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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8045

Trace Level Quantitation of 8 Nitrosamines Including Varenicline Nitroso-Drug Substance Related Impurity (VNDSRI) in Varenicline Tablets Using LCMS-8045

Devika Tupe, Nitish Suryawanshi, Purushottam Sutar, Siddhesh Ghadi, Nitin Shukla, Sujit Patil, Nilesh Patil, Jitendra Kelkar and Pratap Rasam
Shimadzu Analytical (India) Pvt. Ltd.

User Benefits

- A simple and robust LCMS method for the determination of eight Nitrosamines including VNDSRI in Varenicline tablets.
- The LCMS-8045 easily achieves much lower LOQ than prescribed in USFDA-published testing method on VNDSRI.

■ 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 the feelings of craving and withdrawal caused by smoking cessation. It is estimated that Varenicline successfully helps one of every eleven people to be abstinent from tobacco for six months. It is on the World Health Organization's List of Essential Medicines and is also available as a generic medication.

USFDA has identified levels of Varenicline Nitroso-Drug Substance Related Impurity (VNDSRI) which is also known as N-nitroso-varenicline (Fig. 1) above USFDA's acceptable intake limit in some samples of Varenicline finished drugs.

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

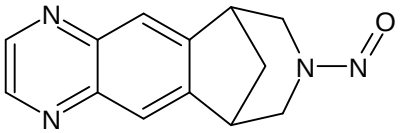

Fig. 1 Structure of VNDSRI

Toxicity/ Regulation/ Method:

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. USFDA 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 found 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.

Considering the need to control the presence of VNDSRI at such low level the USFDA has developed and published an HRMS method to determine VNDSRI in Varenicline tablets. This application note describes an LC-MS/MS method for the determination of not just VNDSRI but also seven other routinely encountered nitrosamines (NSA) prescribed by USFDA and EDQM in Varenicline tablets using Shimadzu LCMS-8045 system.

■ Experimental

A mixture of 8 nitrosamines namely VNDSRI, N-nitrosodimethylamine (NDMA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitrosodiethylamine (NDEA), N-nitrosoethylisopropyl amine (NEIPA), N-nitrosodiiso propylamine (NDIPA), N-nitrosodipropylamine (NDPA), and N-nitrosodibutylamine (NDBA), was analyzed to perform steps such as precursor ion selection and MRM optimization. An LC-MS/MS method with optimum MRMs and their CEs was generated in segments and Ultra High-Performance Liquid Chromatography (UHPLC) Nexera™ X3 coupled with LCMS-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan (Fig. 2) was used for analysis.

LCMS-8045, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

Fig. 2 Nexera™ X3 with LCMS-8045 system

■ Method

The MRM transitions of 8 nitrosamines and 2 internal standards are given in Table 1 and analytical conditions in Table 2.

Table 1 MRM transitions of Nitrosamines

NSA	MRM (Quantifier, Qualifier)
VNDSRI	241>211, 241>181
NDMA	75>43, 75>58
NMBA	147>117, 147>47
NDEA	103>29,103>75
NEIPA	117>75, 117>27
NDIPA	131>43,131>89
NDPA	131>43,131>89
NDBA	159>41, 159>29
NDMA-d6#	81>46
NDEA-d10*	113>34

Internal std. for NDMA
* Internal std. for VNDSRI, NMBA, NDEA, NFIPA, NDIPA, NDPA & NDBA

Table 2 Analytical conditions

HPLC System	: Nexera X3
Column	: Shim-pack™ GIST C18 (150 mm x 4.6 mm, 5 µm; P/N :227-30017-07)
Column Oven	: 40°C
Mobile Phases	: A- 0.1% Formic acid in Water B- 0.1% Formic acid in Methanol
Flow Rate	: 0.7 mL/min
Gradient program (B%)	: 1-9 min → 10 - 70 (%); 9-10 min → 70-90(%); 10-12 min → 90 (%); 12-12.1 min → 90-10 (%); 14 min → STOP.
Injection Volume	: 25 µL
LCMS System	: LCMS-8045 : APCI
Temperature	: Interface: 350°C Desolvation Line: 200°C Heater Block: 200°C
Gas Flow	: Nebulizing Gas: 3 L/min Drying Gas: 5 L/min

Additionally, divert valve function was employed to direct the high concentration API peak to waste. (Fig. 3)

