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Review

Food Sample Preparation for the Determination of Sulfonamides by High-Performance Liquid Chromatography: State-of-the-Art

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Abstract: Antibiotics are a common practice in veterinary medicine, mainly for therapeutic purposes. Sectors of application include livestock farming, aquacultures, and bee-keeping, where bacterial infections are frequent and can be economically damaging. However, antibiotics are usually administered in sub-therapeutic doses as prophylactic and growth promoting agents. Due to their excessive use, antibiotic residues can be present in foods of animal origin, which include meat, fish, milk, eggs, and honey, posing health risks to consumers. For this reason, authorities have set maximum residue limits (MRLs) of certain antibiotics in food matrices, while analytical methods for their determination have been developed. This work focuses on antibiotic extraction and determination, part of which was presented at the "1st Conference in Chemistry for Graduate, Postgraduate Students and PhD Candidates at the Aristotle University of Thessaloniki". Taking a step further, this paper is a review of the most recent sample preparation protocols applied for the extraction of sulfonamide antibiotics from food samples and their determination with high-performance liquid chromatography (HPLC), covering a five-year period.

Keywords: food; sample preparation techniques; sulfonamides; high-performance liquid chromatography; HPLC; ultra-high-performance liquid chromatography; UHPLC

1. Introduction

The scope of this state-of-the-art review is to cover the literature regarding the sample preparation protocols developed for the extraction of sulfonamides (SAs) from food samples followed by high-performance liquid chromatography (HPLC) determination, covering the last five years. The review is divided in two main sections. In this first main section, a theoretical background on veterinary drugs and antibiotic use, sulfonamides and their applications, the reported sample preparation techniques, as well as the chemical composition of the reported food matrices and official methods for the determination of antibiotics/sulfonamides in foodstuff samples are provided for the reader to have a prompt introduction to basic terminology. More details can be found in the cited review articles and book sections. In the second main section, the reported sample preparation protocols are provided in full detail for each food matrix.

1.1. Veterinary Drugs—Antibiotics

The European Union Council Directive 96/23/EC "on measures to monitor certain substances and residues thereof in live animals and animal products" divides all pharmacological substances used for veterinary purposes and their corresponding residues into two main groups, A and B. Group A includes substances with anabolic effect, such as antithyroid agents, steroids, and β-agonists, as well as unauthorized substances, such as chloramphenicol, chlorpromazine, metronidazole, and nitrofurans. Group B includes veterinary drugs, such as antibiotics, anthelmintics, anticoccidials and non-steroidal anti-inflammatory drugs, and environmental contaminants, such as organochlorine and organophosphorus compounds [1].

Antibiotics are used in veterinary medicine in order to improve the health and increase the productivity of the food-producing animals and at the same time reduce the morbidity and mortality rates among the livestock. Animals are administered with antibiotics not only for therapeutic purposes but also as prophylactic and metaphylactic measures [2]. Prophylaxis is a preventative measure, where animals are administered with sub-therapeutic doses and in some cases full doses of antibiotics through feed or water and is a common practice in massive livestock production. Metaphylaxis is a measure taken when a number of animals exhibits some of the disease symptoms, and all the animals are administered in order to prevent the disease from spreading. However, both practices are not always effective due to the fact that some antibiotic groups are active during bacterial cell proliferation [3]. The most common routes of administration are through water and feed medication, followed by injection [2]. Antibiotics can also be used as feed additives. Their intake favors the natural intestinal flora of the animals by inhibiting the harmful microorganisms, and as a result, nutrient absorption and assimilation are increased, thus providing a growth promoting effect [3]. Aminoglycosides, β-lactams (penicillins and cephalosporins), macrolides, phenicols, quinolones, SAs, and tetracyclines are among the most common antibiotic substances in veterinary medicine [4].

The presence of veterinary drug residues in animal-originated products relies on the physicochemical properties of each compound that affect the absorption, distribution, metabolism, and excretion of the drug from the animal body. For this reason, a withdrawal period, between the last drug administration and the slaughter or milk, egg, and honey collection, is established in order to ensure the existence of animal-originated products with low drug residues [4]. However, the extensive use of antibiotics besides therapeutic purposes has led to the presence of antibiotic residues in animal originated food products and the development of bacterial drug resistance [3]. The main reasons for antibiotic residues is the illegal use and uncontrolled administration of veterinary drugs to healthy animals, as well as not taking into consideration the withdrawal period, the lack of professional advice, extra label use, frequent drug administration, and increased dosing, as well as contaminated housing, water, and animal feed [4–6]. Antibiotic residues can cause adverse effects to consumers, such as acute allergic or toxic reactions, chronic toxic effects from prolonged exposure to antibiotic residues, and natural intestinal flora disruption [5].

Liver, kidney, and fat are animal tissues with high antibiotic residue concentration, while muscle tissue has relatively lower residues [5]. The European Union Commission Regulation No 37/2010 sets the legislative basis for the "pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin", by classifying the substances into the categories of allowed and prohibited. For the allowed substances, a maximum residue limit (MRL) is provided for each target tissue expressed in micrograms per kilogram (μg/kg) of fresh target tissue. Target tissues include muscle, fat, liver, and kidney, as well as milk, eggs, and, in a few cases, honey. The MRLs usually refer to a specific compound, a metabolite, or a compound mixture [7].

1.2. Sulfonamides

SAs constitute a wide-spectrum synthetic antibiotic category effective against a wide range of bacterial species, such as *Bacillus* spp., *Brucella* spp., *Streptococcus* spp., staphylococci gram-positive aerobes, and enterobacteriaceae gram-negative aerobes, as well as protozoa, parasites, and fungi.

SAs are derivatives of sulfanilamide and the various SA analogues, that result from the various **R** radicals of the $-SO_2NH\mathbf{R}$ group (Figure 1). Each SA analogue has different physicochemical and pharmacokinetic properties and as a result antibiotic effect. SAs are relatively insoluble with solubility increasing in alkaline pH, which is an important factor for the type of administration and disease treatment they are intended for [8].

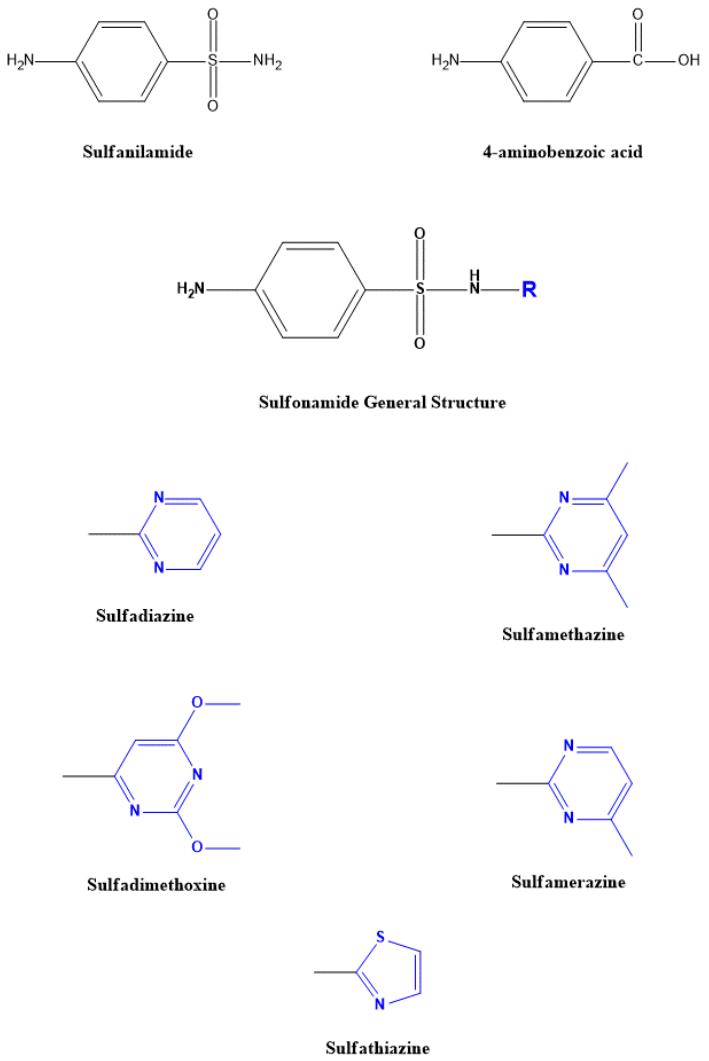

Figure 1. Diagrams of the chemical structure of sulfonamide, sulfanilamide, 4-aminobenzoic acid, and the chemical structure of the most common SA analogues in veterinary medicine.

The SA antibacterial effect relies on their ability to inhibit the conversion of folic acid to tetrahydrofolic acid by competitively antagonizing 4-aminobenzoic acid (Figure 1) for the dihydropteroate synthetase enzyme. Tetrahydrofolic acid is essential for the nucleic acid synthesis, thus SAs inhibit the bacterial DNA and RNA synthesis and subsequently the protein synthesis. Additionally, SAs reduce the bacterial cell permeability for glutamic acid that is essential for the folic acid synthesis. SAs are effective against bacterial species that synthesize the required 4-aminobenzoic acid, but ineffective in the presence of increased amounts of 4-aminobenzoic acid and against species that receive the required 4-aminobenzoic acid from other sources or have antibiotic resistance [6].

SA formulations usually consist of a SA analogue and a diaminopyrimidine, such as aditoprim, baquiloprim, ormetoprim, or trimethoprim, that have a synergistic interaction. The liver and kidney are animal tissues with the highest concentrations of SAs and their metabolites [4].

Sulfamethoxazole, sulfacetamide, and sulfasalazine are SA analogues commonly used in human medicine, while sulfadiazine (SDZ), sulfamethazine (SMZ), sulfadimethoxine (SDMX), sulfamerazine, and sulfathiazole are commonly used in veterinary medicine [6]. The European Union Commission Regulation No 37/2010 sets the MRL for all SA analogues to 100 µg/kg for muscle, fat, liver, and kidney from all food-producing species and bovine, ovine, and caprine milk, while their use is prohibited for animals that produce eggs for human consumption. In the case of more than one SA analogue, the sum of the SA residues should not exceed the provided MRL value (Table 1) [7]. Honeybees are also considered food-producing species and antibiotics are used in beekeeping in order to treat two of the most severe diseases for bees, the American/European foulbrood, and nosemosis. More specifically, the SA analogue sulfothiazole is used against the American foulbrood caused by *Paenibacillus larvae* in order to prevent the spread of the disease, suppress the symptoms, and inhibit the spore germination. SAs can also be used prophylactically against nosemosis. However, a beehive lacks in metabolic pathways for the elimination of antibiotic residues and a withdrawal period does not apply in the case of bees. For this reason, the European Union has not established MRLs for antibiotic residues in honey and only veterinary drugs with zero residues are authorized for beekeeping [9].

