An Automated Solid Phase Extraction (SPE) Method for the Determination of Chloramphenicol in Honey

Application Note FB0111

Keywords

Automation, Chloramphenicol, GX-271 ASPEC™, Honey, LC/MS, LC/MS-MS, Sample Preparation, Solid Phase Extraction, SPE

Introduction

This study was performed in collaboration with Curtis Hedman at the WI State Laboratory of Hygiene, Madison, WI, USA

Chloramphenicol (CAP) is a potent broad-spectrum antibiotic that can cause potentially serious side effects in humans. The use of this antibiotic in foods is highly regulated. Recent reports of honey contaminated with CAP has increased the need for laboratories to determine the presence of CAP in imported honey samples (Helle, N. and Gil, C., 2006; McGrath, T., 2006). The presence of CAP in honey is typically determined by liquid chromatography-mass spectrometry or LC/MS-MS (Quon, D. et al., 2006; Turnipseed, S. et al., 2002; Zhao, L. and Ball, C., 2009). Since honey is a complex matrix and can cause ion suppression and other matrix interferences, CAP is typically extracted by liquid/liquid extraction(LLE) followed by purification using one or two solid phase extraction (SPE) steps prior to analysis.

These steps can be very time-consuming, labor intensive and subject to poor reproducibility. The purpose of this study was to develop and evaluate an automated sample preparation procedure for the determination of CAP in honey. Three SPE cartridges were compared to determine their suitability for purification after the liquid/liquid extraction step. LC/MS with Selected Ion Monitoring (SIM) was utilized to evaluate and compare the overall effectiveness of removing interferents from honey extracts using the three types of SPE cartridge. CAP recovery was compared for the 3 brands of SPE cartridge using LC/MS-MS. SPE for CAP in honey from published methods (Turnipseed, S. et al., 2002; Zhao, L. and Ball, C., 2009) was readily amenable to automation.

Gilson, Inc. World Headquarters
Middleton, WI 53562 USA
Telephone: 800-445-7661

Figure 1. Gilson GX-271 ASPEC™ System with 406 Syringe Pump (part no. 2614007).

Materials & Methods

Materials

All solvents were suitable for GC, HPLC, LC/MS and spectrophotometry. All reagents were ACS grade quality or better. Chloramphenicol (> 98% purity) was obtained from Sigma Aldrich. Chloramphenicol standards were prepared in methanol and stored at 4° C. Honey was obtained from a local honey farm in Wisconsin.

The GX-271 ASPEC system (Figure 1) was utilized to perform solid phase extraction (SPE). The GX-271 ASPEC was controlled using TRILUTION® LH Software. This software also allowed for the addition of select liquid handling steps and the ability to integrate user prompts for manual steps in the procedure (Figure 2).

Sample Preparation Steps Prior to SPE

1. Accurately weigh 2.5g of honey in a 16 x 100 mm screw cap tube.
2. Add 2.5 mL of ultrapure water to the honey (used Dispense Task in TRILUTION LH).
3. Fortify honey (except blanks) with 250 µL of a 1µg/mL chloramphenicol standard solution in methanol.
4. Mix honey using vortex and hand shaking (Figure 2).
5. Add 5 mL ethyl acetate and cap tubes. Let stand for 1 minute and then shake carefully for 5 minutes.
6. Centrifuge at 3500 rpm for 10 minutes and the transfer upper organic layer to another tube.
7. Repeat steps 4 and 5 using 2.5 mL ethyl acetate.
8. Take the final tube (13 x 100mm) containing the organic extract and evaporate with N_2 at 45°C.

9. Reconstitute in 5 mL water, allow the extract to stand for 5 min, and vortex for 2 min. The extract is now ready for SPE clean-up.

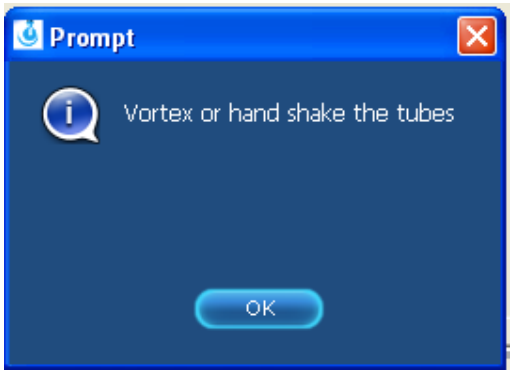


Figure 2. Prompts were used in TRILUTION® LH software to notify a user which steps to perform manually in the extraction method.

The SPE procedure used Agilent SampliQ™ OPT (6mL/170mg) cartridges, Macherey-Nagel CHROMABOND® HR-X (6mL/200mg) cartridges and Waters Oasis™ HLB (6mL/200mg) cartridges. The cartridges were sealed with Gilson 6mL DEC sealing caps.

The solid phase extraction protocol was automated using the Gilson GX-271 ASPEC™ system. The SPE steps are summarized with the general schematic provided in the GX-271 ASPEC control software, TRILUTION LH (Figure 3).

Figure 3. TRILUTION LH Basic SPE Tasks for Solid Phase Extraction of chloramphenicol from Honey

The summary of each step are as follows:

- Condition the cartridge with 5 mL methanol at 6mL/min.
- Condition the cartridge with 6mL water at 6mL/min.
- Load the honey extract in water at a flow rate of 3mL/min.
- Wash the cartridge with 5mL of water at a flow rate of 3mL/min. Follow this with an air push of 9mL to remove any excess water.
- Move the Gilson Mobile SPE Rack over the collection tubes (Figure 4).
- Elute the chloramphenicol with 5mL of 2:8 ethyl acetate: methanol at a flow rate of 1 mL/min.
- Evaporate to 0.25 mL with N_2 at 45°C and then add 0.75 mL methanol; vortex for 2 min. and transfer to an autosampler vial for injection.

