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Liquid Chromatography Mass Spectrometry LCMS-8045RX

Application News

Determination of Nitroso Bumetanide in Bumetanide by LCMS-8045RX

Qianzhan Yang
Shimadzu (China) Co.,LTD.

User Benefits

- This method can accurately quantify the sample in just 8 minutes, which is more efficient than the FDA method.
- The linear minimum concentration is 0.2 ng/mL, which is lower than the nonbinding recommendations of 1 ng/mL by the FDA, indicating greater sensitivity.

■ Introduction

Bumetanide is a derivative of melamine benzene sulfonamide and is a potent diuretic. It is widely used in the treatment of edema associated with heart failure, liver disease, and kidney disease. It is mainly used for various refractory edemas and acute pulmonary edema.

It is particularly suitable for patients with acute and chronic renal failure. Because its structure contains a secondary amine group, it provides an amino unit for the formation of nitrosamines. In the production process, so-called NDSRI (nitroso drug substance-related impurities) and nitrosated bumetanide are produced. These impurities pose a risk of carcinogenicity or mutagenicity and have led to the recall of multiple medications. FDA has quantified it in its guidance for nitroso bumetanide impurities using high-resolution mass spectrometry, with a method detection limit (LOD) = 0.5 ppm. Quantitation limit (LOQ) = 1.0 ppm.

This article uses the LCMS-8045RX to establish a detection method for Nitroso Bumetanide in Bumetanide. After methodological validation, the results show that the analytical method can accurately and rapidly determine the amount of nitrosated bumetanide in the API, which can be used for reference by relevant testing personnel.

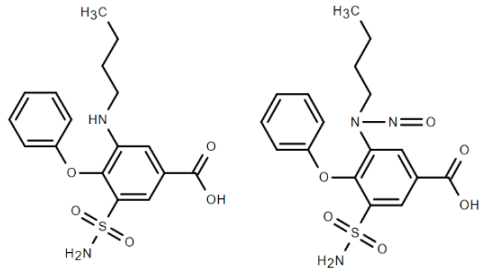

Fig. 1 The structural formula of Bumetanide and Nitroso Bumetanide

■ Sample Preparation

Weigh the raw material medicine and dissolve it in methanol to prepare a sample solution of 1 mg/mL. Then, the solution was filtered through a 0.22 μm filter head. The filtered solution is the one used for detection.

Standard samples were serially diluted with methanol, prepared at concentrations of 0.2, 0.5, 1, 5, 10, 50 and 100 ng/mL, followed by using for preparation of the calibration curve.

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

■ Analysis Conditions

Table 1 Analysis Conditions

System	: Nexera™-LC40B X3
Column	: Shim-pack Velox™ SP-C18 (100 mm × 2.1 mm I.D., 1.8 μm)*1
Temperature	: 40 ˚C
Injection volume	: 2 μL
Mobile phases	: A-0.1% FA in Water B-Methanol
Flow rate	: 0.4 mL/min
Mode	: Gradient elution
Time program (%B)	: 20% (0-0 .5min) → 95% (5 -6min) →20% (6.01-8 min)
FCV Valve Position	: 0 (0 min) → 1 (3min) →0 (4.3min)*2
System	: LCMS-8045RX (ESI Positive)
Focus voltage	: 2 KV
Probe position	: 4 mm
Nebulizing gas	: 3 L/min
Drying gas	: 3 L/min
Heating gas	: 18 L/min
DL temp	: 250 ˚C
Heat block temp	: 450 ˚C
Interface temp	: 350 ˚C

*1 P/N: 227-32001-03
*2: "1" indicates to mass spectrum, and "0" indicates that the flow path is switched to waste liquid

Table 2 MRM Transition

Compound	Precursor *m/z*	Product *m/z*	CE(V)
Bumetanide	394.00	364.20	-12.0
		321.10	-16.0
		240.10	-24.0
Nitroso Bumetanide	364.90	240.20	-16.0
		284.20	-14.0
		348.10	-14.0

■ Method Optimization

Taking the chromatogram peak area at a standard concentration of 100 ng/mL and an injection volume of 1 μL as the indicator, single-factor optimization was performed under different conditions of the mass spectrometer. The optimized parameters included: (1) the temperature of the DL tube, interface, and Heat block; (2) the flow rate of the nebulizing gas, heating gas, and drying gas; (3) the focus voltage . The optimization results are shown in Figure 2:

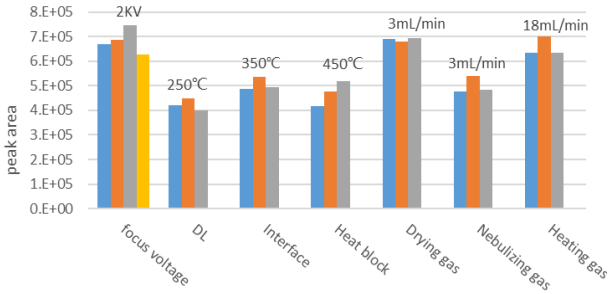

Fig. 2 Optimization of mass spectrum parameters

■ Specificity

Fig. 3 shows the MRM chromatogram of the blank and the standard solution (0.2 ng/mL). There is no obvious interference at the target peak, and the method has good specificity.

