

Detection of hormones (E1, E2, EE2) according to the requirements of the EU Water Framework Directive using an online-SPE-HPLC-MS/MS

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1. Introduction & objectives

The watch list was established by the European Union as part of the EU Water Framework Directive. It includes the estrogen active substances estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) [1]. Necessary detection limits (LODs) are 400 pg/L for E1 as well as E2 and 35 pg/L for EE2. A recent status report by the EU showed the progress, especially in the detection of EE2. So far, no laboratories have been able to achieve the requirements for EE2 [2]. The challenge for a robust method for quantification of estrogens in the ultra-trace range in water is due to the separation of interfering matrix compounds [3]. In our study, we developed a highly selective sample preparation in combination with online-SPE-HPLC-MS/MS.



35 pg/L EE2 → possible?

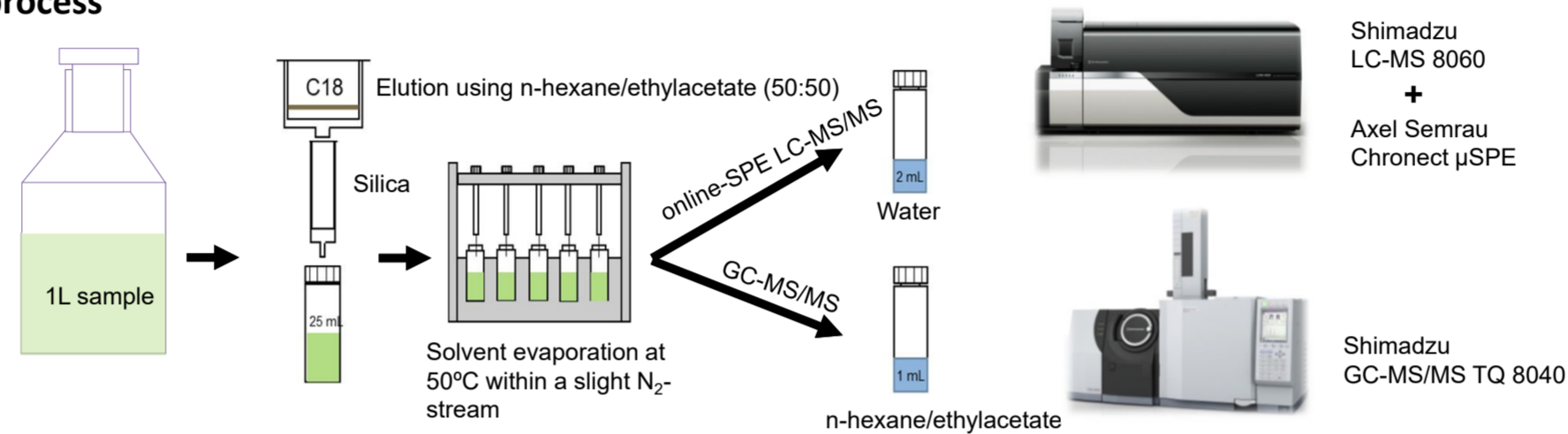
Name of substance/group of substances	CAS number (*)	EU number (*)	Indicative analytical method (*) (*)	Maximum acceptable method detection limit (ng/l)
17-Alpha-ethinylestradiol (EE2)	57-63-6	200-342-2	Large-volume SPE - LC-MS-MS	0,035
17-Beta-estradiol (E2), Estrone (E1)	50-28-2, 53-16-7	200-023-8	SPE - LC-MS-MS	0,4

Source: EU 2018/840, Commission implementing decision of June 2018

2. Methods

As sample, 1 L of surface water was collected as a random sample and 1 ng/L internal standard were added prior to the sample pre-treatment. An offline enrichment was performed by solid phase extraction. For this purpose, Speedisk C-18® cartridges were applied. The elution was performed using n-hexane/ethyl acetate. For an additional clean-up, silica cartridges were used. Subsequently, the solvent was evaporated and the sample re-dissolved in 1 mL water. The measurement was performed by coupling online-SPE and HPLC-LCMS/MS in ESI negative mode using an injection volume of 1 mL. 0.05 mM ammonium fluoride and acetonitrile was used as HPLC solvent. To achieve a high selectivity during MS, specific mass transitions for the individual analytes were selected.