Table 1. MRLs for sulfonamide antibiotics, provided by the EU, Codex Alimentarius and FDA.

Sulfonamide	Animal Species	MRL	Target Tissue
Commission Regulation (EU) No 37/2010 [7]			
Sulfonamides	All food producing species	100 µg/kg	Muscle Fat Liver Kidney
	Bovine Ovine Caprine	100 µg/kg	Milk
Codex Alimentarius [10]			
Sulfadimidine	Cattle	25 µg/L	Milk
	Not specified	100 µg/kg	Muscle Liver Kidney Fat
CFR—Code of Federal Regulations—U.S. Food & Drug Administration (FDA) [11]			
Sulfabromomethazine sodium	Cattle Not specified	100 µg/kg 10 µg/L	Uncooked edible tissue Milk
Sodium sulfachloropyrazine monohydrate	Chicken	0	Uncooked edible tissue
Sulfachlorpyridazine	Calves Swine	100 µg/kg	Uncooked edible tissue
Sulfadimethoxine	Chickens Turkeys Cattle Ducks Salmonids Catfish Chukar partridges	100 µg/kg	Uncooked edible tissue
	Not specified	10 µg/L	Milk
Sulfaethoxypyridazine	Cattle Swine Not specified	100 µg/kg 0 0	Uncooked edible tissue Uncooked edible tissue Milk
Sulfamerazine	Trout	0	Uncooked edible tissue
Sulfamethazine	Chickens Turkeys Cattle Swine	100 µg/kg	Uncooked edible tissue
Sulfaquinoxaline	Chickens Turkeys Calves Cattle	100 µg/kg	Uncooked edible tissue

The most crucial problem arising from the uncontrolled use of SAs is the development and spreading of drug resistance, rather than the presence of SA residues in animal originated products itself. SA drug resistance derives from mutations in the dihydropteroate synthase gene that results in enzymes with structural alterations with decreased affinity towards the SAs. Drug resistance genes can be transferred between bacterial strains or genera during a horizontal gene transfer in plasmids, transposons, or integrons. For this reason, SA resistant strains are higher than tetracyclines and other antibiotics, while bacterial species with more than one gene for SA resistance have been observed. Furthermore, drug resistance in an antibiotic group can favor the development of cross-resistance [6].

1.3. Sample Preparation

Food samples are complex heterogenous matrices, where all analytes are distributed in a random manner. Food analysis involves sampling, homogenization, and sample preparation that increase the analytical accuracy and precision. Focusing on sample preparation it usually involves storage, particle size reduction, homogenization, weighting, dilution, filtration, extraction, clean-up, and derivatization. Proper sample preparation protocols result in matrix interference elimination and analyte preconcentration, thus affecting the selectivity, sensitivity, detection capability, and the overall performance of an analytical technique. The most time-consuming step in analytical method development is the optimization of the sample preparation protocol that includes analyte extraction and clean-up. Some of the most common sample preparation techniques used in food analysis are liquid-liquid extraction (LLE), solid-liquid extraction (SLE), solid-phase extraction (SPE), solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), ultrasonic, and Soxhlet extraction [12].

1.3.1. LLE

LLE is one of the most used sample preparation techniques, along with SPE, and probably the oldest. In LLE the analytes are extracted from an aqueous sample into a water immiscible solvent, according to relative solubility. Despite the wide use of LLE, there are many disadvantages, such as increased time and solvent requirements, analyte lose, sample contamination, and low sensitivity, that reduce LLE applications in modern analytical chemistry. Liquid-phase microextraction (LPME) is an LLE-based microextraction technique that has been developed in order to overcome LLE disadvantages. Extraction takes place between the aqueous sample phase (donor phase) and a water immiscible solvent extraction phase (acceptor phase). LPME can be divided into single-drop microextraction (SMDE), hollow fiber liquid-phase microextraction (HF-LPME), and dispersive liquid-liquid microextraction (DLLME). DLLME is a ternary extraction system that involves the aqueous sample, an extraction solvent, and a disperser solvent. Microliters of the organic extraction solvent and the dispersive solvent are injected into the sample solution and extraction solvent droplets are formed with the help of the dispersive solvent, resulting in a cloudy solution. After the extraction equilibrium between the sample and the extraction solvent is achieved, the cloudy solution is centrifuged and the lower organic phase is collected for analysis. The extraction solvent should be water immiscible, such as chloroform, carbon tetrachloride, and dichloromethane, while the dispersive solvent should be miscible in both aqueous solution and organic solvent, such as ethanol, methanol, acetonitrile, and acetone. Following the principles of green analytical chemistry, conventional extraction solvent can be replaced by ionic liquids that are liquid organic salts, combinations of organic cations with inorganic anions with unique physicochemical properties. Ionic liquids are characterized by low volatility and high density, thus forming more stable droplets and better phase separation than the conventional organic solvents [13].

1.3.2. SLE

The principals of SLE are similar to LLE, except that the analytes are extracted from solid samples. The solid sample is mixed with extraction solvent, the two phases interact, and the soluble

sample components diffuse into the extraction phase. Various organic solvents can be used in SLE, however, SLE is usually laborious and has analogous disadvantages as LLE, such as increased solvent requirements, partial extraction, solvent impurities, and emulsion formation. In order to increase the extraction efficiency of the organic solvent, heating and pressure or ultrasounds can be applied during the extraction. In pressurized liquid extraction (PLE), also known as ASE, the solid sample and the extraction solvent are transferred into an extraction cell and the extraction takes place under high temperature (40–200 °C) and pressure (500–3000 psi) for 5–15 min. After the extraction is complete, the sample extract is collected and purged. The increased temperature and pressure applied in PLE result in reduced extraction time, enhanced analyte's solubility and mass transfer, better solvent penetration, and overall improved extraction yields. However, PLE requires expensive equipment and high temperature solvent reduces the extraction selectivity and applicability of PLE for the extraction of less thermal stable compounds. Ultrasound-assisted extraction (UAE) is a less extreme extraction approach in which extraction is assisted by the application of ultrasounds. Compared with other sample preparation techniques, UAE is relatively faster with reduced solvent requirements, and at the same time enables the extraction of analytes in room temperature. However, this technique lacks selectivity and enrichment capability when extraction of trace amounts is required and is usually combined with other clean-up techniques for improved extraction efficiency [14].

1.3.3. Salting-Out Extraction

The salting-out effect has been exploited in sample preparation techniques, such as Quick Easy Cheap Effective Rugged Safe (QuEChERS) and salting-out liquid-liquid extraction (SALLE), in order to improve analyte's extraction from aqueous samples. The salting-out effect is based on the decrease of solubility of water soluble organic analytes when salt concentration in the aqueous sample solution is increased. This effect favors the partition of the analytes into a water-miscible organic solvent and separation between the two phases. Various water-miscible solvents can be used, but acetonitrile is the most convenient water-miscible organic solvent for the application of the salting-out effect due to being chemically inert with organic analytes and the most common mobile phase component in liquid chromatography, and at the same time, acetonitrile has the ability to precipitate matrix proteins. Salting-out agents are usually inorganic and organic salts that provide cations (Mg^{2+}, Sr^{2+}, Ca^{2+}, Ba^{2+}, K^{+}, Na^{+}, NH_4^{+}, Li^{+}) and anions (SO_4^{2-}, CH_3COO^{-}, Cl^{-}, NO_3^{-}, Br^{-}, I^{-}, CNS^{-}), such as $MgSO_4$, NaCl, CH_3COONH_4, CH_3COONa, and $(NH_4)_2SO_4$, which promote the transfer of the hydrophilic compounds to the organic phase. These salts should be soluble in the aqueous sample but have negligible solubility in the organic phase [15]. In either QuEChERS and SALLE procedures, a water-miscible solvent and a salting-out agent are sequentially added to the sample and the mixture is shaken and centrifuged. The formed organic phase is then collected and can be either injected directly for analysis or further treated for water removal and analyte isolation [14]. Aqueous two-phase system (ATPS) extraction is another alternative based on the partitioning of the analytes between two phases and is used for the extraction of analytes from aqueous samples. The most common biphasic system in food analysis is polymer/salt, while ionic liquid/salt systems were also reported. Polyethylene glycol is usually the polymer of choice combined with phosphate, sulfate, or citrate salts. Parameters that affect the extraction include the molecular weight and concentration of the polymeric phase, the solution pH, and temperature. ATPS does not require the use of organic solvents in contrast with other sample preparation techniques, while sample deproteinization or defatting is not always required [16].

1.3.4. SPE

SPE is a widely used sample preparation technique that provides high enrichment factors and recoveries with reduced sample volume requirements and automation capability. SPE can be used for the daily laboratory routine and in many cases SPE has replaced LLE. In SPE the components of the sample solution interfere with a solid phase (sorbent material) and separation between the

target analytes and the matrix interferences can be achieved. The sorbent is usually packed into SPE cartridges between two frits. SPE cartridges are commercially available for specific applications or can be manually prepared with the selected sorbents. In a typical SPE procedure, the SPE cartridges are conditioned/activated with a solvent or a solvent mixture, the sample solution is loaded into the cartridge, the loaded cartridge is washed to remove the retained interreferences, and the retained analytes are eluted with an appropriate solvent (Figure 2a). However, SPE applications are restricted by the type of the sorbent and the characteristics of the sample components, while the tightly packed SPE cartridges increase the extraction time and cause backpressure [13]. These problems can be eliminated with dispersive solid-phase extraction (DSPE) where the sorbent is dispersed in the sample solution and not packed into a cartridge. After the dispersion, the solution is shaken, and when the extraction is complete, the sorbent is collected by centrifugation or filtration and the analytes are eluted by repeating the previous step with the use of an appropriate solvent (Figure 2b) [17]. C18 and OASIS® HLB are two of the most widely applied commercially available materials for SPE. C18 is a nonpolar sorbent that consists of octadecylsilane bonded to silica particles and is suitable for the reversed-phase binding of hydrophobic analytes. OASIS® HLB is a water-wettable hydrophilic-lipophilic-balanced polymeric reversed-phase sorbent that consists of N-vinylpyrrolidone and divinylbenzene and can be used for the binding of acidic, basic, or neutral analytes [14]. Novel SPE sorbents include carbon nanotubes (CNTs) [13], molecularly imprinted polymers (MIPs) [18], and magnetic materials. Magnetic solid-phase extraction (MSPE) is a SPE-based technique that employs magnetic sorbent materials. The magnetic materials comprise of a magnetic metal oxide nanoparticle core, usually Fe_3O_4, coated with inorganic or organic materials, such as silica, alumina, chitosan, or polypyrrole, while the coating can be modified with functional groups for improved sorption capability. A MSPE application is similar to DSPE, with the difference that the sorbent can be collected by means of a magnet (Figure 2b) [19]. While solid-phase techniques can be used for aqueous samples or sample solutions, matrix solid-phase dispersion (MSPD) can be applied directly for the extraction of analytes from solid, semi-solid, and viscous samples. Typically, the sample is mechanically blended with solid support in order to achieve matrix disruption and the development of interactions between the analytes and the sorbent material. The mixture is then transferred and packed into a SPE cartridge and the analytes are eluted with an appropriate sorbent. Apart from the applicability on solid samples, MSDP provides a simple and selective approach for sample extraction and clean-up in a single step [14].

