SHIMADZU

FP 259

Quantitation of Nitrosamines in Metformin Active Pharmaceutical Ingredient (API) using static and dynamic headspace GCMS/MS

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1. Overview

Health Sciences Authority (HSA) of Singapore on 4th December 2019[1], recalled 3 out of 46 locally marketed Metformin medicines after detecting presence of NDMA “above the internationally acceptable level” and this subject came into mainstream. Subsequently, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have released regular updates into their investigations for the causes of medicine contamination.

Metformin is the first-line drug control of high blood sugar levels in patients with type 2 diabetes, particularly in people who are overweight.

2. Introduction

Nitrosamines, are organic compounds of the chemical structure R2N−N=O, where R is usually an alkyl group. These molecules are of concern because they are probable human carcinogens.

According to USFDA guidance on Nitrosamines, for determination of more than 1 nitrosamines it is imperative to have an analytical method with LOQ ≤ 0.03 ppm for combined Nitrosamines. The static headspace (HS) GCMS/MS easily meets the current regulatory requirements whereas dynamic HS-GCMS/MS can achieve lower LOQ, making it a future ready solution for Nitrosamines.

Shimadzu HS-20 headspace autosampler is equipped with both static and dynamic mode of HS wherein, static is a conventional technique & dynamic is a much more improved and advance technique. The dynamic mode HS involves a combination of multiple sampling of headspace followed by cold trapping which result in trace level detection.(Figure 1)

Here, a comparative study between static and dynamic headspace GCMS/MS has been demonstrated for the quantitation of commonly found Nitrosamines namely N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodibutylamine (NDBA) and N-Nitrosodi-n-propylamine (NDPA).

Combined limit of quantitation (LOQ) of 5.0 ng/mL for static and 0.5 ng/mL for dynamic mode of HSGCMS/MS analysis in Metformin drug substance.

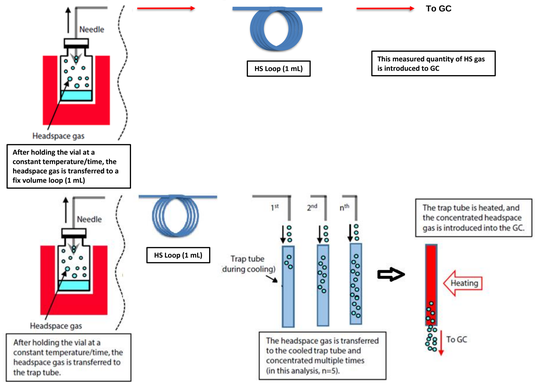

Figure 1 Working of static and dynamic modes of headspace

3. Methods

3-1. GC-MS/MS analysis (Static & Dynamic)

Nitrosamine analysis was performed using GCMS-8050 NX, a triple quadrupole mass spectrometer coupled with HS-20 headspace autosampler with static and dynamic mode of operation from Shimadzu Corporation, Japan (Figure 2).

Individual standards for Nitrosamines were procured from USP. About 5 ppm standard mixture of 6 Nitrosamines namely NDMA, NDEA, NDIPA, NEIPA, NDBA and NDPA were analysed in scan mode and identified with NIST-17 library. Steps such as precursor ion selection and MRM optimization at different Collision Energies (CE) were performed. MRM method with optimized CE was generated in segments which was further used for MRM analysis. For quantitation linearity standards, samples and spike samples were analyzed on static and dynamic mode HSGCMS/MS using conditions described in table 1 & table 2, respectively. The calibration range, LOQ and repeatability at LOQ is shown in table 3. The representative calibration curve, overlay of linearity standards and overlay of LOQ solution (5.0 ng/mL) of NDEA for static & (0.5 ng/mL) for dynamic mode HSGCMS/MS analysis is shown in figure 3 & 4. Further accuracy in terms of recovery was also calculated in both static and dynamic mode and is shown in table 4.

Figure 2 GCMS triple quadrupole mass spectrometer with headspace autosampler

3-2-1. Analytical conditions (Static)

Table 1. Instrument parameters for static mode HSGCMS/MS

GCMS System	**: GCMS-TQ8050 NX with HS-20**		
Column	: Wax ms 30 m, 0.25 mm I.D., 1.0 µm df		
HS Oven Temp.	:135 ºC		
Equilibrating Time	:10.0 min		
Injection Time	:1.0 min		
Injection Mode	: Splitless		
Flow Control Mode	: Column Flow (1 mL/ min)		
	Ramp Rate (ºC/min)	Temp. (ºC)	Hold Time (min)
Temp. Program	-	40.00	0.50
	20	200.00	2.00
	30	240.00	5.00
MS Parameters			
Ionization Mode	: Electron Ionization (EI)		
Interface Temp.	: 250 ºC		
Ion Source Temp.	: 230 ºC		

