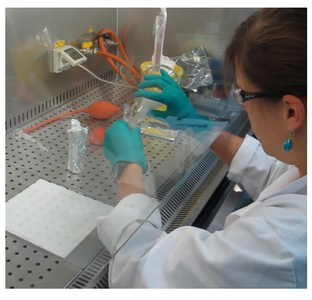

planar-YES

steps towards a fast, robust and sensitive tool for effect-directed analysis of estrogenic activity in water samples

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Introduction

Objectives of the study

- Estrogens/estrogenic activity: may be one reason for observed increase of breast- and testicular cancer [1]
- Drinking water is one possible route of human exposure
- planar-YES: new method for detection of estrogenic activity
- This study: first application of planar-YES bioassay to extracts of raw water samples for drinking water production

planar YES bioassay – innovative elements

- Based on Yeast Estrogen Screen [2], adapted to HPTLC (High Performance Thin-Layer Chromatography) [3][4][5]
- AIM:
  - Effect-directed non-target-screening of water samples
  - Identification of substances causing biological effects
- NEW:
  - Combination of good separation and sensitive detection
  - Potential link to analytical chemistry
  - Direct testing of water samples possible (no extraction)

Material and methods [2]

Sample provenience:

- 2 river waters (Limmat and Rhine)
- Riverbank filtration water (Limmat)
- STP water, Werdhölzli , Zurich (Treated wastewater)
- Mineral water (Evian, Control)

- Water samples divided in 2 parts
- Part 2 spiked w. 7 estrogens and xenoestrogens to 10 ng/l each
- Extraction 3 times with SPE on consecutive days.
- Application to HPTLC-plates with ATS 4
- Automatic development with ADC2.
  Mobile phase Chloroform : Acetone : Petrolether 55:20:25
- 17-β-estradiol (E2) as positive control and standard
- Fluorogen: 4-methylumbelliferyl β-D-galactopyranoside (MUG)

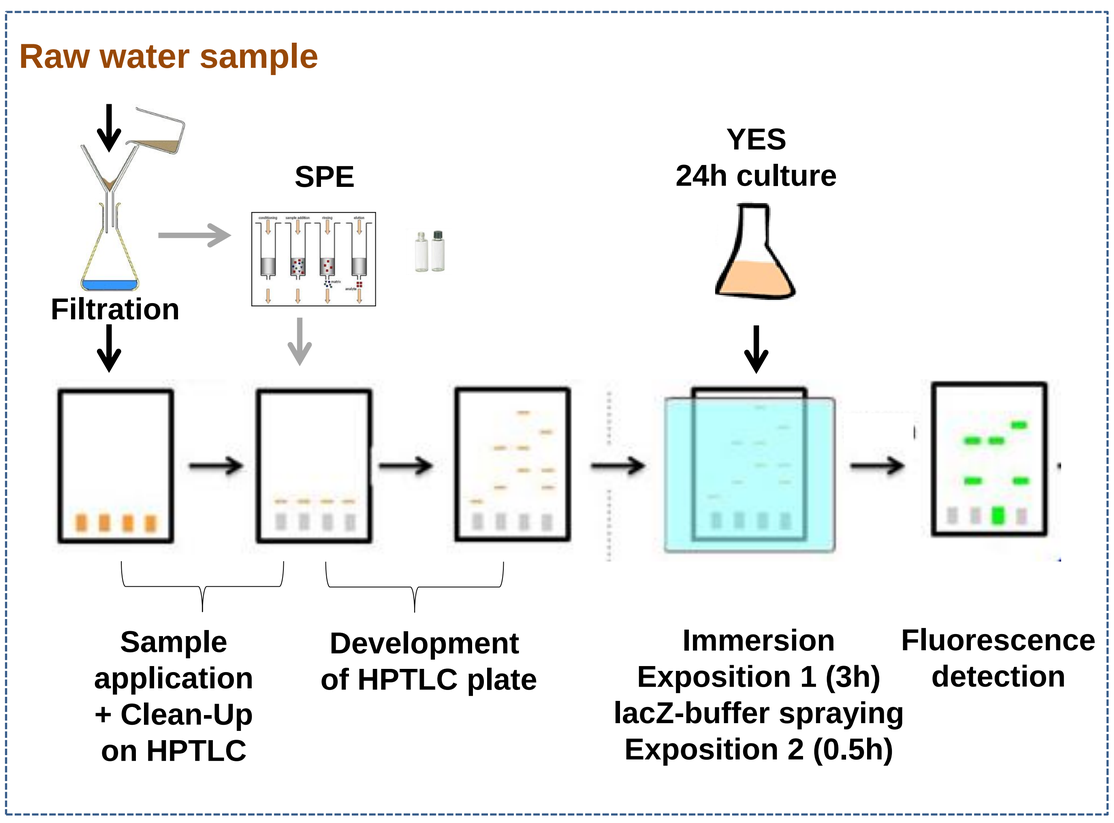

Fig. 1: planar-YES procedure

Results and discussion

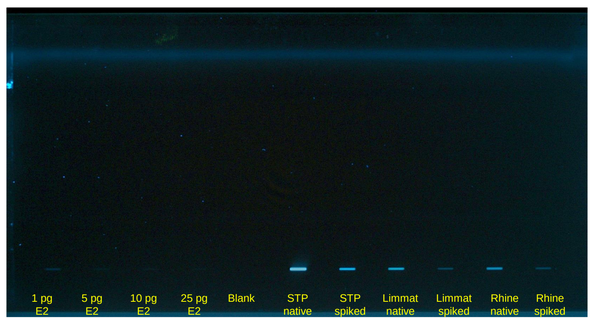

Fig. 2: HPTLC plate after application (366 nm)

