A ß-lactam LC-MS/MS multi-method including penicillins, cephalosporins and carbapenems

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Background

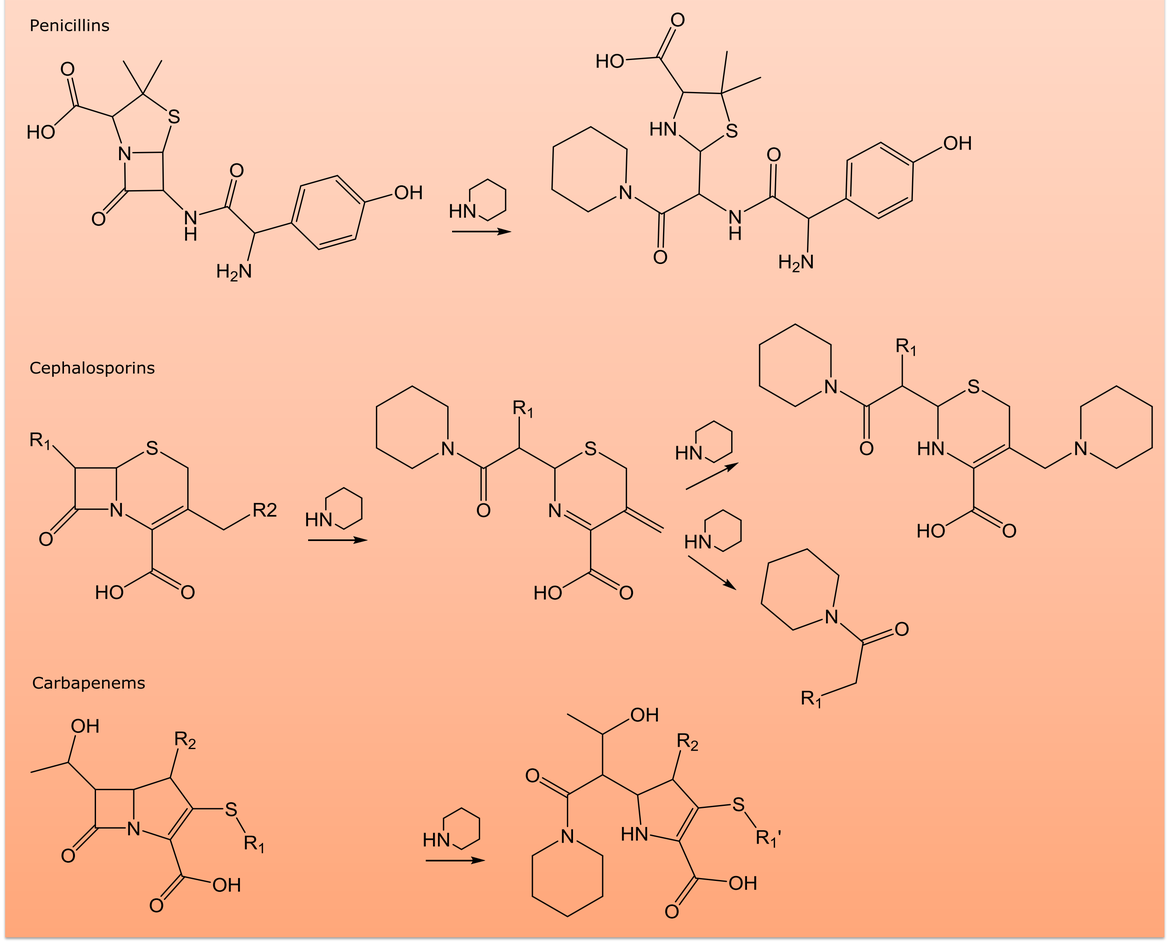
Antimicrobial resistant bacteria are emerging and have become an important public health threat. Nowadays, most penicillins are found to be ineffective against ESBL producing bacteria; cephalosporins are more effective and were assigned as critically important for human health in 2007. Even though these compounds should be used sparingly, resistance towards cephalosporins is rapidly emerging leaving carbapenems as a final ß-lactam resource.

