Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Metformin Drug Substance and Drug Product

Background: Metformin is a prescription drug used to control high blood sugar in patients with type 2 diabetes. Metformin was suspected of being contaminated with N-nitroso-*di*-methylamine (NDMA), a probable human carcinogen, according to the testing results reported by international health authority laboratories. Accordingly, a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method was developed and validated by the Agency to determine the level of NDMA in metformin drug products and drug substances to assist the on-going investigations.

Conclusions:

A LC-HRMS method was developed and validated following ICH Q2 (R1) for the detection and quantitation of NDMA in metformin drugs. The limit of detection (LOD), limit of quantitation (LOQ) and range of the method are summarized below:

	NDMA
LOD (ng/mL)	1.0
(ppm)	0.01
LOQ (ng/mL)	3.0
(ppm)	0.03
Range (ng/mL)	3.0 - 10
(ppm)	0.03 – 0.1

LC-HRMS Method for the Determination of NDMA in Metformin Drug Substance and Drug Product

Purpose
This method is used to quantitate N-nitroso-*di*-methylamine (NDMA) impurity in metformin drug substance or drug product.

Principle
N-nitroso-*di*-methylamine (NDMA) impurity is separated from metformin by reverse phase chromatography and is detected by a high-resolution and high-mass accuracy (HRAM) mass spectrometer. High sensitivity detection is achieved by monitoring the accurate *m/z* value of the protonated NDMA ion. Quantitation is performed by comparing the peak area of the NDMA impurity in extracted ion chromatogram of the samples to the peak area of the NDMA reference standard in external calibration standard.

Reagents
- NDMA Reference Standard
- Formic acid, LC/MS grade (Fisher A117-50 or equivalent)
- Methanol, LC/MS grade (Fisher A456-4 or equivalent)
- Water, LC/MS grade or equivalent

Equipment/Instrument
- HPLC or UHPLC system equipped with temperature-controlled autosampler and column compartment
- Q Exactive™ hybrid quadrupole-orbitrap mass spectrometer (Thermo-Fisher Scientific)
- HPLC column: XSelect CSH C18 2.5 µm, 3.0 x 150 mm, P/N 186006728
- Analytical Balance
- Vortex Mixer
- 15 mL glass centrifuge tubes
- Wrist action shaker
- 0.22 µm PVDF syringe filters
- Centrifuge
- HPLC vials

Mobile phase preparation
- Mobile phase A (0.1% formic acid in water): mix formic acid and water at a volume ratio of 1:1000
- Mobile phase B (0.1% formic acid in methanol): mix formic acid and methanol at a volume ratio of 1:1000

Diluent and Blank: Methanol

NDMA Intermediate Stock Standard preparation (100 ng/mL)
Prepare a 100 ng/mL intermediate stock standard solution in methanol using commercially

available NDMA reference stock standard solution.

Working Standard Preparation (3.0 ng/mL)
Transfer a 3.0 mL aliquot volume of the intermediate stock standard into a 100 mL volumetric flask and dilute to volume with methanol. Prepare fresh daily.

Drug substance sample preparation
Accurately weigh 500 mg of drug substance into a 15 mL glass centrifuge tube. Add 5.0 mL of methanol and mix the solution using a vortex mixer. Shake the sample for 40 minutes using a mechanical wrist action shaker.

Drug product sample preparation
Crush the appropriate number of tablet(s) to obtain a target concentration of 100 mg/mL of API in methanol, and transfer into a 15 mL glass centrifuge tube. Add methanol and mix for about a minute using a vortex mixer. Shake the sample for 40 minutes using a mechanical wrist action shaker.

After extraction, centrifuge the sample for 15 minutes at 4500 rpm. Filter the supernate using a 0.22 µm PVDF syringe filter, discard the first 1 mL and transfer the filtered sample into an HPLC vial for analysis.

Chromatographic Conditions

HPLC Column	XSelect CSH C18 2.5 µm, 3.0 x 150 mm (Waters, Part No. 186006728)		
Column Temp.	30 °C		
Flow Rate	0.3 mL/min		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in methanol		
Gradient	Time (min)	A%	B%
	0	90	10
	5.0	90	10
	6.0	10	90
	9.0	10	90
	9.1	90	10
	14.0	90	10
Injection Volume	3 µL		
Autosampler Temp.	20 °C		
Needle Wash	80:20, Methanol:Water with 0.1% Formic Acid		

Mass spectrometer conditions

- Instrument

Q Exactive™ mass spectrometer (Thermo-Fisher)

- Ion Source Settings
  Note: Ion source parameters can be adjusted to achieve the desired sensitivity.

Sheath Gas Flow Rate	50 arbitrary units
Aux Gas Flow Rate	15 arbitrary units
Sweep Gas Flow Rate	0 units
Spray Voltage	3.5 kV
Capillary Temp.	350 °C
Aux Gas Heater Temp.	350 °C

- Scan Settings
  Note: 1) The scan start-end time should be adjusted for the user's HPLC system since the retention time of the NDMA impurity may vary between different HPLC systems, 2) The divert valve can be used to divert the eluent to waste when a scan is not performed.

