SPECIAL GUEST EDITOR SECTION

Biosensor Screening for Veterinary Drug Residues in Foodstuffs

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The advent of the surface plasmon resonance (SPR) biosensor has led to many applications in diverse fields from the pharmaceutical industry to the life sciences and other areas within biotechnology. One area that has seen a significant increase in applications is the testing for veterinary drug residues in foodstuffs. These include tests for antibiotics, β-agonists, and antiparasitic drugs. The introduction of the Biacore®Q in the late 1990s, an SPR biosensor dedicated to the food industry, and the complementary development of kits to test for these residues mean that end users have a viable alternative screening test to the established enzyme-linked immunosorbent assay (ELISA) techniques. This paper reviews many SPR biosensor veterinary drug tests that have been developed, with particular emphasis placed on kit-based assays.

Animal production in modern society is carried out on an industrial scale. This intensive farming has great economic benefit for the producer as long as the animals remain disease-free and have optimal growth. Licensed veterinary drugs can be administered, under prescription, to food-producing animals for therapeutic, prophylatic, or diagnostic purposes. Medication usually involves individual animal treatment by injection, oral bolus, or drench. In this consumer-led society, the quality of foodstuffs has become a major issue. Emphasis has been placed on ensuring that, if veterinary drugs have been administered to animals, they are not present above their safe maximum residue limit (MRL) or, if banned from use in veterinary medicine, are not present at all.

Antibiotics are among the most widely used veterinary drugs. Administered in low dosage, mass medication aimed at healthy animals helps to improve feed conversion efficiency, promote growth, and prevent disease. Producers who make use of these drugs must adhere to strict withdrawal periods before the animals are sent to slaughter so that the risk of drug residues remaining in food matrixes is minimized. High levels of antibiotic residues have led to allergic reactions in sensitive people and the development of resistant strains of bacteria to commonly used antibiotics (1). Treatment of parasitic diseases involves the use of drugs such as anthelmintics (e.g., ivermectin, levamisole, and benzimidazole) and coccidiostats (e.g., nicarbazin) which, when used for the treatment/prevention of parasitic diseases, require that producers adhere to strict withdrawal periods before the animals are taken to the abattoir.

There are also many unlicensed and illegal veterinary drugs available. β-Adrenergic agonists (β-agonists) are a group of synthetic compounds that have found therapeutic use in human and veterinary medicines, including treatment of chronic obstructive airway diseases such as asthma and bronchitis, and other applications such as tocolysis (uterine relaxation). β-Agonists are orally active and, when fed at high doses (approximately 10 times the therapeutic dose), are effective growth promoters, increasing protein deposition and decreasing fat mass (2). The economic benefits of this practice can be enormous but can give rise to the presence of toxic residues in meat. Within the European Union (EU), the family of β-agonist drugs has been banned for use as growth promotants under Council Directive 96/22/EC (3). However, several β-agonist compounds have been approved for use in livestock production in several regions of the world by local regulatory bodies, including ractopamine (RCT) in the United States (4) and zilpaterol in South Africa and Mexico (5). This has repercussions for import of meat products into the EU. Therefore, screening methods for the detection of β-agonists must be as sensitive as possible.

Analytical techniques that are used for the screening of veterinary drug residues include radioimmunoassay (RIA), enzyme immunoassay (EIA), agar diffusion, and thin-layer chromatography (TLC). Confirmatory tests applied to screening samples that require further investigation are usually based on mass spectrometric (MS) techniques such as liquid/chromatography–MS (LC/MS) and give both unequivocal analyte identification and quantification.

