Disk-based solid phase extraction of large volume samples for the determination of estrogens and diclofenac listed in the WFD Watch list

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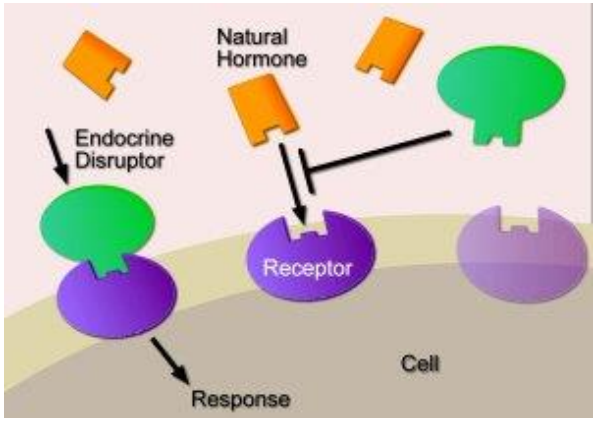
INTRODUCTION

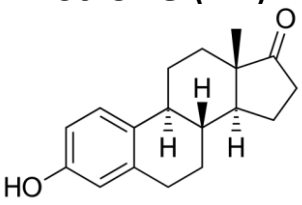
Two groups of environmental contaminants



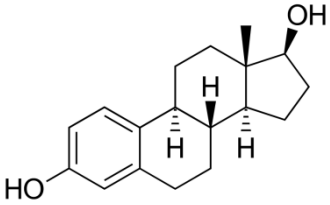
Endocrine disrupting compounds (EDCs)

Active pharmaceutical ingredients (APIs)

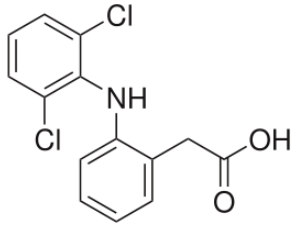
Estrone (E1)



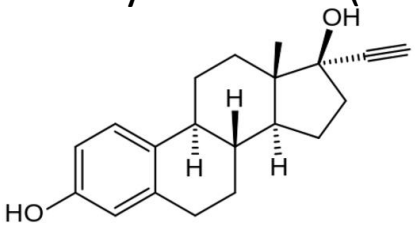
17β-estradiol (E2)



Diclofenac (DF)



17α-ethinyl estradiol (EE2)



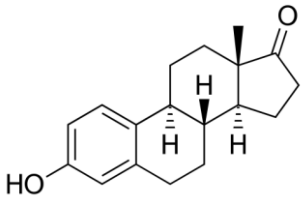
One of the highest estrogenic activity among EDCs

INTRODUCTION

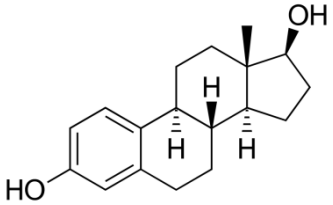
Watch list within WFD (Directive 495/2015)

Known adverse effects on aquatic biota
Confirmed environmental occurrence

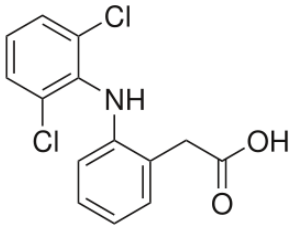
Estrone (E1)



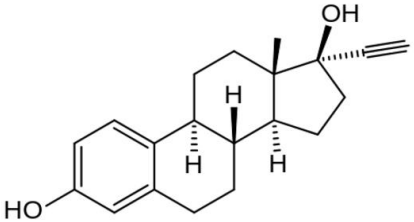
17β-estradiol (E2)



Diclofenac (DF)



17α-ethinyl estradiol (EE2)



INTRODUCTION

Watch list (Directive 495/2015)

- ✓ An indicative analytical method:

  E1, E2, DF: SPE + LC-MS/MS for

  EE2: **large** volume SPE + LC-MS/MS

- ✓ **Whole** water samples → NO filtration

- ✓ Maximum acceptable LOQs:

  E1: 0.4 ng L^{-1}

  E2: 0.4 ng L^{-1}

  EE2: 0.035 ng L^{-1}

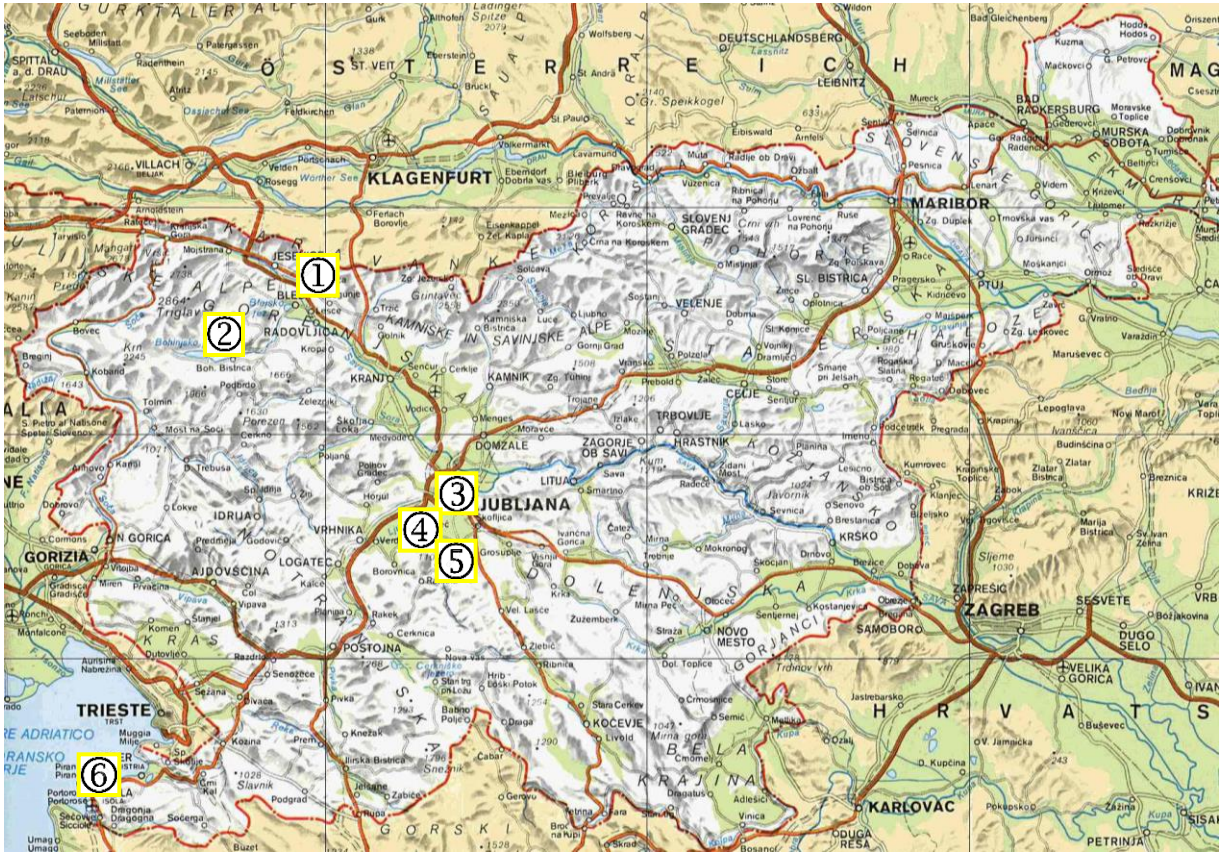
  DF: 10 ng L^{-1}

AIM:
Analytical method using disk-based SPE system
+
application to actual samples

EXPERIMENTAL

SAMPLE COLLECTION (10 L) in July and August 2016:

- Lakes: Bohinj (1) Bled (2), Podpeč (4)
- Rivers: Iška (5), Gradaščica (3)
- Seawater: Portotož (6)



EXPERIMENTAL

OPTIMIZATION OF SAMPLE PREPARATION

I. Sample extraction

•Conventional SPE:

HLB cartridges

•SPE system SPE-DEX® 4790

operated by Envision® Controller

with **HLB-L Horizon® disks**:

✓*Filtration + extraction at once*
✓*Automatic processing (all steps)*

EXPERIMENTAL

OPTIMIZATION OF SAMPLE PREPARATION

I. Sample extraction

STEP 1

- 1 L sample
- Filtration
- HLB cartridges
- Elution: EtAc

STEP 2

- As Step 1 but ↑ V (10 L)

STEP 3

- 10 L
- HLB disks
  - Optimisation of *drying, holder size, elution*
- Validation
- Application to real samples

II. Derivatization of analytes (GC/MS)

- *Various times, T and agents*

RESULTS

DERIVATIZATION

*TMS derivatives of E1 in chromatograms of EE2!

