Identification and quantification of selected plastics in biosolids by pressurized liquid extraction combined with double-shot pyrolysis gas chromatography-mass spectrometry

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Highlights

- Alternative and complementary quantitative analytical method for the analysis of selected plastics in complex environmental samples.
- Pressurized liquid extraction (PLE) combined with double-shot pyrolysis gas chromatography-mass spectrometry (Pyr-GC/MS) allows for the identification and quantification of polyethylene, polystyrene, poly-methyl methacrylate, polypropylene and polyvinyl chloride.
- The use of the double-shot feature for the effective thermal desorption of potentially interfering co-extracted compounds from samples provides an improved alternative for the identification and quantification of selected plastics in complex organic rich samples.
- Rapid measurements and good repeatability without a time consuming sample pre-treatment.
- Total plastic concentration of between 2.8 and 6.6 mg/g dw (median = 4.1 mg/g dw) in Australian biosolids
- PE was the predominant plastic detected (mean concentration of 2.2 mg/g dw), contributing to 50 % of the total of all plastics.

Abstract

The identification and quantification of selected plastics (polystyrene (PS), polycarbonate (PC), poly-(methyl methacrylate) (PMMA), polypropylene (PP), polyethylene terephthalate (PET), polyethylene (PE) and polyvinyl chloride (PVC)) in biosolids (treated sewage sludge) was performed by pressurized liquid extraction (PLE) combined with double-shot pyrolysis gas chromatography-mass spectrometry. Validation of the method yielded recoveries of between 85 and 128 % (mean RSD 11 %) at a linear range of between 0.01 and 2 µg. The distribution of plastics within 25 biosolid samples from a single wastewater treatment plant in Australia was assessed. The mass concentration of PE, PVC, PP, PS and PMMA was between 0.1 to 4.1 mg/g dry weight (dw) across all samples, with a total plastic concentration $\Sigma_{Plastics}$ of between 2.8 and 6.6 mg/g dw (median = 4.1 mg/g dw). PE was the predominant plastic detected (mean concentration of 2.2 mg/g dw), contributing to 50 % of the total of all plastics. Overall, this study demonstrates that pressurized liquid extraction (PLE) combined with double-shot pyrolysis gas chromatography-mass spectrometry can be used to identify and quantify PE, PP, PVC, PS, and PMMA in biosolids.

Keywords: Plastics; Pressurized liquid extraction; Double-shot Pyr-GC/MS; Quantification; Sewage sludge; Biosolids

1. Introduction

Awareness that the natural environment is contaminated with small plastic particles has increased over the past years [1,2,3]. Large amounts of plastic debris accumulate in the environment due to a combination of high production, low recycling volumes and the highly stable nature of most plastic materials [4,5]. Environmental factors such as sunlight, mechanical stress and an oxidizing atmosphere [2], can fragment plastic debris into smaller pieces, with fragments < 5 mm typically defined as microplastics and fragments < 1 µm termed nanoplastics [6]. Plastic particles have been reported to occur in marine waters [7,8], freshwaters [9], terrestrial environments [4,10,11], air [12,13] and living organisms [5,9,14], with their potential risks to organisms and human health identified as an emerging concern [15-17].

Biosolid (treated sewage sludge), a product of wastewater treatment, is an important and potentially significant source of plastic to the terrestrial environment [18-20]. They are applied to vast expanses of agricultural land in order to recycle organic matter, nutrients and to improve soil quality for cropping [18,21]. Between 80 and 99 % of the plastic particles entering wastewater treatment plants (WWTPs) have been reported to be retained in biosolids [22-25]. In Australia, Europe and North America, approximately 50 to 75 % of biosolids are recycled via agricultural land application [18,26] potentially releasing 2,800 to 430,000 tons of plastic annually onto farmlands [18,19,27,28] with unknown consequences for sustainability and food security [18].

Several recent studies have tried to subjectively quantify the amount of plastics in biosolids [19,29-33]. This has typically been performed by visual inspection and particle counting, followed by identification using spectroscopic techniques such as Raman Spectroscopy and Fourier-transform infrared (FT-IR) spectroscopy on a subset of particles [23,25,31,33-36]. While these approaches provide data on the types of plastic, the number of particles, size, shape and color,

they can't quantify the mass concentration of the plastics in the samples [37]. In addition, the above mentioned techniques are size dependent, and in many cases, not able to detect smaller sized plastics – potentially underestimating amount of plastics [19]. For example, FT-IR and Raman require particle sizes of > 20 and > 1 µm respectively [38]. For complex matrices, e.g. biosolids, these techniques require exhaustive and laborious pre-treatment procedures to remove interferences (organic materials) [19,23,39,40].

