BAM
Bundesanstalt für Materialforschung und -prüfung

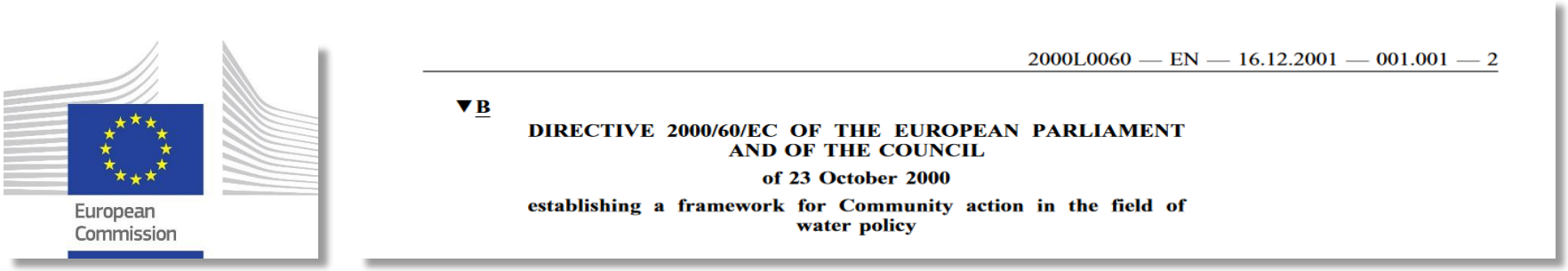
EMPIR EURAMET
The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

”Whole water reference material”

