Quantification of genotoxic nitrosamines in a telmisartan drug product

Using the SCIEX 5500+ system

Sandeep Choudhary,[1] Pankaj Partani,[1] Manoj Pillai,[1] Jack Steed[2]
[1]SCIEX, India; [2]SCIEX, UK

Telmisartan is a drug substance that is susceptible to the presence of nitrosamine impurities. Based on results from animal testing, nitrosamines are classified as a probable carcinogen by the World Health Organization/International Agency for Research on Cancer. Since 2018, when the US Food and Drug Administration (FDA) issued an alert on the presence of nitrosamines in the angiotensin receptor blockers losartan and valsartan, nitrosamines have been detected in several other active pharmaceutical ingredients (APIs), including telmisartan, candesartan, ranitidine and metformin. Recently, the FDA released industry guidance regarding the "control of nitrosamine impurities in human drugs."[1] The nitrosamine compounds currently in scope with this guidance include N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethylisopropylamine (NEIPA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitrosomethylphenylamine (NMPA) and N-nitrosodibutylamine (NDBA), with N-nitrosodipropylamine (NDPA) being included to show separation with its isomer, NDIPA. This list may also expand as more drug products are tested. A recent example is the detection of 1-cyclopentyl-4-nitrosopiperazine (CPNP) in rifapentine and 1-methyl-4-nitrosopiperazine (MNP) in rifamycin, with regulatory authorities now requiring testing for these drug products.

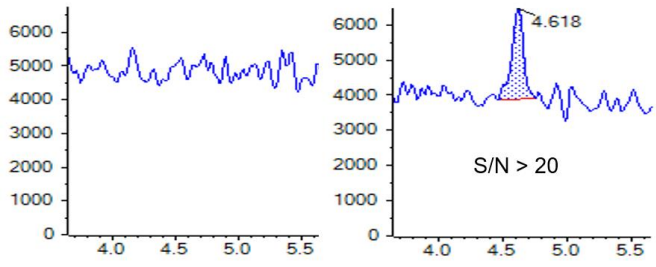

Figure 1. Low-level detection of NDMA in a telmisartan drug product. MRM chromatograms for blank matrix (left) and the lower limit of quantification **(**LLOQ), which is 0.25 ng/mL (right), are shown for NDMA in a telmisartan drug product.

The FDA recommends that manufacturers prioritize the evaluation of APIs and drug products based on factors such as maximum daily dose, duration of treatment, therapeutic indication and the number of patients treated.

This technical note describes a method for the detection and quantification of 8 nitrosamines in telmisartan using the SCIEX 5500+ system, which enables robust and accurate monitoring of nitrosamines at levels below regulatory action limits. While the method described here was developed for the telmisartan drug product, it can also be applied to other sartan API/drug products with slight chromatographic modifications. See figure 1 which highlights the sensitivity of the SCIEX 5500+ system.

Key features of the method for nitrosamine detection and quantification

- A single method for the simultaneous quantification of 8 nitrosamine impurities was developed for telmisartan tablets.
- Optimized chromatography separates the 8 nitrosamine impurities from the telmisartan, lowering ion suppression and minimizing matrix effects.
- Excellent sensitivity allows for the detection of nitrosamines down to the 0.005 ppm (µg/g) level with the ability to go lower if needed by increasing sample concentration.

Methods

Sample preparation: Working standards were prepared in water by serial dilution from a 1.0 mg/mL stock solution to provide a mixed standard with a concentration range of 0.1–100 ng/mL.

Sample and spiked sample preparation: A sufficient amount of powdered tablet was weighed and diluted to create a 20 mg/mL solution in water that was then vortexed for 20 mins to mix. Similarly, the powered tablet solution at 20 mg/mL was spiked with all nitrosamines analyzed to provide a final spike concentration of 0.60, 0.20 and 0.10 ng/mL.

Chromatography: The gradient was developed to separate all of the analytes in the panel from the drug product and excipients to prevent ion suppression. A divert step was also included post-column to prevent large amounts of the drug product from entering the mass spectrometer (MS) system. The column used was a Phenomenex Kinetex 2.6 µm biphenyl, 150 x 4.6 mm. Details of the chromatography are outlined in the supplementary information.[3] A representative chromatogram is shown in Figure 2.

