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Review

Determination of pharmaceuticals in environmental and biological matrices using pressurised liquid extraction—Are we developing sound extraction methods?

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ABSTRACT

Pressurised liquid extraction (PLE) is now a well established and extensively applied extraction technique in environmental analysis for pollutants such as persistent organic pollutants (POPs). During the past decade, an emerging group of environmentally interesting analytes are pharmaceuticals that are continuingly released into the environment. This class is comprised with compounds of various properties. As the field of the analysis of these compounds grows, an increasing number of PLE methods for pharmaceuticals of varying quality are developed and published. This review summarises the critical PLE parameters during PLE method development and highlight them with examples from recently published papers utilising pressurised liquid extraction for the determination of pharmaceuticals in environmental and biological matrices. These recent methods are summarised and critically discussed with the aim to provide important reflections to alleviate in future PLE development for pharmaceuticals in environmental matrices.

Contents

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1. Introduction

PLE is today a well-established technique and has been used for the extraction of a great variety of compounds from numerous matrices [1,2]. In the field of environmental analysis PLE has been applied intensively and many accurate, precise and robust methods have been developed for persistent organic pollutants (POPs) such as DDTs, PCBs, dioxins, furans and PAHs. Many of these compounds are well suited for PLE since they are very stable and therefore high temperatures can be utilised which improves the desorption and mass transfer processes. The analytes are also rather uniform in the sense that they are lipophilic with few or no functional groups which simplifies the development of methods. Furthermore, many of these compounds are stable in harsh chemical surroundings, which means that strong acids or bases often can be used to remove the co-extracted unwanted matrix components [3,4]. Analysis of POPs also has a long tradition in the analytical chemical laboratory which means that there are many well-established techniques to compare PLE with during method development [3,5]. In addition, there are for these pollutants national monitoring programs since several decades, which existence were a result of demands from authorities requesting such programs. Consequently have a large number of reference materials been produced over the years and are today available for method development at reasonable costs and help elevate the quality of these analytical methods [6]. Various labelled standards are also commercially available for nearly all pollutants which means that isotope dilution can be performed which allows for very accurate and precise methods. Consequently PLE in POP analysis has reached a fairly high level of maturity.

In contrast to POPs, a rather new group of emerging contaminants are pharmaceuticals [7–10]. Even though many of these compounds are degraded to a great extent in the environment compared to POPs, they are still considered pseudo-persistent due to the constant input of the compounds from society. Since these compounds are designed to affect biological systems to protect humans and animals, they evidently pollute the environment when they unintentionally end up in different recipients such as soils, water bodies and sediments. Yet another problem is the large number of registered pharmaceuticals worldwide containing different chemical structures which complicates method development. For pharmaceuticals there are several extraction methods available, but when comparing the classical methods to PLE it is often found that the classical techniques are not as exhaustive as PLE, just as was concluded during the introduction of PLE in POP analysis [1,11,12]. PLE has for example been shown to extract herbal drugs much more efficiently, compared to a number of classical pharmacopoeia monograph methods based on reflux extraction, turbo-extraction and others, with increased drug gain up to 340% [13]. Of major concern in extraction of pharmaceuticals is that a very limited choice of reference materials is available. Such materials have been requested by Zeleny et al. in a recent review dealing with analysis of veterinary drug-residues in food producing animals [14]. Such materials are of need to come up with more reliable analytical methods. As the field of pharmaceutical analysis grows there will be more labelled standards available, when authorities demand residue control of pharmaceuticals in different compartments, making analysis more accurate and precise.

In recent pharmaceutical review papers PLE is only briefly mentioned as one out of a many techniques, discussed in a few words [15–17] and without any given key how to utilise the technique. Yet in other studies the optimisation of PLE parameters is not described and consequently it is not always possible to evaluate if the choice of PLE settings are appropriate. In a PLE review from 2005, which summarises analysis of food and biological samples, several important papers dealing with pharmaceutically active compounds were listed together with applied extraction conditions [18]. However, only limited discussion of appropriate applied conditions was made in that review. For example was no information given regarding the usage of spiked versus native analytes in the matrices, neither had the authors listed obtained recoveries (absolute and/or relative) [18]. The lack of such information makes it difficult to judge whether the applied conditions were suitable or not.

This review directs focus to important aspects that should be considered during the development of new methods using PLE. Recent papers which employ PLE for the extraction of pharmaceuticals from a number of environmental and biological matrices are reviewed. Major findings, such as recoveries, are highlighted and shortcomings of the published methodologies are brought forward. The intention is to in this way support with information for future improvement in PLE method development of pharmaceuticals in environmental matrices.

2. Strategies for evaluation of PLE performance

2.1. Choice of extraction strategy during method development

One of the major goals for the analytical chemist is to transfer as many of the analyte molecules as possible from the matrix placed inside the extraction cell to the collection tube without matrix species that potentially could interfere with the detection of the analyte(s). Sample preparation is simply intended to transfer the analytes into a measurable form [19]. This goal requires that the choice of extraction parameters is thoroughly investigated in order to produce an exhaustive methodology. Exhaustiveness assures that as many analyte molecules as possible are available in the final analytical step, which lowers the limit of detection of the entire analytical procedure. In contradiction to this stands the fact that exhaustiveness sometimes leads to co-extraction of unwanted matrix components, which causes problems later in the analytical chain, such as ion suppression in LC–MS [16]. Considering the great complexity of environmental and biological matrices, combined with the large differences in chemical structure between various groups of pharmaceuticals, the final choice of strategy is sometimes a matter of taste and often the individual analytical chemist ends up with a case-to-case situation. In this respect three scenarios, described in the following three sections, can be envisioned based on experience from POP extraction.

2.1.1. Exhaustive extraction

The most common scenario is the utilisation of conditions as harsh as possible, often with the application of high temperatures

which leads to improved analyte desorption from matrix active sites and increased mass transfer rates [16,20]. In this case, however, caution must be taken not to degrade the analytes since some pharmaceuticals are temperature sensitive [16,21]. This harsh strategy often also leads to excess unwanted matrix components which puts stress on the external clean-up and detection system [20,22–24]. Choice of solvent is evidently also of great importance as discussed in detail below (Section 3.3). Some solvents might seem appropriate in terms of releasing analytes from matrix active sites as well as for dissolving the compound. Still some solvents might be "too efficient" in the sense that they co-extract too much of the matrix, which makes the external clean-up procedure very challenging and injection into the chromatographic system impossible [25].

2.1.2. Selective extraction

A second strategy is to try to perform a more selective extraction by fine tuning the extraction conditions in favour of the analytes compared to matrix components. Such a strategy evidently requires that there are chemical differences between analytes and matrix components, which might not always be the case. Caution should also be taken so that not too many analytes are left behind in the matrix. The advantage of this strategy, on the other hand, is that a reduced external clean-up might be achievable or in the best case might even be excluded. A good example of selective extraction is one by Herranz et al. who thoroughly investigated a temperature interval from 60 to 80 °C for the extraction of fluoroquinolones from eggs [26]. Since a large fraction of soluble organic matter was extracted from the eggs above these temperatures, and caused extremely dirty extracts which could not be dissolved in the mobile phase, a final temperature of 70 °C was chosen—as a compromise between extraction efficiency, precision and amount of co-extracted components. Solvent must also be considered as demonstrated by Chu and Metcalfe for the extraction of selective serotonin reuptake inhibitors (SSRIs) from fish tissue [25]. Chu and Metcalfe found methanol to be the best solvent available with good extraction efficiency with a relatively low occurrence of interferences as a result. Similar solvent considerations have been done for the extraction of avermectins from soil samples [27]. In a recent review on advances in LC–MS residue analysis of veterinary medicines in environmental samples it was concluded that up to now these types of extractions – with improved selectivity – have been less of an issue; since such selectivity mainly has been achieved later in the overall analytical procedure [16]. Even so, these types of strategies can be worthwhile despite the longer time invested during method development.

2.1.3. In-cell clean-up

A third strategy is to perform internal clean-up by the addition of adsorption material to the cell, which hinders the co-extraction of unwanted matrix components [28]. Such strategies have been successful in POP and pesticide analysis [4,29–35], but require substantial method development. However, if successful, external clean-up can be completely avoided. To date such strategies have been investigated only to a limited extent and most pharmaceutical application have been based on the first strategy, which utilises as strong conditions as possible without degrading analytes, as will be shown later in this text. One in-cell clean-up application, however, has been published; in which 1 g of food matrix was blended with 2 g of C_{18} as a solid support material, packed into the extraction cell and extraction with 10 mL of hot water at 160 °C at 1500 psi [36]. A volume of 100 μL of these extracts was directly injected into the LC–MS system. Other interesting approaches are removal of matrix components, such as fat from pork with pure hexane prior to extraction of analyte with acetonitrile [37]. Such strategies have been used previously in supercritical fluid extraction (SFE) and then called inverse-SFE. Carbon dioxide was applied, to remove a majority of the matrix, followed by a modified step in which the compounds of interest were released [38–41]. As the field of PLE of pharmaceuticals matures it is likely that in-cell clean-up strategies for pharmaceuticals will increase.

2.2. Matrix consideration and recovery evaluation

In order to optimise extraction conditions a relevant matrix containing the analytes of interest must be available. The best option in this respect is a certified reference material (CRM). In most cases such materials are not available and the development must be based on materials produced in-house. This is often done by spiking material with known concentrations. In these types of experiments it is simply assumed that a reasonable recovery of spiked analytes is good enough to ensure the ability to perform a quantitative analysis of naturally incurred compounds. For some matrices this might be acceptable, however, it is well known that aging – in for example soils and sediment – causes analytes to bind harder to the matrix with time [42–47]. Since spiked analytes do not always mimic naturally incurred analytes, spiking should be done with some caution [48,49]. Dramatically decreased recoveries were observed for sulfonamide antibiotics spiked to soil when the contact time was increased from 90 min to 17 days. In the former case recoveries ranged from 62 to 93%, while in the latter decreased to 13–35%. The authors suggested rapid increase of non-extractable amount with time or transformation of the sulfonamides as two explanations to the different recoveries [50]. Two important strategies can be brought forward when CRMs are lacking and they have been more or less successfully used in pharmaceutical PLE method development described below (Section 2.2.1). These two strategies can also be combined to further strengthen the method development.

2.2.1. Spiked matrices and absolute recoveries

Spiking matrix-blank materials is common in the absence of certified reference materials. Such an approach is often termed "the second best approach" due to differences in extraction behaviour of an incurred material and an added spike [14]. Still such an approach is of value and gives information about basically all steps of the extraction step apart from specific release mechanisms of analytes from strong sorption sites on the matrix surface or entrapped analytes molecules hindered by diffusion within the matrix [1]. Nieto et al. spiked sewage sludge to optimise their method for 11 different conventional pharmaceuticals [51]. They used freeze-dried material, ground to small particles (less than 125 μm), which they spiked. Intensive stirring was employed to assure spreading out of analytes and sufficient contact with the matrix. Such a scheme, will evidently contribute to the analytes having increased possibility for being exposed and bound to matrix active sites and, have been used by others [20]. Time is of importance to mimic naturally incurred analytes, as discussed in Section 2.2 for sulfonamide antibiotics spiked to soil above [50], and differs in various studies. Many studies perform direct extractions [26,51–56] while others allow overnight or 24 h equilibration time [23,37,57,58]. Yet others age the matrix for several days [59,60], up to two weeks [50], where the latter is likely to give a somewhat more realistic extraction scenario when compared to naturally aged samples.

Once a realistic matrix is achieved the task is to evaluate the extraction performance by investigating the recovery. In this respect the absolute recovery is the most important. Absolute recovery gives an estimate of the percentage of analytes present in the matrix that were transferred from the matrix to the test tube. In most cases this is overseen, as observed in the various pharmaceutical specific tables below in which mainly relative recoveries are reported, or sometimes not stated at all. Relative recoveries primarily give information how well a released native analyte is

transferred from the extraction cell (not the matrix) in relation to the freshly added internal standard. This of course compensates for losses that might occur during the various analytical steps, however, the absolute recovery can still be very low. This means that the PLE method in itself is rather unsatisfactorily developed. An easy way of evaluating the absolute recovery is to make a pre-spike of an analyte-free matrix, allow aging, and extract. Further, an extract of the same type of analyte-free matrix should be spiked, i.e. post spiked. Direct comparison of the analytical signals from the two extracts will give a direct measure of the absolute recovery at the applied conditions [61]. Such an approach has been utilised with success for the evaluation of appropriate solvent composition for the extraction of 25 different pharmaceuticals from fish tissue using homogenisation extraction [62]. Evidently such recovery data might be matrix dependent, but gives an idea about the performance at the chosen conditions. Another good example is the work of Herranz et al. [26] in which the extraction of sarafloxacin, enrofloxacin, ciprofloxacin and lomefloxacin (internal standard) spiked in eggs was studied. It was clearly stated that for quantification of the absolute recoveries during method optimisation and validation a six-point matrix matched calibration curve was used. This was achieved by fortification of control egg extracts with each of the target fluoroquinolones thus assuring a perfect match between analysed samples and standard curves. Recoveries were calculated as the ratio of response obtained from spiked egg samples and those measured for the corresponding matrix matched standard. These recoveries gave information of losses occurring only due to the PLE step since they compensated for all losses occurring after the PLE step—such as evaporation, clean-up and possible ion suppression in LC–MS.

2.2.2. Utilisation of native analytes followed by spiking for method accuracy and precision

Once a preliminary method has been developed on spiked matrices it can be further tested on native unknown concentrations of pollutants in the matrix by multiple extraction cycles [20]. Within the field of POP analysis this procedure has been utilised for the extraction of pesticides in soil [63] but later also for herbal drugs from plants [13]. This approach is probably one of the best estimates of exhaustiveness of the applied conditions when CRMs are lacking. Golet et al. [59] used native ciprofloxacin and norfloxacin in sewage sludge and sludge treated soils where the concentration was not known beforehand. Another fluoroquinolone, tosufloxacin, was added to all PLE extracts after PLE to compensate for losses during sample clean-up and thereby be sure to evaluate only PLE in itself and not other losses. A relative comparison of concentrations was done to achieve a realistic scenario of extraction efficiency at different conditions. A similar exhaustive approach was utilised for the extraction of various drugs from sewage where several consecutive extractions were performed on the same matrix until no further analytes were released [20]. It must be emphasised though that the absolute amount present in the sample remains unknown and it is therefore not known when 100% extraction efficiency is obtained during method development. For native analytes this is always the case since it is impossible to know total amounts in an unknown sample. Hawthorne et al. [11] demonstrated that analytes often can be recovered for many hours when applying dynamic PLE at extreme conditions. However, such analytes are most likely of limited relevance from an environmental risk perspective [42–47]—a topic that will not be addressed within this context.

3. Major PLE aspects and parameters

The binding or entrapment of analytes in the matrix will depend on the physicochemical properties of the analytes as well as the matrix [15,19]. In order to overcome these interactions several major PLE parameters must be considered, as touched upon briefly (Sections 2.1.1–2.1.3). It is important to have some knowledge about the basic physiochemical properties of the analytes, such as pK_a values, water solubility, octanol–water distribution (K_{ow}), solid matrix-water distribution (K_d), and possibly complexation properties to metals or organic components [15,61]. A complete list of all these parameters for all possible types of pharmaceuticals is beyond the scope of this text, but useful information can often be found in various analytical overview articles such as those reported for tetracyclines [24], ionophores [61] as well as for over 40 pharmaceuticals in two recent analytical review articles [10,15]. Such knowledge aids in the process of choosing appropriate extraction conditions even though it does not give a direct answers to which final parameter settings are the optimal ones. Often the analytical chemist experiences a great deal of "trial-and-error" during the method development process, simply because there are too many unpredictable aspects when dealing with inhomogeneous environmental matrices.

