SHIMADZU
Excellence in Science

Application News

No. LCMSMS-157E

Liquid Chromatography Mass Spectrometry

Determination of Amantadine Residues in Chicken by Ultra-High-Performance Liquid Chromatography and Triple Quadrupole Mass Spectrometry

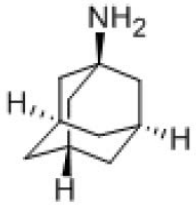
This application note describes a method for the determination of amantadine in chicken using Shimadzu Triple Quadrupole Mass Spectrometer LCMS-8050. After extraction and cleanup, the commercially bought chicken sample was separated by Ultra-High-Performance Liquid Chromatograph Nexera X2 and then quantified by LCMS-8050. The calibration curve of amantadine exhibited good linearity within the concentration range of 0.02-10 µg/L, and the correlation coefficient of calibration curve was 0.9996. Six consecutive analysis at low-, mid- and high-concentration standard working solutions (0.5, 1 and 5 µg/L) were conducted and the RSD of retention time and peak area was 0.07-0.19% and 0.85-1.32% respectively, indicating good instrument precision.

Keywords: Chicken; Amantadine; Triple quadrupole mass spectrometry

■ Introduction

In recent years, with the large-scale breeding of livestock and poultry, a huge amount of anti-bacterial and anti-viral drugs have been used for the prevention and treatment of animal diseases. In December 2012, it was revealed that some chicken farms in Shandong illegally used anti-viral veterinary drugs like amantadine to raise fast growing chickens, which were later supplied to KFC and McDonald's. This incident caused a huge impact and concern. As early as 2005, the Ministry of Agriculture of China have released "Emergency Notice on Checking Amantadine and Other Anti-Viral Drugs" and explicitly require the immediate cessation of the production, operation and use of such drugs (e.g. amantadine and rimantadine). Violators would be punished due to the production and use of fake as well as banned veterinary drugs.

Currently, amantadine is mainly detected by HPLC-fluorescence method or GC method after pre-column derivatization. These methods consist of laborious multi-step procedures which are time-consuming and less sensitive. In China, there are only a few published studies and reports on amantadine residues in animal-derived food. In this application note, a high-sensitivity and high-selectivity UHPLC – tandem MS method is established for the determination of amantadine residues in chicken. It serves as a comprehensive reference for many applications and regulatory personnel.



Chemical structure of amantadine

■Experimental

1.1. Instruments

Shimadzu Ultra-High-Performance Liquid Chromatograph (UHPLC) Nexera X2 and Triple Quadrupole Mass Spectrometer LCMS-8050 was used. The specific configuration included LC-30AD×2 infusion pumps, DGU-20A$_5$ Online Degasser, SIL-30AC Autosampler, CTO-30AC Column Oven, CBM-20A System Controller, Triple Quadrupole Mass Spectrometer LCMS-8050, and LabSolutions Ver. 5.60 Chromatography Workstation.

1.2. Analytical Conditions

Liquid chromatography (LC) parameters

- Chromatographic column: Shimadzu Shim-pack XR-ODS III, 2.0 mm (I.D.) x 150 mm (L) x 2.2 µm
- Mobile phase A: solution containing 0.1% formic acid and 5mM ammonium acetate
- Mobile phase B: Acetonitrile
- Elution mode: gradient elution; the initial concentration of phase B was 5%; Refer to Table 1 for the gradient program.
- Flow rate: 0.4 mL/min
- Column temperature: 30°C
- Injection volume: 2 µL

Table 1: Gradient Program

Time(min)	Module	Command	Value
1.00	Pumps	Pump B Conc.	30
2.00	Pumps	Pump B Conc.	30
2.40	Pumps	Pump B Conc.	95
3.10	Pumps	Pump B Conc.	95
3.11	Pumps	Pump B Conc.	5
4.50	Controller	Stop	

Mass spectrometry (MS) parameters

- Ionization mode: ESI (+)
- Ion spray voltage: 2 kV
- Heating gas: Dry air 10 L/min
- Nebulizing gas: Nitrogen 2.0 L/min
- Drying gas: Nitrogen 10 L/min
- Collision gas: Argon
- Ion source heating temperature: 300°C
- DL temperature: 250°C
- Heating module temperature: 400°C
- Scan mode: Multiple Reaction Monitoring (MRM)
- Dwell time: 100 msec
- Pause time: 3 msec
- MRM parameters: Refer to Table 2

Table 2: MRM Parameters

Compound name	CAS No.	Precursor ion	Product ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Amantadine	768-94-5		135.2* 93.2	-30 -30	-22 -28	-30 -17

Note: * represents quantifier ion.

