Veterinary Drug Analysis: The Role of Isomers and Metabolites

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Introduction

In recent years, it is shown that the presence of isomers and metabolites of veterinary drugs can affect the quantification and identification of compounds present in a sample. If not handled with care, results of a substance may be reported incorrectly, and subsequent enforcement may be incorrect. WFSR has conducted a study to investigate the analytical consequences of possibly present isomers and metabolites on the analysis of the marker molecules. The collected information was used to assess whether these compounds could interfere in the official analysis, *e.g.,* by yielding false-positive or false-negative findings.

Here, two cases are presented that illustrate the analytical challenges when it comes to isomers in official analyses. Each of these cases poses different challenges and was chosen to demonstrate the importance of being aware of the possible issues that can arise when (un)knowingly dealing with isomers.

D-ampicillin and L-ampicillin

An interesting case of stereoisomers was identified in D-ampicillin and L-ampicillin (fig. 1). Findings resulted in the suspicion of conversion of active D-ampicillin to the impurity L-ampicillin in bovine urine (fig. 1). This could result in potential false-negative results for D-ampicillin due to a difference in retention time between the two compounds. Therefore, the conversion of L-ampicillin was confirmed in bovine urine with an analytical standard. Moreover, an experiment was conducted to rule out this conversion in routine analyses performed by WFSR in cheese, milk, kidney, and muscle (fig. 2).

