

## Detection and Quantitation of Nitrosamine Impurities in Drug Substances by LC-HRMS on LCMS-9030

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### User Benefits

- ◆ Simultaneous analysis of up to eight *N*-nitrosamine impurities in drug substances by LC-HRMS method on LCMS-9030.
- ◆ A targeted MS/MS (TOF) method with 2 *m/z* isolation window by the quadrupole was optimized to obtain best sensitivity.
- ◆ A mass tolerance of ( $\pm$ )15 ppm was adopted to produce extracted-ion chromatograms (XICs) for quantitation.

### ■ Introduction

Since 2018, the presences of *N*-nitrosamine impurities in some drug substances and products have been alerted by the US FDA and regulatory agencies in other countries. Nitrosamines (NSA, see Table 1) are toxic chemicals and some of them such as NDMA and NDEA are classified as probable human carcinogens. NDMA and NDEA were found presence first in drug substances and products of Angiotensin II receptor blockers (ARB) like losartan etc. NDMA was found in ranitidine and metformin drug products and recalls of the products occurred due to the content of NDMA above the Acceptable Intake limit (AI, 96 ng/day). Detection and quantitation of NDMA and other concerned nitrosamines at trace levels in drug products are established by using highly sensitive and selective mass spectrometry methods on GC-MS, LC-MS/MS [1] and LC-HRMS [2-4].

Most concerned nitrosamines (see Table 1) are small and polar compounds, which may be interfered by co-eluting species present in the testing samples in LC-MS/MS and LC-HRMS analysis. High-resolution MS methods are capable of distinguishing co-eluting interferences. An example was reported by Yang et al [4] recently, which revealed that the presence of *N,N*-dimethylformamide (DMF) may affect the quantitation results of NDMA in metformin products if the mass accuracy and mass resolution are not sufficient. To date, both dedicated methods for NDMA only and simultaneous analysis method for more NSA are reported and used. In this application note, an orthogonal method for detection

and quantitation of up to eight nitrosamines as listed in Table 1 by LC-HRMS on LCMS-9030 is described.

### ■ Experimental

#### Standard and sample preparation

Eight nitrosamine standards (Table 1) were prepared into individual stock solutions of 100 µg/mL (ppm) in methanol. Mixed stocks of all or selected NSA with each 100 ng/mL were prepared for making calibration series of different concentration levels in a diluent (MeOH:H<sub>2</sub>O = 15:85 (v/v) with 0.1% formic acid). Sample preparation of drug substance refers to the FDA testing method posted in 2019 and 2020 [2,3]. For metformin, 150 mg of metformin hydrochloride (solid) was dissolved 1.5 mL of the diluent in a 2 mL micro-centrifuge tube. The sample tube was vortexed for 3 min followed by shaking on a Vortex-Genie 2 mixer for 30 min at room temperature. The sample tube was centrifuged at 15,000 rpm for 5 min. Then, the supernatant was transferred and filtered with a 0.22 µm nylon syringe filter into a 1.5 mL HPLC sample vial.

#### Analytical conditions

A Shimadzu LCMS-9030 Q-TOF system was employed for the sample analysis. Details of the system and analytical conditions are compiled in Table 2.

**Table 2** Analytical conditions on LCMS-9030

LC Conditions	
Column	Shim-pack™ Solar C18 (4.6 X 250 mm, 5 µm, P/N: 227-30600-02)
Flow Rate	0.8 mL/min
Mobile Phase	A: Water with 0.1% formic acid B: Methanol with 0.1% formic acid
Elution gradient	15% B (0-2.0 min) -> 40% B (5.0 min) -> 95% B (9.5-14.5 min) -> 15% B (14.6-21 min)
Oven Temp.	45°C
Injection Vol.	40 µL
Interface Conditions (LCMS-9030)	
Interface	DUIS, ESI 4.0 kV, Corona needle 4.5 kV
Interface Temp.	400°C
DL Temp.	250°C
Heat Block Temp.	250°C
Nebulizing Gas	2.0 L/min
Heating Gas Flow	5 L/min
Drying Gas Flow	10 L/min

