

# Untersuchungen zur Analytik und zum Einfluss technologischer Prozesse auf Penicillinrückstände in Milch

Fachbereich Chemie der Bergischen Universität Gesamthochschule Wuppertal  
Ludger Grunwald

## Abstract

Even in low concentrations, veterinary medicinal products can have specific effects on organisms and harmful effects on the environment. Inflammation of the teat in agricultural cows (mastitis), but also of the breast in breastfeeding mothers, cause large amounts of pharmaceuticals to be released into the environment.

An analytical method for the determination of penicillins in water and milk was developed, as well as the ability to bind to proteins.

For treating infected animals special preparations, preferably penicillins, are injected directly into the milk channel of the cow in order to obtain a sufficiently high concentration of the antibiotic in the udder. As a consequence of such a medication, the active substances are excreted with the milk for a certain period of time after application.

Four cows suffering from mastitis were treated with Eumacid, a common veterinary drug for mastitis therapy with cloxacillin as active compound. The residue depletion via milk was studied with four qualitative microbiological or immunochemical screening tests (Delvotest SP®, BR-AS special®, Penzym S® test and Snap® test) in comparison with an automated quantitative HPLC method to investigate how good the results from these tests correlate and if they allow the detection of cloxacillin concentrations at or near the MRL value. Data analysis shows excellent correlation between all four qualitative tests and also in comparison with the quantitative HPLC results.

For the penicillin nafcillin a maximum residue limit of 30 µg/kg for milk was established by the European Commission. Flucloxacillin is a penicillin used in human medicine. An automated reversed-phase liquid chromatographic method with UV detection at 300 nm after on-line coupled solid-phase extraction and photochemical post-column derivatization for oxacillin, cloxacillin and dicloxacillin, was extended to include nafcillin and flucloxacillin. This modified method allows a simultaneous analysis of isoxazolyl penicillins and nafcillin or flucloxacillin in one sample. The detection limits were 10 µg/kg for nafcillin and 8 µg/kg for flucloxacillin. The recovery was 67 % for both antibiotics with coefficients of variation of 9 % for nafcillin and 6 % for flucloxacillin.

Milk samples spiked with benzylpenicillin and cloxacillin at and above maximum residue limit concentrations were used to produce yoghurt according to the typical industrial process. As expected, coagulation was negatively influenced by increasing concentrations of residual penicillins. Main degradation products of penicillin G as identified by LC-MS/MS were penillic, penicilloic, and penilloic acid.

To investigate penicillin-binding to milk proteins, a dialysis was performed to eliminate unbound penicillins. Afterwards the donor solutions were tested by indirect competitive ELISA, which utilized antibodies being sensitive to penicillins with intact as well as hydrolysed beta-lactam ring, to determine the amount of penicillins. 1.5 % of ampicillin, 5 % of penicillin G, and 20 % of cloxacillin were bound to milk proteins.