

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

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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).

Due to the highly lipophilic behavior of many endocrine disrupting chemicals (EDCs), including the more oestrogenic compounds, it is expected that these chemicals tend to associate strongly to particulate matter, and therefore to the sludges and sediments.

In addition, the use of sewage sludges 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.

The sampling was carried out in two WWTPs located in the north-east of Gran Canaria Island, Spain. 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.

This method provides recoveries between 75% and 102% with relative standard deviation lower than 9%, and limits of detection ranging from 0.7 to 3.5 ng·g⁻¹. All compounds under study were found in all samples in concentrations ranging from 0.07 to 1.2 μg·g⁻¹. Moreover, concentrations obtained in AST plant were significantly higher than those observed in MBR plant.

EXPERIMENTAL PROCEDURE

- Instrumentation

The apparatus was a Varian 320-MS TQ Mass Spectrometer (Varian Inc., California, USA). Chromatographic separation was performed on a Pursuit XRs Ultra-C8 reversed phase column (2.8 μm particle size, 50 x 2 mm i.d.) from Varian Inc. (California, USA).

- Chromatographic Conditions

The injection volume was 10 μl and the flow rate was 200 μl·min⁻¹. The temperature into the thermostated column compartment was set at 40° C. Mobile phases consist of Methanol (0.1% acetic acid and 15 mmol ammonium acetate) (A) and Water (B).

Time (min)	Gradient profile	Solvent A (%)	Solvent B (%)
-3:00 (equilibration)	Isocratic	30	70
0:00		30	70
9:00	Linear	0	100
10:00		0	100

Table 1. Solvent programme for HPLC-MS/MS analysis at flow rate of 1 mL·min⁻¹.

