LC-ELISA as a contribution to the validation of immunoassays

HOLGER HOFFMANN[1,2], ULRICH PANNE[1,2], RUDOLF J. SCHNEIDER[1,3]

[1] **Bundesanstalt für Materialforschung und -prüfung (BAM), Unter den Eichen 87, 12205 Berlin**
[2] **Humboldt-Universität zu Berlin, Institut für Chemie, Brook-Taylor-Str. 2, 12489 Berlin**
[3] **Technische Universität Berlin, Straße des 17. Juni 135, 10623 Berlin, Germany**

BAM
Bundesanstalt für Materialforschung und -prüfung

Introduction

Enzyme-linked immunosorbent assay (ELISA) is a highly selective and sensitive method for quantitative analysis.

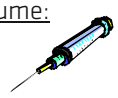
High selectivity is achieved through the 3-dimensional binding site of an antibody, but with small target analytes, structurally related substances might also bind: cross-reactivity (CR). This will negatively impact accuracy. Cross-reactants can be known unknowns or unknown unknowns.

A contribution to validation of ELISAs is provided by a non-target approach of fractionating real-world samples and analysing individual fractions for compounds binding to the antibody: LC-ELISA. Identification is afterwards sought for by (HR)LC-MS/MS.

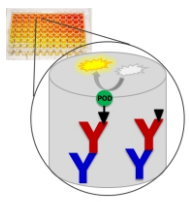
Principle

SPE:
- AutoTrace
- solid phase: Strata-X or BB C18
- load 100-1000 mL sample
- elute with 10 mL methanol
- blow down (N_2), take up in 1 mL H_2O/MeOH 70:30, centrifuge.

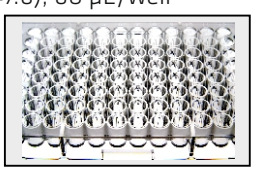
Injection volume:
30 µL



HPLC or LC-MS/MS:
for E1; RP-HPLC:
- Kinetex X_B-C18 (Phenomenex)
- 150 x 3 mm; 2.6 µm + guard c.
- 40 °C; flow rate 0.30 mL/min
- H_2O/ACN(+0.00025% NH_3) grd.



Collect:
into low-bind microtiter plate;
resolution ≈ 0.3 min; neutralize with buffer (pH=7.6), 60 µL/well

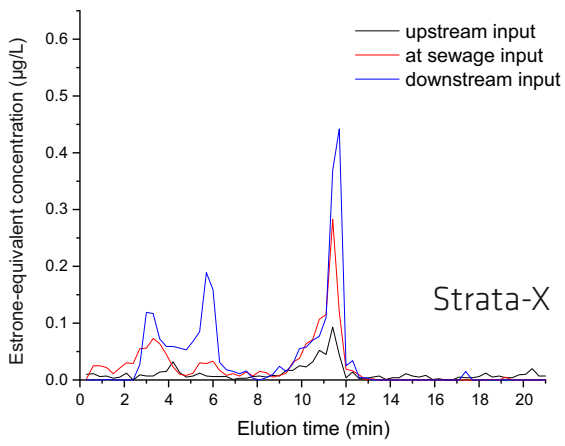


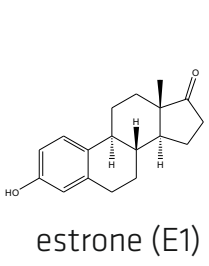
Solvent exchange:
- evaporate to dryness under N_2
- reconstitute in 150 µL water (let soak for 15 min, shake)

ELISA:
- calibrate with E1 standards
- plot equiv. concentration over retention time

⇨ ELISAgram

Results





estrone (E1)

Fig. 1 Overlay of LC-ELISAgrams of samples from 3 sampling sites of a surface water ("Teltowkanal"), pre-concentration 200-fold by SPE on Strata-X

