Test method for the determination of NDMA by LC/MS/MS in Valsartan finished products

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1. Purpose / Scope of application

Detection and quantitative determination of nitrosamine N-nitrosodimethylamine (NDMA) in Valsartan finished products by APCI-UHPLC-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 instruction for CMR substances must be observed.

2.2 Protective material

2.2.1 Gloves

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 the carcinogenic substance N-nitrosodimethylamine 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 with 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 (APCI-UHPLC-MS/MS) in multi-reaction monitoring mode (MRM). The quantification of NDMA is performed using an isotopic labeled internal standard according to the internal standard method. The confirmation is provided by the ion ratios of two mass transitions.

4 Chemicals/materials

4.1 Reference material:

CMR / toxic	Substance	Abbreviation	CAS-No.
X	N-nitrosodimethylamine	NDMA	62-75-9

4.2 Internal Standard

CMR / toxic	Substance	Abbreviation	CAS-No.
X	N-nitrosodimethylamine-d6	NDMA-d6	17829-05-9

4.3 Chemicals

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

4.4 Required Solutions

4.4.1 LC-MS/MS

Eluent A: 0.1 vol-% formic acid in water
dissolve 1 ml formic acid in 1000 ml ultrapure water

Eluent B: methanol HPLC grade

5 Devices

5.1 LC-MS/MS

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

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 screw cap (plastic)
- 5 ml disposable syringes
- different piston and/or direct displacement pipets for volume 3 µl – 10.000 µl
- laboratory glassware (volumetric flask, cylinders, solvent bottles etc.)

6 ProcedureCalibration

6.1.1 Stability / Storage

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

6.1.2 Reference-Substance

Substance is purchased as reference solution (=stock solution):
NDMA: 5000 µg/ml in methanol

Standard solution
From stock solution: 20 µl / 10 ml each with methanol (c = 10 µg/ml)

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

6.1.3 ISTD

ISTD stock solution
NDMA-d6: approx. 10 mg / 10 ml with methanol (c = approx. 1000 ng/ml)

ISTD- Solution
From ISTD stock solution: 25 µl / 50 ml each with methanol (c = 500 ng/ml)

6.2 Calibration solutions

6.2.1 Working range from 0.1 ppm until 3.0 ppm

Description	calibration-solution [µl]	ISTD-solution [µl]	methanol [µl]	water [µl]	concentration [ng/ml]
Blank + ISTD	0	200	300	9500	0
K1	10	200	300	9490	0.5
K2	40	200	300	9460	2

K3	200	200	300	9300	10
K4	400	200	300	9100	20
K5	1000	200	300	8500	50
K6	2000	200	300	7500	100

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

6.2.2 Working range from 3.0 ppm until 30.0 ppm

Description	calibration-solution [µl]	ISTD-solution [µl]	methanol [µl]	water [µl]	concentration [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 in each case: approx. 10 ng/ml measuring solution

If applicable the equal concentrated calibration solutions K4, K5 and K6 from working range 0.0 until 3.0 ppm can be used as calibration solutions K1, K2 and K2 for working range 3.0 until 30.0 ppm.

6.3 Sample Preparation

6.3.1 Blank value

Control blank value

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 value (blank sample) using all solutions and equipment (i.e. sample preparation according to 6.3. without sample weighing) has to be analyzed. The prepared solution is filled into a vial for the subsequent measurement.

6.3.2 Control sample (quality assurance measure)

As control sample, a sample with NDMA is used:

- ► approx. 50 mg (± 0.1 mg) homogenized sample (based on the active component = NDMA) are weighed in a plastic centrifuge tube (for material refer to 5.2)
- ► + 200 µl ISTD solution
- ► + 300 µl methanol
- ► mix using the vortex, followed by 5 minutes in the ultrasonic bath

- add 9.5 ml ultrapure water
- mix again, followed by 5 minutes in the ultrasonic bath
- centrifugate the solution by ultracentrifugation followed by membrane filtration into a GC vial

6.3.3 Sample preparation

- Ca. 50 mg (± 0,1 mg) homogenized sample (based on the active component = NDMA) are weighed in a plastic centrifuge tube (for material refer to 5.2)
- + 200 µl ISTD solution
- + 300 µl methanol
- mix with the vortex, followed by 5 minutes in the ultrasonic bath
- add 9.5 ml ultrapure water
- mix again, followed by 5 minutes in the ultrasonic bath
- centrifugate the solution by ultracentrifugation followed by membrane filtration into a GC vial

6.4 Chromatography

The specified parameters are the default parameters for this method:

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

Elution program (gradient program):

Time [min]	Flow [ml/min]	Eluent A [%]	Eluent B [%]
0	0.5	95	5
3.6	0.5	95	5
4.5	0.5	5	95
7	0.5	5	95

Stop Time: 7 min
Post Time: 4 min

Divert Time [min]	Switchover valve **(valve state)**
0	Waste
2	Detector
3.5	Waste

6.5 Ionization conditions and data acquisition

The mass spectrometer settings may vary depending on the used device and the device condition. Subsequently examples for optimized tune settings are given. The actual settings are stored with the respective sequence data files.

Source: **APCI**

Parameter	Adjustment
Gas Temp.	300°C
APCI Heater	350°C
Gas Flow	6 L/min
Nebulizer	55 psi
Capillary	2000 V
Corrona	8 µA

MRM method:

Name	RT (ca.)	precursor ion / product ion pair (transition) [m/z]	Resolution	Fragmentor	Collision energy [V]	CAV [V]
NDMA	**2.6**	**75 / 58***	unit/unit	37	9	2
		75 / 43	unit/unit		17	2
		75 / 44	unit/unit		13	2
NDMA-d6	**2.6**	81 / 64	unit/unit	30	12	2
		81 /46*	unit/unit		18	2

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

Comment:

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

7 Quality assurance procedure

Refer to 6.3.2 control sample.

8 Data interpretation

8.1 Evaluation of the measured data

8.1.1 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.

8.1.2 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)

2.1 Calculation

The NDMA content in the sample is calculated based on the following formula:

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

X = ng nitrosamine per ml measuring solution

DF = dilution factor

W = sample weight in g

9 Remarks

1. The method is validated for two working ranges (relating to tablet mass, without considering measurement uncertainty):
   - 0.1 – 3.0 ppm
   - 3.0 - 30.0 ppm
2. The limit of quantification (LoQ) of NDMA is 0.2 ppm (working range 0.1 - 3.0 ppm).
3. The limit of detection (LoD) of NDMA is 0.08 ppm (working range 0.1 - 3.0 ppm).
4. If interferences appear further validation can be required.