In recent years, the demand for increased testing with regard to food safety has led to the development of new technologies. The advent of a surface plasmon resonance (SPR)-based biosensor is one such technology. In the late 1990s, Biacore AB (Uppsala, Sweden) introduced the Biacore®Q SPR biosensor, an instrument dedicated to use within the food industry. This fully automated, wizard-driven instrument is capable of analysis of a wide range of analytes,

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Table 1. Validation data for antibiotic biosensor assays[a, b]

Analyte	Sample matrix	Limit of detection, ppb	Intra-assay precision RSD_r, %	Interassay precision RSD_R, %	Recoveries, %
SMT	Muscle	7.4	3.1 (100 ppb)	6.7 (100 ppb)	>80
SDZ	Muscle	5.6	1.8 (100 ppb)	3.5 (100 ppb)	>80
Streptomyacin	Milk	30	2 (200 ppb)	4.3 (200 ppb)	94–102
	Honey	15	2.6 (40 ppb)	13.3 (40 ppb)	85–108
	Kidney	50	4.6 (1000 ppb)	7.1 (1000 ppb)	88–103
	Muscle	70	5.7 (500 ppb)	7.1 (500 ppb)	78–90
CAP	Milk	0.025	4.6 (0.1 ppb)	7.6 (0.1 ppb)	90–109
	Muscle	0.02	9.1 (0.05 ppb)	3.0 (0.05 ppb)	94–98
	Honey	0.07	5.0 (0.1 ppb)	4.7 (0.1 ppb)	107–114
	Prawn	0.073	8.8 (0.15 ppb)	5.5 (0.15 ppb)	76–86
Sulfonamides	Muscle	16.9	3.6 (100 ppb, SMT), 3.1 (100 ppb, SDZ)	3.8 (100 ppb, SMT) 2.8 (100 ppb, SDZ)	>95

[a] The value in parentheses refers to the spiked level at which the precision was measured.

[b] RSD_r = Repeatability relative standard deviation; RSD_R = reproducibility relative standard deviation; SMT = sulfamethazine; SDZ = sulfadiazine; CAP = chloramphenicol.

and the technology is supported by in vitro diagnostic kits for the screening of a range of veterinary drug residues.

Veterinary Drug Screening by SPR Biosensor in Foodstuffs

The first SPR biosensor method (6) for the detection of an antibiotic was developed in milk in 1995 as an assay for screening sulfamethazine (SMT), a member of the sulfonamide family, and compared with conventional techniques (7). In the late 1990s, biosensor assays for the screening of SMT and sulfadiazine (SDZ) residues in porcine bile were developed at the Northern Ireland statutory testing laboratory, where levels found in bile samples were indicative of the amount of residues found in tissues (8, 9). These assays have since replaced enzyme-linked immunosorbent assay (ELISA) methods at that institute. The SMT assay was further evaluated at an abattoir (10), where the biosensor was tested under extremely harsh conditions and where bile samples from about 10% of the pigs processed were tested. Although the study was successful and represented the first report of a biosensor being used for on-site drug screening, testing frequency could not keep pace with the number of pigs on the kill-line. To overcome this, a prototype high throughput screening SPR biosensor with 8 parallel flow cells was placed in an abattoir as part of the EU demonstration project FoodSENSE (Fair-CT98-3630; 11). Again, under the extremely harsh conditions of the abattoir, the system was used to detect, simultaneously, SMT and SDZ in porcine bile, where the analysis time for 48 samples was reduced from 9 h to 50 min (12). Between 1999 and 2001, 3 assays for the screening of specific antibiotics (SMT, SDZ, and streptomycin; 13) were developed for use with the biosensor. Table 1 shows validation data for these assays, which could be used with a wide range of sample matrixes, including muscle, kidney, honey, and milk. For SMT and SDZ, muscle samples were prepared by homogenization with buffer followed by centrifugation, and 40 muscle samples were analyzed within 8 h. For analysis of streptomycin, milk was used directly, honey was solubilized in buffer with pH adjusted to 7–8, and kidney and muscle were homogenized in buffer and centrifuged. Including sample preparation time, 40 milk

