Determination of multi-class emerging contaminants in sludge and recovery materials from waste water treatment plants: development of a modified QuEChERS method coupled to LC-MS/MS

Barbara Benedetti , Mauro Majone , Chiara Cavaliere , Carmela Maria Montone , Francesco Fatone , Nicola Frison , Aldo Laganà , Anna Laura Capriotti

PII: S0026-265X(19)33592-1
DOI: https://doi.org/10.1016/j.microc.2020.104732
Reference: MICROC 104732

To appear in: *Microchemical Journal*

Received date: 17 December 2019
Revised date: 10 February 2020
Accepted date: 10 February 2020

Please cite this article as: Barbara Benedetti , Mauro Majone , Chiara Cavaliere , Carmela Maria Montone , Francesco Fatone , Nicola Frison , Aldo Laganà , Anna Laura Capriotti , Determination of multi-class emerging contaminants in sludge and recovery materials from waste water treatment plants: development of a modified QuEChERS method coupled to LC-MS/MS, *Microchemical Journal* (2020), doi: https://doi.org/10.1016/j.microc.2020.104732

Highlights

- -A QuEChERS-HPLC-MS method for selected emerging contaminants in sludges was developed
- Optimization of the QuEChERS protocol led to quantitative recoveries from complex samples
- The method was successfully applied to innovative recovery materials from wastewaster treatment plants
- Knowledge on contamination put the basis for a risk assessment in the use of recovery materials

Determination of multi-class emerging contaminants in sludge and recovery materials from waste water treatment plants: development of a modified QuEChERS method coupled to LC-MS/MS

Barbara Benedetti[a], Mauro Majone[a], Chiara Cavaliere[a]*, Carmela Maria Montone[a], Francesco Fatone[b], Nicola Frison[c], Aldo Laganà[a,d], Anna Laura Capriotti[a]

[a] *Department of Chemistry, Università di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy*

[b] *Department of Science and Engineering of Materials, Environment and Urban Planning-SIMAU, Marche Polytechnic University, Via Brecce Bianche 12, 60131 Ancona, Italy*

[c] *Department of Biotechnology, University of Verona, Cà Vignal 1, Strada Le Grazie 15, 37134 Verona, Italy*

[d] *CNR NANOTEC, Campus Ecotekne, University of Salento, Via Monteroni, 73100 Lecce, Italy*

***Corresponding author**

Address: Department of Chemistry, Università di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185, Rome, Italy

E-mail: chiara.cavaliere@uniroma1.it

tel: +39 06 4991 3062

Abstract

Recycling and recovering valuable resources from wastewater treatment plants is an important aspect in circular economy. The safe use of sludge and sludge-related products deriving from wastewater treatment strictly depends on their chemical contamination, especially by emerging pollutants. In this work, an analytical method was developed for the determination of a range of selected compounds, included in a recent European watch-list (macrolides, fluoroquinolones, neonicotinoids, carbamates and estrogens), in recovery materials from innovative pilot systems. Both the instrumental analysis by high-performance liquid chromatography-tandem mass spectrometry and the pre-treatment strategy (quick, easy, cheap, effective, rugged and safe technique) were optimized for the purpose. The final method performance were evaluated, revealing determination coefficients (R2) of 0.993-0.9999 for the matrix-matched calibration curves, good accuracy (recovery 68-104% and matrix effect 70-123%), satisfactory precision (relative standard deviation <20%) and limits of detection and quantitation in the low ng g^{-1} levels. Ten different recovery material samples were analysed, showing contamination by few analytes, mainly antibiotics and estrone; ciprofloxacin and azithromycin were the most abundant compounds (up to 500-600 ng g^{-1}). On the contrary, neonicotinoid pesticides were not detected, except for one sample (sample 10, the only compost material). The application of the described method is an essential part of a broader investigation on the suitability and safety of innovative materials coming from waste water treatment plants, in the view of a risk assessment related to their usage.

Keywords

Sewage sludge; emerging contaminants; wastewater treatment plant; analytical method optimization.

Abbreviations

ACET-d, acetamiprid N-desmethyl; AZY, azithromycin; CEC, contaminants of emerging concern; CLARI, clarithromycin; CIPRO, ciprofloxacin; CLOTH, clothianidin; E1, estrone; E2, 17-β-estradiol; EE2, 17-α-ethinyl estradiol; ERY, erythromycin; IMI, imidacloprid; METH-s, methiocarb sulfone; PSA, primary secondary ammine; QuEChERS, quick, easy, effective, rugged and safe; SMART-Plant, Scale-up of low-

carbon footprint Material Recovery Techniques; THIAC, thiacloprid; THIAM, thiamethoxam; WWTPs, waste water treatment plants.

1. Introduction

In the latest years, in view of circular economy, the recovery of different resources from wastewater treatment plants (WWTPs) is raising increasing interest [1]. During wastewater remediation, solid matter is obtained mainly by filtration of influent waters (primary treatment) and secondary treatments, which produce sewage sludges [2]. The possible fates of the solid materials deriving from these processes include land application, composting, landfilling, anaerobic digestion and combustion [3]. Among them, the treatments which create the opportunity of re-using sludge and generating new resources take on crucial importance, bringing doubtless environmental and economical advantages [4].

In this framework, the European project SMART-Plant (Scale-up of low-carbon footprint MAterial Recovery Techniques in existing wastewater treatment Plants) deals with several resource recovery approaches in wastewater management [5]. A range of processes applied during the different stages of wastewater treatment were developed and scaled up to real pilot systems, integrating them in existing WWTPs. As a result, several products of relevant interest in the field of circular economy, called SMART products, were generated, e.g. cellulose, nutrients-rich sludges, biopolymers and biocomposites [6]. No regulations could be directly applied to the use of these innovative products, but environmental issues are related to sludge re-use, especially as fertilizer; indeed, this semi-solid matrix could be contaminated by a wide range of chemicals, including heavy metals and organic pollutants [7]. European legislation only poses limits to the heavy metal content [8]; nevertheless, recent papers reported sludge contamination by numerous organic substances, including contaminants of emerging concern (CEC) [9,10]. This class deserves particular attention, since CEC removal or degradation in WWTPs is not always effective, with consequent discharge in the environment by effluent waters and excess sludge [11,12]. The monitoring of CEC levels is not officially regulated; nevertheless, the European Union (EU) proposed some watch-lists of selected contaminants in waters in the latest years. The most recent document concerns the draft of a list of 15 substances which may pose a significant risk to or via aquatic environment, although monitoring data are insufficient to evaluate the actual hazard [13]. This list includes antibiotics, pesticides, mainly belonging to the neonicotinoid class, and estrogens. The determination

