CURRENT STATUS OF ANALYTICAL METHODS FOR THE DETECTION OF RESIDUES IN BEE PRODUCTS

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The objective of this paper is to review shortly the current status of the analytical methods, used for the examination of bee products. There will be a focus on the main contaminants in bee products. Details about the different method can be found in the references.

ANTIBIOTICS IN HONEY

Presently antibiotics are the main contaminants of honey. Following antibiotics have been been detected in honey (see Reference list)

- Sulfonamides: sulfathiazole, sulfamethazine, sulfamethaxazole, sulfanilamide
- Aminoglycosides: streptomycine
- Tetacyclines: Oxytetracycline, chlortetracycline
- Amphenicols: Chloramphenicol

Following antibiotics have been reported to be used in apiculture, but there are no published works on their detection:

Beta-lactams (penicillins) and macrolides: tylosine, erythromycin

In routine honey analysis antibiotics are generally tested by:

1. Screening, determination of positive samples: Charm II Test, ELISA
2. Quantitative determination of positive samples by HPLC, LC-MS

Screening methods

EIISA

Elisa a radio-immuno assay, in which antibiotics are quantitatively assayed by specific antibodies. It has been tested successfully in honey (Usleber et al.1995, Heering et al. 1998). The detection limit is 10 to 50 ppb. With this method the concentration range of individual antibiotics can be quantitatively determined. Because of cross reactivity with other antibiotics from the same group the method can be used only for semi-quantitative determination. Now this screening method has been replaced in many cases by the Charm II test.

Charm II Test

The Charm II test is produced by Charm Sciences Inc. USA. It is a screening test, used for different food as meat and milk, which has been adapted for honey testing. There are published reports on the use of the method (Koch et al. 1996, Edder and Corvi, 2001). It is based on the specific binding of antibiotics to receptors. The quantitation is determined by measuring of radioactivity: H^3 or C^{14} . The Charm II test can detect the whole group of antibiotics. There are tests for testing in honey of: sulfonamides, tetracyclines, beta-lactams, macrolides, amphenicols and aminoglycosides of the streptomycin type. The screening by the Charm test will exclude the negative samples. The positive samples have to be confirmed by other methods (HPLC, LC-MS, see below). The rate of the correctly classified positive samples depend on the experience of the laboratory. Thus 0 to 30 % of the positively tested samples will be confirmed by other methods. Also, the tests for some antibiotics (e.g. streptomycine) are more safe for others (e.g. sulfonamides).It has been found out, that Para Amino Benzoic Acid (PABA), a natural honey constituent, can disturb the test for sulfonamides and yield "false positive" results. Also, there are matrix effects, especially in honeydew and chestnut honey, which lead to increased rate of "false positive" samples. It seems that the detection level for each antibiotic group depends on the honey type. As a whole, the Charm test for honey is a valuable tool for routine testing for honey producers and packers. Its main advantages are:

- It tests a whole group of antibiotics
- It is considerably cheaper than the other quantitative methods

Quantitation

The results of the Charm test are qualitative to semi-quantitative. The positively tested samples are further analysed by HPLC and LC-MS methods for quantitation of the residues.

HPLC

Only the recent literature will be considered, as older methods have detection limits in the ppm determination range. References from the older literature can be found in Kaufmann et al., 2002. The HPLC is good for routine work, where known antibiotics are analysed. The drawbacks of HPLC are:

- Each antibiotic class has to be tested separately
- There is no 100 % certainty regarding the safe identification of the antibiotics

Recently automatic HPLC systems which include sample extraction and HPLC determination have been developed (http://www.applica-analytik.de). This is a promising development, aiming at a less labour intensive and cheaper antibiotic determination.

Sulfonamides are bound to honey sugars. That is why an acid hydrolysis step is included in the method. The sulfonamides are derivatised for fluorimetric detection and afer separation by reversed-phase HPLC are detected by Diode Array Detector or by a fluorescence detector (Schwaiger and Schuch, 2000, Diserens and Perroud, 2002). The limit of detection is about 5 ppb, the limit of quantitation lies at 10-15 ppb, the recoveries of 11 different substances vary from 70 to 100 %.

