MACHEREY-NAGEL
Chromatography application note

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Determination of chloramphenicol from honey

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Abstract

This application note describes the determination of chloramphenicol in honey using solid phase extraction for analyte enrichment and for sample clean-up. The eluates from SPE are finally analyzed by HPLC-MS/MS.

Introduction

Chloramphenicol (CAP) is a widely used antibiotic in food of animal origin. It is often used as a bacteriostatic antimicrobial agent in honey production. Negative impacts upon human health by the consumption of contaminated food are well known which makes it an unacceptable substance for use with any food producing animals like honey bees [1].

The European Union (EU), as well as many other countries including The United States and Canada, have completely banned the usage of CAP in the production of food. To protect human health, the European food law sets maximum residue limits of CAP at 0.3 µg/kg honey [2]. This leads to an increasing demand for the development of accurate and sensitive analytical methods for the quantification of CAP from honey. The BVL has published a procedure used by German Federal States control authorities for analyzing food and feed in the official collection of test methods under part L. 40.00-17 (§ 64 LFGB) [3].

In this application note an automated SPE method for the determination of CAP from honey using CHROMABOND® HLB on LCTech Free-Style™ SPE module was developed. Identification and quantification of CAP were finally carried out by ESI mass spectrometry on a NUCLEODUR® π^2 column.

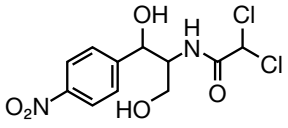

Figure 1: Compound of interest.

Sample pretreatment for solid phase extraction (SPE)

- Weigh 5 g sample into a centrifuge tube
- Add 4 mL water and internal standard solution
- Agitate for 0.5 min
- Add 1 mL of internal standard solution (chloramphenicol and chloramphenicol-d_5, for each c = 5 ng/mL) and agitate
- Add 15 mL of ethyl acetate and agitate
- Centrifuge at room temperature at 3000 rpm for 10 min
- Take 12 mL of the organic phase (ethyl acetate) and evaporate to dryness at 40 °C under a stream of nitrogen
- Redissolve residue in 10 mL water for SPE

Solid phase extraction (manual procedure)

Column:

CHROMABOND® HLB, 3 mL, 200 mg, (REF 730924)

Conditioning:

3 mL methanol, 5 mL water

Sample application:

10 mL sample extract with a flow rate of 3 mL/min

Washing:

10 mL water with a flow rate of 3 mL/min

Drying:

5 min with vacuum

Elution:

5 mL ethyl acetate / methanol (80:20, v/v)

Eluent exchange:

Evaporate eluate to dryness at 40 °C under a stream of nitrogen and redissolve in 1 mL water – acetonitrile (95:5, v/v)

Automated solid phase extraction (LCTech FreeStyle™ SPE module)

Step		Volume	Dispensing speed	Waiting time after dosage	Dispense into
Conditioning	with methanol	3 mL	2 mL/min	5 s	waste
	with water	3 mL	2 mL/min	5 s	waste
Load	Sample solution	9 mL	2 mL/min	150 s	waste
Washing	with water	5 mL	3 mL/min	0 s	waste
Drying	air	100 mL	100 mL/min	0 s	waste
Elution	ethyl acetate – methanol (80:20, v/v)	5 mL	2 mL/min	0 s	vial type 1@16 mL
Drying	air	100 mL	100 mL/min	0 s	sample vial of elution

Table 1: Automated procedure for solid phase extraction with LCTech FreeStyle™ SPE module.

Chloramphenicol from honey

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions

Column:

EC 150/2 NUCLEODUR® π^2, 5 µm, (REF 760624.20)

Eluent A:

water

Eluent B:

acetonitrile

Gradient:

from 5 % B to 95 % B in 7.5 min, hold for 1.0 min, back to 5 % B in 1.0 min, hold for 5.0 min

Flow rate:

0.3 mL/min

Temperature:

35 °C

Injection volume:

5 µL

MS conditions

API 5500, ion source ESI, negative ionization mode, scan type MRM, curtain gas 35 psig, ion spray voltage – 4500 V, temperature 450 °C, nebulizer gas 45 psig, turbo gas 45 psig, CAD medium

MRM transitions

Analyt	$[M-H]^-$	Q_1 (Quantifier)	Q_2 (Qualifier)
Chloramphenicol	320.9	152.0	256.0
Chloramphenicol-d_5	325.9	156.6	261.8

Table 2: MRM transitions for chloramphenicol and chloramphenicol-d_5.

