Quantification of N-nitrosodimethylamine (NDMA) in azithromycin drug products

Using the QTRAP 4500 system

Jack Steed[1], Jessica Smith[1], Ferran Sanchez[2] and Jianru Stahl-Zeng[3]
[1]SCIEX, UK; [2]SCIEX, Spain; [3]SCIEX, Germany

NDMA is a genotoxic compound that belongs to a group of compounds deemed the “cohort of concern” by the European Medicines Agency (EMA) due to its possible impact on human health.[1]

Authorities have determined that it is necessary to test azithromycin drug products for the presence of impurities, including NDMA. This request follows the discovery that numerous active pharmaceutical ingredients (APIs) and drug products potentially contain nitrosamine impurities, beginning in 2018 with sartan-containing medications.[2] Since then, several other drug products have been implicated, with more and more continuing to be added.[3]

As more drug products continue to be implicated, sensitive and robust analytical methods are needed to quantify the nitrosamine impurities in these products at trace concentrations.

Here, a method for NDMA analysis in multiple azithromycin drug products is presented that achieved accurate detection and quantification. See Figure 1, which highlights the sensitivity and reproducibility achieved by this method at 1 ng/mL (0.025 µg/g).

Key features of the NDMA analysis in azithromycin drug products using the QTRAP 4500 system

- Robust quantification of NDMA at 0.5 ng/mL in solution (0.013 µg/g in sample) was achieved, which is below the limit of 0.030 µg/g for drug products with a maximum daily dosage of 880 mg/day
- Excellent accuracy and reproducibility were attained for spike-recovery studies using various drug product matrices, demonstrating feasibility of the assay for quantification
- NDMA was quantified across a linear range that spanned 0.5 to 100 ng/mL, with an r value >0.99

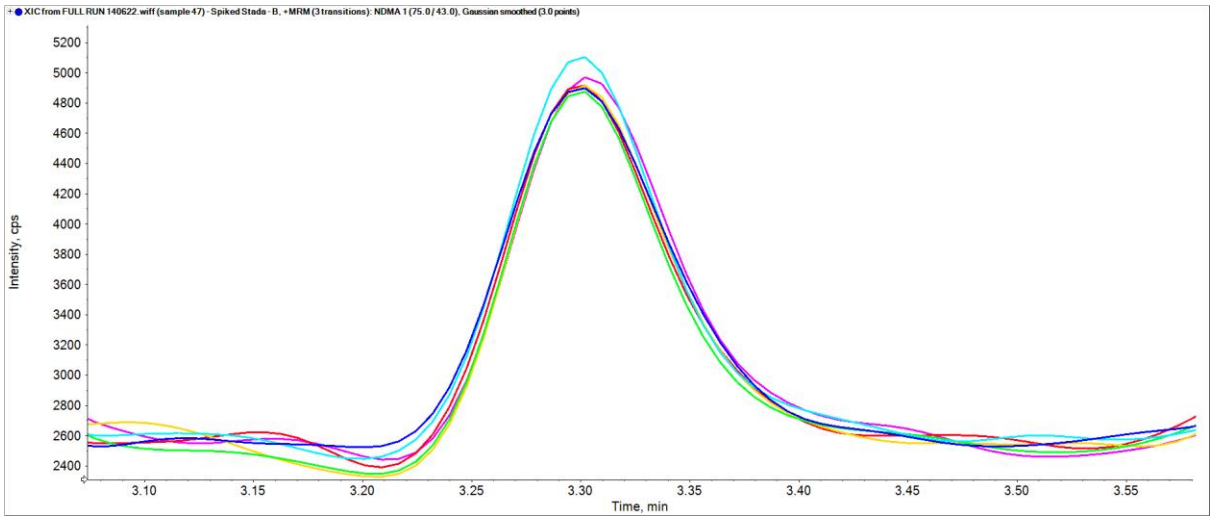

Figure 1. Extracted ion chromatograms (XICs) of 6 consecutive injections of 1 ng/mL (0.025 µg/g) of NDMA spiked in an azithromycin drug product. High reproducibility was observed for the quantification of NDMA. The %CV was 3.40%, well within set specifications for trace analysis.

Methods

Standard preparation: A 1000 µg/mL NDMA stock solution was diluted in water to cover a range between 0.5 and 100 ng/mL.

Sample preparation: Approximately 100 mg of the crushed azithromycin tablet was weighed and diluted with water to achieve a concentration of 40 mg/mL. The solution was vortexed for 30 seconds before sonication for 15 minutes. The solution was again vortexed for 30 seconds before centrifugation for 5 minutes at the highest centrifuge speed (10,416 rcf). The supernatant was filtered through a 0.2 µm cellulose acetate filter and transferred to a HPLC vial for analysis.

