Enrichment and clean-up of steroid hormones from water samples

MACHEREY-NAGEL application department
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Application benefits

- Sensitive determination of steroid hormones from water samples
- High recovery rates were achieved with solid phase extraction using CHROMABOND® HR-X and CHROMABOND® SiOH column
- Fast and sensitive HPLC analysis on a NUCLEOSHELL® RP18 plus column

MN products

REF 730938
CHROMABOND® HR-X, 85 µm, 6 mL, 200 mg

REF 730075
CHROMABOND® SiOH, 45 µm, 6 mL, 1000 mg

REF 760666.20
EC 100/2 NUCLEOSHELL® RP18plus, 2.7 µm

REF 702402
Screw closure, N 9, PP, yellow, center hole, Silicone white/PTFE red, 1.0 mm

REF 702079
Screw neck vial, N 9, 11.6 × 32.0 mm, 1.5 mL, label, flat bottom, amber, silanized

MN application numbers

SPE: 307010
HPLC: 129600

Keywords

steroid hormones, SPE, water, PS/DVB copolymer, Silica gel, LC-MS/MS

Introduction

Synthetic hormones such as 17α ethinyl estradiol (EE2), known as an active ingredient of the birth control pill, but also the natural or nature-identical hormone estradiol (E2) are specifically used to regulate hormone balance in humans. Both substances, as well as their common degradation product estrone (E1), are excreted by the human body and are therefore found in the environment. They are known to have a lasting negative effect on fish reproduction even in the very low ng/l range [1].

By the publication of the COMMISSION IMPLEMENTING DECISION (EU) 2015/495 of 20 March 2015 a first 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 was established [2]. In the first EU Watch List describes very challenging detection requirements for these endocrine disruptors. The LLOQ for estrone and 17-beta-estradiol were set at 0.4 ng/l and for 17-alpha-ethinyl estradiol at 0.035 ng/l. The EU Commission's predicted no-effect concentration (PNEC) values are 3.6 ng/l for estrone, 0.4 ng/l for 17-beta-estradiol, and 0.035 ng/l for 17-alpha-ethinylestradiol.

To achieve the challenging EU LLOQ required for these compounds, a combination of solid phase extraction (SPE), clean-up and concentration, combined with a large volume injection were utilized. Finally, the extracts are analyzed using HPLC-MS/MS on a NUCLEOSHELL® RP18plus column.

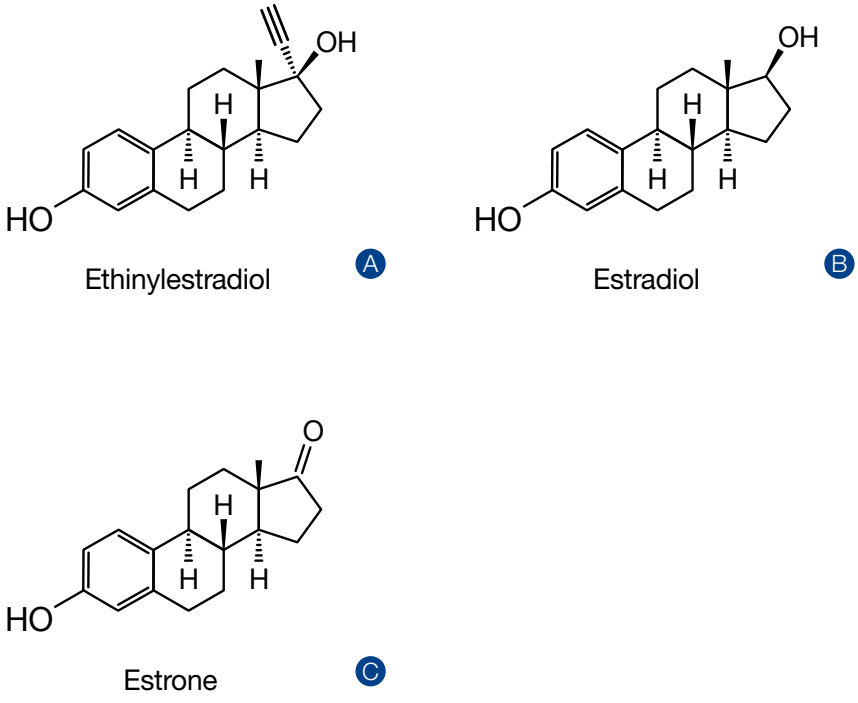

Figure 1: Steroid hormones: A = 17α-Ethinylestradiol, B = 17β-Estradiol, C = Estrone

Sample pretreatment

MN Appl. No. 307010

Solid phase extraction, clean-up, and concentration methodology:

Spike 10 µL of isotopic labeled standard solution (ß = 50 ng/mL in methanol for each component) and spike volume of methanolic native standard solution (ß = 10 ng/mL for each component) directly into the sample. Mix sample by swirling the sample container.