3.1. Sample pre-treatment

The sample is often pre-treated prior to being loaded in the extraction cell [1]. This may for example involve air-drying or freeze-drying, grinding, homogenisation and sieving [20,51,57–59,64,65], all depending on the type of sample. Drying of the sample is important since water in the matrix sometimes may reduce the extraction efficiency [18,66]. Grinding, homogenisation and sieving are mainly performed to decrease diffusion distances of analytes from sample to extraction solvent [1,18]. Particles sizes of 125 μm have been utilised for the extraction of various pharmaceuticals from sewage sludge, sediment and meat [51,52,65], even though sample grinding to even smaller sizes (<15 μm) might be advantageous owing to even shorter diffusion path-lengths [67]. Additional drying can be achieved by adding or grinding the matrix with desiccants such as sodium sulphate [52] or diatomaceous earth [26,37] which also act as dispersion media (see Section 3.2). It should be noted, though, that sample handling, such as air drying and freeze-drying, has been shown to cause losses of semi-volatile compounds [68,69]—a process often overseen. Care should be taken during method development to minimise such losses.

3.2. Packing of the cell—influence of dispersing agent and sample size

PLE is often performed by dispersion of the sample with an inert material in order to avoid sample aggregation, prevent clogging of the extraction cell and allow a greater exposure surface area and thereby improved contact between solvent and matrix. A number of different dispersion agents have been utilised in pharmaceutical analysis such as sand [20,55,56,59,64,70,71], aluminium oxide [51,65], sodium sulphate [52], diatomaceous earth [26,37] and Hydromatrix® (a type of cleaned and sieved diatomaceous earth) [23,25,53,58,72], although some do not mix their samples with the dispersion agent [50,60,73]. It is important to evaluate possible interactions between dispersion media and analytes and therefore analytes can be spiked directly on the dispersion media itself. This was tested for fluoroquinolones, by spiking on diatomaceous earth and sand, giving recoveries above 88%, which demonstrated negligible adsorption to the dispersion media [26]. The dispersing agents were then mixed with the matrix (eggs) to obtain a porous mixture that enabled the extraction solvent to flow through the sample during extraction. In this case, 8 g of quartz sand and 2 g of diatomaceous earth were investigated at ratios of sample/dispersion media of 1:4 and 1:1, respectively. Interestingly, recoveries for diatomaceous earth ranged from 74 to 88%; while corresponding figures

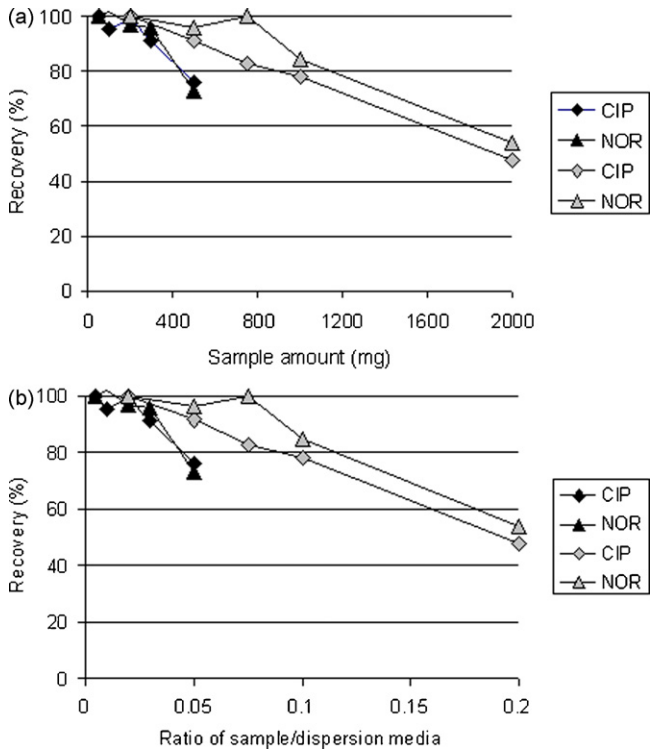

Fig. 1. (a and b) Recovery of ciprofloxacin (CIP) and norfloxacin (NOR) extracted from soil (grey) and sewage sludge (black). Recoveries are plotted versus (a) sample amount and (b) ratio of sample/dispersion media. Data modified from Golet et al. [59].

for quartz sand were only 39 to 42%, despite a lower ratio of sample/dispersing media for diatomaceous earth. This nicely illustrated the better capacity of diatomaceous earth to sorb egg matrix due to its much large porosity which greatly improved the extraction process.

An illustrative example on the effects of dispersion media and sample size was performed by Golet et al., who thoroughly mixed their dried samples (sewage sludge or sludge treated soil) with sand [59]. The reason for this was to allow a greater exposure surface area and hence a better diffusion of solvent into the matrix pores. Sample sizes ranged from 50 to 500 mg for sewage sludge and from 200 to 2000 mg for sludge-treated soil—all mixed with 10 g of sand [59]. From this experiment it could be seen that the absolute amount of extracted native analytes decreased with increased sample size, and there seemed to be a limit to how much sample could be extracted without a decrease in the extraction efficiency. For sewage sludge was 200 mg found optimal while corresponding figure for soil was 500 mg (Fig. 1a).

That samples size has such a dramatic impact on recovery is most likely due to the increase in ratio between sample and supporting matrix that occurs when changing the sample size (Fig. 1b) and not on the sample size as such. This effect is more pronounced for sewage sludge than soil. Sewage sludge, which is wetter than soil, requires more dispersion media per amount sample and hence must be better dispersed than soil. Dispersion of matrix as an aid in drying it and to increase accessibility of matrix to solvent should therefore not be overlooked [4]. The type of dispersion material is also important: In fact, sand might not be the best option to create the best possible dispersion of the sample as illustrated in the previous section with the example in egg, sand and diatomaceous earth by Herranz et al. (Fig. 1b) [26]. It can be worthwhile to test the suitable sample amount and its ratio to the support material, although it is a balance between required amounts for decent analyte detection, and ensuring good extraction efficiency. There is one example of the significance of this ratio in a study where the samples (10 g) were not mixed with any type of dispersion media [60], and notably in this study were reported recoveries only between 30 and 50%. Though a single study is too little to draw any general and certain conclusions from, it might indicate that these types of packing strategies should be avoided since they are likely to cause lowered extraction efficiency due to lowered accessibility of sample to solvent.

3.3. Solvent

A common strategy in finding the optimal extraction solvent is to first optimise the solvent at a moderate temperature during a short extraction time and a few cycles. Thereafter is the temperature investigated followed by time and cycles [20,26,51,59]. The two major criteria for a good solvent are that the solvent must be able to solubilise the analyte and minimise co-extraction of other matrix components. A third aspect is its ability to aid in the release of analytes from analyte matrix sites, which might require specific functionalities of the solvent as well as pH control. Many applications utilise a single solvent such as methanol [25,56], acetonitrile [37] or a combination of two organic solvents (for example methanol/acetone; 1:1) [52]. Since many compounds contain polar groups there are also several applications combining an organic solvent with water in various ratios such as methanol/water (1:1) [20,55] or (1:2) [58]; acetonitrile/water (7:3) [57] and acetone/water (3:7) [53]. In some published applications the choice of solvent seems arbitrary, however, a logical starting point when testing solvents is to look at solvents previously applied with success [20,23]. O'Connor and colleagues tested a number of solvents and solvent/buffer combinations with pH-control based on literature data when extracting tetracyclines from soil [23]. To their surprise were the solvents that had been winning in various standard methodologies not so successful under PLE conditions, probably due to differences when performing extractions at elevated temperatures. There are a number of other applications where pharmaceuticals have been extracted under pH control with the use of, for example, phosphate buffer combined with methanol (1:1) [26,51,59,71]. Nieto et al. [51] demonstrated that such a solvent was better than various water/organic solvent mixtures for the extraction of 11 pharmaceuticals from sewage sludge and investigated the effects of differing ratio between the phosphate buffer and acetonitrile (Fig. 2).

The same solvent combination has also been applied with success to compounds with zwitterion-character, such as fluoroquionolones [26,59,71]. The reason for this is that protonated and unprotonated analytes might bind very differently to matrices and the matrix itself might have altered characteristics depending on the pH [19]. Choosing extraction solvent should therefore be done with care in order to obtain good extraction efficiency.

3.4. Temperature

Temperature is a very important parameter in PLE which adds to the often observed increased recoveries compared to other extraction techniques [1]. In the pioneering paper by Richter et al. [74] this was explained by the different physicochemical properties of the solvents at increased temperatures with reduced solvent viscosity causing increased ability to wet the matrix and solubilise analytes. Other important aspects are elevated diffusion rates and increased ability of the solvent to disrupt matrix–analyte interactions. This speeds up the release of analytes from active sites in the matrix, which is considered the rate-limiting step in many environmental applications [75–77]. A commonly observed effect when increasing the temperature is the increase in extraction efficiencies

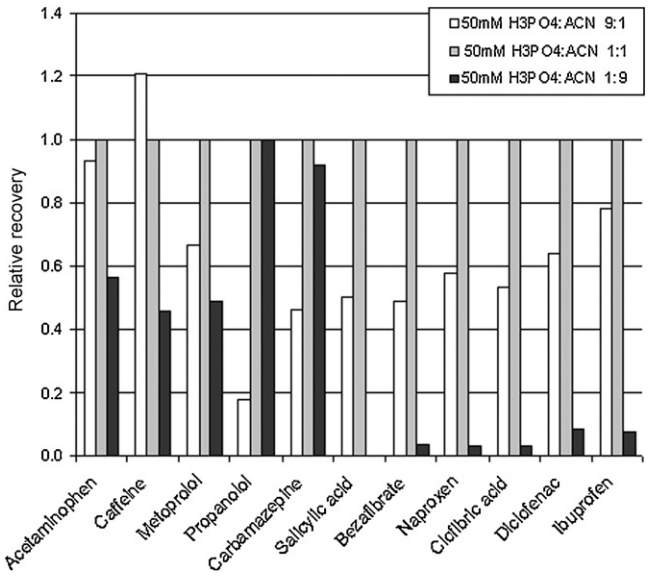

Fig. 2. Effect of various combinations of phosphate buffer/methanol on recovery relative to a ratio of 1:1 (v/v) for various pharmaceuticals spiked to sewage sludge. Data modified from Nieto et al. [51].

which starts to level off above a certain temperature. This effect is exemplified with flouroquinolones in sewage sludge [59].

Substantial improvement in the amounts recovered analyte was achieved when temperature was increased from 50 to 100 °C above which very limited amounts of analytes were recovered. However, there are also examples of dramatic changes in extraction efficiencies beyond 100 °C. Stoob et al. found that temperature strongly influenced the extraction process [50]. Extraction of sulfonamides from soil samples showed a five-fold increase in extraction efficiency at 200 °C compared to 100 °C. Extraction at such high temperatures requires testing of thermal stability of the analytes and indeed proved by quantitative extraction of spiked sulfonamides on diatomaceous earth at 200 °C which showed no tendency of thermal break down. An important consideration made by Stoob and colleagues was the higher matrix load (as shown by darker extracts) observed at elevated temperatures which might be critical for the quantification using LC–MS/MS due to ion suppression. The researchers demonstrated an ion suppression factor of about 1.2–1.8 higher for the samples extracted at 200 °C compared to them from extraction at 100 °C. However, ion suppression was compensated for by the corresponding internal standards and did not affect the quantification apart from a sensitivity reduction of between 20 and 45%. All together, the researchers concluded that the advantage of higher extraction yields outweighed the disadvantage of the slightly smaller sensitivity [50].

Extractions at such high temperatures as 200 °C are not always suitable and there are several applications performed below 100 °C. Some examples are fluoroquinolones from eggs at 70 °C [26], carbamazepine from biosolids at 80 °C [53] and antimicrobials from feedstuff also at 80 °C [70]. Too low temperatures may, however, cause decreased extraction efficiency as demonstrated by Radjenovic and co-workers who observed decreased recoveries below 75 °C when extracting a multitude of pharmaceutical compounds from sludge. Finally a temperature of 100 °C was chosen [58]. Similar observations where observed by Ferrer et al. [57] who showed that recoveries increased above 75 °C up to 120 °C, but then no further increase was seen. In fact 100 °C is the most commonly applied temperature overall. Improved effects were observed up to 100 °C for fluoroquionolones, thereafter levelling off, which indicated no further influence of temperature [59]. Other applications employing 100 °C are several publications for a range of pharmaceuticals extracted from soils and sediments [52,56,59], sewage sludge [20,51,59,71], pork [37] and fish tissue [25]. This is most likely caused by a good balance between good extraction efficiency with relatively moderate co-extraction of undesired matrix components.

3.5. Pressure

Pressure is mainly used to keep the organic solvents as liquids at temperatures above their atmospheric boiling points as well as moving the solvent through the system [36]. Since the applied pressures always are above this threshold value, pressure does not appear to be a critical parameter [36]. Some researches therefore no longer optimise pressure but simply use preset values from the systems available [26,53]. The negligible effect of pressure has been observed in many applications, such as for the extraction of various drugs from sewage, where pressure had no influence in the range 870–2175 psi [20]. Similar pressure intervals have been investigated for soil [59], sediment [57], sewage [71] and meat [36], with equally negligible effects.

3.6. Time and cycles

The duration of the static extraction time is important: A prolonged solvent exposure permits increased swelling with enhanced matrix wetting and increased penetration of solvent into the sample interstices. Thereby, an enhanced possibility of the solvent breaking specific analyte–matrix interactions is ensured [59]. By running many cycles fresh solvent enters the system, which is important to maintain a suitable solvent-to-sample equilibrium, and improves partitioning of the analytes into the liquid phase [59]. Consequently, the final method is a balance between static time, cycles and an acceptable total run time of the entire extraction step. To evaluate the optimal number of cycles may several single extraction steps be performed; once solvent, temperature and time has been investigated. Analysis of extracts from each individual extraction cycle gives information about the number of cycles required to obtain an efficient extraction. Golet et al. demonstrated improved effects of increased static time from 5 to 10 min when extracting fluoroquinolones [59]. The effect of time then levelled off between 15 and 25 min. The optimal number of cycles may also differ depending on the matrix. Golet and co-workers showed that 4 cycles was suitable for sewage sludge, while sewage treated soil required 6 cycles [59], possibly due to stronger interactions between analytes in soil than in sewage and/or aging effects. Although a 10 min extraction offered optimal extraction efficiency; 15 min was selected as operating conditions (combined with 4 or 6 cycles depending on matrix) to ensure quantitative extraction even for “difficult-to-extract” samples. On the other hand, the extraction efficiency was rather unaffected by the number of cycles between 2 and 4 (5 min static steps) for extraction of fluoroquinolones from egg [26]. Since fluoroquinolone from sewage sludge and soil discussed above required longer time and more cycles than from egg, these results demonstrates that some matrices are easier to extract.

