Application Note: 473

Measurement of Chloramphenicol in Honey Using Automated Sample Preparation with LC-MS/MS

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Key Words

- Aria TLX-1
- TurboFlow technology
- TSQ Vantage mass spectrometer
- Food safety

Introduction

Chloramphenicol (CAP) (Figure 1) is a bacteriostatic antimicrobial previously used in veterinary medicine. CAP has been found to be potentially carcinogenic, which makes it an unacceptable substance for use with any food-producing animals, including honey bees. The United States, Canada, and the European Union (EU), as well as many other countries, have completely banned the usage of CAP in the production of food. The EU has set a minimum required performance level (MRPL) for CAP in food of animal origin at a level of 0.3 µg/kg[1].

Currently sample preparation for the detection of CAP in honey by liquid chromatography-mass spectrometry (LC-MS/MS) involves complex offline extraction methods such as solid phase extraction, QuEChERS, or liquid/liquid extraction, all of which require additional sample concentration and reconstitution in an appropriate solvent. These sample preparation methods are time-consuming, often taking 2 hours or more per sample, and are more vulnerable to variability due to errors in manual preparation. To offer a high sensitivity (low ppbs) CAP detection method and timely, automated analysis of multiple samples, our approach is to use the Thermo Scientific Aria TLX-1 system powered by TurboFlow™ automated sample preparation technology coupled to the detection capabilities of a high-sensitivity Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

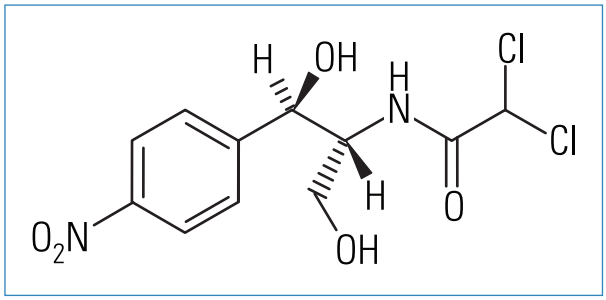

Figure 1: Chemical structure of chloramphenicol

Goal

Develop a quick, automated sample preparation, LC-MS/MS method for chloramphenicol (CAP) in honey by negative ion heated electrospray ionization (H-ESI) using a deuterated internal standard (CAP-d5).

Experimental

Sample Preparation

Organic wildflower honey used in this analysis for the preparation of blanks, QCs, and standards was obtained from a local supermarket. CAP was obtained from Sigma-Aldrich, US (Fluka) and CAP-d5 (100 µg/mL in acetonitrile) from Cambridge Isotope Labs, Inc. (Andover, MA, USA). A CAP working solution was prepared in 1:1 methanol/water at 100 ng/mL. The honey was diluted by adding 30 mL of purified water to 10 g of honey (1:3 w/v). CAP standards and QC standards were serially diluted to the target concentrations with 1:3 honey/water containing 250 pg/mL CAP-d5 as an internal standard. Target standard concentrations ranged from 0.024 µg/kg to 1.5 µg/kg. Four samples of honey obtained internationally and one sample obtained from a local grocery store were analyzed as "samples" and prepared by dissolving 5 g of honey in 15 mL of purified water. The internal standard was added to a final concentration of 250 pg/mL. The injection volume was 25 µL.

Method

The honey extract clean-up was accomplished using the Thermo Scientific TurboFlow method run on an Aria™ TLX-1 LC system using a TurboFlow Cyclone polymer-based extraction column. Simple sugars were un-retained and moved to waste during the loading step, while the analyte of interest was retained on the extraction column. This was followed by organic elution to a Thermo Scientific Hypersil GOLD end-capped silica-based C18 reversed-phase analytical column and gradient elution to a TSQ Vantage™ triple stage quadrupole MS with a H-ESI source. CAP precursor *m/z* 321 → 257, 152, and 194 high resolution selective reaction monitoring (H-SRM) transitions were monitored in the negative ionization mode. The 257 *m/z* product ion for CAP was used for quantitation and the 152 and 194 *m/z* product ions were used as confirmation. Precursor 326 *m/z* → 157 *m/z* and 262 *m/z* H-SRM transitions were monitored for CAP-d5. The total LC-MS/MS method run time was about 5 minutes.

