MACHEREY-NAGEL
Chromatography application note

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Determination of chloramphenicol in prawns

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen

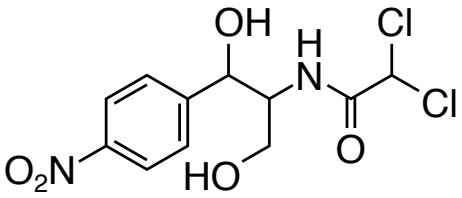
Abstract

This application note describes the determination of chloramphenicol (CAP) from prawns in lower µg/kg range using liquid-liquid extraction of prawn tissue followed by SPE for sample clean up and analyte concentration with CHROMABOND® HR-X columns. After eluent exchange, the eluates from SPE are finally analyzed by HPLC-MS/MS with a NUCLEOSHELL® RP 18plus core-shell column.

Introduction

CAP is a widely used antibiotic in food of animal origin. It is often used in aquaculture as a disinfectant or as a chemotherapeutic agent. Negative impacts upon human health by the consumption of contaminated food are well known. To protect human health, the European food law sets smaller maximum residue limits of CAP at 0.3 µg/kg than China with a maximum residue level of 0.5 µg/kg shrimp [1]. This leads to an increasing demand for the development of accurate and sensitive analytical methods for the quantification of CAP in prawns.

Compounds of interest



Analyte	Formula	Mass [g/mol]
CAP	$C_{11}H_{12}Cl_2N_2O_5$	323.41

Table 1: Molecular formular and mass of the analyte.

Sample preparation

Sample pretreatment – liquid-liquid extraction

Centrifuge tubes:

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

- Weigh out 5 g homogenized prawn
- Spike with a) 50 µL of standard solution (c = 1 µg/mL) and
  b) 100 µL of standard solution (c = 1 µg/mL)
- Add 10 mL of ethyl acetate and shake rigorously for 30 sec
- Centrifuge at 9000 rpm for 15 min at room temperature
- Take supernatant extract for eluent exchange

Sample pretreatment – eluent exchange

Eluent exchange is performed manually. Extracts are evaporated to dryness at 40 °C under a stream of nitrogen and then redissolved in methanol – water (1:10, v/v) for SPE.

Solid phase extraction

Column type:

CHROMABOND® HR-X polypropylene columns (85 µm), 3 mL, 200 mg, (REF 730931)

CHROMABOND® empty columns, 6 mL, PP, (REF 730161)

CHROMABOND® adaptor for combination of 1, 3, 6 mL PP columns with disposable syringes, (REF 730100.4)

Conditioning:

4 mL methanol, 4 mL water

Sample application:

With a flow of 1–2 mL/min

Washing:

1 mL water, 4 mL methanol – water (1:10, v/v)

Elution:

3 mL acetone

Eluent exchange

Eluent exchange is performed manually. Eluates from SPE are evaporated to dryness at 40 °C under a stream of nitrogen and then redissolved in an acetonitrile/water mixture for the subsequent analysis. For HPLC-MS/MS 1 mL of acetonitrile/water (5:95, v/v) is used.

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander Str. 6–8 · 52355 Düren · Germany
www.mn-net.com · info@mn-net.com

Determination of chloramphenicol in prawns

Subsequent analysis: HPLC-MS/MS

Chromatographic conditions:

Column:
EC 50/2 NUCLEOSHELL® RP 18plus 2.7 µm, (REF 763232.20)

Flow rate: 0.3 mL/min

Eluent:
A) 0.005 M ammonium formiat/NH_3, pH 8.5
B) acetonitrile

5–95 % B in 3.6 min, 95 % B for 1 min, 95–5 % in 0.4 min, 5 % B for 5 min

Temperature: 30 °C

Injection volume: 10 µL

MS conditions:

API 3200, ion source ESI, negative ionization mode

Curtain gas 15 psig, ion spray voltage ~4500 V, temperature 550 °C, nebulizer gas 50 psig, turbo gas 50 psig, CAD 3.0 psig

MRM transitions

Analyte	$[M+H]^+$	Q_1 (Quantifier)
Chloramphenicol (CAP 1)	320.96	151.9
Chloramphenicol (CAP 2)	322.93	152.1

Table 2: MRM transitions for the analysis of chloramphenicol, the fragment pattern of the CAP-isotop 322 is used instead of a qualifier because the intensity of other fragments was to low using the API 3200 with negative ionization mode.

