CHEMISCHES UND VETERINÄR-
UNTERSUCHUNGSAMT KARLSRUHE

# Test method for the determination of NDMA by LC-MS/MS in Ranitidine Drug Substance and Film Coated Tablets

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## 1 Purpose / Scope of application

Detection and quantitative determination of the nitrosamine N-nitrosodimethylamine (NDMA) impurity in ranitidine drug substance and 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 is performed using an isotopic labeled internal standard according to the internal standard method. The qualitative confirmation of the analyte 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 |

### 3.2 Internal standard

| CMR / toxic | Substance | Abbreviation | CAS-No. |
|---|---|---|---|
| X | N-nitrosodimethyl-$d_6$-amine | NDMA-d6 | 17829-05-9 |

### 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 (Agilent Infinity 1290 UHPLC + Agilent 6460 APCI-QQQ-MS or equivalent)
- Column: Waters HSS-T3 100 x 3.0 mm, 1.8 µm, 100 Å or equivalent

## 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 10000 µl
- laboratory glassware (volumetric flasks, cylinders, solvent bottles etc.)

# 5 Procedure

## 5.1 Reference substances and solutions

**Substances**

NDMA is 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 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)

**ISTD solution**

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

## 5.2 Calibration working solutions

| Description | Calibration solution [µl] | ISTD solution [µl] | MeOH [µl] | Water [µl] | c [ng/ml] |
|---|---|---|---|---|---|
| Blank + 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 NDMA. 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 sample.

### 5.3.2 Sample preparation for drug substance and solid dosage drug product

- ► approx. 100 mg of a homogenized sample 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
- Autosampler temperature: 10 °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) |
| 3.2 min: | to waste |

Please note:

The scan start and end time should be adjusted for the user's HPLC system since the retention time of the NDMA impurity and drug substance may vary between different HPLC systems. For that purpose a sample is prepared according to 5.3.2. Subsequently, the retention time of the active ingredient is determined by detection with a diode array detector while the mass spectrometer is decoupled. On the basis of the determined retention time the valve settings should be adjusted.

By conducting this procedure, a contamination of the mass spectrometer with active ingredient can be avoided. This is important since ranitidine is able to generate NDMA *in situ*. For the actual measurement the diode array detector should stay coupled to prove that no drug substance enters the MS-device.

## 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. The actual settings are stored with the respective sequence data files.

**APCI Source Settings:**

| Parameter | Setting |
|---|---|
| Gas Temp. | 300°C |
| Vaporizer | 350°C |
| Gas Flow | 6 l/min |
| Nebulizer | 55 psi |
| Capillary | 2000 V |
| Corona Current | 8 µA |

**Scan Settings for the MRM:**

| 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 |

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

# 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 content in the sample (without correction of the recovery rate) is calculated based on the following formula:

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

X = ng NDMA 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

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) are derived from experimental data obtained under repeatability conditions. More precisely, we obtained the performance characteristics by comparing the spiking of ranitidine drug substance at 8 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. The obtained performance characteristics are shown in the following table:

| NDMA | LOD | LOQ | Range |
|---|---|---|---|
| in sample solution | 0,5 ng/ml | 1,2 ng/ml | 0,5 ng/ml – 30 ng/ml |
| in drug substance and film coated tablets | 0,05 ppm | 0,12 ppm | 0,05 ppm – 3 ppm |

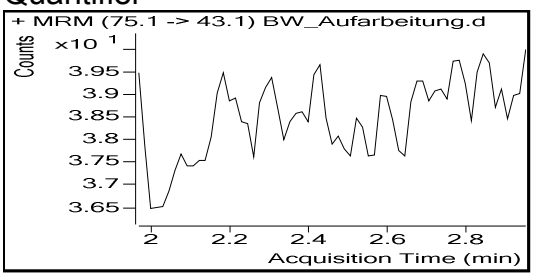
# 8 System Suitability

- No disturbing signals in the blank solutions (diluent with and without internal standard) and sample blank solution
- RT of NDMA approx. 2.5 min
- The S/N ratio should be ≥ 3 for NDMA in calibration solution K2 and verification by at least one qualifier
- Correlation coefficient of calibration should be ≥ 0.998

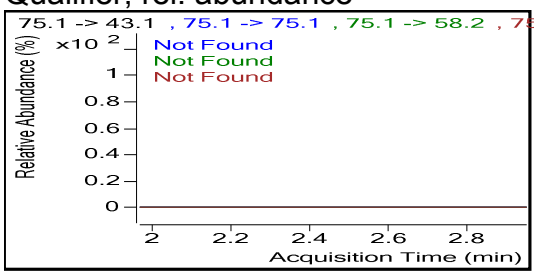
# 9 Example Chromatograms

## Blank Sample

Quantifier

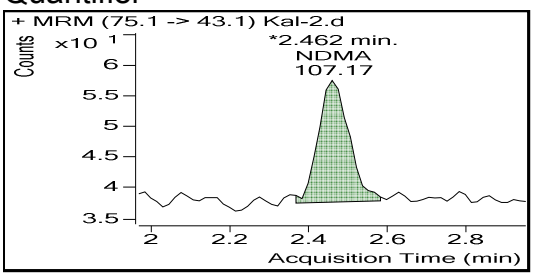



Qualifier, rel. abundance

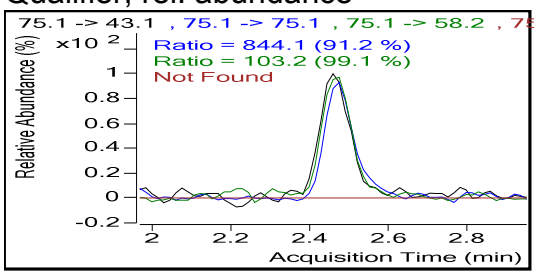



## NDMA Standard (0,5 ng/ml)

Quantifier

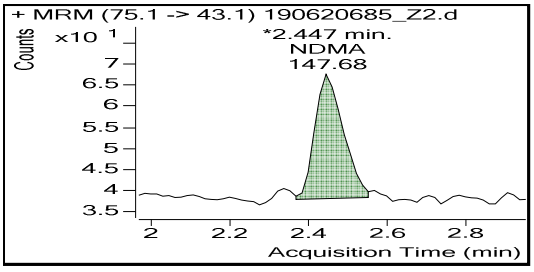



Qualifier, rel. abundance

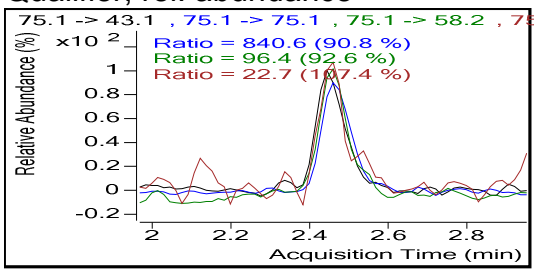



## Spiked ranitidine Sample (0,05 ppm NDMA)

Quantifier




Qualifier, rel. abundance




*there was no NDMA free ranitidine sample available for the validation study, the matrix contained traces of NDMA (< LOD)