Journal of Chromatography A, 1339 (2014) 34–41

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A quick, easy, cheap, effective, rugged and safe extraction method followed by liquid chromatography-(Orbitrap) high resolution mass spectrometry to determine benzotriazole, benzothiazole and benzenesulfonamide derivates in sewage sludge☆

Pol Herrero, Francesc Borrull, Eva Pocurull*, Rosa M. Marcé

Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Sescelades Campus, Marcel·lí Domingo s/n, 43007 Tarragona, Spain

ARTICLE INFO

Article history:
Received 22 January 2014
Received in revised form 25 February 2014
Accepted 27 February 2014
Available online 5 March 2014

Keywords:
Benzotriazoles
Benzothiazoles
Benzenesulfonamides
QuEChERS
Liquid chromatography-high resolution mass spectrometry
Sewage sludge

ABSTRACT

A Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method followed by liquid chromatography-(Orbitrap) high resolution mass spectrometry was developed for the simultaneous determination of five benzotriazole, four benzothiazole and five benzenesulfonamide derivates in sewage sludge. While the method was being developed, several buffers and dispersive solid-phase extraction clean-up (dSPE) sorbents were tested. Citrate buffer and Z-sep+ (zirconium-based sorbent) were the most effective extraction buffer and dSPE clean-up material. The absolute recoveries were higher than 80% for all compounds (100 ng/g (d.w.)) and the matrix effect was less than −20% for most compounds. The limits of detection were between 0.5 and 10 ng/g (d.w.) and the limits of quantification (LOQ) were between 1 and 25 ng/g (d.w.). Repeatability and reproducibility were lower than 15% (%RSD, $n = 5$). Several sludge samples from five sewage treatment plants in Catalonia were analysed and the most abundant compounds were 2-hydroxybenzothiazole (<LOQ-181.2 ng/g (d.w.)) and 4-methyl-1-H-benzotriazole (<LOQ-82.3 ng/g (d.w.)). Benzenesulfonamide (n.d.-70.2 ng/g (d.w.)) and toluenesulfonamide (n.d.-83.9 ng/g (d.w.)) were also determined.

1. Introduction

Sewage sludge is the solid residue resulting from sewage treatment in sewage treatment plants (STPs) and it is often reused, particularly for agricultural applications, one of the most sustainable options. Sewage comes from such discharging sources as households, industry and hospitals, and sludge contains organic contaminants or their metabolites and transformation products. Therefore, this option for managing sewage sludge may cause the intrusion of organic contaminants into agricultural land, resulting in an ecological and/or health risk [1].

Benzotriazole (BTRs), benzothiazole (BTs) and benzenesulfonamide (BSAs) derivates are well-known aquatic contaminants released from industries and households in, for example, metal corrosion inhibitors or plasticizers. Their presence in sewage has been thoroughly investigated [2–11] but the information about their occurrence in sewage sludge is limited to a few papers [5,12–15] on BTRs and BTs. No information about BSAs has been reported. Some of these contaminants have been classified as toxic to aquatic organisms and bacteria with long-term adverse effects [3,16,17] and they are resistant to biodegradation [18].

Nowadays, laboratories monitor concentrations of several chemical pollutants in a variety of environmental samples. Nevertheless, depending on the analytical method used this work can be tedious so several analytical methods have focused on reducing time and costs. One of these methods is QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), an extraction technique based on solid–liquid extraction that revolutionized the determination of pesticide residues in food matrices [19]. Some authors have suggested that it can be used in various solid matrices [20–25].

The extraction techniques that have most commonly been used with sewage sludge samples are pressurized liquid extraction (PLE), microwave assisted extraction (MAE) and ultrasound assisted solvent extraction (USAE) [1,26]. These techniques provide good extraction efficiencies for several compounds but most of them require sophisticated equipment, take a relatively long time or consume considerable amounts of solvent. Moreover, a solid-phase

☆ "Presented at the XIII Scientific Meeting of the Spanish Society of Chromatography and Related Techniques (SECyTA2013), 8–11 October 2013, Puerto de la Cruz, Tenerife, Canary Islands, Spain.".

