SCREENING OF HONEY FOR RESIDUES OF ANTIBIOTICS BY AN OPTICAL BIOSENSOR

Stefan Weigel, Robert Gatermann, Wolfgang Harder

Eurofins/Wiertz-Eggert-Jörissen, Stenzelring 14b, 21107 Hamburg, Germany; Tel.:+49-(0)40-752709-81, Fax: +49-(0)40-752709-35, robert.gatermann@wej.de

ABSTRACT

Residues of antibiotics and chemotherapeutics are frequently detected in honeys of various origin. In order to handle large numbers of samples, automated screening by biosensors can be applied. An efficient analytical procedure for the determination of some prominent antibiotics (chloramphenicol, streptomycin, sulfonamides, tetracyclines) is the combination of a biosensor based screening with a subsequent confirmatory analysis by chromatographic/mass spectrometric methods. This article focusses on chloramphenicol. Screening is carried out on a surface plasmon resonance biosensor (Biacore Q®) in an immunochemical inhibition assay while confirmatory analysis is performed after derivatisation by a highly sensitive gas chromatography-mass spectrometry (GC-MS) or GC-MS/MS method in negative chemical ionisation (NCI) mode. The specified detection limits for both methods are set to 0.1 µg/kg although lower limits can be achieved. Results from routine samples obtained by both methods in parallel are compared to characterise the quality of the screening method. A good correlation of the results is observed.

Keywords: chloramphenicol/biosensor/screening/GC-MS-MS

INTRODUCTION

Within the European Community the use of antimicrobial drugs in beekeeping is not accepted. Thus, no maximum residue limits (MRLs) for these substances are given by European legislation (EC-regulation 2377/90). Nevertheless, certain antibiotics are approved for the treatment of bacterial brood diseases in countries outside the European Union. Furthermore, the illicit use of antibacterial compounds is a current practice in many countries as shown by frequent notifications in the EC Rapid Alert System for Food and Feed (RASFF). Among the detected drugs are tetracyclines, streptomycin, chloramphenicol, sulfonamides and recently also nitrofurans. For monitoring authorities, importers, retailers and commercial laboratories this situation results in the need for suitable detection methods for the banned substances. While highly sensitive and reliable confirmatory methods are needed for the unambiguous quantitative determination of these antibiotic agents in honey, the large number of samples to be analysed requires fast, cost-effective and reliable screening methods for the identification of positive samples. The most widely accepted confirmation methods currently in use are high performance liquid chromatography (HPLC) with either UV or fluorescence detection, liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS) [1-5]. Screening is mostly based on affinity recognition mechanisms such as radio-immunoassays (RIA) or

enzyme-immunoassays (EIA) [1], but recently also some biosensor approaches have been presented [6].

MATERIALS AND METHODS

SCREENING

According to the Qflex kit chloramphenicol handbook [7], 5 g of honey were dissolved in 10 mL extraction buffer (phosphate-buffered saline, 20 mM phosphate, 0.15 M NaCl, pH 7.2). 12 mL of ethyl acetate were added and the sample was shaken for 30 min. After centrifugation, 8 mL of the organic layer were removed and evaporated to dryness. The residue was dissolved in 0.5 mL running buffer which is contained in the kit (HBS-EP: 0.01 M HEPES, 0.15 M NaCl, 0.005 % surfactant P20, 3 mM EDTA, pH 7.4). In addition to the handbook method, the dissolved sample extract was centrifuged for 10 min at 14000 rpm prior to analysis in order to remove matrix interferences.

The biosensor assay was performed on a surface plasmon resonance (SPR) instrument Biacore Q operated with the Xenosense Qflex kits chloramphenicol (containing running-buffer HBS-EP, sensor chip, chloramphenicol calibration solution and chloramphenicol binding protein solution) which were supplied by Biacore AB (Uppsala, Sweden).

The limit of detection is 0.07 µg/kg, the within run precision 5.0 % (at 0.1 µg/kg), the between run precision 4.7 % (at 0.1 µg/kg) [8].