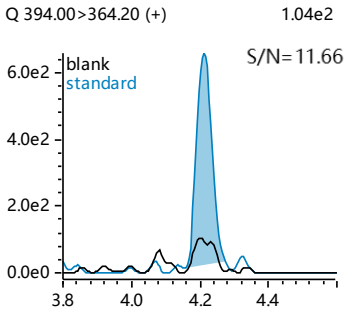

Fig. 3 MRM chromatogram of blank and standard solution

■ Calibration Curve

Different concentrations of the mixed standard working solution were measured under the same conditions. The concentration (C) was plotted on the x-axis, and the peak area (A) was plotted on the y-axis, with a weight of 1/C. A calibration curve was established using the external standard method. The target compounds have a good linear relationship within the machine concentration range, with a correlation coefficient (R) of 0.9988. The accuracy at each calibration point ranging from 91.2-105.3%. Fig. 4 shows the calibration curve.

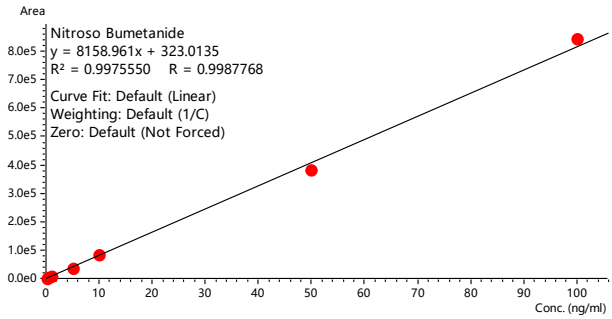

Fig. 4 Calibration curve(0.2-100 ng/mL)

■ Repeatability test

Table3 shows the repeatability for the Nitroso Bumetanide standard solution which concentration of 1, 10 and 50 ng/mL (n=7).

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
Nitroso Bumetanide	0.008	5.396	0.008	2.680	0.069	2.386

■ Recovery

The test sample was analyzed on the LCMS-8045RX, and the quantitative result was that every 1 mg of Bumetanide contains Nitroso Bumetanide 3.94 ng. The recovery experiments was prepared using sample solution (1 mg/mL)spiked with 1 and 5 ng of the Nitroso Bumetanide(n=3) . The average recovery rate of the spiked sample were 98.49% and 101.85% and RSD% as shown in Table 4. Fig. 5 shows the mass chromatograms of spiked solutions.

Table 4 The result of the spiked sample （n=3）

No	Spike （ng）	Recovery rate %	Average recovery rate %	RSD%
1	1	97.00 97.11 101.36	98.49	2.522
2	5	103.24 102.71 99.59	101.85	1.934

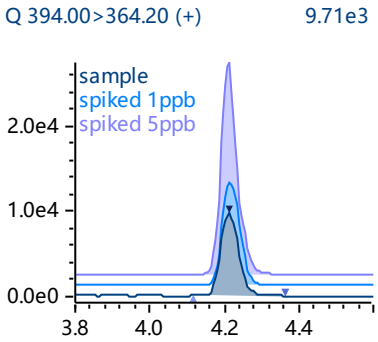

Fig. 5 MRM Chromatogram of spiked sample

■ Probe position

In order to investigate the difference in stability between the RX series instruments and the traditional ESI source, a comparison of relative response values at different horizontal positions was conducted. The results are shown in Fig. 6: The experiment indicates that for Nitroso Bumetanide , the mass spectrometry response is optimal when the spray needle is at a horizontal position of 4-5 mm, and the response slightly decreases as the distance increases further. This suggests that a greater distance can help reduce mass spectrometry contamination and improve system stability, while ensuring the necessary response intensity. At the same time, the results also show that on the new RX series ESI source, the compound response sensitivity to Probe position is reduced, stability is improved, and a balance between sensitivity and robustness has been achieved.

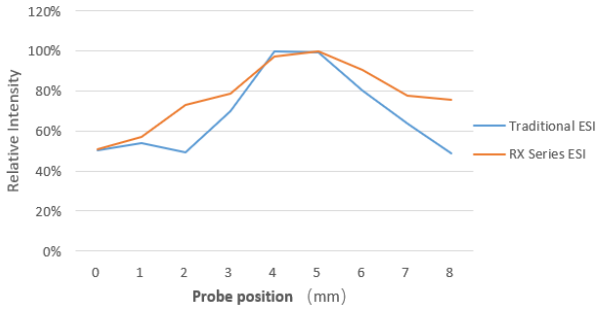

Fig. 6 The relationship between probe position and signal intensity

■ Conclusion

Utilizing the LCMS-8045RX system for quantitative analysis of Nitroso Bumetanide in Bumetanide that the method can accurately determine Nitroso Bumetanide levels within a broad concentration range of 0.2 to 100 ng/mL. In the methodological examination, the method's linearity, repeatability, recovery rate of spiked samples all meet the requirements for detection, making it suitable for the analysis of actual samples.

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