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Application News

No. LCMSMS-179E

Liquid Chromatography Mass Spectrometry

Determination of Olaquindox Metabolites in Chicken by Ultra-High-Performance Liquid Chromatography coupled with Triple Quadrupole Mass Spectrometry

A method for the determination of olaquindox metabolites (3-methyl-quinoxaline-2-carboxylic acid, MQCA) in chicken using Shimadzu Nexera X2 and Triple Quadrupole Mass Spectrometer LCMS-8040 was developed. After extraction and cleanup, the commercially bought chicken sample was separated by Ultra-High-Performance Liquid Chromatograph and then quantified by Triple Quadrupole Mass Spectrometer LCMS-8040. The calibration curve of MQCA showed good linearity within the concentration range of 1-100 µg/L, with correlation coefficient of 0.9999. Six consecutive analyses were conducted for each of the low-, mid- and high-concentration standard working solutions (2 µg/L, 5 µg/L and 10 µg/L); and the RSD of retention time and peak area was 0.02-0.08% and 0.83-1.94% respectively, indicating good instrument precision.

Keywords: Chicken; Olaquindox metabolites; Triple quadrupole mass spectrometry

■ Introduction

Olaquindox, also known as Bay-o-nox, is a feed additive synthesized by Bayer pharmaceutical factory in the early 1970s. With moderate to high cumulative toxicity, olaquindox not only has an obvious teratogenic effect on most animals but also is potentially teratogenic, mutagenic and carcinogenic to humans. Therefore, it is banned or restricted worldwide for use in feed additives. As a major metabolite of olaquindox with relatively high in-vivo stability, 3-methyl-quinoxaline-2-carboxylic acid (MQCA) is one of the marker residues recognized by Codex Alimentarius Commission and can be used as a target analyte for olaquindox residue analysis and monitoring.

Currently, the detection of MQCA mainly uses kit assay, HPLC or HPLC coupled with tandem MS. The use of kit assay and HPLC are generally easy to use and fast, however the methods are not sensitive and may generate false negative results. With the use of LC-tandem MS (LC-MS/MS), it can achieve high selectivity, accuracy and sensitivity, and is suitable for the trace analysis of organic substances in complex matrices. In this application note, a method for the determination of olaquindox metabolites in chicken using Shimadzu Nexera X2 and LCMS-8040 was established, it serves as a reference for many applications and users (e.g. inspection personnel).

■ Experimental

1.1. Instruments

Shimadzu Nexera X2 UHPLC and Triple Quadrupole Mass Spectrometer LCMS-8040 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-8040, and LabSolutions Ver. 5.60 Chromatography Workstation.

1.2. Analytical Conditions

Liquid chromatography (LC) parameters

- Chromatographic column: Inertsil ODS-4 HP, 2.1 mm (I.D.) x 150 mm (L), 3 µm
- Mobile phase A: solution containing 0.1% formic acid
- Mobile phase B: Methanol
- Elution mode: gradient elution; the initial concentration of phase B was 30%; refer to Table 1 for the gradient program.
- Flow rate: 0.4 mL/min
- Column temperature: 40°C
- Injection volume: 2 µL

Table 1: Gradient Program

Time(min)	Module	Command	Value
0.20	Pumps	Pump B Conc.	30
2.80	Pumps	PumpB Conc.	95
2.81	Pumps	Pump B Conc.	30
4.50	Controller	Stop	

Mass spectrometry (MS) parameters

- Ionization mode: ESI (+)
- Ion spray voltage: 4.5 kV
- Nebulizer gas: Nitrogen 3.0 L/min
- Drying gas: Nitrogen 15 L/min
- Collision gas: Argon
- DL temperature: 250°C
- Heating module temperature: 400°C
- Scan mode: Multiple Reaction Monitoring (MRM)
- Dwell time: 80 msec
- Pause time: 3 msec
- MRM parameters: Refer to Table 2

Table 2: MRM Parameters

Compound name	CAS	Precursor ion	Product ion	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
3-Methyl-quinoxaline-2-carboxylic acid (MQCA)	74003-63-7	189.15	145.10* 143.05	-14 -14	-15 -16	-26 -26
Quinoxaline-2-carboxylic acid-D4 (IS)	-	179.15	133.1	-13	-18	-23

Note: * represents quantifier ion

1.3. Preparation of Standard Solutions and Sample Pretreatment

Preparation of standard working solutions:
Standard stock solution at concentration of 10 mg/L was prepared using methanol. The standard stock solution is sequentially diluted with blank extract to give a series of standard working solutions at concentrations of 1, 2, 5, 10, 50 and 100 µg/L.

Chicken sample pretreatment:
The sample was treated and prepared according to the methods stated in "GB/T 20746-2006: Determination of Residues of Carbadox and Olaquindox and the Metabolites in Bovine and Porcine Liver and Muscles."