Other solid-phase extraction variations include SPME and SBSE. In both techniques, the sorbent material is coated on a substrate, and extraction/analyte desorption follow the same principles. SPME employs silica or stainless-steel fibers coated with the sorbent material that can be used for the extraction of analytes from gaseous, liquid, and solid samples. A typical SPME procedure involves the partitioning of the analytes between the sorbent and the sample matrix and analyte desorption directly into the analytical instrument. SPME can be coupled with HPLC by means of a six-port injector combined with a desorption chamber, and desorption can be performed with an organic solvent or the mobile phase in static or dynamic mode. SMPE fibers can be coated with various sorbent materials, thus SMPE applicability can be expanded for the extraction of a wide range of analytes and sample matrices [14]. SBSE employs magnetic stir bars coated with polydimethylsiloxane that is a polar polymeric material and develops hydrophilic interactions with the analytes, such as hydrogen bonds and van der Waals forces. In a typical SBSE procedure, a coated stir bar is introduced into the sample solution and the analytes are absorbed onto the coating by continuous stirring. After the extraction, the bar is collected, washed with deionized water, and dried, while analytes can be desorpted, either thermally by thermal or liquid desorption [13]. Both SPME and SBSE are less time-consuming and have reduced sample and solvent requirements in comparison with SPE.

1.3.5. FPSE

Fabric phase sorptive extraction (FPSE) is a recently introduced novel microextraction technique that employs reusable cellulose or polyester fabric substrates homogenously coated with sol-gel hybrid

sorbents. In a typical FPSE procedure, the coated fabric, along with a magnetic stir bar, are introduced into a vial that contains the sample or sample solution, and analyte extraction is conducted under stirring. The coated fabric is collected and placed for 4–10 min into a second vial that contains the eluting solvent (Figure 2c). The eluate can be centrifuged prior to analysis. The coated fabric is washed with an appropriate organic solvent and rinsed with deionized water between extractions. Reported FPSE coatings include polydimethylsiloxane, poly(ethyleneglycol), C18, and graphene. The FPSE protocol is simple and fast, with reduced solvent requirements, while the coated fabric can be introduced directly to the liquid samples and is compatible with a wide range of organic solvents. FPSE substrates are characterized by increased sorbent loading and improved adsorption capacity in comparison with SPME, providing high analyte preconcentration [20].

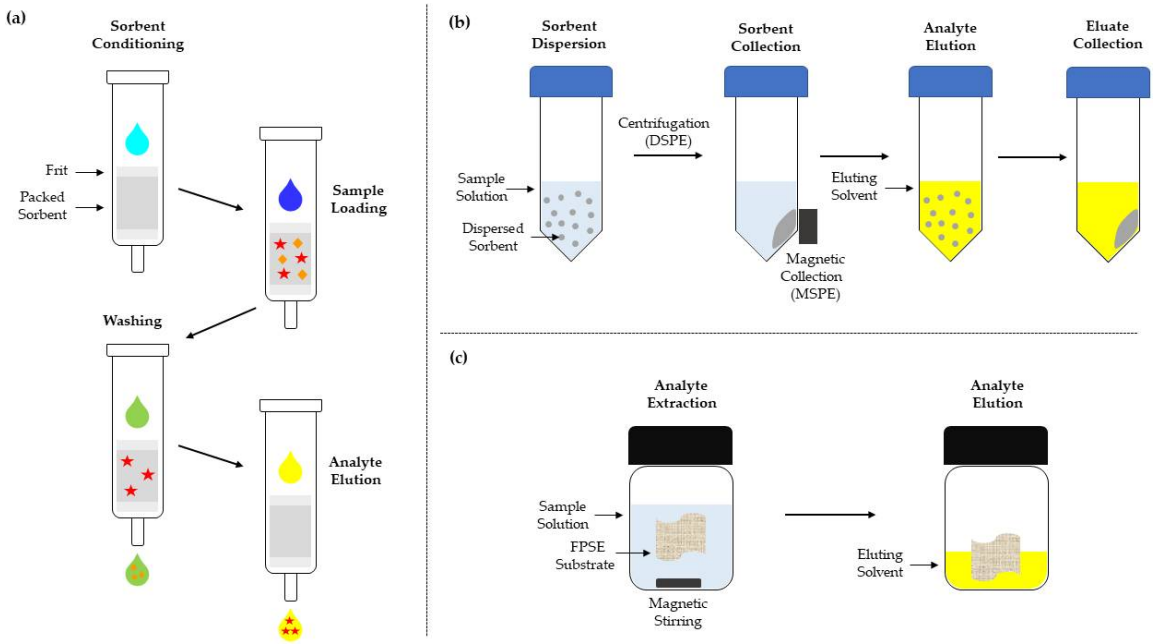

Figure 2. Schematic representation of the basic steps in (**a**) SPE, (**b**) DSPE and MSPE, and (**c**) FPSE.

1.4. Food Composition

Meat contains 72–75% water, 19% proteins, 2.5–5% lipids, 1% vitamins and carbohydrates, and 1% ash. Lipids can vary between 1% and 15%. The main edible animal tissue is the muscle, while other edible parts include organs, such as liver and kidneys, fat, and blood. Edible animal livestock species include bovine and porcine species and poultry. Animal lipids are mostly deposited under the skin (subcutaneous fat), between the muscles (intermuscular fat), and around organs, such as kidneys and heart, but varies between animal species [21]. The chemical composition of fish varies among species, with 50–60% of fish weight being muscle. Fish muscle contains 52–82% water, 16–21% proteins (or 10–25% for farmed species), 0.5–2.3% lipids, 1.2–1.5% ash, and 0.5% carbohydrate content. In comparison with red meat, fish lipid content is lower and ranges between 0.2% and 30%, while lipids are deposited in the liver, muscle, perivisceral, and subcutaneous tissues. Fatty fish species deposit fat all over the muscle tissue that is colored grey, yellow, or pink, such as salmon, and more than 50% of the skin consists of lipids (in other species it ranged between 0.2% and 3.9%) [22]. Milk is a heterogenous mixture that consists of water, emulsified fat, caseins and whey proteins, lactose, minerals, and vitamins. The gross cow milk composition is 86.3% water, 4.9% fat, 3.4% proteins, 4.1% lactose, and 0.7% ash. Buffalo, sheep, and goat milk can also be consumed by humans. Fat is the most important milk component, both organoleptically and commercially, and ranges from below 3% to more than 6% [23]. Eggs consist of white and yolk for 60% and around 30–33% of the total egg weight,

respectively. The egg white or albumen is a protein solution that contains over 40 different proteins, with ovalbumin constituting the 54% of the total white proteins. The egg yolk contains lipoproteins, with low-density lipoproteins constituting the 65% of the total yolk proteins. Egg fat is mainly present in egg yolk as triacylglycerol and phospholipids and comprises the 9–10% of the total egg weight. Other egg components include minerals and vitamins that are also located in the egg yolk [24].

1.5. Official Methods of Analysis

Official methods for the determination of antibiotics/sulfonamides in food samples are available by regulating agencies. The website of the FDA provides a "Laboratory Methods—Drug & Chemical Residues Methods" section [25] that includes analytical methods for the determination of multiple phenicol residues in honey samples with electrospray liquid chromatography—mass spectrometry (LC-MS) and chloramphenicol residues in crustacean species (shrimp, crab, crawfish) samples with liquid chromatography—tandem mass spectrometry (LC-MS/MS) [26], as well as the determination of fluoroquinolone residues in milk samples with LC-MS/MS [27]. Two multi-class and multi-residue LC-MS/MS methods for the determination of drug residues in milk and aquaculture samples (fish, shrimp) are provided in the "Field Science and Laboratories—Laboratory Information Bulletins" section of the FDA website [28]. A high-performance liquid chromatography—ultraviolet detection (HPLC-UV) method for the determination of sulfamethazine in milk samples is also provided by the FDA [29]. The Association of Official Analytical Chemists (AOAC) provides the "AOAC Official Method 993.32" for the determination of eight sulfonamide residues in raw bovine milk samples with liquid chromatography—ultraviolet detection (LC-UV) [30].

2. Extraction of Sulfonamides from Food Samples

Meat, milk, eggs, and honey are animal-originated products with increased demand. In this section the detailed sample preparation protocols are provided for each reported paper.