of these chemicals in sewage sludge becomes essential to provide a picture of their distribution in the environmental compartment, after wastewater treatments, as well as to estimate the risk associated to the usage of sludge-deriving materials. A limited number of papers reported the simultaneous analysis of these contaminants in sludge samples. Antibiotics and estrogens are determined more frequently than neonicotinoids [14–16] and, often, few compounds from the list are incorporated in large multi-residual methods, which deals with endocrine disruptors, pharmaceuticals or pesticides [16–19]. The sample treatment of sludge and similar matrices usually involves different extraction and clean-up steps, including solid-liquid extraction, pressurized liquid extraction and solid phase extraction [18,20,21]. Among these procedures, the quick, easy, effective, rugged and safe (QuEChERS) method, introduced in 2003 [22], has become popular for its versatility and easy application to diverse solid matrices. Several variables are involved in this technique, whose optimization is strictly dependent on the kind of sample and the selected chemicals.

From these premises, we developed a simple and accurate method for the multi-class screening of selected contaminants belonging to the 2018 EU watch-list, as well as two pesticide metabolites, in recovery resources coming from innovative pilot systems in wastewater treatment plant. Since sludge and sludge-deriving samples are rather complex and heterogenous matrices and chemicals belonging to different classes were involved, the analytical method was carefully optimized. A QuEChERS procedure was applied for the extraction and purification of the analytes, after performing different tests to find the optimal conditions. Instrumental determination was accomplished by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS), to achieve high sensitivity and specificity. For the first time, a method specifically suited for the EU watch-list compounds was developed for the application to sludge samples and innovative recovery materials from WWTPs.

2. Materials and methods

2.1 Chemicals and Materials

Chromatographic grade water and acetonitrile (ACN) were obtained from VWR International (Milan, Italy), whereas methanol (MeOH) of the same purity was provided by Merck (Darmstadt, Germany). Magnesium sulfate (purity 97%, anhydrous) was purchased from Carlo Erba Reagenti (Rodano, MI, Italy). Sodium chloride (purity ≥99%), formic acid (ACS reagent, ≥98%), ammonium hydroxide solution (ACS reagent,

28-30% NH_3 basis), and ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2EDTA, ACS reagent, purity ≥99%) were from Sigma Aldrich (St. Louis, MO, USA). End-capped C18 bonded silica loose sorbent and Primary Secondary Ammine (PSA) loose sorbent were purchased from Phenomenex (Torrance, CA, USA). The analytical standards erythromycin (ERY, purity ≥96%), clarithromycin (CLARI, purity ≥98%), azithromycin (AZY, purity ≥95%), ciprofloxacin (CIPRO, purity ≥98%), thiacloprid (THIAC, purity ≥98%), imidacloprid (IMI, purity ≥98%), thiamethoxam (THIAM, purity ≥98%), clothianidin (CLOTH, purity ≥98%), acetamiprid N-desmethyl (ACET-d, purity ≥99%), methiocarb sulfone (METH-s, purity ≥95%), estrone (E1, purity ≥98%), 17-β-estradiol (E2, purity ≥97%) and 17-α-ethinylestradiol (EE2, purity ≥98%) were purchased from Sigma Aldrich . Single stock standard solutions were prepared in pure MeOH at a concentration of 1 mg mL^{-1}, except for THIAM and CIPRO which were dissolved in MeOH/H_2O (50/50, *v/v*) at a concentration of 0.5 mg mL^{-1} and in MeOH/H_2O (25/75, *v/v*) at a concentration of 0.25 mg mL^{-1}, respectively. All stock standard solutions were stored at -20°C. A working standard solution containing all analytes at a concentration of 50 ng mL^{-1} was prepared from the stock standard solutions periodically and injected in the HPLC-MS system to check for standard stability.

Syringe filters Acrodisc 13 mm minispike with 0.2 µm GHP membrane, were from PALL (Pall Italia SrL, Buccinasco, MI, Italy)

2.2 Instrumental analysis

All analyses were performed by HPLC-MS/MS. The LC system was a Ultimate 3000 chromatograph (Thermo Fisher Scientific, Bremen, Germany) equipped with a binary pump, a thermostated column compartment and an autosampler (kept at 14 °C). A C18 Kinetex XB column (100 × 2.1 mm i.d., 2.6 µm particle size, Phenomenex, Torrance, CA, USA) was employed for chromatographic separation. Two analyses were performed, one for antibiotics and pesticides and one for estrogens, since the latter required different mobile phases to enhance sensititvity. An injection volume of 10 µL, a column temperature of 40 °C and a flow rate of 0.2 mL min^{-1} were used in both cases. In the first method, phase A and phase B were water and MeOH, respectively, both containing 0.1% (*v/v*) of formic acid. The elution gradient started from an initial 10% of phase B, hold for 2 min, then phase B was increased up to 95% in 10 min, hold for 2 min;

the return to the initial conditions was performed in 1 min and the column was re-equilibrated for 7 min (total analysis time: 25 min). In the method used for estrogens, phase A and phase B were neutral water and MeOH, respectively. The elution gradient started from an initial 50% of phase B, hold for 1 min, then phase B was increased up to 95% in 3 min, hold for 3 min; the return to the initial conditions was performed in 1 min and the column was re-equilibrated for 7 min (total analysis time: 15 min). A solution of NH_3, at a concentration of 60 mmol L^{-1} in MeOH/H_2O (50/50, *v/v*), was infused post-column, at a flow rate of 50 μL min^{-1} by an auxiliary external micropump (Perkin-Elmer, series 200, Norwalk, CT, USA) [23].

The mass spectrometer was a TSQ Vantage (Thermo Fisher Scientific), constituted by a triple quadrupole analyzer and coupled to the LC by a heated electrospray (ESI) ion source. The mass detection was performed by applying either positive or negative polarity ionization and the multiple reaction monitoring (MRM) mode was chosen to achieve maximum sensitivity and specificity. Vaporizer temperature, capillary temperature and auxiliary gas pressure were set at 300 °C, 275 °C and 25 arbitrary units (a.u.), respectively. Two MRM transitions were selected for each compound and optimized MS settings were used for the acquisition; all parameters are summarized in Table 1. The most abundant MRM transition was used as the quantifier, while the less abundant was used for qualitative confirmation. Triplicate injections were performed for all samples and calibration solutions.

