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Current Trends in Sample Preparation for Growth Promoter and Residue Analysis

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Abstract

A comprehensive review is presented on the current trends in sample preparation for isolation of veterinary drugs and growth promotors from foods. The objective of the review is to firstly give an overview of the sample preparation techniques that are applied in field. The review will focus on new techniques and technologies, which improve efficiency and coverage of residues. The underlying theme to the paper is the developments that have been made in multi-residue methods and particularly multi-class methods for residues of licensed animal health products, which have been developed in the last couple of years. The role of multi-class methods is discussed and how they can be accommodated in future residue surveillance.

Keywords:

Veterinary drugs

Growth promoters

Extraction

Clean-up

Sorbents

Mass spectrometry

Contents

1. Introduction

Sample preparation is the process of extracting chemical residues from a sample and the subsequent purification of the extract to isolate the residues of interest and remove any matrix interferents that may affect the detection system. Even with the advancement of separation and detection techniques, sample preparation is a vital part of the analytical process and effective sample preparation is essential for achieving reliable results and maintaining instrument performance. Sample preparation in residue analysis is often not covered well in literature with many review papers focusing on detection systems. However, a number of good general review papers have reported on the topic of sample preparation specifically [1-4]. Some book chapters provide a more in-depth analysis of the area, but with the rapid progression of sample preparation in recent years, most book chapters are a little outdated at present [5,6].

There have been quite a number of changes in the approach to preparing samples in recent years due to the widespread application of mass spectrometry. While in the past, methods were only capable of analysing lower numbers of residues (usually a single class of drug) [7-1 1], mass spectrometry now offers the possibility to analyse vast numbers of residues in a single run [12-15]. As a result, there is now a tendency to focus towards more generic extraction and clean-up procedures to cover the wide range of veterinary drugs that can be found in food of animal origin [15-17]. Although the use of mass spectrometry permits the use of simpler generic clean-up methods, effective removal of matrix constituents is necessary as these may affect the performance of the mass spectrometer, particularly ion suppression and enhancement effects [18]).

Besides classical liquid-liquid extraction, liquid-liquid partitioning and the well-established solid-phase extraction, a number of new formats have now found applications in residue analysis. QuEChERS [16,17], ultra-filtration [19], on-line SPE [20] and high throughput approaches such as 96-well plates are now beginning to find application in the area. There is a constant need for new techniques that are faster, cheaper, require less solvent and are amenable to automation.

This paper will firstly present a brief overview of the main techniques currently being applied in this area, particularly with a focus on new developments which improve efficiency. Coverage of specific techniques is not intended to be comprehensive and readers seeking a more detailed discussion should refer to the reference papers or books cited in the text. The paper will then review a selection of methods for isolating residues in different biological matrices. The paper largely focuses on multi-residue or multi-class assays that employ selective LC-MS detection.

2. The Sample

2.1 Sample selection

Several edible tissues from food producing animals can be selected for residue surveillance including muscle, liver, kidney, skin and fat, which are normally taken at slaughter houses. In addition, further sample matrix types can be taken on farm or at production sites, including milk, honey, eggs and fish. The approach normally adopted in residue surveillance is to target the matrix where residues are most persistent for Group A substances (banned substances) and at their highest concentration for Group B substances (licensed veterinary drugs). Sample matrix selection for imported foods is limited to traded commodities such as muscle, honey, milk and eggs. Muscle is a particularly advantageous tissue for residue surveillance because it is the main tissue consumed and can be used to analyse both imported and domestic samples, thereby reducing laboratory validation requirements. However, muscle can present analytical difficulties because of variability in residue distribution [21-23], particularly in the area surrounding injection sites [24-27]. There is also the concern of lower probability of finding non-compliant samples compared to matrices such as liver and kidney [28].

Samples for Group A include plasma/serum, urine, faeces, H_2O, feed, bile (abattoir) and thyroid gland (abattoir), which can be taken on-farm or at abbattoir. Alternative matrices allow detection of residues for (i) longer periods post treatment (e.g. β-agonists in retina [29-33] and steroids in hair [34]), (ii) discrimination between endogeneous and exogeneous sources of anabolic agents (steroid esters in urine [35])

or (iii) to allow detection of residues using less complicated equipment (e.g. HPLC detection of semicarbazide in retina [36]).

2.2 Sample storage and preservation

Sample storage is an important step, because of the lag time between sample collection and analysis. Both physico-chemical factors (oxidation, proteolysis and precipitation) and biological factors (microbiological and enzymatic reactions) need to be considered when storing samples. Some studies have reported on the presence of micro-organisms which produce the enzyme penicillinase, which are capable of reducing the concentration of penicillin in kidney tissue stored at 4°C [37]. However, preservation can be achieved through the addition of enzyme inhibitors (e.g. piperonyl butoxide inhibits cytochrome $_{P450)}$. A number of studies have highlighted the degradation of residues during frozen storage, namely β-lactam antibiotics in milk [38], ampicillin in pig muscle [39], chlortetracycline in incurred pig muscle, liver and kidney [40], sulphamethazine in incurred pig muscle and bovine milk [41], and gentamicin residues in egg [42]. EU validation criteria describe guidelines for stability studies to be carried out during method validation [43]. Stability should be determined for the analytes in matrix and in solution at various stages of the sample preparation process. Whenever possible, incurred tissue should be used, otherwise matrix fortified material is used. A practical approach is to run a test to see how long a sample and/or analyte can be held without degradation and then to complete the analysis within that time.

2.3 Sample pre-treatment

The variation of residues within a single organ or tissue is an important factor to consider prior to sample preparation but is often ignored. For example, residue variations may occur in the kidney between the medulla and the cortex [44-46]. Therefore, it is important to take a representative aliquot of the sample, which may require removal of several portions throughout the composite sample to give a representative sample. Homogenisation with a blender is often advantageous for obtaining a homogenous sample but can result in the release of enzymes, which can degrade residues and provide inaccurate results. Liquid samples (blood, plasma, serum, milk, bile or H_2O) are generally easier to process than solid samples and residues are more homogenously distributed throughout.

3. Sample extraction

3.1 Target residue

3.1.1 Free residues and conjugates

The residues present can vary greatly between target tissues due to the extensive metabolism in animals after administration. The target residue for analysis is not always the parent drug but can be comprised of the parent drug and/or metabolites. The free parent and metabolite residues are readily extracted by organic solvents, H_2O or aqueous buffers. However, many residues are present in the conjugated forms (glucoronides or sulfates) and require liberation through enzymatic or chemical hydrolysis prior to extraction. Hydrolysis conditions (namely pH, temperature and time) have to be carefully optimised to ensure efficient deconjugation of residues. Enzymatic hydrolysis generally ensures milder conditions than acid or alkaline hydrolysis. Common enzymatic preparations used for hydrolysis include *Helix promatia* juice (a mixture of β-glucuronidase and arylsulphatase) and *E. Coli* β-glucuronidase.

3.1.2 Bound residues

Residues bound through weak interactions can be easily extracted after dialysis, proteolysis or denaturation of proteins by heat or acid treatments. In practice, analysis of bound residues is applied to very few drugs, namely nitrofurans, florfenicol and triclabendazole. Nitrofuran antibiotics are rapidly metabolised to form bound residues, which persist for many weeks after treatment [47]. These bound metabolites pose a health risk and are used as marker residues to monitor for nitrofurans [48]. It is proposed that binding of residues occurs through cleavage of the nitrofuran ring by stomach acid, leaving the specific tail group covalently bound to tissue [49]. The bound metabolites are cleaved from tissue samples under mildly acidic conditions before undergoing derivatisation to increase the sensitivity of detection [50]. Metabolism studies of florfenicol depletion demonstrated that non-extractable residues of florfenicol were pre-dominant in tissues [51]. Acid hydrolysis of non-extractable residues not only liberates bound residues, it also converts them to florfenicol amine (FFA), which is the marker residue for florfenicol [52,53].

O'Keeffe et al. investigated the release of bound thiabendazole residues, finding optimum yields under alkaline conditions [54]. Acid hydrolysis, Raney nickel catalysis and enzymatic hydrolysis (cystathionine β-lactase) were found to be largely unsuccessful [55].

3.2 Tissue disruption

Disruption of tissue is normally achieved using a probe blender or through enzymatic digestion with proteolytic enzymes such as subtilisin A and ronase E [56]. Several tissue disruption apparatus are available, including probe blenders, ultrasonic probes and stomachers. The ultrasonic probe uses pulsed, high frequency sound waves to agitate and disperse cells. The Stomacher® is an alternative apparatus which extracts residues from samples using crushing action. Stomachers® offer an advantage over probe blending techniques because they eliminate the risk of cross-contamination, as each sample is contained in separate bags.

3.2.1 High-throughput tissue disruption

A number of automated apparatus have been developed that allow unattended disruption of samples, while significantly improving sample throughput and reproducibility. The Omni Prep Multi-Sample Homogenizer™ is a multi-probe blender with a specially designed oscillating sample rack capable of simultaneously processing batches of six samples at a time. The Tomtec Autogizer™ is a more sophisticated system that allows unattended processing of large batches of tissue samples using either five probe blenders or two ultrasonic probes at a time. A major advantage of the system is a three staged automated cleaning program using (a) aqueous, (b) organic and (c) ultrasonic cleaning cycles. The FASTH 21™ homogeniser system is an alternative tissue disruption system, which uses disposable tubes containing rotating blades. Samples are fed in racks, four at a time, in a conveyor belt system and homogenised at high speed. In theory, the system can process as many as 250 samples per hour. In practice, the FASTH 21™ system provides excellent homogenisation of samples but drawbacks of the system include the tendency of the propeller stem to break during homogenisation and the inability to undergo further sample manipulation such as shaking (due to leakage). However, it is proposed through future improvements in tube design that these problems can be resolved.

3.2.2 Evaluation of disruption techniques

The majority of methods in the literature report extraction efficiency (or recovery) using fortified samples. While these artificial systems may demonstrate recovery efficiency during sample preparation, they may not accurately represent the true residue content from a naturally incurred test sample. The total residue concentration in a naturally incurred sample may be difficult to measure due to tight or irreversible binding of residues to matrix components. McCracken et al. compared the extractability of chlortetracycline (CTC), sulphadiazine (SDZ) and flumequine (FMQ) residues from incurred and spiked chicken muscle using four different disruption techniques (probe blender, Stomacher®, ultrasonic bath and end-over-end mixer) [57]. Results showed that extractability of residues from fortified samples were similar for each technique. In contrast, the highest extraction efficiency for all three residues from incurred tissue was achieved using probe blending.

3.3 Sample extraction techniques

3.3.1 Manual sample extraction techniques

Residues are typically extracted from samples using simple solvent extraction or liquid-liquid extraction (LLE). The extraction technique adopted may depend on the nature of the samples (i.e. liquid or solid) and the physico-chemical properties of the residues (polarity and pKa). Simple extraction with aqueous buffer is advantageous for highly polar residues because they reduce non-polar matrix components (e.g. lipids) and extracts can be enriched on reversed phase SPE, while eliminating time consuming evaporation steps. A disadvantage is that strongly protein-bound residues are not fully extracted and polar matrix components are co-extracted. In general, the majority of methods employ more efficient organic solvents as extracting agents [15]. ACN is the preferred extraction solvent as it gives good yields of residues but low levels of matrix co-extractives and is effective at denaturing proteins and inactivating enzymes [15]. MeOH and EtOAc are also widely used solvents but result in the extraction of additional matrix components [15]. However, in the area of multi-residue analysis there is always a compromise between recovery and the purity of sample extracts.

Liquid-liquid extraction (LLE) was the most widely applied extraction procedure in residue analysis due to its high selectivity compared to simple solvent extraction. LLE applications can also include polar ionisable compounds, which can be extracted by non-polar organic solvents using the ion-pair technique: transforming positively charged substances into non-polar neutral compounds in the presence of organic anions, or vice-versa. Examples of the successful application of ion-pair extraction are β-agonists [58], aminoglycosides [59] and oxytetracycline [60]).

However, LLE has been replaced in recent years due to the difficulties in automating LLE, the development of SPE and most importantly the widespread application of more selective LC-MS/MS detection systems.

Anastassiades and co-workers developed a variation of LLE in the QuEChERS sample preparation procedure (standing for ―quick, easy, cheap, effective, rugged and safe), which has been successfully applied to the analysis of hundreds of pesticide residues [61]. In QuEChERS, the high-moisture sample (H_2O is added to dry foods) is extracted with an organic solvent (ACN, EtOAc, or acetone) in the presence of salts ($MgSO_4$, NaCl and/or buffering agents). The addition of salts induces phase separation of the solvent from the aqueous phase. The residues of interest and matrix co-extractives are separated into the relevant liquid phase based on their polarity with the residues partitioning into the organic phase and matrix co-extractives into the aqueous phase. Upon shaking and centrifugation, an aliquot of the organic phase is subjected to further purification using dispersive-SPE, which entails mixing sorbents with the extract. The approach is very flexible, and since its development there have been several modifications to the technique depending on residues, matrices, instrumentation, and analyst preferences [16,17,62-72]. The approach uses very little labware and generates little waste. The technique provides high recovery for many LC- and GC-amenable residues, gives high reproducibility, and costs less than many typical sample preparation approaches [61]. Several groups have adapted the method to analyse residues in a variety of matrices. HAc (1%) has been widely used to adjust pH and promotes stability and recovery of base-sensitive residues [73]. HAc was used to adjust pH by Stubbings and Bigwood to determine residues in chicken muscle [17] and by Aguilera-Luiz et al. to determine 18 antibiotics in milk [72]. QuEChERS low cost, coupled to its flexibility and ease of use will no doubt result in an increase in its application to residue analysis.

Kaufmann et al. developed a =bi-polarity extraction‘ method based upon similar principles as the QuEChERS technique [15]. Whereas QuEChERS is used to extract residues of similar polarity, Kaufmann‘s aim was to develop a method capable of extracting residues of diverse polarities (polar and non-polar). The residues (polar and non-polar) remain in the aqueous phase, which undergoes clean-up by SPE on a mixed-mode Oasis HLB cartridge and subsequently analysed by UPLC-MS/MS.

A major disadvantage of the bi-polarity approach is that matrix components are extracted along with residues. The sample therefore needs to undergo a lengthy SPE procedure to isolate the residues. However, this lengthy clean-up procedure produces a highly pure sample extract ready for analysis. In contrast, the QuEChERS method suspends numerous matrix components in the aqueous phase, which is discarded. The cleaner organic phase can subsequently undergo a much simpler clean-up step (dispersive-SPE). Although the bi-polarity extraction method was able to extract residues with a wide polarity range, it was unable to sufficiently extract very non-polar residues without losing polar residues in the SPE clean-up.

An alternative to QuEChERS is Matrix solid-phase dispersion (MSPD); which was a popular sample preparation technique in residue analysis in the late 1980s and 1990s, which combined both sample extraction and isolation in one step. Barker [74] defined MSPD procedures as those that use dispersing sorbents with chemical modification of the silica surface (e.g. $C_{18,}$ C_8, etc.). For most applications, particles with diameters of 40-100 µm are used [75]. A sample is blended and dispersed with a sorbent using a glass mortar and pestle (**Fig. 1**). Sample/sorbent ratios typically range from 1:1 to 1:4; with 0.5 g sample and 2 g sorbent being the most commonly used quantities. It is important to use a glass or agate mortar and pestle as the use of ceramic/clay can result in loss of analytes [76]. After dispersion, the sample is air-dried (5 to 15 min) prior to compression between two frits in a syringe barrel with a syringe plunger. In recent years, many groups have used non-bonded silica based dispersion agents such as Na_2SO_4 or silica [77-80]. This is approach is advantageous because it eliminates the air-drying step from the procedure.

The choice of wash and elution solvent are key for successful MSPD applications. Lipophilic matrix interferents can be removed through washing with non-polar solvents like hexane. For veterinary drugs, polar solvents, such as dichloromethane (DCM), alcohols and hot H_2O, are typically used. Hot H_2O has been successfully used by Bogialli et al. to extract several classes of drugs from various matrices [82-88]. However, care must be taken when using hot H_2O as some analytes can thermally degrade. The major advantages of MSPD are that (a) the technique can be applied to a wide range of residues, (b) it eliminates the need for protein precipitation steps and (c) it eliminates the need for centrifugation. In addition, because the surface area of the entire sample (including proteins, connective tissues, etc.) is exposed to the solvents, more effective washing and elution of extracts can be achieved. Another advantage is that residues can be sequentially eluted using different solvents of increasing or decreasing polarity. There has been a resurgence of the technique in recent years for the preparation of veterinary samples for drug residue analysis. However, the technique has not found widespread application for routine residue surveillance.

3.3.2 Instrumental-based extraction techniques

A number of instrumental-based extraction procedures have been developed to isolate residues from food, including microwave, supercritical fluid and pressurised liquid extraction systems. Advantages in using such technology include the potential for automation, more selective isolation of residues through tuning of instrument parameters and online clean-up of samples. Disadvantages include the limited number of commercially available instruments, additional extraction costs and instrumental downtime. Pressurised liquid extraction (PLE) is the most widely used instrumental extraction technique. Several applications have also been developed using SFE and MAE but these techniques are not widely used in routine laboratories.

3.3.2.1 Microwave-assisted extraction

Microwave-assisted extraction (MAE) uses microwave energy to heat the solvent/sample mixture in order to partition analytes from the sample matrix into the solvent. Using microwave energy allows the solvent to be heated rapidly: an average extraction takes 15-30 min [89]. MAE offers high sample throughput (several samples can be extracted simultaneously) with low solvent consumption (10-30 mL).

A good review of MAE is available by Eskilsson et al. [90]. MAE systems can

operate in two modes, open (focused MAE) or closed (pressurized MAE) vessels. Open vessels operate at atmospheric pressure, while closed vessels are sealed and operate under higher pressures. Closed vessel MAE operates somewhat like PLE, since the temperature of the solvent can be increased by increasing the pressure. MAE therefore, offers many advantages. However, solvent choice is limited, care must be taken not to overheat the sample, additional clean-up of the samples is generally necessary prior to analysis and MAE is not amenable to automation (on-line extraction and detection) [89]. Akhtar et al. developed a method for MAE extraction of fortified and incurred chloramphenicol residues in freeze-dried egg [91]. Sample extraction time was 10 s using a binary solvent mixture consisting of ACN and 2-propanol. Akhtar also compared MAE with conventional extraction (homogenisation, vortexing) for the determination of incurred salinomycin in chicken eggs and tissues [92].

3.3.2.2 Supercritical fluid extraction

A supercritical fluid (SF) is defined as any substance that is above its critical temperature and pressure [93]. The physical properties of a supercritical fluid are intermediate between those of the liquid and gas phases; the solvating power (density) of an SF is similar to that of a liquid and its diffusivity and viscosity are similar to that of a gas [94]. The effectiveness of SFE is due to large changes in solvating power achieved with minor changes in density (i.e. temperature and pressure) of the SF around the critical point. Higher pressures are necessary to obtain liquid-like densities for temperatures further above the critical limit. Carbon dioxide (CO_2) is the most widely used SF because of its inertness, low cost, high purity, low toxicity and low critical parameters (CO_2: T_c = 31.3°C, P_c = 72.9 atm) [95]. However, the solvating power of supercritical CO_2 at high density is not always sufficient to extract an analyte [89]. If the analyte is not soluble or is strongly bound to the matrix a more polar SF (e.g. N_2O or CHF_3) can be used, otherwise a polar modifier (MeOH, EtOH or H_2O) may be added to the SF in order to increase the solvating power [93]. Several SFE applications have been reported in peer reviewed literature for selective isolation of residues from food (**Table 3-23)**. This demonstrates that the SFE is an effective technique and can extract a wide range of residues from complex matrices. However, particular disadvantages are the lack of automated SFE systems and limited pressure

range of some systems. This has resulted in reduced interest in the area of residue analysis in the last 10 years.

3.3.2.3 Pressurised liquid extraction

Pressurised liquid extraction (PLE) has received numerous names, such as accelerated solvent extraction (ASE), pressurized fluid extraction (PFE), pressurised hot solvent extraction (PH SE), subcritical solvent extraction (S SE) and hot H_2O extraction (HWE) [96]. PLE is carried out at temperatures above the boiling point of the solvent and uses high pressure to maintain the solvent in the liquid phase and achieve fast and efficient extraction of analytes from the solid matrix [96]. HWE is increasingly being used in residue analysis due to low cost, low toxicity and ease of disposal. At ambient temperature and pressure H_2O is a polar solvent, but if the temperature and pressure are increased the polarity decreases considerably and H_2O can be used to extract medium to low polarity analytes [96,97].

A schematic of ASE system is shown in **Fig. 2**. At elevated temperature and pressure, the PLE extraction process proceeds faster but selectivity decreases [98] and the analytes are not the only compounds solubilised. Even after optimisation of all the extraction parameters, matrix interferents (e.g. lipids, collagen, protein) are frequently co-extracted, thereby requiring the sample to undergo further clean-up. Post-extraction clean-up steps can be done manually, although automation of the process is favoured. In particular, *in-situ* clean-up steps have been developed to offer a fast and efficient link between extraction and analysis. The most widely used clean-up methods are pre-PLE, MSPD and SPE. Pre-PLE involves an initial PLE extraction with a non-polar solvent (e.g. hexane) to eliminate the hydrophobic compounds present in the sample prior to extracting analytes of interest. SPE may be coupled on-line to the extractor outlet and can provide clean-up and concentration in one step. When dealing with fatty samples, addition of fat-retaining sorbents, such as Florisil® (synthetic magnesium silicate), alumina or silica gel, prior to analysis can prevent lipids and other interferents from being co-extracted. Although preparation of the extraction cell is time-consuming and tedious [98], the short extraction times, the use of low solvent volumes, the ability to use H_2O (cheap and environmentally friendly) as extraction solvent and the fact it is amenable to automation makes PLE a very

attractive option for residue analysis. A number of PLE applications have been developed in residue analysis and are listed in **Table 3-23**.

4. Sample purification

4.1 Solid phase extraction

SPE is the most important sample purification technique in residue analysis and has gradually replaced LLE and LLP. The objective of this section is to give a brief overview of SPE and sorbent materials. A number of books and review papers have already been written on this topic and can be consulted for more detail [6,99-105]. Conventional SPE phases used in residue analysis and their characteristics are described in **Table 1**. However, these are being replaced more and more by polymeric sorbents that offer advantages for analytes that are difficult to purify on conventional phases shown in **Table 2** [104]. It is expected that this trend will continue in the future with the development of multi-class residue methods. However, continued advantages of SPE include difficulties in achieving consistent flow and plugging of cartridges, which can lead to difficulties in automation. Disk extraction format overcomes these difficulties but have yet to find widespread application in residue analysis. In addition, there is a question if they provide sufficient sample load capacity and sufficient analyte retention, particularly when dealing with multi-residues representing different chemical classes. The use of disks should increase in the future as they offer not only better performance but also faster extraction speeds [1].

4.2 Dispersive-SPE

Dispersive-SPE (DSPE) is a clean-up technique that involves mixing sorbent with a sample that has been pre-extracted with an appropriate solvent. It is typically part of the QuEChERS method where it follows the bi-polarity extraction step. The appropriate sorbent adsorbs matrix co-extractives onto its surface, leaving analytes of interest in the solvent. $MgSO_4$ is added to provide additional clean-up by removing residual H_2O and some other compounds via chelation [73]. Afterwards, the mixture is centrifuged and the resulting supernatant can be analyzed directly or can be subjected to a concentration and/or solvent exchange step if necessary. It is an

extremely fast, simple and inexpensive process that provides high recovery and reproducibility for many LC- and GC-amenable analytes [63].

The principal of the process is the removal of matrix compounds, while leaving the analytes of interest in the solvent. The physico-chemical properties of the analytes and matrix compounds determine the choice of sorbent. In pesticide analysis, primary secondary amine (PSA) is the most common sorbent used. PSA is effective at retaining fatty acids and other organic acids present in food [61]. For food of animal origin, which has higher lipid content, $_{C18}$ or a combination of PSA/C_{18} is more effective because $_{C18}$ removes lipophilic compounds. In recent research, our research group found that the combination of PSA/C_{18} to provide better clean-up than PSA or C_{18} alone for 38 anthelmintics in liver and milk [16]. However, PSA/C_{18} gave a lower recovery for some analytes (due to PSA), compared with $_{C18}$ which gave sufficient clean-up and good recovery for all analytes and was therefore chosen as the preferred sorbent. Graphitised carbon black (GCB) has been reported to be a highly effective sorbent for sample clean-up [63]. However, GCB also removes structurally planar analytes and is therefore not useful in many applications. Addition of HAc to the extraction solvent may help to improve recovery of analytes but it also inhibits PSAs ability to retain acidic matrix compounds [73]. Several papers have reported the use of $_{C18}$ for DSPE in veterinary residue analysis [16,62,64,71]. PSA, NH_2 and silica have also been reported [17,67,71]. DSPE does not provide the same degree of clean-up as SPE. However, it does provide good recovery and reproducibility, coupled with practical and cost advantages [61].

4.3 Immunoaffinity chromatography

A number of good review papers have been published on immunoaffinity chromatography (IAC) and should be consulted for a more detailed overview of this topic [116-121]. A number of IAC applications from peer reviewed literature are shown in **Tables 3-23**. It can be seen that IAC is particularly advantageous when low detection levels in the µg kg^{-1} to ng kg^{-1} are required for banned substances, particularly when using less selective HPLC based detection systems. However, it is difficult to see the practical application of this technique in the isolation of residues of licensed veterinary drug from food where there is now a trend to move towards multi-class residue methods with detection by LC-MS/MS. Despite this, there are some

very good multi-residue applications of IAC in residue analysis [122-124]. While most of these IAC applications have been developed by researchers, a wide range of IAC columns are commercially available from vendors such as Rhone Diagnostics Technologies, Biocode, r-Biopharm, Tecna, Randox and Euro-Diagnostica.

4.4 Molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) are engineered cross-linked polymers that exhibit high affinity and selectivity towards a target compound or class of structurally related compounds (**Fig. 3**) [125,126]. MIPs can be tailored to selectively extract analytes present in complex matrices such as blood, urine, tissue or feed [127]. These materials have demonstrated binding to trace levels of target analytes, and display high selectivity in the presence of other compounds that have similar physico-chemical properties, as well as being extremely stable [128]. A drawback of the technique is the potential leaching or template remaining in the MIP [129], and also binding site heterogeneity, leading to a range of binding affinities for the target analyte. Aqueous samples, such as milk or urine, generally require purification prior to M IP clean-up. However, there are applications reported that apply the aqueous sample directly to MIP without undergoing an initial extraction step [130].

4.5 Molecular weight cut-off devices

4.5.1 Ultra-filtration

The development of multi-residue assays using LC-MS/MS detection has resulted in the alternative purification systems in the field of residue analysis such as ultra-filtration (UF). In residue analysis of food, UF is primarily used to separate analytes of interest from macro-molecules, such as proteins, peptides, lipids and sugars, which may interfere with analysis, particularly affecting ionisation in mass spectrometry. In residue analysis, molecular weigh cut-off devices or spin filters coupled to micro centrifuge tubes are the most commonly used formats. Alternative formats are also available such as 96-well plate, but require dedicated vacuum manifolds and pumps. However, all residue applications use centrifugal devices. Examples of applications include sulphonamides in milk [19,132-134], eggs [132,135,136], plasma [137] and edible tissues [138,139], benzimidazoles in milk [19], tetracyclines in egg [140], β-agonist in urine [141], penicillin G in muscle, kidney and liver [142], and spiramycin (a macrolide) in egg and chicken muscle [143].

