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Liquid Chromatography Mass Spectrometry LCMS-8045

Application News

Determination of NTTP in Sitagliptin by LCMS-8045

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User Benefits

- This method exhibits high sensitivity with a quantitation limit of 5 ng/day, significantly surpassing the acceptable intake limit of 37 ng/day.
- The pretreatment process for this method is straightforward and easy to perform, requiring only ultrasonication.

■ Introduction

Sitagliptin phosphate is designed for patients with type 2 diabetes and can be used alongside metformin. This compound functions as a dipeptidyl peptidase-4 (DPP-4) inhibitor, which enhances the naturally occurring incretin by suppressing the enzyme's activity, ultimately leading to a reduction in blood glucose concentration.

However, the US Food and Drug Administration (FDA) discovered the presence of a genotoxic impurity, NTTP, in some samples of sitagliptin. Consequently, it mandated that relevant companies conduct rigorous testing on their products. The FDA has established strict guidelines, stipulating that the short-term acceptable intake (AI) limit of NTTP in sitagliptin should not exceed 246.7 ng/day, while the lifetime exposure AI limit is set at 37 ng/day.

To ensure compliance with these regulations, a method for analyzing NTTP in sitagliptin was developed utilizing the LCMS-8045 triple quadrupole tandem mass spectrometry system. This method demonstrates remarkable sensitivity, surpassing even the 37 ng/day limit, and boasts excellent system adaptability and accuracy. It is thus ideal for routine analysis of NTTP in both raw materials and formulations of sitagliptin.

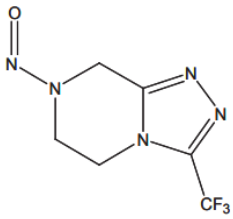

Fig. 1 The structural formula of NTTP

■ Sample Preparation

Ten tablets of a specific brand of sitagliptin were accurately weighed, ground, and thoroughly mixed. An appropriate quantity of the fine powder, approximately equivalent to 100 mg of sitagliptin, was precisely measured into a 10 mL volumetric flask. An adequate amount of water was added, and the mixture was vigorously shaken to ensure complete dissolution. The resulting solution was then centrifuged for 10 minutes to separate any solid particles, followed by filtration. The filtered solution was subsequently utilized as the test solution for further analysis.

Standard samples were serially diluted with water, prepared at concentrations of 0.5,1,4,10,25 and 50 ng/mL, followed by using for preparation of the calibration curve.

■ Analysis Conditions

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

Table 1 Analysis Conditions of Nexera™ and LCMS-8045

System	: Nexera™ XS
Column	: Shim-pack™ GIST C18 (150 mm × 3.0 mm I.D., 3 µm)*1
Temperature	: 30 ˚C
Injection volume	: 5 µL
Mobile phases	: A-0.1% FA in Water B-0.1% FA in ACN
Flow rate	: 0.35 mL/min
Mode	: Gradient elution
Time program (%B)	: 40% (0 min) → 100% (8 min) →40% (8.1-12 min)
FCV Valve Position	: 1 (0 min) → 0 (2.75 min) →1 (8min)*2
System	: LCMS-8045 (ESI Positive)
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
DL temp	: 250 ˚C
Heat block temp	: 400 ˚C
Interface temp	: 400 ˚C

*1 P/N：227-30009-07
*2: "0" indicates to mass spectrum, and "1" indicates that the flow path is switched to waste liquid

Table 2 MRM Transition

Compound	Precursor *m/z*	Product *m/z*	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
NTTP	222.05	192.15	-11.0	-11.0	-20.0
		165.10	-12.0	-24.0	-30.0

■ Specificity

Fig. 2 shows the MRM chromatogram of the blank and the standard solution (0.5 ng/mL). There is no obvious interference at the target peak, and the method has good specificity.

