Limit-test of NDMA and NDEA in Sartans by GC-MS (Liquid-direct-injection)

1 Purpose and scope

The method is used to test the permissible limits of N-nitrosodimethylamine and N-nitrosodiethylamine in various sartans (API and finished products). Since there are a variety of products with different compositions, depending on the test sample, co-elution with matrix components or problems in phase separation may occur. In this case the test method has to be adapted situationally (chromatographic conditions) and the influence of these changes on the validation have to be assessed.

2 Principle of the Method

Extraction of sodium hydroxide suspended tablets or API with dichloromethane and subsequent GC-MS analysis in SIM mode.

3 Validation

See Validation Report ***31_VA_163 Nitrosamine in Sartanen VA***

4 General references

- GC-MS Method for N-Nitrosodiethylamine (NDEA) in Losartan Potassium, Zhejiang Huahai Pharmaceutical
- A-016102 NDEA Method Development in Sartane

5 Definitions

See ***Glossar OMCL***

Term	Description	Note
API	Active Pharmaceutical Ingredient	-
NDEA	N-Nitrosodiethylamin	Carcinogenic
NDMA	N-Nitrosodimethylamin	Carcinogenic
VK	Volumentric falsks	-
SIM	Single Ion Monitoring	-

6 Special instruction / Safety instruction

NDMA and NDEA are potentially carcinogenic. Appropriate protective measures must be taken.

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

7.1 Reference substance

Description	Content / Purity	S-Nr. LIMS	Manuf./Supplier / Art.-Nr. (e.g.)
NDEA	99.9 %	S-3482	Sigma / 442687
NDMA	98.9 %	S-3469	LGC / DRE-C15604000

7.2 Control substances

None

7.3 Equipment and Material

Description
HS-GC-MS/FID (Agilent 7697A-7890-5975C)

7.4 Chemicals

Description	S-Nr. LIMS	Manuf./Supplier / Art.-Nr. (e.g.)
Dichlormethan	S-1803	Merck / 1.06050.1000
Sodiumhydroxide 1 mol/L	S-1916	Merck / 1.09137.1000

7.5 Solutions

Description	Preparation
NDMA Stock-Solution	Weigh in 5 mg NDMA into a 10 mL volumetric flask (VF) and dilute to 10mL with MilliQ H_2O
NDMA Solution 1	Pipet 0,6 mL of NDMA Stock-Solution into a 10 mL VF and dilute to 10mL with MilliQ H_2O
NDEA Stock-Solution	Weigh in 5 mg NDEA into a 10 mL (VF) and dilute to 10mL with MilliQ H_2O
NDEA Solution 1	Pipet 200 µL of NDEA Stock-Solution into a 10 mL VF and dilute to 10mL with MilliQ H_2O
Spiking-Solution	From the NDMA and NDEA Solution 1, a Spiking-Solution will be prepared, so that the respective Nitrosamine limit of the product can be spiked with a reasonable volume of solution

8 Procedure

8.1 Sample preparation

For each test sample, an unspiked and an NDMA / NDEA spiked test solution is prepared.

A representative sample will be prepared from about 10 grinded tablets. A defined 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 g of total mass. The difference of weighing between the spiked and unspiked testing solution must not exceed 1%.

In case API is tested, then 250mg of API will be transferred into a 15mL centrifuge tube.

This sample powder is mixed with 10 mL 1M sodium hydroxide solution and well shaken or vortexed for at least 5 minutes.

2.0 mL of dichloromethane are added to this suspension and shaken again for 5 minutes or vortexed. The suspension is centrifuged for 5 min at about 4000 g. The aqueous phase is removed by means of Pasteur pipette, so that the lower organic phase can be better removed. If a part of the solid-phase get resuspended during the organic phase removal, the separated organic phase is briefly centrifuged again in an Eppendorf vial. The clear organic phase is used for analysis.

For the spiked sample, to the same amount of mixed tablet powder, an amount of spiking solution is added according to the limit and diluted to 10 mL with sodium hydroxide solution, suspended and extracted with dichloromethane as described above.

To estimate the recovery and Assay (for information only), a 3-point linearity determination (including blank) covering the range of spiked test solution at the beginning of every analysis sequence is performed. The sample preparation is analogous to the spiked samples, but without sample weighing.