CONFIRMATION

10 g of honey were dissolved in 15 mL deionised water and 0.2 mL of the internal standard (d5-CAP, 0.1 µg/mL in methanol) were added. The solution was poured onto an diatomaceous earth/kieselgur column (14.5 g, Chromabond XTR, Macherey-Nagel, Düren, Germany). After 45 min the column was washed twice with 40 mL of hexane/ethyl acetate (95:5) and then eluted twice with 40 mL of hexane/ethyl acetate (50:50). The eluate was reduced almost to dryness and 100 µL of the derivatisation solution were added (1,1,1,3,3,3,-hexamethyldisilazane/HMDS + trimethylchlorosilane/TMCS + pyridine; Sylone HTP, Supelco, Steinheim, Germany). The sample was reacted for 45 min at 50 °C in a closed vial and 200 µL of toluene were added before analysis.

GC-MS analysis was carried out an Agilent GC6890N (split/splitless-injector 270 °C, 1 min splitless, temperature programme: 150 °C (0 min), 30 °C/min to 300 °C (5 min), column: J&W DB 5 MS 30m x 0.25 mm x 250 µm, flow 1.0 ml/min), coupled to an Agilent MSD 5973 (NCI, 7.0 Torr methane, 200 °C, -70 eV, 300 µA, SIM mode, monitored ions: CAP 466, 468, 467, 376 and d5-CAP 471, 473).

GC-MS/MS analysis was carried out on a second Agilent GC6890N (split/splitless-injector 270 °C, 1 min splitless, injection volume 2 µL, temperature programme: 150 °C (0 min), 30 °C/min to 300 °C (5 min), column: J&W DB 5 MS 30m x 0.25 mm x 250 µm, flow 1.0 ml/min), coupled to a Kodiak 800 GC-MS/MS (NCI, 7.0 Torr methane, 200 °C, -70 eV, 300 µA, collision cell 1.2 mTorr argon, monitored transitions: CAP 466 > 232, 304, 322 and 468 > 304, 322, 394; d5-CAP 471 > 308, 327 and 473 > 308).

RESULTS

VALIDATION

The biosensor assay was carried out as proposed by the supplier of the ready-to-use Qflex kits chloramphenicol. Therefore, the provided validation data was only verified by own measurements and found to be in the same range.

The confirmatory GC-MS method was fully validated. The limit of detection is 0.1 µg/kg, the limit of quantification 0.3 µg/kg, the precision 1.2 % (at 40 ng/mL), the repeatability 4.8 % (at 0.3 µg/kg), the reproducibility 14.7 % (at 0.3 µg/kg) and the accuracy 93 %. The linearity of the instrument was investigated and given between 20 and 140 ng/mL (correlation coefficient r2=0.9960) while the linearity of the method was investigated and given between 0.1 and 3.0 µg/kg (correlation coefficient r2=0.9997). For samples in which matrix interference hampered the reliable quantification the described MS/MS method was applied, resulting in higher specificity for the analyte, an improved signal-to-noise ratio (s/n) and thus lower detection limits. Figure 1 shows the GC-MS/MS chromatogram of an incurred honey sample (sample no. 8 in table 1) containing approximately 0.1 µg/kg CAP. As seen from the observed s/n of 350 (466>232) and 800 (466>322), quantifications at 0.05 µg/kg and below may be carried out with a high degree of reliability.

