J. Biol. Res. 2011 n.1 - Vol. LXXXIV - Rubbettino - Soveria Mannelli

Determination of Endocrine Disrupting Compounds in surface waters by means of chromatographic techniques coupled to mass spectrometry

M. Di Carro, C. Scapolla, E. Magi*

Department of Chemistry and Industrial Chemistry
Via Dodecaneso 31, 16146 Genoa, Italy
*emanuele.magi@unige.it

KEY WORDS: Endocrine Disrupting Compounds, Chromatography, Mass Spectrometry, Stir Bar Sorptive Extraction, Passive Sampling

Abstract

Two analytical methods were developed to study five endocrine disrupting compounds (4-n-nonylphenol, bisphenol A, estrone, 17β-estradiol and 17α-ethinylestradiol) in waters. One method includes a fast liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS-MS) analysis, while the second comprises a Stir Bar Sorptive Extraction (SBSE) followed by a headspace derivatization and gaschromatography-mass spectrometry (GC-MS) analysis. Passive samplers POCIS (Polar Organic Chemical Integrative Samplers) were used as sampling and preconcentration steps in order to reach the very low levels of the analytes in environmental waters. Both methods were then applied to the determination of the analytes in different water samples.

Introduction

Endocrine disrupting compounds (EDCs) are environmental ubiquitous contaminants that can alter the normal functioning of the endocrine system in wildlife and humans and have several adverse effects, mainly on sex-specific characteristics [1-2].
Passive sampling enables a preconcentration of target analytes. POCIS (Polar Organic Chemical Integrative Samplers) [3] contain a sorbent phase sandwiched between two microporous polyethersulphone membranes, which allow chemicals of interest to pass through the sorbent.

Materials and methods

LC-ESI-MS-MS

Two POCIS were deployed for four weeks in the influent and another two in the effluent of a drinking water treatment plant; another set of two was deployed for 14 days in the influent [6, 7].
The sorbent phase was eluted with tetrahydrofurane/methanol/acetone; the eluate was reduced in a rotary evaporator to 5 mL. An aliquot of 1 mL was dried down and redissolved in 50% CH_3OH containing bisphenol A-$d1_6$ as internal standard and analysed in LC-MS-MS using a Pinnacle DB Biphenylic column at a temperature of 60 °C and a flow rate of 0.35 ml/min, using a gradient starting from 30% acetonitrile in Milli-Q water to 100% acetonitrile at 5 min.
MS parameters were optimized and quantitative analysis was performed in Multiple Reaction Monitoring to ensure maximum sensitivity.
Calibration curves using internal standard method were drawn in the range 1-100 pg, showing good correlation coefficients (0.9993-0.9998). Limits of detection and limits of quantitation were in the range 0.7-1.5 pg and 2.3-4.9 pg, respectively.

GC-MS

Duplicate POCIS were deployed in three Ligurian rivers for 28 days [8] and extracted as reported above; 1 mL of the extract with pyridine and acetic anhydride as a derivatization reagent was subjected to SBSE (room temperature, overnight). The second derivatization step was performed adding pyridine and acetic anhydride to the stir bar (80°C, 30 minutes), which was then desorbed in the Thermal Desorption Unit for the GC-MS analysis.
Quantitative analysis was performed in Single Ion Monitoring mode in order to maximize sensitivity.
Calibration curves showed good correlation coefficients (0.9973-0.9997) in the range 3-100 ng; limits of detection and limits of quantitation were in the range 0.1-1.0 ng and 1.2-2.6 ng, respectively.

Results

LC-MS-MS analyses. Analytes were detected only in the influent: BPA was present in POCIS deployed for two and four weeks (432 and 923 ng/POCIS, respectively); E1 and NP concentration in POCIS deployed for four weeks were 105 and 25 ng/POCIS, respectively. Values represent the average of data obtained from the two POCIS deployed in the same site.
GC-MS analyses. Data of the five EDCs in the three sampling sites are reported in Tab. 2.

	NP (ng/ POCIS)	BPA (ng/ POCIS)	E1 (ng/ POCIS)	E2 (ng/ POCIS)	EE2 (ng/ POCIS)
Site C	3.8	378	< LOQ	36.5	14.7
Site L	7.9	430	< LOQ	< LOQ	< LOQ
Site M	< LOQ	192	19.3	< LOQ	< LOQ

Table 2. EDC concentration in passive samplers determined by GC-MS (ng of analyte per g of sorbent phase). Values represent the average of data obtained from the two POCIS deployed in the same site.

Discussion

With LC-MS-MS analyses, BPA was measured in the samplers deployed in the influent for both two and four weeks, being the concentration in four weeks about the double of that of two weeks, as expected from integrative samplers. The integrative behaviour of POCIS seems to be confirmed also from the data obtained for E1 and NP, which were detected only in the POCIS deployed for four weeks. In the effluent the concentration of all analytes was negligible, indicating their removal by the water treatment plant.
GC-MS data highlight the presence of the analytes in the rivers; in particular, BPA proved to be the most abundant, in good agreement with data from the literature. Estrogen concentrations seem to be in the same range reported for European rivers.

References

[1] Routledge E.J., Sumpter J.P. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environ. Toxicol. Chem., 15: 241-248.
[2] Madsen S.S., Skovbolling S., Nielsen C., Korsgaard B. 2004. 17-β Estradiol and 4-nonylphenol delay smolt development and downstream migration in Atlantic salmon, *Salmo salar*. Aquat. Toxicol., 68: 109-120.
[3] Alvarez D.A., Petty J.D., Huckins J.N., Jones-Lepp T.L., Getting D.T., Manahan S.E. 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem., 23: 1640–1648.
[4] Di Carro M., Scapolla C., Liscio C., Magi E. 2010. Development of a fast liquid chromatography–tandem mass spectrometry method for the determination of endocrine-disrupting compounds in waters. Anal. Bioanal. Chem., 398: 1025–1034.
[5] Magi E., Scapolla C., Di Carro M., Liscio C. 2010. Determination of endocrine-disrupting compounds in drinking waters by fast liquid chromatography–tandem mass spectrometry J. Mass Spectrom., 45: 1003–1011.
[6] Magi E., Di Carro M., Liscio C. 2010. Passive sampling and stir bar sorptive extraction for the determination of endocrine-disrupting compounds in water by GC-MS. Anal. Bioanal. Chem., 397: 1335-1345.