

Quantification of hormones (E1, E2, EE2) according to the requirements of the EU Water Framework Directive using on-line-SPE-HPLC-MS/MS [LCMS-8060NX]

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User Benefits

- ◆ Method meets the requirements of the EU Water Framework Directive's Watch List
- ◆ Sensitive detection
- ◆ Full water sample analysis

▪ **Introduction**

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), 17-β-estradiol (E2) and 17α-ethinylestradiol (EE2) [1]. Necessary detection limits (LODs) are 0.4 ng/L for E1 as well as E2 and 0.035 ng/L for EE2. Development of a robust method for quantification of estrogens in the ultra-trace range in water is challenging due to the separation of interfering matrix compounds [2]. In our study, we developed a highly selective sample preparation in combination with on-line-SPE-HPLC-MS/MS.

▪ **Materials and Methods**

As sample, surface water taken from a river with high waste water content was chosen. 1 l of sample was collected and internal standards were added prior to the sample pre-treatment. An off-line 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 removed and the residue redissolved in 2 ml. The measurement was performed by coupling on-line-SPE and LC-MS/MS in ESI negative mode using an injection volume of 1.5 ml. On-line SPE is used as a further clean-up step here. 0.05 mM ammonium fluoride and acetonitrile were used as eluents. To achieve high selectivity during MS, specific mass transitions for the individual analytes were selected. To demonstrate the performance at trace level concentrations a level below the required LOD of EE2 and a level three times higher were spiked before sample pretreatment. Despite the necessary higher LOD for E2 and E1, the same low concentrations as for EE2 were chosen here to demonstrate the performance of this method. LCMS-8060NX combines superior sensitivity with outstanding robustness due to the IonFocus-technique and is well suited for routine labs analysing dirty samples. Despite clean extracts are injected here, LCMS-8060NX is used to demonstrate it's sensitivity.

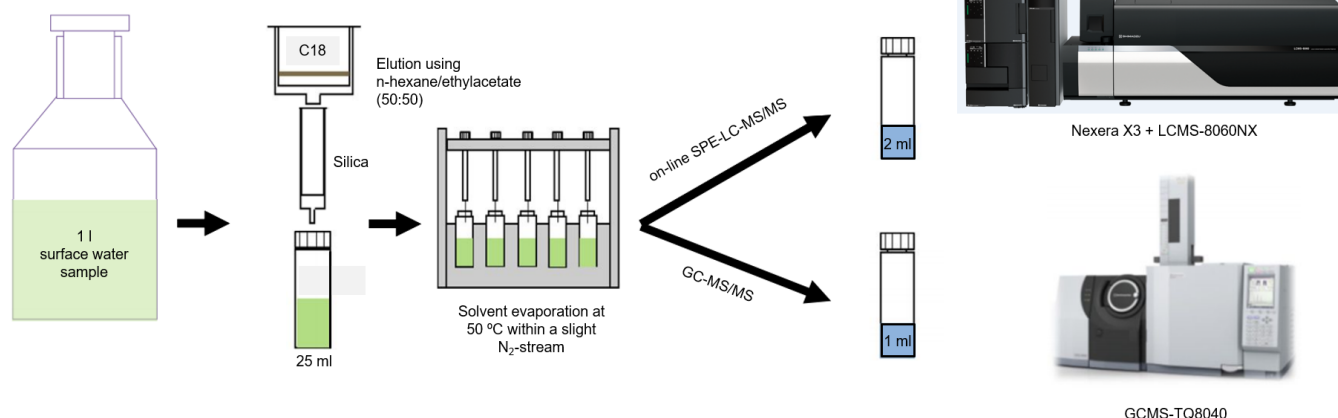


Fig. 1: In this study a Shimadzu LCMS-8060NX LC-MS/MS detector coupled to a Nexera X3 on-line-SPE-UHPLC system was used. As an alternative GC-MS/MS can be used.

Table 1: Method performance in surface water sample. Repeatability is calculated over the whole method.

^a Noise includes the 17- α -Estradiol or 17- β -Estradiol peak, please refer to fig. 2.

