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PV-ZSL-585-01

GC-MS/MS method for the detection and determination of nitrosamine impurities in medicinal products

Distribution:	ZSL, ARZ		
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In force on:

Replaces:

Approved: **on:**

Read by: (*Not applicable in case of electronic notice)* **on:**

1 Objective/scope

This document describes a GC-MS/MS method for the detection and determination of trace amounts of the nitrosamine *N*-nitrosodimethylamine (NDMA) in solid dosage forms containing metformin and in the metformin API. It describes how to prepare the necessary stock and reference solutions as well as the samples and test solutions. The method can be extended to other nitrosamines and medicinal products if necessary.

2 Abbreviations

NDMA: *N*-nitrosodimethylamine

NDEA: *N*-nitrosodiethylamine

DCM: dichloromethane

3 Background

NDMA can be present as an impurity in medicinal products through contamination of the starting materials and solvents used during synthesis, as a by-product of synthesis and possibly as a degradation product of the active substance or excipients. There is a particular risk of nitrosamine formation when using N,N-dimethylformamide (DMF) and other nitrogenous solvents in the presence of a nitrosating agents such as sodium nitrite. The concentrations of nitrosamines are in ppb-ppm range. Most nitrosamines have been found to be carcinogenic in animals after oral administration and inhalation. Many *N*-nitrosamines are such potent carcinogens that even the smallest amounts can cause cancer and therefore no safe toxicological threshold can be established. NDMA is one of the most potent carcinogens and is classified in the EU as a presumed human carcinogen (category 1B).

3.1 Traceability to existing standardised methods (e.g. § 64-Method, commercial kits, official method, etc.)

☒ No suitable standardised method exists.

☐ The following standardised method was significantly modified:

Standardised method, version No.	Title

☐ Presentation changed for better readability of the SOP, the standardised method below was employed without any significant changes (any deviations from the standardised method must be clearly indicated in the text):

Standardised method, version No.	Title

4 Safety advice

In addition to the usual laboratory safety rules and guidelines, the following precautions apply:

4.1 Workstation

Nitrosamines are known carcinogens: use a fume cupboard and wear gloves during handling. Follow the standard safety instructions for CMR substances.

4.2 Personal protective equipment

4.2.1 Gloves

Sample processing and preparation of calibration standards

StarGuard® Comfort disposable nitrile gloves, or equivalent. In the event of contamination, change gloves immediately.

Dichloromethane handling

Ansell™ Barrier™ chemical resistant gloves, or equivalent.

4.3 Substitution testing and exposure minimisation

No substitution testing is possible. Minimising exposure: the method requires the use of nitrosamines as the reference substance. Exposure can be significantly reduced by purchasing commercial reference solutions. The risk of contamination with nitrosamines can also be reduced by purchasing “exact weight” products.

5 Brief description of the method

The homogenised sample is mixed with an internal standard (NDMA-d_6). The extraction solution (dichloromethane with NDEA-d_{10}) is then added and the sample is extracted in an ultrasonic bath. The resulting solution is filtered through a membrane filter and its components are separated by gas chromatography (GC) and analysed by tandem mass spectrometry (MS/MS). NDMA is quantified according to the internal standard procedure using an isotopically labelled internal standard. Verification is done via the ion ratios of another mass transition (qualifier ion). Measurement uncertainty and recovery correction as a rule are determined by statistical analysis of the validation data.

For determination of NDMA, the method has been validated over the working range 20-200 µg/kg of NDMA in the matrix (medicinal product and API). Determination of measurement uncertainty and recovery is based on the validation data.