Thermo-analytical techniques, such as pyrolysis-gas chromatography coupled with mass spectrometry (Pyr-GC/MS), provide an alternative for the identification and quantification of plastic – independent of particle size [8,19,41-44]. Pyr-GC/MS uses polymer specific decomposition compounds for identification and facilitates semi-quantitative to quantitative analyses of plastics in environmental samples [41,42,45]. Nonetheless, the pre-concentration of plastic particles in environmental samples to meet the requirement of analysis by Pyr-GC/MS is not trivial [6]. Similar to the use of FT-IR and Raman, various studies for example have pre-treated environmental samples by either degrading organic materials using chemical or enzymatic digestion and/or by pre-concentrating and separation of plastic particles prior to Pyr-GC/MS analysis [8,41,42,45,46]. Such clean-up procedures are time-consuming and may be prone to errors [6].

Sequential pressurized liquid extraction (PLE) has been recently reported as a promising technique for the extraction of selected plastics (polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polypropylene (PP) and polyethylene terephthalate (PET)) from municipal waste, soil and sediment samples [45,47]. To reduce the interference from the complex organic sample matrix, methanol was used at 100 °C as an initial extraction step and discarded allowing the plastics to be extracted with dichloromethane (DCM) at 180 °C [47] or tetrahydrofuran (THF)

105 at 185 °C [45]. The extract was then either analyzed gravimetrically on the evaporated extracted
106 residues with plastic identification confirmed by FT-IR [47] or alternatively by Pyr-GC/MS [45]. While
107 a sequential extraction clean-up is promising, there is the potential that an initial pre-extraction
108 step with methanol at 100 °C may remove some plastics leading to underestimation.

109 In this paper we present a quantitative method for the extraction and analysis of seven plastics
110 (PS, PC, PMMA, PP, PET, PE and PVC) in biosolid samples by combining a single step PLE with a
111 two-stage (double-shot) Pyr-GC/MS method that negates the need for a pre-extraction clean-
112 up step or sample pre-treatment. Here, the first-shot component of a double-shot Pyr-GC/MS
113 method was used to thermally desorb the potentially interfering organic compounds/materials
114 co-extracted from biosolid samples, with plastic identification and quantification performed in
115 the second-shot. The validated method was applied to a set of biosolid samples collected from
116 a single treatment train and the distribution of plastics evaluated.

117 2. Experimental section

118 2.1 Reference plastic standards and chemicals

119 Plastic reference standards: PE (30-530 µm), PS (40-510 µm), PMMA (30-530 µm), PC (20-520
120 µm), PET (20-510 µm) and PP (20-520 µm) were provided by the Norwegian Institute for Water
121 Research (NIVA, Oslo, Norway). PVC powder with size ≤ 50 µm was purchased from Sigma-
122 Aldrich (St. Louis, MO, USA). Each plastic type was confirmed by FT-IR spectrometer
123 (PerkinElmer, UATR Two) and Pyr-GC/MS prior to use (see Supporting Information (SI) Figure
124 S13 and Table S3). Analytical grade dichloromethane (DCM) was purchased from Merck
125 (Darmstadt, Germany). Hydromatrix was purchased from Agilent Technologies (Santa Clara,
126 CA, USA) and pre- extracted with DCM before use.

2.2 Sample collection

A total of 25 biosolid samples were collected from the solar hall of a WWTP located in South-East Queensland, Australia. For sampling purposes the solar hall was divided into a grid (9 columns by 30 rows) with each row representing approximately 1 day of drying. Samples were collected from approximately every third day of drying (see SI Figure S1 for sampling design) using a stainless-steel shovel and collected into a pre-rinsed (distilled water and acetone) glass jar and stored at - 20 °C until analysis. Ten samples were collected along the days of drying in column 5 (referred to as T1 to T10) and 15 samples collected randomly across the hall (R1 to R15) (SI Figure S1). The purpose of this sampling strategy was to 1) evaluate and understand the natural distribution in plastics content among biosolid samples from a single WWTP; 2) provide a more accurate assessment of the identity and concentrations of plastics present; and 3) provide guidance on the timing and frequency of sample collection.

