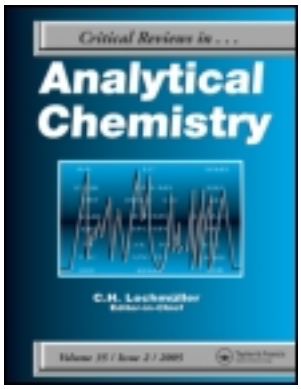


Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/batc20

A Critical Review of Screening Methods for the Detection of Chloramphenicol, Thiamphenicol, and Florfenicol Residues in Foodstuffs

Jeanne V. Samsonova [a], Andrew Cannavan [b] & Christopher T. Elliott [c]

[a] Faculty of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russia

[b] Food and Enviromental Protection Laboratory, FAO/IAEA Agriculture and Biotechnology Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria

[c] Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland

Available online: 11 Jan 2012

To cite this article: Jeanne V. Samsonova, Andrew Cannavan & Christopher T. Elliott (2012): A Critical Review of Screening Methods for the Detection of Chloramphenicol, Thiamphenicol, and Florfenicol Residues in Foodstuffs, Critical Reviews in Analytical Chemistry, 42:1, 50-78

To link to this article: http://dx.doi.org/10.1080/10408347.2012.629951

Critical Reviews in Analytical Chemistry, 42:50–78, 2012

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408347.2012.629951

A Critical Review of Screening Methods for the Detection of Chloramphenicol, Thiamphenicol, and Florfenicol Residues in Foodstuffs

Jeanne V. Samsonova,[1] Andrew Cannavan,[2] and Christopher T. Elliott[3]

[1]*Faculty of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russia*
[2]*Food and Enviromental Protection Laboratory, FAO/IAEA Agriculture and Biotechnology Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria*
[3]*Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland*

This article gives an extensive overview of the wide range of analytical procedures developed for the detection of amphenicol antibiotic residues (chloramphenicol, thiamphenicol, and florfenicol) in many different types of foodstuffs (milk, meat, eggs, honey, seafood). Screening methods such as microbial inhibition methods, antibody-based immunoassays using conventional and biosensor-based detection systems, and some methods based on alternative recognition systems are described. The relative advantages and disadvantages of these methods are discussed and compared. The current status and future trends and developments in the need for accurate and rapid detection of this group of antimicrobials are also discussed.

Keywords amphenicols, chloramphenicol, thiamphenicol, florfenicol, residues, immunoassay, biosensors, screening, food

INTRODUCTION

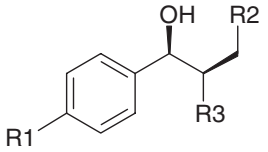
Chloramphenicol (CAP), thiamphenicol (TAP), and florfenicol (FF) are members of the amphenicol family of antibacterial agents (Table 1). Amphenicols exhibit a broad-spectrum antibacterial activity. They are effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. One of the oldest antibacterial agents is CAP, which was first isolated from *Streptomyces venezuelae* in 1947 and named chloromycetin (Erlich et al., 1947; Smadel and Jackson, 1947). Nowadays CAP is produced by means of chemical synthesis.

CAP is a relatively cheap to produce and highly effective broad-spectrum antibiotic with excellent antibacterial and pharmacokinetic properties. These properties underline the reasons why CAP was widely used for many years in veterinary practice both therapeutically and prophylactically. However, CAP is hematotoxic for humans and may cause serious adverse effects such as bone marrow aplasia (loss of ability to produce blood cells) and therefore aplastic anemia. These effects are unrelated to dose and are generally fatal (Yunis, 1989; Holt et al., 1993). Since the size of the CAP dose that may lead to these pathologies is still not defined, the use of this antibiotic for the treatment of food-producing animals in many countries has been prohibited (EU, U.S., Canada, and others). Currently TAP and FF are used as alternatives to CAP for animal treatment. However, because of high efficiency, broad spectrum of action, relatively low cost, and availability, CAP is still in use, often illegally, for the treatment and prevention of some infectious diseases in mammals, birds, bees, and aquaculture. It is also widely used in developing countries. For instance, in recent years CAP residues have been found in shrimp and honey imported from Asia into Europe (Food Standards Agency, 2002a, 2002b; Commission Decision 2001/699/EC, 2001; Commission Decision 2001/705/EC, 2001; Commission Decision 2002/249/EC, 2002). In addition, products of animal origin can contain residues of CAP that are not due to (illegal) use of the drug, but rather due to the natural occurrence of CAP (Hanekamp et al., 2003; Berendsen et al., 2010, 2011). Collectively, all these facts have required the development of highly sensitive, reliable, and available methods for CAP detection for food safety and quality control.

The EU has stringent requirements for methods for detection of prohibited substances for which no maximum residue

Address correspondence to Jeanne V. Samsonova, Dept. of Chemical Enzymology, Faculty of Chemistry, M.V. Lomonosov Moscow State University, 119991, Moscow, Russia. E-mail: jvs@enz.chem.msu.ru

TABLE 1
Chemical structure of CAP, TAP, and FF and their derivatives



Substance	R1	R2	R3
CAP	NO_2	OH	NH-CO-$CHCl_2$
Amino-CAP	NH_2	OH	NH-CO-$CHCl_2$
CAP base	NO_2	OH	NH_2
CAP succinate	NO_2	COO-$(CH_2)_2$-COOH	NH-CO-$CHCl_2$
CAP glucoronide	NO_2	$C_6H_5O_7$	NH-CO-$CHCl_2$
TAP	SO_2CH_3	OH	NH-CO-$CHCl_2$
TAP glycinate	SO_2CH_3	COOH	NH-CO-$CHCl_2$
FF	SO_2CH_3	F	NH-CO-$CHCl_2$
FF amine	SO_2CH_3	F	NH_2

level (MRL) is established (zero tolerance), such as CAP (European Commission Regulations 1430/94, 1994). In particular, the "minimum required performance limit" (MRPL) corresponding to "minimum content of an analyte in a sample which at least has to be detected and confirmed" by a reference analytical method was introduced in 2002 (Commision Decision 2002/657/EC, 2002). For CAP residue detection in food, the MRPL is set at 0.3 μg/kg (Commission Decision 2003/181/EC, 2003). Therefore, very sensitive analytical methods are required for monitoring food for CAP residues. MRLs have been set for TAP and the sum of FF and its metabolites measured as FF amine (Table 2). Recently European Regulation No. 470/2009 of 6 May 2009 (European Council Regulation 470/2009, 2009) has set procedures for the establishment of MRLs for pharmacologically active substances in foodstuffs of animal origin, repealing Regulation No. 2377/90 (European Council Regulation 2377/90, 1990). However, Annex I and IV of Regulation (EEC) No 2377/90 shall continue to apply until entry into force of a new regulation.

Effective drug residue control requires cost-effective routine screening with the capability of high sample throughput, followed by more expensive chemical confirmation if required. False negative results are not acceptable in screening assays, although a limited number of false positives may be tolerated. Presently there is a wide variety of methods for the detection of CAP, TAP, and FF residues in foodstuffs that have been developed (Tables 4, 6, and 7). It should be noted that vast majority of the methods were developed for CAP detection. The impetus for this came from finding multiple contaminated samples in the 1990s and the subsequent introduction of the MRPL in 2003.

Microbiological-based methods, immunoassays, biosensors, and microarrays are all considered suitable for screening purposes in that they can detect antibiotic residues at low concentrations in relatively quick and low-cost ways and are "user-friendly" procedures. Physicochemical methods such as gas chromatography (GC), liquid chromatography (LC), and high-performance liquid chromatography (HPLC) coupled with highly sensitive detection systems, e.g., mass spectrometry, are used as confirmatory methods for the purposes of substance identification and quantification. The combination of screening and confirmatory methods in this two-tier system for drug residue control represents a powerful tool for food safety and quality control. This work overviews the screening methods published for CAP, TAP, and FF detection in various foodstuffs.

MICROBIOLOGICAL METHODS

Microbiological methods are based on the inhibition of bacterial growth due to the presence of antibacterial agents. Results are obtained by measuring the size of inhibition zones, but photometric measurement can be performed as well (Althaus et al., 2003). These forms of bioassay are able to detect a broad spectrum of antimicrobials. They are cheap, easy to perform, and suitable for screening purposes, but are rather labor intensive and slow. Moreover, these kinds of methods are only qualitative in nature. Another major problem associated with the use of such broad-spectrum assays is a lack of sensitivity and specificity for some drugs, in particular CAP (Calderón et al., 1996; Gaudin et al., 2004).

The EC four-plate test (FPT) designed for meat analysis has been recognized in Europe as a valuable method for the detection of antibiotic residues in foodstuffs (Bogaerts and Wolf, 1980). In the FPT different families of antimicrobial agents give rise to different patterns of inhibition zones across the four plates, and it is possible with experience to differentiate between some groups of antimicrobials. A modification of the original EC FPT based on microbial growth inhibition of *Bacillus subtilis* and

TABLE 2
MRL (MRPL) assessment for CAP, TAP, and FF in the EU in the context of Council Regulation (EEC) No. 2377/90

Amphenicol	Marker residue	Animal species	MRPL or MRL, μg/kg	Target tissue	Other provisions	Status	Regulation amending Annex of Regulation 2377/90
CAP	CAP	All food producing species	0.3 (MRPL)			Annex IV	Regs. 675/92; 1430/94
TAP	TAP	All food producing species[a]	50 (MRL)	Muscle[b] Fat[c] Liver Kidney Milk		Annex I	Regs. 895/93; 1798/96; 1000/98; 804/99; 1299/2005; 1805/2006
FF	Sum of FF and its metabolites measured as FF amine	All food producing species except bovine, ovine, caprine, porcine, poultry, and fin fish	All MRLs: 100 200 2000 3000	Muscle Fat Liver Kidney		Annex I	Regs. 2703/94; 613/98; 1942/99; 2385/99; 1322/2001; 1181/2002
		Bovine, ovine, caprine	200 3000 300	Muscle Fat Kidney	Not for use in animals from which milk is produced for human consumption		
		Porcine	300 500 2000 500	Muscle Skin and fat Liver Kidney			
		Poultry	100	Muscle	Not for use in animals which eggs are produces for human consumption		
			200 2500 750	Skin and fat Liver Kidney			
		Fin fish	1000	Muscle and skin in natural proportions			

[a]Not for use in animals whose eggs are produced for human consumption; MRLs for fat, liver, and kidney do not apply to fin fish.
[b]For fin fish muscle relates to "muscle and skin in natural proportions."
[c]For porcine and poultry species this MRL relates to "skin and fat in natural proportions."

TABLE 3
Details of immunoreagent synthesis, antibody production, and their specificity

Antibody	Animal	Coupling derivative	Carrier protein	Coupling reaction	Labeled antigen/coating antigen; coupling reaction	Method	Specificity (CAP as 100%)	Reference
Polyclonal	Rabbit	Reduced CAP (nitro group was reduced to the amine)	BGG	Diazotization	Reduced CAP-RSA	Complement fixation (inhibition) reaction		Hamburger, 1966
Polyclonal	Rabbit	CAP succinate (CAP + succinic anhydride)	KLH	Carbodiimide reaction (EDC)	CAP succinate-BSA mixed anhydride reaction (Tributhylamine + isobutylchloroformate)	Indirect ELISA (tubes)	CAP succinate 853% TAP 0.43%	Campbell et al., 1984
Polyclonal	Sheep	Monoiodoacetyl analog of CAP			^{3}H-N-propionyl analog of CAP	RIA	CAP base 1.2%	Arnold et al., 1984
Polyclonal	Rabbit	3′-CAP-β-monoglucuronid	TG	Mixed anhydride reaction	^{14}C-CAP	RIA	CAP-monoglucoronid 100% CAP 86% CAP succinate 56% CAP palmitate 59% TAP 3.5%	Hock and Liemann, 1985
Polyclonal	Rabbit	CAP base + dimethyladipimidate	HSA		CAP succinate-HRP CAP + succinic anhydride Carbodiimide reaction (NHS + DCC)	Direct ELISA	CAP base 0.5% TAP 0.05%	Märtlbauer and Terplan, 1987
Monoclonal	Mouse	CAP base + SAMSA	BSA	No information	CAP succinate-BSA Mixed anhydride reaction (Tributhylamine + isobutylchloroformate)	Indirect ELISA	CAP succinate 3.13% CAP base 0.05% p-Nitrobenzyl alcohol 0.02% Tetracycline 0.01%	van de Water et al., 1987
Monoclonal	Mouse	CAP base + dimethyladipimidate	HSA		CAP succinate-HRP CAP + succinic anhydride Carbodiimide reaction (NHS + DCC)	Direct ELISA	CAP base 5% p-Nitrobenzyl alcohol 0.94% p-Nitrophenol 0.007%	Hack et al., 1989

(Continued on next page)

TABLE 3
Details of immunoreagent synthesis, antibody production, and their specificity (*Continued*).

Antibody	Animal	Coupling derivative	Carrier protein	Coupling reaction	Labeled antigen/coating antigen; coupling reaction	Method	Specificity (CAP as 100%)	Reference
Polyclonal	Rabbit	CAP succinate	BSA	Activated ester method (NHS + CMCT)	CAP succinate-OVA Activated ester method (NHS + CMCT)	Indirect ELISA	CAP succinate-BSA: CAP succinate 1428% CAP base < 1%	Kolosova et al., 2000
		Reduced CAP (nitro group was reduced to the amine)		Diazotization			Reduced CAP-BSA: CAP succinate 24% CAP base < 1%	
Polyclonal	Rabbit	CAP succinate	BSA	Carbodiimide method	CAP base	SPR biosensor	CAP base chip: FF 0.4% FF amine < 0.1% TAP 0.2; FF amine chip: FF 107% FF amine < 0.1% TAP 26%	Dumont et al., 2006
		FF amine	KLH	MBS spacer to conjugate FF amine to KLH	FF amine			
Polyclonal	Rabbit	CAP succinate	BSA	Activated ester method (NHS + CMCT)	CAP succinate-OVA Activated ester method (NHS + CMCT)	Indirect CL-ELISA	CAP succinate 722% TAP 0.1% FF 0.1%	Zhang et al., 2006

Monoclonal	Mouse	CAP succinate	BSA	Activated ester method	CAP succinate-OVA	Indirect TR-FIA	CAP succinate 300% TAP < 0.1% FF < 0.1%	Shen et al., 2006
Polyclonal	Rabbit	TAP succinate	BSA	Mixed anhydride method	TAP succinate-OVA Mixed anhydride method	Indirect ELISA	TAP 100% CAP 0.43%	Hao et al., 2006
Polyclonal	Camel, donkey, goat	CAP succinate	HSA	Mixed anhydride reaction (N-methyl morpholine + isobutylchloroformate)	CAP succinate-HRP (Mixed anhydride reaction) TAP-DSC-HRP Biosensor chip: CAP base TAP-DSC	Direct ELISA SPR biosensor	ELISA: CAP-HRP: TAP < 0.1–1.2% FF < 0.1–18% FF amine < 0.1–0.3% TAP-HRP: TAP 22–53% FF 49–82% FF amine < 0.1–0.2% SPR biosensor: CAP chip: TAP < 0.1– 0.3% FF < 0.1–0.56% FF amine < 0.1–0.3% TAP chip: TAP < 0.2–56% FF < 0.2–129% FF amine < 0.1–0.9%	Fodey et al., 2007

(Continued on next page)

TABLE 3
Details of immunoreagent synthesis, antibody production, and their specificity (*Continued*).