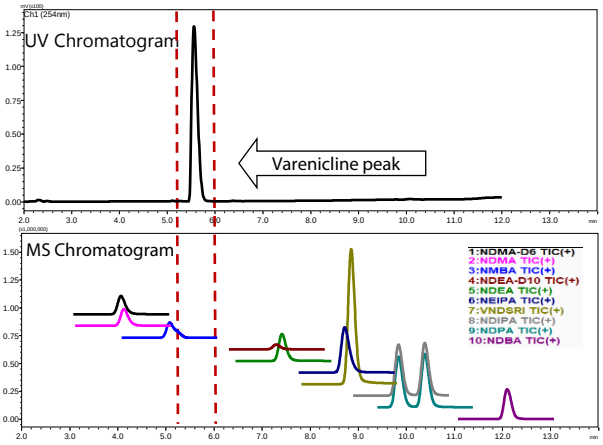

Fig. 3 Representative LCMS chromatogram of 8 nitrosamines along with internal standards and its overlapping with UV chromatogram

Linearity and quantitation

For quantitation, multi-point calibration curves for all 8 nitrosamines were prepared and plotted after analysis using the conditions described in Table 2. Limit of Quantitation (LOQ) for VNDSRI, NDEA, NDIPA is 0.1 ppb; for NMBA, NDIPA, NDPA, NDBA is 0.2 ppb whereas, for NDMA it is 0.5 ppb. The S/N, coefficient of regression and % RSD at LOQ are shown in Table 3. (All concentrations mentioned above are as such)

Fig. 4 to 11 depict the calibration curves, overlay of linearity standards and LOQ solution chromatograms for VNDSRI, NDMA, NMBA, NDEA, NEIPA, NDIPA, NDPA, & NDBA, respectively.

Table 3 Summary of calibration curves

NSA	r^2	LOQ		S/N
		Conc. in ppb	%RSD (n=6)	
VNDSRI	0.999	0.1	15.0	48
NDMA	0.998	0.5	19.3	45
NMBA	0.999	0.2	17.3	13
NDEA	0.999	0.1	17.1	24
NEIPA	0.999	0.1	15.0	21
NDIPA	0.999	0.2	13.0	19
NDPA	0.999	0.2	19.0	62
NDBA	0.999	0.2	11.6	32

Note: All of the above concentrations are as such.