Data files were acquired by Xcalibur software (version 2.2, Thermo Fisher Scientific), which was also used for the peak areas integration.

Table 1: Mass spectrometric detection parameters for the selected analytes; the product ion of the quantifier MRM transition is indicated in bold.

Compound	ESI Polarity	Spray Voltage (*V*)	Sheath gas pressure (a.u.)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (*V*)	S-lens Voltage (*V*)
ERY	+	3900	25	734	**158**	31	154
					576	19	
CLARI	+	3900	25	748	**590**	18	124
					158	21	
AZY	+	4000	10	749.5	**591**	28	187
					83	41	
CIPRO	+	2600	45	332	**288**	18	94
					245	24	

THIAC	+	4000	10	253	**126** 99	23 42	93
IMI	+	2600	45	256	**209** 175	15 17	83.5
THIAM	+	2600	45	292	**211** 132	12 20	79
CLOTH	+	2600	45	250	**169** 132	11 14	57
ACET-d	+	2600	45	209	**126** 90	17 32	87
METH-s	-	2600	45	199	**120** 184	20 17	71
E1	-	3000	25	269	**145** 143	43 55	106
E2	-	3000	25	271	**145** 183	37 61	120
EE2	-	3000	25	295	**145** 159	57 37	125

2.3 Pilot systems and samples

The sludge and sludge-related samples (SMART products) came from the pilot systems (SMARTech) developed within the SMART-Plant project [6]. Table 2 summarizes the type of sample and the site of origin.

Table 2: considered samples coming from the different SMART-Plant pilot systems.

Sample number	Sample type	SMARTech site	WWTP treatment
1	Calcium phosphate-batch 1	SMARTech3 (Cranfield, UK)	IEX- Ion Exchange process for ammonia and phosphorus removal from wastewater
2	Calcium phosphate-batch 2		
3	Calcium phosphate-batch 3		
4	P-rich sludge	SMARTech4a (Carbonera, Italy)	SCENA- Short-Cut Enhanced Nutrient Abatment
5	Excess sludge	SMARTech4b (Psyttalia, Greece)	SCENA- Short-Cut Enhanced Nutrient Abatment (after thermal hydrolisis)
6	Struvite	SMARTech5 (Carbonera, Italy)	SCEPPHAR- Short-Cut Enhanced Phosphorus and PHA Recovery

7	PHA-rich sludge		(in sidestream)
8	PHA-rich sludge	SMARTech2b (Manresa, Spain)	SCEPPHAR- Short-Cut Enhanced Phosphorus and PHA Recovery (in mainstream)
9	Cellulose	SMARTech1 (Geestmerambacht, Netherlands)	Cellvation- Cellulose Recovery with dynamic sieving in primary treatment
10	P-rich compost	SMARTechB (Manresa, Spain)	Compost process on P-rich sludge from SCENA (Carbonera)

More details about the developed SMARTech processes are presented in Supplementary material.

The sludge and related matrices were subjected to drying processes, depending on their characteristics, and sampled at different times during the implementation of the pilot systems. After proper homogenization, a pool of these samples was used for the extraction and determination of the contaminants levels. The dried samples were stored refrigerated (4 °C) until pre-treatment and analysis.

2.4 Sample pre-treatment

The final procedure for sample pre-treatment was a modified QuEChERS method. One hundred mg of dried sludge was weighted in a 50 mL polyethylene centrifuge tube and 10 mL of ACN/H_2O (50/50, *v*/*v*), with 0.1% (*v*/*v*) of formic acid and 0.2% (*w*/*v*) Na_2EDTA, were added and vigorously shaken for 2 min. Four g of $MgSO_4$ and 1 g of NaCl were added to obtain phase separation: the solution was immediately agitated for 1 min and centrifuged (ALC multispeed refrigerated centrifuge, mod. PK 131R, Thermo Electron Corporation) at 3200 ×g for 5 min. Afterwards, two aliquots of the ACN layer were collected: 1 mL was dried at room temperature by a Centrivap concentrator (Labconco, Kansas City, MO, USA), reconstituted with 300 µL of MeOH/H_2O (50/50, *v*/*v*), filtered through a GHP Acrodisc 0.2 µm syringe filter and analyzed by UHPLC-MS/MS for determination of CIPRO.

A second aliquot of 2 mL of the ACN extract was transferred into a 15 mL polyethylene centrifuge tube containing 300 mg of $MgSO_4$ and 100 mg of PSA sorbent for the clean-up; the solution was shaken for 1 min and centrifuged at 3200 ×g for 5 min. One mL of the supernatant was collected, evaporated to dryness and

reconstituted with 200 µL of MeOH/H_2O (50/50, *v*/*v*). The sample was then filtered through a GHP Acrodisc 0.2 µm syringe filter and analyzed by HPLC-MS/MS for determination of all the other analytes.

2.5 Method performance

The developed method was evaluated in terms of the following figures of merit: linearity range, limit of detection (LOD), limit of quantitation (LOQ), intra-day and inter-day precision and trueness.

Standard solutions containing all analytes were prepared at different concentrations by diluting stock solutions in MeOH/ H_2O (20/80, *v*/*v*). The concentration levels ranged from the LOQ (different for each analyte) to 125 ng mL^{-1}. A pooled sample extract was divided into aliquots and spiked with the same concentrations levels to perform a matrix-matched calibration. Therefore, two sets of calibration curves were built by the linear regression method, and linearity was determined by computing the determination coefficient of the curves in the chosen concentration range. LOD and LOQ were defined as the concentrations corresponding to a signal to noise ratio of 3 and 10 respectively, considering the chromatogram of a real sample. Standard solutions at these concentrations were injected for confirmation.

Intra-day and inter-day procedural precision were assessed by performing 3 replicate extractions in the same day (*n*=3), and 2 replicate extractions in 3 different days (*n*=6), respectively.

