

AAFCO Laboratory Methods and Services Committee Tylosin Update

Tylosin has shown to be linear over the range of 1 ppm to 489 ppm using three different columns, Phenomenex Luna ODS(2) 250x4.6 mm 120 A 5 µm, Waters NovaPak C18(1) 250x4.6 mm 100 A 5 µm, and Agilent Zorbax XDB C18(2) 250x4.6 mm 120 A 5 µm. This would equate to 1.0 gm/ton to 20 gm/ton, without adjusting sample size or volumes used to increase the dynamic range of the analysis. When adjusting the sample weight, it can reach up to 60 to 70% Tylosin.

The detection and quantitation limit studies have been done. Using LC-DAD will be insufficient to meet the detection criteria of 0.03 ppm (mg/kg) and the requirement for the quantitation criteria, 0.100 ppm (mg/kg) without method modification. This data is without increasing the sample size or changing the volumes used. Trace level studies have been finished using LC-MS/MS and LC/MS. Both of these techniques will meet the criteria set by the method's need statement.

Cleanup experiments have been completed. Several different SPE column packings will work for this drug. The columns tested, thus far, are Water's OASIS HLB, Sulpelco's DSC-18, DSC-CN, and Sulpelco's Envi-18. Other packings under consideration are PSA, GCB, or combinations of these two with ODS.

Currently, I have requested, via phone calls and AGLABS, actual samples that have been analyzed, along with the microbiological results of the samples, in order to proceed further, and correlate the LC data with the actual potency determined by the official AOAC method. QuEChER'S with the use of the Geno-Grinder may prove a viable extraction and cleanup method for tylosin. This would significantly reduce the time of the analysis significantly.

Please find attached the following:

Attachment A: Proposed method based on Wisconsin's SOP

Attachment B: HPLC-MS results

Attachment C: HPLC-MS/MS results

Respectfully Submitted,

T. Phillips