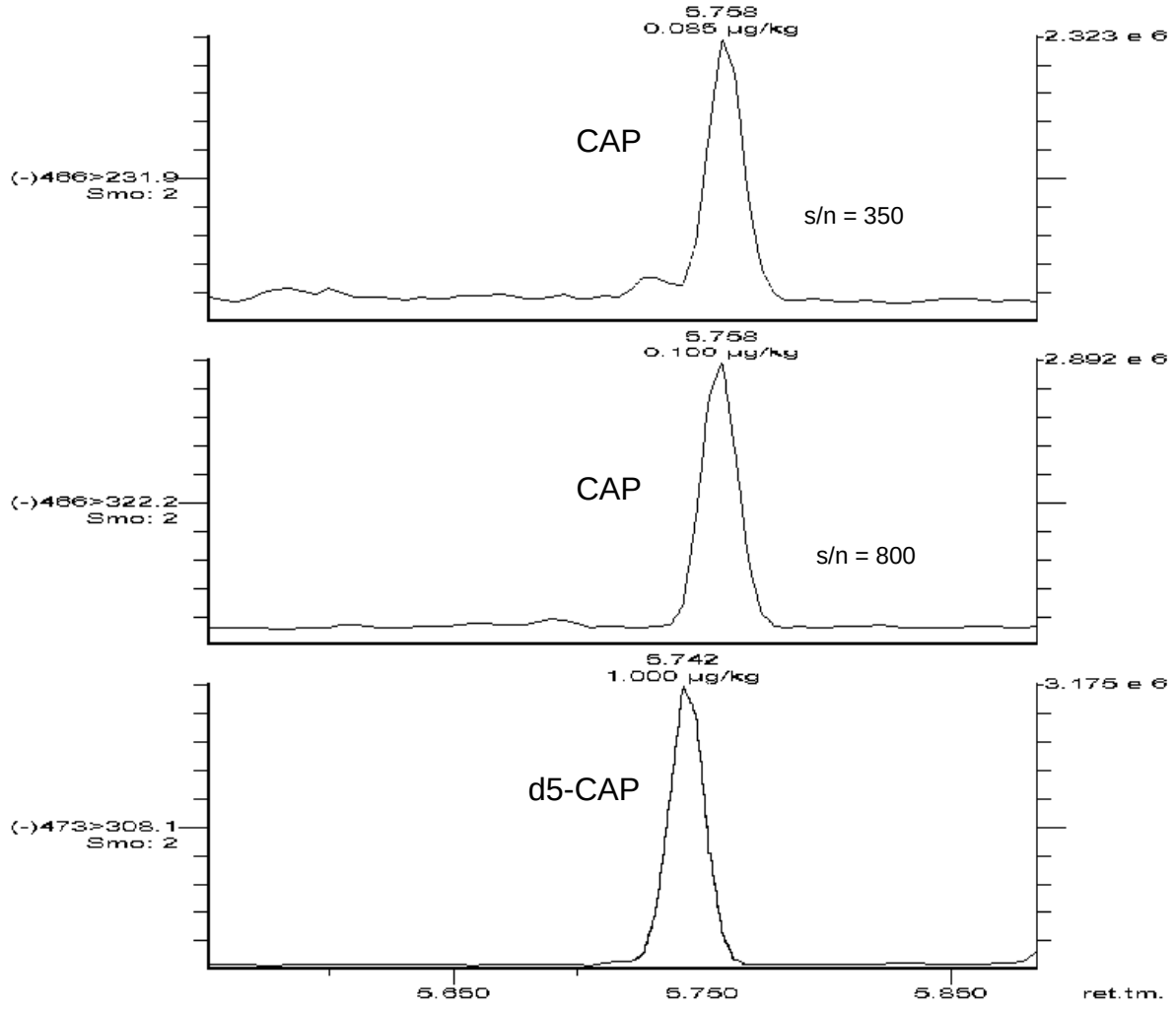

Fig. 1: GC-MS/MS (NCI) chromatograms (selected transitions) and signal-to-noise (s/n) ratios of honey sample no. 8, containing approximately 0.1 µg/kg chloramphenicol (CAP) and 1 µg/kg of the internal standard d5-CAP

PERFORMANCE OF THE BIOSENSOR SCREENING ASSAY

In order to assess the quality of the novel biosensor screening a variety of routine samples differing in geographical and botanical origin were analysed in parallel by the biosensor method and the GC-MS method. The results are shown in table 1. The rate of false results out of a random set of 25 routine samples, as compared to the confirmation method, was no false positive and no false negative (0 %). However, one sample (no. 3) initially was screened positive, but turned out to be negative upon repeated extraction and measurement. Thus, this error has to be attributed more likely to the sample preparation than specific matrix effects of the particular sample. If this sample were regarded as false positive this would correspond to a rate of 14% of the positives (n = 7) in this series of 25 samples. Within a larger set of 18 positive samples (data shown in fig. 1) this would correspond to a rate of 5 %. It can be stated that the method meets the criteria set by the EC decision 2002/657/EC for the validation of quantitative screening methods also under routine conditions.

