EDC WFD

Metrology for monitoring endocrine disrupting compounds under the Water Framework Directive_EDC-WFD

22 February 2023

EDC WFD

PRESENTATION/ CONTEXT

THE CONSORTIUM

- 8 partners / 6 European countries
- 5 NMI/DI, one DI operating outside of its designation, one academic research laboratory, one research institute

- Consortium brings together **scientific excellence** in research institutes and experience in **ultra-trace measurements of micropollutants**

- **Balance of expertise**: development and certification of RM, proficiency tests / interlaboratory comparison design, method development and validation, standardisation

Start date: 1st September 2019
Duration: 36+6 months
Budget: 800K€

THE PROJECT

AIMS:

- ❖ Address the standardization lack for harmonised measurement methods for estrogens in whole water
- ❖ Ensure that measurements of estrogens are traceable, well defined, meet the requirements of the WFD, and thus are comparable across Europe (and worldwide)

- ➢ Chief Stakeholder: Ulrich BORCHERS Chairman of CEN/TC 230
- ➢ Chief Stakeholder and DIN secretary (Andreas Paetz) are kept inform of the progress

- ➢ Support from AFNOR (Arnaud Gaudrier: Secretary of SC «Water Quality»

CONTEXT

- **Estrogens:** group of chemicals of similar structure mainly responsible for female sexual development and reproduction.
- In water ecosystem:
  - **Pseudo-ubiquitous** and occur at **ultra-trace level** (<<ng L^{-1} to tens ng L^{-1})
  - Level at which they can have effects in natural species
    - **Threat for biodiversity**

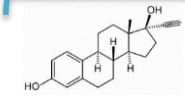
Included in the First Watch List of the Water Framework Directive WFD → *Under review to become priority substances (PS) of the WFD*

- **No EN or ISO standard for MS-based methods** currently available or in progress
- (Accredited) testing laboratories **develop and validate in-house methods** according to internal criteria
- Most of (accredited) testing laboratories **failed to achieve the very low LOQ** to enable monitoring of estrogens at relevant level
- **Metrological endpoints** have been highlighted of particular importance if effect-based method (EBM) results are to be used in a regulatory context
- **Lack/absence of reference materials and ad'hoc proficiency tests**

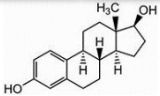
THE PROJECT

Targeted substances

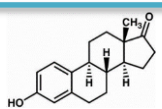
Candidate PS



17α-ethinylestradiol

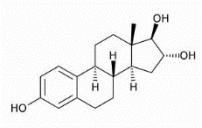


17β-estradiol



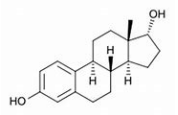
Estrone

+



Estriol

Stability + cross reactivity of EBM



17α-estradiol

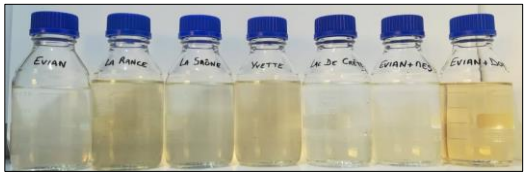
Specificity of MS-based method + cross reactivity of EBM

Targeted level of concentrations (EQS)

Inland waters	PNEC beginning of the project (µg L^{-1})	EQS SCHEER final decision march 2022 (µg L^{-1})
17α-ethinylestradiol	0.000035	0.000017
17β-estradiol	0.0004	0.00018
Estrone	3.6	0.00036

Target Matrix

- Inland freshwaters: surface water and ground water (opt. drinking water)
- Representative of European inland waters panel



AIM

Natural and pharmaceutical estrogens are key Endocrine Disrupting Chemicals (EDC) which are monitored differently depending on the country, and for which standardised reference methods are currently not available.