Column:

CHROMABOND® HR-X, 85 µm, 6 mL, 200 mg (REF 730938)

Conditioning:

Add 5 mL of hexane-ethyl acetate (90:10,v/v), 5 mL of ethanol and 3 × 5 mL of water to the cartridge.

Sample application:

Add 1000 mL water sample with a flow rate of 5 mL/min to 10 mL/min to the cartridge. (Do not let the sorbent material in the cartridge go dry and ensure it is immersed in water at all times.)

Washing step 1:

Add 5 mL 2 % NH3 in methanol:water (5:95).

Drying step:

30 min with vacuum.

Elution:

Add 2 × 5 mL hexane-ethyl acetate (90:10,v/v).

Column:

CHROMABOND® SiOH, 45 µm, 6 mL, 1000 mg (REF 730075)

Conditioning:

Add 3 × 4 mL of ethyl acetate, 3 × 4 mL of hexane-ethyl acetate (90:10,v/v) hexane-ethyl acetate (90:10,v/v) to the cartridge.

Sample application:

Eluate from first SPE enrichement.

Elution:

Add 2 × 4 mL hexane-ethyl acetate (60:40,v/v).

Eluent exchange:

Evaporate eluate to dryness at 35 °C under a stream of nitrogen and dissolve residue in 1 mL water LC-MS grade.

Analysis by HPLC-MS / MS

MN Appl. No. 129600

Chromatographic conditions

Column	EC 100/2 NUCLEOSHELL® RP18plus, 2.7 µm (REF 760666.20)
Eluent A	0.2 mM/l ammonium fluoride in water
Eluent B	0.2 mM/L ammonium fluoride in methanol
Gradient	hold 45 % B for 0.5 min, in 0.5 min from 45 % B to 65 % B, in 4 min from 65 % B to 95 % B, hold 95 % B for 1 min, in 0.1 min to 45 % B, hold 45 % B for 3.9 min
Flow rate	0.35 mL/min
Temperature	40 °C
Injection volume	50 µL

MS conditions for Shimadzu 8050 – Triple Quadrupole MS

Acquisition mode	MRM
Interface	ESI
Polarity	negative
Nebulizing gas flow	2.8 L/min
Heating gas flow	10 L/min
Interface temperature	400 °C

DL Temperature	200 °C
Heat Block Temperature	400 °C
Drying gas Flow	5 L/min
CID gas	270 kPa Argon
Interface Voltage	-3 kV

MRM transitions

Analyte	Abbreviation	CAS number		Q1 mass [Da]	Q3 mass [Da]	Retention time [min]
17α-Ethinylestradiol	EE2	57-63-6	295.1	145.0	143.3	2.46
17β-Estradiol	E2	50-28-2	271.3	144.9	185.0	2.47
Estrone	E1	53-16-7	269.3	145.2	143.2	2.87
Surrogates						
17α-Ethinylestradiol-2,4,16,16-d4	EE2-d4	350820-06-3	299.0	147.2	145.4	2.43
17β-Estradiol-2,4,16,16,17-d5	E2-d5	221093-45-4	276.0	147.3	187.3	2.44
Estrone-2,4 – 16,16-d4	E1-d4	53866-34-5	273.0	147.3	145.2	2.86

Table 2: MRM transitions and retention times of steroid hormones and surrogates.

