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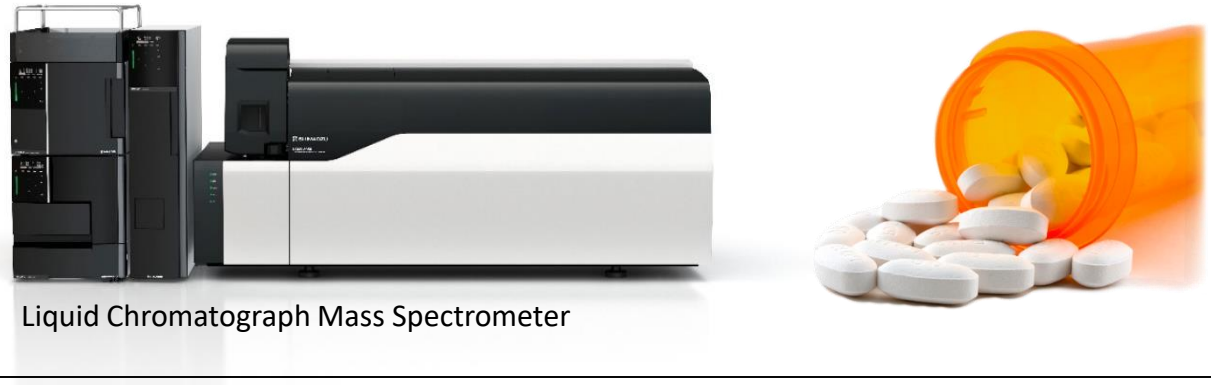
Application News

SSI-LCMS-

Liquid Chromatography Mass Spectrometry

Simultaneous analysis of nitrosamines impurities in Metformin drug substance and drug product using Shimadzu LCMS-8050 triple quadrupole mass spectrometer



Liquid Chromatograph Mass Spectrometer

■ Summary

Due to their genotoxic potential, nitrosamine impurities are of concern to the regulatory agencies. Several ARB drug products (Valsartan, Losartan), Metformin, Ranitidine, Rifampin have been recalled due to the presence of one or more of nitrosamine impurities.[1] This application note describes a highly sensitive and specific determination of eight nitrosamine impurities in Metformin drug substance and drug product using Shimadzu LCMS-8050 triple quadrupole mass spectrometer. The limit of quantitation (LOQ) were estimated and compared with current Food and Drug Administration (FDA) guidelines for the analysis in metformin drug substance and drug product. All the N-nitrosamines met or exceeded the range, linearity, and LOQ criteria set by the FDA.[2]

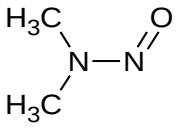
■ Background

Recently, FDA released guidelines for monitoring nitrosamine impurities present in pharmaceuticals. The nitrosamine impurities are present at sub-ng levels in the pharmaceuticals. Shimadzu LCMS-8050 is capable of achieving the desired sensitivity and selectivity required for the analysis of nitrosamines. Figure 1 shows the structures of the nitrosamines analyzed in this report. Furthermore, these impurities were quantitated at LOQ levels in Metformin drug substance and drug product and the percent recovery was calculated.

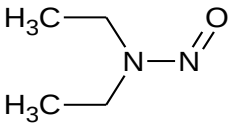
■ Method

The nitrosamine standards were purchased from Sigma Aldrich (St. Louis, MO), LGC standards (Manchester, NH), Cayman Chemical (Ann Arbor, MI) and ChemService (West Chester, PA). LCMS grade solvents were purchased from Honeywell (Charlotte, NC).

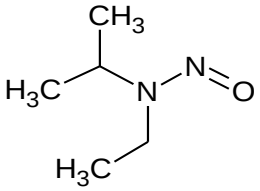
Nexera LC-40 liquid chromatograph was coupled to a LCMS-8050 mass spectrometer. The MS interface and chromatographic details are provided in Table 1 and 2, respectively. Table 3 shows the list of nitrosamines and their MRM transitions used for the analysis. The standards were injected in triplicate. Linear calibration curve along with percent accuracy calculations were performed using LabSolutions v. 5.99 software.



