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Improved sample treatment for the determination of bisphenol A and its chlorinated derivatives in sewage sludge samples by pressurized liquid extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

A selective, sensitive, robust and accurate method for the determination of bisphenol A (BPA) and its chlorinated derivatives in sewage sludge samples using liquid chromatography–tandem mass spectrometry (LC–MS/MS) is presented. Prior to instrumental analysis, an extraction procedure using pressurized liquid extraction (PLE) was carried out in order to obtain the highest recoveries and improve sensitivity. After LC separation, the MS conditions, in negative atmospheric pressurized chemical ionization (APCI) mode, were individually optimized for each analyte to obtain maximum sensitivity in the selected reaction monitoring (SRM) mode. The use of two reactions for each compound allowed simultaneous quantification and identification in one run. The analytes were separated in less than 6 min. BPA-d_{16} was used as internal standard. The limits of detection of the method ranged from 4 to 8 ng g^{-1} and the limits of quantification from 14 to 26 ng g^{-1}, while inter- and intra-day variability was under 6% in all cases. Due to the absence of certified materials, the method was validated using matrix-matched calibration and a recovery assay with spiked samples. Recovery rates ranged from 97.7% to 100.6%. The method was satisfactorily applied for the determination BPA and its chlorinated derivatives in sewage sludge samples collected from wastewater treatment plants (WWTPs) located in the province of Granada (Spain). The sludge samples came from a conventional activated sludge (AS) plant and from a membrane bioreactor (MBR) pilot plant.

1. Introduction

The impact on human health and environment of endocrine disrupting chemicals (EDCs), even at ng L^{-1} levels, is increasingly becoming an important focus for scientific research [1]. EDCs encompass a wide variety of synthetic and natural chemicals that have the ability to mimic hormones and might, therefore, interfere or disrupt normal hormonal functions [2]. Exposure to EDCs has become a highly controversial public health issue. Although sexual differentiation has been the major endpoint for the toxicological assessment of EDCs, concern with these substances also stems from their potential to affect reproductive, metabolic, immune and development functions, growth, behaviour and memory [3]. Effects of EDCs are associated with reduced fertility, congenital malformations of the reproductive tract, and increased incidence of cancer in estrogen-responsive tissues [4]. Recent studies are focused on anthropogenic EDCs, such as synthetic hormones used as contraceptives, a variety of pharmaceuticals and personal care products, as well as large amounts of industrial chemicals, with bisphenol A (BPA), PCBs, dioxins, pesticides, phthalates, alkylphenols and alkylphenol ethoxylates being of special importance [5].

In recent years, most attention has focused on exposure to BPA, a widely used industrial plasticizer with known estrogenic properties. Over 2.000 million tons/year of BPA are used in the manufacture of epoxy resins and polycarbonate plastics, which are, in turn, used in a wide variety of domestic products [6]. BPA is present in dental fillings, plastic food and water containers, baby bottles, food wrap, as well as in the lining of beverage and food cans, presenting a large number of routes for human exposure. Numerous studies have confirmed leaching of BPA from food containers, and detectable levels of BPA have been found in a wide range of packaged foods [7] being oral exposure the primary source of human exposure to BPA [8]. BPA also accounts for most estrogenic activity that leaches from landfills into the surrounding ecosystem; effluent from industrial activity, including treatment of leachate, may serve as an additional route of human exposure, particularly if it finds its way into aquatic species [9]. Wastewater containing BPA is also a source of contamination of aquatic environments from where BPA could reach ground

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waters, rivers, streams and, eventually, drinking water, resulting in a continuous low-level exposure to BPA [6,7]. Even the low levels of BPA found in aquatic ecosystems pose a serious threat to aquatic life [10].

On the other hand, due to its highly lipophilic behaviour, BPA also tends to strongly associate with particulate matter and can, therefore, be found in solid matrices, such as sewage sludge produced during wastewater treatment [11,12]. This represents a serious hazard because the use of sewage sludge as organic amendment of soils has become a common practice in Europe since the last decade, facilitating the "arrival" of these pollutants to humans through the food chain [13]. Given the ubiquity of BPA in human environments, it is not surprising that exposure to BPA is virtually universal. It is also known that BPA easily reacts with sodium hypochlorite – used as bleaching agent in paper factories and in water disinfection – to produce chlorinated derivatives of BPA (mono-, di-, tri- and tetra-chlorobisphenol A) (ClBPAs) that can be released into the environment [14]. BPA chloroderivatives, especially tri- and tetra-chlorobisphenol A, also represent a serious concern, because in both, in-vitro and in-vivo studies, they have proved to be even more active than BPA in competing with 17β-estradiol for human estrogen receptors-α and -β (ER-α and ER-β) binding sites [15], even at lower concentrations than BPA [14,16]. Furthermore, using human breast carcinoma MCF7 cells, it was determined that ClBPAs had greater potential to stimulate growth than BPA [17], which was also confirmed in in-vivo experiments performed with ovariectomized female rats. In addition to their strong estrogenic activity, BPA and their chloroderivatives caused a significant increase in the weight of the uterine tissue and endometrium, leading to an increased risk of cancer. Chronic exposure to ClBPAs even at very low doses may cause more uterotrophic activity than BPA [17]. In chicken and frogs, it was reported that ClBPAs inhibited the binding of 3,3′, 5-triiodothyronine (T3) to transthyretin (TTR), responsible for the plasma transport of thyroid hormone, more strongly than BPA [18].