2.1. Animal Tissue Samples

2.1.1. SLE

The same team developed two SLE protocols for multi-class antibiotic extraction, including 15 SAs, from bovine tissue [31] and fish tissue samples [32] followed by ultra-high-performance liquid chromatography—mass spectrometry (UHPLC-MS/MS). In the first protocol, spiked bovine tissue (2 g) was mixed with acetonitrile (ACN) (10 mL) and ethylenediaminetetraacetic acid (EDTA) (0.1 M, 1 mL) and the mixture was shaken for 20 min and centrifuged at 3100 *g* for 15 min. The supernatant was collected, mixed with *n*-hexane (3 mL), vortexed for 30 s, centrifuged at 3100× *g* for 15 min, and the *n*-hexane layer was discarded. The extract was evaporated under nitrogen stream at 40 °C to 0.5 mL final volume, dissolved in mobile phase (400 μL), and passed through a 0.45 μm filter, prior to analysis. The authors emphasized the simplicity, as well as the reduced cost and time requirements of the developed sample preparation protocol. The addition of EDTA, the sample defatting with *n*-hexane, and the evaporation to 0.5 mL were employed in order to increase the recoveries of all analytes [31]. The same protocol was applied for the fish tissue samples, except for the *n*-hexane treatment step. Spiked fish tissue (2 g) was mixed with ACN (10 mL) and EDTA (0.1 M, 1 mL), and the mixture was shaken for 20 min and centrifuged at 3100× *g* for 15 min. The supernatant was collected and evaporated under nitrogen stream at 40 °C and the residue was dissolved in mobile phase (400 μL) [32]. In both cases, a SPE sample clean-up step was omitted due to increased selectivity over specific antibiotics that prohibits multi-class extraction, while the sample preparation protocol cost and time requirement were decreased and the number of samples analyzed in a daily routine was increased. Another SLE protocol was reported for the extraction of SDZ, SMZ, SIX, SDMX, and sulfaquinoxaline (SQX) from shrimp tissue samples. Spiked tissue (0.5 g) was mixed with methanol (MeOH)-ACN (50:50, *v*/*v*; 1 mL) and the mixture was vortexed, sonicated for 15 min, and centrifuged at 3500 rpm for

10 min. The extraction step was repeated twice with MeOH-ACN (50:50, *v*/*v*; 1 mL) and twice with 0.1% CH_3COOH-MeOH (60:40, *v*/*v*; 0.5 mL) and the supernatants were collected between extractions. All collected supernatants were combined, evaporated to dryness under nitrogen stream, and the residue was dissolved in MeOH (500 μL) and passed through a 0.20 μm syringe filter. Analysis was carried out by high-performance liquid chromatography—diode array detection (HPLC-DAD). SPE and MSPD were also tested but higher recoveries were achieved with the developed sample preparation protocol [33].

Two ASE protocols were reported for the extraction of 15 SAs and metabolites from baby food samples [34], and multi-class antibiotic extraction, including 9 SAs, from fish tissue samples [35]. In the first protocol, spiked baby food sample (5 g) was transferred into an ASE extraction cell and mixed with 1% CH_3COOH in MeOH-ACN (4:1, *v*/*v*). Extraction was carried out at 70 °C and 1500 psi with 5 min preheating time and 5 min static time, while flush volume was 60% and purge time 60 s. Extraction was repeated for 3 cycles. The extracts (3 × 18 mL) were diluted with extraction solvent (3 × 20 mL), placed in the freezer at −18 °C overnight and centrifuged at 4 °C and 4000 rpm for 5 min. The supernatant was collected and passed through a 0.45 μm filter and analyzed with ultra-high-performance liquid chromatography—orbitrap high-resolution mass spectrometry (UHPLC-Orbitrap-MS). The developed sample preparation protocol provided higher recoveries in comparison with an official AOAC QuEChERS extraction protocol [34]. In the second report, the fish tissue samples were purified with C18 resin inside the ASE extraction cell. The sample extracts were evaporated to dryness, dissolved in mobile phase, and centrifuged at −4 °C and 10,000 rpm prior to high-performance liquid chromatography—mass spectrometry (HPLC-MS/MS) analysis. However, information could be collected only from the abstract and the further details are not available because the rest of the paper is written in Chinese [35].

Two extraction protocols, PLE and USE, were reported for the extraction of 16 SAs and metabolites from chicken, sheep, fish, and horse tissue samples. In both cases, analysis was carried out by high-performance liquid chromatography—quadrupole linear ion trap—mass spectrometry (HPLC-QqLIT-MS/MS). For the PLE protocol, spiked tissue (5 g) was defatted with hexane, mixed with diatomaceous earth, and transferred inside a PLE cell. The extraction solvent was 0.2% CH_3COOH in ACN, the preheating period was 8 min, and extraction was achieved at 90 °C and 1500 psi for 7 min. The total flush volume was 80%, 60 s of purging under nitrogen stream was applied and the extraction step was repeated three times. The collected extracts were placed in the freezer at −18 °C for 1 h and centrifuged at 3500 rpm for 10 min in order to remove the tissue proteins. The supernatant was evaporated to dryness under nitrogen stream at 40 °C and the residue was dissolved in H_2O-ACN (85:15, *v*/*v*; 1 mL). For the UAE protocol, spiked tissue (5 g) was mixed with ACN (10 mL) and the mixture was vortexed for 10 s and placed inside an ultrasonic bath for 60 min. Then, the mixture was placed in the freezer at −18 °C for 1 h and centrifuged at 3500 rpm for 10 min, in order to remove the tissue proteins. The supernatant was collected and evaporated to dryness under nitrogen stream at 40 °C. The residue was dissolved in H_2O-ACN (85:15, *v*/*v*; 2 mL), hexane (2 mL) was added, and the mixture was vortexed for 5 s and centrifuged at 3500 rpm for 10 min. The lower phase was used for analysis. Although both developed protocols could efficiently extract the SAs from the tissue samples, UAE is simpler and less solvent and time-consuming [36].

2.1.2. Salting-Out Extraction

Three QuEChERS extraction protocols were reported in the literature. In the first report, a QuEChERS extraction protocol was developed for the extraction of 22 SAs and metabolites from bovine, chicken, pork, and sheep tissue samples. Spiked tissue (5 g) was mixed with H_2O (5 mL), vortexed for 1 min, and 1% CH_3COOH in ACN (10 mL) was added to the mixture. Sodium hydrogen citrate sesquihydrate (0.5 g), sodium citrate (1 g), $MgSO_4$ (4 g), and NaCl (1 g) were added in sequence and the mixture was shaken and vortexed for 1 min between each addition. The mixture was centrifuged at 3500 rpm for 5 min, the supernatant (6 mL) was collected, mixed with primary

secondary amine (PSA) (150 mg) and anhydrous $MgSO_4$ (900 mg), and the mixture was shaken, vortexed, and centrifuged as described. The supernatant (4 mL) was collected and evaporated to dryness under nitrogen stream at 35 °C. The residue was dissolved in 0.01% formic acid in 5% MeOH aqueous solution (500 µL), passed through a 0.22 µm nylon filter, and centrifuged at 13,500 rpm for 10 min prior to high-performance liquid chromatography—high-resolution mass spectrometry (HPLC-HRMS) analysis [37]. In the second report, a modified QuEChERS extraction protocol was developed for the extraction of eight SAs from chicken muscle and egg sample. The extraction protocol employed a commercially available sorbent material Z-Sep$^+$, consisting of C18 and zirconia both bonded to silica particles, for the chicken samples and PSA for the egg samples. Spiked tissue (5 g) was mixed with H_2O (5 mL) and 1% CH_3COOH in ACN (10 mL) and the mixture was shaken for 10 min. $MgSO_4$ (4 g) and CH_3COONa (1 g) were added and the mixture was shaken for 1 min, vortexed for 2 min, and centrifuged at 5000 rpm for 5 min. The supernatant (3 mL) was collected and mixed with C18-zirconia sorbent (300 mg) and the mixture was shaken for 30 s and vortexed for 1 min. The supernatant (2 mL) was collected, evaporated under nitrogen stream, and the residue was dissolved in MeOH-H_2O (50:50, v/v; 1 mL), vortexed for 1 min and filtered prior to high-performance liquid chromatography—fluorescence detection (HPLC-FLD) analysis. A similar protocol was used for the egg sample (5 g), where it was mixed only with 1% CH_3COOH in ACN (10 mL) and PSA was used instead of the C18-zirconia sorbent [38]. Lastly, a fully automated on-line QuEChERS extraction protocol was reported for the extraction of 27 SAs and metabolites from salmon tissue samples. Spiked tissue (1 g) was mixed with 1% CH_3COOH in ACN-H_2O (84:16, v/v; 5 mL) and the mixture was vortexed at 3200 rpm for 1 min. $MgSO_4$ (1 g) and CH_3COONa (0.1 g) were added and sample extract (2 mL) was aspirated three times, followed by 30 s equilibrium time and centrifuged at 2264× g for 5 min. The supernatant (1 mL) was collected and mixed with Z-Sep$^+$ (45 mg), PSA (32 mg), and Na_2SO_4 (0.25 g) and the mixture was vortexed for 1 min and centrifuged at 2264× g for 5 min. The supernatant (200 µL) was collected and mixed with MeOH (300 µL) and ammonium formate solution (0.008 M, 500 µL) prior to ultra-high-performance liquid chromatography—electrospray ionization—quadrupole Orbitrap high-resolution mass spectrometry (UHPLC-ESI-Q-Orbitrap-MS) analysis [39].

Two SALLE protocols were also reported. In the first protocol SALLE was reported in combination with magnetic separation for the extraction of eight SAs from fish tissue samples. Spiked tissue (2 g) was mixed with 0.1% formic acid in ACN (5 mL) and the mixture was vortexed for 1 min. NaCl (0.5 g), $MgSO_4$ (2 g) and Fe_3O_4 (100 mg) were added and the mixture was vortexed for 1 min. The magnetic particles were collected by means of a magnet and the supernatant (1 mL) was collected and evaporated to dryness under nitrogen stream at 45 °C. The residue was dissolved in 0.1% formic acid aqueous solution (1 mL), mixed with *n*-hexane (1 mL), and the mixture was centrifuged at 8000 rpm for 3 min. The aqueous phase was collected and passed through a 0.22 µm syringe filter and analyzed with HPLC-MS/MS. The authors emphasized the good recoveries and the reduced time requirements of the developed sample preparation protocol [40]. In the second report, the samples were treated with ethyl acetate and concentrated under vacuum. The analytes were extracted with HCl solution (2 M), the extract was defatted with *n*-hexane, filtered, and mixed with MeOH-ACN-CH_3COONa (5:5:20, $v/v/v$). Analysis was carried out by HPLC-FLC. However, information could be collected only from the abstract and the further details are not available because the rest of the paper is written in Chinese [41].

