

Application News

LCMS-9050 Quadrupole Time-of-Flight Liquid Chromatograph Mass Spectrometer

Quantification of N-Nitroso Varenicline in Varenicline Tartrate by LC/MS-QTOF

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User Benefits

- ◆ High mass accuracy of LCMS-9050: This feature effectively avoids interference from other ions. Simple switching to the Nexera™ ultra-high performance liquid chromatograph is possible.
- ◆ Ion accumulation technology: This technology enhances the sensitivity of target compounds by approximately 30 times, with sensitivity exceeding regulatory limits by more than 100 times.

■ Introduction

Varenicline Tartrate is a highly selective receptor stimulant of the $\alpha 4 \beta 2$ nicotinic acetylcholine. It is primarily used for the treatment of tobacco dependence in adults. It represents a new class of smoking cessation medicine.

The secondary amine structure in the varenicline can react with trace amounts of nitrites introduced during the synthesis process or production environment, leading to the formation of nitrosamine drug substance-related impurities (NDSRI). In July 2021, the U.S. Food and Drug Administration (FDA) issued a recall for certain batches of varenicline tartrate due to the detection of *N*-nitroso varenicline, a genotoxic impurity, exceeding the maximum daily allowable intake. Following this, the FDA released an LC-ESI-HRMS method for detecting *N*-nitroso varenicline in both the active pharmaceutical ingredient (API) and finished drug products.

According to the Environmental Protection Agency (EPA), the limit for *N*-nitroso varenicline in varenicline tartrate at the maximum recommended dose of 2 mg is 18.5 ppm.

This study established a method for determining the content of *N*-nitroso varenicline in varenicline tartrate using a Shimadzu ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS) system. This method can serve as a reference for relevant professionals in the field.

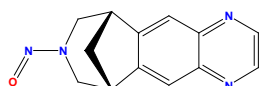


Fig. 1 The structural formula of *N*-nitroso varenicline

■ Sample Preparation

(1) Pre-treatment of the API (Active Pharmaceutical Ingredient): Accurately weigh 48 mg of varenicline tartrate API sample into a centrifuge tube. Add 50 mL of methanol and vortex to dissolve. Filter the solution through a membrane filter to obtain the test solution for the API.

(2) Pre-treatment of the Drug product: Take an appropriate number of varenicline tartrate tablets, grind them into a fine powder, and accurately weigh an appropriate amount of the powder. Dissolve the sample in methanol to prepare a solution with a varenicline concentration of 0.5 mg/mL. Sonicate the solution for 40 minutes, then centrifuge at 4500 rpm for 15 minutes. Filter the supernatant through a membrane filter to obtain the test solution for the finished product.

■ Analytical Conditions

The analytical conditions for HPLC and MS are shown in Table 1. The MRM transitions are shown in Table 2.

Table 1. Analytical Conditions of Nexera and LCMS-9050

System	: Nexera X3
Column	: Shim-pack Velox™ C18 (100 mm x 2.1 mm I.D., 1.8 μ m) ^{*1}
Temperature	: 35 °C
Injection volume	: 5 μ L
Mobile phases	: A-0.1% FA in Water B-0.1% FA in MeOH
Flow rate	: 0.30 mL/min
Mode	: Gradient elution
Time program (%B)	: 20% (0-1 min) → 80% (6-9.50 min) → 100% (10-11 min) → 20% (11.1-15 min)
Switching the Diverter Valve	: To the drain (0 min) → To the MS (3.50 min) → To the drain (6.0 min)
System	: LCMS-9050 (ESI Positive)
Nebulizing gas	: 2 L/min
Drying gas	: 5 L/min
Heating gas	: 15 L/min
DL temp	: 300 °C
Heat block temp	: 200 °C
Interface temp	: 400 °C

*1 P/N: 227-32007-03

Table 2. MRM Transition

Compound	CAS. No.	Precursor <i>m/z</i>	Product <i>m/z</i>	Q1 Pre (V)	CE (V)
N-Nitroso varenicline	2755871-02-2	241.1084	211.1105	-20	-14

■ Results and Discussion

UF-Accumulation can enhance detection sensitivity by accumulating ions in the Q2 collision cell and synchronizing the emission of ions with the injection of ions from the orthogonal accelerator. The ion accumulation process is shown in Fig 2. When measuring genotoxic impurities in pharmaceuticals, even extremely low levels of these impurities can cause irreversible damage to human health. Therefore, improving the sensitivity of the detection method is crucial for accurately quantifying trace or ultra-trace impurities.