Mass spectrometry: The SCIEX 5500+ system was operated in positive atmospheric pressure chemical ionization (APCI) mode using Analyst software. The source-dependent and compound-dependent parameters (DP, EP, CE and CXP) were optimized as necessary. Details of the MS conditions are outlined in the supplementary information.[3]

Data processing: SCIEX OS software was used for data processing.

Optimized chromatography

Using the established gradient, good separation was achieved between all of the nitrosamines analyzed as well as between the excipients and telmisartan drug product (Figure 2). Due to the high level of drug product (20 mg/mL), it is critical to achieve separation to reduce the risk of ion suppression. The telmisartan eluted after the elution of the last nitrosamine, NDBA. This allows an eluant divert step to be included in the method, diverting the high concentration of telmisartan to waste rather than into the MS system.

Quantitative accuracy

Concentration curves were generated in water to evaluate the sensitivity of the developed assay. Linearity, precision and accuracy were all evaluated as part of the method verification as well as the recovery in matrix at the limit of quantification and at the FDA-specified limit of daily exposure (0.03 ppm in API).

Linearity of all 8 compounds was assessed, with all compounds showing >0.99 r values. Table 1 shows the linearity range achieved, lower limit of detection (LLOD), lower limit of quantification (LLOQ) and correlation values of all 8 nitrosamines. The current specification level (0.03 ppm or 0.6 ng/mL) is easily achieved with this method.