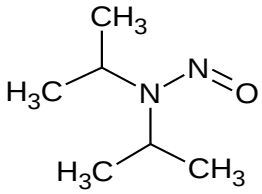
N-nitroso-dimethylamine
(NDMA)
Molar mass: 74.08 g/mol



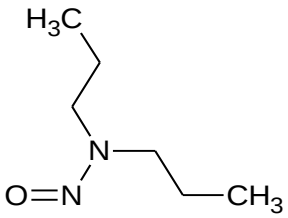
N-nitroso-diethylamine
(NDEA)
Molar mass: 102.14 g/mol



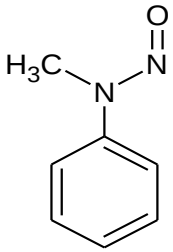
N-nitroso-ethylisopropylamine
(NEIPA)
Molar mass: 116.16 g/mol



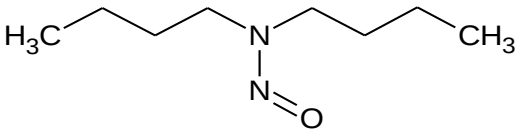
N-nitroso-*di*isopropylamine
(NDIPA)
Molar mass: 130.19 g/mol



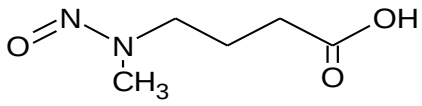
N-nitroso-*di*-n-propylamine
(NDPA)
Molar mass: 130.19 g/mol



N-nitroso-methylphenyllamine
(NMPA)
Molar mass: 136.15 g/mol



N-nitroso-*di*-n-butylamine
(NDBA)
Molar mass: 158.24 g/mol



N-nitroso-N-methyl-4-aminbutyric acid
(NMBA)
Molar mass: 146.14 g/mol

Figure 1. Structures and molar mass for the nitrosamines of interest to the FDA and analyzed in this report.

The stock solutions of all nitrosamine standards were prepared in methanol. The stock solutions were further diluted with 1:1 methanol:water to obtain a nitrosamine standard mixture containing all 8 nitrosamines at 3.0 ng/mL concentration.

The Metformin drug substance and drug product samples were prepared according to the FDA guidelines[2] with some modifications. Briefly, 400 mg drug substance was weighed and transferred to a 15 mL falcon tube. To the sample was added 5 mL methanol. The sample was shaken, centrifuged twice (4500 rpm followed by 15000 rpm), and filtered using a 0.2 µm PVDF filter. The filtrate was stored at -20 °C refrigerator prior to analysis. The filtrate was diluted 1:1 with water for LC-MS analysis.

Metformin drug product sample was prepared by crushing tablet to obtain a target concentration of 200 mg/mL with methanol. The sample was shaken, centrifuged twice (4500 rpm followed by 15000 rpm), and filtered using a 0.2 µm PVDF filter. The filtrate was stored at -20 °C refrigerator prior to analysis. The filtrate was diluted 1:1 with water for LC-MS analysis. For the LC-MS analysis, the time-segmented MRM was performed with diverting excipients to waste.

Table 1. Snapshot of mass spectrometric interface parameters used for the analysis of nitrosamines.

MS	Parameter
Ion Source	APCI
Nebulizing Gas Flow	3 L/min
Interface Temperature	350°C
DL Temperature	200°C
Heat Block Temperature	200°C
Drying Gas Flow	5 L/min

Table 2. Chromatographic conditions used for the analysis of nitrosamines.

	Conditions	
Column[2]	Phenomenex Biphenyl 150x3.0mm, 2.6µm	
Flow rate (mL/min)[2]	0.4	
Mobile Phase A	0.1% formic acid in water	
Mobile Phase B	0.1% formic acid in methanol	
Gradient[2]	**Time (min)**	**%B**
	0.00	5
	3.00	5
	5.00	10
	6.00	60
	10.00	60
	13.00	18
	13.10	100
	15.0	100
	15.1	5
	18	Stop
Injection volume (µL)	15	
Autosampler temperature (°C)	5	
Column Oven (°C)[2]	40	
Diluent	1:1 MeOH:H_2O	

Table 3. MRM transitions used for nitrosamines. Quantifier ions are represented in bold.

