Valisure LC-HRMS Method for Determination of NDMA in Metformin

Revised as of February 27, 2020

Attachment A

Background

U.S. Food and Drug Administration (FDA) published a method titled "Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Metformin Drug Substance and Drug Product". [ref: FY20-058-DPA-S, https://www.fda.gov/media/134914/download, accessed on 2/23/2020]. Based on the testing principle of this method, Valisure scientists made improvements and optimizations using Valisure's in house instrumentation and achieved a lower limit of detection (LOD), a lower limit of quantitation (LOQ), and wider reportable range. Table 1 shows method performance comparisons.

Table 1. Testing Scope Validated by FDA and Valisure using LC-HRMS.

NDMA	FDA	Valisure
LOD (ng/mL)	1.0	0.3
(ppm)	0.01	0.003
LOQ (ng/mL)	3.0	1.0 (Figure 3)
(ppm)	0.03	0.01
Range (ng/mL)	3.0 - 10	1.0 - 200
(ppm)	0.03 - 0.1	0.01 - 2.0

Valisure's Materials and Methods

SCIEX EXIONLC AD (LC) coupled with X500R time of flight high resolution mass spectrometry (HRMS) was purchased from SCIEX (Framingham, MA). Luna Omega PS C18 HPLC column (3 µm, 4.6 × 100 mm) was purchased from Phenomenex (Torrance, CA). Certified reference material of N-Nitrosodimethylamine (NDMA) was purchased from Sigma-Aldrich (St. Louis, MO). Isotopic labeled NDMA standard $^{13}C_2$-D_6-NDMA was purchased from Cambridge Isotope Laboratories (Tewksbury, MA).

Sample Preparation

Sample preparation is same as FDA method. At the end of sample preparation, a known amount of $^{13}C_2$-D_6-NDMA is spiked into each sample extract before instrumental analysis.

Standard Calibration

A 9-point calibration curve is generated by isotopic dilution method which corrects the matrix effects due to the complex sample matrices.

Chromatographic Conditions

HPLC Column	Luna Omega PS C18 3µm, 4.6 x 100mm (Phenomenex, part number 00D-4758-E0)		
Column Temperature	40 °C		
Flow Rate	0.3 - 0.75 mL/min (see gradient for detail)		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in methanol		
Gradient	Time (min)	Flow Rate (mL/min)	B %
	0	0.3	2.5
	2	0.3	2.5
	7	0.3	50
	7.01	0.75	50
	12	0.75	97.5
	12.9	0.75	97.5
	13	0.75	2.5
	15	0.75	2.5
Injection Volume	10 µL		
Autosampler Temperature	5 °C		
Needle Wash	80:20, Methanol:Water with 0.1% Formic Acid		

Mass Spectrometry Conditions

Instrument	SCIEX X500R QToF
Ionization Mode	APCI +
Source Temp.	325 °C
Nebulizer Current	3 µA
Data Acquisition	MRMHR
TOF MS Scan	50 - 450 da
Mass Resolution	> 25,000
Mass Accuracy	15 ppm
NDMA MRM	75.0553 > 75.0553

Summary of Method Differences and Assessment of Data Quality

	FDA Method	Valisure Method	Assessment of Results
Mass Spectrometry	Orbi-Trap HRMS with optimized parameters	Q-TOF HRMS with optimized parameters	Both HRMS can achieve desired mass resolution and accuracy for the analytical purpose
HPLC Method	XSelect CSH C18 2.5 µm, 3.0 x 150 mm with optimized flow and gradient	Luna Omega PS C18 HPLC column 3 µm, 4.6 × 100 mm with optimized flow and gradient	Valisure's method generates increased chromatographic resolution and sharper peaks, thus resulting in higher signal to noise ratio at same concentration than FDA method (Figure 1 and Figure 2)
Quantification	comparing sample peak area with average of six 3 ng/mL standard injections	Isotopic dilution calibration curve with r^2 greater than 0.999, sample concentration is corrected by internal standard (Figure 4)	Without use of an internal standard, the FDA method has the potential to underestimate the NDMA concentration due to matrix effects.

