MULTIRESIDUE AND MULTI-CLASS DETERMINATION OF ANTIBIOTICS AND ANTHELMINTICS IN FEED BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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I. Introduction

The veterinary drugs most broadly used in medicated feed are antimicrobials and anthelmintics. Although these antimicrobials are authorized, traces are undesirable in non-medicated feed. As medicated and non-medicated feeds are often manufactured in the same production lines, carry-over of antimicrobials can occur when a feed miller switches from producing one feed to the next one. To decrease the level of cross-contamination in feed in Belgium, the Federal Agency for the Safety in agreement with Belgian feed producers, have decided to authorize a provisional carry-over at 1 % until 31/12/2016.

An analytical strategy was developed for high-throughput analysis of multiple antibiotics and anthelmintics determination in feed. Because of the large differences in physicochemical properties of these compounds, samples preparation was a simple, fast and primarily focused on the extraction of colistin. After acidic extraction, samples were centrifuged, purified on SPE, and analyzed by UHPLC-MS/MS in MRM mode.

A quantitative validation was done for amoxicillin, chlortetracycline, colistin, doxycycline, fendendazole, flubendazole, ivermectin, lincomycin, oxytetracycline, sulfadiazine, trimethoprim, tiamulin and tilmicosin and performances were in accordance with European Commission Decision 2002/657/CE. Matrix-matched calibration with internal standards were used to reduce the matrix effects. This method has been successfully used to routine monitoring residues in feeds since 3 years.

II. Methods

II.1. Extraction

5 g feed + 50 µl IS + 20 ml HCl 0.5 M
IS =Demeclocycline, Sulfadiazine-$^{13}C_6$,Triclabendazole-d_3, Trimethropim-d_9

Shake 15 min

Centrifuge 5 min at 4000 g

Load 6 ml on SPE

Wash with 5 ml Water

Elute : MeOH/HAc (90/10)
MeOH

Evaporate under N_2 at 40°C

Dissolve in 1 ml H_2O/ACN (90/10)

Centrifuge 5 min at 11500 g

Transfer to a vial prior UHPLC-MS/MS analysis

II.2. Validation

- According to EU Decision 2002/657/EC
- 3 days : 1 calibration curve with 8 levels (0, ¼, ½, ¾, 1, 2, 3 and 4 % carry over level) + 7 blanks + 21 QC spiked at 3 levels (each day)
- Evaluated parameters : selectivity, specificity, linearity, intra-day precision, inter-day precision and trueness (recovery)
- Determination of expanded uncertainty measurement (U, with k=2)

II.3. Chromatography

- Acquity UPLC (Waters)
- Column : Acquity HSS T_3 (Waters, 150x2.1 mm, 1.7µm)
- Injection volume : 20 µl
- Flow rate :0.5 ml/min
- Gradient :

Time (min)	% A Water+0.05% FA	% B ACN
0	90	10
0.5	90	10
2.5	25	75
4	0	100
5	0	100
6	90	10
7	90	10

II.4. Mass spectrometry

- Xevo TQ-MS (Waters,Triple quadrupole mass spectrometer)
- MRM mode : 2 MRM/ compoundsin ESI+ or ESI-
- Post-column delivery of 5% NH_4OH

III. Results

Table 1 : Validated compounds and calibration range

Compound	Lowest level 0.25 %	Mid level 1 %	Highest level 4 %
	(1 % = authorized carry over level µg /kg)		
Amoxicillin	750	3000	12000
Chlortetracycline	750	3000	12000
Colistin	637.5	2550	10200
Doxycycline	625	2500	10000
Fenbendazole	82.5	330	1320
Flubendazole	75	300	1200
Ivermectin	25	100	400
Lincomycin	110	440	1760
Oxytetracycline	500	2000	8000
Sulfadiazine	35	140	560
Tiamulin	100	400	1600
Tilmicosin	1250	5000	20000
Trimethoprim	87.5	350	1400