4.5.2 Size exclusion or gel permeation chromatography

This mode of purification is widely used in the area of pesticide residue analysis. These materials usually have the appearance of a gel, resulting in the generic name gel permeation chromatography (GPC). Purification is achieved through molecular sieving, which occurs through pores on the surface of a solid sorbent. Size exclusion is not very specific and lacks resolving power, with frequent overlap between similar sized molecules, independent of their chemical structure or properties. However, it can be successfully used to separate low molecular weight drugs from larger interferents (proteins, carbohydrates, triglycerides, etc). Few publications report the use of GPC in residue analysis, although it has been successfully used in the analysis of sulphonamides in shrimp [144], thyreostats in thyroid samples [145] and sulphonamides, nitrofurans and growth promoters in animal feed [146].

4.6 High-throughput sample preparation

Recently, there has been a move from slow manual sample preparation techniques to faster automated techniques. Automated sample preparation can be carried out on-line (connected directly to the analysis system) or off-line (sample preparation is automated, but the sample has to be manually transferred to the analysis system). Automated sample preparation offers the ability to perform sample clean-up, concentration and analyte separation in a closed system. This reduces the sample preparation time and the whole sample becomes available for analysis, while sensitivity and limits of detection are improved accordingly. It also removes some of the human element from a procedure, thereby improving precision and reproducibility. Furthermore, automated sample preparation reduces cost by using less solvent and fewer personnel. However, there is an increase in initial capital expenditure. Other advantages include reduced risk of sample contamination and elimination of analyte losses by evaporation or by degradation during sample pre-concentration.

4.6.1 Automated off-line/on-line SPE

Off-line is more common than on-line systems because it can be applied to traditional SPE clean-up. A disadvantage is that extracts require concentration and have to be transferred manually to the analytical system. A particular advantage over some on-

line SPE systems is that memory or sample carry over effects are eliminated through single use SPE cartridges. The Gilson ASPEC XL™ is a typical example of an automated off-line SPE system that can process four samples in parallel in cartridge and 96-well format. A number of applications have been developed using this platform including anabolic steroids in urine [147,148]; quinolones in animal feed [149], seafood [150], bovine plasma, milk and tissues [151]; stilbenes in animal tissues [152]; sulphonamides in ovine plasma [153]; macrocyclic lactones in liver [154] and plasma [155,156]; benzimidazoles in bovine liver [106]; halofuginone in chicken liver and eggs [157]; malachite green in trout muscle [158]; carbadox and olaquindox in porcine liver [159].

As an alternative, automated purification of samples can be achieved through on-line SPE. In this process the extraction cartridge is inserted in place of the sample injection loop, thus allowing simultaneous samples preparation and chromatographic analysis. On-line SPE offers better control of the sample preparation process and improved sensitivity through more selective isolation of target residues. A disadvantage of this approach is that some substances can carryover between injections and result in a memory effect. Spark Holland have developed an on-line system (Symbiosis®) based on disposable single-use-cartridges, which are automatically replaced for each sample to eliminate memory effects (**Fig. 4**). The Symbiosis automated SPE unit has been successfully used in the analysis of β-lactams in bovine milk [160], benzimidazoles in milk [161], tetracyclines in milk [162] and chloramphenicol in egg [163].

4.6.2 Turbulent flow chromatography

Turbulent flow chromatography (TFC) is a high-throughput sample preparation technique that utilizes high flow rates (4-6 mL) and an analytical column containing sorbent particles with large pore sizes (30-60 µm). Due to the large pore size, there is only moderate back-pressure on the column, which serves as both extraction and analytical column. At the higher flow rate, solvent doesn‘t exhibit laminar flow but exhibits turbulent flow instead. This leads to the formation of eddies which promote cross-channel mass transfer and diffusion of the analytes into the particle pores. Samples are applied to the column using aqueous mobile phase (**Fig. 5**). Small

molecules diffuse more extensively than macromolecules (e.g. proteins, lipids, sugars) and are driven into the pores of the sorbent. Due to the high flow rate, the larger molecules are flushed to waste and don‘t have an opportunity to diffuse into the particle pores. The trapped analytes are desorbed from the TFC column by back-flushing it with a polar organic solvent and the eluate can be transferred with a switching valve onto the HPLC system (normal low flow rate) for further separation and subsequent detection (usually by MS/MS). During LC-MS/MS analysis, the TFC column is reconditioned and primed for the next sample.

Although tissue samples need to be extracted with an organic solvent/buffer, liquid sample preparation can be kept to a minimum and usually involves centrifugation, internal standard addition and transfer to a vial or 96-well plate. Simple HPLC pumps and switching valves can be used to carry out TFC, although specialist equipment, termed high turbulent liquid chromatography (HTLC), is also available. The columns used for TFC contain common HPLC sorbents but of larger particle sizes. The chromatographic efficiency of TFC is similar to that of laminar flow but at much lower flow rates. TFC is also effective at separating residues that are bound to sample proteins [165]. The use of TFC eliminates time-consuming sample clean-up in the laboratory and results in a much shorter analysis time, higher productivity and reduced solvent consumption without sacrificing sensitivity or reproducibility.

Mottier et al. carried out quantitative analysis of 16 quinolones in honey using TFC coupled on-line to LC-MS/MS [166]. Sample preparation involved simple dilution with H_2O followed by filtration and transfer of an aliquot into a vial. Sample extraction time was 4.5 min, while the overall analysis took 18.5 min. Recovery of the method ranged from 85 to 127%, while the LOD of the method was 5 µg kg^{-1}. Krebber et al. used TFC-MS/MS for the rapid determination of enrofloxacin and ciprofloxacin in edible tissues [167]. Tissue samples (bovine, porcine, turkey, rabbit) were extracted with ACN:H_2O:formic acid, filtered and an aliquot injected onto the TFC-MS/MS system. The HTLC column consisted of a Cyclone (50 µm) styrene-divi nylbenzene copolymer. The run time for the analysis was 4 min. The LOQ of the method was 25 µg kg^{-1} in all matrices. The recovery of the method ranged between 72 and 105%.

4.6.3 96-well technology

96-well SPE was developed by researchers at Pfizers in the early 1990s to increase sample throughput in clinical analysis [168]. The technique allows the simultaneous extraction of up to 96 samples and reduces the sample preparation time drastically. It furthermore reduces handling errors and limits labour input. This technique can also be automated to improve precision and accuracy. Rubies et al. developed a 96-well Oasis HLB SPE method for isolating nine quinolone residues from bovine muscle prior to LC-MS/MS [169]. CCα values of as low as 2 μg kg^{-1} could be achieved for norfloxacin. Surprisingly, the sample throughput of the method was limited to 24 test samples per day. Pinel et al. have developed a 96-well SPE procedure on C_{18} chemistry to investigate the profile the 17β-estradiol 3-benzoate and 17β-nandrolone laureate ester metabolites in calves urine [170]. Typical CCα values for analytes were less than 0.10 μg kg^{-1}. The same group also investigated the application of 96-well SPE for the detection of the pesticide, fipronil in ovine plasma [171].

4.6.4 Dialysis

Dialysis in combination with trace enrichment and LC is a relatively simple on-line sample preparation technique. Although it is not very selective, the dialysis cell is easy to construct and the technique is very efficient at removing macromolecules that may interfere with the subsequent separation and detection process. Commercial systems, such as the Gilson ASTED XL, are also available for online dialysis. A more detailed description of dialysis can be obtained through consultation of peer reviewed literature [172-174]. Few applications have been reported in the literature on the use of dialysis in residue analysis. The majority of these applications have been applied to the isolation of antibiotic residues from meat [175], fish [176] and egg [177].

5. Applications

5.1 Silbenes

Stilbenes are non-steroidal estrogenic growth promoters which are banned in the EU for use in food producing animals. They include diethylstilbestrol, hexestrol and dienostrol. Diethylstilbestrol can exist in two forms; cis and trans isomer. Stilbenes

are often analysed in conjunction with other steroids. Stilbenes are partially protein-

bound and require a hydrolysis step in order to achieve high extractability [178]. In urine, stilbenes are present as conjugates (mainly as glucuronic acid form) [179].

Xu et al. developed a method for isolating stilbenes from animal tissues using automated SPE on silica [178]. Enzymatic hydrolysis was found to be necessary to achieve high recovery and good sensitivity, and resulted in a 30% increase in peak areas. Bagnati et al. developed an IAC method for isolating stilbenes from urine and plasma [180]. Samples were applied directly to the IAC columns and subsequently derivatised prior to analysis by GC-MS. Dickson et al. developed a screening method capable of identifying dienostrol and hexestrol from bovine urine using IAC and GC-MS detection [181]. Urine samples were divided into two test portions which were processed separately for stilbenes and zeranol. The urine samples were applied directly to the IAC columns, which could be used up to 10 times before being discarded. Msagati et al. developed a supported liquid membrane (SLM) method for the isolation of stilbenes from bovine kidney, liver, urine and milk [182]. Schmidt et al. developed a method for isolating three stilbenes and six resorcyclic acid lactones from bovine urine [183]. After enzymatic hydrolysis, the pH of the samples was adjusted to pH 9: stilbenes contain a phenolic hydroxyl group which provides a stable anion at high pH. Extraction was carried by LLE and purification was performed by SPE.

5.2 Thyreostats

Thyreostats (TSs) have been banned for use in animal husbandry in the EU since 1981 (Council Directive 81/602/EC) due to their use as growth promoting agents and their potential teratogenic and carcinogenic effects. They act by inhibiting the production of hormones in the thyroid gland, which results in weight gain caused by the increased filling of the gastro-intestinal tract and the retention of H_2O in edible tissues [185]. TSs are amphoteric, highly polar, low molecular weight molecules that are known to undergo rapid tautomerisation and oxidation, which makes them difficult isolate and analyse by MS. 4(6)-R-2-thiouracil, tapazole and 2-mercaptobenzimidazole are the most powerful TS agents and therefore of most interest. However, naturally occurring sulphur compounds also exhibit TS action, namely the thiocynates and oxazolidine-2-thiones. The EU has set a MRPL of 100 μg kg^{-1} for all TS residues. Van den Bussche et al. recently published a review paper on TSs that covers their analysis over

the past 35 years and is a must-read for anyone who wants to better understand the area [185]. Mercurated affinity columns were widely used for the clean-up of extracts, although samples are now purified using conventional SPE.

Pinel et al. were able to isolate seven TS residues from various biological matrices, including urine, muscle, liver, thyroid, animal feed, faeces and hair [186]. Solid samples were freeze-dried, extracted with MeOH and evaporated. Urine and solid samples were diluted with buffer and derivatised. The sample was adjusted to pH 2-3 using HCl (3 5%) and extracted with diethyl ether. Extracts were dried over Na_2SO_4 and evaporated to dryness. Sample clean-up for all matrices was carried out by reconstituting the samples in DCM, adding cyclohexane and performing SPE clean-up with silica cartridges. TS residues were eluted with hexane/EtOAc (40:60, v/v) and evaporated to dryness prior to reconstitution with mobile phase. Tissue and feed required an additional reversed-phase SPE step prior to silica cartridge clean-up. Abuin et al. later developed a simple UPLC-MS/MS method capable of detecting six TSs in thyroid tissue [187]. Samples were extracted with MeOH, evaporated and reconstituted in DCM/cyclohexane. Clean-up was performed on silica SPE cartridges. This sample preparation method is easier and faster than other methods and avoids the derivatisation step, while UPLC reduces analysis time.

5.3 Synthetic steroids and resorcyclic acid lactones

In general, steroid hormones can be divided into three principal groups; estrogens, gestagens and androgens (EGAs). Estrogens are so called because of the important role they play in the estrous cycle, 17 beta-estradiol is the most active of these compounds, its synthetic equivalent is ethinylestradiol. Resorcyclic acid lactones (zeranol and taleranol) are structurally similar to estradiol and exhibit estrogenic effects also and have been used in animal fattening. Directive 81/206/EEC prohibited within the EU the use of certain substances having an hormonal action (testosterone, progesterone, melengestrol acetate, zeranol, trenbolone acetate and 17 beta estradiol) [190]. Further to this Council Directive 96/22/EC prohibits in animal husbandry the administration of substances having thyreostatic, estrogenic, androgenic or gestagenic effects [191]. The EU treats all the above mentioned substances as Group A substances hence there is a zero tolerance policy adopted: regardless of level, no

concentration is permitted in the matrices tested. Steroid compounds are difficult to analyse due to the broad range of substances, the complexity of the matrices and the low levels that must be reached. As a result, it is not possible to have a very specific extraction technique. MeOH is the most widely used solvent for extraction of steroids from tissue samples.

Impens et al. described a procedure for isolating 26 EGAs from muscle and kidney fat with GC-MS/MS detection [192]. Samples were extracted with a mixture of NaAc : MeOH and defatted with n-hexane, prior to partitioning into diethyl ether and silica and NH_2 SPE purification. Blasco et al. extracted 22 EGAs from bovine and porcine muscle using MeOH. However method development activities in this study indicated that CAN was a more selective extraction solvent [193]. In urine, steroids can be present in free, glucuronic acid and sulphate forms, which necessitates the inclusion of enzymatic hydrolysis to liberate conjugates. Shao et al. reports that the portion of cleavable conjugated forms of steroids in tissue are very low, which calls into question the requirement for a deconjugation step [194]. Impens et al. reported a method for 22 EGAs in urine, based on simple dilution with H_2O, adjustment to pH 7 and $_{C18}$ SPE [195]. Subsequently, hydrolysis was carried out by incubation with abalone acetone powder and further clean-up on amino SPE cartridges. Helix Pomatia is widely used to deconjugate both glucuronide and sulphate forms of steroidal compounds. However, some problems have been noted with its use, especially conversion of steroids into other forms because Helix Pomatia possesses oxidoreductase enzyme activity, capable of converting the steroid 3-ol group to a 3-oxo group through oxidation [196]. Also the method is prone to interferences arising from the chromatography step [197].

More advanced techniques for the extraction of EGAs from matrices of animal origin have been reported but these are far less common than the classical techniques previously described. Hooijerink et al. described a method for isolating six gestagens from kidney fat using accelerated solvent extraction (ASE) [198]. Huopalathi et al. extracted seven steroids from bovine tissue samples using supercritical carbon dioxide [199]. Stolker et al. extrated 13 residues from bovine muscle, skin and fat using unmodified supercritical CO_2 and in-line alumina trapping [200]. Surprisingly few applications have been reported in literature for isolating steroids from serum/plasma.

In this area, LLE followed by SPE is widely applied. Ferretti et al. extracted both alpha and beta oestradiol from bovine serum with acetate buffer prior to $_{C18}$ SPE clean-up [201]. Biddle et al. developed a method for isolating estrogens from serum, prior to GC-MS/MS [202]. Samples were deconjugated and acylated in one-step derivatisation with 2,3,4,5,6-pentafluorobenzoylchloride (PFB). Samples were then partitioned with ACN and dried on Na_2SO_4 columns. Fedeniuk et al. developed a method for isolating trenbolone and estradiol from bovine serum prior to GC-MS [203]. Residues were extracted from serum by LL E with 1-chlorobutane and purified on Bond-Elut silica SPE. Draisci et al. extracted five steroids from serum with acetate buffer and purified extracts on $_{C18}$ SPE [204].

5.4 β-agonists

There are several review papers available outlining the extraction and purification techniques used in β-agonist (βA) analysis [210-212]. Target samples for residue analysis include edible tissues, plasma and urine, but also include retina and hair. βAs are divided into two groups: the substituted anilines, including clenbuterol, and the substituted phenols, which include salbutamol. It is necessary to carry out enzymatic or acid hydrolysis of the substituted phenols as they contain conjugated esters, particularly in the form of glucuronides and sulphates [210]. Solvent extraction generally offers good recovery of the substituted anilines but not for the more polar substituted phenols. Adjusting the sample pH to a higher value (usually >9) may be necessary to obtain good recovery of the anilines. However, at high pH significant losses may occur with the phenols, but the use of an ion-pairing reagent can help to overcome this [211]. A range of clean-up procedures can be used to isolate residues including LLP, IAC and MSPD. SPE is the most widely used technique with reversed phase and mixed-mode sorbents.

Moragues et al. developed a method capable of isolating seven βAs from animal liver and urine using a $_{C18}$ SPE clean-up [213]. Fesser et al. developed a method to isolate 12 βAs from liver and retina after protease digestion [33]. Sample extracts were purified on Oasis HLB SPE. A number of mixed mode clean-up procedures have been developed to isolate β-agonist residues. Nielen et al. developed a generic method capable of isolating 22 βAs using Bond Elut mixed-mode [214]. Extraction of urine samples was carried out by enzymatic deconjugation with arylsulphatase/β-

glucuronidase and NaOAc buffer (pH 4.8). Extraction of feed samples involved an acid hydrolysis step (phosphoric acid/MeOH), shaking and the addition of NaOAc buffer to an aliquot of the supernatant. Hair samples were digested with NaOH and later neutralised with 1 M HCl and NaOAc buffer. Williams et al. developed a method for isolating nine βAs from bovine liver and retina [32]. Extracts were purified to mixed-mode HCX 96-well SPE cartridges, which combine strong cation exchange and C_8 reversed phase interactions. Retina samples contained fewer interfering peaks and less ion suppression, which resulted in lower LOQs compared to the liver samples.

MIPs has found widespread application in the βA from bovine muscle [215] and urine [216]. Fiori et al. evaluated M I P and non-endcapped $_{C18}$ SPE columns for the isolation of eight βAs from calves‘ urine, with a special focus on minimizing ion suppression [107]. $_{C18}$ SPE achieved better overall recovery (71 -82%), but suffered from matrix enhancement effects (1.59-2.47%). MIPs had lower recovery (29-63%) but also had much lower matrix interferents (0.23-1.00%). In addition, there was a progressive loss in MS signal intensity for the $_{C18}$ extracts, due to a build-up of matrix on the ESI interface. Wang et al. developed an on-line MIPs method for the selective isolation of ractopamine in pork [217].

A number of more simple procedures have been prepared for isolating βA residues prior to screening analysis. Haughey et al. developed a biosensor-based assay for isolating clenbuterol from bovine urine [218]. Samples extracted with NaOH/MTBE, frozen using an aluminium block precooled in liquid nitrogen and the MTBE layer way carried through to analysis. Other groups have developed screening procedures based on LLE [219] and/or SPE [220,221]. Haasnoot et al. developed a novel immunofiltration sample clean-up for isolation of 10 βAs from urine [141]. Urine samples were mixed with polyclonal antibodies raised against salbutamol and isolated by ultra-filtration. The antibody bound βAs were freed from the antibodies by washing with MeOH/0.1 M HAc and analysed by ELISA. The LOD was 30 times lower than that achieved with urine applied directly to the ELISA.

5.5 Amphenicols

The amphenicol class of antibiotics consist of chloramphenicol (CAP), thiamphenicol (TAP), and florfenicol (FF). While CAP is included in annex IV of Council Regulation 2377/90 (banned substance), TAP and FF are approved for use in all food producing species. Once administered, FF is rapidly metabolized to the more persistent florfenicol amine (FFA). The marker residue of FF is described as the sum of FF and its metabolites measured as FFA [28]. Amphenicols are generally analysed in multi-residue methods and numerous papers have been published for their analysis in food. Zhang et al. developed a method for the isolation of FF and FFA in fish, shrimp and pig muscle based on Oasis MCX clean-up [225]. Zhang et al. latered extended the scope of the method to four amphenicols in chicken muscle [226]. Shen et al. developed a method to isolate CAP, TAP, FF and FFA in poultry and porcine muscle and liver based on Oasis HLB clean-up after EtOAc extraction at alkaline pH [227]. This group introduced a -20°C incubation step to removed lipids.

Van de Riet et al. also developed a simple method to isolate the four amphenicols from aquatic species based acetone extraction and LLP clean-up [228]. Boyd et al. used MIPs to selectively isolate CAP in honey, urine, milk and plasma [229]. Isolation of CAP in honey was compared with MIPs, HLB SPE and LLE. By performing a total ion scan the cleanliness of the extracts were determined. MIPs displayed superior sample clean-up compared to LLE and SPE, as there were fewer interference mass ions in the scan. Ion-suppression effects were also investigated and the MIP extracts displayed minor ion-suppression effects compared to LLE and SPE.

A number of screening assays have been developed to isolate amphenicol residues. Shakila et al. developed a microbial screening assay for the detection of CAP in shrimp after extraction with with EtOAc/NH_4OH and ACN [230]. Luo et al. developed an ELISA method for screening FF residues in fish feed [231]. Sample were extracted with EtOAc, concentrated and purified by simple LLP. The method was later adapted to swine muscle [232]. Huang et al. carried out enzymatic digestion to isolate conjugated CAP residues in carp serum and muscle [233]. The results of this study showed the need to carry out enzymatic or chemical hydrolysis prior to extraction. However, metabolism of CAP varies between species with the conjugated CAP present in pigs but free residues present in cattle and chickens.

5.6 Nitrofurans

Nitrofuran (NF) residues have been banned in the EU since the late 1990s due to their carcinogenic and mutagenic properties. In the early 2000s, methodologies were developed that allowed more effective monitoring of NF residues. The analysis of NF residues has recently been reviewed in detail by Vass et al. [47]. Two approaches can be adopted in NF analysis, namely, extraction of total (free and bound) or bound residues. The total residue approach has been applied by several groups and offers advantages in terms of speed as it eliminates the need for time-consuming washing of tissue [242-247]. NF metabolites (AHD, AOZ, AMOZ and SEM) are usually released from tissue by acid hydrolysis and derivatised (overnight incubation with HCl and 2-nitrobenzaldehyde (NBA)). After neutralization, tissue extracts are typically extracted with EtOAc and undergo solid phase extraction prior to determination by LC-MS/MS. A particular disadvantage of analysing total NF residues is that lower sensitivity can be achieved due to matrix effects.

As an alternative, the bound residue approach can be adopted. This involves the labour intensive washing of tissues to remove free residues and matrix components but produces a cleaner extract [50,248,249]. This approach is used to confirm the presence of NF residues in samples because total residues are now widely considered to be insufficiently specific to identify illegal use of NF residues, especially in the case of nitrofurazone abuse (monitoring of the SEM metabolite). Samples are disrupted in the presence of MeOH:H_2O followed by subsequent washings with ice-cold MeOH, EtOH and diethyl ether. Diethyl ether is allowed to evaporate overnight and the sample pellet is hydrolysed, derivatised and neutralized prior to extraction with EtOAc. A disadvantage of this protocol is that an extra day is required to allow evaporation of the diethyl ether, which increases sample turnaround time. Verdon et al. developed an alternative approach for the determination of bound residues based on two MeOH:H_2O (50:50 and 75:25, v/v) washes followed by a pure MeOH and a pure H_2O wash [247]. The advantage of this approach is that the sample pellet can proceed to the hydrolytic derivatisation step on the same day as washing, reducing assay time by one day.

Recent improvements have been made in NF analysis in honey and milk. Honey is particularly challenging because matrix components interfere with the derivatisation process and result in a lower yield of NF derivatives. Several groups have highlighted

the need to include an SPE clean-up to ensure good yield of the derivatives [250-252]. Jenkins and Young dissolved honey samples in 0.12M HCl (30°C for 30 min) prior to purification on Oasis HLB cartridges [251]. It was proposed that this step removed interfering compounds such as polyphenolic constituents, waxes and organic contaminants. The purified extract containing NF metabolites and sugars was subsequently derivatised overnight with NBA and purified on a second Oasis HLB cartridge prior to analysis. Lopez et al. recently developed an improved method for isolating NFs from honey based on the Jenkins and Young method [252]. Honey samples were dissolved in 10% NaCl instead of 0.1 2M HCl prior to HLB purification. This approach gave higher absolute recoveries than those obtained when the sample was dissolved in 0.1 2M HCl. After derivatisation and pH adjustment, NaCl was added to samples to reduce emulsion formation and enhance the partitioning of NF residues into the EtOAc phase. A hexane wash step was included to remove bee wax and unreacted NBA. This group found that the second HLB SPE step was unnecessary and NF residues could be successfully isolated with EtOAc. Groups have recently reported methods to isolate NF residues from milk [253,254]. Chu et al. derivatised whole milk samples and defatted them with hexane prior to HLB SPE [253]. Rodziewicz et al. developed a simple procedure to isolate NF residues from defatted milk samples [254]. Samples were derivatised and subsequently extracted with EtOAc prior to analysis.

One of the major obstacles for NF analysis is the identification of a suitable marker residue for nitrofurazone abuse. The suitability of SEM as a definitive marker for nitrofurazone misuse has been questioned in light of the discovery that SEM in food may arise from sources (azodicarbonamide and carrageenan) other than this illegal veterinary antibiotic. In response to this problem, Cooper and Kennedy investigated retina as an alternative matrix for verification of NF abuse [36]. This group found that total NF antibiotic metabolites could be detected at mg kg^{-1} levels in the retina of pigs due to the accumulation of drug residues in the eye. It was proposed that retinal analysis may allow detection of NF abuse in animals at any point from birth to slaughter. Cooper et al. also investigated the metabolism of NFs in chicken and found that the intact nitrofuran parent compounds could be detected in the eyes of treated birds [255]. A major advantage of retinal analysis comes from the high

concentrations of NFs that can occur in the retina which allows samples to be analysed by HPLC rather than LC-MS/MS.

5.7 Nitroimidazoles

Nitroimidazoles are imidazole heterocycles with a nitrogen group incorporated in the structure. They can be used for the prophylactic and therapeutic treatments of diseases such as histominiasis and coccidiosis in poultry, genital tricchoniasis in cattle and hemorrhagic enteritis in pigs. These compounds are metabolised extensively in bovine, porcine and avian species [256] and the main metabolism route is through oxidation of the side chain on the C-2 position of the imidazole ring to form hydroxy metabolites. Although ronidazole has a different degradation pathway than dimetrimadazole, they form an identical metabolite [256]. Nitroimidazoles are believed to be carcinogenic and mutagenic to humans [257-259] and as a consequence were banned for the use in food producing animals within the European Union under Regulation 2377/90 [28]. Previously the analysis of these compounds was carried out in liver and muscle [260,261] but studies on the stability and homogeneity of nitroimidazoles in incurred muscle [262,263] show that there is not a homogenous distribution of analyte in turkey muscle and also there is a rapid reduction in analyte concentration in muscle stored for prolonged periods above 4°C. In contrast, nitroimidazole residues are stable in plasma, retina and egg matrices. As a result, plasma, retina and egg have been recommended as target matrices for the residue control of nitroimidazoles [262,263].

A number of methods have been developed to isolate nitroimidazole residues from egg, most based on ACN extraction [256,260,264,265]. Two groups found that after extraction no additional purification was necessary [260,265]. Other groups have found that addition of NaCl and SPE clean-up on Oasis HLB or MIPs was required [256,264]. Many methods exist for the determination of nitroimidazoles in tissue samples [260,266-268]. Polzer et al. developed a method to isolate seven nitroimidazoles from poultry and porcine muscle based on enzymatic hydrolysis followed by purification on kieselguhr SPE cartridges [261]. Xia et al. developed an interesting method that allowed the simultaneous isolation of nitroimidazoles as well as a number of nitrofurans in porcine muscle [269]. A number of groups have developed screening methods to detect nitroimidazole residues based on HPLC-UV

[112] and immunobiosensor detection [270]. Han-Wen Sun et al. reported a sensitive HPLC-UV screening method capable of detecting seven nitroimidazoles in porcine and poultry muscle to <0.8 μg kg^{-1} [112]. Fraselle et al. developed a method to detect seven nitroimidazoles in porcine plasma using NaCl/potassium phosphate buffer and protease solution [271]. Digested samples were purified by SPE. Cronly et al. reported a simple method to isolate 10 nitroimidazole residues from serum [272]. Samples are extracted with a mixture of NaCl and ACN and subsequently defatted with hexane.