In this experiment, as shown in Fig 3, the performance of the detection method was compared with ion accumulation or not (UF-Accumulation is enabled when the ID Function is turned off.). It is found that enabling the ion accumulation mode significantly increased the response of the target compound by approximately 30-fold, thereby the sensitivity of the detection method is enhanced.

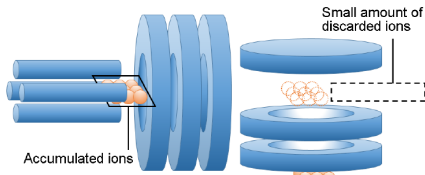


Fig. 2 The ion accumulation process

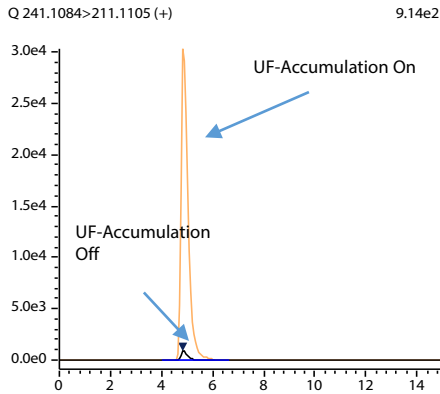


Fig. 3 Comparison of Two Modes

■ Calibration Curve

The calibration curve (external standard method) prepared using the standard sample showed good linearity in a wide dynamic range from 0.1-50 ng/mL with a coefficient of determination (R) of 0.9994. The accuracy at each calibration point ranging from 94.4 - 104.0%. Fig.4 shows the calibration curve (n=6).

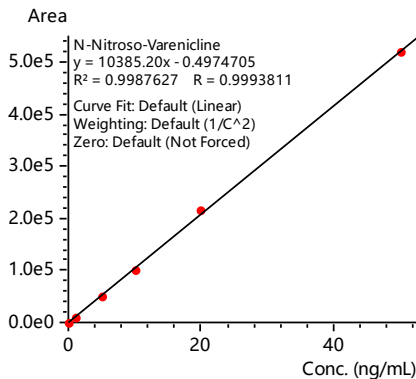


Fig. 4 Calibration curve (0.1-50 ng/mL)

■ Reproducibility

Table 3 shows the reproducibility for the N-Nitroso varenicline standard solution which concentration of 1, 5 and 20 ng/mL (n=6).

Table 3 RSD% of R.T. and Area

Compound	1.00 ng/mL		5.00 ng/mL		20.00 ng/mL	
	RT	Area	RT	Area	RT	Area
N-Nitroso varenicline	0.30	2.96	0.15	3.50	0.13	0.37

■ Recovery

The recovery experiments was prepared using test solution spiked with the N-Nitroso varenicline in the API and Drug product. The recovery rate of the spiked sample were ranging from 95.86 - 106.65% as shown in Table 4. Fig.5 shows the mass chromatograms of spiked solutions.

Table 4 The recovery rate(%) of the spiked sample

Sample	Spiked Amount mg/kg	Recovery %	RSD%
Varenicline Tartrate API sample	5.2	99.79	1.21
	8.6	106.65	0.78
	10.4	105.17	2.11
Varenicline Tartrate tablets	6.4	98.58	0.48
	8.0	97.63	0.95
	9.6	95.86	1.34

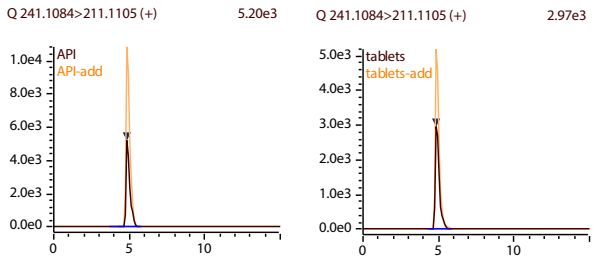


Fig. 5 The MRM chromatograms of N-nitroso varenicline in samples and spiked samples

■ Conclusion

Utilizing the LCMS-9050 system for quantitative analysis of N-Nitroso Varenicline in varenicline tartrate revealed that the method can accurately determine N-Nitroso varenicline levels within a broad concentration range of 0.1 to 50 ng/mL. Furthermore, spiked samples demonstrated excellent accuracy, indicating the reliability of the method. This approach exhibits several notable advantages, including high sensitivity, excellent repeatability, and reliability. As a result, it serves as a valuable reference for inspection personnel in related industries.

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Pharmaceutical

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