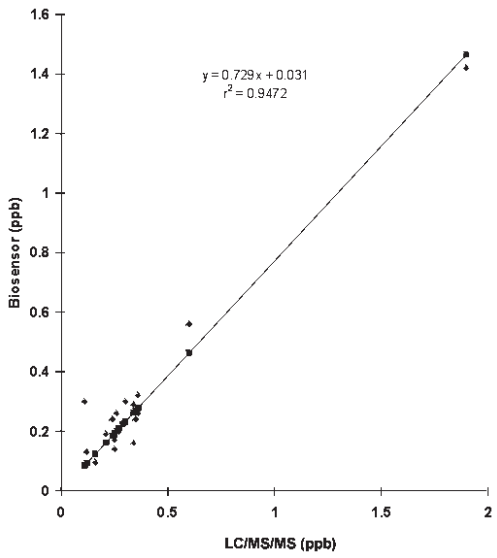

Figure 1. Comparison of biosensor results and LC/MS/MS for muscle samples (n = 25) containing chloramphenicol.

Table 2. Validation data for the antiparasitic drug biosensor assays[a]

Analyte	Sample matrix	Limit of detection, ppb	Intra-assay precision RSD_r, %	Interassay precision RSD_R, %	Recoveries, %
Ivermectin	Liver	19.1	14.9 (100 ppb)	9.7 (100 ppb)	82
Levamisole	Milk	0.49	8.5 (2 ppb)	12.4 (2 ppb)	—[b]
	Liver	6.81	7.2 (100 ppb)	6.7 (100 ppb)	—
Benzimidazole	Serum	2.6	1 (50 ppb)	—	—
Nicarbazin	Eggs	18.9	3.7 (100 ppb)	2.1 (0.1 ppb)	—
	Liver	17.1	7.2 (200 ppb)	7.4 (200 ppb)	—

[a] The value in parentheses refers to the spiked level at which the precision was measured.
[b] — = Not determined.

samples and 20 muscle/kidney samples could be analyzed in 8 h, while 40 honey samples could be analyzed in 8.5 h.

Many researchers strive to develop screening tests that can analyze a particular sample for a family of veterinary drugs, with associated reductions in labor costs and analysis time. In 2003, an assay was developed which could detect at least 19 members of the sulfonamide family of drugs, including SMT, SDZ, sulfathiazole, and sulfaquinoxaline, in porcine muscle (14) with validation data as shown in Table 1. An attribute of this assay was the absence of cross-reactivity with the inactive *N*-acetyl metabolites, thus reducing the possibility of false-positive results. Sample preparation was minimal, involving homogenization in buffer followed by centrifugation, allowing 40 samples to be analyzed in less than 8 h.

Chloramphenicol (CAP) is a broad-spectrum antibiotic with excellent antibacterial and pharmacokinetic properties. In human medicine, however, its use is limited to the treatment of typhoid fever, bacterial meningitis, and conjunctivitis. It has been prohibited from use in food-producing animals, including honeybees, in the EU, the United States, Canada, and many other countries. Recently, the detection of CAP residues in various foodstuffs imported into the EU from Asian countries had a major impact on international trade, and restrictions were placed on the importation of these products. The products affected included poultry, shrimp, and honey. As a prohibited substance, zero tolerance applies, and a minimum required performance limit (MRPL) of 0.3 μg/kg was set under European legislation (15). In response to this requirement, an assay for the detection of CAP was developed in 2003 (16). Table 1 shows the validation performance for the assay. The assay was applicable to a wide range of foods, including honey, chicken, milk, and prawns with a facile sample preparation.

Thus, milk was used directly, muscle and honey were homogenized in buffer and extracted with ethyl acetate, and prawn was homogenized with ethyl acetate, centrifuged, and defatted with cyclohexane. Following sample preparation, results for 40 milk samples or 20 honey/prawn samples were available in 10 h and 80 muscle samples could be prepared and analyzed in 24 h. Each assay demonstrated sensitivity close to the MRPL, and CAP was therefore detected to low levels. A comparison of the biosensor assay with an LC/MS/MS confirmatory method for incurred muscle samples showed a correlation (r^2) of 0.94 with a slope of 0.73 as illustrated in Figure 1. This result demonstrated a reasonable equivalence between independent analytical techniques using radically different detection principles.