5.8 Antibiotics

5.8.1 Aminoglycosides

Aminoglycoside (AMG) residues are basic residues that are soluble in aqueous solvent but are poorly soluble in organic solvent. It has reported that AMG residues are difficult to extract from tissue due to tight binding to proteins and require release by aqueous solution containing strong acid or bases [5]. In recent years, extraction with trichloroacetic acid and subsequent purification on SCX has found widespread application. However, this approach results in low recovery of some residues such as streptomycin because of strong retention on the SCX. Alternative methods have been proposed for the purification of extracts based on MSPD, WCX and ion-pair chromatography [83,114,276]. Recent developments suggest that it is possible to isolate the most important aminoglycoside residues from biological tissues using a single extraction procedure and clean-up on multiple SPE cartridges [114]. The methods have improved significantly but still do not include some residues such as framycetin. In total, from the methods described there are a total of 14 target drugs, with 16 residues if the isomers of gentamicin are included.

Bogialli et al. developed an MSPD method for isolating nine AMG residues from milk based on dispersion on Na_2EDTA-treated sand and hot H_2O extraction at 70°C [83]. Kaufmann and Maden give an excellent report on the development of a WCX method that delivers adequate recovery of streptomycin and 11 other residues from liver, meat and fish prior to LC-MS/MS analyses [276]. Zhu et al. developed a simple method for isolating 13 AMG residues from muscle, liver and kidney based on extraction with 5% TCA and ion pair SPE [114].

5.8.2 β-lactams

β-lactams (βLs) represent a broad class of antibiotics, the most significant of which are the cephalosporins and penicillins. These compounds are typically H_2O-soluble, but are degraded by extremes of pH and elevated temperature. In addition, the - lactam ring structure itself can readily undergo methanolysis, breaking the ring and leading to the formation of methyl ester or penicilloic acids. As a result, MeOH is an unsuitable solvent for extraction and/or analysis of βLs. Consumer MRLs have been laid down for these substances under EU regulation 2377/90 [28]. Issues pertaining to the lack of stability in milk and tissues of certain βLs has been reported in literature [39]. The degradation products of two cephalosporins, ceftiofur and cephapirin, in kidney extract and in acidic and basic solutions was described in recent work by Berendsen et al. [277].

A number of groups have reported the extraction of βLs from milk [278]. Mastovska and Lightfield reported a method for isolating 11 βL antibiotics from bovine kidney based on ACN:H_2O extraction DSPE clean-up on $_{C18}$ [279]. The authors highlighted that extracts should not be diluted in solutions containing formic

acid because they observed rapid degradation of penicillin G and nafcillin. The group report lower recovery for desfuroylceftiofur cysteine disulfide (DCCD), a metabolite of ceftiofur. Other groups have developed direct injection methods for analysing βLs [280,281]. Ito et al. found that significant improvements in sensitivity could be achieved through application of ion-exchange clean-up for isolating penicillins from bovine liver. Katiani et al. developed an on-line SPE method for measurement of sub-ppb levels of βLs in milk by LC-MS/MS [160]. However, the authors noted that matrix effects were evident, leading to ion suppression ranging from 5 to 75%. Oliveira and Cass exploited restricted access media (RAM) columns to separate cepaholsporin residues from milk [282]. Becker et al. developed a comprehensive method for isolating 15 βLs from bovine muscle, kidney and milk prior to LC-MS/MS analysis [283]. Samples were extracted with ACN:H_2O and purified on Oasis HLB cartridge. Daeseleire et al. extracted 11 βLs from milk with ACN and analysed samples without purification by LC-MS/MS [284]. Bruggeman et al. showed that an appropriately imprinted polymer matrix could be used to separate oxacillin from other

penicillin compounds in a mixture [285], which highlights the potential of this separation technology.

5.8.3 Macrolides and lincosamides

Macrolides and lincosamides are two classes of antibiotic with similar antibacterial activity, but differing in chemical structure [5]. The macrolides may be described as being multi-membered lactone rings with one or more sugar moieties attached; the most commonly used members of this compound class are erythromycin and tylosin. The lincosamide antibiotics consist of lincomycin and semi-synthetic derivatives thereof, such as clindamycin and pirlimycin. The structure of lincomycin itself is of a five-membered cyclic amino amide, attached to a thioglycoside side-chain. Both classes of compound are used primarily in food-producing animals for the treatment of bacterial infections, such as mastitis [289]. The broad range of chemical functionalities associated with these compounds can thus pose a challenge to sample preparation. Macrolides are soluble in MeOH, and with isolated or conjugated double bonds, exhibit a somewhat hydrophobic profile. They are unstable in acid, and are typically extracted from alkalinised matrices [290]. Both classes of compound have been extracted using MeOH, aqueous buffer, ACN, or mixtures of ACN and aqueous buffer [291]. A number of sample preparation methods for the isolation of macrolides in food matrices have been reported, as described by Wang in a comprehensive review on analysis of macrolides in samples of food, biological and environmental origin [292]. A range of methods have been developed for the isolation of macrolides and lincosamides using SPE, LLE, direct injection (―dilute and shoot□) and matrix-assisted solid phase dispersion samples preparation approaches.

There has been particular interest in detecting macrolide antibiotic residues in honey in recent years. Benetti et al. extracted five macrolides and lincomycin from honey with tris buffer and purified extracts on Oasis HLB prior to LC-MS/MS [293]. Thompson et al. investigated the fate of tylosin residues in the honey from treated bees [294]. This study showed that tylosin A degrades to yield the antimicrobially active degradation product tylosin B, also known as desmycosin. Wang and Leung later developed a method to detect the residues of seven macrolide residues (including Tylosin B) in eggs, honey and milk prior to LC-MS/MS [295].

A number of methods have been developed for detecting macrolide residues in animal tissue and fish. Bogialli et al. developed a method for hot H_2O extraction isolating six macrolides from milk and yogurt dispersed on sand [85]. Berrada et al. evaluated the suitability of EDTA-McIlvaine buffer and PLE for isolating macrolides from liver and kidney [296,297]. Horie et al. extracted nine ML residues from meat and fish with 0.2% metaphosphoric acid:MeOH (6:4, v/v, 100 mL) [298]. Martos et al. developed a method to measure nine macrolides in animal tissues [299]. Samples were extracted ACN, diluted with H_2O and defatted with hexane prior to LC-MS/MS. This is an interesting application because it contains all three lincosamides. The method developed by Kaufmann is probably the most comprehensive to date because it includes 18 different macrolides and lincosamides [15]; although the total number of macrolides and lincosamides that have been analysed in food in different peer reviewed papers approaches 30 compounds. However, many of these are probably not widely used as veterinary drugs.

5.8.4 Quinolones

Quinolones are antibacterials used for the treatment of infections in both human and veterinary medicine [301]. Their structure consists of an eight-membered heterocyclic system bearing one aromatic ring, a carboxylic acid and a ketone. Modifications to improve antibacterial activity and selectivity have been made, including introduction of fluoro- groups, as well as alkyl and aryl groups. The range of substituents, configurations and chemical properties which quinolones may contain possess challenges to the development of multi-residue methods and the sample preparation steps associated with these compounds must be optimised extensively [302].

Conventional procedures for the isolation of quinolone residues are normally based on solvent extraction with H_2O, acidic aqueous or polar organic solvents (MeOH or ACN). Samples are normally purified on bonded silica or polymer-based SPE phases. Jiménez-Lozano et al. compared seven different kinds of SPE sorbent, including Zorbax $_{C18,}$ Bond Elut $_{C18,}$ Isolute ENV+, Oasis HLB, Oasis MAX, SDB-RPS, and MPC-SD, for the isolation of eight quinolones from animal tissue [303]. The superior performance of the polymer-based sorbents was highlighted. Best results were obtained using SDB-RPS and Oasis MAX cartridges. Similar recovery was observed

for all quinolones on both polymeric sorbents, with the exception of ciprofloxacin which was best recovered on an Oasis HLB cartridge (87%). Christodoulou et al. extracted 10 quinolones from various tissues with 0.1% TFA in MeOH prior to C_{18} SPE clean-up [304]. Christodoulou et al. in the same paper evaluated the suitability of different SPE sorbents including silica and polymeric (DSC- 18, a LiChroLutRP- 18, an Adsorbex C_8 and Abselut NEXUS). LiChroLut RP-18 showed highest analyte recovery, followed closely by Abselut NEXUS.

Zhang et al. analysed 22 quinolones in bovine milk using UPLC-MS/MS as the detection system [305]. Samples were extracted with EDTA-McIlvaine buffer (2 x 10 mL) and the supernatant was applied to a pre-conditioned BondElut Plexa SPE cartridge, which is designed to minimise retention of proteins on the surface of the polymer stationary phase. The quinolones were then eluted with MeOH, the extract was evaporated and reconstituted in 2 mL of mobile phase. Toussaint et al. developed a method for isolating 11 quinolones from pig kidney [306]. Other groups have developed methods for isolating quinolone residues from animal tissues [307]. More novel methods have also been developed including hot water extraction [87]. An effective alternative to quinolone pre-concentration via conventional SPE bonded phases is described by Li et al. [122]. Using an immunoaffinity column the group successfully developed a method to isolate 13 quinolones and six SAs in swine and chicken muscle. Zhao and Li et al. used the same method to isolate 10 quinolones in chicken muscle but used HPLC-FL for analysis [123]. The authors note the possibility of greater selectivity when using such IA columns, compared with the smaller range of interaction mechanisms available for exploitation with more conventional SPE formats.

5.8.5 Sulphonamides

Sulphonamides (SAs) are amphoteric molecules containing different pKa values. They are poorly soluble in H_2O and non-polar solvents, but readily soluble in polar organic solvents. Extraction is typically carried out with DCM, acetone, EtOAc or ACN. The most widely used isolation method for SAs involves LLE followed by SPE clean-up. When extracting SAs from an organic phase into an aqueous phase, it is important to adjust the pH of the aqueous phase to obtain high recovery [10]. This is due to SAs ionic nature, which is caused by the inductive effect of the SO_2 group

[316]. Between pH 5.0 and 5.2 the commonly used SAs are uncharged. The MRL for SAs is reported for muscle, fat, liver, kidney and milk, although other matrices are frequently also analysed (bile, urine and blood serum). The MRL is expressed as the sum of the parent drugs and the combined residues of all substances in the sulphonamide group should not exceed 100 µg kg^{-1}. Hence, it is critical to have methods that are capable of isolating a wide range of SAs. Many sulfonamide formulations are supplied as combination products having two main components, a sulfonamide and a diaminopyrimidine (e.g. trimethoprim and ormethoprim) [317]. These combinations are believed to act synergistically on specific targets in bacterial DNA synthesis. Hence, it is common to analyse diaminopoyrimidines together with SA [276-281]. Recent review papers by Wang et al. [10] and Samanidou et al. [318] provide an excellent overview of SAs in foodstuff of animal origin. The authors discuss the chemistry, antimicrobial activity, legislation and provide a comprehensive review of published methods.

Cai et al. extracted 24 SA residues from muscle with ACN, defatted with hexane and partitioned with H_2O and EtOAc prior to UPLC-MS/MS [319]. Di Sabatino et al. isolated 10 SAs from meat samples using LLE and cation-exchange SPE purification [320]. Gamba et al. used a similar procedure to isolate seven SAs from milk [321]. Forti et al. isolated 10 SAs in egg by extracting with a mixture of acidified (HAc) DCM/acetone and purification on cation-exchange SPE cartridge [322]. Farooq et al. extracted SAs from meat using an ACN/1-propanol solvent system [323]. Sample extracts were purified on Cleanert PEP-SPE cartridges prior to analysis by capillary zone electrophoresis Zou et al. developed a method capable of isolating 12 SAs from animal tissues [324]. Zou et al. used the same derivatisation procedure to determine eight SAs from honey [325]. However, extraction was carried out by MSPD using C_{18} as the dispersant. Sergi et al. developed an MSPD method to isolate 13 SAs from bovine muscle and meat containing baby food using $_{C18}$ as dispersant and chilled MeOH (0°C) as eluting solvent [326]. Li et al. developed an IAC method capable of isolating nine SAs from chicken tissues [327]. Van Rhijn et al. developed a simple ultra-filtration method capable of isolating six SA residues in milk [19]. Samples were mixed with ACN to precipitate proteins and solubilise the SA residues. After ultra-filtration, the extracts were analysed by LC-MS/MS. Koesukwiwat et al.

developed a method for the simultaneous isolation of six SAs, three tetracyclines and pyrimethamine in milk [328].

5.8.6 Tetracyclines

The tetracyclines (TCs) are broad spectrum antibiotics and some of the most widely used veterinary drugs in animal husbandry [334]. Members of the TC group have similar chemical and physico-chemical properties, and are soluble in acids, bases and polar organic solvents (particular alcohols), but insoluble in saturated hydrocarbons. They are amphoteric molecules and only achieve a neutral state as zwitterions. TCs are prone to degradation under strongly acidic and alkaline conditions where they form reversible epimers, namely 4-epi-TCs, anhydro-TCs and iso-TCs. MRLs are established based on the sum of the parent compound and 4-epimer. TCs form chelation complexes with multivalent cations and bind with proteins and silanol groups [335]. Aqueous-based extraction is the primary extraction system for tetracyclines. EDTA is widely used in aqueous extraction and pre-treatment of C_{18} SPE cartridges to minimise TCs interaction with chelating complexes or adsorption onto free silanol groups. Deproteination is normally carried out under mildly acidic conditions using HCl, trichloroacertic or phosphoric acids. LLE from an aqueous phase into an organic phase is difficult to perform due to TCs charge and low affinity for organic solvents. However, ion-pairing reagents can be used to transfer TCs into the organic phase.

Polymeric SPE cartridges have found widespread application in tetracycline analysis in recent years. Pena et al. isolated TC, OTC and CTC residues from porcine tissues using EDTA-McIlvaine buffer (pH 4) [336]. Extracts were deproteinated with 20% TCA and purified on Oasis HLB cartridges. Similar approaches have been adopted by other groups to isolate TCs from milk [337,338]. Nikolaidou et al. developed a method capable of isolating seven TCs from bovine and porcine muscle based on extraction with oxalate buffer (pH 4) prior to purification on Nexus SPE [339]. The same group developed a similar method to isolate seven TCs from bovine liver and kidney [334]. Samples were extracted with 0.4M oxalate buffer (pH 4) and 20% TCA and purified on Discovery (kidney) and LiChrolut (liver) SPE cartridges.

Li et al. developed a simple automated SPE method for on-line extraction of five TCs from honey [340]. The honey samples were diluted in 0.1 M Na_2EDTA-McIlvaine buffer (pH 4) filtered prior to on-line SPE on a $_{C18}$ column. Bogialli et al. developed a simple MSPD method for isolating four TC residues (TC, OTC, CTC, DC) and 3 of their 4-epimers from bovine, porcine and poultry muscle [84]. MSPD was performed with Na_2EDTA treated crystobalite and hot H_2O extraction at 70°C. Some groups have investigated the suitability of MIPs for isolation of TC residues from kidney [341] and animal tissue [342]. Jing et al. encountered difficulties when isolating TC residues from egg and required an alternative MSPD procedure [342]. Bogialli et al. also found that MSPD combined with a hot water extraction was a suitable technique for isolating TCs from muscle tissue [84]. Blasco et al. developed a more automated hot water extraction on an ASETM system [343]. Other groups have developed more novel clean-up procedures using dispersive SPME [344] and metal chelate affinity chromatography (MCAC) [345].

5.9 Anthelmintics

Anthelmintic drugs are used to treat parasitic infections and include benzimidazoles (BZs), flukicides (FCs), levamisole, macrocylic lactones (MLs), and morantel. A number of specific methods exist for the determination of anthelmintic residues. Danaher et al. isolated five M Ls from liver with ACN extraction prior to clean-up on deactivated alumina and $_{C18}$ [348]. Wang et al. developed a similar method but did not carry out the $_{C18}$ SPE [349]. Milk methods require greater sensitivity due to the lower M RLs and because many drugs are not approved for use in lactating species. Some groups have developed sensitive methods based on ACN extraction with C_8 or C_{18} SPE clean-up [350,351]. BZs are more difficult to extract due to the possible presence of some 21 key residues. In addition, it is desirable to include levamisole when testing for BZs but this necessitates LC-MS/MS detection. Dowling et al. developed a multi-residue method for isolating 12 BZs from bovine liver [106]. Samples were extracted by LLE with EtOAc and purified by LLP and automated C_{18} SPE. More comprehensive LC-MS/MS methods have been developed recently. Albin et al. isolated 22 BZs from meat with 1% formic acid and ACN without further clean-up [352]. Radeck et al. subjected milk samples to acid hydrolysis and extracted 23 anthelmintics, including all the main BZ residues and levamisole, with ACN [353]. Extracts were defatted with hexane prior to analysis. Van Holthoon et al. extracted 17

BZs (including levamisole) from milk with ACN and extracts were purified by on-line SPE clean-up using Oasis MAX cartridges [354]. The same groups developed a method to isolate 24 BZs (including levamisole) from egg [20]. Extraction was carried out with ACN and different clean-up procedures were evaluated, including ultra-filtration, off-line SPE and on-line SPE on Oasis MAX cartridges. Ultra-filtration was found to be the preferred extraction technique due to its ease of operation. Few methods have been reported in the literature for the analysis of flukicide residues and msot are mainly single residue methods. Caldow et al. developed an LC-MS/MS assay for phenolic and salicylanilide flukicides in bovine kidney and muscle [355]. Samples were extracted with 1% HAc in acetone and purified on mixed-mode anion-exchange SPE. However, the method was not sufficiently sensitive to allow reliable detection and quantification of oxyclozanide. Kinsella et al. developed and validated a multi-class method capable to isolate 38 anthelmintic residues from bovine milk and liver based on the QuEChERS technique [16].

5.10 Anticoccidials

Anticoccidials (or coccidiostats) are used for treating infections in a range of food producing animals. However, they are most widely used in intensively reared animals (poultry and pigs), followed by calves and lambs. They can be broadly described as polyether ionophores or chemical anticoccidials. Traditionally, anticoccidials were analysed by single residue methods using HPLC or immunochemical assays.

Gerhardt et al. developed one of the first multi-residue methods that allowed detection of three ionophore residues (monensin, salinomycin and narasin) by HPLC with post-column derivatisation UV [362]. Since early 2000s, LC-MS/MS has found widespread application in the analysis of anticoccidials and has allowed the simultaneous detection of their residues at low levels [212]. In addition, some groups have developed assays that additionally include nitroimidazoles [363,364]. However, the nitroimidazoles are normally analysed separately. Blanchflower and Kennedy reported an early method to isolate three ionophore residues from tissue and eggs [365]. Matabudul et al. dispersed egg and liver samples on anh. Na_2SO_4 and extracted four ionophore residues with prior to ACN prior to silica SPE [366]. This method has since been applied by several groups to the analysis of multiple anticoccidials residues from egg, liver and muscle [363,367,368]. Mortier et al. developed a simple

procedure for isolating anticoccidials residues from egg samples without the need for sample purification [364,369].

Difficulties faced in the analysis of anticoccidial residues include the detection of amprolium, semduramicin and toltrazuril residues. Amprolium and semduramicin can present difficulties due poor chromatographic retention and poor peak shape, respectively. In the case of semduramicin, this problem can be offset by the exclusion of Na_2SO_4 from the sample preparation process. Toltrazuril residues (particularly toltrazuril sulphone) present a challenge because of poor response in MS compared to other anticoccidials. As a result, few multi-residue methods have been reported in literature for these residues. Some groups have successfully developed methods to measure toltrazuril residues in eggs [370,371]. Hormazabal and Yndestad developed a complex LLP method for isolating anticoccidial residues including amprolium from tissue, plasma and egg [7]. Amprolium, ethopabate and ionophore extracts were injected separately onto the LC-MS. Olejnik et al. recently developed a comprehensive method for 12 anticoccidial residues from liver based on ACN extraction with purification on neutral alumina and Oasis HLB [372]. One criticism of this method is the exclusion of amprolium, ethopabate and toltrazuril residues. However, the method contains the most important coccidiostat residues, which are outlined in new legislation 2009/124/EC [373] and one can conclude that this is one of the better anticoccidial methods reported in literature to date. Future targets in anticoccidial analysis are the development of methods that will allow the analysis of residues to new non-target MRLs that have been listed for eggs, milk and tissue, while possibly simultaneously detecting nitroimidazoles residues.

5.11 Carbamates

Carbamates are a group of highly effective insecticides sharing the functional group -NH(CO)O-. Several methods have been developed to determine carbamates in a range of food commodities. Carbamate residues may be detected by GC-MS, HPLC fluorescence following post-column derivatisation, or more recently LC-MS. A particular challenge faced in the analysis of carbamate residues is their thermal lability, which highlights the need for careful control of temperature during sample preparation. Ali developed a multi-residue method to detect 10 carbamate residues in liver [374]. Partially frozen liver was mixed with anh. Na_2SO_4 and extracted by

homogenisation in the presence of DCM prior to GPC and aminopropyl Bond Elut SPE purification. Ali highlighted the need to carry out all evaporation steps at 30ºC to ensure satisfactory recovery of the thermally labile carbamate residues [374]. Voorhees et al. subsequently addressed the problem of thermal lability by using on-line SFE - supercritical fluid chromatography coupled to MS (SFC-MS) [375]. Chicken and beef muscle samples were extracted with supercritical CO_2 and trapped on 7% diol on $_{C18,}$ prior to detection by SFC-MS. The system offered advantages in terms of eliminating time consuming solvent evaporation steps. However, disadvantages of the method were that frequent clogging of the cryogenic retention gap resulting in significant downtime and lower recovery when compared with solvent extraction. Argauer et al. extracted carbamate residues from ACN extracts of ground meat using SF-CO_2 (329 bar, 60°C) with off-line trapping on $_{C18}$ [376].

Blasco et al. isolating Pesticide residues (including five carbamates) by dissolved honey in H_2O and applying extracts to $_{C18}$ SPE [377]. Zhen et al. extracted seven carbamate and other pesticide residues from honey samples with MeOH-EtOAc [378]. Extracts were concentrated on an SPE column prepared from Florisil® and anh. Na_2SO_4. Lehotay et al. evaluated the suitability of QuEChERS, traditional SPE and MSPD for isolating 32 pesticides (including carbabryl and propoxur) from milk and egg [63]. The QuEChERS method involved extraction with 1% HAc in ACN followed by addition of anh. $MgSO_4$ (6 g) and anh. NaOAc (1.5 g). Extracts were purified over PSA and $_{C18.}$ SPE clean-up was carried out on $_{C18}$ and PSA in series. MSPD method, involved dispersing samples $_{C18}$ and Na_2SO_4. Dispersed samples were transferred to an empty SPE reservoir, which was stacked on top of a Florisil® cartridge. Analytes were eluted with ACN and concentrated prior to analysis. The methods were found to be comparable although some overestimation was seen using the MSPD method, which was also more time consuming due to requirement of a concentration step.

5.12 Pyrethroids

Pyrethroids are synthetic insecticides derived from naturally occurring pyrethrin compounds which combine efficacy, safety, low environmental hazard and photostability [379]. The widespread use of pyrethroids in crop protection and animal husbandry can lead to the transfer of residues to animal tissues, milk, eggs and honey.

The majority of methods are based on fat analysis because pyrethroids residues accumulate in this matrix [380]. Pyrethroid residues may be purified through adsorption chromatography using Florisil® , silica or alumina, which retain the lipid component of the samples [379]. Sun et al. described a multi-residue method to analyse beef fat for the presence of 18 pyrethroids [381]. Fat was mixed with deactivated Florisil® and packed into a disposable column which was then stacked tandem with a $_{C18}$ SPE column and eluted with ACN. Argauer et al. developed a method to isolate ten pyrethroids from ground meat using SF-CO_2 [382]. Rissato et al. reported a SFE method that combined extraction and Florisil® clean up of pyrethroids residues from honey samples [383]. More recently solid-phase microextraction (SPME), MSPD and QuEChERS have been used in the analysis of pyrethroids. Fernandez-Alvarez et al. developed a multi-residue method to isolate pesticides, including pyrethroids from bovine milk based in SPME [384]. The same group developed an MSPD method for the isolation of 32 pesticide residues, including pyrethroids, from cattle feed using alumina blended with anh. Na_2SO_4. An adsorbent (co-column); Florisil®, was packed at the bottom of the main column to offer a further degree of fractionation and clean-up. The use of the QuEChERS technique for the analysis of permethrins in milk and egg was described by Lehotay and Mastovska [63]. The method involved shaking the sample in a tube with acidified ACN, anh. $MgSO_4$ and anh. NaOAc. Extracts were purified by DSPE using PSA, $_{C18}$ and anh. $MgSO_4$. Stefanelli et al. used automated solvent extraction to isolate pyrethroid residues from ground beef [385]. Beef samples were mixed with anh. Na_2SO_4 and sea sand prior to extraction with light petroleum at 70°C. After H_2O removal (Na_2SO_4), the extract was concentrated and resuspended in hexane. SPE clean-up was performed by a tandem-cartridge system consisting of an Extrelut NT3 (diatomaceous earth) cartridge combined with a Sep-Pack $_{C18}$ cartridge and a Florisil® mini cartridge. The final extract was evaporated and reconstituted in isooctane.

5.13 Sedatives

Sedatives and -adrenergic receptor blockers, such as carazolol and propranolol, are used in farming to reduce the stress levels of animals during transportation, particularly pigs. The most frequently used sedatives include the phenothiazines (such as chlorpromazine, acetopromazine) and butyrophenones (such as azaperone).

The use of phenothiazine sedatives is not permitted (primarily licensed for use in companion animals) in the EU, while the MRL for carazolol in bovine or porcine muscle has been set at 5 g kg^{-1} and the MRL for azaperone residues has been set at 50 g kg^{-1} in animal muscle (including the metabolite azaperol). Despite the varying molecular characteristics of these compounds, multi-residue methods of analysis have been developed.

Govaert et al. isolated five tranquilisers and the beta-blocker carazolol from pig muscle using ACN and purified extracts on SepPak $_{C18}$ [387]. Olmos-Carmona and Hernández-Carrasquilla isolated seven tranquilisers from urine using $_{C18}$ SPE cleanup [388]. Samples required dilution in TEA to minimise residual silanol effects. Zhang et al. investigated the suitability of silica, NH_2, $_{C18}$ and Oasis HLB sorbents for isolating 19 -blockers and 11 sedatives from animal tissue prior to LC-MS/MS [389]. Satisfactory purification of ACN extracts were achieved using NH_2. Polymeric bonded stationary phases for SPE cartridges are often preferred for sedatives due to the absence of secondary retention effects associated with free silanol groups found on $_{C18}$ bonded phases. Kaufmann and Ryser samples extracts on Oasis HLB prior to LC-MS/MS [390]. Delahaut et al. similarly exploited Oasis HLB SPE in their work on tranquilisers and -blockers in pig tissues [391,392]. Some alternative methods have been developed for the isolation of these compounds other than hydrophobic sorbents. Cerkvenik-Flaj s investigated the suitability of mixed-mode cationic exchange sorbent (Oasis MCX SPE) for isolating azaperone and azaperol from kidney prior to HPLC fluorescence detection [393]. Cooper et al. extracted three phenothiazines with acidified ACN and purified extracts by LLP prior to ELISA detection [394].