6 Chemicals/Materials

6.1 Reference materials

CMR / Toxic	Substance	Abbreviation	CAS registry No.	LIMS ref. No.
X	*N*-nitrosodimethylamine	NDMA	62-75-9	6782

6.2 Internal standards

CMR / Toxic	Substance	Abbreviation	CAS registry No.	LIMS ref. No.
X	*N*-nitrosodimethyl-d_6-amine	NDMA-d6	17829-05-9	6784
X	*N*-nitrosodiethyl-d_{10}-amine	NDEA-d10	1219794-54-3	6795

6.3 Chemicals

CMR / Toxic	Substance	Abbreviation	CAS registry No.	LIMS Ref. No.
X	Dichloromethane	DCM	75-09-2	2674

7 Equipment/system

7.1 GC-MS/MS

- Autosampler (CTC Combi PAL, or equivalent)
- Gas chromatograph coupled with a tandem mass spectrometer with an EI source (Agilent 7890B gas chromatography system with Agilent MSD 5977A and Evolution 3 MS/MS upgrade, or equivalent)
- Gas chromatography column: Rtx-624Sil MS, 60 m, 0.32 mm inner diameter, 1.8 µm film thickness

7.2 Laboratory equipment and consumables

- Conical bottom GC vials
- Glass beads (0.25 – 0.5 mm diameter, Roth Art. A553.1, or equivalent)
- Syringe filter, 0.45 µm pore size (Acrodisc 13 mm; Waters GHP Minispike WAT200830, or equivalent)
- Analytical balance with 0.01 mg resolution
- Vortex mixer
- Ultrasound bath
- 15 ml polypropylene centrifuge tubes (Sarstedt 62.55.009; "Ausgabetisch", or equivalent)
- 1 ml disposable syringes (Wicom 7301999-A, or equivalent)
- Disposable cannulas, 0.9 x 40 mm (Wicom cannulas 0.9x40mm WIC7303000, or equivalent)
- Standard laboratory equipment

8 Procedure / calibration

8.1 Solutions

8.1.1 Solution stability/storage

Solution	Storage	Stability
Stock solutions	Refrigerator	According to manufacturer's instructions
Intermediate dilution	Refrigerator	6 months
Internal standard solution	Refrigerator	6 months
Calibration standard solution	Portioned, refrigerator	4 weeks

The working solutions are checked regularly during the analysis by injecting a control. If any signs of degradation or contamination are detected, new solutions must be prepared. NDMA degrades during storage, especially when exposed to light. The samples and solutions must therefore be stored protected from light.

8.1.2 NDMA stock solution

For example, 5000 µg/mL solution of NDMA in methanol, ideally purchased as a ready-to-use reference solution.

8.1.3 Intermediate dilution

Preparation of NDMA intermediate dilution (10 µg/mL NDMA)

In a 10 mL volumetric flask, introduce about 2-3 mL of methanol and add 20 µL of NDMA stock solution. Rinse with methanol, equilibrate the temperature to 20 °C and dilute to volume with the same solvent.

8.1.4 Internal standards

Stock solutions

NDMA-d_6: about 10 mg diluted to 10 mL with **MeOH** (c=1000 µg/mL)

NDEA-d_{10}: about 10 mg diluted to 10 mL with **MeOH** (c=1000 µg/mL)

Intermediate dilutions (NDMA-d_6 and NDEA-d_{10} intermediate dilutions)

50 µL of the corresponding stock solution diluted to 5 ml with MeOH (c=10 µg/mL)

Internal standard solution

250 µL of the NDMA-d_6 intermediate dilution diluted to 5 ml with **ultrapure H_2O** (c=25 ng/50µL)

8.1.5 Preparation of the extraction solution

Example for the preparation of the extraction solution:

Extraction solution in dichloromethane

	NDEA-d_{10}
NDEA-d_{10} intermediate dilution 10000 ng/mL [µL]	250
Total volume [mL] CH_2Cl_2	100
NDEA-d_{10} content [ng/mL]	25

8.1.6 Calibration solutions and blank solution

An example for the preparation of the calibration standards and the blank solution from the intermediate dilutions is given below.

Calibration solutions

For example, 5 µL of the NDMA intermediate dilution diluted to 10 mL with **dichloromethane** (c= 5 ng/mL).