BSTFA* / **MSTFA** + Pyr 22°C < **60°C** > 70°C > 80°C 0.5 h < **1 h** - 6 h > 8-16 h

SPE

Calibration #2:
↑ sensitivity for EE2

(ng L^{-1})	E1	E2	EE2	DF
Required LOQs*	0.4	0.4	0.035	10
Calibration #1	**0.4**	0.4	0.035	**0.5**
	0.8	**0.8**	0.07	**1**
	1.2	**1.2**	0.1	**2.5**
	2.4	**2.4**	0.2	**5**
	5	**5**	0.5	**10**
	7	**7**	1	**15**
	10	**10**	2	**20**
	25	**25**	5	**40**
	50	**50**	10	**80**
Calibration #2	**0.4**	0.4	0.035	**0.5**
	0.8	**0.8**	0.07	**1**
	1.2	**1.2**	0.1	**2.5**
	2.4	**2.4**	0.2	**5**
	5	**5**	**0.5**	**10**

*Directive 2015/495/EU

RESULTS

DERIVATIZATION

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SPE

Sensitivity not sufficient to reach the LOQs for E2 and EE2

(ng L^{-1})	E1	E2	EE2	DF
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Calibration #1	**0.4**	0.4	0.035	**0.5**
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*Directive 2015/495/EU

RESULTS

STEP 3

Cartridges → Disks: **solids retained**

drying for 16 h at 25 °C

- Disks not dried by air drying

- Small holder (**A**): disk clogged after loading 2.5 L of river sample

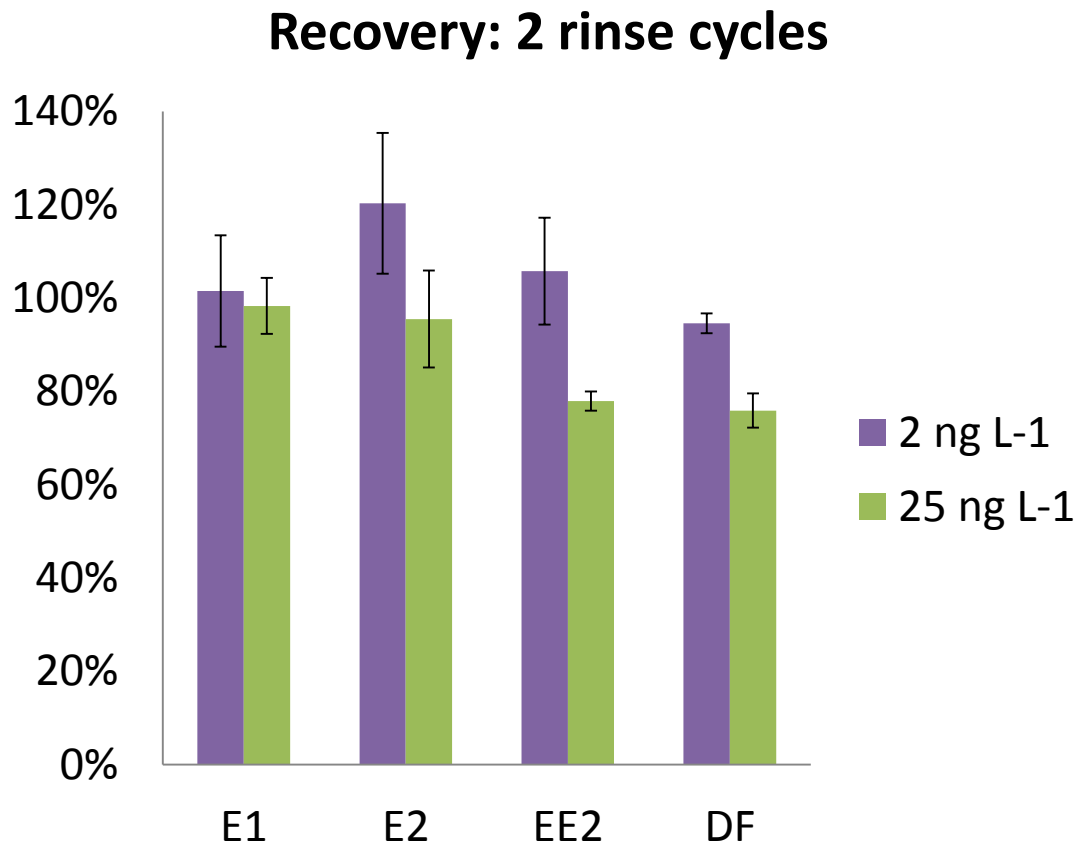
Wider holder (**B**) +
fine mesh screen + 1 µm glass fibre filter + glass wool

RESULTS

STEP 3

SPE recovery:

- Elution with EtAc
- Rinsing cycle (elution) – n = 3
- 3rd cycle: negligible amounts of analytes



