Chloramphenicol and nitrofuran metabolites – a combined method for fast and reliable monitoring of shrimps

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Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic exhibiting activity against both gram-positive and gram-negative bacteria as well as other groups of micro-organisms. It exerts its action through protein inhibition and is effective in the treatment of several infectious diseases. This, together with its low cost and ready availability, has made it extensively used since the 1950th in the treatment of animals all over the world, including shrimp and seafood production. However, at certain susceptible individuals, CAP is associated with serious toxic effects in humans in the form of bone marrow depression, particularly severe in the form of fatal aplastic anaemia [1].

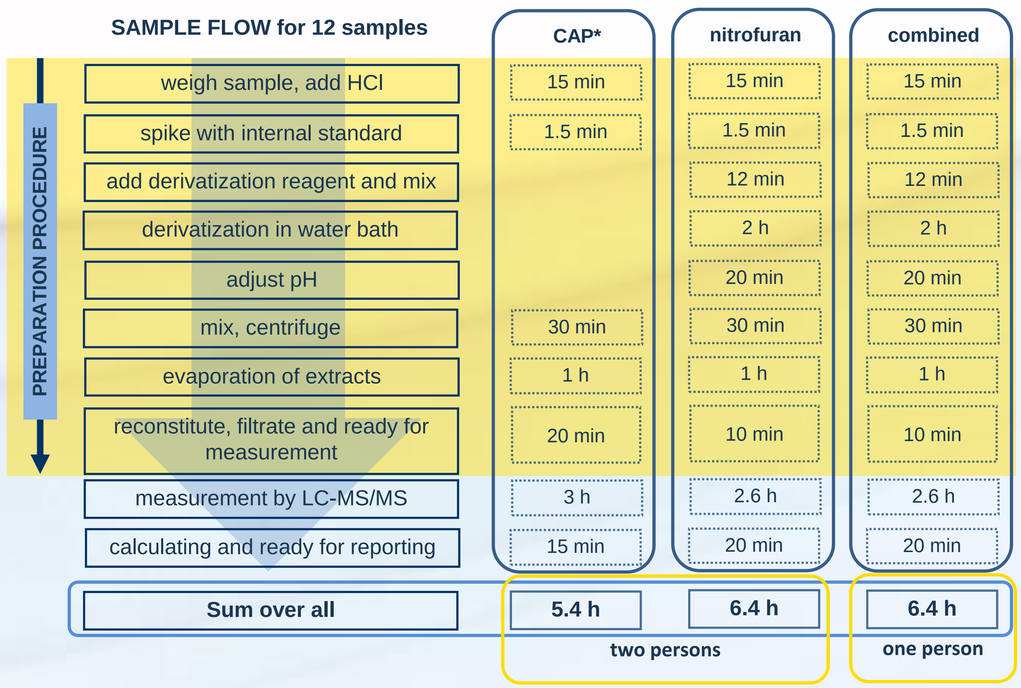
Nitrofurans comprise a group of antibiotic substances that have been used widely in intensive farming of pigs, poultry, fishes and shrimps. Studies in the late 1980th and early 1990th have proven that they are metabolised shortly after administration and form persistent residues that could be detected in the animal tissues for weeks after treatment. Both, the nitrofurans as well as special metabolites, have been classified as genotoxic compounds.

Since their toxicological data did not support the derivation of an ADI and due to a lack of data no maximum residue limit (MRL) could be fixed for CAP or the nitrofuran metabolites. Both are banned in many countries and the EU and included in Annex IV of Council Decision 2077/90 [2]. European Commission established low minimum required performance levels (MRPL) of 0.3 µg/kg for chloramphenicol and 1 µg/kg for each nitrofuran metabolite in aquaculture and other products (Decision 2003/181/EC) [3].

Within industry self-control especially raw materials have to be analysed just in time to avoid delay at import. In former days the analyses of nitrofuran metabolites requires two days for hydrolyses, derivatization, extraction and measurement. In this poster we present a quick and easy one-day method with one LC-MS/MS run for the analysis of residues of chloramphenicol and nitrofuran metabolites in accordance with the requirements of the Commission Decision 2002/657/EC [4].

Method

The figure below shows the preparation procedure for CAP and nitrofurane metabolites. The single preparation for each method takes almost one working day with two persons for preparation and finishing the results. With the combined method only half the time and manpower is necessary. This fast combined method allows the analysis of one sample in less than four hours (not shown). Also a batch of twelve samples could be prepared, measured, checked and finished for a customer in less than one working day.