Chromatograms

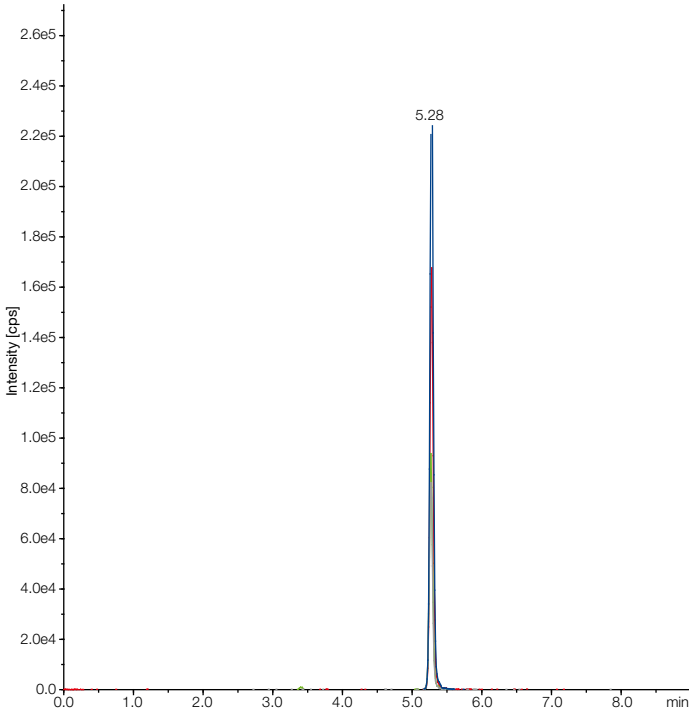

Figure 2: Chromatograms of CAP standard solution (c = 5 ng/mL).

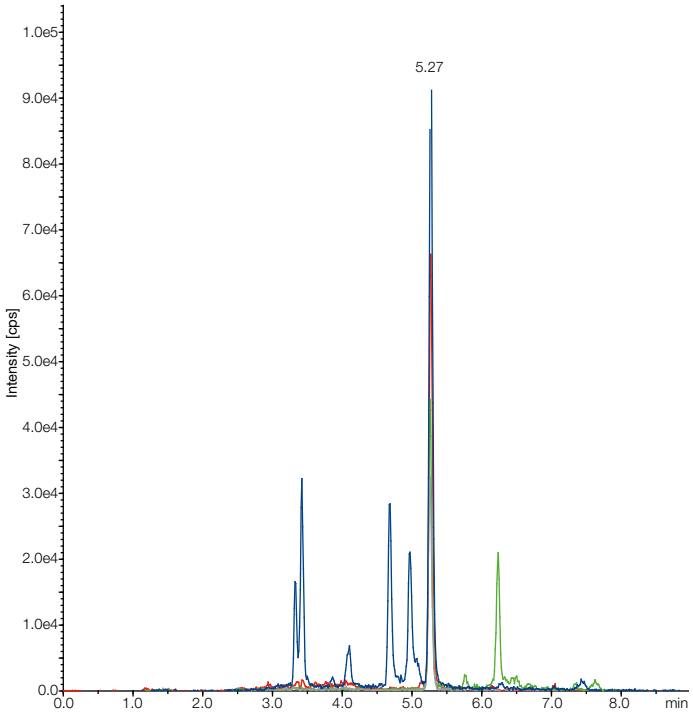

Figure 3: Chromatograms of honey sample spiked with 1 µg/kg CAP and 1 µg/kg CAP-d_5.