2.1.3. SPE

Two SPE protocols were reported for multi-class antibiotic extraction, including 16 SAs, from bovine liver samples [42] and the extraction of SAs from chicken and pork tissue samples [43]. The first extraction protocol employed Oasis HLB cartridges (3 mL, 200 mg) (Waters, Milford, MA, USA). Spiked liver tissue (2 g) was mixed with ACN (10 mL) and EDTA (0.1 M) and the mixture was shaken for 10 min, sonicated for 20 min, and centrifuged at 4000× g for 10 min. The supernatant was collected and evaporated under nitrogen stream to 1 mL final volume. H_2O (5 mL) was added

and the mixture was vortexed for 15 s and loaded into a SPE cartridge preconditioned with ACN (10 mL) and H_2O (10 mL). The loaded cartridge was washed with H_2O (5 mL) and dried under reduced pressure for 5 min. The analytes were eluted with ACN (10 mL) and the eluate was evaporated under nitrogen stream to 0.5 mL final volume. The reduced eluate was dissolved in mobile phase (400 μL), *n*-hexane (2 mL) was added, and the mixture was vortexed for 30 s and centrifuged at 4000× *g* for 10 min. The mixture was passed through a 0.45 μm filter and analyzed with UHPLC-MS/MS. The developed sample preparation protocol included a SPE clean-up step in order to reduce the liver tissue interferences and utilized SPE cartridges with wide range selectivity in order to achieve multi-class antibiotic extraction [42]. The second extraction protocol employed multi-walled carbon nanotubes (MWCNTs) as the sorbent material. The samples were treated with ACN and the sample extract was dissolved in Na_2HPO_4 buffer (pH 5.5–6.0) and loaded into the SPE cartridges. The cartridges were washed with acetone-hexane (5:95, *v*/*v*) and the analytes were eluted with acetone-dichloromethane (1:1, *v*/*v*). Analysis was carried out by HPLC-UV. However, information could be collected only from the abstract and the further details are not available because the rest of the paper is written in Chinese [43].

Two variations of SPE, a MSPE and a DSPE protocol, were reported for the extraction of SDZ, sulfathiazole, sulfamerazine, SMZ, and SMP from chicken, pork, and shrimp tissue samples [44] and for multi-class antibiotic extraction, including 21 SAs, from animal tissue samples [45], respectively. The MSPE protocol employed a magnetic Fe_3O_4@JUC-48 nanocomposite as the sorbent material. Spiked tissue (2 g) was mixed with ACN (20 mL) and the mixture was vortexed for 5 min, sonicated for 30 min, and kept overnight. The mixture was centrifuged at 10,000 rpm and the supernatant was collected and stored at 4 °C. Sample extract (8 mL) was mixed with Fe_3O_4@JUC-48 (25 mg) and the mixture was vortexed for 8 min. The magnetic sorbent was collected by means of an external magnet and the supernatant was discarded. The analytes were eluted with MeOH-CH_3COOH (95:5, *v*/*v*; 0.8 mL) and sonication for 10 min. The sorbent was separated by means of an external magnet and the eluate was collected, passed through a 0.22 μm nylon filter, and analyzed with HPLC-DAD. Sorbent reusability was studied by applying the sorbent material in several extraction cycles. Between the extractions, the sorbent was washed with MeOH-CH_3COOH (95:5, *v*/*v*; 3 × 1 mL) and MeOH (3 × 1 mL) and dried at 60 °C. The sorbent could be reused for seven extraction cycles without significant adsorption capacity reduction. The authors emphasized the increased sensitivity and the higher recovery values achieved, as well as the reduced extraction time and sorbent requirements of the developed sample preparation protocol [44]. In the DSPE protocol, the samples were treated with Na_2EDTA (0.1 M) and 1% CH_3COOH in ACN, followed by a DSPE clean-up step. Analysis was carried out by HPLC-MS/MS. However, information could be collected only from the abstract and the further details are not available because the rest of the paper is written in Chinese [45].

Other reported approaches included a combined MSPD-homogeneous ionic liquid microextraction (HILME) protocol for the extraction of seven SAs from bovine, chicken, and pork tissue samples [46], an on-line SPME protocol for the extraction of five antimicrobials, including sulfametoxydiazine, sulfamethoxazole, and SQX from chicken and pork tissue and egg samples [47] and a SBSE protocol for the extraction of ten SAs from chicken and pork tissue samples [48]. For the MSPD-HILME protocol, the ionic liquid employed acted both as elution solvent in MSPD and extraction solvent in HILME. Spiked tissue (0.2 g), silica gel (1 g), and 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM][BF_4]$) (200 μL) were mixed with a pestle and mortar and the mixture was transferred and placed inside a glass column between absorbent cotton layers. Pure H_2O was passed through the packed mixture and the eluate (3 mL) was mixed with NaCl (0.45 g) and ammonium hexafluorophosphate (2.4 M, 1 mL). The cloudy mixture was centrifuged at 5 °C and 10,000 rpm for 5 min, the supernatant was discarded and the remaining ionic liquid phase was diluted with ACN (300 μL), passed through a 0.22 μm polytetrafluoroethylene membrane filter and analyzed with HPLC-DAD. The authors emphasized the simplicity and the higher extraction recoveries achieved, as well as the reduced reagent requirements of the developed sample preparation protocol [46]. In the on-line SPME

protocol, spiked tissue or egg (5 g) was mixed with Na_2SO_4 (5 g) and ACN (10 mL) and the mixture was sonicated for 10 min and centrifuged at 8000 rpm for 5 min. This step was repeated twice and the three sample extracts were combined and evaporated to dryness. The residue was dissolved in ACN-toluene-*n*-hexane (1:4:45, *v*/*v*/*v*; 25 mL) and used for the on-line SPME. The extraction protocol employed molecularly imprinted monolithic capillary columns with SQX as the template molecule. Activated capillaries were treated with propyltrimethoxysilane and filled with the polymerization mixture that consisted of methacrylic acid (functional monomer), ethylene glycol dimethacrylate (cross-linker), *N*,*N*-dimethylformamide, isooctane, and paraxylene (polymerization and porogenic solvents), while polymerization occurred at 60 °C for 70 h. A prepared monolithic capillary column preplaced the sample loop in an on-line HPLC-UV system and extraction consisted of three steps. In the first step, the sample extract passed through the capillary at a flow rate of 0.15 mL/min so that the analytes came in contact with the imprinted polymeric phase and extracted. In the second step, nitrogen was passed through the capillary so that the residual sample solution was completely removed. Finally, the analytes were eluted from the capillary with 400 μL of mobile phase at a flow rate of 0.15 mL/min. The authors emphasized the increased selectivity and sensitivity, as well as the simplicity and the environmental friendliness of the developed sample preparation protocol [47]. Finally, the SBSE protocol employed poly(vinylphthalimide-*co*-*N*,*N*-methylenebisacrylamide) monolith coated stir bars. Spiked tissue (0.5 g) was mixed with ACN (5 mL), the mixture was sonicated for 15 min and centrifuged at 3000 rpm for 5 min and the supernatant was collected and passed through a 0.45 μm filter. This step was repeated and both supernatants were diluted with Milli-Q H_2O (100 mL). For SBSE, sample solution pH was adjusted (pH 4.0) and NaCl (5%, *w*/*v*) was added, while extraction time was 120 min and liquid desorption time was 60 min. Analysis was carried out by HPLC-MS/MS [48].

All reported literature for the extraction of SAs from animal tissue samples is summarized in Table 2, including recoveries, limit of detection (LOD), limit of quantification (LOQ), or/and decision limit (CC_α), decision capability (CC_β) values.

Table 2. Extraction of SAs from animal tissue samples.

Food Sample	Analytes	Sample Preparation	Analytical Technique/Run-Time	LOD-LOQ	Recovery (%)	Ref.
bovine tissue	41 antibiotics (15 SAs)	SLE	UHPLC-MS/MS 12 min	CC_α (μg/kg): 104–132 (SAs) CC_β (μg/kg): 108–164 (SAs)	91–109 (SAs)	[31]
fish tissue	41 antibiotics (15 SAs)	SLE	UHPLC-MS/MS 12 min	CC_α (μg/kg): 110.6–126.9 (SAs) CC_β (μg/kg): 121.2–153.7 (SAs)	92–111 (SAs)	[32]
shrimp tissue	SDZ, SMZ, SIX, SDMX and SQX	SLE	HPLC-DAD 40 min	LOD (μg/kg): 15 LOQ (μg/kg): 50	88.6–108.4	[33]
baby foods (combinations of powdered milk, cereal, vegetable, honey and meat)	15 SAs and metabolites	ASE	UHPLC-Orbitrap-MS 10 min	LOD (μg/kg): 0.03–0.17 LOQ (μg/kg): 0.10–0.55	75.5–96.6	[34]
fish tissue	19 antibiotics (9 SAs)	ASE	HPLC-MS/MS N/A	LOD (ng/g): 0.003–0.6	55.2–113 (all analytes)	[35]
chicken, sheep, fish and horse tissue	16 SAs and metabolites	PLE, USE	HPLC-QqLIT-MS/MS 11 min	CC_α (μg/kg): 111.2–161.4 (PLE), 119.3-142.7 (USE) CC_β (μg/kg): 122.4–222.8 (PLE), 138.6-185.5 (USE)	N/A	[36]
bovine, chicken, pork and sheep tissue	22 SAs and metabolites	QuEChERS extraction	HPLC-HRMS 17 min	LOD (μg/kg): 3–26 LOQ (μg/kg): 11–88 CC_α (μg/kg): 101–111 CC_β (μg/kg): 102–122	88–107 (beef muscle)	[37]
chicken tissue and egg	8 SAs	QuEChERS extraction	HPLC-FLD 23 min	LOD (μg/kg): 5.8–19.9 (chicken muscle), 4.1–25.6 (egg) LOQ (μg/kg): 19.2–66.2 (chicken muscle), 13.6–85.4 (egg)	66.9–86.8 (chicken muscle), 65.9–88.1 (egg)	[38]
salmon tissue	27 SAs and metabolites	on-line QuEChERS extraction	UHPLC-ESI-Q-Orbitrap-MS 7 min	CC_α (μg/kg): 0.04–1.34 CC_β (μg/kg): 0.07–2.33	83–109	[39]
fish tissue	8 SAs	SALLE and magnetic separation	HPLC-MS/MS 8 min	LOD (μg/kg): 2.5–10 LOQ (μg/kg): 5–25	74.87–104.74	[40]
shrimp tissue	14 SAs	SALLE	HPLC-FLD N/A	LOD (μg/kg): 1.0–5.0	77.8–103.6	[41]
bovine tissue	39 antibiotics (16 SAs)	SPE	UHPLC-MS/MS 12 min	CC_α (μg/kg): 65–125 (SAs) CC_β (μg/kg): 81–150 (SAs)	85–110 (SAs)	[42]
chicken and pork tissue	SAs	SPE	HPLC-UV N/A	LOD (mg/L): 0.003 LOQ (mg/L): 0.01	> 70	[43]
chicken, pork and shrimp tissue	SDZ, sulfathiazole, sulfamerazine, SMZ and SMP	MSPE	HPLC-DAD 16 min	LOD (ng/g): 1.73–5.23 LOQ (ng/g): 3.97–15.89	81.4–101.3 (chicken), 76.1–102.6 (pork), 79.2–102.5 (shrimp)	[44]
animal tissue	63 veterinary drugs (21 SAs)	DSPE	HPLC-MS/MS N/A	LOD (μg/kg): 0.1–3.0 (all analytes) LOQ (μg/kg): 0.5–10.0 (all analytes)	62.2–112.0 (all analytes)	[45]
bovine, chicken and pork tissue	7 SAs	MSPD-HILME	HPLC-DAD 30 min	LOD (μg/kg): 4.3–13.4 LOQ (μg/kg): 14.2–44.8	85.4–95.1 (kidney), 104.5–118.0 (liver), 85.5–112.3 (muscle)	[46]
chicken and pork tissue and egg	5 antimicrobials (sulfametoxydiazine, sulfamethoxazole and SQX)	on-line SPME	HPLC-UV 20 min	LOD (μg/L): 0.10–0.14 (SAs) LOQ (μg/L): 0.39–0.47 (SAs)	84.1–99.6 (SAs in chicken), 87.0–108.2 (SAs in pork), 80.1–101.4 (SAs in egg)	[47]
chicken and pork tissue	10 SAs	SBSE	HPLC-MS/MS N/A	LOD (μg/kg): 0.0012–0.0061 (pork), 0.0021–0.0146 (chicken) LOQ (μg/kg): 0.0040–0.0203 (pork), 0.0066–0.0487 (chicken)	62.4–109.9 (pork), 55.2–109.1 (chicken)	[48]