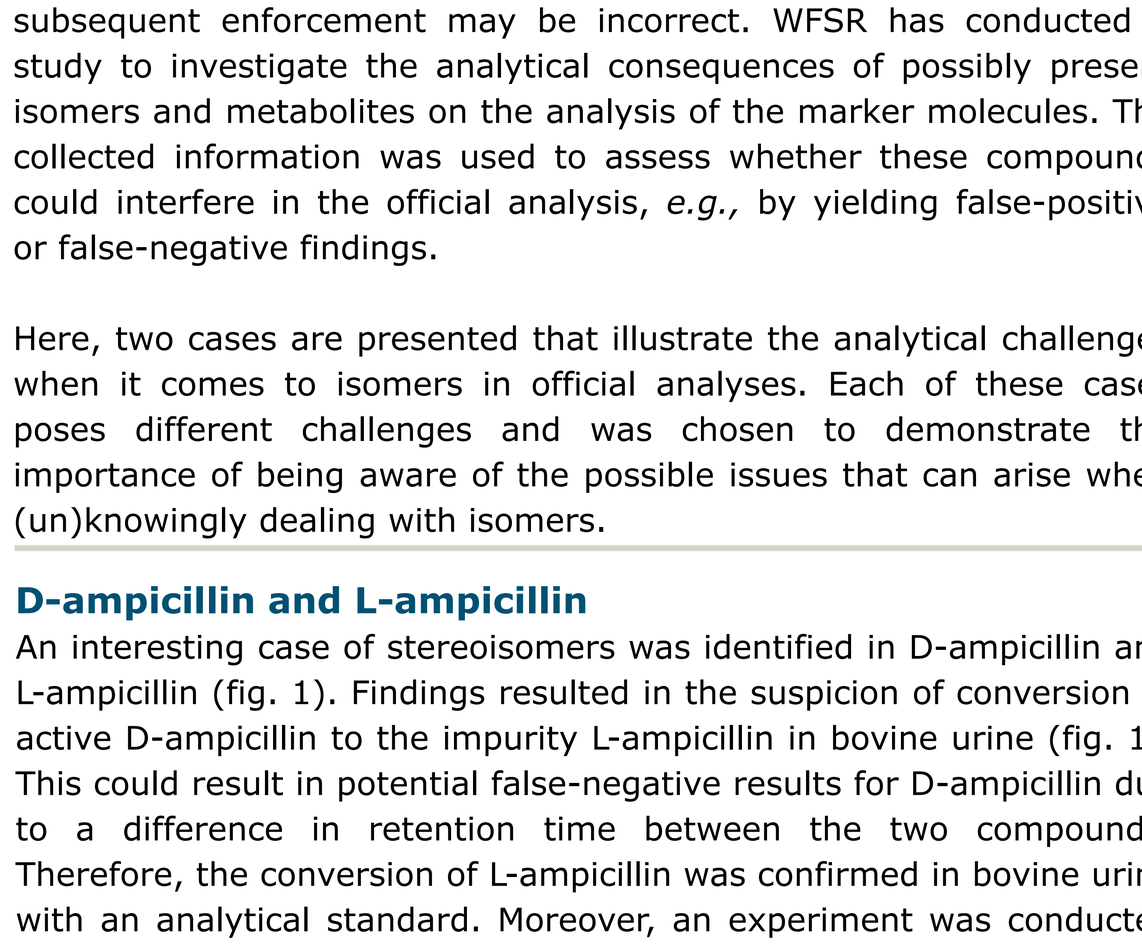

Figure 1. Structural formulas of D-ampicillin and L-ampicillin (top) and the chromatogram of a standard solution containing both compounds.

It is concluded that the conversion of D-ampicillin to L-ampicillin occurs at pH ± 7, and not in acidic or alkaline conditions that are used during routine analyses performed by WFSR. Thus, care should be taken if the pH is ±7 during clean-up because of the conversion of D-ampicillin to L-ampicillin.

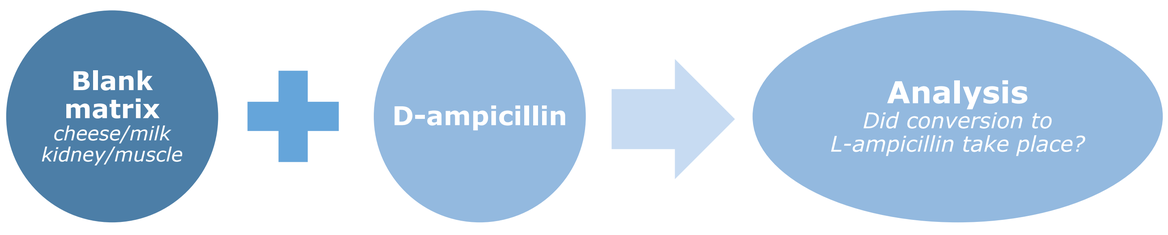

Figure 2. Overview of an experiment that was carried out to determine if D-ampicillin is converted to L-ampicillin during routine analyses performed by WFSR.

3-O-acetyltylosine

3_O-acetyltylosin is included in the sum MRL for tylvalosin (EU/2010/37) and is therefore included in the monitoring program of WFSR. After the analyses of two analytical standards of 3-O-acetyltylosin obtained from two suppliers (P1 and P2), it was noted that these analytical standards produced similar product ions, but with a slight difference in retention time (0.01 min) and a difference in ion ratio. Following this, both standards were analysed using LC-hrMS for identification. It was found that:

- The retention time differed slightly with 0.04 min.
- All fragments of P1 are 42 Da higher than fragments of P2.
- P1 was identified as 3-O-acetyltylosin (fig. 3).
- For P2, the acetyl group is positioned either on R1 or R2 in the mycarose sugar (fig. 3).