Spiked sample preparation: Approximately 100 mg of the crushed azithromycin tablet was weighed and 2475 µL of water was added before adding 25 µL of a 100 ng/mL standard solution. The solution was vortexed for 30 seconds before being sonicated for 15 minutes. The solution was again vortexed for 30 seconds before centrifuging for 5 minutes at the highest centrifuge speed (10,416 rcf). The supernatant was filtered through a 0.2 µm cellulose acetate filter and transferred to a HPLC vial for analysis (40 mg/mL sample concentration, 1 ng/mL spike concentration).

Chromatography: An ExionLC system was used with a Phenomenex Kinetex biphenyl analytical column (2.6 µm, 150 x 4.6 mm). Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in methanol. The flow rate was 0.8 mL/min and the injection volume was 30 µL. The column temperature was set to 40°C. The gradient used is summarized in Table 1.

Table 1. LC gradient.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.00	*80*	*20*
1.00	*80*	*20*
6.00	*55*	*45*
7.00	*1*	*99*
12.00	*1*	*99*
12.10	*80*	*20*
15.00	*80*	*20*

Mass spectrometry: A QTRAP 4500 system, operating in positive ion mode and using atmospheric pressure chemical ionization (APCI), was used for analysis. Table 2 outlines the compound-specific MRM parameters used for analysis.

Table 2. MRM conditions for NDMA analysis.

Compound	Q1 (*m/z*)	Q3 (*m/z*)	Dwell (ms)	DP (V)	CE (V)	CXP (V)
NDMA 1	*75.0*	*43.0*	*200*	*50*	*21*	*19*
NDMA 2	*75.0*	*58.0*	*200*	*40*	*17*	*6*

Note: *NDMA 1 was used as the quantifier transition and NDMA 2 was used as the qualifier transition. The DP for NDMA 1 is slightly higher than NDMA 2 to provide background reduction.*

Data processing: All data were processed using SCIEX OS software and the autopeak integration algorithm. A weighting of 1/x was applied for quantification.

Table 3. Standard criteria assessed. Overview of the criteria assessed for the analysis when using an external standard calibration curve with a 1/x weighting. S/N was assessed using the peak-to-peak calculation. All data presented are based on the quantifier transition.

Parameter	Result
r value	*>0.99*
Range	*0.50–100 ng/mL*
LLOQ	*0.50 ng/mL*
%CV – LLOQ (n=3)	*6.57*
%CV – 1 ng/mL (0.025 µg/g) (n=6)	*4.90*
S/N – LLOQ	*17.3*
Average % accuracy at 1 ng/mL (0.025 µg/g) (n=6)	*99.9*
Average % accuracy at the LLOQ (n=3)	*103*

Results

In nitrosamine analysis, the limit of quantification is important to meet the limits set by regulatory agencies, such as the Federal Drug Administration (FDA) and the European Medicines Agency (EMA). According to these agencies, nitrosamine impurities in drug products must not exceed 0.03 µg/g when the maximum daily dosage is <880 mg/day.[2] This limit was considered to assess the presented method to ensure this level was met or exceeded. Figure 1 shows extracted ion chromatograms (XICs) from 6 consecutive injections of NDMA spiked into sample near the regulatory limit. An XIC of the quantifier transition for NDMA at the LLOQ of 0.5 ng/mL is shown in Figure 2. With this preparation, a concentration of 0.5 ng/mL equates to a concentration of 0.013 µg/g, demonstrating that NDMA can be quantified in sample at levels lower than the 0.03 µg/g limit.

