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Comparison of Two Analytical Methods for the Determination of Traces of Veterinary Antibiotics and Steroid Hormones in Soil Based on Pressurised Liquid Extraction (PLE) and Quick, Easy, Cheap, Effective, Rugged, Safe (Modified-Quechers) Extraction

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Abstract

Veterinary antibiotics and steroid hormones can be present at low levels in soil. Analytical methodologies are therefore necessary to analyse these compounds at the trace level in such a complex matrix.

The goal of this work was to compare Pressurised Liquid Extraction (PLE), which is usually used to extract drugs from soil, and a modified-QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. Furthermore, several clean-up methods were evaluated. Selective Pressurised Liquid Extraction (SPLE) and Dispersive Solid Phase Extraction (dSPE) used after PLE and QuEChERS, respectively, were tested. These techniques permit a fast and simple purification step. SPE, which is frequently used, was also evaluated. To perform this comparison, both recoveries and matrix effects were compared and the analyses were performed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

SPLE and dSPE did not significantly decrease matrix effects. A tandem SPE using SAX and Strata-X cartridges offered the best efficiency. Regarding the comparison between PLE and QuEChERS, the modified-QuEChERS led to better recoveries for certain substances. No significant differences were noted in term of matrix effects. Therefore, the modified-QuEChERS method is recommended.

Keywords: Soil; Pressurised liquid extraction; QuEChERS; LC-MS/MS; Antibiotics, Steroid hormones

Introduction

Several chemical substances are increasingly used in daily life, as well as for industrial and agricultural activities. Therefore, these compounds are being dispersed in environmental compartments. For instance, many drugs and active principles are present in the environment. These pollutants are known as “emerging contaminants”. The pollutants can be defined as contaminants that have not yet been submitted to any regulation and are not necessarily persistent in the environment but are introduced continuously at low concentrations. Veterinary antibiotics and the steroid hormones are among these substances that concern the scientific community. Antibiotics are used in veterinary medicine to treat diseases but also to promote growth [1]. Steroid hormones are administered to both humans and animals for therapeutic reasons, in addition to those produced by the organism [2]. These compounds are subsequently excreted by humans and animals and pass through treatment plants where they are only partially eliminated [1,3,4]. Therefore, these substances are found in waters, sludge and manure spread on agricultural lands.

The dissemination of antibiotics and steroid hormones into the environment can have adverse effects over time. Indeed, they can induce pathogen resistance to antibiotics [1,5] and be harmful to ecosystems. As for steroid hormones, some of them are endocrine disruptors and can produce effects on growth, reproduction and other finely tuned hormonally regulated processes of species [6]. Moreover, their presence is suspected to be linked to an increase of the incidence of breast and testicular cancers in humans noted in recent years [7].

Several methods have already been established to analyse veterinary antibiotics and steroid hormones in water [8-20]. However, few procedures exist for soil [10,17,21-27], which is a more complex matrix, because of the presence of numerous interfering substances and the necessity of breaking interactions between target compounds and soil. Furthermore, the methodologies are generally set up for only one category of compounds, and few inter-families methods have been developed for the soil matrix. Nevertheless, detection and quantification with few steps and in a reasonable time of various chemical families in a sample is a necessity. Therefore, it is necessary to develop sensitive analytical methodologies to detect simultaneously traces of veterinary antibiotics and steroid hormones in soil.

When setting up such analytical procedures, the most difficult and time consuming task is the sample preparation step, which permits the specific extraction of target substances from soil. This step combines one or more extractions and purifications. Concerning the extraction, conventional techniques are based on either mixing the solid with an organic solvent or using a Soxhlet extraction procedure. More recently, other procedures, such as pressurised liquid extraction (PLE) [22, 28-37], supercritical fluid extraction (SFE) [32], ultrasound [22,30,31,38-

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40] or microwaves [31,32], have been used. In 2003, a new extraction procedure named QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) was described. It was initially established to extract pesticides from food matrices [41]. This extraction method is simple, rapid and requires low solvent consumption, which is an advantage in the era of green chemistry. This procedure is relatively new for the soil matrix, and only a few studies based on QuEChERS have been conducted previously on the extraction of compounds [42-45]. Very recently, we developed a modified-QuEChERS method for the extraction of antibiotics and steroid hormones from soil [46].

After the extraction, a purification step is often necessary. It can be conducted with solid phase extraction (SPE) [22,31,47,48], liquid-liquid extraction (LLE) [47], gel permeation chromatography (GPC) [47] or semi-preparative liquid chromatography [47]. SPE is the most often used procedure, as it is fast, efficient and a large variety of purification sorbents is available. Moreover, it requires a low quantity of solvent and presents low risk of sample contamination. Recently, a purification technique, selective pressurised liquid extraction (SPLE), was developed. The technique involves the addition of a purification phase into the PLE cell, which thus avoids the co-extraction of interfering substances. The SPLE method was previously developed to extract PCB (polychlorobiphenyl) from sludge and organochlorine pesticides [49] and oestrogens from soil [50]. This method is rarely used for antibiotics. One application is the extraction of pharmaceuticals from a food matrix [34]. Furthermore, to the best of our knowledge, no multi-family methods using SPLE clean-up have been developed to extract both veterinary antibiotics and steroid hormones from soil.