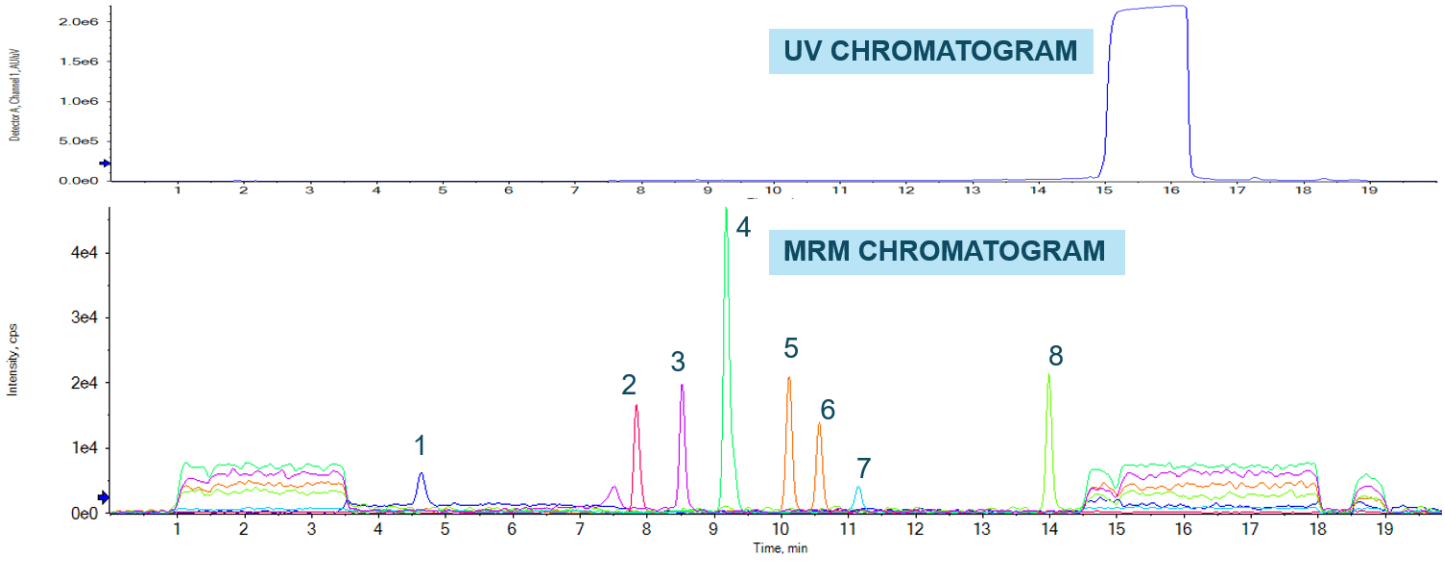

Figure 2. High-quality separation of nitrosamines from a telmisartan drug product. A representative UV chromatogram of 20 mg/mL of telmisartan (top) and overlaid extracted ion chromatograms (XICs) of each nitrosamine analyzed at the specification level of 0.60 ng/mL, or 0.03 ppm (bottom). 1. NDMA, 2. NMBA, 3. NDEA, 4. NEIPA, 5. NDIPA, 6. NDPA, 7. NMPA, 8. NDBA.

Table 1. Linearity range, LLOD, LLOQ, signal to noise (S/N) and r value for all the nitrosamines analyzed. S/N was calculated using the standard deviation algorithm within SCIEX OS software.

Component name	LLOD (ng/mL)	LLOQ (ng/mL) [S/N]	Linearity range (ng/mL) [ppm]	r correlation
NDMA	*0.10*	*0.25 [22]*	*0.25–100 [0.01–5]*	*0.99863*
NMBA	*0.05*	*0.10 [88]*	*0.10–100 [0.005–5]*	*0.99956*
NDEA	*0.05*	*0.10 [24]*	*0.10–100 [0.005–5]*	*0.99748*
NEIPA	*0.05*	*0.10 [280]*	*0.10–100 [0.005–5]*	*0.99917*
NDIPA	*0.05*	*0.10 [28]*	*0.10–100 [0.005–5]*	*0.99887*
NDPA	*0.05*	*0.10 [15]*	*0.10–100 [0.005–5]*	*0.99997*
NMPA	*0.05*	*0.10 [21]*	*0.10–100 [0.005–5]*	*0.99633*
NDBA	*0.05*	*0.10 [52]*	*0.10–100 [0.005–5]*	*0.99912*

Table 2. Accuracy, precision and recovery at the specification level, which is 0.03 µg/g, equivalent to 0.6 ng/mL in solution (N=6).

Analyte	Concentration (ng/mL)	Accuracy (%)	Precision (% CV)	Spike recovery (%)
NDMA	*0.60*	*95.08*	*9.63*	*98*
NMBA	*0.60*	*97.49*	*3.59*	*107*
NDEA	*0.60*	*99.75*	*3.53*	*96*
NEIPA	*0.60*	*102.69*	*1.66*	*85*
NDIPA	*0.60*	*103.05*	*3.44*	*84*
NDPA	*0.60*	*100.69*	*4.32*	*82*
NMPA	*0.60*	*89.24*	*8.78*	*105*
NDBA	*0.60*	*99.93*	*2.88*	*82*

Accuracy, precision and recovery results obtained at the specification level are shown in Table 2. Similarly, the accuracy, precision and recovery results obtained at the determined LLOQ level are shown in Table 3. MRM signal obtained at the LLOQ is also highlighted in Figure 3. The accuracy of the method is between 85% and 110% and precision is <15% at LLOQ levels for all the nitrosamines, while at specification the accuracy was between 89% and 103% and precision is <10%. This shows that the method is easily capable of achieving specification limits for a trace level analysis.

