Nitrosamines by GC-MS/MS

Disclaimer: This is only an English translation of the official document 31_PV_171 and lies outside our quality management system. The officially valid document is the one published on www.swissmedic.ch website.

1 Purpose and scope

This test procedure is for testing for nitrosamines by GC-MS / MS. The test procedure allows the determination of the following nitrosamines at a concentration level of 15 ppb (LOQ):

Substance	Abbreviation
N-Nitrosodimethylamine	NDMA
N-Nitrosodiethylamine	NDEA
Ethylisopropylnitrosamine	EIPNA
N-Nitroso-di-iso-propylamine	DIPNA
N-Nitrosodi-n-propylamine	DPNA
N-Nitrosodi-n-butylamine	DBNA

The focus of the method is on the sartan drug group, which is why only sartan preparations (valsartan, losartan, irbesartan, olmesartan, candesartan) have been validated. For other APIs or finished products, in-situ validation with a focus on extraction, specificity and quantification is required.

2 Principle of the Method

Extraction of sodium hydroxide suspended tablets or API with dichloromethane and subsequent GC-MS/MS analysis in MRM-Mode.

3 Validation

See Validation Report ***31_VA_171 Nitrosamine mittels GC-MS/MS***

4 Definitions and Abbreviations

See ***Glossar OMCL***

Term and abbreviation	Description	Note
API	Active Pharmaceutical Ingredient	-
MK	Volumetric Flask	-
MRM	Multiple Reaction Monitoring	-
ISTD	Internal Standard	EMNA is used as ISTD

5 Special instruction / Safety instruction

Nitrosamines are potentially carcinogenic. Appropriate protective measures must be taken.

6 Reference and control substances, testing equipment, materials, chemicals and solutions

6.1 Reference substances

Description	Content / Purity	Manuf./Supplier / Art.-Nr. (e.g.)
N-Nitrosodimethylamine (NDMA)	99.9 %	Sigma / 442687
N-Nitrosoethylmethylamine (EMNA)	98.9 %	LGC / DRE-C15604000
N-Nitrosodiethylamine (NDEA)	97%	Toronto Research Chemicals / N525950
Ethylisopropylnitrosamine (EIPNA)	95%	MuseChem / M079119
N-Nitroso-di-iso-propylamine (DIPNA)	99%	LGC / DRE-C15604700
N-Nitrosodi-n-propylamine (DPNA)	99.9%	Sigma Aldrich / 48554
N-Nitrosodi-n-butylamine (DBNA)	99.8%	Sigma Aldrich / 442685

6.2 Control substances

None

6.3 Equipment and Material

Description
GC-MS/MS (Agilent 7890B_7000D)

6.4 Chemicals

Description	Manuf./Supplier / Art.-Nr. (e.g.)
Dichloromethane	Merck / 1.06050.1000
Sodium hydroxide 50%	Sigma Aldrich / 415413
Acetonitrile	NeoFroxx / LC-5413.1
H_2O MilliQ	OMCL

6.5 Solutions

Description	Preparation	Stability / Storage temp.
Nitrosamine stock-solutions 1 & 2	Approximately 5 mg of each nitrosamine are weighed into individual 10 mL volumetric flasks, dissolved with methanol and diluted to 10 mL. For each nitrosamine, two solutions (Nitrosamine stock-solution 1 & 2) are prepared.	14 days / 25°C
Nitrosamine mix 1 & 2	For each nitrosamine stock-solution 1, pipette 200 µL into a 10 mL volumetric flask and dilute to 10 mL with H2O MilliQ (Nitrosamine mix 1). A similar mix solution is prepared from stock-solutions 2 (Nitrosamine mix 2). These solutions are stored as a 1mL aliquot at -20 ° C.	14 days / 25°C
Spiking solution A 1 & 2	300 µL of Nitrosamine Mix 1 are diluted with H2O MilliQ to 20 mL (Spiking solution A 1). The same solution is prepared from the Nitrosamine Mix 2 (Spiking solution A 2).	14 days / 25°C
Spiking solution B	300 µL of Nitrosamine Mix 1 are diluted to 10 mL with H2O MilliQ.	14 days / 25°C
ISTD stock-solution	Approximately 5 mg of EMNA are weighed into a 10 mL volumetric flask, dissolved with methanol and diluted to 10 mL.	14 days / 25°C
ISTD dil-solution	500 µL of the ISTD stock-solution are diluted to 10 mL with H2O MilliQ.	14 days / 25°C
1M NaOH solution (ACN/ISTD)	52.6 mL of NaOH 50% are transferred into a 1L volumetric flask and dissolved with H2O MilliQ. 100 µL ISTD-dil solution and 50 mL ACN are added to this solution. The solution is then diluted to 1 L with H2O MilliQ.	14 days / 25°C