2.2. Milk Samples

2.2.1. LLE

An LLE protocol was reported for the extraction of nine SAs from milk samples. Spiked milk (100 μL) was mixed with acidified dichloromethane (800 μL) and the mixture was sonicated for 10 min and centrifuged at 93,000× *g* for 10 min. The organic phase was collected and the step was repeated. The combined organic phases were evaporated to dryness under nitrogen stream at 40 °C and the residue was dissolved in acidified MeOH (100 μL) and filtered prior to HPLC-MS/MS analysis. The authors emphasized the reduced sample and reagent requirements of the developed sample preparation protocol, while a SPE clean-up step was omitted in order to simplify and reduce the cost of the protocol [49]. DLLME and modified QuEChERS extraction, were reported for the extraction of nine SAs from milk samples. In both cases off-line derivatization was conducted with fluorescamine (50 μL) and sonication for 15 min prior to HPLC-FLD analysis. For the DLLME protocol, spiked milk (30 mL) was mixed with 20% trichloroacetic acid aqueous solution (15 mL) and the mixture was vortexed for 10 s and centrifuged at 6000 rpm for 5 min. The supernatant was passed through a 0.2 μm filter and pH was adjusted to pH 4.0–4.5. Treated supernatant (5 mL) was mixed with chloroform (1000 μL, extraction solvent) and ACN (1900 μL, dispersive solvent) and the mixture was shaken until a cloudy solution was formed and centrifuged at 6000 rpm for 5 min. The chloroform phase was collected with a syringe, evaporated under nitrogen stream and the residue was dissolved in Tris buffer (pH 7.0, 1.5 mL) and passed through a 0.2 μm nylon filter. For the QuEChERS protocol, spiked milk (2 mL) was mixed with H_2O (8 mL), vortexed for 10 s, 5% CH_3COOH in ACN (10 mL) was added, and the mixture was shaken for 30 s. The QuEChERS (C18, $MgSO_4$ and PSA) were added and the mixture was shaken for 1 min and centrifuged at 4000 rpm for 5 min. The supernatant (1.5 mL) was collected, evaporated under nitrogen stream and the residue was dissolved in Tris buffer (pH 7.0, 1.5 mL) and passed through a 0.2 μm nylon filter. Both developed sample preparation protocols were simple, fast, and environmentally friendly, providing good recoveries. When compared, DLLME gave lower LOD values and higher recoveries, while QuEChERS extraction was more reproducible with higher throughput [50].

2.2.2. Salting-Out Extraction

SALLE, combined with SPE [51] and a miniaturized SALLE protocol [52], were reported for multi-veterinary drug extraction, including 26 SAs, from milk samples and sulfonamide from tea beverage, water, milk, honey, plasma, blood, and urine samples, respectively. The first protocol employed Oasis HLB Plus cartridges (225 mg) (Waters, Milford, MA, USA). For the SALLE step, spiked milk (5 g) was mixed with oxalic acid-EDTA buffer (pH 3.0, 5 mL) and ACN (10 mL) and the mixture was shaken for 30 s and centrifuged at 3000 rpm for 5 min. The supernatant was collected, $(NH_4)_2SO_4$ (1 g) was added and the mixture was shaken for 2 min, left for 2 min and centrifuged at 3000 rpm for 3 min. Three layers resulted after centrifuging, and the upper ACN and the lower aqueous layer were used for the SPE clean-up step, while the middle layer was the milk fat. The SPE cartridges were preconditioned with MeOH (10 mL), H_2O (10 mL), and oxalic acid-EDTA buffer (pH 3.0, 2 mL), loaded with the aqueous layer, washed with buffer (2 mL) and the analytes were eluted with the ACN layer (10 mL) and MeOH (5 mL). The eluate (3 ML) was evaporated to 0.1–0.2 mL final volume under nitrogen stream at 50 °C for 20 min and the residue was dissolved in CH_3COONH_4 solution (0.1 M, 1 mL), vortexed for 30 s, and passed through a 0.45 μm filter device prior to UHPLC-ESI-Q-Orbitrap-MS analysis [51]. The second protocol employed two 1 mL syringes coupled via their tips that contained the sample solution and extraction solution, respectively. For the preparation of milk samples, spiked milk (1 mL) was mixed with ACN-MeOH-H_2O (40:20:20, *v*/*v*/*v*; 1 mL), the mixture was centrifuged at 3000 rpm for 10 min. For the preparation of honey samples, spiked honey was diluted with H_2O at a concentration of 0.1 g/mL and the mixture was homogenized and centrifuged at 4000 rpm for 20 min. Milk or honey supernatant (0.5 mL) was retracted with the sample syringe A, NaCl (250 mg/mL) was

added, and the mixture was vortexed for 20 s and adjusted to pH 7.0 with NaOH solution (0.1 M). ACN (250 mL) was retracted with the extraction solution syringe B and both syringes were coupled and held vertically. The extraction was conducted by injecting the extraction solvent from syringe B into syringe A, forming a cloudy solution and the content was pumped back to syringe B. This step was repeated five times and the mixture was left in syringe B vertically for 2 min. After phase formation, the upper phase was collected and injected for HPLC-UV analysis. The authors emphasized the reduced organic solvent and sample requirements, as well as the simplicity and the improved extraction efficiency of the developed sample preparation protocol [52].

Two ATPS extraction protocols were reported in the literature for the extraction of SAs from milk. A modified ATPS protocol was reported for the extraction of SDZ and SMZ from milk, egg, and water samples. The extraction protocol employed polyoxyethylene lauryl ether and $Na_2C_4H_4O_6$ in order to form the polymer-organic salt extraction system. For the preparation of milk and egg samples, spiked milk (50 mL) or homogenized spiked egg (50 mL) was mixed with 10% trichloroacetic acid solution (20 mL) and H_2O to 100 mL final volume and the mixture was shaken and centrifuged at 4000 rpm for 30 min. The supernatant was collected and passed through a 0.45 μm filter in order to remove the proteins. The filtrate was added to a mixture of polyoxyethylene lauryl ether (0.027 g/mL) and $Na_2C_4H_4O_6$ (0.180 g/mL) and filled with H_2O to 10 mL final volume. The mixture was placed in a heated water bath under continuous stirring for 20 min and after the phase formation, the upper phase was collected and analyzed with HPLC-UV [53]. An ionic liquid ATPS extraction protocol was reported for the extraction of six SAs from milk samples. Spiked milk (5 mL) diluted with pure H_2O (2.5 mL) was mixed with 10% $HClO_4$ aqueous solution (500 μL) and the mixture was shaken for 2 min and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and passed through a 0.45 μm filter. Butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM][BF_4]$) (300 μL) and $C_6H_5Na_3O_7{\cdot}2H_2O$ (3 g) were added and the mixture was shaken and centrifuged at 10,000 rpm for 10 min. The upper IL phase was collected, diluted with ACN at 1:1 ratio, sonicated, and passed through a 0.22 μm filter. Analysis was carried out by HPLC-UV. The authors emphasized the reduced organic solvent consumption of the developed sample preparation protocol in comparison with classic LLE, as well as the combination of sample preconcentration and clean-up in one step [54].

2.2.3. SPE

Two SPE protocols were reported for multi-veterinary drug extraction, including 18 SAs [55], and the extraction of six SAs [56] from milk samples. The first extraction protocol employed Oasis MCX cartridges (3 mL, 60 mg) (Waters, Milford, MA, USA). Spiked milk (2 g) was mixed with 1% CH_3COOH in ACN (5 mL) and the mixture was vortexed for 30 s and centrifuged at 5000 rpm for 12 min. The supernatant was evaporated to dryness under nitrogen stream at 40 °C and the residue was dissolved in HCl solution (0.1 M, 3 mL) and loaded to a SPE cartridge, conditioned with MeOH (3 mL) and HCl solution (0.1 M, 3 mL). The loaded cartridge was washed with HCl solution (0.1 M, 3 mL) and MeOH (3 mL) and the analytes were eluted with 10% ammonia in ACN (4 mL). The eluate was evaporated to dryness under nitrogen stream at 40 °C and the residue was dissolved in 0.1% CH_3COOH in CH_3COONH_4-MeOH (90:10, v/v; 1 mL) and passed through a 0.22 μm filter prior to ultra-high-performance liquid chromatography—electrospray ionization—mass spectrometry (UHPLC-ESI-MS/MS) analysis [55]. The second extraction protocol employed multi-template MIPs prepared by sol-gel synthesis. Spiked milk (1 g), deproteinized with ACN, was loaded into MISPE cartridges packed with the prepared MIPs (30 mg) and left for 15 min to equilibrate. The loaded cartridge was washed with MeOH (2 mL) and the analytes were eluted with 1% CH_3COOH-MeOH-ACN (50:10:40, $v/v/v$; 2 mL) at a flow rate of 1 mL/min and the eluate was evaporated to dryness under nitrogen stream. Analysis was carried out by HPLC-DAD. MISPE cartridge conditioning was omitted as an unnecessary step, thus reducing the time of the developed sample preparation protocol [56].

