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:	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** stock solution with CH_2Cl_2:

	conc.	Vol. Working Standard sol. **1**	Vol. Working Standard sol. **2**	Vol. Internal Standard working sol.	Fill up to (CH_2Cl_2)
K0	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

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_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 %.

4. 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

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.

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

⇒ Linearity should be not less than 0.995

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.