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NDMA impurity in Ranitidine / LCMS™-9030

Application News

Detection and Quantitation of NDMA Impurity in Ranitidine Drug Substances and Products by LC-HRMS on LCMS-9030

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User Benefits

- Determination of NDMA in ranitidine API and drug products by an orthogonal LC-HRMS method on LCMS™-9030.
- NDMA was found in all the six samples and their levels increased significantly in comparison with the results measured in 10 months ago by LC-MS/MS (TQ) method.

■ Introduction

Among the nitrosamines listed by US FDA and EMA, NDMA is the most-frequently found one in sartans, ranitidine and metformin drug products. Dedicated LC-MS/MS and LC-HRMS methods [1,2] were developed and used for monitoring of NDMA in metformin and ranitidine APIs and drug products. In Apr. 2020, US FDA requested removal of all ranitidine products from the US market, which have been used as for 30 years. In Sep. 2020, EMA recommended to suspend all ranitidine medicines in the Europe [3]. This is because investigation reveals that the level of NDMA increases significantly with time of storage even under normal conditions, which may lead to the level of NDMA in the ranitidine product above the acceptable daily intake limit.

In this Application News, an LC-HRMS method is introduced as an orthogonal method to the LC-MS/MS method [4] for the determination of NDMA in ranitidine products.

■ Experimental

Standard and sample preparation

A NDMA stock of 1000 ng/mL was prepared in methanol from NDMA CRM (Sigma-Aldrich, 5000 µg /mL). A NDMA-d6 stock solution of 800 ng/mL diluted from Surrogate Standard (Restek, 1000 ug/mL) was used as the internal standard (IS). A calibration series of 1, 3, 5, 20, 50, 100, 200 and 500 ng/mL of NDMA with IS of 40 ng/mL were prepared in mixed solvent of MeOH/H_2O (5:95, with 0.1% FA). Standards of 0.3 and 0.5 ng/mL with IS of 40 ng/mL were prepared for testing sensitivity of the method (LOD and LOQ).

Ranitidine drug substance (API) and products (tablet, syrup and injection) obtained from manufacturers were analyzed for NDMA. The powders (API or crushed tablets) was weighted and added to extraction solvent in a ratio of 28.5 mg of ranitidine in 1 mL of the mixed solvent of MeOH/H_2O (5:95, with 0.1% formic acid). IS was added to a final concentration of 40 ng/mL. The mixture was shaken for 30 min at room temperature followed by centrifugation at 15,000 rpm for 10 min. The extract was filtered with 0.22 um PVDF syringe filter and the clear solution was collected in an HPLC sample vial for analysis.

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 1. A targeted MS/MS TOF method was used to achieve high sensitivity, which details are shown in the bottom part in Table 1. Under the LC conditions, the ranitidine eluted after 4.8 min, which was not overlapped with the NDMA and IS peaks.

Table 1 Analytical conditions on LCMS-9030

LC Conditions	
Column	Shim-pack™ Scepter C18-120 (3.0 X 150 mm, 1.9 µm; 227-31013-04)
Flow Rate	0.5 mL/min
Mobile Phase	A: Water with 0.1% formic acid B: Methanol with 0.1% formic acid
Gradient Elution	4% B (0-1.8 min)->35% B (1.8-4.5 min)-> 95% B (5-12 min)->4% B (12.1-15 min)
Oven Temp.	45°C
Injection Volume	10 µL (5 µL for up to 500 ng/mL)
Interface Conditions	
Interface	APCI, 4.5 kV
Interface Temp.	350°C
DL Temp.	280°C
Heat Block Temp.	200°C
Nebulizing Gas	2.0 L/min.
Drying Gas Flow	5.0 L/min.
Data acquisition and data analysis	
MS Mode	Positive, Targeted MS/MS TOF
Isolation window (Q)	(+/-) 2 *m/z*
NDMA (target)	75.0533
NDMA-d6 (IS)	81.0930
TIC range (TOF)	m/z 40 ~ 90
CE spread (V)	0~10
Event time	100 ms
MS start - end	2 - 4.5 min.
XIC mass tolerance	(+/-) 15 ppm

■ Results and Discussion

Calibration curve and LOD/LOQ

Figure 1 shows the IS calibration curves of NDMA from 1 ng/mL to 200 ng/mL. The linearity R2 is 0.9996. Based on S/N of 0.3, 0.5 and 1 ng/mL, the LOD and LOQ of the method are 0.4 and 1.3 ng/mL, respectively. The peak area repeatability (RSD) with 1.0 ng/mL standard for six injections is 9.2%. The XIC peak of 1 ng/mL with IS (40 ng/mL) is displayed in Figure 2.