Chromatogramms

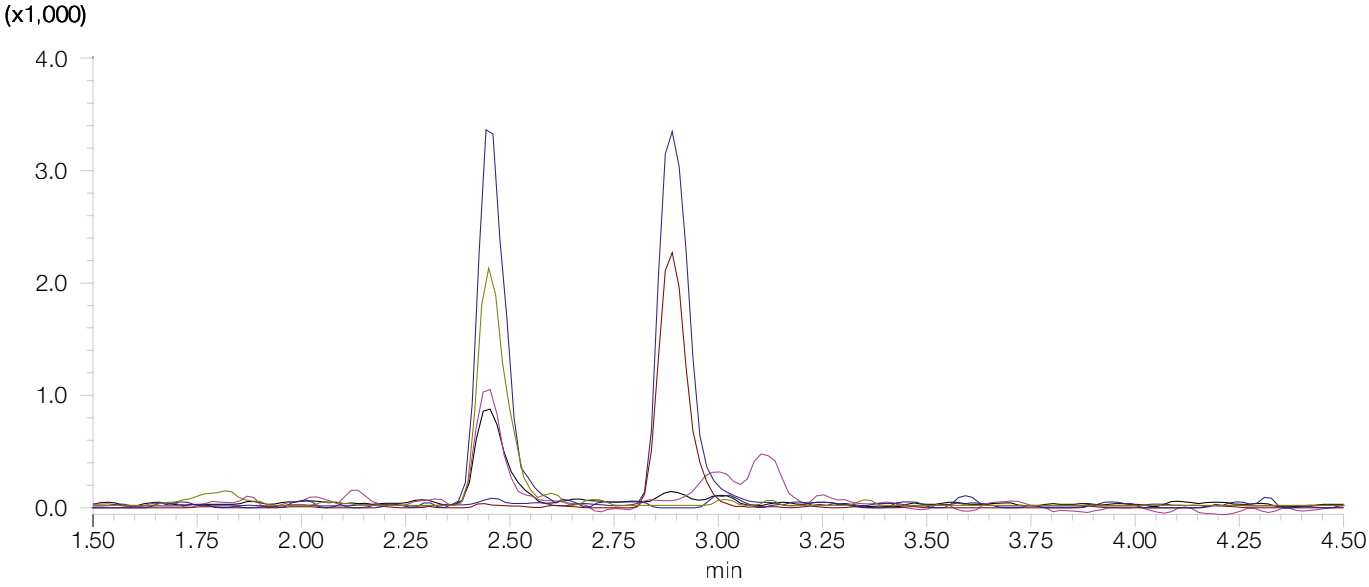

Figure 2: Chromatogram of a sample eluate (concentration: 17α-Ethinylestradiol β = 400 pg/mL, 17β-Estradiol β = 100 pg/mL, Estrone β = 100 pg/mL)