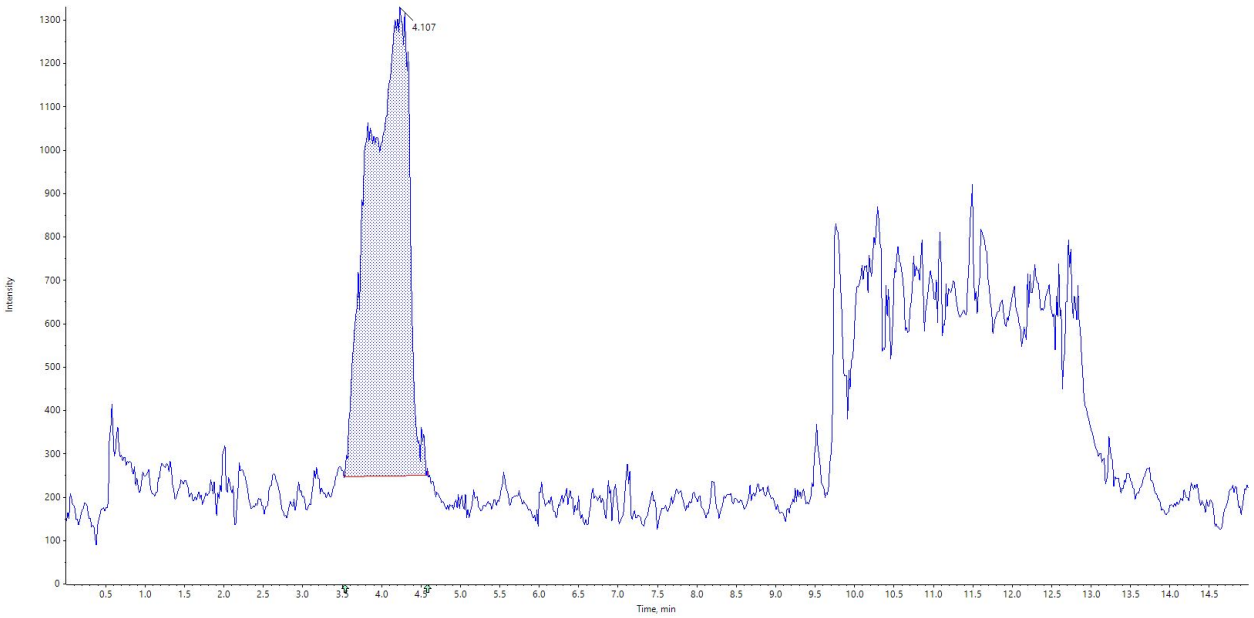

Figure 1. Identified peak of 20 ng/mL NDMA standard solution using FDA method FY20-058-DPA-S, with broadened peak and signal to noise ratio of 106.

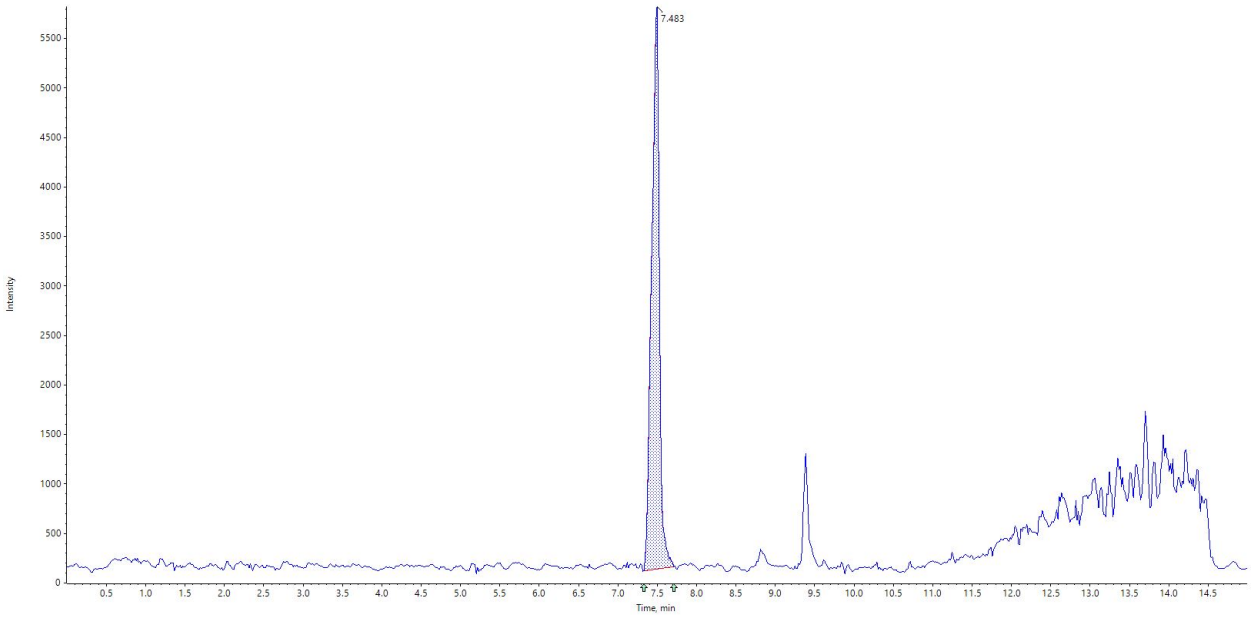

Figure 2. Identified peak of 20 ng/mL NDMA standard solution by Valisure's improved LC-HRMS method, with greater chromatography resolution and signal to noise ratio of 797.