5.14 Non steroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous group of mostly acidic drugs that can be divided into four main sub-classes: (i) salicyclic acid derivatives (ii) propionic acid derivatives (iii) pyrazoles derivatives and (iv) aniline derivatives, including both anthracilic and nicotinic acid derivatives. They all share common pharmacological activity, but they are not structurally related, making universal extraction and purification techniques difficult. Effective detection of a

range of NSA IDs may be achieved through the analysis of residues in liver or kidney, while residues in live animals may be monitored in biological fluids and milk. In order to get the best possible overview of the use of NSA IDs in food-producing animals and of their residues in food of animal origin, a broad control of as many substances of this group as possible should be aspired to. ACN is a commonly used extraction solvent for these substances and further purification is often carried out using SPE and or LLP. In recent years there have been a small number of multi-residue methods which detect quite a wide range of NSA IDs. Daeseleire et al. developed a simple method for the detection of three NSAIDs in milk based on ACN extraction and concentration prior to LC-MS/MS [395]. Van Hoof et al. reported a method for the determination of six NSAIDs in bovine tissue using ACN extraction and Oasis HLB purification [396]. Metamizole (Dipyrone) is one of the few basic NSAIDs and very few methods report the analysis of this substance in foods of animal origin. Penney et al. reported an LC-MS/MS method for the detection of metamizole and its marker metabolites of which 4 Methyl Amino Antipyrine (4-MAA) is the most important in milk and muscle [397]. Samples were extracted with MeOH defatted with hexane prior to analysis.

Malone et al. developed a method for detecting all licensed NSAIDs including 4-MAA (and all licensed corticosteroids) in milk by LC-MS/MS [398]. Samples were extracted with ACN and NaCl was added to aid partitioning of the milk and ACN phase. ACN extracts were simply defatted with hexane prior to analysis. Vinci et al. reported a method for 14 NSA IDs in plasma and serum based on protein denaturation prior to $_{C18}$ SPE purification [399]. Gallo et al. reported a method for detecting 13 NSAIDs in plasma and serum based on the previous method prior to HPLC-PDA. Dowling et al. developed a method for the detection of four NSAIDs in milk. Milk samples were extracted with ACN and purified on IsoluteTM $_{C18}$ SPE cartridges [400]. Gallo et al. developed another method for the detection and confirmation of 16 NSAIDs in cattle and buffalo milk [401]. Milk samples were extracted using a mixture of ACN:MeOH (90:10, v/v). Gonzalez et al. developed a LLE method to isolating 17 NSAIDs from equine plasma and urine samples [402]. NSAIDs have also been analysed as part of multi-class multi-residue methods. Stolker et al. developed a method to analyse 20 NSAIDs (and other drugs) in milk using UPLC-ToF MS [13]. Chrusch et al. developed a method to analyse 10 NSAIDs in bovine

muscle and kidney by LC-MS/MS [403]. Other groups have developed methods for analysing NSAIDs in equine plasma and urine [404,405]. These multi-class methods are discussed in greater detail in a later section *(5.16 multi-class multi-residue analysis*).

5.15 Triphenylmethane dyes

Triphenylmethane dyes (TPMs), including malachite green (MG) and crystal (gentian) violet (CV), are used illegally in aquaculture to treat and prevent fungal and parasitic infections [409]. MG and CV are rapidly metabolised to their non-polar leuco-MG (LMG) and leuco-CV (LCV) forms, which have a longer half-life in fish than the parent compounds [410]. MG is not registered as a veterinary drug and has potential toxicity, teratogenicity and carcinogenicity effects [411]. As a result it is not authorised for use in animals that are produced for human consumption and the EU has set a MRPL of 2 μg kg^{-1} (sum of MG and LMG) in aquaculture [412]. TPMs are acidic molecules that are readily ionisable and suitable for ion-exchange clean-up [413]. The non-chromophorous leuco analogues are often oxidised to their chromophorous parent compound using PbO_2 or other oxidising reagents [414]. It has been reported that MG and LMG are rapidly degraded (most likely through demethylation) during sample preparation [411,415].

Andersen et al. developed a method capable of isolating MG, CV, brilliant green (BG) and leucobrilliant green (LBG) [416]. Fish samples were extracted with ammonium acetate buffer (pH 4.5), hydroxylamine hydrochloride, *p*-toluene sulfonic acid and ACN. Samples were shaken in the presence of alumina and the supernatant was subsequently mixed with H_2O and diethylene glycol. The mixture was extracted with DCM in a separatory funnel, evaporated to dryness and reconstituted in ACN and 2,3-dichoro-5,6-dicyanobenzoquinone (DDQ). The addition of DDQ converts leuco compounds to their charged form which enhances sensitivity. The samples then underwent SPE clean-up with alumina and propylsulfonic acid cartridges in series. Jiang et al. later modified this method and applied it to the analysis of TPMs in fish [417]. A number of groups have developed alternative methods for isolating TPM residues using McIlvaine buffer and ACN prior to purification on aromatic sulphonic acid [413], SAX [418] or alumina-MCX SPE sorbents [415,419]. Other groups have

used an alternative ammonium acetate and ACN extraction solvent prior to PRS [409,420] or alumina-SCX [410] clean-ups.

5.16 Multi-class multi-residue analysis

Yamada et al. reported the first multi-class LC-MS/MS method for >100 residues in 2006 [12]. A number of other groups have followed this trend. While these methods offer many advantages (simplicity, high sample-throughput, reduced cost) there are compromises to be made when developing these methods (lack of specificity or sensitivity, crude extracts that can cause problems for the detection system). These methods may be broken into the following categories – qualitative [421] or quantitative screening assays [15], which use ToF MS, and quantitative confirmatory assays using LC-MS/MS. Therefore, in some cases samples may require additional confirmatory analysis using a second technique. Another disadvantage is that while cost per analyte is greatly reduced, the overall cost per sample is more expensive due to the comprehensive information provided from the analysis. In the EU, there is no advantage in applying such methods due to the present structure of European residue control plans. One can conclude that if such methods were applied in residue surveillance that the current number of samples tested for Group B substances could be reduced. However, one expects that many stakeholders will request modifications to legislation to accommodate such methodology in the future.

The main obstacle in multi-class residue analysis is the development of a generic sample preparation step. Most multi-class methods involve simple liquid extraction while clean-up is carried by LLP or SPE on a generic cartridge (e.g. HLB). The degree of clean-up provided by many of these methods is usually limited because extensive purification would invariably result in total loss of some residues. Other groups have developed simple approaches such as simple ―dilute-and-shoot□, ultra-filtration or on-line column switching. Yamada et al. developed a screening method capable of isolating 130 residues from bovine, porcine and chicken muscle [12]. Residues isolated included antibiotics, antibacterials, anthelmintics and hormonal agents. Samples were dispersed on Na_2SO_4, extracted with ACN/MeOH and defatted with hexane-saturated with ACN, prior to LC-MS/MS.

Stolker et al. isolated 101 veterinary drugs from milk prior to UPLC-ToF MS analysis [13]. Milk samples were extracted with ACN and purified on Strata-X SPE cartridges prior to analysis. Peters et al. adopted a similar approach to isolate 100 residues from egg, fish and meat prior to UPLC-ToF MS analysis [421]. Ortelli et al. isolated 150 veterinary drugs from milk prior to UPLC-ToF MS detection [14]. Samples were deproteinated with ACN, centrifuged and an aliquot of supernatant underwent ultra-filtration. This group found that recovery exceeded acceptable values for several residues, most noticeably MLs (≤10%), quinolones (98-807%), TCs (141-258%), cefquinome (661%) and some benzimidazoles (>436%). The low recovery for MLs was attributed to their larger molecular weight, while other poor recovery results were attributed to ion enhancement/suppression effects.

Kaufmann et al. reported a method for isolating 100 residues from muscle, liver and kidney prior to UPLC-ToF MS analysis [15]. Samples were extracted using the bipolarity approach (see **Fig. 6**). Kaufmann identified difficulties in the isolation of some polar residues, which adsorbed on precipitated proteins or glassware. This was overcome by rinsing the sample and glassware with DMSO and complexing buffer.

A number of other multi-class methods have been developed by other groups but not for such a wide range of analytes. Stubbings and Bigwood developed a QuEChERS based method to isolate 41 residues from tissues prior to LC-MS/MS analyses [17]. Purification was carried out by dispersive-SPE on Bondesil NH_2. An additional SCX-SPE purification step was required to allow satisfactory detection of nitroimidazole residues. Stubbings et al. also developed a method to isolate basic drugs, including BZs, SAs, tranquilizers, quinolones, nitroimidazoles, levamisole, MG and LMG from animal tissue [112]. Samples were extracted using a similar approach to their QuEChERS assay but were purified on Bond Elut SCX SPE cartridges. MG and LMG required an alternative extraction with citrate buffer/ACN and LLP with DCM and NaCl prior to SCX SPE. Aguilera-Luiz et al. also developed a simple QuEChERS based extraction procedure to isolate 18 drug residues from milk [72]. No further clean-up was performed.

Yang et al. developed a method for the analysis of 50 anabolic steroids in muscle, liver and milk using UPLC-MS/MS [422]. Samples were hydrolysed and extracted

with MeOH prior SPE clean-up. HLB and $_{C18}$ cartridges gave similarly high recovery, but GCB-NH_2 clean-up offered superior clean-up and lower matrix suppression effects.

Chrusch et al. describe a multi-class, multi-residue method that is capable of analysing 29 veterinary drugs, including NSAIDs, corticosteroids and anabolic steroids, in bovine muscle and kidney [403]. After acid hydrolyis and protease digestion, samples underwent LLE and purification by SPE. Yu et al. report a method for the detection of 66 acidic and neutral drugs, including NSA IDs, corticosteroids and anabolic steroids, in equine plasma by LC-MS/MS [404]. Plasma samples were deproteinated, diluted with buffer and purified on Bond Elut Certify SPE cartridges prior to analysis.

Several multi-class methods have been published for the analysis of antibiotics, Chico et al. developed a simple method for isolating 39 antibiotics in tissue [423].

Samples were extracted with MeOH:H_2O containing 0.1 M EDTA and diluted in H_2O prior to U PLC-MS/MS. Shao et al. developed a method to isolate 21 antibiotics (7 TCs, 14 quinolones) from porcine kidney, liver and muscle [424]. Samples were extracted with EDTA-McIlvaine buffer and purified on Oasis HLB cartridges prior to UPLC-MS/MS analysis. McDonald et al. developed a method for isolating 19 veterinary drugs (TCs, SAs, trimethoprim and dapsone) from muscle [425]. Residues analysed include TCs, SAs, trimethoprim and dapsone. Samples were extracted with 0.1 M EDTA/ACN, concentrated and reconstituted in H_2O prior to UPLC-MS/MS analysis. Granelli and Branzell developed a screening method to isolate 19 antibiotics from animal tissues [426]. Samples were simply extracted with 70% MeOH and diluted with H_2O prior to analysis by LC-MS/MS. Carretero et al. used automated PLE to isolate 31 antibiotics from tissue [427].

Turnipseed et al. developed a rapid method for analysing 25 antibiotics, including βLs, SAs, TCs, quinolones and macrolides, in milk [428]. Samples were extracted with ACN and purified on Oasis HLB SPE cartridges. Additional purification was performed by ultra-filtration prior to LC-MS/MS analysis. Li et al. developed a screening method to isolate 18 drugs in shrimp [429]. Residues, including SAs,

quinolones, TPM dyes, OTC and toltrazuril sulphone, were extracted by LE and purified on Oasis HLB cartridges.

6. Conclusions/future trends

In the area of banned substances there is a continued trend towards the development of assays to detect ultratrace levels of residues of illegal substances. These methods generally involve more intensive preparation of samples to allow detection of residues to ng kg^{-1} levels. It is expected that the trend to develop more selective isolation procedures using IAC and MIPs will continue but will be limited to illegal substances. In contrast to licensed veterinary drugs, banned substances can not always be combined in methods due to a number of specific sample preparation steps, such as derivatisation (e.g. release of bound residues or deconjugation) and matrix type (retina, thyroid, hair, etc.).

It can be seen from this paper that there is a growing trend to pack more and more residues into methods particularly for veterinary drugs. Groups are now applying less specific sample preparation approaches including ―dilute-and-shoot or protein precipitation combined with ultra-filtration. In addition, more generic clean-up procedures are being adopted that provide more basic clean-up such as QuEChERS or the bipolar extraction approach for isolation and enrichment of polar and non-polar residues. These new approaches coupled to modern mass spectrometry based detection systems allow the analysis of >100 drug residues in food and provide significant benefits. The authors of this review can testify to the benefits of multi-class detection, where the implementation of an assay for anthelmintic drug residues has resulted in fivefold reduction in solvent usage, through the redundancy of traditional SPE and HPLC analysis. This has led to an increase in the output for these substances in our laboratory from approximately 10,000 to 40,000 results per annum for individual analytes. One can conclude that the scope of drug residue methods is fast approaching that of pesticide residue methods.

A major problem with some of these multi-class methods is that there are often compromises observed in LODs, chromatography and quantitation due to poor linearity. As such many of these methods can be categorised as satisfactory for

screening purposes, particularly when analysis is carried out by Tof MS. Going forward one can expect that future assays in this area may follow the route taken by pesticide residue scientists, where assays might be divided into two groups for higher polar and medium to non-polar compounds. In addition, a number of hurdles will have to be overcome because the consolidation of methods will lead to increased numbers of samples being passed through multi-class methods. This will initially result in an increase in testing costs because residue analysis is traditionally a low throughput technique. The current bottlenecks particularly in the area of sample homogenisation of animal tissue will need to be addressed. Although, there are some systems that can provide high throughput processing of tissue, these have not been extensively evaluated in the field of residue analysis. Also the current 24 at a time footprint adopted in traditional residue analysis purification is not ideally suited to automated handling systems. The transfer of assays to 96-well format would be advantageous to improve throughput of samples. Inevitably, this would require adaptation in the areas of SPE, centrifugation, filtration and injection of samples. There has been intense development in the area of sorbent technologies in recent years leading to the development of generic polymeric HLB type sorbents, which appear to offer the best all round generic SPE clean-up solution. Although the development of a HILIC sorbent would be welcome for polar compounds.

If the above obstacles are overcome, one can expect that routine application of screening assays for licensed veterinary drugs will be reduced. The analysis of antibiotic residues using inhibitory assays will only have application in an industry environment. Regulatory agencies will have to reconsider the approach to the design of the national residue surveillance programs. The increase in the scope of residue methods will invariably lead increased rates of non-compliant residue detection in food similar to that in the field of pesticide residue analysis. As a result, decision makers will have to consider the sampling numbers and cost benefit. It can be concluded that developments in multi-class sample preparation procedures will provide us with a clearer picture of the incidence of contaminant residues in food. This may lead to a future cycle of development of targeted methods that will address unique residue problems.

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References

[1] P.L. Buldini, L. Ricci, J.L. Sharma, J. Chromatogr. A 975 (2002) 47.
[2] C. Yu, L.H. Cohen, Lc Gc Europe 17 (2004) 96.
[3] K. Ridgway, S.P.D. Lalljie, R.M. Smith, J. Chromatogr. A 1153 (2007) 36.
[4] Y. Chen, Z. Guo, X. Wang, C. Qiu, J. Chromatogr. A 1184 (2008) 191.
[5] S.B. Turnipseed, A.R. Long, Analytical Procedures For Drug Residues In Food Of Animal Origin, Science Technology System, 1998.
[6] M. O'Keeffe, Residue Analysis in Food: Principles and Applications, CRC Press, 2000.
[7] V. Hormazabal, M. Yndestad, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 1585.
[8] C.R. Anderson, H.S. Rupp, W.H. Wu, J. Chromatogr. A 1075 (2005) 23.
[9] M. Danaher, L.C. Howells, S.R.H. Crooks, V. Cerkvenik-Flajs, M. O'Keeffe, J. Chromatogr. B 844 (2006) 175.
[10] S. Wang, H.Y. Zhang, L. Wang, Z.J. Duan, I. Kennedy, Food Addit. Contam. 23 (2006) 362.
[11] M. Danaher, H. De Ruyck, S.R.H. Crooks, G. Dowling, M. O'Keeffe, J. Chromatogr. B 845 (2007) 1.
[12] R. Yamada, M. Kozono, T. Ohmori, F. Morimatsu, M. Kitayama, Biosci., Biotechnol., Biochem. 70 (2006) 54.
[13] A.A.M. Stolker, P. Rutgers, E. Oosterink, J.J.P. Lasaroms, R.J.B. Peters, J.A. van Rhijn, M.W.F. Nielen, Anal. Bioanal. Chem. 391 (2008) 2309.
[14] D. Ortelli, E. Cognard, P. Jan, P. Edder, J. Chromatogr. B In Press, Corrected Proof.
[15] A. Kaufmann, P. Butcher, K. Maden, M. Widmer, J. Chromatogr. A 1194 (2008) 66.
[16] B. Kinsella, S.J. Lehotay, K. Mastovska, A.R. Lightfield, A. Furey, M. Danaher, Anal. Chim. Acta 637 (2009) 196.
[17] G. Stubbings, T. Bigwood, Anal. Chim. Acta 637 (2009) 68.
[18] J.P. Antignac, K. de Wasch, F. Monteau, H. De Brabander, F. Andre, B. Le Bizec, Proceedings of the EuroResidue V Conference, Noordwijkerhout, The Netherlands (2004) 129.
[19] J.A. van Rhijn, J.J.P. Lasaroms, B.J.A. Berendsen, U.A.T. Brinkman, J. Chromatogr. A 960 (2002) 121.
[20] F.L. van Holthoon, P. Aqai, T. Zuidema, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 867.
[21] H. De Ruyck, E. Daeseleire, K. Grijspeerdt, H. De Ridder, R. Van Renterghem, G. Huyghebaert, J. Agric. Food Chem. 49 (2001) 610.
[22] H. De Ruyck, E. Daeseleire, K. Grijspeerdt, H. De Ridder, R. Van Renterghem, G. Huyghebaert, Br. Poult. Sci. 45 (2004) 540.

[23] I. Reyes-Herrera, M.J. Schneider, K. Cole, M.B. Farnell, P.J. Blore, D.J. Donoghue, J. Food Prot. 68 (2005) 2217.
[24] J.M. Delmas, A.M. Chapel, V. Gaudin, P. Sanders, J. Vet. Pharmacol. Ther. 20 (1997) 249.
[25] J.L. Nappier, G.A. Hoffman, T.S. Arnold, T.D. Cox, D.R. Reeves, V.L. Hubbard, J. Agric. Food Chem. 46 (1998) 4563.
[26] A. Lifschitz, F. Imperiale, G. Virkel, M.M. Cobenas, N. Scherling, R. DeLay, C. Lanusse, J. Agric. Food Chem. 48 (2000) 6011.
[27] C. Prats, G. El Korchi, R. Francesch, M. Arboix, B. Perez, Res. Vet. Sci. 73 (2002) 323.
[28] Off. J. Eur. Union. L224 (1990) 1.
[29] D.R. Doerge, M.I. Churchwell, C.L. Holder, L. Rowe, S. Bajic, Anal. Chem. 68 (1996) 1918.
[30] F. Ramos, P. Gonzalez, A. Oliveira, A. Almeida, C. Fente, C. Franco, A. Cepeda, M.I.N. da Silveira, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 251.
[31] J.H.W. Lau, C.S. Khoo, J.E. Murby, J. AOAC Int. 87 (2004) 31.
[32] L.D. Williams, M.I. Churchwell, D.R. Doerge, J. Chromatogr. B 813 (2004) 35.
[33] A.C.E. Fesser, L.C. Dickson, J.D. MacNeil, J.R. Patterson, S. Lee, R. Gedir, J. AOAC Int. 88 (2005) 61.
[34] A. Gleixner, H. Sauerwein, H.H.D. Meyer, Food. Agric. Immunol. 9 (1997) 27.
[35] B. Le Bizec, F. Courant, I. Gaudin, E. Bichon, B. Destrez, R. Schilt, R. Draisci, F. Monteau, F. Andre, Steroids 71 (2006) 1078.
[36] K.M. Cooper, D.G. Kennedy, Analyst 130 (2005) 466.
[37] M.D. Rose, J. Bygrave, W.H.H. Farrington, G. Shearer, Analyst 122 (1997) 1095.
[38] S. Riediker, A. Rytz, R.H. Stadler, J. Chromatogr. A 1054 (2004) 359.
[39] E. Verdon, R. Fuselier, D. Hurtaud-Pessel, P. Couedor, N. Cadieu, M. Laurentie, J. Chromatogr. A 882 (2000) 135.
[40] J.D.G. McEvoy, J.P. Ferguson, S.R.H. Crooks, D.G. Kennedy, L.A. van Ginkel, G. Maghuin-Rogister, H.H.D. Meyer, M.W. Pfaffl, W.H.H. Farrington, M. Juhel-Gaugain, in 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St Jan, Belgium, 1998, p. 2535.
[41] E.P. Papapanagiotou, D.J. Fletouris, E.I. Psomas, Proceedings of the EuroResidue V Conference, Noordwijkerhout, The Netherlands (2004) 305.
[42] U.T. Sireli, A. Filazi, O. Cadirci, Union of Scientists National Conference, Stara Zagora, Bulgaria (2005) 441.
[43] Off. J. Eur. Union. L221 (2002) 8.
[44] J.D.G. McEvoy, S.R.H. Crooks, C.T. Elliott, W.J. McCaughey, D.G. Kennedy, in 2nd International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St Jan, Belgium, 1994, p. 2603.
[45] A.D. Cooper, J.A. Tarbin, W.H.H. Farrington, G. Shearer, Food Addit. Contam. 15 (1998) 637.
[46] D.N. Heller, J.O. Peggins, C.B. Nochetto, M.L. Smith, O.A. Chiesa, K. Moulton, J. Chromatogr. B 821 (2005) 22.
[47] M. Vass, K. Hruska, M. Franek, Vet. Med. (Praha). 53 (2008) 469.
[48] http://www.emea.europa.eu/pdfs/vet/mrls/nitrofurans.pdf.

[49] L.A.P. Hoogenboom, M.C.J. Berghmans, T.H.G. Polman, R. Parker, I.C. Shaw, Food Addit. Contam. 9 (1992) 623.
[50] A. Conneely, A. Nugent, M. O'Keeffe, P.P.J. Mulder, J.A. van Rhijn, L. Kovacsics, A. Fodor, R.J. McCracken, D.G. Kennedy, Anal. Chim. Acta 483 (2003) 91.
[51] http://cpharm.vetmed.vt.edu/VM8784/ANTIMICROBIALS/FOI/141063.htm.
[52] C.L. Wrzesinski, L.S. Crouch, R. Endris, J. AOAC Int. 86 (2003) 515.
[53] J.E. Wu, C. Chang, W.P. Ding, D.P. He, J. Agric. Food Chem. 56 (2008) 8261.
[54] M. O'Keeffe, J.J. Finnegan, Proceedings of the EuroResidue IV Conference, Veldhoven, The Netherlands (2000) 792.
[55] E. Horne, T. Coyle, M. O'Keeffe, M. Alvinerie, P. Galtier, D.L. Brandon, J. Agric. Food Chem. 51 (2003) 5552.
[56] E. Daeseleire, A. Deguesquiere, C. Vanpeteghem, Z. Lebensm. Unters. Forsch. 192 (1991) 105.
[57] R.J. McCracken, D.E. Spence, D.G. Kennedy, Food Addit. Contam. 17 (2000) 907.
[58] A. Koole, J. Bosman, J.P. Franke, R.A. de Zeeuw, J. Chromatogr. B 726 (1999) 149.
[59] Y. Babin, S. Fortier, J. AOAC Int. 90 (2007) 1418.
[60] D.J. Fletouris, E.P. Papapanagiotou, 14th European Conference on Analytical Chemistry, Antwerp, Belgium (2007) 1189.
[61] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[62] A. Posyniak, J. Zmudzki, K. Mitrowska, in 25th International Symposium on Chromatography, Paris, France, 2004, p. 259.
[63] S.J. Lehotay, K. Mastovska, S.J. Yun, J. AOAC Int. 88 (2005) 630.
[64] C.K. Fagerquist, A.R. Lightfield, S.J. Lehotay, Anal. Chem. 77 (2005) 1473.
[65] K. Mastovska, S.J. Lehotay, J. Agric. Food Chem. 54 (2006) 7001.
[66] F. Plossl, M. Giera, F. Bracher, J. Chromatogr. A 1135 (2006) 19.
[67] C. Pan, H. Zhang, S. Chen, Y. Xu, S. Jiang, Acta Chromatographica 17 (2006) 320.
[68] S.J. Lehotay, J. AOAC Int. 90 (2007) 485.
[69] T.D. Nguyen, B.S. Lee, B.R. Lee, D.M. Lee, G.H. Lee, Rapid Commun. Mass Spectrom. 21 (2007) 3115.
[70] P. Paya, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva, A. Barba, Anal. Bioanal. Chem. 389 (2007) 1697.
[71] T.S. Thompson, J.P. van der Heever, D.K. Noot, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 549.
[72] M.M. Aguilera-Luiz, J.L.M. Vidal, R. Romero-González, A.G. Frenich, J. Chromatogr. A 1205 (2008) 10.
[73] S.J. Lehotay, K. Mastovska, A.R. Lightfield, J. AOAC Int. 88 (2005) 615.
[74] S.A. Barker, A.R. Long, C.R. Short, J. Chromatogr. 475 (1989) 353.
[75] E.M. Kristenson, L. Ramos, U.A.T. Brinkman, Trends Anal. Chem. 25 (2006) 96.
[76] S.A. Barker, J. Chromatogr. A 885 (2000) 115.
[77] S. Bogialli, A. Di Corcia, J. Biochem. Biophys. Methods 70 (2007) 163.
[78] L. Zhang, Y. Liu, M.X. Xie, Y.M. Qiu, J. Chromatogr. A 1074 (2005) 1.
[79] Q.H. Zou, Y. Liu, M.X. Xie, J. Han, L. Zhang, Anal. Chim. Acta 551 (2005) 184.