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Aria TLX-1 System Parameters

Columns
Thermo Scientific TurboFlow Cyclone column (0.5 x 50 mm)
Thermo Scientific Hypersil GOLD (3 x 50 mm, 3 µm particle size)
The analytical column was kept at 30 °C

Mobile Phases

Loading Pump	
Mobile Phase A:	0.02% Acetic Acid (aq)
Mobile Phase B:	Methanol
Mobile Phase C:	1:1:1 Acetone/Acetonitrile/Isopropanol with 0.3% Formic Acid

Elution Pump	
Mobile Phase A:	0.02% Acetic Acid (aq)
Mobile Phase B:	Methanol

Mass Spectrometer Parameters

MS analysis was carried out on a TSQ Vantage™ triple stage quadrupole mass spectrometer. The MS conditions were as follows:

Ion Polarity:	Negative ion mode
Spray Voltage:	1000 V
Vaporizer Temperature:	526 °C
Capillary Temperature:	225 °C
Sheath Gas Pressure (N_2):	60 units
Auxiliary Gas Pressure (N_2):	35 units
Ion Sweep Gas Pressure (N_2):	0.500 units
Scan Type:	H-SRM
Chrom Filter Peak Width:	5.0 s
Collision Gas Pressure:	1.1 mTorr
Declustering Voltage:	0 V
Scan Width:	0.002 *m/z*
Scan Time:	0.200 s
Q1 Resolution:	0.200 Da FWHM
Q3 Resolution:	0.700 Da FWHM
S-Lens (*m/z* 321):	65 V
Collision Energy (*m/z* 321 > 257):	12 V

The entire experiment was controlled by Aria operating software 1.6.2. The data was processed with Thermo Scientific LCQUAN 2.5.6 quantitative software using Thermo Scientific Xcalibur 2.0.7 SP1 data system software.

Results and Discussion

Figure 2 shows comparison chromatography of CAP and CAP-d5 in 1:3 honey/water matrix pre-blank, at the lower limit of quantitation (LLOQ), the upper limit of quantitation (ULOQ), and a post-high standard blank. By comparing pre- and post- blanks, it is clearly indicated that the carryover level has been minimized by using TurboFlow technology. Matrix-matched calibration standards of CAP showed a linear response at greater than two orders of magnitude with $r^2 = 0.9944$ (Figure 3). All %CVs (n=3) were less than 19% for the LLOQ and less than 8% for all other points of the curve. Internal standard % relative standard deviation (RSD) was less than 9%. Chloramphenicol was not detected in any of the honey samples obtained internationally nor from the US. The calculation of actual concentrations of CAP in honey was based on a density of honey equal to 1.367 kg/L[2]. Signal suppression effects were examined by comparing the recovery of CAP and CAP-d5 in three neat (purified water) standards (0.19, 0.38, and 1.5 µg/kg) with their counterparts in 1:3 honey/water. The average recovery corrected by the internal standard was 80.9%, 96.0%, and 92.1% for 0.19, 0.38, and 1.5 µg/kg respectively.

Table 1 highlights current published results of detection methods for chloramphenicol in honey by LC-MS compared to the results of this study. Sample preparation in our study was between 7 and 24 times faster (estimated) than the three current alternative methods discussed. The LC-MS method run time was equal to or as much as four times faster. The limit of detection (LOD) was between 5.7 and 20 times lower than those that reported their LOD. Finally, the LLOQ was between 3.7 and 27 times lower.