2.3 Pressurized liquid extraction

Samples were freeze-dried, milled to fine powder with a commercial grinder for 30 minutes (Extech equipment Pty. Ltd, Victoria, Australia) and shaken using an overhead shaker at 140 rpm for 2 h to homogenize. Following this, ~ 1 g of each sample was extracted by PLE in pre-cleaned 34 ml ASE cells on an ASE 350 system (Dionex, Sunyvale, CA). Extraction was performed with DCM at 180 °C and 1500 psi with a heat and static-time of 5 minutes using three extraction cycles. Extraction parameters used in this study are presented in Table 1, with detailed PLE optimization and validation procedure presented in SI Table S1. The extracts (in solvent) were weighed and 80 µL transferred to a pyrolysis cup (Eco-Cup LF, Frontier Laboratories, Japan) for Pyr-GC/MS analysis. The solvent was evaporated at room temperature for 30 minutes prior to

analysis. It should be noted that the evaporation of the solvent was done in a fume hood to avoid airborne contamination.

2.4 Pyrolysis-GC/MS analysis

Plastic identification and quantification was performed using a double-shot component of a multi-shot micro-furnace pyrolyzer (EGA/PY-3030D) equipped with an auto-shot sampler (AS-1020E) (both Frontier Lab Ltd., Fukushima, Japan) coupled to a GC/MS - QP2010-Plus (Shimadzu Corporation, Japan). The double-shot Pyr-GC/MS technique provides the opportunity for selective sample purification [48,49], as it allows a single sample to be analyzed twice under different temperature conditions, providing a fast and reliable procedure to remove organic materials and analyze plastics in samples [49,50]. The first-shot (thermal desorption), was conducted with a starting temperature of 100 °C, ramped up to 300 °C at 20 ° C min^{-1}, then held at 300 °C for 1 min. The second shot (pyrolysis) was conducted at 650 °C for 0.20 seconds. The pyrolyzer interface and GC injection port temperatures were set at 320 and 300 °C, respectively. The samples were injected with a split of 50:1 on an Ultra Alloy® 5 capillary column (30 m, 0.25 mm I.D., 0.25 µm film thickness) (Frontier Lab). The GC oven column temperature program was as follows: held at 40 °C for 2 min, increased to 320 °C at 20 °C min^{-1}, and held for 14 min. Helium was used as the carrier gas at a 1.0 mL/min with a constant linear velocity. The ion source temperature was kept at 250 °C with an ionization voltage of 70 eV. Scan mode was used with a mass range from 40 to 600 *m/z* (See SI Table S2 for Pyr-GC/MS optimization procedure).

2.5 Plastic indicator compound selection

To identify and quantify single plastics in complex environmental samples, specific indicator compounds for each plastic are required [41,42,44,45,51]. Plastic specific indicator compounds for

PC, PE, PET, PMMA, PP, PS and PVC were determined by analyzing individual reference standards and comparing the pyrograms against literature data [6,41,42,44,52], as well as assessing their specificity against a number of natural materials (chitin isolated from prawns), wood, pine needles, humic acid, cellulose (lab filter paper), fish filet, engine oil, rice and leaves. 1-decene (*m/z* 83) was chosen as the indicator compound for PE as it was the most representative pyrolysis product present at a high abundance, although subject to some minor bias from natural materials (Section 3.3). For PP, 2, 4-dimethyl-1-heptene (*m/z* 126) was specific and was selected as an indicator compound. Methyl methacrylate (*m/z* 100) was specific for PMMA and was selected as an indicator compound. Although styrene (*m/z* 104) was the most abundant indicator compound from the pyrolysis of PS, natural products, such as chitin, wood (lignin) and fish protein, also released styrene as pyrolysis product, hence, styrene trimer (*m/z* 91) was chosen as the PS specific indicator compound [6,41] (see Section 3.3). Benzene (*m/z* 78) was selected as the indicator compound for PVC due the low intensity and sensitivity of the other pyrolysis products. For PC and PET, bisphenol A (*m/z* 213) and vinyl benzoate (*m/z* 105) were selected as the indicator compounds, respectively. (See Table 2 for summary and SI Table S3 for further detail).