**Table 1** Information of eight *N*-Nitrosamine impurities

No.	Compound Name	Abbr.	CAS No	Formula	MW
1	<i>N</i> -Nitroso dimethylamine	NDMA	62-75-9	C <sub>2</sub> H <sub>6</sub> N <sub>2</sub> O	74.1
2	<i>N</i> -nitroso- <i>N</i> -methyl-4-aminobutyric acid	NMBA	61445-55-4	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>	146.2
3	<i>N</i> -Nitroso diethylamine	NDEA	55-18-5	C <sub>4</sub> H <sub>10</sub> N <sub>2</sub> O	102.1
4	<i>N</i> -Nitrosoethyl isopropylamine	NEIPA	16339-04-1	C <sub>5</sub> H <sub>12</sub> N <sub>2</sub> O	116.2
5	<i>N</i> -Nitrosodiisopropylamine	NDIPA	601-77-4	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O	130.2
6	<i>N</i> -Nitrosodi- <i>N</i> -propylamine	NDPA	621-64-7	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O	130.2
7	<i>N</i> -Nitrosomethyl phenylamine	NMPA	614-00-6	C <sub>7</sub> H <sub>8</sub> N <sub>2</sub> O	136.2
8	<i>N</i> -Nitrosodi-butylamine	NDBA	924-16-3	C <sub>8</sub> H <sub>18</sub> N <sub>2</sub> O	158.2

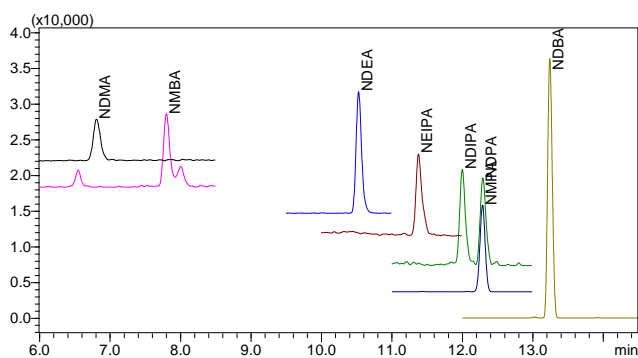
## Results and Discussion

### Targeted MS/MS method

Instead of direct MS TIC method, a targeted MS/MS TIC method was established for the detection of the analytes on LCMS-9030. The quadrupole was set to target for each analyte with a mass window of  $\pm 2$   $m/z$ , while full spectra were collected by TOF MS. With an optimized CE, enhanced sensitivity could be obtained as compared to direct MS TIC acquisition. The details of the acquisition parameters are compiled into Table 3.

**Table 3** Key parameters of targeted MS/MS method for NSA

No.	Abbr.	[M+H] <sup>+</sup> ( $m/z$ )	Q Isolation Window	CE Spread (V)	TOF Mass Range ( $m/z$ )
1	NDMA	75.0553	2 $m/z$	4 - 8	60 - 90
2	NMBA	147.0764	2 $m/z$	2 - 6	125 - 160
3	NDEA	103.0866	2 $m/z$	4 - 8	85 - 120
4	NEIPA	117.1022	2 $m/z$	4 - 8	100 - 125
5	NDIPA	131.1179	2 $m/z$	3 - 7	115 - 150
6	NDPA	131.1179	2 $m/z$	3 - 7	115 - 150
7	NMPA	137.0709	2 $m/z$	5 - 9	127 - 147
8	NDBA	159.1492	2 $m/z$	5 - 9	140 - 170



