DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR QUANTIFICATION OF SOME ENDOCRINE DISRUPTING PHYTOESTROGENS IN WATER

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Environmental concern about chemicals that can alter endocrine functions is increasing due to their wide occurrence in the aquatic environment and their potencial hazard to both aquatic and human life [1].

Presently, most experimental studies about the biological effect of endocrine disruptors are focused in the quantification of pesticides and other synthetic compounds. In contrast, natural occurring substances like phytoestrogens, found in plants and in many food products, have received less attention [2]. However, several studies reported that high concentrations of those polyphenols, structurally similar to natural and synthetic estrogens, are responsible for many biological effects [3]. Moreover, recent studies have shown that isoflavone compounds, including genistein, daidzein and formononetin, are discharged in effluents from sewage treatment plants, from pulp mills and are present in agricultural runoff from intensive livestock operations [4]. Since there is a lack of information about the levels of the cited isoflavones in Portuguese waters and because we anticipate their occurrence in the environment, this study reports the development and validation of a HPLC-DAD method for the quantification of genistein, daidzein and formononetin in aqueous environmental samples.

In a summarized form, the extraction procedure used for the preconcentration of the above referred phytoestrogens from water samples occurred in Oasis Hydrophilic-Lipophilic Balanced (HLB) cartridges [5,6]. During this procedure, distilled water (2 L) was spiked with 800 µg/L of daidzein, 1080 µg/L of genistein

and 1160 µg/L of formononetin. Recoveries in Oasis cartridges exceeded 90% for all compounds. The HPLC-DAD method for quantification and presumptive identification of the phytoestrogens was developed in a LichroCart RP-18 analytical column and followed 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 above mentioned phytoestrogens. The CCs were established using standard mixtures from stock solutions. Each calibration standard was assayed in triplicate and the concentrations used ranged 400-1200 µg/L for daidzein, 540-1350 µg/L for genistein and 580-1450 µg/L for formononetin. The correlation coefficients of the last CCs, for all compounds, were higher than 0,9990.

In conclusion, we developed and validated a not expensive analytical method (HPLC-DAD) for the simultaneous quantification of daidzein, genistein and formononetin, showing that it can be easily adjusted for their quantification at a ng/L level in water samples.

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