Calibration solutions in dichloromethane

	CAL 0	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6
NDMA 10000 ng/mL [µL]	-	5	5	10	25	50	75
NDEA 10000 ng/mL [µL]	-	5	5	10	25	50	75
NDMA-d_6 10000 ng/mL [µL]	25	62.5	25	25	25	25	25
NDEA-d_{10} 10000 ng/mL [µL]	25	62.5	25	25	25	25	25
Total volume [mL] CH_2Cl_2	10	25	10	10	10	10	10
NDMA content [ng/mL]	-	2	5	10	25	50	75
NDMA [ng/mL]	-	2	5	10	25	50	75
NDMA-d_6 content [ng/mL]	25	25	25	25	25	25	25
NDEA-d_{10} content [ng/mL]	25	25	25	25	25	25	25

8.2 Controls

8.2.1 Control blank

All equipment and solutions used must be checked to ensure that they are free of interferences. This is performed through analysis of a control blank prepared as described under 8.2.3 for the sample using the same solutions (including the internal standard solution) and the same equipment (except sample weighing) and introducing the solution into a vial for subsequent measurement.

8.2.2 Control

A medicinal product spiked with NDMA and prepared as described in section 8.2.3 below (1 weighing) is used as a control.

8.2.3 Sample preparation for medicinal products and APIs (including the control) – direct extraction

► weigh out about 0.5 g of glass beads in a polypropylene centrifuge tube;

► crush and homogenise typically 10 tablets (minimum 5);

► weigh **400 mg** of the homogenised sample (medicinal product or API) in the centrifuge tube with the glass beads and record the weight to within 0.1 mg;

► add 50 µL of the internal standard solution;

► close the tube and shake;

► add 1000 µL of the extraction solution;

► vortex to suspend and then **sonicate for 10 min**;

► ultracentrifuge the solution then filter through a membrane filter into a vial.

8.3 GC-MS/MS determination

The GC-MS/MS quantitative determination is carried out according to a documented instrumental test method file (e.g. “Nitrosamine.m”).

The parameters shown below are the standard settings for this method. The actual operating conditions applied are to be recorded (including details of the column used).

Injection conditions-conditions: see actual method, e.g.:

Injection mode:	pulsed injection (splitless)
Injection volume:	3 µL
Pressure:	40 psi for 1.5 min
Purge Flow:	60 mL/min at 1.5 min
Temperature:	250 °C
Gas Saver:	20 mL/min as of 3 min
Injection speed:	50 µL/s
Pre-injection delay:	500 ms
Post-injection delay:	500 ms
GC run time:	36 min

GC-MS conditions see actual operating conditions

Column	see actual column description (e.g. Rtx-624Sil MS, 60 m, internal diameter 0.32 mm, film thickness 1.8 µm)
Column pressure	see actual conditions (e.g. 7.1 psi)
Temperature programme	see actual conditions (e.g. 40 °C / 1 min.- to 160 °C at 6 °C/min., to 240 °C at 15 °C/min / 10 min)
Solvent delay	14 min (end of recording after 24 min)
MS coupling	direct, see actual method
Ionisation	70 eV

8.4 Injection sequence

The following injection sequence should be applied:

- Blank + internal standard (CAL 0)
- Calibration standard + internal standard
- Control blank as described in 8.2.1
- Sample (once or twice)

- After every 10 samples, control blank as described in 8.2.1
- Control as described in 8.2.2
- End with blank + internal standard (CAL 0) and partial calibration

Sequence length approx. 30 injections since longer run times have been shown to cause an increase in measurement fluctuations.

8.5 Ionisation and fragmentation conditions

The mass spectrometer settings may vary depending on the instrument used and the status of the instrument. Optimised settings are given below as a guide. The actual settings are to be recorded with the corresponding sequence data.

MRM mode

Name	RT [min]	Mass to charge ratio [m/z]	Collision energy [V]	Resolution
NDMA	15.54	74 / 44* 74 / 42	5 11	1.5 1.5
NDMA-d_6	15.46	80 / 50* 80 / 46	5 12	1.5 1.5
NDEA-d_{10}	20.35	112 / 94* 112 / 64	5 9	1.5 1.5

* = Quantifier ion (in case of interference, another m/z ratio can be used).

