Development and validation of a confirmatory method for the analysis of avilamycin in animal feed
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Introduction
With the ban of the use of antimicrobial growth promoters in animal feed a need has risen for the development of confirmatory methods. Quantitative microbiological methods are available for the detection of avilamycin, but they are not sufficient because of the lack of confirmation. Avilamycin A (prevalent factor, >60%) and B (<18%) constitute up to 99% of microbiological activity of avilamycin. An HPLC method¹ and the detection with MS² have been described previously, but a combination of both techniques has not been described.

Sample clean up, extraction and analysis
Feed samples were ground and homogenized. The sample was extracted with acetonitrile and shaken with hexane. An aliquot of the ACN layer was diluted with water and concentrated on an Oasis® HLB solid phase extraction cartridge. After dilution with water the sample is injected on an LC-MS/MS system. The analysis is carried out using a Waters Xbridge™ C₁₈ analytical column using a mobile phase of ammonium acetate, water and ACN. The mass spectrometer operated in electrospray positive mode and data acquisition performed in multiple reaction monitoring mode (MRM). The monitored precursor/product ions are shown in Figure 2.

Validation
For validation the following parameters were considered to be important: specificity, decision limit (CC₃), detection capability (CC₁), ruggedness and stability². CC₃ and CC₁ were determined by analysing 20 blank feeds, 20 feeds fortified at 0.5 mg kg⁻¹ and 20 feeds fortified at 1.0 mg kg⁻¹. Ruggedness was determined by introducing small variations in the sample pretreatment procedures. The stability of avilamycin A in stock solutions was determined by storing batches at +4°C and -70°C for different periods of time.

Results
LC-MS/MS traces of a blank and a suspect sample are shown in Figure 3. One sample, out of the 20 that were fortified at 1 mg kg⁻¹, could not be confirmed as avilamycin A (5%). In this case the concentration level, where only ≤ 5 % false compliant results remain, equals the detection capability (CC₁) of the method. Therefore, CC₁ was set at 1 mg kg⁻¹. Extraction time, SPE wash volume and other sample pretreatment procedures were varied to determine ruggedness of the method. The method is found to be sufficiently specific and rugged when the sample preparation procedure is followed accurately. The standard solution is found to be stable for at least three months when stored at +4°C. The analysis of 20 blank feeds showed that the method was specific for avilamycin A.

Sample
The developed method was used to confirm the identity of a suspect sample that was found during microbiological screening in 2006 (see Figure 3). The sample contained 5.5 mg kg⁻¹ (based on microbiological quantification).

References

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