⇒ Main Objective: Develop reliable and harmonized measurement methods for estrogens, to comply with the WFD Directive requirements

⇒ **Outcomes: to be disseminated to CEN/ TC 230 and ISO/ TC 147 to be fed into the documentary standards they develop**

OBJECTIVES

- The overall objective of this project is to **develop traceable measurement methods** for endocrine disrupting chemicals, with a specific focus **on three estrogens of the first watch list: 17-beta-estradiol (17βE2), 17 alpha ethinylestradiol (17αEE2), and estrone (E1)**)

- Estrogens 17-alpha-estradiol (17αE2) and estriol (E3) will be included to demonstrate the reliability of the developed methods

⇒ **to support the requirements of Directive 2013/39/EC, Directive 2009/90/EC and Commission Implementation Decision (EU) 2018/840,**

⇒ **improve the comparability and compatibility of measurement results within Europe**

The specific objectives of the project are to:

1. **Optimize and validate traceable aqueous reference Mass Spectrometry-based methods for the analysis of 5 estrogenic** compounds prioritizing 17βE2, 17αEE2, and E1 in **whole water samples** at environmental quality standard (EQS) levels. **Methods will have limit of quantification (LOQ) not exceeding 30% EQS with a measurement uncertainty of ≤50 % at EQS**

2. **Develop production methods for aqueous reference materials (RM),** which are as close as possible to real water samples, with proven homogeneity, short- and long-term stability

3. **Improve the comparability of estrogen measurements with selected Effect-Based Methods (EBM)** in whole water samples at EQS level. Methods will have been correctly calibrated and information on uncertainty will be provided

4. **Organize and perform an interlaboratory comparison (ILC)** to demonstrate the performance of the developed methods using the reference material (RM) for the selected estrogen substances

5. **Contribute to the work of key European and international standardization organizations e.g. CEN TC 230 and ISO TC 147**

Ultratraces estrogens measurements < 0,1ng/L In whole water

⇒ New dimension

Every detail counts

WHAT DO WE NEED TO MEASURE?
WHAT ARE WE MEASURING?

WHOLE WATER MEASUREMENTS WHAT ARE WE TALKING ABOUT ?

- What we have in essence developed and validated are whole water analysis methods
- Also **Water Framework Directive** requires to analyze whole water
- Whole water is composed of **liquid phase** and **particulate phase**. **Analytes can exist in both phases**.
- When analyzing whole water, one collects, concentrates, extracts both liquid and particulate phases so that **analytes in both phases are captured and analyzed**.

WHOLE WATER MEASUREMENTS WHAT ARE WE TALKING ABOUT ?

- The concentration result obtained is **a sum of the analyte concentration in both phases**.

- Sample preparation is started by adding internal standards and by letting them to **equilibrate between liquid and particulate phases** for at least 12 hours.

- This improves the probability that results are accurate even if analyte is not fully recovered from the particulate phase in sample extraction.

SAMPLING

SAMPLING

- Comply with general principles of ISO 5667 guidelines
- Implement general principles for ultratrace analysis
- No specific risk or source of contamination was identified during field sampling in the project

- Recommendations:

  - use glass bottle and prevent photodegradation of analytes (amber, green bottles, alumina foil, …)
  - Avoid extra steps, intermediate containers in order to minimize risk of cross-contamination and adsorptive losses
  - Sample container cleaning procedures should be implemented: For example calcination of glassware, rinsing containers with solvents

Avoid plastic containers in case of EBMs

Illustration of adsorption phenomena

Table 2. Relative mass lost over 24 h and first-order sorption rates for selected r

Material	%E2†	%EE2	%E1
Type 304 stainless steel	24.9 ± 5.5	53.4 ± 2.0	52.7 ± 2.4
Type 316 stainless steel	30.5 ± 1.8	56.1 ± 3.7	54.3 ± 3.6
Glass (culture tubes)	1.0 ± 0.7	0.7 ± 0.6	0.6 ± 0.4
PolyCarbonate	8.7 ± 5.1	51.2 ± 8.3	44.1 ± 6.8
PVC‡	4.5 ± 2.5	5.0 ± 2.1	7.7 ± 4.5
Teflon	2.3 ± 1.3	4.2 ± 0.6	2.2 ± 0.8
Autoclaved			
Type 304 stainless steel	2.3 ± 7.0	33.7 ± 7.6	28.4 ± 6.1
Type 316 stainless steel	6.8 ± 17.7	33.2 ± 6.2	20.9 ± 5.8
Glass (culture tubes)	−4.9 ± 0.6	−0.2 ± 1.2	0.5 ± 0.8