Streptomycin is analysed by reversed-phase HPLC, followed by post-column derivatisation to enable fluorescence detection (Kocher, 1996, Klementz and Pestermer, 1996, Etter and Corvi,1998). The limit of detection is about 5 ppb, the limit of quantitation is 10 ppb.

Tetracycline and oxytetracycline were determined by reversed-phase HPLC with UV detection in a study of the degradation in honey, but the limit of detection was quite high, about 1 ppm (Sporns et al., 1986). Oxytetracyline (terramycine) degrades within 5-9 weeks (Sporns et al. 1986, Aargauer and Moats, 1991) into different metabolites (Sporns et al., 1986). In a recent ring trial, conducted by FAPAS (2002) different HPLC methods with fluorescence detection has been used, the detection limit being about 50 ppb (Iwaki, 1992, Kaufmann, 1999). The residue level of 0.6 ppm chlortetracycline tested in this collaborative study was far above the levels, expected in the praxis.

LC/MS-MS

This is the most modern and promising method. The analysis is based on clean up by SPE, separation by reversed-phase HPLC and subsequent detection by MS-MS. The MS-MS insures an almost “100 %-safe” specific detection with a very low background noise. The method was developed by Kaufmann et al. (2002) for sulfonamides, tetracyclines (tetracyclines, oxytetracycline and chloroxytetracyclines) and flumequine. The limit of detection is 0.5 to 10 ppb, depending on the substance. The method is presently extended to include also chloramphenicol and streptomycine. The objective is the development of a LC/MS-MS method, capable of detecting all antibiotic residues in honey.

ACARICIDES AND PESTICIDES

Acaricides and pesticides in honey

A list of the recent methods used is given below. Both GC and HPLC methods have been used. GC methods with MS detection seem to be most promising.

Gas Chromatography

In most cases the techniques use extraction of honey by SPE or organic solvents, followed by separation on capillary chromatography with ECD, NPD or MS detection. For methods for the detection of the most commonly used acaricides and pesticides see the reference list below. The limit of determination lies between 1 and 10 ppb. The limit of detection of thymol lies at about 0.02 ppm, the limit of determination being around 0.1 mg/kg (Bogdanov et al., 1998).

HPLC

Reversed-phase HPLC with Photo Diode Array Detection is successfully used. The determination limit for synthetic acaricides is higher than the one achieved by GC methods and lies typically between 10 and 50 ppb. The limit of detection of organic acids lies at about 1 ppm.

Enzyme analysis

A combined determination of oxalic and formic acid can be carried out with the Boerhinger Kit, Germany (Bogdanov et al. 2002). Oxalic acid can also be determined with a Sigma Kit (Mutinelli et al. 1997). The limit of detection of these methods lies between 3 and 5 ppm.

Acaricides and propolis in beeswax

The method, mostly used for the determination of the most common acaricdes bromopropylate, coumphos, fluvalinate and flumethirne is: extraction by hexane, separation from high-molecular compound by freezing centrifugation, purification on Florisil columns and subsequent determination by capillary GC and ECD detection (Zimmerman et al. 1993, Bogdanov et al., 1998). The detection limit is 0.1-0.25 ppm. The objective is to enlarge the deermination scope by including other varroacides as amitraz, cymiazole etc, as proposed by Korta et al. 2002. Only GC-MS detection will allow the detection of all acaricides, used for varroa control.

VOLATILE CONTAMINANTS

Volatile contaminants can be tested with head-space or SPME techniques (Conte et al. 1997, Volante et al. 1998, Piasenzotto et al. 2002):

- Repulsive substances used in honey harvest (phenol, nitrobenzene)
- Anti-wax moth substances: para-dichlorbenzene, naphthalene
- Components of essential oils: thymol

QUALITY ASSURANCE

The introduction of Quality Assurance in analytical laboratories according to the EN 45001 and ISO 9000 norms allow precise and reproducible measurements of trace amounts of residues in bee products. Collaborative trials should be regularly carried out to ensure good performance of the testing laboratories. Three collaborative trials have been carried out recently with bee products : suflonamides in honey, tetracycline in honey and acaricides in beeswax (see reference list).

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