■ Results and Discussion

2.1. Q1 Scan and Product Ion Scan Mass Spectra of the Standard Sample

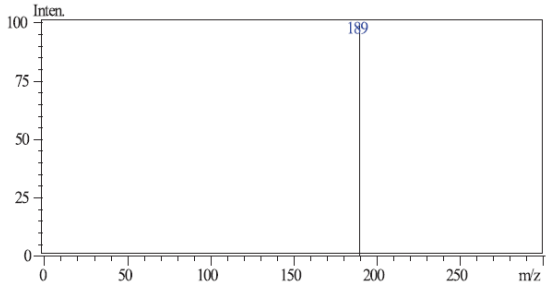

Figure 1: The Q1 scan mass spectrum of MQCA

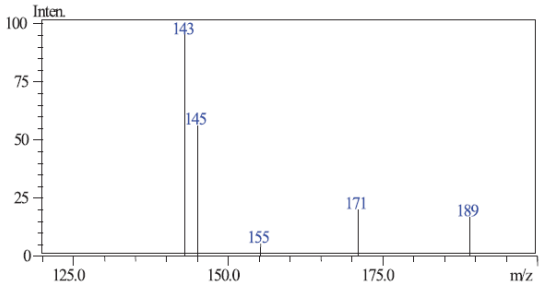

Figure 2: Product ion scan mass spectrum (CE value was -16V) of MQCA

2.2. MRM Chromatogram of the Standard Sample

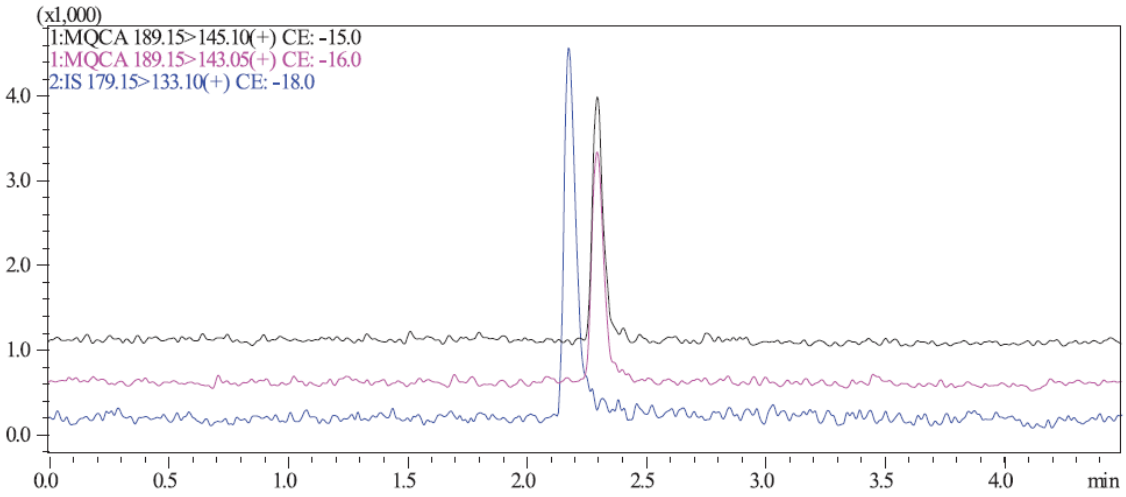

Figure 3: MRM Chromatogram of the Standard MQCA Sample (1 µg/L)

2.3. Calibration and Linearity

Calibration is performed using a 5-point curve at concentrations of 0.5, 5, 10, 50 and 100 µg/L. External standard method was used for quantitation and the calibration curve of MQCA is shown in Figure 4. The calibration curve obtained had good linear relationship with the linear equation, Y = (0.560453) X, and correlation coefficient R of 0.99990.

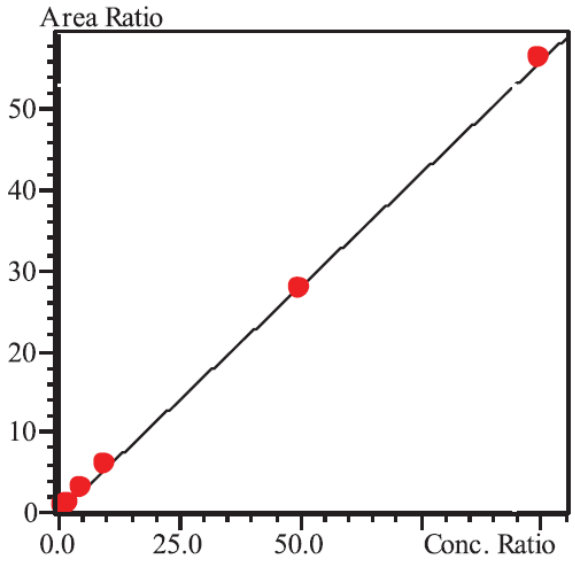

Figure 4: Calibration Curve of MQCA

2.4. Precision Experiment

The standard working solutions at low-, mid- and high- concentrations of 2, 5 and 10 µg/L were determined six times successively to examine instrument precision. The results shown in Table 3 indicated that the RSD of retention time and peak area of standards was 0.02-0.08% and 0.83-1.94% respectively, indicating good instrument precision.

