Food Additives and Contaminants, April 2006; 23(4): 362–384

Taylor & Francis
Taylor & Francis Group

Analysis of sulphonamide residues in edible animal products: A review

S. WANG[1], H. Y. ZHANG[1], L. WANG[1], Z. J. DUAN[1], & I. KENNEDY[2]

[1]*Tianjin Key Laboratory of Food Nutrition and Safety, Faculty of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Tianjin 300222, P. R. China and* [2]*Faculty of Agriculture, Food and Natural Resources, University of Sydney, Sydney, NSW 2006, Australia*

(Received 30 July 2005; revised 30 September, 14 and 27 November 2005; accepted 27 November 2005)

Abstract
The methods of analysis for sulphonamide residues in edible animal products are reviewed. Sulphonamides are widely used for therapeutic and prophylactic purposes in both humans and animals, sometimes as growth promoters as additives in animal feed. As a result of their widespread use, there is concern about whether the levels used of these drugs can generate serious problems in human health, e.g., allergic or toxic reactions. Several methods for the determination of sulphonamides have been reported in the literature and this review considers high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC/MS), gas chromatography (GC), thin-layer chromatography (TLC), high-performance capillary electrophoresis (HPCE), enzyme-linked immunosorbant assay (ELISA), biosensor immunoassay (BIA) and microbiological methods. Specific aspects of analysing sulphonamides, such as sample handling, chromatographic conditions and detection methods are discussed. Methods for drug residue monitoring should be accurate, simple, economical in both time and cost, and capable of detecting residues below the maximum residue limits (MRL). The current sulphonamide detection technologies are based on chromatographic methods or bacteriological growth inhibition. The instrumental methods such as HPLC and GC are both sensitive and specific, but are laborious and expensive. Because of the labour-intensive processes, only a few cases of GC methods applied to residue analysis have been published. These methods are suitable for confirmation but not for screening of large numbers of samples. Microbiological methods do not require highly specialized and expensive equipment. They also use highly homogeneous cell populations for testing and thus result in better assay precision. Although HPCE has powerful separation ability, the precision is poor and the instrument still needs to be improved. To date, this technique has not been widely applied to routine analysis. Currently, TLC has been almost replaced by other instrumental analysis. A rapid, sensitive and specific assay is required to detect positive samples in routine analysis, which can then be confirmed for the presence of sulphonamides by HPLC. Immunochemical methods such as ELISA can be simple, rapid and cost-effective, with enough sensitivity and specificity to detect small molecules. This review can be considered as a basis for further research aimed at identifying the most efficient approaches.

Keywords: *Review, analysis, sulphonamides, veterinary drugs, antibiotics, animal feed*

Introduction

The chemical class of sulphonamides shares a common *p*-aminobenzoyl ring moiety with an aromatic amino group at the N4-position, differing in the substitution at the N1-position. The backbone and structures of the sulphonamides described in this review are shown in Figure 1. Sulphonamides are widely used for therapeutic and prophylactic purposes in both people (Kim & Park 1998) and animals (Schwarz & Chaslus-Dancla 2001), sometimes being used as additives in animal feed because prolonged ingestion of sulphonamides may have a growth-promoting effect (Long et al. 1990). If the proper withdrawal periods are not observed before slaughtering or milking of the medicated animals, meat and milk from these animals may be contaminated with residual sulphonamides (Saschenbrecker & Fish 1980, Franco et al. 1990, McEvoy et al. 1999). At present, sulphonamides and other drugs (chlortetracycline, penicillin and several ionophores) are the most common contaminating antimicrobials in animal feed, generating potentially serious problems in human health, such as allergic or toxic reactions. Furthermore, the main risk from the

Correspondence: S. Wang. E-mail: s.wang@tust.edu.cn

ISSN 0265–203X print/ISSN 1464–5122 online/06/00000362–23 © 2006 Taylor & Francis
DOI: 10.1080/02652030500499359

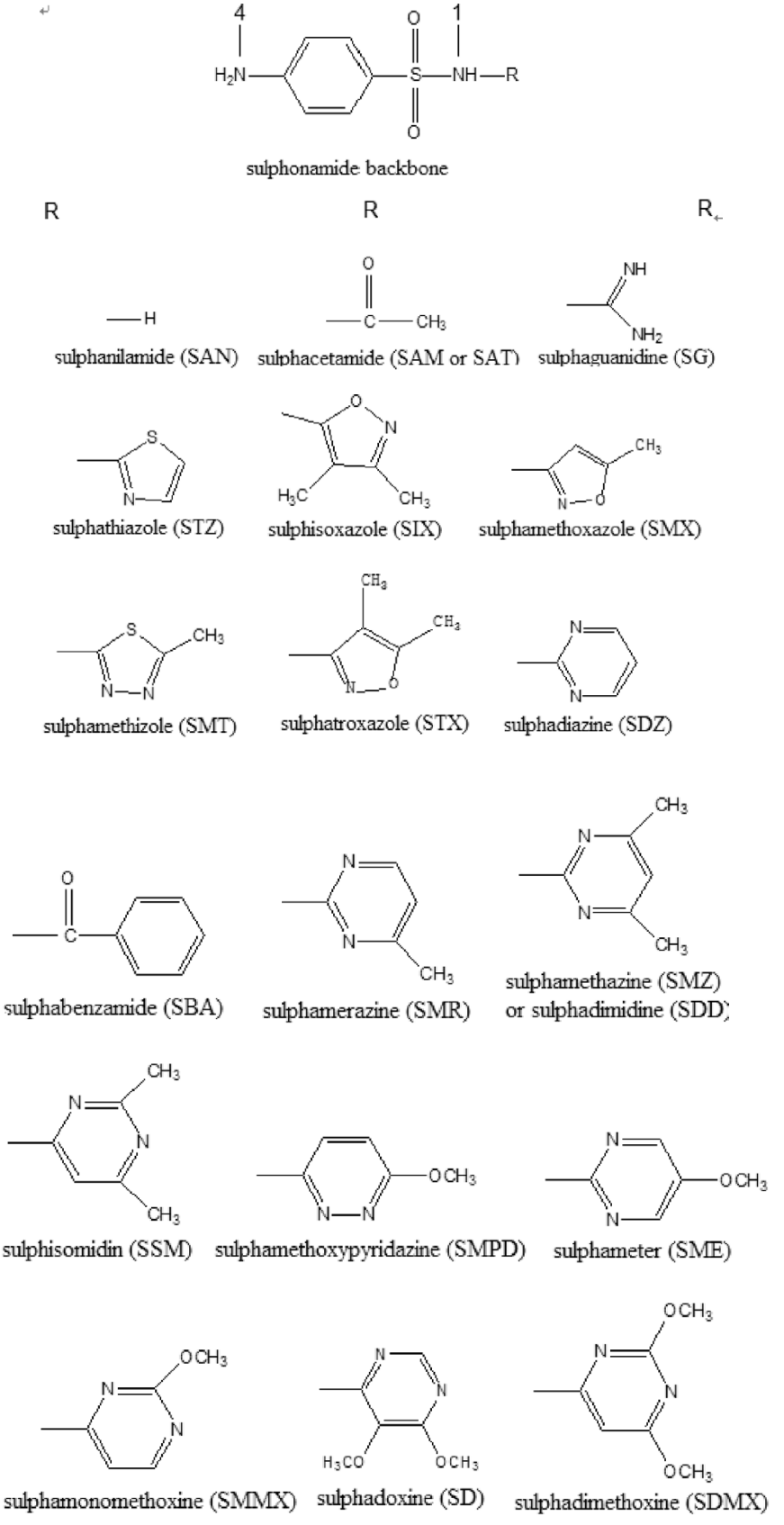

Figure 1. Structures of sulphonamides.

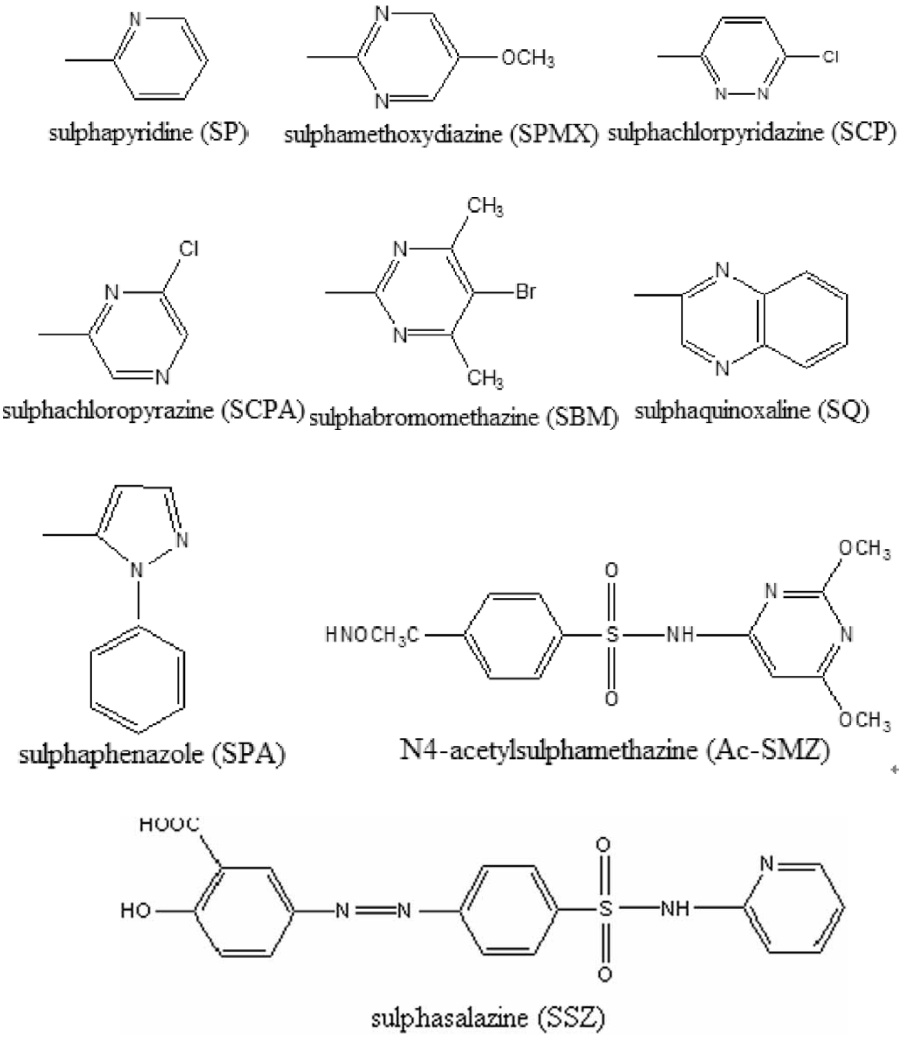

Figure 1. Continued.

excessive use of antimicrobials in animals is that bacteria may develop resistance. In addition, some sulphonamides have been found to be potentially carcinogenic and this fact has become a cause for considerable debate in food safety. It has been estimated that approximately 5% of human patients medicated with sulphonamides received unwanted effects from the drugs (Bevill 1984, Montanaro 1998). As a result, the presence of sulphonamide residues in food was considered harmful to consumers. In order to protect consumers from risks related to drug residues, maximum residue limits (MRL) have been established by law in many countries. In Europe, Canada and the USA, the MRL for the total sulphonamide concentrations in edible tissues is $100\,\mu g\,kg^{-1}$ (Food and Drug Regulation 1991, EU Regulation 1999), and $20\,\mu g\,kg^{-1}$ in Japan (Pastor-Navarro et al. 2004).

Two reviews on the analysis of antibiotics published to date (Agarwal 1992, Guggisber et al. 1992, Shaikh & Moats 1993, Zomer et al. 1996, Marzo & Dal Bo 1998) concentrate on sulphonamides, mainly on high-performance liquid chromatography (HPLC) methods for the determination of sulphonamides in tissue, milk, meat and eggs. The present review extends the scope to include other methods such as, enzyme-linked immunosorbent assay (ELISA), biosensor immunoassay (BIA) and high-performance capillary electrophoresis (HPCE), and has added new progress of the microbiological methods, gas chromatography (GC), thin-layer chromatography (TLC) and HPLC methods.

