

INTRODUCTION

Chloramphenicol (CAP) is a broad-spectrum antibiotic effective against a wide range of microorganisms, but it is well known to have serious toxic effects on humans such as aplastic anemia, and a suspected carcinogenicity! CAP is therefore totally prohibited in food in most countries. EU has determined for CAP a minimum required performance limit (MRPL) of 0.3 µg/kg in food of animal origin. Many methods have been recently published for determining CAP residues in food at and below the MRPL, especially for shrimps, milk and honey.²

We present a method for the detection of CAP in honey, using a new commercially available technology based on Laser Diode Thermo Desorption (LDTD) of the analyte, followed by atmospheric pressure chemical ionization (APCI) and MS/MS analysis. The sample preparation is an SPE extraction using Molecularly Imprinted Polymer cartridge (MIP), and the analysis of each sample by the instrument takes no more than 8 seconds.

A linear regression shows a correlation of $R = 0.9966$ over a range of 0.1 to 3 ppb. 5 different honeys, including a robust dark buckwheat one, were spiked at 0.3 and 1.0 ng/g, and back calculation obtained show a maximal deviation of 17 % (table 1) from expected values. Relative standard deviation (RSD) is 7.2 % at 0.3 ng/g and 8.8 % at 1.0 ng/g.

LDTD Technology

LDTD (Figure 1)

- Plug-and-play ionization source can be interfaced to most popular mass spectrometers.
- Thermal desorption is induced by a laser diode.
- The sample is carried by a carrier gas to a corona discharge region for APCI.
- Loader holds up to 10 LazWell™ plates (960 samples).

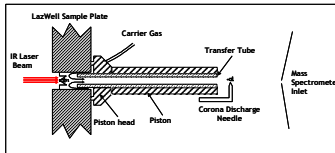


Figure 1 Schematic of the LDTD ionization source.

LazWell™ Plate (Figure 2)

- Standard 96-well plate format.
- Low volume delivery (from 1 to 10 µL of sample per well).
- No carryover.
- No sample desalting needed.
- No mobile phase needed.
- Sample dried at room temperature.

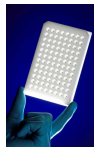


Figure 2 LazWell™ sample plate.

METHOD

Instrumentation (Figure 3)

- LDTD model T-960, Phytronix Technologies
- TSQ® Vantage™ Triple-quadrupole Mass spectrometer, Thermo Fisher Scientific



Figure 3 LDTD-MS/MS analytical system.

Sample preparation:

*MIP steps are based on recommended procedure from data sheet provided by SUPELCO with SupelMIP™ SPE – Chloramphenicol cartridge used in this experiment.

- Weigh 1 g of honey
- Add d5-CAP as Internal Standard
- Dilute with 1 mL water

Cartridge conditioning and loading :

- 1mL methanol
- 1mL ultra pure water
- Load 1 mL of diluted sample

Wash #1:

- 2 x 1mL ultra-pure water
- 1 mL 5 % ACN in 0.5 % acetic acid
- 2 x 1 mL 1 % ammonia (aq)
- 1 mL 20 % ACN in 1 % ammonia

Wash #2:

- 2 x 1mL 2 % acetic acid in dichloromethane

Analyte Elution:

- 2 x 1 mL 10 % methanol in dichloromethane (v/v)

- Evaporation of the elution solvent to dryness
- Reconstitution in 180 µg/mL stearic acid solution in Methanol : Ethyl acetate (60:40)

- Transfer manually 2.0 µL onto LazWell™ to perform LDTD-MS/MS analysis

MS Parameters

- APCI (-)
- Scan time : 0.02 s
- Q1 and Q3 width : 0.50 amu
- Q2 CID : 1.5 mTorr (Ar)

SRM transitions:

- CAP : 321 → 152
- CE = 20 eV
- S-Lens = 71 V

- d5-CAP (IS) : 326 → 157
- CE = 19 eV
- S-Lens = 72 V

LDTD Parameters

- Laser power pattern :
 - Increase laser power to 45 % in 1.0 s
 - Hold at 45 % for 2.0 s
 - Decrease laser power to 0 %