The regular use of antibiotics in veterinary practice contributes to the occurrence of resistant bacteria. Penicillins are the most frequently sold antibiotic group for treatment of broilers. Cephalosporins and carbapenems are not registered for use in poultry production within the EU, but due to their high effectiveness, their use cannot be ruled out. To prevent off-label use of ß-lactams in animal breeding and thus to limit the dissemination of bacterial resistance, the monitoring of poultry muscle for penicillins, cephalosporins as well as carbapenems at levels as low as reasonably possible is of importance.

Hydrolysis

- 10 mL borate buffer + 0.5 mL piperidine, 1h, 60 °C

Figure 1. Penicillin, cephalosporin and carbapenem structure and hydrolysis reaction products as determined by HRMS



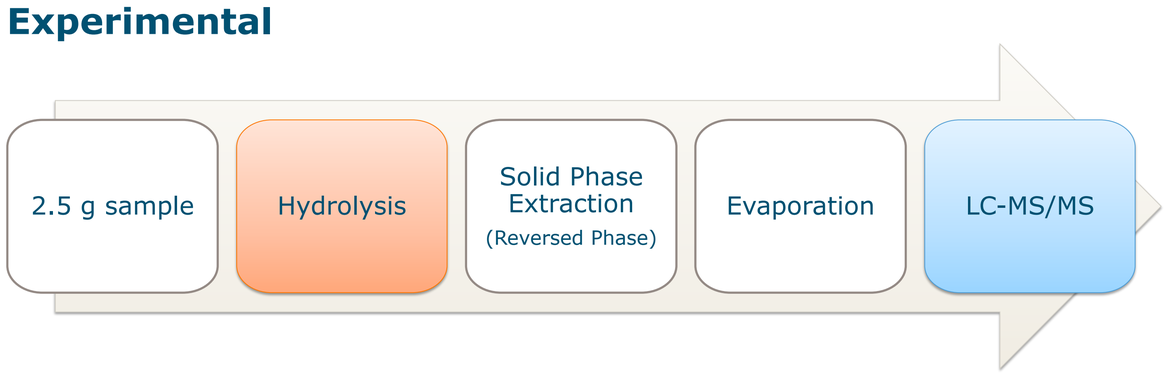
LC-MS/MS

- LC column: Waters Acquity UPLC CSH C_{18}, 2.1 x 100, 1.7 µm
- Solvent A: 0.0032 % ammonia in water
- Solvent B: 0.0032 % ammonia in water/acetonitrile (1:9 v/v))
- 12.5 min. gradient elution
- Flow rate: 0.4 mL min^{-1}.
- Waters Xevo TQS or AB Sciex Qtrap 6500
- Electrospray Ionisation, Selected Reaction Monitoring

Objectives

- Development of an LC-MS/MS method for the analysis of 8 penicillins, 8 cephalosporins, 5 carbapenems and faropenem.
- Include all relevant metabolites of ceftiofur and cephapirin.
- Full validation according to CD 2002/657/EC

Experimental



Results

Figure 2. Chromatograms of a poultry muscle sample spiked at target level (between brackets, in µg kg^{-1}) showing the least abundant product ion of each compound's hydrolysis reaction product. Multiple peaks are proposed to be caused by isomeric reaction products.

