2. BIOSENSOR SYMPOSIUM TÜBINGEN 2001

Detection of Clenbuterol in Urine of Meat Stock and Detection of Sulfamethazine in Pig Meat with the Biosensor Biacore Q

Dominik Guggisberg, Tanja Widmer[1] and Herbert Koch
Veterinary Office, Chemistry Section,
Schwarzenburgstrasse 161, CH-3003 BERN
Dominik.Guggisberg@bvet.admin.ch www.bvet.ch

Abstract

The biosensor-tool *BIACORE Q*[a] was evaluated and validated to find out if it could be applicated on-site in an abattoir for the detection of veterinary drug residues. The study was focused on the following questions:

- **Is the biosensor-instrumentation robust enough for the on-site application in an abattoir?**
- **Are reliable results fast enough available?**
- **How many false-negative and false-positive results can be found?**

This feasibility study was carried out based on the examples clenbuterol and sulfamethazine. A total of 500 urine samples for clenbuterol residues (calf, cattle, pig, and sheep) as well as 160 meat samples for sulfamethazine residues (pig) was randomly taken at two different abattoirs in Switzerland. The samples were analysed with the Biosensor and commercially available Qflex Kits for clenbuterol and sulfamethazine. Positive samples were confirmed using LC-MS.

Quantitative detection of clenbuterol in urine of meat stock

Beta-agonists are sympathomimetic-drugs with predominant affinity for beta-adrenoreceptors. Clenbuterol and salbutamol are used as bronchodilatator for the treatment of respiratory diseases of horses and cattle and to relax the uterus of cows at parturition. By administration of these substances to the fodder, the growth of animals can be accelerated. In the last years, beta-agonists have been used illegally as growth promoters in fattening animals. Several cases of food intoxications in Europe in the nineties caused by the consumption of clenbuterol-contaminated bovine liver indicate that the misuse of clenbuterol can be a serious risk to human health [1].

In Switzerland the maximum residue limit for clenbuterol in animal-derived food is 1 µg/kg in meat (EU: 0,1 µg/kg). In order to control the illegal use of beta-agonists, the EU Member States carry out regular

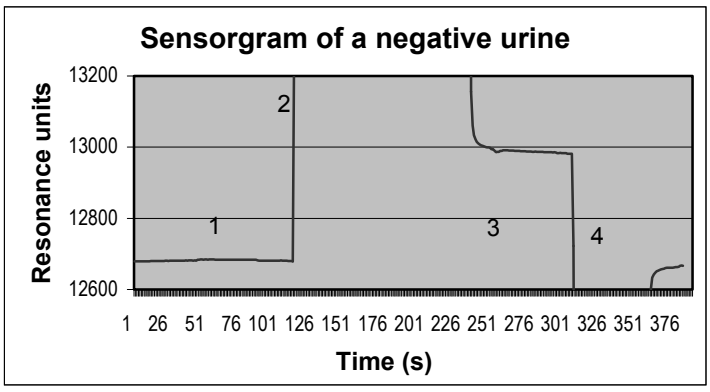
[1] Part of the thesis of T.W.

examinations on drug residues. There is an increasing need for fast and automated screening-systems [2].

The biosensor *BIACORE Q* [3] can be used for the detection of antigen-antibody reactions. A derivative of the molecule to be detected is immobilised on the sensor-chip surface. The autosampler mixes the antibody with the sample before injection; afterwards a microfluid-system transports the sample to the sensor-chip surface, where the antibody binding finally takes place. The quantity of the antibody that binds to the clenbuterol-derivative on the sensor surface is measured by surface plasmon resonance. The detection limit in urine is 0,3 ppb. After the clean-up 40 prepared samples are pipetted on a microtiter plate and given in the autosampler. Then the analysis, which runs fully automatic to the end, can be started. The clean-up (pH-adjustment, liquid/liquid extraction with tert.-butylmethylether, centrifugation and evaporation) for 40 urine samples lasts approximately 3-4 hours, the following analysis [*Fig. 1 & 2*] 6 h 40 min.

Results

Out of 500 urine samples there were 3 positive ones. These positive samples were confirmed as false-positive with LC-MS. The instrument was also tested with incurred urine samples (urine from a horse treated with clenbuterol) and showed positive results which were confirmed using LC-MS [4]. The biosensor-system was compared with two commercially available ELISA-testkits for beta-agonists



regarding time of analysis, costs per sample, number of false-positive measurements.