2.6 Quality Assurance and Quality Control

Prior to each PLE extraction batch, the PLE cells were conditioned with the optimized parameters to remove possible plastic contamination. Similarly, prior to each Pyr-GC/MS analysis batch, a new pyrolysis cup blank (instrument blank) and a procedural blank were used to demonstrate the absence of detectable plastics in the instrument or method before sample analysis. Procedural blanks were prepared by extracting hydromatrix with the PLE method and then treating the clean hydromatrix as a real sample by including in each batch of biosolid

samples to undergo all procedures. No plastic type was identified in the procedural or instrument blanks (SI Figure S7). Additionally, all plastics components of the PLE instrument were sampled directly into pyrolysis cups and analyzed on the Pyr-GC/MS but no potential contamination was found. Three validation criteria were used for confirming the presence of PE in samples:- (1) the presence of a homologous series of the characteristic PE triplets (alkadiene, n-alkene and n-alkane); (2) the presence of a homologous series of > 5 triplets within $C_7 - C_{41}$ of PE standard and (3) the standard deviation of the peak areas of the individual C_{10} triplet is within 2 times the standard deviation of PE standard (*n* = >5).

3. Results and discussion

3.1 Optimization of experimental parameters

The extraction of seven common plastics (PC, PE, PET, PMMA, PP, PS and PVC) by PLE was optimized and validated individually via modification of the method previously reported by [47] as outlined in SI Table S1. A single step extraction procedure using DCM at 180 °C and 1500 psi was validated because PC, PMMA and PS were quantitatively recovered (> 93 %, *n*= 5) at 100 °C whereas PE, PET, PP and PVC required 180 °C to be quantitatively recovered (> 90 %) (Table 3, and SI Table S4). Recoveries were measured by dry weight of the extracts. FT-IR and Pyr-GC/MS analysis of the extracted residues were similar to that of the original spiking standards, indicating that no significant chemical changes had occurred during the extraction processes [47] (see SI Figure S13 and SI Figure S4). Average recoveries > 80 % were obtained for plastics-spiked biosolid samples (Table 3, and SI Table S5). The recovery results confirm that PLE extraction is suitable for the rapid extraction of plastics from biosolids [47]. Reproducibility analysis of the optimized PLE conditions performed on 3 separate occasions was between 1.4

and 10.6 % (RSD, %) (SI Table S6), confirming that the PLE extraction conditions are accurate and reproducible over time.

Given that PE, PET and PP are poorly soluble in DCM at room temperature, a dissolution analysis of PLE extracted PE, PET and PP was performed over 3 hours post extraction. PE, PET and PP remained in solution over this time (RSD of < 20 % for each) allowing for sufficient time to aliquot into the pyrolysis cup for analysis (see Figure 1 for dissolution analysis of PLE extracted PS, PVC, PP, PMMA, PC, PET and PE at multiple time points post extraction).

Double-shot Pyr-GC/MS was optimized and validated for the analysis of PC, PE, PET, PMMA, PP, PS and PVC. The first-shot was optimized to thermally desorb co-extracted potential interferences (organic materials) that may potentially interfere with the pyrogram without degrading the target plastics. This was conducted through progressive heating of the sample extract (100 - 300 °C, 100 - 320 °C and 100 - 340 °C) and confirming that no plastic was measured. Similarly, the second-shot (pyrolysis) temperature was optimized to pyrolyze plastics – PC, PMMA and PS (550, 600, 650 and 700 °C) and assessed by comparing peak areas of their indicator compounds. The decomposition products of all plastic standards were stable up to the pyrolysis temperature of 650 °C (e.g. SI Figure S2). None of the decomposition products of plastics-spiked in biosolids were observed in the ion chromatogram of the thermally desorbed organic fraction. An optimum temperature of 100 - 300 °C was chosen for the thermal desorption step (first-shot) as it had no measurable effect on the accuracy of plastic identification and quantification. The absolute peak areas of all the pyrolysis indicator compounds of PC, PMMA and PS were significantly impacted by changes to the pyrolysis furnace temperatures. A rise in response was observed when the pyrolysis temperature was increased from 550 to 650 °C but declined at 700 °C (SI Figure S3). The optimum temperature

for pyrolysis analysis was found at 650 °C since it revealed higher peak areas for all the indicator compounds.

