Development and validation of a rapid liquid chromatography-tandem mass spectrometry method for quantification of cefquinome, ceftiofur and its active metabolite, desfuroylceftiofur, in porcine plasma

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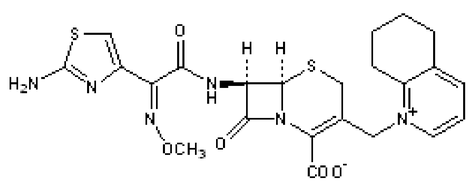
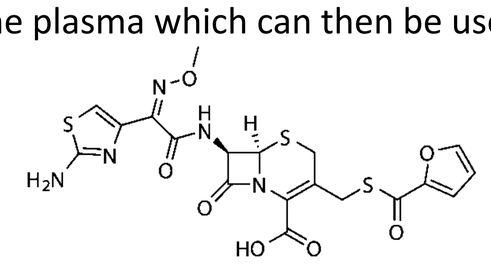
INTRODUCTION AND AIMS

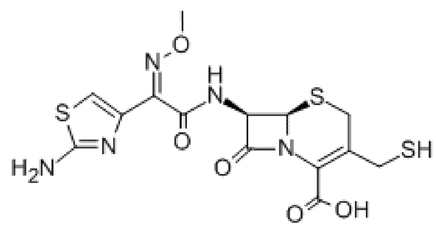
Ceftiofur and cefquinome are cephalosporins used in veterinary medicine. Nevertheless, the use of third and fourth generation cephalosporins in animal husbandry is controversial due to their critical role in human healthcare and the emergence of antimicrobial resistance. Several analysis methods for different matrices have been developed based on derivatisation of ceftiofur and its metabolites including desfuroylceftiofur, to obtain desfuroylceftiofur acetamide. However, these methods are elaborate and cause an overestimation of the antimicrobial potency. To date, no methods have been reported for determination of ceftiofur and desfuroylceftiofur in plasma without derivatizing ceftiofur and in combination with another cephalosporin. The aim of this study was to develop and validate a rapid, sensitive and specific high-performance LC-MS/MS method for determination of cefquinome, ceftiofur and desfuroylceftiofur in porcine plasma which can then be used for pharmacokinetic studies.



Cefquinome



Ceftiofur



Desfuroylceftiofur

MATERIALS AND METHODS

Sample extraction :

250 µL plasma

+ 25 µL ceftiofur-d3 and cefquinome-d7 (10 µg/mL)

+ 60 µL 10 % trichloroacetic acid in water

extraction + centrifugation

275 µL of supernatant

+ 50 µL 5 % TRIS in H_2O
+ 37.5 µL 1 M acetate buffer pH 5
+ 31 µL acetonitrile

analysis 10 µL-aliquot

HPLC-MS/MS

Instrument settings:

- HPLC-MS/MS Instrument: Waters Alliance 2695 system (Zellik, Belgium) in combination with a Quattro Ultima® triple quadrupole mass spectrometer (Waters, Millford, MA, USA).
- HPLC-column: Zorbax Eclipse Plus column (Reversed Phase C18, 100 mm x 2.1 mm i.d., dp: 3.5 µm) combined with a guard column of the same type both from Agilent Technologies (Diegem, Belgium)
- Mobile Phase: (A) 0.1% (v/v) formic acid and 2 mM ammonium formate in H_2O, (B) 0.1% (v/v) formic acid in acetonitrile.
- Gradient elution program is shown in Table 1.
- MS/MS settings are shown in Table 2.

Table 1: LC-gradient

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 1.0	95	5
1.0 – 5.0	95 – 60	5 – 40
5.0 – 8.0	60 – 5	40 – 95
8.0 – 10.0	5	95
10.0 – 10.1	5 – 95	95 - 5
10.1 – 15.0	95	5

Table 2: MS/MS ESI+ settings

Analyte	MM[a] (g/mol)	Precursor ion (m/z)[b]	Product ions[c] (m/z)	CE[d] (eV)	Cone (V)	Retention time (min)
Cefquinome	528.6	529.0	**134.0** / 396.3	15	20	4.81
Ceftiofur	523.6	524.0	**240.9** / 126.0	20	20	8.67
Desfuroylceftiofur	429.5	430.1	**241.0** / 126.0	20	20	7.54
Ceftiofur-d3	526.6	527.0	244.1	20	20	8.65
Cefquinome-d7	535.6	536.4	141.0	15	20	4.79

Note: [a] MM = molecular mass, [b] m/z = mass to charge ratio, [c]bold = ion used for quantification [d] CE = collision energy

Method validation:

Linearity, limit of quantification (LOQ), limit of detection (LOD), within- and between-run precision and accuracy, carry-over, specificity, SSE and robustness[1].

