## GC-MS Method ´NDMA in Metformin-HCl API and Finished dosage forms´ (Shimadzu GC-MS QP 2020plus):

Scope: This method is understood as screening method for NDMA in Metformin API and Finished dosage forms (FDF) by GC-MS liquid injection. Positive samples (NDMA > 32 ppb) must be quantified by standard addition with internal standard calibration. The method should be validated with respect to different matrices.

GC-MS Parameter:

| | |
|---|---|
| Column: | Restek Rtx-624 with guard-column<br>(30 m x 0.32 mm I.D., 1.8 µm) |
| Inj. volume: | 2 µl |
| Inj. temp.: | 240 °C |
| Column flow (He): | 1.5 ml/min |
| Oven temperature: | 60.0 °C, hold: 2.00 min<br>15 °/min → 240.0 °C, hold: 10 min<br>Runtime: 24.00 min |
| Split: | 10.0 |
| Ion source temp.: | 230 °C |
| Interface temp.: | 240 °C |
| Detector voltage: | 1 kV (absolute) |
| SIM @ (*m/z*): 74.00, 42.00 [NDMA]; 80.00, 46.00 [NDMA-d6] | |
| ~ Retention time: | 6.56 min [NDMA-d6]<br>6.59 min [NDMA] |

| | |
|---|---|
| Solvent: | $CH_2Cl_2$ (Dichlormethane for residue analysis, min 99.9 %, |
| | e. g. Th. Geyer Chemsolute 2311.2500 ) |
| | Water, Millipore |
| | Methanol (e. g. 34966 LC-MS Chromasolv, Honeywell) |
| | 1 M HCl (e. g. diluted from 25 % HCl p.a. Chemsolute, Th. Geyer) |
| Reagents: | NDMA (N-Nitrosodimethylamine), e. g. LGC standards DRE-C15604000, 0.1 g |
| | NDMA-d6 (N-Nitrosodimethyl-d6-amine), e. g. CDN-Isotops, D-2937, 0.1 g |

**Standard stock solution (NDMA) = 1 mg/ml in MeOH**

For example, weigh 10 mg of NDMA in a 10.0 ml volumetric flask and dilute to volume with MeOH.

Working standard solution **1** (c = 1 µg/ml)

Dilute 20 µl Standard stock solution to 20.0 ml with $CH_2Cl_2$.

Working standard solution **2** (c = 10 µg/ml) // Individual volumes can also be used as Spiking solution.

Dilute 200 µl Standard stock solution to 20.0 ml with $CH_2Cl_2$.

**Internal Standard stock solution NDMA-d6 = 1.0 mg/ml in MeOH**

For example, weigh 10 mg of NDMA-d6 in a 10.0 ml volumetric flask and dilute to volume with MeOH.

Internal Standard working solution (c = 5 µg/ml)

Dilute 100 µl Internal Standard stock solution to 20.0 ml with $CH_2Cl_2$.

**Reference sample amount:** max. 400 mg API or less (depending on the grade of contamination)

## 1. Linearity

Prepare the following concentrations by dilution of the **NDMA** standard solutions with $CH_2Cl_2$:

| | conc. (ppm) | Conc. (µg/ml) | Vol. Working Standard sol. **1** | Vol. Working Standard sol. **2** | Vol. Internal Standard working sol. | Fill up to ($CH_2Cl_2$) |
|---|---|---|---|---|---|---|
| K0 | 0 | 0 | 0 | -- | 40 µl | 2.0 ml |
| K1 | 0.025 ppm | (0.010 µg/ml) | 20 µl | -- | 40 µl | 2.0 ml |
| K2 | 0.050 ppm | (0.020 µg/ml) | 40 µl | -- | 40 µl | 2.0 ml |
| K3 | 0.100 ppm | (0.040 µg/ml) | 80 µl | -- | 40 µl | 2.0 ml |
| K4 | 0.250 ppm | (0.100 µg/ml) | -- | 20 µl | 40 µl | 2.0 ml |
| K5 | 0.500 ppm | (0.200 µg/ml) | -- | 40 µl | 40 µl | 2.0 ml |
| K6 | 1.000 ppm | (0.400 µg/ml) | -- | 80 µl | 40 µl | 2.0 ml |
| K7 | 2.000 ppm | (0.800 µg/ml) | -- | 160 µl | 40 µl | 2.0 ml |
| K8 | 4.000 ppm | (1.600 µg/ml) | -- | 320 µl | 40 µl | 2.0 ml |
| K9* | 6.000 ppm | (2.400 µg/ml) | -- | 480 µl | 40 µl | 2.0 ml |