[80] Y. Liu, Q.H. Zou, M.X. Xie, J. Han, Rapid Commun. Mass Spectrom. 21 (2007) 1504.
[81] C. Ferrer, M.J. Gómez, J.F. García-Reyes, I. Ferrer, E.M. Thurman, A.R. Fernández-Alba, J. Chromatogr. A 1069 (2005) 183.
[82] S. Bogialli, R. Curini, A. Di Corcia, M. Nazzari, M.L. Polci, J. Agric. Food Chem. 51 (2003) 4225.
[83] S. Bogialli, R. Curini, A. Di Corcia, A. Lagana, M. Mele, M. Nazzari, J. Chromatogr. A 1067 (2005) 93.
[84] S. Bogialli, R. Curini, A. Di Corcia, A. Lagana, G. Rizzuti, J. Agric. Food Chem. 54 (2006) 1564.
[85] S. Bogialli, A. Di Corcia, A. Lagana, V. Mastrantoni, M. Sergi, Rapid Commun. Mass Spectrom. 21 (2007) 237.
[86] S. Bogialli, G. D'Ascenzo, A. Di Corcia, G. Innocenti, A. Lagana, T. Pacchiarotta, Rapid Commun. Mass Spectrom. 21 (2007) 2833.
[87] S. Bogialli, G. D'Ascenzo, A. Di Corcia, A. Laganà, S. Nicolardi, Food Chem. 108 (2008) 354.
[88] S. Bogialli, G. D'Ascenzo, A. Di Corcia, A. Laganà, G. Tramontana, J. Chromatogr. A 1216 (2009) 794.
[89] V. Camel, Analyst 126 (2001) 1182.
[90] C.S. Eskilsson, E. Bjorklund, J. Chromatogr. A 902 (2000) 227.
[91] M.H. Akhtar, L.G. Croteau, C. Dani, K. AbouElSooud, in 109th AOAC International Meeting, Ios Press, Nashville, Tn, 1995, p. 33.
[92] M.H. Akhtar, J. Environ. Sci. Health. B. 39 (2004) 835.
[93] J.L. Hedrick, L.J. Mulcahey, L.T. Taylor, Mikrochim. Acta 108 (1992) 115.
[94] L.T. Taylor, Supercritical Fluid Extraction, Wiley-Interscience, 1996.
[95] M. Zougagh, M. Valcarcel, A. Rios, Trends Anal. Chem. 23 (2004) 399.
[96] R. Carabias-Martinez, E. Rodriguez-Gonzalo, P. Revilla-Ruiz, J. Hernandez-Mendez, J. Chromatogr. A 1089 (2005) 1.
[97] L. Ramos, E.M. Kristenson, U.A.T. Brinkman, J. Chromatogr. A 975 (2002) 3.
[98] H. Giergielewicz-Mozajska, L. Dabrowski, J. Namiesnik, Crit. Rev. Anal. Chem. 31 (2001) 149.
[99] E.M. Thurman, M.S. Mills, Solid-Phase Extraction: Principles and Practice, Wiley-Interscience, 1998.
[100] J.S. Fritz, Analytical Solid-Phase Extraction, Wiley-VCH, 1999.
[101] C.W. Huck, G.K. Bonn, J. Chromatogr. A 885 (2000) 51.
[102] M.C. Carson, J. Chromatogr. A 885 (2000) 343.
[103] V. Camel, Spectrochim. Acta, Part B 58 (2003) 1177.
[104] N. Fontanals, R.M. Marcé, F. Borrull, Trends Anal. Chem. 24 (2005) 394.
[105] A. Zwir-Ferenc, M. Biziuk, Pol. J. Environ. Stud. 15 (2006) 677.
[106] G. Dowling, H. Cantwell, M. O'Keeffe, M.R. Smyth, Anal. Chim. Acta 529 (2005) 285.
[107] M. Fiori, C. Civitareale, S. Mirante, E. Magaro, G. Brambilla, Proceedings of the EuroResidue V Conference, Noordwijkerhout, The Netherlands (2004) 207.
[108] J.P. Antignac, B. Le Bizec, F. Monteau, F. Poulain, F. Andre, J. Chromatogr. B 757 (2001) 11.
[109] M. Danaher, M. O'Keeffe, J.D. Glennon, Analyst 125 (2000) 1741.
[110] M. Cossu, M.C. Alamanni, Ital. J. Food Sci. 15 (2003) 541.

[111] P.A. Blackwell, H.C.H. Lutzhoft, H.P. Ma, B. Halling-Sorensen, A.B.A. Boxall, P. Kay, Talanta 64 (2004) 1058.
[112] G. Stubbings, J. Tarbin, A. Cooper, M. Sharman, T. Bigwood, P. Robb, Anal. Chim. Acta 547 (2005) 262.
[113] V.F. Samanidou, K.I. Nikolaidou, I.N. Papadoyannis, J. Sep. Sci. 28 (2005) 2247.
[114] W.-x. Zhu, J.-z. Yang, W. Wei, Y.-f. Liu, S.-s. Zhang, J. Chromatogr. A 1207 (2008) 29.
[115] H.W. Sun, F.C. Wang, L.F. Ai, J. Chromatogr. A 1175 (2007) 227.
[116] D.S. Hage, J. Chromatogr. B 715 (1998) 3.
[117] N. Kobayashi, J. Goto, Bunseki Kagaku 47 (1998) 537.
[118] M.C. Hennion, V. Pichon, J. Chromatogr. A 1000 (2003) 29.
[119] N. Delaunay-Bertoncini, M.C. Hennion, J. Pharm. Biomed. Anal. 34 (2004) 717.
[120] L.K. Amundsen, H. Siren, Electrophoresis 28 (2007) 99.
[121] N.A. Guzman, T. Blanc, T.M. Phillips, Electrophoresis 29 (2008) 3259.
[122] C. Li, Z.H. Wang, X.Y. Cao, R.C. Beier, S.X. Zhang, S.Y. Ding, X.W. Li, J.Z. Shen, J. Chromatogr. A 1209 (2008) 1.
[123] S.J. Zhao, X.L. Li, Y.K. Ra, C. Li, H.Y. Jiang, J.C. Li, Z.N. Qu, S.X. Zhang, F.Y. He, Y.P. Wan, C.W. Feng, Z.R. Zheng, J.Z. Shen, J. Agric. Food Chem. 57 (2009) 365.
[124] J.S. Li, C.F. Qian, J. AOAC Int. 79 (1996) 1062.
[125] I.A. Nicholls, K. Adbo, H.S. Andersson, P.O. Andersson, J. Ankarloo, J. Hedin-Dahlström, P. Jokela, J.G. Karlsson, L. Olofsson, J. Rosengren, S. Shoravi, J. Svenson, S. Wikman, Anal. Chim. Acta 435 (2001) 9.
[126] A. Cameron, S.A. Håkan, I.A. Lars, J.A. Richard, K. Nicole, A.N. Ian, O.M. John, J.W. Michael, J. Mol. Recognit. 19 (2006) 106.
[127] G. Brambilla, M. Fiori, B. Rizzo, V. Crescenzi, G. Masci, J. Chromatogr. B 759 (2001) 27.
[128] J. Svenson, I.A. Nicholls, Anal. Chim. Acta 435 (2001) 19.
[129] A. Ellwanger, C. Berggren, S. Bayoudh, C. Crecenzi, L. Karlsson, P.K. Owens, K. Ensing, P. Cormack, D. Sherrington, B. Sellergren, Analyst 126 (2001) 784.
[130] R. Mohamed, J. Richoz-Payot, E. Gremaud, P. Mottier, E. Yilmaz, J.C. Tabet, P.A. Guy, Anal. Chem. 79 (2007) 9557.
[131] C.Y. He, Y.Y. Long, J.L. Pan, K. Li, F. Liu, J. Biochem. Biophys. Methods 70 (2007) 133.
[132] N. Furusawa, Fresenius. J. Anal. Chem. 364 (1999) 270.
[133] N. Furusawa, J. Chromatogr. A 898 (2000) 185.
[134] N. Furusawa, K. Kishida, Fresenius. J. Anal. Chem. 371 (2001) 1031.
[135] N. Furusawa, J. AOAC Int. 85 (2002) 848.
[136] N. Furusawa, Anal. Chim. Acta 481 (2003) 255.
[137] N. Furusawa, Chromatographia 52 (2000) 653.
[138] M.T. Muldoon, S.A. Buckley, S.S. Deshpande, C.K. Holtzapple, R.C. Beier, L.H. Stanker, J. Agric. Food Chem. 48 (2000) 545.
[139] N. Furusawa, Biomed. Chromatogr. 15 (2001) 235.
[140] N. Furusawa, Chromatographia 53 (2001) 47.
[141] W. Haasnoot, A. Kemmers-Voncken, D. Samson, Analyst 127 (2002) 87.
[142] N. Furusawa, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 161.
[143] N. Furusawa, Talanta 49 (1999) 461.

[144] J.E. Roybal, A.P. Pfenning, S.B. Turnipseed, S.A. Gonzales, Anal. Chim. Acta 483 (2003) 147.
[145] S. Abuín, R. Companyó, F. Centrich, A. Rúbies, M.D. Prat, J. Chromatogr. A 1207 (2008) 17.
[146] G. Biancotto, R. Angeletti, R.D.M. Piro, Analyst 121 (1996) 229.
[147] S.A. Hewitt, M. Kearney, J.W. Currie, P.B. Young, D.G. Kennedy, Anal. Chim. Acta 473 (2002) 99.
[148] G. Kaklamanos, G. Theodoridis, T. Dabalis, J. Chromatogr. B 877 (2009) 2330.
[149] I. Pecorelli, R. Galarini, R. Bibi, A. Floridi, E. Casciarri, A. Floridi, Anal. Chim. Acta 483 (2003) 81.
[150] L. Johnston, L. Mackay, M. Croft, J. Chromatogr. A 982 (2002) 97.
[151] N. Mestorino, M.L. Marchetti, E. Turic, J. Pesoa, J. Errecalde, Anal. Chim. Acta 637 (2009) 33.
[152] X. Hong, G. Long, H. jia, L. Anqing, T. Danzhou, J. Chromatogr. B 852 (2007) 529.
[153] P. Hubert, P. Chiap, B. Evrard, L. Delattre, J. Crommen, J. Chromatogr. B: Biomed. Appl. 622 (1993) 53.
[154] G. Scarano, H. Esposito, L. Grasso, V. Soprano, G. Oliviero, in 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St Jan, Belgium, 1998, p. 2551.
[155] L. Antonian, P. DeMontigny, P.G. Wislocki, J. Pharm. Biomed. Anal. 16 (1998) 1363.
[156] A. Lifschitz, J. Sallovitz, F. Imperiale, A. Pis, J. Jauregui Lorda, C. Lanusse, Vet. Parasitol. 119 (2004) 247.
[157] S. Yakkundi, A. Cannavan, C.T. Elliott, T. Lövgren, D.G. Kennedy, J. Chromatogr. B 788 (2003) 29.
[158] K. Halme, E. Lindfors, K. Peltonen, Food Addit. Contam. 21 (2004) 641.
[159] M.J. Hutchinson, P.B. Young, D.G. Kennedy, J. Chromatogr. B 816 (2005) 15.
[160] L. Kantiani, M. Farre, M. Sibum, C. Postigo, M. Lopez de Alda, D. Barcelo, Anal. Chem. 81 (2009) 4285.
[161] F.L. van Holthoon, Spark Holland European Users Meeting UM07_TH05 (2007).
[162] Spark Holland Application Note 30 (2000).
[163] Spark Holland Application Note 53074 (2007).
[164] Y. Xu, K.J. Willson, D.G. Musson, J. Chromatogr. B 863 (2008) 64.
[165] D. Zimmer, V. Pickard, W. Czembor, C. Muller, in 15th Montreux Symposium on LC-MS, SFC-MS, CE-MS and MS-MS, Elsevier Science Bv, Montreux, France, 1998, p. 23.
[166] P. Mottier, Y.A. Hammel, E. Gremaud, P.A. Guy, J. Agric. Food Chem. 56 (2008) 35.
[167] R. Krebber, F.-J. Hoffend, F. Ruttmann, Anal. Chim. Acta In Press, Corrected Proof.
[168] R.F. Venn, J. Merson, S. Cole, P. Macrae, J. Chromatogr. B 817 (2005) 77.
[169] A. Rubies, R. Vaquerizo, F. Centrich, R. Compañó, M. Granados, M.D. Prat, Talanta 72 (2007) 269.
[170] G. Pinel, L. Rambaud, G. Cacciatore, A. Bergwerff, C. Elliott, M. Nielen, B. Le Bizec, J. Steroid Biochem. Mol. Biol. 110 (2008) 30.
[171] E. Bichon, C.A. Richard, B. Le Bizec, J. Chromatogr. A 1201 (2008) 91.

[172] N.C. van de Merbel, U.A.T. Brinkman, Trends Anal. Chem. 12 249.
[173] N.C. van de Merbel, J.J. Hageman, U.A.T. Brinkman, J. Chromatogr. A 634 (1993) 1.
[174] J.A. Jonsson, L. Mathiasson, J. Sep. Sci. 24 (2001) 495.
[175] M. McGrane, M. O'Keeffe, M.R. Smyth, Anal. Lett. 32 (1999) 481.
[176] T. Agasoster, K.E. Rasmussen, J. Pharm. Biomed. Anal. 10 (1992) 349.
[177] M. Lolo, S. Pedreira, C. Fente, B.I. Vazquez, C.M. Franco, A. Cepeda, Anal. Chim. Acta 480 (2003) 123.
[178] H. Xu, L. Gu, J. He, A.Q. Lin, D.Z. Tang, J. Chromatogr. B 852 (2007) 529.
[179] J. Cooper, W. Currie, C.T. Elliott, J. Chrom. B: Biomed. Sci. Appl. 757 (2001) 221.
[180] R. Bagnati, M.G. Castelli, L. Airoldi, M.P. Oriundi, A. Ubaldi, R. Fanelli, J. Chromatogr. B: Biomed. Appl. 527 (1990) 267.
[181] L.C. Dickson, J.D. MacNeil, J. Reid, A.C.E. Fesser, J. AOAC Int. 86 (2003) 631.
[182] T.A.M. Msagati, M.M. Nindi, Annali Di Chimica 96 (2006) 635.
[183] K. Schmidt, C. Stachel, P. Gowik, 14th European Conference on Analytical Chemistry, Antwerp, Belgium (2007) 1199.
[184] R. Bagnati, M.G. Castelli, L. Airoldi, M.P. Oriundi, A. Ubaldi, R. Fanelli, J. Chrom. B: Biomed. Sci. Appl. 527 (1990) 267.
[185] J. Vanden Bussche, H. Noppe, K. Verheyden, K. Wille, G. Pinel, B. Le Bizec, H.F. De Brabander, Anal. Chim. Acta 637 (2009) 2.
[186] G. Pinel, E. Bichon, K. Pouponneau, D. Maume, F. Andre, B. Le Bizec, J. Chromatogr. A 1085 (2005) 247.
[187] S. Abuín, F. Centrich, A. Rúbies, R. Companyó, M.D. Prat, Anal. Chim. Acta 617 (2008) 184.
[188] K. De Wasch, H.F. Be Brabander, S. Impens, M. Vandewiele, D. Courtheyn, J. Chromatogr. A 912 (2001) 311.
[189] P.E. Asea, J.D. MacNeil, J.O. Boison, J. AOAC Int. 89 (2006) 567.
[190] Off. J. Eur. Union. L222 (1981) 32.
[191] Off. J. Eur. Union. L125 (1996) 3.
[192] S. Impens, K. De Wasch, M. Cornelis, H.F. De Brabander, J. Chromatogr. A 970 (2002) 235.
[193] C. Blasco, C. Van Poucke, C. Van Peteghem, J. Chromatogr. A 1154 (2007) 230.
[194] B. Shao, R. Zhao, J. Meng, Y. Xue, G. Wu, J. Hu, X. Tu, Anal. Chim. Acta 548 (2005) 41.
[195] S. Impens, J. Van Loco, J.M. Degroodt, H. De Brabander, Anal. Chim. Acta 586 (2007) 43.
[196] E. Vanluchene, W. Eechaute, D. Vanderkerckhove, J. Steroid Biochem. Mol. Biol. 16 (1982) 701.
[197] V. Ferchaud, B.L. Bizec, F.M.F. Andre, P. Courcoux, The Analyst 125 (2000) 2255.
[198] H. Hooijerink, E.O. van Bennekom, M.W.F. Nielen, Anal. Chim. Acta 483 (2003) 51.
[199] R.P. Huopalahti, J.D. Henion, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 69
[200] A.A.M. Stolker, P.W. Zoontjes, L.A. van Ginkel, in 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St Jan, Belgium, 1998, p. 2671.

[201] G. Ferretti, C. Ferranti, T. Crovella, M. Fiori, C. Civitareale, C. Marchiafava, F.d. Quadri, P. Cammarata, L. Palleschi, J. Chromatogr. B 871 (2008) 135.
[202] S. Biddle, P. Teale, A. Robinson, J. Bowman, E. Houghton, Anal. Chim. Acta 586 (2007) 115.
[203] R.W. Fedeniuk, J.O. Boison, J.D. MacNeil, J. Chromatogr. B 802 (2004) 307.
[204] R. Draisci, L. Palleschi, E. Ferretti, L. Lucentini, P. Cammarata, J. Chromatogr. A 870 (2000) 511.
[205] Y. Yang, B. Shao, J. Zhang, Y.N. Wu, J. Ying, J. Chromatogr. B 870 (2008) 241.
[206] J. Seo, H.-Y. Kim, B.C. Chung, J. Hong, J. Chromatogr. A 1067 (2005) 303.
[207] M. -R. Fuh, S. -Y. Huang, T. -Y. Lin, Talanta 64 (2004) 408.
[208] E.M. Malone, C.T. Elliott, D.G. Kennedy, L. Regan, Anal. Chim. Acta 637 (2009) 112.
[209] P.R. Kootstra, P.W. Zoontjes, E.F. van Tricht, S.S. Sterk, Anal. Chim. Acta 586 (2007) 82.
[210] D. Boyd, M. Okeeffe, M.R. Smyth, Analyst 121 (1996) R1.
[211] F.J. dos Ramos, J. Chromatogr. A 880 (2000) 69.
[212] A.A.M. Stolker, U.A.T. Brinkman, J. Chromatogr. A 1067 (2005) 15.
[213] F. Moragues, C. Igualada, Anal. Chim. Acta 637 (2009) 193.
[214] M.W.F. Nielen, J.J.P. Lasaroms, M.L. Essers, J.E. Oosterink, T. Meijer, M.B. Sanders, T. Zuidema, A.A.M. Stolker, Anal. Bioanal. Chem. 391 (2008) 199.
[215] P.R. Kootstra, C. Kuijpers, K.L. Wubs, D. van Doorn, S.S. Sterk, L.A. van Ginkel, R.W. Stephany, Proceedings of the EuroResidue V Conference, Noordwijkerhout, The Netherlands (2004) 75.
[216] C. Widstrand, F. Larsson, M. Fiori, C. Civitareale, S. Mirante, G. Brambilla, J. Chromatogr. B 804 (2004) 85.
[217] S. Wang, L. Liu, G.Z. Fang, C. Zhang, J.X. He, J. Sep. Sci. 32 (2009) 1333.
[218] S.A. Haughey, G.A. Baxter, C.T. Elliot, B. Persson, C. Jonson, P. Bjurling, J. AOAC Int. 84 (2001) 1025.
[219] J. Pleadin, T. Gojmerac, I. Bratos, Z. Lipej, D. Novosel, A. Vulic, Food Technol. Biotechnol. 47 (2009) 67.
[220] R. Granja, A.M.M. Nino, F. Rabone, R.E.M. Nino, A. Cannavan, A.G. Salerno, in Sask Val Workshop 2007, Saskatoon, Canada, 2007, p. 1475.
[221] S. Boyd, H.H. Heskamp, T.F.H. Bovee, M.W.F. Nielen, C.T. Elliott, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 24.
[222] N. Van Hoof, D. Courtheyn, J.P. Antignac, M. Van de Wiele, S. Poelmans, H. Noppe, H. De Brabander, Rapid Commun. Mass Spectrom. 19 (2005) 2801.
[223] P. Gallo, G. Brambilla, B. Neri, M. Fiori, C. Testa, L. Serpe, Anal. Chim. Acta 587 (2007) 67.
[224] J. Cai, J. Henion, J. Chrom. B: Biomed. Sci. Appl. 691 (1997) 357.
[225] S. Zhang, F.Y. Sun, J.C. Li, L.L. Cheng, J.Z. Shen, J. AOAC Int. 89 (2006) 1437.
[226] S.X. Zhang, Z.W. Liu, X. Guo, L.L. Cheng, Z.H. Wang, J.Z. Shen, J. Chromatogr. B 875 (2008) 399.
[227] J.Z. Shen, X. Xia, H.Y. Jiang, C. Li, J.C. Li, X.W. Li, S.Y. Ding, J. Chromatogr. B 877 (2009) 1523.
[228] J.M. Van De Riet, R.A. Potter, M. Christie-Fougere, B.G. Burns, J. AOAC Int. 86 (2003) 510.

[229] B. Boyd, H. Bjork, J. Billing, O. Shimelis, S. Axelsson, M. Leonora, E. Yilmaz, J. Chromatogr. A 1174 (2007) 63.
[230] R.J. Shakila, R. Saravanakumar, S.A.P. Vyla, G. Jeyasekaran, Innov. Food Sci. Emerg. Technol. 8 (2007) 515.
[231] P.J. Luo, X.Y. Cao, Z.H. Wang, H.Y. Jiang, S.X. Zhang, X. Chen, J.P. Wang, C.M. Feng, J.Z. Shen, Food. Agric. Immunol. 20 (2009) 57.
[232] P.J. Luo, H.Y. Jiang, Z.H. Wang, C.M. Feng, F.Y. He, J.Z. Shen, J. AOAC Int. 92 (2009) 981.
[233] Z.Y. Huang, Q.P. Yan, Q. Zhang, A.H. Peng, Aquac. Int. 17 (2009) 69.
[234] H. Kubala-Drincic, D. Bazulic, J. Sapunar-Postruznik, M. Grubelic, G. Stuhne, J. Agric. Food Chem. 51 (2003) 871.
[235] B. Boyd, H. Bjork, J. Billing, O. Shimelis, S. Axelsson, M. Leonora, E. Yilmaz, in 9th International Symposium on Advances in Extraction Technologies, Alesund, Norway, 2007, p. 63.
[236] C. Van De Water, N. Haagsma, J. Chromatogr. A 411 (1987) 415.
[237] C. Van De Water, D. Tebbal, N. Haagsma, J. Chromatogr. 478 (1989) 205.
[238] T. Gude, A. Preiss, K. Rubach, J. Chrom. B: Biomed. Sci. Appl. 673 (1995) 197.
[239] S.X. Zhang, J.H. Zhou, J.Z. Shen, S.Y. Dnc, J.C. Li, J. AOAC Int. 89 (2006) 369.
[240] R. Stidl, M. Cichna-Markl, J. Sol-Gel Sci. Technol. 41 (2007) 175.
[241] J.M. Hayes, R. Gilewicz, K. Freehauf, M. Fetter, J. AOAC Int. 92 (2009) 340.
[242] A. Leitner, P. Zollner, W. Lindner, J. Chromatogr. A 939 (2001) 49.
[243] M. O'Keeffe, A. Conneely, K.M. Cooper, D.G. Kennedy, L. Kovacsics, A. Fodor, P.P.J. Mulder, J.A. van Rhijn, G. Trigueros, in 1st International Symposium on Recent Advances in Food Analysis, Elsevier Science Bv, Prague, Czech Republic, 2003, p. 125.
[244] P. Mottier, S.P. Khong, E. Gremaud, J. Richoz, T. Delatour, T. Goldmann, P.A. Guy, J. Chromatogr. A 1067 (2005) 85.
[245] J.K. Finzi, J.L. Donato, M. Sucupira, G. De Nucci, J. Chromatogr. B 824 (2005) 30.
[246] C. Bock, P. Gowik, C. Stachel, J. Chromatogr. B 856 (2007) 178.
[247] E. Verdon, P. Couedor, P. Sanders, in 5th International Symposium on Hormone and Veterinary Drug Residue Analysis, Elsevier Science Bv, Antwerp, Belgium, 2006, p. 336.
[248] R.J. McCracken, D.G. Kennedy, J. Chromatogr. B 691 (1997) 87.
[249] E. Horne, A. Cadogan, M. Okeeffe, L.A.P. Hoogenboom, Analyst 121 (1996) 1463.
[250] S.P. Khong, E. Gremaud, J. Richoz, T. Delatour, P.A. Guy, R.H. Stadler, P. Mottier, J. Agric. Food Chem. 52 (2004) 5309.
[251] K.M. Jenkins, M.S. Young, Lc Gc Europe (2005) 19.
[252] M.I. Lopez, M.F. Feldlaufer, A.D. Williams, P.S. Chu, J. Agric. Food Chem. 55 (2007) 1103.
[253] P.S. Chu, M.I. Lopez, J. Agric. Food Chem. 55 (2007) 2129.
[254] L. Rodziewicz, J. Chromatogr. B 864 (2008) 156.
[255] K.M. Cooper, R.J. McCrack-En, M. Buurman, D.G. Kennedy, Food Addit. Contam. 25 (2008) 548.
[256] P. Mottier, I. Hure, E. Gremaud, P.A. Guy, J. Agric. Food Chem. 54 (2006) 2018.

[257] Summary Reports, Committee for Veterinary Medicinal Products, The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, http://www.emea.eu.int/index/indexv1 .htm#.
[258] IARC Monographs of the Evaluation of Carcinogenic Risks to Humans. Overall Evaluation of Carcinogenicity. Supplement 7 (1987).
[259] 34th Report of the Joint FAO/WHO Expert Committee of Food Additives. Evaluation of Certain Veterinary Drug Residues in Food, World Health Organisation, Geneva (1989).
[260] X. Xia, X. Li, J. Shen, S. Zhang, S. Ding, H. Jiang, J. AOAC Int. 89 (2006) 94.
[261] J. Polzer, P. Gowik, J. Chrom. B: Biomed. Sci. Appl. 761 (2001) 47.
[262] J. Polzer, C. Stachel, P. Gowik, Anal. Chim. Acta 521 (2004) 189.
[263] J. Polzer, P. Gowik, Anal. Chim. Acta 529 (2005) 299.
[264] R. Mohamed, P. Mottier, L. Treguier, J. Richoz-Payot, E. Yilmaz, J.-C. Tabet, P.A. Guy, J. Agric. Food Chem. 56 (2008) 3500.
[265] E. Daeseleire, H.D. Ruyck, R.V. Renterghem, The Analyst 125 (2000) 1533.
[266] X. Xia, X. Li, S. Zhang, S. Ding, H. Jiang, J. Shen, Anal. Chim. Acta 586 (2007) 394.
[267] C. Ho, D.W.M. Sin, K.M. Wong, H.P.O. Tang, Anal. Chim. Acta 530 (2005) 23.
[268] J.E. Matusik, M.G. Leadbetter, C.J. Barnes, J.A. Sphon, J. Agric. Food Chem. 40 (1992) 439.
[269] X. Xia, X. Li, S. Zhang, S. Ding, H. Jiang, J. Li, J. Shen, J. Chromatogr. A 1208 (2008) 101.
[270] L. Connolly, C.S. Thompson, S.A. Haughey, I.M. Traynor, S. Tittlemeier, C.T. Elliott, Anal. Chim. Acta 598 (2007) 155.
[271] S. Fraselle, V. Derop, J.-M. Degroodt, J. Van Loco, Anal. Chim. Acta 586 (2007) 383.
[272] M. Cronly, P. Behan, B. Foley, E. Malone, L. Regan, J. Chromatogr. B 877 (2009) 1494.
[273] H.W. Sun, F.C. Wang, L.F. Ai, J. Chromatogr. B 857 (2007) 296.
[274] A. Aerts, I. Egberink, C. Kan, H. Keukens, W. Beek, J. AOAC Int. 74 (1991) 76.
[275] C.S. Thompson, I.M. Traynor, T.L. Fodey, S.R.H. Crooks, Anal. Chim. Acta 637 (2009) 259.
[276] A. Kaufmann, K. Maden, J. AOAC Int. 88 (2005) 1118.
[277] B.J.A. Berendsen, M.L. Essers, P.P.J. Mulder, G.D. van Bruchem, A. Lommen, W.M. van Overbeek, L.A.M. Stolker, J. Chromatogr. A In Press, Corrected Proof.
[278] W.A. Moats, J. Agric. Food Chem. 31 (1983) 880.
[279] K. Mastovska, A.R. Lightfield, J. Chromatogr. A 1202 (2008) 118.
[280] Y. Ito, Y. Ikai, H. Oka, H. Matsumoto, Y. Miyazaki, K. Takeba, H. Nagase, J. Chromatogr. A 911 (2001) 217.
[281] Y. Ito, T. Goto, H. Oka, H. Matsumoto, K. Takeba, J. Chromatogr. A 1042 (2004) 107.
[282] R.V. Oliveira, Q.B. Cass, J. Agric. Food Chem. 54 (2006) 1180.
[283] M. Becker, E. Zittlau, M. Petz, Anal. Chim. Acta 520 (2004) 19.
[284] E. Daeseleire, H. De Ruyck, R. Van Renterghem, in SIMSUG 2000 Meeting, Cumbria, England, 2000, p. 1404.