Conclusion

A quick, automated online extraction, LC-MS/MS method has been developed here that is sensitive enough to detect 0.023 µg/kg (LOD) and quantify 0.047 µg/kg (LLOQ) of CAP in honey for screening purposes. This is significantly lower than the MRPL of 0.3 µg/kg (ppb) set by the EU. This method eliminates the need for time-consuming sample preparation procedures such as solid phase extraction, QuEChERS, and liquid-liquid extraction. Dilution with water to reduce sample viscosity is the only pretreatment required. The LC-MS/MS method run time is 5 minutes, and the sample throughput can be improved by multiplexing on an Aria TLX-4 system.

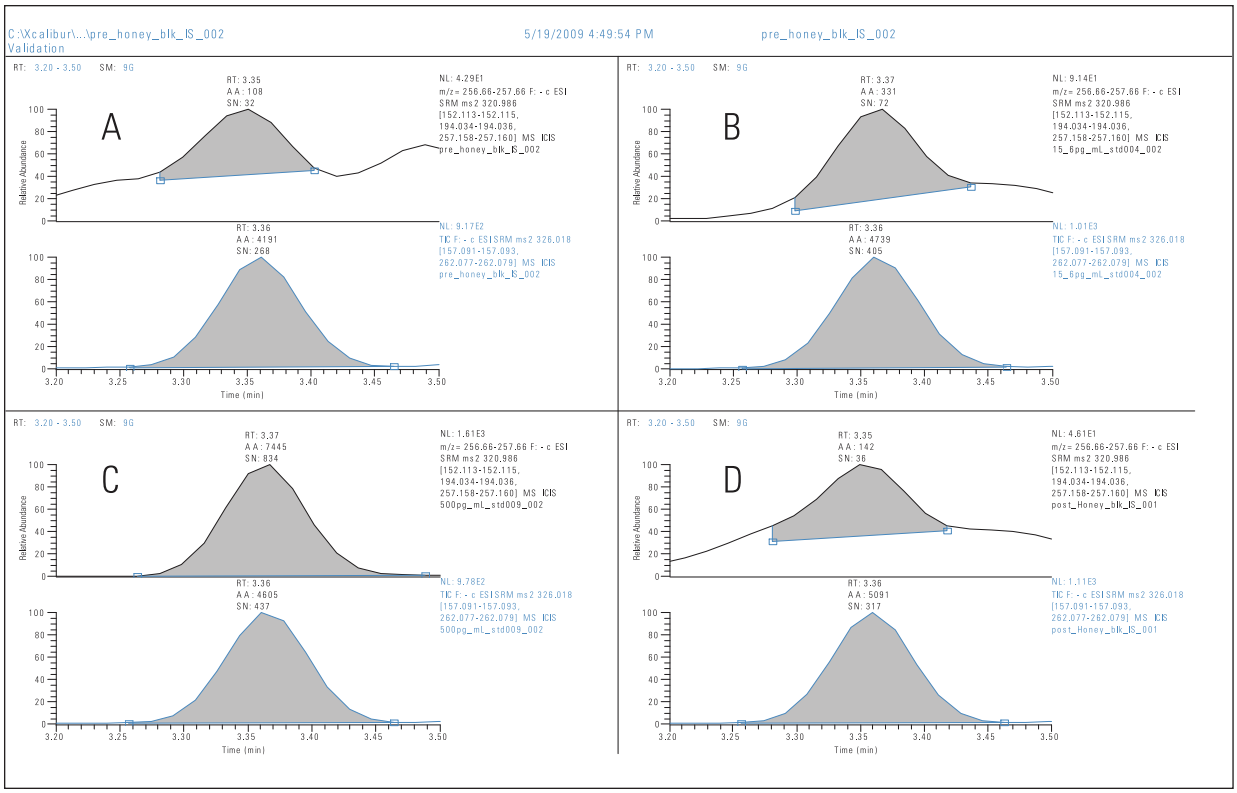

Figure 2: Chromatography comparison of CAP SRM *m/z* 257 transition (upper traces) and CAP-d5 (lower traces) in Pre-Blank Honey Matrix (panel A), at LLOQ of 0.047 µg/kg (panel B), at ULOQ of 1.5 µg/kg (panel C), and in Post-High Standard Blank (panel D)

