

Development of a monitoring-system for identification and quantification of antibiotics and corresponding conversion products in honey by means of LC-MS/MS

Honey as a natural finish product has to fulfill strict criteria. Therefore national authorities in the European Union and Switzerland have passed laws regarding the quality of honey. The use of antibiotics in the apiculture against the American fire blight and other bacterial diseases can cause residues in the food-grade honey. The occurrence of antibiotic residues can lead to allergic reactions and furthermore promote antibiotic-resistances. Under these circumstances it is very important to detect veterinary residues in food stuff, in special honey and other bee products and withdraw products containing antibiotics from the market.

Aim of this work was to develop a monitoring-system to detect residues of antibiotic application in the apiculture. To determine the antibiotics in honey, liquid-liquid extraction and solid phase extraction coupled to LC-MS/MS technique was used. Beside the main antibiotics furthermore a couple of metabolite (degradation and conversion products formed in vivo and in vitro) can be detected. This work describes the method development for in total 63 antibiotic substances. The following antibiotic classes were included in the monitoring-system: aminoglycosides, tetracyclines, sulfonamides, macrolides, fluoroquinolones, nitrofurans and the single agent's chloramphenicol and trimethoprim. An online-SPE (solid phase extraction) was introduced in the LC-MS/MS system to get better reproducibility and to lower the cost of each analysis. The methods meet the criteria according to the EU-regulation 470/2009 in conjunction with regulation 37/2010/EU and were validated on the basis of the regulation 2002/657/EU.

Within a market survey (N=70) of honeys from regional supermarkets no antibiotic residues could be observed. However, during the routine control of raw honeys in some cases sulfonamides ($50 \pm 2 \mu\text{g/kg}$ sulfathiazole; $0.5 \pm 0.03 \mu\text{g/kg}$ sulfadimidine), tetracyclines ($3 \pm 0.3 \mu\text{g/kg}$ demeclocycline) and in two samples chloramphenicol were confirmed. The collected analysis data of the routine monitoring was also implemented by using chemometric software to classify honeys regarding the botanical and geographical origin. The utilized principal component analysis (PCA) differs between honeys sorts for example acacia, honeydew, rape and mountain blossom honey. In addition to the classical pollen analysis the chemometric evaluation is a reliable tool for the classification of raw honeys.

The LC-MS/MS methods for the determination of antibiotics in honey include a broad spectrum of substances. In particular the analysis of tetracyclines provides data about the main substances and also involves degradation and conversion products. In comparison to published methods the analysis of tetracyclines in honey is the most comprehensive method. The requirement of a routine control monitoring-system is shown by positive findings of antibiotics in raw honey. To ensure the high quality of honey the developed needs to be updated by new antibiotics and/or metabolites in the future. In the long-range the lower occurrence of antibiotics in food and in this case honey leads to a reduction of risk factors for antibiotic resistances.