Antibody	Animal	Coupling derivative	Carrier protein	Coupling reaction	Labeled antigen/coating antigen; coupling reaction	Method	Specificity (CAP as 100%)	Reference
Polyclonal	Camel, goat, donkey	CAP succinate	HSA	No information	CAP succinate-HRP Carbodiimide method (EDAC)	Direct ELISA	No cross-reaction with TAP and FF	Wesongah et al., 2007
Polyclonal	Rabbit	CAP succinate (CAP + succinic anhydride)	KLH	No information	CAP succinate-EDF CAP succinate-HRP	CEIA-LIF; Direct ELISA	CEIA-LIF: FF, TAP, ampicillin < 0.01% Direct ELISA: FF 0.07% TAP 0.01% Ampicillin 0.01%	Zhang et al., 2008b
Polyclonal	Rabbit	FF amine	BSA	Glutaraldehyde coupling	FF amine-OVA Glutaraldehyde coupling	Indirect ELISA	FF amine 100% FF 10.88% TAP 4.33% CAP 1.56%	Wu et al., 2008
Monoclonal	Mouse	FF succinate	HSA	Activated ester method (NHS + DCC)	FF succinate-OVA Mixed anhydride method	Indirect ELISA	FF 100% FF amine 0.04% TAP 2.2% CAP < 0.1%	Luo et al., 2009a
Polyclonal	Rabbit	FF amine	BSA	Formaldehyde coupling	FF-OVA (Formaldehyde coupling) TAP glycinate-OVA (Active ester method, NHS+ EDC) FF glutarate-OVA (FF+ glutaric anhydride; Mixed anhydride reaction (tri-*n*-butylamine + isobutylchlorocarbonate))	Indirect ELISA	FF amine 100% FF 225; 133; 97% TAP 31; 5; 6% CAP 0.6; 0.2; < 0.1% Data for three coating conjugates respectively	Luo et al., 2009b

Polyclonal	Rabbit	CAP succinate	KLH	Activated ester method (NHS + DCC)	CAP succinate-HRP Activated ester method (NHS + EDC)	Direct ELISA	CAP succinate-BSA: CAP succinate 1056%	Samsonova et al., 2010
		CAP base	BSA	Carbodiimide method (EDC)			CAP base < 0.1% TAP < 0.1% FF < 0.1% CAP succinate-KLH: CAP succinate 251% CAP base < 0.1% TAP < 0.1% FF < 0.1% CAP base-BSA: CAP succinate 11.3% CAP base 4.6% TAP < 0.1% FF < 0.1%	
Polyclonal	Rabbit	CAP succinate	KLH	Activated ester method (NHS + DCC)	CAP succinate-OVA Activated ester method (NHS + DCC)	Indirect BS-ELISA	FF 0.05% TAP 0.008%	Wang et al., 2010
Polyclonal	Rabbit	FF succinate	HAS	Activated ester method (NHS + DCC)	FF maleate-OVA Mixed anhydride method	Indirect ELISA	FF 100% TAP 40% FF amine 0.3% CAP 0.1%	Luo et al., 2011

TABLE 4
Antibody-based methods for CAP, TAP, and FF detection in foodstuffs

Method	Analyte	Object	Sample pretreatment	Limit of detection	Reference
			RIA		
RIA	CAP	Swine muscle, eggs, milk	Solvent extraction	200 ng/kg	Arnold et al., 1984
RIA	CAP	Meat, eggs, milk,	Solvent extraction	200 ng/kg	Arnold and Somogyi, 1985
RIA	CAP	Urine, plasma	Dilution	10^{-11} M (3.2 ng/L)	Hock and Liemann, 1985
		Milk, rabbit tissues	Solvent extraction		
RIA	CAP	Swine muscle, blood, urine, liver, kidney, bile, fat	Solvent extraction	1–5 μg/kg	Boertz et al., 1985
RIA	CAP	Eggs	Solvent extraction	1 μg/kg	Scherk and Agthe, 1986
RIA	CAP	Swine, bovine, veal muscle	Solvent extraction	1 μg/kg	Agthe and Scherk, 1986
RIA	CAP	Eggs	Solvent extraction	0.5 μg/kg	Beck et al., 1987
RIA	CAP	Animal tissue	Enzyme digestion, SPE	0.2 ng/g	Freebairn et al., 1988
			ELISA		
Direct ELISA	CAP	Milk	Dilution with buffer	500 ng/kg	Märtlbauer and Terplan, 1987
Indirect ELISA	CAP	Swine muscle	Solvent extraction, SPE	5 μg/kg	van de Water et al., 1987
Indirect BS-ELISA	CAP	Swine muscle	Aqueous extraction	10 μg/kg	van de Water and Haagsma, 1990a
Indirect BS-ELISA	CAP	Milk	Defatting, filtration	1 μg/kg	van de Water and Haagsma, 1990b
Indirect ELISA	CAP	Milk	Dilution with buffer;	8 μg/kg	Kolosova et al., 2000
		Meat, eggs	Buffer extraction		
ELISA test (Euro-Diagnostica B.V.)	CAP	Shrimp	Buffer extraction; Solvent extraction	0.25 μg/kg[b] 0.1 μg/kg[b]	Impens et al., 2003
ELISA test (Ridascreen, R-Biopharm)	CAP	Porcine tissues (muscle, kidney)	Solvent extraction, SPE	0.1 ng/g	Posyniak et al., 2003
ELISA test (Euro-Diagnostica B.V.)	CAP	Muscle, eggs, honey	Solvent extraction, SPE	0.018; 0.0076; 0.063 μg/kg[a]	Scortichini et al., 2005
		Milk	Defatting/defatting, solvent extraction	0.22/0.11 μg/kg	

ELISA test (Ridascreen, R-Biopharm)	CAP	Seafood, meat, honey	Solvent extraction	0.3 μg/kg	Shen and Jiang, 2005
Direct CL-ELISA	CAP	Skim milk	Dilution with buffer	0.05 ng/mL	Lin et al., 2005
Indirect CL-ELISA	CAP	Shrimp	Solvent extraction	0.01 ng/mL	Xu et al., 2006
Indirect CL-ELISA	CAP	Chicken muscle	Solvent extraction	6 ng/L	Zhang et al., 2006
Indirect ELISA	TAP	Eel	Solvent extraction	0.16μg/L (in extract)	Hao et al., 2006
Direct ELISA	CAP	Sheep serum	No pretreatment	0.1 ng/mL	Wesongah et al., 2007
Indirect ELISA	FF amine	Swine muscle, chicken muscle, fish	Acid hydrolysis, solvent extraction	3.08; 3.3; 3.86 μg/kg	Wu et al., 2008
Indirect ELISA	FF	Fish feed	Solvent extraction	2.5 ng/mL (in extract)[c]	Luo et al., 2009a
Indirect ELISA	FF, FF amine	Swine muscle	Extraction with acidic buffer, dilution	1.6 ng/g	Luo et al., 2009b
Indirect BS-ELISA	CAP	Milk	Solvent extraction	0.042 ng/mL	Wang et al., 2010
Indirect ELISA	FF, TAP	Swine feed	Solvent extraction	0.02 mg/kg	Luo et al., 2011
		Fast immunoassay with visual detection			
Dipstick EIA	CAP	Milk	No pretreatment	1 μg/kg	Schneider et al., 1994
Immunofiltration EIA				0.7 μg/kg	
Immunofiltration EIA (Quik-card® test)	CAP	Eggs	Solvent extraction, SPE	2 μg/kg	Nouws et al., 1987a
Immunofiltration EIA (Quik-card® test)	CAP	Urine; Liver, kidney, muscle	Buffer extraction	2.5–5 μg/kg; 15–50 μg/kg	Nouws et al., 1987b
Immunofiltration EIA (Quik-card® test)	CAP	Milk	Deproteinization without extraction;	5–10 μg/kg;	Nouws et al., 1988
			Deproteinization, solvent extraction, SPE	0.1 μg/kg	
Immunofiltration EIA (La Carte® test)	CAP	Muscle tissue	Buffer extraction, filtration	1–3 μg/kg	Laurensen and Nouws, 1990
Immunochromatographic assay	CAP	Fish	Solvent extraction	10 ng/g (visual)	Li et al., 2007
Immunochromatographic assay	CAP	Milk	No pretreatment	10 ng/mL (visual)	Byzova et al., 2010

(Continued on next page)

TABLE 4
Antibody-based methods for CAP, TAP, and FF detection in foodstuffs (*Continued*).

Method	Analyte	Object	Sample pretreatment	Limit of detection	Reference
			Other immunoassays		
CEIA-LIF	CAP	Milk	Solvent extraction	0.1 ng/mL	Blais et al., 1994
Indirect TR-FIA	CAP	Shrimp, chicken muscle	Solvent extraction	0.05 ng/g	Shen et al., 2006
ELISA with voltammetric detection	CAP	Milk	Deproteinization, solvent extraction	0.064 μg/L	Song et al., 2007
CEIA-LIF	CAP	Chicken, fish	Solvent extraction	0.035 μg/kg	Zhang et al., 2008b
		Milk	Defatting, solvent extraction		
Indirect mesofluidic fluorescent immunoassay	CAP	Milk	Defatting	0.008 μg/L	Zhang et al., 2008a
		Meat	Solvent extraction		
Direct hapten-coated BS-ELISA	CAP	Shrimp	Solvent extraction	0.2 ng/mL	Sai et al., 2010
			Biosensor assay		
SPR biosensor assay	CAP	Milk	Defatting	0.1 μg/L	Gaudin and Maris, 2001
SPR biosensor assay (Qflex® kit)	CAP, CAP glucoronide	Milk	No pretreatment	0.005 μg/kg; [a]	Ferguson et al., 2005
		Poultry muscle, honey, prawns	Solvent extraction	0.02; 0.04; 0.04 μg/kg[a]	
SPR biosensor assay	CAP glucoronide, CAP	Porcine kidney, prawns, dairy products	Solvent extraction, SPE;	0.05 μg/kg (CAP glucoronide, kidney)[a]	Ashwin et al., 2005
		Honey	Buffer extraction	0.04 μg/kg (CAP, prawns)[a]	
SPR biosensor assay	TAP, FF, FF amine, CAP	Shrimp	Solvent extraction	0.1; 0.2; 250; 0.5 μg/kg[b]	Dumont et al., 2006
SPR biosensor assay	CAP	Honey	Dilution with a buffer	17.5 fg/mL	Yuan et al., 2008
SPR biosensor assay	CAP	Pork meat	Solvent extraction	0.5 ng/mL	Dong et al., 2008
SPR biosensor assay	CAP	Honey	Dilution with a buffer	42.4 pg/mL	Yuan et al., 2009
Flow-injection fluorescence sensor	FF	Liver, pork, chicken, fish	Solvent extraction	< 2.5 μg/kg	Ge et al., 2010

[a]Decision limit (CCα).
[b]Detection capabilities (CCβ).
[c]IC_{50}: inhibition concentration at 50%.

TABLE 5
Bio- and immunosensor techniques employed for CAP detection

Biosensor technique	Support	Immobilized substance	Method of immobilization	Labeled component	Detection limit	Measurements in food	Reference
Piezoelectric immunosensor (QCM)	Gold surface covered with thiol or sulfide SAM	Antibodies	Carboxyl-amine coupling	Label-free	10^{-5} M (3.2 mg/kg)	No	Park et al., 2004
Chemiluminescent immunosensor	Nylon membrane	Antibodies	Adsorption	CAP labeled with HRP	10^{-8} M (3.2 μg/kg)	Pork, beef, chicken, shrimp, milk	Park and Kim, 2006
Lactate oxidase-based amperometric biosensor	Cylindrical membrane-covered oxygen sensor	—	—	—	Ppm range (μg/kg)	Diluted milk	Rinken and Riik, 2006
Piezoelectric immunosensor (QCM)	Gold surface covered with SAM (MPA)	Antibodies	Carboxyl-amine coupling	Label free	3×10^{-6} M (969 μg/kg)	No	Adanyi et al., 2006
OWLS immunosensor	Glass surface silanized by APTS	Antibodies	Glutaraldehyde coupling	Label free	10^{-7} M (32.3 μg/L)	No	Adanyi et al., 2006
Amperometric immunosensor	Glassy carbon electrode covered by poly-TTCA/AuNPs/Den/CdS layer[a]	Anti-CAT antibodies	Carboxyl-amine coupling	CAP labeled with hydrazine	45 pg/mL	Beef, chicken, pork	Kim et al., 2010
Microcantilever immunosensor	Gold surface covered with protein A	Antibodies	Interaction with protein A	Label-free	0.2 ng/mL	No	Tan et al., 2010
Impedimetric immunosensor	Gold electrode covered by SATUM/AuNPs/MSA layer[b]	Antibodies	Glutaraldehyde coupling	Label-free	1.6 ng/kg	Shrimp	Chullasat et al., 2011

[a]Poly-TTCA/AuNPs/Den/CdS: poly 5, 2′:5′,2′′-tethiophene-3′-carboxyl acid/gold nanoparticle/dendrimer/cadmium sulfide nanoparticles.
[b]SATUM/AuNPs/MSA: a self-assembled thiourea monolayer/gold nanoparticle mercaptosuccinic acid.