Nieto et al. [51] demonstrated that 2 cycles were sufficient to recover 10 different spiked pharmaceuticals from sewage. The most polar analytes were recovered in the first cycle of 15 min with the use of a 50 mM phosphoric acid buffer in methanol (pH 2), while the least polar analytes where recovered in the second fraction. Since the solvent is highly polar, the partitioning of analytes from the matrix to the solvent is probably more favourable for the polar analytes who consequently elute first comparable to what occurs in a chromatographic process: The solvent is better at breaking polar interactions and “similar dissolves similar”. Similar observations were made by Radjenovic and colleagues who optimised extraction time and cycles for 31 pharmaceuticals from sludge [58]. About 80%

of the compounds were found in the first extract, while the rest were mainly retrieved in the second. Some analyte groups, however, were found also in the third extract. Hence, 3 cycles were applied.

As exemplified above, the extraction time and the number of cycles can evidently vary greatly. Of the reviewed publications is one of the shortest optimised extraction times 3 min in 1 cycle. In that study 6 and 9 min gave lower extraction efficiency, which is quite uncommon and no explanation was given to these results [64]. There are also reports of 5 min static extraction with the use of a single cycle [36,70]. Most commonly, the applied extraction time ranges from 5 to 10 min with between 2 and 3 cycles, as shown for biosolids [53], sewage sludge [51,58], soil [23,73], sediment [52], pork [37], fish tissue [25] and eggs [26]. In many of those cases only limited amounts of pharmaceuticals were recovered from the sample in the third and fourth cycle [20,51,71,73]. This result is illustrated in the work by Schlüsener et al., who extracted antibiotics from solid matrices [73]. In their study 82% of azithromycin was recovered from digested sludge in the first cycle while 12% was present in the second cycle. Only small amounts could be found in the third and forth cycle giving 4% and 2% recovery, respectively. Consequently two extraction cycles were chosen in the final extraction method to reduce sample preparation time.

4. Applications

4.1. Pharmaceuticals

4.1.1. Single analyte and group specific methods—pharmaceuticals

A number of single analyte or group specific methods have been developed the last few years (Table 1). Diphenhydramine has been used for treating allergies for more than 50 years [57]. Due to its physicochemical properties it has been expected to bind to sediments and there cause negative effects in bottom-dwelling organisms. Due to these suspicions did Ferrer et al. developed an extraction method for the determination of this compound in sediments samples [57]. The sampled sediments came from streams receiving water from wastewater treatment plants. Blank sediment samples were fortified with the compound and the internal standard atrazine-d_5 followed by incubation for 24 h. The samples were not mixed with any kind of support material. A number of solvent combinations were evaluated with a final composition of acetonitrile/water (7:3). Investigated temperatures ranged from 75 to 200 °C, but no improvements were observed over 120 °C which was chosen in the final settings. Other information regarding development of extraction parameters were not given. The final method applied a static extraction step of 5 min in 3 cycles. Despite that method development was performed on freeze-dried material, the final extraction was performed on 10 g wet sediments (60% water content) without mixing with any type of drying material. This is somewhat surprising considering the large negative effects sometimes observed due to inappropriate mixing and insufficient drying of samples as discussed in Section 3.2. It can therefore not be excluded that the recoveries for the wet natural aged samples were lower than the 75% recovery reported for dry spiked sediment obtained through an external calibration curve. The developed method is also somewhat unusual in the sense that no external clean-up apart from filtration was performed prior to injection into the chromatographic system. Nor were any attempts made to evaluate possible ion suppression effects.

Carbamazepine is used to treat epilepsy, but also in a number of psychotherapy applications [53]. It is known to be detected in various waste water treatment effluents and river water together with its metabolites [78]. To study the presence of carbamazepine and

Table 1
Single analyte and group specific methods—pharmaceuticals.

Analyte	Sample matrix	Cell matrix	Solvent	Temperature (°C)	Pressure (psi)	Static time (min)	Cycles	Spiking	IS	Abs Rec (%)	Rel Rec (%)	Clean-up Analysis	Ref
Diphenhydramine	Sediment 10g	None, wet matrix	Acetonitrile/water (7:3)	120	1500	5	3	Spiking on freeze-dried matrix	atrazine-d_5	75 (8)[a]	–	Filtration LC/TOF–MS LC–ES–MS/MS	[57]
Carbamazepine	Biosolid 10g	Hydromatrix	acetone/water (3:7)	80	1500	5	3	Spiking on centrifuged sludge material	caffeine-$^{13}C_3$		91 (6)[b]	92 (4)[c]	SPE HLB cartridges	[53]
CBZ-EP											86 (8)	88 (5)		
CBZ-DiOH											84 (4)	83 (8)		
CBZ-2OH											84 (7)	89 (4)		
CBZ-3OH											84 (8)	80 (7)	LC–ES–MS/MS	
CBZ-10OH											88 (5)	90 (5)		
Caffeine											82 (6)	83 (6)		
Barbital	Pork tissue 5g	Diatomaceous earth	Acetonitrile	100	1500	6	2	Spiking and homogenisation of blank pork tissue	–		91 (7)[d]		SPE C_{18} cartridges GC–MS	[37]
Amobarbital											95 (5)			
Phenobarbital											103 (6)			

[a] Recovery of spiked analyte versus external calibration curve; [b] Raw biosolid; [c] Treated biosolid; [d] Only one spiking level out of three reported in the table.

five metabolites together with caffeine in biosolids in a municipal wastewater treatment plant, an extraction method was developed by Miao et al. [53]. Raw and treated biosolids were spiked and equilibrated for 2 h. Prior to loading the samples into the cells they were centrifuged to reduce the water content. To further dry the samples, reduce clumping and channelling of solvent the biosolids were mixed with Hydromatrix®. The amount of Hydromatrix® used depended on sample water content [53]. Various mixtures of acetone and water were investigated and when the extraction solvent consisted of more than 80% water extraction efficiency was low. Optimal combination was acetone/water (3:7). Temperature was investigated, although the temperature interval was not stated. Optimal temperature was 80 °C. No further details were given about optimisation of extraction parameters. In the final method a static extraction step of 5 min in 3 cycles was used. At the final conditions recoveries were high and always exceeded 80% for all compounds in both raw and treated biosolids. Recoveries were estimated from triplicate extractions of spiked samples compared to five spiked samples in a dilution series called calibration by standard addition according to the authors. It is not clear from the text exactly how this was done. However, if the samples in the dilution series were spiked prior to extraction the recoveries reported are only relative and do not assure that quantitative recoveries were obtained during the optimization of the extraction procedure. Furthermore did the recoveries include the SPE step (Solid Phase Extraction) on HLB Oasis cartridges (Hydrophilic Lipophilic Balance) and were not explicitly devoted to the PLE step. It also seems most likely that the reported data were relative recoveries considering the high recoveries and low standard deviations.

Barbiturates have previously been used to reduce anxiety, respiration, blood pressure and heart rate [37]. They have been prohibited for human use and as additives in animal feed, but still need to be monitored due to misuse as feed additives in animal butchery. Zhao et al. developed a method for the determination of barbiturates in pork tissue [37]. Blank, homogenized pork samples were spiked and left overnight at room temperature. Samples were mixed with diatomaceous earth. Prior to extraction of the barbiturates, fat was removed by using a single PLE step of pure hexane at room temperature for 5 min. Several extraction temperatures were investigated between 50 and 125 °C, and 100 °C was chosen as optimal. Acetonitrile was tested alone and at two different solvent combinations (acetonitrile/acetone and acetonitrile/dichloromethane) and different extraction temperatures were applied. However, the evaluation was not very systematic and from the data it is difficult to know which parameter actually affected the recoveries. In the end pure acetonitrile was chosen as extraction solvent with the use of a static time of 6 min in 2 cycles. To assess the recovery of the final method six blank pork replicates as well as samples spiked at three different concentration levels were used. These results were compared to a calibration curve obtained from spiked blank pork samples undergoing the same procedure as the samples (matrix matched calibration). Just as for the study by Miao et al. [53], it is difficult to judge exactly how this was done; however, if the samples were spiked prior to extraction the obtained recoveries are relative. Consequently it can not be assured that the developed exaction procedure is quantitative. Even in this study the SPE step on C_{18} cartridges was included in the evaluation of recoveries and therefore not only the PLE step is investigated.

The reported methods for single pharmaceuticals are quite similar in terms of temperature, time and cycles, though the solvents differ somewhat in chemical properties (Table 1). From the published reports it is, however, somewhat difficult to evaluate the exhaustiveness of the final PLE methods since it is unclear what the presented recoveries represent. The published papers can serve as a good starting point for developing new methods, but require additional method development and validation for a successful PLE method.

4.1.2. Multi class methods—pharmaceuticals

Several multi-class residue methods have been developed recently to determine a large number of pharmaceuticals in biosolids in a single analysis (Table 2). Nieto and colleagues analysed sewage sludge for its content of 10 different pharmaceuticals [51]. The method was developed by spiking lyophilized samples. A total of 5 g of sludge were mixed with aluminium oxide. Initially were various solvent combinations between water, phosphoric acid buffer, acetonitrile and methanol evaluated. A combination of 50 mM phosphoric acid/methanol (1:1) was found optimal and yielded recoveries ranging from 71 to 125% for 10 out of 11 pharmaceuticals (salicylic acid had a recovery of only 3%). A static time of 15 min was utilized based on previous studies [59], but the number of cycles was varied between 1 and 4. Final setting was 2 cycles for 15 min. Temperature was investigated (though not shown), although the initial setting of 100 °C was still chosen based on previous findings [59]. It is not clear how recoveries were obtained, but it seems as if they were based on spiking experiments and compared to a conventional external calibration curve. The final recoveries when spiking 400 μg/kg were higher than 68% for all pharmaceuticals (Table 2). Surprisingly no ion suppression was observed despite that no clean-up could be conducted due to very low recoveries (<10%) in the SPE step.

In a later study by Barron et al., 27 pharmaceuticals were determined in sludge and soil [55]. Method development was performed on spiked sewage sludge or soil. This material was mixed with sea sand and spiked immediately prior to extraction. Several solvent combinations were tested where acetone/water and methanol/water showed the best results. Finally methanol/water (1:1) was chosen due to decreased losses during the following SPE step using HLB cartridges. Static time and cycles were optimised and 5 min in 2 cycles were applied. A temperature interval of 40–100 °C was investigated and 60 °C was chosen, since some pharmaceuticals were degraded above this temperature. The absolute recovery of the entire extraction and clean-up procedure was determined by performing pre-, and post-spiking of sewage and soil extracts, thereby compensated for any ion suppression that might have occurred. Thereafter, the recovery could be evaluated. For both matrices the majority of pharmaceuticals showed recoveries between 70 and 130%, even though several analytes showed rather low recoveries. The ion suppression was also tested by post-spiking of sewage and soil extracts which were compared to pure standards injected into the chromatographic system. Ion suppression was rather limited for soil, while salient for the analytes from sewage sludge.

Recently Radjenović and colleagues determined 31 pharmaceuticals in various types of sewage sludge [58]. The applied methodology was developed by spiking freeze-dried sewage sludge which were stirred intensively and equilibrated for 24 h. The biosolids (1 g) were mixed with Hydromatrix® prior to extraction. Both methanol/water and acetonitrile/water were evaluated as extraction solvents at different ratios of the organic solvent and water. Methanol/water (1:2) was chosen at a temperature of 100 °C. This temperature was better than 75 °C, which caused recoveries to decrease. A 5 min static time was used in three cycles. The first fraction contained roughly 80% of the total analytes extracted in three cycles. In principle did the second fraction contain the remaining 20%, though; some of the less polar pharmaceuticals only were recovered in the third cycle. This is not surprising considering the high water content in the solvent causing decreased solubility of hydrophilic compounds in the solvent requiring increased elution volumes. The recoveries of the extraction were evaluated by performing pre- and post-spiking of sewage and soil extracts. How-

Table 2
Multi-class methods—pharmaceuticals.

No. of analytes	Sample matrix	Cell matrix	Solvent	Temperature (°C)	Pressure (psi)	Static time (min)	Cycles	Spiking	IS	Abs Rec (%)	Rel Rec (%)	Clean-up Analysis	Ref
10	Sewage sludge	Aluminum oxide	50 mM phosphoric acid/methanol (1:1)	100	1450	15	2	Spiking on freeze-dried matrix.	–	68–120[a]	–	Filtration LC–MS	[51]
27[b]	Sewage sludge	Sea sand	Methanol/water (1:1)	60	1500	5	2	Spiking on matrix	–	70–130[c] 40–69[d] <2[e]	–	SPE HLB cartridges LC–ES-MS/MS	[55]
27[f]	Soil									70–13[g] 50–69[h] <30[i]	–		[55]
31[j]	Sewage sludge		Methanol/water (1:2)	100	1500	5	3	Spiking on freeze-dried matrix	^{13}C-phenacetin, mecoprop-d_3, ibuprofen-d_3, atenolol-d_7, carbamazepin-d_{10}, diazepam-d_5, phenobarbitol-d_3	70–125[k] 44–69[l] <30[m]	–	SPE HLB cartridges filtration LC-ES-MS/MS	[58]

[a]Recovery range for 10 of the pharmaceuticals in the final spiking experiment. It is not clear from the paper if these are absolute or relative recoveries, but it seems that they are compared to a conventional external calibration curve; [b]27 pharmaceuticals investigated but not all could be fully recovered in sewage sludge. The different analytes recoveries are grouped in c, d and e. All data reported are absolute recovery for the entire method including SPE where samples are pre- and post-spiked with analytes compensating for ion suppression; [c]17 out of 27 pharmaceuticals showed recoveries in this range; [d]7 out of 27 pharmaceuticals; [e]3 out of 27 pharmaceuticals; [f]27 pharmaceuticals investigated but not all could be fully recovered in soil. The different analytes recoveries are grouped in g, h and i. All data reported are absolute recovery for the entire method including SPE where samples are pre-, and post-spiked with analytes compensating for ion suppression; [g]18 out of 27 pharmaceuticals showed recoveries in this range; [h]5 out of 27 pharmaceuticals; [i]4 out of 27 pharmaceuticals; [j]31 pharmaceuticals investigated but not all could be fully recovered in sewage sludge. The different analytes recoveries are grouped in k, l and m. The reader should consult the paper for details on recovery estimations due to difficulties understanding how these experiments were performed. The presented data only refers to treated sewage sludge, but data are available for 4 more types of sewage sludge in the original paper; [k]12 out of 31 pharmaceuticals showed recoveries in this range; [l]13 out of 31 pharmaceuticals; [m]6 out of 31 pharmaceuticals.

ever, it is not clear to us whether the actual recoveries should be considered absolute or relative and if they refer only to the PLE step or if they also include the SPE step using HLB cartridges. Nevertheless, the authors carried out a thorough method validation and also presented several data on ion suppression, which was quite severe for nearly all pharmaceuticals. The presented data demonstrated that 2/5 of the pharmaceuticals were recovered to between 70 and 125%, while another 2/5 showed recoveries between 44 and 69%. The last 1/5 of the investigated pharmaceuticals was recovered to less than 30%.

The development of multi-class methods requires substantial work due to the great complexity in both matrices and differences between analytes of interest. Large efforts have been made by the various research groups to overcome obstacles associated with these methods (Table 2), but in some cases further development is necessary. Information is sometimes also lacking to be able to better understand the actual performance of the PLE step, and complementary experiments might sometimes be of use to assure a quantitative extraction step.

