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Application News

SSI-LCMS-

Liquid Chromatography Mass Spectrometry

Got DMF? Chromatographic separation and identification of NDMA and DMF using LCMS-9030.

Liquid Chromatograph Mass Spectrometer
LCMS-9030

■ Summary

Shimadzu's LCMS-9030 high resolution accurate mass instrument coupled to LC-40 liquid chromatograph was used for the chromatographic separation and identification of N-nitrosodimethyl amine (NDMA) with N,N-dimethylformamide (DMF). Mass accuracy of less than 10 ppm was used to further ensure the specificity of NDMA and DMF. NDMA and DMF were identified and quantitated in Metformin tablet formulation.

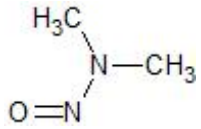
■ Background

DMF is a commonly used solvent in pharmaceutical manufacturing and can be present at low levels in the active pharmaceutical ingredient (API) and/or final drug product. NDMA along with other nitrosamines are common impurities found in drug products. FDA reported that elevated levels of NDMA impurity can be estimated due to coelution of NDMA with DMF.[1] We utilized LCMS-9030 to perform baseline chromatographic separation of NDMA and DMF followed by unequivocal identification and quantitation of NDMA and DMF. The recovery of NDMA in Metformin tablets was also calculated. The method can be extended to other pharmaceutical formulations with minor chromatographic modifications.

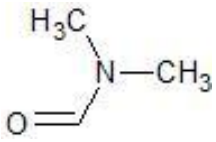
■ Method

NDMA and DMF were purchased from Sigma Aldrich (St. Louis, MO). LCMS grade solvents were purchased from Honeywell (Charlotte, NC).

Nexera LC-40 high pressure binary pump was coupled to LCMS-9030 QTOF high resolution accurate mass spectrometer. The chromatographic separation was performed on a Restek Raptor C18 column (2.1x100 mm, 2.7 µm). The mobile phase A was 0.1% formic acid in water and B was 0.1% formic acid in methanol (Table 1). LCMS-9030 was custom tuned specifically for NDMA and DMF analysis to achieve a mass accuracy of 7 ppm or better. Divert valve was used to divert excipients to waste when not collecting MS data. The standards were injected in triplicate. Linear calibration curve along with percent accuracy calculations was performed using LabSolutions v. 5.99 SP2 software.



N-nitroso-dimethylamine **(NDMA)**
Monoisotopic mass: 74.0480
$[M+H]^+$: 75.05529



N,N-dimethylformamide **(DMF)**
Monoisotopic mass: 73.0528 g/mol
$[M+H]^+$: 74.06004

Figure 1. Chemical structure for NDMA and DMF. Also presented here are the monoisotopic masses and the protonated adduct for NDMA and DMF.

The stock solutions of NDMA and DMF standards were prepared in methanol. Further dilutions of stock solution were accordingly prepared in 1:3 methanol:water to be used for calibration curve and for spiking into the tablet matrix.

The Metformin tablet matrix was prepared according to the FDA guidelines[2] with minor modifications. Briefly, the Metformin drug product sample was prepared by crushing the 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 divided into two portions: unspiked tablet matrix (blank) and 10 ng/mL NDMA spike. The overall composition of filtrate was 1:3 methanol:water for LCMS analysis.

Table 1. Snapshot of LCMS parameters.

LCMS-9030	Parameters	LC-40	Parameters
Ion Source	APCI	Column	Restek Raptor C18 4.6x100mm, 2.7µm
Nebulizing Gas	3 L/min	Flow rate (mL/min)	0.6
Interface Temperature	350°C	Mobile Phase A	0.1% formic acid in water
DL Temperature	200°C	Mobile Phase B	0.1% formic acid in methanol
Heat Block Temperature	200°C	Injection volume (µL)	15
Drying Gas	5 L/min	Autosampler temperature (°C)	5
Isolation window	+/- 1.4 Da	Column Oven (°C)	25
Polarity	Positive	Elution	Gradient
Scan type	Extracted Ion Chromatogram (EIC)	Rinse type	External only
Scan Start-End time (min)	2.75-4.75	Divert valve	Divert to waste, except when collecting MS data