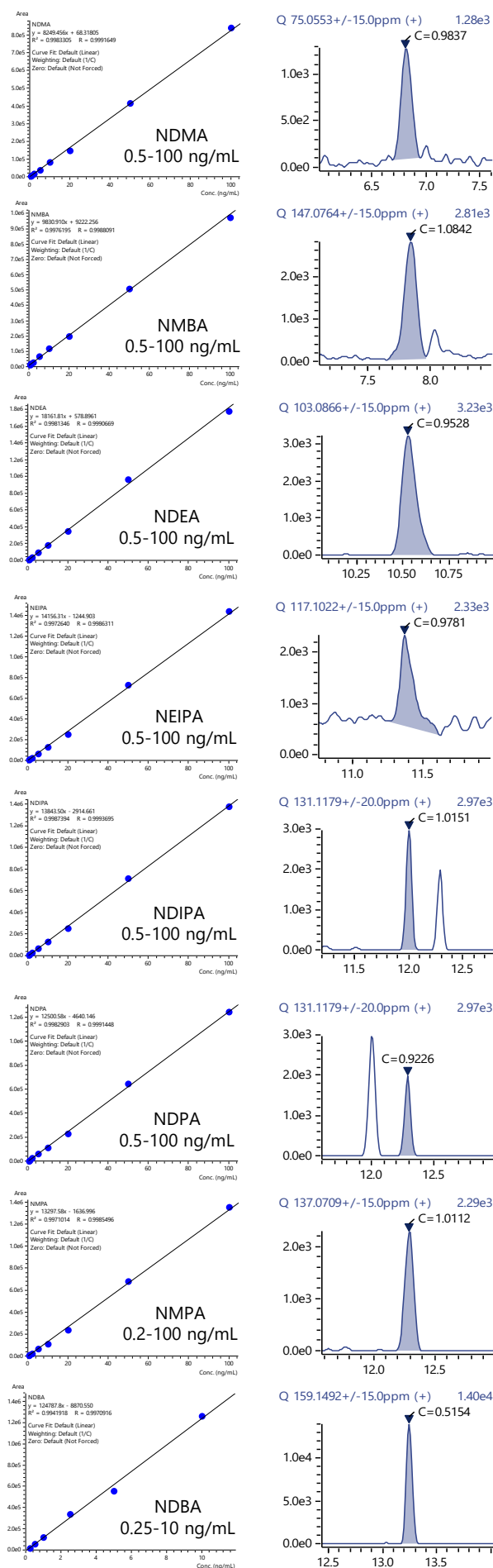
**Figure 1** XICs of eight NSA mixed standards each at 5 ng/mL except NDBA at 2.5 ng/mL.

Extracted-ion chromatograms (XICs) of mixed standard are shown in Figure 1. The mass tolerance set to obtain the XICs is ( $\pm$ )15 ppm (NDPA ( $\pm$ )20 ppm), which is with reference to the recent publication by Yang et al [4]. The isotopic ion of the interference DMF ( $m/z$ 75.0571, with  $^{15}\text{N}$ ) is very closed to the monoisotopic ion of NDMA ( $m/z$ 75.0553), a mass tolerance of less than 20 ppm is required for distinguishing co-eluted NDMA and DMF.

### Calibration curves & quantitation method

Mixed standards of the eight NSA of 0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL (NDBA: 0.1 ~ 50 ng/mL) were used to establish calibration curves and tested for sensitivity (LOD and LOQ). Figure 2 shows the calibration curves and XIC peaks of 1 ng/mL (NDBA 0.5 ng/mL). All calibration curves are linear type with  $R^2$  greater than 0.99 for a wide range from 0.5 ng/mL to 100 ng/mL except NDBA (0.25~10 ng/mL).

The performance parameters of the quantitation method established are summarized in Table 4. The LOQs for the eight NSA for standards in solvent achieved 1 ng/mL or lower with accuracy at 92.3% - 108.4% and repeatability at 1.9% - 15.2% for the 1 ng/mL level (NDBA 0.5 ng/mL).



**Figure 2** Calibration curves of 8 NSA mixed standards and XIC peaks of 1 ng/mL (NDBA 0.5 ng/mL)

**Table 4** Calibration curves and quantitation preformation for eight *N*-nitrosamines by targeted MS/MS method on LCMS-9030

Performance Parameter	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDPA	NMPA	NDBA
LOD (ng/mL)	0.5	0.5	0.2	0.5	0.5	0.5	0.1	0.1
LOQ (ng/mL)	1	1	0.5	1	1	1	0.2	0.25
Range (ng/mL)	1 ~ 100	1 ~ 100	0.5 ~ 100	1 ~ 100	1 ~ 100	1 ~ 100	0.2 ~ 100	0.25 ~ 10
R <sup>2</sup>	0.9983	0.9976	0.9981	0.9973	0.9987	0.9983	0.9971	0.9942
Accuracy at LOQ (%)	98.3	108.4	107.4	97.8	101.5	92.3	116.5	106.8
Accuracy at 1 ng/mL (%)	98.3	108.4	95.3	97.8	101.5	92.3	96.7	104
Accuracy at 5 ng/mL (%)	94.5	115.6	104.8	92.4	104.7	103.8	98.3	90.4
Accuracy at 50 ng/mL (%)	100.6	101.3	106.4	102.8	103.5	103.7	102.7	101.7 (10 ng/mL)
Conc. %RSD at 1 ng/mL (n=7)	7.39	15.18	5.87	12.27	8.20	9.95	3.19	1.89
Conc. %RSD at 5 ng/mL (n=7)	2.65	3.42	1.85	1.47	1.81	2.67	2.41	0.61