Table 1: Qualitative comparison of biosensor and GC-MS results

Sample	Result Biacore [µg/kg]	Result GC-MS(-MS) [µg/kg]	Qualitative match
1	< 0.07	< 0.05	+
2	< 0.07	< 0.05	+
3	< 0.07	< 0.05	+
4	0.08	0.06	+
5	< 0.07	< 0.05	+
6	< 0.07	< 0.05	+
7	< 0.07	< 0.05	+
8	0.1	0.09	+
9	0.2	0.15	+
10	< 0.07	< 0.05	+
11	< 0.07	< 0.05	+
12	0.23	0.14	+
13	< 0.07	< 0.05	+
14	< 0.07	< 0.05	+
15	< 0.07	< 0.05	+
16	< 0.07	< 0.05	+
17	< 0.07	< 0.05	+
18	< 0.07	< 0.05	+
19	< 0.07	< 0.05	+
20	< 0.07	< 0.05	+
21	< 0.07	< 0.05	+

22	0.18	0.2	+
23	0.16	0.2	+
24	< 0.07	< 0.05	+
25	< 0.07	< 0.05	+

The accuracy of the biosensor method was checked by the parallel determination of incurred samples by the screening and the confirmatory method. The comparison of the concentrations determined for positive samples by biosensor and by GC-MS or GC-MS/MS showed a good agreement between the two methods. An excellent correlation of the biosensor results with the confirmatory data was observed for the investigated concentration range (fig. 2). The correlation coefficient r2 of the linear regression curve was 0.939, with deviations of the screening value from the GC-MS value in the range of 0 % to 40 %. Even at the concentration range below the European minimum required performance limit (MRPL) of 0.3 µg/kg a satisfactory correlation (r2 = 0.657) was observed (fig. 3), although the linear regression coefficient was lower than for the entire concentration range.

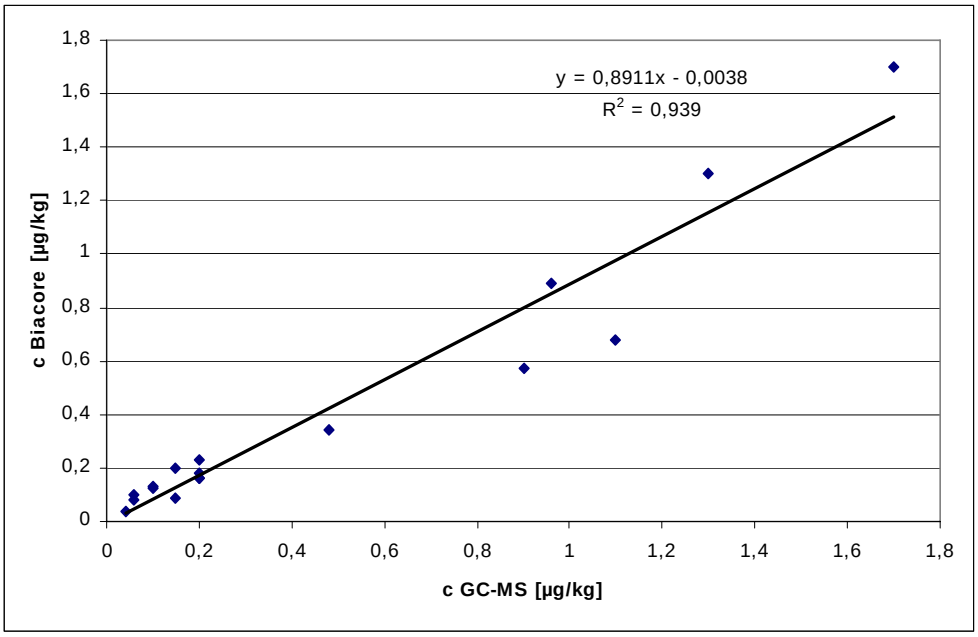

Fig. 2: Comparison of screening (Biacore) and confirmatory (GC-MS) concentration results for positive samples (n = 17)

