEIPA			
NDIPA			
NDPA			
NDBA			

2. Method development

2-1. GC-MS/MS analysis with dynamic headspace technique

Individual Certified Reference Standard (CRS) for all 6 N-nitrosamines from United States Pharmacopeia (USP) were procured. 10 ppm mixture of NDMA, NDEA, NEIPA, NDIPA, NDPA & NDBA standards was prepared using CRS and analysed in scan mode for identification. Steps such as precursor ion selection & Multiple Reaction Monitoring (MRM) method optimization at different Collision Energies (CE) were performed to obtain MRMs and their optimum CEs.

Quantitation of above mentioned NSAs was performed using Shimadzu's GCMS-TQ8050 NX system with HS-20 NX autosampler (Figure 1).

Figure 1: Shimadzu GCMS-TQ8050 NX system with HS-20 NX headspace sampler

2-2. Analytical conditions

Acquisition parameters were optimized to get accurate and precise results. Refer instrument parameters as per mentioned in Table 2.

Table 2. Instrument parameters for Headspace GC-MS/MS

GC parameters			
Column	: SH-PolarD 60 m, 0.32 mm I.D., 0.5 µm df		
Injection Mode	: Split @5		
Flow Control Mode	: Linear Velocity @44 cm/sec		
Carrier Gas	: Helium		
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° (9 min)		
MS parameters			
Ionization Mode	: Electron Ionization (EI)		
Ion Source Temp.	: 250 ºC		
Interface Temp.	: 235 ºC		
HS parameters			
Oven Temp.	: 110 ºC	Equilibrating Time	: 20.00 min
Sample Line Temp.	: 120 ºC	Pressurizing Time	: 0.30 min
Transfer Line Temp.	: 130 ºC	Pressure Equilib. Time	: 0.10 min
Trap Cooling Temp.	: 80 ºC	Load Time	: 0.30 min
Trap Desorb Temp.	: 280 ºC	Load Equilib. Time	: 0.10 min
Trap Equilib. Temp.	: 80 ºC	Dry Purge Time	: 0.00 min
Shaking Level	: 5	Injection Time	: 10.00 min
Multi Inj. Count	: 3	Needle Flush Time	: 10.00 min
Pressurizing Gas Pressure	: 160 kPa	GC Cycle Time	: 45.00 min

MRM Transitions				
Impurity	**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

2-3. Sample preparation

Diluent: Prepared 0.5% Sodium Hydroxide in MS grade water.

Linearity standard stock solution: Linearity standard stock was prepared using above listed CRMs which contains 3.63 ppb of NDMA and 0.99 ppb of NDEA, NEIPA, NDIPA, NDPA and NDBA.

Linearity solutions: Transferred 0.3 mL, 0.5 mL, 0.75 mL, 1.0 mL and 1.5 mL of Linearity standard stock solution into five different 10 mL volumetric flask and diluted with diluent. As such linearity concentration for all impurities were as per reported in Table 3.

Table 3. Concentrations of each NSA in linearity Levels:

Name of Impurity	Concentration in ppb				
	Level-1	Level-2	Level-3	Level-4	Level-5
NDMA	0.11	0.18	0.27	0.36	0.54
NDEA, NEIPA, NDIPA, NDPA & NDBA	0.03	0.05	0.075	0.1	0.15

Test solution: Weighed 33 mg (± 10%) of Deferiprone API and add 300 mg (± 10%) of sodium carbonate in a 20 mL headspace vial. Add 1 ml of diluent, crimp the vial tightly and inject.

Spiked sample solution: Weigh 33 mg (± 10%) of Deferiprone API and add 300 mg (± 10%) of sodium carbonate in a 20 mL headspace vial. Add 1 ml of respective linearity level, crimp the vial with cap septa tightly and inject.

Linearity level-1 was considered as LOQ level and range for the calibration was up to 150% of limit level. Method was validated with respect to Specificity, LOQ precision, Linearity, Method Precision at working level and Accuracy at four levels i.e. at LOQ, 50%, 100% and 150%.