For ratios close to the limit (> 0.33, which corresponds to 50% of the limit, see chapter 10.1 Evaluation), additional analysis must be carried out to ensure that the limits are not exceeded.

- Two more unspiked sample preparations with a weight of about 250 mg of active ingredient or max. 1g of mixed tablet powder
- One sample preparation each with 70% or 130% of the weight of the normal sample preparation
- Two additional, spiked with the limit, sample preparation with a weight of about 250 mg of active ingredient or max. 1g of mixed tablet powder

If the content of the test sample must be determined, a standard addition of at least 3 points must be performed.
If the ratio is significantly above the limit (> 0.60, which corresponds to 150% of the limit), the additional experiments can be avoided and a standard addition can be carried out directly to determine the content (quantification).

8.2 Instrument parameters

GC-parameters

Column	DB-624, 30m x 0.32 mm, 1.8 µm			
Carrier gas	Helium			
Flow rate	1.5 mL / min			
Injector port temp.	160 °C			
Injection volume	1.5 µL			
Split Ratio	2:1			
Oven-Program	Start Temp / °C	Heat-rate / °C/min	End Temp / °C	Hold / min
	60	0	60	2
	60	15	195	0
	195	25	260	4.4

MS-Parameter

Ion Source	EI
Source Temp.	230 °C
Quad Temp.	150 °C
Fixed Electron Energy	70.3 eV
Acquisition Type	SIM
Solvent Delay	3 min
Trace Ion Detection	True
Gain Factor	1.2
SIM Segment 1	Start Time 3 min, m/z 74, Dwell Time: 300
SIM Segment 2	Start Time 7.5 min, m/z 102, Dwell Time: 300

9 Evaluation and uncertainty of measurement

9.1 Evaluation

To evaluate the samples, the peak areas for NDMA and NDEA are compared in the unspiked and spiked test solutions. If the ratio of the peak areas is smaller than the limit of 0.50, the limits of NDMA and NDEA are not exceeded (safety threshold).

Peak area (unspiked) / peak area (spiked) <0.50

At ratios close to the limit (> 0.33, corresponding to 50% of the limit), additional analysis must be performed to ensure that the limit is not exceeded.
If the ratio is significantly above the limit (> 0.60, corresponding to 150% of the limit), the additional analyses can be omitted and a standard addition can be carried out directly to determine the content (quantification).

The evaluation of the additional experiments is performed as follows:

Evaluation of the extraction:

From the unspiked testing solution, a linear regression between the weights and the peak areas is calculated. If the correlation coefficient corresponds to r <0.95, the extraction is insufficient and must be repeated with smaller weights. If the spiked / unspiked ratio is already above 0.5, reanalysis with smaller sample weights can be avoided since, despite incomplete extraction, the limit is already exceeded.

Evaluation of the limits:

The peak areas of the spiked and unspiked test solution are each averaged and the ratio calculated according to the formula peak area (unspiked) / peak area (spiked). The individual values may not deviate more than 10% from the mean value.

For the final evaluation of the sample, only the multiple sample preparation is used. The first analysis serves as a rough estimate.

Determination of recovery and content estimation (for information only):

The 3-point linearity is used to calculate the recovery in the spiked sample. If the recovery is in the range of 80% - 120%, the content of the analytes can be estimated via the 3-point linearity.

If the recovery is outside this limit, there are not negligible matrix effects and the content must be estimated if necessary via a standard addition.

9.2 uncertainty of measurement

Not applicable for a limit test.

10 Documentation

The documentation requirements are described in the corresponding SOP.
If the limit of a test sample is not exceeded, in LIMS will be documented as "< {Limit} ppb".

Examples:
< 300 ppb NDMA
< 177 ppb NDEA

If the limits are exceeded, the results obtained in the content determination will be documented in LIMS as "> {Limit} ppb".

Examples:
> 300 ppb NDMA
> 177 ppb NDEA

11 System Suitability Test

- No disturbing signals in the Blank-Solution
- No significant co-elution in the spiked test solutions
- RT of NDMA ca. 6.0 min, RT für NDEA ca. 8.0 min
- S/N of spiked test solutions is at least 10 for both signals (NDMA / NDEA)