Other biosensor applications for the screening of antibiotics in milk have included enrofloxacin/ciprofloxacin (17),

Table 3. Validation data for β-agonist biosensor assays[a, b]

Analyte	Sample matrix	Limit of detection, ppb	Intra-assay precision RSD_r, %	Interassay precision RSD_R, %	Recoveries, %
CBL	Urine	0.3	7 (0.3 ppb)	9.2 (0.3 ppb)	88–103
RCT	Liver	0.17	9.3 (0.4 ppb)	14.5 (0.4 ppb)	—[c]
	Urine	0.22	7.3 (0.4 ppb)	6.9 (0.4 ppb)	—
β-Agonists	Liver	0.194 (Salbutamol)	19.0 (1 ppb)	14.0 (1 ppb)	—

[a] The value in parentheses refers to the spiked level at which the precision was measured.
[b] RSD_r = Repeatability relative standard deviation; RSD_R = reproducibility relative standard deviation; CBL = clenbuterol; RCT = ractopamine.
[c] — = Not determined.

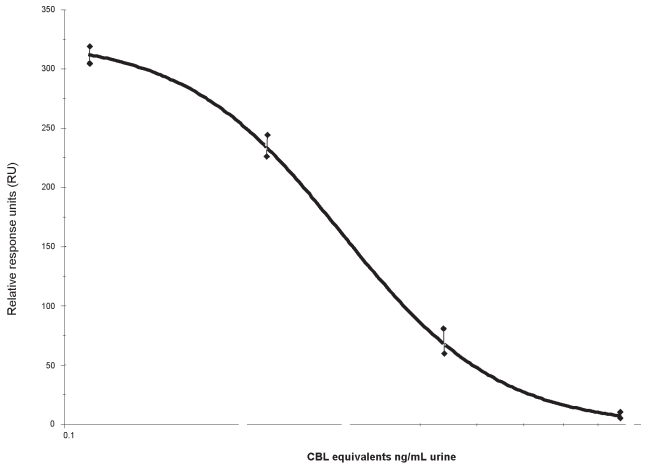

Figure 2. Typical calibration curve (*n* = 4) with error bars for CBL urine assay.

gentamicin (18), and β-lactams (19, 20). The β-lactam assays are of particular interest as a receptor protein, R39, is used—which binds β-lactams in their parent form and does not recognize the hydrolyzed form of this family of drugs.

SPR biosensor assays have also been developed to screen for the antiparasitic anthelmintic agents ivermectin (21, 22), levamisole (23), benzimidazole (24), and for the coccidiostat nicarbazin (25) as summarized in Table 2. Sample preparation was minimal, and involved acetonitrile extraction and solid-phase extraction (SPE; ivermectin), acetonitrile extraction (levamisole and nicarbazin), or cleanup by precipitation (benzimidazole) with the number of samples that could be tested in 1 working day as follows: ivermectin, about 20; levamisole, 18–50; and nicarbazin, 20–30.