(*CAP: first step without HCL)

Each sample is spiked with the internal standard (IS) solution (containing CAP-D5, SEM-13C3, AOZ-D4, AMOZ-D5, AHD-13C3). Experience has revealed that matrix effects on the sensitivity of the MS/MS signals differ strongly. Therefore, the recovery rate for each sample and each analyte is determined individually.

HPLC	Agilent 1100 (pump, degasser, autosampler, column oven)
Column	RESTEK Raptor Biphenyl, 150 x 4.6 mm, 5 µm, 50°C
Eluent	0.5 mM Ammoniumacetat and methanol
Gradient	linear, 0.700 mL/min
MS/MS	AB Sciex API 4000, TurbolonSpray®Source
Mode	ESI positive / negative, MRM mode

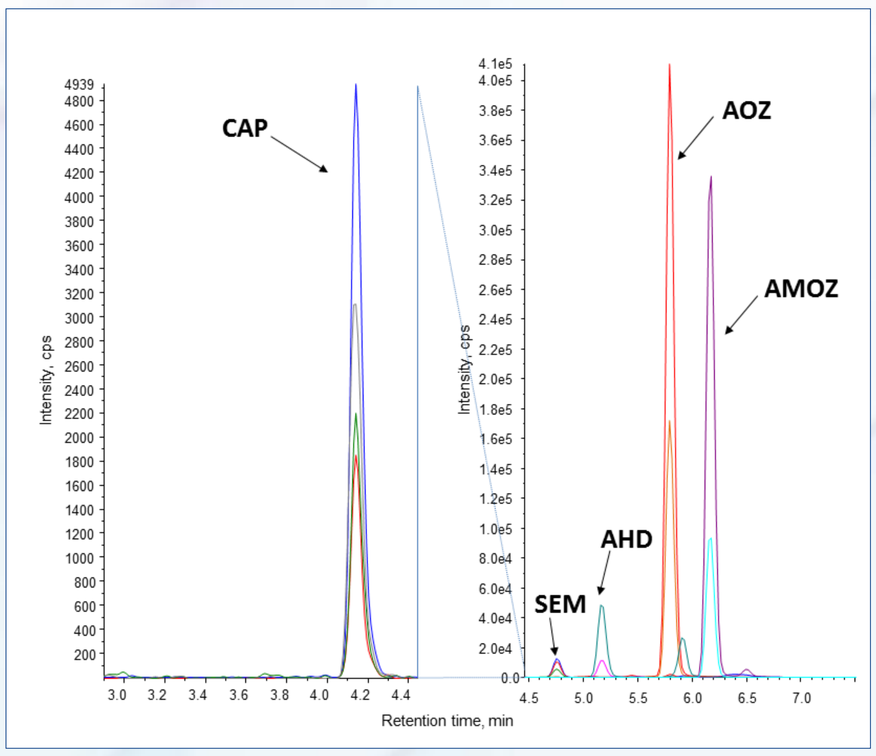
Conclusion

The validation of this method was carried out according to the commission decision (2002/657/EC) [3]. To determine cc α and cc β at a low level, validation was done with shrimps spiked at 0.1 µg/kg CAP and 1 µg/kg of each nitrofuran metabolite. The method shows recovery rates between 90 and 107 % and very good reproducibility. A summary of the validation data is shown in the table below.

Analyte	cc α	cc β	LOD	LOQ	MRPL [3]	recovery	repeatability (CV)	spiking level
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg // aquaculture products	%	%	µg/kg
SEM	0.6	0.8	0.2	0.6	1	98	17	1
AHD	0.6	0.7	0.1	0.4	1	107	14	1
AOZ	0.7	0.9	0.1	0.4	1	103	14	1
AMOZ	0.6	0.7	0.2	0.5	1	90	29	1
CAP	0.1	0.1	0.01	0.03	0.3	103	7.2	0.1

Results

Figure show chromatogram obtained by applying the proposed method to routine analysis of fish sample, spiked with 5 µg/kg for each nitrofuran metabolite and 0.5 µg/kg for chloramphenicol.



References

[1] K.N. Woodward, in: D.H. Watson (Ed.), Pesticide, Veterinary and Other Residues in Food, Woodward Publisher Limited, Cambridge, 2004, p. 176, Chapter 8.

[2] Council Regulation (EEC) No. 2377/90-laying down a Community procedure for establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, Off. J. Eur. Comm. L224 (1990).

[3] Commission Decision 2003/181/EC.

[4] Commission Decision of 12 August 2002 (2002/657/EC).

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