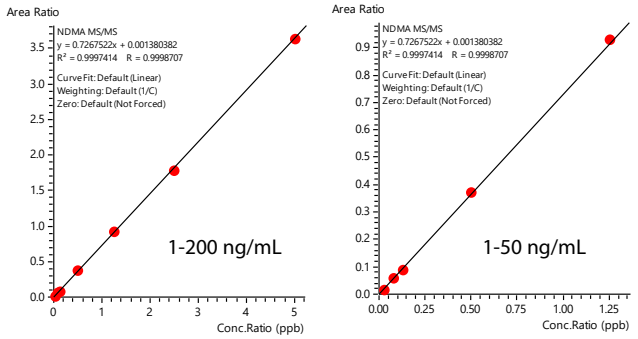

Figure 1 Calibration curve of NDMA at 1, 3, 5, 20, 50, 100, 200 ng/mL with IS of 40 ng/mL

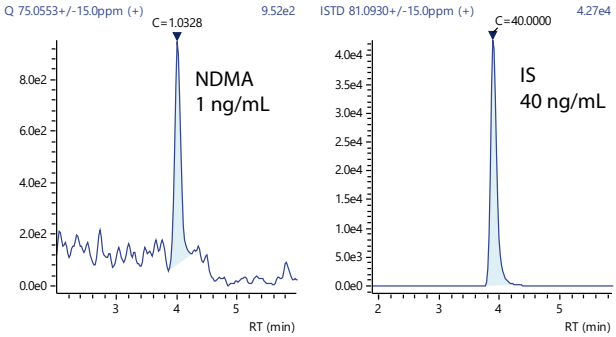

Figure 2 XICs of NDMA of 1 ng/mL and IS (NDMA-d6) of 40 ng/mL.

Quantitation of NDMA in ranitidine drug substance (API) and products

Six ranitidine samples were analyzed using the LC-HRMS method, which include one API powders, three tablet product, one injection product and one syrup product. The content of these ranitidine products are described in Table 2. The liquid samples (injection and syrup) were analyzed directly with only adding IS (5% of the sample volume).

The quantitative results of NDMA in these samples are shown Table 2. It is noted that the liquid samples (Injection and Syrup) were not diluted before analysis, except adding IS to the samples (5% volume of the sample volume). As a result, the contents of ranitidine in

Table 2 Results of NDMA in ranitidine samples by LC-HRMS method on LCMS-9030

Sample Description	Ranitidine in Extract (mg/mL)	NDMA Measured (ng/mL)	NDMA (ppm)
Injection (50mg/ 2mL per vial)	23.8	17.9	0.75
Syrup (75mg/5mL per dose)	14.3	301.3	21.07
API (powders)	28.5	42.2	1.48
Tablet 1 (150mg/T)	28.5	518.7	18.19
Tablet 2 (150mg/T)	28.5	321.6	11.3
Tablet 3 (150mg/T)	28.5	60.2	2.11

23.8 and 14.3 mg/mL, respectively. It can be seen from Table 2 that NDMA was found in all these samples. The levels of NDMA in these samples increased significantly except the Injection as in comparison with that measured in about ten months ago by MRM method on LCMS-8060 [4]. This observation is in accordance with the remark by EMA [3] that there is some evidence that NDMA may form from the degradation of ranitidine itself with increasing levels seen over its shelf life.

■ Conclusion

In this study, a targeted MS/MS TOF method was used to determine NDMA on LCMS-9030. The LOD, LOQ and linearity are comparable with the FDA reference LC-HRMS method. Six samples of ranitidine drug substance and products were analyzed and NDMA was found in all these samples. The levels of NDMA are increased significantly (except the injection sample) in comparison with the results measured in 10 months ago by LC-MS/MS method [4].

■ References

1. US FDA posted on 17 Oct 2019, "LC-MS/MS Method for the Determination of NDMA impurity in Ranitidine Drug Substance or Solid Dosage Drug Product'; https://www.fda.gov/media/131868/download
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3. EMA on 27 Nov 2020, "EMA confirms recommendation to suspend all ranitidine medicines in the EU"; https://www.ema.europa.eu/en/documents/referral/ranitidine-article-31-referral-ema-confirms-recommendation-suspend-all-ranitidine-medicines-eu_en.pdf
4. Shimadzu Application News, AD-0222, "Quantitative Determination of NDMA in Ranitidine Drug Products – Examples of Actual Samples Analysis"; (April 2020)

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