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Application News

Gas Chromatograph Mass Spectrometer GCMS-QP™2020 NX, AOC-30i

Quantitation of NDMA and NDEA in Metformin and 5 Sartan APIs as per the EDQM method Procedure B

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User Benefits

- This application note demonstrates applicability of EDQM method (Procedure B) for Metformin in addition to Sartan APIs.
- The GCMS-QP2020 NX with AOC-30i system achieves LOQ lower than the EDQM method (Procedure B) meeting the defined criteria of S/N and recovery.

■ Introduction

Overview : The Drug Regulatory Authorities first noticed the presence of the N-Nitrosamine impurity (NSA), N-Nitrosodimethylamine (NDMA) in products containing valsartan in July 2018. Valsartan is an Angiotensin II Receptor Blocker (ARB) and belongs to a family of analogue compounds commonly referred to as the Sartans. Similarly, NSA has also been detected in other drug products such as Metformin. Metformin is a prescription drug used to control high blood sugar in patients with Type 2 diabetes. Considering the significance of these drugs, it is crucial to make Sartans and Metformin available with safe levels of NSA.

Like USFDA, European Directorate for the Quality of Medicines and Healthcare (EDQM) ensure access to good quality medicines in Europe. EDQM has been working actively at various levels to address the presence of Nitrosamines in active substances and medicines. EDQM has been regularly informing all stakeholders, from national authorities to manufacturers, on the state of the works and on initiatives taken.

EDQM procedure for NSA: EDQM enlists 3 procedures for determination of NSA viz procedure A, B and C for LC-MS/MS, GC-MS and GC-MS/MS, respectively. Procedures A and B have been validated as limit tests (30 ppb) with recovery demonstrated at limit level. The procedure C has been validated as a quantitative test wherein a three-point calibration is plotted with recovery performed at limit level (30 ppb). This application news describes analytical procedures for the detection of 2 commonly found N-Nitrosamines namely N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) in Sartan and Metformin APIs by procedure B. The procedure B further describes 2 sample preparations procedures namely sample preparation 1 and 2. The sample preparation 1 is used for Valsartan, Losartan and Olmesartan additionally Metformin API is also analysed with this method. The sample preparation 2 is used for Candesartan and Irbesartan. When a procedure is applied to substances outside of the scope covered by the initial validation or to medicinal products or if procedure A or B is used quantitatively, then it must be validated.

■ Experimental

A mixture of NDMA, NEMA and NDEA standards was analyzed using scan mode for identification. By referring to the parameters mentioned in procedure B, a GC-MS quantitation method was created (Table 1). EDQM method B uses a two single point calibration methods each for sample preparation -1 and 2. However, in this application news two different calibrations (three-point) each for sample preparation 1 and 2 were plotted.

The calibration for sample preparation 1 is ranging from 3.75 ppb to 7.5 ppb whereas, for sample preparation 2 is ranging from 1.5 ppb to 6.0 ppb. Quantitation of NDMA and NDEA was performed using Shimadzu GCMS-QP2020 NX system with AOC-30i autosampler (Figure 1).

Note = All above concentrations are as such.

Figure 1: GCMS-QP2020 NX system with AOC-30i autosampler

■ Method

Table 1: Instrument configuration and analytical conditions

GCMS System	: GCMS-QP2020 NX with AOC-30i
Column	: SH-I-624Sil MS 30 m, 0.25 mm I.D., 1.4 µm df (P/N: 221-75962-30)
Injection Mode	: Splitless (Sampling time 0.5 min)
Flow Control Mode	: Column Flow
Injector Port Temp.	: 250 °C
Carrier Gas	: Helium
Column Flow	: 1.0 mL/min
Injection Volume	: 2.0 µL*

Temp. Program

Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
-	40	0.5
58.8	140	2
20	180	0.5
30	240	1.8
40	280	2.5

Ionization Mode	: Electron Ionization (EI)
Interface Temp.	: 240 °C
Ion Source Temp.	: 230 °C
Acquisition Mode	: SIM
Ions	: 74, 88 and 102 amu

*: EDQM mentions injection volume of 2.5µL

Note: Below concentrations are with respect to sample concentration.

■ Sample Preparation-1 (Valsartan, Losartan and Olmesartan)

Internal standard solution: Dissolve 5.0 mg of N-nitroso-ethylmethylamine (NEMA) in methanol and dilute to 10.0 mL with the same solvent. Dilute 500 µL of the solution to 10.0 mL with water for chromatography.

Extraction solution: Dissolve 40.0 g of sodium hydroxide in 800 mL of water for chromatography. Add 100 µL of the internal standard solution and dilute to 1000 mL with water for chromatography.

N-Nitrosamines spiking solution: For each N-nitrosamine concerned, use the corresponding Certified Reference Standard (CRS). In a single volumetric flask, dilute 200 µL of each of these CRS to 20.0 mL with water for chromatography. Dilute 300 µL of this solution to 20.0 mL with water for chromatography.