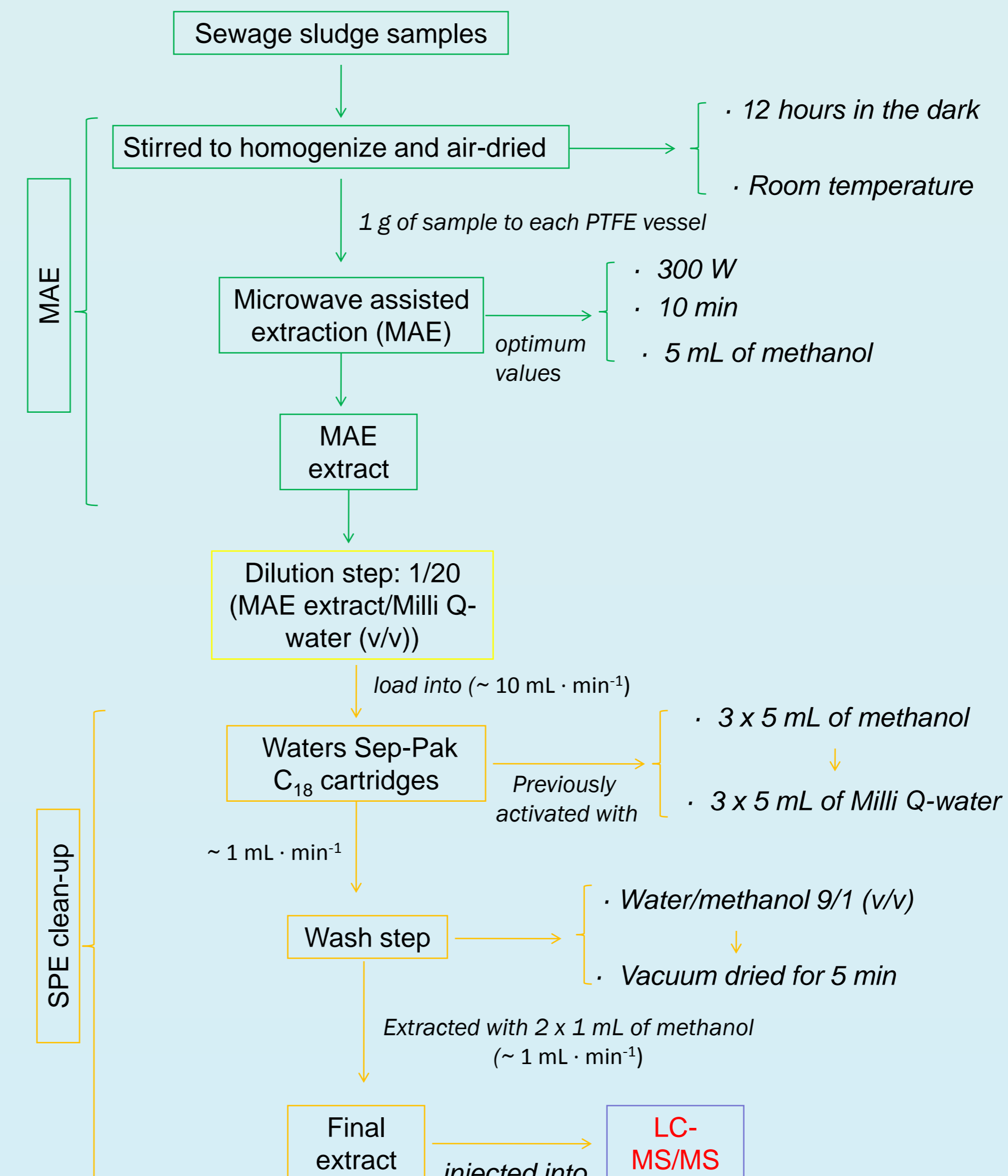


Fig. 1. Varian 320-MS TQ Mass Spectrometer (Varian Inc., California, USA).



Fig. 2. Microwave oven with 6 MF100 vessels (Anton Paar, Graz, Austria).



Fig. 3. Varian Vac Elut extraction manifold (Varian Inc., California, USA).

RESULTS

Compound	m/z Precursor [M+NH ₄] ⁺	m/z Precursor [M-H] ⁻	Cone ^a (V)	Fragment ions ^b	Ion mode
NP ₁ EO	282.3	-	30	265.3 (6) ^c , 127.1 (8)	ESI+
NP ₂ EO	326.3	-	30	183.1 (9) ^c , 121 (20)	ESI+
OP ₁ EO	268.1	-	30	251.1 (6) ^c , 113 (7.5)	ESI+
OP ₂ EO	312.3	-	30	183.0 (9.5) ^c , 121 (19.5)	ESI+
NP	-	218.7	-64	105.7 (20.5) ^c	ESI-
OP	-	204.7	-72	134 (16.5) ^c , 106 (19.5)	ESI-
BPA	-	226.7	-60	211.7 (17.5) ^c	ESI-
E2	-	271.1	-60	183.5 (14.5) ^c , 145.2 (20.5)	ESI-
E3	-	287.2	-67	171.0 (16.5) ^c , 145.2 (19.5)	ESI-
EE	-	295.3	-71	159.5 (17.5) ^c , 145.2 (21.5)	ESI-

Table 2. Characteristic of ESI/MS/MS parameters for each compound studied. a: Capillary voltage.; b: Collision energy in brackets. c: Fragment ion used for quantitation (MRM).

Compound	10 ng g ⁻¹ a		100 ng g ⁻¹ a		LOD ng g ⁻¹ b	LOQ ng g ⁻¹ c
	Recovery (%)	RSD ^d (%)	Recovery (%)	RSD ^d (%)		
E3	99.7	7.1	102.1	5.1	1.2	4.0
BPA	102.5	9.5	92.4	11.1	0.7	2.3
E2	75.9	5.4	71.7	5.1	1.5	5.0
EE	72.5	7.5	78.2	3.9	0.9	3.0
OP ₁ EO	82.6	6.0	88.1	5.1	2.3	7.6
OP ₂ EO	79.3	6.3	82.2	6.2	2.4	7.9
OP	80.0	10.2	85.1	6.6	2.2	7.3
NP ₁ EO	87.1	6.1	90.0	4.9	3.3	10.9
NP ₂ EO	82.1	6.1	81.1	9.1	0.8	2.6
NP	85.1	4.8	77.7	4.1	3.5	11.6

Table 3. Analytical parameters. a: n=6; b: Limits of detection; c: Limits of quantification; d: Relative standard deviation.