- Carrier gas flow : 3 L/min (Air)
- Corona voltage value : 5 kV

Chloramphenicol precursor and product ion spectra:

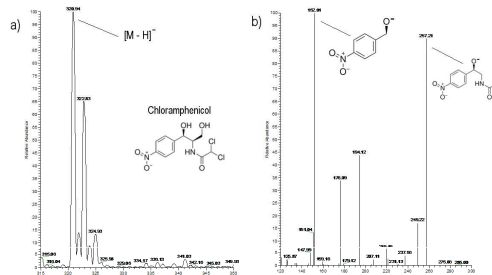


Figure 4: (a) Full scan spectra, in negative APCI mode, of a 2 µL deposit of a 1 µg/mL CAP in methanol (2 nanograms). (b) Product spectra of parent mass 321 with a collision energy of 15 eV, from a 2 nanograms deposit of CAP.

Desorption Profiles

In figure 5 are displayed desorption profile typical of LDTD methods, where time is not a parameter of interest. The abscissa represents the laser firing sequence on 4 wells containing extracted CAP from 4 samples of blueberry honey spiked at low levels.

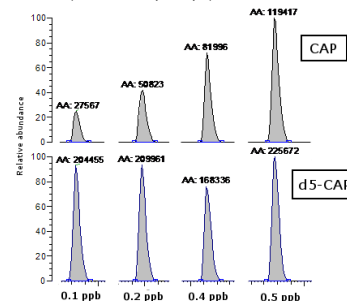


Figure 5: Desorption profile of extracted CAP from a blueberry honey spiked at 4 low levels

RESULTS

Linearity and reproducibility

A blueberry honey was spiked at concentrations over a 0.1 to 3.0 ng/g range to check the linearity of this method. We obtained the linear plot displayed in figure 6, with a correlation of $R = 0.9966$.

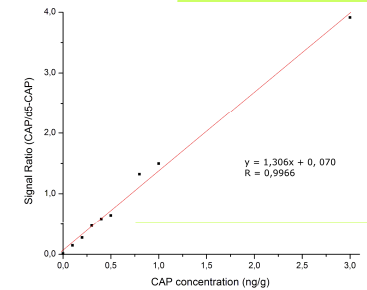


Figure 6: Linear fit over a 0.1 to 3.0 ng/g range, for a blueberry spiked honey.

Five different honeys, including a robust dark buckwheat one, were each spiked at 0.3 and 1.0 ng/g, together with a blank, and were analyzed to check the validity of this method for different types of honeys. Calculated concentration for these samples using linear fit shown in figure 6, and RSD values for those 5 honeys are presented in table 1.

Concentration (ng/g)	Calculated concentration (ng/g)	RSD (%)
0	-0,04 ; -0,02	N/A
0,3	0,28 ; 0,32	7,2
1,0	0,92 ; 1,17	8,8

Table 1: Calculated concentrations and relative standard deviation for 5 different honeys spiked at 0.3 and 1.0 ng/g

CONCLUSIONS

- Very easy sample preparation.
- Ultra-fast Chloramphenicol thermal desorption in 3 seconds.
- Analysis by the instrument takes less than 8 seconds for each sample.
- Reproducible for different honeys, even for robust dark ones.
- Suitable method for rapid detection of CAP in honey with an expected limit of detection well below 0.1 ppb.

REFERENCES

1. J. A. Turton, D. Yallop, C. M. Andrews, R. Fagg, M. York, T. C. Williams *Hum. Exp. Toxicol.* 18, 566, 1999
2. L. Santos, F. Ramos *Current Pharma. Anal.* 2, 53, 2006
3. J. Wu, C. S. Hughes, P. Picard, S. Letarte, M. Gaudreault, J. F. Levesque, D. A. Nicoll-Griffith, K. P. Bateman *Anal. Chem.* 79, 4657 – 4665, 2007