Fig.1 *Typical sensorgram of a urine sample containing no analyte*
1: Baseline with HBS-EP Buffer
2: Injection of the urine sample
3: The new baseline after the injection of the urine sample. The computer calculates the difference between the two baselines 1 & 3 in resonance units
4: Regeneration

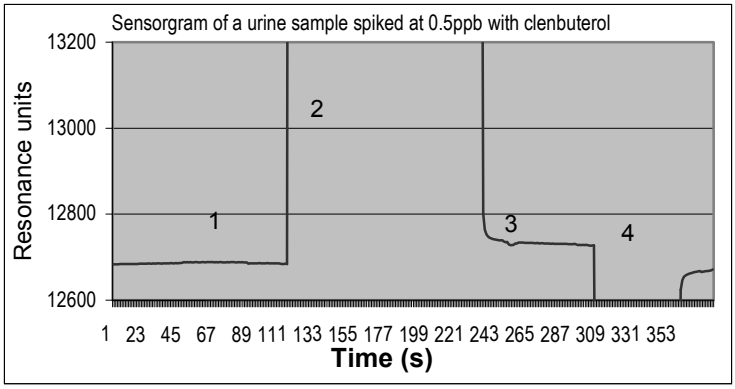


Fig.2 Typical sensorgram of a urine sample containing clenbuterol

1: Baseline with HBS-EP Buffer

2: Injection of the urine sample

3: The new baseline after the injection of the urine sample. The computer calculates the difference between the two baselines 1 & 3 in resonance units

4: Regeneration

Validation

The *Biacore Q* and the Qflex Kit for clenbuterol was tested with an "inhouse-validation" procedure. The results are shown in Table 1.

Urine: spiked value (ppb)	*Number*	*Mean (ppb)*	*Standard-deviation (ppb)*	*Relative standard-deviation (%)*	*Mean recovery (%)*
0,3	5	0,26	0,01	4,1	85,3
0,4	5	0,39	0,07	16,8*	97,9
0,5	5	0,52	0,03	5,9	103,2

(* one outlier; Calculation without outlier: 7%)

Table 1 Recoveries with urine samples spiked with clenbuterol

The results for cross-reactivities are shown in Table 2.

Cross-reactivities for different beta-agonists **Mean**	
1. Clenbuterol	**95%**
2. Mabuterol	**55%**
3. Cimaterol	**12%**
4. Salbutamol	**6%**

Table 2 Cross-reactivities for different beta-agonists, spiked in urine samples

Quantitative detection of sulfamethazine in pig-meat

Sulfamethazine belongs to the group of the sulphonamides (antimicrobially effective chemotherapeutic agents). The effect of the sulphonamide is based on a substrate competition with the p-aminobenzoic-acid, which is an important substance for many bacteria during the biosynthesis of the folic acid. Sulfamethazine is used against general infections for cattle and pigs. The use of chemotherapeutic agents in veterinary medicine in order to combat bacterial infections can cause drug residues in animal-derived food, in particular, if the withdrawal periods before slaughtering are not paid attention to [5]. Antimicrobial residues in the meat can endanger the health of the consumers (danger of developing antimicrobial resistance). In Switzerland the maximum concentration for sulphonamide in meat is 0,1 mg/kg (100 ppb). A total of 160 samples (muscles and kidney) were randomly taken at the slaughterhouse and analysed by the *Qflex kit Sulfamethazine* on the biosensor *BIACORE Q*. One meat sample (135 ppb) and one kidney sample (158 ppb) were positive. The results were confirmed using LC-MS (muscle: 149 ppb, kidney: 188 ppb).

Conclusion

From a technical point of view it is possible to use the biosensor directly at the slaughterhouse for screening of clenbuterol residues in urine samples and sulfamethazine residues in meat samples. Out of 500 urine samples, 3 positive samples were tested negative for clenbuterol using LC-MS. Two samples tested positive for sulfamethazine out of 160 samples were confirmed using LC-MS. The analysis method is rapid and reliable.

Literature

[1] Salleras, L., Dominguez, A., Mata, E., Taberner, J., Moro, I., Salva, P.*:* Epidemiologic Study of an Outbreak of Clenbuterol Poisoning in Catalonia (1995) *Spain. Public Health Reports* **110**, 338-342
Maistro, S., Chiesa, E., Angeletti, R., Brambrilla, G.*:* Beta blockers to prevent clenbuterol poisoning (1995) *Lancet* **346**, 180
Pulce, C., Lamaison, D., Keck, G., Bostvironnois, C., Nicolas, J., Descotes, J.*:* Collective Human Food Poisonings by Clenbuterol Residues in Veal Liver (1991) *Vet Hum. Toxicol.* **33**, 480-481
Brambilla, G., Cenci, T., Franconi, F., Galarini, R., Macri, A., Rondoni, F., Strozzi, M., Loizzo, A.*:* Clinical and pharmacological profile in a clenbuterol epidemic poisoning of contaminated beef meat in Italy (2000) *Toxicology Letters* **114**, 47-53

[2] Elliot, C.*:* Fast automated screeening systems for veterinary drug residues (2000) *EuroResidue* **5**, 54-62

[3] Schindler, F.*:* Surface Plasmon Resonance – was steckt dahinter? (2000) *Laborpraxis*, 38-42 (Oktober)

[4] Van Vyncht, G., Preece, S., Gaspar, P., Maghuin-Rogister, G., DePauw, E.: Gas and liquid chromatography coupled to tandem mass spectrometry for the multiresidue analysis of β-agonists in biological matrices. (1996) *Journal of Chromatography A*., **750**, 43-49

[5] Baxter, G. A., O'Connor, M., Haughey, S. A., Crooks, S. and Elliot, C. T.: Evaluation of an immunobiosensor for the on-site testing of veterinary drug residues at an abattoir. Screening for sulfamethazine in pigs. (1999) *Analyst*, **124**, 1309-1314

[a] http://www.biacore.com