

ASSESSMENT OF THE PRESENCE OF SOME EDCs IN SEWAGE SLUDGE SAMPLES USING A MAE-SPE-LC-MS/MS METHOD

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Abstract

A microwave assisted extraction followed by liquid chromatography–electrospray ionization tandem mass spectrometry method (MAE-LC-ESI-MS/MS), has been successfully applied to the determination of some endocrine disrupting chemicals (EDCs) in sewage sludge samples. This method provides recoveries between 75% and 102% with relative standard deviation lower than 9%, and limits of detection ranging from 0.1 to 0.5 ng·g⁻¹. In this work, we show the results of applying this methodology to sludge samples from two wastewater treatment plants (WWTPs) located on the island of Gran Canaria, Spain. All compounds under study (nonylphenol (NP), octylphenol (OP), and their short ethoxylated chains APnEOs (n≤2), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE) and bisphenol A (BPA)) were found in all samples in concentrations ranging from 0.07 to 1.2 μg·g⁻¹.

Keywords: Endocrine-disrupting chemical, mass spectrometry, wastewater treatment, environment

Introduction

Nowadays, it is widely demonstrated that endocrine system of wildlife and humans can be altered by many chemical substances which can come from both natural and anthropogenic source (Schwarzenbach et al, 2006). The list of chemicals observed to be capable of disrupting the endocrine system continues to grow, and this growth may continue while the study of the so-called emerging contaminants remains a subject of scientific and environmental interest.

Due to the highly lipophilic behavior of many EDCs, including the more oestrogenic compounds, it is expected that these chemicals tend to associate strongly to particulate matter, and therefore to the sediments. So, the determination of these substances in the sewage sludges has a great importance since the WWTPs act like a sink of organic compounds, and therefore, like a potential emissary of these substances to the environment if the removal is not complete. In addition, the use of sewage sludge from wastewater treatment plants as organic amendment has become usual in Europe during the last

decade to mitigate the low productivity or profitability of several agriculture soils (Andreu et al, 2007), which facilitates the “arrival” of these pollutants to humans through the food chain.

Given the above, the aim of this work was the application of a simple, rapid and sensitive methodology for the determination of NP, OP, and their short ethoxylated chains AP_nEOs (n≤2), BPA and the steroidal hormones 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE) in sewage sludge samples collected from two WWTPs in the island of Gran Canaria (Spain). The first of the two treatment plants under study had a conventional activated sludge treatment (AST), while the second treatment plant had an advanced membrane bioreactor treatment (MBR).

Methods

The apparatus was composed of a Varian 320-MS TQ Mass Spectrometer (Varian Inc., CA, USA) equipped with a Varian LC system consisting of a binary pump, autosampler and temperature controlled column compartment. The optimisation of MS/MS conditions for each compound was performed by direct infusion of pure standards (see Table 1). For the chromatographic separation, a Pursuit XRs Ultra-C18 reversed phase column (2.8 μm particle size, 50mm×2mm i.d.) from Varian Inc. (CA, USA) was employed. The mobile phase consisted of water (solvent A) and methanol (solvent B) with 0.1% (v/v) glacial acetic acid and 15mM ammonium acetate. Gradient elution consisted of a solution of 30:70 (v/v) methanol:water for 9min, followed by an increase in methanol to 100 % over 10 min. The injection volume was 10 μL and the flow rate was 200 μL·min⁻¹ for 10 min. The temperature in the column compartment was set to 40° C.

The optimum conditions for the microwave assisted extraction were as follow: sample amount, 1 g; extraction solvent volume (methanol), 5 mL; power, 200 W; extraction time, 5 min. MAE sample extracts were filtered through a 45μm pore size nylon filter and were mixed with 95 mL of Milli-Q water prior to the SPE clean-up procedure. The optimum conditions for the solid phase extraction clean-up procedure were as follow: cartridge, Sep-Pak C18 from Waters (Mi, USA); adsorption volume, 100 mL; desorption volume, 2 mL of methanol; adsorption flow rate, ~ 10 mL·min⁻¹; desorption flow rate, ~ 2 mL·min⁻¹.

Table 1. Characteristic of ESI/MS/MS parameters for each compound studied;^a fragment ion used for quantitation (MRM).