Most of the papers reviewed refer to the analysis of animal tissues such as muscle, liver, kidney, skin and fat. Other matrices analysed are salmon muscle, skin and liver tissues (Zheng et al. 1994, Kitts et al. 1995, Gehring et al. 1996). With respect to analytes, most papers deal with sulphamethazine (SMZ), sulphadiazine (SDZ), sulphamerazine (SMR) and sulphathiazole (STZ) using other examples with lower frequency. Methods described in the literature usually allow

the multiresidue determination of one or more analytes, because of their similarity in behaviour.

Analytical methods

Sulphonamide residue analysis generally involves extraction with an appropriate solvent followed by one or more clean-up processes and then quantitative determination. Current conventional sulphonamide detection methods capable of measuring a wide spectrum of different sulphonamides include HPLC, TLC, HPCE, ELISA, BIA and microbiological assays. HPLC has been used most frequently as it provides a sensitive and specific, but very laborious and expensive, analytical method. The method is more suitable for confirmation of contamination than for screening of large numbers of test samples.

Tables I–IV summarize detailed relevant information about the analytical methods (HPLC, TLC, HPCE, ELISA, immunochemical assays) for animal tissues. Tables I–III include sample treatment for extraction and clean-up steps, specify the separation and detection techniques, columns and mobile phases in HPLC, TLC and HPCE, and include recovery data from spiked experiments, indicating the recovery for each sulphonamide. Table IV summarizes the sample treatment, conjugation methods for immunogens, antibodies and comments on the methods.

Extraction methods

Traditionally, the extraction of sulphonamides from meat, milk and eggs has been performed with organic solvents. Sulphonamides are not very soluble in non-polar solvents, but have good solubility in more polar solvents. Extraction is generally carried out with chloroform, methylene chloride, acetone, acetonitrile or ethyl acetate (Papapanagiotou et al. 2000, Stoev & Michailova 2000, Roudaut & Garnier 2002). Some organic solvents also denature the sample protein, which results in a cleaner extraction and helps release any drug residues bound to proteins (Barbieri et al. 1995). Liquid–liquid extraction (LLE) often followed by solid-phase extraction (SPE) clean-up step (Kim et al. 2003, Maudens et al. 2004, Pecorelli et al. 2004). Some of the newer methods have replaced the traditional liquid extraction step with ultra-filtration (Furusawa et al. 1999a, 2001, 2003a), matrix solid-phase dispersion (Boulaire et al. 1997, Bogialli et al. 2003, Kishida & Furusawa 2003). The ultra-filter unit eliminates many steps and problems associated with classical clean-up techniques, e.g., emulsion and reduced recoveries, and consequently increase clean-up yields. The technique also reduces analytical costs. Matrix solid-phase dispersion (MSPD) offers distinct advantages over classical sample treatment procedures in that (1) the analytical protocol is drastically simplified and shortened; (2) the possibility of emulsion formation is eliminated; and (3) solvent consumption is substantially reduced. SPE can be applied to liquid samples directly or used as a clean-up step after the extraction of the solid samples, and eliminates the use of large amounts of solvent for extraction. Supercritical fluid extraction has also been used to the extraction of sulphonamides from eggs (Pensabene et al. 1997), meat (Din et al. 1997), beef and chicken liver (Ashraf et al. 1996, Combs et al. 1996), and spray-dried milk powder (Malik et al. 1994).

Clean-up procedures

Because of the complex nature of the sample matrices, a clean-up step is required before the chromatographic determination. Sample clean-up procedures include column chromatography, TLC, LLE, SPE and MSPD. The application of these techniques in residue analysis has been adequately addressed in both the scientific and commercial literature. Most of the treatments use the consistency of LLE (Papapanagiotou et al. 2000, Roudaut & Garnier 2002, Szymanski et al. 2003) and/or SPE (Kim et al. 2003, Pang et al. 2003, Garcia et al. 2004, Maudens et al. 2004). For the initial extraction of analyte from the sample matrix, most workers carry out homogenization of the sample with solvent. Some workers have employed MSPD where the sample is directly blended with chromatographic material such as bonded silica (McCracken et al. 2000). For a clean-up or enrichment method for sulphonamides in milk, precipitation, ultra-filtration, LLE and SPE-ion exchange, C_{18}, cychlodextrin-bonded phase, alumina and cyclohexyl were used. Bondapak Cl*-Corasil gave very good results with milk samples, but meat and egg samples were not cleaned up enough on this packing material (Agarwal 1992).

However, clean-up procedures vary widely and do not necessarily depend on either the sample matrix or the solvent used in the previous extraction step. The purpose of these additional clean-up steps is to extract sulphonamides selectively while leaving other interfering compounds behind. As sulphonamides are ionic in nature, when extracting sulphonamides from an organic phase into an aqueous phase, the adjustment of the pH of the aqueous phase to the proper range is critical to obtain complete recoveries (Unruh et al. 1990, Pang et al. 2003).

Determination techniques

For the determination of sulphonamides, GC/MS, LC/ultraviolet-visible (UV), LC/fluorescence

Table I. HPLC methods for the determination of sulphonamide residues in edible animal product.

Sample	Analyte	Sample treatment	Determination technique	Recovery	Comments	Reference
Honey	SG, SAN, SAM, SDZ, STZ, SP, SMR, SME, SMZ, SMPD, SCP, SDD	Sample pretreatment included acidic hydrolysis, followed by liquid–liquid extraction and SPE on a strong cation exchanger	HPLC-fluorescence, with an RP-18 column (15 cm × 4.6 mm i.d., 5 μm), radient elution: 2% solvent B → 35% solvent B in 31 min → 75% solvent B in 10 min. A: 0.020 mol l^{-1} acetate buffer (0.0819% (w/v), sodium acetate in water, pH 4.75)–acetonitrile (98:2, v/v); B: the same acetate buffer–acetonitrile (68:32; v/v); detection at an excitation wavelength of 420 nm and an emission wavelength of 485 nm	40–67%	LOD and LOQ had values of 1 or 2 and 2 or 5 μg kg^{-1}, respectively.	Maudens et al. (2004)
Chicken plasma	SMMX, SDMX	Mixed with 4 mol l^{-1} ammonium sulfate solution then centrifuged	HPLC with a polyethylene glycol reversed-phase column (15 cm × 4.6 mm i.d., 5 μm); mobile phase: 0.001 mol l^{-1} sodium acetate solution; photodiode array detection at 267 nm	> 78%	LOD ≤ 90 ng ml^{-1}	Kishida et al. (2004)
Muscle	SDZ, STZ, SP, SMR, SMZ, SCP, SMMX, SMX, SQ, SDMX	Extracted with ethyl acetate, centrifuged, the residue was suspended in ethyl acetate, purification using Speedisk column, eluted with methanol–ammonia (97.5 : 2.5, v/v).	HPLC with a C_8 (25 cm × 3 mm i.d., 5 μm); mobile phase: A (acetonitrile) (%), B (acetate buffer, pH 4.5) (%) in gradient mode: t_0: A% = 15%; t_{22}: A% = 41%; t_{24}: A% = 15%; t_{30}: A% = 15%; UV detector was set at 270 nm	In the range 72–92% except for sulfathiazole with 63%	LOD: 30–70 ng ml^{-1}	Pecorelli et al. (2004)
Swine, cattle muscle, liver, sheep kidney, horse muscle, kidney; chicken muscle, liver	SCP, SDZ, SDMX, SD, SMZ, SMPD, SQ, STZ	Extracted using a modified version of AOAC Official Method 983.31 (Sulfonamide Residues in Animal Tissues)	HPLC with a C_{18} column; mobile phase: 0.02 mol l^{-1} phosphoric acid–acetonitrile (60.5/39.5); fluorescence (excitation, 405 nm; emission, 495 nm). The sample is a derivatized pre-column with fluorescamine	No relative information	LOD for all sulfonamides was 10 μg kg^{-1}, with the exception of SQ, for which the LOD was 15 μg kg^{-1}	Salisbury et al. (2004)

Water, urine, milk, bovine liver and kidney tissues	5-SMC, SG, SMX, SMR, SMT, SMZ, SAM, SP, SBA, SMPD, SQ, SMMX, SDMX, SSZ, SDZ, STZ	SLM made up of 5% TOPO dissolved in hexyl amine was used as a sample clean-up and/or enrichment technique	HPLC coupled to MS	No relative information	LOD: 1.8 ng ml^{-1} for SG and SMR, 3.3–10 ng ml^{-1} for the other in bovine liver and kidney; 2.1 ng ml^{-1} for SG and SMR, 7.5–15 ng ml^{-1} for the other in cow's urine, 12.4 ng ml^{-1} for SG and SMR, 16.8–24.3 ng ml^{-1} for the other in milk	Msagati & Nindi (2004)
Porcine kidney	SMPD, SMX, SDMX	Added phosphoric acid in the solid-phase extraction step, and using methanol to elute the analytes	HPLC–DAD	No relative information	No information	Garcia et al. (2004)
Milk	SDZ, SMR, SMZ, SMX, SMMX, SDMX	Isolated by a hollow dialysis fibre	HPLC with a Zorbax Eclipse XDS-C8 column (150 × 4.6 mm i.d., 5 μm); mobile phase: 25:75 (v/v) of acetonitrile and 0.01 mol l^{-1} phosphate buffer (pH 4.0); UV detector was set at 260 nm	97.8–100.4%	LOD in sub-ng ml^{-1} levels	Yang et al. (2004)
Milk	SMZ	Extracted with acetonitrile then cleaned up using SPE	HPLC/APCI-MS with an ODS-2 column, mobile phase: acetonitrile–water: 0.1 mol l^{-1} aqueous ammonium acetate solution (17:33:50 or 13:37:50, v/v/v)	No relative information	LOD: 8.2 ng ml^{-1}	Kim et al. (2003)
Meat: chicken, beef, pork	SDZ, SDD, SMMX, SMX, SQ, SDMX	Sample preparation is carried out by MPSD with an aqueous ethanol solution	HPLC with a Mightysil RP-4 GP column; mobile phase: 2% (v/v) acetic acid solution (pH 2.7, in water)–ethanol (75:25, v/v); photodiode array detection	>85%	LOQ: 6–40 ng ml^{-1}	Kishida & Furusawa (2003)
Honey	STZ	Liquid-phase extraction using 1 mol l^{-1} HCl; precolumn derivatization	HPLC with Hypersil BDS C_{18} (25 cm × 4.6 mm i.d., 5 μm) column; mobile phase: 2% acetic acid/acetonitrile (60:40, v/v) (pH 4.5); fluorimetric detection, using an excitation wavelength of 405 nm and an emission wavelength of 495 nm	60.6–71.8%	LOQ: 10 μg kg^{-1}	Anne-Claire & Zeggane (2003)
Honey	SAM, SP, SMR, SMPD, SME, SCP, SMX, SDMX	Dissolved in phosphoric acid solution (pH 2), then cleaned by SPE, an aromatic sulfonication-exchange cartridge and an Oasis HLB cartridge; derivatized with fluorescamine	HPLC with fluorescence detection at excitation and emission wavelengths of 405 and 495 nm	73.5–94.1%	LOD was 2 μg kg^{-1} for SAM, SPY, SMR, SMP and 5 μg kg^{-1} for SME, SCP, SMX and SDM	Pang et al. (2003)

(*continued*)

Table I. Continued.

