Test method for the determination of NDMA and NDEA by LC-MS/MS in Sartan containing film coated tablets

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1 Purpose / Scope of application

Detection and quantitative determination of the nitrosamines N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamin (NDEA) in Sartan containing film coated tablets by UHPLC-APCI-MS/MS.

2 Safety instructions

The generally applicable guidelines of the laboratory apply, as well as in addition:

2.1 Workplace instructions

Due to the carcinogenic properties of nitrosamines, all work must be carried out with gloves and under fume cupboard. The workplace instructions for CMR substances have to be followed.

2.2 Protective material

Sample processing and preparation of standards for calibration:

- single use nitrile gloves: StarGuard Comfort.
- In case of contamination, change gloves immediately.

Transfer of methanol:

- Chemical protection glove: Ansell Barrier

2.3 Minimization- and Substitution-Check

A substitution of carcinogenic nitrosamines is not possible. To minimize the workplace concentration: Nitrosamines are needed as a standard substance. By purchasing reference solutions, the possible risk of contamination is reduced as much as possible.

3 Brief description

The homogenized sample is suspended in methanol, extracted in an ultrasonic bath and diluted with water. The membrane-filtered sample is separated by ultra-high performance liquid chromatography (UHPLC) and analyzed by chemical ionization under atmospheric pressure and tandem mass spectrometry (UHPLC-APCI-MS/MS) in a multiple reaction monitoring mode (MRM). The quantification of NDMA and NDEA is performed using isotopic labeled internal standards according to the internal standard method. The qualitative confirmation of each substance is provided by ion ratios of two mass transitions.

4 Chemicals and materials

4.1 Reference materials

CMR / toxic	Substance	Abbreviation	CAS-No.
X	N-nitrosodimethylamine	NDMA	62-75-9
X	N-nitrosodiethylamine	NDEA	55-18-5

4.2 Internal Standard

CMR / toxic	Substance	Abbreviation	CAS-No.
X	N-nitrosodimethyl-d_6-amine	NDMA-d6	17829-05-9
X	N-nitrosodiethyl-d_{10}-amine	NDEA-d10	1219794-54-3

4.3 Further Chemicals

CMR / toxic	Substance	Abbreviation	CAS-No.
X	Formic acid LC/MS grade	HCOOH	64-18-6
X	Methanol HPLC grade	MeOH	67-56-1

4.4 Required Solutions for LC-MS/MS

Eluent A: 0.1 vol-% HCOOH in water
dissolve 1 ml HCOOH in 1000 ml of ultrapure water

Eluent B: MeOH HPLC grade

5 Devices

5.1 LC-MS/MS

- UHPLC-System coupled with a tandem mass spectrometer with APCI source (i.e. Agilent Infinity 1290 UHPLC + Agilent 6460 APCI-QQQ-MS)
- Column: Waters HSS-T3 100 x 3.0mm, 1.8 µm, 100 Å

5.2 Laboratory devices

- brown glass GC-Vials
- syringe attachment filter with 0.20 µm PET membrane
- analytical balance, 0.1mg precision
- vortex mixer

- ultrasonic bath
- 12 - 15 ml centrifuge tubes with plain bottom and plastic screw cap
- 5 ml disposable syringes
- different piston and/or direct displacement pipets for volumes of 3 µl up to 10.000 µl
- laboratory glassware (volumetric flasks, cylinders, solvent bottles etc.)

6 ProcedureCalibration

6.1.1 Stability / Storage

Solution	Storage	Stability
stock solutions	refrigerator	at least 1 year
standard-/mix-/additional solutions	refrigerator	1 year
calibration and sample solutions	room temperature	at least 1 week
internal standards (ISTD)	refrigerator	unlimited

6.1.2 Reference substances and solutions

Substances and stock solutions

NDEA and NDMA are purchased as solution (= stock solution): 5000 µg/ml in MeOH

Standard solution

From stock solutions: 20 µl of each stock solution / 10 ml MeOH (c = 10 µg/ml)

Calibration solution:

From standard solution: 500 µl / 10 ml of water (c = 500 ng/ml)

6.1.3 ISTD

ISTD stock solutions

NDMA-d6: approx. 10 mg / 10 ml MeOH (c = approx. 1000 µg/ml)
NDEA-d10: approx. 10 mg / 10 ml MeOH (c = approx. 1000 µg/ml)

ISTD solution

From ISTD stock solutions: 25 µl of each stock solution/ 50 ml MeOH (c = 500 ng/ml)

6.1.4 Calibration working solutions

Working range from 0 ppm up to 3.0 ppm

Description	calibration-solution [µl]	ISTD-solution [µl]	MeOH [µl]	water [µl]	C_{NDEA} [ng/ml]	C_{NDMA} [ng/ml]
Blank value + ISTD	0	200	300	9500	0	0
K1	4	200	300	9496	0.2	0.2
K2	10	200	300	9490	0.5	0.5

K3	20	200	300	9480	1	1
K4	40	200	300	9460	2	2
K5	200	200	300	9300	10	10
K6	400	200	300	9100	20	20
K7	1000	200	300	8500	50	50
K8	2000	200	300	7500	100	100

- Concentration of internal standard: approx. 10 ng/ml in each case

Working range from 3.0 ppm up to 30.0 ppm

Description	calibration-solution [µl]	ISTD-solution [µl]	MeOH [µl]	water [µl]	C_{NDMA} [ng/ml]
Blank value + ISTD	0	20	30	950	0
K1	40	20	30	910	20
K2	100	20	30	850	50
K3	200	20	30	750	100
K4	300	20	30	650	150
K5	400	20	30	550	200
K6	600	20	30	350	300

- Concentration of internal standard: approx. 10 ng/ml in each case
- NDEA concentration is not specified in that table although contained in the solutions as the method is not validated for this NDEA working range.