4.2. Antimicrobials

4.2.1. Single analyte and group specific methods—antimicrobials

4.2.1.1. Sulfonamides. Sulfonamides are employed in food-producing animals to avoid bacterial proliferation and infections and in the livestock industry to promote growth. Sulfonamides are inexpensive and readily available [79] and represent one of the substance groups most frequently applied in the EU [50]. The presence of sulfonamide residues in food is a toxicological and regulatory concern as some sulfonamides could be carcinogenic, cause allergic hypersensitivity reactions and reduce the therapeutic effectiveness of these drugs on humans [36]. Sulfonamides are amphoteric compounds with two pK_a-values which play an important role for the environmental behaviour as well as for their extraction from soil [50]. The methods review has been divided into soil and food matrices (Table 3).

PLE used for the extraction of five sulfonamide antibiotics (sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, and sulfathiazole) and their N^4-acetyl conjugates in agricultural soils was described by Stoob et al. [50]. The samples were milled to a particle size of 2 mm and homogenised. A thorough investigation was performed by using several different types of samples which were spiked and aged in intervals of a few hours over a few days up to a period of 3 months. Extraction cells (11 mL) were prepared with a cellulose filter and about 500 mg of diatomaceous earth. After adding the sample (4 g), the cell was filled up with diatomaceous earth. The sulfonamides were quantified by the ratio between the peak areas of the analytes and the peak area of the corresponding deuterium labelled internal standard. Initial experiments demonstrated that absolute extraction recoveries were 62–93% of the sulfonamides when the spiking had been 90 min prior to extraction, except for sulfamethoxazole which was recovered only to 41%. These percentages decreased significantly after a contact time of 6 and 17 days (resulting in absolute recoveries ranging from 36 to 55 and 13 to 35, respectively). Stoob et al. concluded that a short contact time (90 min) was not sensitive to extraction parameters and therefore not representative for real world samples. As a consequence, the extraction was developed with an aged (contact time of several days) composite soil sample from a grassland field site treated with manure containing the five sulfonamides. When optimising the PLE method the following parameters were varied; temperature, extraction time, pressure, flush volume, and the number of sequential extractions as well as the composition and the pH of the extraction solvent. The most important parameter for the extraction efficiency was temperature, whereas the pH of the extraction solvent did not significantly influence extraction efficiency between pH 4.1 and 8.8. A temperature increase from 100 to 200 °C improved the absolute amounts analytes released up to a factor of six for aged residues in soils. In contrast, no temperature dependence was observed during short-term spike experiment. Buffered water (pH 8.8) mixed with acetonitrile (85:15) used at 200 °C and 1450 psi with a, 5 min static extraction time in a single cycle were found optimal [50].

Pressurized liquid extraction has also been used in the quantification of sulfonamides in food samples. Gentili et al. described a method for the rapid extraction and unequivocal confirmation of 13 sulfonamides in raw meat and infant foods [36]. The instrumental parameters (pressure, time, and extraction cycles) were optimised at 70 °C by using a fortified blank matrix and water as a solvent. Optimal temperature was 160 °C at a pressure of 1470 psi, applying a 5 min extraction step in one cycle. When using these settings on three meat matrices (bovine, porcine, and poultry) each fortified with a total of 50, 100, and 150 pbb of total level of sulfonamide residues, it was concluded that the absolute recoveries were independent of the applied fortification levels. The homogenised food samples were mixed with C_{18}-material in a 1:2 ratio. The absolute recoveries of 13 sulfonamides were determined using mixed samples of the three matrices spiked at a 100 ppb level. Recoveries for meat samples and baby food samples were between 70 and 99% and 71 and 101%, respectively [36].

In a recent study by Font et al. pressure, temperature and extraction time were investigated and it could be concluded that temperature was the most important parameter with optimal conditions 160 °C and 1500 psi using water as solvent [79]. Pork meat (10 g) was spiked with variable volumes of stock solutions of sulfonamides (from 10 to 100 μl) and was left for 15 min at room temperatures before blended with 10 g diatomaceous earth. The absolute recoveries were calculated by comparing peak areas of the pork tissue samples spiked before the PLE procedure with peak areas of pork tissue samples spiked after the PLE procedure. The recoveries for 12 sulfonamides were between 76 and 100% [79].

In general extraction of sulfonamides seems to gain from high temperatures (above 150 °C) applying a solvent containing primarily of water at 1500 psi. On the other hand time has in principal been shown to be of less importance as has the number of cycles (Table 3).

4.2.1.2. Tetracyclines. The tetracycline class of antibiotics is one of the most extensively used as growth promoters and therapeutic drugs in animal production. The most widely used compounds within this group are tetracycline, oxytetracycline, chlortetracycline and doxycycline [80]. Depending on the animal species treated, up to 75% of a single dose of tetracycline is excreted in non-metabolised form in urine or faeces [21]. More than 20,000 tons of antibiotics are produced each year and half of that amount is used in animal production [24]. Residues of these drugs have been found in soils, where manure from treated animals has been applied, at concentration levels as high as 20 μg/kg [23]. The application of antibiotics to agricultural lands through repeated fertilization with animal manure potentially poses an ecological and environmental threat because the excreted antibiotics could contaminate soils, streams, and groundwater [21]. The strong interaction of tetracyclines with natural organic matter (NOM) and with clay components in soil – due to their general complex binding properties with di- and trivalent cations [81] – makes the extraction difficult and quantification is often not reproducible. To alleviate these problems, pressurised liquid extraction (PLE) has been used recently to extract tetracycline antibiotics from soils [23]. Due to the widespread use of tetracyclines in the animal industry, the literature on the analysis of these compounds is fairly extensive and includes methods for extraction and analysis in various matrices (e.g., animal tissues, urine, faeces, groundwater, surface waters, and

Table 3
Single analyte and group specific methods—antimicrobials.

Analyte	Sample matrix	Cell matrix	Solvent	Temperature (°C)	Pressure (psi)	Static time (min)	Cycles	Spiking	IS	Abs recovery (RSD) (%)		Rel. recovery (%)	Conc. level (μg/kg)	Clean-up and analysis	Ref.
Sulfonamides															
										Meat	Baby food				
Sulfisomidine										94–99	97–100				
Sulfadiazine										92–94	91–95				
Sulfapyridine										89–92	90–94				
Sulfamerazine										98–99	99–101				
Sulfamoxole										70–76	71–75				
Sulfamethazine	meat[a] and baby food	40% infant food + 60% rice starch, maize starch, sunflower oil and water 3 g of homogenized sample 1:2 C18	Hot H_2O	160	1500	15	1	1 g spiked with analytes after homogenisation. Aged 30 minutes	Sulfameter (SME)	88–92	89–95		100	Lipid removal at −18 °C, centrifugation and LC-ESI(+)–MS/MS (MRM)	[36]
Sulfamethizole										86–91	87–93				
Sulfathoxypiridazine										85–88	84–87				
Sulfamonomethoxine										90–94	91–95				
Sulfachloropyridazine										79–85	83–86				
Sulfamethoxazole										92–96	93–95				
Sulfaquinoxaline										81–85	82–87				
Sulfadimethoxine										85–93	88–92				
Sulfadiazine										93					
Sulfathizole										69					
Sulfamethazine										81					
Sulfamethoxazole										41					
Sulfadimethoxine	aged soil samples	soil sample (4 g), and filled up with diatomaceous earth	Buffered water (pH 8.8) 17:3 acetonitrile	200	1450	5	1	Spiking blank soil sample	isotope labeled 5 ng/μL	62			1250	Filtration on 0.45 LC-ESI(+)–MS/MS (SIM)	[50]
Acetylsulfadiazine										N/A					
Acetylsulfathizole															
Acetylsulfamethazine															
Acetylsulfamethoxazole															
Acetylsulfadimethoxine															
Sulfasalazine										79–82					
Sulfabenzamide										76–83					
Sulfisoxazole										82–87					
Sulfadimethoxyne										84–90					
Sulfachloropyridazine										90–95					
Sulfadiazine	Pork meat	10 g portion of chopped and minced pork muscle tissue	Hot water	160	1500	5	1	10 g pork meat spiked with variable volume of stock solutions of sulfonamides	none	91–96			50–200	SPE (HLB) CE-MS	[79]
Sulfamethoxypyridazine										91–93					
Sulfathiazole										98–100					
Sulfadimidine										82–87					
Sulfapyridine										97–100					
Sulfaguanidine										79–85					
Sulfanilamide										91–93					
Tetracyclines															
Oxytetracycline (OTC)	manure-borne oxytetracycline in soil	5 g of soil was mixed with a sufficient amount of Hydromatrix (Dionex) to fill the 11-mL extraction cell	Two step: (a) 100% McIlvaine buffer pH 7.8 with 100 mM EDTA; (b) water 3:2 metanol (v/v) ved 30 grader	2 step: (1) 60; (2) 30	1500	5	2	N/A	Demeclocycline	N/A		N/A	N/A	Standard addition ELISA vs SPE (HLB) LC–MS/MS	[21]

Table 3 (*Continued*)

Analyte	Sample matrix	Cell matrix	Solvent	Temperature (°C)	Pressure (psi)	Static time (min)	Cycles	Spiking	IS	Abs recovery (RSD) (%)	Rel. recovery (%)	Conc. level (μg/kg)	Clean-up and analysis	Ref.
Tetracycline Oxytetracycline Chlortetracycline Doxycycline	Different spiked soil age for 24 h. Are tested.	5 g of soil and inert diatomaceous earth	5% (w/v) sodium acetate, 100 mM EDTA 1:1 methanol, (adjusted to pH 8 with NaOH)	60	1500	5	2	soils were spiked using a slurry method	None	99 99 92 99		1000 for PLE development otherwise 25, 100	SPE (StrataX-SAX) LC–MS/MS	[23]
Tetracycline Chlortetracycline Oxytetracycline Doxycycline	bovine, swine, poultry and lamb	11 g EDTA washed Sea sand	Water and methanol–water (1:1, v/v)	70	1500	10	N / A	spiked with the four TCs at three levels aged for 10 min	demeclocycline (DMC)		90–91 (5–18) 89–93 (9–13) 92–93 (11–16) 95–98 (6-13)	1, 100, 200	SPE (HLB) LC-ESI–MS/MS (SRM)	[80]
Quinolones Ciprofloxacin/ Norfloxacin	sewage sludge (dried for 72 h at 60 °C)/Sludge-treated soils (dried at 40 °C) for 72 h)	500 mg of sample mixed with ~10 g of quartz sand	50 mM phosphoric acid (aq; pH 2) 1:1 acetonitril (v/v)	100	1450	15	4/6	Spiked over night	tosufloxacin surrogate standard (TOS-IS),		89/88[a] (ciprofloxacin) and 80[a]/84[a] (norfloxacin)	250–2000 and IS: 100 μg/mL	SPE (MPS discs) LC-FLD	[59]
Cinoxacin Ciprofloxacin Danofloxacin Difloxacin Enoxacin Enrofloxacin Flumequine Nalidixic acid Norfloxacin Ofloxacin Oxolinic acid Pipemidic acid Rufloxacin	Fish and swine feed (1 g)	None	metaphosphoric acid 0.2% in water/ acetonitrile, 70/30 (v/v), pH 2.6.	N/A	N/A	N/A	N/A	1 g fish feed spiked and mixed and aged for 30 min	None	[a]84–90 (4) 58–71 (9) 63–73 (10) 59–85 (9) 31–51 68–86 (9) 75–86 (7) 75–92 (10) 61–72 (10) 88–99 (9) 73–90 (7) 55–66 (8) 77–103 (8)		5000–25000	5 μm filter and SPE (HLB)-LC-DAD or FLD	[82]
Enrofloxacin Ciprofloxacin Sarafloxacin	egg (2 g)	Quartz sand and diatomaceous earth	Phosphate 50 mM pH 3.0 1:1 acetonitrile (v/v)	70	1500	5	3	2 g spiked and mixed with dispersion agent	Lomefloxacin		68–88 67–90 71–87	50–1000 and IS at 500	No clean-up LC-FLD	[26]
Danofloxacin Sarafloxacin Ciprofloxacin Marbofloxacin Enrofloxacin Difloxacin Oxolinic acid Flumequine	Chicken (1 g)	diatomaceous earth (1.5:1) and sand (dead volume)	Dichloromethane	50	1700	0	1	Spiked prior to dispersion	5 mg/L of lomefloxacin	86–105 (16) 66–81 (16) 63–67 (16) 67–85 (16) 82–90 (14) 87–94 (5) 91– 12 (8) 93–110 (12)		100 for PLE opt and 0.5–8 for validation	in-line SPE-CE-MS/MS with PLE	[83]

Analyte	Sample matrix	Cell matrix	Solvent	Temperature (°C)	Pressure (psi)	Static time (min)	Cycles	Spiking	IS	Abs recovery (RSD) (%)	Rel. recovery (%)	Conc. level (μg/kg)	Clean-up and analysis	Ref.
Macrolides														
Erythromycin	Meat	5 g sample mixed with 7 g AlO_2	MeOH	80	1500	15	2	Homogenized sample dissolved in 10 mL acetone with analyte	None		58–68	200	Evaporation and filtration (0.45 μm). LC–MS in SIM mode.	[65]
Josamycin											77–91			
Roxithromycin											78–86			
Spiramycin											75–84			
Tilmicosin											79–87			
Troleandomycin											69–75			
Tylosin											84–90			
β-Lactams														
Amoxicillin	Animal feed	5 g sample	MeCN:H_2O (1:3)	50	1500	5	1	Top of cell. Matrix matched calibration	None	86 (6)		200	Filtration. LC-UV	[88]
Penicillin V			MeCN:H_2O (1:1)							95 (2)				
Anticoccidials														
Robenidine	Animal feed	8 g sample mixed with 8 g beach sand	MeOH (1% acetic acid)	100	1500	3	3	Spike in homogenized sample	None		85–91	30–90	SPE (Al_2O_3)-LC-MS (SIM)	[64]
Miscellaneous														
Triclosan	Sludge	0.1 g sample mixed with 3 g HM	DCM	60	1500	5	3	Spike in homogenized sample and aged for 4 h	^{13}C		98 (6)	100	SPE (HLB)-LC–MS/MS	[93]
Triclocarban	Sediment	10 g sample (<0.5 mm) mixed with 2 g HM	DCM	100	1500	5	1	Spike in homogenised sample and aged for 24 h	^{13}C	100 (8)[a]	98 (6)	10	SPE (SiO_2)-LC-MS/MS (SRM) or SPE (SiO_2)-GC–MS (SIM)	[95]
Triclosan									None					
Biphenylol	Sludge and sediment	1 g sample mixed with sand	DCM	100	1500	5	3	Spike prior to PLE, prior to SPE, prior to derivatisation		73 (12)[a]		1	SPE (SiO_2)-GC–MS/MS	[96]
Triclosan									^{13}C	99 (2)		100		

[a] Recoveries are for the entire analytical procedure, not only the PLE step.

soil) [24]. A number of methods with PLE have been published for tetracyclines (Table 3).

