COMPARISON OF HPLC AND ELRA METHOD IN CASE OF ANALYSIS OF 17α-ETHINYLESTRADIOL IN THE WATER OF AQUATIC MICROCOSMS

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Introduction

The synthetic hormone 17α-Ethinylestradiol (EE) was added to aquatic microcosms (230 L water, 10 cm sediment from Lake Ammersee, Bavaria, Germany) in order to investigate endocrine disrupting effects on the plankton biocenosis[1]. Water samples were taken and the EE-concentrations were on the one hand measured with HPLC and UV-detection, on the other hand an enzyme linked receptor assay (ELRA)[2] was applied.

Methods and Materials

In surface waters EE is found in dissolved and bound state. The bound form of EE is released at pH 2-3. The internal standard (17β-estradiol) is added to the acidified water sample which is then sucked through a SPE cartridge (Bond Elut (Varian)). This cartridge type is very suitable for the enrichment of phenols in water. After extraction the analytes are eluted with ethylacetate and the solvent is evaporated to nearly dryness. The residues are dissolved in dichloromethane and drawn on a LiChrolut Adsorbex NH_2 (Merck) cartridge. First the semi to unpolar impurities are washed with dichloromethane and discarded. EE is eluted with methanol, the methanol fraction is concentrated nearly to dryness. The residues are dissolved in acetonitrile/water (1:1) and analysed with HPLC/UV-detection (λ=220 nm)[2].

The Enzyme Linked Receptor Assay (ELRA) was performed as published elsewhere[3]. In brief, all reactions were carried out in polystyrene 96-microwell plates. Plates were coated and left to incubate overnight with 17ß-estradiol-BSA at 4°C. All subsequent reactions were carried out at room temperature. Solutions were prepared in PBS (80 mM, pH 7.6). After washing plates three times with PBS-Tween 20 and blocking for 1h, 17ß-estradiol solutions of known concentrations (0

µg l-1 – 1000 µg l-1; 50 µl per cavity) or samples were added together with the estrogen receptor. The estrogen receptor was produced in a recombinant yeast expression system and diluted 1:50 in PBS for measurement. This was followed by a 1h incubation at rt. After a further washing step, a biotinylated mouse anti-ER-antibody was added to each well prior to a 1h incubation. An additional washing-step followed and a streptavidin-POD-biotin complex was pipetted into each well and incubated for 1h. After washing the plate, peroxidase substrate was added. The substrate solution consisted of a 200 mM citrate buffer containing 1.2 mM TMB and 0.004 % H_2O_2. The substrate reaction was terminated after 20 min with 2M H_2SO_4 and absorbances measured at 450 nm with an ELISA reader). Curves were fitted applying the 4-parameter logistic function. Estradiol equivalent for unknown samples were calculated from the calibration curve.

Results and Discussion

The HPLC data were plotted against the results of the ELRA measurements (Fig. 1). Above EE-concentrations of 3-5 µg/L the HPLC and ELRA data show good correlations which have to be statistically analysed. Below 3 µg/L the ELRA method detected higher concentrations of EE than the HPLC. This may be due to the fact that the very specific HPLC method could not identify compounds that were causing a signal at the ELRA method because they were binding to the estrogen receptor. The total estrogenity might thus be caused by other analytes than 17α-Ethinylestradiol, possibly metabolites or degradation products.

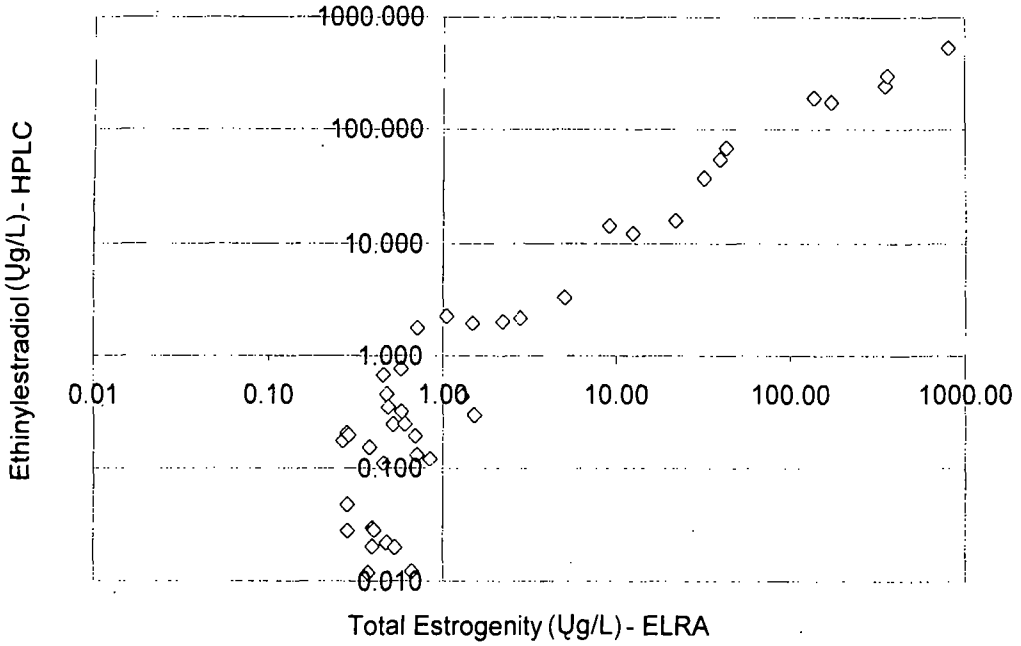

Fig. 1: Correlation of total estrogenity (ELRA method) and Ethinylestradiol concentrations in the water of 230 L microcosms (HPLC method)

Conclusions:

The measurement of EE in natural aqueous systems can be properly done by ELRA and HPLC method in the ppb range. Below ppb range other effects seem to influence the determination of EE in case of the ELRA method.

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