



Antibiotics in the chain; analyses of feces to study the life-cycle of antibiotics

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Background

To prevent further dissemination of resistance, the use of antimicrobial compounds in animal husbandry should be decreased. Therefore, instead of monitoring food products related to MRL regulations, antibiotic usage in general should be monitored in an effective way. The analysis of feces is a promising option.

Objectives

- Effective monitoring of antibiotic usage
- Non-invasive sampling (at-farm and in the slaughterhouse)
- Development of an LC-MS/MS multi-method that is able to detect antibiotics belonging to different classes (10 quinolones, 19 sulfonamides, 4 tetracyclines and 14 macrolides) in animal feces.
- Full validation according to CD 2002/657/EC

Experimental

- 2 g feces
- Extraction with EDTA-McIllvain buffer + ACN
- Protein removal by PbAc₂
- Dilution by EDTA (0.1 M)
- Solid Phase Extraction, Strata-X RP
- Evaporation of the solvent, 45°C, N₂
- Reconstitution in H₂O/MeOH

LC-MS/MS

- LC column: Phenomenex Kinetex C₁₈ 2.1 x 100, 1.7 µm
- Solvents: 2 mM NH₄Ac + 0.016% HCOOH in water and MeOH
- 10 min. gradient elution, flow rate: 0.4 mL min⁻¹
- MS: AB Sciex Qtrap 6500, Electrospray Ionisation, SRM