1.3. Preparation of Standard Solutions and Sample Pretreatment

Preparation of standard working solutions:
Standard working solution at the concentration of 10 mg/L was prepared using methanol. It was sequentially diluted with 5% acetonitrile solution to obtain a series of standard working solutions at concentrations of 0.02 µg/L, 0.1 µg/L, 0.5 µg/L, 5 µg/L and 10 µg/L.

Chicken sample pretreatment:
2 g (± 0.02 g) of chicken sample was accurately weighed in a 50 mL centrifuge tube. 10 mL of acetonitrile: 1% trichloroacetic acid solution (1:1) was added to the chicken sample. The mixture was homogenized for 1 minute and centrifuged at 10,000 rpm for 20 minutes. Solid phase extraction was conducted where 4 mL of the supernatant was transferred to WondaSep (InertSep) MPC column for cleanup. The detailed SPE cleanup procedure is shown below:

Activation: 5 mL of methanol; 5mL of water
Load sample
Rinsing: 3 mL of 2% HCl; 3mL of methanol
Elution: 5 mL of 5% ammonium hydroxide-methanol-isopropanol (5:80:15)
Dry the eluent under N_2 and redissolve it to 1mL, followed by filtering
LC-MS/MS analysis

■ Results and Discussion

2.1. Q1 Scan and Product Ion Scan Mass Spectra of Amantadine Standard

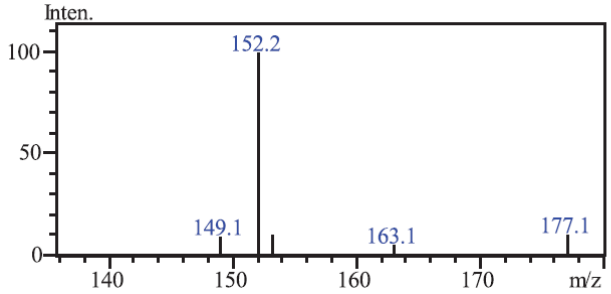


Figure 1: The Q1 scan mass spectrum of amantadine standard

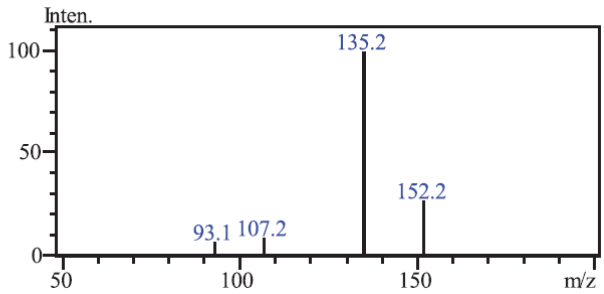


Figure 2: Product ion scan mass spectrum (CE value was -18V) of amantadine

2.2. MRM Chromatogram of Amantadine Standard

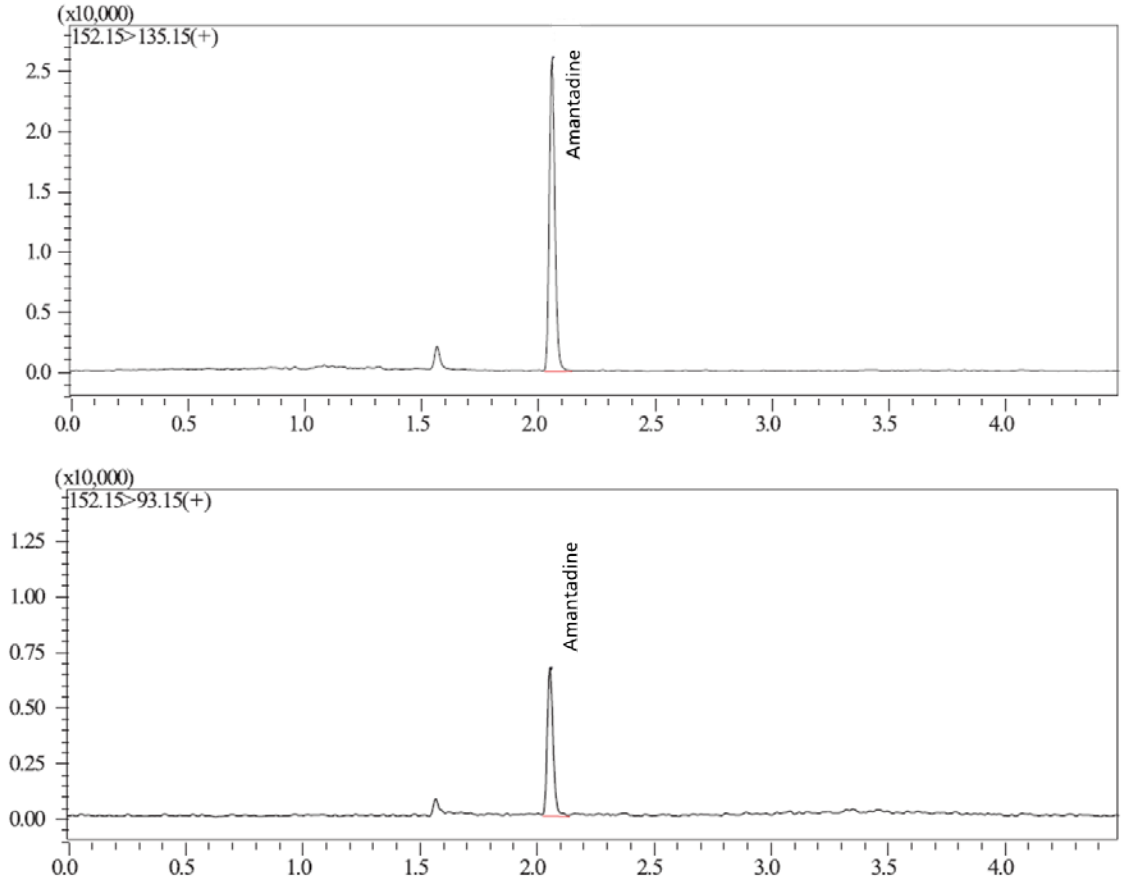


Figure 3: MRM Chromatogram of Amantadine Standard (1 µg/L)

2.3. Calibration and Linearity

Calibration was performed using a 5-point curve at concentrations of 0.02 µg/L, 0.1 µg/L, 0.5 µg/L, 5 µg/L and 10 µg/L. Quantitation was conducted using the external standard method and the calibration curve of amantadine is shown in Figure 4. The calibration curve obtained had good linearity with the linear equation Y = (38915.5) X + (1207.4), and correlation coefficient R of 0.99960.