Trueness was estimated by calculating recovery and matrix effect (ME), with 6 replicate tests, according to Matuszewski et al. [24]. In these experiments, the proper amount of pooled solid sample was spiked with 200 µL of a standard mix at a concentration of 250 ng mL^{-1} in MeOH (addition of 50 ng of each standard) and kept in contact overnight; then, the solvent was evaporated, and the fortified sample was used for recovery evaluations. In particular, recovery (R) was calculated as follows:

$$R\ (\%) = \ 100 * \frac{A_B - A_{NS}}{A_A - A_{NS}}$$

with A_{NS}, A_B and A_A representing the peak areas of a non-spiked sample (NS), a sample spiked before QuEChERS treatment (B) and a sample spiked after treatment (A), respectively. On the other hand, ME was estimated by the following calculation:

$$ME\ (\%) = \ 100 * \frac{A_A - A_{NS}}{A_P}$$

with A_{NS}, A_A and A_P representing the peak areas of extracts NS, A and a neat standard solution in solvent (P), respectively.

3. Results and discussion

3.1 Optimization of the HPLC-MS/MS method

In order to obtain an accurate and sensitive analysis of the selected compounds in the recovery material samples, both the instrumental method and the sample preparation were optimized.

Firstly, the mass spectrometric detection was evaluated, by direct infusion of single standard solutions (concentration of 1 µg mL^{-1} in MeOH) into the ion source. To find the best MRM transitions, a full scan spectra was acquired and different mass settings were tuned. The S-lens potential, which is the voltage applied to focus specific ions from the ion transfer tube into the first quadrupole, was regulated to maximize the response of the most intense ion (precursor ion). Subsequently the precursor ion was fragmented in the collision cell by applying collision energies (CE) in a defined range; the two most intense product ions and the related optimal CE were chosen to define the quantifier and qualifier transitions. Once the MRM transitions were selected, two ESI source settings, namely the spray voltage and the sheath gas pressure, were optimized. These variables were supposed to have a significant influence on the mass signal; indeed, they influence the ESI spray stability, as well as the ionization efficiency. Therefore, a 3 levels, full factorial experimental design was performed, to evaluate these variables in a multivariate fashion, investigating their relationships with the response (peak areas of the MRM transitions) and among each other: nine experiments, with different combinations of the considered variables were carried out. Subsequently, based on the responses of the experiments, response surfaces were built and the optimal values of spray voltage and sheath gas pressure, which maximized the MS signal, were determined. More details on the experimental design (variable levels and performed experiments) are provided in Supplementary material (Tables S1-S2). All the optimized parameters of the MS detection are shown in Table 1 and they allowed to obtain a high sensitivity and specificity of the analysis.

In parallel, starting from a literature method which involved all the analytes herein considered [20], the chromatographic method was improved, mainly in terms of sensitivity, analysis time and repeatability. A

mix containing all the analytes, at a concentration of 50 ng mL^{-1} was used for chromatographic optimization. Some modifications of the literature gradient were tested, and a compromise between peak separation and rapidity was achieved with a run of 25 min ; thanks to the use of the MRM mode, a perfect separation among the analytes peaks was not necessary (see Fig. S1 in Supplementary Material). Based on the literature data, the solvents set for phase A and B were water and MeOH [18,20,25,26], respectively; for sensitivity enhancement, the effect of the acidity was investigated by testing neutral phases and phases with the addition of 0.01% (*v*/*v*) and 0.1% (*v*/*v*) of formic acid. Best results were achieved by employing water and MeOH with 0.1% (*v*/*v*) of formic acid for all analytes, except for E1, E2 and EE2. These analytes, which required the negative ESI polarity, were not detected when using acid mobile phases and showed poor sensitivity with neutral mobile phases. Therefore, a compromise for the simultaneous analysis of all 14 compounds was not feasible, and a chromatographic method specific for estrogens was developed. A rapid gradient of 15 min was implemented, using neutral water and MeOH as mobile phases, and a freshly prepared solution of NH_3 60 mmol L^{-1} in MeOH/H_2O (50/50, *v*/*v*) was added at the exit of the column flow, with the aid of an auxiliary pump system. This setup was optimized in a previous work [23], and allowed to promote the negative ionization of estrogens in the ion source, leading to a great enhancement of the chromatographic peak areas. Thanks to the instrumental optimization, the LODs (referred to neat standard solutions) reached extremely low values, in the range of 4-120 pg mL^{-1}.

3.2 Extraction procedure from sludge samples

When the simultaneous determination of different classes of chemicals is required, it is fundamental to develop an accurate extraction-purification method . The analytes selected in this work (Table 1) belong to the classes of macrolide antibiotics (ERY, CLARI and AZY), fluoroquinolones (CIPRO), neonicotinoids (THIAC, IMI, THIAM, CLOTH, ACET-d,), carbamates (METH-s) and hormones (E1, E2, EE2). In order to develop a fast and reproducible pre-treatment method for their determination in sludges and recovery materials, the QuEChERS methodology was selected. Several variables were studied with the aim of maximizing recovery and limiting ME. Indeed, both parameters need to be carefully evaluated when dealing with complex matrices, to ensure a high accuracy of the method [27,28]. All the tests were performed on a spiked pooled sample and are presented in the scheme in Fig. 1. The starting point (test A)

was a QuEChERS method by Nannou *et al.* [29], used for neonicotinoids extraction from sediments, whose extraction solvent composition was modified to make it suitable for antibiotics. In particular, 1 g of spiked sludge was extracted with 10 mL of ACN/H_2O (50/50, *v*/*v*) with the addition of 0.5% (*v*/*v*) of formic acid and 0.1% (*w*/*v*) of Na_2EDTA; these additives were used according to Gago-Ferrero *et al*. [18], which employed them to enhance the extraction of antibiotics from sludge. Afterwards, a clean-up step was carried out with 100 mg of PSA and 100 mg of C18 loose sorbents, as shown in Fig.1. This preliminary test led to satisfactory recoveries of pesticides and estrogens (60-90%), but rather poor recovery of antibiotics (0-49%). Moreover, despite the clean-up step, a strong ion suppression was observed for most analytes, except for ACET-d and METH-s. These results suggested that the sample amount and pre-concentration factor could lead to insufficient clean-up and massive presence of interferent species. In addition, the recovery of antibiotics could benefit from the use of a higher amount of Na_2EDTA; in fact, Na_2EDTA can be used to chelate the metal ions present in sludge and modify the equilibrium between these ions and antibiotics, causing their release, with consequent improvement of the recovery [21]. Therefore, in all the subsequent tests (B1-B5), the sample weight was reduced to 0.1 g and the percentage of Na_2EDTA in the extraction phase was set at 0.2% (*w*/*v*). On the other hand, different amounts of PSA and C18 loose sorbents were selected in 5 distinct protocols (B1-B5), to investigate their effect on the efficiency of the clean up as well as on the analyte recovery.