c (Internal Standard) = 0.100 µg/ml; prepare K9 if needed

Fill each solution in a GC-Vial

## 2. System suitability:

### 2.1 Linearity:

$R^2 \geq 0{,}995$

## 2.2 LOQ

*S/N* (from K1) > 10

## 3. Sample preparation

### 3.1 Sample solution (API samples) for screening purposes:

Metformin-HCl:

Weigh a max. of 400 mg API in a suitable glass vial. Add 20 µl of Internal Standard working solution. Add 5.0 ml water and dissolve by vortexing for 2 min, then add 1.0 ml of $CH_2Cl_2$, and vortex for 2 x 2 min with intermediate shaking. After standing and layering, the lower layer was taken for injection. If the separated organic phase is not completely clear, it should be centrifuged first, and then the clear supernatant is injected. Prepare in duplicate.

A third sample is prepared following the above procedure, but 40 µl of Working standard solution **2** is added directly before the Internal Standard. Recovery should be within 70 - 130 %.

### 3.2 Standard Addition (for quantification)

Sample solution (API): Weigh a max. of 1,600 mg API in a 5.0 ml volumetric glas flask and add 1 M HCl. Vortex for 2 min with intermediate shaking until the sample is completely dissolved. Bring up to volume.

Prepare the following Standard addition by adding the volumes from the table (concentrations and volumes may be adapted accordingly, depending on the contamination of the sample). Vortex each prepared level for 2 x 2 min with intermediate shaking after the addition of $CH_2Cl_2$:

| | Vol. of Sample solution | Vol. Working Standard sol. **1** | Vol. Internal Standard working sol. | Spiked amount | Vol. $CH_2Cl_2$ added |
|---|---|---|---|---|---|
| STD-K0 | 1.0 ml | 0 µl | 20 µl | 0 | 980 µl |
| STD-K1 | 1.0 ml | 25 µl | 20 µl | 0.025 µg | 955 µl |
| STD-K2 | 1.0 ml | 100 µl | 20 µl | 0.100 µg | 880 µl |
| STD-K3 | 1.0 ml | 400 µl | 20 µl | 0.400 µg | 580 µl |

⇒ Linearity should be not less than 0.995

### 3.3 Sample solution (Drug products):

For example weigh an equivalent of a max. of 1,600 mg API of the fine powdered matrix in a suitable glas flask and add 25.0 ml 1 M HCl. The sample is placed for 10 min in an ultrasonic bath and then vortexed for 2 min with intermediate shaking. It is then centrifuged for 5 min at 4,500 rpm. The supernatant is used as Sample solution.

### 3.4 Standard addition (FDF)

Prepare the following Standard addition by adding the volumes from the table (concentrations and volumes may be adapted accordingly, depending on the contamination of the sample). Vortex each prepared level for 2 x 2 min with intermediate shaking after the addition of $CH_2Cl_2$:

| | Vol. of Sample solution | Vol. Working Standard sol. **1** | Vol. Internal Standard working sol. | Spiked amount | Vol. $CH_2Cl_2$ added |
|---|---|---|---|---|---|
| STD-K0 | 5.0 ml | 0 µl | 20 µl | 0 | 980 µl |
| STD-K1 | 5.0 ml | 25 µl | 20 µl | 0.025 µg | 955 µl |
| STD-K2 | 5.0 ml | 100 µl | 20 µl | 0.100 µg | 880 µl |
| STD-K3 | 5.0 ml | 400 µl | 20 µl | 0.400 µg | 580 µl |

$\Rightarrow$ Linearity should be not less than 0.995

## 4. Calculation

Plot the ratio of the peak areas of NDMA/NDMA-d6 against the concentration of NDMA (ratio of NDMA/NDMA-d6, if necessary). Determine the intercept and slope of the calibration curve and calculate the amount of NDMA in STD-K0.