22nd February 2023

EDC WFD

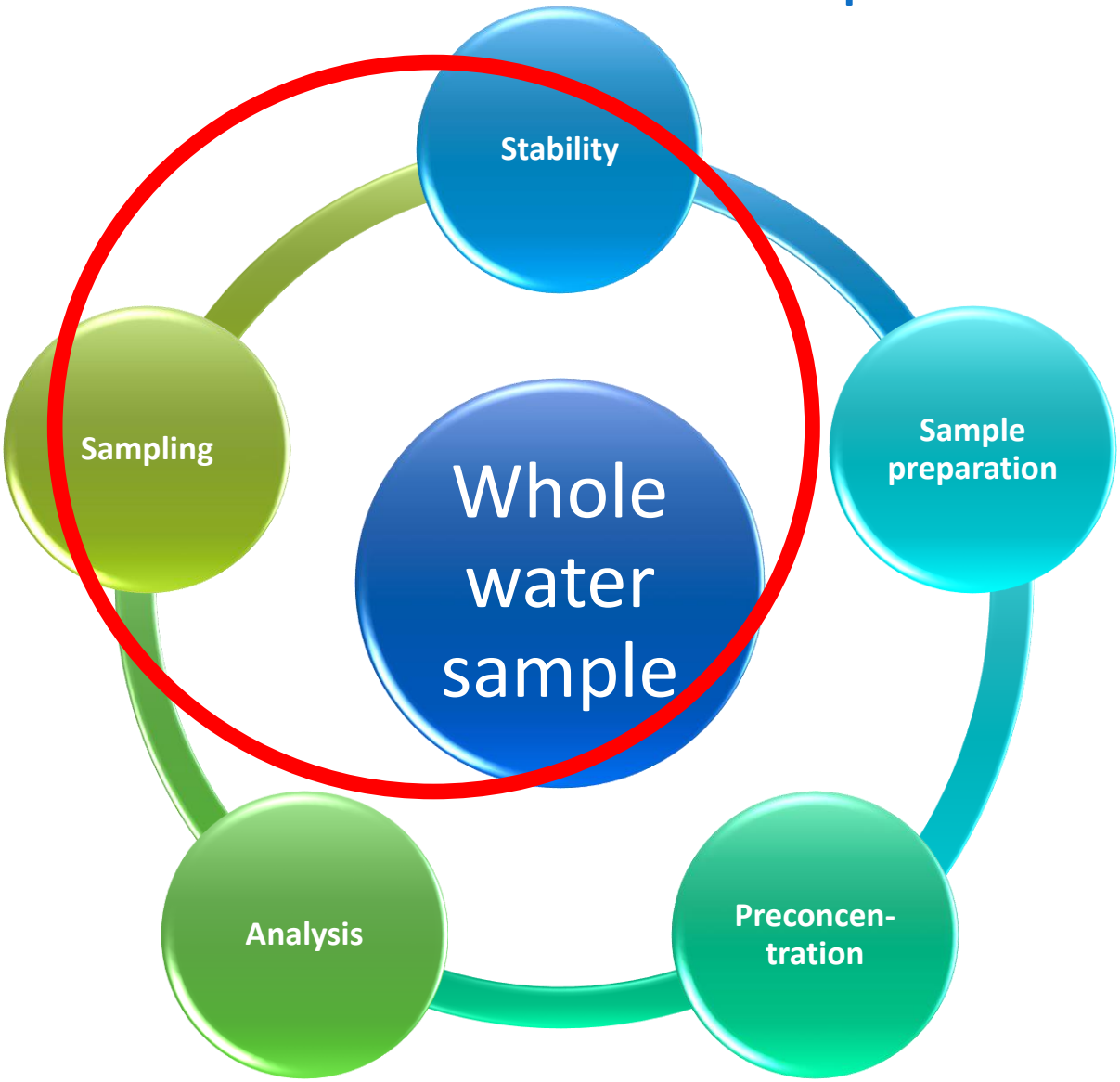
General aspects



"Main objective: Develop reliable and harmonized measurement methods for estrogens, to comply with the EU-WFD requirements (whole water samples)"

The consequence: Analysis of non-filtered water samples or separate analysis of filtered water and SPM (suspended particulate matter) . With a typical load of SPM of 50 mg/L it's challenging to apply preconcentration methods like SPE (solid phase extraction) or to do direct injection with regards to mass spectrometric based methods

General aspects



Need and proof of concept for whole water reference material