* Corresponding author. Tel.: +34 977 55 84 92; fax: +34 977 55 84 46.
E-mail address: eva.pocurull@urv.cat (E. Pocurull).

http://dx.doi.org/10.1016/j.chroma.2014.02.081
0021-9673/

extraction (SPE) is usually performed after solvent extraction to reduce matrix interferences and improve limits of detection (LODs).

The QuEChERS method has recently been applied to determine a wide range of pharmaceutical compounds [21] in sewage sludge for the first time with very promising results. It has also been used to extract polycyclic aromatic hydrocarbons [27] and chlorinated compounds [23] from soil samples. Nonetheless, it has not yet been applied to other compound families such as BTRs, BTs and BSAs. QuEChERS methods use a single step buffered acetonitrile extraction and simultaneously salt out water from the aqueous sample using anhydrous magnesium sulphate to induce liquid–liquid partitioning. Subsequently, a clean-up step using a dispersive solid-phase extraction (dSPE) is often conducted to reduce matrix interferences [20]. The most frequent analytical techniques used to determine BTRs, BTs and BSAs are LC [2,3,28] and GC [7,29,30] coupled to MS or MS/MS and HRMS [18,30,31] by Orbitrap based mass analysers or hybrid Q-TOF analysers.

This study, then, focuses on developing a method that uses QuEChERS extraction (including the study of various dSPE sorbents to reduce the matrix effect in sludge samples) followed by LC-(Orbitrap) HRMS to determine five BTRs, four BTs and five BSAs in sewage sludge. Little is known about the occurrence of these compounds in this kind of sample and this study will extend our knowledge by analyzing several sludge samples from different STPs.

2. Experimental

2.1. Reagents and standards

The chemical standards of five benzotriazole derivates, four benzothiazole derivates and five benzenesulfonamide derivates were purchased from Sigma–Aldrich (St. Louis, USA). They were, respectively 1-H-benzotriazole (BTR), 4-methyl-1-H-benzotriazole (4TTR), 5-methyl-1-H-benzotriazole (5TTR), 5,6-dimethyl-1H-benzotriazole (XTR) and 5-chloro-1-H-benzotriazole (ClBTR); benzothiazole (BT), 2-aminobenzothiazole (NH_2BT), 2-hydroxybenzothiazole (OHBT) and 2-(methylthio)benzothiazole (MeSBT); and benzenesulfonamide (BSA), *orto*-toluenesulfonamide (*o*-TSA), *para*-toluenesulfonamide (*p*-TSA), *N*-methyl-*para*-toluenesulfonamide (Me-*p*-TSA) and *N*-ethyl-*para*-toluenesulfonamide (Et-*p*-TSA). Stock solutions of individual standards at 1000 mg/L were prepared in methanol and stored at −20 °C. A mixed working solution of 10 mg/L was prepared weekly in methanol. The chemical structures of the compounds studied are shown in Fig. S1. 2-chlorobenzothiazole (ClBT) and 4-bromobenzenesulfonamide (BrBSA) were tested as internal standards (IS) and were from Sigma–Aldrich. Stock solutions of each IS at 1000 mg/L and a mixed working IS solution of 100 mg/L were prepared in methanol and stored at −20 °C.

Ultrapure water was obtained using an ultrapure water purification system from Veolia Waters (Sant Cugat del Vallés, Spain). Acetonitrile (ACN) and methanol (MeOH) were of HPLC grade and supplied by Prolabo (VWR, Llinars del Vallès, Spain). Acetic acid, ammonium acetate and ammonium hydroxide (LC-MS grade) were purchased from Sigma–Aldrich and nitrogen gas was provided by Carburos Metálicos (Tarragona, Spain).