3. Materials & process



4. Results

Sample enrichment and clean-up using offline SPE and a silica showed already a high selectivity for matrix separation, resulting in an increased signal-to-noise ratio. A further removal of interfering matrix compounds was achieved by the use of a cyano-phase during the online-SPE and chromatographic separation on a biphenyl HPLC phase. MS/MS-detection was done after negative electrospray ionisation. By calculating the signal-to-noise ratio of one of the smallest detectable standards (20 pg/mL), theoretical instrument detection limits (LOD) of 0.7 pg/L for EE2, 0.4 pg/L for E2 and 0.3 pg/L for E1 can be reached. As such values are not representing the reality due to matrix interferences in real samples, experiments with surface water were done. Thus, based on the signal-to-noise ratios, LODs of 30 pg/L for EE2, 30 pg/L for E2 and 10 pg/L for E1 were obtained. To verify the calculated LODs (especially for EE2) surface water without a background contamination was spiked with 30 pg/L of EE2. The results confirm the previously theoretically derived LODs of 30 pg/L for EE2

Online-SPE LC-MS/MS

Column: Restek Raptor Biphenyl (2,7 µm, 50 x 2,1 mm) + Guard Column (2,7 µm, 5 x 2,1 mm)
HPLC: Flowrate 0.4 mL/min, Acetonitril / Water (+ 0,05 mM NH₄F)
Online-SPE Cartridge: Cyano (CN)-Phase, Elution by the HPLC gradient
MS: ESI⁻ Mode, Multiple reaction monitoring (MRM) for the target analytes (E1, E2, EE2)

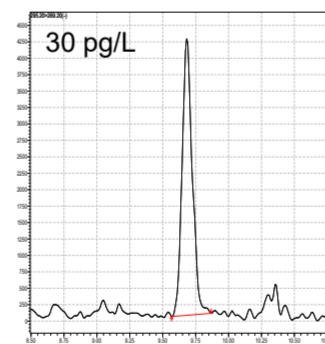


Figure 1: 30 pg/L of EE2 spiked in 1L river water before sample treatment.

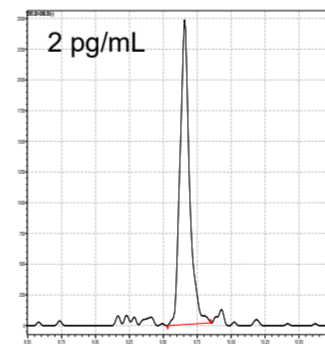


Figure 2: 2 pg/mL of a EE2 standard.

GC-MS/MS

Column: Restek 5Sil-MS 30 m, 0,25 mm ID, 0,25 µm df + 10 m Guard Column
Injection volume: 3 µL
Carrier gas: Helium 5.0
Derivatisation: 50:50 Pyridin : BSTFA; Multiple reaction monitoring (MRM) for target analytes (E1, E2, EE2)

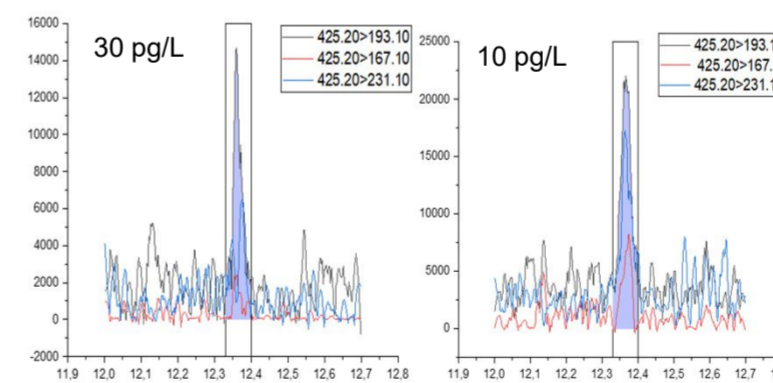


Figure 2: 30 pg/L / 10 pg/L of EE2 spiked in river water before sample treatment.

5. Conclusions

- Requirements of the EU Water Framework Directive for E1, E2 (data not shown here) and EE2 successfully achieved using online-SPE-LC-MS/MS as well as GC-MS/MS.
- GC-MS/MS as an alternative to the proposed LC-MS/MS method.
- As next step, more samples from different water bodies should be investigated to get more information about the robustness of the methods

6. Acknowledgement

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Literature

- EU 2018/840. "COMMISSION IMPLEMENTING DECISION (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495".
- Loos, R., D. Marinov, I. Sanseverino, D. Napierska, and T. Lettieri. 2018. "Review of the 1st Watch List under the Water Framework Directive and recommendations for the 2nd Watch List, EUR 29173 EN, Publications Office of the European Union, Luxembourg, 2018, ISBN 978-92-79-81839-4, doi:10.2760/614367, JRC111198."
- Itzel, F., L. Gehrmann, T. Teutenberg, T. C. Schmidt, and J. Tuerk. 2019. "Recent developments and concepts of effect based methods for the detection of endocrine activity and the importance of antagonistic effects." Trends in Analytical Chemistry 118:699-708.