Linearity solution-1 (15.0 ppb): Add 50 µL of the N-nitrosamines spiking solution to 10.0 mL of the extraction mixture. Vortex for 5 min. Add 2.0 mL of dichloromethane (DCM) and shake well for at least 5 min, then centrifuge at about 5000 rpm for 5 min. Use the lower organic layer for GCMS injection.

Linearity solution-2 (30.0 ppb): Add 100 µL of the N-nitrosamines spiking solution to 10.0 mL of the extraction mixture. Vortex for 5 min. Add 2.0 mL of DCM and shake well for at least 5 min, then centrifuge at about 5000 rpm for 5 min. Use the lower organic layer for GCMS injection.

Linearity solution-3 (60.0 ppb): Add 200 µL of the N-nitrosamines spiking solution to 10.0 mL of the extraction mixture. Vortex for 5 min. Add 2.0 mL of DCM and shake well for at least 5 min, then centrifuge at about 5000 rpm for 5 min. Use the lower organic layer for GCMS injection.

Test solution: Suspend 250.0 mg of the API to be examined in 10.0 mL of the extraction mixture. Vortex for 5 min. Add 2.0 mL of DCM and shake well for at least 5 min, then centrifuge at about 5000 rpm for 5 min. Use the lower organic layer for GCMS injection.

Spiked solution: Suspend 250.0 mg of API in 10.0 mL of the extraction mixture. Add 50 µL of the N-nitrosamines spiking solution to prepare spiked solution with concentrations of 15 ppb. Vortex for 5 min. Add 2.0 mL of DCM and shake well for at least 5 min, then centrifuge at about 5000 rpm for 5 min. Use the lower organic layer for GCMS injection.

■ Sample Preparation-2 (Candesartan and Irbesartan)

Internal standard solution: Dissolve 5.0 mg of N-nitroso-ethylmethylamine (NEMA) in methanol and dilute to 10.0 mL with the same solvent. Dilute 100 µL of the solution to 10.0 mL with methanol.

Extraction solution: Dilute 100 µL of internal standard solution in 100 mL of dichloromethane.

N-Nitrosamines spiking solution: For each N-nitrosamine concerned, use the corresponding Certified Reference Standard (CRS). In a single volumetric flask, dilute 200 µL of each of these CRS to 10.0 mL with methanol. Further, dilute 300 µL of this solution to 20.0 mL with methanol.

Linearity solution-1 (15.0 ppb): Add 50 µL of the N-nitrosamines spiking solution to 5.0 mL of the extraction mixture. Shake well for 5 min, then centrifuge at about 5000 rpm for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (0.45 µm) to obtain a clear solution. Use the clear solution for GCMS injection.

Linearity solution-2 (30.0 ppb): Add 100 µL of the N-nitrosamines spiking solution to 5.0 mL of the extraction mixture. Shake well for 5 min, then centrifuge at about 5000 rpm for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (0.45 µm) to obtain a clear solution. Use the clear solution for GCMS injection.

Linearity solution-3 (60.0 ppb): Add 200 µL of the N-nitrosamines spiking solution to 5.0 mL of the extraction mixture. Shake well for 5 min, then centrifuge at about 5000 rpm for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (0.45 µm) to obtain a clear solution. Use the clear solution for GCMS injection.

Test solution: Suspend 500.0 mg of the API in 5.0 mL of the extraction mixture. Add 100 µL of methanol. Shake well for at least 5 min, then centrifuge at about 5000 rpm for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (0.45 µm) to obtain a clear solution. Use the clear solution for GCMS injection.

Spiked solution: Suspend 500.0 mg of the API in 5.0 mL of the extraction mixture. Add 100 µL of N-nitrosamines spiking solution. Shake well for at least 5 min, then centrifuge at about 5000 rpm for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (0.45 µm) to obtain a clear solution. Use the clear solution for GCMS injection.

■ Results and Discussion

Relative retention time (RRT): Both NDMA and NDEA matched the expected RRT criteria (Table 2).

Table 2: RRTs of N-nitrosamines

N-Nitrosamines	Expected RRT	Found RRT
NDMA	0.9	0.9
NEMA (ISTD)	1.0	1.0
NDEA	1.1	1.1

System suitability : For each N-Nitrosamine

Signal-to-noise (S/N) ratio: S/N for the peak due to each N-nitrosamine for the spiked solution, should be minimum of 10. (Table 3)

Table 3: S/N ratio for linearity solution-1 (15.0 ppb)

APIs	S/N for spiked solution	
	NDMA	NDEA
Candesartan	19	56
Irbesartan	17	29
Losartan	24	28
Olmesartan	66	16
Valsartan	26	16
Metformin	23	75

Repeatability : Ratio between area of the peak due to the concerned N-Nitrosamine and the area of the peak due to the internal standard of the reference solution should be less than 20%. The repeatability test passed the criteria (Table 4).