In order to rule out errors in the weighing or dilution of the solutions, the spiking solutions A are prepared in duplicate. To compare the two solutions, blank spikes are prepared using 200 µL spiking solution A (1) and spiking solution A (2) (see paragraph 9.1). Both solutions are analyzed 3 times each. The mean values of the response (area / concentration) of the two triplicate analyzes may not deviate from each other by more than 5%.

7 Procedure

7.1 Sample preparation

A representative sample is prepared from about 10 ground tablets. The quantity of mixed tablet powder is weighed into a 15 mL centrifuge tube, which corresponds to an amount of active ingredient of approx. 250 mg, paying attention to not exceed 1.5 g of total mass. For API samples, 250 mg of active ingredient are weighed in each case.
This sample powder is mixed with 10 mL NaOH solution, vortexed briefly and then shaken well for at least 5 minutes. 2.0 mL of dichloromethane are added to this suspension, vortexed briefly and then shaken well for at least 5 minutes. The suspension is then centrifuged at about 10’000 g for at least 5 min. The upper aqueous phase is gently removed, so that the lower organic phase can be withdrawn more easily.

For the spiking test solution, the same amount of mixed tablet powder is weighed into a 15 mL centrifuge tube, an amount of Spiking solution, corresponding to the limit (30 ppb in the case of

the Sartans) is added along with 10 mL NaOH solution, suspended and extracted with dichloromethane as described above.

Together with each measurement series, a 3-point linearity plot is generated. The corresponding calibration standard solution preparation is analogous to the spiking test solution without weighing the mixed tablet powder. In general, concentrations of 50% / 100% / 200% of the limits (15 ppb, 30 ppb and 60 ppb in the case of the Sartans) are used for the calibration. The 100% linearity standard solution is re-analyzed after regular intervals (approximately every 5 samples) as a standard check.

For Nitrosamine levels above the limit, additional analyses must be performed, in order to confirm the result. In this case an additional triplicate sample preparation is carried out. If the nitrosamine content is outside the calibrated range, the calibration line must be extended accordingly.

7.2 Sequence example

After every five test samples (spiked and unspiked), a blank injection followed by the 100% linearity standard solution is performed. Depending on the matrix load of the test samples, additional blanks might be carried out.

Example:

1. Dichlormethane (DCM) Blank
2. Blank Extract
3. Linearity sol. 1 50%
4. Linearity sol. 2 100%
5. Linearity sol. 3 200%
6. Blank Extract
7. TestSample1
8. TestSampleSpiked1
9. TestSample2
10. TestSampleSpiked2
11. TestSample3
12. TestSampleSpiked3
13. TestSample4
14. TestSampleSpiked4
15. TestSample5
16. TestSampleSpiked5
17. DCM Blank
18. Lin 2 100%
19 DCM Blank
20. TestSample6
21 TestSampleSpiked6
22 TestSample7
23. TestSampleSpiked7