[285] O. Brüggemann, K. Haupt, L. Ye, E. Yilmaz, K. Mosbach, J. Chromatogr. A 889 (2000) 15.
[286] M. McGrane, M. O'Keeffe, M.R. Smyth, in 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St Jan, Belgium, 1998, p. 2779.
[287] S.-H. Hsieh, H. -Y. Huang, S. Lee, J. Chromatogr. A In Press, Corrected Proof.
[288] L.K. Sørensen, L.K. Snor, J. Chromatogr. A 882 (2000) 145.
[289] T.A. McGlinchey, P.A. Rafter, F. Regan, G.P. McMahon, Anal. Chim. Acta 624 (2008) 1.
[290] R.W. Fedeniuk, P.J. Shand, J. Chromatogr. A 812 (1998) 3.
[291] B. Shaikh, W.A. Moats, J. Chromatogr. 643 (1993) 369.
[292] J. Wang, Mass Spectrom. Rev. 28 (2009) 50.
[293] C. Benetti, R. Piro, G. Binato, R. Angeletti, G. Biancotto, Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk. Assess. 23 (2006) 1099
[294] T.S. Thompson, D.K. Noot, J. Calvert, S.F. Pernal, Rapid Commun. Mass Spectrom. 19 (2005) 309.
[295] J. Wang, D. Leung, Rapid Commun. Mass Spectrom. 21 (2007) 3213.
[296] H. Berrada, F. Borrull, G. Font, J.C. Molto, R.M. Marce, J. Chromatogr. A 1157 (2007) 281.
[297] H. Berrada, F. Borrull, G. Font, R.M. Marce, J. Chromatogr. A 1208 (2008) 83.
[298] M. Horie, H. Takegami, K. Toya, H. Nakazawa, Anal. Chim. Acta 492 (2003) 187.
[299] P.A. Martos, S.J. Lehotay, B. Shurmer, J. Agric. Food Chem. 56 (2008) 8844.
[300] H.C. Higgins, J.D.G. McEvoy, Food Addit. Contam. 19 (2002) 819.
[301] J.A. Hernández-Arteseros, J. Barbosa, R. Compañó, M.D. Prat, J. Chromatogr. A 945 (2002) 1.
[302] V. Andreu, C. Blasco, Y. Picó, Trends Anal. Chem. 26 (2007) 534.
[303] E. Jimenez-Lozano, D. Roy, D. Barron, J. Barbosa, Electrophoresis 25 (2004) 65.
[304] E.A. Christodoulou, V.F. Samanidou, I.N. Papadoyannis, J. Sep. Sci. 30 (2007) 2676.
[305] H. Zhang, Y.P. Ren, X.L. Bao, J. Pharm. Biomed. Anal. 49 (2009) 367.
[306] B. Toussaint, G. Bordin, A. Janosi, A.R. Rodriguez, J. Chromatogr. A 976 (2002) 195.
[307] B. Toussaint, M. Chedin, G. Bordin, A.R. Rodriguez, J. Chromatogr. A 1088 (2005) 32.
[308] J.H. Shim, M.H. Lee, M.R. Kim, C.J. Lee, I.S. Kim, Biosci., Biotechnol., Biochem. 67 (2003) 1342.
[309] F.J. Lara, A.M. Garcia-Campana, F. Ales-Barrero, J.M. Bosque-Sendra, Electrophoresis 29 (2008) 2117.
[310] E. Caro, R.M. Marce, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 562 (2006) 145.
[311] H.Y. Yan, F.X. Qiao, K.H. Row, Anal. Chem. 79 (2007) 8242.
[312] S.J. Zhao, L. Cun, H.Y. Jiang, B.Y. Li, J.Z. Shen, Chin. J. Anal. Chem. 35 (2007) 786.
[313] M.P. Heymo, E. Nemutlu, S. Kir, D. Barron, J. Barbosa, Anal. Chim. Acta 613 (2008) 98.
[314] V. Samanidou, E. Evaggelopoulou, M. Trotzmuller, X.H. Guo, E. Lankmayr, J. Chromatogr. A 1203 (2008) 115.

[315] M.P. Hermo, D. Barrón, J. Barbosa, J. Chromatogr. A 1201 (2008) 1.
[316] D. Guggisberg, A.E. Mooser, H. Koch, J. Chromatogr. 624 (1992) 425.
[317] H.F. De Brabander, H. Noppe, K. Verheyden, J. Vanden Bussche, K. Wille, L. Okerman, L. Vanhaecke, W. Reybroeck, S. Ooghe, S. Croubels, J. Chromatogr. A In Press, Corrected Proof.
[318] V.F. Samanidou, E.P. Tolika, I.N. Papadoyannis, Sep. Purif. Rev. 37 (2008) 327.
[319] Z.X. Cai, Y. Zhang, H.F. Pan, X.W. Tie, Y.P. Ren, J. Chromatogr. A 1200 (2008) 144.
[320] M. Di Sabatino, A.M. Di Pietra, L. Benfenati, B. Di Simone, J. AOAC Int. 90 (2007) 598.
[321] V. Gamba, C. Terzano, L. Fioroni, S. Moretti, G. Dusi, R. Galarini, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 18.
[322] A.F. Forti, G. Scortichini, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 214.
[323] M.U. Farooq, P. Su, Y. Yang, Chromatographia 69 (2009) 1107.
[324] G.H. Zou, M.X. Xie, X.F. Wang, Y. Liu, J. Wang, J. Song, H. Gao, J. Han, J. Sep. Sci. 30 (2007) 2647.
[325] Q.H. Zou, J. Wang, X.F. Wang, Y. Liu, J. Han, F. Hou, M.X. Xie, J. AOAC Int. 91 (2008) 252.
[326] M. Sergi, A. Gentili, D. Perret, S. Marchese, S. Materazzi, R. Curini, Chromatographia 65 (2007) 757.
[327] B.Y. Li, C. Li, H.Y. Jiang, Z.N. Wang, X.Y. Cao, S.J. Zhao, S.X. Zhang, J.Z. Shen, J. AOAC Int. 91 (2008) 1488.
[328] U. Koesukwiwat, S. Jayanta, N. Leepipatpiboon, J. Chromatogr. A 1140 (2007) 147.
[329] S. Bogialli, R. Curini, A. Di Corcia, M. Nazzari, R. Samperi, Anal. Chem. 75 (2003) 1798.
[330] K. Kishida, N. Furusawa, J. Liq. Chromatogr. Relat. Technol. 26 (2003) 2931.
[331] J.R. Perkins, D.E. Games, J.R. Startin, J. Gilbert, J. Chromatogr. 540 (1991) 239.
[332] A. Gentili, D. Perret, S. Marchese, M. Sergi, C. Olmi, R. Curini, J. Agric. Food Chem. 52 (2004) 4614.
[333] G. Font, A. Juan-Garcia, Y. Pico, J. Chromatogr. A 1159 (2007) 233.
[334] K.I. Nikolaidou, V.F. Samanidou, I.N. Papadoyannis, J. Liq. Chromatogr. Relat. Technol. 31 (2008) 2523.
[335] H. Oka, Y. Ito, H. Matsumoto, J. Chromatogr. A 882 (2000) 109.
[336] A. Pena, C.M. Lino, R. Alonso, D. Barcelo, J. Agric. Food Chem. 55 (2007) 4973.
[337] Z. Yue, Y. Qiu, X. Liu, C. Ji, Chin. J. Anal. Chem. 34 (2006) 1255.
[338] H. De Ruyck, H. De Ridder, Rapid Commun. Mass Spectrom. 21 (2007) 1511.
[339] K.I. Nikolaidou, V.F. Samanidou, I.N. Papadoyannis, J. Liq. Chromatogr. Relat. Technol. 31 (2008) 3032.
[340] J.T. Li, L.G. Chen, X. Wang, H.Y. Jin, L. Ding, K. Zhang, H.Q. Zhang, Talanta 75 (2008) 1245.
[341] E. Caro, R.M. Marce, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 552 (2005) 81.

[342] T. Jing, X.D. Gao, P. Wang, Y. Wang, Y.F. Lin, X.Z. Hu, Q.L. Hao, Y.K. Zhou, S.R. Mei, 15th Biennial Conference on Applied Surface Analysis, Soest, Germany (2008) 2009.
[343] C. Blasco, A.D. Corcia, Y. Picó, Food Chem. 116 (2009) 1005.
[344] W.H. Tsai, T.C. Huang, J.J. Huang, Y.H. Hsue, H.Y. Chuang, J. Chromatogr. A 1216 (2009) 2263.
[345] E. Cristofani, C. Antonini, G. Tovo, L. Fioroni, A. Piersanti, R. Galarini, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 40.
[346] S. Bogialli, C. Coradazzi, A. Di Corcia, A. Lagana, M. Sergi, J. AOAC Int. 90 (2007) 864.
[347] Z.F. Yue, Y.M. Qiu, X.Y. Lin, C.N. Ji, Chin. J. Anal. Chem. 34 (2006) 1255.
[348] M. Danaher, M. O'Keeffe, J.D. Glennon, L. Howells, Analyst 126 (2001) 576.
[349] H. Wang, Z.J. Wang, S.Y. Liu, Z.R. Liu, Bull. Environ. Contam. Toxicol. 82 (2009) 395.
[350] B.J.A. Berendsen, P.P.J. Mulder, H.A. van Rhijn, Anal. Chim. Acta 585 (2007) 126.
[351] D.A. Durden, J. Wotske, J. AOAC Int. 92 (2009) 580.
[352] K. Albin, L. Hartig, K. von Czapiewski, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) Poster.
[353] W. Radeck, P. Gowik, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 1181.
[354] F.L. van Holthoon, T. Zuidema, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 849.
[355] M. Caldow, M. Sharman, M. Kelly, J. Day, S. Hird, J.A. Tarbin, J. Chromatogr. A In Press, Corrected Proof.
[356] X.L. Hou, Y.N. Wu, J.Z. Shen, L. Wang, S.Y. Ding, Chromatographia 65 (2007) 77.
[357] J.H. He, X.L. Hou, H.Y. Jiang, J.Z. Shen, J. AOAC Int. 88 (2005) 1099.
[358] X.L. Hou, X.W. Li, S.Y. Ding, J.H. He, H.Y. Jiang, J.Z. Shen, Chromatographia 63 (2006) 543.
[359] M. Danaher, M. O'Keeffe, J.D. Glennon, J. Chromatogr. B 761 (2001) 115.
[360] M. Danaher, M. O'Keeffe, J.D. Glennon, in 4th International Symposium on Hormone and Veterinary Drug Residue Analysis, Antwerp, Belgium, 2002, p. 313.
[361] M.S. Ali, T. Sun, G.E. McLeroy, E.T. Phillippo, J. AOAC Int. 83 (2000) 31.
[362] G.C. Gerhardt, C.D.C. Salisbury, H.M. Campbell, Food Addit. Contam. 12 (1995) 731.
[363] B. Shao, X.Y. Wu, J. Zhang, H.J. Duan, X.G. Chu, Y.N. Wu, Chromatographia 69 (2009) 1083.
[364] L. Mortier, E. Daeseleire, P. Delahaut, Anal. Chim. Acta 483 (2003) 27.
[365] W.J. Blanchflower, D.G. Kennedy, J. Chromatogr. B 675 (1996) 225.
[366] D.K. Matabudul, B. Conway, I. Lumley, S. Sumar, Food Chem. 75 (2001) 345.
[367] D.K. Matabudul, I.D. Lumley, J.S. Points, Analyst 127 (2002) 760.
[368] M. Dubois, G. Pierret, P. Delahaut, J. Chromatogr. B 813 (2004) 181.
[369] L. Mortier, E. Daeseleire, C. Van Peteghem, Rapid Commun. Mass Spectrom. 19 (2005) 533.

[370] P.P.J. Mulder, P. Balzer-Rutgers, E.M. te Brinke, Y.J.C. Bolck, B.J.A. Berendsen, H. Gerçek, B. Schat, J.A. van Rhijn, Anal. Chim. Acta 529 (2005) 331.
[371] V. Hormazabal, M. Yndestad, O. Ostensvik, J. Liq. Chromatogr. Relat. Technol. 26 (2003) 791.
[372] M. Olejnik, T. Szprengier-Juszkiewicz, P. Jedziniak, J. Chromatogr. A In Press, Corrected Proof.
[373] Off. J. Eur. Union. L40 (2009) 7.
[374] M.S. Ali, J. AOAC Int. 72 (1989) 586.
[375] K.J. Voorhees, A.A. Gharaibeh, B. Murugaverl, J. Agric. Food Chem. 46 (1998) 2353.
[376] R.J. Argauer, K.I. Eller, M.A. Ibrahim, R.T. Brown, J. Agric. Food Chem. 43 (1995) 2774.
[377] C. Blasco, M. Fernandez, A. Pena, C. Lino, M.I. Silveira, G. Font, Y. Pico, J. Agric. Food Chem. 51 (2003) 8132.
[378] J. Zhen, L. Zhuguang, C. Meiyu, M. Yu, T. Jun, F. Yulan, W. Jiachen, C. Zhaobin, T. Fengzhang, Chin. J. Chromatogr. 24 (2006) 440.
[379] Z.M. Chen, Y.H. Wang, J. Chromatogr. A 754 (1996) 367.
[380] A.W. Chen, J.M. Fink, D.J. Letinski, G.P. Barrett, J.C. Pearsall, J. Agric. Food Chem. 45 (1997) 4850.
[381] F. Sun, F.-Y. Lin, S.-S. Wong, G.-C. Li, J. Food Drug Anal. 11 (2003) 258.
[382] R.J. Argauer, K.I. Eller, R.M. Pfeil, R.T. Brown, J. Agric. Food Chem. 45 (1997) 180.
[383] S.R. Rissato, M.S. Galhiane, F.R.N. Knoll, B.M. Apon, J. Chromatogr. A 1048 (2004) 153.
[384] M. Fernandez-Alvarez, M. Llompart, J.P. Lamas, M. Lores, C. Garcia-Jares, R. Cela, T. Dagnac, Anal. Chim. Acta 617 (2008) 37.
[385] P. Stefanelli, A. Santilio, L. Cataldi, R. Dommarco, J. Environ. Sci. Health. B. 44 (2009) 350.
[386] M. Fernandez-Alvarez, M. Llompart, J.P. Lamas, M. Lores, C. Garcia-Jares, R. Cela, T. Dagnac, J. Chromatogr. A 1216 (2009) 2832.
[387] Y. Govaert, P. Batjoens, K. Tsilikas, J.M. Degroodt, S. Srebrnik, in 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St Jan, Belgium, 1998, p. 2507.
[388] M.L. Olmos-Carmona, M. Hernandez-Carrasquilla, J. Chromatogr. B 734 (1999) 113.
[389] J. Zhang, B. Shao, J. Yin, Y. Wu, H. Duan, J. Chromatogr. B 877 (2009) 1915.
[390] A. Kaufmann, B. Ryser, Rapid Commun. Mass Spectrom. 15 (2001) 1747.
[391] D. Fluchard, S. Kiebooms, M. Dubois, P. Delahaut, J. Chromatogr. B 744 (2000) 139.
[392] P. Delahaut, C. Levaux, P. Eloy, M. Dubois, Anal. Chim. Acta 483 (2003) 335.
[393] V. Cerkvenik-Flajs, Anal. Chim. Acta 586 (2007) 374.
[394] J. Cooper, P. Delahaut, T.L. Fodey, C.T. Elliott, Analyst 129 (2004) 169.
[395] E. Daeseleire, L. Mortier, H. De Ruyck, N. Geerts, Anal. Chim. Acta 488 (2003) 25.
[396] N. Van Hoof, K. De Wasch, S. Poelmans, H. Noppe, B. De, Hubert, Rapid Commun. Mass Spectrom. 18 (2004) 2823.
[397] L. Penney, C. Bergeron, B. Coates, A. Wijewickreme, J. AOAC Int. 88 (2005) 496.

[398] E.M. Malone, G. Dowling, C.T. Elliott, D.G. Kennedy, L. Regan, J. Chromatogr. A In Press, Corrected Proof.
[399] F. Vinci, S. Fabbrocino, M. Fiori, L. Serpe, P. Gallo, Rapid Commun. Mass Spectrom. 20 (2006) 3412.
[400] G. Dowling, P. Gallo, S. Fabbrocino, L. Serpe, L. Regan, Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk. Assess. 25 (2008) 1497
[401] P. Gallo, S. Fabbrocino, F. Vinci, M. Fiori, V. Danese, L. Serpe, Rapid Commun. Mass Spectrom. 22 (2008) 841.
[402] G. González, R. Ventura, A.K. Smith, R. de la Torre, J. Segura, J. Chromatogr. A 719 (1996) 251.
[403] J. Chrusch, S. Lee, R. Fedeniuk, J.O. Boison, Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk. Assess. 25 (2008) 1482
[404] N.H. Yu, E.N.M. Ho, F.P.W. Tang, T.S.M. Wan, A.S.Y. Wong, J. Chromatogr. A 1189 (2008) 426.
[405] E.N.M. Ho, D.K.K. Leung, T.S.M. Wan, N.H. Yu, J. Chromatogr. A 1120 (2006) 38.
[406] P. Gallo, S. Fabbrocino, F. Vinci, M. Fiori, V. Danese, A. Nasi, L. Serpe, J. Chromatogr. Sci. 44 (2006) 585.
[407] M. McDonald, K. Granelli, P. Sjoberg, Anal. Chim. Acta 588 (2007) 20.
[408] P. Delahaut, P. Jacquemin, Y. Colemonts, M. Dubois, J. De Graeve, H. Deluyker, J. Chrom. B: Biomed. Sci. Appl. 696 (1997) 203.
[409] K. Halme, E. Lindfors, K. Peltonen, J. Chromatogr. B 845 (2007) 74.
[410] J.A. Tarbin, D. Chan, G. Stubbings, M. Sharman, Anal. Chim. Acta 625 (2008) 188.
[411] A.A. Bergwerff, P. Scherpenisse, J. Chromatogr. B 788 (2003) 351.
[412] Off. J. Eur. Union. L6 (2004) 38.
[413] P. Scherpenisse, A.A. Bergwerff, Proceedings of the EuroResidue V Conference, Noordwijkerhout, The Netherlands (2004) 173.
[414] J.T. Yuan, L.F. Liao, X.L. Xiao, B. He, S.Q. Gao, Food Chem. 113 (2009) 1377.
[415] X.L. Wu, G. Zhang, Y.N. Wu, X.L. Hou, Z.H. Yuan, J. Chromatogr. A 1172 (2007) 121.
[416] W.C. Andersen, S.B. Turnipseed, C.M. Karbiwnyk, R.H. Lee, S.B. Clark, W.D. Rowe, M.R. Madson, K.E. Miller, Proceedings of the EuroResidue VI Conference, Egmond aan Zee, The Netherlands (2008) 279.
[417] Y. Jiang, P. Xie, G.D. Liang, Aquaculture 288 (2009) 1.
[418] M.C. Yang, J.M. Fang, T.F. Kuo, D.M. Wang, Y.L. Huang, L.Y. Liu, P.H. Chen, T.H. Chang, J. Agric. Food Chem. 55 (2007) 8851.
[419] W.C. Andersen, S.B. Turnipseed, J.E. Roybal, J. Agric. Food Chem. 54 (2006) 4517.
[420] L.G. Rushing, H.C. Thompson, J. Chrom. B: Biomed. Sci. Appl. 688 (1997) 325.
[421] R.J.B. Peters, Y.J.C. Bolck, P. Rutgers, A.A.M. Stolker, M.W.F. Nielen, J. Chromatogr. A In Press, Corrected Proof.
[422] Y. Yang, B. Shao, J. Zhang, Y. Wu, H. Duan, J. Chromatogr. B 877 (2009) 489.
[423] J. Chico, A. Rúbies, F. Centrich, R. Companyó, M.D. Prat, M. Granados, J. Chromatogr. A 1213 (2008) 189.
[424] B. Shao, X. Jia, Y. Wu, J. Hu, X. Tu, J. Zhang, Rapid Commun. Mass Spectrom. 21 (2007) 3487.

[425] M. McDonald, C. Mannion, P. Rafter, J. Chromatogr. A In Press, Corrected Proof.
[426] K. Granelli, C. Branzell, Anal. Chim. Acta 586 (2007) 289.
[427] V. Carretero, C. Blasco, Y. Pico, J. Chromatogr. A 1209 (2008) 162.
[428] S.B. Turnipseed, W.C. Andersen, C.M. Karbiwnyk, M.R. Madson, K.E. Miller, Rapid Commun. Mass Spectrom. 22 (2008) 1467.
[429] H. Li, P.J. Kijak, S.B. Turnipseed, W. Cui, J. Chromatogr. B 836 (2006) 22.
[430] S. LeBoulaire, J.C. Bauduret, F. Andre, J. Agric. Food Chem. 45 (1997) 2134.
[431] K. Granelli, C. Elgerud, Å. Lundström, A. Ohlsson, P. Sjöberg, Anal. Chim. Acta 637 (2009) 87.
[432] A. Kaufmann, P. Butcher, K. Maden, M. Widmer, Anal. Chim. Acta 586 (2007) 13.
[433] Y.-A. Hammel, R. Mohamed, E. Gremaud, M. -H. LeBreton, P.A. Guy, J. Chromatogr. A 1177 (2008) 58.
[434] H.P.O. Tang, C. Ho, S.S.L. Lai, Rapid Commun. Mass Spectrom. 20 (2006) 2565.

List of Figures:

Figure 1

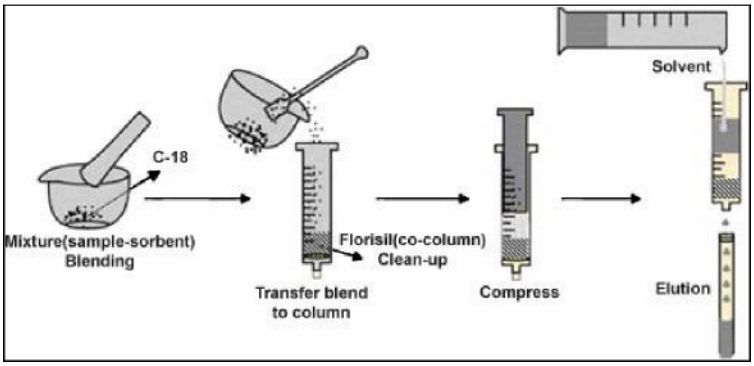

C-18
Mixture(sample-sorbent)
Blending
Transfer blend
to column
Florisil(co-column)
Clean-up
Compress
Solvent
Elution

Figure 2

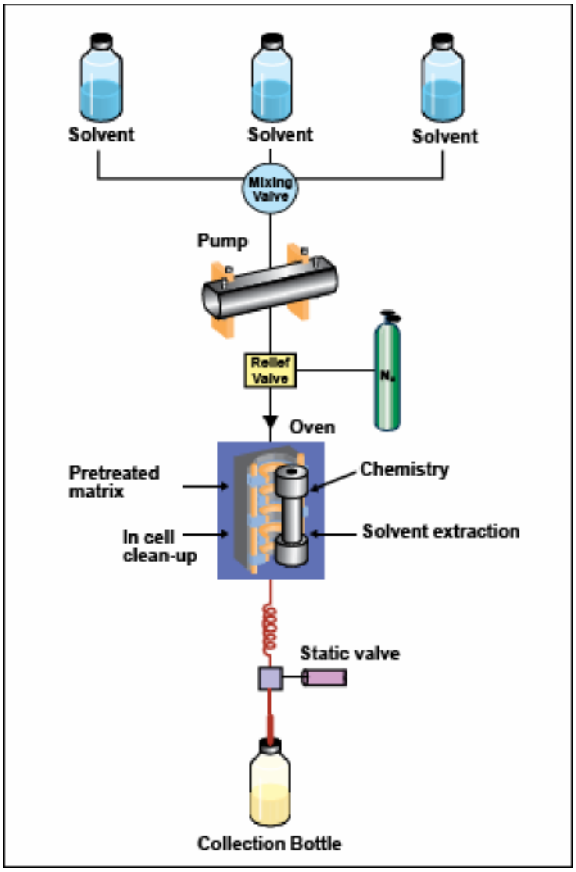

Solvent
Solvent
Solvent
Mixing Valve
Pump
Relief Valve
N_2
Oven
Pretreated matrix
In cell clean-up
Chemistry
Solvent extraction
Static valve
Collection Bottle

Figure 3

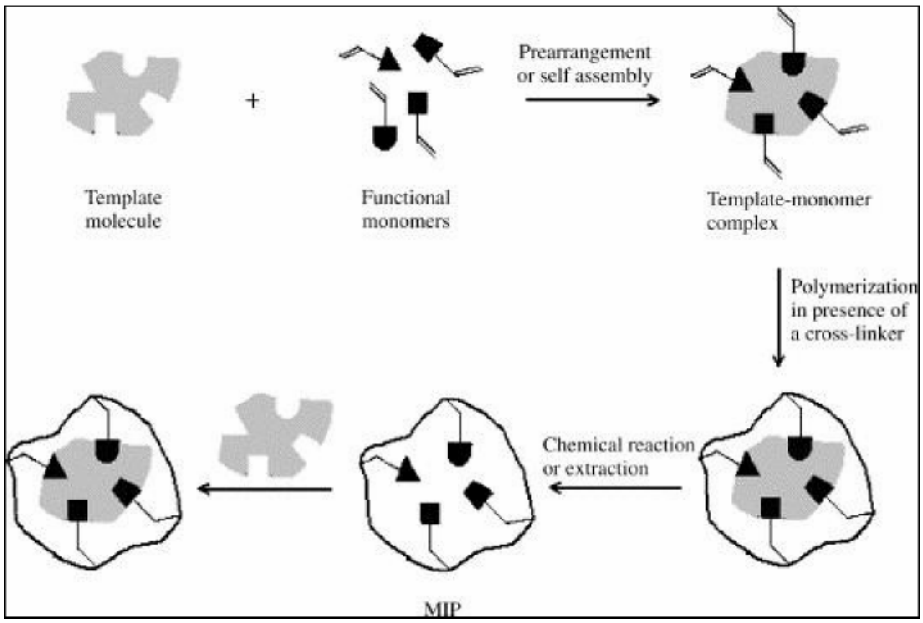

Prearrangement
or self assembly
Template
molecule
Functional
monomers
Template-monomer
complex
Polymerization
in presence of
a cross-linker
Chemical reaction
or extraction
MIP