N-Nitrosamine	MRM Transitions	CE
NDMA	**75.10>43.10** 75.10>58.10	**-16.0** -16.0
NMBA	**147.15>44.15** 147.15>117.10	**-13.0** -9.0
NDEA	**103.15>29.10** 103.15>75.10	**-14.0** -13.0
NEIPA	**117.10>75.05** 117.10>41.05	**-11.0** -22.0
NDIPA	**131.15>43.00** 131.15>89.10	**-12.0** -12.0
NDPA	**131.15>43.00** 131.15>89.10	**-12.0** -12.0
NMPA	**137.10>66.15** 137.10>77.00	**-18.0** -26.0
NDBA	**159.15>57.15** 159.15>41.10	**-14.0** -21.0

■ Results and Discussion

Eight N-nitrosamine standards of interest to the FDA were selected for development of a LC-MS/MS method on the LCMS-8050 instrument.

Q1 and Q3 scans were performed on the standards to obtain the *m/z* value for precursor ion for each standard. Flow injection analysis was used to perform the MRM optimization on the obtained precursor ions. The MRM optimization workflow generated the optimized parameters for the product ions for individual nitrosamines. Table 3 shows the qualifier and quantifier ions used for all the N-nitrosamines used in this analysis.

Phenomenex Biphenyl (150x3.0 mm, 2.6 µm) column and gradient provided in the FDA document was used for the analysis. A time-segmented MRM method was developed based on the retention time of individual analyte. Figure 2 represents the time segmented total ion chromatogram obtained for NDMA, NMBA, NDEA, NEIPA, NDIPA, NDPA, NMPA, and NDBA. Note that the NDIPA and NDPA have similar product ions but were well separated chromatographically (Figure 2). This allowed for easy identification and quantitation of both analytes.

The stock standards for all the N-nitrosamines was prepared in methanol and further diluted in 1:1 methanol:water in the range 0.3-100 ng/mL. A linear calibration curve was generated for all nitrosamines. The r^2 value was calculated using the LabSolutions (v. 5.99 SP2) software to assess the linear range. The percent accuracy was calculated with reference to the theoretical value. The limit of quantitation was determined based on lowest calibration point that can be included in the calibration curve with a percent accuracy between 70-130%. The LOQ obtained on LCMS-8050 was compared with the desired LOQ by the FDA. All nitrosamines either met or exceeded the range and LOQ criteria set by the FDA.