The results of the comparison among the performed test, in terms of recovery, are shown in Fig. 2.

A major difference was observed in the recovery of the antibiotic class, compared to neonicotinoids and estrogens. In particular, ERY and CIPRO exhibited a poor recovery in all the tests which involved the clean-up step (tests A-B1-B2-B3-B4), with a slight improvement when only C18 was used (test B3). This suggests a probable adsorption on the clean-up phases, with a particular affinity to PSA. In fact, when no clean-up was performed, the recovery of the two antibiotics increased (51% and 71% for CIPRO and ERY respectively). On the other hand, AZY revealed a stronger affinity to the C18 phase, showing a better recovery when only PSA was employed for purification (test B2). As far as neonicotinoids are concerned, an improvement in recovery was observed when decreasing the amount of sample, maybe due to other species, which could interfere with the extraction. On the contrary, the clean-up step did not affect their recovery,

which was always in the range 80-120%, with only slight differences among the tests. Estrogens recoveries were satisfactory in all cases and ranged from 75 to 111%, without significant differences.

As far as ME is concerned, results are shown in Fig. 3. The best option for most compounds was procedure B2, characterized by a decreased amount of sample and the use of the sole PSA (100 mg) for the clean-up step. By applying this method, the ME was 50-100% for all analytes. C18 sorbent revealed to be less effective in reducing ion suppression, since no significant improvement was observed with its use (tests B1, B3 and B4).

In order to achieve a compromise between recovery and ME, method B2 was selected as the best for all compounds, except ERY and CIPRO. Indeed, to avoid massive loss of these analytes, their determination should be performed in a sample not subjected to clean-up. To improve the signal of the two antibiotics, three tests involving different reconstitution volumes of the extract, namely 200, 300 and 400µL, were compared. The ME of CIPRO had a benefit from dilution and the intermediate value of 300 µL was selected as the optimal, to avoid an excessive penalisation of sensitivity. Unfortunately, ERY revealed a strong ion suppression, despite the dilution of the sample. The quantitation of this substance was therefore not possible, due to low accuracy and reproducibility caused by matrix interferents.

Given all the results, the optimal procedure involved the split of the sample extract and the analysis of both a purified aliquot (prepared by following protocol B2) and a not purified aliquot (as described in "materials and methods").

3.3 Method performance and quantitation

The whole final method was evaluated in terms of the classical analytical performances. All figures of merit, obtained as described in "materials and methods", are summarized in Table 3.

Table 3: performances of the proposed analytical method.

analyte	External calibration		Matrix-matched calibration		LOD* (ng g $^{-1}$)	LOQ* (ng g $^{-1}$)	ME (%)	REC ± SD (%)	RSD(%) (Intra-day, n=3)	RSD(%) (Inter-day, n=6)
	Linearity range (ng mL^{-1})	R^2	Linearity range (ng mL^{-1})	R^2						
CLARI	0.1-125	0.9993	0.1-125	0.9901	0.3	1.7	79	80 ± 4	5.5	11.8
AZY	0.4-125	1.0000	0.5-125	0.9980	1.4	4.6	123	71 ± 6	8.8	20.0
CIPRO	0.3-125	0.9964	1.8-125	0.9992	7.9	26.5	103	68 ± 5	7.7	17.0
THIAC	0.05-125	1.0000	0.2-125	0.9960	0.5	1.8	70	100 ± 4	4.2	7.8
IMI	0.3-125	0.9965	0.6-125	0.9970	1.9	6.5	87	103 ± 5	5.0	6.1
THIAM	0.2-125	0.9978	0.5-125	0.9985	1.6	5.5	70	89 ± 6	7.1	13.0
CLOTH	0.2-125	0.9941	0.8-125	0.9932	2.4	7.9	76	100 ± 2	2.4	3.5
ACET-d	0.05-125	0.9961	0.3-125	0.9972	0.8	2.6	83	104 ± 5	5.1	14.0
METH-s	0.1-125	0.9998	0.2-125	0.9999	0.6	2.1	90	98 ± 4	3.9	6.3
E1	0.1-125	0.9996	0.4-125	0.9936	1.3	4.2	104	99 ± 7	7.0	9.3
E2	0.2-125	0.9996	3.8-125	0.9947	11.4	37.9	103	86 ± 2	2.2	12.7
EE2	0.3-125	0.9990	7.1-125	0.9959	21.4	71.4	102	89 ± 3	3.2	2.9

*LOD and LOQ values are calculated by considering the background noise in real sample extracts

Linearity was assessed in a range from the LOQ to 125 ng mL^{-1}; this range was slightly narrower in the matrix matched curves, being the LOQ values in matrix higher than in neat solvent. A determination coefficient of 0.990-1 was achieved for all compounds for both the solvent-based (external) and matrix-matched calibration curves. Very good LOD and LOQ values were reached, in the ranges of 0.3-21.4 ng g^{-1} and 1.7-71.4 ng g^{-1}, considering dry weight of sludge samples. Intra-day and inter-day procedural precision, estimated as relative standard deviation (RSD, %), were in the ranges 2.2-8.8% and 2.9-20%, respectively. The only exception was ERY, which exhibited very low precision (RSD >50%).

Recovery (REC) of 68-104% was obtained for all analytes, with neonicotinoids and estrogens exhibiting the highest values (89-104%). The estimation of ME, calculated by comparing the signal of single standards in neat solvent and added to a pooled extract [24], led to values ranging from 70 to 120%. Nevertheless, when applying the final procedure to the recovery material samples, standard additions on single extracts were performed and the MEs in each sample were evaluated: some differences among the samples aroused.

Therefore, when the observed MEs substantially differed from the values of the pooled extract, the standard addition method was used for quantitation.

3.4 Determination of contamination levels in the recovery materials

The optimized analytical method was applied to the recovery materials obtained from the SMART-Plant pilot systems. A total of 10 samples were analysed, including recovery cellulose, different sludge samples, compost and calcium salts (Table 2 and Fig. S2 in Supplementary material). A slight contamination of the samples was highlighted, as shown in Table 4, which presents the results of the analyses expressed as ng per g of dry weight.

Table 4: quantitation results, expressed as ng per g of dry weight of each sample.