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7.3 Instrument parameters

GC-Parameters

Column 1 (Inlet → EPC)	VF-624ms, 30m, 0.25 mm ID, 1.4 µm Film			
Column 2 (EPC → MS)	Deactivated Fused Silica, 1.35m, 0.15 mm ID.			
Carrier gas	Helium			
Flow rate column 1	1.3 mL/min / Post run -1.57 mL/min			
Flow rate column 2	1.45 mL/min / Post run 5.34 mL			
Injector port temp.	250 °C			
Injection volume	3.0 µL (Draw Speed 75 µL/min, Viskosity Delay 7s)			
Pulse Pressure Purge Time Gas Saver	40 psi for 0.5 min 60 mL/min at 0.5 min 20 mL/min after 1.5 min			
Liner	Splitless, single taper with wool (Agilent 5183-4694)			
Oven-Programme	Start Temp / °C	Heating-rate / °C/min	End Temp / °C	Hold / min
	40	0	40	0.5
	40	60	140	2
	140	20	180	0.5
	180	30	240	1.8
Post Run Oven	280°C for 2.5 min			
Transfer Line	240°C			

MS-Parameters

Ion Source	Extractor Source
Source Temperature	230°C
Quad Temperature	150°C (both)
Fixed Electron Energy	40 eV
Acquisition Type	MRM
Solvent Delay	4.5 min
Gain Factor	15
Collision Gas	Nitrogen / 1.5 mL/min
Quench Gas	Helium / 2.25 mL/min

MRM Transitions

Subst.	Q1	Resolution	Q2	Resolution	RT / min	RT Delta left	RT Delta Right	Dwell Time	CE
NDMA	74	Unit	44.1	Unit	4.766	0.25	0.25	199	5
NDMA	74	Unit	42.1	Unit	4.766	0.25	0.25	199	22
ENMA	87.9	Unit	71	Unit	5.509	0.4	0.4	199	5
ENMA	87.9	Unit	42.1	Unit	5.509	0.4	0.4	199	22
NDEA	102	Unit	85.1	Unit	6.201	0.25	0.25	199	3
NDEA	102	Unit	56	Unit	6.201	0.25	0.25	199	19
EIPNA	115.9	Unit	99.1	Unit	6.881	0.25	0.25	199	5
EIPNA	115.9	Unit	44.1	Unit	6.881	0.25	0.25	199	14
DIPNA	129.9	Unit	88.1	Unit	7.472	0.25	0.25	199	5
DIPNA	129.9	Unit	71	Unit	7.472	0.25	0.25	199	14
DPNA	129.9	Unit	113.1	Unit	7.999	0.25	0.25	199	1
DPNA	129.9	Unit	88	Unit	7.999	0.25	0.25	199	1
DBNA	157.9	Unit	141	Unit	9.67	0.25	0.25	199	1
DBNA	157.9	Unit	99.1	Unit	9.67	0.25	0.25	199	7

Due to the high matrix load of some samples (eg candesartan), it is recommended to replace or purify the liner and the inlet after a large series of measurements or when the efficiency of the method decreases.

8 Evaluation and uncertainty of measurement

8.1 Evaluation

For the calculation of the calibration line, the ratios of the analyte peak areas to the ISTD peak area are plotted against the concentrations of the analytes.

The results of the samples are used to calculate the analyte concentration in the sample solution and in the spiked sample solution. The calculated recovery in the spiked sample solution must be between 70% and 130%.

If a nitrosamine content above the limit is detected, the finding is confirmed by an additional triplicate test solution preparation. As a final result, the mean value of the triplicate preparation is entered in the LIMS.

In the case of a poor recovery, the assay determination is carried out via a standard addition. The standard addition must be validated in-situ.

9 Documentation

If the limit of a test sample is not exceeded, in LIMS will be documented as "< {Limit} ppb".

Examples:
< 30 ppb NDMA
< 30 ppb NDEA

If the limits are exceeded, the results obtained in the content determination will be documented in LIMS as "XX ppb".

Examples:
50 ppb NDMA
80 ppb NDEA

10 System Suitability Test

- No interfering signals in the Blank-Solution
- No significant co-elution in the spiked test solutions
- S/N of spiked test solutions is at least 10 for all spiked nitrosamine signals
- Correlation of the calibration line r > 0.995
- The 100% linearity standard solution must have a recovery of 70% - 130%.
- Quantifier / Qualifier Ratios must be comparable with the values of the standard solutions.

11 Document change history

Version-Nr.:	Change Datum/Visum:	Changes to the previous version:
1.0	17.Nov.2019 / cma	First version of the document (EN translation)