RESULTS

STEP 3

VALIDATION

LOQ estimation approach not defined within Directive 2015/495/EU

	R^2	Accuracy (%)		LOQ (ng L^{-1})				Method repeat.	Instr. repeat.	
		L	H	I. s/n	II. 10×SD/slope	III. $\bar{x}$ + 10×SD	Acceptable LOQ		L	H
E1 0.1-50ng L^{-1}	0.995	33.5	3.42	1.00	2.27	**0.290**	0.4	0.262-22.3	4.18	2.18
E2 0.1-50ng L^{-1}	0.995	29.9	5.78	**1.00**	3.67	1.37	0.4	1.89-35.4	2.62	1.93
EE2 0.5-50ng L^{-1}	0.993	57.5	8.15	1.00	3.74	**0.724**	0.035	9.81-15.8	7.78	2.22
DF 0.05-50ng L^{-1}	0.993	14.7	6.94	1.00	0.284	**0.119**	10	0.721-28.9	7.69	1.23

Lowest LOQs using I. or III.
DF: values < required LOQ
E1: values < required LOQ (III.)

RESULTS

STEP 3

VALIDATION

LOQ estimation approach not defined within Directive 2015/495/EU

Sample	DF (ng L^{-1})
Bled	2.11
Bohinj	0.313
Iška	< LOQ
Portorož	1.93
Podpeč	< LOQ
Gradaščica	5.69

	R^2	Accuracy (%)		LOQ (ng L^{-1})				Method repeat.	Instr. repeat.	
		L	H	I. s/n	II. 10×SD/slope	III. $\bar{x}$ + 10×SD	Acceptable LOQ		L	H
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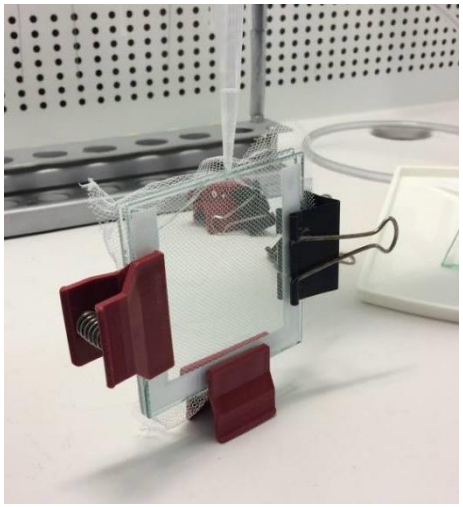
Lowest LOQs using I. or III.
DF: values < required LOQ
E1: values < required LOQ (III.)

ONGOING WORK

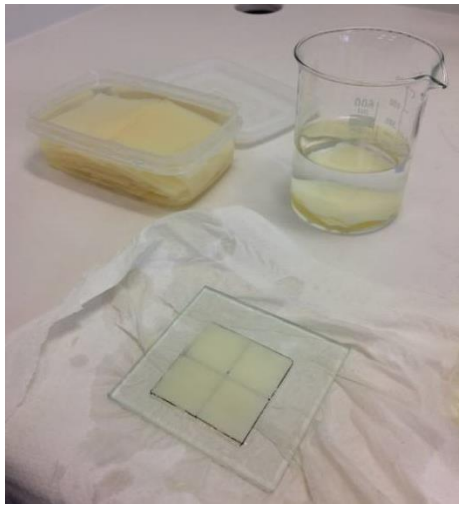
Alternative way to ↑ sensitivity is being investigated in collaboration with **RECETOX (supervision: prof. Vrana):**

passive sampling → **diffusive gradient in thin films** (DGT) sampler for organic contaminants

I. Sampler preparation



Diffusive gel



Binding gel
(with HLB sorbent)

II. Laboratory experiment

- *Rs determination*
- *Field experiments*

CONCLUSIONS

- The optimization of an analytical method for determining E1, E2, EE2 and DF in large whole SW samples
- Method based on SPE using HLB disks on a SPE-DEX® system
- The required LOQs in the Decision 2015/495/EU were reached for E1 and DF
- The LOQ for EE2 set in Directive 2015/495/EU (0.035 ng L^{-1}) was not achieved
- Alternative sampling technique is being investigated to detect low sub ng L^{-1} concentrations of the analytes

THANK YOU FOR YOUR ATTENTION

GLOBAQUA

mass twin

European Commission

Horizon 2020
European Union funding
for Research & Innovation

instrumentalia

Research centre
for toxic compounds
in the environment

Slovenian Research Agency

Jožef Stefan Institute
Ljubljana, Slovenia

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