		EE2	E2	E1	17- α -Estradiol
MRM for quantification		295.2>269.2	271.2>145.2	269.2>145.1	271.35>145.2
Calibrated range (concentration in sample) [ng/l]		0.005-45	0.009-9	0.009-1.8	0.009-1.8
r²		0.9997	0.9992	0.9992	0.9997
Datapoints per peak at 0.030 ng/l		16	16	20 (0.14 ng/l)	16
Repeatability (%RSD peak area ISTD, n = 12)		20	15	12	same ISTD as E2
S/N 0.030 ng/l (n = 4)		8	1 ^a	contained in sample	
S/N 0.090 ng/l (n = 4)		21	2 ^a	(0.14 ng/l)	
Recovery (n = 4), calibration range 0.005-0.9 ng/l	0.030 ng/l	126	76	98	92
	0.090 ng/l	95	73	91	109
Required LOD [ng/l] [1]		0.035	0.4	0.4	Not regulated

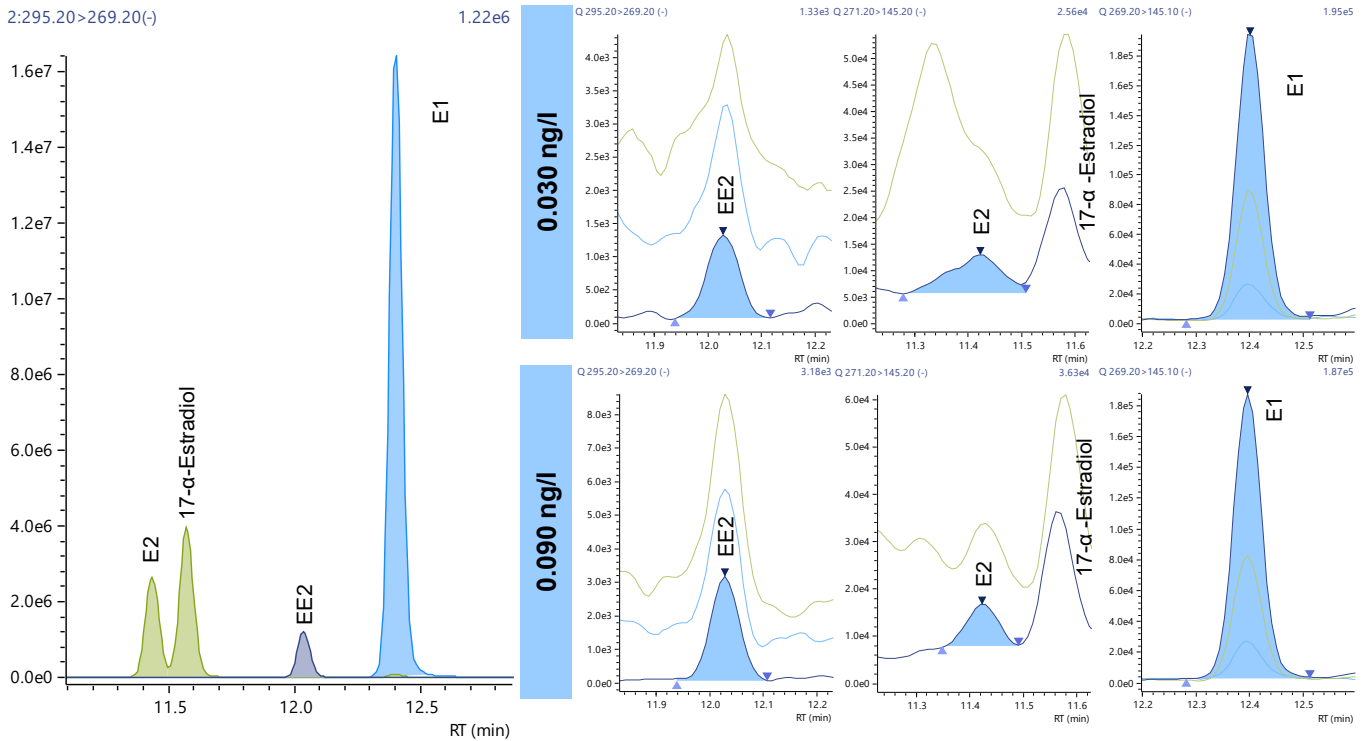


Figure 2: Chromatograms of a surface water sample spiked with 0.030 ng/l and 0.090 ng/l (right) and a calibration standard at 1.8 ng/l (left). No smoothing.