In this context, the goals of the work described in this paper were (i) to develop a method based on PLE to extract both veterinary antibiotics and steroid hormones from soil, (ii) to compare the method with the modified-QuEChERS extraction method we previously developed and validated [46] and (iii) to evaluate SPLE, dSPE and SPE for the purification step. For these purposes, both recoveries and matrix effects were compared. The compound separation and detection were achieved with liquid chromatography-tandem mass spectrometry (LC-MS/MS), which permits both sensitive and selective analyses.

To perform this comparison, 11 natural or synthetic steroid hormones were chosen. These are the natural androgens androstenedione and testosterone; the natural progestagen progesterone and the synthetic progestagens norethindrone, gestodene and levonorgestrel; the natural oestrogens oestriol, oestrone, 17α-oestradiol, 17β-oestradiol and the synthetic oestrogen 17α-ethinyloestradiol. As target veterinary compounds, the sulphonamides sulphanilamide, sulphadiazine, sulphathiazole, sulphameter, trimethoprim, sulphadimidine, sulphabenzamide, sulphadimethoxine; the macrolides erythromycin, tylosin and roxithromycin; the β-lactam penicillin G; the antiparasitic dicyclanil and the phenicol florfenicol were chosen. Finally, six compounds that we considered as markers of human contamination were also included: sulphamethoxazole, carbamazepine, fluvoxamine, paracetamol, ibuprofen and bisphenol A.

Experimental

Chemicals and materials

Vetranal (V) or pestanal (P) analytical standards were used in addition to others that meet United States Pharmacopeia testing specifications (USP). Sulphanilamide (V), sulphadiazine (V), sulphathiazole (99%), sulphameter (V), trimethoprim (V), sulphadimidine (V), sulphabenzamide, sulphadimethoxine, erythromycin (USP), tylosin tartrate (V), roxithromycin (>90%), penicillin G potassium salt (V), dicyclanil (V), florfenicol, androstenedione (V), testosterone (V), progesterone (>99%), norethindrone (V), levonorgestrel (99%), oestriol (V), oestrone (>99%), 17α-oestradiol (V), 17β-oestradiol (> 98%), 17α-ethinyloestradiol (V), sulphamethoxazole, carbamazepine (USP), fluvoxamine, paracetamol (99%), ibuprofen (V) and bisphenol A (99%) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). The steroid gestodene (98%) was purchased from AK Scientific (California, USA).

Individual stock solutions were prepared at concentrations of 200 mg/L in methanol and stored at -23°C. Working solutions were prepared by the appropriate mixture and dilution of the stock solutions.

Methanol (MeOH), acetonitrile (ACN) (LC-MS grade) and acetone (HPLC grade) were purchased from Sigma-Aldrich. Pure water was obtained from a MilliQ device from Millipore (Saint-Quentin-en-Yvelines, France). Formic acid (98%, LC-MS grade), ammonium hydroxide solution (NH3, aq; 25% in water) and citric acid monohydrate were purchased from Sigma-Aldrich. Phosphoric acid (min 85%) and sodium hydroxide were obtained from Prolabo (Paris, France). Acetic acid and anhydrous sodium salt were acquired from Arcos (Geel, Belgium).

For PLE, diatomaceous earth from Sartorius (Germany) was purchased.

QuEChERS extract tubes (AOAC method) were obtained from Agilent Technologies (Massy, France). They contain 1.5 g of NaOAc and 6 g of $MgSO_4$.

The florisil phase was purchased from SDS (Peypin, France). The aluminium oxide phase was obtained from Sigma–Aldrich, and SampliQ Silica SAX SPE was purchased from Agilent Technologies. The Bondapak HC18 (Waters, Saint Quentin Yvelines, France) sorbent was also tested.

Solid-phase extractions were performed using Agilent Technologies SAX (Strong Anion eXchange) (500 mg, 3 mL) cartridges or Phenomenex (Le Pecq, France) Strata-X (200 mg, 3 mL) cartridges.

Soil sample

For the various experiments, a clay-loam soil that was not treated with manure or sludge was used. It contained 32.4% clay, 45.1% loam, 22.5% sand and 2.99% organic matter.

The soil sample was passed through a 3-mm sieve to remove coarse particles. It was subsequently ground in a mortar and passed through a 0.63 mm sieve to obtain a homogeneous sample.

For both PLE and QuEChERS procedures, 5 g of soil was spiked at 50 ng/g with a MeOH solution containing the 31 substances. The sample was then left either 7 hours at room temperature (PLE) or one night under a nitrogen stream (modified-QuEChERS) before the extraction.

Pressurised Liquid Extraction (PLE)

PLE experiments were performed with Thermo Scientific™ Dionex™ ASE 200 Accelerated Solvent Extractor. An 11-mL PLE cell was filled with 5 g of soil and diatomaceous earth, and cellulose filters were put at the two cell extremities (Figure 1A and1B).