	CLARI	AZY	CIPRO	IMI	E1
			ng g^{-1}		
Sample 1	- [a]	-	11 ± 6 [b]	-	-
Sample 2	-	-	14 ± 5 [b]	-	-
Sample 3	-	-	8 ± 6 [b]	-	-
Sample 4	50 ± 8	506 ± 18	597 ± 6	-	22 ± 11
Sample 5	-	30 ± 19	507 ± 6	-	27 ± 12
Sample 6	30 ± 8	29 ± 19	54 ± 6	-	24 ± 12
Sample 7	56 ± 8	302 ± 19	144 ± 6	-	24 ± 12
Sample 8	-	18 ± 12	273 ± 50	-	-
Sample 9	-	72 ± 16	304 ± 5	-	20 ± 10
Sample 10	31 ± 8	342 ± 19	217 ± 8	6 ± 1 [b]	-

a- not detected peaks or peak area below the method LOD

b- concentrations below LOQ; they were estimated by extending the calibration curve below the lower limit of the linearity range

Only few analytes from the list were detected, mainly belonging to the antibiotics class, with CIPRO quantified in all samples, at higher levels than the other compounds (up to 600 ng g^{-1}). Low levels of E1 (approximately 20-30 ng g^{-1}) were detected in sample 4, 5, 6, 7 and 9; on the contrary, the other hormones were under the LOD in all samples. Pesticides were not detected in any of the samples, except for sample 10,

in which a very low concentration of IMI was determined (6 ng g^{-1}). This value was at the LOQ concentration level, therefore characterized by a high uncertainty.

Some considerations can be made by comparing the quantitation results for samples coming from the same SMARTech, from similar processes, or from totally different processes.

Samples 1, 2 and 3 were calcium phosphate samples, coming from three different procedures of SMARTech 3; they exhibited only a negligible contamination by CIPRO, whose concentration could be only estimated, with low precision, being below the LOQ of the method. In a previous work, fluoroquinolones were found to favourably adsorb onto calcium phosphate [30]; therefore, the low detected levels could be due to efficient removal of this antibiotic from water before the SMARTech 3 treatment, in agreement with the absence of the other contaminants in these samples.

Sample 4 was generated by a particular treatment of sidestream liquors from secondary wastewater treatment in SMARTech 4a. This sample revealed the highest concentration of antibiotics, with AZY and CIPRO reaching a concentration of 500-600 ng g^{-1}. This result suggests that antibiotics could be adsorbed onto the activated sludge used in the SCENA treatment, thus accumulating in the final P-rich sludge.

Sample 5 derived from the same SCENA approach, but, before the treatment, the sidestream liquor was subjected to thermal hydrolysis (SMARTech 4b). In this sample, CLARI was not detected and AZY concentration was 10-fold lower compared to sample 4; on the contrary, a similar concentration of CIPRO was detected. It was observed that fluoroquinolones are quite stable to heat degradation [31], which could explain CIPRO higher concentration, despite the thermal process applied.

Samples 6 and 7, namely struvite ($(NH_4)MgPO_4 \cdot 6(H_2O)$) and polyhydroxy alcanoate (PHA)-rich sludge, were the two products obtained by the SCEPPHAR technology (SMARTech5). Struvite (sample 6) was recovered after treating waters derived from primary cellulosic sludges with magnesium, in a precipitation reactor. Compared to sludge samples from the other SMARTechs, the contamination from CIPRO was one order of magnitude lower, suggesting that this inorganic salt is less prone to adsorb CIPRO from treated wastewaters, compared to organic matter [32]. In sample 7, which derived from aerobic PHA accumulation within the SCEPPHAR system, all antibiotics exhibited higher concentrations than in sample 6, with AZY being approximately one order of magnitude higher. The PHA-rich sludge is obtained by treating the supernatant (liquid phase) collected after struvite recovery (solid phase); therefore, the concomitant presence

of antibiotics, although at different levels, in both SMART products suggests their distribution between the two phases.

Sample 8 derived from a SCEPPHAR process applied to wastewater mainstream (SMARTech 2b), in order to directly obtain PHA-rich sludge from untreated wastewater. Only AZY and CIPRO were detected in this sample: AZY revealed a one order of magnitude lower concentration, compared to sample 7, while CIPRO was found at a doubled concentration. This result could be ascribed to the different input sources (sidestream liquors and primary wastewater for SMARTech5 and SMARTech2b, respectively) of the two SCEPPHAR processes.

Sample 9 was constituted by a fluffy cellulose matter, recovered after primary wastewater filtering (SMARTech1). This material derived from a completely different treatment compared to the other SMART products, and was not subjected to any bio-chemical process; hence, a higher contamination from organic substances could be expected [33]. Nevertheless, the only detected contaminants were AZY, CIPRO and E1, at similar concentrations compared to the other samples, thus suggesting a low contamination of the influent water.

Sample 10 was the only compost material among all samples (coming from SMARTechB); in particular, it derived from a dynamic composting process applied to P-rich sludges coming from a SCENA treatment (sample 4 type). Compared to sample 5, lower concentrations of all the detected contaminants were observed, suggesting that the composting process could cause pollutants degradation to some extent [34]. Nevertheless, the observed decrease of contamination levels was not substantial; the water loss during composting might cause a pre-concentration which makes the contaminants degradation less evident [33]. The described results indicate a complicated mechanism of absorption, degradation and distribution of the contaminants across the innovative processes of the SMART-Plant project, which is not easy to unravel. Indeed, the complex processes involved in the production of the described recovery materials make it difficult to interpret the results of contaminants quantitation. Moreover, to better compare the samples, as well as the sample treatments, the knowledge on influent levels of the pollutants would be helpful.

4. Conclusions

The present work proposes a method for the determination of selected emerging contaminants in sewage-sludges and other similar materials, coming from innovative and sustainable processes in WWTPs. The careful study and optimization of both the instrumental analysis and the pre-treatment steps allowed to obtain a fit-for-purpose method, which provides reliable quantitative results on the presence of estrogens, antibiotics and neonicotinoid pesticides. This analysis can represent the basis for the risk assessment related to the usage of innovative recovery materials. By this evaluation, the actual potential in circular economy represented by the innovative systems belonging to the SMART-Plant project can be appraised.

Moreover, the developed method, particularly suitable for sludge, compost and other recovery materials from WWTPs could be applied for the screening of emerging contaminants coming from the 2018 European watch-list, thus providing additional information regarding their diffusion, distribution and removal by wastewater and sludge treatments.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This study was carried out within the framework of the 'SMART-Plant' Innovation Action, which has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 690323.