Three MSPE protocols were reported for the extraction of nine SAs [57], SMP, SMZ, sulfamethoxazole and sulfachloropyridazine [58], and five SAs [59] from milk samples. The first protocol employed silica-based magnetic sorbent material. Magnetic sorbent (0.1 g), conditioned with MeOH (5 mL) and sonication for 5 min and washed with deionized H_2O (2 × 10 mL), was added to spiked milk (10 mL). The mixture was sonicated for 15 min, the magnetic sorbent was collected by means of a magnet and the supernatant was discarded. The sorbent was washed with acetate buffer (pH 4.0, 3 × 5 mL) and the analytes were with 10^{-3} M NaOH in MeOH (5 mL) for 5 min. The eluate was collected, evaporated to dryness under nitrogen stream and the residue was dissolved in 1% formic acid aqueous solution (500 μL) and passed through a 0.2 μm nylon filter prior to HPLC-DAD analysis. The authors emphasized the simplicity, higher recovery values, and the lower organic solvent requirements of the developed sample preparation protocol in comparison with classic SPE [57]. The second protocol employed a magnetic hyper cross-linked polystyrene composite as the sorbent material. Spiked milk (25 mL) was agitated for 15 min and the magnetic sorbent was added (20 mg). The sample pH was adjusted to pH 5.0, extraction/stirring time was 10 min and analytes were eluted with ACN (2 × 1 mL) and sonication for 5 min. Analysis was carried out by high-performance liquid chromatography—amperometric detection (HPLC-AD). The proposed magnetic composite combined large surface area, high adsorption, and magnetic separation, while a small amount could be used for the extraction from large volumes of untreated milk. The authors emphasized the good recovery's simplicity of the sample preparation protocol, as well as the reduced time and solvent requirements in comparison with classic sample preparation techniques [58]. The last MSPE protocol employed a magnetic graphene-based composite ($CoFe_2O_4$-graphene) as the sorbent material. Spiked milk (1.5 mL) was mixed with 15% $HClO_4$ aqueous solution (0.2 mL) and the mixture was vortexed for 30 s and centrifuged at 14,000 rpm for 5 min. The supernatant was collected, diluted with deionized H_2O (100 mL), and the pH was adjusted to pH 4.0 and mixed with the magnetic sorbent (15 mg). The magnetic sorbent was previously conditioned with MeOH (5 mL), H_2O (5 mL) and sonication for 5 min. The mixture was shaken for 20 min and vortexed for 2 min. The magnetic particles were collected by means of a magnet and the supernatant was discarded. The analytes were eluted with 5% CH_3COOH in MeOH (0.5 mL) and the eluate was passed thought a 0.22 μm filter prior to HPLC-UV analysis. The authors emphasized the simplicity, low LOD values, and improved recoveries of the developed sample preparation protocol in comparison with other reported methods. The magnetic sorbent displayed increased extraction efficiency for the analytes, while it could be reused after washing with ACN and ultrapure H_2O [59].

2.2.4. Other Extraction Techniques

Interesting approaches reported for the extraction of SAs from milk samples include FPSE, graphene-modified melamine sponge (GMeS) microextraction and miniaturized syringe assisted extraction (mini-SAE). The FPSE protocol was reported for the extraction of SMZ, SIX, and SDMX from milk samples. The extraction protocol employed highly polar sol-gel poly(ethylene glycol) coated cotton cellulose fabric segments as the sorbent material. FPSE media incubated in MeOH-ACN (50:50, v/v; 2 mL) for 5 min and rinsed with H_2O (2 mL), was introduced into spiked whole milk (1 g) for 30 min. The fabric-milk system was stirred by means of a magnetic stirrer for 30 min and the extraction media was transferred and incubated in MeOH (250 μL) for 8 min and ACN (250 μL) for 5 min. The extract was filtered prior to HPLC-UV analysis. The coated fabric was washed with ACN-MeOH (50:50, v/v; 2 mL) for 5 min, left to dry for 5–10 min, and kept in an air-tight container between extractions and could be reused for up to 30 times. The developed sample preparation protocol eliminated deproteinization and evaporation/reconstitution, thus reducing the extraction time and the errors resulting from these steps. The proposed fabric sorbent could be applied directly into the untreated milk sample offering a simpler extraction protocol and higher recoveries. Furthermore, the fabric sorbent displayed high chemical and solvent stability that allows the use of the suitable extraction solvent for sample analysis with multiple chromatographic techniques [60]. The GMeS

microextraction protocol was reported for the extraction of eight SAs from milk, egg, and water. The extraction protocol employed novel graphene-modified melamine sponges as the sorbent material. For the preparation of milk samples, spiked milk (15 mL) was defatted with centrifuging at 4000 rpm and 4 °C for 10 min and deproteinized with 15% trichloroacetic acid solution (1 mL for every 10 mL defatted sample solution), vortexing for 1 min, and centrifuging at 4000 rpm for 5 min. The supernatant was collected and mixed with NaCl (6% *w*/*v*) and centrifuged. The supernatant was used for the GMeS extraction. For the preparation of egg samples, homogenized spiked egg (1 g) was mixed with double distilled H_2O (8.7 mL), 15% trichloroacetic acid solution (0.3 mL), and NaCl (6% *w*/*v*) and stirred for 1 min prior to extraction. GMeS cubes conditioned with MeOH and distilled H_2O were placed inside the sample solution (10 mL) and stirred at 600 rpm for 30 min. The cube was collected, placed into a syringe cartridge, rinsed with H_2O, and squeezed in order to remove the absorbed sample. The analytes were eluted with 5% ammonia in ACN (2 × 1 mL), the eluate was evaporated to dryness under nitrogen stream and the residue was dissolved in H_2O-ACN (70:30, *v*/*v*; 100 μL) and sonicated for 1 min prior to HPLC-DAD analysis. The authors emphasized the simple and rapid preparation and easier handling, as well as the improved recoveries and environmental friendliness of developed GMeS material in comparison with other sorbents found in the literature [61]. The mini-SAE protocol was reported for the extraction of SDZ and sulfamonomethoxine from milk samples. The extraction protocol employed a poly (hydroxyethyl methacrylate) polymer as the sorbent material. Spiked milk (50 g) was mixed with 16% lead acetate aqueous solution (3 mL) and the mixture was stirred for 5 min and centrifuged at 4000 rpm for 4 min. The supernatant was collected, 16% lead acetate aqueous solution (2 mL) was added, and the mixture was centrifuged at 4000 rpm for 4 min. The supernatant (1 mL) was loaded into the mini-SAE device packed with the polymer sorbent (50 mg) and conditioned with MeOH (2 mL) and H_2O (2 mL). The device was washed with H_2O (1 mL) and the analytes were eluted with 5% CH_3COOH in MeOH (3 mL). The eluate was evaporated to dryness under nitrogen stream and the residue was dissolved in phosphate buffer (pH 4.0, 1 mL) and derivatized with fluorescamine prior to HPLC-FLD analysis [62].

2.3. Milk Product Samples

Three protocols were also reported for the extraction of SAs from milk products (baby formula, cheese, and butter). Firstly, a SLE protocol was reported for multi-veterinary drug extraction, including 24 SAs, from baby formula samples. Spiked formula (1 g) was mixed with EDTA aqueous solution (0.05 M, 10 mL), the mixture was vortexed, 0.1% formic acid in ACN (10 mL) was added, and the mixture was vortexed, shaken for 15 min and centrifuged at 2000 rcf for 10 min. The supernatant (2 mL) was collected, evaporated to dryness under nitrogen stream at 40 °C, and the residue was dissolved in H_2O-ACN (75:25, *v*/*v*; 1 mL). Analysis was carried out by UHPLC-MS/MS. A clean-up step was omitted due to low recoveries for β-lactams, tetracyclines and dyes, and variable recoveries for the other analytes [63]. A QuEChERS extraction protocol was reported for multi-veterinary drug extraction, including sulfachloropyridazine, sulfadimidine, SDMX, and SQX, from cheese samples. Spiked cheese (10 g) was mixed with 1% CH_3COOH in ACN (10 mL) and Na_2EDTA solution (0.1 M, 10 mL) and the mixture was vortexed for 1 min. $MgSO_4$ (4 g) and CH_3COONa (1 g) were added and the mixture was stirred for 1 min and centrifuged at 4500 *g* for 5 min. The supernatant (2 mL) was collected and passed through a 0.2 μm nylon filter and the filtrate (1 mL) was diluted with 0.01% formic acid solution-MeOH (50:50, *v*/*v*; 1 mL) prior to analysis with UHPLC-MS/MS. The developed sample preparation protocol enabled the extraction of multiple veterinary drugs, in comparison with other similar protocols, that were used for the extraction of a single antibiotic or antibiotic group [64]. An ionic liquid—magnetic bar—liquid-phase microextraction (IL-MB-LPME) was reported for the extraction of eight SAs from butter samples. The extraction protocol employed magnetic hollow fibers as the extraction configuration and 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$) immobilized on the hollow fiber micropores as the extraction solvent. Spiked butter (30 g) was added into a vessel containing eight magnetic fibers and Na_2SO_4 aqueous solution (3 M, 6 mL) and the

vessel was sealed and placed into a water bath at 45 °C and magnetic stirring at 500 rpm for 25 min. The magnetic fibers were collected by means of a magnet, washed with hexane (1 mL), and the analytes were eluted with MeOH (200 µL) and sonication for 3 min. The eluate was collected, mixed with Na_2SO_4 (100 mg) and the supernatant was passed through a 0.22 µm filter. Analysis was carried out by HPLC-UV. The Na_2SO_4 aqueous solution acted both as the extraction solvent for the extraction of the SAs from the butter and as the sample solution for the IL magnetic hollow fibers, thus the developed sample preparation protocol combined analyte extraction, clean-up, and preconcentration in one step [65].