The extraction was performed with the following conditions: temperature of 80°C, pressure of 120 bars, 10 min of static time and 2 cycles. MeOH was chosen as the extraction solvent. The extract was

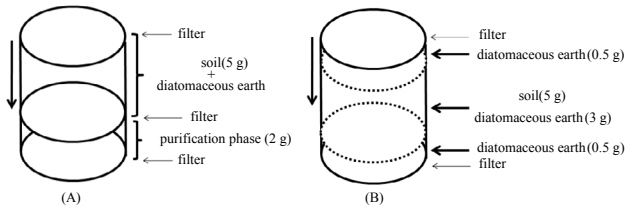

Figure 1: PLE (A) and SPLE (B) cell filling.

subsequently evaporated to dryness under vacuum at a temperature of 40°C (miVac, Genevac LTD, Ipswich, England).

QuEChERS extraction

Ten millilitres of MilliQ water and 15 mL of ACN were added to the tube that contained the spiked soil. The tube was shaken with a vortex device (Vortex Fischer Scientific FB15013 TopMix). The AOC acetate buffer was then added, and the tube was immediately manually shaken for 30 s and swirled on a vortex mixer for 30 s. The tube was then shaken for 3 min at 750 rpm in a sample homogeniser (SPEX Sample Prep, 2010 GenoGrinder, Delta Labo, Avignon, France). After centrifugation at 5,000 rpm for 2 min (Sigma Laboratory Centrifuges 3K30H, Fisher Bioblock Scientific), 10 mL of the ACN layer was transferred into a 12 mL glass tube. The extract was then evaporated to dryness under a gentle stream of nitrogen at a temperature of 40°C.

Solid Phase Extraction (SPE) clean-up

After PLE or QuEChERS extraction, a clean-up was performed using SPE (Rapid Trace SPE Workstation, Caliper), and the clean-up involved two steps. First, after pre-conditioning of the SAX cartridge with MeOH and 0.04 M citric acid, the extract (dissolved in 97/3 citric acid 0.04 M/MeOH) was passed through the cartridge at a flow rate of 1 mL/min and recovered in a tube. Second, a Strata-X cartridge was also conditioned with both MeOH and 0.04 M citric acid, and the previously recovered extract was passed through the cartridge at 1 mL/min. The Strata-X cartridge was subsequently washed with 2 mL of 0.04 M citric acid and 2 mL of NaOAc 0.1 M. The cartridge was then dried for 30 min under a stream of nitrogen and eluted with 10 mL of MeOH at a flow rate of 1 mL/min. Finally, the recovered extract was evaporated to dryness under a gentle stream of nitrogen at a temperature of 40°C.

The dry residue was dissolved in 200 µL of 95/5 H_2O/MeOH and mixed for 20 s prior to LC-MS/MS analysis. All of the extractions were performed in duplicate.

Analysis by liquid chromatography-tandem mass spectrometry

A HP1100 chromatographic system (Agilent Technologies) equipped with a degasser, a binary pump, an autosampler and a column oven was used. The LC system was coupled to a triple-stage quadrupole mass spectrometer 3200 QTrap (ABSciex, Les Ulis, France) with an electrospray ion (ESI) source (TurboV, ABSciex). The analytes were identified by both their chromatographic characteristics and their specific multiple reaction monitoring (MRM) fragmentation patterns. The chromatographic conditions as well as the parameters used for the detection have been previously described [46]. Figures 2A and 2B display chromatograms showing the separation of the 23 positively and 8 negatively ionised compounds, respectively. Data processing was performed with Analyst software (version 1.5.1).

Data analysis

To evaluate recoveries, the signal obtained for soil samples spiked at 50 ng/g (Sspiked) was compared with the signal obtained for soil extracts spiked after the sample preparation with a MeOH solution containing the 31 substances at the same concentration (Sref), where Sblank corresponds to the signal of a non-spiked extract of soil:

Matrix effects were determined by comparison of the signal (Sref) with the signal of the standards in a solvent (Ssolvent) at the same concentration. The matrix effect was determined with the following equation:

Results and Discussion

Development of the PLE

PLE: During the development of a method based on PLE, several parameters had to be optimised such as the temperature, the pressure, the static time and the number of cycles, as well as the nature of the extraction solvent. The solvent is the parameter that most affects the extraction efficiency [51]. Therefore, the temperature, the pressure, the static time and the number of cycles were chosen based on literature data and to optimise the extraction solvent.

Several studies have been dedicated to the extraction of antibiotics or steroid hormones from in soil using PLE.

For veterinary antibiotics, relatively low temperatures are typically used (between 70°C and 80°C) because of the thermal degradation of some substances [28,37]. Pressures between 100 and 140 bars are usually mentioned [28,37] as well as static times between 5 and 10 minutes [37]. Finally, authors often recommend 2 or 3 cycles [28,37].

For steroid hormones, temperatures above 100°C (usually 120°C) [52], pressures between 100 and 140 bars [52,53], a static time of 10 minutes [52] as well as 2 cycles [53] are often mentioned.

Considering all these data, the following parameters were fixed: temperature of 80°C, pressure of 120 bars, 10 min of static time and 2 cycles.