RESULTS

Table 3: Evaluation of validation parameters for cefquinome, ceftiofur and desfuroylceftiofur in plasma (n=6).

Compound	Linearity (µg/mL)	LOQ (µg/mL)	LOD (µg/mL)	Spiked conc. (µg/mL)	Found concentrations (µg/mL, mean ± SD[a])	Within-run / between-run accuracy (%)	Within-run / between-run precision (RSD[b], %)	Validation result	SSE [c] (%)	Robustness [d]
Cefquinome	0.20 – 15.0	0.20	0.003	0.20 1.0 15.0	0.19 ± 0.01 0.98 ± 0.02 15.07 ± 0.90	-8.9 / -7.0 -4.5 / -2.0 -6.6 / +0.5	+2.5 / +3.2 +1.4 / +2.5 +5.5 / +5.9	✓	83 ± 8.2	✓
Ceftiofur	0.01 – 1.0	0.01	0.003	0.01 0.1 1.0	0.01 ± 0.00 0.09 ± 0.00 1.00 ± 0.04	-7.2 / -8.2 -8.8 / -6.6 -0.3 / +0.5	+6.4 / +5.3 +3.0 / +3.8 +2.4 / +3.6	✓	116.9 ± 7.6	✓
Desfuroylceftiofur	0.20 – 15.0	0.20	0.028	0.20 1.0 15.0	0.20 ± 0.01 1.05 ± 0.03 15.34 ± 0.99	-4.7 / -2.5 +6.4 / +5.5 -5.3 / +2.2	+5.2 / +6.2 +3.5 / +3.2 +6.5 / +6.5	✓	197.5 ± 24.5	✗

Note: [a] SD = standard deviation, [b] RSD = relative standard deviation, [c] SSE = signal suppression enhancement, [d] new column

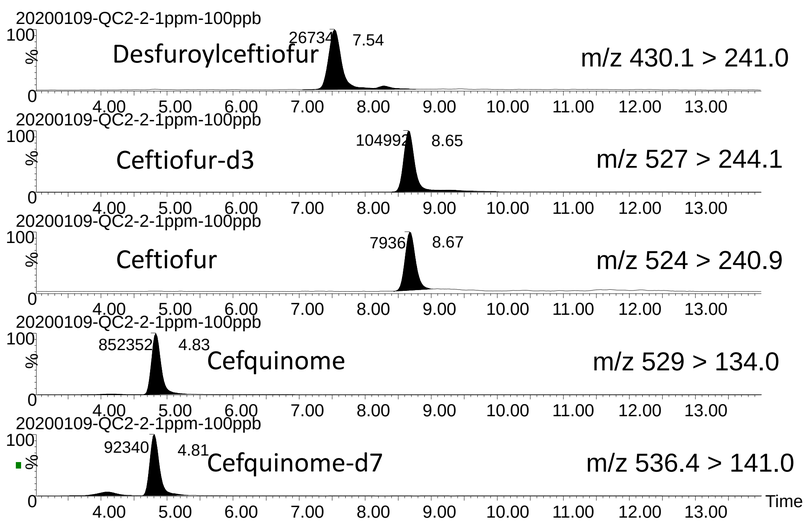


Figure 1: LC-MS/MS chromatograms of spiked porcine plasma (concentration: 1 µg/mL cefquinome and desfuroylceftiofur; 0.1 µg/mL ceftiofur)

DISCUSSION AND CONCLUSIONS

The LC-MS/MS parameters resulted in good chromatographic properties during method development (Figure 1). Furthermore, the method was successfully validated for cefquinome and ceftiofur according to European guidelines (Table 3). For desfuroylceftiofur the robustness was not as expected. Hence, further research is needed (i.e. different column types, different mobile phases,…) to improve this aspect. The validated LC-MS/MS method will be used to investigate ceftiofur and cefquinome in porcine plasma after IM administration according to the leaflet.

References: [1] Commission Decision 2002/657/EC, *Off. J. Eur. Communities* **2002**, *L221*, 29.