Figure 4

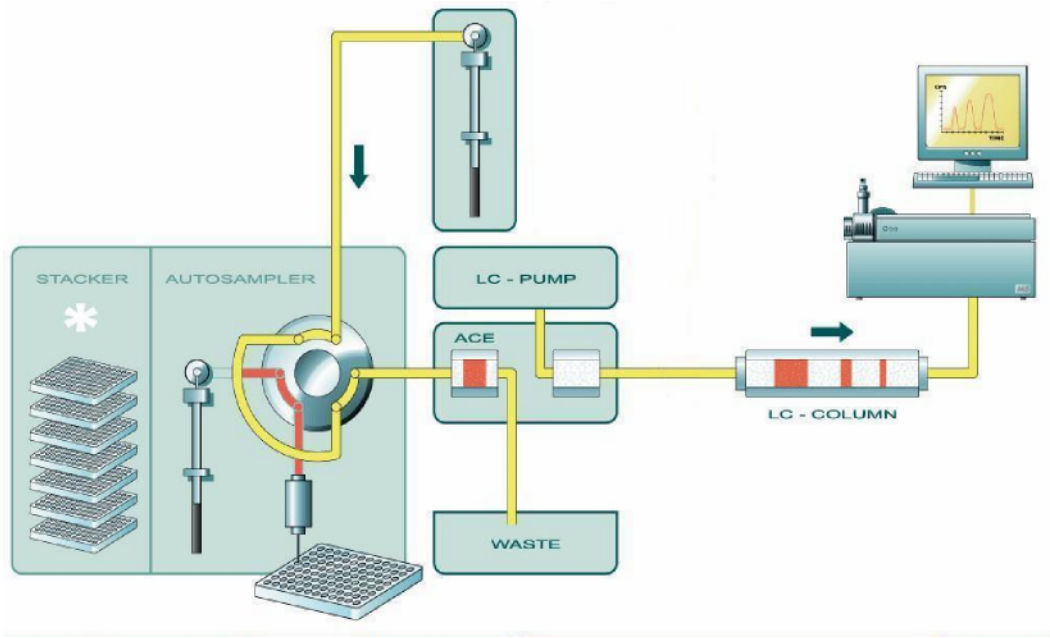


Figure 5

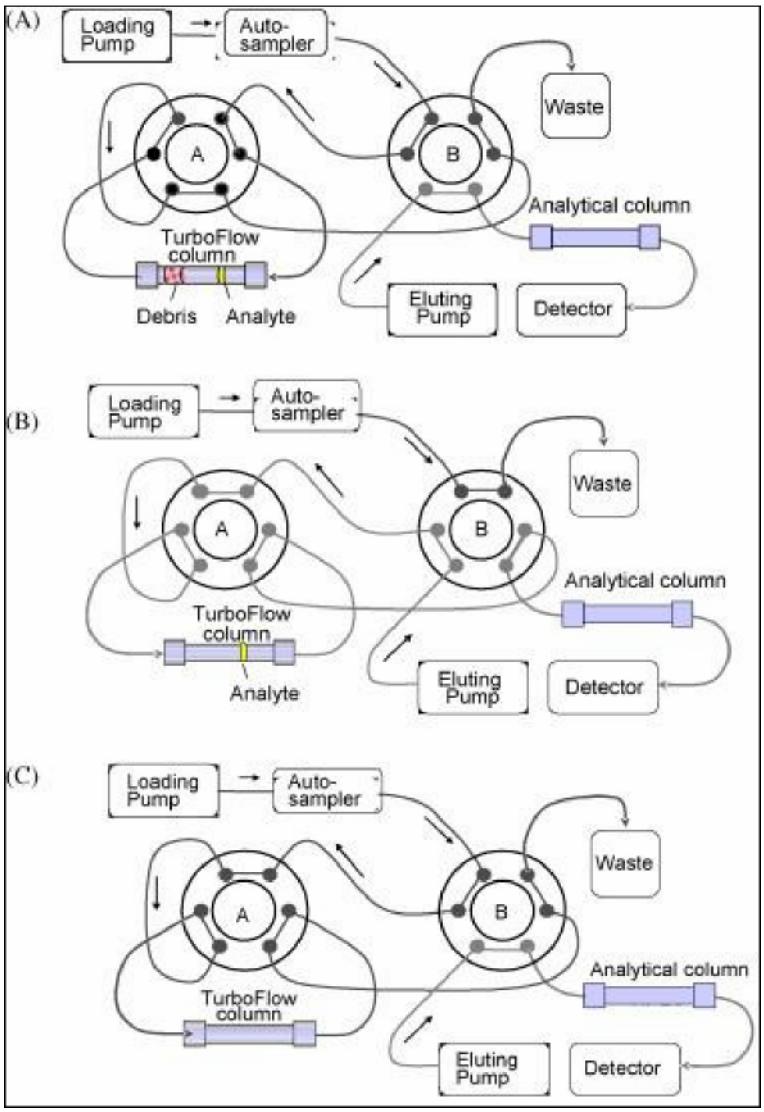

(A)
Loading Pump
Auto-sampler
Waste
A
B
Analytical column
TurboFlow column
Debris
Analyte
Eluting Pump
Detector
(B)
Loading Pump
Auto-sampler
Waste
A
B
Analytical column
TurboFlow column
Analyte
Eluting Pump
Detector
(C)
Loading Pump
Auto-sampler
Waste
A
B
Analytical column
TurboFlow column
Eluting Pump
Detector

Figure 6

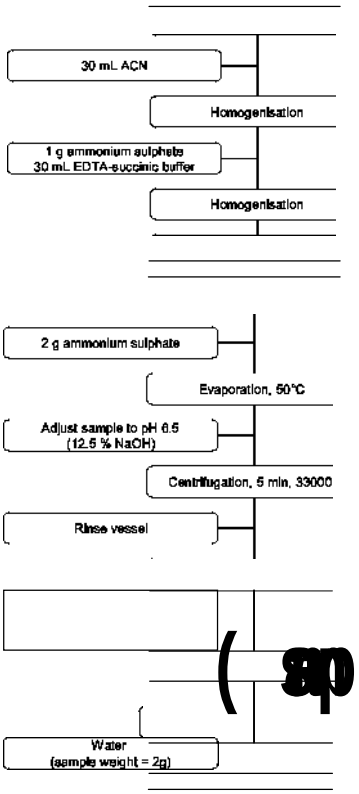

30 mL ACN
Homogenisation
1 g ammonium sulphate
30 mL EDTA-succinic buffer
Homogenisation
2 g ammonium sulphate
Evaporation, 50°C
Adjust sample to pH 6.5
(12.5 % NaOH)
Centrifugation, 5 min, 33000
Rinse vessel
Water
(sample weight = 2g)

Table 1. Conventional sorbent phases used in solid phase extraction

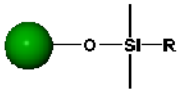
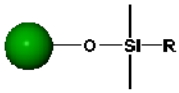
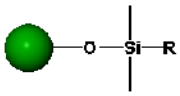
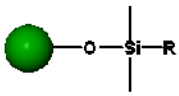
Mode	Sorbent Type	Advantages/ Disadvantages	Applications
Reversed Phase	 R=-(CH_2)n-CH_3 (n=1,3...17) R= C_6H_{11} R= C_6H_5	*Advantages:* • Robust, stable • General clean-up • Broad application range • Suitable for polar extracts *Disadvantages:* • Non-specific • Residual silanol group effects • Not suitable for highly polar analytes	Benzimidazoles (ref. [106] – C18). B-agonists (ref. [107] – C18)
Normal Phase	 R=OH R=CN R=Diol Al_2O_3 MgO_3Si	*Advantages:* • Suitable for a range of analytes • Generic clean-up • Suitable for polar analytes *Disadvantages:* • Non-selective elution can occur • Residual silanol group effects • Limited operational pH range	Corticosteroids (ref. [108]– SiOH) Macrocyclic Lactones (ref. [109] – alumina) Pyrethroids (ref. [110] – MgO_3Si)
Anion Exchange	 R=NH_2 R=PSA_1 (Primary/Secondary Amine) R=WAX (weak anion exchanger) R=SAX (strong anion exchanger)	*Advantages:* • Greater specificity than RP/NP SPE • Suitable for extraction of acidic drugs *Disadvantages:* • Slower kinetics than reverse-phase or normal-phase interactions	Oxytetracycline, sulfachloropyridazine (ref. [111] – Isolute SAX)
Cation Exchange	 R=Diethylaminopropyl R=PSA2 (Propylsulphonic Acid) R=WCX (weak cationic exchanger) R=SCX (strong cationic exchanger)	*Advantages:* • Greater specificity than RP/NP SPE • Suitable for extraction of basic drugs *Disadvantages:* • Slower kinetics than reverse-phase or normal-phase interactions	17 basic drugs (ref. [112] – Bond Elut SCX)

Table 2. Examples of selected polymeric sorbent phases used in solid phase extraction

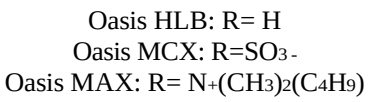
Sorbent Type	Advantages/Disadvantages	Applications
Metharcylate-Divinyl-benzene (Nexus)	*Advantages:* No secondary silanol effects Higher surface area than RP/NP bonded phases, thus less sorbent required Large range of compounds can be extracted Extraction may be performed at extremes of pH No need for pre-wetting	Tetracyclines (Ref. [113])
Styrene Divinyl-benzene (SDVB)		Hydrophobic analytes
Pyrrolidone-based Copolymeric Adsorbents  Oasis HLB: R= H Oasis MCX: R=SO_3^- Oasis MAX: R= $N^+(CH_3)_2(C_4H_9)$	*Disadvantages:* Hydrophobicity of most copolymers limits retention of polar compounds Irreversible adsorption of highly acidic/basic drugs on mixed mode sorbents	Multi-class methods (Ref. [15] - Oasis HLB) Aminoglycosides (Ref. [114] - Oasis HLB) Closantel (Ref. [115] - Oasis Max)

Table 3. Examples of methods used for the analysis of stilbenes.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
3	Tissues	LSE (MTBE, acetate buffer + β-glucuronidase)	Automated SPE (Sep-Pak silica)	-	LC-MS/MS	59-108	0.03-0.3	[178]
3	Urine, plasma	-	IAC (sepharose gel, antibodies raised against DES)	-	GC-MS	28-96	0.04-0.45	[180]
3 (+ zeranol)	Urine	-	IAC	-	GC-MS	63-109	0.15-0.84	[181]
3	Kidney, liver, milk, urine	LE (MeOH/1% HAc, EtOAc)	SLM (5% tri-n-octylphosphine oxide in n-undecane/di-n-hexylether, 0.2 µm Teflon filter)	-	LC-MS	60-93	1.3-4.3	[182]
3 (+ 6 resorcyclic acid lactones)	Urine	LLE with diethylether, defatting with hexane.	SPE (Oasis HLB + NH_2)	Enzymatic hydrolysis (helix promatia in sodium phosphate buffer)	LC-MS/MS	95-108	<1	[183]
3	Urine, plasma	IAC (sepharose 4B)	Elution solvent: Acetone:H_2O (95:5, v/v)	-	GC-MS	28-96	-	[184]

Table 4. Examples of methods used for the analysis of thyreostats.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
5	Tissue	MSPD (silica gel)	Wash solvent: chloroform	Elution solvent: (MeOH:chloroform)	GC-MS	47-97 72-97	10-50 2-10	[78] [80]
5	Milk, urine	MSPD (silica gel)	Wash solvent: MeOH:chloroform (5:95, v/v)	Elution solvent: MeOH:chloroform (20:80, v/v)	GC-MS	50-103	1.6-4	[79]
6	Thyroid	LSE (EtOAc)	Automated GPC	-	UPLC-MS/MS	80-109 (except tapezole-20%)	50	[145]
7	Muscle, liver, thyroid, urine, feed, faeces, hair	Samples were diluted with buffer, adjusted to pH 2-3 (35 % HCl), derivatised and extracted with diethyl ether.	Tissue and feed required an initial C_{18} SPE step SPE (silica)	Solid samples were initially freeze-dried and extracted with MeOH.	LC-MS/MS	-	15 (muscle, liver, thyroid) 5 (faeces) 50 (hair) 0.1-5.1 (urine)	[186]
6	Thyroid	LSE (MeOH), evaporated and reconstituted in DCM/cyclohexane	SPE (silica)	-	UPLC-MS/MS	40-79	25	[187]
5	Thyroid	LSE (MeOH)	SPE (silica)	-	LC-MS/MS	-	0.9-1.9	[188]
6	Thyroid, muscle	LSE (EtOAc), LLP (hexane)	SPE (NH_2)	TAP + MBI required an additional SPE (alumina) step	HPLC-UV or LC-MS	-	5	[189]

Table 5

Table 5. Examples of methods used for the analysis of synthetic steroids and resorcyclic acid lactones.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (µg kg^{-1})	Ref
22	Urine	Enzymatic hydrolysis (Helix Promatia)	Automated SPE (C_{18} + NH_2)	-	LC-MS/MS	74-114	≤0.8 µg L^{-1}	[147]
26	Muscle, kidney fat	LSE (MeOH + NaOAc),	SPE (silica + NH_2)	After LSE, defatting with hexane, LLP (diethylether)	GC-MS/MS	-	≤2.5	[192]
22 anabolic steroids	Muscle	LSE (MeOH)	SPE (C_{18} + NH_2) Elution solvent: ACN	Enzymatic hydrolysis (subtilisine)	LC-MS/MS	-	<0.5	[193]
11	Kidney , muscle, liver, milk	LLE (MeOH), defatting with hexane	SPE (Oasis HLB, Silica, NH_2)	Enzymatic hydrolysis (Helix Promatia)	LC-MS/MS	64-104	≤0.12	[194]
22	Urine	Dilution with H_2O, adjustment to pH 7	SPE (C_{18}) Hydrolysis (abalone acetate powder) SPE (NH_2)	-	GC-MSn	-	2.5 (10, α-trenbolone)	[195]
7	Kidney fat	PLE (containing alumina + anh. Na_2SO_4) 50°C, 1 cycle, 5 min	SPE (C_{18})	Pre-PLE: defatting with hexane. Elution with ACN	LC-MS/MS	17-58 (100-135% with i.s.)	≤0.1	[198]
7	Tissues	SFE (CO_2)	-	-	LC-MS/MS	-	10	[199]
13	Muscle, skin, fat	SFE (CO_2) with in-line alumina trapping	Alkaline hydrolysis, MTBE extraction	-	GC-MS	-	2 (5, melengestrol)	[200]
Estradiol	Serum	LLE (acetate buffer)	SPE (C_{18})	-	LC-MS/MS	86-93	≤0.1	[201]
Estradiol	Serum	Enzymatic hydrolysis (β-glucuronidase) Acylation with 2,3,4,5,6-pentafluorobenzoylchloride	LLP (ACN)	Drying on Na_2SO_4 columns	GC-MS/MS	-	≤5	[202]
2	Serum	LLE (1-chlorobutane)	SPE (Bond-Elut silica)	-	GC-MS	80-120	<83	[203]
5	Serum	LLE (acetate buffer)	SPE (C_{18})	-	LC-MS/MS	90-97	100	[204]
8 progestogens	Eggs	MSPD (C_{18})	Wash solvent: MeOH:H_2O (1 :9, v/v). Elution solvent: MeOH	SPE (GCB)	LC-MS/MS	84-111	0.2-2	[205]
10	Muscle	LSE (MeOH)	Freezing-lipid filtration. SPE (C_8, silica, NH2)	-	GC-MS	68-106	0.1-0.4	[206]
10	Tissues	LSE (ACN), defatting with hexane	SPE (C_{18})	-	GC-MS/MS	79-104	0.1-0.4	[207]
13	Muscle	LLE (EtOAc:diethyl ether)	SPE (Strata-X)	Enzymatic hydrolysis (Helix Promatia)	UPLC-MS/MS	98-102	≤0.3	[208]
18	Urine	Enzymatic hydrolysis (Helix Promatia)	SPE (C_{18} + HLB)	-	GC-MS	60-122	<1	[209]

Table 6. Examples of methods used for the analysis of β-agonists.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (jig kg^{-1})	Ref
9	Bovine liver, retina	LSE (sodium citrate buffer)	96-well SPE (HCX mixed-mode)	Enzymatic hydrolysis (Helix Promatia)	LC-MS/MS	56-114	0.02-0.5	[32]
12	Bovine liver, retina	Protease enzyme digestion LLP (DCM :hexane)	SPE (Oasis HLB)	LLE (MTBE + NaCl/EtOAc:IPA)	LC-MS/MS	92-118	0.5-5	[33]
4 β-agonists	Urine	IAC (Fractoprep, Merck)	Elution solvent: EtOH :0.03M acetate buffer, pH 4 (8:2, v/v)	-	HPLC-EC	65-75	-	[58]
8 β-agonists	Bovine urine	LLE (0.05M acetate buffer, pH5.2)	SPE (C_{18})	Elution solvent: MeOH + 1% TEA	LC-MSn	71-82	-	[107]
Clenbuterol	Feed, liver, urine, milk	MIPs (clenbuterol template)	Elution solvent: MeOH:TFA (99:1, v/v)	-	H PLC-PDA	91-93	5 (20 for liver)	[127]
10	Urine	Urine was mixed with polyclonal antibodies raised against salbutamol	Ultra-filtration	-	ELISA	-	0.14	[141]
7	Liver, urine	LE (acetate buffer) NaOH hydrolysis	SPE (C_{18}) LLE (MTBE)	Hexane wash in SPE step.	LC-MSn	-	0.05-0.2	[213]
22	Bovine and porcine urine, feed, hair	LE (NaOAc buffer)	SPE (Bond Elut mixed-mode)	Enzymatic hydrolyis (urine), acid hydrolysis (feed), NaOH digestion (hair)	LC-MS/MS UPLC-MS/MS	85-111 (urine)	≤10	[214]
10	Bovine muscle	Enzymatic digestion. LLE (EtOAc)	MIPs	Defatting with heptane	LC-MSn	84-134	0.13-1	[215]
8 β-agonists	Bovine urine	Dilution with H_2O Enzymatic hydrolysis	MIPs Elution solvent: MeOH:HAc (9:1, v/v)	Enzymatic hydrolysis	LC-MS/MS	32-66	0.01-1.9	[216]
Ractopamine	Porcine	LE (ACN + 4M potassium carbonate)	MIPs (ractopamine template)	Defatting with hexane MIPs coulple on-line to HPLC	HPLC-FL	56-67	4.6	[217]
15 β-agonists	Urine	MIPs (MIP4SPE ,MIP Technologies, Lund, Sweden)	Elution solvent: MeOH:HAc (9:1, v/v)	-	LC-MS/MS	72-111	<3	[222]
6 β-agonists	Bovine urine, hair	LE (PBS)	SPE (Extrelut 5)	Elution solvent: hexane:DCM (8:2, v/v)	GC-MS	65-85	0.5-2.5	[223]
5 β-agonists	Bovine urine	IAC (sepharose)	Elution solvent: 2% HAc	Coupled on-line via HPLC (C_{18})	LC-MS/MS	94-108	-	[224]

Table 7. Examples of methods used for the analysis of amphenicols.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (rig kg^{-1})	Ref
CAP	Honey	QuEChERS (ACN)	DSPE (PSA)	-	LC-MS/MS	83-89	0.2	[67]
CAP	Milk	MIPs	Elution solvent: H_2O:MeOH (1:9, v/v) + 1% HAc	-	LC-MS/MS	96-102	0.06	[130]
FF + FFA	Fish, shrimp, porcine muscle	LSE (fish + shrimp; ACN:EtOAc, porcine; EtOAc:NaOH)	SPE (Oasis MCX)	Defatting with hexane	GC-ECD	72-110	0.5-1	[225]
4	Chicken muscle	LSE (EtOAc)	SPE (Oasis MCX)	Defatting with hexane	LC-MS/MS	95-107	0.1-1	[226]
4	Poultry, porcine muscle, liver	LSE (EtOAc + 2% NH_4OH)	SPE (Oasis HLB)	Defatting with hexane	GC-MS	79-105	0.1-0.5	[227]
4	Aquatic species	LSE (acetone)	LLP (DCM)	Defatted with hexane	LC-MS	71-107	0.1-1	[228]
CAP	Shrimp	LSE (EtOAc:NH_4OH + ACN)	LLP (hexane + EtOAc)	-	Microbial assay	96	1	[230]
FF + FFA	Fish feed	LSE (EtOAc)	LLP (hexane + PBS buffer)	Dilution with buffer	ELISA	98-121	2000	[231]
FF + FFA	Porcine muscle	LSE (PBS buffer)	-	Dilution with buffer	ELISA	5 8-97	4	[232]
CAP	Muscle	MSPD (C_{18})	Wash solvent: hexane + ACN:H_2O (5:95, v/v). Elution solvent: ACN:H_2O (1:1, v/v)	LLP (EtOAc)	GC-ECD + GC-MS	93-98	1.6	[234]
CAP	Honey, urine, milk, plasma	MIPs (chloramphenicol template)	Elution solvent: Honey: MeOH:DCM (1:9, v/v). Urine: MeOH. Milk + plasma: MeOH:HAc:H_2O (89:1:10, v/v/v)	-	LC-MS/MS	57-120	0.02-0.03	[235]
CAP	Porcine muscle	IAC (sepharose 4B)	Elution solvent: MeOH	-	HPLC-UV	66-75	-	[236]
CAP	Milk, egg	IAC (silica)	Elution solvent: Glycine 0.2M:NaCl 0.5M (pH 2.8)	-	HPLC-UV	80-100	-	[237]
CAP	Porcine liver, kidney, muscle, urine	IAC (agarose)	Elution solvent: EtOH: H_2O (7:3, v/v)	-	GC-ECD	54-96	-	[238]
CAP	Chicken liver, muscle	IAC (sepharose 4B)	Elution solvent: MeOH	-	GC-EC	74-97	-	[239]
CAP	Shrimp	IAC (silica [sol-gel])	Elution solvent: ACN: H_2O (4:6, v/v)	-	HPLC-UV	68	-	[240]
FF	Swine feed	LSE (ACN:H_2O)	SPE (Envi-Carb; GCB)	-	HPLC-UV	99-101	-	[241]

Table 8. Examples of methods used for the analysis of nitrofurans.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
4	Porcine retina	LLE (EtOAc)	-	Overnight hydrolysis + derivatisation	LC-MS/MS	-	-	[36]
AOZ	Porcine liver	LLE (EtOAc)	SPE (MAX + HLB)	Samples were pre-washed and derivatised (HCl) overnight	HPLC-UV + LC-MS/MS	84-90	1	[50]
5	Poultry muscle	LLE (EtOAc)	Ultra-centrifugation	Samples were derivatised (HCl) overnight prior to LLE Samples were pre-washed (protein-bound residues only)	LC-MS/MS	-	0.5	[247]
4	Honey	Dissolve samples in 0.12M HCl	SPE (Oasis HLB)	-	LC-MS/MS	-	0.2-0.3	[251]
4	Honey	Dissolve samples in 10% NaCl	SPE (Oasis HLB)	Hydrolysis, derivatisation, LLP (EtOAc + hexane)	LC-MS/MS	92-103	0.25	[252]
4	Milk	LLP (hexane)	SPE (Oasis HLB)	Overnight hydrolysis + derivatisation	LC-MS/MS	83-104	$\leq$0.2	[253]
4	Milk	LLE (EtOAc)	-	Overnight hydrolysis + derivatisation	LC-MS/MS	91-107	0.12-0.29	[254]
4	Chicken tissues, eyes	LLE (EtOAc)	-	Samples were derivatised (HCl) overnight prior to LLE Samples were pre-washed (protein-bound residues only)	LC-MS/MS	-	-	[255]

Table 9. Examples of methods used for the analysis of nitroimidazoles.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
7	Poultry muscle, egg	LSE (potassium phosphate buffer + EtOAc)	Defatting with hexane	-	LC-MS/MS	88-111	0.07-0.36	[256]
7	Poultry, porcine muscle	Protease digestion LLE (NaCl:KH_2PO_4 buffer)	SPE (EXtrelut NT20)	Defatting with hexane	GC-MS	94-118	0.65-2.8	[261]
4	Porcine liver	LSE (EtOAc)	SPE (Oasis MCX)	Defatting with hexane	LC-MS/MS	83-98	0.1-0.5	[266]
2	Poultry, porcine tissues	LSE (Na_2SO_4 + toluene)	SPE (NH_2)	-	GC-MS	72-106	0.1-1.5	[267]
6 + 4 NFs	Porcine muscle	Acid hydrolysis (0.2M HCl)	SPE (Oasis HLB)	-	LC-MS/MS	93-106	0.01-0.2	[269]
5	Poultry muscle	LSE (EtOAc)	-	-	SPR	-	2	[270]
7	Porcine plasma	Protease digestion LLE (NaCl: KH_2PO_4 buffer)	SPE (Chromabond XTR)	Defatting with hexane	LC-MS/MS	93-123	0.25-1	[271]
10	Bovine, porcine, ovine, avian, equine plasma	LLE (ACN + NaCl)	LLP (hexane)	-	LC-MS/MS	101-108	0.52-1.52	[272]
7	Muscle	LSE (EtOAc)	SPE (SCX)	Elution solvent: 28% NH_3OH:ACN (5:95, v/v)	HPLC-UV	71-100	0.2	[273]
4	Egg, plasma, faeces	LE (aqueous buffer)	SPE (EXtrelut)	LLP (isooctane)	HPLC-UV	-	10	[274]
7	Kidney, liver, serum, milk, egg	LE (ACN)	Ultra-centrifugation	-	SPR	-	1-3	[275]

Table 10. Examples of methods used for the analysis of aminoglycosides.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
9	Milk	MSPD (sand)	PLE (H_2O) 70°C, dynamic mode (1 mL min^{-1}), 4 min	-	LC-MS/MS	70-92	2-13	[83]
13	Various	LSE (5% TFA)	Ion-pair SPE (Oasis HLB)	Elution solvent: ACN:0.2M HFBA (8:2, v/v)	LC-MS/MS	61-116	2-25	[114]
11	Muscle, liver	LSE (5 % trichloroacetic acid)	SPE (WCX)	-	LC-MS/MS	40-80	15-40	[276]

Table 11. Examples of methods used for the analysis of β-lactams.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (rig kg^{-1})	Ref
10 β-lactams	Bovine Kidney	LSE (ACN:H_2O, 8:2, v/v)	DSPE (C_{18})	-	LC-MS/MS	58-75	<MRL	[64]
10	Milk	Centrifugation	On-line SPE (C_{18})	-	LC-MS/MS	83-139	0.0144-0.5115	[160]
3 penicillins	Milk	LLE (ACN)	LLP (phosphate buffer, DCM, petroleum ether)	-	HPLC-UV	94-103	10	[278]
11	Bovine kidney	LSE (ACN:H_2O, 4:1 v/v)	DSPE (C_{18})	-	LC-MS/MS	87-103	-	[279]
6 penicillins	Bovine liver, kidney, muscle	Muscle – LSE (2% NaCl) Liver, kidney – LSE (5% sodium tungstate, 0.17 *M* sulfuric acid, 2% NaCl)	Ion-exchange SPE (Bond Elut C_{18} + Sep-Pak Accell Plus QMA)	-	LC-MS/MS	77-101	2-10	[281]
5	Milk	-	RAM (C8, C18, phenyl, cyano)	-	HPLC-UV	91-94	50-100	[282]
15	Bovine muscle, kidney, milk	LE (ACN:H_2O)	SPE (Oasis HLB)	-	LC-MS/MS	71-116	4.6-359	[283]
11	Milk	LLE (ACN)	-	-	LC-MS/MS	57-88	1-25	[284]
5 penicillins	Porcine muscle	MSPD (C_{18})	Wash solvent: hexane. Elution solvent: MeOH (4°C)	SPE $_{(C18)}$ cleanup	HPLC-UV	45-130	20	[286]
8 penicillins	Milk, porcine liver, kidney	Milk - LLE (phosphate buffer, pH8 + hexane) Tissue – LSE (MeOH)	Milk – SPE (C_{18})	-	HPLC-UV + LC-MS	90-111 (UV) 83-95 (MS)	2.27-4.06 (UV) 0..01-0.51 (MS)	[287]
4 cephalasporins	Milk	LLE (ACN)	SPE (C_{18})	-	HPLC-UV	69-93	7-11	[288]

