Varenicline nitrosamine drug substance-related impurity (NDSRI) quantification in a varenicline drug product

Using the QTRAP 5500+ system

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

Varenicline tartrate is an active pharmaceutical ingredient (API) found within some smoking cessation medications. In 2021, it was discovered that varenicline tartrate can form a nitrosamine-related impurity that can potentially be carcinogenic.[1] Therefore, the medication was voluntarily recalled to mitigate the adverse effects of administering the drug product. In response, the FDA determined an acceptable daily intake level of 37 ng/day[1] and released a method to analyze the varenicline NDSRI in both a varenicline drug substance and drug product.[2]

The current limit of 37 ng/day equates to 18.5 µg/g (ppm) when using the equation below and taking the maximum daily dose of 2 mg (2 x 1 mg tablet) per day into account.[3,4]

$$\mu g/g\ (PPM) = Acceptable\ Intake\ (AI, ng)/Maximum\ Daily\ Dose\ (MDD, mg)$$

Due to the possible carcinogenicity of the impurity, it is important to provide a sensitive and robust method to analyze the compound within a varenicline drug product to therefore control the amount of the impurity delivered to the patient.

Here, an assay was developed that can quantify a varenicline NDSRI specifically and sensitively in a varenicline drug product using the QTRAP 5500+ system. This assay meets or exceeds current guidelines.

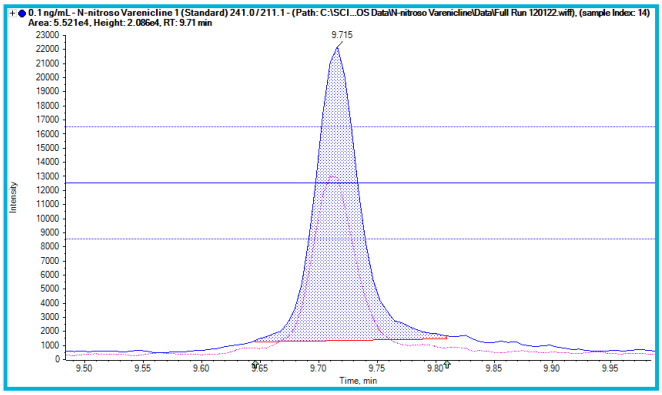

Figure 1. Lower limit of quantification for varenicline. An overlaid XIC of the quantifier and qualifier transitions for varenicline NDSRI at the LLOQ of 0.1 ng/mL. The ion ratio lines show the ±30% tolerance allowed for the qualifier ion to ensure accurate quantification.

Key features of the QTRAP 5500+ system for varenicline NDSRI analysis

- High sensitivity was achieved on the QTRAP 5500+ system, evidenced by LLOD and LLOQ values as low as 0.02 and 0.10 ng/mL, respectively
- Varenicline NDSRI was quantified at levels below the limit of 37 ng/day, as outlined by the FDA
- An average spike recovery value of 102.0% was within typical specifications
- A high level of specificity was achieved when using 2 MRM transitions and ion ratio values
- A simple sample preparation provided consistent results across multiple preparations of the un-spiked sample
- A linear range was achieved between 0.1 and 50 ng/mL with an r value >0.99

Methods

Standard preparation: Approximately 5 mg of the varenicline NDSRI (purchased from LGC) was weighed and diluted with methanol to achieve a concentration of 1 mg/mL. The solution was vortexed for 30 seconds before sonicating for approximately 10 minutes to dissolve. Once dissolved, solutions were prepared, ranging in concentration from 0.02 to 50 ng/mL.

Sample preparation: A suitable amount of varenicline tablets were crushed. Approximately 5 mg of the crushed tablet was weighed and diluted with methanol to achieve a concentration of 1 mg/mL. 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 PTFE filter and transferred to a HPLC vial for analysis.

Spiked sample preparation: A suitable amount of varenicline tablets were crushed. Five mg of the crushed tablets was weighed, and 4.9 mL of methanol was added before adding 100 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 PTFE filter and transferred to a HPLC vial for analysis (1 mg/mL sample concentration, 2 ng/mL spike concentration).

Chromatography: An ExionLC AD system was used with a Phenomenex Kinetex biphenyl analytical column (2.6 µm, 150 x 4.6 mm). Refer to the SCIEX How method for more chromatographic details.[5]

Mass spectrometry: A QTRAP 5500+ system, operating in positive ion mode and using electrospray ionization (ESI), was used for analysis. See the SCIEX How method for MRM details.[5]

Data processing: All data were processed using SCIEX OS software.