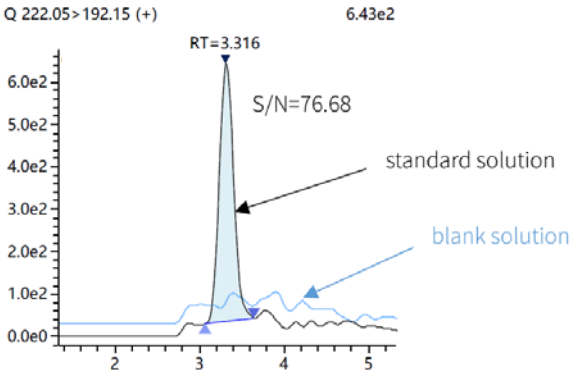

Fig. 2 MRM chromatogram of blank and standard solution

■ Calibration Curve

The calibration curve(external standard method) prepared using the standard sample showed good linearity in a wide

dynamic range from 0. 5 - 50 ng/mL with a coefficient of determination (R^2) of 0.9996. The accuracy at each calibration point ranging from 95.94 – 105.11%. Fig. 3

shows the calibration curve.

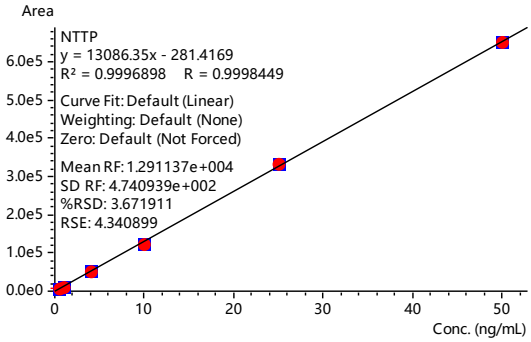

Fig. 3 Calibration curve(0.5-50 ng/mL)

■ Reproducibility recovery

Table3 shows the reproducibility for the NTTP standard solution which concentration of 1, 10 and 50 ng/mL (n=6).

Table 3 RSD% of R.T. and Area

Compound	1 ng/mL		10 ng/mL		50 ng/mL	
	R.T	Area	R.T	Area	R.T	Area
NTTP	0.13	2.77	0.09	1.54	0.05	0.65

■ Recovery

The test solution was analyzed on the LCMS-8045, and the quantitative result was that every 100 mg of sitagliptin phosphate contains NTTP 93.12 ng. Fig. 4 shows the mass chromatograms of the test solution.

The recovery experiments was prepared using test solution spiked with 10, 100, and 250 ng of the NTTP. The recovery rate of the spiked sample were 104.3% ,107.2% and 104.3% as shown in Table 4. Fig. 5 shows the mass chromatograms of spiked solutions.

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

Compound	Spiked 10 ng	Spiked 100 ng	Spiked 250 ng
NTTP	104.3	107.2	104.3

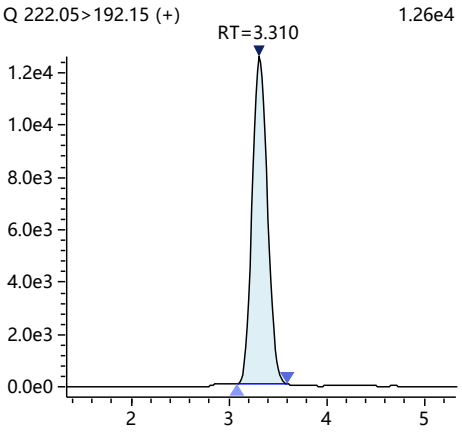

Fig. 4 MRM Chromatogram of test solution

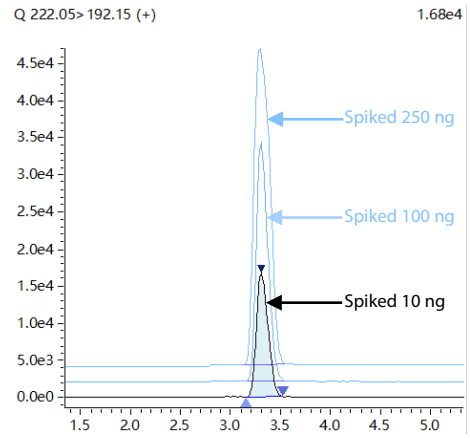

Fig. 5 MRM Chromatogram of spiked solutions

■ Conclusion

Utilizing the LCMS-8045 system for quantitative analysis of NTTP in sitagliptin revealed that the method can accurately determine NTTP levels within a broad concentration range of 0.5 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, shortened analysis time, stability, and reliability. As a result, it serves as a valuable reference for inspection personnel in related industries.

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