MRM Transitions

Comp.	MRM-1	CE-1	MRM-2	CE-2
NDMA	74.00>44.10	5	74.00>42.10	14
NDMA (d6)	82.00>48.00	20	NA	NA
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	130.00>42.10	14
NDBA	116.00>99.10	5	158.00>99.10	7

3-2-2. Analytical conditions (Dynamic)

Table 2. Instrument parameters for dynamic mode HSGCMS/MS

GCMS System	**: GCMS-TQ8050 NX with HS-20**		
Column	: Wax ms 30 m, 0.25 mm I.D., 1.0 µm df		
Mode	: Dynamic headspace with Multi Injection Count (MIC) of 1		
HS Oven Temp.	: 110 ºC		
Equilibrating Time	: 20.0 min		
Injection Time	: 25.0 min		
Injection Mode	: Split (10:1)		
Flow Control Mode	: Column Flow (4 mL/ min)		
	Ramp Rate (ºC/min)	Temp. (ºC)	Hold Time (min)
	-	35.00	2.00
Temp. Program	5	105.00	1.17
	10	200.00	0.00
	30	240.00	12.00
MS Parameters			
Ionization Mode	: Electron Ionization (EI)		
Interface Temp.	: 240 ºC		
Ion Source Temp.	: 240 ºC		

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	130.00>42.20	14
NDPA	130.10>113.10	6	130.10>43.20	18
NDBA	116.00>99.10	5	158.00>99.10	7

3-3-1. Sample preparation (Static)

Metformin API sample preparation
Accurately weighed 300 mg of API and 100 mg NaCl into a 20 mL headspace vial. Further added 1 mL of dimethyl sulfoxide and 0.5 mL of Milli Q water. Crimped the vial with cap-septa and injected.

Metformin API spike sample preparation
Accurately weighed 300 mg and 100 mg NaCl of API into a 20 mL headspace vial. Further added 1 mL of linearity standards prepared in dimethyl sulfoxide and 0.5 mL of Milli Q water. Crimped the vial with cap-septa and injected.

3-3-2. Sample preparation (Dynamic)

Metformin API sample preparation
Accurately weighed 100 mg of Metformin API and 300 mg Na_2CO_3 in a 20 mL headspace vial. Further added 1 mL of water. Crimped the vial with cap-septa and injected.

Metformin API spike sample preparation
Accurately weighed 100 mg of Metformin API and 300 mg of Na_2CO_3 in a headspace vial. Further added 1 mL of respective linearity standard solution. Crimped the vial with cap-septa and injected.

4. Results

Table 3. Standard summary for static and dynamic mode analysis

Comp.	Calibration range		r²		Conc. of LOQ level		% RSD of area at LOQ	
	Static	Dynamic	Static	Dynamic	Static	Dynamic	Static	Dynamic
NDMA	5.0 to 66.6 ng/mL	1.0 / 0.5 to 4.0 ng/mL	0.999	0.997	5.0 ng/mL	1.0 ng/mL	4.8	7.5
NDEA			0.999	0.994		0.5 ng/mL	2.2	6.5
NEIPA			0.999	0.997			5.8	5.7
NDIPA			0.999	0.995			3.7	14.6
NDBA			0.998	0.989			6.9	10.7
NDPA	NA		NA	0.991	NA		NA	10.6

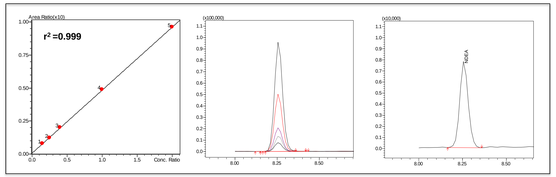

Figure 3 Representative calibration curve, overlay of linearity standards and LOQ solution (5.0 ng/mL) of NDEA for static mode HSGCMS/MS analysis

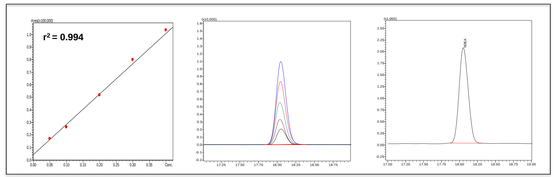

Figure 4 Representative calibration curve, overlay of linearity standards and LOQ solution (0.5 ng/mL) of NDEA for static mode HSGCMS/MS analysis

Table 4. Summary of recovery at LOQ Level for static and dynamic mode of analysis

Compound	% Recovery at LOQ	
	Static (5.0 ng/mL)	Dynamic (0.5 ng/mL)
NDMA	109	NA
NDEA	91	86
NEIPA	100	106
NDIPA	91	106
NDBA	106	106
NDPA	NA	114

5. Conclusion

- Static mode HSGCMS/MS can easily achieve LOQs lesser than the USFDA prescribed 0.03 ppm limit.
- Dynamic mode in HSGCMS/MS can achieve 5 times lower LOQ than static mode, making it future ready solution for Nitrosamines

6. References

[1] HSA Press Release “HSA Recalls Three out of 46 Metformin Medicines", December, 2019.