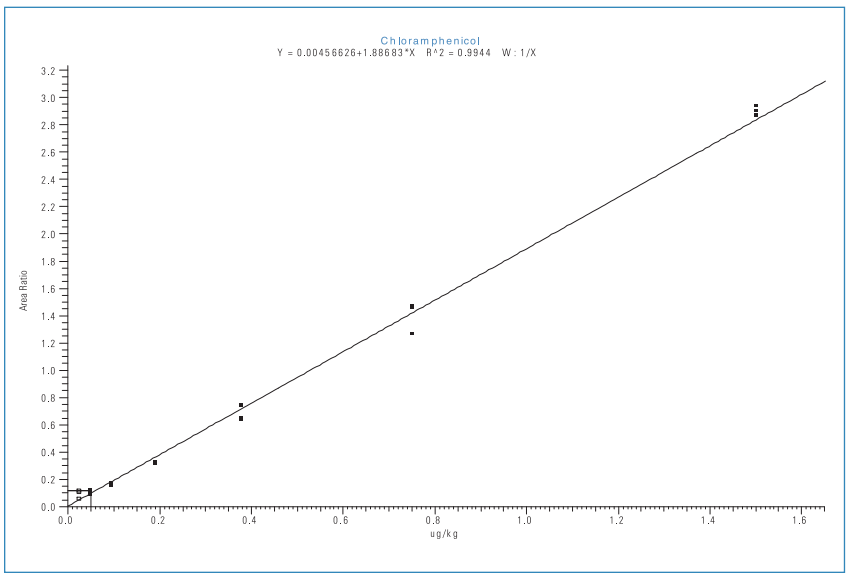

Figure 3: Linear regression curve of CAP in honey:water matrix standards based on area ratio with internal standard CAP-d5 (1/X weighting) showing linearity over two orders of magnitude using the TurboFlow method.

	TurboFlow Method (on-line)	SPE (off-line)	QuEChERS (off-line)	Liquid/Liquid Extraction (off-line)
Sample prep time (min)	5	120 (estimated)	35 (estimated)	60 (estimated)
LC/MS Method Runtime (min)	5	12	5 or 10	20
Sample Extraction	TurboFlow Cyclone column (0.5 X 50 mm) on-line LC extraction	J.T. Baker 500-mg Bakerbond C18 SPE	"Modified" QuEChERS	Hexane/Acetonitrile Extraction, Evaporation, and Redissolution
Analytical Column	Thermo Scientific Hypersil GOLD, 3 x 50 mm, 3 um	Macherey-Nagel Nucleosil 100-5 C18 HD column, 2 X 70 mm	100 mm × 4.6 mm RP-18e monolithic column (Merck USA) or a 4.6 mm × 250 mm, 5-µm particle, XDB conventional column (Agilent)	Phenomenex C18 Luna column, 2 X 150 mm, 5 µm
Injection volume (µL)	25	10	10	20
HPLC system	TLX-1	HP 1100 Binary pump	Agilent 1100 Binary pump	Agilent 1100 Binary pump
Detector	Thermo Scientific TSQ Vantage Triple Quadrupole MS	Micromass QuattroMicro Triple Quadrupole MS	ESI-MS (Not specified)	Applied Biosystems API 3000 Triple Quadrupole MS
LLOD (µg/kg)	0.024	0.2	Not specified.	0.11
LLOQ (µg/kg)	0.047	0.5	0.20	0.14
Reference	Data presented herein.	2004 by Ortelli et al. (3)	2006 by Pan et al. (4)	2007 by Rodziewicz et al. (5)

Table 1: Comparison of CAP detection in honey by TurboFlow method with current sample prep alternatives.

References and Acknowledgements

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