Aim

- All project partners should evaluate sample preparation techniques, MS- or EB- methods, or do validation studies with exactly the same whole water matrix with a defined, constant, and stable composition.

Stability

- Ensure the stability of the whole water samples from sampling until the sample preparation and analysis in terms of degradation, metabolism or transformation of the analytes.

SOP

- A detailed and easy-to-use standard operation procedure is needed for the reliable and safe use of a developed whole water reference material.

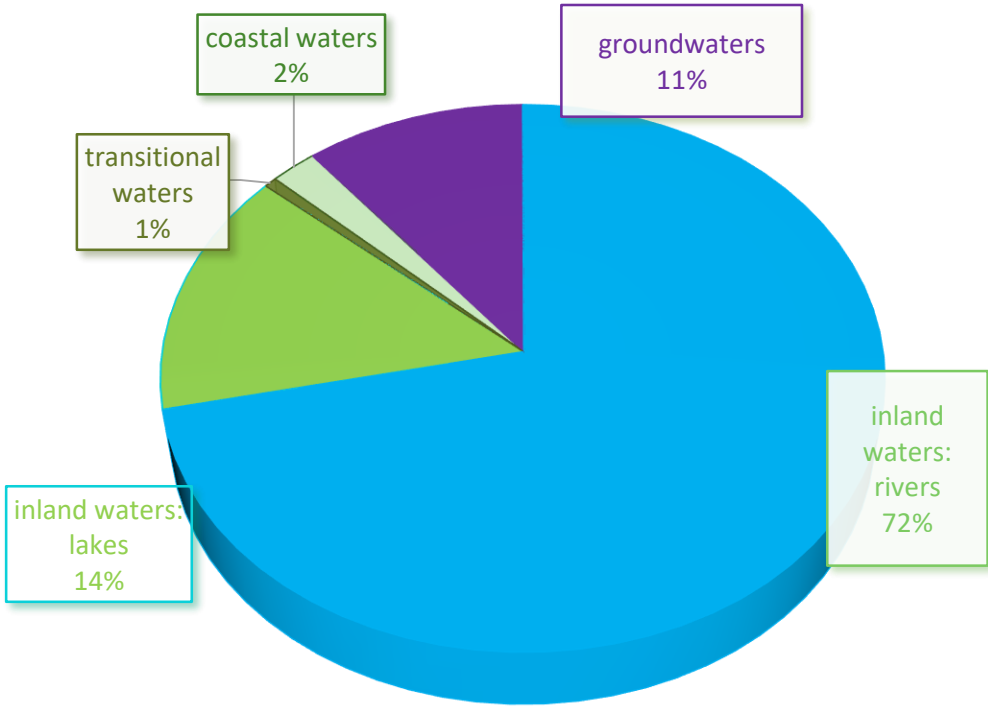
Need and proof of concept for a whole water reference material

