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Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8050 NX, AOC-20i+s Plus

Quantitation of 6 N-Nitrosamines in Metformin and 5 Sartan APIs as per the EDQM method Procedure C

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User Benefits

- EDQM method (Procedure C) applicability for Nitrosamine determination in Metformin in addition to Sartan APIs.
- The GCMS-TQ8050 NX with AOC-20i+s Plus system achieves LOQ much lower than the EDQM method (Procedure C) 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.

What are NSA? : NSA are organic compounds of the chemical structure $R_2N–N=O$, where R is usually an alkyl group. These compounds are listed as Class 1 mutagens in ICH M7: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit potential carcinogenic risk. As a result of the significant toxicity associated with these impurities, it is recommended to take steps to control and limit their presence in pharmaceutical materials.

Like USFDA, European Directorate for the Quality of Medicines & 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 & C for LCMS/MS, GCMS & GCMS/MS, respectively. Procedures A and B have been validated as limit tests (30 ppb) and procedure C has been validated as a quantitative test. This application note describes analytical procedures for the detection of various N-Nitrosamines in Sartan & Metformin APIs by procedure C. The scope of each procedure is defined in Table 1. With these three procedures, it is possible to analyse the following N-nitrosamines: N-nitroso-dimethylamine (NDMA); N-nitroso-diethylamine (NDEA); N-nitroso-dibutylamine (NDBA); N-nitroso-N-methyl-4-aminobutyric acid (NMBA); N-nitroso-diisopropylamine (NDIPA); N-nitroso-ethyl-isopropylamine (NEIPA) and N-nitroso-dipropylamine (NDPA). Procedure A uses deuterated N-nitroso-diethylamine (NDEA-d10) as internal standard. Procedures B and C use N-nitroso-ethylmethylamine (NEMA) as internal standard. When a procedure is applied to substances outside of the scope covered by the initial validation (Table 1) or to medicinal products or if procedure A or B is used quantitatively, then it must be validated.

Table 1: Scope of the validation

Active Substances	NDMA	NDEA	NDBA	NMBA	NDIPA	NEIPA	NDPA
Candesartan cilexetil	ABC	ABC	C	A	AC	AC	C
Irbesartan	ABC	ABC	C	A	AC	AC	C
Losaratan potassium	ABC	ABC	C	A	AC	AC	C
Olmesartan medoxomil	ABC	ABC	C	A	AC	AC	C
Valsartan	ABC	ABC	C	A	AC	AC	C

■ Experimental

A mixture of NDMA, NEMA, NDEA, NEIPA, NDIPA, NDPA & NDBA standards was analyzed using scan mode for identification. Steps such as precursor ion selection & MRM optimization at different Collision Energies (CE) were performed to obtain optimum MRMs and their CE in segments. By referring GC parameters mentioned in procedure C & using optimized MRMs, a GCMS/MS quantitation method was created (Table 2). Optimized MRM and CE (Table 3) are very important for trace level determination & may vary for different instrument make. Quantitation of above-mentioned NSAs was performed using Shimadzu GCMS-TQ8050 NX system with AOC-20i+s Plus autosampler (Fig. 1).

Fig. 1: GCMS-TQ™ 8050 NX system with AOC-20i+s Plus autosampler

■ Method

Table 2: Instrument configuration and analytical conditions

GCMS System	: GCMS-TQ8050 NX with AOC-20i+s Plus
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	: 1.3 mL/min
Injection Volume	: 1.0 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

*= EDQM procedure mentions injection volume of 3 µL however, optimum sensitivity was achieved with 1 µL.

Table 3: MRM transitions of N-nitrosamines

Compound	Principal	CE-1	Qualifier	CE-2
NDMA	74.00>44.10	6	74.00>42.10	15
NEMA (ISTD)	88.00>71.00	5	88.00>43.00	7
NDEA	102.00>85.10	6	102.00>56.10	17
NEIPA	116.00>99.10	6	116.00>42.10	23
NDIPA	130.00>88.10	6	130.00>42.00	11
NDPA	130.00>113.00	5	130.00>43.10	13
NDBA	116.00>99.10	6	158.00>99.00	10

Note: Below concentrations are with respect to sample concentration.

■ Sample

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 500 mL of water for chromatography. Add 100 µL of the internal standard solution, then 50 mL of acetonitrile, 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 100 µL of each of these CRS to 10.0 mL with water for chromatography. Dilute 300 µL of this solution to 20.0 mL with water for chromatography.

Linearity solution-1 (7.5 ppb): Add 25 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) & Shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Use the lower organic layer for GCMS/MS injection.

Linearity solution-2 (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 DCM & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Use the lower organic layer for GCMS/MS injection.

Linearity solution-3 (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 & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Use the lower organic layer for GCMS/MS injection.