Previously published PLE methods were used for extracting tetracyclines from soils in a study by Aga et al. [21]. As no method development was performed, the study is not reviewed in detail here although the settings are included in Table 3. Recently, an extraction and clean-up method was described for the analysis of chlortetracycline, doxycycline, oxytetracycline and tetracycline in soil [23]. The soil samples were spiked (10 μg/g soil) and aged for 24 h prior to extraction. Prior to extraction 5 g of soil was mixed with Hydromatrix® to fill the 33 mL cell. Different solvents were tested in order to determine the best recoveries and reproducibility. A solvent mixture of 1:1 (v/v) methanol/acetate buffer (pH 8) at 60 °C and 1500 psi with a 5 min static step in two cycles proved to give the best extraction efficiency. The absolute recoveries for the isolated PLE step were 92–99% for all the compounds. Despite the high recovery rates obtained without sample clean-up, SPE was surprisingly carried out prior to LC–MS analysis [23].

Recently, Blasco et al. applied PLE to extract four tetracyclines (tetracycline, chlorotetracycline, oxytetracycline and doxycycline) from different types of meat [80]. All parameters affecting the PLE extraction efficiency, such as temperature, pressure, treatment of sand, static time, cell size, number of extraction cycles and flush volume, were carefully evaluated. The presented data were absolute recoveries obtained by samples spiked at 10 μg/kg without adding the IS. The best results were obtained using sea sand as dispersant and a mixture of methanol/water (1:1) at 70 °C and 1500 psi. However, extraction with water, instead of with methanol–water, also provided very good recoveries (only 4–10% lower than those obtained for the extraction with methanol/water). Therefore, water was finally selected as the optimal solvent with a 10 min static step applied in one time cycles. Bovine, swine, poultry and lamb muscle tissues (1 g), were spiked with the four tetracyclines at the three levels (1, 100 and 200 μg/kg) and left for 10 min before adding the IS. The relative recoveries were all in the range of 89–98% for the entire analytical procedure [80].

Overall, optimal temperature for tetracyclines appears to be in the range 60–70 °C using pure water or a combination of water/organic solvent as extraction solvent at a pressure of 1500 psi. The static step should have durations of 5 min in 2 cycles or 10 min in 1 cycle (Table 3).

4.2.1.3. Quinolones. Fluoroquinolones are highly useful antibacterial agents, applied in both in human and veterinary medicine worldwide [26], particularly because of their broad activity spectrum and good oral absorption [59]. These drugs belong to the family of gyrase inhibitors and they are a group with different chemical structures and spectra of activity [82]. Today ciprofloxacin is the most widely prescribed fluoroquinolones in the world, followed by ofloxacin [17]. In the last 10 years PLE has been applied for different matrices, which is reviewed here (see also Table 3).

Golet et al. have extracted norfloxacin and ciprofloxacin in sewage sludge and soil by PLE [59]. Dried samples of 200 mg of sewage sludge and 500 mg of sludge-treated soil, respectively, mixed with approximately 10 g of quartz sand. They were extracted for 4 and 6 × 15 min static extraction respectively, with 5 min preheat, at 100 °C, 1450 psi, 300 s purge and with 150% flush volume. An IS was added prior to sample clean-up on MPC (mixed phase cation exchange) cartridge discs. The samples were analysed with LC-fluorescence detection. During PLE development temperatures from 50 to 150 °C were applied, but between 100 and 150 °C, the extraction efficiency remained constant. The pressure was investigated in the range from 725 to 2175 psi. A diluted phosphoric acid (50 mM, pH 2.0), acetonitrile mixture (1:1) was used as extraction solvent. The buffer was chosen after investigation of solvent composition and pH effect. Extraction efficiencies altered with organic solvent/water ratio and improved at extreme pH values. A 1:1 organic solvent/water ratio and acidic conditions yielded the highest recoveries probably due to better solubility of fluoroquinolones or appearing advantageous ionic interaction at low pH. Data was presented as obtained concentrations, although the spiking level was unclear. The static time was tested at four settings from 5 to 20 min and the operation conditions were set to 15 min. On spiked samples 2–4 cycles were tested, although no dramatic differences were observed between them. Up to 8 cycles was tested on soil and sludge containing native analytes to evaluate percentage of extractable amounts per cycle. Since no detection was observed above 3 (sludge) and 6 (soil) cycles, the final settings were set to 4 and 6 cycles respectively. For method validation for the entire extraction and analytical procedure samples were spiked, although not specified how. Recoveries rates – probably relative, since internal calibration with IS added after PLE was employed – for fluoroquinolones ranged from 82 to 94% for sewage sludge and from 75 to 92% for sludge-treated soil. A standard addition quantification procedure was compared to the internal calibration but the results were not stated other than a stated difference in RSD between the different approaches (15% < LOQ < 8–11%) [59].

Pecorelli et al. carried out a study on feed samples and assessed the ability to extract 13 quinolones (pipemidic acid, rufloxacin, enoxacin, ofloxacin, norfloxacin, ciprofloxacin, danofloxacin, enrofloxacin, difloxacin, cinoxacin, oxolinic acid, nalidixic acid and flumequine) in different animal feeds [82]. The matrices were extracted with metaphosphoric acid (0.2%) in water/acetonitrile (7/3; v/v) at pH 2.6 while temperature, pressure, extraction time and number of cycles was not descried. Fish feed was spiked in the range 5–25 mg/kg by the addition of an appropriate volume of standard stock solution (50, 100 and 250 μl) to 1 g feed test portions. The spiked samples were left for 30 min at ambient temperature before extraction. For the complete analytical procedure, an average absolute recovery range of 58–103% was reported for 12 of the fluoroquinolones—all but enoxacin, for which the corresponding range was only 31–51% for not specified reasons [82].

Herranz et al. extracted 3 fluoroquinolones (enrofloxacin, ciprofloxacin and sarafloxacin) from eggs [26]. The results showed that recoveries depended mainly on solvent composition. A 50 mM aqueous phosphoric acid/acetonitrile mixture (1:1, v/v) was used as the optimum extraction solvent at 1500 psi, while temperature and time did not play an important role in the extraction efficiency. To determine the linearity and the reproducibility of the whole analytical method 2 g of egg sample was spiked with the analytes in the range 50–1000 and 500 ng/g of lomefloxacin added as IS. Mean relative recovery of the IS at the spiked level was 75. Mean relative recovery values were 68–88% for enrofloxacin, 67–90% for ciprofloxacin and 71–87% for sarafloxacin with RSDs lower than 11% in all cases.

In a later study by Lara et al. eight regulated quinolone antibiotics in chicken muscle were extracted by dichloromethane (pH 5) [83]. Portions of 1 g of spiked samples were mixed with 1.5 g of pelletized diatomaceous earth and loaded into a 10 mL cell with a cellulose filter at the bottom. The dead volume was filled with quartz sand. The specifications for optimisation of extraction parameters was not mentioned, however the final conditions selected were, dichloromethane as extraction solvent at 1700 psi and 50 °C. The preheating time was 5 min and the static extraction time was set to 0 min with 1 static cycles and 1 min purging. Absolute recoveries between 63 and 112% were obtained for the entire SPE-CE-MS procedure, with RSD lower then 16% in all cases [83].

For quinolones there is no clear consensus regarding extraction parameters, and in some cases they are not specified. Temperatures vary from 50 to 100 °C, while solvents range from phosphoric acid/acetonitrile mixtures to dichloromethane. Extraction time also varies to a great extent where some perform a single cycle

below 5 min and other set their extraction to 15 min at 4 cycles (Table 3).

4.2.1.4. Macrolides. Macrolide antibiotics are heavily used in both human and veterinary medicine. Due to their extensive use in food producing animals maximum residue levels (MRLs) have been established for these meats [84], why there is an apparent requirement for good extraction and analytical methodologies are required. In total five PLE methods have been published with macrolides [20,65,70,73,85–87], where one of these methods is solely dedicated to macrolides and reviewed in this section [65]. That method determines seven macrolides (erythromycin, josamycin, roxithromycin, spiramycin, tilmicosin, troleandomycin and tylosin) in meats. The other methods are reported in the multi-class method section for antimicrobials (Section 4.2.2).

In the optimisation of a method for beef, pork, chicken and fish meat, lyophilised and sieved (<125 μm) samples of 5 g of beef meat were spiked by adding 10 mL acetone containing seven macrolides [65]. The samples were thereafter vigorously shaken. Subsequently, the organic solvent was evaporated (20 °C). Different extraction solvents were tested—acetonitrile, methanol, methanol–water mixtures and acidic water. The stated recovery of the different solvents ranged from 32 to 90% for the seven macrolides. Pure methanol was found to be the best solvent with recoveries ranging from 58% (erythromycin) to 90% (tylosin). Unfortunately, it is not clear how these recoveries were obtained, nor if these values only represent the recovered fraction of the freshly spiked macrolides. Temperature (40–120 °C), pressure (500–2500 psi), flush-percentage (50–150%) and cycle (1–3) variables were likewise optimised . Recoveries ranged from 42 to 90% and optimal conditions were 80 °C, 1500 psi, 150% flush volume and 2 cycles. When increasing the temperature to 100 °C a cloudy extract-suspension was observed likely caused by co-eluting lipids. The final optimised method yielded macrolide-recoveries of 58–91% in bovine, porcine, poultry and fish meats. It should be noted that these macrolide analysis were performed using standard addition technique from one bovine matrix thus establishing an external calibration curve for all samples and matrices [65].

4.2.1.5. β-Lactams. This class of antibiotics is used in the veterinary sector and can be orally applied via animal feed. Cross-contamination problems between different veterinary medicines at feed mills could be a potential dilemma as some antibiotics exert adverse mixture effects [61], for this reason good and reliable analytical methods are required to analyse animal feed. One method using PLE is published to determine two β-lactams (penicillin V and amoxicillin) in medicated swine feed samples was published by Benito-Peña et al. (Table 3) [88]. In that study the thermal stability of the compounds was evaluated at 50 °C in acetonitrile, methanol and water, and dissipation was observed in methanol [88]. This finding is consistent with others' who investigated alcoholysis of β-lactams [89]. In the optimisation of PLE method of Benito-Peña et al. [88], 5 g of the homogenised and sieved (<850 μm) blank feed samples were spiked with amoxicillin and penicillin V at two levels (200 and 500 μg/kg), mixed and aged for 15 h before PLE-extraction. Diatomaceous earth was tested as a dispersing agent in the cell-packing but gave insignificant improvements and was discarded. All PLE-extracts were passed through a filter (0.22 μm) prior to LC-UV analysis. The PLE extraction was optimised with the use of the matrix-matched pre- and post-spike approach, which is highly recommendable: Standard curves were acquired in blank PLE feed -extracts of the different tested extraction solvents. Acetonitrile, water and three different mixtures between acetonitrile/water (1:3, 1:1, and 3:1) were tested as extraction solvents. Other evaluated test-parameters were temperature (25 and 50 °C), cycles (1 and 2), flush volume (60–120%) and cell size (11 and 22 mL). The optimal parameters were; acetonitrile/water (1:3 for amoxicillin and 1:1 for penicillin V), 50 °C, one cycle and 60% flush volume of 11 mL cells with overall absolute recoveries (and RSD) of 86 (6)% and 95 (2)%, respectively, at 200 mg/kg. . Benito-Peña et al. also investigated the potential sorption to cellulose acetate, glass and nylon membrane PLE-filters, and found no sorption to any filter [88]. This approach is very recommendable to assess any loss of analyte to filters.

4.2.1.6. Anticoccidials. This class of compounds is used as growth promoters and to prevent microbial infections in livestock [61,90,91]. One method is published to determine salinomycin in soils along with four other antibiotics (discussed under multi-class methods Section 4.1.1) [73]. Two methods were developed to determine robenidine in animal feed by PLE-SPE-LC–MS by Kot-Wasik et al. and Wilga et al. [64,92]. The PLE method by Kot-Wasik et al. was optimised using 22 mL cells and fractional factorial design with respect to temperature (40–140 °C), time (2–9 min), pressure (500–2500 psi) and four different solvent compositions (methanol or acetonitrile each with 1% acetic acid or formic acid) on pre-spiked feed samples [64]. The recovery from the samples was compared to an external calibration curve. It was stated to be similar to a curve obtained from spiked blank feed samples, thus matrix interference where claimed to be minimal, although this was not shown in any other way. Hence, it is difficult to judge weather the obtained recoveries are absolute or relative. If the assumption of no matrix effect is correct, however, the recoveries are absolute and are 66% for the for the combined PLE and sample prep step. The optimal parameters were 3 min extractions in 3 cycles with methanol containing 1% acetic acid at 100 °C, 1500 psi and 60 s purge. To the PLE extract a drying agent (molecular sieve, 3 Å) was added. A 2 mL aliquot of this mixture was passed through 1 g aluminium oxide and eluted with 10 mL methanol preceding the LC–MS analysis [64].

More recently, a developed PLE method used for the determination of robenidine in animal feed was compared with other extraction techniques (viz Soxhlet, Soxtec, ultrasonic, microwace-assisted and shake-flask) by the same research group [92]. Extraction temperature (60–140 °C), pressure (1000–3000 psi), time (1–5 min), flush volume and different solvents (methanol and acetonitrile combined with formic or acetic acid) were optimised. Once again, the PLE extract was purified by afore mentioned molecular sieve drying agent and cleaned by aluminium oxide [64,92]. The optimal parameters in this more recent method were 1500 psi, 3 cycles of 4 min at 80 °C with 100% flush volume with methanol containing 1% acetic acid. Recovery of around 85% yielded for manufacturer feed containing 66 mg/kg robenidine.

4.2.1.7. Miscellaneous. A PLE-SPE-LC–MS/MS method intended for the determination of the two bactericides or antiseptics triclosan and triclocarban in waste water sludge has been published [93] (Table 3). These bactericides are widely used in personal-care products [94]. In the study, the waste water effluent's discharge potential into the aquatic environment was investigated [93]. The loaded 33 mL PLE cells were spiked on top with 20 ng ^{13}C-labelled versions of both triclosan and triclocarban prior to the extraction. The PLE extract was cleaned by SPE for which several materials were tested and HLB was found optimal [93]. Traces of carry-over in the PLE system was observed when procedural blanks were run between samples. The researchers made good matrix matched relative recovery studies of the entire method (PLE-SPE) and nicely, evaluated the matrix effect. Unsurprisingly, the authors found relative recoveries to the ^{13}C-labelled internal standards of 98% for both bactericides. The absolute recovery could have been demonstrated by the spiking of ^{13}C-labelled internal standards in PLE extracts and comparison of these with other (normal) pre-spiked samples. In a sampling campaign, the researchers observed decrease of the

^{13}C-labelled internal standards (from 106% down to 44%) in some samples compared to external standards (without matrix effect) reflecting the combined value of the absolute recovery and the matrix effect. This observed decrease probably demonstrates the high variability of the matrix composition between samples; however, with the employed setup it is not possible to clarify if the losses were due to matrix effects or unsuccessful extractions.

Another method to determine the two antiseptics triclosan and biphenylol in marine sediments was published in 2003 [95]. The researchers investigated temperature and pressure dependency in the PLE extraction. Samples of 10 g sieved (<0.5 mm) and freeze dried sediment mixed with 2 g Hydromatrix® in 22 mL cells with dichloromethane as extraction solvent were used. The optimal conditions were found to be 100 °C and 1500 psi. The recovery experiments on triclosan and biphenylol were performed by using pre-analysed sediment with a concentration lower than 0.5 μg/kg that was spiked to 10 and 1 μg/kg, respectively, and aged for 24 h prior to PLE-extraction [95]. The PLE-extract was reduced to 5 mL (nitrogen) and further cleaned on silica, reconstituted in ethyl acetate and filtered (0.45 μm PTFE) prior to GC–MS injection or reconstituted once again to acetonitrile/water (1:1) if LC–MS/MS technology was utilised [95]. The recoveries (and RSD) for the whole method were reported as 100 (8)% for triclosan (10 μg/kg) and 73 (12)% for biphenylol (1 μg/kg). It is unclear whether the researchers used matrix matched calibration or external standard curves in the recovery experiments. Consequently it is uncertain if the reported recoveries are absolute or if matrix effect has given a negative contribution to them.