EUROPEAN WATER BODIES

Europe

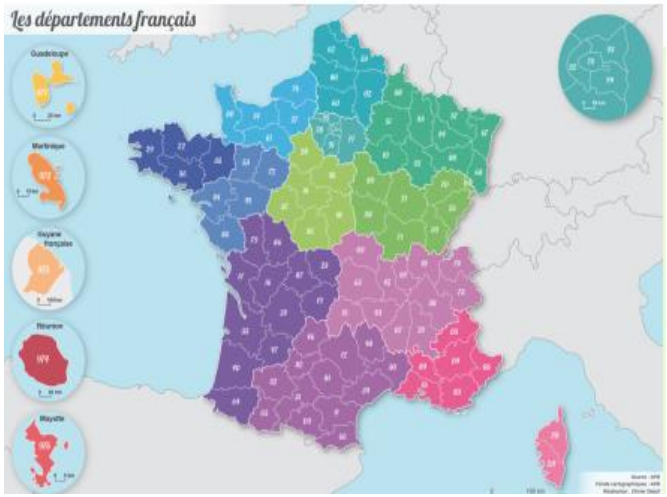
Source:
https://www.eea.europa.eu/themes/water/european-waters

TYPOLOGY OF WATER BODIES

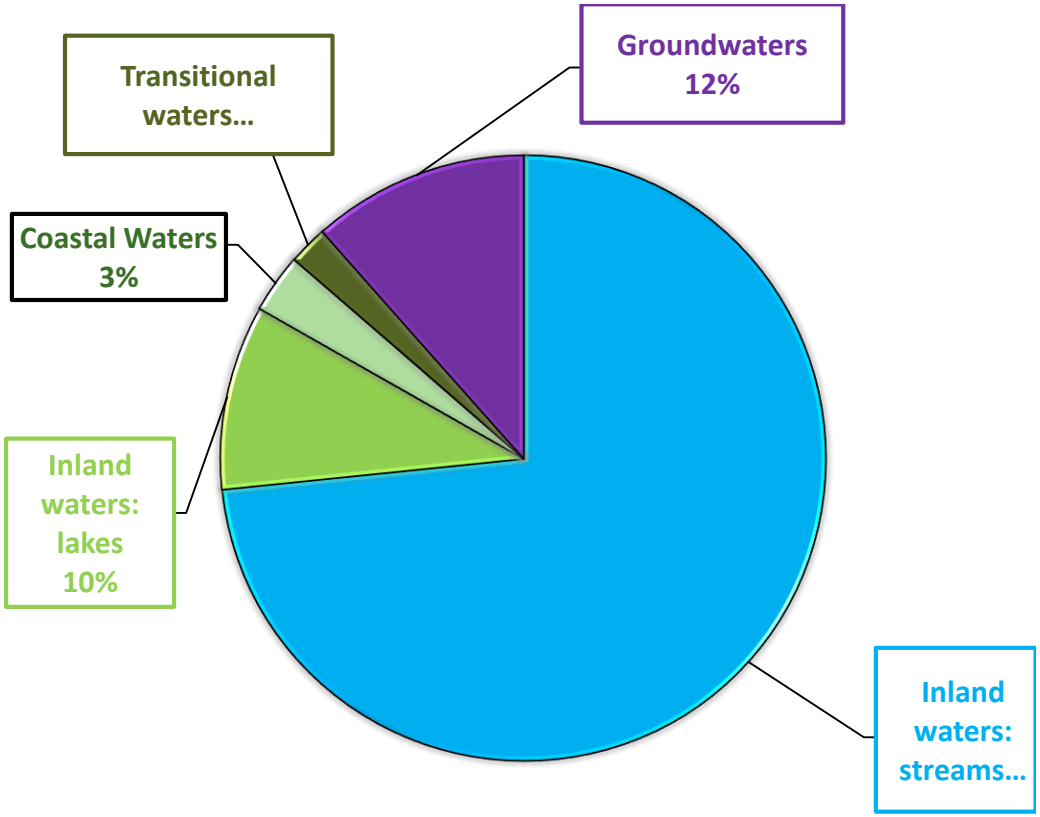


Need and proof of concept for a whole water reference material

FRENCH WATER BODIES



Source FNE



Need and proof of concept for a whole water reference material

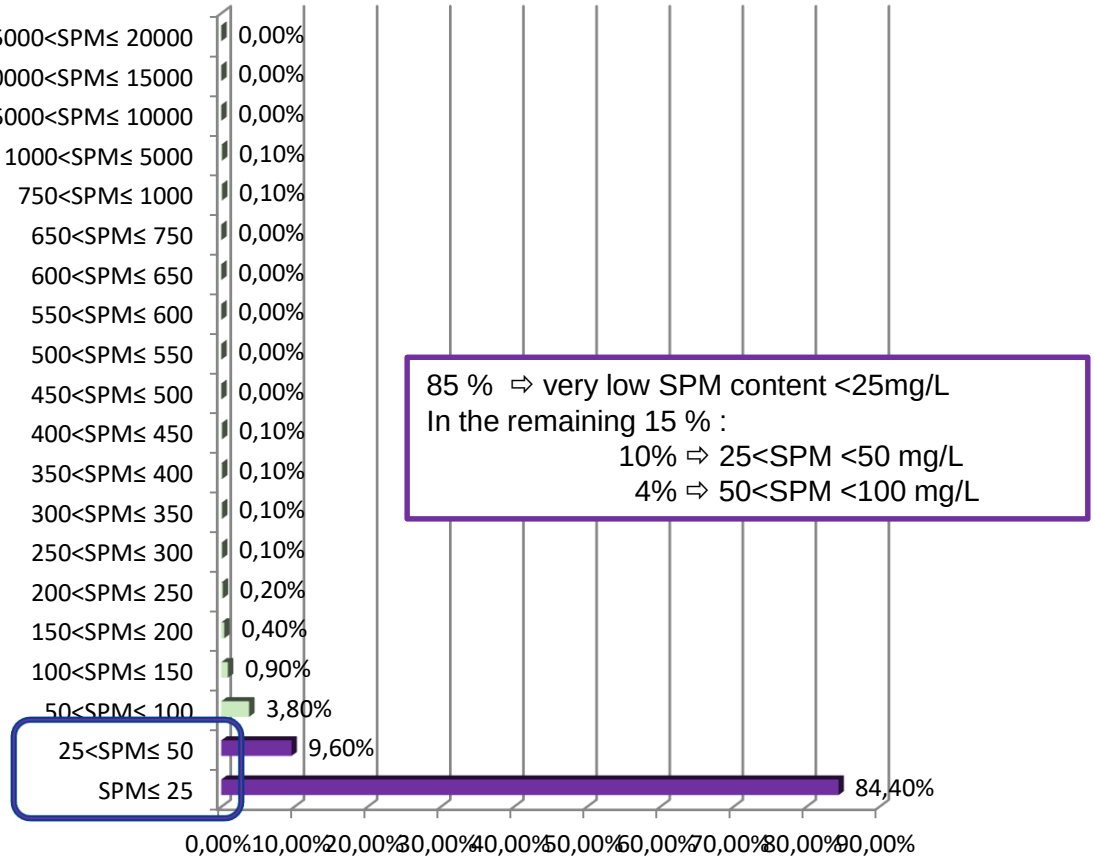
INLAND SURFACE WATERS

Min	0.06 mg L^{-1}
Max	16,000 mg L^{-1}
Average	21.7 mg L^{-1}
Median	9 mg L^{-1}
Quartile 25	4.8 mg L^{-1}
Quartile 75	17 mg L^{-1}

Number of data = 188 283.according to EN 872 with few exceptions

Period : 03/01/2011 01/01/2018

SUSPENDED PARTICULATE MATTER



Need and proof of concept for a whole water reference material

Total Organic Carbon

Min	0.09 mg C L^{-1}	Median	3.1 mg C L^{-1}
Max	190 mg C L^{-1}	Quartile 25	2.0 mg C L^{-1}
Average	3.80 mg C L^{-1}	Quartile 75	4.6 mg C L^{-1}

Conductivity

Min	0.131 µS cm^{-1}	Median	381 µS cm^{-1}
Max	60,500 µS cm^{-1}	Quartile 25	181 µS cm^{-1}
Average	490 µS cm^{-1}	Quartile 75	602 µS cm^{-1}

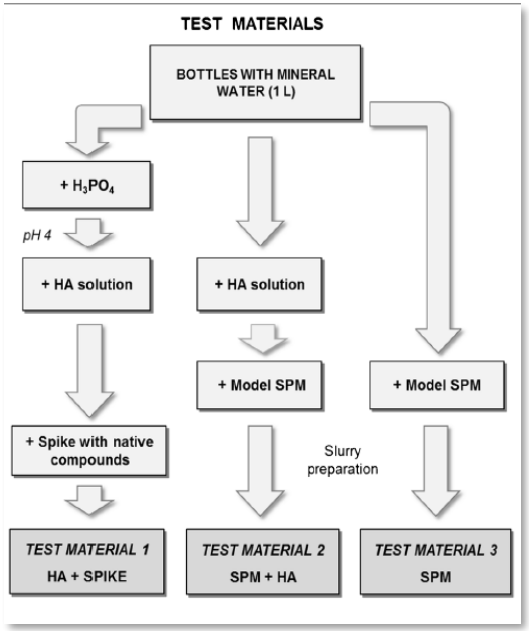
pH

Min	5.3	Median	7.9
Max	12.96	Quartile 25	7.5
Average	7.88	Quartile 75	8.2

Reference material candidate

Need for a representative water matrix

- Setup of a “synthetic real water matrix”
- Defined composition consists of commercial available mineral water with known ingredients (inorganics, pH, and “one source water”)
- Simulated dissolved organic carbon (DOC): commercially available humic acid (CAS-No. 68131-04-4, Sigma-Aldrich) at 7 mg/L level
- Defined pH-value given by the mineral water: pH = 7.3
- Model suspended particulate matter (SPM) estrogen-free and heat sterilized: 50 mg/L
- Estrogen spiking solution containing all five estrogens at desired concentration level (e.g., EQS)



Type	still
Calcium (Ca)	80
Chloride (Cl^-)	6.8
Magnesium (Mg)	26
Potassium (K)	1
Sodium (Na)	6.5
TDS	309

Reference material candidate

Representative water matrix – synthetic real water matrix

- The water matrix can be provided as a kit to all the project partner; all constituents were handled separately; the mineral water in glass bottles can be purchased worldwide by all the partners

- Setting up the individual water samples in each laboratory by a SOP

- Each individual water samples has the same distinct composition and can be used by each partner for method development, sample preparation evaluation and validation

- In comparison to naturally contaminated water this synthetic whole water matrix can be spiked to every desired estrogen level

