U.S. FOOD & DRUG ADMINISTRATION

Contains Nonbinding Recommendations

Liquid Chromatography-High Resolution Mass Spectrometry (LC-ESI-HRMS) Method for the Determination of Varenicline Nitrosamine Drug Substance-Related Impurity (NDSRI) in Varenicline Drug Product and Drug Substance

Background:

Varenicline tartrate is the active pharmaceutical ingredient (API) in varenicline drug products. The potential for the presence or formation of *N*-nitroso-varenicline has been identified in the drug product. To help ensure the safety and quality of varenicline tartrate drug products and drug substance, the agency has developed and validated a method to determine the presence or absence of varenicline Nitrosamine Drug Substance-Related Impurity (NDSRI). The structure for varenicline NDSRI is shown in Figure 1 below.

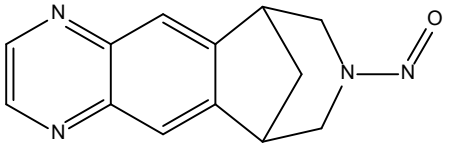

Figure 1: Varenicline NDSRI

Conclusions:

A reverse phase LC method with HRMS detection was developed and validated for the determination of varenicline NDSRI in varenicline tartrate drug product and drug substance. The method was validated according to ICH Q2 (R1). Method verification and/or re-validation is recommended prior to use to demonstrate that the method is suitable for its intended purpose. The limit of detection (LOD), limit of quantitation (LOQ) and range of the method are summarized below:

	Varenicline NDSRI
Limit of Detection (LOD)	0.2 ppm
Limit of Quantitation (LOQ)	1.0 ppm
Range	1.0 – 200 ppm

LC-ESI-HRMS Method for the Determination of Nitrosamine Drug Substance-Related Impurity (NDSRI) in Varenicline Tartrate Drug Product and Drug Substance

Purpose
This method was developed and validated to quantitate varenicline NDSRI in varenicline tartrate drug product and drug substance.

Principle
Varenicline NDSRI was separated from varenicline tartrate by reverse phase chromatography and was detected by a high-resolution and high-mass accuracy (HRAM) mass spectrometer. High sensitivity detection was achieved by monitoring the accurate *m/z* value of the protonated impurity ion. Quantitation was performed by comparing the peak area of the varenicline NDSRI in extracted ion chromatogram (with *m/z* tolerance of ± 15 ppm) of the samples, to the peak area of the varenicline NDSRI reference standard in an external standard calibration.

Reagents
- *N*-Nitroso-Varenicline Reference Standard
- Methanol, LC/MS grade
- Water, LC/MS grade or equivalent
- Formic Acid, LC/MS grade

Equipment/Instrument
- HPLC or UHPLC system equipped with temperature-controlled autosampler and column compartment
- Q Exactive™ hybrid quadrupole-orbitrap mass spectrometer (Thermo-Fisher Scientific) or equivalent
- HPLC column: XSelect CSH Phenyl-Hexyl XP, 2.5 µm 130 Å, 150 x 4.6 mm (Waters Part No. 186006735 or equivalent)
- Analytical Balance
- Vortex Mixer
- 15 mL glass centrifuge tubes
- Wrist action shaker
- 0.22 µm PVDF syringe filters
- Centrifuge
- HPLC vials

Mobile Phase A: Water, 0.1% Formic Acid

Mobile Phase B: Methanol, 0.1% Formic Acid

Diluent and Blank: Methanol

Stock Standard Preparation
Accurately weigh 10 ± 3 mg of varenicline NDSRI reference standard and transfer into a 100 mL volumetric flask. Dilute to volume with methanol and mix using a stir bar and plate until

dissolved. Prepare in duplicate. Label as Stock Std #1 and Stock Std #2.

Intermediate Stock Standard A
Transfer the appropriate aliquot volume of each of the stock standards into separate volumetric flasks to get a target concentration of 1000 ng/mL. Dilute to volume with methanol.

Intermediate Stock Standard B (100 ng/mL)
Transfer 5.0 mL aliquot volume of each of the intermediate stock standard A into separate 50 mL volumetric flasks and dilute to volume with methanol.

Working Standard and QC Standard Preparation (1 ng/mL)
Transfer 1.0 mL aliquot volume of each of the intermediate stock standard B into separate 100 mL volumetric flasks and dilute to volume with methanol. Designate one standard as the working standard and the other as the QC standard. Prepare fresh daily.

Drug substance sample preparation
Accurately weigh 43 ± 4 mg of varenicline tartrate drug substance and quantitatively transfer into a 50 mL volumetric flask. Dilute to volume with methanol and mix the solution using a stir bar and plate until fully dissolved. Filter the solution using a 0.22 µm PVDF syringe filter and transfer the filtered sample into an hplc vial for LC/MS analysis.