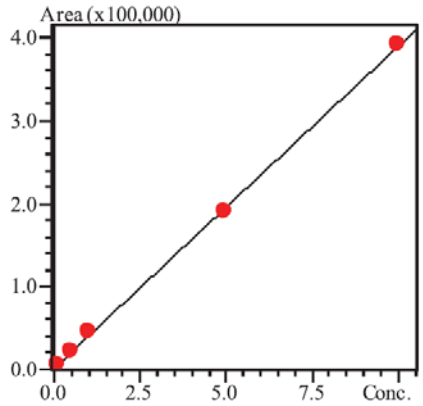


Figure 4: Calibration Curve of Amantadine

2.4. Precision Experiment

The prepared standard working solutions at concentrations of 0.5 µg/L, 1 µg/L and 5 µg/L were determined successively six times to evaluate instrument precision. The repeatability results of retention time and peak area are shown in Table 3. The results indicated that the RSD of retention time and peak area of standards at the specified concentrations were 0.07-0.19% and 0.85-1.32% respectively, indicating good instrument precision.

Table 3: Repeatability Results of Retention Time and Peak Area of Amantadine (n=6)

Conc.(µg/L)	RSD% (R.T.)	RSD% (Area)
0.5	0.12	1.32
1	0.19	1.13
5	0	0.85

2.5. Method Performance

In order to determine the sensitivity of the instrument, 7 replicates were prepared at the concentration of 0.02 µg/L and analyzed. The method detection limit (MDL) and lower limit of quantitation (LOQ) were calculated based on the standard deviations (S) determined from 7 injections where MDL = 3.14 x S and LOQ = 4 x MDL. The results are shown in Table 4.

Table 4: Detection Limit and Lower Limit of Quantitation of Amantadine

Compound Name	Standard deviation (S)	MDL (µg/L)	LOQ (µg/L)
Amantadine	0.0013	0.0041	0.0163

2.6. Matrix Spike and Recovery Experiment

A small amount of amantadine standard working solution was added to 2 g of blank chicken sample to achieve a matrix spike solution at concentration of 1 µg/kg. The matrix spike sample was extracted and purified, and analyzed according to the conditions in 1.2. Figure 5 and 6 show the MRM chromatogram of blank chicken, and matrix spike sample (1 µg/kg). The recovery was determined to be 77%.

