Application News

Liquid Chromatography Mass Spectrometry LCMS-8050

Determination of NDSRI in Chlorpromazine Tablets by LCMS-8050

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User Benefits

- Accurate quantification of the sample can be completed in just 5.5 minutes.
- The linear minimum concentration is 0.1 ng/mL, which meets the requirement for high sensitivity of the instrument to detect low-limit impurities.

■ Introduction

Chlorpromazine, a phenothiazine derivative, acts as an antagonist at dopamine, histamine, and cholinergic receptors. Its hydrochloride salt is clinically used to treat psychosis, emesis, hypothermic anesthesia, and artificial hibernation.

Due to its tertiary amine structure, chlorpromazine may form nitrosamine impurities (NDSRIs) such as N-Nitroso-desmethyl-chlorpromazine during manufacturing. These impurities are potentially carcinogenic/mutagenic and have prompted drug recalls. FDA classifies N-Nitroso-desmethyl-chlorpromazine as a high-risk Class I nitrosamine, with an Acceptable Intake (AI) limit of 26.5 ng/day. For a maximum daily dose of 600 mg, this corresponds to a limit of 1.1 ng per 25 mg tablet.

This study developed an analytical method using LC-MS/MS (Shimadzu LCMS-8050) to quantify N-Nitroso-desmethyl-chlorpromazine in chlorpromazine tablets. The validated method enables accurate and rapid determination of this impurity, providing a reliable approach for quality control.

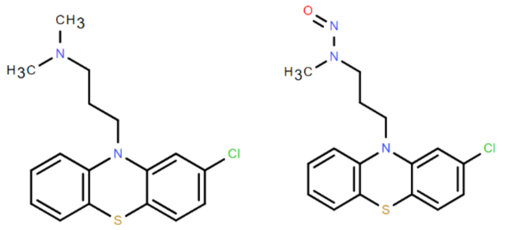

Fig. 1 The structural formula of Chlorpromazine and N-nitroso-desmethyl-chlorpromazine

■ Sample Preparation

Take two tablets of the preparation sample (specification: 25 mg), add 5 mL of a 50% acetonitrile-water solution, and perform ultrasonic extraction for approximately 30 minutes. Centrifuge at 5000 r/min for 5 minutes to achieve stratification. Take the supernatant and pass it through a 0.22 μm nylon filter membrane for instrumental analysis for further analysis.

Standard samples were serially diluted with acetonitrile, prepared at concentrations of 0.1,0.2,0.5,1,5,10,20,50 and 80 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

System	: Nexera™ XS
Column	: Shim-pack™ GISS HP-C18 (100 mm x 2.1 mm I.D., 3 μm)[*1]
Temperature	: 30 ˚C
Injection volume	: 2 μL
Mobile phases	: A-0.05% Formic acid in Water B-Acetonitrile
Flow rate	: 0.3 mL/min
Mode	: Gradient elution
Time program (%B)	: 55% (0-1 .5min) → 100% (1.51 -3.5min) →55% (3.51-5.5 min)
FCV Valve Position	: 0 (0 min) → 1 (2.8 min) →0 (3.4 min)[*2]
System	: LCMS-8050 (ESI Positive)
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
ESI position	: 1 mm
DL temp	: 200 ˚C
Heat block temp	: 400 ˚C
Interface temp	: 350 ˚C
IF voltage	: 1 KV

*1 P/N: 227-30084-02

*2: "1" indicates to mass spectrometer, and "0" 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)
N-nitroso-desmethyl-chlorpromazine	332.80	303.10	-18.0	-16.0	-15.0
		246.10	-26.0	-19.0	-17.0
		214.15	-24.0	-35.0	-16.0
Chlorpromazine	318.90	86.20	-12.0	-21.0	-17.0
		214.10	-16.0	-44.0	-23.0
		246.10	-16.0	-26.0	-12.0

■ Method Optimization

Taking the chromatogram peak height at a standard concentration of 50 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; (2) the IF voltage; (3) the ESI position. The optimization results are shown in Fig. 2.

