Liquid Chromatography Mass Spectrometry LCMS-2050

Application News

Determination of N-Nitroso-Nebivolol in Nebivolol Drug Substance by LCMS-2050

Tao Liu
Shimadzu (China) Co.,LTD.

User Benefits

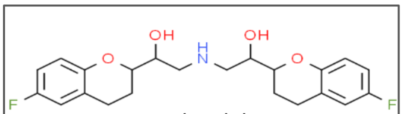
- A new method for the determination of NDSRI impurity in nebivolol raw material was established using LCMS-2050.
- The method has high sensitivity, short analysis time and high accuracy, which can fully meet the FDA's AI limit for N-Nitroso-nebivolol.

■ Introduction

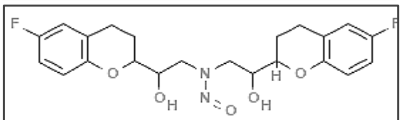
On September 4, 2024, the FDA issued the final guidance on the control of nitrosamine impurities in human drugs (Nitrosamine Guidance Version 2). The revised guidance describes two categories of nitrosamines: small-molecule nitrosamines and nitrosamine drug substance-related impurities (NDSRIs). Unlike small-molecule nitrosamine impurities that are structurally dissimilar to the active pharmaceutical ingredient (API), NDSRIs generally exhibit structural similarity to the API and may form through interactions between the API and nitrosating agents, such as nitrite impurities present in drug excipients.

Nebivolol is a potent and selective third-generation β-blocker clinically used for the treatment of patients with moderate-to-severe hypertension, as well as angina pectoris and congestive heart failure, with a recommended daily dose of 5 mg. Due to its structural characteristics, the synthesis of nebivolol may involve nitrosation reactions, leading to the introduction of the NDSRI impurity N-nitroso-nebivolol. According to the FDA's nitrosamine guidance, based on the Carcinogenic Potency Categorization Approach (CPCA), the acceptable intake (AI) limit for N-nitroso-nebivolol, a potential NDSRI in nebivolol, is set at 1500 ng/day.

This study established an LC-MS (LCMS-2050) method for the determination of N-nitroso-nebivolol in nebivolol drug substance. The method demonstrates high sensitivity, short analysis time, and accurate results, providing a reference for the detection of related nitrosamine impurities.



Nebivolol



N-nitroso-nebivolol

■ Sample Preparation

0.5 mg of a brand of nebivolol drug substance was precisely weighed and placed in a 1 mL volumetric flask. 50% methanol-water was used to adjust the volume to the mark, followed by thorough shaking and ultrasonication for 10 minutes. The volumetric flask was taken out and cooled to room temperature. 50% methanol-water was added to make up to the mark. The mixture was homogenized and passed through a 0.22 µm nylon filter membrane before instrumental analysis.

The standard stock solution (10 µg/mL) was taken and serially diluted with 50% methanol-water to obtain standard working solutions at concentrations of 1, 5, 10, 50, 100 and 180 ng/mL for subsequent determination.

■ Analysis Conditions

The analytical conditions are shown in Table 1. A Nexera XR UHPLC system with a photodiode array detector (PDA) was coupled to the single quadrupole mass spectrometer (LCMS-2050). The LCMS-2050 is equipped with a heated DUIS™ ion source for ionization, which combines the advantages of both ESI and APCI sources.

Table 1 Analysis Conditions

System	: Nexera™ XR
Column	: Shim-pack™ GIST-HP C18 (100 mm × 2.1 mm I.D., 2 µm)*1
Detection	: PDA at 190 to 800 nm
Temperature	: 40 °C
Injection volume	: 10 µL
Mobile phases	: A-0.1% formic acid in Water B-acetonitrile
Flow rate	: 0.35 mL/min
Mode	: Gradient elution
Time program (%B)	: 40% (0.00 min) → 75% (1.50 min) → 90% (3.50 min) →90% (3.50-4.00 min) → 40% (4.01-8.00 min)
FCV Valve Position	: 0 (0.00 min) → 1 (2.80 min) →0 (4.30 min)*2
System	: LCMS-2050 (DUIS Negative)
Mode	: SIM
Interface Voltage	: -2.0 kV
Nebulizing gas	: 3 L/min
Drying gas	: 7 L/min
Heating gas	: 5 L/min
DL temp	: 200 °C
Desolvation temp	: 500 °C

*1 P/N：227-30001-04
*2: "1" indicates to mass spectrum, and "0" indicates that the flow path is switched to waste liquid