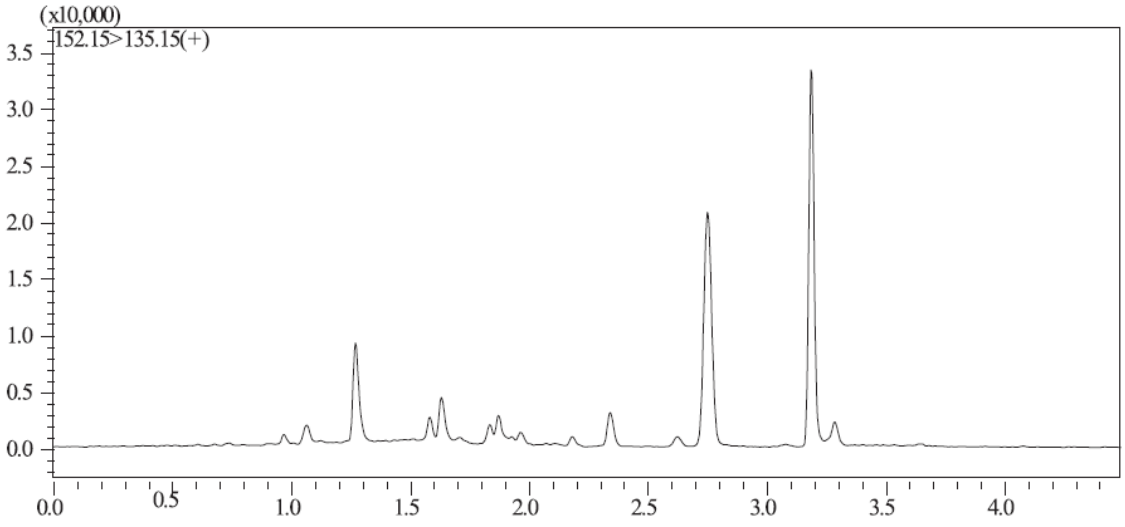


Figure 5: MRM Chromatogram of the Blank Chicken Sample

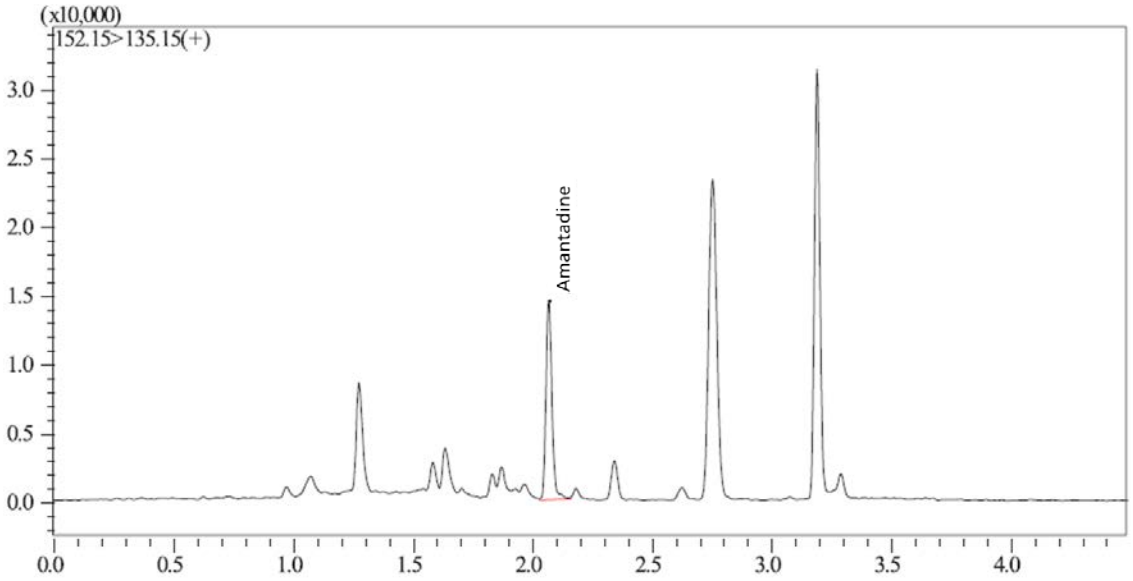


Figure 6: MRM Chromatogram of the 1 µg/kg Matrix Spike Sample

■ Conclusion

This application note describes a method for the determination of amantadine residues in chicken using Shimadzu Triple Quadrupole Mass Spectrometer LCMS-8050. After extraction and cleanup, the chicken sample was separated by Nexera X2 UHPLC and identified and quantified by LCMS-8050.

The calibration curve of amantadine exhibited good linearity within the range of 0.02-10µg/L. Six consecutive analyses were conducted at each of the three concentrations, and the RSD of retention time and peak area were below 0.19% and 1.32% respectively. This simple method can be used for the rapid and high-sensitivity detection of amantadine in chicken.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. Established in 1975, Shimadzu Scientific Instruments (SSI), the American subsidiary of Shimadzu Corporation, provides a comprehensive range of analytical solutions to laboratories throughout North, Central, and parts of South America. SSI maintains a network of nine regional offices strategically located across the United States, with experienced technical specialists, service and sales engineers situated throughout the country, as well as applications laboratories on both coasts.

For information about Shimadzu Scientific Instruments and to contact your local office, please visit our Web site at www.ssi.shimadzu.com

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS, INC.
Applications Laboratory
7102 Riverwood Drive, Columbia, MD 21045
Phone: 800-477-1227 Fax: 410-381-1222
URL http://www.ssi.shimadzu.com

First Edition: August 2018