- Minimum losses with glass container and materials

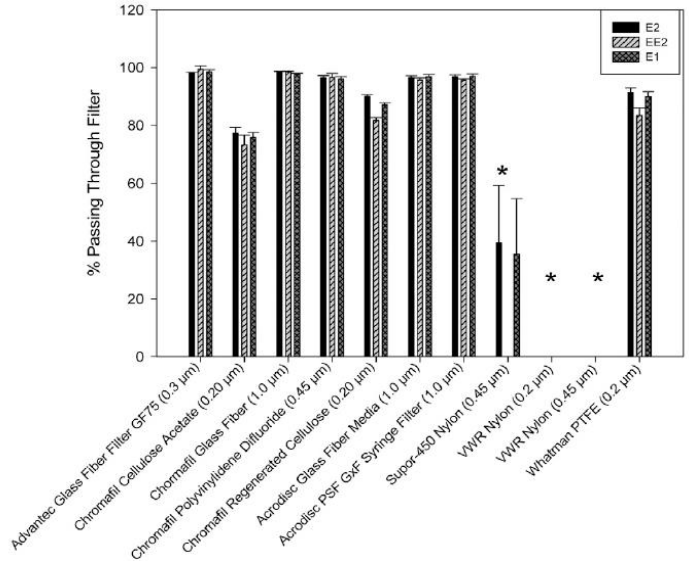


Fig. 3. Percent passing through different filter types. *Significant differences ($p < 0.05$) between the percent passing and the initial concentration. Error bars represent 1 SD of the mean for triplicate samples. E1, estrone; E2, 17β-estradiol; EE2, 17α-ethynylestradiol.

Charles W. Walker* and John E. Watson, 2010

STABILITY

Stability

Main drivers

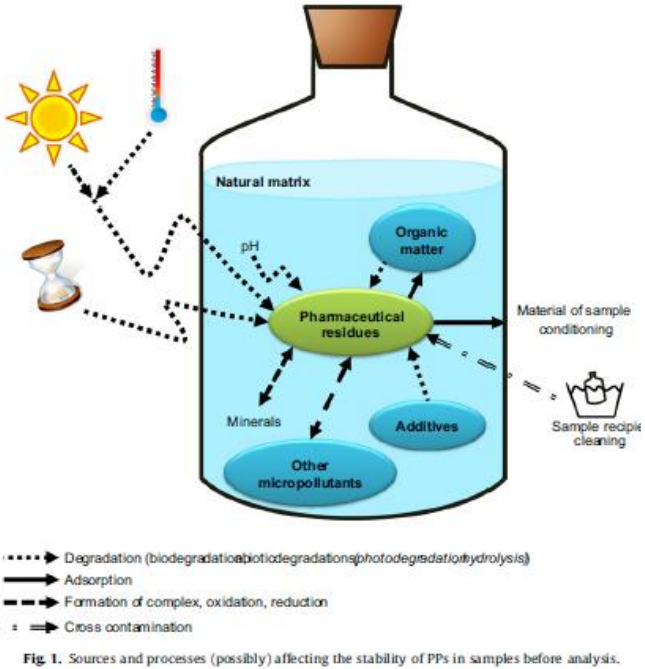


Fig. 1. Sources and processes (possibly) affecting the stability of PPs in samples before analysis.

