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Development and Validation of an LC-MS/MS Method for the Determination of Total Florfenicol Residues as Florfenicol Amine in Kidney

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Background

Florfenicol is a broad-spectrum antibiotic for which a Maximum Residue Limit (MRL) is defined in Commission Regulation (EU) No. 37/2010. According to this MRL definition, the marker residue of florfenicol is defined as the *"sum of florfenicol and its metabolites measured as florfenicol amine"*.

Introduction

In treated animals florfenicol (FF) is metabolised to florfenicol amine (FFA) via several other metabolites. Both the parent compound and its metabolites can be present as residues in different tissues. Therefore, analytical methods should include a hydrolysis step to yield free FFA.

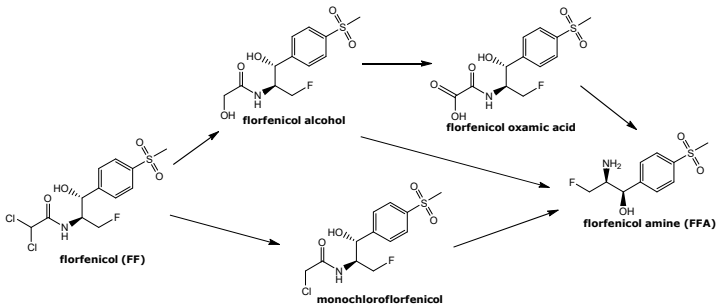
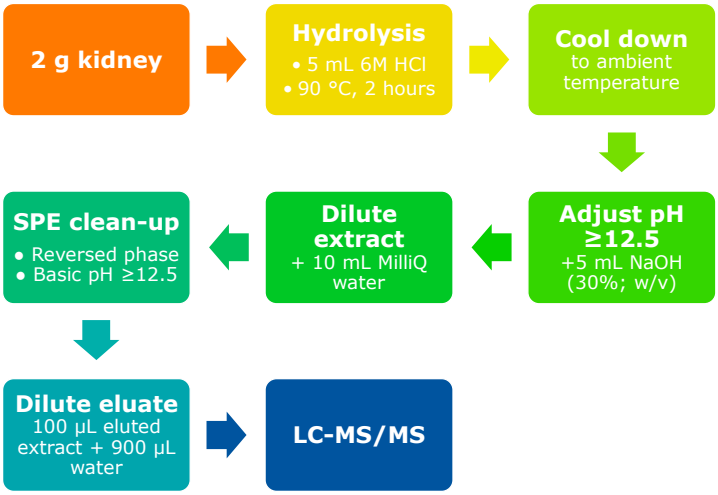


Figure 1. FF is metabolised to FFA via several other metabolites.

Objective

- To develop a method for total florfenicol residues as florfenicol amine in kidney at MRL concentrations.

Sample preparation



Quantification

For quantification matrix fortified samples were used that were fortified with FFA. Every analysis the completeness of the hydrolysis was checked by analysing a blank sample which was fortified with florfenicol

LC-MS/MS analysis

- Analytical column: Acquity BEH C18 (2.1 x 100mm, 1.7 µm)
- MRM transitions (ESI+):
  - FFA: *m/z* 248.0 → 130.1; *m/z* 248.0 → 91.0
  - FFA-d_3: *m/z* 251.0 → 132.0

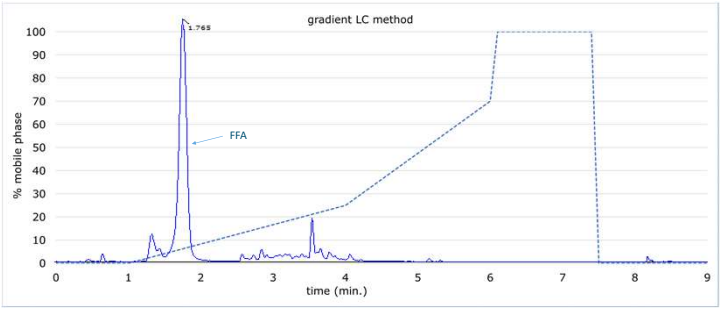


Figure 2. Mobile phase gradient of the LC-method and a chromatogram of a kidney sample spiked with FFA. Flow rate of 0.4 mL min^{-1}. Mobile phase A: 0.01% formic acid in water (v/v). Mobile phase B: 0.01% formic acid in acetonitrile (v/v).

Validation

Table 1. Results validation study in porcine and bovine kidney. The validation procedure was performed in accordance with EU criteria (2002/657/EC).

Species	Level (µg kg^{-1})	Accuracy (%)	Repeatability (CV%)	Within-laboratory reproducibility (CV%)	CC_α (µg kg^{-1})	CC_β (µg kg^{-1})
Porcine (n=21)	250	98	2.4	4.1		
	500	100	3.3	3.9	532	563
	750	102	2.5	2.7		
Bovine (n=7)	150	103	1.3	2.1		
	300	102	0.9	1.4	307	314
	450	102	0.7	1.2		

Conclusion

The developed method is suitable for the quantitative analysis of total florfenicol as free FFA from 125-2500 µg kg^{-1} in porcine kidney and 75-1500 µg kg^{-1} in bovine kidney.

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