Table 2 SIM Conditions

ID	Compound	CAS	Molecular formula	Polarity	*m/z*
1	N-Nitroso-nebivolol	1391051-68-5	$C_{22}H_{24}F_2N_2O_5$	-	479.02

■ Separation Evaluation

To ensure high sensitivity in impurity analysis and prevent contamination of the mass spectrometer by high-concentration active pharmaceutical ingredient (API) components, flow path switching technology was employed. This method diverts the main nebivolol peak to waste, allowing only impurities to enter the mass spectrometer for detection. Under the optimized chromatographic conditions, The retention times of nebivolol and N-nitroso-nebivolol were 1.952 min and 3.408 min, respectively, so the mass spectrometry acquisition was initiated after 2.8 min, ensuring selective detection of the target impurity. This approach effectively enabled accurate and interference-free analysis of trace-level impurities.

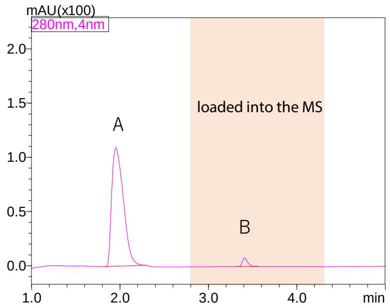


Fig.1 PDA Chromatogram (A, nebivolol Concentration 500 µg/mL
and B, N-nitroso-nebivolol Concentration 16 µg/mL)

■ Specificity

Fig. 2 shows the SIM chromatogram of the blank and the standard solution (5 ng/mL). There is no obvious interference at the target peak, so the method has good specificity.

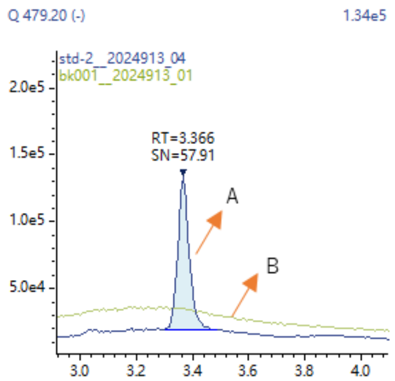


Fig. 2 SIM chromatogram of blank and standard solution
(A, standard solution concentration 5 ng/mL and B, blank)

■ Calibration Curve

The calibration curve(external standard method) prepared using the standard sample showed good linearity in a wide dynamic range of 180-fold for. Fig. 3 shows the calibration curve.

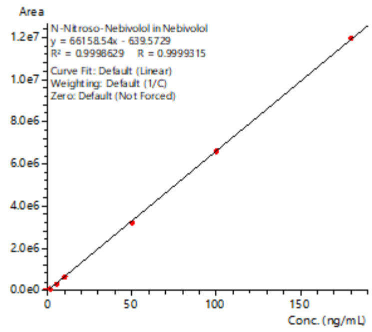


Fig. 3 Calibration curve(1-180 ng/mL)

■ Repeatability

Table3 shows the repeatability value for the standard solution which concentration of 1, 10 and 100 ng/mL (n=6).

Table 3 RSD% of R.T. and Area

Compound	1 ng/mL		10 ng/mL		100 ng/mL	
	R.T	Area	R.T	Area	R.T	Area
N-nitroso-nebivolol	0.40	2.02	0.09	1.49	0.12	1.36

■ Recovery

Standard solutions of various concentrations were spiked to the nebivolol drug substance for the recovery experiments, and each addition amount was determined in triplicate. The recovery rates are presented in Table 4, and the results indicate that the recovery rates comply with the requirements of the Pharmacopoeia.

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

Compound	Background content (µg/mg)	0.03 µg/mg		0.3 µg/mg	
		Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
N-nitroso-nebivolol	0.0022	100.4	0.77	100.2	1.89

■ Conclusion

A novel method for determining the content of N-nitroso-nebivolol in nebivolol drug substance was established by using the LCMS-2050. Within the concentration range of 1 - 180 ng/mL, the correlation coefficients of the N-nitroso-nebivolol was 0.9999. When the spiked concentration of 0.03 µg/mg and 0.3 µg/mg, the recovery rate of N-nitroso-nebivolol was 100.4 and 100.2, respectively, and the recovery rate was good and met the requirements of pharmacopoeia. The method has high sensitivity, linearity, precision and recovery rate can meet the detection requirements, and which can be used for the detection of nitrosamine impurity N-nitroso-nebivolol in nebivolol drug substance.

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Shimadzu Corporation
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03-LCMS-055-EN First Edition: Sep. 2025

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