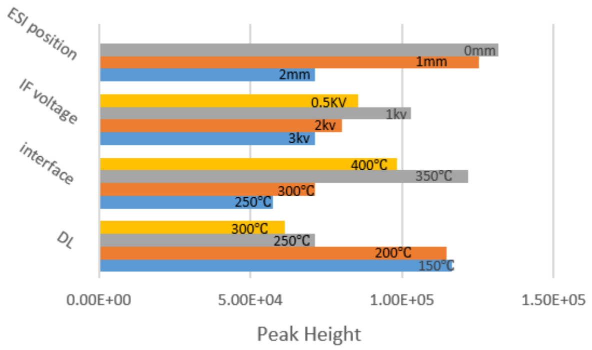

Fig. 2 Optimization of mass spectrum parameters

The target compound showed optimal response at 350° C interface temperature and 1 kV IF voltage. Peak height variation was negligible between ESI positions (0 mm and +1 mm) and DL temperatures (150° C and 200° C). To improve contamination resistance for high-concentration samples, +1 mm ESI position and 200° C DL temperature were selected.

■ Specificity

Fig. 3 shows the MRM chromatogram of the blank and the standard solution (0.1 ng/mL). There is no obvious interference at the target peak, and the method has good specificity.

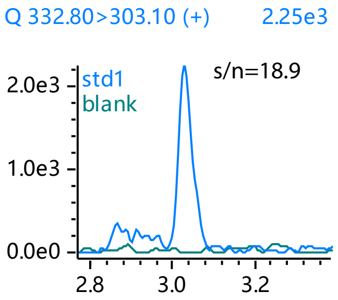

Fig. 3 MRM chromatogram of blank and standard solution

■ 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 0.9992. The accuracy at each calibration point ranging from 94.3-109.9%.

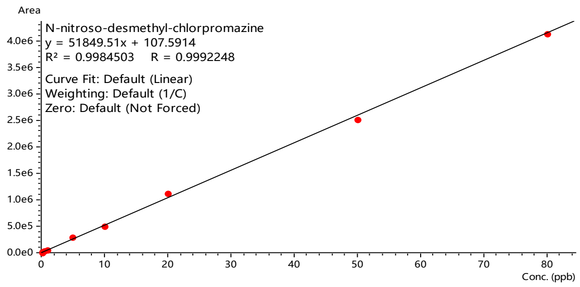

Fig. 4 Calibration curve(0.1-80 ng/mL)

■ Repeatability test

Table3 shows the repeatability for the N-nitroso-desmethyl-chlorpromazine standard solution which concentration of 0.5 and 5 ng/mL (n=6).

Table 3 RSD% of R.T. and Area

Compound	0.5 ng/mL		5 ng/mL	
	R.T	Area	R.T	Area
N-nitroso-desmethyl-chlorpromazine	0.103	5.756	0.096	4.462

■ Recovery

The test sample was analyzed on the LCMS-8050, and the quantitative result was that every 10 mg of Chlorpromazine contains N-nitroso-desmethyl-chlorpromazine 0.56 ng. The recovery experiments was prepared using sample solution (10 mg/mL)spiked with 0.4 and 4 ng of the N-nitroso-desmethyl-chlorpromazine(n=3) . The average recovery rate of the spiked sample were 81.79% and 91.80% 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	0.4	83.80 80.23 81.35	81.79	2.23
2	4	94.07 92.20 89.11	91.80	2.73

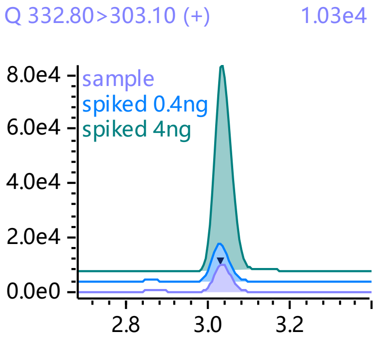

Fig. 5 MRM Chromatogram of spiked sample

■ Conclusion

Utilizing the LCMS-8050 system for quantitative analysis of N-nitroso-desmethyl-chlorpromazine in chlorpromazine tablets that the method can accurately determine N-nitroso-desmethyl-chlorpromazine levels within a broad concentration range of 0.1 to 80 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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