Linearity solution-4 (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 & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Use the lower organic layer for GCMS/MS 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 & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Use the lower organic layer for GCMS/MS injection.

Spiked solution: Suspend 250.0 mg of API in 10.0 mL of the extraction mixture. Add 50 & 100 µL of the N-nitrosamines spiking solution to prepare spiked solution with concentrations of 15 & 30 ppb, respectively. Vortex for 5 min. Add 2.0 mL of DCM & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Use the lower organic layer for GCMS/MS injection.

■ Results and Discussion

Fig. 2 to 7 depicts the calibration curve, overlay of all linearity standards, overlay of 15.0 ppb and 30.0 ppb replicate standards (n=6) for all NSAs.

N-Nitrosodimethylamine (NDMA)

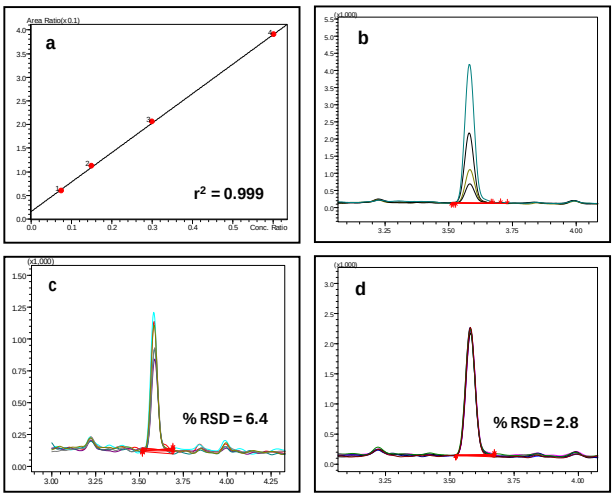

Fig. 2: a) Calibration curve, b) Overlay of linearity standards, c) Chromatogram of 15.0 ppb & d) chromatogram of 30.0 ppb for NDMA

N-Nitrosodiethylamine (NDEA)

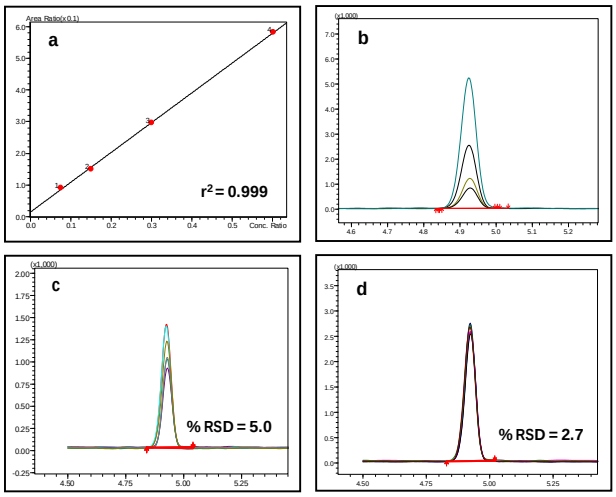

Fig. 3: a) Calibration curve, b) Overlay of linearity standards, c) Chromatogram of 15.0 ppb & d) Chromatogram of 30.0 ppb for NDEA

N-Nitrosoethylisopropylamine (NEIPA)

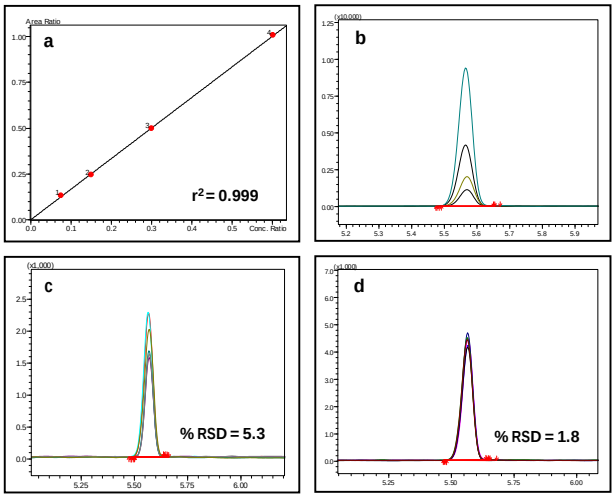

Fig. 4: a) Calibration curve, b) Overlay of linearity standards,
c) Chromatogram of 15.0 ppb & d) Chromatogram of 30.0 ppb for NEIPA

N-Nitrosodipropylamine (NDPA)

