ANALYSIS OF TRACE AMOUNTS OF ESTROGENIC COMPOUNDS IN HIGH VOLUME WATER SAMPLES BY SPE-UPLC-MS/MS

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Abstract

In this work, a solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and optimized for the simultaneous analysis of six different estrogenic compounds in water. The developed method is suitable for the determination of the selected compounds at the low ng/L level. Real environmental water samples will be analyzed using this method.

Introduction

Today's increasing urbanization and rapidly growing industrial and agricultural activity release more and more contaminants into the environment. Freshwater pollutants of anthropogenic origin are a global health concern now. A diverse group of these emerging contaminants consists of endocrine disrupting compounds (EDCs). EDCs disturb the endocrine system of aquatic and terrestrial organisms, causing decreased fecundity, altered mating behaviour, developmental disorders, and thyroid dysfunction [1], [2]. Epidemiological studies suggest associations between chronic human exposure to EDCs and reproductive dysfunctions or civilization diseases, as well. Thus, there is a growing interest towards quantitative information about endocrine disruptors in freshwaters.

Experimental

The aim of this work was to develop a solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of six different EDCs, including three estrogens (17-□-estradiol, 17-□-ethinylestradiol, estrone) and three industrial chemicals (bisphenol A, bisphenol F, bisphenol S) in water. Ultrapure water was acidified to pH=3 and spiked with the target analytes at 100 ng/L. Solid phase extraction (SPE) was carried out by an automata SPE instrument (Dionex AutoTrace 280, ThermoFisher Scientific). SPE conditions were optimized by testing different types of SPE cartridges and eluents. For signal enhancement, chemical derivatization was carried out. Target analytes were incubated with dansyl chloride at 65 °C for 10 minutes under alkaline conditions. Instrumental analysis of the target compounds was performed by a Waters ACQUITY UPLC H-Class System, coupled with an Xevo TQ-S micro triple quadrupole mass spectrometer equipped with Electrospray Ionization source operated in negative ion mode detecting native compounds and in positive ion mode detecting dansylated derivates. The chromatographic separation was performed on Waters Aquity UPLC BEH C18 column. The mobile phase consisted of water and methanol, containing 0,1% formic acid when analyzing dansylated derivates. The quantitative analysis of the target compounds was performed in multiple reaction monitoring (MRM) mode.

Results and discussion

Limits of detection (LOD) for the selected native EDCs varied from 0,05 to 7,5 ng/L detected in negative ion mode. Chemical derivatization of the target compounds with dansyl chloride improved the sensitivity of the method. As the electrospray ionization technique is generally more effective for ionization of polar or ionic substances than non-polar compounds, chemical derivatization of native steroid estrogens with low polarity enhances their poor ionization efficiency. The reaction of steroid estrogens with dansyl chloride produces derivates containing easily ionizable basic N-atoms, resulting in enhanced sensitivity in ESI positive ion mode [4], [5]. The signal intensities of dansyl derivates were remarkably higher compared to native compounds. The LOD values were by an order of magnitude lower in positive ion mode after derivatization.

Conclusion

The developed method is suitable for the simultaneous determination of trace amounts of the selected six EDCs in water. Chemical derivatization with dansyl chloride significantly improved the sensitivity of the method. The LOD values of the target analytes were an order of magnitude lower in positive ion mode after derivatization compared to the related native compounds analyzed in negative ion mode. The developed method will be applied to real environmental samples.

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