6.2 Sample preparation

6.2.1 Blank sample

All equipment used for sample preparation and handling, e.g. centrifuge tubes, pipet tips, membrane filters, have to be checked for possible contamination with nitrosamines. Therefore a blank sample using all solutions and equipment (i.e. sample preparation according to 6.2.2 without sample weighing) has to be analyzed. The prepared solution is transferred into a vial for the subsequent measurement to obtain a blank value.

6.2.2 Sample preparation

- ► approx. 100 mg of a homogenized sample of the finished product are weighed into a plastic centrifuge tube
- ► addition of 200 µl of ISTD solution
- ► addition of 300 µl of MeOH
- ► vortexing, followed by treatment for 5 minutes in an ultrasonic bath
- ► addition of 9.5 ml of ultrapure water
- ► vortexing, followed by treatment for 5 minutes in an ultrasonic bath
- ► ultracentrifugation of the sample followed by membrane filtration into a GC vial

A quality assurance sample is regularly treated in the same way.

6.3 Chromatographic conditions

The specified parameters are the default parameters for this method:

- Column: HSS-T3 100 x 3,0 mm, 1.8 µm, 100 Å
- Column oven temperature: 30 °C
- Injection volume: 20 µl

Elution gradient:

Time [min]	Flow [ml/min]	Eluent A [%]	Eluent B [%]
0	0.5	95	5
3.0	0.5	95	5
8.0	0.5	40	60
9.0	0.5	5	95
12	0.5	5	95

Stop Time: 12 min
Post Time: 4 min

Divert Time setting for the switchover valve:

0 min: to waste valve
1.5 min: to MS valve for NDMA and NDMA-d6 detection (time segment 2)
4.0 min: to MS valve for NDEA and NDEA-d10 detection (time segment 3)
8.5 min: to waste valve

6.4 Ionization conditions and data acquisition

The mass spectrometer settings may vary depending on the used device and the device condition. Thus, examples for optimized settings are given subsequently. For the ionization of NDMA and NDEA separate source parameters are used and applied to the time segments 2 and 3 respectively.

APCI source parameter:

Parameter	Time segment 2 (NDMA)	Time segment 3 (NDEA)
Gas Temp.	300°C	300°C
APCI Heater	350°C	350°C
Gas Flow	6 l/min	5 l/min
Nebulizer	55 psi	45 psi
Capillary	2000 V	1500 V
Corrona	8 µA	8 µA

MRM method:

Name	RT [min]	precursor ion / product ion pair (transition) [m/z]	Resolution	Dwell time [ms]	Fragmentor [V]	Collision energy [V]	CAV [V]
NDMA	**2.6**	**75 / 58***	unit/unit	200	37	9	2
		75 / 43	unit/unit	200	37	17	2
		75 / 44	unit/unit	200	37	13	2
NDMA-d6	**2.5**	81 / 64	unit/unit	200	30	12	2
		81 /46*	unit/unit	200	30	18	2
NDEA	**7.3**	**103 / 75***	unit/unit	200	76	9	3
		103 / 47	unit/unit	200	76	17	3
		103 / 29	unit/unit	200	76	13	3
NDEA-d10	**7.1**	113 / 81	unit/unit	200	81	9	3
		113/ 34*	unit/unit	200	81	17	3

*=Quantifier (in case of interferences, it is also possible to quantify by using another precursor ion / product ion pair (transition))

Comment: transition m/z 75 - 43 usually has a higher background (noise)

7 Data interpretation

7.1 Evaluation of the measured data

7.1.1 General

Analysis is carried out by the integration of the peak areas of the respective mass traces and calculation according to the method of internal standard.

7.1.2 Qualifier

At least a second precursor ion / product ion pair (transition) is used to verify the results (qualifier). The relative intensity of the quantifier / qualifier (qualifier ratio) from the calibration measurements is compared to the qualifier ratio of the samples (software determines the intensity ratio and issues the „qualifier ratio“).

The maximum accepted relative ion intensity tolerance is set as follows (taken from the Commission Decision (2002/657/EG) amending Directive 96/23/EG):

Deviation +/- 20 % (Qualifier Ratio)

7.1.3 Calculation

The NDMA and NDEA contents in the sample are calculated based on the following formula:

$$nitrosamine\ [mg/kg] = \frac{X * DF}{W * 1000}$$

X = ng nitrosamine per ml measuring solution

DF = dilution factor

W = sample weight in g

Furthermore the recovery rate obtained from the validation data is included in a final calculation step.

8 Remarks

The method is validated for two working ranges for NDMA (0 – 3 ppm and 3 – 30 ppm) and one working range for NDEA (0 – 1.5 ppm).

The limit of quantification (LoQ) and the limit of detection (LoD) is related to tablet mass, without considering measurement uncertainty:

- LoQ of NDMA: 0.2 ppm (working range 0 - 3.0 ppm).
- LoD of NDMA: 0.08 ppm (working range 0 - 3.0 ppm)
- LoQ of NDEA is 0.04 ppm (working range 0 - 1.5 ppm)
- LoD of NDEA is 0.02 ppm (working range 0 - 1.5 ppm)

The validation data were obtained with valsartan film coated tablets as matrix.