3.2 Method performance and validation

An external calibration curve was prepared by weighing plastic standards (from 0.5 to 100 µg), mixing with pre-washed hydromatrix (milled to fine powder), PLE extracted and aliquoted into pyrolysis cups. With a split of 50:1 the calibration range of each plastic was from 0.01 to 2 µg on column having $R^2 \geq 0.93$ (Table 3 and SI Figure S11). Plastic standards were measured individually and in mixtures. The limit of quantification (LOQ) for each plastic was defined as the lowest detectable standard (lowest concentration) of the calibration curve and also in pooled biosolid which produced a signal 10 times the baseline noise (*S/N* 10) and where the RSD of 7 replicate injections was < 20 % (Table 3). Intra- and inter day variation was calculated as the RSD % of five repeated analyses of a plastic (5 µg) on the same day and over 5 days respectively, with precision between 3.1 – 12.0 % (Table 3). Three recovery experiments containing all seven reference plastics at known concentrations (20 to 100 µg), were included in this study and underwent the same treatment as the biosolid samples (see SI Table S10 for detail spiking procedure). Acceptable mean recoveries were between 84 % and 109 % (n = 3) for all plastics (SI Table S7). Matrix interferences were investigated by spiking four of the analyzed biosolid samples with concentrations of plastics (20 to 100 µg) (see SI Table S10 for detail spiking procedure). The mean recoveries of the spiked plastics ranged from 85 % to 128 % (n = 4) (SI Table S8) indicating that the results were not influenced by matrix interferences. Method performance was evaluated by analyzing extracted PS standard and PS spiked biosolid, with an acceptance criterion of less than 20 % drift from the spiked concentration. Method

reproducibility was assessed by a laboratory duplicate analysis of selected biosolid samples, with comparable levels observed for all samples (Figure 3).

3.3 Potential Matrix Interferences

Natural materials are a potential source of interfering indicator compounds during pyrolysis [41,45]. The potential for their formation was evaluated by analyzing a number of organic materials and biogenic polymers such as chitin isolated from prawn, wood (lignin), pine needles, humic acid (organic matter), cellulose (lab filter paper), fish filet (proteins, fat), engine oil (hydrocarbons), rice (carbohydrate) and leaves (cellulose, organic matter) as previously reported by [41,45]. None of the natural materials produced interfering indicator compounds for PP, PET, PC, PMMA and PS when using thermal desorption (first-shot of the double-shot method) as a clean-up step (Table 4). Chitin, wood and fish protein released styrene (PS monomer) during the pyrolysis process as previously reported [6,41,45], hence, PS trimer is therefore used as the indicator compound for PS. Phenylalanine is a known precursor for styrene formation during pyrolysis with no PS trimer formed [41,45].

The formation of various indicator compound interferences for PE has previously been reported following the pyrolysis of a range of natural products [45]. Commonly, biogenic materials such as natural fats (e.g. fish protein) and waxes that are rich in long alkyl chains have been reported to produce n-alkanes and n-alkenes during pyrolysis [41,45,53]. Most of the materials tested produced traces of 1-decene, however these background interferences were typically at or below the LOQ (0.03 mg/g) following using thermal desorption as a clean-up step (Table 4). 1-decene was chosen as an indicator compound in this study due to its higher sensitivity and lower LOQ compared with other alkanes and alkadiene pyrolysates of PE [45]. A check of all samples against the three PE validation criteria outlined in Section 2.6 were found

285 to conform, with chain lengths in the range of 7 – 36 carbon atoms. This indicates that the
286 measured PE concentration is free of positive or negative bias from matrix effects and
287 interfering environmental sources of the matrix compounds.

288 Humic acid (organic matter), rice (carbohydrate), fish filet (proteins, fat), engine oil
289 (hydrocarbons) and wood (lignin) caused an increased background interference for the analysis
290 of PVC, however these interferences were below the LOQ (0.03 mg/g) following the thermal
291 desorption clean-up step (Table 4). We therefore accept that the quantification of PE and PVC
292 in the biosolid samples are potentially subject to a minor source of positive bias, however these
293 are typically at or below LOQs (Table 4).