With these conditions, the extraction solvent was chosen. As the studied compounds exhibit different physical/chemical properties, compromises were necessary. The solvents usually mentioned in the literature to extract veterinary antibiotics from soil are MeOH, acetone, acetone/MeOH (50/50) mixtures, as well as MeOH and ACN mixed with aqueous solutions [28,29,31,37]. Citric acid- or phosphoric acid-based solvents are also employed to enhance the extraction of antibiotics from soil when PLE is used. The citric acid permits limiting the complexation of target analytes with cations (Ca^{2+}, Mg^{2+}) as well as the cation exchange process, which makes it easier to extract the target compounds [29,54]. Citric acid is preferred to EDTA, which can precipitate in the apparatus.

For steroid hormones, solvents such as MeOH, acetone and acetone/MeOH (50/50) mixture are the most often mentioned [52,53].

Finally, the following solvents were evaluated: acetone, MeOH, acetone/MeOH (50/50), MeOH/ACN/0.2 M citric acid (40/40/20) adjusted to pH=4 (with either sodium hydroxide or ammonium hydroxide) [29] and MeOH/ACN/aqueous solution of phosphoric acid (40/40/20) + 1 mM citric acid. For the latter solvent, different percentages of phosphoric acid in aqueous solution were tested: 0.10% (pH=3.1), 0.35% (pH=2.8) and 0.875% (pH=2.7). In all cases, after the

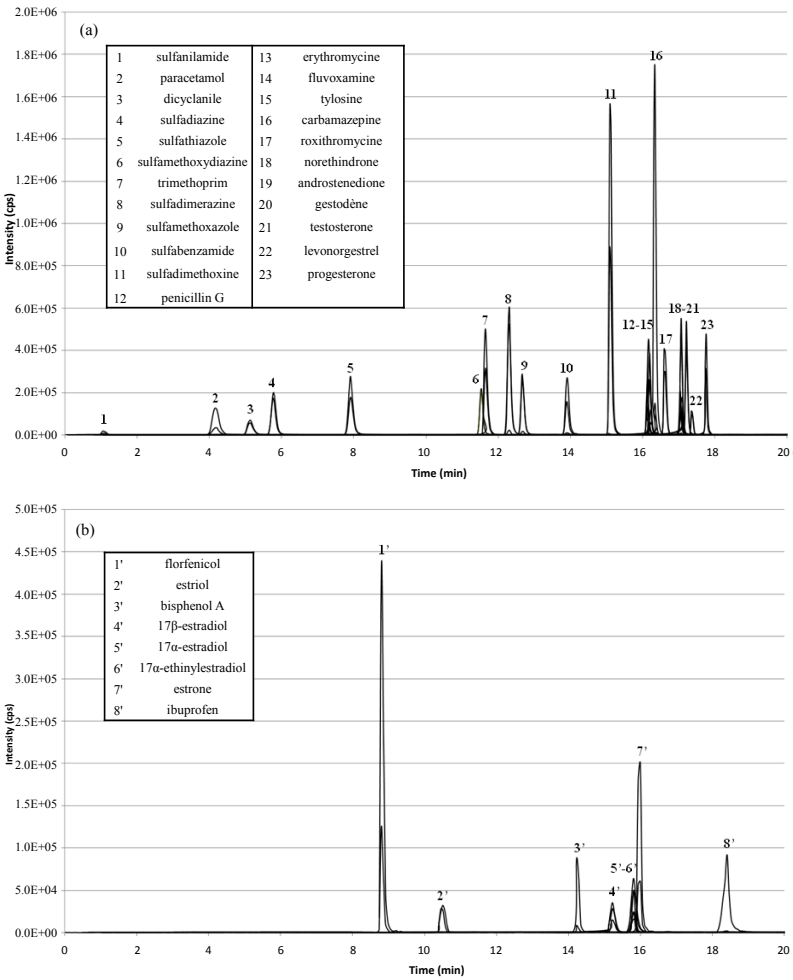

Figure 2: Chromatograms showing the separation of the (a) 23 positively ionised compounds and (b) 8 negatively ionised compounds.

PLE step, the extract was evaporated to dryness and dissolved in 1.5 mL of MilliQ water before the injection in LC-MS/MS.

The extraction solvent choices were evaluated for both recoveries and matrix effects. The recovery values are compiled in the table 1.

As shown in this table, acetone was not useful for extracting antibiotics and human drugs from the soil samples. Indeed, recoveries were lower than 50% for most of the sulphonamides, lower than 10% for macrolides and penicillin G was not detected. For human drugs, recoveries were lower than 5% for fluvoxamine and ibuprofen. Furthermore, only 40% of paracetamol was extracted from the matrix with this solvent. Acetone was only suitable for steroid hormones, as recoveries between 70% and 80% were obtained for androgens and progestagens. As for oestrogens, they exhibited recoveries between 60% and 80%.