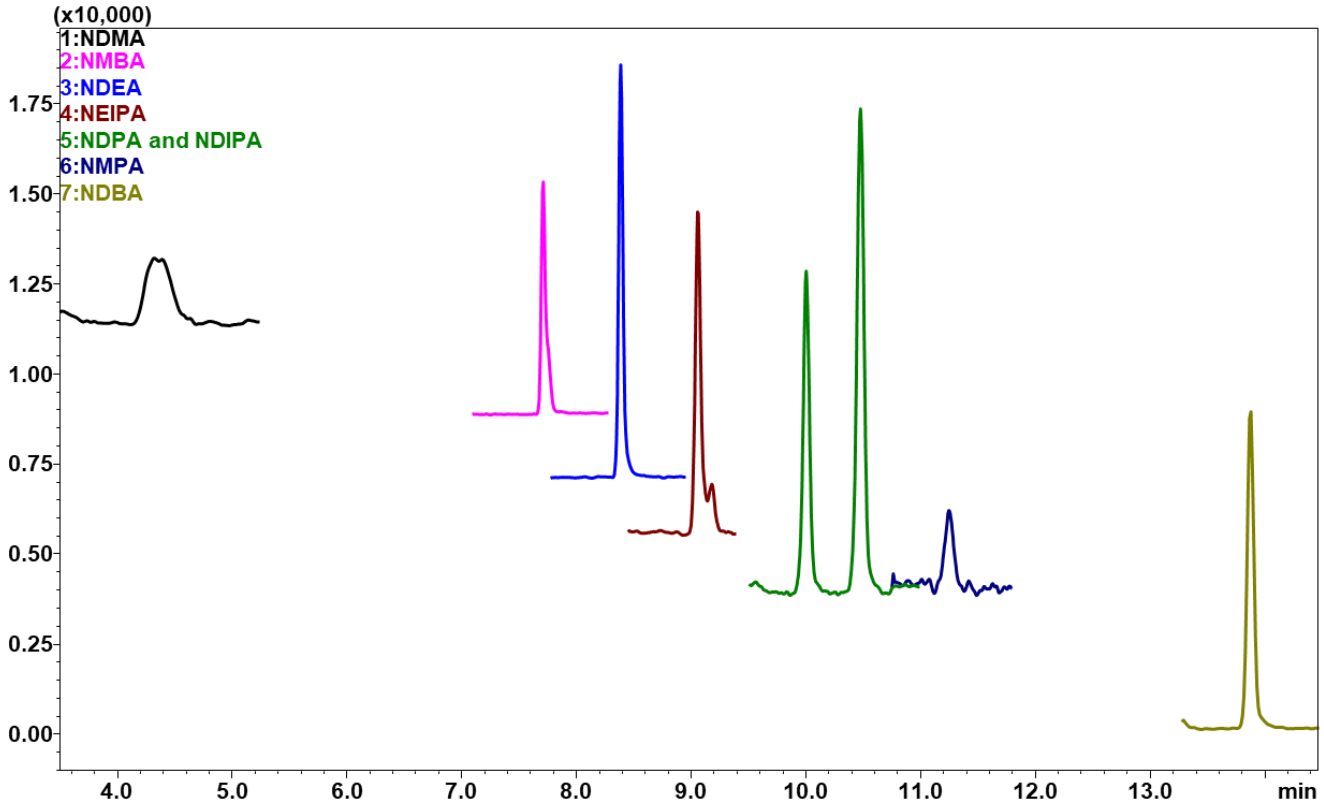


Figure 2. Total ion chromatogram for eight nitrosamine standards. Represented here is the 3 ng/mL standard at 15 µL injection volume.

Table 4. The LOQ and percent accuracy for the entire calibration range obtained for nitrosamines. Also, listed here is the LOQ criteria set by the FDA.

Nitrosamine	LOQ FDA (ng/mL)	LOQ LCMS-8050 (ng/mL)	% accuracy (n=3, for the range)	Meets FDA LOQ criteria
NDMA	1	**1**	86.9-106.7	**YES**
NDEA	2	**0.3**	86.4-121.8	**YES**
NEIPA	2	**0.5**	84.3-106.6	**YES**
NDIPA	2	**0.5**	78.7-106.3	**YES**
NDPA	0.5	**0.3**	86.0-115.8	**YES**
NMPA	0.5	**0.5**	81.1-130.0	**YES**
NDBA	0.5	**0.3**	87.7-119.5	**YES**
NMBA	0.5	**0.3**	81.0-130.0	**YES**