Sample	Analyte	Sample treatment	Determination technique	Recovery	Comments	Reference
Milk and eggs	SDZ, STZ, SP, SMR, SME, SMZ, SMT, SCP, SMPD, SMX, SQ, SMMX, SDMX	Using the matrix solid-phase dispersion technique with hot water as extractant	HPLC/MS with C_{18} reversed column (25 cm × 4.6 mm i.d., 5 μm), gradient elution: (where t is time in min) t_0, A = 10%; t_{20}, A = 36%; t_{29}, A = 50%; t_{30}, A = 100%; t_{35}, A = 100%; t_{37}, A = 10%; t_{45}, A = 10%, phase A: methanol and B: water. Both contained 5 mmol l^{-1} of formic acid. MS data acquisition was performed in the positive-ion mode	77–92%	LOQ: 1–3 μg kg^{-1} SAs in milk and 2–6 μg kg^{-1} in eggs	Bogialli et al. (2003)
Eggs	SMMX, SDMX, SQ	Homogenized with perchloric acid solution using a handy ultrasonic-homogenizer followed by a centrifugal ultrafiltration unit	HPLC with a reversed-phase C_4 column (15 cm × 4.6 mm i.d., 5 μm); mobile phase: 0.18 mmol l^{-1} citric acid; photodiode array detection was set at 267 nm	80.3–88.4%	LOD: < 50 μg kg^{-1}	
Cows milk	SDD	Homogenization with an inorganic acid solution by means of a handy ultrasonic homogenizer, followed by centrifugation	HPLC was performed on a C_4 column; mobile phase: 1.25 mmol l^{-1} succinic acid solution; photodiode array detection	No relative information	LOQ: 23 ng ml^{-1}	Furusawa (2003a)
Milk and eggs		Using different sample pretreatment: Direct injection and extractive condensation of the analyte in the solid–solid and liquid–liquid system	Micellar liquid chromatography	96–105%	LOD: 100 ng ml^{-1} (DSJ), 50 ng ml^{-1} (SPE), and 25 ng ml^{-1} (LLE) in milk, 25 ng ml^{-1} (SPE and LLE) in eggs	Szymanski (2003)
Eggs	SDMX, SDT	Albumen: extracted with ethyl acetate; yolk: extracted with ethyl acetate and the extract purified twice by liquid–liquid partition with methanol and isooctane	HPLC with a Nova-Pak C_{18} column (15 cm × 3.9 mm i.d., 4 μm), mobile phase: acetonitrile–0.01 mol l^{-1} ammonium acetate, pH 6.0 (12:88 v/v for SDM; 14:86 v/v for SDT)	87.0 and 77.4% for the albumen, 64.6 and 67.4% for the yolk, respectively	LOQ: 5 μg kg^{-1}	Roudaut & Garnier (2002)
Milk honey	SAM, SDZ, SDMX, SMR, STZ, SMMX, SAN, SP, SQ, SIX	Mixed with a micellar solution of 0.10 mol l^{-1} SDS and sonicated during 10 min; filtered into the autosampler vials through 0.45 μm nylon membranes	HPLC with an ODS-Hypersil column (10 cm × 4.6 mm i.d., 5 μm); mobile phase: 0.019 N SDS/5.8% acetonitrile at pH 3.0; UV-visible detector was set at 275 nm	87–108 and 72–119% for milk and honey, respectively	LOD: 100 ng ml^{-1}	Caballero et al. (2001)
Milk	SDZ, SMR, SDD, SQ, SMMX, SMX, SDMX	Cleaned up using an Ultrafree®-MC/PL centrifugal ultrafiltration unit and 25% (v/v) aqueous ethanol as solvent	HPLC with a Mightysil® RP-4 GP column (25 cm × 4.6 mm i.d., 5 μm); mobile phase: 25% (v/v) aqueous ethanol solution, photodiode array detector	> 82%	LOD: 5–20 ng ml^{-1}	Furusawa (2001)

Edible tissues from cattle, pigs, chickens and sheep	SMZ	Homogenized with 30% (v/v) ethanol in water followed by an Ultrafree-MC/PL as a centrifugal ultrafiltration unit	HPLC with a reversed-phase C_4 column (25 cm × 4.6 mm i.d., 5 μm); mobile phase: 15% (v/v) ethanol in water: photodiode array detector was set at 263 nm	> 80%	LOQ: 57–60 μg kg^{-1}	Furusawa (2001)
Veterinary commercial formulations	SMPD, SMX, SDMX	Prepared by direct dilution of the stock solutions	HPLC with a Kromasil C_{18} column (15 cm × 4.6 mm i.d., 5 μm), mobile phase: 10 mmol l^{-1} citrate buffer (pH 3.0)–methanol (from 31:69 to 69:31) in gradient mode: t_0: CH 3OH% = 31%; t_4: CH 3OH% = 69%; t_{14}: CH 3OH% = 69%; t_{16}: CH 3OH% = 31%; UV detector was set at 255 nm	No relative information	LOD: 110, 130, 50 ng ml^{-1} for SMP, SMX and SDM respectively; LOQ: 400, 400 and 200 ng ml^{-1} for SMP, SMX and SDM, respectively	Berzas-Nevado et al. (2001)
Chicken and swine muscles	SDZ, STZ, SMR, SMZ, SMMX, SMX, SQ, SDMX	Extracted with acetonitrile and filtered, then partitioned with acetonitrile-saturated *n*-hexane, evaporated to dryness, the residue passed through a Sep-Pak Clr cartridge for sample clean-up	HPLC method equipped with a photodiode array detector using a Luna C_{18} (25 cm × 4.6 mm i.d., 5 μm) column; gradient elution of acetonitrile and 0.05 mol l^{-1} sodium dihydrogen phosphate	In the range of 71.9 similar to 96.9% and 71.1 similar to 99.6%, respectively	LOD: 40 ng ml^{-1} for STZ and 20 ng ml^{-1} for other 12 drugs	Kao et al. (2001)
Gilthead sea bream tissues (muscle skin, liver, gills, fat, and kidney)	SDZ	Extracted with dichloromethane and the analytes are partitioned into hydrochloric acid	HPLC with a Nucleosil 100 RP C_{18} column (25 cm × 4.6 mm i.d., 5 μm), mobile phase: acetonitrile: 10 mM phosphoric acid (16:84, v/v); UV monitored at 271 nm	82.1% in muscle and skin, 77.8–87.4% in the other tissues	LOD: 3.1 μg kg^{-1}	Papapanagiotou et al. (2000)
Liver and kidney (Swine, Bovine, Chicken)	SDZ, SMR, SDD, SIZ, SMPD, SMMX, SMX, SIX, SDMX, SQ	Extracted with ethyl acetate; evaporated and then dissolved in 50% (v/v) ethyl acetate *n*-hexane, then applied to a Bond Elut PAS (primary/secondary amine cartridge), eluted with 20% (v/v) acetonitrile–0.05 mol l^{-1} ammonium formate	HPLC (LC-MS-MS) with an L-column ODS column (25 cm × 4.6 mm i.d., 5 μm); mobile phase: methanol–acetonitrile–0.05 mol l^{-1} formic acid (10:15:75); UV detection was set at 272 nm	70.8–98.2%	LOD: 30 μg kg^{-1}	Ito et al. (2000)
Swine tissues (kidney, liver, muscle, fat and fat + skin)	SDZ	LLE with acetone and ethyl acetate, followed by a clean-up using a SPE column (aminopropyl and benzenesulfonic acid)	HPLC with a Spherisorb ODS-2 column (25 cm × 4.6 mm i.d., 5 μm); mobile phase: 1% acetic acid in water–acetonitrile (85:15, v/v); UV detection at 270 nm and atmospheric pressure chemical ionization mass spectrometry in the positive APCI-MS/MS mode	No relative information	LOQ: 50 μg kg^{-1}; LOD of 12, 38, 15 and 17 μg kg^{-1} for SDA in kidney, liver, muscle and fat, respectively	Baere et al. (2000)

(*continued*)

Table I. Continued.

Sample	Analyte	Sample treatment	Determination technique	Recovery	Comments	Reference
Swine urine	SMZ Ac-SMZ	Lyophilized urine was thawed, reconstituted adding Milli-Q grade water and left for 30 min at room temperature, gently shaking every 10 min	HPLC/MS/MS with column (Zorbax SB-C_{18} 30 cm × 2.1 mm i.d., 3.5 μm), using a rapid gradient (from 20 to 80% methanol in 3 min), a PE Sciex API 365 triple quadruple (QqQ), operated in the SRM mode, or a Finnigan LCQ ion-trap (IT) mass spectrometer, operated in narrow-range product ion scan, used as the final detector. Electrospray was used as the ionization technique	No relative information	LOQ: 500 ng ml^{-1}	Bartolucci et al. (2000)
Meat and kidney	SAN, SDZ, SMR, SDD, SMPD, SCP, SD, SMX, SQ, SDMX,	First extraction with ethyl acetate, then acetone and further purified by partitioning three times with water–methylene chloride	HPLC with a Chrompack column (25 cm × 4.6 mm i.d., 5 μm), mobile phase: acetonitrile–water (35/65, v/v) pH 3.0 containing 0.01 mol l^{-1} K_2HPO_4 and fluorescence detection ($\lambda_{ex} = 405$ nm and $\lambda_{em} = 490$ nm)	64–75%	LOQ: 1 μg kg^{-1} for SQ and 0.5 μg kg^{-1} for the other	Stoev & Michailova (2000)
Milk eggs	SDD	Shaking with 20% (w/v) trichloroacetic acid–methanol (4:1, v/v); ultrafiltration using Molcut II®. A LiChrospher® 100 RP_8 column and using mobile phase of 4% (v/v) acetic acid solution–acetonitrile (6:4, v/v)	HPLC with LiChrospher® 100 RP-8 column (25 cm × 4.6 mm i.d., 5 μm); mobile phase: 4% (v/v) acetic acid solution–acetonitrile (6:4, v/v). photodiode array detector was set at 268 nm	80.8–88.0%	LOD in milk and eggs were 0.01 g ml^{-1} and 10 μg kg^{-1}, respectively	Furusawa (1999b)
Edible fish and shrimp	SMMX	Tissue was homogenized with acetonitrile–tetrahydrofuran (95:5) containing 0.5% EDTA, and then centrifuged	HPLC with a Hisep shielded hydrophobic phase column (15 cm × 4.6 mm i.d., 5 μm); mobile phase: 0.05 mol^{-1} citric acid–0.2 mol l^{-1} disodium hydrogen phosphate buffer, pH 2.5, in 10 mmol^{-1} tetra-*n*-butyl ammonium bromide–acetonitrile (85:15); UV detector was set at 265 nm	80%	LOD: 20–40 μg kg^{-1}	Ueno et al. (1999)
Meats (beef, pork, chicken) and eggs	SDD	Extraction of SDD was performed using a Sep-Pak(R) CN cartridge	HPLC method with a LiChrospher (R) 100 RP_8 end-capped column; mobile phase: acetonitrile–acetic acid–water (28:4:68, v/v/v); photodiode array detector	> 80.2%	LOD: 20 μg kg^{-1}	Furusawa (1999b)

Edible fish and shrimp	SMMX	Tissue was homogenized with acetonitrile–tetrahydrofuran (95:5) containing 0.5% EDTA, and then centrifuged	HPLC with a Hisep shielded hydrophobic phase column (15 cm × 4.6 mm i.d., 5 μm); mobile phase: 0.05 mol $^{-1}$ citric acid–0.2 mol l^{-1} disodium hydrogen phosphate buffer, pH 2.5, in 10 mmol $^{-1}$ tetra-*n*-butyl ammonium bromide–acetonitrile (85:15); UV detector was set at 265 nm	80%	LOD: 20–40 μg kg^{-1}	Ueno et al. (1999)
Meats (beef, pork, chicken) and eggs	SDD	Extraction of SDD was performed using a Sep-Pak(R) CN cartridge	HPLC method with a LiChrospher (R) 100 RP_8 end-capped column; mobile phase: acetonitrile–acetic acid–water (28:4:68, v/v/v); photodiode array detector	> 80.2%	LOD: 20 μg kg^{-1}	Furusawa (1999)
Swine muscle, liver and kidney	SMZ	Extraction was achieved by irradiating the sample in methanol for 25 s in a household microwave oven, commonly referred to as MAE	HPLC using a C_{18} column and detected at 450 nm after derivatization with 4-dimethylaminobenzaldehyde in a heated rector at 40°C	No relative information	LOD: 2.5 μg kg^{-1}	Akhtar et al. (1998)
Pork veal	STZ, SMR, SCP, SMZ, SMPD, SMX, SQ, SDMX	SPE extraction eluted with methylene chloride and ethyl acetate successively	HPLC with an column (Spherisorb C_{18} ODS II; 25 cm × 4.6 mm i.d., 5 μm); solution A: an ammonium acetate buffer 0.01 mol l^{-1}, pH 5.2, and solution B: acetonitrile–methanol (70/30; v/v). 14% B in A for 5 min → 20% B in A from 5 to 22 min → 54% B in A from 22 to 30 min → 14% B in A for 5 min, a photodiode array and fluorescence detectors	45–85%	LOD: 3.0–7.5 μg kg^{-1} for pork except SMP (66.5 μg kg^{-1}) and 3.5–7.5 μg kg^{-1} for veal except SMP (35 μg kg^{-1})	Boulaire et al. (1997)
Muscle and skin from salmon	SDZ	Cleaned-up by a PRS cartridge, eluted with 0.2 mol l^{-1} phosphoric acid, the eluate was loaded onto a C_{18} cartridge, eluted with methanol	HPLC (LC-APCI/MS) with column (15 cm × 4.6 mm i.d., 5 μm), mobile phase: acetonitrile–2% acetic acid (20/80) and postcolumn derivatization with fluorescamine	averaged 75.4%	LOD: 0.2 μg kg^{-1}; LOQ: 1.0 μg kg^{-1}	Gehring et al. (1996)
Meat	SMZ	Extraction by acetone/methylene chloride, liquid–liquid defatting and clean-up	HPLC with gradient pump and fluorimetric detector, after derivatization with fluorescamine at pH 3.0	No relative information	LOD: 2–10 μg kg^{-1}	Barbieri et al. (1995)

(*continued*)

Table I. Continued.

