# DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR QUANTIFICATION OF SOME NATURAL, PHARMACEUTICAL AND INDUSTRIAL COMPOUNDS IN RIVER WATER

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The increasing level of organic compounds released in the environment has raised the global concern about the contamination of water resources. Some of those chemicals, either of natural or synthetic origin, can interfere with the normal function of the endocrine system by binding to oestrogen receptors. This fact may induce the presence of estrogenic effects, disturbing the reproductive physiology of the organisms, compromising immunity, or even promoting carcinogenesis [1,2]. As there is a lack of information about the levels of those compounds in Portuguese waters, and we anticipate their occurrence in the environment, this study reports the development and validation of a HPLC-DAD method for the quantification in water of several estrogenic compounds of natural (biochanin A, BIO-A), pharmaceutical (17α-ethinylestradiol, EE2) and industrial (bisphenol A, BPA and 4-nonylphenol, 4-NP) origin. The importance of these compounds comes from the fact that: BIO-A is a potent phytoestrogen found in plants and many food products which, in high levels, produce biological damages [3]; EE2 is used in birth control medication and hormone replacement therapy and its levels are usually high in polluted environments [4]; BPA is used as additive in insecticides, pharmaceutical drugs, plastics, coating and adhesives; and 4-NP is the main microbial degradation product of nonylphenol polyethoxylates used in detergents, paints, herbicides and cosmetics [5].In the present study, water samples containing the mentioned xenoestrogens suffered a preconcentration treatment in Oasis Hydrophilic-Lipophilic Balanced (HLB) cartridges [5,6], before HPLC-DAD analysis. For evaluating the recoveries, distilled water (1 L) was spiked with 1350 µg/L of BPA, 2250 µg/L of EE2, 180

µg/L of BIO-A and 4500 µg/L of 4-NP. Our data revealed that recoveries exceeded 90% for BPA, EE2 and BIO-A and 70% for 4-NP. The chromatographic system consisted of a Merck Hitachi HPLC system with diode array detection and a LichroCart RP-18 analytical column. The HPLC-DAD method for quantification and presumptive identification of the last xenoestrogens was developed following published validation criteria (ICH) [7]. Here, among other measured parameters, we report the detection limits (DL), the quantification limits (QL) and the calibration curves (CC) for the four mentioned xenoestrogens. Based on a Signal-to-Noise (S/N =3) computation, the DLs were 200 µg/L for BPA, 380 µg/L for EE2, 20 µg/L for BIO-A and 1000 µg/L for 4-NP. The QLs determined by Signal-to-Noise (S/N=10) were 450 µg/L for BPA, 1000 µg/L for EE2, 80 µg/L for BIO-A and 2500 µg/L for 4-NP. The CCs of the last xenoestrogens were established using standard mixtures from stock solutions. In this study each calibration standard was assayed in triplicate and the concentrations used range from 900 - 2700 µg/L for BPA, 1500 – 4500 µg/L for EE2, 120 – 360 µg/L for BIO-A and 3000 - 9000 µg/L for 4-NP. The correlation coefficient of the calibration curves for BPA, EE2, BIO-A and 4-NP were 0,9966; 0,9974; 0,9985; 0,9990, respectively.Preliminary studies revealed that this method can be successfully applied for the determination of the referred xenoestrogens in Douro River. The preconcentration was carried out with 1 L of river water and BPA, EE2 and BIO-A were detected at level of µg/L.

In conclusion, we developed and validated an economical analytical method (HPLC-DAD) for the simultaneous quantification of several xenoestrogenic compounds in water samples, showing that it can be easily adjusted for their quantification at ng/L or µg/L levels.


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