Between 1999 and 2002, biosensor assays for β-agonists were developed to detect clenbuterol (CBL) residues in urine from cattle at low levels (26), and RCT residues in liver and urine from pigs (unpublished data, 2002). Table 3 shows validation data for these assays. The CBL assay was unique in that a calibration curve in buffer (Figure 2) had been developed which mimicked a curve that had been extracted from urine. Sample preparation involved the extraction of basified urine using *tert*-butyl methyl ether, evaporation, and reconstitution in buffer that allowed 40 samples to be analyzed in 9 h. Typical RCT calibration curves from extracted liver and urine are illustrated in Figure 3. The preparation of liver samples for the RCT assay involved extraction of homogenized liver with acetonitrile, which was evaporated to dryness, reconstituted in buffer, and filtered, a protocol that allowed 20 samples to be analyzed in 17 h. Urine samples were deconjugated with β-glucuronidase before extraction with ethyl acetate, SPE cleanup, evaporation, and reconstitution in buffer, which facilitated analysis of 20 samples in 19 h. Comparison of the biosensor results for urine containing incurred RCT over 4 orders of magnitude against a confirmatory LC/MS/MS technique (27), as illustrated in Figure 4, gave a correlation (r^2) of 0.98. The observed bias of the biosensor assay for RCT is significantly reduced over the range of levels more typical of contaminated urine (RCT < 600 ppb; slope = 1.4) and provides confidence in the use of the biosensor assay for screening.

To support these 2 screening tests, a generic, multi-β-agonist assay (28) was developed which can detect at least 13 members of the β-agonist family, including CBL, salbutamol, and mabuterol in liver derived from cattle, sheep, and pigs with validation data shown in Table 3. In addition, it was found that zilpaterol could also be detected by this method. In combination, these assays were complementary and reduced routine analysis time for the screening of β-agonists. Screening samples for β-agonists was achieved with reference to a salbutamol calibration curve, as illustrated in Figure 5. Samples are homogenized, digested, deconjugated, purified by SPE, evaporated, and reconstituted

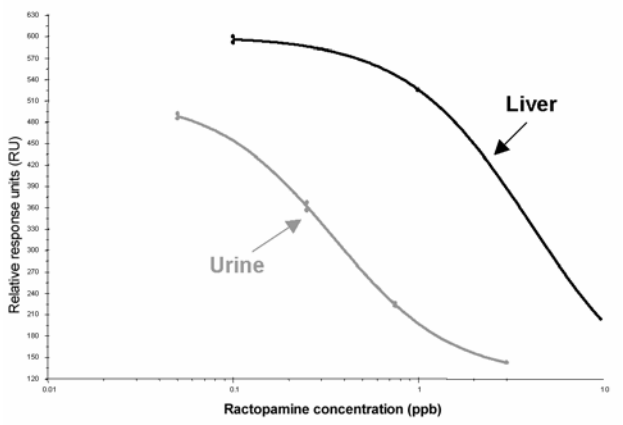

Figure 3. Typical calibration curves obtained for ractopamine in liver and urine.

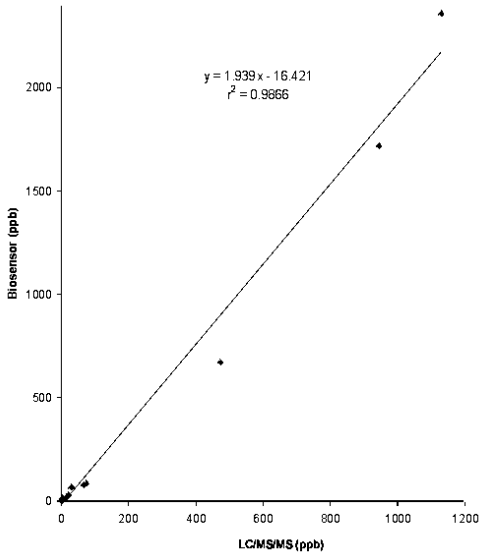

Figure 4. Comparison of biosensor results with LC/MS/MS for ractopamine-incurred urine samples (*n* = 20).