2.4. Egg Samples

Two SPE protocols were reported for the extraction of SAs from egg samples. The first SPE protocol was reported for the extraction of 13 SAs from egg samples. The extraction protocol employed Strata-X SCX cartridges (Phenomenex, Macclesfield, UK). Homogenized spiked egg (10 g) was adjusted to pH 5.0–6.0 with 10% CH_3COOH solution (900 µL) for 15 min. Chloroform-acetone (50:50, *v*/*v*; 30 mL) was added and the mixture was shaken for 10 min and sonicated for 20 min. NaCl (3 g) and Na_2SO_4 (3 g) were added and the mixture was centrifuged at 2209 *g* and 10 °C for 10 min and placed at −70 °C for 30 min. The organic phase was collected (25 mL), mixed with CH_3COOH (2.5 mL), and loaded into the SPE cartridge conditioned with *n*-hexane (2 × 3 mL) and acetone-5% CH_3COOH in chloroform (50:50, *v*/*v*; 2 × 3 mL). The loaded cartridge was washed with H_2O (5 mL) and MeOH (5 mL) and the analytes were eluted with MeOH-ammonia solution (97.5:2.5, *v*/*v*; 13 mL). The eluate was evaporated to dryness under nitrogen stream at 45 °C and the residue was dissolved in mobile phase (0.5 mL), mixed with *n*-hexane (0.5 mL), and centrifuged at 2209× *g* and 20 °C for 10 min. The lower phase was collected, centrifuged for another 10 min, and the supernatant was analyzed with HPLC-DAD [66]. The second SPE protocol was reported for the extraction of SDZ from egg samples. The extraction protocol employed SDZ imprinted microspheres (100 mg) packed into a glass syringe conditioned with MeOH (5 mL) and Milli-Q H_2O (5 mL). Spiked egg yolk (2 g) and white (2 g) were respectively mixed with MeOH (10 mL) and the mixture was sonicated for 10 min. The supernatants were collected and the step was repeated for both egg yolk and white. All collected supernatants were combined, concentrated to 10 mL final volume, and loaded to the MISPE cartridge. The loaded cartridge was washed with MeOH-H_2O (30:70, *v*/*v*; 1 mL) and the analyte was eluted with MeOH (1 mL). The eluate was analyzed directly with HPLC-DAD. The authors emphasized the clean-up efficiency and analyte preconcentration achieved by the developed extraction protocol, while sample defatting was not necessary [67]. Additionally, QuEChERS extraction [38], on-line SPME [47], ATPS [53], and GMeS microextraction [61] were reported for the extraction of SAs from egg samples and sample preparation protocols are given in detail in Sections 2.1 and 2.2.

2.5. Honey Samples

An on-line SPE protocol was reported for the extraction of 15 SAs from honey samples. The extraction was achieved in a Zorbax Extended C-18 (12 mm × 4.6 mm; 5 µm) column (Agilent, Santa Clata, CA, USA). Spiked honey (1 g) was hydrolyzed with HCl solution (3 M, 800 µL) for 90 min and neutralized with citrate buffer (pH 3.5, 200 µL) and NaOH solution (10 M, 240 µL). The SAs were derivatized with 0.2% fluorescamine (200 µL) and the sample solution was passed through a 0.22 µm filter and injected to the on-line SPE-HPLC-FLD system. The authors emphasized the reduced organic solvent and sample requirements, as well as the simplicity, environmental friendliness, and increased selectivity and sensitivity of the automated SPE protocol [68]. Additionally, a miniaturized SALLE [52] protocol was reported for the extraction of sulfonamide from honey samples and details are provided in Section 2.2.

All reported literature for the extraction of SAs from milk and milk product, egg, and honey samples is summarized in Table 3.

Table 3. Extraction of SAs from milk and milk product, egg, and honey samples.

Food Sample	Analytes	Sample Preparation	Analytical Technique/Run-Time	LOD-LOQ	Recovery (%)	Ref.
milk	9 SAs	LLE	HPLC-MS/MS 30 min	LOQ (μg/kg): 12.5–45 CC_α (μg/kg): 106–122 CC_β (μg/kg): 112–145	89–105	[49]
milk	9 SAs	DLLME, QuEChERS extraction	HPLC-FLD 15 min	LOD (μg/L): 0.60–1.21 (DLLME), 1.15–2.73 (QuEChERS) LOQ (μg/L): 2.01–4.02 (DLLME), 3.85–9.09 (QuEChERS)	90.8–104.7 (DLLME), 83.6–104.8 (QuEChERS)	[50]
milk	105 veterinary drugs (26 SAs)	SALLE and SPE	UHPLC-ESI-Q-Orbitrap-MS 14 min	LOQ (μg/kg): 1.0 (all analytes)	71–120 (all analytes)	[51]
tea beverage, water, milk, honey, plasma, blood and urine	sulfonamide	miniaturized SALLE	HPLC-UV 7 min	LOD (ng/mL): 0.3 LOQ (ng/mL): 1.0	96.66 (tea beverage), 76.67 (milk), 43.33 (honey)	[52]
milk, egg and water	SDZ and SMZ	ATPS extraction	HPLC-UV N/A	LOD (pg/mL): 2.92–3.64 (milk), 2.90–3.49 (egg) LOQ (pg/mL): 9.73–12.15 (milk), 9.66–11.62 (egg)	97.14–99.52 (milk), 96.90–99.30 (egg)	[53]
milk	6 SAs	ATPS extraction	HPLC-UV 30 min	LOD (ng/mL): 2.04–2.84 LOQ (ng/mL): 6.73–9.37	72.32–108.96	[54]
milk	38 veterinary drugs (18 SAs)	SPE	UHPLC-ESI-MS/MS 13 min	CC_α (μg/kg): 109–114 (SAs) CC_β (μg/kg): 116–123 (SAs)	87–119 (all analytes)	[55]
milk	6 SAs	SPE	HPLC-DAD 15.3 min	LOD (μg/kg): 1.9–13.3 LOQ (μg/kg): 5.6–42.2 CC_α (μg/kg): 101.9–113.5 CC_β (μg/kg): 114.4–135.4	N/A	[56]
milk	9 SAs	MSPE	HPLC-DAD 35 min	LOD (μg/L): 7–14 CC_α (μg/kg): 108.86–117.16 CC_β (μg/kg): 117.73–134.32	81.88–114.98	[57]
milk and water	SMP, SMZ, sulfamethoxazole and sulfachloropyridazine	MSPE	HPLC-AD N/A	LOD (ng/mL): 2.0–2.5 (milk) LOQ (ng/mL): 6.0–7.5 (milk)	92–105 (milk)	[58]
milk	5 SAs	MSPE	HPLC-UV 8 min	LOD (μg/L): 1.16–1.59 LOQ (μg/L): 3.52–4.81	62.0–104.3	[59]
milk	SMZ, SIX and SDMX	FPSE	HPLC-UV 6.5 min	CC_α (μg/kg): 114.4–116.5 CC_β (μg/kg): 104.1–118.5	93–107	[60]
milk, egg and water	8 SAs	GMeS microextraction	HPLC-DAD 30 min	LOQ (μg/kg): 0.31–0.91 (milk), 0.96–1.32 (egg)	90–105 (milk), 90–108 (egg)	[61]

Table 3. *Cont.*

Food Sample	Analytes	Sample Preparation	Analytical Technique/Run-Time	LOD-LOQ	Recovery (%)	Ref.
milk	SDZ and sulfamonomethoxine	mini-SAE	HPLC-FLD N/A	LOD (ng/g): 0.19–0.26 LOQ (ng/g): 0.67–0.87	85.6–100.3	[62]
baby formula	150 veterinary drugs (24 SAs)	SLE	UHPLC-MS/MS 17.5 min	LOQ (ng/g): 1–10 (all analytes)	50–120 (all analytes)	[63]
cheese	17 veterinary drugs (sulfachloropyridazine, sulfadimidine, SDMX and SQX)	QuEChERS extraction	UHPLC-MS/MS 8.5 min	LOD (µg/kg): 0.2–1.7 (SAs) LOQ (µg/kg): 0.7–5.5 (SAs) CC_{α} (µg/kg): 3.4–5.8 (SAs) CC_{β} (µg/kg): 5.7–10.2 (SAs)	72.5–106.3 (SAs)	[64]
butter	8 SAs	IL-MB-LPME	HPLC-UV 30 min	LOD (µg/kg): 1.20–2.17 LOQ (µg/kg): 4.00–7.25	73.25–103.85	[65]
egg	13 SAs	SPE	HPLC-DAD 45 min	LOD (µg/kg): 0.30–1.29 LOQ (µg/kg): 0.92–3.92 CC_{α} (µg/kg): 11.3–18.5 CC_{β} (µg/kg): 13.2–27.3	45.2–87.5	[66]
egg	SDZ	SPE	HPLC-DAD N/A	LOD (µg/L): 0.06 (egg yolk), 0.05 (egg white) LOQ (µg/L): 0.20 (egg yolk), 0.17 (egg white)	78.22–86.10	[67]
honey	15 SAs	on-line SPE	HPLC-FLD 30 min	LOD (ng/g): 0.1–1.0	76–108	[68]

3. Conclusions

The most recent literature regarding the extraction of SAs from food samples was successfully reported. In the case of animal tissue samples, SLE [31–33], ASE [34–36], QuEChERS extraction [37,38], SALLE [40,41], and SPE [42] were the most reported methodologies for the extraction of SAs, along with other antibiotic or veterinary drugs, followed by reports of USE [36], DSPE [45], and SBSE [48]. Reports of on-line techniques include a fully automated on-line QuEChERS extraction protocol [39] and an on-line SPME protocol that utilized molecularly imprinted monolithic capillary columns [47]. Reported novel solid-phase sorbent materials include MWCNTs utilized in a SPE protocol [43], and Fe_3O_4@JUC-48 nanocomposite utilized in a MSPE protocol [44]. An ionic liquid application was also reported in a MSPD-HILME protocol [46]. SPE [55], MSPE [57], and ATPS extraction [53,54] were the most reported approaches for the extraction of SAs from milk samples. Other approaches include LLE [49], DLLME [50], as well as a modified QuEChERS extraction protocol that employed C18, $MgSO_4$ and PSA [50], SALLE combined with SPE [51], and a miniaturized SALLE protocol [52]. Interesting approaches include FPSE [60], GMeS microextraction [61], and mini-SAE [62]. Reported novel materials include a magnetic hyper cross-linked polystyrene composite [58] and a magnetic graphene-based composite [59] utilized in MSPE protocols and multi-template MIPs prepared by sol-gel synthesis utilized in a SPE protocol [56]. Furthermore, SLE [63], QuEChERS extraction [64], and IL-MB-LPME [65] were reported for the extraction of SAs from milk products. In the case of egg samples, SPE [66,67] was the main reported technique, while QuEChERS extraction [38], on-line SPME [47], ATPS [53], and GMeS microextraction [61] protocols included eggs, along with other food matrices. Finally, for honey samples only an on-line SPE [68] and a miniaturized SALLE [52] protocol were reported in the recent literature.

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