The MeOH/ACN/0.2 M citric acid (40/40/20) mixture adjusted to pH=4 was also not suitable. Indeed, this mixture did not display a significant increase of recoveries compared to the other solvents tested. Moreover, this solvent led to important matrix effects: between -70% and -90% for most of the antibiotics and between -60% and -85% for steroid hormones. This could perhaps be explained by the presence of citric acid, which most likely favours the extraction of interfering substances.

Concerning phosphoric acid-based mixtures, an increase of the matrix effects was noted as the acid percentage increased. As for citric acid, phosphoric acid may induce a strong extraction of interfering compounds. As good recoveries were obtained with the mixture containing only 0.10% phosphoric acid while limiting matrix effects, the MeOH/ACN/0.10% phosphoric acid (40/40/20) + 1 mM citric acid (pH=3.1) mixture was selected for further tests (mixture further designated 0.10% PA).

Thus, 0.10% PA, an acetone/MeOH (50/50) mixture and MeOH were compared. The obtained recoveries are displayed in table 1. The results indicate that 0.10% PA permitted to obtain good recoveries for veterinary antibiotics. Indeed, dicyclanil, as well as trimethoprim, had their recoveries clearly improved with this solvent. Sulphonamide and florfenicol recoveries did not vary significantly with the nature of the solvent. Most of them were included between 80% and 100%. Macrolide recoveries were 1.5- to 2-fold higher with the 0.10% PA solvent compared with the two others. It was also the case for penicillin G, which was not extracted a lot with the acetone/MeOH (50/50) mixture and MeOH.

On the other hand, the phosphoric acid-based solvent led to a huge decrease of paracetamol and bisphenol A recoveries. However,

Compounds	Acetone	MeOH	Acetone/ MeOH 50/50	MeOH/ ACN/citric acid (pH=4)	MeOH/ACN/ phosphoric acid (pH=3.1)
ANTIBIOTICS					
sulfanilamide	46 ± 1.6	84 ± 4.4	101 ± 20.7	70 ± 1.8	73 ± 12.1
sulfadiazine	18 ± 0.2	91 ± 2.1	90 ± 5.0	106 ± 0.6	89 ± 1.2
sulfathiazole	19 ± 0.3	78 ± 2.2	96 ± 4.6	110 ± 1.21	82 ± 0.7
sulfameter	37 ± 1.3	91 ± 4.3	100 ± 8.2	109 ± 0.3	92 ± 4.1
trimethoprim	9 ± 0.1	15 ± 0.8	23 ± 2.0	79 ± 2.4	82 ± 1.3
sulfadimidine	64 ± 0.3	90 ± 2.3	94 ± 2.8	109 ± 1.1	89 ± 0.5
sulfabenzamide	17 ± 0.3	75 ± 1.0	87 ± 8.3	110 ± 3.3	99 ± 2.0
sulfadimethoxine	57 ± 1.5	88 ± 2.4	99 ± 6.8	110 ± 8.7	94 ± 3.5
erythromycin	4 ± 0.2	16 ± 2.0	16 ± 0.6	40 ± 18.8	33 ± 2.1
tylosin	7 ± 0.2	42 ± 2.6	65 ± 7.6	95 ± 8.5	86 ± 2.9
roxithromycin	7 ± 0.4	64 ± 2.0	48 ± 2.3	100 ± 3.8	97 ± 2.5
penicillin G	nd	6 ± 1.4	14 ± 0.1	82 ± 1.23	75 ± 0.3
dicyclanil	60 ± 0.1	62 ± 3.8	63 ± 3.0	82 ± 1.9	102 ± 9.3
florfenicol	83 ± 5.0	91 ± 6.7	105 ± 6.0	109 ± 1.1	96 ± 6.3
STEROID HORMONES					
androstenedione	75 ± 3.0	92 ± 7.2	96 ± 8.1	97 ± 0.7	99 ± 5.6
testosterone	76 ± 1.5	112 ± 2.8	104 ± 18.9	100 ± 3.8	101 ± 4.2
progesterone	77 ± 1.0	135 ± 3.5	117 ± 22.9	91 ± 2.1	108 ± 13.7
norethindrone	81 ± 0.8	102 ± 0.9	109 ± 1.4	96 ± 5.8	100 ± 4.2
gestodene	69 ± 11.7	108 ± 14.1	96 ± 16.2	100 ± 2.5	101 ± 13.5
levonorgestrel	74 ± 2.6	98 ± 1.6	112 ± 5.4	99 ± 2.7	113 ± 20.8
estriol	59 ± 4.2	28 ± 1.9	33 ± 3.0	20 ± 1.8	8 ± 2.4
estrone	78 ± 3.7	89 ± 3.4	80 ± 3.6	60 ± 2.2	62 ± 3.5
17α-estradiol	75 ± 1.7	69 ± 2.9	73 ± 1.0	50 ± 2.0	47 ± 2.0
17β-estradiol	75 ± 1.2	90 ± 3.7	78 ± 5.2	50 ± 3.3	36 ± 4.4
17α-ethinylestradiol	69 ± 11.0	81 ± 1.3	70 ± 0.1	60 ± 1.8	64 ± 0.7
HUMAN CONTAMINANTS					
sulfamethoxazole	48 ± 0.3	99 ± 3.2	102 ± 0.8	110 ± 2.2	100 ± 3.2
carbamazepine	90 ± 0.5	99 ± 1.9	97 ± 1.7	96 ± 1.6	101 ± 3.2
fluvoxamine	3 ± 0.1	38 ± 2.0	nd	60 ± 1.1	60 ± 18.9
paracetamol	40 ± 0.1	18 ± 0.4	17 ± 1.3	8 ± 0.2	4 ± 0.4
ibuprofen	1 ± 0.16	43 ± 7.3	68 ± 7.9	97 ± 7.0	98 ± 7.7
bisphenol A	100 ± 7.1	77 ± 5.7	70 ± 5.0	42 ± 1.7	43 ± 5.4

