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Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8040 NX, AOC™-20i+s Plus

Quantitation of Varenicline Nitroso-Drug Substance Related Impurity (VNDSRI) in Varenicline API by GC-MS/MS

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User Benefits

- A GC-MS/MS method for the trace level determination of VNDSRI in Varenicline API

■ Introduction

Varenicline is a prescription medication used to treat smoking addiction. It is a high-affinity partial agonist for the α4β2 nicotinic acetylcholine receptor subtype (nACh) and has the capacity to reduce cravings and withdrawal symptoms. It is estimated that varenicline successfully helps, one of every eleven people who smoke; to remain abstinent from tobacco for six months. It is on the World Health Organization's List of Essential Medicines and is available as a generic medication.

Varenicline Nitroso-Drug Substance Related Impurity (VNDSRI) is also known as N-nitroso varenicline. (Figure-1) USFDA has identified higher levels of this impurity in some Varenicline finished drugs to be above FDA's acceptable intake limit in some samples of Varenicline finished drugs.

To ensure the safety and quality of varenicline tartrate drug product and drug substance, the USFDA has developed and validated a method to determine the presence or absence of VNDSRI.

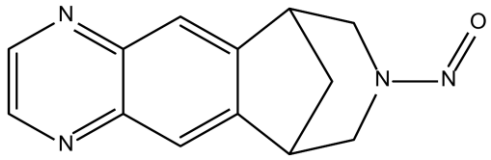

Figure 1: Structure of VNDSRI

Toxicity/ Regulation/ Methods: Consumption of VNDSRI up to the acceptable intake limit of 37 nanograms per day is considered reasonably safe for humans, based on lifetime exposure. VNDSRI may increase the risk of cancer if people are exposed to it above the acceptable intake limit and over a long period of time, but a person taking a drug that contains VNDSRI at-or-below the acceptable intake limit every day for 70 years is not expected to have an increased risk of cancer. Agency scientists have evaluated the risk of exposure to VNDSRI at interim acceptable intake levels up to 185 ng per day (92.5 ppm) and have determined that it presents a minimal additional cancer risk when compared to a lifetime of exposure to VNDSRI at the 37 ng per day (18.5 ppm) level.

N-nitroso varenicline is a solid with boiling point reported as 400.6±40.0° C at 760 mmHg. Due to such high boiling nature, LC-MS/MS has been the preferred analytical choice. The USFDA has published an HRMS method for the determination of VNDSRI in Varenicline tablets.

However, an attempt was made to develop an application where selective extraction of VNDSRI is done by using liquid-liquid extraction technique which minimize the high boiling API matrix. Similarly, with the help of high temperature compatible column & advanced GC features unwanted traces of impurity is removed thus reducing any possibility of carryover. This application note describes a GC-MS/MS method for the determination of VNDSRI in Varenicline API using GCMS-TQ8040 NX with AOC-20i+s Plus system. (Figure 2)

Figure 2: GCMS-TQ™8040 NX with AOC™-20i+s Plus system

■ Experimental

Standard VNDSRI was prepared in a suitable diluent and analyzed using scan mode for identification. Once identified, steps such as precursor ion selection & MRM optimization were performed. This step helps to derive optimized MRMs and their optimized Collision Energies (CE) which are very crucial for GC-MS/MS quantitation. A GC-MS/MS method with optimum MRM and their CE was generated and used for analysis.

For quantitation, a three-point calibration curve ranging from 2.0 to 10.0 ppm for VNDSRI was plotted. The conditions used for analysis are described in Table 1. The limit of detection (LOD) & limit of quantitation (LOQ) was found to be 0.5 ppm & 2.0 ppm, respectively. The S/N & % RSD at LOQ are shown in Table 2.