3. Results and Discussion

Figure 2 & 3 depicts calibration curve (a), overlay of all linearity standards (b), chromatogram of LOQ standard solution (c) and overlayed chromatogram of LOQ precision (d) for representative analytes. The chromatographic overlay of test and spiked sample solutions for six nitrosamines is shown in the figure 4.

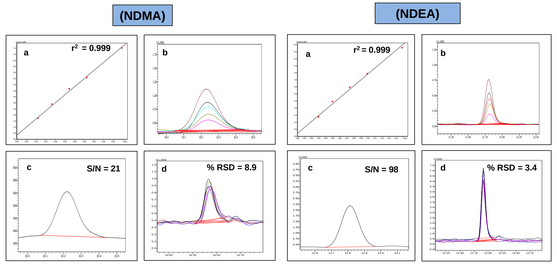

Figure 2: Data for NDMA

Figure 3: Data for NDEA

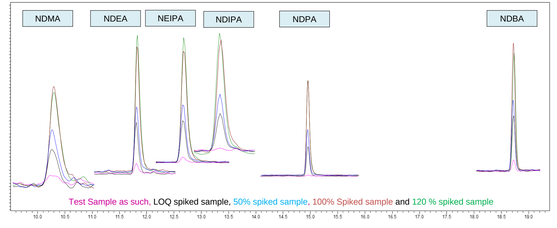

Figure 4: Chromatographic overlay for spiked solutions at each accuracy level with test sample

System suitability and method evaluation:

1) **System Precision :** 100 % linearity level standard solution (Level-4) were injected in six replicates. %RSD of area observed for each nitrosamine impurity was less than 5%.

2) **Linearity and range:** Standard solutions from LOQ level to the 150 % of working level were injected in triplicate injection to evaluate the linearity. Correlation coefficient observed for all the nitrosamines were more than 0.995.

3) **LOQ prediction and confirmation:** LOQ was evaluated based on S/N method (considering S/N>10) and confirmed with precision and accuracy. Linearity level-1 (LOQ standard) was injected in six replicates and %RSD of area for this injection was below 10%.

4) **Accuracy:** Accuracy was evaluated in terms of spike-recovery method at LOQ, 50% 100% and 150% concentration. %recoveries observed for each nitrosamine impurity were lie in between the range of 80 % to 120%**.**

5) **Repeatability / Method precision :** Method precision was performed by injecting six injections of 100 % spiked sample solution and %RSD of content in these injections was below 10%.

Nitrosamine impurities which are analysed in this method, was found to be below the limit of quantification. Hence, Method precision was performed with spiked sample analysis.

Results from all above validation parameter[1] proves that the method complies with the acceptance criteria's which are specified in the method validation guidelines and suitable for quantification of mentioned nitrosamines at ultra trace level, meeting specification criteria's with USP <1469> [2] . Table-4, Table-5 and Table-6 represents results for system suitability, Accuracy and Method precision, respectively.

Table 4: Results of System Suitability parameters

Suitability parameter	Nitrosamines Impurities					
	NDMA	NDEA	NEIPA	NDIPA	NDPA	NDBA
System Precision (%RSD, n=6)	4.6	2.6	3.5	3.0	3.0	3.0
Linearity (r^2)	0.999	0.999	0.999	0.999	0.999	0.994
LOQ precision (%RSD, n=6)	8.9	3.4	3.3	5.2	4.8	3.8
S/N at LOQ (Peak to peak)	21	98	178	50	10	252

Table 5: Results of accuracy parameter

Accuracy levels	Average recovery (%)					
	NDMA	NDEA	NEIPA	NDIPA	NDPA	NDBA
Level-1 (LOQ)	117	97	91	92	93	119
Level-2 (50%)	102	87	85	88	88	107
Level-3 (100%)	100	87	86	90	94	97
Level-4 (120%)	93	77	78	84	85	87

Table 6: Results of Method precision parameter

Parameter	%RSD of observed content (n=6)					
	NDMA	NDEA	NEIPA	NDIPA	NDPA	NDBA
Method precision	5.5	7.3	7.0	6.2	6.2	11.1

4. Conclusion

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

5. References

[1] ICH Q2 (R2): Validation of analytical procedures: Test and Methodologies

[2] USP <1469> General chapter for Nitrosamine Impurities