Abstract

Estrogens are considered to belong to chemicals that negatively affect the endocrine system, even if present at very low concentrations. They are discharged into environment as a result of an increasing application of drugs etc.

This work is focused on the separation and quantification of five estrogens, namely estrone (E1), 17β-estradiol (βE2), 17α-estradiol (αE2), 17α-ethynylestradiol (EE2) and estriol (E3) in natural water samples by HPLC-UV method. The chromatographic system consisted of a C18 stationary phase (SunFire® C18, 150 x 4.6 mm, octadecyl bounded to silica gel, particle size 5 μm) and binary mobile phase of acetonitrile/water in various ratios in isocratic separation mode. The effect of acetonitrile content in the mobile phase and flow rate of the mobile phase on retention and separation parameters was tested. Under the optimized separation conditions (acetonitrile/water 40/60 (v/v), 1.3 ml/min), all the compounds were baseline resolved and eluted within 15 min. These experimental conditions were applied to the calibration measurements which were carried out within the concentration range from 0.001 to 1 mg/ml. Limits of detection (LOD) and limits of quantification (LOQ) for the individual estrogens and their mixture (standards dissolved in methanol) were determinated. The detection limits are within the range of 0.40 – 2.10 μg/ml. To improve the sensitivity of UV detection, the preconcentration step was included prior to the HPLC separation. The solid-phase extraction (SPE) tested new sorption materials based on C18 bound to the silica support by monomeric and polymeric mechanisms and was concentrated on the effect of the experimental parameters on the preconcentration yield. Under the optimized conditions, this yield equals 97 to 103 % for mixture of estrogens and 85 to 98 % for individual estrogens when using an endcapped, monomer-bound preconcentration column (Discovery DSC-18Lt) . The optimized SPE-HPLC-UV method permitted analyses of natural waters with a very good correlation of the calibration dependences ($R^2 > 0.9977$) within a concentration range from 0.01 to 1 μg/ml for all the estrogens studied. The detection limits including preconcentration step are within the range of 3.50 – 14.40 ng/ml.