Reference material candidate

Stability of a whole water sample from sampling until analysis

- Stability study for the determination of the short-time stability (e.g., transport and analysis of sample in the lab) of five selected estrogens (Estrone (E1), 17α-Estradiol (aE2), 17β-Estradiol (bE2), Estriol (E3) and 17α-Ethinylestradiol (EE2)) in common water matrices

- Representative synthetic real water matrix: mineral water, DOC of 7 mg/L, pH 7.3 without SPM and the estrogens at a concentration level of 10 ng/L for each species. Two stabilizing reagents (methanol and ascorbic acid) were evaluated within this study

- Reference temperature; -20°C, storage temperature +4°C and room temperature.

- Sampling time: first week daily and one sample at the day fourteen

- Storage of all samples at -20°C. Isochronous sample preparation and analysis

Reference material candidate

Stability of a whole water sample from sampling until analysis

- First stability study shows best results for sample conservation at +4°C without any stabilizing reagent
- Second study was designed analogously but SPM was used to have a complex water matrix in terms of a whole water sample
- Reference temperature: -20°C, storage temperature +4°C
- Sampling time: day one, five and one sample at the day fourteen
- Storage of all samples at -20°C. Isochronous sample preparation and analysis
- The results of the first study can be confirmed: **best results for sample conservation at +4°C without any stabilizing reagent**

Reference material candidate

Stability of a whole water sample from sampling until analysis

- For the evaluation of effects resulting from a possible microbial activity in whole water samples a microorganism containing spiking solution was added to the synthetic real water samples containing SPM. As model microorganisms selected iron and manganese oxidizing bacteria were used (Sphingomonas spec. and Sphaerotilus spec.)

- Reference temperature: -20°C, storage temperature +4°C

- Sampling time: day one, five and one sample at the day fourteen

- Storage of all samples at -20°C. Isochronous sample preparation and analysis

- Impact of microbial activity showed only a small influence on the decrease of the estrogen concentration. Effect should be taken into account when calculating the uncertainty budget for the whole analytical procedure.

Reference material candidate

Homogeneity of the reference material candidate

- Due to the fact that the complete prepared RM is used for further extraction and analysis and no sub-sampling is allowed the “intra-bottle” homogeneity is not of interest and was not determined.