Table 12. Examples of methods used for the analysis of macrolides and lincosamides.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
5	Milk, yoghurt	MSPD (sand)	Elution solvent: H_2O (70°C, 30mM formic acid)	-	LC-MS/MS	86-117	0.2-7	[85]
5	Honey	LLE (tris buffer, pH 10.5)	SPE (Oasis HLB)	-	LC-MS/MS	77-103	<0.25	[293]
6 macrolides	Eggs, honey, milk	LLE (ACN or 0.1 M phosphate buffer, pH 8)	SPE (Oasis HLB)	-	UPLC-QToF MS or LC-MS/MS	88-115	0.01-0.5	[295]
7 macrolides	Liver, kidney	LSE (EDTA:McIlvaine's buffer)	SPE (Oasis HLB)	Elution solvent: MeOH	HPLC-PDA	40-93	15-50	[296]
9 macrolides	Meat, fish	LSE (0.2 % metaphosphoric acid:MeOH, 6:4)	SPE (Oasis HLB)	-	LC-MS	70-93	10	[298]
9	Bovine, porcine, poultry muscle	LSE (ACN)	Dilution (H_2O) + defatting with hexane	-	LC-MS/MS	62-117	0.36-0.7	[299]
5	Feed	PLE (Acetone:H_2O (65 :35, v/v) pH 2.0)	80°C, 2 cycles, 5 min	Mix sample + sand SPE (HIILIC)	IST (screening test)	57-96	5400-10000	[300]

Table 13. Examples of methods used for the analysis of quinolones.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
7	Bovine tissues	MSPD (sand)	Elution solvent: H_2O (100°C)	-	LC-MS/MS	87-109	2-9	[86]
8	Milk	MSPD (sand)	Elution solvent: H_2O (90°C)	-	LC-MS/MS	93-110	0.3-1.5	[87]
7	Eggs	MSPD (sand)	Elution solvent: H_2O + 50 mM formic acid (1 00°C)	-	LC-MS/MS	89-103	0.2-0.6	[88]
13 quinolones + 6 sulphonamides	Muscle	LSE (MeOH:H_2O, 8:2)	IAC (sepharose 4B)	Elution solvent: MeOH:H_2O:NH3 (90:9.8:0.2, v/v/v)	LC-MS/MS	75-104	0.5-3	[122]
10	Chicken muscle	LSE (0.1M PBS, pH 7)	IAC (sepharose 4B)	Elution solvent: 70% MeOH in PBS	HPLC-FL	82-101	0.1-0.15	[123]
13	Feed	PLE (0.2% MPA H_2O:ACN (70:30, v/v) pH 2.6)	-	Automated SPE (Oasis HLB)	LC-PDA; LC-FI	31-103	400-1500	[149]
10	Bovine, porcine, ovine muscle	LSE (0.1 % TFA in MeOH)	SPE (LiChroLut RP-18)	-	HPLC-UV	92-107	10-18	[304]
22	Milk	LLE (EDTA-McIlvaine buffer)	SPE (Bond Elut Plexa)	-	UPLC-MS/MS	41-95	0.01-0.34	[305]
11	Pig kidney	LSE (ACN)	SPE (SDB-RPS)	-	LC-MS/MS	99-104	0.3-1.5	[307]
4	Eggs	SFE (CO_2 + 20% MeOH)	300 bar, 60°C	-	HPLC-FL	83-96	10	[308]
8	Avian muscle	PLE (DCM) In-line SPE (Oasis MCX)	50°C, 1 cycle, 0 min	Mix sample + diatomaceous earth. SPE (Oasis HLB)	CE-MS/MS	63-112	0.040-0.140	[309]
4	Urine, tissue	MIPs (enrofloxacin template)	Elution solvent: ACN + 4% formic acid	SPE (Oasis HLB)	HPLC-UV	70-96	30	[310]
5	Eggs and tissue	MIPs (ofloxacin template)	Elution solvent: ACN:TFA (99:1, v/v)	M I-MSPD	HPLC-FL	86-105	0.05-0.09	[311]
7	Muscle	LSE (PBS)	SPE (Oasis HLB)	Elution solvent: MeOH	HPLC-FL	70-106	0.1-0.3	[312]
5	Milk	LLE (ACN)	SPE (Strata X)	Elution solvent: ACN:1% TFA (75:25, v/v)	LC-UV, LC-FL, LC-MS, LC-MS/MS	80-100	9-13, 3-8, 1-5, 0.5-1	[313]
7	Gilthead seabream	LSE (0.1M NaOH)	SPE (Oasis HLB)	Elution solvent: 0.1% TFA in ACN + ACN	LC-MS/MS	90-132	2-2.7	[314]
9	Pig kidney	LSE (0.35% m-phosphoric acid:ACN, 73:27)	SPE (ENV+ Isolute)	-	LC-ToF MS	≥63	0.1-2	[315]

Table 14. Examples of methods used for the analysis of sulphonamides.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
6	Milk	LLE (ACN)	Ultra-filtration	-	LC-MS/MS	90-125	5-10	[19]
6	Chicken muscle	LSE (ACN)	DSPE (C_{18})	-	HPLC-FL	90-95	1-5	[62]
12	Milk, eggs	MSPD (sand)	Elution solvent: H_2O (milk 75°C, egg 100°C)	-	LC-MS	77-92	1-3	[82]
24	Muscle	LLE (ACN)	LLP (H_2O: EtOAc)	Defatting with hexane	UPLC-MS/MS	68-114	0.04-0.37	[319]
10	Tissues	LLE (acetone: chloroform)	SPE (cation-exchange)	-	HPLC-UV	63-77	3-14	[320]
7	Milk	LLE (acetone: chloroform)	SPE (cation-exchange)	-	HPLC-PDA	56-8 1	20	[321]
10	Egg	LLE (DCM: acetone + HAc)	SPE (cation-exchange)	-	LC-MS/MS	100	15	[322]
4	Muscle, liver	LSE (ACN:1-propanol)	SPE (Cleanert-PEP)	-	CZE	83-95	4-6	[323]
12	Tissues	LSE (ACN)	SPE (C_{18})	Derivatisation + SPE (silica)	HPLC-UV	65-103	3-5	[324]
8	Honey	MSPD (C_{18})	Wash solvent: hexane Elution solvent: EtOAc	Derivatisation + SPE (silica)	HPLC-UV	63-96	4	[325]
13	Bovine muscle, baby food	MSPD (C_{18})	Elution solvent: MeOH	-	LC-MS/MS	87-101	0.06-0.35	[326]
9	Chicken muscle, liver	LSE (MeOH:H_2O)	IAC (sepharose 4B)	-	HPLC-UV	74-109	2	[327]
6 SAs + 3 TCs + pyrimethanime	Mild	LLE (20% TCA + McIlvaine buffer)	SPE (Oasis HLB)	-	LC-MS/MS	72-79	0.48-2.64	[328]
12	Cattle, fish muscle	MSPD (sand)	Elution solvent: H_2O (80°C)	-	LC-MS/MS	73-104	3-15	[329]
6	Muscle	MSPD (alumina)	Elution solvent: H_2O:EtOH (7:3, v/v)	-	HPLC-PDA	85-101	6-40	[330]
10	Porcine Kidney	SFE (CO_2 + MeOH)	361 bar, 90 °C	-	SFC-MS	-	-	[331]
13	Meat and infant food	PLE (H_2O)	160°C, 1 cycle, 15 min	MSPD	LC-MS/MS	70-101	0.4-2.6	[332]
12	Pork muscle	PLE (H_2O)	160°C, 1 cycle, 5 min	Mix sample + diatomaceous earth. SPE (Oasis HLB)	CE-MSn	76-98	1.56-12.5	[333]

Table 15. Examples of methods used for the analysis of tetracyclines.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
7	Muscle	MSPD (sand)	Elution solvent: H_2O (70°C)	-	LC-MS/MS	88-109	1-9	[84]
5	Bovine Muscle	LSE (0.3M citrate buffer, pH 4)	SPE (Abselut Nexus)	Elution solvent: MeOH:ACN:0.05M oxalic acid (3:3:4, v/v/v)	HPLC-PDA	99-103	25-40	[113]
7	Bovine liver, kidney	LSE (15% TFA + 0.4M oxalate buffer, pH 4)	SPE (Discovery [liver], Lichrolut [kidney])	Elution solvent: MeOH:ACN:oxalic acid (30:30:40, v/v/v)	HPLC-PDA	92-125	10-54	[334]
3	Porcine muscle, liver	LSE (EDTA-McIlvaine buffer, pH 4)	SPE (Oasis HLB)	-	LC-MS/MS + HPLC-FL	65-90	50 (muscle) 100 (liver)	[336]
10	Milk	LLE (0.1 M EDTA-McIlvaine buffer)	SPE (Oasis HLB)	-	LC-MS/MS	74-101	0.5-10	[337]
7	Milk	LLE (20% TCA)	SPE (Oasis HLB)	-	LC-MS/MS	90-101	5-25	[338]
7	Bovine, porcine muscle	LSE (0.4 M oxalate buffer, pH 4)	SPE (Nexus)	-	HPLC-PDA	89-114	3-14	[339]
5	Honey	LLE (0.1 M Na_2EDTA-McIlvaine buffer, pH 4)	Automated SPE (C_{18})	-	HPLC-UV	84-1 21	5-12	[340]
4	Porcine Kidney	MIPs (TC + OTC template)	Elution solvent: MeOH:1M KOH (9:1, v/v)	-	HPLC-UV	-	-	[341]
4	Lobster, duck, honey, egg	Lobster – LSE (McIlvaine buffer) Duck, Honey – LE (5% HCl + HAc:MeOH) Egg - MSPD (silica)	MIPs (TC template) – lobster, duck, honey Elution solvent: MeOH:0.1M KOH)	-	LC-MS/MS	95-103	0.1-0.3	[342]
4	Bovine, porcine, poultry, lamb muscle	Dionex ASE 200® PLE (H_2O)	70°C, 1 cycle, 10 min	Samples were pre-mixed with sand SPE (Oasis HLB)	LC-MS/MS	>89	0.5-1	[343]
7	Cheese	MSPD (sand)	Elution solvent: H_2O (70°C)	-	LC-MS/MS	96-117	1-2	[346]
10	Milk	LLE (0.1M EDTA:McIlvaine's buffer)	SPE (Oasis HLB)	Elution solvent: MeON:EtOAc (9:1, v/v)	LC-MS/MS	74-101	0.5-10	[347]

Table 16. Examples of methods used for the analysis of anthelmintics.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
38	Bovine liver, milk	QuEChERS (ACN + $MgSO_4$)	DSPE (C_{18})	-	LC-MS/MS	61-115	5	[16]
12 BZs	Bovine liver	LSE (EtOAc)	SPE (C_{18})	Elution solvent: EtOAc	HPLC-UV	25-100	-	[106]
5 MLs	Liver	LSE (isooctane)	SPE (alumina)	Elution solvent: MeOH:EtOAc (7:3, v/v)	HPLC-FL	80-9	2	[109]
5 MLs	Bovine liver	LSE (ACN)	SPE (alumina + $_{C18}$)	Elution solvent: ACN	HPLC-FL	73-97	2	[348]
3 MLs	Milk	LLE (ACN)	SPE (alumina)	-	HPLC-FL	72-8 1	1	[349]
5 MLs	Milk	LLE (ACN)	SPE (C_8)	-	HPLC-FL	-	0.1	[350]
6 MLs	Milk	LLE (ACN)	SPE (C_{18})	-	LC-MS/MS	>95	0.14-0.25	[351]
22 BZs	Muscle	LSE (ACN + 0.1% formic acid)	Centrifugation	-	LC-MS/MS	-	5-10	[352]
23	Milk	LLE (ACN)	Defatting with hexane	Acid hydrolysis	LC-MS/MS	-	1-5	[353]
17 BZs	Milk	LLE (ACN)	On-line SPE (Oasis MAX)	-	LC-MS/MS	68-107	2	[354]
8	Bovine kidney	LSE (ACN + 1% HAc)	SPE (Oasis MAX)	-	LC-MS/MS	77-8 1	-	[355]
4 MLs	Bovine liver, muscle	LSE (ACN)	SPE (C_{18})	Elution solvent: ACN	HPLC-FL	70-94	0.5-1	[356]
4 MLs	Bovine liver	IAC (sepharose 4B)	Elution solvent: MeOH	-	HPLC-FL	79-116	-	[357]
4 MLs	Bovine liver, muscle	IAC (sepharose 4B)	Elution solvent: MeOH	-	LC-MS/MS	63-84	-	[358]
5 MLs	Liver	SFE (CO_2)	300 bar, 100°C	SPE (alumina)	HPLC-FL	76-97	2	[359]
10 BZs	Liver	SFE (CO_2)	690 bar, 80°C	SPE (SCX)	HPLC-UV	51-115	50	[360]
5 MLs	Bovine liver	LSE (ACN)	SPE (alumina (NP), C_{18} (RP))	Elution solvent: ACN	HPLC-FL	72-86	-	[361]

Table 17. Examples of methods used for the analysis of anticoccidials.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
6	Chicken egg, fat, liver, muscle, plasma	LLE (acetone:THF, 6:4)	LLP	SPE (silica)	LC-MS	61-114	1-7 (4-10, plasma)	[7]
14	Chicken muscle, egg	LLE (ACN)	-	Samples were pre-mixed with Na_2SO_4	LC-MS/MS	78-125	0.1-0.2	[363]
5	Egg	LLE (ACN)	-	-	LC-MS/MS	42-1 13	0.75-6	[364]
3	Tissue, egg	LE (H_2O + MeOH)	LLP (hexane:toluene)	-	LC-MS/MS	73-117	1	[365]
4	Liver, eggs	LLE (ACN)	SPE (silica)	Samples were dispersed on Na_2SO_4	LC-MS/MS	93-106	2.5-50	[366]
5	Liver, eggs	LLE (ACN)	SPE (silica)	Samples were dispersed on Na_2SO_4	LC-MS/MS	86-118	2.5	[367]
9	Muscle, egg	LLE (ACN)	SPE (silica)	Samples were dispersed on Na_2SO_4	LC-MS/MS	40-60	0.07-0.6	[368]
4 ionophores	Egg	LLE (ACN)	-	-	LC-MS/MS	85-120	1	[369]
Toltrazuril + halofuginone	Egg	LLE (EtOAc)	SPE (Oasis HLB)	LLP (hexane:trisodium phosphate buffer)	HPLC-UV or LC-MS/MS	-	10-30 (UV) 1 (MS)	[370]
Toltrazuril + toltrazuril sulphone	Muscle, egg	LLE (acetone:THF, 6:4)	LLP (DCM)	Defatting with hexane	LC-MS	91-98	<2.5	[371]
12	Poultry liver	LLE (ACN)	SPE (Oasis HLB)	Defatting on alumina SPE columns	LC-MS/MS	81-129	0.04-10.9	[372]

Table 18. Examples of methods used for the analysis of carbamates.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (µg kg^{-1})	Ref
10	Liver	LSE (DCM)	GPC (SX-3 resin, 200-400 mesh) SPE (NH_2)	Samples were pre-mixed with Na_2SO_4	HPLC-FL	>80	-	[374]
3	Chicken, beef muscle	Online-SFE (CO_2) (219 atm, 90°C)	-	-	SFC-MS	53	175-200	[375]
6	Muscle	LLE (ACN) LLP (hexane)	SFE (CO_2) (329 bar, 60°C)	-	HPLC-FL or GC-MS	>80	1	[376]
5	Honey	Dissolved in H_2O	SPE (C_{18})	-	LC-MS	>75	<20	[377]
7	Honey	LLE (MeOH:EtOAc)	SPE (Florisil® + Na_2SO_4)	-	GC-MS	>75	≤8.7	[378]

Table 19. Examples of methods used for the analysis of pyrethroids.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (µg kg^{-1})	Ref
32 pesticides	Milk, egg	QuEChERS (ACN + 1% HAc, $MgSO_4$, NaOAc)	DSPE (PSA, C_{18}, $MgSO_4$)	-	GC-MS + LC-MS/MS	70-120	<10	[63]
4	Honey	LLE (H_2O:acetone, 1:1)	SPE (C_{18}, Florisil®)	Elution solvent: MeOH, ACN	HPLC-UV	96-99	0.2-1.6	[110]
18 pesticides	Beef fat	Melted fat was mixed with Florisil®	SPE (Florisil® + C_{18})	-	GC-ECD	88-137	3-118	[381]
3	Honey	LLE (hexane:acetone, 6:4) or SFE (CO_2 + 10% ACN) (400 bar, 90°C)	SPE (Florisil®)	-	GC-ECD + GC-MS	75-94	<10	[383]
12	Milk	SPME (100°C)	(polydimethylsiloxane (PDMS)/divinylbenzene (DVB) coating)	Sample was diluted with H_2O	GC-µECD	69-120	-	[384]
26 pesticides	Muscle	PLE (light petroleum) (70°C, 1 cycle, 10 min)	SPE (Extrelut NT3 + C_{18}) + Florisil® mini-cartridge	Samples were pre-mixed with Na_2SO_4 + sand	GC-MS/MS	84-99 (pyrethroids)	1-8	[385]
32 pesticides	Cattle feed	MSPD (alumina + Na_2SO_4)	Elution solvent: EtOAc	Florisil® was added as a co-column	GC-ECD	>75	0.03-1.5	[386]

Table 20. Examples of methods used for the analysis of sedatives.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
6	Porcine muscle	LSE (ACN)	SPE (C_{18})	-	LC-MS/MS	-	5	[387]
7	Urine	Dilution with TEA carbonate-bicarbonate buffer	SPE (C_{18})	LLP (MTBE)	GC-MS	>70	5-50	[388]
11 + 19 β-blockers	porcine kidney, liver, muscle; bovine muscle	LSE (ACN)	SPE (NH_2)	Samples were pre-mixed with Na_2SO_4	LC-MS/MS	84-113	≤0.6	[389]
7	Bovine + porcine kidney	LSE (ACN)	SPE (Oasis HLB)	-	LC-MS/MS	>75	<1	[390]
7	Porcine kidney + muscle	LSE (ACN)	SPE (Oasis HLB)	-	LC-MS/MS	83-109	1-10	[392]
2	Kidney	LSE (ACN)	SPE (Oasis MCX)	-	HPLC-FL	86-94	3-10	[393]
6	Porcine kidney	LSE (ACN:HCl, 75:25)	LLP (NaOH + DCM)	-	ELISA	-	5-20	[394]

Table 21. Examples of methods used for the analysis of non-steroidal anti-inflammatory drugs.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity ($\mu g\ kg^{-1}$)	Ref
12 Corticosteroids	Bovine hair, urine, muscle	LE	SPE $_{(C18}$ (RP), SiOH (NP))	Elution solvent: Diethylether (RP), EtOAc:cyclohexane:HAc (90:5:5, v/v/v)	LC-MS/MS	37.7-66.6%	0.04-0.07 (muscle, urine) 2.9-9.3 (hair)	[108]
3	Milk	LLE (ACN)	Centrifugation	-	LC-MS/MS	92-98	0.5-1	[395]
6	Bovine muscle	LLE (ACN)	SPE (Oasis HLB)	-	LC-MSn	-	21-59	[396]
4	Milk, bovine, porcine muscle	LLE (MeOH)	LLP (hexane)	-	LC-MS/MS	82-128	20-130	[397]
7	Milk	LLE (ACN + NaCl)	LLP (hexane)	-	LC-MS/MS	96-102	0.34-61	[398]
14	Plasma, serum	Hydrolyis + protein denaturisation (HCl)	SPE (C_{18})	-	LC-MSn	72-101	<25	[399]
4	Milk	LLE (ACN)	SPE (C_{18})	-	GC-MS/MS	104-112	0.59-2.69	[400]
16	Bovine, buffalo milk	LLE (ACN:MeOH, 9:1)	SPE (C_{18})	-	HPLC-PDA (screen) LC-MSn (confirm)	69-97	2-15	[401]
17	Equine plasma, urine	Acidification (pH 2-3) + LLE (diethyl ether)	LLP (plasma – sodium hydrogencarbonate buffer) (urine – sodium carbonate:sodium hydrogencarbonate buffer, 2 :1)	-	GC-MS	23-100	5-25	[402]
13	Plasma, serum	Hydrolyis + protein denaturisation (HCl)	SPE (C_{18})	-	HPLC-PDA	73-104	<25	[406]
5 Corticosteroids	Milk	LLE (20% TFA)	SPE (Oasis HLB)	Elution solvent: MeOH	LC-MS/MS	97-111%	≤0.4	[407]
5 Corticosteroids	Bovine liver, milk, urine, faeces	IAC (Tesyl gel)	Elution solvent: MeOH:H_2O (8:2, v/v)	-	GC-MS	50-80%	-	[408]

Table 22. Examples of methods used for the analysis of triphenylmethane dyes.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (jig kg^{-1})	Ref
MG + LMG	Fish	LSE (ACN, ammonium acetate buffer, hydroxylamine HCl, *p*-toluene sulphonic acid)	LLP (DCM)	Automated SPE (alumina + propylsulfonic acid)	LC-MS/MS	5 8-68	0.24	[409]
13	Fish	LSE (ACN + 0.1M ammonium acetate buffer)	LLP (DCM)	SPE (Isolute SCX-2)	LC-MS/MS	-	1.1-14	[410]
4	Aquatic products	LSE (ACN, McIlvaine buffer, *p*-toluene sulphonic acid, *N*,*N*,*N*',*N*'-tetramethyl-1,4-phenylenediamine dihydrochloride)	LLP (DCM)	SPE (alumina + Oasis MCX)	LC-MSn	81-116	0.5	[415]
4	Fish	LSE (ACN, ammonium acetate buffer, hydroxylamine HCl, *p*-toluene sulphonic acid)	LLP (DCM)	SPE (alumina + propylsulfonic acid)	HPLC-PDA (screen) LC-MSn (confirm)	67-9 1	0.07-0.24	[416]
MG + LMG	Fish	LSE (ACN, ammonium acetate buffer, hydroxylamine HCl, *p*-toluene sulphonic acid)	LLP (DCM)	SPE (acidified alumina)	LC-MS/MS	63-90	0.1-0.2	[417]
3	Fish	LSE (McIlvaine buffer + ACN)	LLP (DCM)	Sample mixed with SAX resin	ELISA	62-108	0.05	[418]
4	Fish	LSE (ACN, ammonium acetate buffer, hydroxylamine HCl, *p*-toluene sulphonic acid)	LLP (H_2O, DCM, diethylene glycol)	SPE (alumina + propylsulfonic acid)	HPLC-UV	49-8 8	03.-0.6	[420]

Table 23. Examples of methods used for multi-class multi-residue analysis.

Number of residues	Matrix	Extraction technique	Purification technique	Additional sample preparation	Detection system	Recovery (%)	Sensitivity (µg kg^{-1})	Ref
>100	Muscle	LE (ACN:MeOH, 95:5 v/v)	LLP (hexane)	-	LC-MS/MS	70-120	0.03-3	[12]
101	Milk	LLE (ACN)	Strata-X SPE	-	UPLC-Tof MS	80-120	<7	[13]
150	Milk	LLE (ACN)	Ultrfiltration	-	UPLC-Tof MS	60-120	0.5-25	[14]
100	Meat	Bipolarity extraction	Oasis HLB SPE	-	UPLC-Tof MS	>80	1-5	[15]
38 Anthelmintics	Liver and milk	QuEChERS	DSPE (C_{18})	-	LC-MS/MS	70-120	5	[16]
41	Chicken muscle	QuEChERS (ACN + 1% HAc)	DSPE (NH_2)	-	LC-MS/MS	53-110	0.5 x MRL	[17]
18 antibiotics	Milk	QuEChERS (ACN + 1% HAc: 0.1 M Na_2EDTA, 1:1, v/v)	No clean-up	-	UPLC-MS/MS	70-111	1-4	[72]
14	Bovine Muscle	MSPD (C_{18})	Elution solvent: Hexane, Benzene, EtOAc, MeOH	-	GC + HPLC-PDA	60-94	-	[74]
Antibiotics	Kidney	QuEChERS (ACN or ACN:H_2O [8:2, v/v])	DSPE (Silica or C_{18})	-	LC-MS/MS	-	-	[71]
31 basic drugs	Various	LSE (CAN + Na_2SO_4) MG + LMG from fish – LSE (citrate buffer:ACN)	SPE (Bond Elut SCX) Elution solvent: ACN:35% NH_3 (95:5, v/v) + MeOH:35% NH_3 (75:25, v/v)	Fish – LLP (DCM)	LC-MS, HPLC-UV, HPLC-FL	53-104	-	[112]
10	Milk	LLE (20% TCA)	SPE (Oasis HLB)	Elution solvent: MeOH + 5% MeOH:2% NH_3OH	LC-MS	72-97	0.48-2.64	[328]
29	Bovine muscle and kidney	Enzyme digestion, LE (IPA:H_2O)	C_{18} + Oasis MAX SPE	-	LC-MS/MS	≥50	≤1	[403]
66	Equine plasma	LLE (TrichloroHAc)	Bond Elut SPE	-	LC-MS/MS	≥60	5-500	[404]
100	Egg, fish, meat	LE (ACN:H_2O)	Strata-X SPE	-	UPLC-Tof MS	70-100	-	[421]
50 anabolic steroids	Muscle, liver, milk	Enzymatic hydrolyisis, LE (MeOH)	GCB + NH_2 SPE	-	UPLC-MS/MS	77-121	0.01-0.7	[422]
39 antibiotics	Meat	LE (MeOH:H_2O)	Dilution	-	LC-MS/MS	61-95	10 or 20	[423]
21 antibiotics	Porcine kidney, liver, muscle	EDTA-McIlvaine buffer	Oasis HLB SPE	-	UPLC-MS/MS	85-120	≤10	[424]
19 antibiotics	Muscle	LE (EDTA:ACN)	-	-	UPLC-MS/MS	94-102	≤5	[425]
19 antibiotics	Muscle and kidney	LE (70% MeOH)	Dilution	-	LC-MS/MS	46-121	2-15	[426]
31 antibiotics	Bovine and porcine muscle	PLE (H_2O)	70°C, 1 cycle, 10 min	Mix sample + sand	LC-MS/MS	75-99	3-15	[427]
25 antibiotics	Milk	LLE (ACN)	SPE (Oasis HLB) Elution solvent: ACN	Ultra-filtration	LC-MS/MS	>50	0.25-50	[428]
18	Shrimp	LSE (TCA: hydroxylamine hydrochloride)	SPE (Oasis HLB)	Elution solvent: MeOH + MeOH:ACN (1:1, v/v)	LC-MSn	40-90	≤200	[429]
14	Meat	MSPD (C_{18})	Wash solvent:	Elution solvent:	HPLC-PDA + HPLC-	45-102	0.4-42.5	[430]

			hexane	DCM, EtOAc	UV			
19 antibiotics	Muscle	LE (70% MeOH)	Dilution	-	LC-MS/MS	68-95	1-30	[431]
>100	Urine	Dilute and shoot	-	-	UPLC-Tof MS	130	≤45	[432]
42 antibiotics	Honey	LLE (4 separate extractions)	-	-	LC-MS/MS	40-90	27-80	[433]
13	Muscle	LE (ACN)	On-line SPE (HLB)	-	LC-MS/MS	-	0.03-8.4	[434]