Evaluation of a polyclonal anti‑sulfamethoxazole and a monoclonal anti‑estrone antibody in different types of environmental water samples

Holger Hoffmann1,2, Christian Knizia2, Ulrich Panne2, Rudolf J. Schneider1

1 Bundesanstalt für Materialforschung und -prüfung (BAM), Unter den Eichen 87, 12205 Berlin/D

2 Humboldt-Universität zu Berlin, Institut für Chemie, Brook-Taylor-Str. 2, 12489 Berlin/D

The development of the enzyme-linked immunosorbent assay (ELISA) provided a highly selective and sensitive method for quali- and quantification of analytes. The high selectivity and sensitivity is based on a high affinity between analyte and antibody, but it can be disturbed by different matrix effects. One case of matrix effect is the interference with other, often structurally similar, substances, which are called cross‑reactants. But also the presence of heavy metals, a high content of organic matter or extreme pH values can lead to incorrect results. These influences are, in contrast to cross-reactants, unspecific. One goal of immunoassay evaluation is therefore to discriminate cross-reacting compounds from matrix effects.

Sulfamethoxazole (SMX) and estrone (E1) were used as analytes. SMX is a bacteriostatic antibiotic for use in humans. E1 is a native estrogenic hormone, mainly deriving from estradiol metabolism, and is produced by animals and humans. The typical concentration range of SMX in wastewater or surface water is in the low µg/L and sub µg/L range. Estrone occurs in the mid‑ng/L down to low ng/L range.

The SMX measurements were performed with a polyclonal antibody, which provided in earlier measurements of surface water a 200-fold overestimation compared with the concentration measured by LC-MS/MS. Therefore it is important to identify substances in real samples or get an impression of the matrix influences which are responsible for these false-positive results.

To quantify low concentrations of the analytes, especially of estrone, samples were pre-concentrated by solid phase extraction. Afterwards the extracts were fractionated by high‑performance liquid chromatography. The fractions were tested for “binding” to the anti‑SMX or anti-estrone antibody by ELISA. By this it is possible to get a kind of chromatogram, an “ELISAgram”. The number and intensity of false‑positive signals allows to get an insight of how strong the influences are and how many compounds, respectively which kind of compounds, are responsible for the overestimation.

One identified cross-reacting compound was *N*-acetyl-SMX. It represents the highest signal in the ELISAgram of the SMX ELISA. The second interesting effect is, that after separation of SMX from other sample compounds a satisfactory accordance between the measured concentration by LC‑MS/MS and ELISA was observed. For estrone no known cross-reactants were detected.

Besides the analyte signals some other smaller peaks were occurred. These signals could result from other individual compounds, but the signals are often very broad, which suggests that these are substances which are classified as non-specific matrix effects. A distinction between cross-reactant and unspecific matrix effects remains difficult, because of an often similar behavior.