TABLE 6
Microarrays and approaches to simultaneous multi-analyte detection (including CAP)

Microarray	Support	Immobilized substance	Detected analytes	Detection limit	Matrix (if applied)	Reference
Small molecule microarray	Glass slide	Analyte-protein conjugate	CAP, clenbuterol, tylosin	0.03; 0.01; 0.88 μg/L	Milk, cheese, chicken, pork	Peng and Bang-Ce, 2006
Immunochip	Glass slide	Antibody	Atrazine, nonylphenol, 17-beta estradiol, paraverine, CAP	0.001–5 μg/mL[a]	—	Gao et al., 2009
Suspension microarray	Fluorescent microsphere	Analyte-protein conjugate	CAP, clenbuterol; 17-beta-estradiol	40; 50; 1000 ng/L		Liu et al., 2009
Suspension microarray	Fluorescent microsphere	Analyte-protein conjugate	Tylosin, tetracycline, gentamicin, streptomycin, CAP	0.3; 1.5; 4; 20; 25 ng/mL	Milk	Su et al., 2011
iSPR biosensor microarray	Golden chip	Analyte derivative	Neomycin, gentamicin, kanamycin, streptomycin, sulfamethazine, CAP, enrofloxacin	123.2; 105.4; 0.9; 50.1; 9.2; 30.3; 10.1 ng/mL (10× diluted milk)[b]	10-fold diluted full-fat goat milk powder	Rebe Raz et al., 2009
Multichannel SPR immunosensor	Golden chip with m-SAM	Analyte-protein conjugate	Enrofloxacin, sulfapyridine, CAP	0.34; 0.43; 0.22 μg/L (milk diluted 5 times)	Whole milk	Fernández et al., 2010

[a]Detection range.
[b]IC_{50}: inhibition concentration at 50%.

TABLE 7
Alternative methods for CAP detection

Method	Analyte	Matrix	Sample pretreatment	Detection limit	Reference
Polarographic determination	CAP	Milk	No pretreatment	0.3 μg/mL	Fossdal and Jacobsen, 1971
Differential pulse polarography	CAP	Meat products, milk, eggs	(Enzymatic hydrolysis), solvent extraction	0.5 ng/g	Duda and Kucharska, 1999
Voltammetric determination	CAP	Milk	Solvent extraction	4.7×10^{-8} M (15 μg/L)	Agüí et al., 2002
Voltammetric determination	CAP	Ophthalmic solutions Milk	Dilution; Deproteinization, MIP microcolumn extraction	1.4×10^{-8} M (4.5 μg/L)	Mena et al., 2003
Voltammetric determination	CAP	Milk	Deproteinization, solvent extraction	0.83 μg/L	Chai et al., 2006
Disposable electrochemical sensor strip	CAP Parathion, 2,4,6-trinitrotoluene	Veterinary preparation Lake water	—	0.42 μM (136 μg/L)	Chen et al., 2006
Voltammetric determination	CAP	Milk	Solvent extraction	5×10^{-9} M (1.6 μg/L)	Xiao et al., 2007
Flow-injection assay with amperometric detection	CAP	Eye drops Milk	Dilution; Deproteinization, SPE	3×10^{-8} M (9.7 μg/L)	Chuanuwatanakul et al., 2008
Fluorescent flow-through assay using MIP	CAP	Buffer	—	8 μg/mL	Suárez-Rodríguez and Díaz-García, 2001
Chemiluminescence microfluidic system	CAP	Honey	Dilution with buffer	7.46×10^{-6} μmol/L (2.4 ng/L)	Thongchai et al., 2010
CD-MIS	CAP, furazolidone, enrofloxacin	Chicken meat	Solvent extraction, SPE	19.7; 12.5; 13 μg/kg[a]	Jafari et al., 2007
Capillary electrophoresis	Amoxicillin, doxycycline, streptomycin, TAP, FF, nifursol, enrofloxacin, norfloxacin	Poultry and porcine tissues (muscle, liver, kidney, skin with fat)	Solvent extraction	8 (TAP); 15 (FF) μg/kg	Kowalski et al., 2003
Capillary electrophoresis	Ampicillin, amoxicillin, cloxacillin, penicillin, tetracycline, CAP	Milk	Deproteinization, SPE	0.48; 0.94; 0.93; 1.09; 0.78, 0.72 μg/mL	Santos et al., 2007

(Continued on next page)

TABLE 7
Alternative methods for CAP detection *(Continued)*

Method	Analyte	Matrix	Sample pretreatment	Detection limit	Reference
Capillary electrophoresis	CAP, ciprofloxacin, ampicillin, sulfamethoxazol	Bovine raw milk	Defatting, deproteinization, solvent extraction, SPE	29, 12, 13, 14 μg/L	Vera-Candioti et al., 2010
Capillary electrophoresis (MEKS)	CAP, TAP, FF	Bovine milk	Solvent extraction	5.3; 4.3, 4.8 μg/kg	Pezza et al., 2006
Capillary electrophoresis (MEKS)	CAP, TAP, FF	Poultry muscle	Solvent extraction, SPE	1.5; 3.2; 7.4 ng/g	Kowalski et al., 2008
Capillary electrophoresis (MEKS)	Seven sulfonamides, CAP, TAP, FF	Poultry tissues (muscle, liver, skin with fat)	Solvent extraction, SPE	1.3 (CAP); 2.6 (TAP); 5.7 (FF) ng/g	Kowalski et al., 2011

[a]Limit of quantification.

Micrococcus luteus for the detection of 66 antimicrobial residues in frozen, thawed, and fresh tissues was reported (Currie et al., 1998). With regard to the family of amphenicols, the only compound within this group that can be detected at concentrations lower than the MRL is FF. The modified FPT, together with radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), was used for screening CAP residues in the tissues and fluids of treated cattle (Lynas et al., 1998). The sensitivity of the FPT was shown to be poor (300 ng/mL) compared to the other assays. The authors concluded that the sensitivity of the FPT is inadequate for the detection of CAP residues where only minimal withdrawal periods have been observed. The same observations were made in a comparative study of three bioassays: the swab test on premises, a microbial inhibition test (the same bacteria were used in both bioassays), and thin-layer chromatography/bioautography (Korsrud et al., 1987). In contrast, a comparative study of FPT, ELISA, and HPLC for the detection of CAP residues in chicken revealed the usefulness of this microbiological method as a screening test (Tajik et al., 2010). A total of 17.5% of samples tested were found to be contaminated with the antibiotic. Among all samples found to be positive by FTP, 78.5% (liver), 75% (kidney), and 50% (muscle) of samples were also found to be positive by ELISA and HPLC. The study also confirmed that the illegal use of CAP in the poultry industry continues in some regions.

A modification of FPT—a one-plate method—was used for the detection of antibiotics in kidneys and meat by growth inhibition of *Bacillus subtilis* (Koenen-Dierick et al., 1995). Although in this method β-glucuronidase was used to cleave biologically inactive CAP glucuronide excreted in urine, the sensitivity remained poor in comparison to the CAP MRPL of 0.3 μg/kg: 30 μg/mL for the one-plate test and 12.5 μg/mL for the FPT.

A commercially available microbial inhibitor test Delvotest "SP" (inhibition of *Bacillus stearothermorphilus* var. *calidolactis* growth) was found to be insensitive to CAP in ewe milk with a reported limit of detection of 12000 μg/kg (Althaus et al., 2003). The same situation was observed for CAP detection in bovine milk. Senyk et al. (1990) and Os and Beukers (1980) observed the range of detection limits of 9000–21000 and 8000–10000 μg/kg, respectively. Similarly, the sensitivity of a five-plate test towards CAP and TAP in milk (6000 μg/L for both) (Gaudin et al., 2004) and FF in pig, cattle, sheep, and poultry muscle (between 1000 and 3000 μg/kg, i.e., $\geq$ 10 MRL) was unsatisfactory (Gaudin et al., 2010).

CAP detection in foodstuffs was also performed utilizing *Micrococcus luteus* (Singer and Katz, 1985), *Photobacterium phosphoreum* (Tsai and Kondo, 2001), and *Photobacterium leiognathi* (Shakila et al., 2007). Detection limits in urine and serum of 0.25 μg/mL and 0.025 μg/mL in milk and 0.1 μg/g in muscle tissue were obtained (Singer and Katz, 1985). Tsai and Kondo (2001) achieved sensitivity of 3.12 μg/mL. Shakila et al. (2007) used an improved extraction procedure and a large sample size (100 g) and were able to detect 1 μg/kg of CAP in shrimp tissue. This equates to the lowest detection limit of CAP for microbiological assay reported thus far. Nevertheless, none of these microbiological based tests was able to detect CAP, TAP, and FF at or below MRPL/MRL and thus cannot be considered as suitable screening procedures for these important antibiotics.

ANTIBODY-BASED METHODS

A wide range of methods have been developed for CAP, TAP, and FF detection in foods where unique recognition molecules, i.e., antibodies, were used as the biodetector. Among these are conventional immunoassays and more novel immunoassays with modifications incorporated to improve sensitivity, biosensor techniques, and microarrays (Tables 4–6). Antibodies are the core elements of all these assays and they, more than any other single factor, determine the sensitivity and specificity of the procedure. Amphenicols are low-molecular-weight substances that need to be coupled to a carrier protein to produce antibodies in immunized animals. They also need to be coupled to a protein or an enzyme to produce coating antigens or labeled antigens in ELISA-based methods or they need to be immobilized directly onto the surface of biosensor chips.

Design of Immunoreagents

The CAP molecule (2,2-dichloro-N-((1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)acetamide) can be described as having three main parts: the core nitrophenyl moiety and the propanediol and dicloroacetamido groups (Figure 1). The first anti-CAP antibodies were produced in 1966 (Hamburger, 1966). Later Hamburger and Douglass (1969) used a hapten inhibition method to define the antibody binding sites on the CAP molecule. It should be noted that the original immunizing hapten was coupled through the nitro group to a carrier protein. The authors concluded that the aromatic ring and the dichloroacetamido group accounted for a large portion of the immunological reactivity of the hapten. In the acetamine chain significant changes in activity were noted upon removal of the two chlorine atoms.

For the modification of the parent molecule all three sites have been used (Figure 1, Table 2). Hamburger (1966) modified CAP by reduction of the nitro group to an amine and coupled the resulting amino-CAP to a protein by a diazotization reaction. The same approach was used only by Freebairn et al. (1988) and Kolosova et al. (2000). Recently, Sai et al. (2010) used reduced CAP (amino-CAP) for direct immobilization on a surface of polysterene plates preliminarily treated with glutaraldehyde. Most often protein (enzyme) conjugates have been produced using CAP succinate with a free carboxyl group. This is most likely due to the commercial availability of the CAP succinate derivative. As an alternative, it can be obtained by the reaction of CAP with succinic anhydride (Campbell et al., 1984; Märtlbauer and Terplan, 1987; Hack et al., 1989; Zhang et al., 2008b). The carboxyl group of CAP succinate is easily activated and then

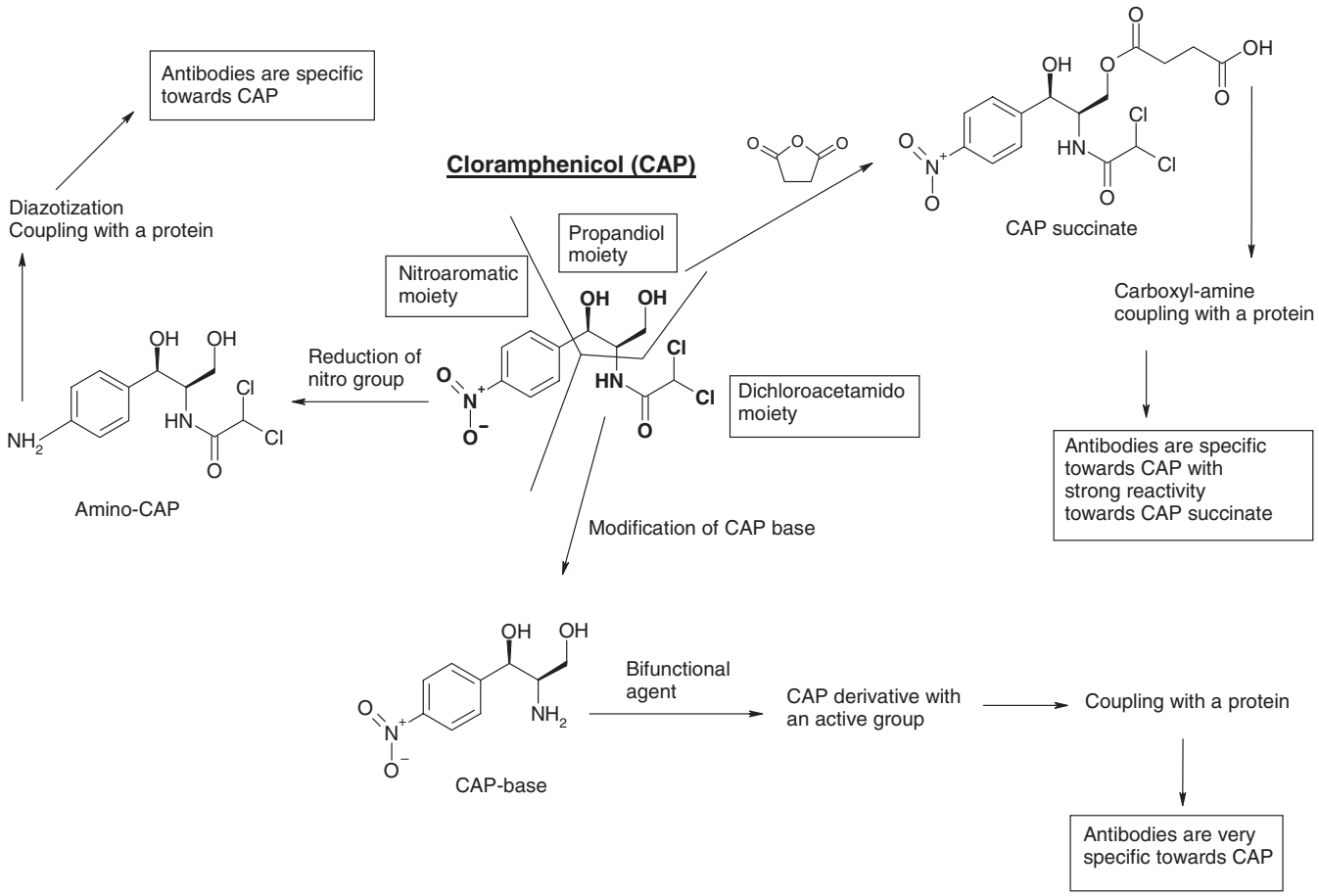


FIG. 1. Chloramphenicol and three pathways of its modification for the following immunoreagent synthesis; antibodies cross-reactivity profile.

conjugated to the amino groups of a protein (enzyme). Activation can be achieved by use of the mixed anhydride reaction (Campbell et al., 1984; van de Water et al., 1987; Fodey et al., 2007) or an activated ester method (Kolosova et al., 2000; Shen et al., 2006; Zhang et al., 2006; Samsonova et al., 2010; Wang et al., 2010; Sai et al., 2010). Direct conjugation of CAP succinate to the free amino groups of a protein or enzyme with the employment of a carbodiimide is also possible (Campbell et al., 1984; Dumont et al., 2006; Wesongah et al., 2007). When CAP succinate is used as an immunizing hapten the nitrophenyl and dichloroacetamido groups, which account for a large portion of the immunological reactivity of the hapten (Hamburger and Douglass, 1969), are well displayed for antibody recognition. CAP succinate was also used for the production of a conjugate with the heterobifunction oligoethylene glycol linker for immobilization on a mixed self-assembled monolayer (m-SAM) sensor surface (Yuan et al., 2008). Among other applications of CAP derivatives with modifications of the propanediol moiety are the use of the 3′-CAP-β-monoglucuronide as an immunizing hapten (Hock and Liemann, 1985) and CAP-polyethylene glycol (PEG)-amine for immobilization onto a carboxymethylated dextran surface of a surface plasmon resonance (SPR) sensor chip (Yuan et al., 2009). The latter was synthesized starting from the reaction of CAP with triphosgene to form a CAP chloroformate, followed by the reaction with PEG-diamine to provide a CAP-PEG-amine derivative.