Drug product sample preparation
Crush the appropriate number of tablet(s) to obtain a target concentration of 0.5 mg/mL as varenicline in methanol, and transfer into a 15 mL glass centrifuge tube. Add the appropriate volume of methanol and mix for about a minute using a vortex mixer. Shake the sample for 40 minutes using a mechanical wrist action shaker.

After extraction, centrifuge the sample for 15 minutes at 4500 rpm. Filter the supernate using a 0.22 µm PVDF syringe filter into an HPLC vial for LC/MS analysis.

Chromatographic Conditions

HPLC Column	XSelect CSH Phenyl-Hexyl XP, 2.5 µm 130 Å, 150 x 4.6 mm (Waters Part # 186006735 or equivalent)		
Column Temp.	30 °C		
Flow Rate	0.5 mL/min		
Mobile Phase A	Water, 0.1% Formic Acid		
Mobile Phase B	Methanol, 0.1% Formic Acid		
Gradient	**Time (min)**	**A%**	**B%**
	0	70	30
	1.0	70	30
	6.0	20	80
	9.5	20	80
	10.0	0	100
	11.0	0	100
	11.1	70	30
	15.0	70	30

Injection Volume	5 µL
Autosampler Temp.	4 - 8 °C
Needle Wash	Methanol

Mass spectrometer conditions

- Instrument
  Q Exactive™ mass spectrometer (Thermo-Fisher)
- ESI Ion Source Settings

Sheath Gas Flow Rate	50 arbitrary units
Aux Gas Flow Rate	15 arbitrary units
Sweep Gas Flow Rate	0 units
Spray Voltage	3.5 kV
Capillary Temp.	350 °C
Aux Gas Heater Temp.	350 °C

- Scan Settings

Parameters	Varenicline NDSRI
Scan Type	PRM
Polarity	Positive
Scan Start -End (min)	0 – 15
Isolation Window	1.0 *m/z*
Microscans	1
Resolution	70,000
AGC target	1e6
Maximum IT	100 ms

Inclusion List

Mass (*m/z*)	Polarity	Start (min)	End (min)	Comment
241.1084	Positive	8.1	9.2	Varenicline NDSRI

Injection Sequence

- Inject Blank (use diluent) at least once at the beginning of a sequence
- Inject the Working Standard for six consecutive times
- Inject the QC Standard before injecting any samples
- Inject the QC Standard once every six injections of the samples and at the end of a sequence.

Example:

Order	Solution	No. of Injections
1	Blank	2
2	Working Standard	6
3	QC Standard	1
4	Blank	1
5	Sample 1	1
6	Sample 2	1
7	Sample 3	1
8	Sample 4	1
9	Sample 5	1
10	Sample 6	1
11	QC Standard	1
…	…	…

System Suitability

- The % RSD (n = 6) of the varenicline NDSRI peak areas for the first six injections of the working standard solution should not be more than 10%.
- The % recovery of the QC Standard should be between 85 – 115%.

Data Processing

- Varenicline NDSRI peak areas from the extracted ion chromatograms (EIC) with a *m/z* tolerance of ±15 ppm are used for quantitation. The varenicline NDSRI *m/z* values to be extracted are listed below:

Varenicline NDSRI	
m/z to be extracted	211.1105 169.0762

- The retention time difference of the varenicline NDSRI peak in the analyzed samples should not be more than 2% of the retention time of the corresponding varenicline NDSRI peak in the reference standard solution.

Calculation

Drug Substance:

$$\text{Varenicline NDSRI (ppm)} = \frac{A_{spl}}{As} \times C_s \times \frac{1\ mg}{1 \times 10^6\ ng} \times \frac{V}{W} \times 10^6$$

where: A_{spl} = Area of the varenicline NDSRI peak in the sample solution
As = Average area (n = 6) of the varenicline NDSRI peak from the first six consecutive injections of the working standard
C_s = Concentration of the varenicline NDSRI in the working standard (ng/mL)
W = Weight of drug substance (mg) as varenicline

Varenicline Tartrate MW = 361.3 g/mol
Varenicline MW = 211.26 g/mol
V = Volume of the diluent in the sample solution (mL)

Drug Product:

$$\text{Varenicline NDSRI (ppm)} = \frac{A_{spl}}{As} \times C_s \times \frac{1\ mg}{1\times10^6\ ng} \times \frac{1}{0.5\ mg/mL} \times 10^6$$

where: A_{spl} = Area of the varenicline NDSRI peak in the sample solution
As = Average area (n = 6) of the varenicline NDSRI peak from the first six consecutive injections of the working standard
C_s = Concentration of the varenicline NDSRI in the standard solution (ng/mL)