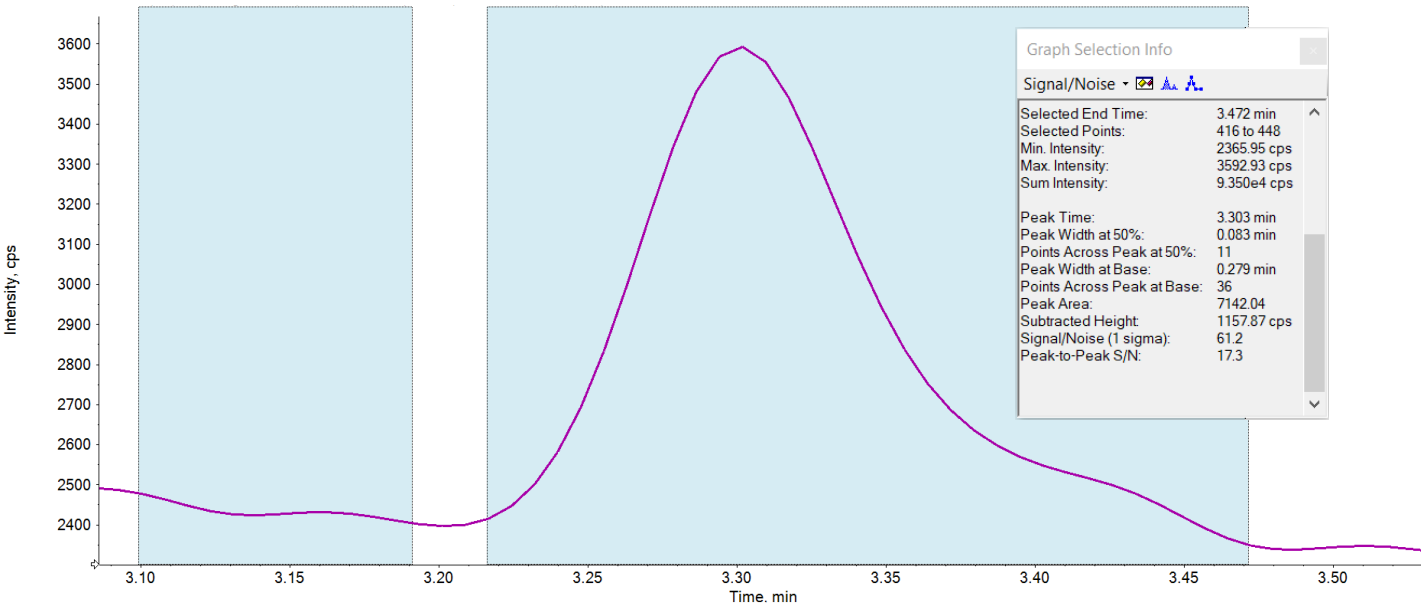

Figure 2. An XIC of the quantifier transition for NDMA at the LLOQ of 0.5 ng/mL. The above XIC shows NDMA at 0.5 ng/mL and includes the peak-to-peak graph selection pane from the Explorer module of SCIEX OS software that highlights a peak-to-peak S/N of 17.3x. According to the ICH Q2(R2) guidelines, a 10:1 S/N is considered acceptable at the quantification limit.[4]

In addition to sensitivity, several other criteria were assessed, including linearity, precision and accuracy. The linearity of the calibration curve spanned 0.5–100 ng/mL with an r value >0.99. At all concentrations measured, accuracy values were between 90% and 110%. Precision and accuracy were assessed at both the LLOQ (0.5 ng/mL) and just below the specification level, at 1 ng/mL (0.025 µg/g in sample, based on a limit of 0.030 µg/g). At both concentration levels, the %CV was <10% and accuracy values were between 95% and 105%. Table 3 summarizes the standard criteria assessed and Figure 3 shows the calibration curve that spans 0.5 to 100 ng/mL.

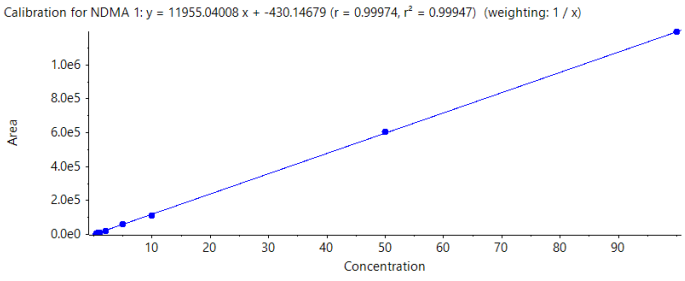

Figure 3. The calibration curve of the quantifier transition for NDMA. Linearity spanned a range of 0.5–100 ng/mL with an r value >0.99.

Ion ratios were assessed using 2 transitions to improve the specificity of the analysis and confirm the identification of the target analyte.

Un-spiked and spiked samples were analyzed to assess the levels of NDMA in the final drug products from 3 different manufacturers. For all batches analyzed, NDMA was not detected. Spike recovery and precision were assessed at 1 ng/mL (0.03 µg/g). The spike recovery for all samples was between 85% and 115%, with %CV <5% across the 3 different manufacturers. These results highlight the high level of recovery and precision achieved using the presented workflow. Table 4 summarizes the % spike recovery and precision for all the spiked samples analyzed.

Table 4. Spike recovery results of samples spiked at 1 ng/mL in 3 drug products. The % spike recovery was between 85% and 115% across all the batches analyzed, with %CV values <5%. N=6.

Manufacturer	% Spike recovery	Average %CV of calculated concentration
A	*104–114*	*3.40*
B	*103–113*	*3.07*
C	*103–112*	*3.16*

Conclusions

- Demonstration of a robust and sensitive quantitative method for NDMA analysis in multiple azithromycin drug products
- Linearity was achieved between 0.5 and 100 ng/mL with an r value >0.99
- Excellent accuracy and precision were reached in external standard and spiked drug product at a concentration of 1 ng/mL

References

1. Assessment report – Nitrosamine impurities in human medicinal products – European Medicines Agency – June 2020
2. Control of Nitrosamine Impurities in Human Drugs – Guidance for Industry - February 2021
3. European Medicines Agency, 2022 – Nitrosamine impurities
4. ICH guideline Q2(R2) on validation of analytical procedures – 31 March 2022

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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