VNDSRI

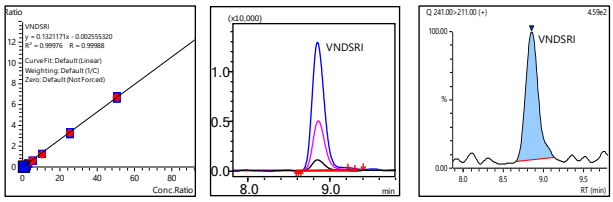

Fig. 4 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for VNDSRI

NDMA

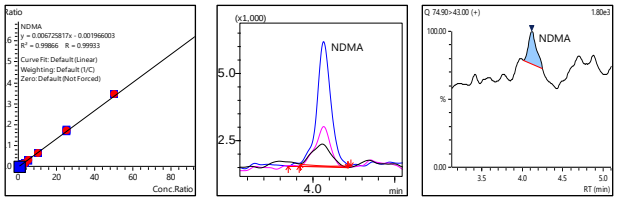

Fig. 5 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for NDMA

NMBA

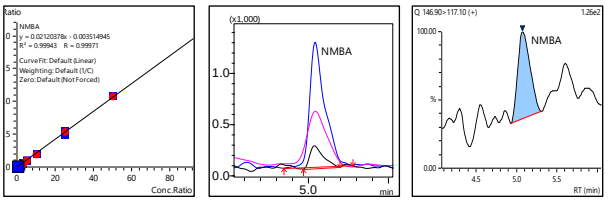

Fig. 6 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for NMBA

NDEA

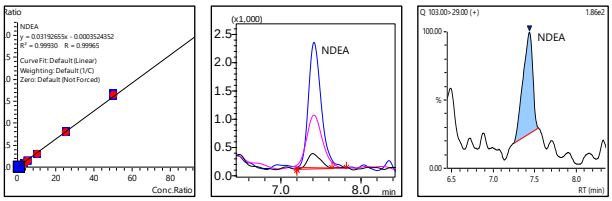

Fig. 7 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for NDEA

NEIPA

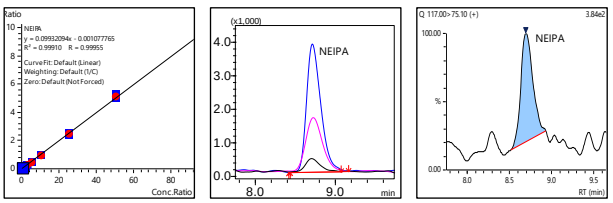

Fig. 8 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for NEIPA

NDIPA

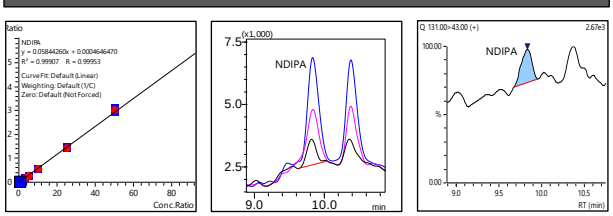

Fig. 9 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for NDIPA

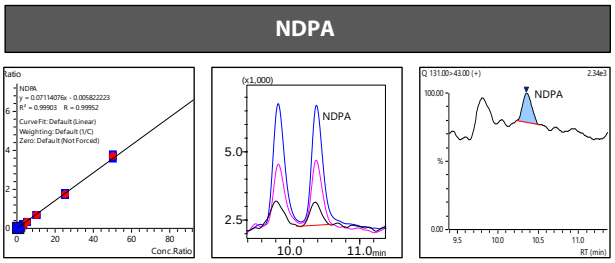

Fig.10 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for NDPA

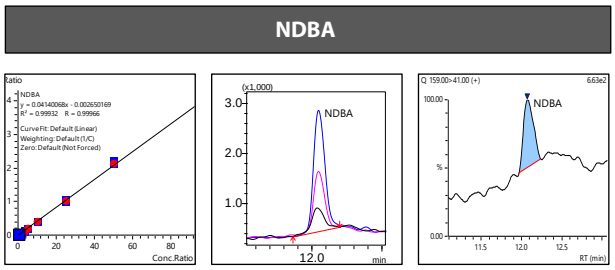

Fig. 11 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for NDBA

■ Sample Preparation

1. Weigh crushed formulation sample and placebo sample equivalent to 5 mg of Varenicline API in two different 15 mL centrifuge tubes.
2. Add 10 mL of water spiked with internal standards to formulation and placebo samples.
3. Vortex the samples and sonicate for 15 min. Centrifuge for 15 min at 5500 rpm.
4. Filter the supernatant using a 0.22 µm nylon syringe filter and analyze the samples using LC-MS/MS.

■ Spiked Sample Preparation

1. Weigh crushed placebo sample equivalent to 5 mg of Varenicline API in 15 mL centrifuge tube.
2. Add 10 mL of water spiked with internal standards and nitrosamine standards* to placebo.
3. Vortex the sample and sonicate for 15 min. Centrifuge for 15 min at 5500 rpm.
4. Filter the supernatant using a 0.22 µm nylon syringe filter and analyze the sample using LC-MS/MS.

* Spiked recovery was performed at 3 different concentrations (0.2, 0.5 and 1.0 ppb).

■ Results

The summary of sample analysis is shown in Table 4, while the % recoveries found for all 8 nitrosamines spiked at 3 different concentrations are shown in Table 5.

Table 4 Summary of sample analysis

NSA	Amount found (ppm)	
	Formulation	Placebo
VNDSRI	16.83	BLOQ
NDMA	BLOQ	BLOQ
NMBA	BLOQ	BLOQ
NDEA	BLOQ	BLOQ
NEIPA	BLOQ	BLOQ
NDIPA	BLOQ	BLOQ
NDPA	BLOQ	BLOQ
NDBA	BLOQ	BLOQ

Note: Amount reported is w.r.t. sample concentration at 0.5 mg/mL.

Table 5 Summary of spiked sample analysis

NSA	% Recovery		
	0.2 ppb	0.5 ppb	1.0 ppb
VNDSRI	113	95	94
NDMA	NA	123	106
NMBA	125	106	105
NDEA	99	107	101
NEIPA	108	104	95
NDIPA	70	70	77
NDPA	126	93	86
NDBA	84	76	73

■ Conclusion

- A single LC-MS/MS quantification method for Varenicline Nitroso-Drug Substance Related Impurity along with seven other routinely analyzed nitrosamines in Varenicline tablets has been successfully developed on the Shimadzu LCMS-8045 system.
- Varenicline placebo tablets were found to be free of any nitrosamine impurity.
- Varenicline tablets were found to contain 16.83 ppm of VNDSRI, which is below the acceptable intake limit of 37 nanograms (18.5 ppm) per day.
- Recovery analysis was performed at three different levels, and it matched to the acceptance criteria between 70 to 130 % for all nitrosamine impurities.

BLOQ = Below limit of quantitation

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