NDMA Impurity	
Scan Type	PRM
Polarity	Positive
Scan Start -End (min)	3.2 – 5.0
m/z Isolated for PRM	75.0553
NCE	80
Isolation Window	1.5 m/z
Microscans	3
Resolution	35,000
AGC target	2e5
Maximum IT	100 ms

Injection Sequence

- Inject Blank (use diluent) at least once at the beginning of a sequence
- Inject working standard solution for six consecutive times before the injection of the first sample
- Inject working standard solution once every six injections of samples and at the end of a sequence.
- Example:

Order	Solution	No. of Injections
1	Blank	1
2	Standard (3 ng/mL)	6
3	Blank	1
4	Sample 1	1
5	Sample 2	1
6	Sample 3	1
7	Sample 4	1

8	Sample 5	1
9	Sample 6	1
10	Standard (3 ng/mL)	1
…	…	…

System Suitability

- The % RSD (n = 6) of the NDMA peak areas for the first six injections of the standard solution (3 ng/mL) should be no more than 10%.
- The cumulative % RSD of the NDMA peak areas for working standard should be no more than 15%. (cumulative % RSD of the peak area is calculated by combining the initial six replicate injections of the standard solution and each subsequent bracketing standard).

Data Processing

- NDMA peak areas from the extracted ion chromatograms (EIC) with a *m/z* tolerance of 15 ppm are used for quantitation. The NDMA *m/z* value to be extracted is listed below:

NDMA	
m/z to be extracted	75.0553

- The retention time difference of the NDMA impurity in the analyzed samples should not be more than 2% of the retention time of the corresponding NDMA peak in the reference standard solution.

Calculation

Drug Substance:

$$\text{NDMA impurity (ng/mg or ppm)} = A_{spl} \times \frac{C_s}{As} \times \frac{V}{W}$$

Where: A_{spl} = Area of the NDMA peak in the sample solution
As = Average area of the NDMA peak in the Working Standard Solution from the first six consecutive injections
C_s = Concentration of the NDMA in Working Standard Solution (ng/mL)
W = Weight of drug substance (mg)
V = Volume of the diluent in the sample solution (mL)

Drug Product:

$$\text{NDMA impurity (ng/mg or ppm)} = A_{spl} \times \frac{C_s}{As} \times \frac{1}{100\ mg/mL}$$

Where: A_{spl} = Area of the NDMA peak in the sample solution
As = Average area of the NDMA peak in the Working Standard Solution from the first six consecutive injections

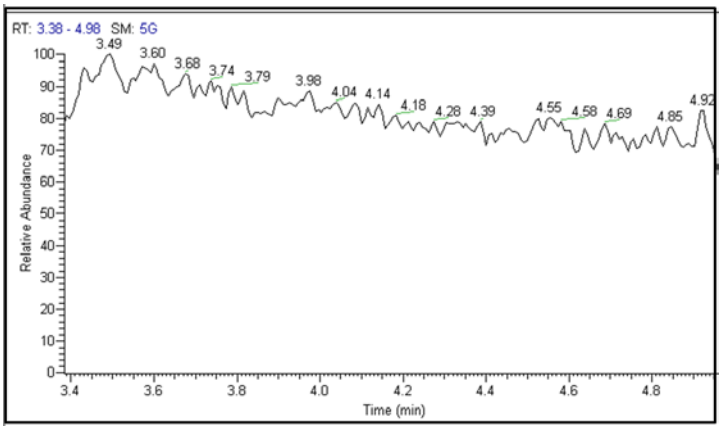
C_s = Concentration of the NDMA in Working Standard Solution (ng/mL)

Report

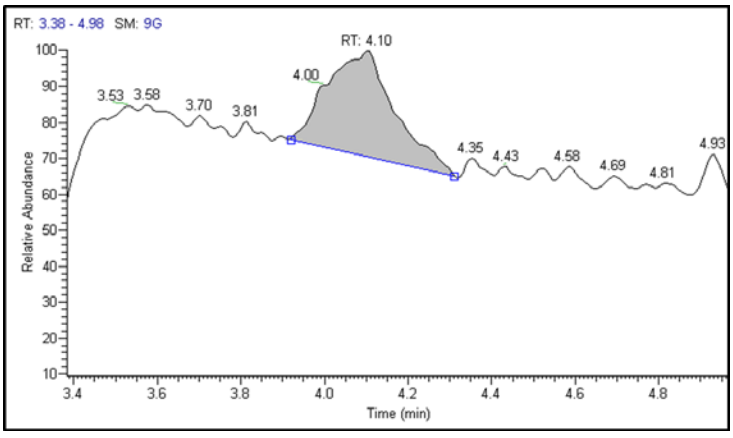
- Report the nitrosamine impurity content in ppm with three significant figures if the value is ≥ LOD
- Report 'not detected' if no nitrosamine impurity is detected or the value is < LOD

Example Chromatograms

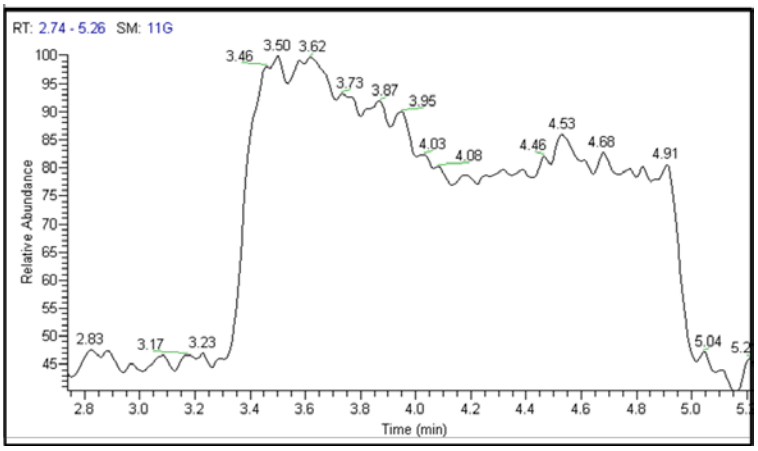
Methanol Blank



NDMA (3.0 ng/mL Standard)



Metformin drug product



Metformin drug product spiked with NDMA standard