Table 1: PLE recoveries (%) for the 31 studied compounds and for the following solvents: acetone, MeOH, acetone/MeOH (50/50), MeOH/ACN/citric acid (pH=4) and MeOH/ACN/phosphoric acid + citric acid (pH=3.1).

it allowed a good extraction of ibuprofen. As for carbamazepine, this compound was not affected by the solvent nature.

Finally, androgen and progestagen recoveries were not influenced by the solvent nature (included between 90% and 110%). Oestrogens recoveries were clearly lower with the 0.10% PA mixture.

In this multi-residues and multi-families method, a compromise had to be made. As fluvoxamine may be slightly extracted only with MeOH, and most of the oestrogens (target compounds with proved endocrine disruptive properties) displayed a slight increase of their recoveries with this solvent, and MeOH was finally selected as the extraction solvent.

Selective Pressurised Liquid Extraction (SPLE) clean-up: A SPLE clean-up corresponding to the incorporation of 2 g of purification phase at the bottom of the PLE cell was considered (Figure 1A). Experiments were conducted with or without adding the phase to evaluate the sorbent efficiency.

Different SPLE tests were performed. Phase/solvent combinations were established to associate a solvent with the phase the most adapted (according to the polarity, pH) to have more chance of decreased matrix effects.

For the acetone/MeOH (50/50) mixture, florisil, silica and aluminium oxide were evaluated. They have already been used to extract oestrogens from soil with an acetone-based solvent [50]. The effect of C18 was evaluated with both MeOH and the 0.10% PA mixture. The C18 phase could retain apolar interferents not brought by the polar solvents. The phosphoric acid-based solvent was adjusted to pH=4 to perform an experiment with the SAX phase. Indeed, at this pH, the carboxylic acids functions of humic and fulvic acids are partially negatively ionised, and therefore, these acids could be retained by the SAX sorbent. In all cases, the final extract was evaporated to dryness and dissolved in 1.5 mL of MilliQ water before the injection in LC-MS/MS.

Concerning acetone/MeOH (50/50) mixture, the results (Figure 3) indicate that florisil did not permit to decrease clearly matrix effects: they were still important for androgens, progestagens and oestrogens. They were only decreased slightly for penicillin G, sulphamethoxazole, carbamazepine, paracetamol and oestriol. With the silica sorbent, the same phenomenon was observed. Matrix effects were even amplified for sulphonamides and ibuprofen. Finally, the use of the aluminium oxide phase led to an increase of matrix effects of some compounds such as sulphonamides and oestrogens. Furthermore, they were not decreased for other compounds, such as progestagens and androgens.

As for the MeOH/C18 combination, the C18 phase did not permit decreased matrix effects. Those obtained for sulphonamides were even increased (Figure 4).

For the 0.10% PA based solvent not adjusted to a specific pH, the C18 phase did not allow reducing clearly matrix effects (Figure 5). Furthermore, the SAX phase used with the phosphoric acid-based solvent adjusted to pH=4 induced an increase of most of the sulphonamide matrix effects. Furthermore, no reduction was observed for the other substances. At the pH values studied, the matrix interferents most likely exhibit more affinity for the solvent than the purification sorbent.

When sorbents were used, matrix effects were sometimes enhanced. It could be due to the extraction of a part of the purification phase, which could interfere with target analytes in the electrospray interface.

Finally, matrix effects differences between the solvents (without purification phase) were also examined (Figure 3 and 5). No obvious differences were observed. MeOH displayed matrix effects slightly lower for some compounds (veterinary antibiotics: dicyclanil, sulphonamides) compared with the other solvents.

In conclusion, no purification phase led to a significant decrease of matrix effects. Moreover, it was observed that recoveries were reduced with the purification sorbents. Considering all these results, the SPLE method was not adapted to the soil.

