

# LIQUID CHROMATOGRAPHY COUPLED TO ION TRAP BASED MASS SPECTROMETRY APPLIED IN THE ANALYSIS OF ESTROGENIC CONTAMINANTS

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Steroid hormones, including sex steroids, play an important role in biological homeostasis. Any disruption to the endocrine balance in steroid hormones can cause adverse effects in both humans and wildlife. Through recent years, considerable attention has been focused on environmental estrogens. Natural, as well as, synthetic compounds that elicit estrogenic activity have already been identified in several environmental matrices.

A precise and reliable method was developed for the determination of three of the most commonly found endogenous estrogens, namely estron, estradiol, and estriol, together with two synthetic estrogens, ethynylestradiol and DES in environmental water samples, using ion trap based mass spectrometry combined with liquid chromatography (LC-ESI-MS).

**In a first stage, mass spectrometer parameters were optimized.** Effects of solvent composition, buffers and flow rate on electrospray ionization of the target compounds were evaluated through direct infusion with a syringe pump. Only operating in negative ion mode the electrospray ionization source gave acceptable results, generating the intact deprotonated molecules [M-H]<sup>-</sup>. Further fragmentation was investigated by altering collision energy. Simultaneously, chromatographic conditions were set to separate the five compounds of interest. Using a narrow bore column, run time could be decreased drastically.

Finally, all estrogens could be separated within a few minutes on a Purospher<sup>®</sup> Star RP-18 column (55 x 2 mm I.D., particle size 3 μm) using a gradient system at a flow rate of 300 μL/min. Mobile phases consisted of acetonitrile and water with no buffer added. The method was validated and found to be linear, selective and robust. Limits of detection (LOD) and quantitation (LOQ) were in the low nanogram range for all compounds.

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