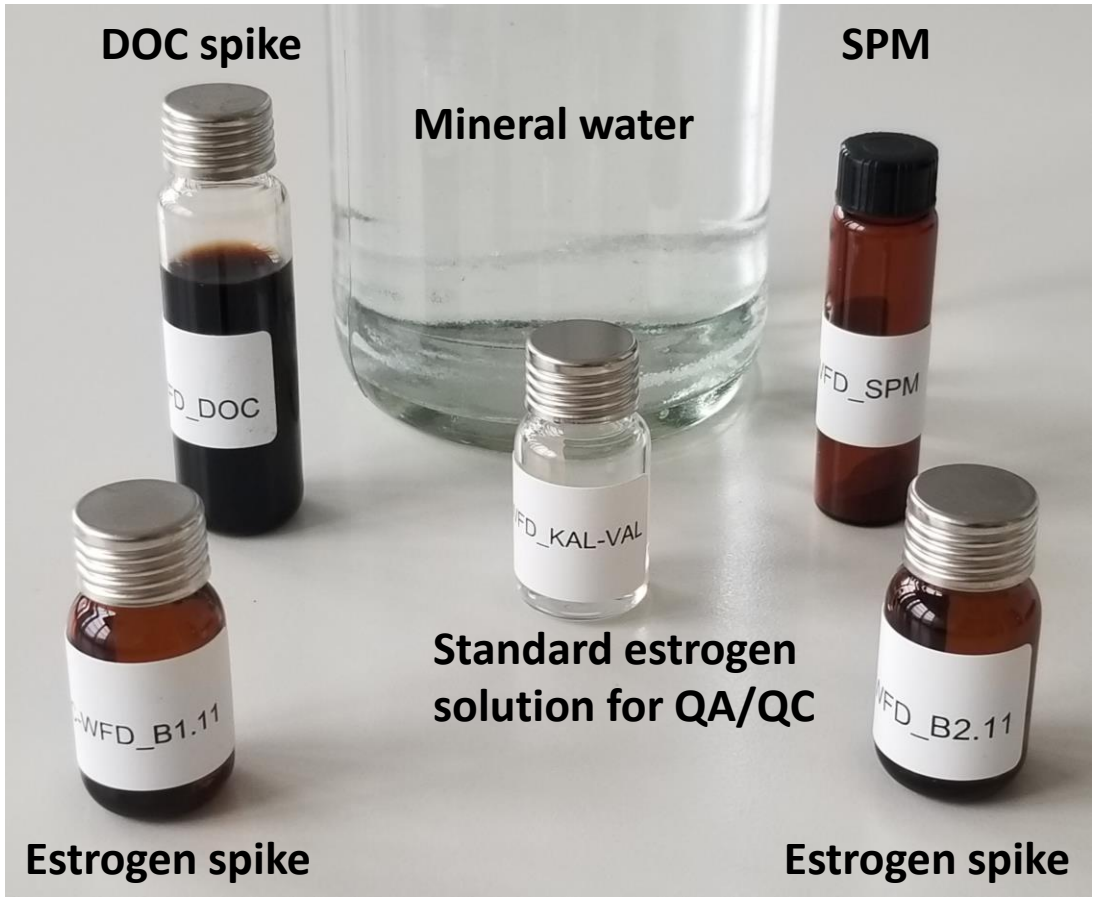
- In a former EMRP project (ENV 08 – “Traceable Measurements for Monitoring Critical Pollutants under the European Water Framework Directive”) the “inter-bottle” homogeneity was evaluated and assessed. Here, no significant inhomogeneities could be observed

- It is strongly recommended to strictly follow the given SOP to have a reliable and reproducible matrix composition and estrogen concentration in the reconstituted RM.

Reference material candidate

SOP for the "Preparation of reference material candidate":