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

Conc.(µg/L)	RSD% (R.T.)	RSD% (Area)
2	0.08	1.94
5	0.08	0.97
10	0.02	0.83

2.5. Method Performance

In order to examine the sensitivity of the instrument, 7 replicates were prepared at the concentration of 1 µg/L. Seven parallel injections were conducted to analyze the results. 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: Method Detection Limit and Lower Limit of Quantitation of MQCA

Compound Name	Standard deviation (S)	MDL (µg/L)	LOQ (µg/L)
MQCA	0.062	0.19	0.78

2.6. Matrix Spike and Recovery Experiment

A small amount of MQCA standard working solution was spiked to 5 g of blank chicken sample to achieve a matrix spike solution at a concentration of 1 µg/kg. The matrix spike sample was extracted, 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 85.7%.

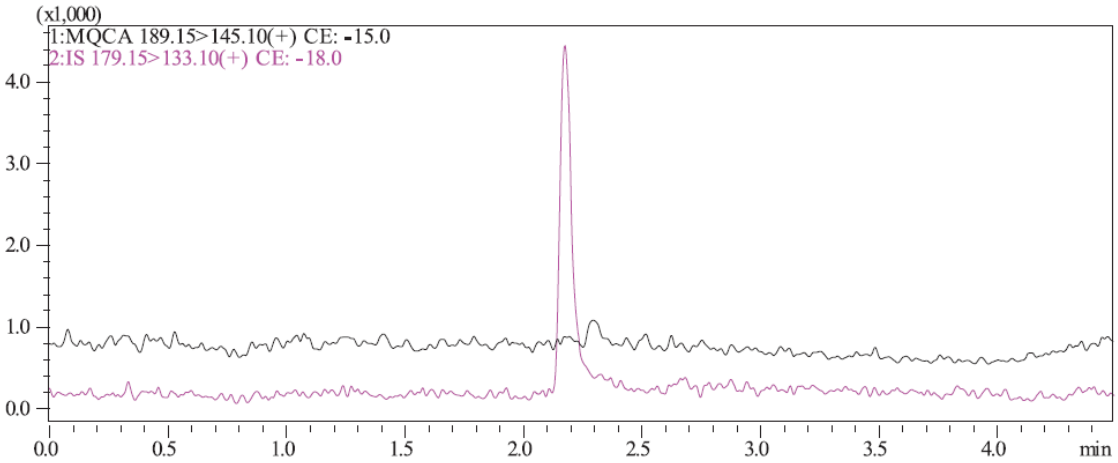

Figure 5: MRM Chromatogram of the Blank Chicken Sample

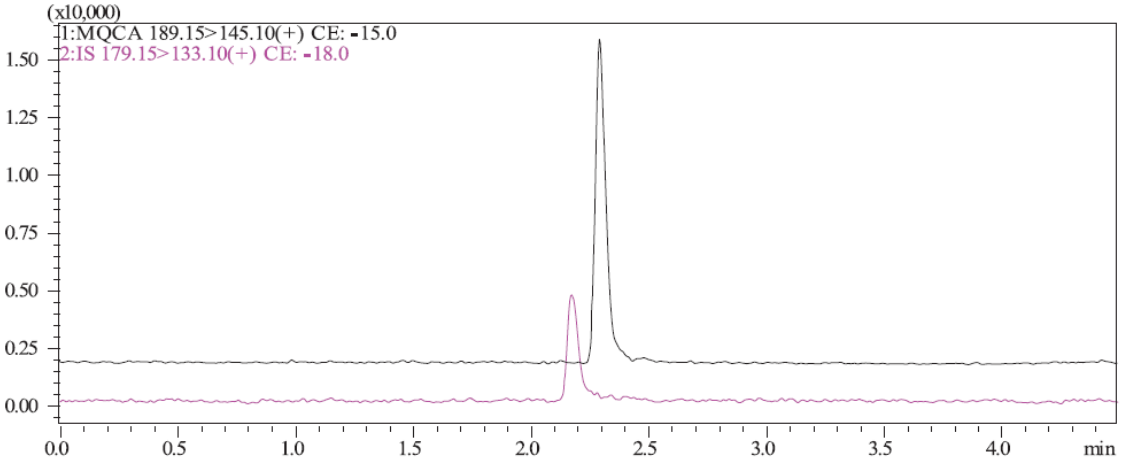

Figure 6: MRM Chromatogram of the 1 µg/kg Matrix Spike Sample

■ Conclusion

A method for the determination of olaquindox metabolites (i.e. MQCA) in chicken using Shimadzu Triple Quadrupole Mass Spectrometer LCMS-8040 was established. After extraction and cleanup, the chicken sample was separated by UHPLC Nexera X2 and quantified by Triple Quadrupole Mass Spectrometer LCMS-8040. The calibration curve of MQCA exhibited good linearity within the concentration range of 1-100 µg/L. Six consecutive analyses were conducted for standard working solutions at different concentrations and the RSD of retention time and peak area was below 0.08% and 1.94% respectively. This simple method meets the LOQ requirements specified in "GB/T 20746-2006: Determination of Residues of Carbadox and Olaquindox and the Metabolites in Bovine and Porcine Liver and Muscles" and can be used for the rapid and high-sensitive determination of olaquindox metabolites in chicken.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

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