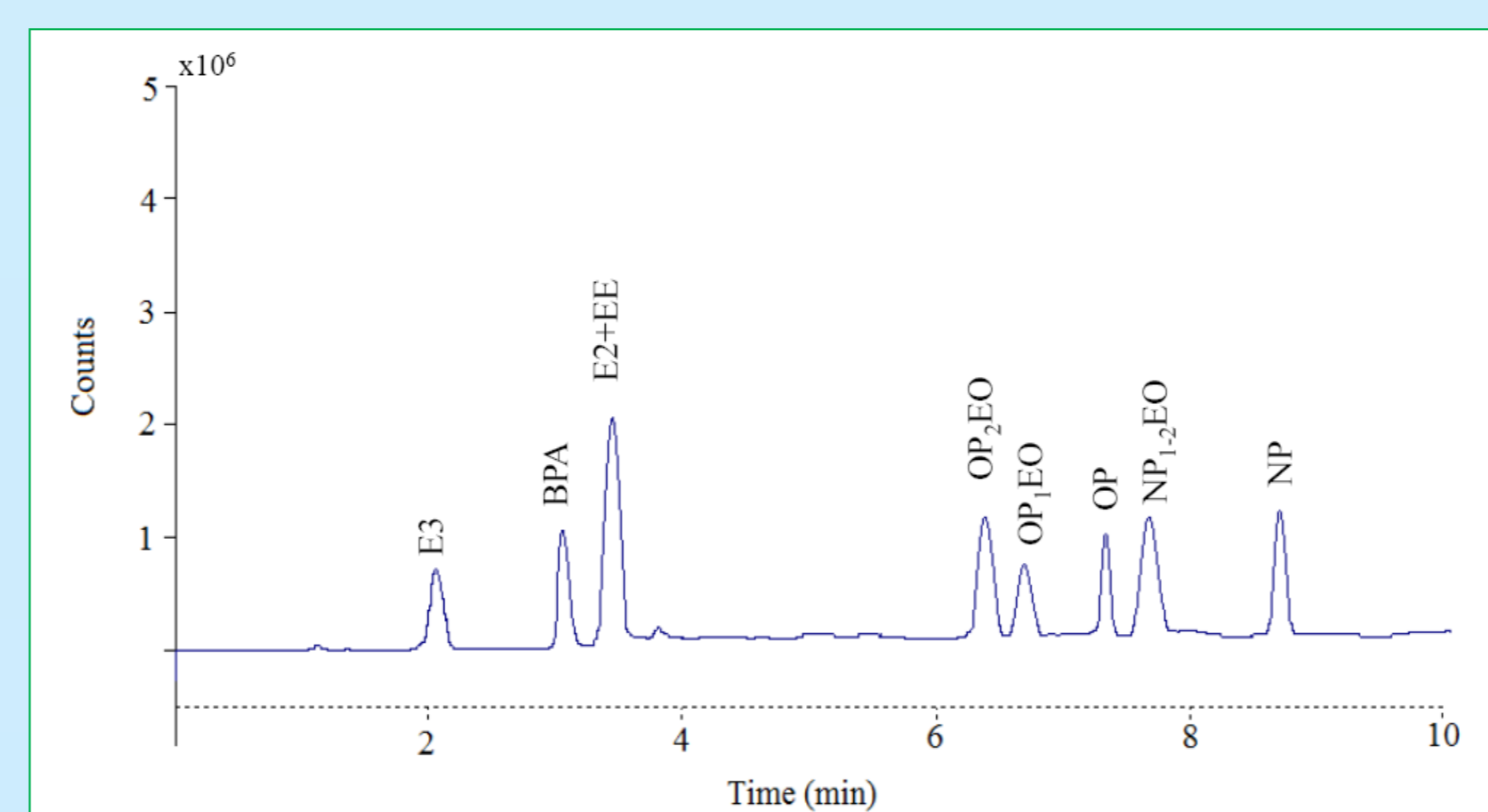