Sample	Analyte	Sample treatment	Determination technique	Recovery	Comments	Reference
Spray-dried milk powder	SMZ	Dry milk powder with measured concentrations of SMZ was treated with supercritical CO_2	HPLC with a Phenomonex Bondclone 10 C-18 (300 × 3.9 mm i.d.) column; mobile phase: 76:24 (v/v) 0.1 mol l^{-1} KH $2PO_3$ to methanol; UV detector was set at 254 nm	No relative information	No relative information	Malik et al. (1994)
Chinook salmon muscle and liver tissues	SDMX	Extracted with dichloromethane, evaporated the solvent, reconstituted in HPLC mobile phase	HPLC (LC/MS/MS) with a Beckman Ultrasphere ion-pair column (25 cm × 4.6 mm i.d., 5 μm); detection at 280 nm	78% in muscle; 61% in liver	LOD: 50 μg kg^{-1} in muscle, 200 μg kg^{-1} in liver	Zheng et al. (1994)
Pigs' lungs tissue and edible tissues	SDMX, SMX	Sample pretreatment consisted of a simple extraction with an aqueous buffer solution	HPLC consisted of an on-line gel-permeation column, a small preconcentration column and an analytical reversed-phase HPLC column and detected by UV absorption	60–85%	LOD: 10–50 μg kg^{-1}	Mengelers et al. (1993)
Milk	SDZ, STZ, SP, SMR, STZ, SMZ, SCP, SQ, SDMX	Diluted with potassium phosphate buffer (1 mol l^{-1}, pH 4.4) and passed through a Cyclobond-ISPE column, eluted with methanol. Further cleaned using an alumina and AGMP-1 ion exchange column	HPLC using a reverse-phase column and diode array detector at 265 nm	No relative information	No relative information	Agarwal (1993)
Milk	SDD	Using SPE on a C_{18} cartridge eluted with sodium acetate buffer/acetonitrile (70/30)	HPLC analysis was performed with LiChroSpher C-18 and SuperSpher C-18 columns. Detection was performed at 270 nm. The mobile phase was sodium acetate buffer (0.01 mol l^{-1}; pH 4.6) and acetonitrile (85:15, 75:25)	130–74%	LOQ: 10 μg kg^{-1}	Hoffmeister et al. (1991)
Milk	SMZ	Extracted with chloroform, then evaporated to dryness, redissolved in potassium phosphate buffer (pH 5.0), passed through a cyclobond I SPE column. eluted with aqueous methanol (50%)	HPLC with UV detection at 265 nm	83.2–88.2%	No relative information	Agarwal (1990)

Table II. TLC methods for the determination of sulphonamide residues in edible animal product.

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Reference
salmon muscle tissue	SDZ, SMR, SMZ, SP, SDMX	MPSD was employed whereby the sample was ground with C_{18}-derivatized silica gel and eluated with dichloromethane	Analysis on a TLC plate using ethyl acetate-*n*-butanol–methanol–aqueous ammonia (35:45:15:2, v/v/v/v) and detected after spraying the plate with a solution of fluorescamine	61, 63, 60, 63 and 57% for SDZ, SMRZ, SMTZ, SDMX, SP, respectively	LOD: 110, 440, 70, 130 and 130 ng ml^{-1} for SDZ, SMRZ, SMTZ, SDMX and SP, respectively	Reimer & Suarez (1991)
milk	SMZ	Homogenized and passed through C_{18} SPE columns, eluted with methanol, then concentrated on a small ion-exchange resin	Analysis on a TLC plate precoated with silica gel 60 and eluted using methanol–acetic acid–acetone (1:5:94). Fluorescence detection was induced with fluorescamine and quantified with a scanning densitometer	88.3–103.2%	LOQ: 510 ng ml^{-1}	Unruh et al. (1990)
animal plasma	SMZ	Membrane filtration and then competitive drug displacement from proteins	TLC plates developed with ethyl acetate and scanned at an excitation wavelength of 310 nm	No information	LOD: 50 ng ml^{-1}	Bevill et al. (1978)

Table III. HPCE methods for the determination of sulphonamide residues in edible animal product.

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Reference
Meat	SMZ, SMR, SDZ, SIX, SDMX, SMMX, SPA, SQ	Solvent extraction was used to extract sulfonamide from meat and the SPE procedure was employed for sample clean-up and preconcentration	HPCE separation using 35 mmol l^{-1} Na_3PO_4-H_3PO_4 buffer (pH 6.5) at an applied voltage of 25 kV. UV detection was set at 205 or 210 nm	80–97%	LOD: 5–10 μg kg^{-1}	Fuh & Chu (2003)
Binary and ternary mixtures	SMZ, SQ, SPM	No information	HPCE separation was carried out in a fused silica capillary (50 cm effective length, 75 μm i.d.), an applied voltage of 20 kV for 5 min, then increased to 30 kV, electrolyte: 30 mmol l^{-1} borate buffer (pH 9.2) plus 6% acetonitrile plus 40 mmol l^{-1} sodium dodecyl sulfate. Diode array detection is set at 214 nm	No information	LOQ: 100 ng ml^{-1}	
Milk	STZ, SMZ, SMPD, SSM, SMR, SME, SDZ, SQ, SMMX, SDMX, SCP, SMX, SIX	Deproteined first, then filtered through a 0.45 μm filter	HPCE separation was carried out in a capillary (44 cm × 50 μm, i.d.), using UV-Vis detector and a phosphate-borate buffer at pH 6.85 added with organic modifier or β-cyclodextrin, an applied voltage of 20 kV	No information	No information	Lin et al. (1996)
Pork meat	SMX, SAN, SDD, STZ, SMR, SDZ, SD, SMPD, SDMX, STX, SQ, SG, SPMX, SCP, SCPA	Homogenized in a household food processor and extracted in a stomacher apparatus using acetonitrile. Then centrifuged and filtered through a 0.45 μm filter	HPCE separation was carried out in a Beckman standard capillary (50 cm effective length, 75 μm i.d.). The wavelength of the UV detection was set at 254 nm, 0.02 mol l^{-1} phosphate 0.02 mol l^{-1} borate buffer is used	No information	LOD: 200–900 ng ml^{-1}	Ackermans et al. (1992)
No sample	Seven sulphonamides	No sample treatment	HPCE separation conditions were 20 mmol l^{-1} sodium tetraborate, 0.5 mmol l^{-1} EDTA, 100 mmol l^{-1} SDS, pH 8.5, 15 kV; a (47 cm effective length × 50 μm i.d.) fused silica capillary; UV detection set at 205 nm	No information	No information	Dang et al. (1992)

Table IV. ELISA methods for the determination of sulphonamide residues in edible animal product.

Sample	Analyte	Sample treatment	Conjugation methods for immunogens	Antibodies	Comments	Reference
Swine	SMZ	Added EDTA and then centrifuged	Diazotization	Polyclonal antibodies	IC_{50}: 100 $\mu g l^{-1}$	Singh et al. (1989)
Animal tissues	SMZ	MPSD (C_{18}, octadecylsilyl-derivatized silica)	Diazotization	Polyclonal antibodies	Muscle: LOD: 4 $\mu g kg^{-1}$; LOQ: 5 $\mu g kg^{-1}$; liver: LOD: 5 $\mu g kg^{-1}$; LOQ: 5 μg kg^{-1}	Renson et al. (1993)
Milk, Plasma, Urine and Edible Tissues	SMZ	Milk, plasma and urine samples were diluted with PBS; tissue samples were homogenized with PBS, then centrifuged	Diazotization; azocoupling	Polyclonal antibodies	IC_{50}: 0.15 $\mu g l^{-1}$; standard range: 0.05–5 $\mu g l^{-1}$	Fránek et al. (1999)
Egg, milk, beef and lamb muscle, chicken, turkey and porcine muscle, porcine kidney, porcine liver, pig feedstuffs	SCP	Mixed with methanol and then centrifuged	Active-ester method	Polyclonal antibodies	LOD: 0.42–3.9 $\mu g l^{-1}$; IC_{50}: 4.5–75.0 $\mu g l^{-1}$	Spinks et al. (2001)
Bee honey	STZ	Diluted with deionized water, without previous extraction or clean-up	Modification of the active-ester method	Polyclonal antibodies	LOQ: 0.03 $\mu g l^{-1}$; IC_{50}: 1.6 $\mu g l^{-1}$	Pastor-Navarro et al. (2004)
No sample was analysis	SP, STZ, SDZ, SMX, SDMX, SMT, SAN	No sample treatment	Cross-linking using EDC	Polyclonal antibodies	IC_{50}: 1.3–21.6 $mg l^{-1}$	Sheth & Sporns (1991)
No sample was analysis	SNT, SP, STZ	No sample treatment	Cross-linking using DCC	Monoclonal antibodies	IC_{50}: sulfanitran: 1.41 $\mu g l^{-1}$; SP: 22.8 $\mu g l^{-1}$; STZ: 322 $\mu g l^{-1}$	Muldoon et al. (1999)
No sample was analysis	STZ, SDZ, SMZ, SCP, SMX, SPMX, SDMX, SP, SD	No sample treatment	active-ester method	Monoclonal antibodies	IC_{50}: < 100 $\mu g l^{-1}$ for eight sulfonamides	Haasnoot et al. (2000b)
No sample was analysis	SMT, SCP, STZ, SPMX, SP, SDMX, SD, SDZ, SMX, SIX	No sample treatment	Diazotization, succiny-lation, glutaraldehyde	Polyclonal, monoclonal antibodies	IC_{50}: < 25 $\mu g l^{-1}$ for 11 sulfonamides	Haasnoot et al. (2000a)
No sample was analysis	SMT, STZ, SCP, SMP, SP, SMX, SDZ, SMZ, SFX, SQ, SDM	No sample treatment		Mutant antibodies	Very for different sulfonamides and libraries	Korpimäki et al. (2002)
No sample was analysis	SDZ, SDMX, STZ, SP, SMX	No sample treatment	Using glutaraldehyde or a succinimide ester as a cross-linker	Monoclonal antibodies	LOQ: < MRL	Cliquet et al. (2003)
Milk, beef, chicken serum	13 sulphonamides	Milk and chicken serum were diluted before analysis. Beef samples were centrifuged		Mutant antibodies	LOQ: < MRL	Korpimäki et al. (2004)

Abstract: reference (Reference), limit of detection (LOD), limit of quantification (LOQ), normal-phase matrix solid-phase dispersion (MPSD), direct sample injection (DSJ), microwave-assisted extraction (MAE), perfluorotributylamine (PFTBA), inside diameter (i.d.), supported liquid membrane (SLM), trinoctylphosphine oxide (TOPO), selected reaction monitoring (SRM), phosphate-buffered saline (PBS), ethylenediaminetetraacetic acid (EDTA), inhibiting concentration 50% (IC_{50}), sulphanitran (SNT), dicyclohexylcarbodiimide (DCC), 1,3-dicyclohexylcarbodiimide (EDC).

detection (FLD), LC/photodiode array detection (DAD) and LC/MS have been used to present. GC/MS and LC/FLD need derivatization; however, LC/MS does not and it has been used more frequently.