Mompelat et al. (2013)

Numerous reviews and publications

⇒ Not all in agreement

- Methodological strategy
- Acceptance criteria
- Missing key information

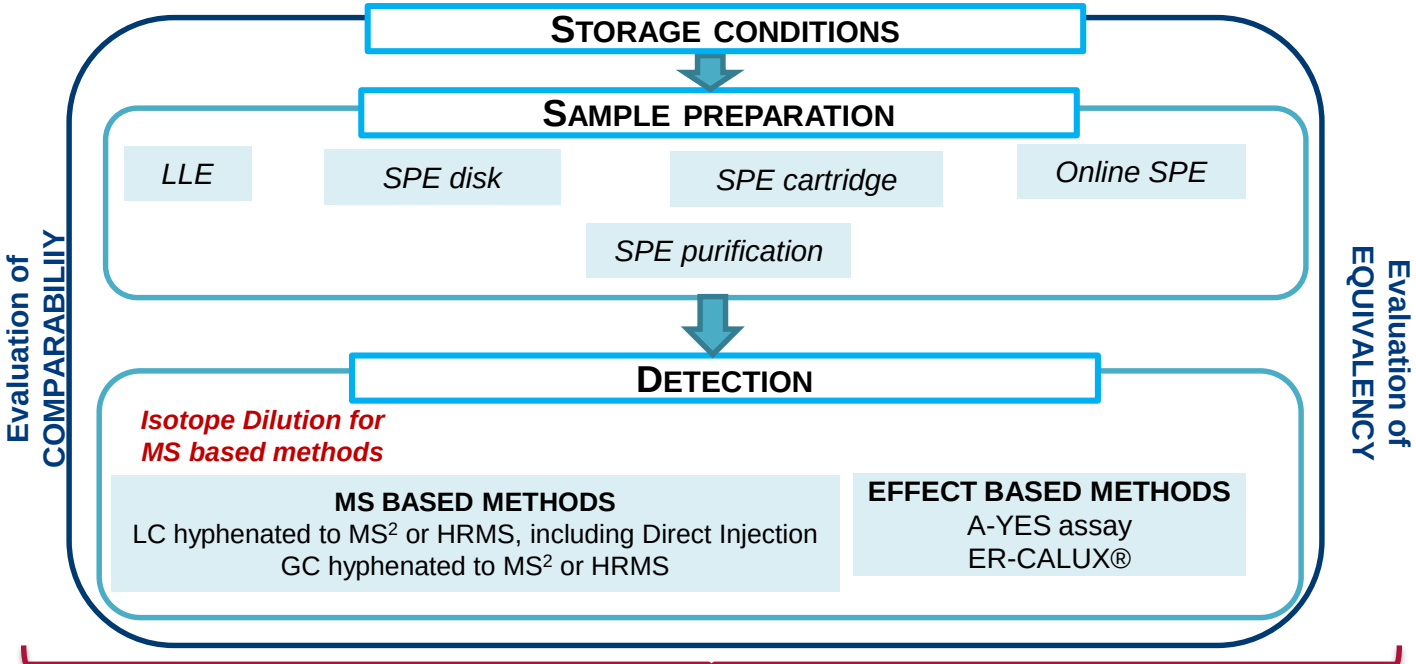
Stability

- Recommendations:

- Glassware (coloured) shall be preferred over other type of containers if very low LOQs (<<0,1ng/L) are targeted, to minimize the risk of analyte losses due to adsorption
- Storage at 4°C is OK for 14 days for synthetic samples of low and high complexity
- Storage time is expected to be shorter for complex natural samples. Addition of 1% MeOH is recommended to avoid biodegradation because it has not shown negative effects on recoveries
- Storing samples at -20°C is possible but it may increase risk of clogging during sample extraction if SPE cartridges are used for extraction.

Plastic container shall be avoided if LOQ <<0,1ng/L targeted

METHOD OPTIMIZATION



Comparison of sample preparation techniques for estrogens in whole water & recommendations on the most appropriate ones

Most promising MS-based method(s) for the measurement of selected estrogens in whole water samples compatible with the requirements of the QA/QC Directive (LOQ≤ 30% EQS with U(k=2) ≤50% at EQS)

Recommendations to improve the comparability of estrogens measurements with the set of selected EBM in whole water samples at EQS levels


QUESTIONS?
COMMENTS?
CONCERNS?