A further method to generate a modification to CAP was the alteration or elimination of the dichloroacetamido group. In several publications a modified CAP base (CAP without the dichloroacetamido group, Table 1) was used for the production of polyclonal (Arnold et al., 1984; Märtlbauer and Terplan, 1987) and monoclonal antibodies (van de Water et al., 1987; Hack et al., 1989). The CAP base was first modified by a bifunctional agent, then the resultant derivative with an active group was conjugated to a carrier protein. As examples, Märtlbauer and Terplan (1987) and Hack et al. (1989) modified the CAP base with dimethyl adipimidate; van de Water et al. (1987) used S-acetyl-mercaptosuccinic anhydride (SAMSA) and Arnold et al. (1984) used a monoiodoacetyl analog of CAP as the immunizing hapten. The CAP base was also used for direct coupling to a protein for antibody production (Freebairn et al., 1988; Samsonova et al., 2010) or for direct immobilization on the surface

of a chip for SPR biosensor assay (Gaudin and Maris, 2001; Dumont et al., 2006; Fodey et al., 2007).

Similar approaches were used for the modification/coupling of structural analogs of CAP-TAP and FF (Tables 1 and 2). TAP was modified via hydroxyl group. N,N′-disuccinimidyl carbonate (DSC), a homobifunctional cross-linking agent, was used to produce a TAP-DSC intermediate, which was then coupled with horseradish peroxidase (HRP) or immobilized onto the surface of an SPR sensor chip (Fodey et al., 2007). TAP succinate (Hao et al., 2006) and TAP glycinate (Luo et al., 2009b) were produced from TAP and an anhydride and then coupled with a protein. With regards to FF and its derivatives, FF amine was most often used as the starting compound. Thus, FF amine (via the free amine group) was directly immobilized onto the surface of an SPR sensor chip (Dumont et al., 2006) or used for the production of a coating antigen by glutaraldehyde coupling (Wu et al., 2008) or formaldehyde coupling (Luo et al., 2009b). FF succinate (Luo et al., 2009a, 2011), FF maleate (Luo et al., 2011), and FF glutarate (Luo et al. 2009b), obtained in a reaction of FF with appropriate anhydride via the free hydroxyl group, were used for production of immunogens and coating antigens.

Antibody Specificity

Since Hamburger (1966) first reported CAP-specific antibody production a wide range of polyclonal and monoclonal antibodies have been produced against CAP (Table 3). Polyclonal anti-CAP antibodies were obtained in rabbits, sheep, and occasionally in animals such as camels, donkeys, and goats (Fodey et al., 2007; Wesongah et al., 2007). To produce antibodies an immunizing hapten was usually coupled to a human or bovine serum albumen (HSA, BSA) and keyhole limpet hemocyanin (KLH) (Table 3). A bovine gamma globulin (BGG) (Campbell et al., 1984) and thyreoglobulin (TG) (Hock and Liemann, 1985) were also used as carrier proteins.

In most cases the generated antibodies were shown to be specific to CAP with some cross-reactivity towards CAP derivatives and analogs; none of them cross-reacted with antibiotics of other groups (Table 3). Antibodies produced against the three main types of CAP derivatives (see Figure 1) showed three different cross-reactivity profiles. Just two groups produced antibodies against a diazo derivative of CAP (Table 3) and only Kolosova et al. (2000) assessed the antibody's cross-reactivity by indirect ELISA. The antibodies produced showed a moderate cross-reactivity towards CAP succinate (24%) and did not recognize CAP without the dichloracetamido group (CAP base). On the contrary, antibodies produced against a conjugate of CAP succinate generally have a very high affinity for CAP succinate as an initial coupling hapten (Table 3). This is a fairly usual finding for polyclonal and monoclonal antibodies produced against such conjugates (Campbell et al., 1984; Kolosova et al., 2000; Shen et al., 2006; Zhang et al., 2006; Samsonova et al., 2010). For instance, cross-reactivity towards CAP succinate of up to 1428% has been obtained (Kolosova et al., 2000). These antibodies also showed no or negligible cross-reactivity towards other related compounds where the immunologically important nitrophenyl (TAP and FF) and dichloroacetamido (CAP base) groups are lacking (Campbell et al., 1984; Kolosova et al., 2000; Shen et al., 2006; Zhang et al., 2006, 2008b; Dumont et al., 2006; Fodey et al., 2007; Wesongah et al., 2007; Samsonova et al., 2010; Wang et al., 2010). Some researchers were able to change the specificity behavior of such antibodies by employing a heterologous competing antigen. For example, Dumont et al. (2006) used FF amine to immobilize on the surface of an SPR biosensor chip. In such a system FF was recognized at 107% cross-reactivity and TAP at 26% relative to CAP. In a later study Fodey et al. (2007) used TAP modified with DSC to produce a conjugate with an enzyme for an ELISA and for immobilization onto the surface of an SPR biosensor chip. Cross-reactivity values of up to 53%/56% for TAP and 82%/129% for FF in ELISA/biosensor assays, respectively, were reported. The only polyclonal antibodies produced against a conjugate of 3′-CAP-β-monoglucuronide with TG (Hock and Liemann, 1985) recognized CAP-monoglucuronide and CAP to similar extents (100 and 86%, respectively). They also showed moderate cross-reactivity towards CAP succinate (56%) and CAP palmitate (59%) but low binding towards TAP (3.5%).

Monoclonal (van de Water et al., 1987; Hack et al., 1989) and polyclonal (Arnold et al., 1984; Märtlbauer and Terplan, 1987) antibodies were produced against a conjugate of modified CAP base with a protein. These antibodies are usually very specific to CAP and showed low cross-reactivity towards all CAP derivatives tested (CAP succinate, CAP base) and analogs (TAP, FF) (Table 2). For the production of specific anti-CAP antibodies the CAP base also can be directly coupled to free carboxyl groups of a carrier protein in a one-step procedure without preliminary modification (Samsonova et al., 2010). Polyclonal antibodies showed low cross-reactivity towards CAP succinate (11.3%) and recognized CAP base to a lesser extent (4.6%). It seems that for antibody recognition the presence of acylamido bond (-NH-CO-) is important but not the length of chemical bridge between the CAP molecule and a protein. However, Märtlbauer and Terplan (1987) modified the CAP base with bifunctional imidoester (dimethyl adipimidate) and introduced an imidoamide (imidine) bond (–NH-C(NH)-). With this approach highly specific antibodies were obtained (cross-reactivity towards CAP base was 0.5%, TAP, 0.05%).

Polyclonal antibodies produced against TAP succinate conjugated with BSA showed negligible cross-reactivity towards CAP (Hao et al., 2006). However, no information was given on how this antibody recognized FF. Two groups produced polyclonal antibodies against a conjugate of FF amine with BSA. In this study FF amine was directly coupled to a carrier protein via free amino groups by glutaraldehyde (Wu et al., 2008) or formaldehyde coupling (Luo et al., 2009b). Whereas the antibody produced by Wu et al. (2008) showed low cross-reactivity to FF (10.88%; FF amine as 100%), antibodies obtained by Luo et al. (2009b) recognized FF molecules very well

(cross-reactivity ranged from 97 to 225% depending on the coating conjugate used). In the latter work one homogolous (FF amine) and two heterologous (FF glutarate and TAP glycinate) coating haptens were used. It should be noted that all antibodies have been shown to recognize TAP and CAP to much lower extents with cross-reactivities ranging from a few percentages to lower than 0.1%. This indicates that the methylsulfonylphenyl and fluorine moieties are important antigenic determinants. In only one combination of an antibody with a homologous coating conjugate (FF amine-ovalbumin (OVA)) was a 31% cross-reaction towards TAP observed (Luo et al., 2009b). Monoclonal (Luo et al., 2009a) and polyclonal antibodies (Luo et al., 2011) produced against a conjugate of FF succinate with HSA showed a different cross-reactivity pattern (Table 3). Whereas a monoclonal antibody was shown to be very specific towards FF with negligible reactivity towards other amphenicols, the polyclonal antibody showed a 40% reactivity towards TAP. It should be noted that in the latter case a heterologous coating conjugate (FF maleate-OVA) was used.

Sample Pretreatment

Amphenicols need to be detected in various food matrices including meat, eggs, milk, honey, and seafood. Sample preparation methods are usually shorter and less complicated than with chromatographic methods (Tables 4 and 7). Milk samples can be analyzed directly (Fossdal and Jacobsen, 1971; Schneider et al., 1994; Ferguson et al., 2005; Byzova et al., 2010) or diluted with a buffer before analysis (Märtlbauer and Terplan, 1987; Kolosova et al., 2000; Lin et al., 2005; Fernández et al., 2010). Su et al. (2011) used ethyl ether to denature milk proteins and extract free fatty acids followed by organic solvent evaporation and filtration by a 0.22 μm ultrafiltration membrane. More complex sample preparation usually involves a combination of deproteinization, defatting, solvent extraction, and solid-phase extraction (SPE). For instance, milk samples were defatted (van de Water and Haagsma, 1990b; Gaudin and Maris, 2001; Scortichini et al., 2005; Zhang et al., 2008a) or deproteinated (Nouws et al., 1988) and then analyzed. More complex procedures include solvent extraction (Blais et al., 1994; Duda and Kucharska, 1999; Agüí et al., 2002; Ferguson et al., 2005; Chai et al., 2006, Pezza et al., 2006; Xiao et al., 2007; Wang et al., 2010), solvent extraction with preliminary defatting (Scortichini et al., 2005; Zhang et al., 2008b), and deproteinization followed by SPE (Nouws et al., 1988; Song et al., 2007; Santos et al., 2007; Chuanuwatanakul et al., 2008) or by purification employing molecular imprinted polymer (MIP) columns (Mena et al., 2003). The most complicated extraction procedure for milk samples, which includes defatting, deproteinization, solvent extraction, and finally SPE, was developed by Vera-Candioti et al. (2010). The sample preparation of different tissues (meat, eggs, shrimp, fish) usually includes solvent extraction (Table 4) sometimes followed by SPE (Nouws et al., 1987a; van de Water et al., 1987; Posyniak et al., 2003; Scortichini et al., 2005; Ashwin et al., 2005; Kowalski et al., 2008, 2011). Quick buffer extraction of CAP residues from meat (Laurensen and Nouws, 1990; van de Water and Haagsma, 1990b; Kolosova et al., 2000) and shrimps (Impens et al., 2003) is also possible, but in these cases sensitivity of the developed assay is not as high as for the more complex extraction procedures. Recently, Chullasat et al. (2011) minimized the matrix effect of a shrimp sample using buffer extraction followed by desalting and dilution of an extract 10,000 times. Honey samples can be diluted with water/buffer (Ashwin et al., 2005; Yuan et al., 2008, 2009; Thongchai et al., 2010) or extracted with an organic solvent (Ferguson et al., 2005) followed by SPE (Scortichini et al., 2005).

CAP is usually extracted with ethyl acetate. The use of mixtures of acetone/dichloromethane (Scortichini et al., 2005), chloroform/isooctane (Arnold et al. 1984, Arnold and Somogyi, 1985), acetonitrile/acetate buffer (Posyniak et al., 2003), acetonitrile/water (Shen et al., 2006; Zhang et al., 2006, 2008a, 2008b; Jafari et al., 2007), methanol/water (Ashwin et al., 2005), and dichloromethane (Vera-Candioti et al., 2010) as extractants is also described. Sometimes *n*-hexane has been used as a final step to remove lipids. TAP, FF, and FF amine are often extracted with ethyl acetate (Hao et al., 2006; Wu et al., 2008; Luo et al., 2009a). Wu et al. (2008) used preliminary acidic hydrolysis of a sample (swine and chicken muscle, fish) to extract FF amine. Extraction of FF was also performed with the use of an acetone/water mixture (Ge et al., 2010). For simultaneous extraction of amphenicols different approaches were used. Luo et al. (2009b) extracted FF and FF amine from swine muscle by phosphate buffer containing trichloroacetic acid for deproteinization to increase FF amine recovery from the samples. FF or TAP were extracted from swine feed with ethyl acetate by Luo et al. (2011). Kowalski et al. (2003) extracted FF and TAP together with other antibiotics from poultry and porcine tissues with ethyl acetate in the presence of 1 M NaOH after sample deproteinization by acetonitrile. All amphenicols were extracted from poultry tissues with acetonitrile followed by SPE (Kowalski et al., 2008, 2011).

Immunoassay

Immunoassays for low molecular substances such amphenicols are based on competition between free analyte in a sample (extract) and labeled analyte for a limited number of binding sites on the specific antibodies. The label can be a radioactive isotope, an enzyme (most often HRP), or a fluorophore. Hamburger (1966) used a complement fixation inhibition technique and was able to reproducibly detect 10^{-5} μg (3.1×10^{-14} mole) of CAP. The first reported immunoassay to detect and quantify CAP was developed in 1984 by Campbell et al. (1984). This enzyme-linked immunoassay was developed in a heterogeneous indirect format with the use of polysterene tubes. A limit of detection of 1 ng/mL was achieved. Detailed cross-reactivity data of an anti-CAP antiserum were also presented for the first time in this study.

