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

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1. Overview

N-nitrosamine impurity (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[1]. 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.

2. Introduction

Like United States Food and Drug Administration (USFDA), European Directorate for the Quality of Medicines & Healthcare (EDQM) ensures access to good quality medicines in Europe. EDQM has been working actively at various levels to address the presence of NSA in active substances and medicines. EDQM enlists 3 procedures for determination of NSA viz procedure A, B & C for LC-MS/MS, GC-MS & GC-MS/MS, respectively. Procedures A and B have been validated as limit tests (30 ppb) and procedure C has been validated as a quantitative test. Procedure A uses deuterated N-nitroso-diethylamine (NDEA-d10) as internal standard whereas Procedures B and C use N-nitroso-ethylmethylamine (NEMA) as internal standard (IS).[2]

This poster describes a validated GC-MS/MS procedure for detection of various N-nitrosamines in Candesartan, Irbesartan, Losartan, Olmesartan, Valsartan & Metformin APIs as per procedure C. With these three procedures, it is possible to analyse the following NSA: 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).

3. Methods

3-1. GC-MS/MS analysis

Individual Certified Reference Standard (CRS) for all 6 N-nitrosamines from United States Pharmacopeia (USP) were procured. A mixture of NDMA, NEMA-IS, NDEA, NEIPA, NDIPA, NDPA & NDBA standards was prepared using CRS and analysed in scan mode for identification. Steps such as precursor ion selection & Multi Reaction Monitoring (MRM) optimization at different Collision Energies (CE) were performed to obtain MRMs and their optimum CEs. By referring GC parameters mentioned in procedure C & using obtained MRMs, a GC-MS/MS quantitation method was created (Table 1). Optimized MRM and CE (Table 2) 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 (Figure 1).

Figure 1: Shimadzu GCMS-TQ8050 NX system with AOC-20i+s Plus autosampler

3-2. Analytical conditions

Table 1. Instrument parameters for GC-MS/MS

GCMS System	: GCMS-TQ8050 NX with AOC-20i+s Plus
Column	: SH-I-624SII MS 30 m, 0.25 mm I.D., 1.4 µm df (P/N: 221-75962-30)
Injection Mode	: Splitless
Flow Control Mode	: Column Flow
Injector Port Temp.	: 250 ºC
Carrier Gas	: Helium
Column Flow	: 1.3 mL/min
Injection Volume	: 1.0 µL (EDQM procedure mentions injection volume of 3 µL)
Temp. Program	

Ramp Rate (ºC/min)	Temp. (ºC)	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 ºC
Ion Source Temp.	: 230 ºC

Table 2: MRM transitions for N-nitrosamines

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

3-3. Sample preparation

Internal standard solution: Dissolve 5 mg of NEMA-IS in methanol and dilute to 10 mL with the same solvent. Dilute 500 µL of this solution to 10 mL with Milli Q water.

Extraction solution: Dissolve 40 g of sodium hydroxide in 500 mL of Milli Q water. Add 100 µL of the internal standard solution, then 50 mL of acetonitrile, and dilute to 1000 mL with Milli Q water.

N-Nitrosamines spiking solution: For each N-nitrosamine, use the corresponding CRS (500 ppm each) in a single volumetric flask, dilute 100 µL of each of these CRS to 10 mL with Milli Q water. Dilute 300 µL of this solution to 20 mL with Milli Q water.

Linearity solutions (7.5, 15.0, 30.0 & 60.0 ppb): Add 25, 50, 100 & 200 µL of the N-nitrosamines spiking solutions to individual 10 mL of the extraction mixture. Vortex for 5 min. Add 2 mL of dichloromethane (DCM) & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Collect lower organic layer from individual tubes and analyze on GC-MS/MS.

Test solution: Weigh 250 mg of API in 10 mL of the extraction mixture. Vortex for 5 min, add 2 mL of DCM & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Collect lower organic layer from individual tubes and analyze on GC-MS/MS.

Spiked solution: Weigh 250 mg of API in 10 mL of the extraction mixture. Add 50 & 100 µL of NSA spiking solution to achieve spiking of 15 & 30 ppb with respect to sample. Vortex for 5 min, add 2 mL of DCM & shake well for at least 5 min, then centrifuge at about 7800 rpm for 5 min. Collect lower organic layer from individual tubes and analyze on GC-MS/MS.

4. Results and Discussion

Figure 2 & 3 depicts the calibration curve, overlay of all linearity standards, overlay of 15.0 ppb and 30.0 ppb replicate standards (n=6) for NDMA & NDEA (Representative chromatograms)

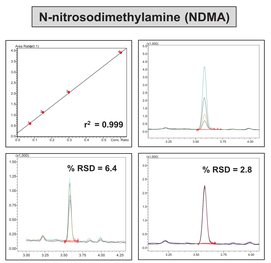

Figure 2: a) Calibration curve, b) Overlay of linearity standards, c) Chromatograms of 15.0 ppb & d) Chromatograms of 30.0 ppb for NDMA

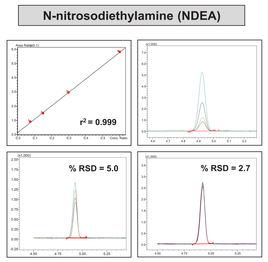

Figure 3: a) Calibration curve, b) Overlay of linearity standards, c) Chromatograms of 15.0 ppb & d) Chromatograms of 30.0 ppb for NDEA

Chromatographic overlay for 6 N-nitrosamines and NEMA-IS in 5 Sartan and Metformin APIs by procedure C: Spiked solution at 30.0 ppb (Figure 4)

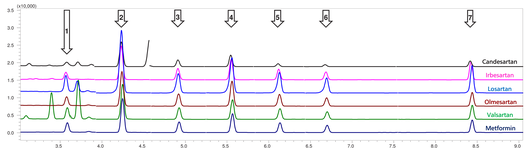

Figure 4: Chromatographic overlay for spiked solutions at 30.0 ppb for 6 APIs

System suitability criteria:

1) Repeatability : Ratio between principal MRM transition and internal standard solution should be less than 20 %. The repeatability test passed the criteria (Table 3).

2) 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 3)

Table 3: Repeatability of area ratio for the reference solutions (n=6) and S/N ratio 1 & 2 (Principal/Qualifier) for linearity solution-1 (7.5 ppb)

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
S/N at 7.5 ppb (peak to peak)						
Principal	24	102	194	125	78	107
Qualifier	23	18	5	37	13	24

Validity criteria for test : For the test solution to be valid, the area ratio between principal & qualifier transition for the test solution should be within 20 % of the ratio calculated for the spiked solution.

Summary of all 6 N-nitrosamines detected are included in Table 4.

Table 4: Sample summary

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

Recovery Study Criteria: The calculated recovery in all spiked solutions should be between 70-130 %. (Table 5 & 6)

Table 5: 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 6: 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

5. 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.
- Combination of noise reduction technology and sensitive detector enables N-nitrosamine determination at 2 times lower LOQ (15 ppb) than prescribed in EDQM procedure C (30 ppb).

6. References

[1] WHO Information Note; Update on Nitrosamine impurities, 20 Nov. 2019.
[2] European Pharmacopoeia (Ph. Eur.) Commission new general chapter on the analysis of N-nitrosamine impurities in active substances (2.5.42)

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