Table 2. Isotopic distribution pattern of DMF and suggested interfering isotopes.

m/z	% isotopic contribution	Chemical Formula	Comment	Relationship with NDMA
74.0600	100%	C_3H_8NO	$[M+H]^+$ Naturally occurring most abundant isotope	More than 1 mass unit apart
75.0571	0.37%	$C_3H_8{}^{15}NO$	Replacement of ^{14}N with ^{15}N	Suggested interference: 23.6 ppm mass difference
75.0634	3.24%	$C_2{}^{13}CH_8NO$	Replacement of one ^{12}C by ^{13}C	Suggested interference: 115 ppm mass difference

■ Results and Discussion

NDMA and related nitrosamines have been reported as contaminants in low amounts in foods, beverages, and other consumer goods. Recently, NDMA was reported to be present at unacceptable levels in several angiotensin receptor blocker (ARB) drugs. This led to a widespread recall of ARB drugs by the regulatory agencies. A recent study by the FDA reported that the NDMA levels may be reported higher than usual if DMF is present as a process impurity.[1]

The protonated adduct for the naturally occurring most abundant isotope of DMF is 1 mass unit apart (Table 1). In this case, a unit resolution instrument such as a triple quadrupole mass spectrometer should be able to potentially differentiate between NDMA and DMF, specifically when MRM transitions are being used. However, there are potentially two other suggested interferences (Table 2): (1) m/z 75.0571 (0.37% contribution) due to substitution of ^{14}N with ^{15}N and (2) m/z 75.0634 (3.24% contribution) due to substitution of ^{12}C with ^{13}C. While the percent contribution of second suggested interference is higher, it is easier to distinguish on a high resolution instrument with a mass accuracy of 20 ppm or better. Despite only 0.37% contribution, the interference observed with ^{15}N isotope cannot be distinguished unless a mass accuracy of 15 ppm or better is used. In light of the above information, it is imperative that chromatographic separation of DMF and NDMA will allow the user to be able to use either a triple quadrupole or QTOF instrument for the analysis of DMF and NDMA.

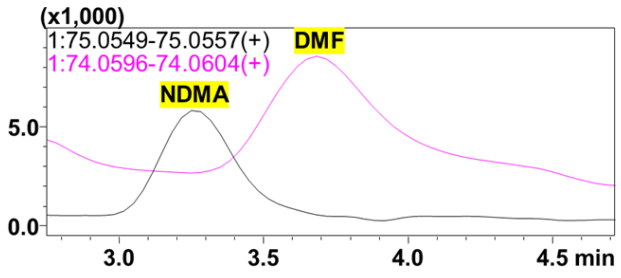


Figure 2. NDMA and DMF (20 ng/mL neat standard) were chromatographically separated. Shown here is LCMS-9030 operated at 5 ppm mass accuracy window.

Figure 2 shows the chromatographic separation (Restek Raptor C18 column) of NDMA and DMF standard solutions prepared in neat solvent. The chromatographic conditions as described in Table 1 were used. NDMA and DMF were baseline separated with this chromatographic method. A mass accuracy of 10 ppm or better allowed for further confirmation of the presence of NDMA and DMF peaks.