Chloramphenicol from honey

Calibration curves

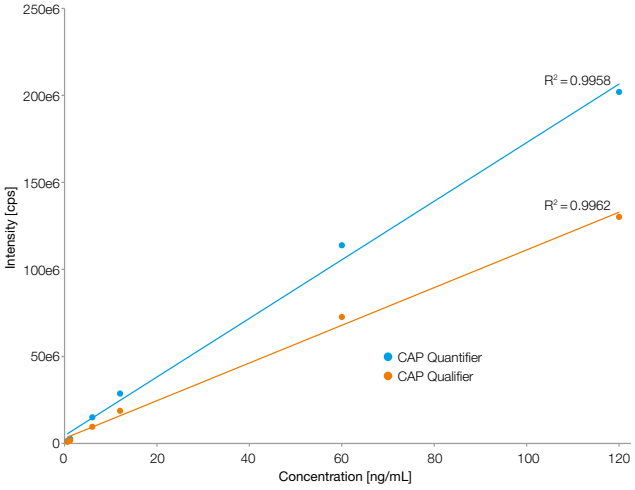

Figure 4: Calibration curve in concentration range between 0.5 ng/mL and 100 ng/mL CAP.

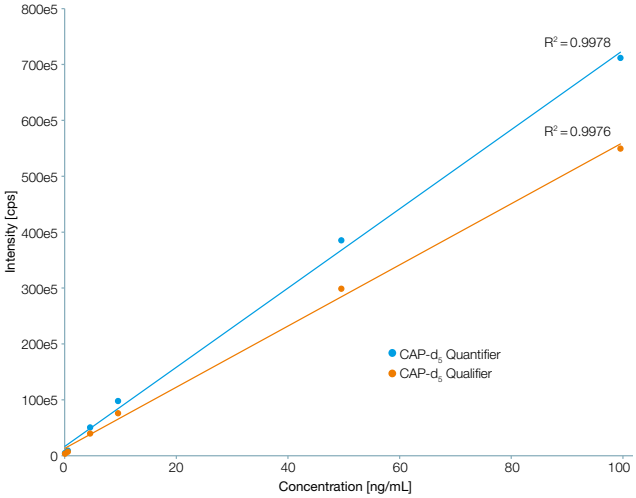

Figure 5: Calibration curve in concentration range between 0.6 ng/mL and 120 ng/mL CAP-d_5.

Recovery rates

Methodology	Recovery rate for CAP [%], (n = 3)	Recovery rate for CAP with correction of the internal standard CAP-d_5 [%], (n = 3)
SPE (manual)	74.6 ± 2.7	92.9 ± 3.4
Automated SPE	86.8 ± 4.4	90.9 ± 5.4

Table 3: Recovery rate for solid phase extraction method.

Conclusion

The results show that the determination of CAP from honey could be carried out successfully with all the tested products. By using SPE with CHROMABOND® HLB it was possible to recover more than 90 % of CAP from honey with good reproducibility (RSD less than 5 %). A correction with the internal standard chloramphenicol-d_5 leads to significant higher recovery rates of the manual performed procedure by eliminating matrix effects. By using the LCTech FreeStyle™ SPE module for solid phase extraction it was possible to reach recovery rates higher than 85 % without internal standard correction. Figure 4 and 5 show calibration curves for CAP and CAP-d_5 which were performed in the concentration range between 0.5 ng/mL to 100 ng/mL and 0.6 ng/mL to 120 mg/mL. The response of standard solutions with concentrations higher than 50 ng/mL CAP presents saturation effects.

The identification and quantification of CAP in the solid phase extracts were carried out by ESI mass spectrometry on an EC 150/2 mm NUCLEODUR® π^2 column. The chromatogram (figure 3) shows the results of the extraction of honey sample spiked with 1.0 µg/kg CAP.

In summary the presented application describes a quick and convenient method for the determination of chloramphenicol in honey with automated and manual SPE procedures.

References

[1] Side-effects of Chloramphenicol and Aureomycin, Br Med J. 1951, 384-2, 388-392.

[2] Verordnung (EG) Nr. 470/2009.

[3] Bestimmung von Antibiotika-Rückständen in Honig L40.00-17.

Additional information

The following applications regarding “Determination of chloramphenicol from honey” and further applications can be found on our online application database at *www.mn-net.com/apps*

SPE: MN Appl. No. 306350

HPLC: MN Appl. No. 128140

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 760624.20, EC 150/2 NUCLEODUR® π^2, 5 µm

REF 730924, CHROMABOND® HLB, 3 mL, 200 mL

REF 730223, CHROMABOND® centrifuge tubes with screw cap, 50 mL

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red

Acknowledgment

We thank the company LCTech GmbH for the cooperation and for providing the robotic system, called FreeStyle™ SPE module.