

Measurement of Chloramphenicol in Honey Using an Online Sample Extraction with LC-MS/MS

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Overview

A quick, automated online extraction LC-MS/MS method was developed for the detection of Chloramphenicol (CAP) in honey by negative ion heated electrospray ionization (HESI) using a deuterated internal standard (CAP-d₅).

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]. Current 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 appropriate solvent. These sample preparation methods are time-consuming often taking 2 hours or more per sample (estimate) and are more vulnerable to variability due to errors in manual preparation. In order to offer a high sensitivity (in the low ppb) CAP detection method and timely, automated analysis of multiple samples, our approach is to use Thermo Scientific TurboFlow online sample extraction technology coupled to the detection capabilities of a high-sensitivity Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Experimental

Sample Preparation:

Organic wildflower honey used in this analysis for 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. Dilution of honey involved the addition of 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 µg/mL CAP-d₅ as internal standard. Target standard concentrations ranged from 0.024 to 1.5 mg/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. Internal standard was added to a final concentration of 250 µg/mL. Injection volume was 25 µL.

Methods:

The honey extract clean-up was accomplished using a TurboFlow™ method run on a Thermo Scientific Aria TLX-1 LC system using a TurboFlow Cyclone polymer-based extraction column. Simple sugars were unretained 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 an end-capped silica-based C18 reversed-phase analytical column (Thermo Scientific Hypersil GOLD) and gradient elution to a TSQ Vantage™ triple stage quadrupole MS with a HESI source. CAP precursor m/z 321 > 257, 152, and 194 H-SRM high resolution selective reaction monitoring) transitions were monitored in 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 to 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.

Aria™ TLX-1 System Parameters

Columns
Thermo Scientific 0.5 x 50 mm TurboFlow Cyclone column
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 Acetone: Acetonitrile: Isopropanol with 0.3% Formic Acid
Elution Pump
Mobile Phase A: 0.02% Acetic Acid (aq)
Mobile Phase B: Methanol

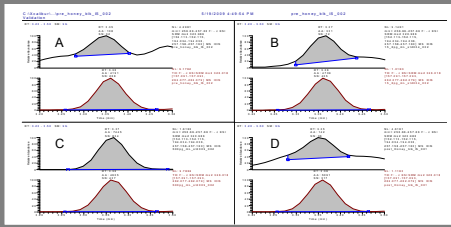
Mass Spectrometer Parameters

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

Ion Polarity: Negative ion mode
Spray Voltage: 1000 V
Vaporizer Temperature: 526 °C
Capillary Temperature: 225 °C
Sheath Gas Pressure (N₂): 60 units
Auxiliary Gas Pressure (N₂): 35 units
Ion Sweep Gas Pressure (N₂): 0.500 units
Scan Type: H-SRM
Chrom Filter Peak Width: 5.0 s
Collision Gas Pressure: 1.1 mTorr
Decustering Voltage: 0 V
Scan Width: 0.002 m/z
Scan Time: 0.200 s
Q1: 0.200 Da FWHM
Q2: 0.700 Da FWHM
S-Lens (m/z): 45 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 using Thermo Scientific LQquan 2.5.6 quantitative software using Thermo Scientific Xcalibur 2.0.7 SP1 data system software.

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 ULQO of 1.5 µg/kg (panel C), and in Post-High Standard Blank (panel D).



Results

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 (ULQO), and a post-high standard blank. Matrix-matched calibration standards of CAP showed linear response at greater than 2 orders of magnitude with $r^2 = 0.9944$ (Figure 3). All % CVs (n=3) were less than 19% for the LLOQ and less than 6% for all other points of the curve. Internal standard %RSD was less than 6%. Chloramphenicol was not detected in any of the honey samples obtained internationally nor from the US. Calculation of actual concentrations of CAP in honey was based on a density of honey equal to 1.367 g/mL [2]. Signal suppression effects were examined by comparing recovery of CAP and CAP-d₅ in 3 neat (purified) water matrices (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 80.0%, 86.0% and 92.1% for 0.19, 0.38 and 1.5 µg/kg respectively.

Figure 3. Linear Regression Curve of CAP in Honey - Water Matrix Standards based on Area Ratio with Internal Standard CAP-d₅ (1X-weighting).

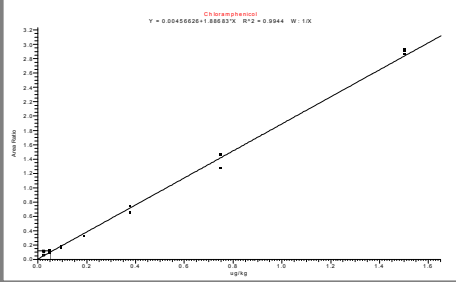


Table 1 highlights current published results of detection methods for chloramphenicol in honey by LC-MS compared to the results of this study. Sample preparation in our study was between 7 and 24 times faster (estimated) than the three current alternative methods discussed. The LC-MS method run time was equal to or as much as 4 times faster. The limit of detection (LOQ) was between 5.7 and 20 times lower than those that reported their LOQ. Finally, the LLOQ was between 3.7 and 27 times lower.

Table 1. Comparison of CAP Detection in Honey by TurboFlow Method with Current Alternatives.

Sample prep time (min)	TurboFlow Online Extraction	Offline SPE	Offline QuEChERS	Liquid/Liquid Extraction
5	120 (estimated)	35 (estimated)	60 (estimated)	60 (estimated)
LC/MS Method Runtime (min)	5	12	5 or 10	20
Sample Extraction	Thermo Scientific Cyclone column (0.5 X 50 mm) online LC extraction	J.T. Baker 500mg Bakerbond C18 SPE column	Modified QuEChERS	Hexane/Acetone/nitrite Extraction, Evaporation, and Redissolution
Analytical Column	Thermo Scientific Hypersil GOLD 3 x 50 mm, 3 µm	Madshey-Nagel Nucleosil 100 S C18 HD column 2 X 70 mm	100 mm x 4.6 mm ID 10 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	Agilent 1200 Binary	HP 1100 Binary pump	Agilent 1100 Binary	Agilent 1100 Binary
Detector	Thermo Scientific TSQ Vantage Triple Quadrupole MS	Micromass QuattroMicro Triple Quadrupole MS	ESI/MS (Not specified)	Applied Biosystems API 3000 Triple Quadrupole MS
LLOQ (µg/kg)	0.024	0.2	Not specified	0.11
LOQ (µg/kg)	0.047	0.5	0.20	0.14
Reference	Data presented herein.	2004 by Orrell et al. (3)	2006 by Pan et al. (4)	2007 by Rodziewicz et al. (5)

Conclusions

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 EU. This TurboFlow 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 sample 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.

References

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