Table 4: Repeatability of area ratio for the reference solutions (n=6)

Linearity solutions	% Repeatability	
	NDMA	NDEA
Linearity solution-1 (15.0 ppb)	1.8	2.1
Linearity solution-2 (30.0 ppb)	1.1	1.4
Linearity solution-3 (60.0 ppb)	1.2	1.4

Figure 2 and 3 depicts the calibration curve and chromatogram of 15.0 ppb standard for NDMA and NDEA.

N-Nitrosodimethylamine (NDMA)

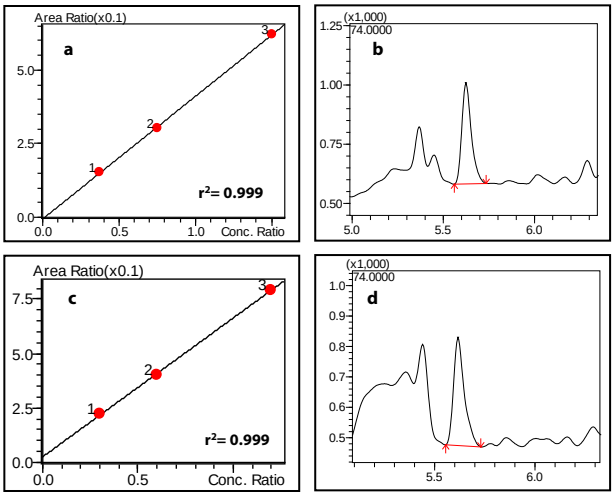

Figure 2: a) Calibration curve as per preparation-1, b) Chromatogram of 15.0 ppb standard as per preparation-1, c) Calibration curve as per preparation-2 d) Chromatogram of 15.0 ppb standard as per preparation-2

N-Nitrosodiethylamine (NDEA)

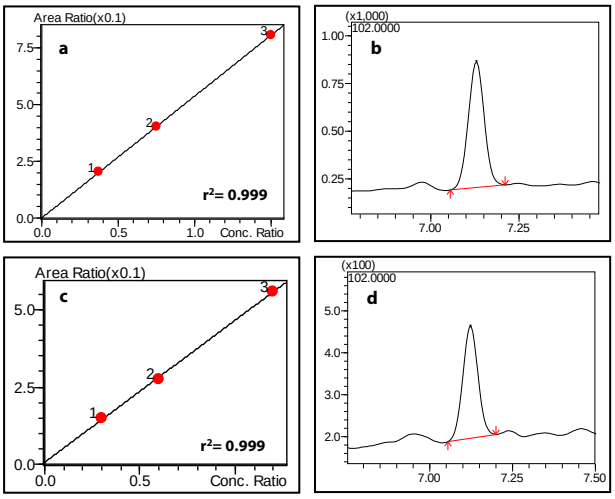

Figure 3: a) Calibration curve as per preparation-1, b) Chromatogram of 15.0 ppb standard as per preparation-1, c) Calibration curve as per preparation-2 d) Chromatogram of 15.0 ppb standard as per preparation-2

The amount calculated in Metformin and 5 Sartan APIs is summarized in table 5.

Table 5: Results for sample summary

APIs	Amount in sample (ppb)	
	NDMA	NDEA
Candesartan	BLOQ	BLOQ
Irbesartan	BLOQ	BLOQ
Losartan	BLOQ	BLOQ
Olmesartan	BLOQ	BLOQ
Valsartan	17.6	BLOQ
Metformin	BLOQ	BLOQ

BLOQ: Below limit of quantitation.

Recovery Study: There is no set criteria for spiked recovery study. The calculated recovery in all spiked solutions is between 70-130 %. (Table 6)

Table 6: Results for recovery study at 15.0 ppb

APIs	% Recoveries at 15.0 ppb	
	NDMA	NDEA
Candesartan	103	122
Irbesartan	115	96
Losartan	95	99
Olmesartan	111	119
Valsartan	107	112
Metformin	98	100

Table 7 depicts the LOQ comparison for Shimadzu application news and EDQM.

Table 7: LOQ comparison of Shimadzu and EDQM

Compound	LOQ Comparison	
	Shimadzu	EDQM
NDMA	15.0 ppb	30.0 ppb
NDEA		

■ Conclusion

- Quantitation of 2 NSAs in Metformin and 5 Sartan APIs as per EDQM procedure B was successfully demonstrated on Shimadzu GCMS-Q2020 NX with AOC-30i autosampler system.
- EDQM procedure B is only applicable to Sartans however, this application news demonstrates method applicability to Metformin API as well.
- The repeatability (n=6) for EDQM LOQ i.e., 30.0 ppb and Shimadzu LOQ i.e., 15.0 ppb was found to be less than 20 %.
- The S/N ratios for both NDMA and NDEA were easily achieved as per EDQM method procedure B.
- Accuracy in terms of recovery fulfills acceptance criteria for LOQ concentration i.e.,15.0 ppb.

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