Chromatograms

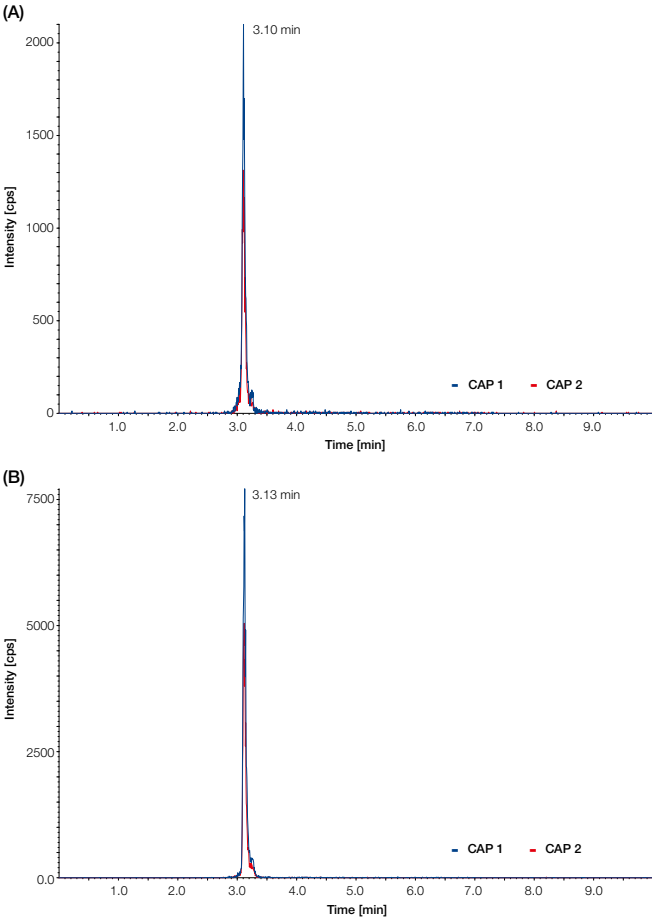


Figure 1: Chromatograms of HPLC-MS/MS analysis, samples spiked with 5 ng CAP (A) and 10 ng CAP (B).

Calibration curve

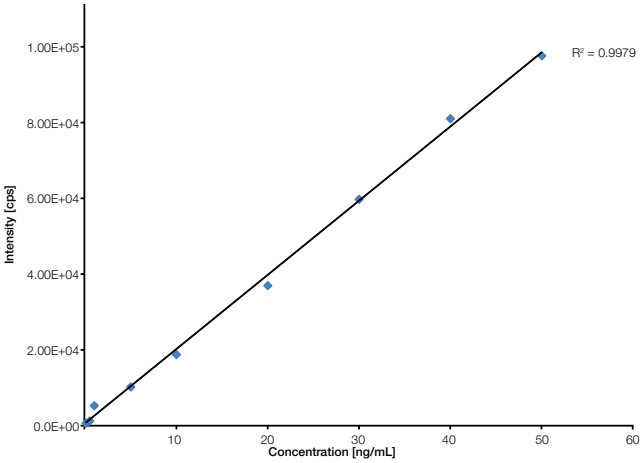


Figure 2: Calibration curve of CAP.

Recovery rates

The recovery rate for CAP was 79.4 % ± 9.1 %.

LOD/LOQ

The limit of detection (LOD) was estimated to be ≤ 0.13 µg/kg and the limit of quantitation (LOQ) was estimated to be ≤ 0.14 µg/kg.

Conclusion

Using CHROMABOND® HR-X cartridges and a subsequent LCMS analysis using NUCLEOSHELL® RP 18plus showed reliable results for the analysis of CAP from prawns. The SPE method proposal leads to clean sample extracts with high recovery rates.

References:

1. COMMISSION DECISION, 2003/181/EC, Official Journal of the European Union, 2003, L 71/17.

Additional information

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

SPE: MN Appl. No. 306 390
HPLC: MN Appl. No. 128 180

Product information

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

REF 730223, CHROMABOND® centrifuge tubes 50 mL
REF 730931, CHROMABOND® HR-X, 3 mL, 200 mg
REF 730161, CHROMABOND® empty columns, 6 mL
REF 730100.4, CHROMABOND® adaptor
REF 702923 Screw neck vials N 13
REF 702052 N 13 PP Screw cap, black, closed top, silicone white / PTFE red
REF 702293 Screw neck vials N 9
REF 702107 N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red
REF 763232.20, EC 50/2 NUCLEOSHELL® RP 18plus, 2.7 µm