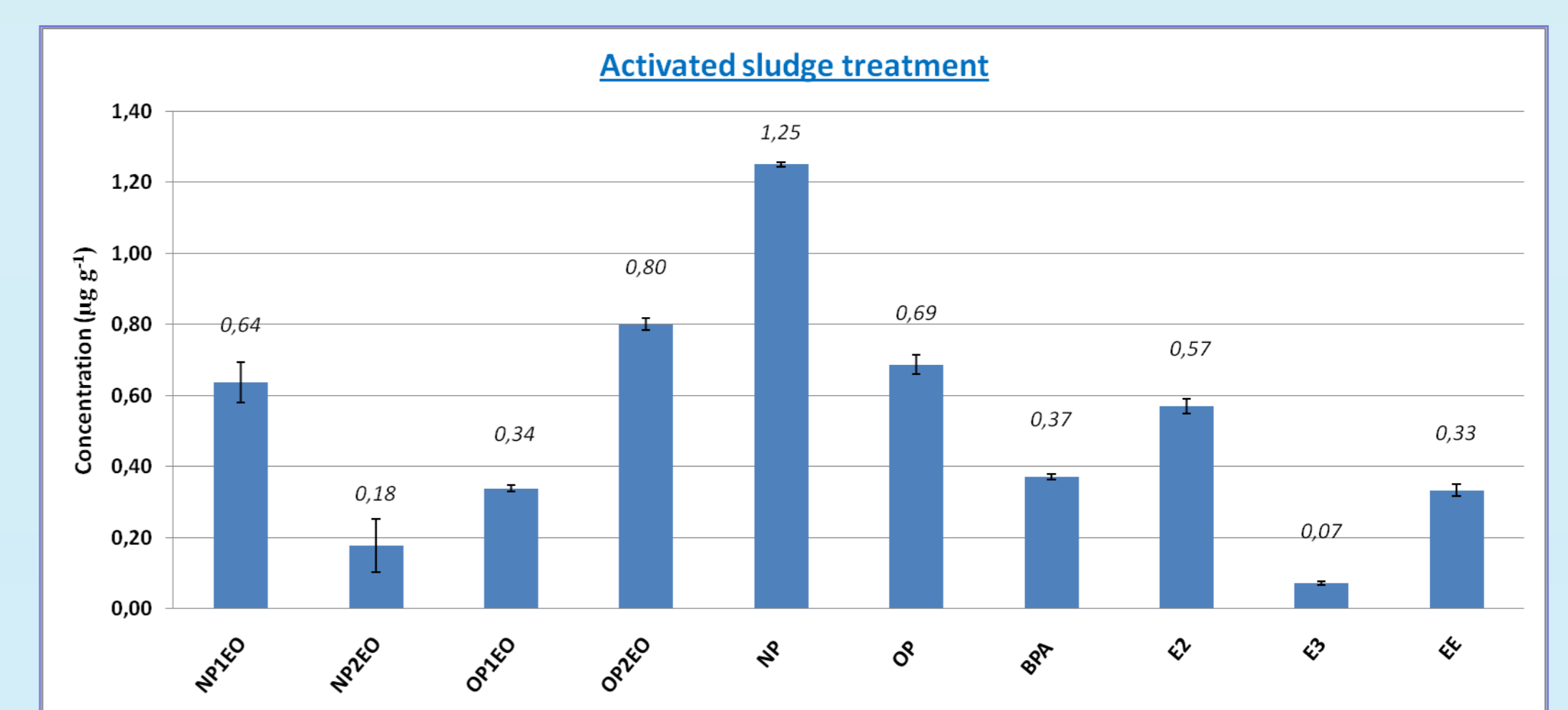