Assessing standard criteria

Several parameters were evaluated to assess the validity of this method for analyzing varenicline NDSRI in a varenicline drug product (tablet formulation). A summary of the standard criteria assessed is presented in Table 1. The analysis was performed using an external calibration curve that spanned concentrations ranging from 0.1 to 50 ng/mL. An overlay of the linearities achieved by quantifier and qualifier transitions is shown in Figure 2. Both quantifier and qualifier transitions achieved r values >0.99.

Table 1. Calibration curve results. Overview of the standard criteria assessed for the analysis of the external standard calibration curve with $1/x^2$ weighting. S/N was assessed using the peak-to-peak calculation. All data presented are based on the quantifier transition.

Parameter	Result
r value	*0.99924*
LLOQ	*0.1 ng/mL*
%CV - LLOQ (n=6)	*1.80*
S/N - LLOQ	*55.7*
LLOD	*0.02 ng/mL*
S/N - LLOD	*8.8*
Average % accuracy at the LLOQ (n=6)	*101.11*

An LLOD of 0.02 ng/mL and an LLOQ of 0.1 ng/mL for varenicline NDSRI were achieved, as shown in Table 1. These values were reliable, based on the peak-to-peak signal-to-noise (S/N) ratio. The S/N ratios at the LLOD and LLOQ were 8.8 and 55.7, respectively. These ratios exceeded the minimum requirements of 3 for LLOD and 10 for LLOQ. Lower LLOD and LLOQ values might have been possible to achieve, however, a conservative approach was used due to a slight hump in the blank extracted ion chromatogram (XIC) at the retention time of the analyte. Figure 3 shows XICs for the blank control and samples at the LLOD and LLOQ.

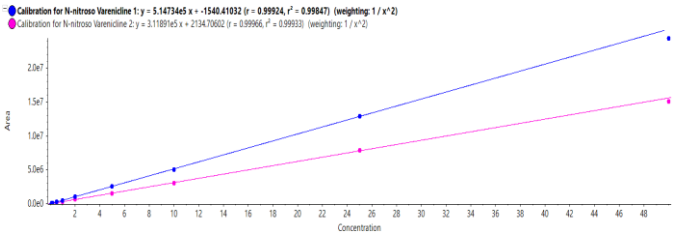

Figure 2. Overlaid linearities of the quantifier (blue) and qualifier (pink) ions. An r value >0.99 was achieved for the linearity of both the quantifier and qualifier ions.

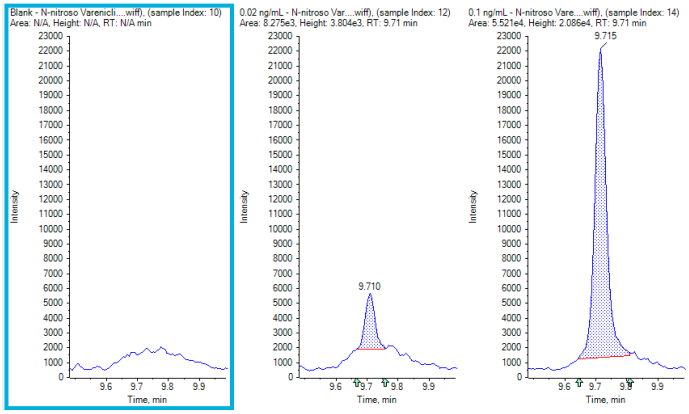

Figure 3. Quantification of varenicline NDSRI in a varenicline drug product. Quantifier ion XICs of the blank (left) and at the LLOD (0.02 ng/mL, middle) and LLOQ (0.1 ng/mL, right). Clear peaks are seen at the expected retention time for the LLOD and LLOQ.

Precision was assessed at the LLOQ of 0.1 ng/mL by performing 6 injections and calculating the %CV of the LLOQ concentration. As shown in Table 1, for this application, the %CV at the LLOQ was 1.80%, indicating that the measurements were highly precise. Moreover, an average accuracy of 101.11% was calculated for the 6 replicate injections analyzed, spanning a range from 99.44 to 104.08%. Both precision and accuracy values calculated were within acceptable criteria, as a %CV less than 10% and accuracy within ±30% of the expected value are considered typical.

Assessing matrix criteria

To maximize the specificity of the analysis, 2 MRM transitions were used. The ion ratios for the varenicline NDSRI-spiked samples were similar to the ratios observed in the standard solution, indicating that the sample contributed minimal interference.