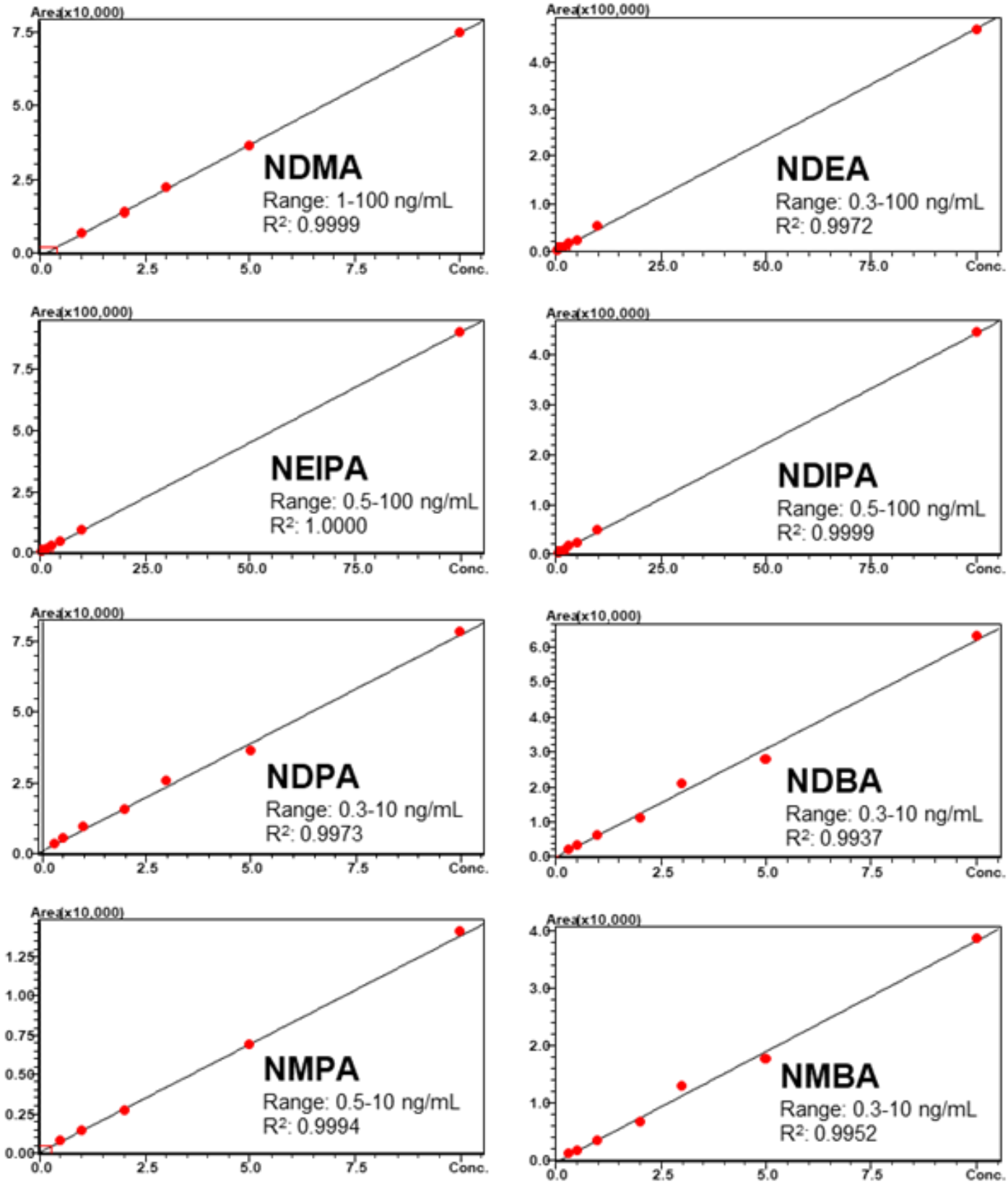


Figure 3. Calibration curve depicting linear range and r^2 value obtained for NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA, NMPA, and NMBA.

Table 5. System suitability test performed using 3 ng/mL nitrosamine standard mix. %RSD was calculated for 6 replicate injections. Also shown here is the cumulative %RSD calculated by combining the initial six injections of the standard solution and each subsequent bracketing standard between the sample injections.

Nitrosamine	%RSD of 6 replicate injections of 3 ng/mL STD (n=6)	Meets FDA criteria	Cumulative %RSD of peak area (3 ng/mL QC check) (n=16)	Meets FDA criteria
NDMA	3.2	**YES**	5.3	**YES**
NDEA	4.2	**YES**	5.6	**YES**
NEIPA	1.5	**YES**	2.7	**YES**
NDIPA	4.3	**YES**	3.7	**YES**
NDPA	2.6	**YES**	4.5	**YES**
NMPA	3.6	**YES**	5.9	**YES**
NDBA	2.5	**YES**	3.7	**YES**
NMBA	2.5	**YES**	8.1	**YES**
FDA criteria	**NMT 10%**		**NMT 15%**	

The LOQ obtained by LCMS-8050 are listed in Table 4. The obtained LOQ was compared with that required by the FDA in accordance to the document FY-20-106-DPA-S. The LOQ for all the N-nitrosamines analyzed either met the criteria or performed better than the LOQ required by the FDA (Table 4). The percent accuracy at LOQ was in 70-130% range.