Radioimmunoassay (RIA)

In RIA, a radioactive isotope is used as the label, and this, for safety reasons, means strict requirements for the handling, utilization, and disposal of reagents. A few RIAs for the detection of CAP in food were developed in 1980s (Table 3). The first immunoassay (RIA) for determination of CAP residues in milk, meat, and eggs was published in 1984 (Arnold et al., 1984). All authors used solvent extraction for sample pretreatment and were able to detect down to 0.2 μg/kg CAP (Arnold et al., 1984; Arnold and Somogyi, 1985; Freebairn et al., 1988). Although the use of RIA is limited, a RIA kit for CAP detection has been commercially available for some time (Lynas et al., 1998; McMullen et al., 2004).

Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) was and remains probably the most often used screening method for ampheniocols. ELISA is a cheap, sensitive, and reliable method for the detection of veterinary drug residues in various foodstuffs. ELISA is very suitable for high-throughput screening, and it has been used for many years for this purpose. The assay can be performed in direct format, i.e., when antibodies are immobilized on a surface of polysterene plates, or in an indirect format when analyte derivative (usually a conjugate with a protein) is immobilized.

ELISA methods for the detection of CAP, FF, and its metabolite FF amine in foodstuffs at MRPL or MRL level and lower have been described (Table 3). Wesongah et al. (2007), for example, developed an ELISA for the detection of CAP in sheep serum that can be used for control of tissues destined for human consumption. Since the 1980s and 1990s the sensitivity of developed ELISAs has been improved substantially. Traditional ELISAs are based on colorimetric detection of a product resulting from the oxidative reaction of a substrate catalyzed by an enzyme. A few approaches have been suggested to improve the sensitivity achievable by the ELISA technique. Among them are the biotin-streptavidin amplification system and more sensitive signal generation systems such as chemiluminescence. Thus, ELISA with monoclonal antibodies allowed the determination of CAP in swine muscle at a concentration of 5 μg/kg (van de Water et al., 1987). Later, the sensitivity of ELISA was significantly improved by using a biotin-streptavidin system and a coating antigen with lower CAP incorporation (van de Water and Haagsma, 1990a, 1990b). Similarly a biotin-streptavidin amplified ELISA (BS-ELISA) was able to detect 0.042 ng/mL of CAP in milk, which is eight-fold more sensitive than the traditional ELISA (Wang et al., 2010). Recently, Sai et al. (2010) developed BS-ELISA, where coating hapten was directly immobilized onto wells of polysterene plates. The surface of a plate was treated with glutaraldehyde followed by a reaction with the free amino group of CAP derivative (the nitro group of CAP was reduced to an amino group). A few ELISAs with chemiluminescent detection were able to detect CAP in milk (Lin et al., 2005), shrimp (Xu et al., 2006), and chicken muscle (Zhang et al., 2006). The best sensitivity was achieved by Zhang et al. (2006) and was as low as 6 ng/L in milk.

ELISAs are often used in combination with a chromatographic method for screening and confirmation purposes (van de Water and Haagsma, 1991; Keukens et al., 1992; Impens et al., 2003; Posyniak et al., 2003; Scortichini et al., 2005; Shen and Jiang, 2005). The results of both methods are usually in good agreement. Various ELISA kits are commercially available (Impens et al., 2003; Posyniak et al., 2003; Scortichini et al., 2005; Shen and Jiang, 2005). Gaudin et al. (2003) in an inter-laboratory study demonstrated that ELISA kits for CAP detection in milk and muscle (commercial and in-house) show good repeatability and accuracy and could be considered as suitable tests for screening purposes. The global rates of false compliant results of 2.2% for milk and 0% for pig muscle were lower than 5%, whichever kit was used. The global rates of false noncompliant results (16.7% and 10% for milk and muscle respectively) were also reasonably satisfactory.

Only a small number of studies using ELISA for the detection TAP, FF, and FF amine in concentrations lower then MRL have been published so far (Hao et al., 2006; Wu et al., 2008; Luo et al., 2009b). Hao et al. (2006) developed an ELISA for TAP detection in eels. A method developed by Wu et al. (2008) was found to be suitable for the detection of FF amine as a residue marker of FF in meat and fish. For this, FF from food samples was first hydrolyzed with hydrochloric acid, then the resulting FF amine was extracted with ethyl acetate. An ELISA for the simultaneous extraction and determination of FF and FF amine in swine muscle tissue was developed by Luo et al. (2009b). The authors used a simple one-stage extraction with phosphate buffer containing trichloroacetic acid. However, it should be stressed that for both methods some underestimation of FF and FF amine concentration measured by ELISA was observed. This fact indicates that the sample preparation procedures were not fully optimized. Two articles were devoted to FF and TAP detection in fish feed (Luo et al., 2009a) and swine feed (Luo et al., 2011).

Other Immunoassays

The requirement for sensitive CAP residue detection stimulated the development of more sensitive analytical methods. Possible ways to increase sensitivity of an immunoassay include the use of an alternative label such as a fluorescent agent (Shen et al., 2006; Zhang et al., 2008a) or signal detection system (Song et al., 2007). Combination with other separation or detection systems is also possible (Blais et al., 1994; Zhang et al., 2008b). These approaches helped to achieve a limit of detection 10–20 times better than the traditional ELISAs. Sensitive time-resolved fluoroimmunoassay (TR-FIA) in microtiter plate format for CAP detection in shrimp and chicken muscle was developed by Shen et al. (2006). Signal detection was based on the unique fluorescent properties of europium chelate (used

as a label for monoclonal antibodies) characterized by narrow-band emission lines, high quantum yields, and long fluorescence lifetime.

Capillary electrophoresis immunoassay (CEIA) was described by Blais et al. (1994) and Zhang et al. (2008b). CEIA combines the specificity and sensitivity of immunoassay performed in solution with the effective separation capability of capillary electrophoresis (CE). In the first stage, CAP from a sample and CAP-fluorescein conjugate competed for binding to specific antibodies. Bound and unbound forms of CAP-fluorescein conjugate were then analyzed by capillary zone electrophoresis with laser-induced fluorescence (LIF) detection. This CEIA-LIF system permits direct visualization of immunocomplex formation and dissociation (due to presence of the analyte), simplifying the interpretation of the test results. The equilibrium for CEIA-LIF was reached in just 15 min. Blais et al. (1994) developed a CEIA-LIF with a reported sensitivity of 0.1 ng/mL of CAP in milk (CAP was extracted from milk with an organic solvent). Later, higher sensitivity was achieved by Zhang et al. (2008b). The detection limit for CAP in solution was 0.0016 μg/L compared to 0.03 μg/L for ELISA (developed using the same immunoreagents), and in animal-derived foods it was 0.035 μg/kg.

An immuno-voltammetric technique was used for CAP detection in milk (Song et al., 2007). The main part of the assay was an indirect ELISA followed by voltammetric measurement after reaction on a plate was terminated. *p*-Nitrophenylphtalate used as substrate was oxidased to p-nitrophenol by alkaline phosphatase used as an enzyme label. Then the reaction was stopped and a small-sized electrochemical sensor with a glassy carbon electrode was inserted into wells of a plate to quantify *p*-nitrophenol by differentiated pulse voltammetry. The method had a sensitivity of 0.064 μg/L for CAP in milk.

A highly selective and sensitive mesofluidic immunoassay system based on competitive immunoassay in poly dimethylsiloxane channels was successfully applied for detection of CAP in meat and milk (Zhang et al., 2008a). The immunoassay was constructed in an indirect format. Glass beads were amino-silane modified, covalently precoated with CAP derivative (CAP succinate), and then infused into the microchannels (ø 300 μm). All other reagents were sequentially emitted into microchannels followed by a short incubation and washing. Fluorescence intensities of beads (Cy5-labeled secondary antibodies anchored by anti-CAP antibody-CAP succinate complex on glass beads) were employed to determine the concentration of CAP. The system showed very high sensitivity (limit of detection 0.008 μg/L) and excellent performance. It took about 30 min to detect CAP, including bead infusing, immunoreactions, and washing steps. The authors stressed that the system can be readily automated and expanded for multi-analyte analysis. To date this is the most sensitive immunoassay for CAP detection in foodstuffs reported.

Fast Immunoassays with Visual Detection

For on-site monitoring purposes simple and quick tests are required. These tests are usually qualitative and give "yes/no" (threshold level) responses. In general, fast tests are less sensitive than quantitative methods (Table 4), but they are quick and easy to perform. They do not require expensive and sophisticated equipment, and the result can be read by eye. On-site CAP residue monitoring has been performed in the form of immunofiltration enzyme immunoassay (EIA) (Nouws et al., 1987a, 1987b, 1988; Laurensen and Nouws, 1990; Schneider et al., 1994), dipstick assay (Schneider et al., 1994), and immunochromatographic assay (Li et al, 2007; Byzova et al., 2010).

Commercial immunofiltration EIA (adaptation of commercial Quik-card® or La Carte® tests) was used for detection of CAP in different objects (Nouws et al., 1987a, 1987b, 1988; Laurensen and Nouws, 1990). All reactions were performed on a card where anti-CAP antibodies were immobilized. The design of the card allows effective diffusion of the added reagents. Sample extract, enzyme solution, and substrate solutions were added onto the card one after another and the result was read by eye after 5 min. CAP was detected at concentrations of a few micrograms per kilogram in urine, eggs, milk, and muscle (Nouws et al., 1987a, 1987b, 1988; Laurensen and Nouws, 1990). A more complex extraction procedure including deproteinization, solvent extraction, and SPE facilitated the detection of CAP at a concentration of 0.1 μg/kg (Nouws et al., 1988). These tests performed well in comparative studies with different screening and quantitative methods (van de Water and Haagsma, 1991; Keukens et al., 1992).

Schneider et al. (1994) developed immunofiltration and dipstick EIAs for CAP detection in milk without preliminary pretreatment. In both assays a nylon membrane was coated with specific antibodies and fixed in a plastic test device in close contact with a filter layer, or mounted on a plastic dipstick. The devices were then incubated with a mixture of sample and CAP-enzyme conjugate, followed by a washing step and reaction with an enzyme-substrate-chromogen solution. Both methods showed similar sensitivity (Table 3).

A fast colloidal gold-based immunochromatographic assay was used to detect CAP in fish (Li et al., 2007) and milk (Byzova et al., 2010). Fish samples were extracted with solvents, whereas milk was analyzed directly. Li et al. (2007) evaluated the detection limit as CAP concentration, which caused a slight but distinguishable difference from the negative control (10 ng/g). In contrast, Byzova et al. (2010) used the CAP concentration at which the test line disappeared (10 ng/mL) as the detection limit. This type of assay allows fast generation of results and is very suitable for on-site monitoring. A test strip is practically a ready-to-use "device"; all necessary reagents are inserted on a strip beforehand and dried. While the liquid containing CAP migrates along the test strip, interaction between free CAP, immobilized CAP-protein conjugate, and specific antibodies

conjugated with colloidal gold occurs. The intensity of the test (colored) line is inversely proportional to the amount of CAP present in a sample. The assay procedure is very simple; a test strip is put into a sample (extract) and the results can be read by eye within 5–10 min. On one hand, qualitative results based on the "yes/no" principle (threshold level) can be considered a drawback of immunochromatographic methods. On the other hand, such methods can also be used in quantitative mode, when test line intensity can be evaluated with a portable scanning device. This helps to achieve assay sensitivity comparable to that of ELISA (Byzova et al., 2010).

Biosensors

Biosensors can help to meet the urgent need for rapid, high-capacity, highly selective and sensitive screening methods for the detection of antibiotic residues in foodstuffs. They allow quick and sensitive measurement of response by using effective sensing elements based on different signal generation principles. A variety of bio- and immunosensor techniques have been developed for amphenicol detection, but few of them have been used for amphenicol detection in real samples (Tables 4 and 5).

Rinken and Riik (2006) developed a lactate oxidase-based amperometric biosensor for the rapid determination of CAP and penicillin residues in raw milk. However, the sensor was able to detect these antibiotics only in the ppm (mg/kg) concentration range. The concentration of antibiotics was determined by two characteristic reaction parameters, calculated from the biosensor transient response with the dynamic biosensor model. The dynamics of the oxidation of lactic acid catalyzed by lactate oxidase was followed with a cylindrical membrane-covered oxygen sensor. The authors observed an antagonistic effect due to the simultaneous presence of these antibiotics.

The only flow-injection fluorescence biosensor developed for FF detection in animal tissues was described by Ge et al. (2010). FF from a sample (liver, meat, fish) was purified using MIP columns. Other interesting applications of MIP technology will be described later (see "Alternative methods"). The assay principle was based on inhibition of fluorescence intensity (fluorescence energy transfer) of 3-p-nitrylphenyl-5-(2′-sulfonophenylazo) rhodanine and BSA in the presence of FF. Experiments with real samples proved that the developed sensor can be used for rapid and sensitive (< MRL) analysis of FF residues.

Immunosensors

Immunosensors which are based on affinity binding between specific antibodies and target analytes have a wide range of applications. Immunosensors can utilize various techniques for signal registration after analyte-antibody interaction occurs. The potential application of a few methods for real-time detection of CAP in solution was demonstrated (Table 5). However, most of the methods lack the required sensitivity for real sample analysis (Table 5).

Label-free methods do not require an additional reagent such as a label for sensor response and can be used as simple, effective, and rapid screening methods. A label-free, antibody immobilized quartz crystal microbalance (QCM) system for detection of CAP was developed by Park et al. (2004) and later by Adányi et al. (2006). To bind an anti-CAP antibody onto the gold electrode surface of piezoelectric crystals, a self-assembled monolayer (SAM) was formed by a chemisorption procedure. A CAP solution was injected into the reaction cell and frequency shifts were measured. Repeated use of the sensor up to eight times was possible (Park et al., 2004). A detection limit of 10^{-5} M (32.3 mg/L) CAP was calculated (Park et al., 2004). Later, Adányi et al. (2006) developed a more sensitive method, with a limit of detection of 3×10^{-6} M (969 μg/L) CAP being reported. A label-free optical waveguide light mode spectroscopy (OWLS) immunosensor system was also developed by Adányi et al. (2006). All experiments were performed using a flow-injection analyzer system with the surface of the waveguide sensor being silanized. The covalent coupling of antibodies was performed in the flow-through cell just before the measurements. The assay was more sensitive than the QCM system described above (Table 5).