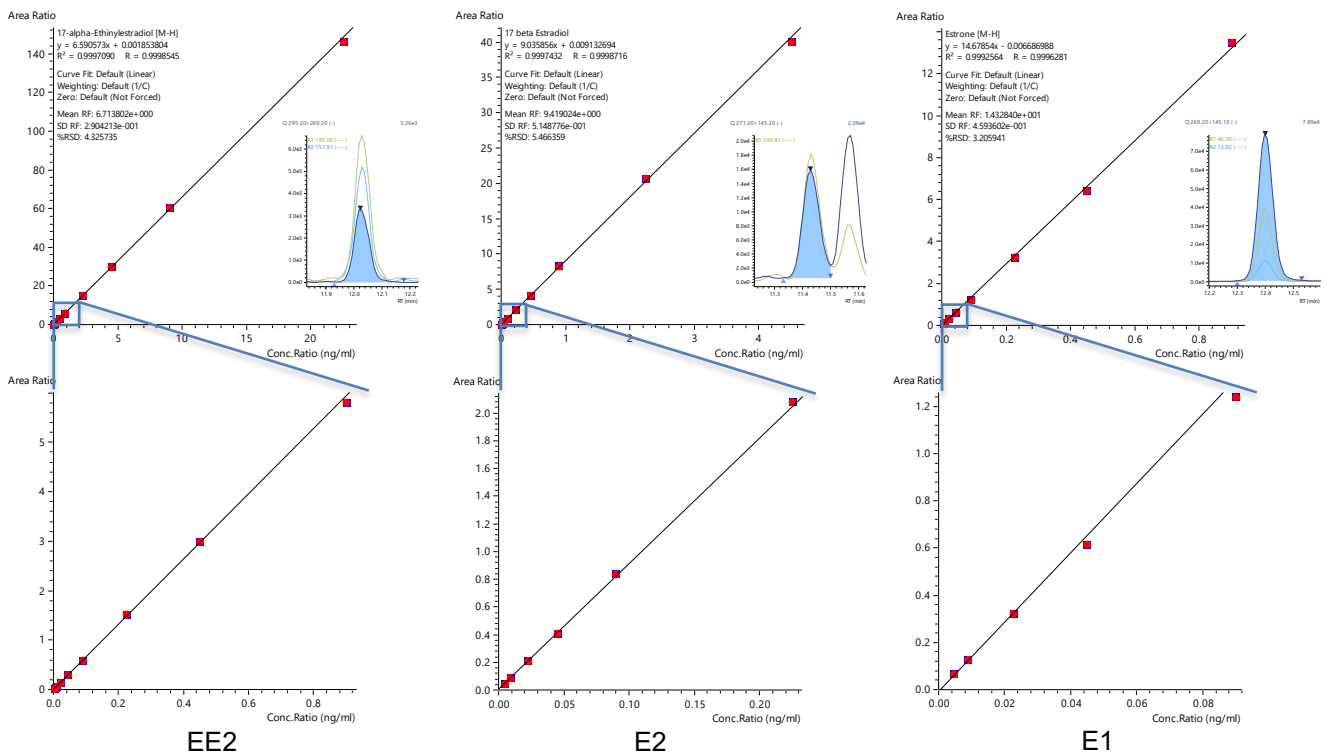


Figure 3: Calibration curves. Calibration ranges see table 1. Chromatograms show the lowest calibration level in 5% acetonitrile.

▪ Results

All Analytes were quantified in surface water at 0.030 ng/l and 0.090 ng/l reaching recoveries between 73 and 126 % (n = 4). Peaks were detected with 2-3 MRMs and 16 datapoints per peak even at 0.030 ng/l. The isobaric compounds E2 and 17- α -Estradiol were separated by chromatography. All results are shown in table 1, figure 2 and 3.

▪ Conclusion

Requirements of the EU Water Framework Directive's Watch List for E1, E2 and EE2 were successfully achieved using on-line-SPE-LC-MS/MS. GC-MS/MS is an alternative to the proposed LC-MS/MS method.

▪ Literature

- [1] EU 2018/840, Official Journal of the European Union, 2018, L141/9-12
- [2] Itzel, F. et. al., 2019, Trends in Analytical Chemistry 118:699-708

▪ Acknowledgements

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