Table 2 : Relative standard deviation (RSD), recovery and lowest R^2

Coumpound	Spiked level (µg/ml)	Recovery (%)	RSD, (%)	RSD_R (%)	Lowest R^2	U (%)
Amoxicillin	1500	99.1	3.4	5.1	0.986	28.2
	3000	93.0	1.9	9.7		
	6000	89.4	6.9	6.9		
Colistin (A+B)	1275	95.5	12.4	14.0	0.997	36.6
	2550	95.4	8.7	11.8		
	5100	99.2	4.5	9.6		
Chlortetracycline	1500	95.8	9.5	8.6	0.997	18.5
	3000	100.2	3.8	5.7		
	6000	94.9	8.0	9.4		
Doxycycline	1250	96.9	10.2	10.0	0.996	21.2
	2500	94.0	3.9	6.6		
	5000	94.0	8.1	7.1		
Flubendazole	150	97.0	1.0	6.7	0.994	27.0
	300	94.5	1.2	9.4		
	600	99.6	0.9	6.3		
Fenbendazole	165	97.4	8.2	6.1	0.989	33.2
	330	96.5	11.9	12.5		
	660	106.7	8.2	11.4		
Ivermectin	50	103.5	6.6	9.3	0.998	35.4
	100	96.0	9.1	7.1		
	200	95.1	7.8	11.0		
Lincomycine	220	101.6	4.1	8.4	0.996	29.4
	440	99.7	3.3	10.0		
	880	93.7	4.3	8.0		
Oxytetracycline	1000	101.7	7.0	9.3	0.998	18.6
	2000	100.1	5.9	7.7		
	4000	94.4	7.2	7.3		
Sulfadiazine	70	102.1	3.9	4.7	0.995	13.4
	140	99.7	2.4	3.9		
	280	101.5	2.4	4.1		
Tiamulin	200	92.3	7.3	4.7	0.999	32.8
	400	93.5	11.7	7.1		
	800	94.5	6.05	6.5		
Tilmicosin	2500	104.4	5.0	6.2	0.990	33.2
	5000	95.6	8.2	5.6		
	10000	94.9	6.6	6.6		
Trimethoprim	175	103.9	7.8	9.1	0.998	23.8
	350	92.9	8.8	8.2		
	700	90.4	3.3	7.1		

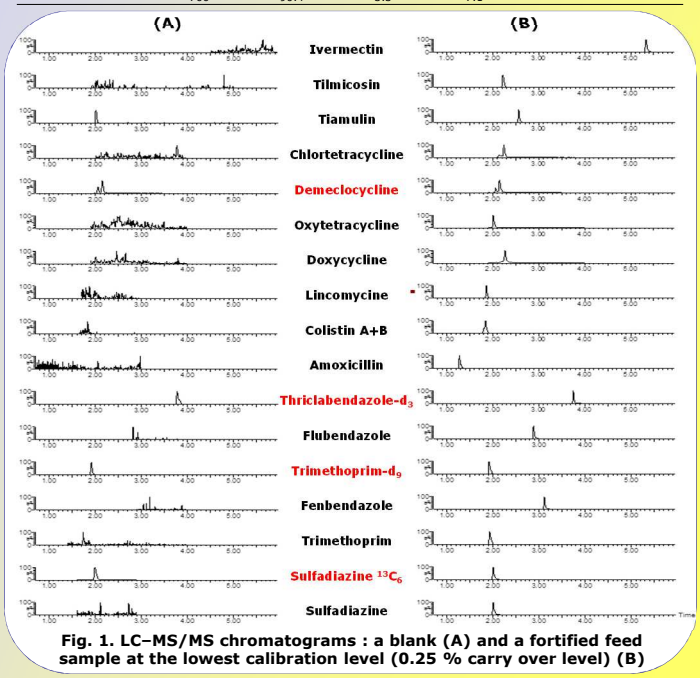

Fig. 1. LC–MS/MS chromatograms : a blank (A) and a fortified feed sample at the lowest calibration level (0.25 % carry over level) (B)

IV. Conclusion

- The method has been used successfully for a surveillance check for the presence of residues in feed for the past 3 years. All results are detailed in the following paper : Development and Validation of Rapid Multiresidue and Multi-class Analysis for Antibiotics and Anthelmintics in Feed by Ultra High Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry, Food Additives and Contaminants, 2016.
- Performances were also demonstrated by participating in PT. (Proficiency test for antibiotics and anthelmintics in compound feed. Wageningen Rikilt. Confidential Rikilt Report 2014-510 44p.)