Another label-free technique, microcantilever immunosensor, was developed for the sensitive detection of clenbuterol and CAP (Tan et al., 2010). The detection limits were 0.1 and 0.2 ng/mL, respectively. Protein A was used to modify the microcantilever gold surface and to immobilize specific antibodies. The deflection of the V-shaped microcantilever was measured by monitoring the position of a laser beam reflected from the microcantelever (the apex). After the analyte was added into the fluid cell, microcantilever deflection was monitored in situ. The authors stressed that microcantilever immunosensors have the noticeable advantages of being label-free, detecting multiple analytes in a single step in real time, being suitable for in situ monitoring, and consequently lowering the analytical cost.

Park and Kim (2006) developed a simple and inexpensive chemiluminescent immunosensor. Specific anti-CAP antibodies were immobilized onto a nylon membrane that was installed in a flow-through cell. A mixture of CAP standard plus CAP-HRP conjugate was injected into the cell, followed by substrate solution. The emitted light was then measured. The analysis time was around 5 min, with high sensitivity reported (10^{-8} M or 3.2 μg/kg). CAP measurements in model samples (pork, beef, chicken, shrimp, milk) were also conducted.

Recently Kim et al. (2010) developed a sensitive amperometric immunosersor for CAP detection at the pg/mL level. The surface of a glassy carbon electrode was enlarged with gold nanoparticles, dendrimers, and cadmium sulfide nanoparticles for further enhancement of assay sensitivity (Table 5). As a recognition element the authors used anti-chloramphenicol acetyltransferase antibody. Free CAP and CAP labeled with hydrazine competed for the binding sites. As a final step, the

hydrazine catalyzed the electrochemical reduction of hydrogen peroxide. The immunosensor was examined in real beef, chicken, and pork samples for the analysis of CAP.

Ultra trace analysis of CAP by real-time, label-free impedimetric immunosensor was described by Chullasat et al. (2011). This technique is based on the change in interfacial property (resistance and/or capacitance) between the electrode surface and analyzed solution when immobilized antibodies react with an antigen. The authors used a multilayer electrode modified with a self-assembled thiourea monolayer followed by gold nanoparticles, mercaptosuccinic acid, and antibody. The modified electrode can be reused up to 45 times. The determination limit in buffer was as low as 1.0×10^{-16} M (3.2×10^{-8} μg/kg). In shrimp it was possible to detect 1.6 ng/kg of CAP, which is much lower than the MRPL.

None of the described bio- and immmunosensors developed so far for CAP detection have been validated and used for routine food analysis. The only biosensor technique used for this purpose at the date of writing is the surface plasmon resonance (SPR)-based biosensor.

Surface Plasmon Resonance (SPR) Biosensor Methods

SPR-based biosensors have become a powerful analytical tool and are currently actively used for routine food analysis (Elliott et al., 1998; Haughey and Baxter, 2006). SPR biosensors utilize the principle of label-free interaction analysis, with interactions between molecules such as antigen and antibody being monitored in real time. The principle of the method is based on the surface plasmon resonance phenomenon. For the analysis to take place a derivative of the target analyte is covalently immobilized on the surface of a sensor chip with a thin gold layer. A mixture of recognition protein (antibody) and the sample to be analyzed is passed over a flow cell on a chip. When the antibody binds to the derivative on the chip surface, this changes the bound mass and generates a proportional response (the change in refractive index), which can be monitored in real time. After the measurement, the chip has to be regenerated with a basic, acidic, high-salt, or detergent solution to remove bound molecules and prepare the chip for the next injection.

SPR biosensor–based methods were developed for CAP and CAP glucuronide detection in various matrices including muscle, kidney, prawns, honey, and milk (Table 4) and the simultaneous qualitative detection of all amphenicols in shrimp (Dumont et al., 2006). For the simultaneous detection of amphenicols a mixture of a sample extract along with polyclonal antibodies produced against a CAP succinate-BSA conjugate was passed over four flow cells of a sensor chip. A flow cell where CAP base was immobilized on the surface was used for CAP detection. The three other flow cells had FF amine immobilized on the chip surface and were used for FF, FF amine, and TAP detection. Thus, a single chip and one polyclonal antibody were used for the detection of all the amphenicols in a single sample extract. Ferguson et al. (2005) used a commercially available Qflex® kit for Biacore® Q biosensor for the highly accurate and reliable detection of CAP and CAP glucuronide in poultry muscle, honey, prawns, and milk in concentrations below the MRPL. The method was well validated for all reported matrices. A portable self-built SPR system was described recently (Dong et al., 2009); the limit of detection of CAP in pork meat extracts was reported to be 0.5 ng/mL.

The latest achievement to be published is a rapid and ultrasensitive SPR biosensor method with limits of detection as low as 0.74 fg/mL for CAP in buffer and 17.5 fg/mL for CAP in honey (Yuan et al., 2008). Such a high sensitivity was achieved through the use of large gold nanoparticles (40 nm) for signal enhancement on an m-SAM sensor surface with an immobilized conjugate of CAP derivative with OVA. The assay was further modified and carried out without any surface regeneration for rapid and sensitive CAP detection (Yuan et al., 2009). This was made possible by the preparation of a new surface with a newly immobilized derivative (CAP-carbamate-PEG-NH_2), which allowed fast association and dissociation between CAP and antibody to occur. Elimination of surface regeneration helped to maintain the stability of the immobilized surfaces for multiple sample injections and surface binding events.

The use of SPR biosensor systems in food safety analysis has been recently reviewed by Huet et al. (2010) and Situ et al. (2010). The widespread use of SPR has been constrained by the high price of the instruments and will become a mainstream screening procedure only when these costs are reduced.

Microarrays and Simultaneous Detection of Multiple Analytes

Immunoassays are usually designed for a specific single-analyte detection, except for some approaches where antibodies with generic specificity have been used. This can be considered as a disadvantage in comparison with chromatographic methods. Over the past decade much effort has been directed toward the miniaturization and increasing the throughput of immunoassay systems, along with reduced sample and reagent consumption, i.e., microarray development. Microarrays are designed for simultaneous detection of a number of different analytes. Usually, microarrays are constructed in the following way. Recognition elements towards different substances (for instance, antibodies) are immobilized on different parts of a chip (usually in spots). The sample is then injected over the surface and where interactions occur signals are generated. Microarrays can provide a highly sensitive and precise technique for obtaining information from biological samples. With the features of high-throughput, miniaturization, and automation, microarrays show great promise for the screening of foodstuffs for the presence of a variety of unwanted chemicals. A few approaches for simultaneous multi-analyte detection (including CAP) were published recently (Table 6).

CAP, clenbuterol, and tylosin were detected simultaneously and quantitatively by small molecular microarray (SMM) (Peng

and Bang-Ce, 2006). For this application three drugs attached to carrier proteins were covalently immobilized onto the surface of modified glass slides. The assay was constructed in an indirect format using three specific antibodies and secondary antibodies labeled with Cy5 fluorescent dye. Although analysis time was comparable to that of ELISA (about 2 h), during this time three analytes were simultaneously detected at concentrations lower then MRL/MRPL. Measurements in various foods (50 samples) were in agreement with ELISA results. A similar approach, but in a direct format with covalently immobilized antibodies, was used by Gao et al. (2009). An immunochip was designed for atrazine, nonylphenol, 17-beta estradiol, paraverine, and CAP detection. However, the detection limits achieved with this application still needs to be decreased to have any practical application.

Suspension array technology for the simultaneous detection of CAP, clenbuterol, and 17-beta-estradiol in a 96-well microplate format has been developed (Liu et al., 2009). The method was based on the Luminex platform technology (χMAP®), where a high-throughput and efficient microsphere format is utilized. A suspension array is simply a transfer of the microarray format from a glass slide (planar and solid microarray) to a microsphere format. The system is flexible and easy-to-use and provides more information with less sample volume required. For analysis, the BSA conjugates of three analytes were coupled to fluorescent microscopic beads with a particular color code (spectral address) to permit discrimination of individual assays (one conjugate per type of bead). The next stage included incubation of a conjugate-coupled bead mixture with a standard solution contained CAP, clenbuterol, 17-beta-estradiol, and three biotin-labeled specific antibodies, followed by a reaction with streptavidin-R-phycoerythrin, and, finally, simultaneous registration of the median fluorescence intensity for each type of bead. The limits of detection were 40, 50, and 1000 ng/L for CAP, clenbuterol, and 17-beta-estradiol, respectively, but no measurements in food matrix were performed. In the next study the authors used the same technology to detect five antibiotics including tylosin, tetracycline, gentamicin, streptomycin, and CAP in milk (Su et al., 2011). The assay format was also modified in that biotinilated secondary antibodies were used instead of biotinilated primary antibodies due to instability issues in using the latter format. This alteration was also found to improve assay sensitivity. However, despite this modification, the limit of detection for CAP was still far from that required to meet MRPL demands.

Rebe Raz et al. (2009) developed a microarray biosensor based on an imaging surface plasmon resonance platform (iSPR). Seven antibiotics (namely, neomycin, gentamicin, kanamycin, streptomycin, sulfamethazine, CAP, and enrofloxacin) were quantitatively and simultaneously detected in a model matrix (10-fold diluted full-fat goat milk powder) using a single round sensor chip. For the assay, a multistandard solution containing all seven antibiotics was mixed with a cocktail of seven specific antibodies and injected over a sensor chip arrayed with antibiotics. The assay of five antibiotics was sensitive enough for milk control at the MRL level. However, CAP and gentamicin could be controlled only at 22xMRPL and 4xMRL, respectively. The authors stressed that the overall performance of the microarray biosensor was comparable to that reported for conventional SPR biosensors with four flow channels in terms of assay sensitivity and robustness.

Recently, a new approach for the simultaneous detection of multiple analytes (including CAP) in milk was proposed. A novel portable SPR immunosensor in a traditional multichannel format for the simultaneous detection of three representatives of important antibiotic families in milk was described (Fernández et al., 2010). The sensor was based on a novel approach to spectroscopy of surface plasmons that utilizes a special diffraction grating. Hapten-protein conjugates were immobilized onto a surface of a sensor chip via m-SAM (two channels per substance). Before analysis, milk was diluted five times, without a clean-up step. Although enrofloxacin and sulfapyridine were detected far below their MRLs, the detection of CAP was slightly compromised by sample dilution. The immunosensor was developed for on-site analysis of antibiotic residues in whole milk. The device is a small, portable, six-channel device connected to a computer and does not need an external power supply. The whole analytical procedure took about 30 min per sample.

ALTERNATIVE ASSAYS

CAP can be also detected directly by methods where its electrochemical or luminescent properties are used (Table 7). These methods are cheap, quick, easy to perform, do not demand extra reagents for derivatisation, and most of them can be realized in portable format, but in general they lack sensitivity for CAP detection in food. Alternative artificial recognition systems such as MIP were also employed (Table 7).

Electrochemical methods use a signal from the electroreduction of the nitro group of CAP to the amine group. Fossdal and Jacobsen (1971) and later Duda and Kucharska (1999) detected CAP in foods by differential pulse polarography with mercury drop electrodes. Voltammetric determination of CAP in milk was also described (Agüí et al., 2002; Mena et al., 2003; Chai et al., 2006; Xiao et al., 2007). Square-wave voltammetry at electrochemically activated cylindrical carbon fiber microelectrodes (Agüí et al., 2002; Mena et al., 2003), differential pulse volammetry at a glassy carbon electrode (Chai et al., 2006), voltammetry at a single-wall carbon nanotube-gold nanoparticle-ionic liquid composite film modified glassy carbon electrode (Xiao et al., 2007), and cyclic voltammetry at a boron-doped diamond thin-film electrode in flow-injection format (Chuanuwatanakul et al., 2008) were utilized. A disposable electrochemical sensor for the determination of nitroaromatic compounds such as CAP (antibiotic), parathion (pesticide), and 2,4,6-trinitrotoluene (explosive) by a single-run approach was described (Chen et al., 2006). Sensor strips incorporated an electrochemically preanodized screen-printed carbon three-electrode configuration for convenient, fast, low sample volume (20 μL drop), and direct

detection of nitroaromatic compounds by square-wave voltammetry. The analysis was done in a single run simply by measuring the ratio of peak currents between analytes of interest and internal standard. The detection limit for CAP was 0.42 μM (136 μg/L). So far the most sensitive electrochemical method developed for CAP detection in food (milk) was described by Chai et al. (2006). The use of a cationic surfactant, cetyltrimethyl ammonium bromide, improved the reductive peak current and therefore significantly improved the sensitivity of the voltammetric determination of CAP (limit of detection was 0.83 μg/L). Although these methods are not sensitive enough to detect CAP in food at the MRPL level, electrochemical, chemiluminescent, and other physicochemical methods have been successfully used for the detection of CAP in pharmaceutical preparations such as ophthalmic solutions where CAP concentrations to be determined are high (Zhao et al., 1998; Wang et al., 1999; David et al., 2000; Icardo et al., 2003; Zhuang et al., 2011; Yang et al., 2011).

A few interesting applications of a new artificial recognition system, MIP, have been described (Suárez-Rodríguez and Díaz-García, 2001; Mena et al., 2003; Thongchai et al., 2010). MIPs are easily obtained by copolimerization of suitable functional monomers and cross-linkers in the presence of the print molecule. Removal of the template leaves a polymer that selectively recognizes the target analyte. MIPs are usually reusable and exhibit high stability. A fluorescent competitive flow-through assay for CAP detection using MIP as recognition phase was developed (Suárez-Rodríguez and Díaz-García, 2001). In this assay CAP base conjugated with a fluorescent dye (dansyl-chloride) was used as a labeled component. Free and labeled CAP competed for the recognition points of the MIP placed into a flow cell. It should be stressed that the system needs further development in terms of improving sensitivity and selectivity.