Figure 3 shows the calibration curves for all eight nitrosamines standards prepared as near solution. The r^2 value for all the analytes was better than 0.99 indicating excellent lineary in the calibration range. Note that the calibration range obtained on LCMS-8050 met or exceeded the criteria required by the FDA.

Table 5 shows the summary of average percent accuracy for the triplicate injection of individual calibration levels. The overall percent accuracy for all calibration levels ranged between 70-130%. Overall, the data demonstrated excellent sensitivity, linearity, and accuracy for the analysis of N-nitrosamines.

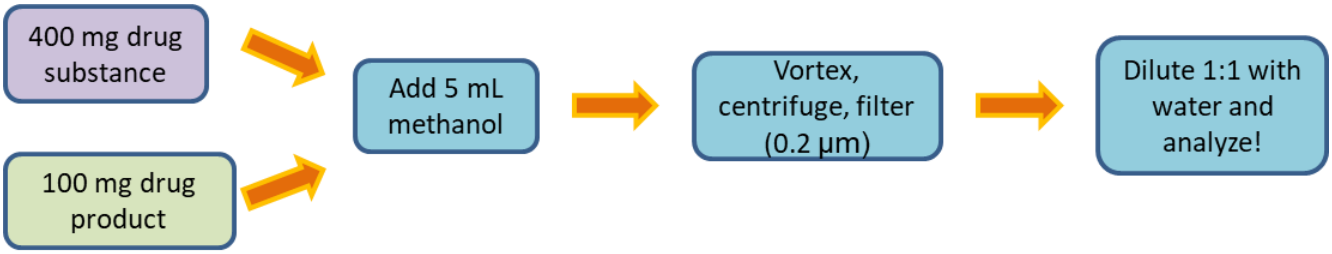


Figure 4. Schematic representation of the sample preparation for the Metformin granules (drug substance) and tablets (drug product).

The Metformin samples were prepared using the method shown in Figure 4 (more details can be found in the Methods section). The samples were then analyzed on the LCMS-8050 following the same method as the standards. Table 6 shows the LOQ levels that were achieved in the drug product and drug substance. Each matrix was spiked with 0.5 ng/mL, 1 ng/mL, and 10 ng/mL of each nitrosamine. Figures 5 and 6 show the overlaid chromatograms of the unspiked (blank) and spiked matrices. NDIPA and NDPA have the same MRM transitions but are chromatographically separated. The percent recovery is shown for individual nitrosamines in each matrix at the achieved LOQ. Note that the samples were spiked at or below the LOQ required by the FDA. All recoveries were between 70-150%.

Table 6. Summary of spike recoveries obtained in metformin tablets and granules for all eight nitrosamines. Note that the granules and tablets were spiked at or below the required LOQ.

Nitrosamines	Desired LOQ (FDA) (ng/mL)	LOQ LCMS-8050 (ng/mL)	Spike level (ng/mL)	% spike recovery (±%RSD) n=3	
				Metformin Granules	Metformin Tablets
NDMA	1.0	1	1.0	76.8 (±4.2)	104.4 (±1.0)
NDEA	2.0	0.3	0.5	116.4 (±7.5)	116.1 (±6.2)
NEIPA	2.0	0.5	0.5	96.3 (±1.8)	102.4 (±7.0)
NDIPA	2.0	0.5	1.0	90.7 (±11.5)	92.5 (±2.9)
NDPA	0.5	0.3	0.5	128.2 (±13.1)	87.8 (±24.1)
NMPA	0.5	0.5	0.5	91.9 (±9.7)	108.7 (±23.3)
NDBA	0.5	0.3	0.5	117.2 (±6.2)	146.6 (±9.1)
NMBA	0.5	0.3	0.5	103.7 (±5.5)	108.9 (±8.1)

