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

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1. Overview

Chromatographic separation and identification of dimethylformamide (DMF) and N-nitroso-dimethylamine (NDMA) was performed to prevent overestimation of NDMA when DMF interference is present. This method can easily be implemented for the impurity evaluation of active pharmaceutical ingredients (API), intermediates, and final drug products irrespective of triple quadrupole or high-resolution mass spectrometers.

2. Introduction

DMF (Figure 1) is a commonly used solvent in pharmaceutical manufacturing. DMF can be present at low levels in the (API) and/or final drug product. NDMA (Figure 1) along with other nitrosamine impurities is a common impurity found in API and drug products. The Food and Drug Administration (FDA) reported that overestimation of NDMA is possible if NDMA and DMF are coeluting.[1] Modern and intelligent analytical tools such as high-resolution mass spectrometers can play a crucial role in accurately identifying the impurity present in the sample.[2] However, a high-resolution instrument may not be readily accessible to every laboratory. Here, advantage of chromatographic separation of NDMA and DMF is emphasized. For proof of concept, the identification was performed on a Shimadzu LCMS-9030 QTOF instrument.

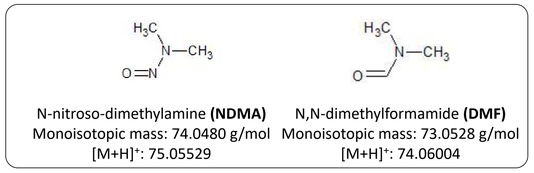

Figure 1 Chemical structure of NDMA and DMF.

3. Method

NDMA and DMF were purchased from Sigma Aldrich (St. Louis, MO). LCMS grade solvents were purchased from Honeywell (Charlotte, NC). LCMS method parameters are shown in Table 1.

Table 1 Snapshot of LCMS parameters

LCMS-9030	Parameters	LC-40	Parameters
Ion source	APCI	**Column**	Restek Raptor C18 4.6x100 mm, 2.7 µm
Nebulizing gas	3 L/min	**Flow rate**	0.6 mL/min
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**	15 µL
Drying gas	5 L/min	**Autosampler temperature**	5 °C
Isolation window	±1.4 Da	**Column oven**	25 °C
Polarity	Positive	**Elution**	Gradient
Scan type	Extracted ion chromatogram (EIC)	**Rinse type**	External only
Scan start-end time	2.75-4.75 min	**Divert valve**	Divert to waste, except when collecting MS data

- Stock solutions of NDMA and DMF standards were prepared in methanol. Further dilutions of stock solutions were prepared in 1:3 methanol:water.
- The calibration curve was run with a 2-50 ng/mL range and the standards were injected in triplicate (Figure 2 inset).
- LCMS-9030 was custom tuned for NDMA and DMF to achieve a mass accuracy of 7 ppm or better.
- Use of a divert valve is recommended when applying the method to API and/or drug product analysis.

4. Results and Discussion

NDMA and related nitrosamines are reported as contaminants in low amounts in foods, beverages, and other consumer goods. NDMA was recently reported to be present at unacceptable levels in several angiotensin receptor blocker (ARB) drugs by regulatory agencies. The FDA published that NDMA levels may be reported higher than usual if DMF is also present in the sample as a process impurity.

Table 2 shows the isotopic distribution pattern for DMF. The naturally abundant isotope (*m/z* 74.0600) of DMF can be easily distinguished from the naturally abundant isotope of NDMA (*m/z* 75.05529) by a triple quadrupole instrument. However, there are 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. Bearing in mind 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.

Table 2 Isotopic distribution pattern of DMF and suggested interfering isotopes

m/z	% isotopic distribution	Chemical formula	Comment
74.0600	100%	**C_3H_8NO**	[M+H]+ naturally occurring most abundant isotope. More than 1 mass unit apart from NDMA
75.0571	0.37%	**$C_3H_8{}^{15}NO$**	Replacement of ^{14}N with ^{15}N. Suggested interference with NDMA (23.6 ppm mass difference with NDMA)
75.0634	3.24%	**$C_2{}^{13}CH_8NO$**	Replacement of one 12C with 13C. Suggested interference with NDMA (115 ppm mass difference with NDMA)

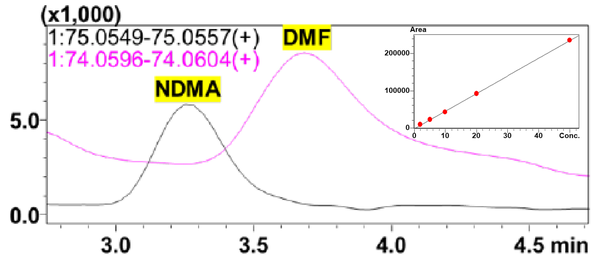

Figure 2 NDMA and DMF (20 ng/mL) were chromatographically separated. Shown here is LCMS-9030 operated at 7 ppm mass accuracy window. (Inset) NDMA calibration curve in 2-50 ng/mL range.

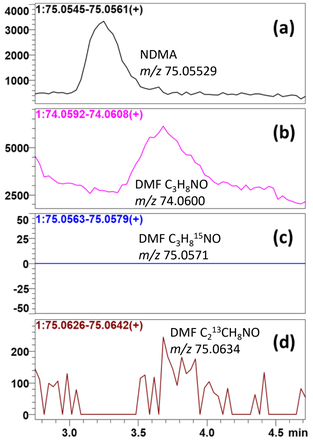

Figure 3. EIC for (a) NDMA, (b) DMF C_3H_8NO, (c) $C_3H_8{}^{15}NO$ (50 ng/mL), and (d) $C_2{}^{13}CH_8NO$ in 10 ng/mL neat solution at 10 ppm mass accuracy.

Figure 3 shows the EIC chromatograms from a 10 ng/mL neat solution containing NDMA and DMF. The mass accuracy range was deliberately increased to 10 ppm to investigate if mass tolerance can play a role in the potential interference of DMF with NDMA quantitation. Figure 3a and 3b show strong signal strength for the naturally abundant isotope of NDMA and DMF, respectively. 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 even at 50 ng/mL concentration. Figure 3d shows the EIC for $C_2{}^{13}CH_8NO$ isotope for DMF. At 10 ppm mass accuracy we observed ~5.6% contribution (based on area count) from ^{13}C isotope.

5. Conclusions

- NDMA and DMF were baseline separated with the new chromatographic method.
- No interference was observed from the ^{15}N isotope of DMF even at 50 ng/mL concentration of DMF (10 ppm mass accuracy).
- The area ratio of ^{13}C to naturally abundant isotope of DMF was at ~5.5%. Since, the mass difference between ^{13}C isotope of DMF and NDMA is >100 ppm a QTOF instrument will be able to easily distinguish the NDMA and DMF at better than 15 ppm mass accuracy.
- Excellent mass accuracy, linearity, and percent accuracy was observed with the reported LCMS method.
- This method can be transferred to triple quadrupole MS instruments where DMF is suspected to be a potential interference for the quantitation of NDMA in pharmaceutical formulations.

References

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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.
3. Wu Q, Kvitko E, Zenzola N, Kucera K, Light D. ChemRxiv. Cambridge: Cambridge Open Engage; 2020;