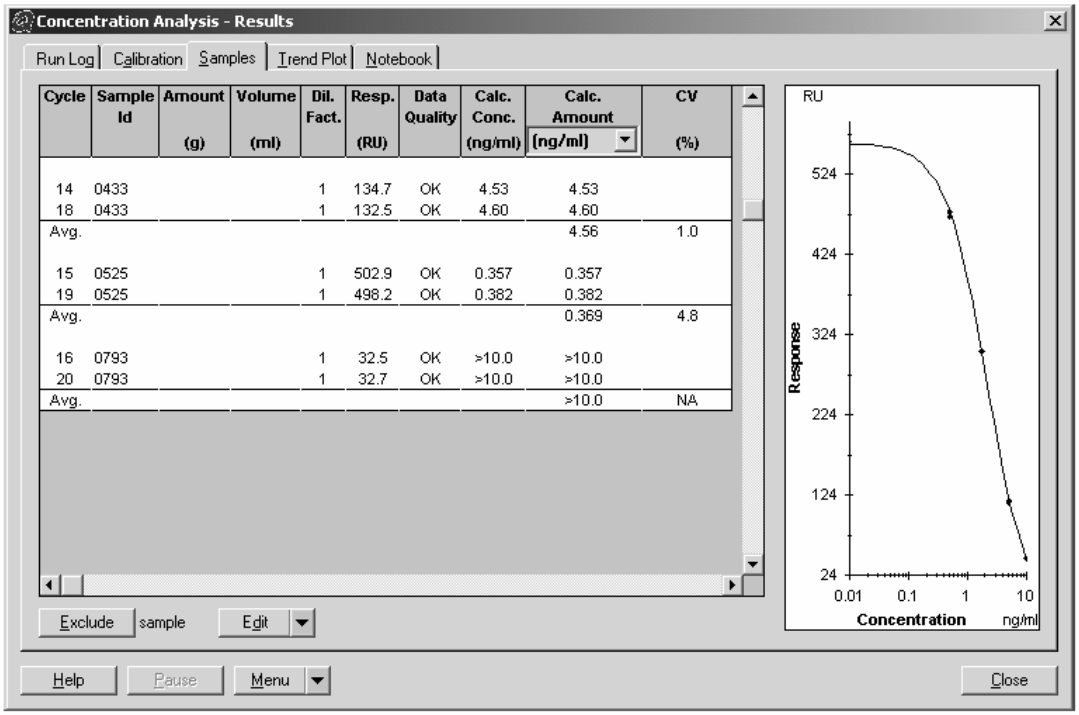

Figure 5. Typical instrument display of a salbutamol calibration curve and indicative levels of β-agonists for liver samples.

in buffer, allowing 16 samples to be prepared and analyzed in 1.5 working days.

Conclusions

Over the last decade, with the advent of the SPR biosensor, assays have been developed for the detection of a wide range of veterinary drug residues. These techniques have found applications for a wide range of sample matrixes, including muscle, urine, honey, and prawns. The assays for the banned veterinary drugs, such as CAP and β-agonists, can screen for residues in samples to <1 ppb, indicating excellent sensitivity. Typically, samples can be prepared and analyzed in <1.5 days. Comparison between biosensor and confirmatory methods indicated a high degree of equivalence, with correlation (r^2) > 0.9. The introduction of the Biacore Q optical biosensor, dedicated for use in the food industry, and the development of test kits for screening of veterinary drugs, have highlighted how this technology can potentially be used for screening samples with regard to food safety in this consumer-led society.

References

(1) Levy, S.B. (1992) *The Antibiotic Paradox*, Plenum Press, New York, NY

(2) Witkamp, R.F. (1995) in *Proceedings of the Scientific Conference on Growth Promotion in Meat Production*, Brussels, Belgium, pp 297–323

(3) EU Council Directive 96/22/EC (1996) *Off. J. Eur. Comm.* **L125**, 3–9

(4) U.S. Food and Drug Administration (FDA) Freedom of Information Report (2002) New Animal Drug Application (*NADA*), 140–863, pp 1–66

(5) Shelver, W.J., & Smith, D.J. (2004) *J. Agric. Food Chem*. **52**, 2159–2166

(6) Sternesjö, Å., Mellgren, C., & Bjorck, L. (1995) *Anal. Biochem*. **226**, 175–181