The authors thank Samuela Guida (Cranfield University, UK), Juan A. Baeza and Natalia Rey Martìnez (Universitat Autonoma de Barcelona, Spain), Simos Malamis (National Technical University of Athens, Greece), Nagore Guerra Gorostegi (Universitat de Vic, Spain) and CirTec B.V. (Purmerend, Netherlands) for providing the samples.

Author Statement

Barbara Benedetti: Investigation, Methodology, Writing - Original Draft

Mauro Majone: Conceptualization, Supervision.

Chiara Cavaliere: Project administration, Validation, Writing - Review & Editing.

Carmela Maria Montone: Investigation, Validation.

Francesco Fatone: Funding acquisition.

Nicola Frison: Funding acquisition.

Aldo Laganà: Project administration, Resources.

Anna Laura Capriotti: Project administration, Resources.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.P. van der Hoek, H. de Fooij, A. Struker, Wastewater as a resource: Strategies to recover resources from Amsterdam's wastewater, Resour. Conserv. Recycl. 113 (2016) 53–64. doi:10.1016/J.RESCONREC.2016.05.012.

[2] S. Raghuvanshi, V. Bhakar, C. Sowmya, K.S. Sangwan, Waste Water Treatment Plant Life Cycle Assessment: Treatment Process to Reuse of Water, Procedia CIRP. 61 (2017) 761–766. doi:10.1016/J.PROCIR.2016.11.170.

[3] L. Zhang, C. (Charles) Xu, P. Champagne, W. Mabee, Overview of current biological and thermo-chemical treatment technologies for sustainable sludge management, Waste Manag. Res. 32 (2014) 586–600. doi:10.1177/0734242X14538303.

[4] M. Smol, J. Kulczycka, A. Henclik, K. Gorazda, Z. Wzorek, The possible use of sewage sludge ash (SSA) in the construction industry as a way towards a circular economy, J. Clean. Prod. 95 (2015) 45–54. doi:10.1016/J.JCLEPRO.2015.02.051.

[5] SMART-Plant (Scale-up of low-carbon footprint MAterial Recovery Techniques in existing wastewater treatment Plants), (2016). https://smart-plant.eu/index.php/the-project/about-smart-plant.

[6] SMART-Plant Technical Factsheets, (2019). https://smart-plant.eu/index.php/the-project/technical-factsheets.

[7] N. Dichtl, S. Rogge, K. Bauerfeld, Novel Strategies in Sewage Sludge Treatment, CLEAN – Soil, Air, Water. 35 (2007) 473–479. doi:10.1002/clen.200720022.

[8] Council of European Communities, Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture, Off. J. Eur. Communities. L181 (1986) 6–12.

[9] K. Fijalkowski, A. Rorat, A. Grobelak, M.J. Kacprzak, The presence of contaminations in sewage sludge – The current situation, J. Environ. Manage. 203 (2017) 1126–1136. doi:10.1016/J.JENVMAN.2017.05.068.

[10] O. Zuloaga, P. Navarro, E. Bizkarguenaga, A. Iparraguirre, A. Vallejo, M. Olivares, A. Prieto, Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: A review, Anal. Chim. Acta. 736 (2012) 7–29. doi:10.1016/J.ACA.2012.05.016.

[11] P. Krzeminski, M.C. Tomei, P. Karaolia, A. Langenhoff, C.M.R. Almeida, E. Felis, F. Gritten, H.R. Andersen, T. Fernandes, C.M. Manaia, L. Rizzo, D. Fatta-Kassinos, Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: A review, Sci. Total Environ. 648 (2019) 1052–1081. doi:10.1016/J.SCITOTENV.2018.08.130.

[12] A. Bacaloni, L. Callipo, E. Corradini, P. Giansanti, R. Gubbiotti, R. Samperi, A. Laganà, Liquid chromatography-negative ion atmospheric pressure photoionization tandem mass spectrometry for the determination of brominated flame retardants in environmental water and industrial effluents, J. Chromatogr. A. 1216 (2009) 6400–6409. doi:10.1016/j.chroma.2009.07.039.

[13] European Commission, Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Impl, Off. J. Eur. Union. 61 (2018) 9–12.

[14] Y. Huang, M. Cheng, W. Li, L. Wu, Y. Chen, Y. Luo, P. Christie, H. Zhang, Simultaneous extraction of four classes of antibiotics in soil, manure and sewage sludge and analysis by liquid chromatography-tandem mass spectrometry with the isotope-labelled internal standard method, Anal. Methods. 5 (2013) 3721. doi:10.1039/c3ay40220g.

[15] M.B.R. Cerqueira, J.R. Guilherme, S.S. Caldas, M.L. Martins, R. Zanella, E.G. Primel, Evaluation of the QuEChERS method for the extraction of pharmaceuticals and personal care products from drinking-water treatment sludge with determination by UPLC-ESI-MS/MS, Chemosphere. 107 (2014) 74–82. doi:10.1016/J.CHEMOSPHERE.2014.03.026.

[16] W. Peysson, E. Vulliet, Determination of 136 pharmaceuticals and hormones in sewage sludge using quick,

easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry, J. Chromatogr. A. 1290 (2013) 46–61. doi:10.1016/j.chroma.2013.03.057.

[17] M. Gorga, S. Insa, M. Petrovic, D. Barceló, Analysis of endocrine disrupters and related compounds in sediments and sewage sludge using on-line turbulent flow chromatography-liquid chromatography-tandem mass spectrometry, J. Chromatogr. A. 1352 (2014) 29–37. doi:10.1016/j.chroma.2014.05.028.

[18] P. Gago-Ferrero, V. Borova, M.E. Dasenaki, N.S. Thomaidis, Simultaneous determination of 148 pharmaceuticals and illicit drugs in sewage sludge based on ultrasound-assisted extraction and liquid chromatography–tandem mass spectrometry, Anal. Bioanal. Chem. 407 (2015) 4287–4297. doi:10.1007/s00216-015-8540-6.

[19] A. Masiá, K. Vásquez, J. Campo, Y. Picó, Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Túria River Basin, J. Chromatogr. A. 1378 (2015) 19–31. doi:10.1016/j.chroma.2014.11.079.

[20] R. Massei, H. Byers, L.-M. Beckers, J. Prothmann, W. Brack, T. Schulze, M. Krauss, A sediment extraction and cleanup method for wide-scope multitarget screening by liquid chromatography–high-resolution mass spectrometry, Anal. Bioanal. Chem. 410 (2018) 177–188. doi:10.1007/s00216-017-0708-9.