Calibration curves

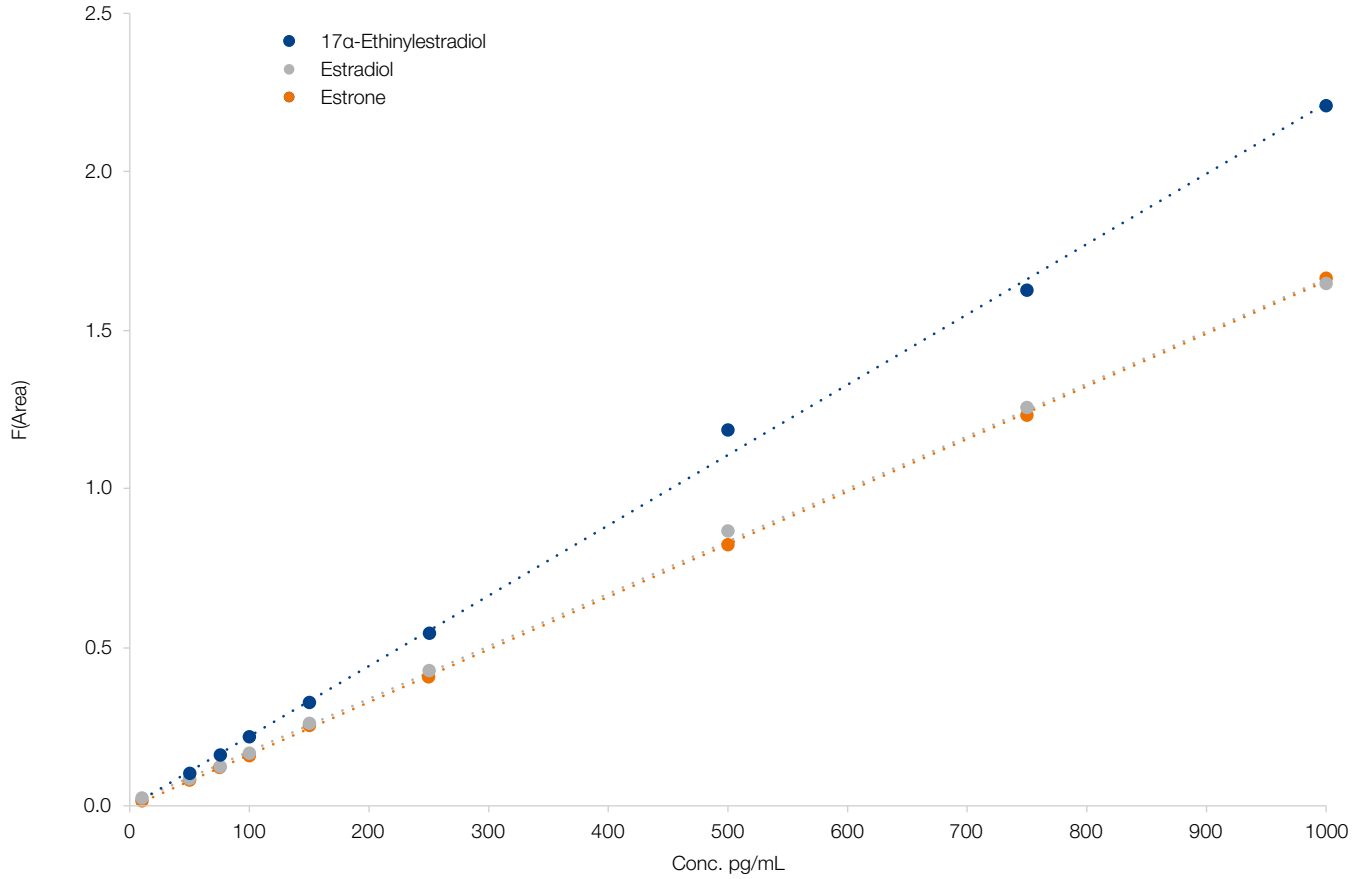

Figure 3: Calibration range for: 17α-Ethinylestradiol (y = 0,0022x - 0,0006, R^2 = 0,9984), 17β-Estradiol (y = 0,0017x + 0,008, R^2 = 0,9994, Estrone (y = 0,0017x - 0,0031, R^2 = 0,9999) between 10 – 1000 pg/mL.

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Recovery rates

Abbreviation	Recovery rate [%] ± RSD [%] (n= 3)
EE_2	86.6 ± 4.8
E2	84.9 ± 13.8
E1	79.7 ± 8.8

Recovery rates for the presented SPE method using CHROMABOND® HR-X and CHROMABOND® SiOH columns (sample concentration ß = 100 pg/L for EE2, E1, and E2).

Conclusion

This essay presents the reliable and successful determination of 17α-Ethinylestradiol, 17β-Estradiol, and Estrone from drinking water. By using a spherical, hydrophobic polystyrene-divinylbenzene resin, CHROMABOND® HR-X, it was possible to efficiently extract the steroid hormones before cleaning the extract with silica gel, CHROMABOND® SiOH. In combination with large volume injection, high recovery rates about 80 % with good reproducibility could be achieved. The elution step from the PS/DVB copolymer was optimized with a hydrophobic solvent mixture (hexane-ethyl acetate (90:10; v/v)). The cleaning procedure could follow directly without timewasting eluent exchange.

The identification and the quantification of the focused analyts was finally carried out by ESI mass spectrometry on a NUCLEOSHELL® column. Using core-shell particle technology allows highest column efficiency and resolution at a short run time with much lower back pressure compared to fully porous particles. The polar monomeric octadecyl modification successfully helps to separate analytes from matrix for a wide calibration range from 10 pg/mL up to 1000 pg/L.

References

[1] Steroid hormones in the aquatic environment, J.O. Ojoghoro, M.D. Scrimshaw, J.P. Sumpter, Science of The Total Environment, Volume 792, 20 October 2021, 148306

[2] COMMISSION IMPLEMENTING DECISION (EU) 2015/495 of 20 March 2015 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.

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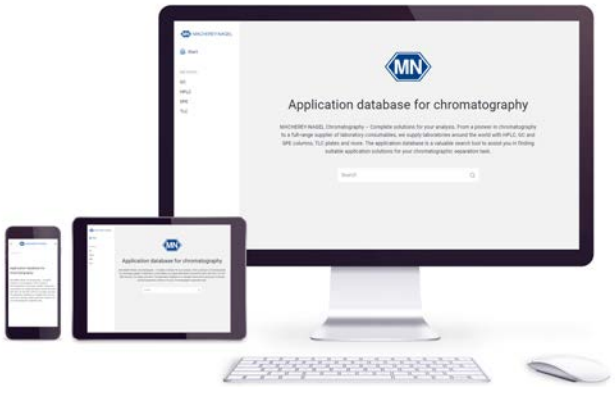

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