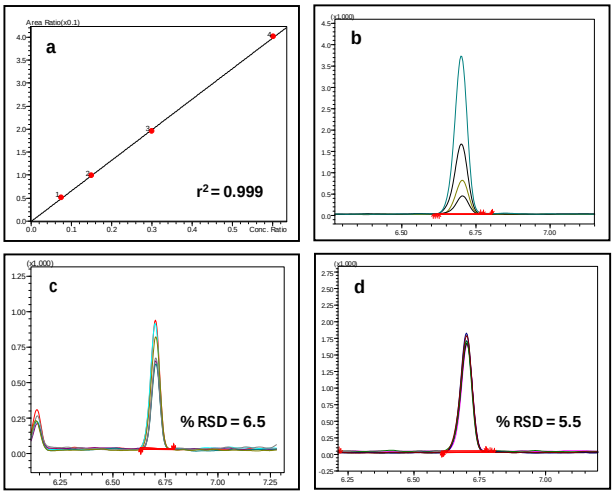

Fig. 6: a) Calibration curve, b) Overlay of linearity standards,
c) Chromatogram of 15.0 ppb & D) Chromatogram of 30.0 ppb for NDPA

N-Nitrosodiisopropylamine (NDIPA)

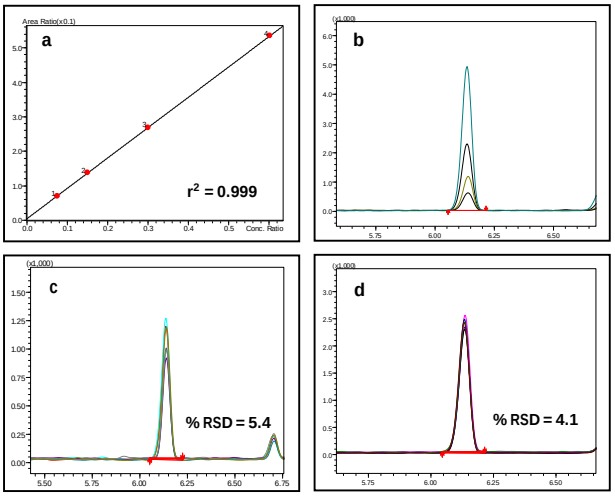

Fig. 5: a) Calibration curve, b) Overlay of linearity standards,
c) Chromatogram of 15.0 ppb & Chromatogram of 30.0 ppb for NDIPA

N-Nitrosodibutylamine (NDBA)

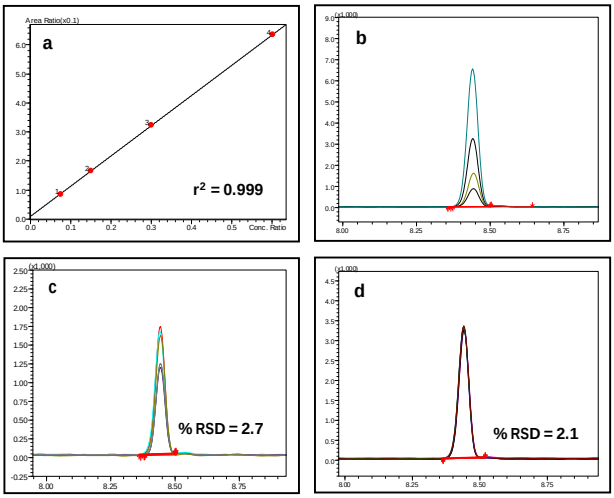

Fig. 7: a) Calibration curve, b) Overlay of linearity standards,
c) Chromatogram of 15.0 ppb & d) Chromatogram of 30.0 ppb for NDBA

Chromatographic overlay for 6 N-Nitrosamines and 1 ISTD in 5 Sartan and Metformin APIs by procedure C: Spiked solution at 30.0 ppb (Fig. 8)

