Method for the determination of NDMA and NDEA by LC-MS/MS in Sartans (drug substance and 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 various sartans (film coated tablets as well as drug substance) 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)**Calibration working solutions**

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

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 weighting) 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 for finished product and Valsartan drug substance

- ► 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.

5.3.3 Sample preparation for Irbesartan, Losartan, Candesartan and Olmesartan drug substance

- ► approx. 50 mg of a homogenized sample is weighed into a plastic centrifuge tube
- ► addition of 200 µl of ISTD solution
- ► addition of 300 µl of MeOH
- ► vortexing, followed by treatment for 30 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
Vaporizer	350°C	350°C
Gas Flow	6 l/min	5 l/min
Nebulizer	55 psi	45 psi
Capillary	2000 V	1500 V
Corrona Current	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]	Cell Accelerator Voltage [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	50	30	12	2
		81 /46*	unit/unit	50	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	50	81	9	3
		113/ 34*	unit/unit	50	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 (without correction of the recovery rate) 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

In a final calculation step, the result is corrected by a concentration depended recovery rate obtained from the validation data.

7 Method validation

7.1 Evaluation of extraction efficiency

Extraction efficiency was tested for Irbesartan and Valsartan drug substance as well as for the corresponding drug products. Because of the solubility of Losartan drug substance, the extraction is expected to be sufficient. The extraction efficiency of nitrosamines from other sartans (i.e. Candesartan, Olmesartan) has not been tested yet due to the lack of contaminated material. However, a similar extraction behavior to Irbesartan drug substance is expected due to a comparable solubility. In case of the detection of NDEA or NDMA in drug substances or products containing Olmesartan, Candesartan or other not tested matrices, the extraction efficiency should be verified.

7.2 Evaluation of performance characteristics

The limit of detection (LoD) and limit of quantification (LoQ) were statistically determined and visually verified by preparing standards and spiked samples of known concentrations. For spiked samples the signal to noise level was generally above 3 at the limit of detection.

The statistically determined performance characteristics (LoD, LoQ, precision and accuracy) derived from experimental data obtained in a comprehensive in-house validation study that was carried out under within-laboratory reproducibility conditions on the basis of a fractional factorial design. Thus, the uncertainty associated performance characteristics are not comparable to those obtained under repeatability conditions. This approach is a worst-case consideration and provides larger values than a repeatability-based approach as, e.g., the DIN32645. More precisely, we obtained the performance characteristics by comparing the spiking of four different matrices at 7 different levels. This approach enabled the determination of concentration dependent measurement uncertainties. At the limit of quantification a measurement uncertainty of 30 % was accepted. Matrices which were not included in the first validation are consecutively integrated in the study by testing selected spiked samples. Up to now, a wide range of sartan containing finished products, also combination products, have been shown to be in line with the obtained performance characteristics. The obtained LoDs and LoQs are shown in the following table:

Drug substance	**NDMA LoD/LoQ [ppm]**		**NDEA LoD/LoQ [ppm]**	
	Drug product	Drug substance	Drug product	Drug substance
Valsartan	0.05/0.1	0.05/0.1	0.02/0.04	0.02/0.04
Irbesartan	0.05/0.1	0.1/0.2	0.02/0.04	0.04/0.08
Losartan	0.05/0.1	0.1/0.2	0.02/0.04	0.04/0.08
Olmesartan	0.05/0.1	0.1/0.2	0.02/0.04	0.04/0.08
Candesartan	0.05/0.1	0.1/0.2	0.02/0.04	0.04/0.08

8 System Suitability

- No disturbing signals in the blank-solutions
- RT of NDMA approx. 2.6 min; RT of NDEA approx. 7.3
- The S/N should be ≥ 3 for NDEA in K1 and verification by at least one qualifier
- The S/N should be ≥ 3 for NDMA in K2 and verification by at least one qualifier
- Correlation coefficient of calibration should be ≥ 0.998