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VALIDATION OF A METHOD FOR TIAMULIN IN LIVER BY LC-MS/MS

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Abstract

The antibiotic Tiamulin is a semisynthetic derivative of pleuromutilin. Pleuromutilins are mainly used in feed or water medication for gastrointestinal disease. In the residue control the marker residue for tiamulin in pig liver is "the sum of the metabolites that may be hydrolysed to 8-α-hydroxymutilin". A screening and confirmatory method validated according to Commission Decision 2002/657/EC for the analysis of the marker residue is presented. The method is a modification of work by Markus and Sherma (1993) and the focus points in the method optimization have been to reduce the amounts of organic solvents used in the extraction procedure and to substitute the solvents incriminating to the environment.

Introduction

The figures in Table 1 shows, that tiamulin is widely used in the Danish pig production, and is at the "top five" of the most popular antibiotics used in pigs. In 2014 the Danish pig producers committed themselves to reduce the consumption of tetracyclines by 50% by the end of 2015 (Danmap 2014, Landbrug og Fødevarer). To ensure animal welfare while reducing the amount of tetracyclines used, one of the strategies mentioned was to replace tetracyclines with tiamulin (Landbrug og Fødevarer).

Table 1. Use of pleuromutilins in Danish pig production (Vetstat). Tiamulin accounts for nearly 100%.

Year	Kg active Component	% of total use of antibacterials
2013	8.963	9.9
2014	8.121	9.4
2015	7.880	9.7

Method

Extraction:
To 1 g liver add 10 mL extraction solution (Acetone:0,5 M HCl) Shake, centrifugate. Repeat with 5 mL extraction solution. Add 2 mL 0,5 M HCl and 10 mL water. Evaporate to 10-15 mL 45 °C
Hydrolysis:
Add 3 mL water and 1 mL 7 M NaOH. Shake. Incubate 20 min 45 °C. Add 5 mL water and 1 mL concentrated HCL. Shake. Add 2 mL heptane. Shake 10 min. Remove heptane.
Clean-up:
Condition OASIS HLB-column. Methanol, Water.
Load sample. Rinse with 3 mL 15% methanol.
Elute with 5 mL of methanol.
Evaporate to dryness and dissolve in 300 µL methanol shake and add 700 µL of water. High speed centrifugate before injection of 10 µL to LC/MS-MS-system. ESI positive mode.

Results and Discussion

The analyses of the validation are done as 6 double determinations in four days by two different technicians. The absolute recovery of the method was calculated found to be only 31 %. A necessary heptane washing step partly accounts to the loss of analyte, but it was concluded that the low recovery was acceptable because the CV% was low (9%), indicating that the recovery was stable. In table 2, an overview of the results of the validation is presented. The MRL in liver is 500 µg/kg and the validation resulted in a CCα of 588 µg/kg and a CCβ of 50 µg/kg.

Table 2. Overview of the results of the validation

Level	CV% repeatability	CV% within-laboratory reproducibility	Reproducibility CV(%) Horwitz equation	Relative recovery %	Absolute recovery %
250 µg/kg	7.0	10.7	19.7	90	-
500 µg/kg	11	11.4	17.8	94	31
750 µg/kg	8.6	10.1	16.7	94	-

Conclusion

The method is accredited and has been applied to the Danish National Residue Control Plan in 2014 and 2015. A total of 204 samples have been analysed. There were no detected residues above CCβ in the analysed samples.

Acknowledgements

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References

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