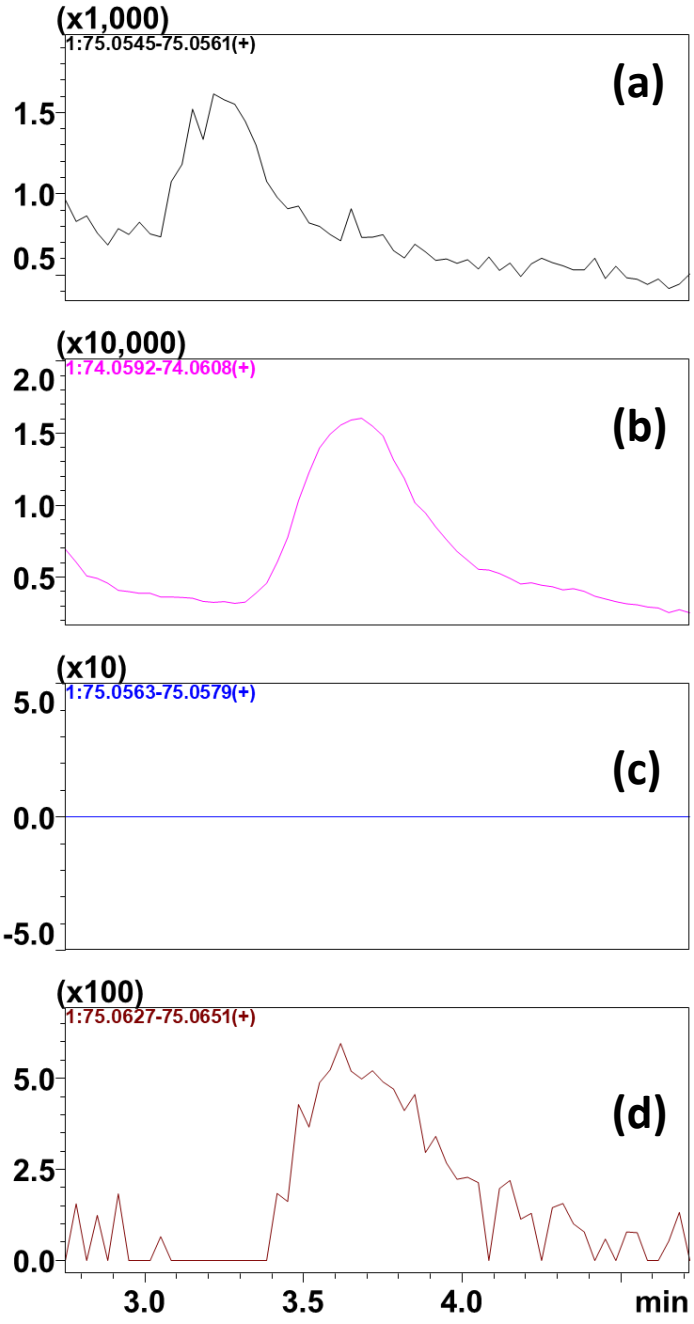


Figure 3. EIC for (a) NDMA (10 ppm mass accuracy), (b) DMF C_3H_8NO (10 ppm mass accuracy), (c) $C_3H_8{}^{15}NO$ (10 ppm mass accuracy), and (d) $C_2{}^{13}CH_8NO$ (15 ppm mass accuracy) in unspiked Metformin tablet.

A Metformin tablet was tested using the above LCMS conditions (Table 1). Figure 3 shows the EIC chromatograms for unspiked Metformin tablet. The mass accuracy range was deliberately increased to 10 ppm to observe any potential interference arising from the presence of DMF. Figure 3a demonstrates the presence of NDMA in this sample. Figure 3b confirms that DMF is also present in this Metformin tablet sample. We were specifically interested in the presence of ^{15}N and ^{13}C substituted DMF isotopic impurities when DMF is present in the sample. Figure 3c clearly demonstrates that no significant quantitative interference can be observed from the $C_3H_8{}^{15}NO$ isotope of DMF at 10 ppm mass accuracy. Figure 3d shows the EIC for $C_2{}^{13}CH_8NO$ isotope of DMF. The mass accuracy for $C_2{}^{13}CH_8NO$ isotopic interference was deliberately increased from 10 ppm to 15 ppm. At 15 ppm mass accuracy we observed ~0.03% contribution (based on intensity) from ^{13}C isotope. We conclude that at the concentration DMF is present in this Metformin sample, there is no interference due to the ^{15}N isotope of DMF. If DMF is coeluting with NDMA, there may be some interference from the ^{13}C isotope of DMF, in this case about 0.03%. However, note that ^{13}C isotope can be easily distinguished from NDMA due to >115 ppm mass difference between ^{13}C DMF and NDMA.