RM kit



Reference material candidate

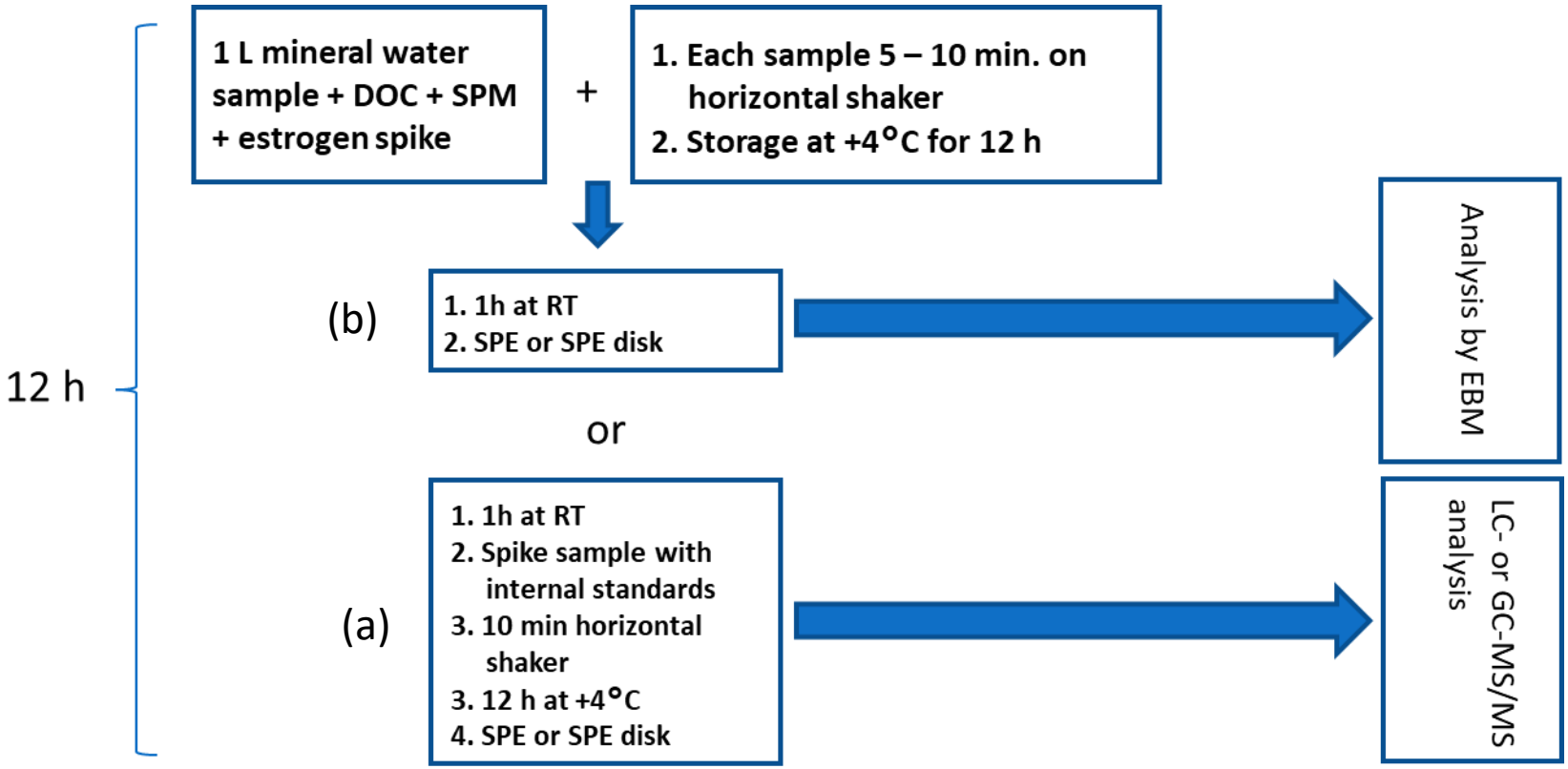
SOP for the "Preparation of reference material candidate":

"Samples should be prepared in amber glass bottles (1000 mL are recommended). A fixed volume of the prepared DOC spiking solution with known DOC concentration (actual 1100 $mg \cdot L^{-1}$) using a 0.45 µm syringe filter (PTFE syringe filters are recommended) giving a final DOC of 7 $mg \cdot L^{-1}$ must be added to the mineral water. Additionally, an aliquot of at least 100 µL of an estrogen spiking solution (e.g., 10 $ng \cdot mL^{-1}$ of E1, alphaE2, betaE2, EE2, and E3 in acetonitrile or methanol) must be added to the DOC containing mineral water. Finally, the desired amount (50 $mg \cdot L^{-1}$) of suspended particulate matter is added. All steps must be controlled gravimetrically. The resulting reference material sample solutions are homogenized on a horizontal shaker or equivalent for at least 10 minutes. Subsequently, an appropriate internal standard mix is used with the needed concentrations (e.g., 1 $ng \cdot L^{-1}$ for each isotopically labeled estrogen). After spiking the samples with the internal standards, they are homogenized again on a horizontal shaker or equivalent for at least 5 to 10 minutes. Store the samples at least for 12 h at +4°C to ensure the equilibrium time. This final RM can be stored for a maximum of two weeks at +4 °C. When analyzing the stored whole water samples allow them to stand at room temperature (+20 °C) for at least one hour."

(Taken from deliverable D5: Recommended production methods for aqueous reference materials, which are as close as possible to real water samples, with proven homogeneity and short- and long-term stability)

Reference material candidate

SOP for the "Preparation of reference material candidate":



- Procedure can be applied for chemical analysis by using internal standards (a) as well as for EB methods without adding internal standards (b).


QUESTIONS?
COMMENTS?
CONCERNS?