(7) Mellgren, C., Sternesjö, Å., Hammer, P., Suhren, G., Bjorck, L., & Heeschen, W. (1996) *J. Food Prot*. **59**, 1223–1226

(8) Crooks, S.R.H., Baxter, G.A., O'Connor, M.C., & Elliott, C.T. (1998) *Analyst* **123**, 2755–2757

(9) Elliott, C.T., Baxter, G.A., Crooks, S.R.H., & McCaughey, W.J. (1999) *Food Agric. Immunol.* **11**, 19–27

(10) Baxter, G.A., O'Connor, M.C., Haughey, S.A., Crooks, S.R., & Elliott, C.T. (1999) *Analyst* **124**, 1315–1318

(11) Crooks, S.R.H., Stenberg, E., Johansson, M.A., Hellenäs, K.E., & Elliott, C.T. (2001) *Proceedings of SPIE-The International Society for Optical Engineering, Vol. 4206 (Photonic Detection and Intervention Technologies for Safe Food)*, Boston, MA, pp 123–130

(12) Situ, C., Crooks, S.R.H., Baxter, G.A., Ferguson, J., & Elliott, C.T. (2002) *Anal. Chim. Acta* **473**, 143–149

(13) Baxter, G.A., Ferguson, J.P., O'Connor, M.C., & Elliott, C.T. (2001) *J. Agric. Food Chem.* **49**, 3204–3207

(14) McGrath, T., Baxter, A., Ferguson, J., Haughey, S.A., & Bjurling, P. (2005) *Anal. Chim. Acta* **529**, 123–127

(15) European Commission Decision 2003/181/EC (2003) *Off. J. Eur. Comm.* **L71**, 17–18

(16) Ferguson, J., Baxter, G.A., Young, P., Kennedy, G., Elliott, C., Weigel, S., Gatermann, R., Ashwin, H., Stead, S., & Sharman, M. (2005) *Anal. Chim. Acta* **529**, 109–113

(17) Mellgren, C., & Sternesjö, Å. (1998) *J. AOAC Int*. **81**, 394–397

(18) Haasnoot, W., & Verheijen, R. (2001) *J. Food Agric. Immunol.* **13**, 131–134

(19) Gustavsson, E., Bjurling, P., Degelaen, J., & Sternesjö, Å. (2002) *Food Agric. Immunol*. **14**, 121–131

(20) Gustavsson, E., Bjurling, P., & Sternesjö, Å. (2002) *Anal. Chim. Acta* **468**, 153–159

(21) Samsonova, J.V., Baxter, G.A., Crooks, S.R.H., & Elliott, C.T. (2002) *J. AOAC Int*. **85**, 879–882

(22) Samsonova, J.V., Baxter, G.A., Crooks, S.R.H., Small, A.E., & Elliott, C.T. (2002) *Biosens. Bioelect.* **17**, 523–529

(23) Crooks, S.R.H., McCarney, B., Traynor, I.M., Thompson, C.S., Floyd, S., & Elliott, C.T. (2003) *Anal. Chim. Acta* **483**, 181–186

(24) Johnsson, L., Baxter, G.A., Crooks, S.R.H., Brandon, D.L., & Elliott, C.T. (2002) *Food Agric. Immunol*. **14**, 209–216

(25) McCarney, B., Traynor, I.M., Fodey, T.L., Crooks, S.R.H., & Elliott, C.T. (2003) *Anal. Chim. Acta* **483**, 165–169

(26) Haughey, S.A., Baxter, G.A., Elliott, C.T., Persson, B., Jonson, C., & Bjurling P. (2001) *J. AOAC Int*. **84**, 1025–1030

(27) Antignac, J.P., Merchand, P., Le Bizec, B., & Andre, F. (2002) *J. Chromatogr. B* **774**, 59–66

(28) Traynor, I.M., Crooks, S.R.H., Bowers, J., & Elliott, C.T. (2003) *Anal. Chim. Acta* **483**, 187–191