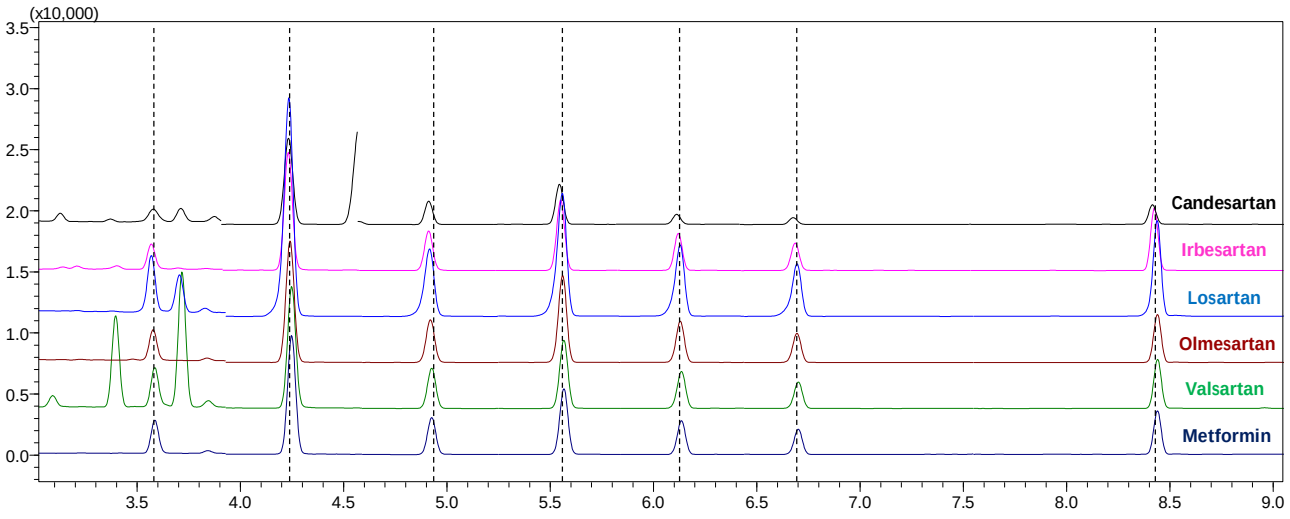

Fig. 8: Chromatographic overlay for spiked solutions at 30.0 ppb for 6 APIs

System suitability : For each N-Nitrosamine

Repeatability : Ratio between area of the peak due to the concerned N-Nitrosamine for principal MRM transition 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)

Repeatability (%)	NDMA	NDEA	NEIPA	NDIPA	NDPA	NDBA
Linearity solution-1 (7.5 ppb)	4.1	7.1	4.0	2.8	6.9	3.7
Linearity solution-2 (15.0 ppb)	6.4	5.0	5.3	5.4	6.5	2.7
Linearity solution-3 (30.0 ppb)	2.8	2.7	1.8	4.1	5.5	2.1

Signal-to-noise (S/N) ratio 1 & 2 (Principal/Qualifier) : The criteria for S/N of principal transition & qualifier transition of linearity solution-1, is minimum 10 & 3, respectively. (Table 5)

Table 5: S/N ratio 1 & 2 (Principal/Qualifier) for linearity solution-1 (7.5 ppb)

S/N (Peak to Peak)		
Compounds	Principal	Qualifier
NDMA	24	23
NDEA	102	18
NEIPA	194	5
NDIPA	125	37
NDPA	78	13
NDBA	107	24

It passed the S/N criteria for the principal and for the qualifiers (Table 5).

Validity criteria for test : The test is not valid unless the ratio between the area of the peak corresponding to the principal transition and the area of the peak corresponding to the qualifier transition for the test solution is within 20 % of the same ratio calculated for the spiked solution.
All the samples showed below LOQ concentrations i.e. 15.0 ppb. Hence, the validity criteria is not applicable to the samples.

Summary of all 6 N-Nitrosamines detected are included in Table 6.

Table 6: Sample summary

APIs	Concentration in ppb					
	NDMA	NDEA	NEIPA	NDIPA	NDPA	NDBA
Candesartan	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Irbesartan	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Losartan	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Olmesartan	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Valsartan	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Metformin	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ

Recovery Study Criteria: The calculated recovery in all spiked solutions is between 70-130 %. (Table 7 & 8)

Table 7: Results for recovery study at 15.0 ppb

APIs	% Recoveries at 15.0 ppb					
	NDMA	NDEA	NEIPA	NDIPA	NDPA	NDBA
Candesartan	112	97	72	77	74	84
Irbesartan	98	98	103	99	101	93
Losartan	94	108	104	104	111	104
Olmesartan	98	108	107	108	108	100
Valsartan	100	97	104	108	102	101
Metformin	75	96	104	98	101	96

Table 8: Results for recovery study at 30.0 ppb

APIs	% Recoveries at 30.0 ppb					
	NDMA	NDEA	NEIPA	NDIPA	NDPA	NDBA
Candesartan	103	99	77	73	70	87
Irbesartan	102	106	107	110	105	105
Losartan	92	108	106	103	103	103
Olmesartan	102	102	105	110	107	105
Valsartan	101	104	108	107	109	104
Metformin	120	102	105	105	109	106

The Comparison between LOQ mentioned in EDQM procedure C and Shimadzu application news is described in Table 9.

Table 9: LOQ comparison of EDQM and Shimadzu

Compound	LOQ Comparison	
	Shimadzu	EDQM
NDMA	15.0 ppb	30.0 ppb
NDEA		
NEIPA		
NDIPA		
NDPA		
NDBA		

■ Conclusion

- Quantitation of 6 NSAs in 6 APIs as per EDQM procedure C was successfully demonstrated on Shimadzu GCMS-TQ8050 NX with AOC-20i+s Plus autosampler system.
- EDQM procedure C 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 ratio criteria for principal and qualifier ion of all NSAs are easily achieved as per EDQM method procedure C.
- Accuracy in terms of recovery fulfils acceptance criteria for both LOQs i.e.,15.0 ppb (Shimadzu) and 30.0 ppb (EDQM).

06-SAIP-GC-029-EN First Edition: Oct. 2021

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