A third method for the determination of triclosan was develpped for sludge and lake sediments. Lyophilised samples of 1 g mixed with sand and packed in 2 mL PLE-cells spiked with ^{13}C-labelled triclosan on top were used [96]. The PLE-extracts were cleaned on silica and derivatised with diazomethane prior to GC–MS/MS analysis. Different solvents (acetone to toluene), temperature, pressure, solvent volume and cycles were tested. The optimal conditions were 100 °C at 5 min and 1500 psi with 2 mL dichloromethane in 3 cycles. An absolute recovery setup on 100 ng/g spiked sediment – spiked not only before PLE-extraction, but also before silica clean-up as well as before the derivatisation – was thoroughly described. Such a spike-recovery approach yields the absolute recoveries of each analytical sample preparation step and is highly recommendable. The absolute recovery (and RSD) of the PLE extraction was 99 (2)%. The derivatisation was well evaluated by the use of a pure commercially acquired reference standard (triclosan methyl ether—the main derivatisation product of triclosan) giving a yield of 58 (5)%. The relative recovery to ^{13}C-triclosan for the whole method was 100 (3)% [96].

4.2.2. Multi-class methods—antimicrobials

Ten multi-class PLE-methods for various matrices have been published between 2002 and to date and are reviewed here. The final PLE-settings of these methods are shown in Table 4.

In the majority of the reviewed methods, recoveries were calculated for the entire analytical method – not for the individual PLE step – and also in varying ways as either relative or absolute recoveries: This makes it somewhat difficult to compare the actual extraction efficiencies of the PLE step between the different methods. Since each method contains compounds from a number of classes, methods have been divided based on matrices for which they were developed.

4.2.2.1. Soil and manure. Three multi-class papers which use PLE for extraction of soil and manure were published between 2003 and 2006 [73,86,87]. One of them is a PLE-SPE-LC–MS/MS method for 4 macrolides and 1 ionophore (erythromycin, oleandomycin, roxithromycin, tiamulin and salinomycin) in soil which was published by Schlüsener et al. [73]. Three additional compounds (tylosin, monensin and ivermectin) were initially included in the methods, but yielded fluctuating and unsatisfactory recoveries (data was not shown). PLE was optimised with regard to solvent, temperature, number of cycles and static time and PLE extracts were analysed directly after centrifugation (n = 3). Recovery data from PLE optimisation was unfortunately presented as ratios to the internal standard methyloxime-erythromycin (relative recoveries) and only for one of the compounds (roxithromycin). For PLE optimisation 10 g of air dried soil was spiked (500 μg/kg) and mixed for 10 min prior to PLE in 11 mL cells. A 24-h aging effect was studied on the spiked soil, but resulted in similar recoveries. PLE was tested in the temperature range 40–120 °C, for acetone, acetonitrile, methanol as well as methanol with 1% (v/v) aqueous ammonia; the latter giving substantially higher recoveries up to and especially at 80 °C. Recoveries for the other solvents were lower and rather constant over the temperature range for roxithromycin. Hence methanol with ammonia was chosen as extraction solvent. Extracts from 3 cycles were analysed separately and since no analytes were present in the extracts from the third cycle, 2 cycles was chosen for the final method (data was only shown for roxithromycin). Impact of duration of static time was investigated (10 and 20 min), but not observed (data not shown). Larger samples and cells (30 g/33 mL) were used for method validation and application than for the PLE optimisation. The soil was not mixed with cell matrix; although the void volume of the PLE cell was filled with Ottawa sand. Validation of the entire PLE-SPE-LC–MS step was made on antibiotic free soil at 1, 6, 20, 200 and 2000 μg/kg soil (n = 3; data not shown). It was not demonstrated if all five analytes had the same recovery as the internal standard in the SPE clean-up. LOQ (S/N 10:1) ranged from 0.6 to 5.3 μg/kg soil. Combined mean relative recoveries (RSD(%)) calculated from all the tested concentration levels were as follows; erythromycin 43(23)%, oleandomycin 38(51)%, roxithromycin 94(19)%, salinomycin 76(32)% and tiamulin 118(18)%. For erythromycin and oleandomycin the recoveries are quite low and only 2 of all the tested compounds had RSD-values below 20% indicating poor repeatability: Especially the result for oleandomycin seems deficient, particularly considering that it is relative and not absolute recoveries that are stated. As mentioned above, 3 compounds were excluded from the initial method due to poorly repeatable recovery rates, although no theories on explaining this behaviour are given in the paper (i.e. thermal degradation). As stated in the published work; soil is a complex matrix why SPE clean up and matrix matched calibration was used. However; the soil used for making the soil matrix extract was different than for the samples themselves. Moreover, standards were diluted 1:1 in soil matrix and not dissolved in 100% matrix extract, which could be part of the problem. In addition, ion suppression might vary more or less with each individual sample due to the nature of the matrix resulting in high RSD-values. One way to avoid this is to use standard addition quantification, which was not used in that work [73]. Mean relative recoveries were calculated by combining results obtained when different concentrations were applied: Hence, the high RSD-values might reflect a difference in extraction efficiencies depending on amount of applied analyte. The data in this study as well as studies presented below indicate that macrolides in general seem to be a challenging class of compounds.

In 2004 a PLE-SPE-LC–MS/MS method for the analysis of 3 veterinary antimicrobial classes sulfonamides, tetracyclines and macrolides in soil and barley was published [86]. Sulfadiazine, chlorotetracycline, oxytetracycline, erythromycin and tylosin with its 3 main metabolites tylosin B, C and D were analysed in 2 agricultural soils [81,86]. The PLE optimisation for soil was described, although without any detailed data in the paper by Jacobsen et al. [86]. Nevertheless, moisture content of sample, sample size and

Table 4
Multi-class methods—antimicrobials.

Class[b] (number of analytes)	Sample matrix	Cell matrix	Solvent	Temp (°C)	Pressure (psi)	Static time (min)	Cycles	Flush volume (%)	Purge time (s)	Spiking	Abs Rec (%)	Rel Rec (RSD) (%)	Clean-up & Analysis	Ref
MLs (4) IPs (1)	Soil (30 g)	Ottawa sand	MeOH with 1% NH4OH (25%)	80	2030	10	2	70	180	Validation: 1–200 μg/kg on 10 g soil; mixed and aged for 24 h. IS: (E)-9-[O-(2-methyloxime)]-erythromycin (added after PLE extraction). Matrix matched internal calibration.		38–118[a] 74[a]	Diol-SPE-RP-HPLC-APCI(+)-MS/MS (SRM) LOQ:1–5 ppb	[73]
SAs (1) TCs (2) MLs (4)	Soil (10 g), barley grain (10 g)	Ottawa sand (1:1)	MeOH/0.2 M citric acid buffer (1:1)	Ambient	1500	10 and 3	2	90	N/A	Validation: spiking of analytes at 5–100 μg/kg soil. External calibration.	52–85[a] 33–119[a] 45–127[a]		SAX-HLB SPE-RP-HPLC-ESI(+)-MS/MS (MRM) LOQ: 1–10 ppb	[86]
SAs (3) TCs (7) MLs (4)	manure (0.75 g)	Ottawa sand (packed cell)	1 × 0.2 M citric acid buffer, pH 4.7; 2 × MeOH/0.2 M citric acid buffer (4:1)	75	2500	5	3	50	60	Validation: analytes spiked at 5–5000 μg/kg soil. Standard addition. Instrument standard; Oleandomycin	59–109[a] 24–228[a] 9–35[a]		SAX-HLB SPE–RP-HPLC-ESI(+)-MS/MS (MRM) LOQ: 10–100 ppb	[87]
SAs (5) MLs (4) TRIM	Sewage sludge (200 mg)	Quartz sand (packed 11 mL cell)	H_2O/MeOH (1:1 v/v)	100	1450	5	2 and 3	120	60	PLE opt: analytes spiked on samples 400 μg/kg d.w. mixed 30 min and lyophilised. Validation: Deterium and Isotope standards, Tylosin and oleandomycin at 500 μg/kg d.w.) spiked on freeze dried sludge prior to PLE. No aging.	55–64[a] 29–45[a] 51[a]	79–106[a] 91–142[a] 78[a]	SPE (Oasis HLB)-RP-HPLC-ESI(+)-MS/MS (MRM) LOQ: 3–40 ppb	[20]
SAs (7)	Sludge (1 g)	Na2EDTA and Hydromatrix (5:1)	acetone/MeOH (1:1, v/v)	75	1500	5	3	75	60	ext strd calibr. PLE opt: analyte spike 500 ng/g sludge. No surr or int strd.	<1–104[a]		SPE (HLB)-RP-HPLC-ESI(+)-MS/MS LOQ:0.5 ppb	[98]
SAs (2) TCs (2) QNs (3)	sewage sludge (4.5 g)	Sand (1:1)	H_3PO_4 (0.35%)/ACN (1:1 v/v) w/ 0.01M citric acid	100–110	1030–1615	10	5 (although 3 was sufficient)	60	40	PLE opt: analyte spike on quartz sand. PLE-SPE opt: analyte spike on sludge. Strd add for some samples. Recoveries calculated from strd addition data.	52–91[a] 27–95[a] 58–84[a]		SCX and HLB SPE-RP-HPLC-ESI(+)-MS/MS LOQ: 0.1–160 ppb	[71]
SAs (10) TCs (3) MLs (5) QNs (8) β-Ls (3) TRIM[b] Ronidazole[c]	Meat (1 g)	Na_2EDTA washed sand (1:11 + packed cell)	H_2O	70	1500	10	1	60	60	1 g portions; frozen. Spiked at 5–2400 μg/kg meat 10 min prior to PLE. IS: Dapsone.	70–100 80–100 60–100 65–90 70–100 95 75	81–98 70–87 79–92 71–96 74–98 72–85 77–98	Centrifugation, no clean-up LC-ESI(+)MS/MS LOQ: 10–50 ppb	[85]

Table 4 (*Continued*)

Class[b] (number of analytes)	Sample matrix	Cell matrix	Solvent	Temp (°C)	Pressure (psi)	Static time (min)	Cycles	Flush volume (%)	Purge time (s)	Spiking	Abs Rec (%)	Rel Rec (RSD) (%)	Clean-up & Analysis	Ref
MLs (3) Glycopeptides Zinc bacitracin	Animal feed		Acetone:H_2O (13:7, pH 2)	80	N/A	5	2	NA	300	Spike in homogenised sample	94–98 57 60		Adjusted to pH 6.5/8.0, microbial growth plate system detection (zone diameter) LOQ: 5–10 ppm	[70]

[a] Recovery for the entire method, not just the PLE step; [b] TCs: tetracyclines, SAs: sulfonamides, MLs: macrolides, QN: quinolones, βLs: β-lactams, IPs: ionophores, TRIM: trimethoprim; [c] A nitroimidazole.

extraction solvent with regards to solvent composition, buffers and concentration were optimised and briefly described. Optimal sample moisture content was found to be 5%. Soil sample size up to 25 g was tried, but since large samples caused problems with clogging, 10 g was chosen for the final method. The composition of the extraction solvent was found to be crucial due to the presence of tetracyclines in the method since they have strong cation complex-binding properties [81]. Hence, a complexating buffer with citric acid and phosphate (McIlvane buffer) and EDTA in methanol was tried as extraction solvent but found to cause clogging due to precipitation. A combination of methanol and citric acid buffer was further investigated with better results and was optimised with regards to buffer concentration (0.2–0.5 M; no significance) and methanol content (50–75%). Final extraction solvent was 0.2 M citric acid (pH adjusted to 4.7)/methanol (1:1, v/v). Higher methanol content yielded darker extracts and heavier clean-up was needed. The PLE was carried out at ambient temperature due to epi-formation of the tetracycline at elevated temperatures. External calibration was used [86]. Absolute recoveries (pre and post extraction spike) were obtained from the validation procedure of the entire analytical procedure by spiking 2 types of soil ($n = 6$) with analytes at 4 concentration levels; 5, 25, 75 and 100 μg/kg soil. Recoveries differed slightly between the soils as well as between the different concentration levels and ranged between 33 and 76% (2–24%) for the tetracyclines, 55–119% (2–8%) for sulfadiazine and 45–186% (2–80%) for the macrolides (RSD(%) in parenthesis). For sulfadiazine the recovery and reproducibility is very good and nice also for the tetracyclines. In general was the reproducibility lower for the highest and lowest concentrations tested and better for the loamy sand soil than for the sandy soil—especially for the macrolides. In the paper there are suggested different sorption mechanisms for macrolides on the two soils as well as choice of extraction buffer as an explanation for the behaviour of the macrolides [86,87]. Thermal lability of the macrolides is discussed in other papers; but since PLE was carried out at ambient temperature in this study, it is not an explanation here. On the other hand, the recovery rates for the macrolides were as high as 186%, which might indicate analytical interferences [97] and perhaps ion signal modification from matrix effects. The method was with some minor alterations successfully also applied to barley grains, but not reviewed further here [86].