Currently, the most widely used analytical methods for sulphonamides are based on a HPLC separation, followed by ELISA, BIA and microbiological assay. Relatively few methods are based on GC, HPCE or TLC.

High-performance liquid chromatography (HPLC)

HPLC has become the most widely used technique for the determination of sulphonamide residues in meat, milk and poultry, and a number of methods have been published. Table I summarizes the HPLC methods that are reviewed here. Separation is usually performed with silica-based reversed-phased columns, mainly C_{18}, C_8 or C_4 (Furusawa et al. 2003b, Maudens et al. 2004, Pecorelli et al. 2004), but in some cases ion-pair column is also used (Zheng et al. 1994).

Mobile phases mainly consist of acetonitrile–water mixtures, but methanol–water or ternary mixtures of acetonitrile–methanol–water are also used. Ethanol–water has also been reported (Furusawa & Kishida 2001, Kishida & Furusawa et al. 2003). In addition, acetate or sodium dodecyl sulfate (SDS) is often added to these systems to improve the separation. The mobile phases containing sodium acetate–acetonitrile could separate all sulphonamides on LiChrospher and CP TMSphere columns (Agarwal 1992). The retention behaviour of sulphonamides was dependent not only on the polarity of the mobile phase, but also on the ionization of sulphonamides. Therefore, the pH of the mobile phase played an important role in the chromatographic separation. The optimum pH of the buffer was 4.4, at which all sulphonamides except sulphanilamide gave excellent retention.

Several spectroscopic techniques, such as UV absorption (Berzas-Nevado et al. 2001, Caballero et al. 2001, Pecorelli et al. 2004), FLD (Anne-Claire & Zeggane 2003, Maudens et al. 2004, Salisbury et al. 2004), DAD (Kishida & Furusawa 2003, Garcia et al. 2004, Kishida et al. 2004) or MS (Bogialli et al. 2003, Kim et al. 2003, Msagati & Nindi 2004) are used for detection in LC. UV detection is often carried out at 270–280 nm, in some cases at 255 nm. Fluorescence detection is carried out at an excitation wavelength of 405–420 nm and an emission wavelength of 485–495 nm. Photodiode array detection is often performed at 267, 268 or 263 nm. MS is normally used for confirmatory analysis since it is highly specific. Fragmentation pathways involving distinct functional groups are preferred because they provide higher selectivity. Several interfaces have been used for LC-MS (or LC-MS/MS), always in the positive mode. Since MS is incompatible with most of the mobile phases, volatile additives must be used when LC is coupled to MS. Thus, ammonium acetate or formic, acetic and trifluoroacetic acid are added to acetonitrile–water mixtures.

Gas chromatography (GC)

It is essential to derivatize sulphonamides to prepare suitable volatile derivatives before GC analysis. The derivatization generally involves either N-methylation or N-methylation followed by acylation of the N4-primary amino function with pentafluoroalkane carboxylic anhydride. Because of these laborious processes, only a few cases of GC methods applied to residue analysis have been published (Nose et al. 1976, Chiavarino et al. 1998, Reeves 1999). Furthermore, there is only one report on the analysis of sulphonamides in food products (Reeves 1999) in which a GC method was established to analyse ten kinds of sulphonamides (SDZ, STZ, sulphapyridine (SP), SMR, SMZ, sulphachlorpyridazine (SCP), sulphadimethoxine (SDMX), sulphaquinoxaline (SQ), sulphamethizole (SMT), sulphabromomethazine (SBM)) in bovine milk. The analysis conditions were as follows: GC column was a DB-1, 30 m, 30.25 mm i.d. 0.25 mm film thickness. The injection port temperature was 240°C, with a septum purge-on time of 1 min at 0.5 ml min^{-1}. The temperature programme was 40°C (1 min), 30°C min^{-1} to 200°C, 6°C min^{-1} to 280°C, and hold for 10 min. MS was turned in positive-ion mode using PFTBA.

Thin-layer chromatography (TLC)

Currently, TLC has been almost replaced by other instrumental analysis. However, the use of TLC to quantitate sulphonamides can reduce solvent use and the cost of the analysis, allowing the screening of a larger number of samples. The sample preparation for TLC often includes SPE (Unruh et al. 1990) and membrane filtration (Bevill et al. 1978).

Unruh et al. presented a method using a TLC plate precoated with Silica Gel 60 and eluting chemicals with 0.5 ml methanol–acetic acid–acetone (1 : 5 : 94, v/v/v) to analyse the SMZ residues in milk. Reimer & Suarez (1991) developed a TLC method with a high-performance TLC plate eluting with ethyl acetate-*n*-butanol–methanol–aqueous ammonia (35 : 45 : 15:2, v/v/v/v) to analysis five sulphonamides (SDZ, SMR, SMZ, SDMX, SP) in salmon muscle tissue. Fluorescence detection with an excitation wavelength of 310 nm is often used for detecting sulphonamides residues in TLC. The method detection limits at the 99% confidence

level were 0.11, 0.44, 0.07, 0.13 and 0.13 mg l^{-1} for SDZ, SMR, SMT, SDMX and SP, respectively. The lowest detectable levels were approximately 0.04 ppm for SDZ, SMT, SDMX and SP, and 0.10 mg l^{-1} for SMR. The average recoveries of analytes were 61, 63, 60, 63 and 57% for SDZ, SMR, SMT, SDMX and SP, respectively, and were analyst-dependent. The method gave linear detector responses for all analytes over spiking levels ranging from 0 to 2 mg l^{-1} (Reimer & Suarez 1991).

High-performance capillary electrophoresis (HPCE)

In recent years, HPCE has become a popular and powerful separation technique that is widely applied to diverse samples because of its high resolution, extremely high efficiency, rapid analysis, and small consumption of both sample and solvent in comparison with HPLC. Thus, HPCE is expected to be an excellent alternative method for the identification and separation of sulphonamides. The separation of sulphonamides by HPCE has been performed using two modes: capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC). A procedure for measuring 16 sulphonamide residues in pork meat samples was described by Ackermans et al. (1992).

The sample preparation procedures for HPCE analysis are similar to those for HPLC. The phosphate or borate buffer is often used as electrolytes in the CZE mode, and SDS or β-cyclodextrin (β-CD) is often added to the phosphate or borate buffer as an electrolyte modifier on the migration behaviour and separation of sulphonamides in MEKC. The migration behaviour and selectivity of sulphonamides in CZE are mainly manipulated by the pH of the buffer. The migration order of sulphonamides depends on the ratios of charge to mass and is primarily determined by their pK$_a$ values. Thus, precise optimization of buffer pH is critical to improve the separation of some closely migrating sulphonamides. On the other hand, buffer pH and micellar concentration greatly affect the migration and selectivity of sulphonamides in MEKC. The migration order of sulphonamides is mainly determined by their pK$_a$ values and the magnitude of the binding constants of solutes-to-micellars. The influences of buffer pH and micellar concentration correlate with each other. The binding constants correlate with the differences between the electrophoretic mobility of sulphonamides measured at a pH below pK$_a$-2 in CZE and that in MEKC. UV-Vis detection with a wavelength of 254 nm and diode array detection with a wavelength of 214 nm are often used in HPCE and sometimes end-column electrochemical detection is used.

Fuh & Chu (2003) described a HPCE method combined with SPE clean-up for the determination of eight commonly used sulphonamides (SMZ, SMR, SDZ, sulphadimethoxine (SDM), sulfamonomethoxine (SMMX), sulphaphenazole (SPA), SQ and sulfisoxazole (SIX)) in meat. Complete separation of eight sulphonamides was achieved by using 35 mmol l^{-1} Na_3PO_4-H_3PO_4 buffer (pH 6.5) at an applied voltage of 25 kV. 1-Naphthoxyacetic acid was used as an internal standard for quantitative analysis. Satisfactory linearity, 0.5–50 μg ml^{-1}, of each compound was obtained. Good recovery rates from 80 to 97% were determined. The detection limits of each sulphonamide approximately ranged from 5 to 10 μg kg^{-1}. Berzas-Nevado et al. (1999) presented a MEKC method to separate SMZ, SQ, menadione and pyeimethamine. Separation was carried out at 20 kV for the first 5 min and then raised to 30 kV until the end came, using 30 mmol l^{-1} borate buffer adjusted to pH 9.2, 6% acetonitrile, and 40 mmol l^{-1} sodium dodecyl sulfate as electrolyte. The limits of quantification were about 1 mg l^{-1} for every component. Lin et al. (1996) investigated the influence of buffer pH and electrolyte modifier on the migration behaviour and separation of thirteen sulphonamides. Ackermans et al. (1992) established a HPCE method to determinate the 16 sulphonamides in pork meat. Calibration graphs for five sulphonamides were constructed, and regression coefficients of at least 0.999 were obtained. The limit of detection for the method varied from 2 to 9 mg l^{-1}. Dang et al. (1992) separated and determined seven sulphonamides and trimethoprim by MEKC successfully, employing SDS as a micellar phase and tetrabutylammonium bromide as additive. The effects of surfactant and modifier concentrations, pH and applied voltage on the retention behaviour of the solutes and the column efficiency were studied.

Enzyme-linked immunosorbent assay (ELISA)

ELISA methods have been developed for several of the sulphonamides (Fleeker & Lovet 1985, Dixon-Holland & Katz 1988, Jackman et al. 1993). Polyclonal antibodies (PAbs) were generated for these methods using immunogens in which the sulphonamide of interest was linked to a carrier protein via the N4-position (Figure 1), resulting in a general assay for the class of sulphonamides. In contrast, N1-substituents, which are specific for individual sulphonamides, were readily exposed to the immune system and this resulted in the production of antibodies, which were highly specific for the particular sulphonamide drug.

Due to the allergic or toxic reactions and the widespread use of SMZ, many ELISA methods have been established to detect this sulphonamide. Fránek et al. (1999) described a highly sensitive immunoassay that exhibited 50% binding inhibition to SMZ in buffer at approximately 150 ng ml^{-1}. Renson et al. (1993) developed an immunoassay for SMZ using an antiserum raised in rabbits by immunization against a sulphamethazine diazo derivative coupled to bovine serum albumin. The method allowed a detection limit well below the tolerance limit (100 μg kg^{-1}) generally applied for SMZ. Singh et al. (1989) developed a competitive solid-phase enzyme immunoassay to measure residues of SMZ in swine plasma. Standard curves were constructed over 10–1000 ng ml^{-1} SMZ. Recoveries of SMZ in spiked plasma samples between 16 and 500 ng ml^{-1} ranged from 97.6 to 106.3% (mean recovery 103%). Spinks et al. (2001) developed an ELISA for the detection of residues of the antibiotic SCP. Their assay had a lower detection limit of 0.65 ng ml^{-1} in assay buffer. The potential application of the assay to detect residues of SCP at the 100 μg kg^{-1} level in eggs, milk, beef, lamb, pork, chicken, turkey, porcine kidney, porcine liver and pig feedstuffs was discussed with regard to the effects of sample extracts on the standard curves.