PLE/QuEChERS comparison

The goal of this part was to compare the optimised PLE method with a modified-QuEChERS extraction that had been previously developed and validated [46]. Both the PLE and QuEChERS technique developments permitted setting of the parameters, particularly the nature of the extraction solvent. Thus, MeOH was retained for PLE, whereas ACN was considered as the most adapted solvent for the modified-QuEChERS extraction. However, the rapid and simple purification methods SPLE and dSPE for PLE and QuEChERS,

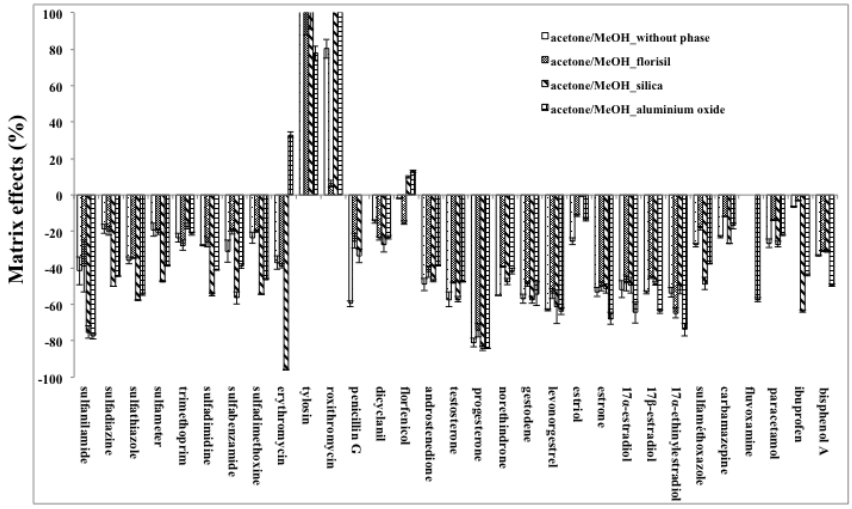

Figure 3 : Matrix effects (%) of the 31 studied compounds during the tests performed with an acetone/MeOH (50/50) mixture.

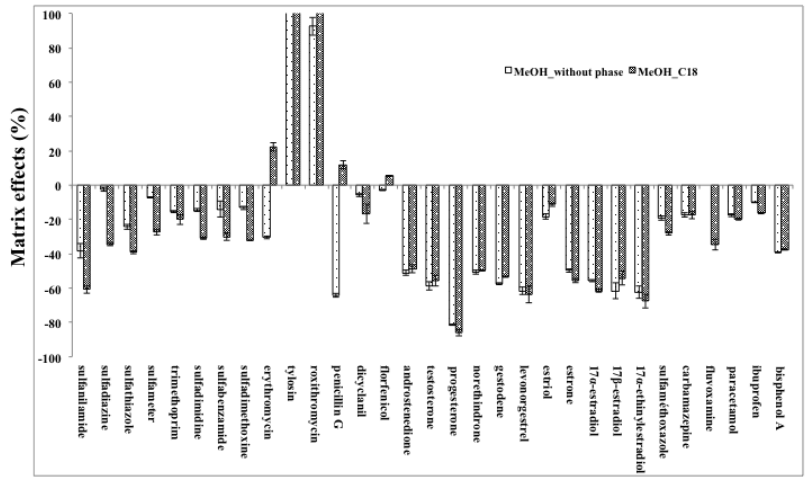

Figure 4: Matrix effects (%) of the 31 studied compounds during the tests performed with MeOH.

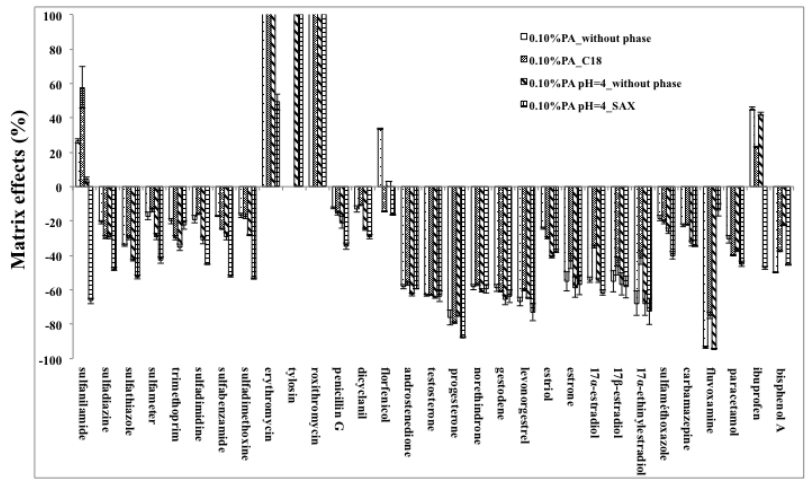

Figure 5: Matrix effects (%) of the 31 studied compounds during the tests performed with 0.10% PA-based solvent.

respectively, were not efficient in the reduction of matrix effects. It was therefore necessary to use a purification step with SPE cartridges.