**Table 5** LOD and LOQ of NSA spiked in metformin drug substance by targeted MS/MS method

	NDMA	NMBA	NDEA	NEIPA	NMPA	NDBA
LOD (ng/mL)	0.5	0.5	0.2	0.5	0.2	0.25
(ppm)	0.005	0.005	0.002	0.005	0.002	0.0025
LOQ (ng/mL)	1	1	0.5	1	0.5	0.5
(ppm)	0.01	0.01	0.005	0.01	0.005	0.005
Range (ng/mL)	1 ~ 100	1 ~ 100	0.5 ~ 100	2 ~ 100	0.5 ~ 100	0.5 ~ 10
(ppm)	0.01 ~ 1	0.01 ~ 1	0.005 ~ 1	0.02 ~ 1	0.005 ~ 1	0.005 ~ 1

### Nitrosamines in metformin API

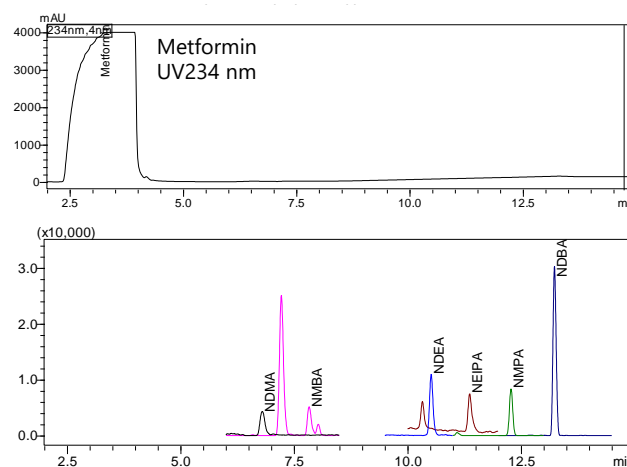
The method established was applied to metformin drug substance (API). Following the sample preparation procedure [3] by US FDA, the spiked samples of 6 nitrosamines (NDIPA and NDPA were not included) were tested for sensitivity (LOD and LOQ). As shown in Figure 3, metformin eluted as a broad peak from 2.4 min to 4.5 min (234 nm UV detector). With a programmed flow switching valve control, the eluent was diverted to the waste to avoid contamination of the extremely high concentration of metformin (100 mg/mL) in the extract samples to the interface.

The LOD and LOQ values of the six nitrosamines spiked in metformin (extract) testing solutions (100 mg/mL) are summarized in Table 5. The values in ng/mL are the concentration of testing solutions and the values in ppm are the content of NSA in metformin sample (w/w).

### Nitrosamines in two ARB APIs

The method established (slight modification in elution program) was applied to losartan and candesartan drug substances. Following the sample preparation procedure [2] by US FDA, the spiked samples of 7 NSA in the drug substance extract (20 mg/mL) were tested for sensitivity (LOD and LOQ). As shown in Figures 4 and 5, losartan and candesartan eluted as a broad peak at 11.4-12.0 min and a peak at 8.6-9.0 min (254 nm UV detector), respectively. With a proper programmed flow switching valve control, the eluent was diverted to the waste to avoid contamination of the high concentration of the APIs (20 mg/mL) in the extract samples to the interface.

The LOD and LOQ values of the seven NSA in solutions (ng/mL) and in drug substances (ppm) are summarized in Table 6.