Another application of MIPs, their use for sample preconcentration and clean-up before analytical detection, has been described (Mena et al., 2003; Thongchai et al., 2010). Mena et al. (2003) used MIP as a selective SPE sorbent prior to the voltammetric determination of CAP. In these studies large volumes of milk sample (17 mL) had to be passed for long time periods (68 min) to achieve a detection limit of 1.4×10^{-8} M (4.5 μg/L). MIPs were also used as a preconcentration step in a microflow chemiluminescent system for the determination of CAP in honey (Thongchai et al., 2010). For this application the MIP was preloaded into a channel in a planar glass microfluidic device. The microfluidic device consisted of a network of channels etched into a solid substrate (glass). The channel networks were connected to a series of reservoirs containing samples and reagents that formed a complete device or "chip" with overall dimensions of a few cm. The sample containing CAP was first preconcentrated on the MIP, then detected by an enhancement effect on the chemiluminescence reaction of tris(2,2′-bipyridyl) ruthenium(II) with cerium(IV) sulfate in sulfuric acid. The microflow sensor could be used more than 300 times/month (100 samples in triplicate) before the chemiluminescence intensity began to decrease. The system developed was simple, selective, and highly sensitive. The detection limit of the method was well below the MRPL for CAP, and this was achieved due to the high sensitivity of the chemiluminescent detection system. This is the only method among those described in this section that was able to detect CAP at concentrations below the MRPL.

Recently, two approaches were proposed for the simultaneous detection of multiple analytes (including amphenicols). Jafari et al. (2007) evaluated positive corona discharge ion mobility spectrometry (CD-IMS) for the determination of three residual veterinary drugs, including furazolidone, CAP, and enrofloxacin, in poultry. Ion mobility spectrometry (IMS) has been developed as an instrumental analytical technique for detecting and identifying volatile organic compounds based upon the mobilities of gas phase ions in a weak electric field. The major advantage of IMS is the fast response and portability of the system. The authors concluded that the CD-IMS method has great potential for the analysis of veterinary drugs and needs to be further investigated. However, the method was not sensitive enough for CAP detection (limit of detection 66xMRPL). Moreover it was not possible to analyze furazolidone by IMS due to its conversion to metabolites.

The second approach, a capillary electrophoresis (CE) method, was proposed for the simultaneous detection of antibiotics in milk (Pezza et al., 2006; Santos et al., 2007; Vera-Candioti et al., 2010) and tissues (Kowalski et al., 2003, 2008, 2011). CE employs the separation of target substances under high voltage followed by UV detection. CE was sensitive enough to detect TAP and FF together with another six frequently used antibiotics at concentrations below their MRLs using a simple solvent extraction of target analytes from poultry and porcine tissues (Kowalski et al., 2003). However, some of the drugs had similar migration times, and the method was validated separately for each compound. Santos et al. (2007) developed a CE method for the simultaneous screening of six antibiotics (ampicillin, amoxicillin, cloxacillin, penicillin, tetracycline, and CAP) and Vera-Candioti et al. (2010) developed a CE coupled with diode array detection for the simultaneous quantification of four antibiotics of different groups, namely CAP, ciprofloxacin, ampicillin, and sulfamethoxazol. Whereas Santos et al. (2007) prepared milk samples by SPE after protein precipitation with trichloroacetic acid, Vera-Candioti et al. (2010) used quite a long sample preparation technique including defatting, deproteinization, and solvent extraction followed by SPE. Although much effort was devoted to preconcentration of the sample, the method developed by Vera-Candioti et al. (2010) was unable to detect CAP and ampicillin in concentrations at or lower than the MRPL and MRL, respectively. In general, this method was more then ten times less sensitive than one developed earlier (Santos et al., 2007). For instance, in the latter method limits of detection obtained for CE were comparable to that obtained for HPLC; CAP can be detected at a concentration of 2.4xMRPL by CE and at 1.5xMRPL by HPLC. Moreover, low recovery values

for amoxicillin and ampicillin were obtained, mainly due to the low affinity of these antibiotics towards the SPE resin.

The separation and determination of substances containing no functional groups that could be ionized in the pH range between 2 and 12, such as FF, by conventional capillary zone electrophoresis are difficult. Recently, CE in a separation mode referred to as micellar electrokinetic capillary chromatography (MEKC) was used for antibiotic detection in foodstuffs (Table 7). MEKC utilizes sodium dodecyl sulfate as a surfactant in a separation buffer to increase the selectivity of electrophoretic separation and to improve the resolution. MEKC for the simultaneous detection of all three amphenicols (CAP, TAP, FF) was developed in milk using a simple solvent extraction (Pezza et al., 2006) and poultry tissues (Kowalski et al., 2008). Later Kowalski et al. (2011) developed MEKC for the simultaneous detection of CAP, TAP, and FF together with seven sulfonamides using solvent extraction followed by SPE. Limits of detection of amphenicols by MEKC were usually a few micrograms per kilogram (Table 7). MEKC was also successfully used for the simultaneous determination of amphenicols in pharmaceutical preparations (Hillaert and Van den Bossche, 2004; Pajchel et al., 2008). To date none of the CE or MEKC methods has been shown to be capable of detecting CAP at concentration equal to or below the MRPL, whereas TAP and FF are easily detected at concentrations far below their MRLs (Table 7). CE has been demonstrated to be a promising, effective, and economic approach for the separation of a large variety of substances. It is fast (analysis time about 10–15 min), simple, and inexpensive. CE offers a real alternative tool to chromatographic separation techniques.

CONCLUSIONS

The evolution of screening procedures capable of detecting amphenicol residues in foodstuffs has taken about 40 years thus far. Over this time period a large range of biological- and bioassay–based procedures have been developed and validated. The clear trend has been towards reduced time and increased sensitivity. The latter of these parameters has been driven by the ever-reducing concentrations of CAP permitted to be in foodstuffs due to the adverse properties associated with this compound. It is at such times that some thought must be given to the future direction of screening for these compounds. For example, should sensitivities be lowered by a factor of 2, 10, or 100? It is likely that emerging bioanalytical techniques may allow femtogram concentrations to be detected. However, to what purpose? The risks associated with consuming CAP residues at the current MRPL value must be considered negligible; should detection limits be driven by the technological abilities or on a risk-assessment basis? Furthermore, with the finding that it is quite likely that CAP residues can occur due to natural contamination of foods via the food web, exactly what will be gained by "chasing zero"? Surely the emphasis in relation to method development should be about speed and cost and giving laboratories and food companies the ability to detect such residues in the developing world, where most of the problems seem to originate.

ABBREVIATIONS

APTS	γ-aminopropyltriethoxysilane
BGG	bovine gamma globulin
BSA	bovine serum albumin
BS-ELISA	biotin-streptavidin-amplified ELISA
CAP	chloramphenicol
CAT	chloramphenicol acetyltransferase
CD-IMS	corona discharge ion mobility spectrometry
CE	capillary electrophoresis
CEIA	capillary electrophoresis immunoassay
CEIA-LIF	capillary electrophoresis immunoassay with laser-induced fluorescence
CL-ELISA	ELISA with chemiluminescent detection
CMCT	1-cyclohexyl-3-(2-morpholino-ethyl) carbodiimide metho-p-toluene sulphonate
DCC	N,N′-dicyclohexylcarbodiimide
DSC	N,N′-disuccinimidyl carbonate
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EDF	ethylenediamine fluorescein thiocarbamyl
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FF	florfenicol
FPT	four-plate test
GC	gas chromatography
HPLC	high-performance liquid chromatography
HRP	horseradish peroxidase
HSA	human serum albumin
IMS	ion mobility spectrometry
KLH	keyhole limpet hemocyanin
LC	liquid chromatography
LIF	laser-induced fluorescence
MBS	3-maleimidobenzoyl-N-hydroxysuccinimide ester
MIP	molecular-imprinted polymer
MPA	3-mercaptopropionic acid
MRL	maximum residue level
MRPL	minimum required performance limit
NHS	N-hydroxysuccinimide
OVA	ovalbumin
OWLS	optical waveguide light mode spectroscopy
PEG	polyethylene glycol
QCM	quartz crystal microbalance
RIA	radioimmunoassay
RSA	rabbit serum albumin
SAM	self-assembled monolayer
m-SAM	mixed self-assembled monolayer
SAMSA	S-acetyl-mercaptosuccinic anhydride
SMM	small molecular microarray
SPE	solid phase extraction
SPR	surface plasmon resonance

iSPR	imaging surface plasmon resonance
TAP	thiamphenicol
TG	thyreoglobulin
TR-FIA	time-resolved fluoroimmunoassay

REFERENCES

Adányi, N.; Váradi, M.; Kim, N.; Szendrö, I. *Curr. Appl. Phys.* **2006**, *6*, 279–286.

Agthe, O.; Scherk, F. *Arch. Lebensmittelhyg.* **1986**, *37*, 97–99.

Agüí, L.; Guzman, A.; Yáñez-Sedeño, P.; Pingarrón, J. M. *Anal. Chim. Acta* **2002**, *461*, 65–73.

Althaus, R. L.; Torres, A.; Montero, A.; Balasch, S.; Molina, M. P. *J. Dairy Sci.* **2003**, *86*, 457–463.

Arnold, D.; Somogyi, A. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 984–990.

Arnold, D.; vom Berg, D.; Boertz, A. K.; Mallick, U.; Somogyi, A. *Arch. Lebensmittelhyg.* **1984**, *35*, 131–136.

Ashwin, H. M.; Stead, S. L.; Taylor, J. C.; Startin, J. R.; Richmond, S. F.; Homer, V.; Bigwood, T.; Sharman, M. *Anal. Chim. Acta* **2005**, *529*, 103–108.

Beck, M.; Martlbauer, E.; Terplan, G. *Arch. Lebensmittelhyg.* **1987**, *38*, 99–103.

Berendsen, B.; Stolker, L.; de Jong, J.; Nielen, M.; Tserendorj, E.; Sodnomdarjaa, R.; Cannavan, A.; Elliott, C. *Anal. Bioanal. Chem.* **2010**, *397*, 1955–1963.

Berendsen, B. J.; Zuidema, T.; de Jong, J.; Stolker, L. A.; Nielen, M. W. *Anal. Chim. Acta* **2011**, *700*, 78–85.

Blais, B. W.; Cunningham, A.; Yamazaki, H. *Food Agric. Immunol.* **1994**, *6*, 409–417.

Boertz, A. K.; Arnold, D.; Somogyi, A. *Z. Ernahrungswiss.* **1985**, *24*, 113–119.

Bogaerts, R.; Wolf, F. *Fleischwirtschaft* **1980**, *60*, 672–674.

Byzova, N. A.; Zvereva, E. A.; Zherdev, A. V.; Eremin, S. A.; Dzantiev, B. B. *Talanta* **2010**, *81* (15), 843–848.

Calderón, V.; González, J.; Díez, P.; Berenguer, J. A. *Food Addit. Contam.* **1996**, *13*, 13–19.

Campbell, G. S.; Mageau, R. P.; Schwab, B.; Johnston, R. W. *Antimicrob. Agents Chemother.* **1984**, *25*, 205–211.

Chai, C.; Xu, M.; Liu, G. *Chin. J. Anal. Chem.* **2006**, *34*, 1715–1718.

Chen, J. C.; Shih, J. L.; Liu, C. H.; Kuo, M. Y.; Zen J. M. *Anal. Chem.* **2006**, *78*, 3752–3757.

Chuanuwatanakul, S.; Chailapakul, O.; Motomizu S. *Anal. Sci.* **2008**, *24*, 493–498.

Chullasat, K., Kanatharana, P., Limbut, W., Numnuam, A., Thavarungkul, P. *Biosens. Bioelectron.* **2011**, *26*, 4571–4578.

Commission Decision 2001/699/EC. Commission Decision of 19 September 2001 concerning certain protective measures with regard to certain fishery and aquaculture products intended for human consumption and originating in China and Vietnam. *Off. J. Eur. Comm.* **2001**, *L251*, 11–12.

Commission Decision 2001/705/EC. Commission Decision of 27 September 2001 concerning certain protective measures with regard to certain fishery and aquaculture products intended for human consumption and originating in Indonesia. *Off. J. Eur. Comm.* **2001**, *L260*, 35–36.

Commission Decision 2002/249/EC. Commission Decision of 27 March 2002 concerning certain protective measures with regard to certain fishery and aquaculture products intended for human consumption and imported from Myanmar. *Off. J. Eur. Comm.* **2001**, *L84*, 73–74.

Commission Decision 2002/657/EC. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Comm.* **2002**, *L221*, 8–36.

Commission Decision 2003/181/EC. Commission Decision of 13 March 2003 amending Decision 2002/657/EC as regard the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin. *Off. J. Eur. Comm.* **2003**, *L71*, 17–18.

Currie, D.; Lynas, L.; Kennedy, D. G.; McCaughey, W. J. *Food Addit. Contam.* **1998**, *15*, 651–660.

David, V.; Saèz, R. M.; Mateo, J. V.; Calatayud, J. M. *Analyst* **2000**, *125*, 1313–1319.

Dong, Z.; Huang, G.; Xu, S.; Deng, C.; Zhu, J.; Chen, S.; Yang, X.; Zhao, S. *J. Microsc.* **2009**, *234*, 255–261.

Duda, J.; Kucharska, U. *Anal. Lett.* **1999**, *32*, 1049–1064.

Dumont, V.; Huet, A.-C.; Traynor, I.; Elliott, C.; Delahaut, P. *Anal. Chim. Acta* **2006**, *567*, 179–183.

Elliott, C. T.; Baxter, G. A.; Hewitt, S. A.; Arts, C. J. M.; Van Baak, M.; Hellenäs, K. E.; Johansson, A. M. *Analyst* **1998**, *123*, 2469–2473.

Erlich, J.; Bartz, Q. R.; Smith, R. M.; Joslyn, D. A.; Burkholder, P. R. *Science* **1947**, *106*, 417.

European Council Regulation 470/2009. Council Regulation (EEC) No 470/2009 of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No. 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. *Off. J. Eur. Comm.* **2009**, *L152*, 16–24.

European Commission Regulation 1430/94. Commission Regulation (EC) No 1430/94 of 22 June 1994 amending Annexes I, II, III and IV of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off. J. Eur. Comm.* **1994**, *L156*, 6–8.

European Council Regulation 2377/90. Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off. J. Eur. Comm.* **1990**, *L152*, 10–32.

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

Fernández, F.; Hegnerová, K.; Piliarik, M.; Sanchez-Baeza, F.; Homola, J.; Marco, M. P. *Biosens. Bioelectron.* **2010**, *26*, 1231–1238.

Food Standards Agency. A Category B Food Hazard Warning: Chloramphenicol in Chinese honey, 19 February **2002a**. http://www.food.gov.uk.

