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-nitrosodiethylamine (NDEA) in Sartan containing film coated tablets by UHPLC-APCI-MS/MS.

2 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.

3 Chemicals

3.1 Reference material

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

3.2 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

3.3 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

3.4 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

4 Devices

4.1 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 3.0 mm, 1.8 µm, 100 Å

4.2 Laboratory devices

- brown glass GC-Vials
- syringe attachment filter with 0.20 µm PET membrane
- analytical balance, 0.1 mg 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.)

5 Procedure

5.1 Reference substances and solutions

Substances

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

Standard mix solution

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

Calibration solution:

From standard mix solution: 1000 µl / 10 ml of MeOH (c = 1000 ng/ml)

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)

5.2 Calibration working solutions

Working range from 0 ppm up to 3.0 ppm (NDMA and NDEA)

Description	calibration solution [µl]	ISTD solution [µl]	MeOH [µl]	water [µl]	c [ng/ml]
Blank value + ISTD	0	200	300	9500	0
K1	2	200	298	9500	0.2
K2	5	200	295	9500	0.5
K3	10	200	290	9500	1
K4	20	200	280	9500	2
K5	50	200	250	9500	5
K6	100	200	200	9500	10
K7	200	200	100	9500	20
K8	300	200	0	9500	30

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

Working range from 3.0 ppm up to 30.0 ppm (NDMA)

Description	standard mix - solution [µl]	ISTD-solution [µl]	MeOH [µl]	water [µl]	C [ng/ml]
Blank value + ISTD	0	20	30	950	0
K1	2	20	28	950	20
K2	5	20	25	950	50
K3	10	20	20	950	100
K4	15	20	15	950	150
K5	20	20	10	950	200
K6	30	20	0	950	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.

5.3 Sample preparation

5.3.1 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 5.3.2 without sample weighing) has to be analyzed. The prepared solution is transferred into a vial for subsequent measurement to obtain a blank value.

5.3.2 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.

5.4 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
1.5 min: to MS for NDMA and NDMA-d6 detection (time segment 2)
4.0 min: to MS for NDEA and NDEA-d10 detection (time segment 3)
8.5 min: to waste

5.5 Ionization conditions and data acquisition

The mass spectrometer settings may vary depending on the used device. 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)

6 Data interpretation

6.1 Evaluation of the measured data

6.1.1 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.

6.1.2 Qualifier

At least one 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)

6.1.3 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.

7 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 – 3 ppm).

The limit of quantification (LoQ) and the limit of detection (LoD) is related to the determination of nitrosamins in 100 mg tablet mass:

- LoQ of NDMA: 0.1 ppm
- LoD of NDMA: 0.07 ppm
- LoQ of NDEA is 0.04 ppm
- LoD of NDEA is 0.02 ppm

The validation data were obtained with valsartan/HCT film coated tablets, irbesartan film coated tablets, losartan film coated tablets and candesartan film coated tablets as matrix. At the limit of quantification a measurement uncertainty of 30 % was accepted. For the detection of NDEA and NDMA in other matrices than specified above, extraction efficiency, LoDs and LoQs have to be verified.