Results

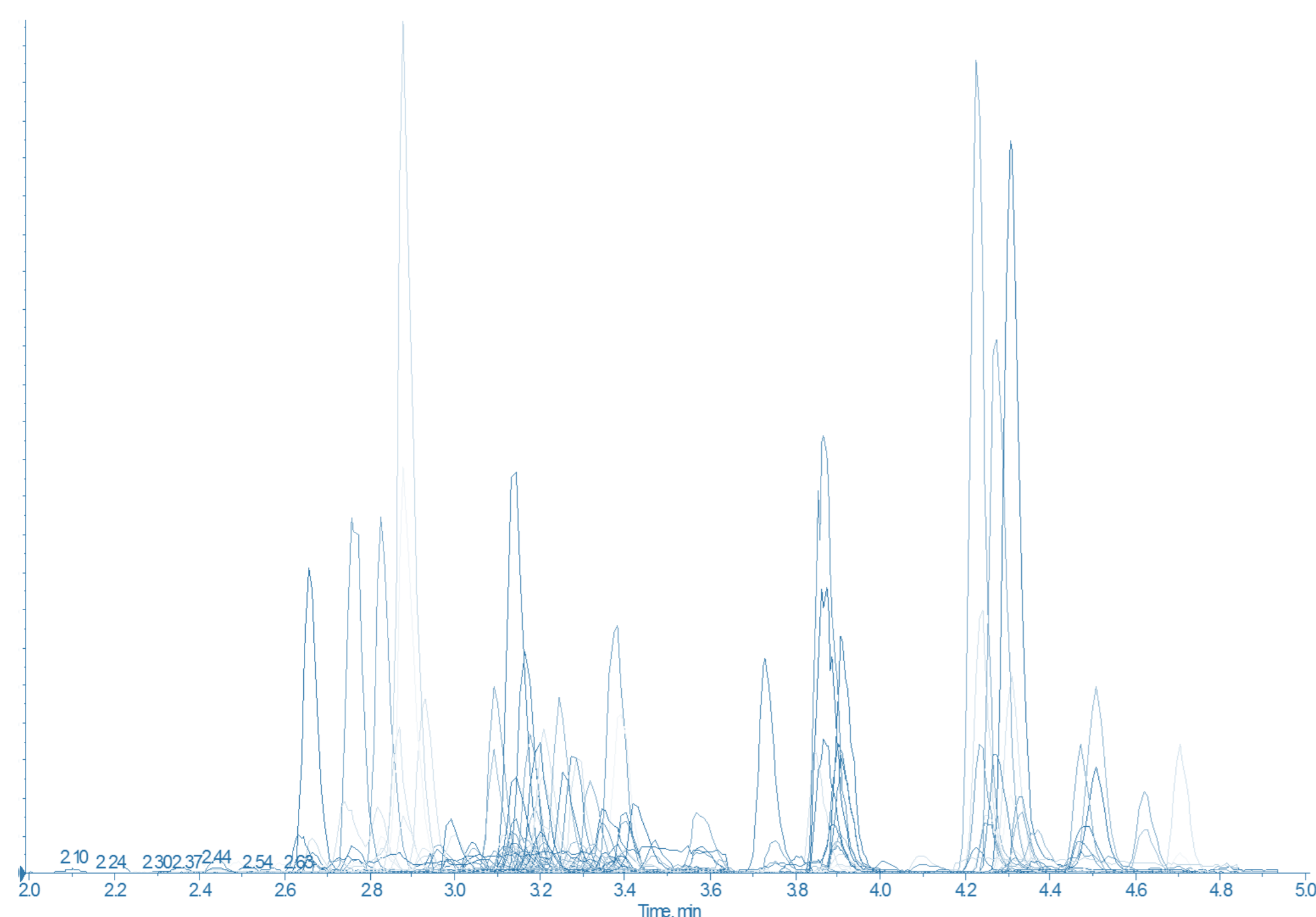


Figure 1. Representative chromatogram of a blank feces sample spiked with tetracyclines, quinolones, macrolides and sulfonamides at target level.

Validation

The method was fully validated for porcine feces according to CD 2002/657/EC.

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

β-lactam group	Target level (µg/kg)	Trueness (%)	RSD _{RL} (%)
Tetracyclines	20	98 - 103	6.7 - 16
Quinolones	20	88 - 103	3.5 - 21
Macrolides*	20	84 - 108	3.9 - 18
Sulfonamides	5	93 - 104	2.9 - 14

* Tylosin and Tylosin show trueness > 110 % and tylosin, tylosin, pirlimycin and tulathromycin show RSD_{RL} > 22 % and therefore the method is considered qualitative for these compounds.

- Chlortetracycline was not detected in spiked samples. This is probably caused by degradation in feces.

Background & objectives

Validation

Experimental

Monitoring study

Monitoring study

Of 20 randomly selected pig and 20 cattle farms, 17 animals were selected of which feces was collected at the slaughterhouse. These 680 samples were analyzed for tetracyclines, quinolones, macrolides and sulfonamides.

Table 2. Overview of monitoring results

	Pigs	Calves
% stables positive	75	95
% animals positive	53	74
Antibiotics found (number of positive animals, Concentration in µg/kg)	Doxycycline (#100, 2-95000) Oxytetracycline (#49, 4-1500) Tylosin (#48, 2-7700) Sulfadiazin (#31, 1-220) Tiamulin (#7, 1-4) Lincomycin (#2, 1-2) Sulfadimethoxin (#1, 6)	Oxytetracycline (#171, 5-17000) Tetracycline (#102, 3-112) Doxycycline (#53, 5-177) Sulfadiazine (#50, 1-81) Flumequin (#38, 1-1803) Lincomycin (#30, 1-149) Tilmicosin (#27, 2-218) Sulfadoxin (#2, 1-5) Sulfamethazin (#1, 24) Ciprofloxacin (#1, 13)

median concentration

Results

- High antibiotic incidence in feces of pigs and calves
- Mixtures of antibiotics were found (up to 7 in a single animal)
- High levels of antibiotics were found in feces. This contributes to the production of resistant bacteria in the gut.

Conclusions

- A method for the analysis of tetracyclines, quinolones, macrolides and sulfonamides in feces was developed and fully validated.
- This strategy is non-invasive and samples can be taken at the farm or in the slaughterhouse.
- Applicable in determination of antibiotic usage and researching the relation between residues and resistance formation in the gut.

Acknowledgement

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