[21] M. Chen, Q. Yi, J. Hong, L. Zhang, K. Lin, D. Yuan, Simultaneous determination of 32 antibiotics and 12 pesticides in sediment using ultrasonic-assisted extraction and high performance liquid chromatography-tandem mass spectrometry, Anal. Methods. 7 (2015) 1896–1905. doi:10.1039/C4AY02895C.

[22] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, J. AOAC Int. 86 (2003) 412–431. doi:10.2478/s11687-011-0011-9.

[23] A.L. Capriotti, C. Cavaliere, P. Foglia, R. Samperi, S. Stampachiacchiere, S. Ventura, A. Laganà, Ultra-high-performance liquid chromatography-tandem mass spectrometry for the analysis of free and conjugated natural estrogens in cow milk without deconjugation, Anal. Bioanal. Chem. 407 (2015) 1706–1719. doi:10.1007/s00216-014-8398-z.

[24] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, Anal. Chem. 75 (2003) 3019–3030. doi:10.1021/ac020361s.

[25] M.E. Dasenaki, N.S. Thomaidis, Multianalyte method for the determination of pharmaceuticals in wastewater samples using solid-phase extraction and liquid chromatography–tandem mass spectrometry, Anal. Bioanal. Chem. 407 (2015) 4229–4245. doi:10.1007/s00216-015-8654-x.

[26] V. Limay-Rios, L.G. Forero, Y. Xue, J. Smith, T. Baute, A. Schaafsma, Neonicotinoid insecticide residues in soil dust and associated parent soil in fields with a history of seed treatment use on crops in southwestern Ontario, Environ. Toxicol. Chem. 35 (2016) 303–310. doi:10.1002/etc.3257.

[27] B. Benedetti, E. Magi, M. Di Carro, The experimental design approach to the optimization of a simple quick easy cheap effective rugged and safe method for the analysis of phytoestrogens in complex soy-based food, J. Sep. Sci. 42 (2019) 3225–3233. doi:10.1002/jssc.201900462.

[28] A.L. Capriotti, C. Cavaliere, C. Crescenzi, P. Foglia, R. Nescatelli, R. Samperi, A. Laganà, Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry, Food Chem. 158 (2014) 392–400. doi:10.1016/j.foodchem.2014.02.130.

[29] C.I. Nannou, V.I. Boti, T.A. Albanis, Trace analysis of pesticide residues in sediments using liquid chromatography–high-resolution Orbitrap mass spectrometry, Anal. Bioanal. Chem. 410 (2018) 1977–1989. doi:10.1007/s00216-018-0864-6.

[30] H. Bouyarmane, I. El Hanbali, M. El Karbane, A. Rami, A. Saoiabi, S. Saoiabi, S. Masse, T. Coradin, A. Laghzizil, Parameters influencing ciprofloxacin, ofloxacin, amoxicillin and sulfamethoxazole retention by natural and converted calcium phosphates, J. Hazard. Mater. 291 (2015) 38–44. doi:10.1016/j.jhazmat.2015.02.049.

[31] L. Tian, S. Khalil, S. Bayen, Effect of thermal treatments on the degradation of antibiotic residues in food, Crit. Rev. Food Sci. Nutr. 57 (2017) 3760–3770. doi:10.1080/10408398.2016.1164119.

[32] Z.L. Ye, Y. Deng, Y. Lou, X. Ye, S. Chen, Occurrence of veterinary antibiotics in struvite recovery from swine wastewater by using a fluidized bed, Front. Environ. Sci. Eng. 12 (2018) 7. doi:10.1007/s11783-018-1015-1.

[33] J. Martín, J.L. Santos, I. Aparicio, E. Alonso, Pharmaceutically active compounds in sludge stabilization treatments: Anaerobic and aerobic digestion, wastewater stabilization ponds and composting, Sci. Total Environ. 503–504 (2015) 97–104. doi:10.1016/j.scitotenv.2014.05.089.

[34] A. Ezzariai, M. Hafidi, A. Khadra, Q. Aemig, L. El Fels, M. Barret, G. Merlina, D. Patureau, E. Pinelli, Human and veterinary antibiotics during composting of sludge or manure: Global perspectives on persistence, degradation, and resistance genes, J. Hazard. Mater. 359 (2018) 465–481. doi:10.1016/j.jhazmat.2018.07.092.

Figure captions

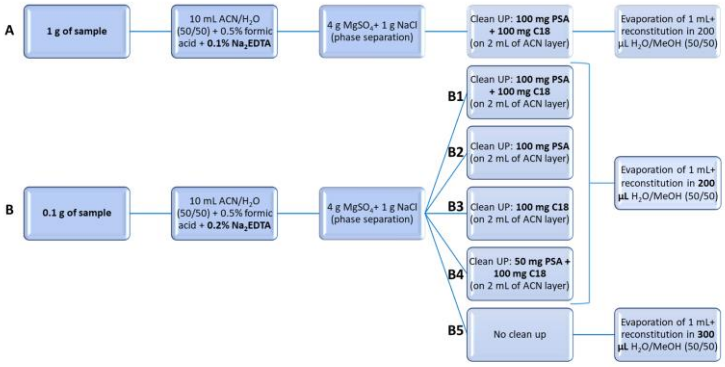

Figure 1: tests performed to optimize the extraction/purification of the analytes from the sludge samples.

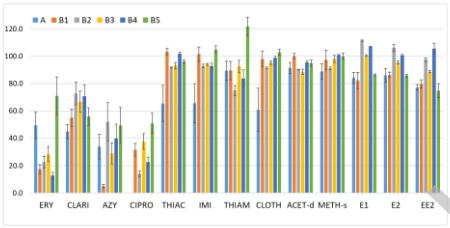

Figure 2: recoveries of the single analytes obtained by the different tests performed, by spiking the solid samples at a concentration of 500 ng g^{-1} of each standard, before extraction.

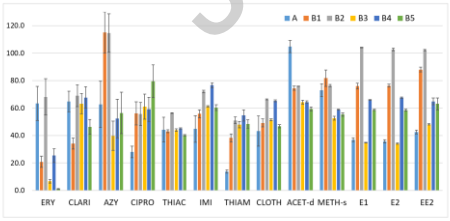

Figure 3: matrix effect of the single analytes obtained by the different tests performed.