Figure 4 shows the overlaid XICs of the quantifier and qualifier ions with added ion ratio lines to indicate the set tolerance of ±30%. This analysis is easily performed in the Analytics module of the SCIEX OS software, which flags samples if the ion ratio tolerances are exceeded.

Table 2. Deviation of individual un-spiked sample injections from the mean.

Sample name	Amount (µg/g)	Average amount (µg/g)	% Difference from the average
Un-spiked sample A1	*1.68*	*1.75*	*3.7*
Un-spiked sample A2	*1.73*		*0.9*
Un-spiked sample B1	*1.75*		*0.3*
Un-spiked sample B2	*1.82*		*4.3*

Spiking of the varenicline drug product was performed at 2 ng/mL (2 µg/g) to assess the recovery and precision in matrix. The sample was spiked at this level because a similar amount was found in the un-spiked sample (1.75 µg/g) therefore, permitting an accurate representation of spike recovery in sample. Figure 4 shows the amount of varenicline NSDRI present in both the un-spiked and spiked samples.

To evaluate the spike recovery of the method, the varenicline drug product was prepared for 2 un-spiked and 2 spiked samples. Each un-spiked sample was injected in duplicate and the spiked preparations were injected in triplicate to determine precision. Table 3 provides an overview of the amount of varenicline NSDRI detected, %CV values and spike recovery values determined. A %CV value of <10% and accuracy within ±30% of the expected value were achieved.

The variability of the un-spiked sample preparation was assessed to ensure that the amount in sample was consistent between different preparations. Table 2 demonstrates high consistency between the 2 un-spiked preparations and 4 injections analyzed, as all values deviated less than 5% from the average. This result indicates that the sample preparation is consistent and within an acceptable deviation from the mean.

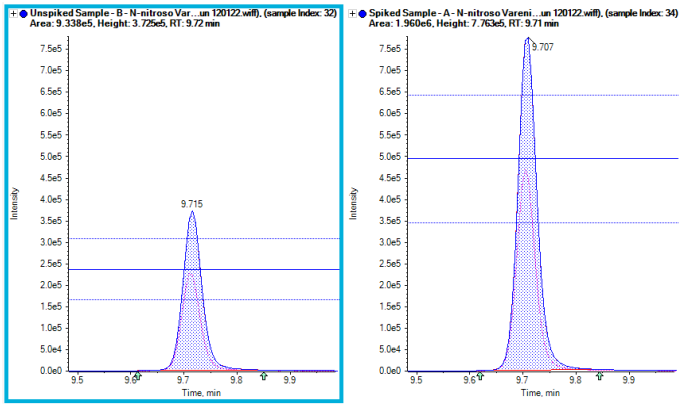

Figure 4. Varenicline NDSRI XICs for un-spiked and spiked samples. (Left) Overlaid XICs of varenicline NDSRI from the un-spiked sample. (Right) Sample spiked with varenicline NSDRI, showing the overlays of the quantifier (blue) and qualifier (pink) ions. Ion ratio lines highlight the ±30% tolerance allocated for the ratio between the quantifier and qualifier fragments. The un-spiked sample was determined to contain 1.75 µg/g varenicline NSDRI. Therefore, the sample was spiked at a suitable level to ensure accurate spike recovery could be calculated (2 µg/g).

Table 3. The un-spiked and spiked sample results.

Sample type	Average amount in un-spiked sample (µg/g)	Average amount in spiked sample (µg/g)	%CV of spiked sample (µg/g, n=6)	Average % spike recovery (n=6)
Varenicline tablet	*1.75*	*3.79*	*1.33*	*102.0*

Conclusions

- A suitable method was developed that can detect varenicline NDSRI in a varenicline drug product and can quantify it at and below the recommended 37 ng/day limit specified by the FDA
- An LLOD value of 0.02 ng/mL and an LLOQ value of 0.1 ng/mL highlight the excellent sensitivity provided by the QTRAP 5500+ system
- An r value >0.99 across the range of 0.1 to 50 ng/mL demonstrates accurate quantification
- An average spike recovery value of 102.0% highlights reliable quantification in the varenicline sample matrix
- The use of ion ratio values increased specificity and ensured accurate quantification in matrix.

References

1. FDA updates and press announcements on nitrosamine in varenicline (chantix) – September 2021
2. Liquid chromatography-high resolution mass spectrometry (LC-ESI-HRMS) method for the determination of varenicline nitroso-drug substance related impurity (NDSRI) in chantix™ drug product and varenicline drug substance – August 2021
3. Control of nitrosamine impurities in human drugs – guidance for industry - February 2021
4. Chantix – how to take chantix
5. Download the SCIEX How method.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices