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Chromatographic Residue Analysis of Sulfonamides in Foodstuffs of Animal Origin

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Abstract: The widespread use of antibiotics in veterinary medicine for prophylactic, therapeutic and growth promoting purposes may result in the presence of residues in foodstuff of animal origin. Sulfonamides, N-derivatives of 4-amino-benzenesulfonamide, belong to a diverse class of synthetic antibiotics consisting of chemically related compounds. A number of them e.g., sulfamethazine, sulfadiazine, sulfaquinoxaline and sulfamethoxypyridazine, are widely used in animal husbandry. Their residues in food are of great concern because of their potential carcinogenic character and because they may contribute to the development of antibiotic resistance in humans. These facts have induced most of the regulatory activities on sulfonamides. Regulatory bodies in the European Union as well as in other countries have set different maximum residue limits in various tissues. These regulations have stimulated the development of fast, accurate and sensitive analytical methods for monitoring sulfonamide residues in food samples of animal origin. Gas chromatography methods tend to be time-consuming due to the required previous cleanup and derivatization procedures, thus, HPLC methods with UV or MS detection have the leading position. These and other aspects of current analytical methodology, including sample preparation and cleanup, are reviewed and discussed. Emphasis is given to confirmatory methods since these comprise useful tools to regulatory agencies, and identifications based on these methods can be used in support of regulatory action.

Keywords: Sulfonamides, residue, international regulation, meat, milk, food of animal origin, limit of detection

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INTRODUCTION

Antibacterial sulfonamides (SAs) also called simply "sulfa drugs" are a group of synthetic antibacterial agents that contain the sulfonamide group widely used in veterinary practice for the treatment of infections and growth promotion of food-producing animals. There is a risk of SAs residues in animal products if these drugs have been improperly administered or if the withdrawal period has not been observed. Monitoring of such residues in products for human consumption and in slaughtered animals has become one of the most important duties for public health agencies. Moreover drug residues may cause allergic or toxic reaction to consumers and promote occurrence of antibiotic-resistant bacteria (1–7).

It has been reported that some SAs e.g., sulfamethazine (SMZ) or sulfadimidine produce tumours in rodent bioassay and also some evidence on the toxicity of SAs on the thyroid gland has been presented (8–10). Due to their potential carcinogenic character regulatory agencies have adopted maximum residue levels (MRL) for SAs in foodstuffs of animal origin, in order to protect consumers' health (11). For unambiguous identification as well as for confirmation of antibiotics residues in food products of animal origin for human consumption, public health agencies in many countries rely on detection by mass spectrometry. Thus LC–MS–MS has become widely used as a complementary technique to GC–MS in residue analysis because of its applicability to the determination of polar and/or non-volatile compounds without derivatization, including both electrospray and atmospheric pressure chemical ionization.

There are several interesting reviews in the literature concerning the analysis of SAs in food. Guggisberg et al. in 1992 wrote a review on methods for the determination of SAs and their metabolites, in meat, using various analytical techniques e.g., HPLC, GC, GC-MS, LC-MS, SFC-MS, TLC and immunological methods (12).

A review on HPLC methods for the determination of SAs in tissue, milk and eggs has been published by Agarwal in 1992. This review focuses on HPLC methods for the determination of SAs in foods of animal origin published in the time period of 1980–1992. The existing methods were critically evaluated and suggestions for future research were made (13).

In 1993 Shaikh and Moats, published a review on chromatographic analysis of antibacterial drug residues in food products of animal origin. SAs that are being reviewed among other antibiotics are determined by liquid chromatographic methods for residue analysis in food products of animal origin, such as meat and milk. This review covers cleanup procedures, such as, ultrafiltration, liquid-liquid

partition, solid-phase extraction, immunoaffinity, and matrix solid-phase dispersion, for use as extraction, deproteinization, and concentration steps (14).

In 1998 Marzo and Dal Bo published a review on the use of chromatography as an analytical tool for selected antibiotic classes, addressed to pharmacokinetic applications. In this review, the authors describe the analytical methods employed in the pharmacokinetics of various antibiotics including SAs (15).

Kennedy et al. published a review in 1988 (16) on the use of liquid chromatography–mass spectrometry in the analysis of residues of antibiotics in meat and milk. In this review, quantitative LC-MS methods for the analysis and confirmation of veterinary drug residues of each of the major classes of antibiotics including SAs are presented.

Niessen, in 1998, wrote a review on the analysis of antibiotics by Liquid Chromatography–Mass Spectrometry. Currently available data on these compound classes are reviewed, with special emphasis given to important aspects, especially the ones relevant to LC–MS and on the obtained mass spectral information. The main application area of LC–MS in this field, which is the confirmation of identity in animal food products for human consumption at maximum residue levels, set by the regulatory authorities, is reviewed. LC–MS for the determination and confirmation of SAs, β-lactam antibiotics, (fluoro)quinolone antibiotics, as well as various other groups including aminoglycosides, chloramphenicol, ionophore antibiotics, 5-nitrofuran derived compounds and macrolide antibiotics are being criticised (17).

In 2002 Di Corcia and Nazzari wrote a review on LC-MS methods for analyzing antibiotic and antibacterial agents in animal food products. These methods have given a strong impulse to develop determinative and confirmatory methods for the medicines mentioned above in foodstuffs. Analytical methods developed for analyzing components of the major classes of the medicines mentioned above are reviewed here. The discussion is focused also on sample treatment and final LC–MS analysis (18).

In 2002 Joshi in his review article on the HPLC separation of antibiotics present in formulated and unformulated samples, presents column and mobile phase conditions for SAs among various classes of antibiotics, developed from April 1998 to November 2000. A brief discussion on chemical structure, spectrum of activity and action mechanism of each class has also been given (19).

In 2003 Balizs and Hewitt wrote a review on the determination of veterinary drug residues by LC-MS/MS. This paper describes the principles, the current technology and the applications of HPLC and tandem mass spectrometry in the analysis of veterinary drug residues (20).

In 2005 Núñez et al. in their paper on the LC–MS/MS analysis of organic toxics in food review the state of the art of LC–tandem MS (LC–MS/MS) for the analysis of organic toxics in food products. They include instrumental aspects, such as ionization sources and analyzers, as well as confirmation and quantification procedures. Moreover, the application of LC–MS/MS to SAs among other antibiotics in a range of food products is discussed (21).

Gentili et al., in 2005, wrote about LC-MS/MS for performing confirmatory analysis of veterinary drugs in animal-food products. This review focuses on recent developments and trends in liquid chromatography coupled to mass spectrometry, with a particular emphasis given to tandem mass spectrometry and the new criteria established by the European Union for performing confirmatory analysis of veterinary drugs in animal-food products. The combination of liquid chromatography and tandem mass spectrometry allows unequivocal identification of traces of antibiotics and antibacterial agents in complex biological matrices, such as honey, eggs, milk and meat. The sensitivity of the coupling is particularly useful for confirming the presence of banned substances that require limits of detection as low as possible (22).

Wang et al., in their 2006 review on the analysis of SA residues in edible animal products, describe the methods of analysis of SA residues in edible animal products. This review refers to HPLC, LC/MS, GC, TLC, HPCE, ELISA, biosensor immunoassay and microbiological methods. Specific aspects of analysing SAs, such as sample handling, chromatographic conditions and detection methods are discussed. The current SA detection technologies are based on chromatographic methods or bacteriological growth inhibition (23).

In 2006 Garcia-Ruiz and Marina published a review on the recent advances in the analysis of antibiotics by capillary electrophoresis. In this review, the main aspects related to the separation of different groups of antibiotics by CE as well as the different applications reported in the literature from the beginning of 2003 through May 2005 are provided to the readers. Firstly, the experimental conditions employed to achieve the analysis of antibiotics by CE are given. Then, the main applications performed in the pharmaceutical, clinical, food, and environmental fields are being reviewed while emphasis is given to sample preparation requirements needed in each case (24).

Finally in 2007, Blasco et al., in their review on the progress in analysis of residual antibacterials in food, cover challenges and achievements in this field, focusing on the developments of the past five years, and the impact of Commission Decision 2002/657/EC and its application in the detection of antibacterial residues in food matrices (25).

The aim of the present review is to provide the state of the art on the analytical strategies concerning the analysis of SAs in food products of

animal origin, with special attention on sample preparation and confirmation according to regulatory demands.

CHEMISTRY-ANTIMICROBIAL ACTIVITY

There are several sulfonamide-based groups of drugs. The original antibacterial SAs are synthetic antimicrobial agents that contain the SA group. The SA functional group is $-S(=O)_2NH_2$, a sulfone group connected to an amine group. The general formula as shown in Figure 1 is RSO_2NH_2, where R is an organic group. Figure 1 illustrates the chemical structures of most commonly used SAs, while Figure 2 the chemical structures of SAs that don't follow the general formula.

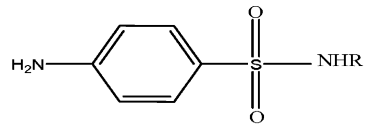
SAs are widely used antibacterial agents chiefly because of their low cost, low toxicity, and excellent activity against common bacterial diseases. They were the first efficient treatment to be employed systematically for the prevention and cure of bacterial infections. The first SA was trade named Prontosil, which is a prodrug. The synergistic action of SA with trimethoprim, which acts against dihydrofolate reductase, has brought about an enormous resurgence in SA use everywhere during the last decade (26–28).

SAs are used in agriculture, aquaculture, animal husbandry, and also as human medicines. SAs have been widely used in animal feed as growth promoters, to prevent and treat a series of diseases in animal feeding, such as infectious diseases of digestive and respiratory tracts and they have also played an important role as effective chemotherapeutics in bacterial and protozoan infections in veterinary medicine practice (29–31).

The basic structure of SA cannot be modified if it is to be an effective competitive "mimic" for p-aminobenzoic acid (PABA). Essential structural features are the benzene ring with two substituents in para positions, an amino group in the fourth position, and the singly substituted 1-sulfonamido group. SAs competitively inhibit the bacterial enzyme, dihydropteroate, which is responsible for incorporation of PABA into dihydrofolic acid, the immediate precursor of folic acid. This inhibition blocks the synthesis of dihydrofolic acid and decreases the amount of metabolically active tetrahydrofolic acid, a cofactor for the synthesis of purines, thymidine, and DNA. Therefore, SAs inhibit the growth and reproduction of bacteria. Trimethoprim, which is a potent inhibitor of the enzyme dihydrofolate reductase in bacteria, interferes competitively with the conversion of dihydrofolic acid to folic acid (26–29).

Organisms susceptible to SAs must synthesize their own folic acid. Mammalian cells use preformed folic acid and, therefore, are not

General formula:



SG 4-amino-*N*-(aminoiminomethyl)-benzenesulfonamide		SME 4-amino-*N*-(5-methoxy-pyrimidin-2-yl)-benzenesulfonamide	
STZ 4-Amino-N-2-thiazolylbenzenesulfonamide		SMMX 4-amino-*N*-(6-methoxy-pyrimidin-4-yl)-benzenesulfonamide	
SIX 4-Amino-N-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide		SD 4-amino-*N*-(5, 6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide	
SMX 4-amino-*N*-(5-methylisoxazol-3-yl)-benzenesulfonamide		SDMX 4-amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide	
SMT 4-amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-benzenesulfonamide		SCZ 4-amino-*N*-(5-chloro-3-pyrazinyl)-benzenesulfonamide	
SDZ 4-amino-*N*-pyrimidin-2-yl-benzenesulfonamide		SQX 4-amino-*N*-quinoxalin-2-yl-benzenesulfonamide	
SBA N-[(4-Aminophenyl)sulfonyl]-benzamide		SPR 4-amino-*N*-[2-pyridyl]-benzenesulfonamide	
SMR 4-amino-*N*-(4-methylpyrimidin-2-yl) benzenesulfonamide		SPZ 4-amino-*N*-(1-phenyl-1*H*-pyrazol-5-yl)-benzenesulfonamide	
SMZ or SDD 4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl) benzenesulfonamide		SCP 4-Amino-N-(6-chloro-3-pyridazinyl)benzenesulfonamide	
SMPD 4-amino-*N*-(6-methoxypyridazin-3-yl)-benzenesulfonamide		SAM N-(4-Aminophenylsulfonyl)acetamide	
SMX 4-Amino-N-(5-methyl-3-isoxazolyl)benzenesulfonamide		SNM 4-Aminobenzenesulfonamide	–H
STR 4-amino-N-(5-methyl-isoxazol-3-yl)-benzenesulfonamide			

Figure 1. Chemical structure of sulfonamides. Code and R-group molecular structure.

susceptible. Cells that produce excess PABA or environments with PABA, such as necrotic tissues, allow for resistance to these antibiotics by competition with the SA (32).

SAs are active against both gram-positive and gram-negative bacteria. Their in vitro spectrum includes *Streptococcus pyogenes*, *Streptococcus pneumoniae*, some strains of *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Haemophilus influenzae*, *Haemophilus ducreyi*, *Brucella* species and *Vibrio cholera*. *Chlamydia trachomatis*, *Actinomyces* and *Nocardia* species, and the protozoans, *Plasmodium falciparum* and *Toxoplasma gondii*, also are susceptible to SAs. *Escherichia coli* frequently are susceptible when present in the urinary tract, particularly if the organism is community acquired and the infection has not been treated previously. *Klebsiella* species, *Proteus mirabilis*, and *Serratia marcescens* vary in their in vitro susceptibility (26).

Most SAs are well absorbed orally with the exception of the enteric SAs, such as sulfaquinoxaline, which are minimally absorbed. Delays in absorption may occur in adult ruminants or when SAs are administered with food to monogastric animals. SAs are widely distributed throughout the body. They cross the placenta, and a few penetrate into the cerebrospinal fluid. SAs may be distributed into milk; however, they vary greatly in their ability to do so. The process depends on several factors, including protein binding and pKa values (32).

SAs are primarily metabolized in the liver but metabolism also occurs in other tissues as well. Biotransformation occurs mainly by acetylation, glucuronide conjugation, and aromatic hydroxylation in many species. The types of metabolites formed and the amounts of each vary depending on the specific SA administered; the species, age, diet, and environment of the animal; the presence of disease; and with the exception of pigs and ruminants, even the sex of the animal. N_4-acetyl metabolites have no antimicrobial activity and hydroxymetabolites have

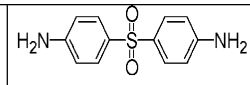
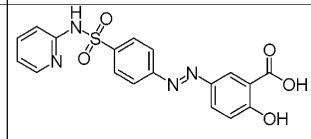
DDS 4-amino-phenyl-sulfone	
SSZ 2-hydroxy-5-[4-(pyridin-2-yl-sulfamoyl)-phenylazo]-benzoic acid	

Figure 2. Chemical structure of SAs not following the general model as given in Figure 1.

2.5 to 39.5% of the activity of the parent compound. Metabolites may compete with the parent drug for involvement in folic acid synthesis but have little detrimental effect on the bacterial cell, and so could lower the activity of the remaining parent drug. Renal excretion is the primary route of elimination for most non-enteric SAs and it occurs by glomerular filtration of parent drug, tubular excretion of unchanged drug and metabolites, and passive re-absorption of non-ionized drug. Alkalization of the urine increases the fraction of the dose that is eliminated in the urine. In general, the metabolites of the parent drug are more quickly eliminated by the kidney than the original SA is, but the proportions of metabolites formed can vary, depending on many factors (32).

Improper, illegal or extra-label use of drugs can result in non-compliant residues. Also, an insufficient withdrawal period can also cause non-compliant residues. Every drug has a set withdrawal period before the residue levels in the animal body drop below the tolerance level. If this withdrawal period is not maintained before slaughter, higher residue level may be present in food. At present, SAs 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 excessive use of antimicrobials in animals is that bacteria may develop resistance. In addition, some SAs have been found to be potentially carcinogenic and this fact has become a cause for considerable debate in food safety (33, 34).

LEGISLATION

In order to protect consumers from risks related to drug residues, maximum residue limits (MRL) have been established by law in many countries (11, 34). The substances with MRLs (permitted) are contained in group B of Annex I of Council Directive 96/23/EC (35). Recently, the European Union (EU) has issued a specific regulation decision (2002/657/EC) concerning the performance of methods and the interpretation of results in the official control of residues in products of animal origin. Some new parameters must be calculated as limit of decision (CCα) and detection capability (CCβ). Decision limit (CCα) means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant. Detection capability (CCβ) means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β. In the case of substances for which no permitted limit has been established, the

detection capability is the lowest concentration at which a method is able to detect truly contaminated samples with a statistical certainty of $1 - \beta$. In the case of substances with an established permitted limit, this means that the detection capability is the concentration at which the method is able to detect permitted limit concentrations with a statistical certainty of $1 - \beta$ (36, 37).

European Union Council Regulation (EEC) No 2377/90 sets MRL values for all substances belonging to the SA group. According to this regulation the combined total residues of all substances within the sulfonamide group should not exceed 100 μg/kg, in muscle, fat, liver, kidney and milk (11). The maximum residue limit (MRL) of SAs in animal foodstuffs established by the Food and Drug Administration (FDA) and the Japanese Ministry of Health and Welfare are shown in Tables 1 and 2, respectively (9, 38).

Table 1. Maximum Residue Level (MRL) values for sulfonamides by Food and Drug Administration Department of Health and Human Services. PART 556 – TOLERANCES FOR RESIDUES OF NEW ANIMAL DRUGS IN FOOD.

Sulfonamides (SAs)	Specific Tolerances for Residues of New Animal Drugs (MRLs)
Sulfachloropyridazine (SCP)	A tolerance of **0.1 ppm** is established for negligible residues of sulfachlorpyridazine in uncooked edible tissues of calves and swine.
Sulfadimethoxine (SDMX)	*Tolerances.* (1) A tolerance of **0.1 ppm** is established for negligible residues of sulfadimethoxine in uncooked edible tissues of chickens, turkeys, cattle, ducks, salmonids, catfish, and chukar partridges. (2) A tolerance of **0.01 ppm** is established for negligible residues of sulfadimethoxine in milk.
Sulfamerazine (SMR)	A tolerance of **zero** (below LOD) is established for residues of sulfamerazine (N^1-[4-methyl-2-pyrimidinyl] sulfanilamide) in the uncooked edible tissues of trout.
Sulfamethazine (SMZ)	A tolerance of **0.1 ppm** is established for negligible residues of sulfamethazine in the uncooked edible tissues of chickens, turkeys, cattle, and swine.
Sulfaquinoxaline (SQX)	A tolerance of **0.1 ppm** is established for negligible residues of sulfaquinoxaline in the uncooked edible tissues of chickens, turkeys, calves, and cattle.
Sulfathiazole (STZ)	A tolerance of **0.1 ppm** is established for negligible residues of sulfathiazole in the uncooked edible tissues of swine.
Sulfachloropyrazine (SCZ)	**0.1 ppm** in uncooked edible tissues of calves and swine

Table 2. Maximum residue limit (MRL) values for sulfonamides in food listed by The Japan Food Chemical Research Foundation (38).

Food	MRL (ppm)
SULFABENZAMIDE (SBA) and SULFACETAMIDE (SAM)	
Cattle, muscle, fat, liver, kidney, edible offal, Other terrestrial mammals, muscle, fat, liver, kidney, edible offal	0.1
Milk	0.01
SULFACHLOROPYRIDAZINE (SCP)	
Cattle, muscle fat, liver, kidney, edible offal	0.1
Pig, muscle fat, liver, kidney, edible offal	0.05
SULFAGUANIDINE (SGN)	
Cattle, muscle fat, liver, kidney, edible offal, Pig, muscle fat, liver, kidney, edible offal, Other terrestrial mammals, muscle fat, liver, kidney, edible offal	0.1
Milk	0.01
SULFAMERAZINE (SMR) and SULFATROXAZOLE (STR)	
Cattle, muscle fat, liver, kidney, edible offal, Pig, muscle fat, liver, kidney, edible offal, Other terrestrial mammals, muscle fat, liver, kidney, edible offal, milk	0.1
SULFADIAZINE (SDZ)	
Cattle, muscle fat, liver, kidney, edible offal, Pig, muscle fat, liver, kidney, edible offal, Other terrestrial mammals, muscle fat, liver, kidney, edible offal, Chicken and other poultry muscle, fat, liver, kidney, edible offal, Salmoniformes (such as salmon and trout)	0.1
Milk	0.07
Chicken eggs, Other poultry eggs	0.02
SULFADIMETHOXINE (SDMX)	
Pig, muscle and liver	0.2
Cattle, muscle fat, liver, kidney, edible offal, Pig, muscle fat, liver, kidney, edible offal, Other terrestrial mammals, muscle fat, liver, kidney, edible offal, chichen, muscle, fat, liver	0.05

Chicken eggs	1
Pig, kidney and edible offal, other poultry, muscle	0.1
SULFATHIAZOLE (STZ)	
Cattle, muscle, fat, liver, kidney, edible offal, Pig, muscle fat, liver, kidney, edible offal, Other terrestrial mammals, muscle fat, liver, kidney, edible offal, Chicken and other poultry muscle, fat, liver, kidney, edible offal.	0.1
Milk	0.9
SULFAMONOMETHOXINE (SMMX)	
Cattle, muscle	0.01
Pig, muscle	0.02
Cattle, fat, liver, kidney, edible offal, pig fat, liver, kidney	0.05
Other terrestrial mammals, muscle, fat, liver, kidney, edible offal	0.1
Pig, edible offal, Chicken, muscle, fat, liver, kidney, edible offal Salmoniformes (such as salmon and trout), Anguilliformes (such as eel), Perciformes (e.g., bonito, horse mackerel, mackerel, sea bass, sea bream, tuna), other fish	
SULFAMETHOXAZOLE (SMX)	
Pig, muscle, fat, liver, kidney, edible offal, Chicken, muscle, liver, kidney, edible offal	0.02
Chicken, fat	0.05
SULFAMETHOXYPYRIDAZINE (SMPD)	
Pig, muscle	0.03
Pig, fat, liver, kidney	0.05
SULFAMOILDAPSONE (SDDS)	
Pig, muscle, fat, liver, kidney	0.1
Pig, edible offal	0.3
SULFANILAMIDE (SNM) and **SULFAPYRIDINE (SPN)**	
Cattle, muscle, fat, liver, kidney, edible offal, Pig, muscle fat, liver, kidney, edible offal	0.1
Milk	0.01

(*continued*)

Table 2. Continued.

Food	MRL (ppm)
SULFAQUINOXALINE (SQX)	
Cattle, muscle fat, liver, kidney, edible offal, Pig, muscle fat, liver, kidney, edible offal, other terrestrial mammals, muscle fat, liver, kidney, edible offal, other poultry fat, muscle	0.1
Milk	0.01
Chicken, muscle, fat	0.05
SULFADOXINE (SD)	
Cattle, muscle, fat, liver, kidney, edible offal Pig, muscle, fat, liver, kidney Other terrestrial mammals, muscle, fat, liver, kidney, edible offal	0.1
Pig, edible offal	0.02
Milk	0.06
SULFADIMIDINE (SDD)	
Cattle, muscle, fat, liver, kidney, edible offal Pig, muscle, fat, liver, kidney, edible offal Sheep, muscle, fat, liver, kidney, Horse, muscle, fat, liver, kidney, Other terrestrial mammals, muscle, fat, liver, kidney, edible offal Chicken, muscle, fat, liver, kidney, edible offal Duck, muscle, fat, liver kidney, Turkey, muscle, fat, liver kidney, Other poultry, muscle, fat, liver kidney, edible offal	0.1
Milk	0.025
Chicken eggs	0.01
Other poultry, eggs	0.01

ANALYTICAL STRATEGIES

Analytical methods can be classified to identification/determination methods and confirmatory methods. The latter are determined as methods that provide full or complementary information enabling the substance to be unequivocally identified and if necessary quantified at the level of interest. Confirmatory methods for organic residues or contaminants shall provide information on the chemical structure of the analyte. Consequently methods based only on chromatographic analysis without the use of spectrometric detection are not suitable on their own for use as confirmatory methods. However, if a single technique lacks sufficient specificity, the desired specificity shall be achieved by analytical procedures consisting of suitable combinations of cleanup, chromatographic separation(s) and spectrometric detection.

When mass fragments are measured using other than full-scan techniques, a system of identification points shall be used to interpret the data. For the confirmation of substances listed in Group A of Annex I of Directive 96/23/EC, a minimum of 4 identification points (IP) shall be required. For the confirmation of substances listed in Group B of Annex I of Directive 96/23/EC, a minimum of 3 IP are required. In order to qualify for the IP required for confirmation and the sum of IP to be calculated:

(a) a minimum of at least one ion ratio shall be measured, and
(b) all relevant measured ion ratios shall meet the criteria described above, and
(c) a maximum of three separate techniques can be combined to achieve the minimum number of IP.

One IP is earned by Low resolution mass spectrometry (LR) and LR-MS^n precursor ion, 1.5 IPs by LR-MS^n transition products, 2 IPs by High Resolution MS and HR- MS^n precursor ion and 2.5 HR-MSn transition products. Each ion may only be counted once.

Different analytes can be used to increase the number of IP only if the derivatives employ different reaction chemistries. For substances in Group A of Annex 1 to Directive 96/23/EC, if one of the following techniques are used in the analytical procedure: HPLC coupled with full-scan diode array spectrophotometry (DAD); HPLC coupled with fluorescence detection; HPLC coupled to an immunogram; two-dimensional TLC coupled to spectrometric detection; a maximum of one IP may be contributed, providing that the relevant criteria for these techniques are fulfilled. Product ions include both daughter and granddaughter products. For example by LC-MS-MS 1 precursor and 2 daughters provide 4 IPs. By LC-MS-MS 2 precursor ions, each with 1 daughter 5 IPs are earned, while and LC-MS-MS-MS 1 precursor, 1 daughter and 2 granddaughters yield 5.5 IPs (36).

Analytical methodologies are further discussed in the next section; while information from all reported methods is summarised in Table 3. It is obvious that LC-DAD is the technique of choice for residue analysis of SAs in food products. However LC-MS and LC-MS^n are nowadays considered as the state of the art in confirmatory methods.

Most published methods deal with the determination of SAs in milk and a great number in chicken and pork tissue. Almost one third of the published methods concern the determination of up to three SAs, one third from four to eight SAs, while two papers deal with nine, and five with ten SAs. There also four papers that propose methods for the simultaneous determination of 11, 12, 15 and 16 SAs each one. These methods are summarized in the next paragraphs.

SNM, STZ, SDZ, SMR, SMZ, SMX, SIX and **SDMX** were determined in milk by Matrix Solid Phase Dispersion (MSPD) after mixing with 2 g C_{18}. Filtered sample was then analysed on a Supelcosil LC-18, 3 μm, analytical column, with 0.017 M orthophosphoric acid-acetonitrile (90:10, v/v) as mobile phase. SMR was used as internal standard. Recoveries obtained were in the range of 73.1 to 93.7%. Photo-diode array detection at 270 nm provided LOD values in the range 31.25–62.5 ng mL^{-1}. The savings in terms of time and solvent requirements, compared to classical extraction techniques, make this procedure attractive. The method presented here isolates eight SAs simultaneously, requires only 0.5 mL sample and requires no extensive extract cleanup steps other than drying the methylene chloride, centrifugation and filtering prior to analysis. Furthermore, use of the MSPD method results in extracts relatively free from interfering co-extractants, which could aid in their detection by other more sensitive means, such as immunoassay techniques, by eliminating cross-reacting compounds (39).

SNM, STZ, SDZ, SMR, SMZ, SMX, SIX and **SDMX** were determined in pork muscle tissue by SMPD after being mixed with 2 g C_{18}. HPLC analysis was performed on a column made by blending C_{18} with muscle tissue matrix. Mobile phase a mixture of 0.017 M H_3PO_4 and acetonitrile, was delivered isocratically. SMR was used as internal standard. Recovery ranged from 70.4 to 95.8%. Photo-diode array detection at 270 nm provided LOD 31.25–62.5 ng/g. The method eliminates many of the problems associated with classical techniques for the isolation of SAs from tissue, and uses small sample sizes, has a minimal number of steps and no chemical manipulations (such as pH adjustments), and requires a minimal amount of solvent. The savings in terms of time and solvent requirements make this procedure an attractive alternative to classical isolations. In addition, this method may be suitable for the isolation of different residues from other tissues or matrices (40).

SMZ was determined in milk after addition of 50 mL chloroform. The extract was further purified by SPE. Chloroform extract of milk was loaded on the SPE column. After washing with buffer, SMZ was eluted with aqueous (50%) methanol. The total eluent was injected into the HPLC. A Supelco LC-M-DB, 5 μm, analytical column was used with a mobile phase of 70% ammonium acetate buffer at pH adjusted to 4.7 with acetic acid and 30% methanol. Average recovery was 86.0%. The proposed method describes a simple, fast and reliable approach for the detection of sulfamethazine residues in milk up to 5 ppb level by UV detection at 265 nm (41).

SDD was determined in milk by SPE on C-18 cartridge conditioned with water and acetonitrile (10% and 15%). Residues were eluted with sodium acetate buffer/acetonitrile. A LiChroSpher C-18 and a SuperSpher C-18 analytical column were used with sodium acetate buffer (0.01 M; pH 4.6) and acetonitrile (85 + 15, 75 + 25) as mobile phase. Mean recovery was 74–130%. UV detection was performed at 270 nm (42).

SMMX and **SDMX** (and their N4-acetylated forms) were determined in fish tissues after adsorption on Sep-Pak Alumina B cartridge, elution with 20 mL of 70 % acetonitrile. Analysis was performed on YMC-Pack C_{18} with 0.05 M phosphate buffer, pH 2.5-acetonitrile (65: 35) as mobile phase. Recovery >80% was achieved. UV detection at 265 nm yielded LOD 0.1 ppm (43).

SDZ, STZ, SPN, SMR, SMT, SMZ, SCP, SDMX and **SQX** were determined in de-creamed milk after dilution with potassium phosphate buffer (pH 4.4). SPE was performed on alumina column. Analytes were separated on an LC 18-DB, 5 μm analytical column with Solvent A: Ammonium acetate buffer (pH 4.7): methanol (850:150, v/v) and Solvent B: Ammonium acetate buffer 2 (pH 8.0): methanol (700:300, v/v) as mobile phase under a gradient program. Recovery obtained was >50%. A diode array detector at 265 nm yielded LOD 10 ppb. This method is a significant improvement over the existing methods for the analysis of SAs in milk. No solvent extraction is required. However it needs further work to improve the recoveries of SAs and also to be able to remove the interferring matrix peak which elutes near the retention time of sulfadiazine (44).

SMMX, SDMX (N_4-acetyl metabolites) were determined in beef, pork, chicken and eggs. In 5 g, 25 mL of 90% (v/v) acetonitrile solution and 20 mL hexane were added to extract the analytes. A LiChrosorb RP-18 (7 μm) column was used for analysis with a mobile phase of acetonitrile-0.05 M phosphate buffer (pH 5.0) (25:75, v/v). Recovery ranged from 85.7 to 95.8%. UV detection at 270 nm yielded practical limits of detection 0.001 ppm. The method presented here is a rapid, sensitive, precise and economical one, with several minor technical

Table 3. Overview of published methods for the determination of sulfonamides in various food matrices of animal origin.

SA Analytes	Sample Type	Sample Preparation	Chromatographic Conditions	R %	Detection	LOD (ng/mL)	Ref.
SNM, STZ, SDZ, SMR, SMZ, SMX, SIX, SDMX	Milk	2 g C_{18} and 0.5 mL spiked milk sample, homog., matrix placed in 10-mL plastic syringe barrel, compressed to a final vol. of 4.5 mL, Col. wash with 8 mL hexane, SAs eluted with 8 mL CH_2Cl_2, CH_2Cl_2 extract dried under dry N_2, add. of 0.1 mL of MeOH and 0.4 mL of 0.017 M H_3PO_4 to dry residue, sonic., centr., filtr., inj. to the HPLC.	**Col.**: Supelcosil LC-$_{18}$, 3 µm, 7.5 cm × 4 mm **MP**: 0.017 M H_3PO_4-ACN (90: 10, v/v) **FR**: 1 for 5 min increasing to 2 at 5 min. **Inj. vol.**: 20 µL **IS**: SMR.	73.1–93.7	DAD: 270 nm	31.25–62.5	39
SNM, STZ, SDZ, SMR, SMZ, SMX, SIX, SDMX	Pork muscle tissue	2 g C_{18} and fortified pork tissue (0.5 g), homog., transfer to a 10-mL syringe barrel, compressed to vol. of 4.5 mL, Col. wash with hexane, SAs eluted with CH_2Cl_2 (8 mL), extract dried under dry N_2, add. of 0.1 mL of MeOH and 0.4 mL of 0.017 M H_3PO_4, sonic., centr., filtr., inj. into the HPLC.	**Col.**: End capped, octadecylsilyl-derivatized silica. **MP**: isocratic: 0.017 M H_3PO_4 to CAN **FR**: 1.0, **Inj. vol.**: 20 µL **IS**: SMR	70.4–95.8	DAD: 270 nm	31.25–62.5 (ng/g)	40
SMZ	Milk	10 mL milk, add. of 50 mL $CHCl_3$, shake, $CHCl_3$ extract, filtr., re-extr., filtr., combine $CHCl_3$ extracts evap., add. of 5 mL K_3PO_4 buf. (pH 5.0), vortex. SPE: cond. with 5 mL dis. H_2O followed by 5 mL K_3PO_4 buf., $CHCl_3$ extract of milk, through SPE Col., wash.with 5 mL of buf., SMZ eluted with 2 mL aq. (50%) MeOH, inj. into the HPLC.	**Col.**: Supelco LC-M-DB, 25 cm × 4.6 mm, 5µm. **MP**: 700 mL buf. (CH_3COONH_4 buf. 3.85 g CH_3COONH_4 in 900 mL H_2O, pH adj. to 4.7 with CH_3COOH, final vol. to 1000 mL with dis. H_2O) and 300 mL MeOH. **FR**: 1.3, **Inj. vol.**: 50 µL.	86.0	UV: 265nm	up to 5	41
SDD	Milk	SPE.: C_{18} Col. wash with H_2O and ACN (10 % and 15 %), SAs eluted with CH_3COONa buf./ ACN (70 + 30).	**Col.**: LiChroSpher C-$_{18}$ and SuperSpher C-$_{18}$. **MP**: CH_3COONa buf. (0.01 M; pH 4.6) and ACN (85 + 15, 75 + 25) **FR**: 1.	74–130	UV: 270 nm		42
SMMX, SDMX (and their N4-acetylated forms)	Fish tissues	adsorption on Sep-Pak Alumina B cartridge, el. with 20 mL of 70 % ACN, eluate evap. residue diss. in 30 % ACN, inj. into HPLC.	**Col.**: YMC-Pack C_{18} **MP**: 0.05 M phos. buf., pH 2.5-a ACN, (65: 35).	>80	DAD: 265 nm	100	43

SDZ, STZ, SPN, SMR, SMT, SMZ, SCP, SDMX, SQX	Milk	5 mL decreamed milk, dil. with 5 mL K_3PO_4 buf. (pH 4.4), SPE: Cond. with 5 mL dis. H_2O followed by 5 mL K_3PO_4 buf., 10 mL diluted milk through the SPE Col., wash with 5 mL, K_3PO_4 buf., 5 mL mixt. of buf./dis. H_2O, el. with 4 mL MeOH, pass eluate through alumina Col., collect eluate, eluate remaining SAs from the alumina Col. with mixt. of H_2O, buf., and MeOH, evap. Dis. in 1 mL MeOH, load to col., el. with 4 mL acidic el. solvent prepared with MeOH, CH_3COOH, and dis. H_2O, inj. to the HPLC.	**Col.**: LC $_{18}$-DB, 250 × 2.1 mm, 5 µm. **MP**: Solvent A: CH_3COONH_4 buf. (pH 4.7): MeOH (850:150, v:v). Solvent B: CH_3COONH_4 buf. 2 (pH 8.0): MeOH (700:300, v:v). 0 min. 0% B, 5 min. 0% B 20 min. 100% B, 40 min. 100% B 45 min. 0% B **FR**: 0.4, **Inj. vol.**: 100 µL.	>50	DAD: 265 nm	10	44
SMMX, SDMX (N_4-acetyl metab.)	Beef, pork, chicken and eggs	5g, with 25 mL of 90% (v/v) ACN sol. and 20 mL hexane, homog., centr. for 10 min, supern. into separating funnel, extr. repeated twice, ACN layer dried by add. of anhydrous sodium sulphate, filtr., apply to alumina Col., washed with 30 mL of ACN, SAs and metab. eluted with 90% (v/v) ACN sol., eluate evap., residue diss. in 1 mL of HPLC MP, 20 µL inj. into the HPLC.	**Col.**:LiChrosorb RP-18 (7 µm) Col. (250 × 4 mm) **MP**: ACN-0.05 M phos. buf. (pH 5.0), (25:75, v/v) **FR**: 1.0, **Inj. vol.**: 20 µL.	85.7–95.8	UV: 270 nm	1	45
SMZ, SDMX	Chicken tissues	SFE, in-line adsorption trap, recovery from neutral alumina with HPLC MP.		77–89		>100	46
SQX, SMPD, SDD, SMMX, SDMX SQR	Chicken, yolk and pork	MSPD: 0.5 g, mixed with 0.7g silica gel and 1.5 mL ACN, mixt. dried, wash with hexane, eluted with MeOH or THF, aliquot of the MeOH or THF sol. Inj. to HPLC.		69.6–93.1		10–40	47

(*continued*)

Table 3. Continued.

SA Analytes	Sample Type	Sample Preparation	Chromatographic Conditions	R %	Detection	LOD (ng/mL)	Ref.
SDZ, SPN, SMX, SGN, SNM	Milk, Trout tissue, Egg	**Milk**: Sample of 3 g, homog. with 0.5 mL of 30% TCA, centr., recovery of the aq. phase, residue re-extr. with 3% TCA (4 mL), aq. phases comb., dil. to 10 mL with TCA, filtr. Through a 0.45-pm Millipore filter, inj. to the HPLC. **Trout tissue and egg**: Sample of 3 g, with 4 mL of 3% TCA, homog., centr., recovery of the aq. phase, residue re-extr. with 4 mL of 3% TCA.), aq. phases comb., dil. to 10 mL with TCA, filtr., inj. to the HPLC.	**Col.**: 15 × 0.46 cm Spherisorb ODS-2, 5 μm **MP**: isocratic ACN-H_2O (3:97) 5 min, lin. grad. from 3:97 to 40:60 over 15 min, back to initial conditions in 1 min for 10 min. **FR**: 0.5, **Inj. vol.** 50 μL.	95	FL: λ_{exc}.302 λ_{em}. 412 nm	11–19	48
15 SAs*	Meat and meat products	Extr. by Acetone/CH_2Cl_2, liquid-liquid defatting and cleanup, deriv. with fluorescamine at pH 3.0.			FL	0.002–0 0.010 (μg/g)	49
SDZ, SMR, SMZ, SME, SMX, SDMX	Milk	Skimmed milk: protein removed, in an acid medium (2N HCl), filtr., pH 3 buf. sol., fluorescamine and sodium acetate are added to filtrate.	**Col.**: C_{18} **MP**: mixt. of ACN/CH_3COOH 2% (40:60).	>90	FL (λ_{exc}. 405 nm, λ_{em}. 495 nm)		50
SDZ, SCP, STZ, SMR, SPN, SMZ, SQX, SDMX	Chicken liver extracts	Collection of the SAs inside the extr. vessel on an alumina trap, alumina in-line trap was rinsed with 50:50 H_2O:MeOH.	**Col.**:.Prodigy C_{18} (250 × 4.6 mm, 5 μm). **MP**: first 8 min: 85:15 8 mM CH_3COONH_4: ACN adj. to pH 6.5 with CH_3COOH., at 15.5 min the MP was 60:40 8 mM CH_3COONH_4: ACN (pH 6.5) after a lin. gr. of 100% A (85:15) to 100% B (60:40), at 23 min, the MP back to initial conditions. **FR**: 1. **Inj. vol.**: 20 μL.		LC/MS-MS	10–100 (ng)	51
SMT, SMM, SDM	Eggs	SPE on silica and C_{18} cartridges.	**C_{18}** Micro Bondapak, Waters (30 cm × 3.9 mm, 10 μm). **MP**: H_2O pH 3.6/CH_3CN 80:20 v/v. **FR**: 1 mL/min.	92.00–103.3	UV: 267 nm	0.056–0.205 ppm	52
SCP	Muscle tissue and liver of broiler chickens	10 g sample, homog. with 60 cm^3 mixt. CH_2Cl_2-MeOH-CH_3COOH (90:5:5 v/v/v), extr. twice, precip. proteins removed by filtr., filtrate heated to the CH_3COOH fraction, the pH was adj. to 7.0 by add. of a sol. of 0.1 mol dm^3 NaOH.	**Col.**: C_8 HL (4.6 × 200 mm) 5 μm **MP**: 60% ACN in H_2O with pH=9.5 adj. with NH_3. **FR**: 1 cm^3/min. **Inj. vol.**: 50 μL.	79.2–87.3	UV: 254 nm	0.02 (μg/g)	53

SMZ, N_4-acetyl-SMZ desamino-SMZ	Egg albumin and egg yolk	**Egg**: 1 g, fortif., add. of 4 mL ACN, homog., centr. 15 min, supern. Transf. into centr. tube, rehomog. with 2 mL ACN, centr., comb. Extracts, evap. residue diss. in 1 mL MP. **albumin**: analyzed directly by HPLC. **yolk extract**: add. of 4 mL hexane, vortex, centr., discard upper hexane layer, add. of 250 mg NaCl to the lower aq. layer, vortex, centr., HPLC.	**Col.**:LiChrosorb RP (150 mm × 4.6 mm) 5 µm. **MP**: grad.: 5% ACN in 0.01 M phos. buf. for 5 min., grad. to 15% ACN in 15 min., for 20 min., and grad. back to 5% ACN in 35 min. **Inj. vol.**: 75 µL.	63–101	UV: 268 nm		54
9 SAs	Porcine muscle	Automated dialysis: blend saline, centr., supern. filtr., dialysis, resulting dialysate conc. on reversed phase trace enrichment cartridge.		>80	UV: 280 nm	40 (ng/g)	55
SMZ, SDM	Milk	0.5 mL of milk with 0.5 mL of 50% (v/v) EtOH sol., ultrasonic, centr., supern., Ultrafree–MC/ Biomax, centr., ultra-filtrate, inj. to the HPLC.	**Col.**: C_{18} Mightysil RP-18 GP Aqua, 250 × 4.6 mm **MP**: 25% (v/v) EtOH sol. **FR**: 0.8, **Inj. vol.**: 50µL.	>83	DAD: 266 nm	3–5	3
SMX, SDZ, SMR, SIX, SDD, SDMX, SMPD, SIZ, SMMX, SQX	Animal liver and kidney	extr. with ethyl acetate, evap. Dissolve in 5 mL of 50% ethyl acetate-n-hexane, Bond Elut PAS (anion cartridge), el. with 5 mL of 20% ACN–0.05 M and ammonium formate.	**Col.**: L-Col. ODS (5 µm, 250 × 4.6 mm) **MP**: MeOH–ACN–0.05 M-HCOOH (10:15:75) **FR**: 1.0, **Inj. vol.**: 20µL.	81.0–98.2	UV: 272 nm	0.03 (µg/g)	56

(*continued*)

Table 3. Continued.

SA Analytes	Sample Type	Sample Preparation	Chromatographic Conditions	R %	Detection	LOD (ng/mL)	Ref.
SNM, SDZ, SMR, SDD, SMPD, SCP,SDZ, SMX, SDMX, SQX	Meat, mix meat and kidney	5 g, homog., extr. twice with 10 mL acetone, filtr., extracts comb. solvent evap. residue diss. in 15 mL H_2O by ultrasonic. for 1 min, filtr., sol. 3x extr. with 5 mL CH_2Cl_2 in sep. funnel, lower layers comb. Evap. under N_2 stream. Deriv.: of 0.1% fluorescamine in acetone and 0.25 mL of 1 M K_2HPO_4 aliquots anal.	**Col.**: Chrompack (250×4 mm, 5 µm 100 RP ODS-2) **MP**: Mixt. of ACN–H_2O (35/65, v/v) of pH 3.0, containing 0.01 M K_2HPO_4 **FR**: 1.5, **Inj. vol.**: 100 µL.	60–72	FL λ_{ex}=405nm λ_{em}=405nm)	0.05 (µg/g)	57
SDMX, OH-SDMs (hydroxy metab.)	Edible chicken tissues (liver and muscle)	1 g sample, with 20 mL 90% (v/v) ACN (saturated with n-hexane) sol. (in H_2O) and 16 mL of n-hexane, homog., 2 min, centr. 5 min, ACN layer, evap.to 2 mL, sol. into Col., wash 3 mL, el. with 1 mL 0.5 N HCl, add. H_2O up to 2 mL, 0.4 mL of eluate, centr., ultrafiltr. inj. to HPLC.	**Col.**: Mightysil® RP-4 GP (5 µm) Col. (4.6 × 250 mm) **MP**: isocratic of 4% (v/v) CH_3COOH sol. – ACN – N,N DMF, (83:12:5, v/v/v) **FR**: 1.0, **Inj. vol.**: 20 µL.	81.3–88	DAD: 273nm	50	58
SDZ, SDD, SMMX, SMX, SDMX, SQX	Chicken muscle tissues	0.5 g in mortar, add. of 2 g alumina N-S, ground, mixt. transferred to a 15-mL syringe barrel, barrel placed on a vacuum manifold, SAs eluted with 10 mL 70% (v/v) aq. EtOH sol., eluate evap., residue diss. in 1 mL of HPLC MP, filtr., inj.	**Col.**: LiChrospher 100 RP-8, 5 mm, 250 × 4.6 mm **MP**: a mixt. of 1% CH_3COOH sol. (pH 3.0, in H_2O)–ACN–DMF (78:22:5, v/v/v), **FR**: 1.0, **Inj. vol.**: 10 µL.	92.2–101.6	DAD: 267 nm	3–16	59
SDZ, SMR, SDD, SMMX, SMX, SDMX, SQX	Milk	0.5-mL sample, in micro-centrifuge tube with 0.5 mL of 25% (v/v) aq. EtOH, ultrasonic 30 s, stir 30 s and centr. 5 min, 0.4 mL of supern. liquid, centr. for 5 min, inj. to the HPLC	**Col.**: Mightysil RP-4 GP (end-capped) (5µm) (4.6×250 mm) **MP**: 25% (v/v) aq. EtOH sol.**FR**: 0.8, **Inj. vol.**: 100 µL.	86.2–92.2	DAD: 269 nm	5–20	60
11 SAs*	Milk, meat and eggs	after extr. with CH_2Cl_2, fat removed over a silica cartridge, SAs el. with a buf. sol., eluate is extract. with ethyl acetate.	**Col.**:C_{18} Col.		DAD: 266 nm	2 (µg/kg)	61

SD, SDMX, SDD, SMX, SDZ, DDS	Milk	1.0 mL, spiking, allow to stand 30 min at RT, mixing with equal vol. of ACN, centr., supern., dil. with H_2O, ultrafiltr., centr., ultrafiltrate analysis.	**Col.**: 15 cm × 3 mm Waters Symmetry C_{18} **MP**: ACN–10 mM CH_3COONH_4 (pH 3.5), 15-min lin. grad. from 0 to 90 vol.% ACN, starting 5 min after inj. **FR**: 0.400, **Inj. vol.**: 100 µL **IS**: d_7 – Sulphadimidine.	69–87	LC/MS-MS	< MRL, CCα: (mean) 102.8–107.1 CC_β: (mean) 107.7–114.8	62
SDD, SDMX	Eggs	**Albumin**: homog., 5 g albumin, add. of 0.5 mL H_2O, 0.3 mL 1 M HCl and 1 mL THF, vortex, extr. with 5 mL ethyl acetate, centr. for 5 min, supern., evap. residue diss. in 0.8 mL 0.01 M CH_3COONH_4 buf. Sonic., 50 µL, inj. HPLC. ***Yolk***: 5 g, homog. 25 mL ethyl acetate sonic. 5 min, centr. 5 min, supern. evap., yolk extract purified by LLE with 2.5 mL MeOH and 10 mL isooctane, org. layer discarded, evap. of MeOH, add. of 10 mL CH_2Cl_2 and 5 mL 0.01 M CH_3COONH_4 buf. to the dry extract, org. phase evap. residue diss. in 1 mL 0.01 M CH_3COONH_4 buf. and 1 mL isooctane, centr. for 5 min, discard supern., inj. HPLC.	**Col.**: 4-µm Nova-Pak C_{18} Col. (150 × 3.9 mm) **MP**: mixt. of ACN and 0.01 m CH_3COONH_4, pH 6.0 (12:88 v/v for SDD 14:86 v/v for SDMX). **FR**: 1. **Inj. vol.**: 50 µL.	64.6–87	UV: 275 nm	**LOQ** 0.005 µg/g	63
SDZ, STZ, SPR, SMR, SMT, SDD, SIX, SMX, STR, SCZ, SPZ, DDS	Bovine, pig and chicken muscle, bovine, and pig liver and kidney	10 g sample, homog., spiking with 1 mg SAs,(0.3 mg dapsone), allow to stand 10 min, add. of 10 mL ACN homog., add. of 5 mL n-hexane, vortex, ultrasonic-bath, centr., discard upper layer, filtr. middle layer (ACN) SPE: cond. with 5 mL MeOH and 5 mL deionised H_2O, after loading, Col. wash with 5 mL 0.1 N HCl and 5 mL MeOH, SAs eluted with 5 mL ammonia sol./ACN (v/v 1/19), allowed to dry under N_2 at 40°C, reconst. 200 µL ACN/H_2O (v/v1/4).	**Col.**:. Phenomenex Luna C_{18} (250 × 2 mm, 5µm) **MP**: grad. el. (5% B lin., descend to 40% within 16 min, back to 5% in 1 min, equil. for 3 min) **FR**: 0.35, **Inj. vol.**: 25 µL.	13.1–65.7	DAD: 260 nm (dapsone at 294 nm)	**LOD**:1 **CCa: (ppb)** DDS: 17–20 Others:104–128 **CCb: (ppb)** 19–24, Others:107–154	64
SMMX, SDMX, SQX	Eggs	0.2 g sample, homog. in 0.4 mL of 10% (v/v) $HClO_4$ sol. (in H_2O) (ultrasonic-homog.) 30 s, centr. 5 min, 0.2 mL of supern. liquid 5 min, ultra-filtrate inj. into HPLC.	**Col.**:. Mightysil RP-4 GP (5µm, 150 mm × 4.6mm) **MP**: MP, 0.18 mol l/L citric acid; **FR**: 1.0 mL/min. **Inj. vol.**: 20 µL.	80.9–87.6	DAD: 267 nm	0.02–0.04 (µg/g)	65

(*continued*)

Table 3. Continued.

SA Analytes	Sample Type	Sample Preparation	Chromatographic Conditions	R %	Detection	LOD (ng/mL)	Ref.
SDZ, SDD, SMMX, SMX, SDMX, SQX	Chicken, beef, and pork tissues	0.3 g for beef, 0.5 g for pork and chicken, blended with 2 g of Alumina N-S, mixt. transferred syringe barrel, el. with 10 mL of 70% (v/v) aq. EtOH sol., eluate evap. residue diss. in 1 mL of the HPLC MP, filtrate inj. into HPLC.	**Col.**: Mightysil RP-4 GP (250 × 4.6 mm) **MP**: mixt. of 2% CH_3COOH sol. (pH 2.7, in H_2O)-EtOH (75:25, v/v) **FR**: 0.8. **Inj. vol.**: 10 µL.	92.7–100.7	DAD: 267 nm	**LOQ**: 6–33	66
SMZ	Cattle, pigs, chickens, sheep edible tissues	Homog. with 30% (v/v) EtOH in H_2O followed by an Ultrafree (R) -MC/PL as a centr. ultra-filtr. Unit.	**Col.**: reversed-phase C_4. **MP**: 15% (v/v) EtOH in H_2O.	80	DAD	**LOQ**: 57–60 µg/g	67
SDZ, STZ, SPR, SMR, SMZ, SMMX, SCP, SMX, SQX, SDMX	Muscle	10 g, extr. with 20 mL of ethyl acetate and 10 g of anhydrous Na_2SO_4, shaking, centr., supern., repeat same extr. twice, combin. of extracts. Evap. under vacuum, suspension of residue in 40 mL of ethyl acetate, cond. with 2 × 3 mL of n-hexane and 2 × 4 mL of ethyl acetate, wash with 5 mL of H_2O and 5 mL of MeOH.	**Col.**:. C_8 250 mm × 3 mm, 5 µm, **MP**: Grad. 0 min A:B 15:85, 22 min 41:59, 24 min 15:85, 30 min 1:85 A = ACN, B = acetate buf. pH 4.5. **FR**: 0.4, **Inj. vol.**: 20 µL.	72–86	DAD: 270 nm	30–70 **CC**α: 105.2–116.2 **CC**β: 110.9–134.9	37
SGN, SAM, SMX, SMR, SMT, SSZ5-SMZ, SMZ, SMPD, SMMX, SDMX, SPN, SBA, STZ, SDZ, SQX	Milk, bovine liver and kidney tissues	**Tissues**: 5 g, sliced, blend, spiking, 2 mL CH_3COOH 0.1M in H_2O, add 10 mL ethyl acetate, extr. of tissues using this acid. ethyl acetate, vortex 20 s, add. of 3 mL acetone, centr. 10 min, org. phase into centr.tube, add. of same amount of org. phase to the remainder of the tissue samples, re-extr. all org. fractions pooled, further purify. and/or enrich. **Milk**: filtr., 10 mL spiking, dil. with MeOH to 10 mL, add. of 0.1 M CH_3COOH in H_2O, vortex for 20 s, add. of 3 mL acetone and 12 mL ethyl acetate, centr. 10 min, org. phase into centr. tube, add. of same amount of org. phase to the remainder of the tissue samples, repeat of extr. process, all org. fractions pooled, kept 1 h before further purif. and/or enrich. with SLM.	**Col.**: C_{18} Clipeus Higgins, 150 mm × 3.0 mm × 5µm **MP**: Isocratic: A = 85% (25mM AcOH in H_2O) and B = 15% (25mM AcOH in MeOH) **FR**: 150 µL.	34–90	MS	1.8–24.3	68

SMZ	Pork	Homog. with 100% H_2O, centr., ultrafiltr.	**Col.**: C_{18}.	81	DAD	**LOQ**: 0.09 µg/g	69
SPN, SDZ	Milk	AOAC: extr. by $CHCl_3$–acetone, dissolve in Tris–buf., fatty residues extract. with hexane, aq. layer, filtr., analyze.	**Col.**:. C_{18} Luna separation Col. (5 µm, 100 A°, 150 mm × 4.6 mm, Phenomenex)	41.47–78.17	CL	6.2–13.6	70
SMZ, SCP, SDZ, SMX, SDMX			**MP** mixt. of tri-sodium citrate hydrate citric acid/monohydrate buf.: ACN: THF (65:22:13). **FR**: 1.0, **Inj. vol.**: 100 µL.				
SDZ, STZ, SMX, SAM-Na, SMMX-Na	Milk	1 mL sample, spiking, add. of 0.6 mL EtOH, ultrasonic 60s, dil. with 0.02 mol/L Na_2HPO_4 buf. sol. (pH 2.5) to 10 mL, centr. for 6 min, extr.	**Col.**: Hypersil ODS Col. (200 × 4.6 mm, 5 µm) **MP**: MeOH-0.02 mol/L buf. Na_2HPO_4 sol. (3:7, v/v; pH 3.0). **FR**: 0.5, **Inj. vol.**: 20.	14.7–95.7	UV: 269 nm	1.7–22	71
SDZ, SMR, SMZ, SMMX, SDMX, SMX	Milk	Microdialysis System: stainless steel tubing, (sample region), connected with two PEEK tee connector at both ends, piece of hollow fiber placed in the system, Col. couplers used to hold the hollow fiber, one end connected with a syringe pump to deliver perfusate, other end connected with a needle placed in the LC injector perfusate delivered through hollow fiber to the sample loop.	**Col.**: Zorbax Eclipse XDS-C_8 Col. (150 mm × 4.6 mm, 5 µm) **MP**: 25:75 (v/v) of ACN and 0.01 M phos. buf. (pH 4.0). **FR**: 0.6, **Inj. vol.**:10 µL.	97.8–100.4	UV: 260 nm	0.08–0.86	72
SMMX, SDMX and their hydroxy/N4-acetyl metab.	Chicken plasma, muscle, liver, eggs	**Chicken plasma, eggs**: 0.2 mL with 0.6 mL of EtOH, homog. for 30 sec, centr. for 5 min, supern., filtr.,filtrate inj. into the HPLC. **Muscle, liver**: 0.2 g with 0.8 mL of EtOH, homog. 30 s., centr. 5 min, supern., filtr., filtrate evap. residue diss. in 0.8 mL of 50% (v/v) EtOH sol. (in H_2O), 0.3 mL portion, centr. 5 min, deprot. with centr. 20 µL of ultra filtrate inj. into the HPLC.	**Col.**: Mightysil RP-4 GP (150 mm × 4.6 mm) **MP**: 10% EtOH in 1% CH_3COOH sol. (in H_2O) at 0 min, grad. to 20% EtOH in 6 min, held for 20 min, after completion of HPLC run, back to initial conditions within 3 min. **FR**: 1.0. **Inj. vol.**: 20 µL.	90–96	DAD: 270 nm	**LOQ** 7.2–29.8	73

(*continued*)

Table 3. Continued.

SA Analytes	Sample Type	Sample Preparation	Chromatographic Conditions	R %	Detection	LOD (ng/mL)	Ref.
10SAs*	Chicken meat	Extr. with mixt. of ACN and $CHCl_3$ (ACN: $CHCl_3$ 10:1).	**Col.**: Hypersil BDS C_{18} **MP**: ACN and 30 mmol/L NaH_2PO_4 (pH 5).Lin. grad. El. ACN from 8. 5% to 37.5% (v/v) in 40 min.	>50	coulometric porous graphite electrode array detector	20–40 (μg/kg)	74
SDZ, STZ, SMPD, SMMX, SDMX, SQX	Chicken liver	SPE. with ethyl acetate, NH_2 Col. to clean up.	**Col.**:Intersil ODS-3 C_{18} **MP**: MeOH/ACN (1/1, V/V) (A) and H_2O/CH_3COOH (90/2, V/V) (B) (15% A to 25% within 8 min, up to 45% in 12 min for 3 min, back to 15% in 2 min).	>69.6	DAD	8–12 (μg/kg)	75
SDZ, SMR, SDD, STZ SMX, SMT, SMPD, SCP, SDZ, SIX	Pork and eggs	5 g homogenized pork (or 5 mL Homog. egg), +10 mL Fe(III) potassium cyanide, add. of 5 mL zinc, 5 min heat in H_2O 75°C, filtr., add. of 10 mL ACN, ultrasonic for 5 min, filtr., syringed with 10 mL ACN, filtr., dil. of filtrate with dis. H_2O on-line SPE, coupled to HPLC.	**Col.**: RP (Alltech-C_{18} 5 μm, 4.6 cm × 25 cm) **MP**: mixt. of MeOH and H_2O (22:78) **FR**: 1.	68.62–81.47	UV: 270nm	4.1–10.0	76
SDZ, SMZ	Eggs	1 g sample, spiking, add. of 0.6 mL, sonic. 60 s., dil. with 0.02 mol/L phos. buf. sol. (pH 4.0) to 10 mL, vortex 30 s, stand 30 min, centr., supern. Extr. by polymer monolith microextr.: poly (methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary as extr. medium, preconditioning with 0.5 mL of MeOH and, 0.5 mL of 20 mmol/L phos. buf. (pH 4.0), extr. by ejecting 1 mL of sample sol. through the monolithic capillary, desorption: 0.05 mL of MeOH-0.02 mol/L phos. buf. sol. (pH 3.0) (40/60, v/v), inj. through the monolithic capillary, sample ready for anal. with HPLC.	**Col.**: Kromasil ODS (5 μm), 150 mm × 4.6 mm, **MP**: MeOH-0.02 mol/L phos. buf. sol. (pH 3.0)(30:70, v/v), **FR**: 0.6, **Inj. vol.**:20 μL.	65.5–67.3	UV: 269 nm.	8.8–11.2 (ng/g)	77
SDZ, SMR, SMZ	Calf and pig tissue	4 g sample, homog. with 20 mL ACN, centr.,. supern.(1 mL), evap. reconst. with 0.5 mL MP, filtr., inj.	**Col.**: Synergy MAX-RP (150 mm × 3.0 mm, 4 μm particle) **MP**: grad. prepared from 0.01 mol L^{-1} CH_3COONH_4 (A) and ACN (B). **FR**: 0.75, **Inj. vol.**:10 μL.	78.4–104.1	DAD: 270nm	3–6	78

SDZ, STZ, SMZ, SMPD, SMMX, SMX, SDMX, SQX	Bovine milk	Filtr., 10 mL milk +15 g of anhydrous sodium sulfate, blend with 15 mL of ethyl acetate 1 min, centr., org. phase into clean centr. tubes, same amount of org. phase added to the remainder of the sample in the centr. tubes, repeat extr. Comb. extracts, evap. at 40°C, dissolve residue in 1 mL of ethyl acetate, apply sol. to anion cartridge (LC-NH_2) pre-washed with 2 mL of ethyl acetate, wash with 2 mL of n-hexane, air dry for 5 min, wash with 2 mL H_2O, SAs eluted with 1.5 mL of a 1:1:8 (v/v/v) mixt. of MeOH, ACN and 1.0% CH_3COOH, inj. to HPLC.	**Col.**: Inertsil ODS-3 C_{18} (250 × 4.6 mm, 5 µm) **MP**: 0.1% CH_3COOH sol. (A) and a 1:1 (v/v) mixt.(B) of MeOH and ACN, grad. el. (15% B at beginning lin. ascend to 50% within 30 min, back to 15% within 4 min, equil. for 4 min) at 25°C. **FR**: 1.0, **Inj. vol.**: 40 µL.	70.5–89.0	DAD: 270 nm	0.8–1.5 **CC*α***: 10.6–11.1 **CC*β***: 11.2–12.3	79
SNM, SDZ, SMR, SMZ,	Cow's milk	0.5 mL sample, + 0.2 mL of EtOH–CH_3COOH (97:3, v/v), homog., centr. For 5 min at 4°C, inj.	**Col.**:.Ether-type C_8 (5 µm) (150 mm × 4.6 mm) **MP**: isocratic el. with ACN–H_2O (5:95, v/v), **FR**: 1.5.**Inj. vol.**: 20 µL.	80.1–87.6	DAD: 270 nm	30–60	80

improvements over previously published procedures. It may be useful for monitoring residual drugs in various foods of animal origin and studying pharmacokinetics (45).

SMZ, SDMX and **SQX** were determined in chicken tissues after SFE, in-line adsorption trap and recovery from neutral alumina with HPLC mobile phase. Mean recovery was 77–95%. LOD was greater than 100 ppb (46).

SMPD, SDD, SMMX, SDMX and **SQR** were determined in chicken, yolk and pork by MSPD. Sample of 0.5 g were mixed with 0.7 g silica gel and 1.5 mL acetonitrile. The mixture was dried, and washed with hexane, eluted with methanol or THF, aliquot of the methanol or THF solution subjected to HPLC. Recovery was 78.1–93.1% in chicken, 74.6–86.2% in yolk and 69.6–87.1% in pork. LOD values obtained ranged from 0.01 to 0.04 ppm (47).

SDZ, SPN, SMX, SGN and **SNM** were determined in milk, trout tissue and egg. Milk sample of 3 g, was homogenised with 0.5 mL of 30% TCA. Trout tissue and egg samples of 3 g, were homogenized with 4 mL of 3% TCA. After extraction samples were analysed on an analytical column Spherisorb ODS-2, (5 μm). The mobile phase, a mixture of acetonitrile-water (3:97) was initially delivered initially isocratically, and then under a linear gradient. Average recovery obtained was 95%. Fluorescence detector with excitation and emission wavelengths of 302 and 412 nm provided LOD (μg/mL) 0.011–0.019. The derivatization reaction proposed considerably lowers the detection limits of sulfonamides in complex food matrices and is suitable for joint use with HPLC separation of these drugs. The method is sensitive enough to be applied to biological samples. Simple sample preparation and automatic derivatization considerably decrease the total analysis time needed (48).

Fifteen **SAs** were determined in meat and meat products after LLE by acetone/methylene chloride. Analytes were determined by fluorimetric detector yielding LOD values in the range 0.002 and 0.010 mg/kg (49).

SDZ, SMR, SMZ, SME, SMX and **SDMX** were determined in skimmed milk after deproteinization with HCl 2 N. Fluorescamine and sodium acetate were added for derivatization. A C_{18} column was used with a mixture of acetonitrile/acetic acid 2% (40:60) (%) as mobile phase. Recovery was better than 90%. Spectrofluorimeter detector was used at excitation wavelength of 405 nm and emission wavelength of 495 nm (50).

SDZ, SCP, STZ, SMR, SPN, SMZ, SQX and **SDMX** were determined in chicken liver extracts. SAs were collected in the extraction vessel on an alumina trap, which was rinsed with 50:50 water:methanol. A Prodigy C_{18} (5 μm) analytical column was used with a mobile phase of 8 mM ammonium acetate: acetonitrile adjusted to pH 6.5 with acetic acid. HPLC APCI-MS, has been shown to produce very low LOQs using both full scan and selected ion modes for the eight regulated SAs investigated.

Selected ion mode yielded an LOQ of 50 pg for three of the eight SAs investigated. The analysis of supercritical fluid extracts of chicken liver containing sulfadimethoxine at 100 pg mL^{-1} or less was found to be feasible by HPLC:APCI-MS. In addition, the method also demonstrated good linearity and reproducibility for the detection of a representative SA in both full scan and single ion modes. HPLC:APCI-MS offers promising results for the routine identification and analysis of SAs from biological matrices (51).

SMT, SMMX and **SDMX** were determined in eggs after extraction with acetone and dichloromethane cleanup. Concentration was performed on a silica cartridge and a C_{18} cartridge. Recovery greater than 90% was reported (52).

SCP was determined in muscle tissue and liver of broiler chickens. Extraction was performed by 60 mL mixture dichloromethane-methanol-acetic acid (90:5:5 v/v/v). Extracts were analysed on a C-8 HL 5 µm column was used with a mobile phase of 60% acetonitrile in water with pH=9.5 adjusted with NH_3. UV detection was performed at 254 nm. The developed HPLC method enables successful quantitative determination of SCP residues in samples of muscle tissue and liver of broilers. The detection limit of 0.02 µg/g and the recovery ranging from 79.2 ± 0.6 to $86.7 \pm 0.2\%$ for the muscle tissue and 81.7 ± 0.8 to $87.3 \pm 0.7\%$ for the liver samples confirm the applicability of the method (53).

A gradient HPLC procedure for the separation of **SMZ**, and its major metabolites, N4-acetyl-SMZ, desamino-SMZ, glucosyl-SMZ, and glucuronyl-SMZ in egg albumin and egg yolk has been developed. Albumin was analyzed directly by HPLC. The yolk extract was extracted by hexane. Extracts were analyzed by a LiChrosorb RP 5 µm column, with a mobile phase of 0.01 M phosphate buffer and acetonitrile delivered by gradient. Recovery obtained was for SMZ in egg albumin was 101%, in egg yolk 79% and for N_4-acetyl-SMZ in egg albumin 88%, in egg yolk 91%, for desamino-SMZ in egg albumin 84% and in egg yolk 63%. UV detection was performed at 268 nm. The main characteristics of the proposed procedure are summarized as follows: shorter analysis time; high precision (RSD<3.7% in the recovery test); harmless to the environment. This procedure may, therefore, be useful for the routine residue monitoring of SMZ and SDM in milk (54).

SDZ, STZ, SPN, SMR, SMT, SMZ, SMPD, SCP and SIX were determined in porcine muscle by automated dialysis, by blend saline, centrifugation, supernatant filtration, dialysis, resulting dialysate concentrated on reversed phase trace enrichment cartridge. Mean recovery was higher than 80%. UV detection was performed at 280 nm providing LOD 40 ng/g (55).

SMZ and **SDM** were determined in milk after deproteinization with 50% (v/v) ethanol solution. After ultrasonic, and ultracentrifugation,

supernatant was injected to the HPLC system using a Mightysil RP-18 GP Aqua column, with a mobile phase of 25% (v/v) ethanol solution. Recovery obtained was >83%. Photo-diode array detector at 266 nm provided LOD for SMZ 3 ng mL^{-1} and for SDM 5 ng mL^{-1} (3).

SMX, SDZ, SMR, SIX, SDD, SDMX, SMPD, SIZ, SMMX and **SQX** were determined in animal liver and kidney after extraction with ethyl acetate. Samples were further purified by SPE on Bond Elut PAS (anion cartridge). Elution of analytes was performed with 20% acetonitrile–0.05 M and ammonium formate. An LC-column ODS (5 μm) was used with a mobile phase: methanol–acetonitrile–0.05 M-formic acid (10:15:75). Different recovery rates were obtained in the range from 81–98.2 %. UV detection was performed at 272 nm. The present method is not only simple, rapid, and reliable, but also permits the simultaneous determination of ten different kinds of SAs with good recoveries (>70%), relative standard deviation (<7.0%), and detection limits (0.03 mg/g). Furthermore, it is applicable to a direct LC–MS–MS analysis. Accordingly, wide spread use of the method presented here is strongly recommend for the routine determination of residual SAs in animal liver and kidney (56).

SNM, SDZ, SMR, SDD, SMPD, SCP, SDZ, SMX, SDMX and **SQX** were determined in meat, mix meat and kidney after LLE with acetone and methylene chloride. For the derivatization of 0.1% fluorescamine in acetone and 0.25 mL of 1 M K_2HPO_4 were added and aliquots were analyzed by a Chrompack analytical column (5 μm 100 RP ODS-2) with a mobile phase of mixture of acetonitrile–water (35/65, v/v) of pH 3.0, containing 0.01 M K_2HPO_4. Mean recoveries ranged from 60–72%. Fluorescence detection (λ_{ex}=405nm, λ_{em}=405nm) yielded LOD: 0.05 mg/kg. The proposed simplified cleanup procedure enables quantitative determination of ten of the most used SAs at levels considerably lower than the level of MRL 100 mg/kg adopted by the European Community (57).

SDMX and **OH-SDMs** (hydroxy metabolites) were determined in edible chicken tissues (liver and muscle). Chicken tissues were treated with 90% (v/v) acetonitrile (saturated with n-hexane) solution (in water) and of n-hexane. Analysis was performed on a Mightysil® RP-4 GP (5 μm) column with an isocratic mobile phase of 4% (v/v) acetic acid solution – acetonitrile – N,N dimethylformamide (83:12:5, v/v/v). Recovery obtained was 81.3–88%. Photo-diode array detection at 273 nm yielded practical limit of detection 0.05 ppm. The HPLC photo-diode array detector allows the separation of target compounds and identification of them by retention time and spectrum. SDM and OH-SDMs could be identified in samples with their retention times and absorption spectra. The proposed cleanup technique removed almost all interfering peaks, and allowed a reliable confirmation by plotting of

absorption spectrum taken at the peak. Since the proposed procedure gives higher efficiency of cleanup and is highly precise, this procedure may be useful for the monitoring residue (58).

SDZ, SDD, SMMX, SMX, SDMX and **SQX** were determined in chicken muscle tissues by MSPD with alumina N-S. SAs were eluted with 10 mL of 70% (v/v) aqueous ethanol solution. A LiChrospher 100 RP-8, 5 mm, analytical column was used with a mobile phase of a mixture of 1% acetic acid solution (pH 3.0, in water)–acetonitrile–DMF (78:22:5, v/v/v). Recovery was 93.0–101.6%. Photo-diode array detection at 267 nm yielded LOD values in ppm ranged from 0.003 to 0.016. The proposed MSPD–HPLC method for the simultaneous determination of SDA, SDD, SMM, SMX, SDMX, and SQX in chicken muscle tissues offers shorter analysis time (1.5 h per sample), low organic solvent consumption (12 mL per sample), high precision (RSD 9%). These findings demonstrate that this method is useful for the routine residue monitoring of these compounds in chicken muscle tissues (59).

SDZ, SMR, SDD, SMMX, SMX, SDMX and **SQX** were determined in milk after microcentrifugation. A Mightysil RP-4 GP (end-capped) (5 μm) analytical column was used with a mobile phase of 25% (v/v) aqueous ethanol solution. Recovery was in the range 85.3–92.2%. Photo-diode array detection at 269 nm provided various practical detection limits (μg/mL) that ranged from 0.005 to 0.02. Shorter analysis time and use of smaller amounts of organic solvents and less toxic solvents and reagents were achieved by the proposed procedure. This procedure is harmless to the analyst/environment and significantly reduces costs. The shorter analysis time (total <40 min per sample), the highly precise (inter- and intra-assay variabilities=2.0–3.1%), no toxic solvents used; low solvent consumption (total solvent consumption <6 mL of ethanol per sample) make the proposed procedure useful for the routine residue monitoring of SDZ, SMR, SDD, SMM, SMX, SDMX, and SQX in milk (60).

Eleven **SAs** were determined in milk, meat and eggs. After extraction with dichloromethane, fat is removed over a silica cartridge. The SAs were eluted with a buffer solution and eluate was extracted with ethyl acetate. A C_{18} column was used with diode array or UV detector at 266 nm providing LOD: 2 μg/kg (61).

SDZ, SMZ, SMX, SDX, SDM and **DDS** were determined in milk after deproteinization with acetonitrile and ultrafiltration. *A* Waters Symmetry C_{18} analytical column was used with mobile phase of acetonitrile–10 mM ammonium acetate (pH 3.5), 15-min linear gradient from 0 to 90 vol.% acetonitrile. d_7 -Sulfadimidine was used as IS. Recovery was in the range 69%–87%. Tandem MS provided LOD: SDZ, SMZ SMX SDX, SDM,<100 mg/kg(<MRL), DDS< 5 mg/kg.(<MRL). The off-line combination of ultrafiltration and LC–MS–MS has been

shown to be a promising technique with good analytical performance for the trace-level determination, i.e. identification and quantification, of veterinary drugs in raw and preserved milk and milk replacers. Although ultrafiltration-based sample preparation is performed off-line, the possibility of treating 24 samples simultaneously ensures that there is no barrier here to high sample throughput. With SAs as test analytes, it is demonstrated that no problems are encountered at or below the MRL levels of such analytes, nor at the much lower concentrations typically required in the case of a banned compound. Proper consideration of analyte characteristics, MS–MS detectability and required sensitivity (MRLs, banned substances) will be a main aspect of such studies aiming at wide-ranging multi-residue methods (62).

SDD and **SDMX** were determined in eggs after deproteinization with 1 M hydrochloric acid and tetrahydrofuran. Extraction from albumen was performed with ethyl acetate. Yolk samples were further purified twice by liquid- liquid partition with methanol and isooctane. HPLC analysis was performed on a 4-μm Nova-Pak C_{18} column used with a mixture of acetonitrile and 0.01 M ammonium acetate, pH 6.0 (12:88 v/v for SDD 14:86 v/v for SDMX) as mobile phase. Mean recovery was for albumen: SDD 87%, SDMX 77.4% and for Yolk: SDD: 64.6% and SDMX: 67.4%. UV detection at 275 nm yielded LOQ 0.005 μg/g (63).

SDZ, STZ, SPN, SMR, SMT, SDD, SIX, SMX, STR, SCZ, SPZ and **DDS** were determined in bovine, pig and chicken muscle, bovine and pig liver and kidney. After extraction with acetonitrile and n-hexane, further clean up was performed by SPE. Cartridges were conditioned with methanol and water. A Phenomenex Luna C_{18} (5 μm) column was used with gradient elution. SAs were eluted with ammonia solution/ acetonitrile (v/v 1/19). Low recovery rates were obtained 13.6–60.9%. Photo-diode array detection at 260 nm (dapsone at 294 nm) provided LOD 1 ppb for all analytes. The extraction method developed for these twelve compounds is more reliable and more sensitive then those previously used. Further a higher sample throughput is achieved and the consumption of the chemicals could be lowered to 25% (64).

SMMX, SDMX and **SQX** were determined in eggs after homogenization with 0.4 mL of 10% (v/v) perchloric acid solution (in water). Analysis was performed on a Mightysil RP-4 GP column (5 μm) with a mobile phase of 0.18 mol/L citric acid. Recovery ranged from 80.9 to 87.6 %. Photo-diode array detection at 267 nm PDL provided (practical detection limits) 0.02–0.04 μg/g. It is a rapid and simple method without use of organic solvents for determination and identification of SMM, SDM, and SQX in eggs. The main advantages of proposed procedure are summarized as follows: (a) by extraction using a handy ultrasonic-homogenizer followed by purification using a micro-centrifugal ultrafiltration unit, the sample preparation is

especially easy/rapid and is able to recover SAs effectively; (b) shorter analysis time, the total time required for the analysis of one sample<30 min; (c) proving reproducible and repeatable recoveries, the R.S.D.s were 3.4–5.8%, and economical; (d) no organic solvents used at all, harmless to the environment and human. The present procedure may be useful for the international harmonized analytical method for routine residue monitoring of SAs in eggs (65).

SDZ, SDD, SMMX, SMX, SDMX and **SQX** were determined in chicken, beef, and pork tissues after MSPD with 2 g of Alumina N-S. Elution was performed with 70% (v/v) aqueous ethanol solution. Separation was achieved on a Mightysil RP-4 GP column with a mixture of 2% acetic acid solution (pH 2.7, in water)-ethanol (75:25, v/v). Various recovery rates were obtained in the range 92.5–98.9%. Photo-diode array detection at 267 nm yielded LOQ within the range from 0.006 to 0.032 ppm. The proposed toxic/harmful solvents-free method uses normal-phase MSPD followed by HPLC, achieved the simultaneous determination of six SAs in meat with high accuracy and confirmation. Therefore, this method can be suitable for routine technique in laboratories (66).

SMZ was determined in edible tissues from cattle, pigs, chickens and sheep. Tissues were homogenized with 30% (v/v) ethanol in water followed by treatment on an Ultrafree (R) -MC/PL as a centrifugal ultrafiltration unit. A reversed-phase C-4 column was used with 15% (v/v) ethanol in water as mobile phase. Average recovery was 80%. A photo-diode array detector provided LOQ: 0.057–0.060 mg/kg (67).

SDZ, STZ, SPN, SMR, SMZ, SMMX, SCP, SMX, SQX and **SDM** were determined in muscle after extraction with ethyl acetate and n-hexane. A C_8 5 μm, (Phenomenex, Torrance, CA, USA) analytical column was used with a mobile phase under a Gradient program. Recovery rates were 72–86%. Photo-diode array detection at 270 nm yielded LOD 0.03–0.07 μg/mL. This approach utilises a traditional detection by HPLC–DAD without the use of a very sophisticated system such as LC–MS, which can be better dedicated to banned substances (group A of Annex I of directive 96/23/EC) (37).

SGN, SAM, SMX, SMR, SMT, SSZ, 5-SMZ, SMZ, SMPD, SMMX, SDMX, SPN, SBA, STZ, SDZ and **SQX**, were determined in milk, bovine liver and kidney tissues (water, urine) after extraction with acetic acid 0.1M in water and ethyl acetate. Further purification and/or enrichment was performed on with SLM. A C_{18} Clipeus Higgins, 5 μm column was used with a mobile phase of A = 85% (25mM AcOH in water) and B = 15% (25mMAcOH in MeOH) delivered isocratically. Recovery ranged in milk: 34–77%, in Liver 42–88% and in Kidney 44–90%. *E*lectrospray ion trap mass spectrometer yielded LOD values 1.8 to 24.3 ppb. The sulfonamide compounds of interest in this work have been

separated and detected by LC-ES-MS and the enrichment and/or cleanup method was by SLM technique. Eleven out of 16 SA compounds were successfully enriched using 5% tri-*n*-octyl phosphine oxide (TOPO) in hexylamine liquid membrane. The high extraction efficiencies of this membrane are probably due to the fact that, it combined both qualities of hexylamine and the TOPO. The use of selected ion monitoring provided additional selectivity and sensitivity in the determination of these compounds (68).

SMZ was determined in pork after homogenization with 100% water, centrifugation, and ultrafiltration. A reversed-phase C_1 was used for separation. Average recovery was 81%. Photodiode-array detector yielded LOQ 0.09 μg/g (69).

SPN, SDZ, SMZ, SCP, SDZ, SMX and **SDMX**, were determined in milk, according to AOAC official method after extraction by chloroform and acetone. Analysis was performed on a C_{18} Luna separation column, 5 μm. Mobile phase was a mixture of tri-sodium citrate hydrate citric acid/monohydrate buffer: ACN: THF (65:22:13) delivered isocratically. Recovery was (%) 41.47–67.09%. Chemiluminescence detection yielded LOD values of μg/L for SPN: 6.2, for SDZ:6.9, for SMZ:7.1, for SCP:13.2, for SDZ:13.6, for SMX:10.4 and for SDMX:9.5. The method has been applied to the analysis of spiked raw milk samples, and the results are comparable to that provided by a collaborative study using the AOAC official method, in terms of both recovery and precision. LOD values are better than those commonly reported for the analysis of these compounds, allowing the determination of SAs in milk in the very low $g\,L^{-1}$ range. Moreover, the time of analysis is shorter that those reported by other chromatographic methods (70).

SDZ, STZ, SMX, SAM-Na and **SMMX-Na** were determined in milk after deproteinization with ethanol. Separation was achieved on a Hypersil ODS column (5 μm) with a mobile phase of methanol-0.02 mol/L buffer Na_2HPO_4 solution (3:7, v/v; pH 3.0). Recovery ranged from 14.7% to 95.7%. UV detection at 269 nm yielded LOD 1.7–2.8 $ng\,mL^{-1}$. In-tube SPME coupled to HPLC with a poly-(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary as the extraction medium was successfully applied to the simple and rapid determination of five sulfonamide antibacterial residues in milk. In comparison to the pre-treatment methods as reported previously, the proposed in-tube SPME-HPLC method is environmentally friendly and inexpensive andeasily realizes on-line analysis. In addition, using the in-tube SPME coupled to HPLC with UV detection, simultaneous analysis was accomplished with high sensitivity. Therefore, the proposed method will be useful and practical in future residue monitoring and in studying the pharmacokinetics of **SDZ, STZ, SMX, SAM-Na** and **SMMX-Na** in milk (71).

SDZ, SMR, SM, SMMX, SDMX and **SMX** were determined in milk using a Microdialysis System of stainless steel tubing, (serving as sample region), connected with two PEEK tee connector at both ends, a piece of hollow fiber placed in the system. Separation was performed on a Zorbax Eclipse XDS-C_8 analytical column (5 μm particle size) with a mobile phase of 25:75 (v/v) of acetonitrile and 0.01 M phosphate buffer (pH 4.0). Recovery ranged from 97.8% to 100.4%. UV detection at 260 nm yielded LOD that ranged from 0.08 to 0.86 (μg/L). The study has demonstrated that the microdialysis-HPLC system is with potential to enrich SAs and lower the LOD in analysis process. It is applicable to routine analysis of SAs in milk with advantages of simple, rapid, small sample volume, low LOD, and organic solvent-free in sample pre-treatment (72).

SMMX, SDMX and their hydroxy/N4-acetyl metabolites were determined in chicken plasma, muscle, liver, eggs after extraction with ethanol. Analysis was performed on a Mightysil RP-4 GP column with a mobile phase of ethanol in 1% acetic acid solution (in water). Various recovery rates were achieved as shown in Table 3. The present study has succeeded in making a simple method without the use of toxic/harmful solvents and reagents at all for simultaneous determination of SMMX, SDMX, and their OH/Ac-metabolites in chicken plasma, eggs, liver, and muscle. This method has been developed to study the pharmacokinetic profiles in chickens and monitor the drug residues of SMMX, SDMX, and their OH/Ac-metabolites in chicken products. The complete procedure, which harms neither the environment nor humans, is economical and provides reproducible recoveries (73).

Ten **SAs** were determined in chicken meat after extraction with mixture of acetonitrile and chloroform (acetonitrile:chloroform is 10:1) using a Hypersil BDS C_{18} with a mobile phase:acetonitrile and 30 mmol/L NaH_2PO_4 (pH 5) using a linear gradient. Recovery was >50%. Coulometric porous graphite electrode array detector provided LOD: 20–40 μg/kg (74).

SDZ, STZ, SMPD, SMMX, SDMX and **SQX** were determined in chicken liver after SPE extraction with ethyl acetate, NH_2 column to clean up. Analysis was performed on an Inertsil ODS-3 C_{18} with a mobile phase: methanol/acetonitrile (1/1, v/v) (A) and water/acetic acid (90/2, V/V) (B). Recovery obtained was greater than 69.6%. Photo-diode array detection LOD: 8–12 μg/kg (75).

SDZ, SMR, SDD, STZ, SMX, SMT, SMPD, SCP, and **SIX** were determined in pork and eggs after extraction with Fe(III) potassium cyanide, zinc and acetonitrile. Further clean up was achieved by on-line SPE coupled to HPLC Column: RP (Alltech-C18 5μm Alltech, USA). Mobile phase was a mixture of methanol and water (22:78). Recovery obtained was in eggs 70.38–81.47% and in pork 68.6–80.84%. UV detection at 270 nm yielded LOD: (ng/L) for SDZ: 5.9, for STZ: 9.3, for

SMR: 4.6, for SMX: 5.1, for SMT: 7.6, for SDD: 4.9, for SMPD: 7.1, for SCP: 4.1, for SDZ: 4.5 and for SIX: 10.0. A method for the determination of residual SAs in eggs and pork was established using carbon nanotubes as sorbent for online SPE coupled with HPLC-UV. The present method is not only simple, rapid, and reliable, but it also permits the simultaneous determination of 10 different kinds of SAs with good recoveries relative standard deviation and LOD. Accordingly, widespread use of the method presented here is recommend for the routine analysis of residual SAs in eggs and pork (76).

SDZ and **SMZ** were determined in eggs. Extraction was performed by polymer monolith microextraction (PMME): poly (methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary as extraction medium. Separation of analytes was achieved on a Kromasil ODS (5 μm) analytical column with a mobile phase of methanol-0.02 mol/L phosphate buffer solution (pH 3.0) (30:70, v/v). Recovery was for SDZ: 67.3% and for SMZ: 65.5%. UV detection of 269 nm yielded LOD for SDZ: 11.2 ng/g and for SMZ: 8.8 ng/g. PMME coupled to HPLC/UV with a poly (methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary as the extraction medium was successfully applied to the simple, rapid and sensitive determination of SDZ and SMZ residues in eggs. Therefore, the proposed method will be useful and practical in residue monitoring and in studying the pharmacokinetics of SDZ and SMZ in eggs in future (77).

SDZ, SMR and **SMZ** were determined in calf and pig tissue after extraction with acetonitrile. Separation was performed on a Synergy MAX-RP (4 μm particle) analytical column (Phenomenex, Torrance, USA). Mobile phase was delivered under a gradient prepared from 0.01 mol L^{-1} ammonium acetate (A) and acetonitrile (B). Recovery ranged from 77.8 to 104.1%. Photodiode array detector at 270 nm provided (ppb): (instrumental LOD) (pH 4.50) for SDZ: 3, for SMR: 5 and for SMZ: 6. It may be concluded that the RP-C12 analytical column with mobile phase of pH 4.5 enabled excellent chromatographic separation of SMZ, SDZ and SRZ. Peak shape and resolution were good and satisfactory quantification of the three SAs in edible calf and pig tissue (muscle and liver) was possible without extract cleanup. The proposed conditions are useful for achieving rapid and reliable screening of the SA content of calf and pig tissue at concentrations below the MRL. A possible further improvement could be complementary use of MS or MS—MS detection to investigate whether the putative metabolites really are derivatives of SMZ, to confirm positive results, and as a useful tool in pharmacokinetic studies (78).

SDZ, STZ, SMZ, SMPD, SMMX, SMX, SDMX and **SQX** were determined in bovine milk after filtration and extraction with ethyl acetate. SPE was used for further cleanup using an anion cartridge

($LC\text{-}NH_2$) pre-washed with ethyl acetate. SAs were eluted from cartridge with a 1:1:8 (v/v/v) mixture of methanol, acetonitrile and 1.0% acetic acid. Separation was performed on an Inertsil ODS-3 C_{18} (5 μm) analytical column with a mobile phase of 0.1% acetic acid solution (A) and a 1:1 (v/v) mixture (B) of methanol and acetonitrile, gradient elution at 25°C. Photodiode-array detection at 270 nm yielded LOD: 0.8–1.5 μg/L. The simplified and effective pretreatment procedure, including extraction with ethyl acetate and cleanup with $LC\text{–}NH_2$ cartridge, enables quantitative determination of eight of the most used SAs at concentrations far below maximum residue level and instrumental analysis is simpler than the AOAC official method. Moreover, the method has been validated according to the European Commission Decision 2002/657/EC. It has good recoveries (70.5–89.0%) and relative standard deviation. The method can be applied for the quantitative confirmation of SAs in residual control (79).

SNM, SDZ, SMR and **SMZ** were determined in cow's milk after deproteinization with ethanol–acetic acid (97:3, v/v). An Ether-type C_8 (particle size 5 μm) analytical column was used with a mobile phase of acetonitrile–water (5:95, v/v) delivered isocratically. Mean recovery was 80.1%–87.6%. Photo-diode array detection at 270 nm yielded LOD for **SNM**: 30 ng mL^{-1} and for SDZ, SMR, SMZ: 60 ng mL^{-1}. A simple and rapid method for determining **SNM, SDZ, SMR** and **SMZ** in milk using Ether-type C_8 as separation column was developed. The main characteristics of the proposed procedure are summarized as follows: shorter analysis time (total less 15 min per sample); high precision (R.S.D. $<6.0\%$ in the recovery test); nearly harmless to the environment (total solvent consumption <1.0mL of ethanol and 0.6mLof acetonitrile, respectively); lowcost. Therefore, this method is useful for practical residue monitoring and studying pharmacokinetics of SAM, SDA, SMA and SMZ in milk (80).

SDZ, STZ, SMR, SMZ, SMMX, SMX, SQX and **SDMX** were determined in pork and chicken muscle tissue by SPME. Desorbed analytes were analysed by LC–MS for analysis. A Supelco LC–18DB (5 μm) analytical column was used with a mobile phase: A (water) and B (80% acetonitrile in water) (70:30, v/v). This study evaluated an integrate method of combining SPME with LC–MS to determine the trace amount of SAs in meat samples. LC–APCI–MS was a very selective and sensitive method for determination of SA. The results indicated that the linearity and precision of the APCI mode are better than those in the ESI mode for analysis of trace SAs. In the APCI method, the LOD for SAs in water and meat are 0.6–7.5 and 16–39 g kg^{-1}, respectively. The analytical curves of SAs were linear in the range of 10–1000 μgL^{-1} for SAs in water and 50–2000 μg kg^{-1} in meat. In addition, the feasibility of applying SPME–LC–MS to determine the amount of SAs in real meat samples from a

local market was tested. The detected SAs in the samples ranged from 52 to 157 $\mu g\,kg^{-1}$. Therefore, the proposed method is precise and offers a high level sensitivity to determine trace amounts of SAs in meat products containing high amount of interferences (81).

SDZ, SMZ, SMPD, SMX, SDMX and **SQX** were determined in kidney after extraction with dichloromethane and petroleum benzene. Analysis was achieved on an XTerra MSC_{18}, 5 μm, analytical column. Mobile phase was a mixture of formic acid (0.1%, v/v) and methanol. Mean recovery was 46–66%. Photo-diode array detector at 268 nm provided capability of detection (μg/kg), that ranged from 135.9 to 186.0 (82).

SMMX, SDMX (and their N4-acetyl metabolites) **AcSMMX, AcSDMX** were determined in Eggs after extraction with saturated ammonium sulfate solution (4 mol/L) and cleanup using RAM (restricted-access media)-HPLC Column: Supelco RAM-HPLC Hisep shielded hydrophobic phase column (5 μm). The mobile phase was 0.3% (v/v) acetic acid solution (pH 2.9, in water)–ethanol (75:25, v/v). Recovery ranged from 91% to 94%. Photo-diode array detector at 267 nm yielded LOD (ppm) 0.01–0.03. The method requires no hazardous-chemicals and is, therefore, safer both for humans and for the environment. The RAM HPLC enabled the simple and rapid analysis which avoided analyte losses and resulted in high reproducibility and reliability. This method might, therefore, be useful for practical residue monitoring and pharmacokinetic studies with eggs (83).

SDZ, SQX, SMTH and **SDM** were separated by HPLC and SQX and SDM were determined in cow's milk. The analytical column, a Kromasil, C_{18} 5 μm, analytical column, was operated at ambient temperature. The mobile phase, a mixture of 0.5% acetic acid as solvent A, CH_3CN as solvent B and CH_3OH was delivered to the analytical column according to a gradient program. DAD detection was performed for detection and confirmation of separated analytes with monitoring at 260 nm. LOD and LOQ were 13 and 40 μg/kg respectively. Solid-phase extraction was applied to remove all matrix interference from milk samples after deproteinization with 8 M HCl. High extraction recoveries (>84%) were achieved using DSC-18 cartridges with CH_3OH-0.5% CH_3COOH as eluent. The method was applied to the analysis of twenty two milk samples from local market. SQX was identified in seven of these samples. The described confirmatory method is a simple validated assay, which can be readily adapted by any laboratory for the quality control and the quantitative determination of residues of the four examined SAs in milk. Validation was performed according to the European Union regulation 2002/657/EC for the validation of an analytical method for residues in animal products. The results of validation process demonstrate that the method is suitable for application in European Union

statutory veterinary drug residue surveillance programmes. The four investigated SAs were resolved within 20 min. LOQ values achieved were matching to MRL values. The CC*a* values calculated by spiking 20 blank milk samples at MRL (100 μg/kg) were 111.8 μg/kg for SDM and 117.1 μg/kg for SQX. CC*b* values calculated by analyzing 20 blank spiked samples at corresponding CC*a* level for each analyte were 116.6 μg/kg and 134.0 μg/kg (84).

Confirmation of antibiotic residues in food is performed by LC-MS, mainly LC-MS^2. When mass fragments are measured using techniques other than full-scan, the system of IPs is applied. Commission Decision 2002/657/EC contains detailed information on the relationship between a range of classes of mass fragments, a range of techniques and combinations there of and IPs earned. Table 4 gives analytical data of a selection of methods found in literature using mass spectrometric detection.

The reported methods all employed positive-ion mode and spectra for all SAs exhibited the protonated molecular ion, $[M+H]^+$, as the base peak; except for sulfanilamide for which the base peak was the $[M+NH_4]^+$ adduct ion in presence of ammonium salts in mobile phase. In tandem MS ions common to all SAs were produced at *m/z* 156, the *p*-aminobenzenesulfonic acid moiety ($[M\text{-}RNH_2]^+$), *m/z* 108 ($[M\text{-}RNH_2\text{-}SO]^+$) and *m/z* 92 ($[M\text{-}\ RNH_2\text{-}SO_2]^+$) and ions derived from the variable amine substituent, RNH_3 ($[MH\text{-}155]^+$). The peak at *m/z* 156, the base peak for several of the SAs, results from the cleavage of the sulfur–nitrogen bond on the SA. Some spectra also exhibited ions at $[MH\text{-}93]^+$ and $[MH\text{-}66]^+$ corresponding to $[O_2SNHR]^+$ and $[MH\text{-}H_2SO_2]^+$, respectively. Table 5 illustrates the main fragments of SAs in tandem MS (16).

CONCLUSION

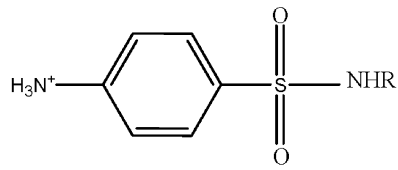
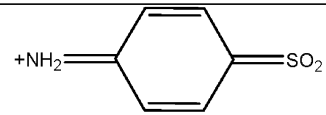
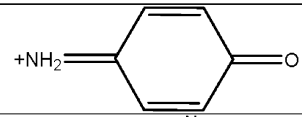
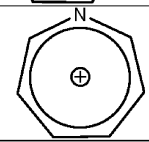
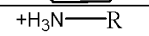
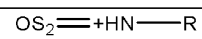
Conventional, and state-of-the art analytical procedures—and their performances—for the various SAs are discussed in this review. Current research issues include the requirement of simple sample preparation procedures, the possibility of multi component analysis with good resolution if DAD is used or even not perfect resolution if MS is applied, low LOD values able to detect residues below MRL values. Methods for drug residue monitoring should be accurate, simple, and economical both in time and cost. Confirmation of analyte identity is of paramount importance, as indicated by regulatory agencies. The current legislation demands detection technologies based on chromatographic methods for confirmation purposes. Bearing in mind that most antibiotics are thermally labile and low-volatile compounds, liquid chromatography coupled to MS (LC–MS) and tandem mass spectrometry (LC–MS/MS) have become the

Table 4. MS data for confirmatory analysis of SAs in food products of animal origin.

Mass spectrometer: Platform single quadrupole **ionisation mode**: APCI in positive mode, **vaporizer temperature**: 400°C, **corona pin voltage**: 3.0 kV, **extraction cone voltage**: 30 V, **source temperature**:120°C, **scan time**: 1s, **damping gas**: nitrogen, **sheath gas**: nitrogen (51)	Compound	TIC (ng)	EIC (ng)
	SDZ	10	0.8 (156)[a]
	SCP	100	10 (156)
	STZ	20	1 (156)
	SMR	10	0.8 (265)
	SPN	10	0.8 (250)
	SMZ	10	0.8 (279)
	SQX	10	0.8 (300)
	SDMX	10	0.8 (310)
Mass spectrometer: equipped with an ESI interface **Ionisation mode**: ESI in the positive ion mode (62)	Sulfonamide	$[M+H]^+$	Product ions *** (*m*/*z*)
	SD	311.1	245, <u>156</u>, 140 108, 92
	SDMX	311.1	245, 218, <u>156</u> 108, 92
	SDD	279.2	204, <u>186</u>, 156, 124 108, 92
	SMX	254.1	188, <u>156</u>, 108, 92
	SDZ	251.1	<u>156</u>, 108, 92
	DDS	249.0	<u>156</u>, 108, 92
Mass spectrometer: ion trap 400V (68)	$[M+H]^+$, $[SQX-Na]^+$		
Mass spectrometer: ESI source or APCI source on a quadrupole ion trap mass spectrometer **ionisation mode**: APCI in positive mode, **vaporizer temperature**: 400°C, **damping gas**: high purity helium, **sheath gas**: nitrogen, **flow-rate of sheath gas**: 1.5 L/min, **discharge current**: 1.0 μA, **capillary temperature**: 120°C, **capillary voltage**: 14V, **multipole 1 offset voltage**: −3V, **lens voltage**: −16V, **multipole 2 offset voltage**: −7V; **multipole RF amplitude voltage**: 400V (81)	$[M+H]^+$, were obtained as the base ions for all sulfonamides in both ESI and APCI mass spectra. A small characteristic fragment ion corresponding to $[NH_2C_6H_4SO_2]^+$ at *m*/*z* 156 which is the fundamental structure of sulfonamides was also observed.		

[a]Number in parentheses is *m*/*z* used for extracted ion chromatograms.
**Minimum detectable quantity from Total Ion Chromatogram.
***Underlined *m*/*z* transition was used for quantification.

Table 5. Main fragments of sulfonamides in MS/MS (16).

Positive fragment	Protonated sulfonamide
	
(*m/z* 156)	
(*m/z* 108)	
(*m/z* 92)	
$(MH\text{-}155)^+$	
$(MH\text{-}93)^+$	
$(MH\text{-}66)^+$	$[MH\text{-}H_2SO_2]^+$

most popular techniques for the determination of these analytes during the last few years, using electrospray (ESI) or atmospheric pressure chemical ionisation (APCI) sources. In this sense, LC coupled to ESI has become a very valuable technique for multiresidue analysis, because it is more sensitive, selective and allow rapid and multiresidue determination in complex matrices, providing structural information.

When mass fragments are measured using techniques other than full-scan, the system of identification points is applied. Commission Decision 2002/657/EC contains detailed information on the relationship between a range of classes of mass fragments, a range of techniques and combinations of IPs earned.

Extraction of these compounds from biomatrices and extract purification is performed mainly by SPE, LLE and MSPD. A decrease in toxic/harmful solvents consumption is a positive direction for

analytical science, because environmental problems are a worldwide concern. For this reason solvent free sample preparation techniques would be the trend in analytical strategies for SA residues determination in animal-derived food.

REFERENCES

1. Calza, P., Medana, C., Pazzi, M., Baiocchi, C., and Pelizzetti, E. (2004) Photocatalytic transformation of sulfonamides on titanium dioxide. *Appl. Catal. B: Environ.*, 53: 63–69.
2. Kishida, K. (2007) Quantification and confirmation of six sulfonamides in meat by liquid chromatography–mass spectrometry with photodiode array detection. *Fd. Contr.*, 18: 301–305.
3. Furusawa, N. (2000) Simplified determining procedure for routine residue monitoring of sulfamethazine and sulfadimethoxine in milk. *J. Chromatogr. A*, 898: 185–191.
4. Franek, M., Kolar, V., and Deng, A. (1999) Determination of sulfadimidine (sulfamethazine) residues in milk, plasma, urine and edible tissues by sensitive ELISA. *P. Fd. Agric. Immunol.*, 11: 339–349.
5. Ikai., Y., Oka, H., Kawamura, N., Hayakawa, J., Yamada, M., Harada, K-I., Suzuki, M., and Nakazawa, H. (1991) Application of an amino cartridge to the determination of residual sulfonamide antibacterials in meat, fish and egg. *J. Chromatogr.*, 541: 393–400.
6. Oka, H., Ikai, Y., Kawamura, N., and Hayakawa, J. (1991) Limited survey of residual tetracyclines in tissues collected from diseased animals in Aichi Prefecture, Japan. *J. Assoc. Off. Anal.Chem.*, 74: 894–896.
7. Collins-Thompson, D.L., Wood, D.S., and Thomson, I.Q. (1988) Detection of antibiotic residues in consumer milk supplies in North America using the Charm II procedure. *J. Food Protect.*, 51: 632–633.
8. Littlefield, N.A., Sheldon, W.G., Allen, R.R., and Gaylor, D.W. (1990) Chronic toxicity and carcinogenicity studies of SMZ in Fischer 344/N rats: two-generation exposure. *Food Chem. Toxicol.*, 28: 157–167.
9. Code of Federal Regulations 21 CFR Parts 510 and 556, US Government Printing Office, Washington, DC, 1987.
10. Federal Register, 53FR 9492, March 23, NCTR (1988) Technical Report for Experiment No. 418, NCTR, March 1988.
11. 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. Commun.*, 1990, *L 224*, 1–8. http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/consleg/1990/R/01990R2377-20051119-en.pdf
12. Guggisberg, D., Mooser, A.E., and Koch, H. (1992) Methods for the determination of sulfonamides in meat. *J. Chromatogr. A*, 624: 425–437.
13. Agarwal, V.K. (1992) High-performance liquid chromatographic methods for the determination of sulfonamides in tissue, milk and eggs. *J. Chromatogr.*, 624: 411–423.

14. Shaikh, B. and Moats, W.A. (1993) Liquid chromatographic analysis of antibacterial drug residues in food products of animal origin. *J. Chromatogr.*, 643: 369–378.
15. Marzo, A. and Dal Bo, L. (1998) Chromatography as an analytical tool for selected antibiotic classes: a reappraisal addressed to pharmacokinetic applications. *J. Chromatogr. A*, 812: 17–34.
16. Kennedy, D.G., McCracken, R.J., Cannavan, A., and Hewitt, S.A. (1998) Use of liquid chromatography–mass spectrometry in the analysis of residues of antibiotics in meat and milk. *J. Chromatogr. A*, 812: 77–98.
17. Niessen, W.M.A. (1998) Analysis of antibiotics by liquid chromatography–mass spectrometry. *J. Chromatogr. A*, 812: 53–75.
18. Di Corcia, A. and Nazzari, M. (2002) Liquid chromatographic–mass spectrometric methods for analyzing antibiotic and antibacterial agents in animal food products. *J. Chromatogr. A*, 974: 53–89.
19. Joshi, S. (2002) HPLC separation of antibiotics present in formulated and unformulated samples. *J. Pharm. Biomed. Anal.*, 28: 795–809.
20. Balizs, G. and Hewitt, A. (2003) Review: Determination of veterinary drug residues by liquid chromatography and tandem mass spectrometry. *Anal. Chim. Acta*, 492: 105–131.
21. Núñez, O., Moyano, E., and Galceran, M.T. (2005) LC–MS/MS analysis of organic toxics in food. *Trends Anal. Chem.*, 24: 683–703.
22. Gentili, A., Perret, D., and Marchese, S. (2005) Liquid chromatography-tandem mass spectrometry for performing confirmatory analysis of veterinary drugs in animal-food products. *Trends Anal. Chem.*, 24: 704–733.
23. Wang, S., Zhang, H., Wang, L., Duan, Z.J., and Kennedy, I. (1996) Analysis of sulfonamide residues in edible animal products: A review. *Food Add. Contam.*, 23: 362–384.
24. García-Ruiz, C. and Marina, M.L. (2006) Recent advances in the analysis of antibiotics by capillary electrophoresis. *Electrophoresis*, 27: 266–282.
25. Blasco, C., Torres, C.M., and Pico, Y. (2007) Progress in analysis of residual antibacterials in food. *Trends Anal. Chem.*, 26: 895–913.
26. Connor, E.E. (1998) Sulfonamides antibiotics. *Honorable mention paper Primary Care Update for OB/GYNS*, 5: 32–35.
27. http://www.elmhurst.edu/~chm/vchembook/653sulfa.html (3-11-2007).
28. http://en.wikipedia.org/wiki/Sulfonamide_%28medicine%29 (3-11-2007).
29. http://lib.bioinfo.pl/meid:30982 (3-11-2007).
30. Lu, K.H., Chen, C.Y., and Lee, M.R. (2007). Trace determination of sulfonamides residues in meat with a combination of solid-phase micro-extraction and liquid chromatography–mass spectrometry. *Talanta*, 72: 1082–1087.
31. Fang, G.Z., He, J.X., and Wang, S. (2006) Multiwalled carbon nanotubes as sorbent for on-line coupling of solid-phase extraction to high-performance liquid chromatography for simultaneous determination of 10 sulfonamides in eggs and pork. *J. Chromatogr. A*, 1127: 12–17.
32. http://www.usp.org/pdf/EN/veterinary/sulfonamides.pdf (3-11-2007).
33. Kishida, K., Nishinari, K., and Furusawa, N. (2005) Liquid chromatographic determination of sulfamonomethoxine, sulfadimethoxine, and their N^4-Acetyl metabolites in chicken plasma. *Chromatographia*, 61: 81–84.

34. Wang, S., Zhang, H., Wang, L., Duan, Z.J., and Kennedy, I. (1996) Analysis of sulfonamide residues in edible animal products: A review. *Fd. Addit. Contam.*, 23: 362–384.
35. 96/23/EC: Council Directive of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC, *Off. J. Eur. Commun.*, **1996**, *L125*, 10–32.
36. 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. Commun.,* **2002**, *L221*, 8–36.
37. Pecorelli, I., Bibi, R., Fioroni, L., and Galarini, R. (2004) Validation of a confirmatory method for the determination of sulfonamides in muscle according to the European Union regulation 2002/657/EC. *J. Chromatogr. A*, 1032: 23–29.
38. http://www.m5.ws001.squarestart.ne.jp/foundation/agrlist.php (22/10/2007).
39. Long, A.R., Short, R., and Barker, S.A. (1990) Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk. *J. Chromatogr.*, 502: 87–94.
40. Long, A.R., Hsieh, L.C., Malbrough, M.S., Short, C.R., and Barker, S.A. (1990) Multiresidue method for the determination of sulfonamides in pork tissue *J. Agric. Food Chem.*, 38: 423–426.
41. Agarwal, V.P. (1990) Detection of SMZ residues in milk by high performance liquid chromatography. *J. Liq. Chromatog. Related Technol.*, 13: 3531–3539.
42. Hoffmeister, A., Suhren, G., and Heeschen, W. (1991) High pressure liquid chromatographic determination of sulfadimidine residues in milk-incidence in consmumer milk from various European countries. *Milchwissenschaft-Milk Sci. Intern.*, 46: 770–774.
43. Ueno, R., Uno, K., Kato, M., Kubota. S.S., and Aoki, T. (1991) Simultaneous determination of sulfamonomethoxine, sulfadimethoxine and their N^4-acetylated forms in fish tissues by high performance liquid chromatography. *Nippon Suisan Gakkaishi*, 57: 549–554.
44. Agarwal, V.P. (1993) Application of solid phase extraction for the analysis of sulfonamides in milk by high performance liquid chromatography. *J. Liq. Chromatogr. Related Technol.*, 16: 3793–3799.
45. Furusawa, N. and Mukai, T. (1994) Simultaneous high-performance liquid chromatographic determination of residual sulfamonomethoxine, sulfadimethoxine and their N4-acetyl metabolites in foods of animal origin. *J. Chromatogr. A*, 677: 81–85.
46. Parks, O.W. and Maxwell, R.J. (1994) Isolation of sulfonamides from fortified chicken tissues with supercritical CO_2 and in-line adsorption. *J. Chromatogr. Sci.*, 32: 290–293.
47. Tamura, H., Yotoriyama, M., Kurosaki, K., and Shinohara, N. (1994) High performance liquid chromatographic analysis of sulfonamides in livestock products using matrix solid-phase dispersion (MSPD) method with silica gel. *J. Food Hyg. Soc. Japan*, 35: 271–275.
48. Viñas, P., López Erroz, C., Campillo N., and Hernández-Córdoba, M. (1996) Determination of sulfonamides in foods by liquid chromatography with post-column fluorescence derivatization. *J. Chromatogr. A*, 726: 125–131.

49. Barbieri, G., Bergamini, C., Ori, E., and Resca, P. (1995) Determination of sulfonamides in meat and meat products. *Industrie Alimentari*, 34: 1273–1276.
50. Laloux, J., Romnee, J.M., Marin, C., and Vanwijnsberghe, D. (1996) Determination of sulfonamides in milk using fluorescamine derivatization and HPLC separation. *Milchwissenschaft-Milk Sci. Intern.*, 51: 517–520.
51. Combs, M.T., Ashraf-Khorassani, M., and Taylor, L.T. (1999) HPLC:atmospheric pressure chemical ionization-mass spectroscopy of eight regulated sulfonamides. *J. Pharm. Biomed. Anal.*, 19: 301–308.
52. Tsai, S.-C., Rau, Y.-H., Hsu, S.-C., Chou, C.C., and Fu, W. (1998) Development of a high performance liquid chromatographic method for detection of sulfonamide residues in egg. *J. Food Drug Anal.*, 6: 509–510.
53. Kostadinovic, L., Pavkov, S., and Gaal, F. (1999) An improved method for the determination of sulfachloropyrazine in meat and liver of broilers during and after their treatment for coccidiosis. *Acta Aliment.*, 28: 311–319.
54. Shaikh, B., Rummel, N., and Donoghue, D. (1999) Determination of SMZ and its major metabolites in egg albumin and egg yolk by high performance liquid chromatography. *J. Liq. Chromatogr. Rel. Technol.*, 22: 2651–2662.
55. McGrane, M., O'Keeffe, M., and Smyth, M.R. (1999) The analysis of sulfonamide drug residues in pork muscle using automated dialysis. *Anal. Lett.*, 32: 481–495.
56. Ito, Y., Oka, H., Ikai, Y., Matsumoto, H., Miyazaki, Y., and Nagase, H. (2000) Application of ion-exchange cartridge clean-up in food analysis. V. Simultaneous determination of sulfonamide antibacterials in animal liver and kidney using high-performance liquid chromatography with ultraviolet and mass spectrometric detection. *J. Chromatogr. A*, 898: 95–102.
57. Stoev, G. and Michailova, A. (2000) Quantitative determination of sulfonamide residues in foods of animal origin by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. A*, 871: 37–42.
58. Furusawa, N. (2000) HPLC determination of sulfadimethoxine and its hydroxyl metabolites following SPE of edible chicken tissues. *J. Liq. Chromatogr. Rel. Technol.*, 23: 1413–1422.
59. Kishida, K. and Furusawa, N. (2001) Matrix solid-phase dispersion extraction and high-performance liquid chromatographic determination of residual sulfonamides in chicken. *J. Chromatogr. A*, 937: 49–55.
60. Kishida, K. and Furusawa, N. (2001) High-performance liquid chromatographic procedure for routine residue monitoring of seven sulfonamides in milk. *Fres. J. Anal. Chem.*, 371: 1031–1033.
61. Diserens, J.M., Renaudbezot, C., and Savoyperroud, M.C. (1991) Simplified determination of sulfonamides residues in milk, meat and eggs. *Deutsche Lebensmittel-Rundschau*, 87: 205–211.
62. van Rhijn, J.A., Lasaroms, J.J.P., Berendsena, B.J.A., and Brinkmanb, U.A.T. (2002) Liquid chromatographic–tandem mass spectrometric determination of selected sulfonamides in milk. *J. Chromatogr. A*, 960: 121–133.
63. Roudaut, B. and Garnier, M. (2002) Sulfonamide residues in eggs following drug administration via the drinking water. *Fd. Addit. Contam.*, 19: 373–378.

64. Hela, W., Brandtner, M., Widek, R., and Schuh, R. (2003) Determination of sulfonamides in animal tissues using cation exchange reversed phase sorbent for sample cleanup and HPLC–DAD for detection. *Fd. Chem.*, 83: 601–608.
65. Furusawa, N. (2003) Rapid high-performance liquid chromatographic determining technique of sulfamonomethoxine, sulfadimethoxine, and sulfaquinoxaline in eggs without use of organic solvents. *Anal. Chim. Acta*, 481: 255–259.
66. Kishida, K. and Furusawa, N. (2003) Toxic/harmful solvents-free technique for HPLC determination of six sulfonamides in meat. *J. Liq. Chromatogr. Rel. Technol.*, 26: 2931–2939.
67. Furusawa, N. (2001) Determining the procedure for routine residue monitoring of SMZ in edible animal tissues. *Biomed. Chromatogr.*, 15: 235–239.
68. Msagati, T.A.M. and Nindi, M.M. (2004) Multiresidue determination of sulfonamides in a variety of biological matrices by supported liquid membrane with high pressure liquid chromatography-electrospray mass spectrometry detection. *Talanta*, 64: 87–100.
69. Kishida, K. and Furusawa, N. (2004) Determining SMZ in pork using HPLC with a 100% water mobile phase. *LC GC N. Amer.*, 22: 1092–1096.
70. Soto-Chinchilla, J.J., Gamiz-Gracia, L., Garcıa-Campana, A.M., Imai, K., and Garcıa-Ayuso, L.E. (2005) High performance liquid chromatography post-column chemiluminescence determination of sulfonamide residues in milk at low concentration levels using bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate as chemiluminescent reagent. *J. Chromatogr. A*, 1095: 60–67.
71. Wen, Y., Zhang, M., Zhao, Q., and Feng, W.Q. (2005) Monitoring of five sulfonamide antibacterial residues in milk by in-tube solid-phase microextraction coupled to high-performance liquid chromatography. *J. Agric. Food Chem.*, 53: 8468–8473.
72. Yang, T.C.C., Yang, I.L., and Liao, L.J. (2005) Determination of sulfonamide residues in milk by on-line microdialysis and HPLC. *J. Liq. Chromatogr. Relat. Technol.*, 27: 501–510.
73. Kishida, K. and Furusawa, N. (2005) Simultaneous determination of sulfamonomethoxine, sulfadimethoxine, and their hydroxy/*N*4-acetyl metabolites with gradient liquid chromatography in chicken plasma, tissues, and eggs. *Talanta*, 67: 54–58.
74. Li, S.G., Zhao, J.M., Wang, W.X., Jin, Y., and Du, Y.G. (2005) Simultaneous determination of ten sulfa drugs in chicken meat by high performance liquid chromatography coulometric array detector. *Chin. J. Anal. Chem.*, 33: 442–446.
75. Wu, Y., Liu, S., Shan, J., and Wang, H. (2005) Simultaneous determination of sulfonamide residues in chicken liver by solid phase extraction and high performance liquid chromatography. *Fenxi Huaxue*, 33: 1713–1716.
76. Fang, G.Z., He, J.X., and Wang, S. (2006) Multiwalled carbon nanotubes as sorbent for on-line coupling of solid-phase extraction to high-performance liquid chromatography for simultaneous determination of 10 sulfonamides in eggs and pork. *J. Chromatogr. A*, 1127: 12–17.
77. Yi, W., Ying, W., and Yuqi, F. (2006) Monitoring sulfadiazine and SMZ residues in eggs using polymer monolith microextraction coupled with high performance liquid chromatography. *Chinese J. Chromatogr.*, 24(5): 471–474.

78. Gratacós-Cubarsí, M., Castellari, M., Valero, A., and García-Regueiro, J. A. (2006) A simplified LC–DAD method with an RP-C12 column for routine monitoring of three sulfonamides in edible calf and pig tissue. *Anal. Bioanal. Chem.*, 385: 1218–1224.
79. Wu, Y.L., Li, C., Liu, Y.J., and Shen, Y.Z. (2007) Validation method for the determination of sulfonamide residues in bovine milk by HPLC. *Chromatographia*, 66: 191–195.
80. Huang, X., Yuan, D., and Huang, B. (2007) Simple and rapid determination of sulfonamides in milk using Ether-type column liquid chromatography. *Talanta*, 72: 1298–1301.
81. Lu, K.H., Chen, C.Y., and Lee, M.R. (2007) Trace determination of sulfonamides residues in meat with a combination of solid-phase micro-extraction and liquid chromatography–mass spectrometry. *Talanta*, 72: 1082–1087.
82. Garcia, I., Ortiz M.C, Sarabia, L., and Aldama, J.M. (2007) Validation of an analytical method to determine sulfamides in kidney by HPLC-DAD and PARAFAC2 with first-order derivative chromatograms. *Anal. Chim. Acta*, 587: 222–234.
83. Kishida, K. (2007) Restricted-access media liquid chromatography for determination of sulfamonomethoxine, sulfadimethoxine, and their N^4-acetyl metabolites in eggs. *Fd. Chem.*, 101: 281–285.
84. Samanidou, V., Tolika, E., and Papadoyannis, I. (2008) Development and validation of an HPLC confirmatory method for the residue analysis of four sulfonamides in cow's milk according to the European Union Decision 2002/657/EC. *J. Liq. Chromatogr. Rel. Technol.*, 31: 1358–1372.

APPENDIX

Abbreviations used throughout text and table

Acid.	Acidified	LLE	Liquid Liquid Extraction
Add.	Addition	LOD	Limit of Detection
Adj.	Adjusted	LOQ	Limit of Quantification
Anal.	Analysis	LR	Linear Range
APCI	Atmosph. Pressure Chem. Ionization	Metab.	metabolites
Aq.	Aqueous	Microextr.	Microextraction
Buf.	Buffer Solution	MIP	Molecularly Imprinted Polymers
CE	Capillary Electrophoresis	Mix.	Mixing
Centr.	Centrifugation	Mixt.	Mixture
Col.	Column	MP	Mobile Phase
Combin.	Combined	MRL	Maximum Residue Limit
Conc.	Concentrated/concentration	MS	Mass Spectroscopy
Cond.	Conditions/conditioned	MSPD	Matrix Solid Phase Dispersion
DAD	Diode array detector	OH-SDMs	(hydroxy metab. of SDM)
DDS	Dapsone	PDA	Photodiode array detector
Deprot.	Deproteinization	Phos.	Phosphate
Deriv.	Derivatization	Precip.	Precipitation
Det.	Detection	Purif.	Purification
Dil.	Dilution	RAM	restricted Access media
Dis.	Dissolution	Reconst.	Reconstitution
Dist.	Distilled	SAM	Sulfacetamide
El.	Eluent/elution	SAs	Sulfonamides
Electr.	Electrochemical	SBA	Sulfabenzamide
ELISA	Enzyme-linked immunosorbant assay	SCP	Sulfachloropyridazine
Em.	Emission	SCZ	Sulfachloropyrazine
Equil.	Equilibration	SD	Sulfadoxine
ESI	Electrospray Ionization	SDD	Sulfadimidine
		SDDS	Sulfamoildapsone
EtOH	Ethanol	SDMX	Sulfadimethoxine
EU	European Union	SDX	Sulfadoxine
Evap.	Evaporation to dryness	SDZ	Sulfadiazine
Exc.	Excitation	Sep.	Separation
Extr.	Extraction	SFC	Supercritical Fluid Chromatography
Filtr.	Filtration	SGN	Sulfaguanidine
FL	Fluorescence	SIM	Sulfisomidine
Fortif.	Fortified	SIX	Sulfisoxazole
FR	Flow Rate (mL/min)	SIZ	Sulfisozole

Appendix Continued

Acid.	Acidified	LLE	Liquid Liquid Extraction
GC	Gas Chromatography	SLM	Solid Liquid Membrane
Grad. El.	Gradient Elution		
Homog.	Homogenization/homogenous	SME	Sulfameter
HPCE	High-performance cap. Electrophor	SMM	Sulfamonomethoxine
HPLC	High-performance liquid chrom.	SMMX	Sulfamonomethoxine
Inj.	Injection	SMO	Sulfamoxole
IP	Identification Point	SMPD	Sulfamethoxypyridazine
IS	Internal Standard	SMR	Sulfamerazine
Is. El.	Isocratic Elution	SMT	Sulfamethizole
Lin.Gr.	Linear Gradient	SQX	Sulfaquinoxaline
SMX	Sulfamethoxazole	SSZ	Sulfasalazine
SMZ	Sulfamethazine	STR	Sulfatroxazole
SNM	Sulfanilamide	STZ	Sulfathiazole
Sol.	Solution	Supern.	Supernatant
Sonic.	Sonication	TCA	Trichloroacetic acid
SPE	Solid Phase Extraction	TLC	Thin Layer Chromatography
SPN	Sulfapyridine	TOPO	tri-*n*-octyl phosphine oxide
SPR	Sulfapyridine	Transf.	Transferred
SPZ	Sulfaphenazole	Ultrafiltr.	Ultrafiltration
SQR	Sulfaquinoxarine	Vol.	Volume