Food Standards Agency. A Category B Food Hazard Warning: Chloramphenicol in Chinese Royal Jelly, 18 March **2002b**. http://www.food.gov.uk.

Fodey, T.; Murilla, G.; Cannavan, A. *Anal. Chim. Acta* **2007**, *592*, 51–57.

Fossdal, K.; Jacobsen, E. *Anal. Chim. Acta* **1971**, *56*, 105–115.

Freebairn, K. W.; Crosby, N. T.; Landon, J. In *Immunoassays for Veterinary and Food Analysis—1*; Morris, B. A., Clifford, M. N., Jackman, R., Ed.; Elsevier Applied Science: London, 1988; pp. 305–309.

Gao, Z.; Liu, N.; Cao, Q.; Zhang, L.; Wang, S.; Yao, W.; Chao, F. *Biosens. Bioelectron*. **2009**, *24*, 1445–1450.

Gaudin, V.; Maris, P. *Food Agric. Immunol.* **2001**, *13*, 77–86.

Gaudin, V.; Cadieu, N.; Maris, P. *Food Agric. Immunol.* **2003**, *15*, 143–157.

Gaudin, V.; Maris, P.; Fuselier, R.; Ribouchon, J. L.; Cadieu, N.; Rault, A. *Food Addit. Contam.* **2004**, *21*, 422–433.

Gaudin, V.; Hedou, C.; Rault, A.; Verdon, E. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2010**, *27*, 935–952.

Ge, S.; Yan, M.; Cheng, X.; Zhang, C.; Yu, J.; Zhao, P.; Gao, W. *J. Pharm. Biomed. Anal.* **2010**, *52*, 615–619.

Hack, R.; Märtlbauer, E..; Terplan, G. *Food Agric. Immunol.* **1989**, *1*, 197–201.

Hamburger, R. N. *Science.* **1966**, *152*, 203–205.

Hamburger, R. N.; Douglass, J. H. *Immunology* **1969**, *17*, 587–591.

Hanekamp, J. C.; Frapporti, G.; Olieman, K. *Environ. Liability* **2003**, *11*, 209–221.

Hao, K., Guo, S. D., Xu, C. L. *Anal. Lett*, **2006**, *39*, 1087–1100.

Haughey, S. A.; Baxter, G. A. *J. AOAC Int.* **2006**, *89*, 862–867.

Hillaert, S.; Van den Bossche, W. *J. Pharm. Biomed. Anal.* **2004**, *36*, 437–440.

Hock, C.; Liemann, F. *Arch. Lebensmittelhyg.* **1985**, *36*, 138–142.

Holt, D.; Harvey, D.; Hurley, R. *Adverse Drug React. Toxicol. Rev.* **1993**, *12*, 83–95.

Huet, A.-C.; Fodey, T.; Haughey, S. A.; Weigel, S.; Elliott, C.; Delahaut, Ph. *Trends Anal. Chem.* **2010**, *29*, 1281–1294.

Icardo, M. C.; Misiewicz, M.; Ciucu, A.; Garca Mateo, J. V.; Calatayud, J. M. *Talanta* **2003**, *60*, 405–414.

Impens, S.; Reybroeck, W.; Vercammen, J. *Anal. Chim. Acta* **2003**, *483*, 153–163.

Jafari, M. T.; Khayamian, T.; Shaer, V.; Zarei, N. *Anal. Chim. Acta* **2007**, *581*, 147–53.

Keukens, H. J.; Aerts, M. M. L.; Traag, W. A.; Nouws, J. F. M.; de Ruig, W. G.; Beek, W. M. J.; Den Hartog, J. M. P. *J. AOAC Int.* **1992**, *75*, 245–256.

Kim, D.-M.; Rahman, Md. A.; Do, M. H.; Ban, C.; Shim, Y.-B. *Biosens. Bioelectron.* **2010**, *25*, 1781–1788.

Koenen-Dierick, K.; Okerman, L.; de Zutter, L.; Degroodt, J. M.; van Hoof, J.; Srebrnik, S. *Food Addit. Contam.* **1995**, *12*, 77–82.

Kolosova, A. Yu.; Samsonova, J. V.; Egorov. A. M. *Food Agric. Immunol.* **2000**, *12*, 115–125.

Korsrud, G. O.; Naylor, J. M.; Salisbury, C. D.; MacNeil, J. D. *J. Agric. Food Chem.* **1987**, *35*, 556–559.

Kowalski, P.; Oledzka, I.; Lamparczyk, H. *J. Pharm. Biomed. Anal.* **2003**, *32*, 937–947.

Kowalski, P.; Plenis, A.; Oledzka, I. *Acta Pol. Pharm.* **2008**, *65*, 45–50.

Kowalski, P.; Plenis, A.; Oledzka, I.; Konieczna, L. *J. Pharm. Biomed. Anal.* **2011**, *54*, 160–167.

Laurensen, J. J.; Nouws, J. F. *Vet. Q.* **1990**, *12*, 121–123.

Li, K.; Liu, L.; Xu, C.-L.; Chu, X.-G. *Anal. Sci.* **2007**, *23*, 1281–1284.

Lin, S.; Han, S. Q.; Liu, Y. B.; Xu, W. G.; Guan, G. Y. *Anal. Bioanal. Chem*. **2005**, *382*, 1250–1255.

Liu, N.; Su, P.; Gao, Z.; Zhu, M.; Yang, Z.; Pan, X.; Fang, Y.; Chao, F. *Anal. Chim. Acta* **2009**, *632*, 128–134.

Luo, P.; Cao, X.; Wang, Zh.; Jiang, H.; Zhang, S.; Chen, X.; Wang, J.; Feng, C.; Shen, J. *Food Agric. Immunol*. **2009a**, *20*, 57–65.

Luo, P.; Jiang, H.; Wang, Zh.; Feng, C.; He, F.; Shen, J. *J. AOAC Int.* **2009b**, *92*, 981–988.

Luo, P. J.; Jiang, W. X.; Chen, X.; Shen, J. Z.; Wu, Y. N. *J. Anim. Sci*. **2011**, *89*, 3612–3616.

Lynas, L.; Currie, D.; Elliott, C. T.; McEvoy, J. D.; Hewitt, S.A. *Analyst* **1998**, *123*, 2773–2777.

Märtlbauer, E.; Terplan, G. *Arch. Lebensmittelhyg.* **1987**, *38*, 3–7.

McMullen, S. E.; Landsden, J. A.; Schenck, F. J. *J. Food Prot.* **2004**, *67*, 1533–1536.

Mena, M. L.; Agüí, L.; Martinez-Ruiz, P.; Yáñez-Sedeño P.; Reviejo, A. J.; Pingarrón, J. M. *Anal. Bioanal. Chem.* **2003**, *376*, 18–25.

Nouws, J. F. M.; Laurensen, J.; Aerts, M. M. L. *Arch. Lebensmittelhyg.* **1987a**, *38*, 7–9.

Nouws, J. F. M.; Reek, F.; Aerts, M. M. L. Baakman, M.; Laurensen, J. *Arch. Lebensmittelhyg.* **1987b**, *38*, 9–11.

Nouws, J. F. M.; Laurensen, J.; Aerts, M. M. L. *Vet. Q.* **1988**, *10*, 270–272.

Pajchel, G.; Michalska, K.; German, R.; Tyski, S. *Chromtographia* **2008**, *68*, 587–591.

Park, I. S.; Kim, N. *Anal. Chim. Acta* **2006**, *578*, 19–24.

Park, I. S.; Kim, D. K.; Adanyi, N.; Varadi, M.; Kim, N. *Biosens. Bioelectron.* **2004**, *19*, 667–674.

Peng, Z.; Bang-Ce, Y. *J. Agric. Food Chem.* **2006**, *54*, 6978–6983.

Pezza, L.; Rios, A.; Nozal, L.; Arce, L.; Valcarcel, M. *Quim. Nova* **2006**, *29*, 926–931.

Posyniak, A.; Zmudzki, J.; Niedzielska, J. *Anal. Chim. Acta* **2003**, *483*, 307–311.

Rebe Raz, S.; Bremer, M. G. E. G.; Haasnoot, W.; Norde, W. *Anal. Chem.* **2009**, *81*, 7743–7749

Rinken, T.; Riik, H. *J. Biochem. Biophys. Methods.* **2006**, *66*, 13–21.

Sai, N.; Chen, Y.; Liu, N.; Yu, G.; Su, P.; Feng, Y.; Zhou, Z.; Liu, X.; Zhou, H.; Gao, Z.; Ning, B. A. *Talanta* **2010**, *82*, 1113–1121.

Samsonova, J. V.; Fedorova, M. D.; Andreeva, I. P.; Rubtsova, M. Yu.; Egorov, A. M. *Anal. Lett.* **2010**, *43*, 133–141.

Santos, S. M.; Henriques, M.; Duarte, A. C.; Esteves, V. I. *Talanta* **2007**, *71*, 731–737.

Scherk, F.; Agthe, O. *Arch. Lebensmittelhyg.* **1986**, *37*, 146–147.

Schneider, E.; Märtlbauer, E.; Dietrich, R.; Usleber, E.; Terplan, E. *Arch. Lebensmittelhyg.* **1994**, *45*, 43–48.

Scortichini, G.; Annunziata, L.; Haouet, M. N.; Benedetti, F. *Anal. Chim. Acta* **2005**, *535*, 43–48.

Senyk, G. F.; Davidson, J. H.; Brown, J. M.; Hallstead, E. R.; Sherbon, J. W. *J. Food Prot.* **1990**, *53*, 158–164.

Shakila, R. J.; Saravanakumar, R.; Vyla, S. A. P.; Jeyasekaran, G. *Innovat. Food Sci. Emerg. Tech.* **2007**, *8*, 515–518.

Shen, H.-Yu.; Jiang, H.-L. *Anal. Chim. Acta* **2005**, *535*, 33–41.

Shen, J.; Zhang, Z.; Yao, Y.; Shi, W.; Liu, Y.; Zhang, S. *Anal. Chim. Acta* **2006**, *575*, 262–266.

Singer, C. J.; Katz, S. E. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 1037–1041.

Situ, C.; Buijs, J.; Mooney, M. H.; Elliott, C. T. *Trends Anal. Chem.* **2010**, *29*, 1305–1315.

Smadel, J. E.; Jackson, E. B. *Science.* **1947**, *106*, 418–419.
Song, W.-W.; Ding, M.-X.; Zhang, N.-W.; Liu, H.-F.; Xu, M.-G.; Liu, G.-Y.; Chai, C.-Y. *Chin. J. Anal. Chem.* **2007**, *35*, 1731–1735.
Suárez-Rodríguez, J. L.; Díaz-García, M. E. *Biosens. Bioelectron.* **2001**, *16*, 955–961.
Su, P.; Liu, N.; Zhu, M.; Ning, B.; Liu, M.; Yang, Z.; Pan, X.; Gao, Z. *Talanta* **2011**, *85*, 1160–1165.
Tan, W.; Huang, Y.; Nan, T.; Xue, C.; Li, Z.; Zhang, Q.; Wang, B. *Anal. Chem.* **2010**, *82*, 615–620.
Tajik, H.; Malekinejad, H.; Razavi-Rouhani, S. M.; Pajouhi, M. R.; Mahmoudi, R.; Haghnazari, A. *Food Chem. Toxicol.* **2010**, *48*, 2464–2468.
Thongchai, W.; Liawruangath, B.; Liawruangrath, S.; Greenway, G. M. *Talanta* **2010**, *82*, 560–566.
Tsai, C. E.; Kondo, F. *J. Food Prot.* **2001**, *64*, 361–366.
van de Water, C.; Haagsma, N. *J. Assoc. Off. Anal. Chem.* **1990a**, *73*, 534–540.
van de Water, C.; Haagsma, N. *Food Agric. Immunol.* **1990b**, *2*, 11–19.
van de Water, C.; Haagsma, N. *J. Chromatogr.* **1991**, *566*, 173–185.
van de Water, C.; Haagsma, N.; van Kooten, P. J.; van Eden, W. *Z. Lebensm. Unters. Forsch*. **1987**, *185*, 202–207.
van Os, J. L.; Beukers, R. *J. Food Prot*. **1980**, *43*, 510–511.
Vera-Candioti, L.; Olivieri, A. C.; Goicoechea, H. C. *Talanta* **2010**, *82*, 213–221.
Wang, A.; Zhang, L.; Fang, Y. *Anal. Chim. Acta.* **1999**, *394*, 309–316.
Wang, L.; Zhang, Y.; Gao, X.; Duan, Z.; Wang, S. J. *Agric. Food Chem.* **2010**, *58*, 3265–3270.
Wesongah, J. O.; Murilla, G.; Guantai, A. N.; Elliot, C.; Fodey, T.; Cannavan, A. *J. Vet Pharmacol. Therap.* **2007**, *30*, 68–73.
Wu, J.-E; Chang, C.; Ding, W.-P.; He, D.-P. *J. Agric. Food Chem.* **2008**, *56*, 8261–8267.
Xiao, F.; Zhao, F; Li, J.; Yan, R.; Yu, J.; Zeng, B. *Anal. Chim. Acta.* **2007**, *596*, 79–85.
Xu, C.; Peng, C.; Hao, K.; Jin, Z., Wang, W. *Luminescence* **2006**, *21*, 126–128.
Yang, X. F., Li, N. B., Luo, H. Q. *Luminescence* **2011**, in press.
Yuan, J.; Oliver, R.; Aguilar, M. I.; Wu, Y. *Anal. Chem.* **2008**, *80*, 8329–8333.
Yuan, J.; Addo, J.; Aguilar, M. I.; Wu, Y. *Anal. Biochem.* **2009**, *390*, 97–99.
Yunis, A. A. *Am. J. Med.* **1989**, *87*, 44N–48N.
Zhang, S.; Zhang, Z.; Shi, W.; Eremin, S. A.; Shen, J. *J. Agric. Food Chem.* **2006**, *54*, 5718–5722.
Zhang, D.; Zuo, P.; Ye, B.-C. *J Agric. Food Chem.* **2008a**, *56*, 9862–9867.
Zhang, C.; Wang, S.; Fang, G.; Zhang, Y.; Jiang, L. *Electrophoresis* **2008b**, *29*, 3422–3428.
Zhao, M.; Hu, J.; Lai, Y.; Li, Q.; Wu, Z. *Anal. Lett.* **1998**, *31*, 237–249.
Zhuang, Y.-F., Zhu, S.-N., Wei, W., Li, J.-L. *Luminescence*. **2011**, in press.