(All concentrations mentioned above are relative to sample)

■ Method

Table 1: Analytical conditions

GCMS System	: GCMS-TQ8040 NX with AOC-20i+s Plus
Column	: Capillary GC column of suitable stationary phase and dimensions
Injection Mode	: Splitless (Topaz liner Cat #23336)
Flow Control Mode	: Column flow
Injector Port Temp.	: 310 ºC
Carrier Gas	: Helium
Column flow	: 1.5 mL
Injection Volume	: 2.0 µL
Temp. Program	: 40 ºC for 0 min hold; heat at 15 ºC/min to achieve 310 ºC and hold for 3 min
Ionization mode	: Electron Ionization (EI)
Interface Temp.	: 310 ºC
Ion Source Temp.	: 250 ºC
Acquisition Mode	: MRM
MRM and CE	: 181.00>127.10 at CE 18; 181.00>154.10 at CE 8; 181.00>145.20 at CE 22

■ Sample Analysis

Linearity Standards Preparation:

Add 2.0 g NaCl to three individual 50 mL centrifuge tubes, to the same tubes add 20 mL of 0.002, 0.005 & 0.01 ppm standards prepared in HPLC grade water and 20 mL of Dichloromethane (DCM), shake the tube for 15 min on cyclomixer, centrifuge at 4500 rpm for 10 min, separate and inject the lower organic layer into GC-MS/MS.

Sample Preparation:

Weigh 20.0 mg Varenicline API and 2.0 g NaCl into a 50 mL centrifuge tube, transfer 20 mL of HPLC grade water and 20 mL of DCM to it, shake the tube for 15 min on cyclomixer, centrifuge at 4500 rpm for 10 min, separate and inject the lower organic layer into GC-MS/MS.

Spiked Sample Preparation:

Weigh 20.0 mg Varenicline API and 2.0 g NaCl into a 50 mL centrifuge tube, transfer 20 mL of 2 ppb VNDSRI standard prepared in HPLC grade water and 20 mL of DCM to it, shake the tube for 15 min on cyclomixer, centrifuge at 4500 rpm for 10 min, separate and inject the lower organic layer into GC-MS/MS.

■ Results and Discussion

Figure 3 depicts calibration curve, overlay of linearity standards & overlay of LOQ standards for VNDSRI

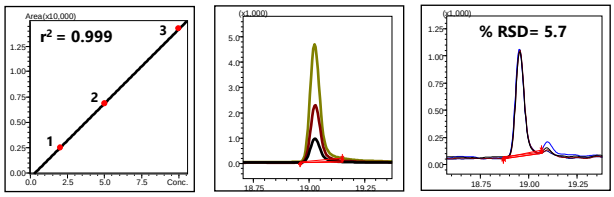

Figure 3: Calibration curve, overlay of linearity standards & chromatogram of LOQ solution for VNDSRI

Overlay of VNDSRI at LOQ level and diluent blanks showcasing no carryover (Figure 4)

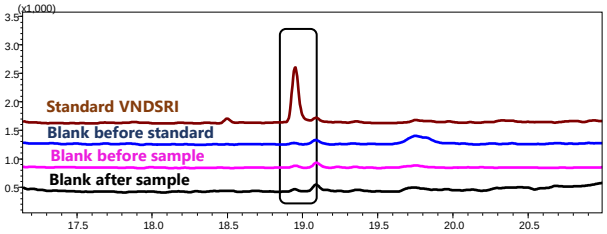

Figure 4: Overlay of standard VNDSRI and diluent blanks at different intervals

The linearity range, LOQ established from S/N and % RSD at LOQ are shown in table 2 whereas results for spiked study are shown in table 3.

Table 2: Summary of validation results for VNDSRI

Linearity range (ppm)	r^2	LOQ			
		Conc. (ppm)	% RSD (n=6)	% RSD (with bracketing)	S/N*
2.0 to 10.0	0.999	2.0	5.7	8.7	95

* = Peak to Peak
Results expressed are relative to sample

Table 3: The sample spiked study at LOQ level (Results expressed are relative to sample)

VNDSRI			
Amt. sample (ppm)	Amt. spiked (ppm)	Amt. in spiked (ppm)	% Accuracy
2.14	2.0	4.2	105

■ Conclusion

- Low level quantitation of VNDSRI in Varenicline API was successfully performed by using Shimadzu GCMS-TQ8040 NX with liquid autoinjector AOC-20i+s Plus
- The Correlation coefficient (r^2) was greater than 0.999
- The repeatability (% RSD) for six replicates of LOQ standards along with bracketing was found to be less than 10%
- Accuracy study in terms of spiked recovery was performed at LOQ level, and it matched the acceptance criteria between 90 to 110%.

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