Compound	<i>m/z</i> Precursor [M+NH ₄] ⁺	<i>m/z</i> Precursor [M-H] ⁻	Cone (V)	Fragment ions (Collision potential)	Ion mode
NP ₁ EO	282.3	-	30	265.3 (6) ^a , 127.1 (8)	ESI+
NP ₂ EO	326.3	-	30	183.1 (9) ^a , 121 (20)	ESI +
OP ₁ EO	268.1	-	30	251.1 (6) ^a , 113 (7.5)	ESI +

OP ₂ EO	312.3	-	30	183.0 (9.5) ^a , 121 (19.5)	ESI+
NP	-	218.7	-64	105.7 (20.5) ^a	ESI-
OP	-	204.7	-72	134 (16.5) ^a , 106 (19.5)	ESI-
BPA	-	226.7	-60	211.7 (17.5) ^a	ESI-
E2	-	271.1	-60	183.5 (14.5) ^a , 145.2 (20.5)	ESI-
E3	-	287.2	-67	171.0 (16.5) ^a , 145.2 (19.5)	ESI-
EE	-	295.3	-71	159.5 (17.5) ^a , 145.2 (21.5)	ESI-

Results and discussion

The optimized MAE-SPE-LC-MS/MS methodology reported recoveries ranging from 75% to 102% with relative standard deviation lower than 9%. The limits of detection (LODs) and limits of quantification (LOQs) were in the range of 0.1–0.5 and 0.3–1.7 ng·g⁻¹ respectively. The sampling was carried out in two WWTPs located in the north-east of Gran Canaria Island. The first one presented a conventional activated sludge treatment (AST), while the second treatment plant had an advanced membrane bioreactor treatment (MBR). Due to the closeness between the two treatment plants, water input to both plants was very similar in nature and composition.

In Fig 1, the concentrations of all EDCs in both WWTPs under study are highlighted. As can be seen, all compounds have been detected and quantified in the two sludge samples, presenting concentrations ranging from 0.1 to 1.2 µg·g⁻¹. The results for both treatment plants showed higher concentrations in the sample from AS treatment plant than in the sample from MBR treatment plant, except for NP₂EO and for OP₁EO. The observed phenomena can be explained attending to the higher removal of the analytes in the MBR and the similarities between the two input sources.

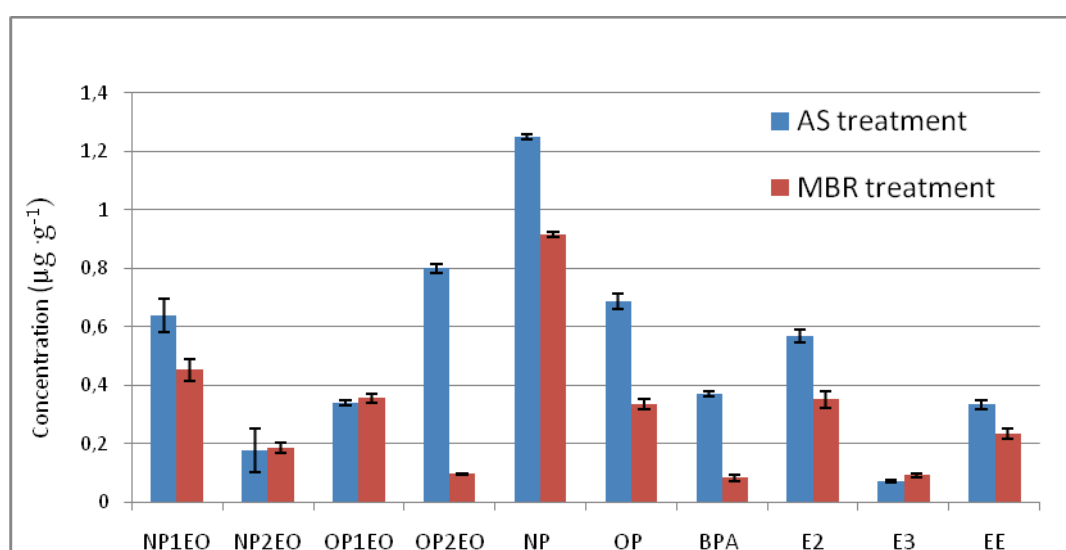


Figure 1. Concentration of each analyte obtained from sewage sludge samples taken from the two WWTPs under study

Conclusions

The application of the methodology to samples from a WWTP revealed that all of the target analytes were present in the two samples analyzed, with concentrations on the order of $\mu\text{g}\cdot\text{g}^{-1}$. This is the first study to report the occurrence and concentration of EDCs in WWTPs sludge samples in Gran Canaria Island. Further investigation regarding chronic effects and their consequences from the exhibition to these pollutants in aquatic organism must be studied.

References

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