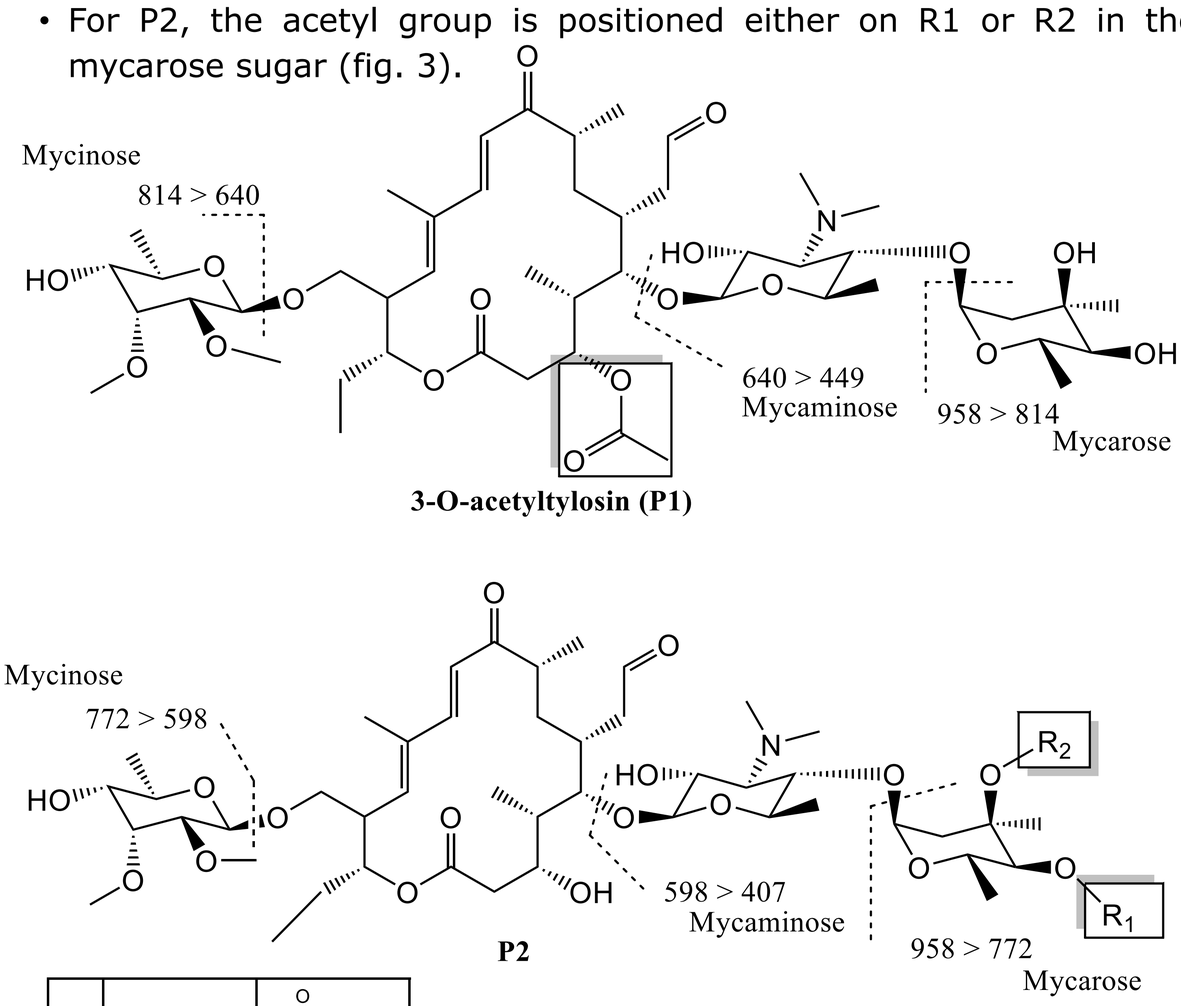

Figure 3. Structural formulas of 3-O-acetyltylosine (top) and P2 (bottom). The proposed fragmentation route is indicated with the dotted line. The difference in functional groups is shown using a black outline. The position of the acetyl group is positioned in the mycarose sugar in P2, positioned at either R1 or R2.

It is concluded that the analytical standard of one of the suppliers is not 3-O-acetyltylosine. This standard is therefore not used in the analyses of 3-O-acetyltylosine performed by WFSR. Moreover, this case shows that an identity-check of analytical standards is required to ensure correct identification and quantification of substances.

Conclusion

WFSR has conducted a study to investigate the analytical consequences of possibly present isomers on the analysis of the marker molecules of veterinary drugs. The two presented cases illustrate the importance of thorough research into isomers, metabolites, and other potentially interfering substances and the possible risks they pose for MRL enforcement.

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