Dipl.-Chem. A. Vockel

Determination of chlortetracycline residues in biological samples of lifestock farming using HPLC-UV-MS/MS

- Development of analytical methods and application in medication studies -

Tetracyclines, broad spectrum antibiotics, are widely used in lifestock farming. Especially chlortetracycline (CTC) is frequently added to feed for the treatment of bacterial diseases in pigfattening. As a consequence, antibiotic residues remaining in animal products may promote the development and distribution of bacterial resistance to antibiotics. It has been recognized in the European Union´s legislation on the control of veterinary drug residues, that tetracyclines produce epimers. The maximum residue limits (MRL) for tetracyclines in edible animal tissues have been defined as the sum of the parent drug and the epimers. CTC gives rise not only to the epimer (e-CTC) but further microbial and toxicological relevant conversion and degradation products (also called "metabolites"). These metabolites are not detectable with previous validated methods.

This work aimed to get more information on the fate of CTC in pig production. To estimate the chlortetracycline levels in excreta and remaining residues in carcass samples two medication studies were carried out under controlled conditions (Haus Düsse, Bad Sassendorf, and FAL Braunschweig). Piglets were reared without antibiotic medication and then treated with chlortetracycline at the limit of the typical therapeutical dose.
Firstly, analytical methods for the quantification of CTC and e-CTC in urine, faeces, and plasma as well as in bones, muscle, liver and kidney were developed and validated according to international guidelines. These methods include the extraction of the samples with McIlvaine-buffer, following by a cleanup-step with solid phase extraction (Oasis HLB) and finally the chromatographic identification and quantification with HPLC-UV-MS/MS.
The results from the medication studies supply evidence for a formation of iso-CTC und e-iso-CTC and small amounts of anhydro-CTC und e-anhydro-CTC in addition to e-CTC. In consequence, the analytical method was extended to include the quantitative determination of these additional metabolites of CTC. A method for the quantification of e-iso-CTC was developed, which is commercially not available as reference standard. The e-iso-CTC can be prepared *in situ* by epimerization of iso-CTC in standard solution. Furthermore another metabolite could be detected. It was identified by mass spectrometry (LC-MSMS-technique) based on typical fragmentation reactions, as the keto-tautomer of e-CTC. Evidence is given, that the various chlortetracycline metabolites can be formed *in vitro*, during sample preparation depending on the conditions of sample pretreatment (e.g. pH, solvent), as well as *in vivo.*

A detailed mass balance study of the antibiotics applied to pigs showed that high amounts of CTC were excreted mainly via faeces (88 %) and to a lesser extent via urine (5 %). The remaining drug amount in the animal predominantly accumulates in bones (2.75 %). Comparatively small amounts were found in muscle (0.09 %), liver (0.005 %) and kidney (0.001 %). Important implications result from the fact that in all carcass samples not only CTC and e-CTC were detected, but also the isomers and small amounts of the anhydro compounds. Therefore, the determination of residual chlortetracycline levels should not be restricted to the sum of the contents of CTC and its epimer. Also the non active compounds iso-CTC and e-iso-CTC and the toxic degradation products anhydro-CTC and e-anhydro-CTC should be taken into account, which can be also potent on tetracycline resistance strains. Based on the data obtained the total sum of CTC concentrations could frequently exceed MRL.

In this work analytical methods were developed and validated which can be used in routine analysis. These methods lead to new findings, relevant to the protection of the consumer, and broaden facilities for the control of food from animal origin.