**Figure 3** XICs of spiked nitrosamines (5 ng/mL mixture) with UV chromatogram (top) of metformin extract (100 mg/mL)

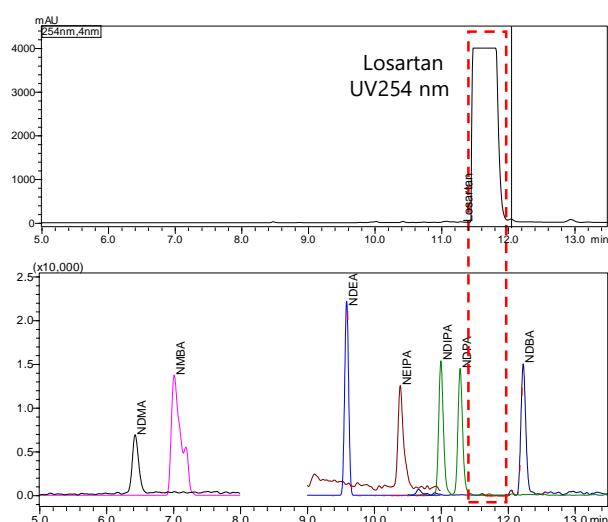
### Conclusion

In this study, a targeted MS/MS (TOF) method was established for detection and quantitation of up to 8 *N*-nitrosamines on LCMS-9030 Q-TOF system. The method was evaluated in terms of LOD and LOQ, linearity, repeatability in reference to the FDA recommended method. As demonstration, the method was applied to determine nitrosamines spiked in drug substances including metformin (100 mg/mL) and two ARB, losartan and candesartan (20 mg/mL).

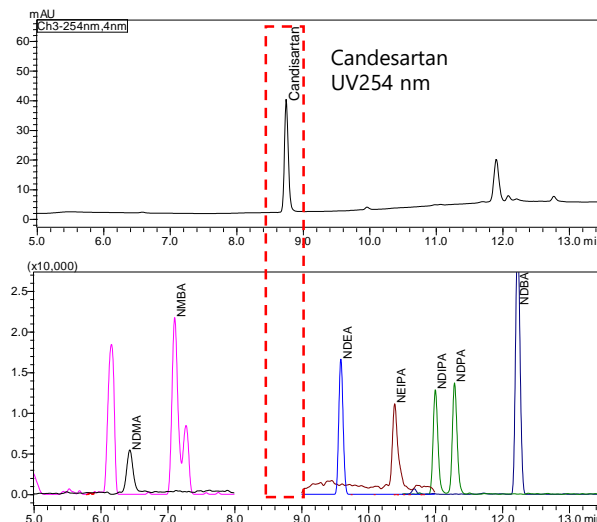
**Table 6** LOD and LOQ of NSA spiked in losartan and candesartan drug substances by targeted MS/MS method

	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDPA	NDBA
LOD (ng/mL)	0.5	0.5	0.2	0.5	0.2	0.2	0.1
(ppm)	0.025	0.025	0.01	0.025	0.01	0.01	0.005
LOQ (ng/mL)	1	1	0.5	1	0.5	0.5	0.25
(ppm)	0.05	0.05	0.025	0.05	0.025	0.025	0.013
Range (ng/mL)	1 ~ 100	1 ~ 100	0.5 ~ 100	1 ~ 100	0.5 ~ 100	0.5 ~ 100	0.25 ~ 10
(ppm)	0.05 ~ 5.0	0.05 ~ 5.0	0.025 ~ 5.0	0.05 ~ 5.0	0.025 ~ 5.0	0.025 ~ 5.0	0.0125 ~ 0.50

Note: The LOD and LOQ measured are same in the losartan and candesartan matrix (20 mg/mL)



**Figure 4** XICs of spiked nitrosamines (5 ng/mL mixture) and UV chromatogram of losartan extract (20 mg/mL)



**Figure 5** XICs of spiked nitrosamines (5 ng/mL) and UV chromatogram of candesartan extract (20 mg/mL)

## References

1. S. Rane, D. Tupe, D. Bhandarkar, S. Saravannan and B. Karthikeyan; "Quantitation of 6 Nitrosamines in 5 Sartans by LC-MS/MS system as per proposed USP General Chapter <1469>; <https://www.shimadzu.com/an/literature/06-saip-082-lc-041-en.html>
2. US FDA posted on 05/21/2019, "LC-HRMS Method for the Determination of Six Nitrosamines in ARB Drugs"; <https://www.fda.gov/media/125478/download>
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4. J. Yang, T. A. Marzan, W. Ye, C. D. Sommers, J. D. Rodriguez and D. A. Keire, The AAPS Journal (2020) 22: 89; <https://pubmed.ncbi.nlm.nih.gov/32613429/>

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