2.2. Sampling

The sewage sludge samples were collected from five STPs in Catalonia (Spain) located in Tarragona (STP1), Reus (STP2), Castell-Platja d'Aro (STP3), Blanes (STP4), and Palamós (STP5). Two sewage sludge samples were collected from each STP in different months. These STPs receive urban sewage and industrial discharges from a population of between 100,000 and 200,000 inhabitants and they use activated sludge for biological treatment. They all use anaerobic digestion, except for STP3 which uses aerobic digestion. STPs 3, 4 and 5 are equipped with tertiary sewage treatments. Sewage sludge was dehydrated by centrifugation, in most of cases, or by press filters. The sludge samples collected were frozen before being lyophilized and were then crushed in a mortar and pestle and sieved (125 μm) to obtain particles with the same diameter.

Spiked samples for purposes of optimization were prepared by adding the stock mixture of standards in acetone (the required volume to wet and cover the sludge). The solvent was slowly evaporated at room temperature inside an extractor hood and the sample was frequently homogenized during the 2 days before extraction to ensure good interaction between the compounds and the matrix.

2.3. LC-(Orbitrap)HRMS analysis

An Accela 1250 UHPLC chromatograph coupled to an Orbitrap/Exactive mass analyser, both from Thermo Fisher Scientific (Bremen, Germany), were used for LC-HRMS measurements. It was equipped with a quaternary pump (1250 bar) and an Accela autosampler, made up of an automatic injector (refrigerated at 10 °C) and a column oven (heated at 50 °C). The electrospray interface was a heated electrospray ionization source (HESI-II). The chromatographic separation was achieved with an Ascentis Express C_{18} column (100 × 2.1 mm, 2.7 μm fused core particle size) from Supelco (Sigma–Aldrich) under gradient elution conditions. The mobile phase was ultrapure water/acetonitrile (98:2) 0.1% CH_3COOH (solvent A) and methanol (Solvent B). It started isocratic at 0.5% B for 5.25 min and then increased to 18% in 3.5 min. After remaining constant for a further 1.25 min, it increased to 35% in 6 min and then up to 95% in another 4 min. It then remained constant for 4 min and returned to initial conditions in 1 min. The flow rate was 800 μL/min and the injection volume was 20 μL. All of the compounds were eluted in less than 18 min.

To optimize HRMS measurements, a mixture of all compounds was infusioned in the source with a syringe pump connected with the column flow conditions. Depending on the compound, either precursor ion $[M+H]^+$ or $[M-H]^-$ were monitored to determine the parameters that lead to best response in each mode, positive or negative, in full-scan at a resolution of 50,000 FWHM over a mass-range of 50–500 Da. Thus, the optimized spray voltage was 4 kV in both ionization modes, the capillary voltage was 37.5 and −40 V, the tube lens voltage was 90 and −90 V and the skimmer voltage was 20 and −25 V, in the positive and negative mode, respectively. Sheath gas was set to 55 AU and auxiliary gas to 20 AU, the transfer tube was set to 350 °C and the heater temperature was set to 400 °C in both ionization modes.

Four time windows were used. Two in negative mode (0–2.3 and 4.8–7.6 min) and two in positive mode (2.3–4.8 and 7.6–20 min) with two scan events in each time window. The scan events were: one in full-scan mode (at 50,000 FWHM with an injection time of 250 ms) and the other in all ion fragmentation mode (at 10,000 FWHM with an injection time of 50 ms) at 25 eV the HCD cell in both negative and positive ionization mode. To follow the guidelines of the European Directive 2002/657/EC [32], at least two product ions for each compound (Table S1) were used for confirmation purposes.

2.4. QuEChERS extraction

One gram of freeze-dried sludge was weighed into a 50 mL centrifuge tube from Scharlab (Sentmenat, Spain), 10 mL of cooled water was added to the tube, and the tube was shaken vigorously for 1 min. Then, 10 mL of ACN was added, followed by an extraction salt packet (Scharlab) for the European Committee for Standardization (CEN) extraction method [33], which contains 4 g of anhydrous