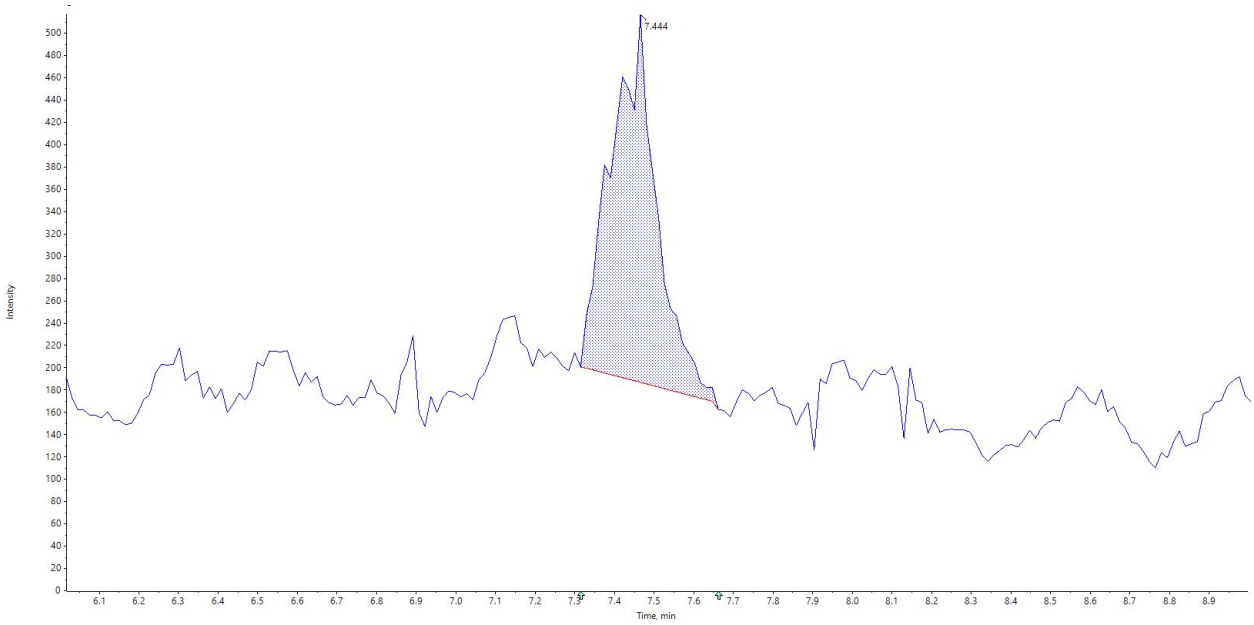

Figure 3. Identified peak of 1 ng/mL (LOQ) NDMA standard solution by Valisure's improved LC-HRMS method, with signal to noise ratio of 42.

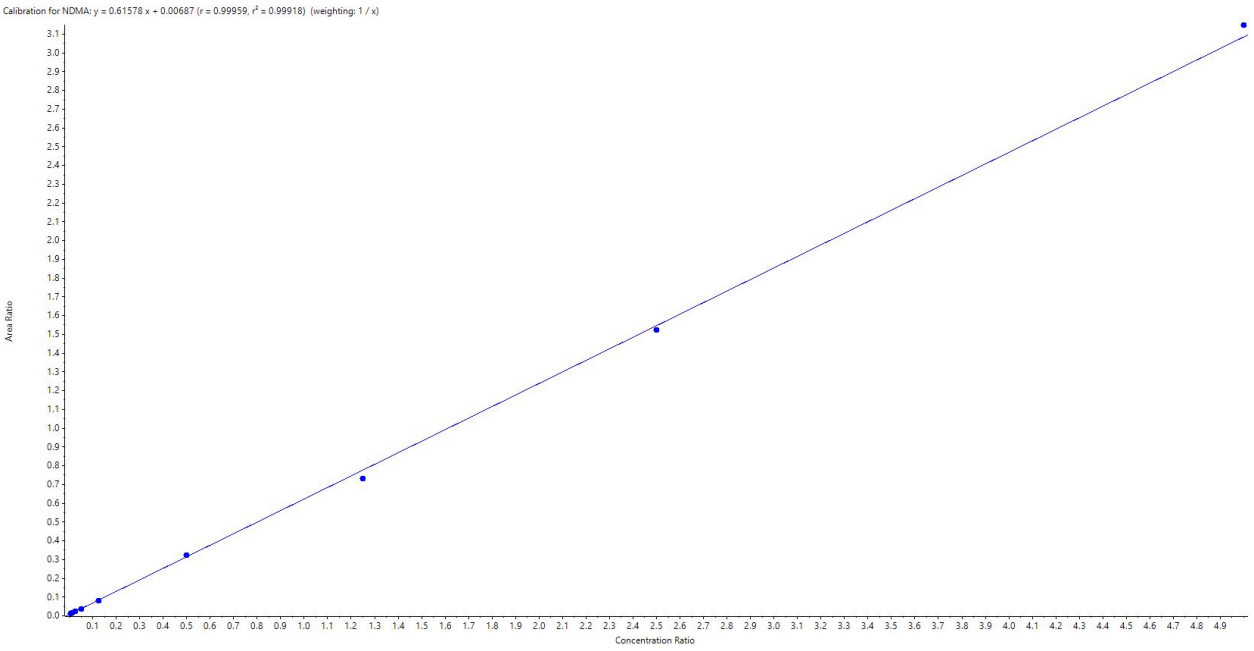

Figure 4. Linear isotopic dilution calibration curve ranged from 0.3 to 200 ng/mL, equivalent to 0.003 to 2 ppm NDMA in API.