In 2006 a similar but more comprehensive method was developed for the challenging matrix swine manure for the same antimicrobial classes but also including the compounds tetracycline, sulfamethazine, sulfadoxine as well as the epi-forms of oxy-, chloro-, and tetracycline and published by Jacobsen and Halling-Sørensen [87]. The method comprises 11 individual compounds and 1 IS added prior to LC–MS analysis (i.e. instrument standard). Presented recoveries were absolute recoveries for the entire PLE-SPE-LC-ESI(+)-MS/MS procedure and obtained from method validation at three concentration levels 50–5000 μg/kg manure. They were 24–228 (3–67%) for the teracyclines, 59–109 (2–13%) for the sulfonamides and 9–35 (3–13%) for tylosin (RSD(%) in parenthesis). The higher RSD-values were obtained at the lowest concentration and for the higher concentration levels they were in the rage of 2–13% for all of the compounds. The complexity of the method is of course constituted by the difficult matrix combined with a large number of compounds. The matrix was frozen, lyophilised and pulverised before extraction. The PLE development is described with limited data and was carried out for the three compounds sulfadiazine, oxytetracycline and tylosin. Sample conservation and sample storage was investigated in order to increase recoveries and PLE was optimised with regards to solvent, buffer, cycles, pressure and temperature, with some of the data presented. Sample size and/or sample and cell matrix ratio was not investigated. The problems in the PLE development seem to have been

the stability of tylosin, why sample conservation with sodium azide before the extraction was tested (0.5, 1.0 and 2.5%; w/v). Significantly higher recoveries, although still low, were observed; why this procedure was not employed. Instead, a stability test was carried out by spiking wet manure at different times prior to freezing (0, 5, 20, 40, 90 min). Oxytetracycline and sulfadiazine were not affected, but rapid degradation of tylosin was observed: 90 min of storage at ambient temperature decreased recovery rates to less than 1/10 compared to when frozen immediately. Hence, samples were frozen immediately after collection. In the PLE method itself, altering the pressure (500–2500 psi) had no significant impact on recoveries. The temperature (ambient to 150 °C) was mostly important for oxytetracycline, which seemed to degrade at temperatures above 75 °C, but only of minor importance to sulfadiazine and surprisingly of none to tylosin. The soil method described above [86] was carried out at ambient temperature with consideration to the epi-forms of the tetracyclines. But here the optimisation was carried out on oxytetracycline why the effect of the chosen temperature on the epi-forms was unfortunately not studied (although these compounds were included in the method validation). As for the soil method [86], extraction solvent composed by methanol and citric acid was used, although the buffers ethyl acetate, EDTA and phosphoric acid were also tested as well as several organic solvents (methanol, acetonitrile, dimethyl sulfoxide, acetone, dichloromethane, tetrahydrofuran, *iso*-propanol, methyl *t*-butyl ether and hexane; data not shown). Methanol, acetone, dimethyl sulfoxide and all the buffers gave satisfactory recoveries, although some kind of precipitation in the PLE extracts was observed when citric acid/ethyl acetate was used. The precipitation was probably because of decreased solvent polarity and precipitating salts (data not shown) [87]. Precipitation also occurred when EDTA was mixed with methanol, resulting in blockage of the PLE equipment. The addition of citric acid to methanol improved recoveries; hence citric acid buffer was further tried (100%) and mixed with methanol at different ratios (20 and 50%). Citric acid concentration (>0.2 M) had no impact on recoveries (data not shown). The 1:1 ratio was excluded and then a numbers of cycles (2–4) combining two solvents with 100% and 20% citric acid were investigated. Increasing number of cycles did not improve recoveries alone, but seemed to be more dependent on the use of citric acid buffer. The difference was most prominent for oxytetracycline, but was also evident for sulfadiazine although a minor decrease in recoveries was observed for tylosin. The final combination of solvents was constituted of 2 cycles with 100% citric acid buffer (0.2 M) followed by 1 cycle with citric acid buffer/methanol solvent (1:4). The instrument standard was used to monitor the response difference between the different samples, and varied immensely between different types of manure. Providentially standard addition prior to LC–MS/MS was used for calibration and quantification, which compensates for such matrix effects.

4.2.2.2. Sludge. For sludge three papers were published between 2005 and 2009 [20,71,98]. Göbel et al. developed and validated a PLE method for activated and digested sewage sludge in 2005. The PLE extracts were subsequently cleaned-up and analysed using Oasis HLB SPE and two different LC–MS/MS procedures [20]. The analytes belonged to the sulfonamide and macrolides antimicrobial classes and were sulfadiazine, sulfathiazole, sulfamethazine, sulfapyridine, sulfamethoxazole, erythromycin, clarithromycin, roxithromycin, azithromycin, dehydro-erythromycin as well as trimethoprim. For the PLE optimisation activated sludge was filtered and the solid phase was retained. Prior to freezing and lyophilisation, the solids were fortified with analytes corresponding to a concentration of 400 μg/kg d.w. and mixed for half an hour. Solvent, solvent pH, temperature, time, pressure and sample size, number of cycles and compound stability on sand was investigated and described in text for 6 of the 10 compounds, although very little actual data of the optimisation was presented. The final PLE method was carried out with the following conditions: 2 or 3 cycles of 5 min static extraction at 100 °C and 1450 psi with H_2O/MeOH (1:1) as extraction solvent. Investigated solvents were methanol, acetonitrile, acetone and water alone or in different combinations. Water combined with an organic solvent gave the best recoveries (given as concentrations) and extraction of macrolides as well as trimethoprim increased with decreased solvent polarity. The final solvent; methanol and water (1:1), was chosen to increase recoveries for trimethoprim and the macrolides, which are in general somewhat more lipophilic compounds than the sulfonamides. A pH alteration from 4 to 10 of the water fraction did not affect recoveries and Göbel et al. speculate that this result might be an indication of dominating hydrophobic matrix-analyte interactions over ionic for the macrolides. Extraction temperatures between 60 to 200 °C were investigated. Extraction efficiencies dropped with 10–20% when temperatures were below 100 °C and with 20–95% above 100 °C, with the highest reduction for sulfamethoxazole at elevated temperatures, possibly due to thermal degradation of the analytes [20]. Considering that sulfonamides are found to be quite stable [99,100] increasing interfering matrix effects could be an alternative explanation to degradation. Problematically darker extracts were obtained with elevated temperatures (which caused clogging of the SPE cartridges) indicating increased amounts of extracted matrix. In the paper results from a stability study of the compounds on sand does not indicated any thermal degradation performed at the final chosen conditions. Altering the extraction pressure (870–2175 psi) did not affect recoveries, although clogging of SPE cartridges also occurred with extracts obtained at elevated pressure levels, and filtration of the extracts lead to significantly lower recoveries for macrolides. Cycle times of 1, 3, 5, 10 and 20 min were investigated with an recovery optimum at 5 min (20% higher recoveries). Varying the sample size (100–400 mg) did not affect extraction efficiencies. Four cycles were performed and analysed separately. Analyte recoveries for each cycle are neither presented as relative nor absolute but as percentage values of the total extracted amount in all of the 4 cycles. The majority of the extraction occurs in the 1st cycle and some in the 2nd. For digested sludge, minor amounts are also extracted in a 3rd and 4th cycle, but nothing more for activated sludge. Surprisingly though, 2 cycles were chosen for digested sludge and 3 for activated sludge. More than 2 cycles for digested sludge resulted in clogging of the system. The entire method was validated on activated sludge, which was spiked with 50–100 ng of analytes and surrogated standards post lyophilisation. Both absolute and relative recoveries were obtained in the method validation. Unfortunately it is not clear weather the external calibration was matrix matched. Moreover, were surrogate standards confusingly used differently in the two analytical methods: The spiking was either performed prior to ("method 2") or after PLE ("method 1") as well as with different surrogate standards. Relative recoveries were determined using "method 1" (spiking after PLE-extraction) and were reported to be 78–106% (3–7%) for the sulfonamides and trimethoprim, and 91–142% (9–16%) for the macrolides. Roughly the same results were said to be obtained for the entire method and for the SPE-LC–MS/MS part, indicating thermal stability of the compounds during the PLE-extraction. However, in "method 1"was spiking apparently performed after the PLE extraction; why relative recoveries from "method 1" should only reflect the SPE-LC–MS/MS step and will in any case only say something about the accuracy of the chosen surrogate standards since they are relative. Results obtained from both "method 1" and "method 2" were also used to calculate absolute recoveries. For sulfonamides/trimethoprim and macrolides respectively they ranged from 51 to 64% (3–17%) and 29 to 45% (7–27%) for "method 1" and from 47 to 83% (7–19%) and 74 to 91% (21–33%) for "method 2"

(pH4). Corresponding numbers for pH 7 ("method 2" only) were 41–51% (3–6%) and 88–90% (3–5%). For the surrogate standards d_4-sulfamthoxazole and oleandomycin the recoveries for "method 2"were 37(15)% and 93(9)% respectively at pH 4, while at pH 7 they were 44(4)% and 95(3)% at pH 7 [20]. This result indicates that d_4-sulfamthoxazole was probably not suitable as a surrogate standard under the conditions used. In general, the method is difficult to evaluate due to interchangeable procedure steps such as the different spiking procedures, use of surrogate standards and different data compensation with recovery results between different analytes.

Another PLE-SPE-LC–MS/MS method for sludge was published in 2006 by Silvia Díaz-Cruz et al. [98]. The intentional analytes were 9 sulfonamides and 2 penicillins (β-lactams), although, particularly poor recoveries (<1%) for both of the penicillins and 2 of the sulfonamides were obtained, which in reality turns the method into a single-class analytical method for 7 sulfonamides: The extracted compounds were sulfamethazine, sulfadimethoxine, sulfapyridine, sulfadiazine, sulfamethoxypyridiazine, sulfathiazole and sulfamethoxazole, with recovery rates ranging from 15 to 104 (9–57)%. The explanation to the poor recovery results for in particular some of the compounds (sulfisoxazole, sulfamethizole, nafcillin and dicloxacillin; <1%) is largely ion suppression. Matrix matched calibration was not employed in the method, although, the ion suppression effects were nicely assessed by a pre- and post extraction spike experiment in PLE extracts compared to standards. Ion suppression was found to be approximately 95–110% for the later 4 excluded compounds [98]. Penicillins belong to the β-lactam antimicrobial class and they seem to degrade easily. For example; during treatment with β-lactams, they are to be dissolved just prior to injection to minimise degradation in the solution. Pharmacokinetics data also show a rapid metabolisation in the body. As mentioned earlier in the text (Section 4.2.1.5), they are found to degrade rapidly in alcohols, which was used for reconstitution of the extracts in the method of Silvia Díaz-Cruz [98]. This lability might also have contributed to low recoveries for the 2 penicillins initially included in the method. The PLE optimisation is not well described in the paper. Testing of chelating agents such as Na_2EDTA, citric acid and McIlvane buffer was performed as well as mixing of the samples with different amounts of Na_2EDTA. The solvents acetone, methanol, isopropanol and acetonitrile were also tried as well as altering the temperature (65–90 °C) and pressure (1200 and 1800 psi), but no data was reported. The final settings were 3 cycles of 5 min static extraction at 75 °C and 1500 psi. Acetone/H_2O (1:1) was used as extraction solvent. The method was validated on analyte spiked sludge at the two concentration levels 0.1and 1 μg/g, with quite different recoveries obtained at the two levels (recoveries and RSD-values are stated above). Instrument repeatability was measured on repeated injections of analyte standard solutions. Quite differently from other publications the limit of quantification was defined as a signal to noise ratio of 1:8 and not to 1:10 [98]. The best results were obtained for sulfamethazine and for sulfapyridine at 1 μg/g which were recovered at 104 (12)% and 85 (10)%. Surprisingly low recoveries combined with high RSD-values were obtained for sulfathiazole and sulfamethoxazole at both of the concentration levels 15–39 (29–54)%).

A comprehensive multi-class PLE-SPE-LC-ESI-MS/MS method for 7 compounds from 3 antimicrobial classes in sludge was developed by Lillenberg et al. [71]. Unlike the other reviewed methods, in which commercially available PLE-systems exclusively are used, an "in-house designed system" was used for this method. The analysed compounds were 3 fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin); 2 tetracyclines (tetracycline and doxycycline); and 2 sulfonamides (sulfadimethoxine and Sulfamethoxazole) and the final conditions were 5 cycles of 10 min static time extraction at approximately 100 °C and 1000–1600 psi. An extraction solvent composed of 0.35% H_3PO_4/acetonitrile (1:1) with 0.01 M citric acid was used. The analytes were spiked on quartz sand and PLE was optimised with regards to solvent, solvent pH, static time, temperature, pressure and number of extraction cycles, although, no detailed data was presented in the paper. Recoveries ranged between 55 and 100% during optimisation. Cell matrix and ratio was not optimised. In line with many of the other reviewed methods above, altering the pressure (1000–1600 psi) had no impact on extraction efficiencies. Aqueous solutions with methanol and acetonitrile were discarded as extraction solvents in favour for a 0.35% phosphor buffer/acetonitrile mixture (1:1); pH 2.5 with 0.01 M citric acid). Extraction time and temperature was set to 10 min and 120 °C respectively since longer times and higher temperature affected the recoveries negatively—especially for the tetracyclines. Lillenberg et al. speculate that thermal degradation or interfering extracted matrix might have been causing these effects [71]. Three cycles seemed to extract the majority of the analytes (2% recovery in a 4th and 0% in a 5th cycle); yet 5 cycles was still chosen for the final settings [71], although minimising the number of cycles would probably have reduced matrix effects. Matrix effects were however said to be compensated for by the use of one standard addition per sample set for quantification, but is not explained in detail. No surrogate or internal standards were used, but data was corrected with obtained recoveries from the method validation. For method validation for the entire method, sludge was spiked with analytes at one concentration level which were different depending on the analyte (0.45–680 ng/g). Good recoveries and RSD-values were obtained for all of the compounds except for tetracycline, for which recovery was rather low. The values were for tetracycline 27(5)%, doxycycline 95(1)%, norfloxacin 58(2)%, ciprofloxacin 61(1)%, ofloxacin 84(1)%, sulfamethoxazole 91(0)% and sulfadimethoxine 52(2)% [71].

4.2.2.3. Meat. An extensive multi-class PLE-LC–MS/MS method intended for antimicrobial residue screening of meat was published in 2008 by Carretero et al. [85]. In total 31 different compounds belonging to the 8 antimicrobial classes β-lactams, lincosamides, macrolides, quinolones, sulfonamides, tetracycline, nitroimidazoles and trimethoprim were included in the method. Homogenised meat was frozen at −80 °C and 1 g of sample was mixed with analytes and the internal standard dapsone. For the PLE step, the following variables were tried; temperature (50, 70, 90 and 100 °C), pressure (1000, 1500, 2000, 2500 psi), cell matrix treatment (EDTA treated sand, hydrazine treated sand, EDTA + hydrazine treated sand), static time (2, 5, 10, 15 and 20 min), number of cycles (1–3), cell size (5, 11, 22 and 33 mL) as well as flush volume (30, 60, 100 and 150%) and a number of data from the optimisation was presented as absolute recoveries and ranged from roughly 65 to 100% (tetracyclines: 80–100%, β-lactams: 70–100%, macrolides: 60–100%, quinolones: 65–90%, sulfonamides: 70–100%, trimethoprim: 95%, ronidazole: 75% and dapsone: 95%). Since no other sample preparation than centrifugation of the extracts were employed prior to LC–MS/MS analysis; the recoveries largely reflect the efficiency of the PLE procedure. The temperatures 70 and 90 °C gave the highest recoveries for almost all of the compounds; lower or higher temperature caused a significant drop of recovery for the majority of the analytes. Pressure did not affect recoveries. Of the sand treatments tested EDTA was superior to the other two, although sand without treatment was not reported to have been tried. The EDTA addition seemed in particular important for the tetracyclines, quinolones and β-lactams. For many of the compounds did usage of the smaller cells result in about half or lesser recoveries compared to when the larger cells were used. As Carretero et al. wrote, the cell size determines the extraction solvent volume and as a consequence the important sample/matrix ratio (if sample size is kept constant). The 22 mL sized cell was chosen due to improved recoveries for

the larger cells. A cell size of 33 mL was not used as it would have delivered larger extracted volumes. Longer static extraction times increased recoveries, but not above 10 min (data not shown). The number of cycles did not affect extraction immensely, although a slight decrease in recoveries occurred when 3 cycles were carried out. For the chosen PLE cell (22 mL), no effect of changing the flush volume was observed. The method was validated according to EU regulation 2002/657/EC. Internal matrix matched calibration was used and relative recoveries were measured and validated at three concentration levels: LOQ, MRL and 2MRL (i.e. 5–50, 50–1200 and 100–2400 μg/kg). They were in the range of 70–87 (7–14)% for the tetracyclines, 74–84 (5–17)% for β-lactams, 77–98 (6–15)% for macrolides, 71–96 (5–15)% for quinolones, 81–98 (5–18)% for sulfonamides and 72–85 (10–12)% for trimethoprim and 77–98 (8–18)% for ronidazole. Only one single compound (dapsone) was used as surrogate standard for all the analytes [85].