On the other hand, the effects of chlorination on the acute toxicity of BPA and its derivatives have raised concerns because they have not been clarified yet. It is known that ClBPAs can photodegrade, producing more toxic oxygen reactive species. Acute cytotoxicity of ClBPAs was increased by UVB and UVC irradiation [19]. These results are interesting, because ClBPAs, which return to the environment from wastewater treatment after a slow and even failed biodegradation [20,21], will be exposed to sunlight that will enhance their cytoxicity by the generation of photodegradation subproducts.

Moreover, it is known that BPA is metabolized to glucuronide in rat liver [22] and the metabolites are rapidly eliminated in the faeces and urine, but ClBPAs are degraded slowly and accumulated through the food chain in the human body where they act as persistent EDCs.

There is a growing need to determine the fate of EDCs in the environment, since it has been reported that EDCs with high estrogenic activity have a great tendency to associate with particulate matter and sediments [23]. However, although there are data regarding the levels of BPA in treated sludge, the same is not true for its chlorinated derivatives, and this information is required to determine which processes could improve the removal of these EDCs from wastewaters in WWTPs. Furthermore, the determination of the partition coefficient between the solid and liquid phases in biological treatment units will certainly help to explain the fate and behaviour of BPA and its derivatives in WWTPs. Since the studies involving the determination of the dissolved phase fraction of these contaminants are much more numerous than those involving the determination in solid matrices, it seems reasonable to develop selective, sensitive and robust analytical methods for determination of these substances in treated sludge.

Until the 1990s, traditional approaches for the extraction of EDCs in solid matrices were based almost exclusively on Soxhlet extraction and steam-distillation. However, these techniques make the analysis procedure excessively time consuming (up to 48 h) and require large amounts of hazardous organic solvents [24]. To overcome these limitations, new extraction approaches have been developed for the extraction of organic pollutants. One of the most widely used techniques for sewage sludge matrices is ultrasound-assisted extraction (USE) [25–28]. Although this technique is considerably faster than Soxhlet extraction, it also requires relative large volumes of toxic and costly organic solvents. More efficient techniques have therefore been developed such as pressurized liquid extraction (PLE) [29–31] or microwave-assisted extraction (MAE) [32]. PLE is a very efficient technique that can be applied to thermally stable compounds. It offers important improvements over other techniques including shorter extraction time, lower amount of solvent, higher level of automation and the ability to perform multiple extractions simultaneously [33]. Moreover, although there are several methods for the determination of BPA in sludge matrices, almost all of them include a solid-phase extraction (SPE) procedure after the extraction process; this improves preconcentration of the analytes and help reduce the matrix effect. However, these extraction methodologies lead to a long, tedious and expensive analytical process without a clear improvement of the final extracts. It is important to highlight that there are not methods for the determination of each and every chlorinated BPA in sewage sludge. Table 1 summarizes the most relevant methods available.

The main objective of the present work is to develop a rapid, robust, sensitive and accurate method for the determination of BPA and its chlorinated derivatives in sewage sludge samples using PLE followed by LC–MS/MS analysis. In addition, the efficiency of the SPE procedure to reduce the matrix effect is also evaluated. The method will allow the analysis of a larger number

Table 1
Analytical methods for the determination of BPA and derivatives in sewage sludge.

Analyte	Analytical technique	LOQ	Reference
BPA	USE–SPE–GC–MS	130 ng g^{-1} (LOD)	[35]
BPA	USE–SPE–GC–MS	108 ng g^{-1}	[25]
BPA	Selective pressurized liquid extraction–GC–MS	35.7 ng g^{-1}	[37]
BPA	USE–SPE–GC–MS	1.84 μg g^{-1}	[27]
BPA	USE–SPE–LC–MS/MS	9.8 ng g^{-1} (LOD)	[28]
BPA	MAE–SPE–LC–MS/MS	2.3 ng g^{-1}	[32]
BPA	USE–SPE–UHPLC–MS/MS	0.7 ng g^{-1}	[36]
BPA and Cl_4-BPA	Soxhlet–SPE–LC–MS/MS	0.15 ng g^{-1} (BPA) 0.03 ng g^{-1} (Cl_4-BPA)	[34]

LOQ, limit of quantification; LOD, limit of detection; SPE, solid-phase extraction; LC, liquid chromatography; MS, mass spectrometry; USE, ultrasound extraction; MAE, microwave assisted extraction; GC, gas chromatography, UHPLC, ultra high performance liquid chromatography.