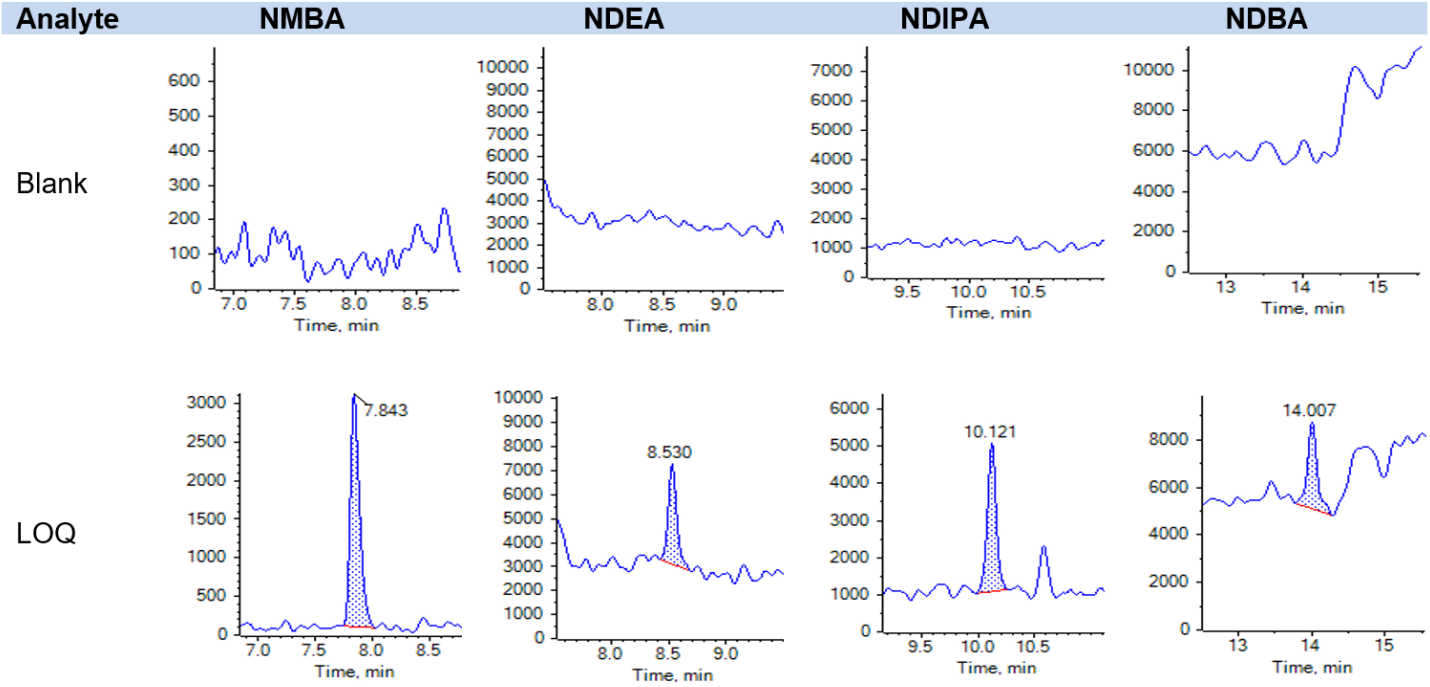

Figure 3. MRM chromatograms highlighting signal obtained at the LLOQ. Representative blank and LLOQ (0.1 ng/mL) level XICs of 4 representative nitrosamines (NMBA, NDEA, NDIPA and NDBA).

Table 3. Accuracy, precision and recovery at the LLOQ for each nitrosamine analyzed (N=6).

Analyte	Concentration (ng/mL)	Accuracy	Precision (% CV)	Spike recovery (%)
NDMA	*0.25*	*109.81*	*14.65*	*97*
NMBA	*0.10*	*105.69*	*6.32*	*109*
NDEA	*0.10*	*101.91*	*11.27*	*102*
NEIPA	*0.10*	*92.31*	*5.58*	*84*
NDIPA	*0.10*	*92.17*	*13.65*	*106*
NDPA	*0.10*	*90.42*	*13.71*	*87*
NMPA	*0.10*	*94.70*	*14.45*	*108*
NDBA	*0.10*	*85.75*	*16.04*	*96*

Conclusions

The method described is capable of accurately detecting and quantifying 8 nitrosamines in a telmisartan drug product at levels well below the current FDA specifications using an external calibration curve, verified by the use of spiked sample to ensure accurate quantification. Implementation of this method on the QTRAP 5500+ system will enable the monitoring of the 8 listed nitrosamine compounds to help ensure fewer costly product recalls and unnecessary patient exposure to these genotoxic compounds.

References

1. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Control of Nitrosamine Impurities in Human Drugs: Guidance for Industry, February 2021.
2. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline. Validation of analytical procedures: text and methodology Q2(R1), June 2014.
3. Download Supplementary information.

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500 Old Connecticut Path | Framingham, MA 01701 USA
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