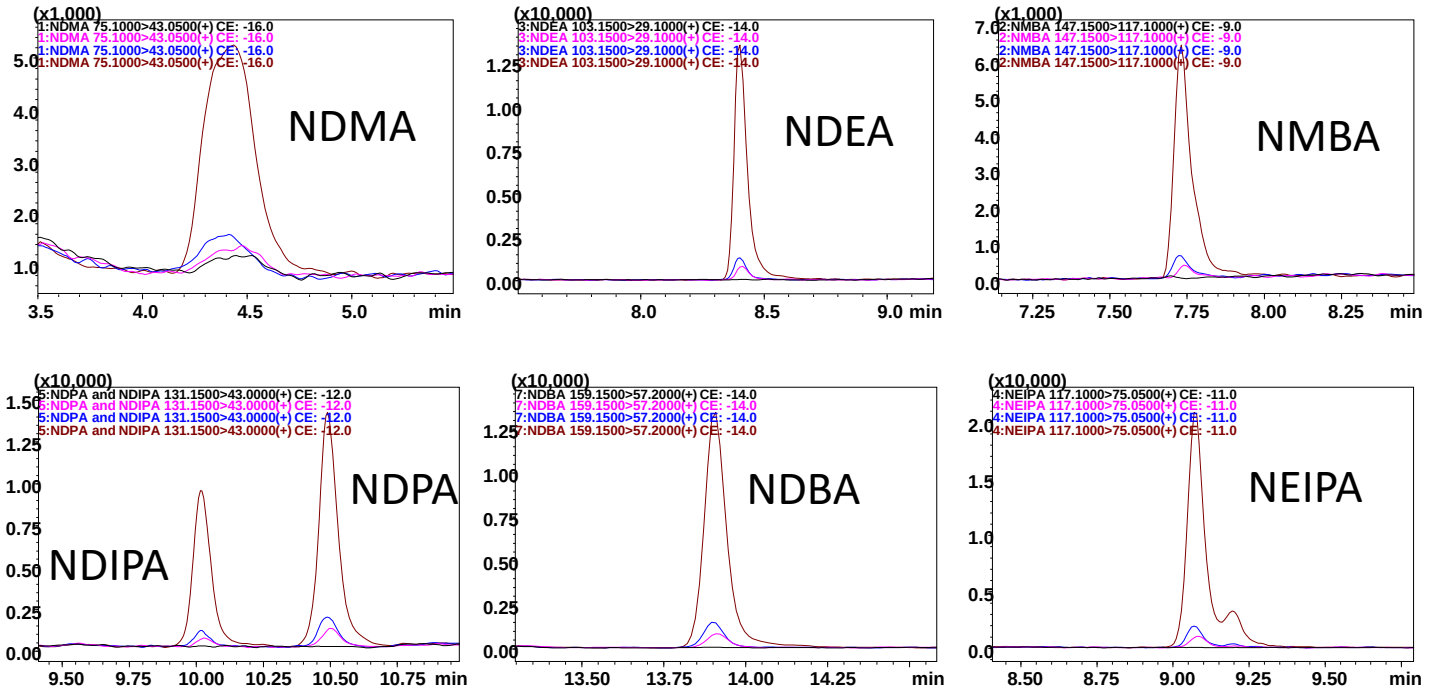


Figure 5. Overlaid chromatograms of nitrosamine impurities in Metformin granules. The following colors represent the corresponding chromatograms: black is the unspiked matrix, pink is 0.5 ng/mL spiked matrix, blue is 1.0 ng/mL spiked matrix, and brown is 10 ng/mL spiked matrix.