294 3.4 Case study- distribution of plastic concentration in a municipal biosolid 295 samples

296 The optimized and validated method was applied to 25 biosolid samples collected from a
297 WWTP (a single treatment train) in South-East Queensland, Australia. The pyrograms of the
298 analyzed biosolid samples featured specific compounds clearly related to the presence of PE,
299 PP, PVC, PS and PMMA (see SI Figure S6 and Table S9 for example). All samples contained PE,
300 PP and PVC, while PS was found in 80 % and PMMA in 50 % of samples. Concentrations of
301 measured plastics ranged from 0.1 to 4.1 mg/g dw and were consistent between all samples
302 (Table 5, Figure 2). PE ranged from 0.7 to 4.1 mg/g dry weight (dw) (median = 1.9 mg/g dw),
303 PP from 0.2 to 1.5 mg/g dw (median = 0.6 mg/g dw), PVC from 0.7 to 1.2 mg/g dw (median =
304 0.9 mg/g dw), PS from 0.1 to 0.9 mg/g dw (median = 0.4 mg/g dw), and PMMA from 0.4 to 0.9
305 mg/g dw (median 0.5 mg/g dw) (Table 5, Figure 2). The average concentrations of total plastics
306 ($\Sigma_{Plastics}$) in the biosolids ranged from 2.8 mg/g dw to 6.6 mg/g dw with a median value of 4.1
307 mg/g dw (Figure 2, Table 5).

PE was the predominant plastic detected in biosolids (mean concentration of 2.2 mg/g dw), contributing to 50 % of the total sum of all plastics (Table 5, SI Figure S12). The total individual sum concentrations of PE, PP, PVC, PS and PMMA in the biosolid samples ranged from 6 to 55 mg/g dw (significantly different from each other ($p < 0.05$, ANOVA)) with a total sum of 109 mg/g dw. (Table 5). Similarly, the total individual sum of plastics observed for the randomly collected samples, (PE = 33 mg/g dw, PP =11 mg/g dw, PVC =13 mg/g dw, PS = 5 mg/g dw and PMMA = 2 mg/g dw) were slightly higher than the samples taken along the days of drying (PE =22 mg/g dw, PVC = 9 mg/g dw, PP = 6 mg/g, PS= 4 mg/g dw, and PMMA = 4 mg/g dw) except for PMMA.

The variance in plastic concentration, both for the sum of all analyzed plastics and for the individual plastics, between all samples was relatively low, hence, the distribution throughout the biosolids treatment train can be considered relatively homogenous. To test for reproducibility and sensitivity of the method, duplicate analysis (i.e., splitting 6 samples into two) was as assessed. Analytical reproducibility was found as RSD = 20 % across all detected plastics (Figure 3). As such analytical variability was only a very minor source of uncertainty.

4. Conclusions

The results of this work demonstrate that single step PLE coupled with double-shot Pyr-GC/MS is a suitable method for the rapid and effective identification and quantification of PE, PP, PVC, PMMA and PS in biosolid samples and may be suitable for other plastic types and environmental matrices. The unique use of the double-shot feature for the effective thermal desorption of potentially interfering co-extracted compounds from the biosolid samples provides an improved alternative for the identification and quantification of plastics in complex organic rich samples. This reduces processing and labor times needed to pre-treat samples.

The sensitivity of the method proves the advantage of using Pyr-GC/MS to measure the mass load concentrations of specific plastics in samples which provides a basis for the uniform reporting of results as compared to the use of conventional FT-IR and Raman. In particular, the results are obtained as mass concentration which is a more standardized and reliable way for comparison of data than particles number which can be difficult when comparing between locations [42,47]. It should however be noted that the mass concentrations provided by this method are at the same time highly complementary to conventional FT-IR and Raman analysis of particle counts that provides information on particle size, shape, and color [42,47].

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6. Supporting Information

Additional information on sampling design; optimization procedure of PLE; optimization procedure of Double-Shot Pyrolysis method; recovery and spike experiments on PLE; thermal desorption and pyrograms of PS, PE, PP PET, PMMA and PC at 100 - 300 °C and 650 °C

respectively; influence of Pyr-GC/MS temperature on peak response of PMMA, PS, and PC; comparison of pyrograms of extracted PP and PE at 180 °C and the original spiked material; comparison of a single shot and a double-shot pyrograms of extracted sewage sludge; recovery and spike experiments on PLE combined with Pyr-GC/MS; and typical characteristics of real biosolid sample on a double-shot Pyr-GC/MS.

7. Conflicts of interest

There are no conflicts of interest to declare.

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