To further confirm the results, Metformin tablet was spiked with 10 ng/mL NDMA. Figure 4a shows the overlaid EIC chromatograms for NDMA. The black trace shows no interference from the solvent blank used for the sample preparation. The pink trace is the 10 ng/mL NDMA standard. The blue trace is unspiked (blank) Metformin tablet. The brown trace is the Metformin tablet spiked with 10 ng/mL NDMA. The overlaid chromatograms show an increase in intensity of the brown trace when compared to the NDMA standard at 10 ng/mL and blank Metformin tablet, hence confirming the presence of NDMA in the tablet formulation.

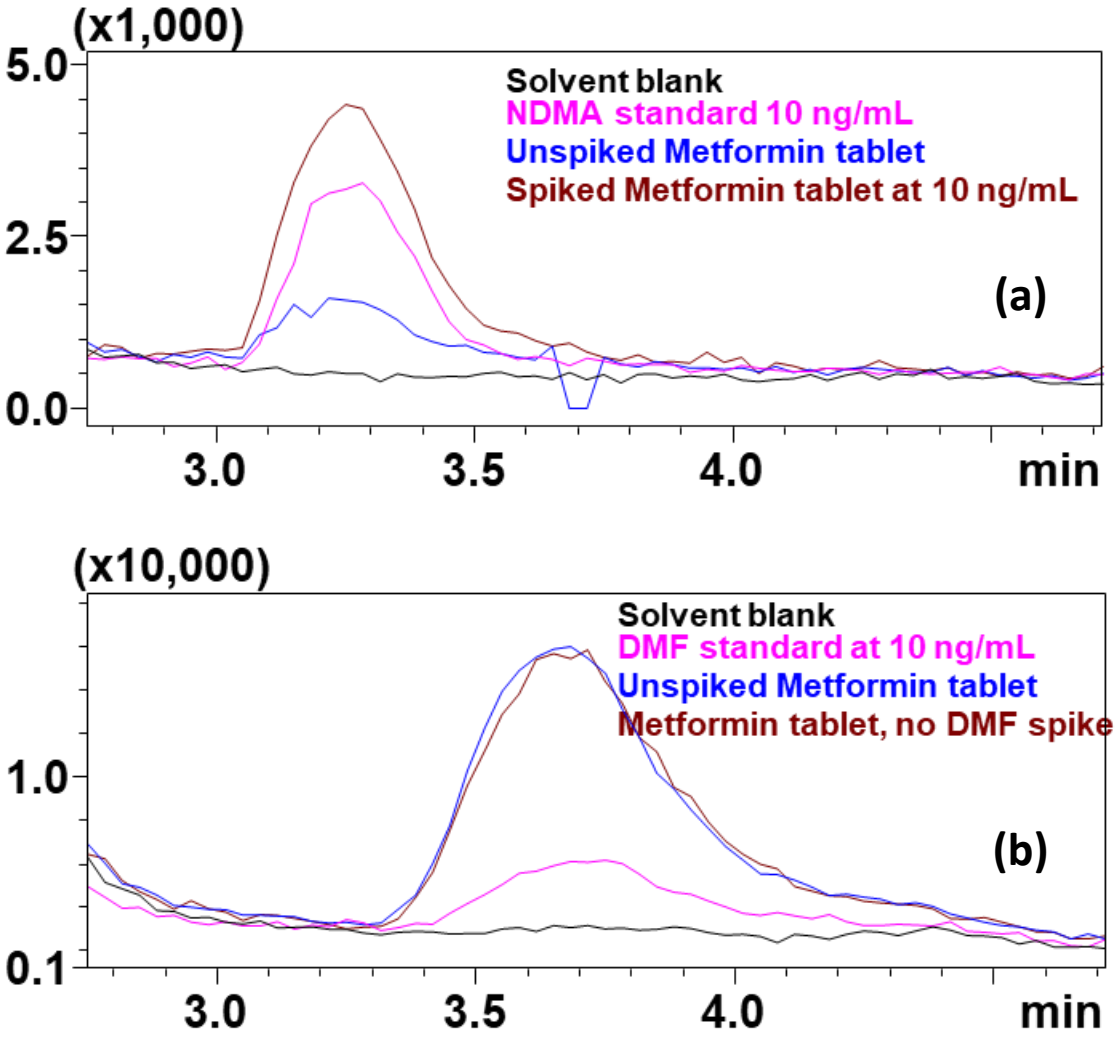


Figure 4. Overlaid EIC chromatograms for (a) NDMA and (b) DMF C_3H_8NO (7 ppm mass accuracy). Figure legend shows the color representation of the individual traces.

Similarly, figure 4b shows the overlaid EIC for DMF. The overlaid chromatograms for DMF demonstrate that there was no interference from solvent blank (black trace). Additionally, the Metformin tablet was spiked only with NDMA, so there is no increase in peak intensity (compare brown and blue trace). The pink trace is 10 ng/mL DMF standard for reference. This data further confirms no significant interference from DMF using the reported LCMS method with LCMS-9030 instrument.

A linear calibration curve was generated for NDMA in the 2-50 ng/mL range (Figure 5). The standards were injected in triplicate. Excellent linearity was noted in this calibration range with an r^2 >0.99. The overall percent accuracy for all calibration points was between 92.3-136.5%. The percent recovery for 10 ng/mL standard was calculated at 89.2%. The amount of NDMA in unspiked Metformin tablet was calculated at 3.1 ng/mL (Table 3).