9 Quality control during analysis

9.1 Use of controls

A control is included in each injection series. The control chart is stored electronically at the following address:

L:\Abt.1\ZSL-GC-MS\Kontrollkarten (internal reference)

A medicinal product spiked with NDMA and prepared as described under 8.2.2 is used as the control. If the limits described in the control chart are exceeded, inform the study director.

10 Evaluation

10.1 Interpretation of the results

10.1.1 General

The evaluation is performed according to the internal standard method by integrating the peaks of the relevant mass traces and using external calibration.

For a result to be positive, the retention time, the mass transfers of the quantifier and qualifier ion as well as the corresponding intensity ratios must comply with the predefined specifications when compared with a standard solution in the medium calibration range. These values must be updated according to selected

calibration solution before starting the result evaluation.

10.1.2 Calibration

All calibration points from 2 mg/mL are typically included in the evaluation.

In the “MassHunter” software, select the linear regression model, with the parameter “ignore origin” and without weighting.

10.1.3 Confirmation

At least a second MRM analysis is carried out to confirm the results with a qualifier ion. The relative intensity of the qualifier ion with reference to the quantifier ion is determined from the calibration data and verified in the samples (the software determines the intensity ratios and gives the “qualifier ratio”).

The maximum tolerance for the relative ion intensity is as follows:

Deviation ± 20% (qualifier ratio)

Inform the study director if any deviations are found to be outside these limits. The study director will decide whether the identity of the analyte is confirmed.

10.2 Calculation of NDMA content

10.2.1 Calculation of the uncorrected sample content

The following data is required for the content to be correctly calculated in the report:

The concentration of the calibration standards must be given in ng/mL. The software then performs the “Calc. Conc” in ng/mL.

Dilution = dilution factor/mass of the sample (e.g. in case of 400 mg, extracted with 1 mL of DCM = 2.5)

The method converts the “Calc. Conc” (ng/mL) into NDMA content using the dilution factor according to the following formula (in µg/kg, not corrected for recovery):

$$NDMA\ [\mu g/kg] = X\ \times D$$

X = ng NDMA per mL of test solution

D = factor calculated from the weighed sample mass in g and the dilution

10.2.2 Calculation of measurement uncertainty

Measurement uncertainty is calculated using characteristic data sheet 1. Relative measurement uncertainty is recorded in LIMS.

10.2.3 Data entry in LIMS

The results for the determination of nitrosamine in the matrix are given as the individual results for the samples, not corrected for recovery, and the calculated relative measurement uncertainty. The date of measurement is recorded in LIMS allowing the raw data to be traced back.

11 Validation data

11.1 Storage of validation data and calculation sheets

The validation data and characteristic data sheet 1 for recovery and calculation of measurement uncertainty are stored under \\CVUA-KA-S001.cvua-ka.mlrbw.net\daten\Abt.1\ZSL-GC-MS\Validierung\PV-ZSL-585-01 (internal reference).

11.2 Validation parameters

The method was validated for medicinal products containing metformin and the active pharmaceutical ingredient metformin hydrochloride. The working range is 20 µg/kg to 200 µg/kg in the matrix. The limit of quantification was found to be 15 µg/kg in the matrix.

11.3 Confirmation of the results for matrices that were not considered in the validation study

11.3.1 Matrices requiring additional validation

The validation study was performed on a medicinal product containing metformin hydrochloride as the API, as well as on the API itself. The transferability of the validation to other active substances must be checked where applicable.

11.3.2 Confirmation of the results using standard addition

For samples that are not covered by the validation study, the content can be determined using the standard addition method and calculated using the standard addition calculation template:

e.g. Fabasoft: QM genehmigte Arbeitshilfen zur Validierung und Berechnung von Analysenergebnissen/Standardaddition.xls

12 Related documents and references

SOP-ZSL108-xx (internal SOP)