Figure 4. Gilson Mobile SPE Rack

LC/MS and LC/MS-MS Analysis

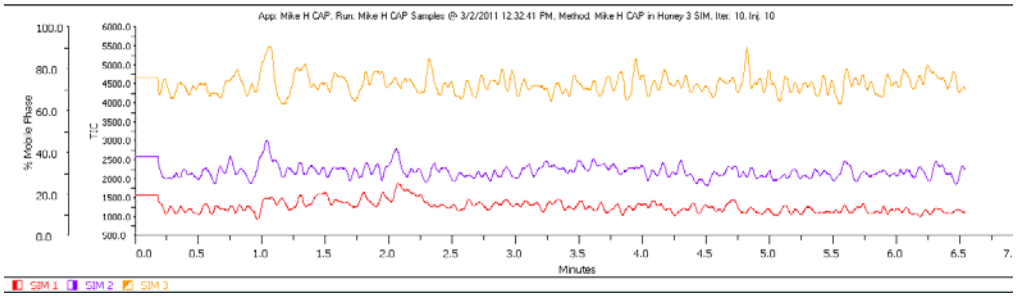
LC/MS analysis was carried out using a Gilson LC/MS Purification System with TRILUTION® LC software and a Perkin Elmer FLEXAR SQ 300 Single Quadrupole MS Detector (Figure 5). This system was used to evaluate the effectiveness of the SPE cartridge to remove interferents that may bias quantitation by LC/MS-MS.

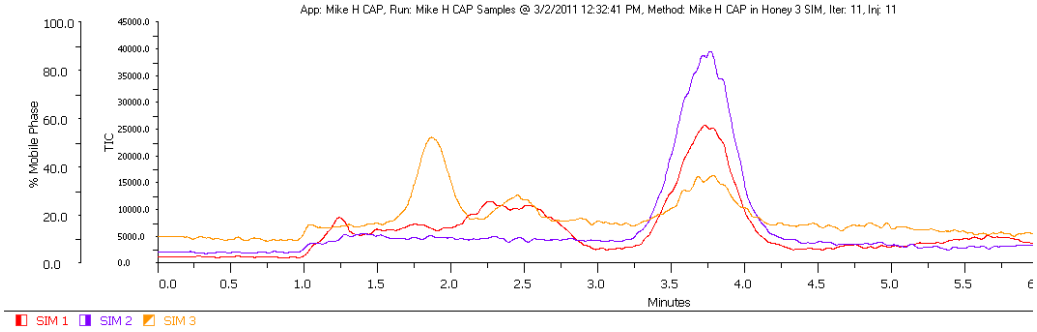
Figure 5. Gilson LC/MS purification system.

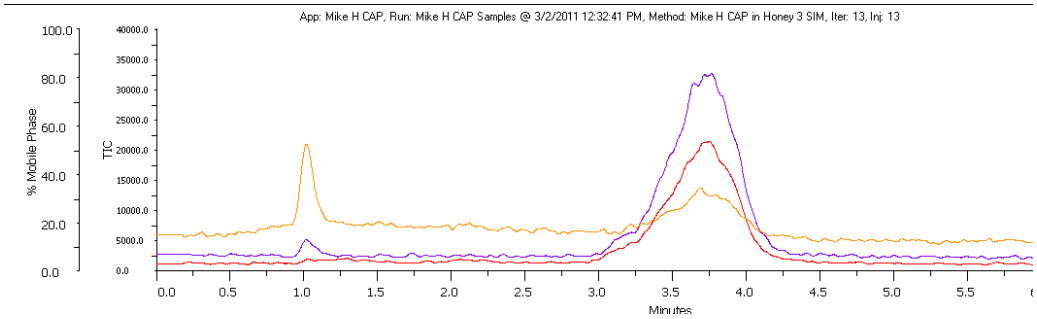
Conditions:

Column: Phenomenex Luna 5µC18, 21.2 x 50mm
Mobile Phase: 60:40 Water: Methanol, ISOCRATIC, 20mL/min
Injection: 500uL with MRA Splitter ratio of 1:500
Make-up pump to MS source flow rate: 0.5mL/min
Mode: Negative ion
Ions monitored (SIM): *m/z*=321(parent), *m/z*=257, *m/z*=152
Countercurrent drying gas temperature: 350°C and flow rate 15L/min
Nebulizing gas pressure: 80 psi of N_2

LC/MS-MS analysis for CAP recovery was performed on an Agilent 1100 HPLC with tandem mass spectrometric detection (Applied Biosystems/MDS SCIEX API 4000).
Conditions:
Column: Phenomenex Luna 3 µ C18, 3.0 x 150mm
Injection and Flow rate: 20 µL, 0.3 mL/min
Mobile Phase: 0 min 0.1% Formic acid: Methanol (80:20)
3 min 0.1% Formic acid: Methanol (80:20)


App: Mike H CAP, Run: Mike H CAP Samples @ 3/2/2011 12:32:41 PM, Method: Mike H CAP in Honey 3 SIM, Iter: 10, Inj: 10
% Mobile Phase
TIC
Minutes
SIM 1
SIM 2
SIM 3


App: Mike H CAP, Run: Mike H CAP Samples @ 3/2/2011 12:32:41 PM, Method: Mike H CAP in Honey 3 SIM, Iter: 11, Inj: 11
% Mobile Phase
TIC
Minutes
SIM 1
SIM 2
SIM 3


App: Mike H CAP, Run: Mike H CAP Samples @ 3/2/2011 12:32:41 PM, Method: Mike H CAP in Honey 3 SIM, Iter: 13, Inj: 13
% Mobile Phase
TIC
Minutes