A method consisting of tandem SPE clean-up using both SAX and Strata-X cartridges was optimised [46]. Good recoveries were obtained (between 60 and 90% for most of the compounds). Furthermore, the substances exhibited reasonable matrix effects: lower than -40% for most of the analytes and between -20 and -60% for steroid hormones (most of them were lower than -50%). This SAX+Strata-X purification was applied to the extracts obtained by both PLE and QuEChERS. The efficiencies of the two whole protocols were compared. Their recoveries are displayed in Figure 6. In the case of the sulphonamides, androgens, progestagens and oestrogens, florfenicol and some human contaminants such as carbamazepine, ibuprofen, bisphenol A, the recoveries obtained with both PLE and modified-QuEChERS were comparable. However, some substances displayed better recoveries with modified-QuEChERS extraction: trimethoprim, macrolides (tylosin and roxithromycin), penicillin G, dicyclanil, oestriol, fluvoxamine and paracetamol. This observation can be explained by the fact that PLE requires a high temperature. A part of the compounds would is perhaps degraded when PLE is used, which could explain the lowest recoveries being found with this technique. Indeed, the degradation of veterinary antibiotics as well, as oestriol, when extraction is performed at high temperature has been previously mentioned in the literature [55,56]. In short, modified-QuEChERS produced the best results in term of recoveries.

Matrix effects were also compared (Figure 7). For most of the compounds, matrix effects were comparable with the two extraction methods. Nevertheless, it was observed that when PLE was employed, matrix effects were slightly lower for some sulphonamides and slightly higher for steroid hormones. In conclusion, the PLE and modified-QuEChERS methods are equivalent in terms of matrix effects. Taking into account both recoveries and matrix effect results, the modified-QuEChERS method is recommended.

Conclusions

It was demonstrated that as dSPE for the QuEChERS extraction, SPLE allowing a simple and rapid clean-up was not adapted to the purification of soil extracts containing veterinary antibiotics and steroid hormones. Therefore, a tandem SPE using both SAX and Strata-X cartridges was employed.

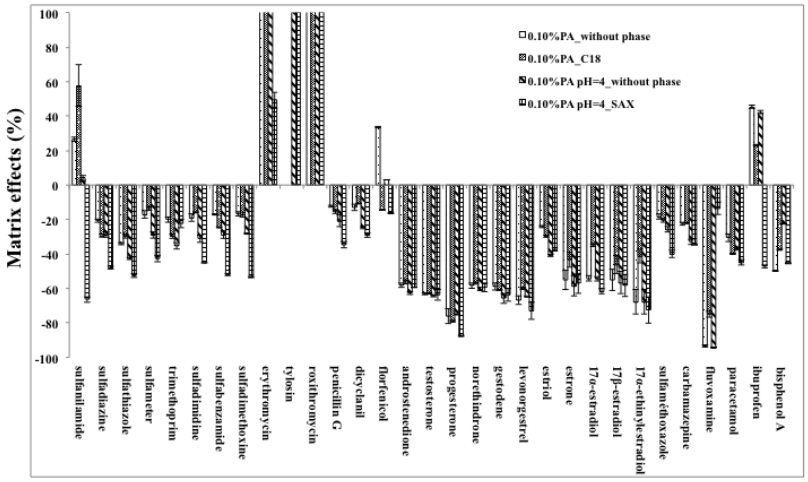

Figure 6: Recoveries (%) for PLE and QuEChERS extractions followed by the SAX+Strata-X purification for the 31 studied compounds.

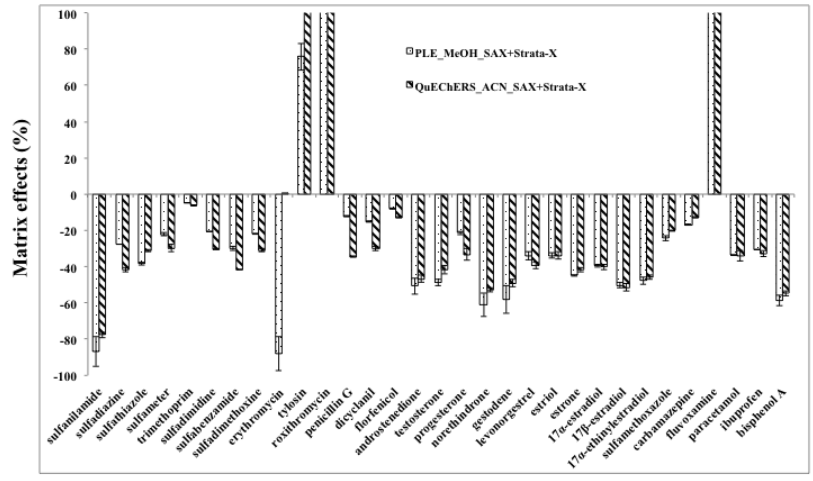

Figure 7: Matrix effects (%) for PLE and QuEChERS extractions followed by the SAX+Strata-X purification for the 31 studied compounds.

The PLE/QuEChERS comparison revealed that the modified-QuEChERS extraction is the most adapted procedure for the examined type of multi-residues and multi-families analysis. Indeed, this technique permitted obtaining of higher recoveries for some substances compared with PLE. Moreover, this method is easier to set up, more rapid and cheaper than PLE, which requires a substantial investment. Furthermore, the PLE cell preparation is time consuming compared with the QuEChERS methodology. In this work, it was demonstrated that in certain cases, the QuEChERS method, which is relatively innovative for soil matrix analysis, is superior to the standard PLE method.

Furthermore, setting up a modified-QuEChERS extraction will allow the analysis of a large number of soil samples rapidly. This method will facilitate large-scale screening of soils.

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