GC-MS Method ´NDMA in Ranitidin-API´ (Shimadzu GC-MS QP 2020plus):

**Scope:** This method is understood as screening method for NDMA in Ranitidin API by GC-MS liquid injection. If NDMA is detected within the working range, a suitable Standard addition with internal standard calibration should be performed on each positive sample. Finished products could also be quantified with this method, but this must be validated with respect to each matrix.

**GC-MS Parameter:**

- **Column:** Restek Rtx-624 with guard-column (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
  15 °/min → 240.0 °C, hold: 10 min
  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]
  6.59 min [NDMA]

**Solvent:**

CH₂Cl₂ (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 CH₂Cl₂.

**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 CH₂Cl₂.

**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 CH₂Cl₂.

**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 stock solution with CH₂Cl₂:

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>K0</td>
<td>0</td>
<td>--</td>
<td>40 µl</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>K1</td>
<td>0.025 ppm (0.010 µg/ml)</td>
<td>20 µl</td>
<td>--</td>
<td>40 µl</td>
</tr>
<tr>
<td>K2</td>
<td>0.050 ppm (0.020 µg/ml)</td>
<td>40 µl</td>
<td>--</td>
<td>40 µl</td>
</tr>
<tr>
<td>K3</td>
<td>0.100 ppm (0.040 µg/ml)</td>
<td>80 µl</td>
<td>--</td>
<td>40 µl</td>
</tr>
<tr>
<td>K4</td>
<td>0.250 ppm (0.100 µg/ml)</td>
<td>--</td>
<td>20 µl</td>
<td>40 µl</td>
</tr>
<tr>
<td>K5</td>
<td>0.500 ppm (0.200 µg/ml)</td>
<td>--</td>
<td>40 µl</td>
<td>40 µl</td>
</tr>
<tr>
<td>K6</td>
<td>1.000 ppm (0.400 µg/ml)</td>
<td>--</td>
<td>80 µl</td>
<td>40 µl</td>
</tr>
<tr>
<td>K7</td>
<td>2.000 ppm (0.800 µg/ml)</td>
<td>--</td>
<td>160 µl</td>
<td>40 µl</td>
</tr>
<tr>
<td>K8</td>
<td>4.000 ppm (1.600 µg/ml)</td>
<td>--</td>
<td>320 µl</td>
<td>40 µl</td>
</tr>
<tr>
<td>K9</td>
<td>6.000 ppm (2.400 µg/ml)</td>
<td>--</td>
<td>480 µl</td>
<td>40 µl</td>
</tr>
</tbody>
</table>

\[ c \text{ (Internal Standard)} = 0.100 \mu g/ml \]

Fill each solution in a GC-Vial

⇒ **Result:** NDMA: \( R^2 = 0.99986 \)

2. **LOQ/LOD**

Limit of quantitation/Limit of detection (LOQ/LOD):

NDMA:
$S/N$ (from K2) = 26.35

⇒ LOQ = 19.8 ppb
⇒ LOD = 5.9 ppb

3. Sample preparation

Sample solution (API samples) for screening purposes:

Ranitidine:

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$_2$Cl$_2$, 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 is added directly before the Internal Standard. Recovery should be within 70 - 130 %.

4. Standard Addition (for quantification)

Sample solution (API): Weigh a max. of 1,600 mg API in a 5.0 ml volumetric glass 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$_2$Cl$_2$:

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<tbody>
<tr>
<td>STD-K0</td>
<td>1.0 ml</td>
<td>0 µl</td>
<td>20 µl</td>
<td>0</td>
<td>980 µl</td>
</tr>
<tr>
<td>STD-K1</td>
<td>1.0 ml</td>
<td>25 µl</td>
<td>20 µl</td>
<td>0.025 µg</td>
<td>955 µl</td>
</tr>
<tr>
<td>STD-K2</td>
<td>1.0 ml</td>
<td>100 µl</td>
<td>20 µl</td>
<td>0.100 µg</td>
<td>880 µl</td>
</tr>
<tr>
<td>STD-K3</td>
<td>1.0 ml</td>
<td>400 µl</td>
<td>20 µl</td>
<td>0.400 µg</td>
<td>580 µl</td>
</tr>
</tbody>
</table>

⇒ Linearity should be not less than 0.995

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 glass 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.

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$_2$Cl$_2$:
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Calculation:

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