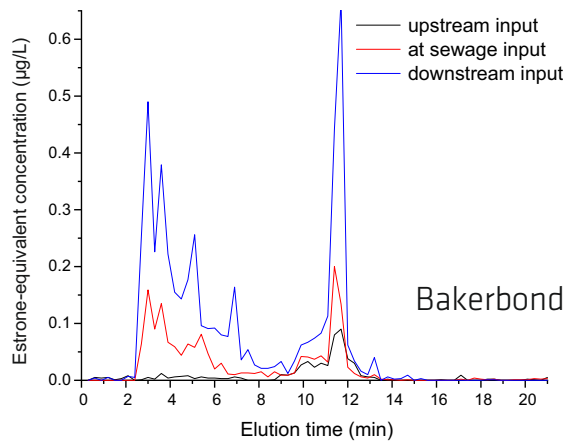
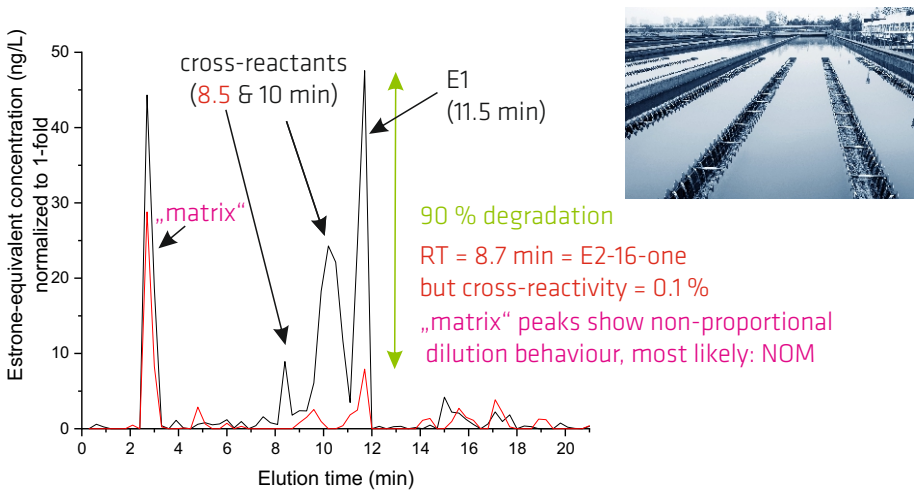
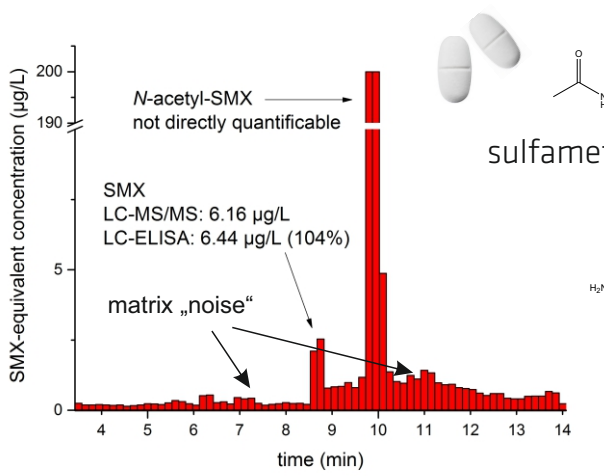


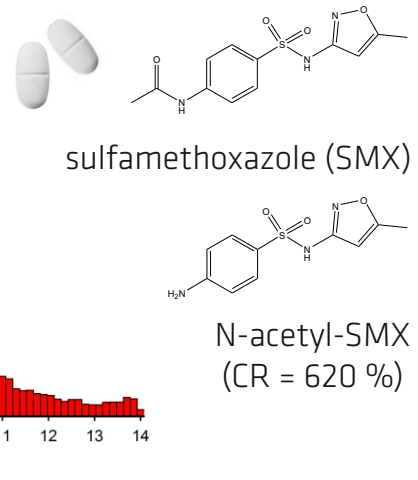
Fig. 2 Overlay of LC-ELISAgrams of samples from 3 sampling sites of a surface water ("Teltowkanal"), pre-concentration 200-fold by SPE on Bakerbond C18



90 % degradation
RT = 8.7 min = E2-16-one
but cross-reactivity = 0.1 %
„matrix" peaks show non-proportional dilution behaviour, most likely: NOM

Fig. 3 Overlay of LC-ELISAgrams for <u>influent</u> and effluent wastewater of a WWTP, pre-concentration factor 12.5, back-calculated concentrations





sulfamethoxazole (SMX)

N-acetyl-SMX
(CR = 620 %)

Fig. 4 SMX equivalent concentrations plotted versus timed fractions of a HPLC run; Berlin WWTP influent sample, polyclonal anti-SMX ab

- Treated sewage input does not only lead to input of estrone into surface waters but also to polar compounds that cross-react with a monoclonal anti-estrone antibody [1].
- Different amounts of compounds (that bind to the antibody) are enriched by solid-phase extraction (SPE) materials Strata-X and C18, respectively (Fig. 1 and 2).

Conclusions

- LC-ELISA as a nontarget approach can contribute to the detection of unknown unknowns in real water samples.
- LC-ELISA can contribute to the validation of antibodies and immunoassays as it allows to predict, for a given matrix, the presence of binding compounds that negatively impact accuracy.
- By observation of their degradation and dilution behaviour in LC-ELISA, true cross-reactants can be distinguished from „matrix" compounds such as natural organic matter (NOM) (Fig. 3).
- A polyclonal anti-sulfamethoxazole antibody [2] gave accurate results when its unexpectedly present metabolite N-acetyl-SMX (cross-reactivity 620 %) was separated beforehand (Fig. 4).

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References
1) H Hoffmann, C Knizia, M Kuhne, U Panne, RJ Schneider: LC-ELISA as a contribution to the assessment of matrix effects with environmental water samples in an immunoassay for estrone (E1). Accred Qual Assur 23 (2018) 349-364
2) H Hoffmann, S Baldofski, K Hoffmann, S Flemig, CP Silva, VI Esteves, F Emmerling, U Panne, RJ Schneider: Strucutral considerations on the selectivity of an immunoassay for sulfamethoxazole. Talanta 158 (2016) 198-207