Fig. 5. Total ion current (TIC) chromatogram of spiked sample (100 ng · g⁻¹) with all analytes after MAE-SPE process.

1)



2)

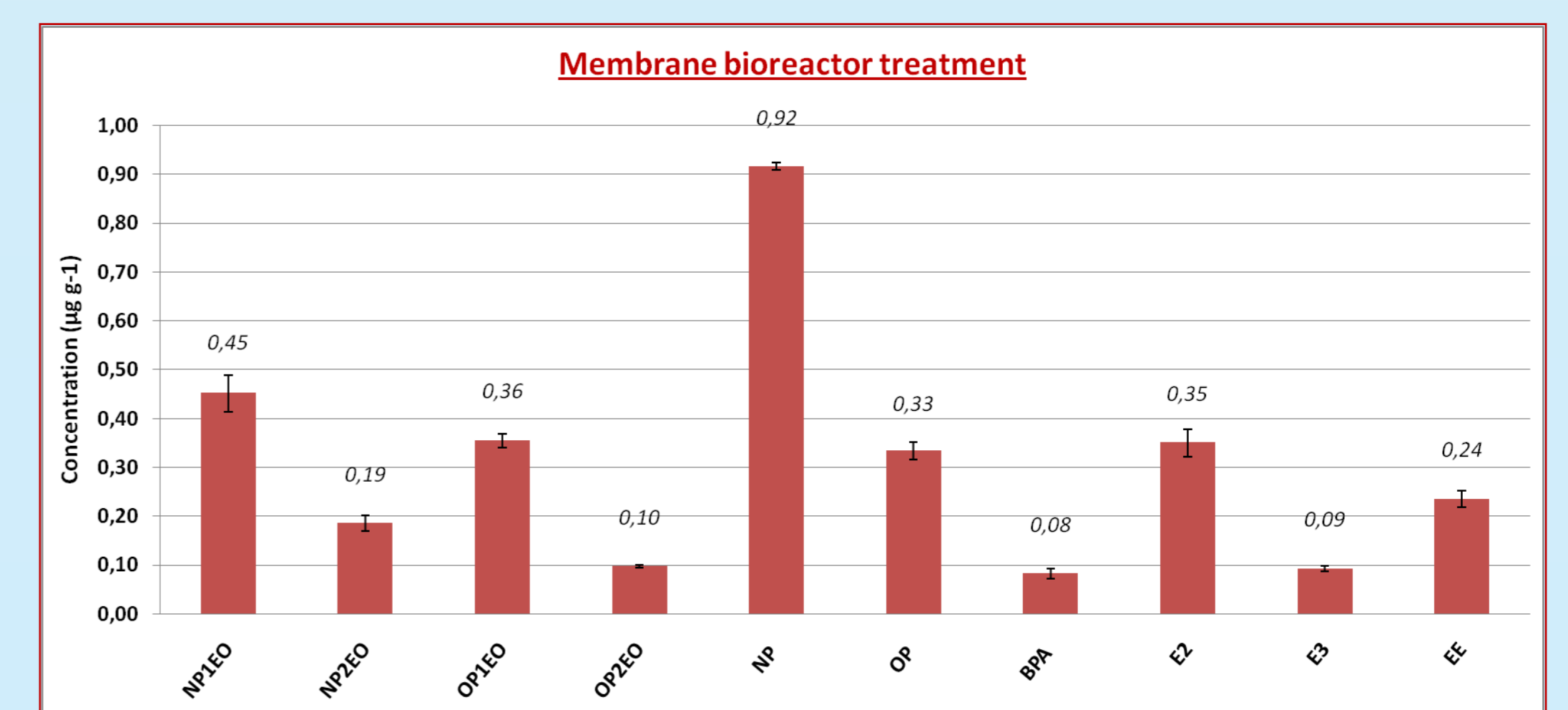


Fig. 4. Concentrations obtained for all of the compounds in the activated sludge treatment plant (AST) (1) and in the membrane bioreactor treatment plant (MBR).