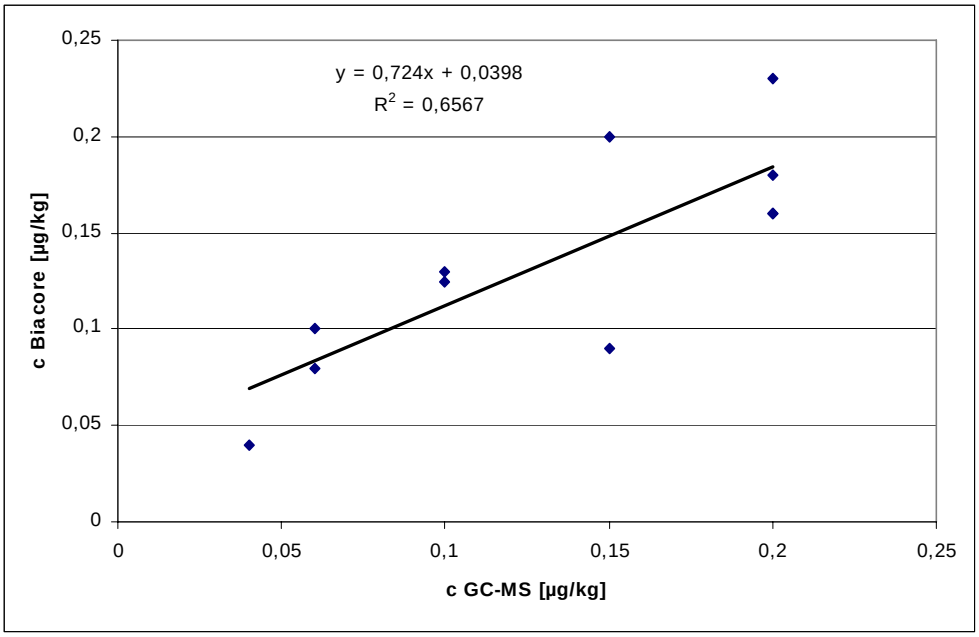

Fig. 3: Comparison of screening (Biacore) and confirmatory (GC-MS) concentration results for positive samples (n = 11) at low concentrations

CONCLUSIONS

The applied biosensor screening showed to be highly reliable under routine conditions. Sample preparation for the screening assay was more straightforward and faster than the one for the confirmatory method. The screening method was characterised by its simple handling, not requiring highly specialised staff for the operation of the instrument and data evaluation, in contrast to chromatographic methods. It enabled the processing of at least 40 samples per technician and day. Short run times on the biosensor (10 min) enable the processing of 80 samples overnight while the capacity of the instrument for 24 h is in the range of 120 real samples (including calibration and quality assurance samples). The full capacity of the instrument could be met by additional staff for the sample preparation. The method meets or exceeds validation criteria for screening. The rate of false positives is low, thus reducing the number of costly and time consuming confirmatory analysis.

REFERENCES

Bogdanov, S., Current status of analytical methods for the detection of residues in bee products, Apiacta 38 (2003) 190-197

Hormazabal, V., Yndestad, M., Simultaneous determination of chloramphenicol and ketoprofen in meat and milk and chloramphenicol in egg, honey, and urine using liquid chromatography-mass spectrometry, J. Liq. Chromatog. Rel. Technol. 24 (2001) 2477-2486

Ortelli, D., Edder, P., Corvi, C., Analysis of chloramphenicol residues in honey by liquid chromatography-tandem mass spectrometry, Chromatographia 59 (2004) 61-64

Verzegnassi, L., Royer, D., Mottier, P., Stadler, R.H., Analysis of chloramphenicol in honeys of different geographical origin by liquid chromatography coupled to

electrospray ionisation tandem mass spectrometry, Food Addit. Contam. 20 (2003) 335-42

Kaufmann, A., Roth, S., Ryser, B., Widmer, M., Quantitative LC/MS-MS determination of sulfonamides and some other antibiotics in honey, J. AOAC Int. 85 (2002) 853-860

Ferguson, J.P., Baxter, G.A., McEvoy, J.D.G., Stead, S., Rawlings, E., Sharman, M., Detection of streptomycin and dihydrostreptomycin reisdues in milk, honey and meat samples using an optical biosensor, Analyst 127 (2002) 951-956

Biacore AB, Qflex kit chloramphenicol handbook, version 1.0, Uppsala 2003

Biacore AB, Product Information Sheet „Qflex kit chloramphenicol“, version 1.0, available from www.biacore.com