The antibodies used for all of the above-mentioned methods are specific for individual sulphonamides. As the number of drugs used for animals is increasing, and immunoassays which act as best rapid screening methods for sulphonamides requiring further confirmation by other more definitive methods, it would be more efficient if an immunoassay could detect a number of different commercial sulphonamides, so more efforts are being concentrated on developing multiresidue methods. In the case of sulphonamides, several multiresidue methods have been developed which can determine a whole range of sulphonamides simultaneously. Sheth & Sporns (1991) were the first to report the development of sulphonamide-specific antibodies. They immunized rabbits with a sulphathiazole derivative (N1-[4-(carboxymethyl)-2-thiazolyl]-sulfonamide, TS) linked at its side chain to keyhole limpet haemocyanin (TS-KLH). The PAbs recognized nine sulfonamides showing 50% inhibition at a concentration of less than 5000 ng ml^{-1}. Assil et al. (1992) synthesized another sulphonamide derivative with a larger side chain (N1-[4-methyl-5-[2-4-carboxyethyl-1-hydroxyphenyl]]-azo-2-pyridyl)-sulphanilamide, PS). The polyclonal serum they obtained was purified by affinity chromatography and the purified fraction showed 50% inhibition with seven sulphonamides at concentrations less than 10 000 ng ml^{-1}. The first published study about sulphonamide-specific monoclonal antibodies (mAb) was from Muldoon et al. (1999). After immunization with an N-sulphanilyl-4-aminobenzoic acid–protein conjugate, only one mAb was obtained that recognized eight sulphonamides at concentration levels less than 10 000 ng ml^{-1}. Haasnoot et al. (2000a, b) used the same sulphonamide derivatives as Sheth & Sporns (1991) and Assil et al. (1992) had used to induce mAb. The best mAb showed 50% inhibition with 18 tested sulphonamides at values less than 10 000 ng ml^{-1}, and with eight at concentrations of less than 1000 ng ml^{-1}. Unfortunately, the relevant sulphonamides SMZ, sulphatroxazole (STX), and SCP were not detected at the MRL (100 ng ml^{-1}). Spinks et al. (1999) carried out a molecular modelling study on the sulphonamide structure and deduced that cross-reactive antibodies could possibly be obtained using a sulphonamide as hapten with a more planar structure (sulphacetamide (SAM)) or a greater bend (SCP). Despite this interesting hypothesis, immunization with such conjugates did not lead to antiserum with a broader specificity for sulphonamides. Li et al. (2000) reported the detection of sulphonamides in swine meat by immunoaffinity chromatography using cross-reactive polyclonal antibodies (PAb) induced with three different sulphonamide haptens. Sulphonamides were best recognized by the antibodies induced with H2-protein conjugates, slightly less with H1-protein and worst with H3-protein conjugates. None of the reported mAbs or PAbs (except those of Li et al. 2000), in which cross-reactivity values were not mentioned) could detect all the relevant sulphonamides (SMZ, sulphadoxine (SD), SCP, SQ, STX) at the level of MRL.

Different strategies for the development of sulphonamide-specific mouse antibodies were compared by Cliquet et al. (2003). With the best mAb (3B5B10E3), two competitive inhibition ELISAs were developed. SDZ, SDMX, STZ, SP and sulphamethoxazole (SMX) were detected with both ELISAs at their MRL (100 ng ml^{-1}) in buffer solution. SDZ, STZ, and SMX could be detected at 10 ng ml^{-1}. Korpimäki et al. (2004) used protein engineering to improve the broad specificity of sulfa antibody 27G3 and improved the best mutant of the previous studies with site-directed mutagenesis. The new mutants recognized different sulphonamides with affinities sufficient for detection of all 13 tested sulphonamides at the levels below the MRLs. They also demonstrated the functionality of one mutant in some real sample matrices (Korpimäki et al. 2002, 2003). There are commercially available test kits for most sulphonamides, but at present there is none for multiresidue analysis.

Biosensor immunoassay

For the 'real-time' detection of sulphonamides in slaughterhouses, body fluids (bile, urine and blood serum) can be employed as markers for residue presence in tissue (Fodey et al. 1997, Crooks et al. 1998, Elliott et al. 1999). These body fluids can normally be analysed directly without extraction. Currently, the technology for performing 'real-time' analysis can be based on a commercially available surface plasmon resonance (SPR) biosensor (Biacore^{TM}).

An SPR-based BIA was described for the determination of SMZ residues in milk. SMZ was covalently immobilized to a carboxymethylated dextran (CM-dextran)-modified gold film. PAbs raised against SMZ were added to the sample and the immobilized surface was used to determine the amount of free antibodies. The LOD of this inhibition assay in raw and defatted milk was less than $1\,\mu g\,kg^{-1}$, which was better than those obtained with microbiological or physical methods. Other advantages of the biosensor assay they described were the lack of a sample preparation, its fully automated operation, the short time for analysis (20 min) and the better specificity (Sternesjö et al. 1995). A more rapid BIA screening assay for the detection of SDZ in pig bile was developed by Elliott et al. (1999). PAbs to SDZ were added to 20 times diluted bile samples. They found that an action level of $600\,\mu g\,kg^{-1}$ in pig bile could be used to control for the presence of SDZ in edible tissue at the MRL level ($100\,\mu g\,kg^{-1}$). Crooks et al. (1998) applied the same system and procedure to detect both SMZ and SDZ in pig bile on two separate biosensor chips and they found that the BIAs showed more reliable results than conventional immunoassay methods. However, due to the specificity of the antibodies used, these BIAs were all specific for one of the sulphonamides and, in order to detect several sulphonamides, different BIAs had to be used. For the screening of more sulphonamides at the same time, several investigations were focused on the development of generic antibodies detecting the group of sulphonamides (Spinks et al. 1999, Haasnoot et al. 2000a, b). However, these approaches were not successful as important sulphonamides (such as SMZ) were not detected or were detected at a high level only. A MAb (21C7) raised against SMZ (Kohen et al. 2000) was identified as an antibody recognizing several important sulphonamides dissolved in buffer (Haasnoot et al. 2000a). This MAb was used for the development of anti-idiotype antibodies and was applied in a BIA for the detection of SMZ in urine (Akkoyun et al. 2000); however, it was not applied for the detection of several sulphonamides in other sample materials.

Haasnoot et al. applied a MAb against SMZ (21C7) in an optical biosensor (Biacore Q) to develop a rapid BIA for the detection of several sulphonamides in chicken serum. Using MAb 21C7, the LOD for SMZ in ten times diluted chicken serum was lower ($10\,ng\,ml^{-1}$). Compared with the PAbs, the MAb-based BIA resulted in a better sensitivity and was found suitable for the detection of eight sulphonamides in ten times diluted chicken serum with LODs between 7 and $20\,ng\,ml^{-1}$. Protein engineering of this antibody (Korpimäki et al. 2002, 2003) produced binders capable of detecting ten of the tested 13 sulphonamides in a buffer system with an adequate sensitivity for screening of sulphonamide contamination below the European Union MRL. The functionality of the best-obtained mutant in different sample matrices was also briefly demonstrated by using it in the analysis of SMZ from semi-skimmed milk, chicken serum and minced beef samples.

Microbiological methods

Microbiological methods do not require highly specialized and expensive equipment. They also provide highly homogeneous cell populations for test and thus result in better assay precision. These methods, therefore, are ideal in situations where the number of tests is likely to be small and, hence, large capital expenditure cannot be justified. Microbiological methods, however, are limited only to those analytes that either promote or inhibit microbiological growth. This fact mandates that no other substance be present in the test sample that either promotes or inhibits growth or modifies the response to the analyte. Microbiological methods using broth cultures (tube methods) and methods on semi-solid culture media (plate methods) offer several advantages. Although yeasts, protozoa and algae can be used for microbiological assays, most methods use bacteria because of the relative ease of use.

Gaudin et al. (2004) presented a microbiological method for the screening of antibiotic residues in milk. The sensitivity of this screening test was established by the analysis of milk samples spiked with 66 antibiotics. The plates were of *Bacillus stearothermophilus* for sulphonamides and beta-lactams, and the sensitivity was at or below the MRL for some sulphonamides. Although initial screening bioassay systems are recognized for their sensitivities to antimicrobial drug groups, none are sensitive to sulphonamides at or near the MRLs set in the Codex Alimentarious. Okerman et al. (1998) used a modified four-plate test to screen 4795 meat samples. The four-plate test is not sensitive enough to detect sulphonamides and quinolones at the

MRL, but higher levels may cause inhibition. Braham et al. (2001) developed a sulphonamide-sensitive rapid assay using *B. stearothermophilus*-inoculated PM indicator agar containing bromcresol purple and trimethoprim. Five sulphonamides plus 16 other antimicrobial drugs were tested in standard concentrations in water, bovine kidney and ground beef. Sulphonamides were detected at concentrations near the MRLs. Vermunt et al. (1993) made some improvements in the tube diffusion method for detecting antibiotics and sulphonamides in raw milk. Samples shown to inhibit growth of *B. stearothermophilus* var. *calidolactis* in the screening method are now heated for 10 min at 80°C to exclude the inhibitory effect of any lysozyme and lactoferrin complex. Inhibition of growth in the heated milks, which can be overcome by adding *p*-amino benzoic acid (PABA), shows the presence of sulphonamides. Possibly some variants of the tube diffusion method will be applied in future as a screening test in order to bring the detection limits closer to or beyond the toxicologically safe levels.

Bermudez-Almada et al. (2001) monitored the sulphonamide residue levels by a microbiological receptor assay (Charm II) and confirmed by HPLC, in porcine muscles collected at federally inspected packing plants of Northwestern Mexico. Molina et al. (2003) determined the detection limits of 24 antimicrobial agents (including SDZ, SMX, sulphamethoxypyridazine (SMPD) and SQ) in ewes' milk by a commercially available version of brilliant black reduction test, BRT inhibitor test with pre-diffusion AiM(R) (BRT AiM(R)).

Other analytical methods

Spectrophotometric methods (Ribone et al. 2001, Nagaraja et al. 2002, Fan et al. 2003) have also been used to analyse sulphonamide residues. Lopez-Martinez et al. (2002) used the bivariate calibration spectrophotometric method to determine the SMX and SMPD in veterinary products. The statistical evaluation of the method bias showed that the proposed procedure is comparable with commonly used first-derivative spectrophotometry. However, the advantage of bivariate calibration is its simplicity due to the minimal spectra manipulation when compared with derivative techniques. Evgeńev et al. (2002) found the operating conditions for the determination of sulphonamides and carbutamide as 4,6-dinitrobenzofuroxane derivatives by flow-injection analysis with spectrophotometric detection. The best results were obtained when flows of ethanol (methanol) and a buffer solution were mixed at a volume ratio of 30 : 70 and pH 6.5–7.0. The calibration range was 0.25–5.5 $\mu g\,ml^{-1}$. The LODs were 0.12–0.24 $\mu g\,ml^{-1}$. Determination of a group of sulphonamides using sequential injection analysis (SIA) technique with chemiluminescence (CL) detection has also been devised (Pasekova et al. 2001). Supercritical fluid chromatography (Ashraf et al. 1997, Combs et al. 1997, Dost et al. 2000) and first-derivative photochemical-induced fluorescence method (Pena et al. 1995) have also been used to analyse the sulphonamide residues.

Conclusions and outlook

The monitoring of sulphonamide residues in slaughtered animals has become one of the most important duties for public health agencies. The analytical methods for drug residue monitoring should be accurate, simple, economical in time and cost, and capable of detecting residues below MRLs. Discharging toxic organic solvents is also a worldwide problem. Therefore, the use of organic solvents, especially chlorinated ones should be minimized. The current sulphonamide detection technologies are based on chromatographic methods or bacteriological growth inhibition. The instrumental methods such as HPLC and GC provide sensitive and specific techniques, but they have the following problems:

- Extraction and clean-up involve numerous different analytical steps that are time-consuming and do not permit the monitoring of a large number of samples.
- Recoveries are sometimes low and variable.
- Toxic solvents such as methylene chloride and acetonitrile are used as extraction solvents and/or HPLC mobile phases.

These methods are suitable for confirmation but not for the screening of large numbers of samples. A rapid, sensitive and specific assay is needed to pick positive samples in routine analysis, which can then be confirmed for the presence of sulphonamides by HPLC. Therefore, during the past decades, a variety of immunochemical methods were developed for the detection of contaminants. ELISA and other immuno-based methods have flexibility and can be designed with appropriate sensitivity so that MRLs or levels of concern are easily detected. Immunochemical methods can be a simple, rapid and cost-effective, with sufficient sensitivity and specificity to detect small molecules, and can provide results at low detection limits without major changes in procedure. Like any other newly developed technique, immunoassays also suffer from disadvantages, such as a significant development time and being generally unsuitable for multiresidue analysis.

Acknowledgement

The authors are grateful for the financial support of the New Century Talent Program of the Ministry of Education, P. R. China (Project No. NECT-04-0243) and the Scientific Research of Refund of Tianjin University of Science and Technology, P. R. China (Project No. 20050204).

References

Ackermans MT, Beckers JL, Everaerts FM, Hoogland H, Tomassen MJH. 1992. Determination of sulphonamides in pork meat extracts by capillary zone electrophoresis. Journal of Chromatography 596:101–109.