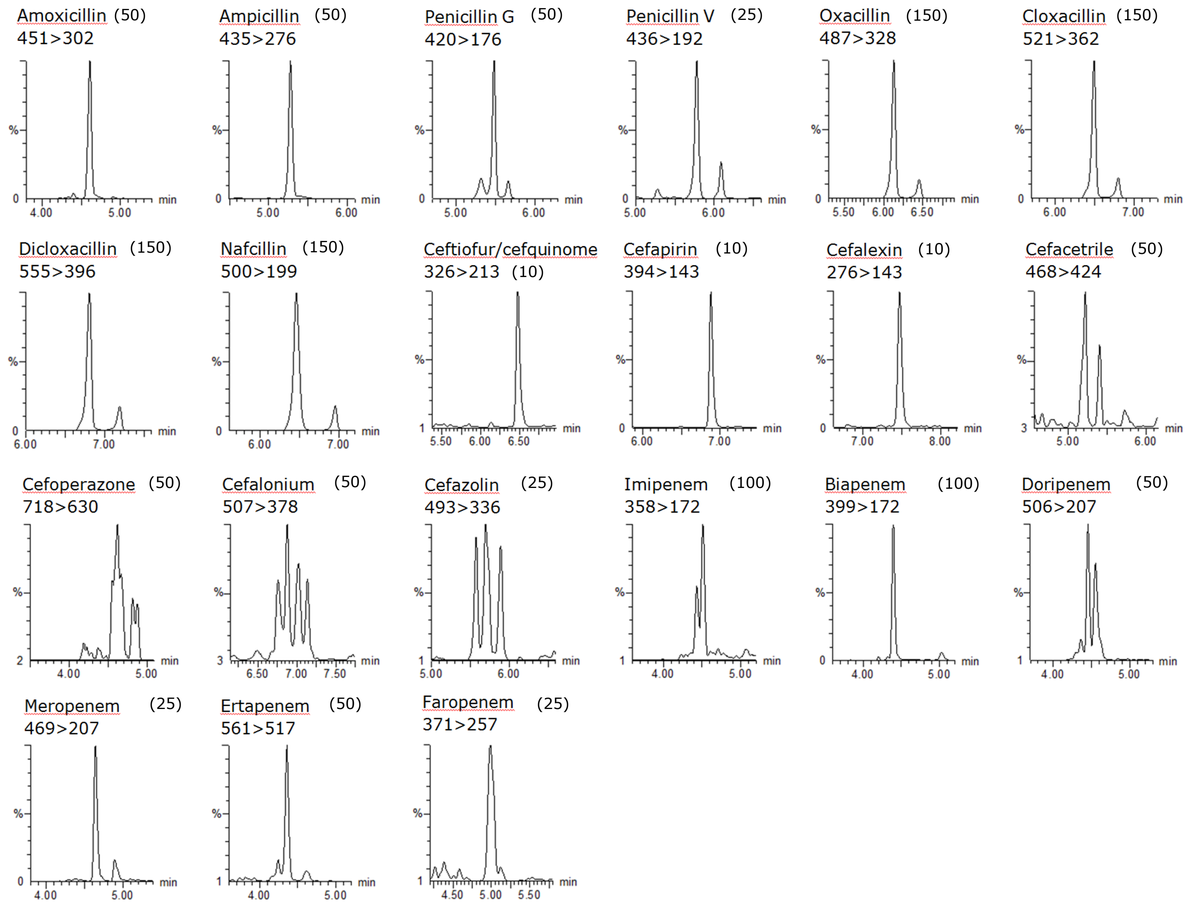


Table 1. Summary of validation results, trueness and RSD_{RL} at target level

ß-lactam group	LoD(µg/kg)	Trueness (%)	RSD_{RL} (%)
Penicillins	≤ 0.25*target	97 - 106	2.8 – 11
Cephalosporins[#]	≤ 0.25*target	96 - 108	7.3 – 19
Carbapenems	≤ 0.25*target	91 - 107	7.0 - 15

[#]Biapenem can only be analyzed qualitatively due to high trueness (118 %) and RSD_{RL} (47 %)

Conclusions

- For the first time an LC-MS/MS method for the analysis of 8 penicillins, 8 cephalosporins, 5 carbapenems and faropenem was developed and fully validated according to CD 2002/657/EC.
- Also applicable for the analysis of porcine and bovine muscle, milk and colostrum.

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Reference: Berendsen *et al.* 2013, Comprehensive analysis of ß-lactam antibiotics including penicillins, cephalosporins, and carbapenems in poultry muscle using liquid chromatography coupled to tandem mass spectrometry, Anal Bioanal Chem (2013) 405:7859–7874