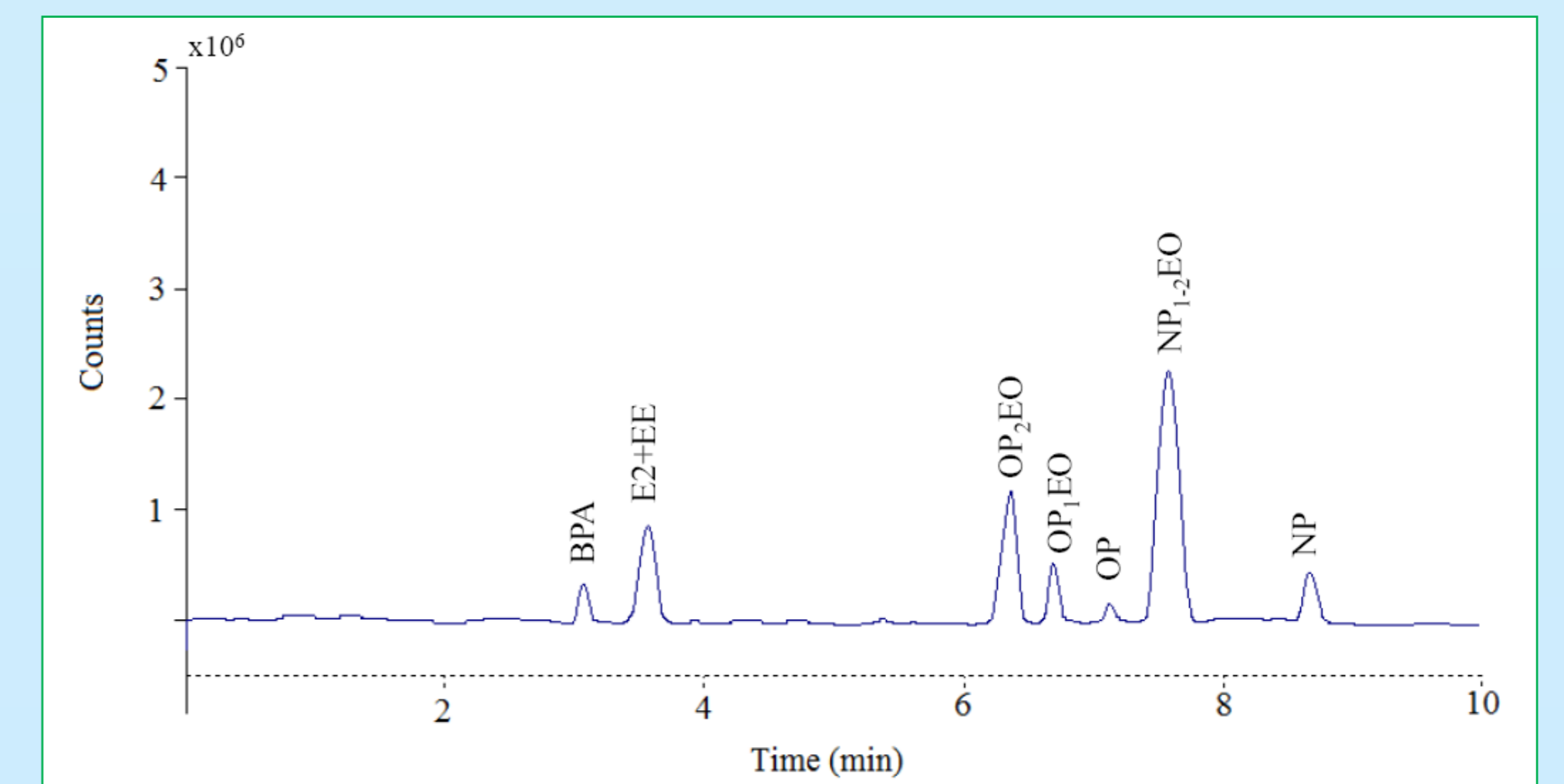


Fig. 6. Total ion current (TIC) chromatogram of the AST sewage sludge sample.

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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 μg·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

- Schwarzenbach R.P. et al (2006), Science, 313, 1072-1077.
- Andreu V. et al (2007), Sci. Total Environ., 378, 124-129.