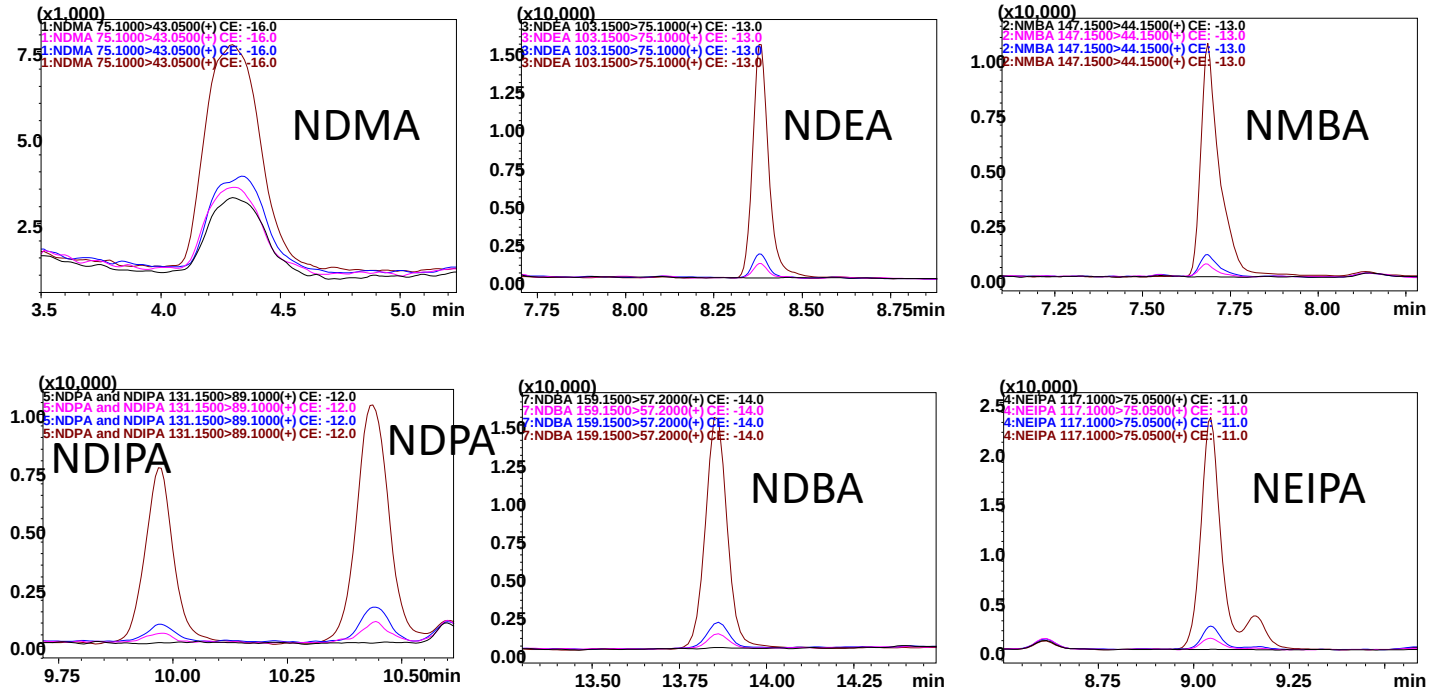


Figure 6. Overlaid chromatograms of nitrosamine impurities in Metformin tablets. The following colors represent the corresponding chromatograms: black is the unspiked matrix, pink is 0.5 ng/mL spiked matrix, blue is 1.0 ng/mL spiked matrix, and brown is 10 ng/mL spiked matrix.

■ Conclusion

Shimadzu's LCMS-8050 instrument was used for the analysis of nitrosamines. The LC-MS/MS analysis demonstrated excellent linearity, accuracy, and sensitivity for NDMA, NMBA, NDEA, NEIPA, NDIPA, NDPA, NMPA, and NDBA. All eight nitrosamines demonstrated r^2 values better than 0.99. Additionally, the overall percent accuracy obtained at all calibration levels was between 70-130%. The obtained LOQs were compared with FDAs current guidance on nitrosamines for these analytes in Metformin tablets and granules. All the analytes either met or performed better than the criteria specified by the FDA. The same LOQs were achieved in both the drug substance and drug product. Excellent quantitation and spike recoveries were obtained in Metformin tablet and granule matrix. The recoveries ranged from 70-150% at the respective LOQs in matrix.

All in all, LCMS-8050 proved to be a robust and reliable instrument for the sensitive and specific identification and quantitation of nitrosamines in Metformin granules and tablets.

References

1. https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications (current as of 02/24/2021)
2. FY20-106-DPA-S_LC-ESI-HRMS Method for the Determination of Nitrosamine Impurities in Metformin Drug Substance and Drug Product. US Food and Drug Administration Published 06/03/2020.

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First Edition: Sept. 24, 2020