4.2.2.4. Animal feedstuff. A multi-class antimicrobial method for feedstuff was published in 2002 by Higgins and McEvoy [70]. PLE extracts of feedstuff were screened for residue-levels of the antimicrobial feed additives spiramycin and tylosin (macrolides), virginamycin (a streptogramin which consists of 2 combined macrolides), avoparcin (a glycopeptide) and zinc bacitracin (a polypeptide), which are banned in the EU since 2006 [101]. The analyte detection was employed with microbial growth inhibition screening, which was optimised and described in the paper. In short, growth inhibition zone diameters were used for measurement of results. All samples were made in replicates. Non-medicated feedstuff (blank matrix) was used for method validation with the control of false positives and false negatives. This matrix was fortified and used to determine extraction recoveries. Minimal detectable concentrations were calculated with the help of a previously determined limit of decision for the plate assay and with results obtained from the application of pure standards on plates prepared with blank sample extract. Unfortunately, the PLE optimisation was not reported but was carried out by using 5 g sample in 33 mL cells with acetone/water (65/35, pH 2) at 80 °C in two 5 min cycles. The recoveries (and SD) of the five antibiotics from the 5 mg/kg (10 mg/kg for zinc bacitracin) fortified feed were 94 (4)%, 94 (3)%, 99 (5)%, 57 (2)%, and 60 (8)%, respectively [70]. The recoveries were remarkably high for the macrolides also compared to many of the other above reviewed multiclass methods which were employing chromatography–mass spectrometry for detection.

4.2.2.5. Summary on multi-class methods for antimicrobials. A number of ambitious multi-class methods for antimicrobials are reviewed here. The obvious challenges with these methods are the simultaneous extraction of many different compounds that differ widely in thermal lability, matrix adsorption and solvent solubility. Most of these multi-class methods are also developed for complicated matrices such as soil, sludge and manure.

During method development focus is often on increasing recoveries for the most challenging class of analytes and is particularly evident in the selection of extraction solvent and temperature. Tetracyclines are for example especially challenging in soil, manure and sludge matrices due to their metal ion complexation abilities: They require the addition of complexating or chelating agents which in their turn could cause clogging in the PLE due to precipitation [87]. Macrolides seem to be difficult to recover to high extents in most of the multi-methods [20,73,85–87] perhaps due their rather lipophilic nature compared to the other classes. In one of the methods where macrolides are included [87] lipid removal with LLE is carried out after PLE that might remove some of the macrolides content, although in the same study, major degradation of tylosin is observed while storing the sample just for 90 min. Suggested reasons in the reviewed publications are also hydrophobic analyte–matrix interactions [20] and thermal lability [86,87] although without certainty, especially since one of the methods with somewhat low recoveries is carried out at ambient temperature [86]. However, better results seemed to have been obtained in the single class methods (Section 4.2.1 and Table 3), although relative recoveries are reported for them. Interestingly, are good recoveries observed in the method by Higgins et al. for macrolides, where microbial growth inhibition is used for detection—not chromatography–mass spectrometry [70]. Thermal lability is also discussed for tetracyclines [71,86] and even sulfonamides [20], although the sulfonamides are successfully extracted at elevated temperatures in the single class methods (Section 4.2.1 and Table 3). Tetracyclines are indeed known to be light sensitive and their epi-forms suspected to be thermally labile [81,86], but as mentioned does the recovery of the tetracycline seem to largely depend on the usage of a successful complexating agent. Different agents like EDTA or McIlvane buffer (phosphorous and citric acid) were applied in varyingly successful ways in the methods either as extraction buffers or added to the cell support material and increased recoveries compared to when no agent was used [71,85–87].

Some general conditions are observed in the reviewed methods in this section. Pressure had little or no significant meaning to extraction efficiencies [20,70,71,73,85–87,98]. In most of the final methods 2 or 3 cycles are employed [20,70,73,85–87,98]. More cycles did not improve recovery due to either suspected degradation of the analytes or ion suppression most likely caused by the enhanced levels of extracted matrix [73,87,85]. In one case increasing number of cycles caused clogging of the PLE system [20]. The majority of the final methods [70,73,85,87] used similar extraction temperatures of about 70–80 °C (the range for all of the methods were ambient to 110 °C). Elevated temperatures cause similar problems as too many extraction cycles; darker extracts and clogging of PLE or SPE, [20] and degradation of analytes [71].

In addition to the need for a sufficiently good PLE method, the final recovery of the analytes in an analytical method depends on subsequent adequate sample clean up. This is especially important for complex matrices such as soil, manure and sludge to minimise ion suppression when LC–MS/MS is used for analysis and illustrated by some of the reviewed methods [20,87,98]. However, an extended discussion around this lies beyond the scope of this review.

4.3. Estrogens

In recent years it has become apparent that large quantities of estrogens of anthropogenic origin are released into the aquatic environment from human house holds. Estrogens are excreted from the human body in urine as conjugates [102]. These conjugates are largely biologically inactive. However, deconjugation often occurs in the sewage treatment plants, which renders the estrogens in their biologically active forms [103,104]. Consequently, conjugated estrogens may be present in the sewage sludge and sewage effluent with the capacity to exert endocrine disrupting effects in animals living in habitats receiving discharges. The estrogens in question are the natural estrogens produced in the human body, like estrone (E1) and 17β-estradiol (E2), but in particular the synthetic estrogen 17α-ethinylestradiol (EE2) widely used in oral contraceptives. 17α-Ethinylestradiol is one of the most widely used pharmaceuticals in the world since well over 100 million women take oral contraceptives on a daily basis.

The impact of estrogens on the aquatic environment has received increased focus during the last decade: It is now widely accepted that these estrogens have the capacity to induce intersex conditions in male and immature fish living in freshwater habitats [105–107]. Since such effects have obvious deleterious impacts on

Table 5
Estrogen methods.

Reference	[108]	[109]	[52]	[56]
Sample matrix	Soil	Sewage sludge	Sediment	Sediment
Cell matrix	–	Aluminium oxide	Sodium sulphate	Sand
Solvent[a]	Acetone	Methanol:acetone (1:1) Water:methanol (1:1)	Methanol:acetone (1:1)	Methanol
Temperature (°C)	20, 40, 60, 80, 100	25, 50, 75, 100, 125	75	100
Cycle Time (min)				
preheat	5		5	
static	5	3, 5	5	15
No of cycles (static)	1	2–6	2	4
Pressure	–	1000, 1500, 2000	1500	1500
IS	17β-estradiol-d_2	–	Equilin-d_4	17β-estradiol-d_2
Flush volume (%)		30		60
Purge time (s)		120		
Optimal conditions	60 °C, 5 min preheat, 1 × 5 min cycle (Acetone)	75 °C, 1500 psi, 4 cycles (2 × 3 min MeOH:Ac; 2 × 3 min H_2O:MeOH)	75 °C, 1500 psi, 5 min pre-heat, 2 × 5 min cycles (methanol:acetone)	100 °C, 1500 psi, 4 × 15 min cycles (methanol)
Relative recovery (RSD) (%)				
17α-estradiol	94 (13)	110[b]	86 (12)	
17β-estradiol	100 (12)	100	81 (11)	
Estrone	103 (10)	98	79 (10)	116 (7)
Estriol	89 (12)	95	87 (13)	
17α-ethinylestradiol		93	72 (12)	
Diethylstilbestrol Progesterone		105	91 (9)	

[a]The specific solvents stated in each column is the optimised solvents for the final method. This solvent was applied to optimise all other extraction parameters listed below;
[b]Standard deviations were <10% in all samples.

fish communities and ultimately the entire ecosystem, there has been an increased demand for monitoring the presence of estrogens in waste water and surface water but also in solid samples such as soil and sediment. The fact that sewage sludge can be used as manure and consequently can be amended to agricultural soils further increase the probability that these compounds may find their way into the food chain directly or through run-off into surface waters.

In order to monitor the impacts of these compounds on the environment, efficient and reliable analytical methods for analysing estrogens in such samples are needed. Implementing PLE for these analytical methods may be a useful approach and has been attempted for different matrices by several authors [52,56,108,109] (Table 5).

Beck et al. studied the influence of extraction temperature on the extraction of estrone (E1), 17α-estradiol (α-E2), 17β-estradiol (E2), and estriol (E3) in soil using a variety of solvents [108]. The obtained results showed that acetone was the best solvent for extracting these estrogens from soil. Relative PLE recoveries for all compounds were in the range of 100%. Similar but, nevertheless, lower recoveries were obtained using ethyl acetate, whereas other solvents (including methanol) and solvent mixtures proved less efficient [108]. The optimal extraction temperature for estrogen PLE proved to be 60 °C, with relative recoveries around 90–100% for all 4 compounds. Extractions at 20, 40, 80 and 100 °C proved to be less efficient. At 80 °C high recoveries were obtained for estrone and estriol but not for EE and E2. These results on temperature optimums are somewhat in agreement with a study be Nieto et al. on sewage sludge [109]. In that study the relative recoveries of E1, E2, E3, α-E2, EE2 and diethylstilbestrol (DES—a synthetic estrogen used to prevent miscarriage and other pregnancy complications) was investigated at different temperatures, pressures and cycles. By testing PLE recoveries at 25, 50, 75, 100 and 125 °C Nieto et al. found 75 °C to be the only extraction temperature resulting in good recoveries for all 6 compounds to be in the range 90–100% [109]. A mixture of methanol/acetone (1:1) was used in order to obtain high recoveries. Extracting estrogens at 75 °C using acetone as solvent resulted in a complete loss of recovery for E1, E2, α-E2, EE2 and E3 and only a 12% recovery for DES. This result is clearly in contrast to the study by Beck et al. [108] in which acetone proved to be the most effective extraction solvent. Apart from the minor difference in temperature optimum between the two studies (60 °C vs. 75 °C), Nieto et al. used two 5 min static cycles rather than a single 5 min static cycle as Beck et al. [108,109]. However, it seems unlikely that these minor differences can explain a nearly 100% difference in estrogen recoveries between the studies when acetone was used as extraction solvent in both cases. It may be speculated that the sorption of the estrogens in the two different sample types (soil and sewage sludge) and/or the sample water content result in the differences in the efficiency so that acetone can extract estrogens from the samples.

In extracting estrogens from sediments Cespedes et al also used a mixture of methanol/acetone (1:1) for studying the PLE recoveries of E1, E2, EE2, E3, DES and progesterone (another female sex steroid) [52]. Using approximately the same PLE conditions (75 °C, 1500 psi, 2 × 5 min static cycle), Cespedes et al observed PLE recoveries similar to that of Nieto et al. [52,109], which indicates that the methanol/acetone mixture is a useful extraction solvent for wet samples.

In a recent study Dussault et al extracted EE2 from sediment using methanol as solvent [56]. The extraction temperature was 100 °C and 4 × 15 min static cycles were used. This resulted in a relative EE2 recovery (RSD) of 116(7)%. The recovery is similar to but higher than the 85% recovery for EE2 in sewage sludge obtained by Nieto et al. [109] using the same solvent at 75 °C. It is, however, in sharp contrast to the results by Beck et al. [108], who obtained recoveries for E1, α-E2, E2 and E3 below 10% using methanol as solvent. This discrepancy may partly be due to differences in the log K_{ow} value for the natural estrogens (2.81–3.94) investigated in the study by Beck et al and EE2 (4.1) [108]. It may also be a result of the difference in the ability of methanol to extract estrogens from soil and sediment. Also, Dussault et al added 10–15 g Ottawa sand in the extraction cells, whereas Beck et al added approximately 30 g soil to the extraction cells with no additional Hydromatrix® [56,108]. Unfortunately, in none of the studies [52,56,103,108] absolute PLE recoveries were reported and it is therefore not possible to assess the absolute efficiency of the PLE extractions.

Nieto et al. also studied the influence of pressure and the number of static extraction cycles on the relative PLE recovery of estrogens [109]. The results showed that 1500 and 2000 psi efficiently extracted estrogens whereas 1000 psi resulted in lower recoveries. The fact that the relative recoveries were low using 1000 psi is somewhat surprising but no suggestions as to why this comparatively low recovery was observed are presented in the paper. The highest relative recovery was found when estrogens were extracted over 4 static cycles for 2×3 min using a mixture of methanol/acetone (1:1) followed by 2×3 min cycles using water/methanol (1:1). However, the recoveries were only marginally better than other tested extraction cycle regimes [109]. With the possible exception of E3, it appears as if significantly better recoveries than the relative recoveries obtained from 2×5 min cycles using methanol/acetone (1:1) as solvent were not obtained. In the study by Beck et al a single 5 min static cycle resulted in good relative recoveries and Dussault et al. used 4×15 min static cycles for the extraction of EE2, but the results presented by Beck et al., Nieto et al. and Cespedes et al. indicate that equally good relative recoveries can be obtained with fewer cycles and extraction times [52,56,108,109].

Overall, the data presented indicate that there is some consensus regarding optimal temperature and pressure for estrogen PLE. Optimum temperature appears to be in the range 60–75 °C and the pressure should be 1500 psi. Also, the results indicate that 2–4 static cycles are suitable for PLE of estrogens. The differences between the relative recoveries for 2 vs. 4 cycles are marginal, and 2 static cycles may be sufficient in most cases. Concerning the choice of extraction solvent, information is conflicting in particular with regard to the use of pure methanol and acetone. This incongruity may be due to differences in the geochemistry and water content, i.e. polarity, between different samples, such as soil and sediment. Such properties may affect the sorption of the estrogens to organic matter and inorganic particles in the samples differently and hence extraction efficiencies. In order to establish which extraction solvents are the most suitable ones for individual sampling matrices a comparative study that investigates absolute recoveries in different matrices with different solvents is needed.

5. Conclusions

From the reviewed research papers, it is evident that several successful PLE methods for pharmaceuticals in environmental matrices have been developed during the last decade. PLE have been applied to various matrices such as soil, sludge, sediment, manure, meat, feeding stuff, baby food and egg for the extraction of antimicrobials, antiseptics, estrogens, antiepileptic drugs and antidepressants. In general, there appears to be some consensus on the PLE settings for specific pharmaceutical groups. Pressure may be less important while solvent composition and temperature are presumably the parameters that influence the extraction efficiencies to the largest extent. Extraction temperature should be well determined since this parameter affects not only the absolute amounts extracted, but also the degradation of analytes and the co-extraction of unwanted matrix components.

Although final PLE settings are stated in most studies, the individual PLE optimisation step is unfortunately often sparsely described and not evaluated. However, such steps could provide useful information as to why the final settings were chosen. Varying validation approaches, not always clearly described in the reviewed literature, have resulted in some difficulties in evaluating the different PLE methods. For instance, while relative recovery offers information about surrogate or internal standard accuracy, the absolute recovery provides an estimate on extraction efficiency of the analyte. Hence, comparison of relative and absolute recoveries of different methods is less meaningful. Moreover, matrix effects are not always investigated or compensated for and consequently such recovery values will reflect not only the extraction efficiency but also the effect of the signal suppression. In most cases, extracts are prepared in sample clean-up steps and subsequently analysed using LC–MS/MS, in which ion suppression of the analyte signal frequently occurs due to the co-elution of matrix components. Thus, the pre- and post-spike approach [87,88,93,96] when optimising and validating the PLE step and analytical method is highly recommendable. We also expect PLE methods with integrated clean-up or inverse PLE to increase in future publications.

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