Agarwal VK. 1990. Detection of sulphamethazine residues in milk by high performance liquid chromatography. Journal of Liquid Chromatography 13:3531–3539.

Agarwal VK. 1992. High-performance liquid chromatographic methods for the determination of sulphonamides in tissue, milk and eggs. Journal of Chromatography 624:411–423.

Agarwal VK. 1993. Application of solid-phase extraction for the analysis of sulphonamides in milk by high-performance liquid chromatography. Journal of Liquid Chromatography 16:3793–3799.

Akhtar MH, Wong ML, Crooks SRH, Sauve A. 1998. Extraction of incurred sulphamethazine in swine tissue by microwave assisted extraction and quantification without clean-up by high-performance liquid chromatography following derivatization with dimethylaminobenzaldehyde. Food Additives and Contaminants 15:542–549.

Akkoyun A, Kohen F, Bilitewski U. 2000. Detection of sulphamethazine with an optical biosensor and anti-idiotypic antibodies. Sensors and Actuators B 70:12–18.

Anne-Claire M, Zeggane S. 2003. HPLC determination of sulphathiazole in French honeys. Journal of Liquid Chromatography and Related Technologies 26:953–961.

Ashraf KM, Gandee MB, Combs MT, Taylor LT, Frey CR. 1997. Semipreparative separation and fractionation of sulphonamides via supercritical fluid chromatography. Journal of Chromatographic Science 35:593–597.

Ashraf KM, Taylor LT, Schweighardt FK. 1996. Comparison of supercritical CHF_3 and CO_2 and methanol-modified CHF_3 and CO_2 for extraction of sulphonamides from chicken liver. Journal of AOAC International 79:1043–1049.

Assil HI, Sheth H, Sporns P. 1992. An ELISA for sulphonamide detection using affinity-purified polyclonal antibodies. Food Research International 25:343–353.

Baere SD, Baert K, Croubels S, Busser JD, Wasch KD, Backer PD. 2000. Determination and quantification of sulphadiazine and trimethoprim in swine tissues using liquid chromatography with ultraviolet and mass spectrometric detection. Analyst 125:409–415.

Barbieri G, Bergamini C, Ori E, Resca P. 1995. Determination of sulphonamides in meat and meat products. Industrie Alimentari 34:1273–1276.

Bartolucci G, Pieraccini G, Villanelli F, Moneti G, Triolo A. 2000. Liquid chromatography tandem mass spectrometric quantitation of sulphamethazine and its metabolites: Direct analysis of swine urine by triple quadrupole and by ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 14:967–973.

Bermudez-Almada MD, Miranda-Vasquez L, Espinosa-Plascencia A, Valenzuela-Quintanar AI, Vazquez-Moreno L. 2001. Sulphonamide residues in muscle of pigs slaughtered in the Northwest of Mexico. Revista Cientifica-Facultad DE Ciencias Veterinarias 11:127–132.

Berzas-Nevado JJ, Castañeda-Peñalvo G, Guzmán-Bernardo FJ. 1999. Micellar electrokinetic capillary as an alternative method for the determination of sulphonamides and their associated compound. Journal of Liquid Chromatography and Related Technology 22:1975–1986.

Berzas-Nevado JJ, Castañeda-Peñalvo G, Guzmán-Bernardo FJ. 2001. Simultaneous determination of sulphamethoxypyridazine, sulphamethoxazole, sulphadimethoxine and their associated compounds by liquid chromatography. Analytica Chimica Acta 442:241–248.

Bevill RF. 1984. Sulphonamides. In: Jukes TH, Dupont HL, Crawford LM, editors. CRC Handbook Series Inzoonoses, Section D, Antibiotics, sulphonamides, and public health. Boca Raton, FL: CRC Press. p. 355–365.

Bevill RF, Schemske KM, Luther HG, Dzierzak EA, Limpoka M, Felt DR. 1978. Determination of Sulphonamides in Swine Plasma. Journal of Agriculture and Food Chemistry 26:1201–1203.

Bogialli S, Curini R, Corcia ADI, Nazzari M, Polci ML. 2003. Rapid confirmatory assay for determining 12 sulphonamide antimicrobials in milk and eggs by matrix solid-phase dispersion and liquid chromatography-mass spectrometry. Journal of Agriculture and Food Chemistry 51:4225–4232.

Boulaire SL, Bauduret JC, Andre F. 1997. Veterinary drug residues survey in meat: An HPLC method with a matrix solid phase dispersion extraction. Journal of Agriculture and Food Chemistry 45:2134–2142.

Braham R, Black WD, Claxton J, Yee AJ. 2001. A rapid assay for detecting sulphonamides in tissues of slaughtered animals. Journal of Food Protection 64:1565–1573.

Caballero RD, Torres-Lapasío JR, Baeza-Baeza JJ, Garćia-Alvarez-Coque MC. 2001. Micellar chromatographic procedure with direct injection for the determination of sulphonamides in milk and honey samples. Journal of Liquid Chromatography and Related Technology 24:117–131.

Chiavarino B, Crestoni ME, Marzio AD, Fornanni S. 1998. Determination of sulphonamide antibiotics by gas chromatography coupled with atomic emission detection. Journal of Chromatography B 706:269–277.

Cliquet P, Cox E, Haasnoot W, Schacht E, Goddeeris BM. 2003. Generation of group-specific antibodies against sulphonamides. Journal of Agriculture and Food Chemistry 51:5835–5842.

Combs MT, Ashraf Khorassani M, Taylor LT. 1996. Comparison of supercritical CHF_3 and CO_2 for extraction of sulphonamides from various food matrices. Analytical Chemistry 68:4507–4511.

Combs MT, Ashraf Khorassani M, Taylor LT. 1997. Method development for the separation of sulphonamides by supercritical fluid chromatography. Journal of Chromatographic Science 35:176–180.

Crooks SRH, Baxter GA, O'Connor MC, Elliott CT. 1998. Immunobiosensor — an alternative to enzyme immunoassay screening for residues of two sulphonamides in pigs. Analyst 123:2755–2757.

Dang Q, Sun Z, Ling D. 1992. Separation of sulphonamides and determination of the active ingredients in tablets by micellar electrokinetic capillary chromatography. Journal of Liquid Chromatography 603:259.

Din N, Bartle KD, Clifford AA, McCormack A, Castle L. 1997. Supercritical fluid extraction of sulphamethazine and its metabolites from meat tissues. Journal of Chromatographic Science 35:31–37.

Dixon-Holland DE, Katz SE. 1988. Competitive direct enzyme-linked immunosorbent assay for detection of

sulphamethazine residues in swine and muscle tissue. Journal of the Association of Official Analytical Chemists 71:1137–1140.

Dost K, Jones DC, Davidson G. 2000. Determination of sulphonamides by packed column supercritical fluid chromatography with atmospheric pressure chemical ionisation mass spectrometric detection. Analyst 125:1243–1247.

Elliott CT, Baxter GA, Crooks SRH, McCaughey WJ. 1999. The development of a rapid immunobiosensor screening method for the detection of residues of sulphadiazine. Food Agriculture and Immunology 11:19–27.

EU Regulation No. 508/1999. 1999. L60, 16–52.

Evgeńev MI, Garmonov SY, Shakirova LS. 2002. Flow-injection determination of sulphanilamides in drugs and biological fluids with spectrophotometric detection. Journal of Analytical Chemistry 57:64–70.

Fan J, Chen YH, Feng SL, Ye CL, Wang JJ. 2003. Flow-injection spectrophotometric determination of sulphadiazine and sulphamethoxazole in pharmaceuticals and urine. Analytical Sciences 19:419–422.

Fleeker JR, Lovet TLJ. 1985. Enzyme immunoassay for screening sulphamethazine residues in swineb blood. Journal of the Association of Official Analytical Chemists 68:172–174.

Fodey TL, Crooks SRH, Elliott CT, McCaughey WJ. 1997. Comparison of porcine urine and bile as matrices to screen for the residues of two sulphonamides using a semi-automated enzyme immunoassay. Analyst 122:165–168.

Food and Drug Regulation. 1991. Canada Gazette Part II, Table 3, Division 15, Part B. 125: 1478–1480.

Franco DA, Webb J, Taylor CE. 1990. Antibiotic and sulphonamide residues in meat: Implication for human health. Journal of Food Protein 53:178–185.

Fránek M, Kolar V, Deng A, Crooks S. 1999. Determination of sulphadimidine (sulfamethazine) residues in milk, plasma, urine and edible tissues by sensitive ELISA. Food and Agricultural Immunology 11:339–349.

Fuh MRS, Chu SY. 2003. Quantitative determination of sulphonamide in meat by solid-phase extraction and capillary electrophoresis. Analytica Chimica Acta 499:215–221.

Furusawa N. 1999a. High-performance liquid chromatographic determination/identification of oxytetracycline and sulphadimidine in meat and eggs. Chromatographia 49:369–373.

Furusawa N. 1999b. Liquid-chromatographic determination of sulphadimidine in milk and eggs. Fresenius Journal of Analytical Chemistry 364:270–272.

Furusawa N. 2001. Determining the procedure for routine residue monitoring of sulphamethazine in edible animal tissues. Biomedical Chromatography 15:235–239.

Furusawa N. 2003a. Clean and rapid liquid chromatographic technique for monitoring of oxytetracycline and sulphadimicline in milk without use of organic solvents. Chromatographia 57:317–320.

Furusawa N. 2003b. Rapid high-performance liquid chromatographic determining technique of sulphamonomethoxine, sulphadimethoxine, and sulphaquinoxaline in eggs without use of organic solvents. Analytica Chimica Acta 481:255–259.

Furusawa N, Kishida K. 2001. High-performance liquid chromatographic procedure for routine residue monitoring of seven sulphonamides in milk. Fresenius Journal of Analytical Chemistry 371:1031–1033.

Garcia I, Sarabia L, Ortiz MC, Aldama JM. 2004. Robustness of the extraction step when parallel factor analysis (PARAFAC) is used to quantify sulphonamides in kidney by high-performance liquid chromatography-diode array detection (HPLC-DAD). Analyst 129:766–771.

Gaudin V, Maris P, Fuselier R, Ribouchon JL, Cadieu N, Rault A. 2004. Validation of a microbiological method: The STAR protocol, a five-plate test, for the screening of antibiotic residues in milk. Food Additives and Contaminants 21:422–433.

Gehring TA, Rushing LG, Churchwell MI, Doerge DR, McErlane KM, Thompson HC. 1996. HPLC determination of sulphadiazine residues in Coho salmon (Oncorhynchus kisutch) with confirmation by liquid chromatography with atmospheric pressure chemical ionization mass spectrometry. Journal of Agriculture and Food Chemistry 44: 3164–3169.

Guggisber GD, Mooser AE, Koch H. 1992. Methods for the determination of sulphonamides in meat. Journal of Chromatography 624:425–437.

Haasnoot W, Cazemier G, Du Pré J, Kemmers-Voncken A, Bienenmann-Ploum M, Verheijen R. 2000a. Sulphonamide antibodies: from specific polyclonals to generic monoclonals. Food Agriculture and Immunology 12:15–30.

Haasnoot W, Du Pré J, Cazemier G, Kemmers-Voncken A, Verheijen R, Janssen BJM. 2000b. Monoclonal antibodies against a sulphathiazole derivative for the immunochemical detection of sulphonamides. Food Agriculture and Immunology 12:127–138.

Hoffmeister A, Suhren G, Heeschen W. 1991. High pressure liquid chromatographic determination of sulphadimidine residues in milk — incidence in consumer milk from various European countries. Milchwissenschaft. Milk Science International 46:770–774.

Ito Yuko OH, Ikai Y, Matsumoto H, Miyazaki Y, Nagase H. 2000. Application of ion-exchange cartridge clean-up in food analysis V. Simultaneous determination of sulphonamide antibacterials in animal liver and kidney using high-performance liquid chromatography with ultraviolet and mass spectrometric detection. Journal of Chromatography 898:95–102.

Jackman R, Brown AJ, Dell AN, Everest SJ. 1993. Production of antisera to 12 sulphonamides and their use in enzyme immunoassays. In: Haagsma N, Ruiter A, Czedik-Eyeenberg PB, editors. Euroresidue II: Conference of Residues of Veterinary Drugs in Foods, University of Utrecht, Utrecht, the Netherlands. pp. 391–394.

Kao YM, Chang MH, Cheng CC, Chou SS. 2001. Multiresidue determination of veterinary drugs in chicken and swine muscles by high-performance liquid chromatography. Journal of Food and Drug Analysis 9:84–95.

Kim DH, Choi JO, Kim J, Lee DW. 2003. Application of a polymeric solid phase extraction for the analysis of sulphonamides in milk by LC/MS. Journal of Liquid Chromatography and Related Technologies 26:1149–1159.

Kim DS, Park MS. 1998. Antibiotic use at a pediatric age. Yonsei Medical Journal 39:595–603.

Kishida K, Furusawa N. 2003. Toxic/harmful solvents-free technique for HPLC determination of six sulphonamides in meat. Journal of Liquid Chromatography and Related Technologies 26:2931–2939.

Kishida K, Nishinari K, Furusawa N. 2004. Liquid chromatographic determination of sulphamonomethoxine, sulphadimethoxine, and their N4-acetyl metabolites in chicken plasma. Chromatographia 61:81–84.

Kitts DD, Zheng M, Burnsflett E, McErlane KM. 1995. Comparison of sulphadimethoxine residue analysis in salmon muscle using HPLC and Charim-II test. Journal of Food Protection 58:678–682.

Kohen F, Gayer B, Amir-Zaltsman Y, O'Keeffe M. 2000. Generation of an anti-idiotypic antibody as a surrogate ligand for sulphamethazine in immunoassay procedures. Food Agriculture and Immunology 12:193–201.

Korpimäki T, Brockmann E-C, Kuronen O, Saraste M, Lamminmaki U, Tuomola M. 2004. Engineering of a broad specificity antibody for simultaneous detection of 13 sulphonamides at the maximum residue level. Journal of Agriculture and Food Chemistry 52:40–47.

Korpimäki T, Rosenberg J, Virtanen P, Karskela T, Lamminmäki U, Tuomola M, Vehniäinen M, Saviranta P. 2002. Improving broad specificity hapten recognition with protein engineering. Journal of Agriculture and Food Chemistry 50:4194–4201.

Korpimäki T, Rosenberg J, Virtanen P, Lamminmäki U, Tuomola M, Saviranta P. 2003. Further improvement of broad specificity hapten recognition with protein engineering. Protein Engineering 16:37–46.

Li JS, Li XW, Yuan JX, Wang X. 2000. Determination of sulphonamides in swine meat by immunoaffinity chromatography. Journal of the Association of Official Analytical Chemistry 83:830–836.

Lin CE, Lin WC, Chlou WC, Lin EC, Chang CC. 1996. Migration behaviour and separation of sulphonamides in capillary zone electrophoresis I. Influence of buffer pH and electrolyte modifier. Journal of Chromatography 755:261–269.

Long AR, Hsieh LC, Malbrough MS, Short CR, Barker SA. 1990. Multiresidue method for the determination of sulphonamides in pork tissue. Journal of Agriculture and Food Chemistry 38:423–426.

Lopez-Martinez L, Lopez-de-Alba PL, De Leon-Rodriguez LM, Yepez-Murrieta ML. 2002. Simultaneous determination of binary mixtures of trimethoprim and sulphamethoxazole or sulphamethoxypyridazine by the bivariate calibration spectrophotometric method. Journal of Pharmaceutical and Biomedical Analysis 30:77–85.

Malik S, Duncan SE, Bishop JR, Taylor LT. 1994. Extraction and detection of sulphamethazine in spray-dried milk. Journal of Dairy Science 77:418–425.

Marzo A, Dal Bo L. 1998. Chromatography as an analytical tool for selected antibiotic classes: A reappraisal addressed to pharmacokinetic applications. Journal of Chromatography 812:17–34.

Maudens KE, Zhang GF, Lambert WE. 2004. Quantitative analysis of twelve sulphonamides in honey after acidic hydrolysis by high-performance liquid chromatography with post-column derivatization and fluorescence detection. Journal of Chromatography 1047:85–92.

McCracken RJ, Spence DE, Kennedy DG. 2000. Comparison of extraction techniques for the recovery of veterinary drug residues from animal tissues. Food Additives and Contaminants 17:907–914.

McEvoy JDG, Mayne CS, Higgins HC, Kennedy DG. 1999. Transfer of sulphamethazine from contaminated dairy feed to cows' milk. Veterinary Record 144:470–475.

Mengelers MJB, Polman AMM, Aerts MML, Kuiper HA, Vanmiert A. 1993. Determination of sulphadimethoxine, sulphamethoxazole, trimethoprim and their main metabolites in lung and edible tissues from pigs by multidimensional liquid-chromatography. Journal of Liquid Chromatography 16:257–278.

Molina MP, Althaus RL, Molina A, Fernandez N. 2003. Antimicrobial agent detection in ewes' milk by the microbial inhibitor test brilliant black reduction test-BRT AiM (R). International Dairy Journal 13:821–826.

Montanaro A. 1998. Sulphonamide allergy. Immunology and Allergy Clinics of North America 18: 843.

Msagati TAM, Nindi MM. 2004. Multiresidue determination of sulphonamides in a variety of biological matrices by supported liquid membrane with high pressure liquid chromatography-electrospray mass spectrometry detection. Talanta 64:87–100.

Muldoon MT, Font IA, Beier RC, Holtzapple CK, Young CR, Stanker LH. 1999. Development of a cross-reactive monoclonal antibody to sulphonamide antibiotics: Evidence for structural conformation-selective hapten recognition. Food Agriculture and Immunology 11:117–134.

Nagaraja P, Yathirajan HS, Sunitha KR, Vasantha RA. 2002. A new, sensitive, and rapid spectrophotometric method for the determination of sulfa drugs. Journal of AOAC International 85:869–874.

Nose N, Kobayashi S, Hirose A, Watanabe A. 1976. Gas–liquid chromatographic determination of sulphonamides. Journal of Chromatography 123:167–173.

Okerman L, Van Hoof J, Debeuckelaere W. 1998. Evaluation of the European four-plate test as a tool for screening antibiotic residues in meat samples from retail outlets. Journal of AOAC International 81:51–56.

Pang GF, Cao YZ, Fan CL, Zhang JJ, Li XM, Li ZY, Jia GQ. 2003. Liquid chromatography–fluorescence detection for simultaneous analysis of sulphonamide residues in honey. Annals of Bioanalytical Chemistry 376:534–541.

Papapanagiotou EP, Iossifidou EG, Psomas IE, Photis G. 2000. Simultaneous HPLC determination of sulphadiazine and trimethoprim in cultured gilthead SEA bream (Sparus aurata L.) tissues. Journal of Liquid Chromatography and Related Technology 23:2839–2849.

Pasekova H, Polasek M, Cigarro JF, Dolejsova J. 2001. Determination of some sulphonamides by sequential injection analysis with chemiluminescence detection. Analytica Chimica Acta 438:165–173.

Pastor-Navarro N, Garcia-Rover C, Maquieira A, Puchades R. 2004. Specific polyclonal-based immunoassays for sulphathiazole. Annals of Bioanalytical Chemistry 379:1088–1099.

Pecorelli I, Bibi R, Fioroni L, Galarini R. 2004. Validation of a confirmatory method for the determination of sulphonamides in muscle according to the European Union regulation 2002/657/EC. Journal of Chromatography 1032: 23–29.

Pena MS, Acedo MJ, Salinas F, Mathedero MC, Aaron JJ. 1995. Analysis of sulphamethazine in the presence of sulphamerazine or sulphadiazine by first-derivative photochemically induced fluorescence. Journal of Pharmaceutical and Biomedicalanalysis 13:1107–1112.

Pensabene JW, Fiddler W, Parks OW. 1997. Isolation of sulphonamides from whole egg by supercritical fluid extraction. Journal of Chromatographic Science 35:270–274.

Reeves VB. 1999. Confirmation of multiple sulphonamide residues in bovine milk by gas chromatography-positive chemical ionization mass spectrometry. Journal of Chromatography B 723:127–137.

Reimer GJ, Suarez A. 1991. Development of a screening method for 5 sulphonamides in salmon muscle-tissue using thin-layer chromatography. Journal of Chromatography 555:315–320.

Renson C, Degand G, Maghum-Roglster G. 1993. Determination of sulphamethazine in animal tissues by enzyme immunoassay. Analytica Chimica Acta 275:323–328.

Ribone ME, Pagani AP, Olivieri AC. 2001. Simultaneous multivariate, spectrophotometric analysis of ear drops containing a ternary mixture of antipyrine, sulphathiazole, and rivanol. Analytical Letters 34:2077–2088.

Roudaut B, Garnier M. 2002. Sulphonamide residues in eggs following drug administration via the drinking water. Food Additives and Contaminants 19:373–378.

Salisbury CDC, Sweet JC, Munro R. 2004. Determination of sulphonamide residues in the tissues of food animals using automated precolumn derivatization and liquid chromatography with fluorescence detection. Journal of AOAC International 87:1264–1268.

Saschenbrecker PW, Fish NA. 1980. Sulphamethazine residues in uncooked edible tissues of pork following recommended oral administration and withdrawal. Canadian Journal of Comparative Medicine 44:338–345.

Schwarz S, Chaslus-Dancla E. 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Veterinary Research 32:201–225.

Shaikh B, Moats WA. 1993. Liquid-chromatographic analysis of antibacterial drug residues in food-products of animal origin. Journal of Chromatography 643:369–378.

Sheth HB, Sporns P. 1991. Development of a single ELISA for detection of sulphonamides. Journal of Agriculture and Food Chemistry 39:1696–1700.

Singh P, Ram BP, Sharkov N. 1989. Enzyme immunoassay for screening of sulphamethazine in swine. Journal of Agriculture and Food Chemistry 37:109–114.

Spinks CA, Schut CG, Wyatt GM, Morgan MRA. 2001. Development of an ELISA for sulphachlorpyridazine and investigation of matrix effects from different sample extraction procedures. Food Additives and Contaminants 18:11–18.

Spinks CA, Wyatt GM, Morgan MRA. 1999. Molecular modelling of hapten structure and relevance to broad spectrum specificity immunoassay for sulphonamide antibiotics. Bioconjugate Chemistry 10:583–588.

Sternesjö A, Mellgren C, Björk L. 1995. Determination of sulphamethazine residues in milk by a surface plasmon resonance-based biosensor assay. Analytical Biochemistry 226:175–181.

Stoev G, Michailova AL. 2000. Quantitative determination of sulphonamide residues in foods of animal origin by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography 871:37–42.

Szymanski A. 2003. Micellar liquid chromatography for the determination of sulphonamide residues in milk and eggs. Chemia Analityczna 48:429–438.

Ueno R, Sangrungruang K, Miyakawa M. 1999. A simplified method for the determination of several fish drugs in edible fish and shrimp by high-performance liquid chromatography. Food Research International 32:629–633.

Unruh J, Piotrowski E, Schwartz DP, Barford R. 1990. Solid-phase extraction of sulphamethazine in milk with quantitation at low ppb levels using thin-layer chromatography. Journal of Chromatography 519:179–187.

Vermunt AEM, Stadhouders J, Loeffen GJM, Bakker R. 1993. Improvements of the tube diffusion method for detection of antibiotics and sulphonamides in raw-milk. Netherlands Milk and Dairy Journal 47:31–40.

Yang TCC, Yang IL, Liao LJ. 2004. Determination of sulphonamide residues in milk by online microdialysis and HPLC. Journal of Liquid Chromatography and Related Technologies 27:501–510.

Zheng M, Liu HY, Hall SF, Kitts DD, McErlane KM. 1994. High-performance liquid chromatographic analysis of Romet-30 in Chinook salmon (Oncorhynchus tshawytscha): Wash-out time, tissue distribution in muscle, liver and skin, and metabolism of sulphadimethoxine. Journal of Chromatography 670:77–88.

Zomer E, Quintana J, Scheemaker J, Saul S, Charm SE. 1996. High-performance liquid chromatography-receptorgram: A comprehensive method for identification of veterinary drugs and their active metabolites. Veterinary Drug Residues ACS Symposium Series 636:149–160.