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LC-MS/MS – Points of attention when using isotope labelled standards

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Introduction

The use of isotope labelled internal standards is of common use in LC-MS/MS analysis of antibiotics and other veterinary drugs. The advantages are various like extraction recovery correction and elimination of matrix effects during LC-MS/MS analysis. However, there are some points of attention when using isotope labeled internal standards. If the number of labeled atoms is too low or when the number of C-atoms of the analyte is high, it's is possible that the unlabeled analyte of interest will contribute on the signal of the internal standard. For that reason, it's advisable to consider if the concentration of the internal standard is appropriate in relation to the concentration of the native compound.

Case study: Spiramycin in bovine muscle

Due to an unsatisfactory z-core of 2.3 for Spiramycin in a proficiency test for macrolides in bovine muscle a follow up was started to determine the cause of this deviation. It was noted that the correlation coefficient of the curve from 0.9924 was sufficient, but that the peak area of the internal standard Spiramycin-d3 increased when increasing the concentration of Spiramycin. It turns out that the increasing peak area of Spiramycin-d3 was caused by the third isotope peak of Spiramycin. A Spiramycin molecule contains 43 atoms of carbon which means that the 3rd isotope peak is present for 3.2 % and will contribute on the signal of Spiramycin-d3 during LC-MS/MS analysis (Figure 1 and 2).
At a concentration of 100 µg/kg for Spiramycin-d3 the contribution of Spiramycin at 400 µg/kg on the Spiramycin-d3 is significant with 12.8%. This reflects in a flattened calibration curve and overestimation of the Spiramycin concentration (Figure 3).

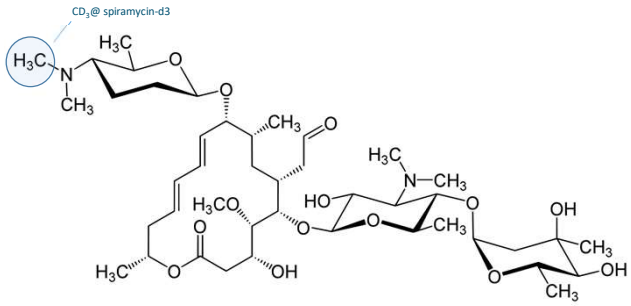

Figure 1. Structure of Spiramycin ($C_{43}H_{74}N_2O_{14}$) with molar mass 843.5 ; LC-MS/MS detection $[M+2H]^{2+}$ Spiramycin @ *m/z* 422.3 and Spiramycin-d3 @ *m/z* 423.8

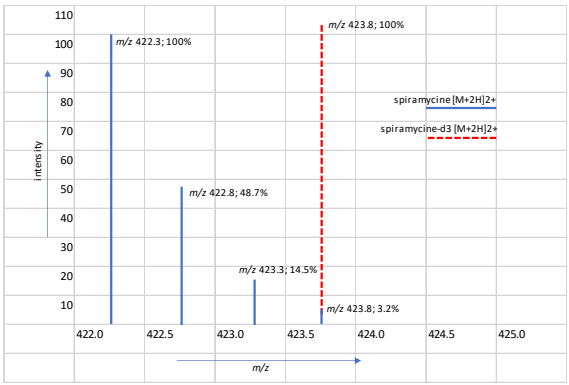

Figure 2. Contribution from Spiramycin 3rd isotope peak on Spiramycin-d3 @ *m/z* 423.8

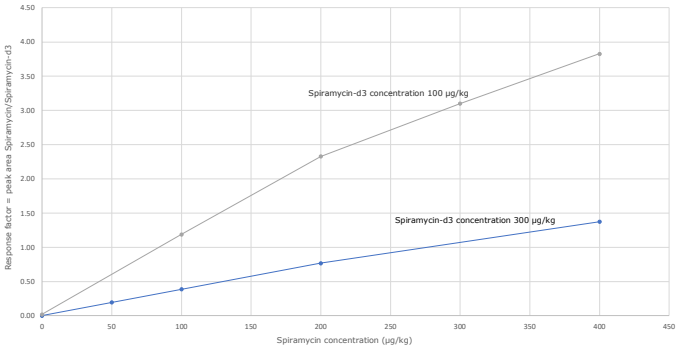

Figure 3. Effect of the concentration Spiramycin-d3 on the calibration curves for Spiramycin

Conclusions and recommendations

During LC-MS/MS detection contribution from the native compound on the signal of the isotope labelled internal standard is possible when concentrations are not well chosen. This will effect the calibration curve and thus the quantification, overestimation is for real concern.
When using isotope labelled internal standards it is advisable to determine whether contribution from the native compound can be expected. If so, the right concentration for the isotope labelled internal standard has te be calculated. A contribution less than 1 % is considered to be sufficient. For the quantification of Spiramycin in bovine muscle a calibration range up to 400 µg/kg is used. The ideal concentration for Spiramycin-d3 in this case would be 1500 µg/kg.

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