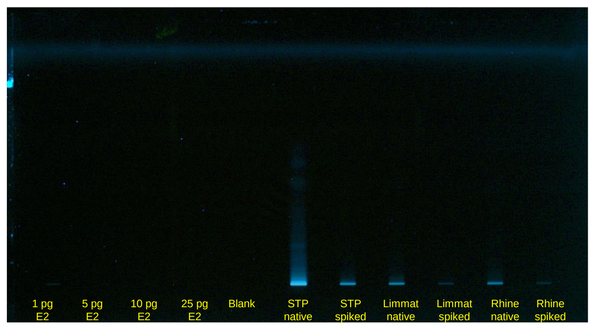

Fig. 3: HPTLC plate after development (366 nm)

- Fluorescent areas: autofluorescence of water extracts before (Fig. 2) and after (Fig.3) development

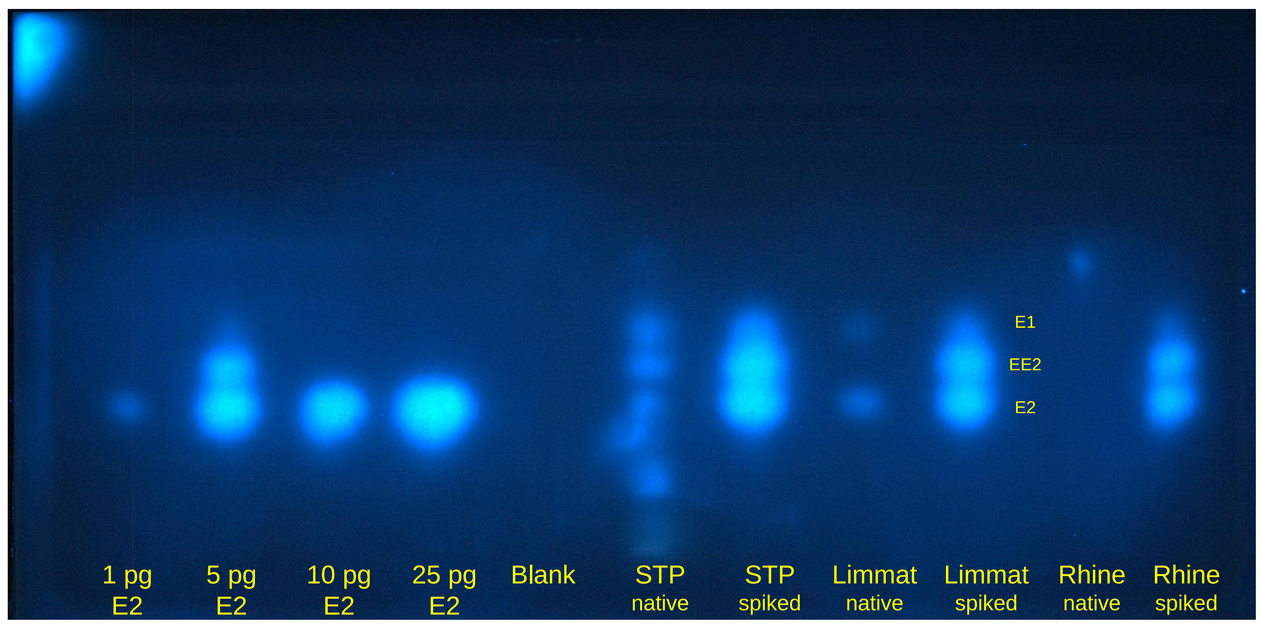

Fig. 4: Fluorescence detection of estrogenic activity on HPTLC plate (366 nm)

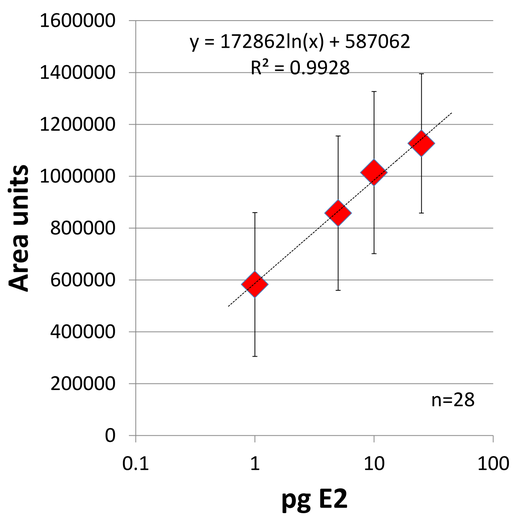

Fig. 5: Dose-response curve of peak area vs. mass per spot of E2 standards

- Estrogenic activity found in Limmat water and STP runoff (Werdhölzli)
- Possible estrogenic activity in Rhine water
- No estrogenic activity found in water from river bank infiltration and Evian water
- Rf-value: approximation of substances
- Peak height and –area of E2 spots allow for quantification of activity (Fig. 5)
- STP effluent & Limmat water contain E2 and unknown estrogenic substances

- Direct processing of native water samples on HPTLC plates is feasible (data not shown)

Conclusion and outlook

- Robust, reproducible assay, up to 11 samples simultaneously
- LOQ < 1 pg/band EEQ (0.1 pg/band within reach)
- Potential to detect 1 ng/l EEQ in native water samples
- Low time demand, compared to classical YES
- Link to analytical chemistry possible (e.g. TLC-MS interface)
- Application to other matrices (cosmetics, plastics exudates …)
- Further refinement of procedure under way
- Aim: ISO certification of planar-YES

References

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