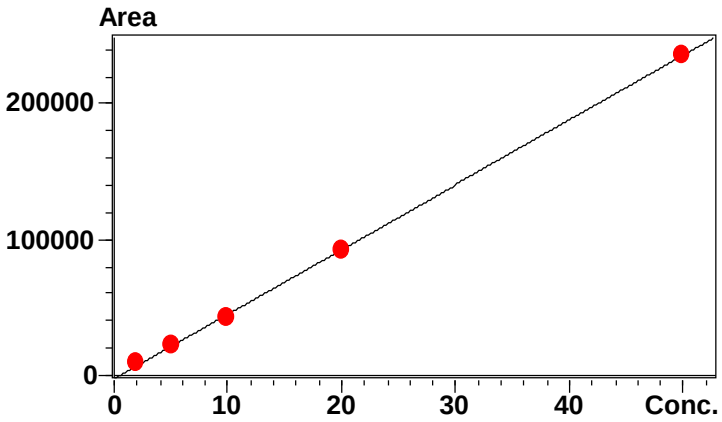


Figure 5. NDMA calibration curve (range: 2-50 ng/mL)

Table 3. Summary of calibration curve and recovery data.

	NDMA
Range (ng/mL)	2-50
r^2	0.9997
Weighting	None
% Recovery (10 ng/mL spike)	89.2
% Accuracy	92.3-136.5
NDMA in unspiked metformin tablet	3.1 ng/mL

■ Conclusion

Shimadzu's LCMS-9030 instrument was used for the analysis of NDMA and DMF. A new chromatographic method was developed on LCMS-9030 to chromatographically separate NDMA and DMF. A narrow mass accuracy tolerance window was used to investigate any potential interference arising from the presence of DMF in Metformin tablet sample. While DMF was found to be present in the tablet sample, it was not found to be a potential interference using the current method. Excellent mass accuracy, linearity, and overall recovery in matrix samples was observed with the reported LCMS method.

The universal nature of this method provides an added advantage of being able to be transferred to triple quadrupole MS instruments such as LCMS-8060 and LCMS-8050 where DMF is suspected to be a potential interference for the quantitation of NDMA in pharmaceutical formulations.

References

1. Yang, J., Marzan, T.A., Ye, W. et al. A Cautionary Tale: Quantitative LC-HRMS Analytical Procedures for the Analysis of N-Nitrosodimethylamine in Metformin. AAPS J 22, 89 (2020).
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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LCMS-8050


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Q-TOF LCMS-9030

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