Determination of Tamoxifen and its Major Metabolites in Exposed Fish

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Tamoxifen (TAM), (Z)-1-(p-dimethylaminoethoxyphenyl)-1, 2-diphenyl-1-butene, is a nonsteroidal agent that has been used in breast cancer treatment for decades. Its major metabolites are 4-hydroxytamoxifen (4-OHT), N-desmethyltamoxifen (DMT), and endoxifen. While TAM and metabolites have been measured in humans, rats, and chickens, we know of no studies conducted in fish, although fish are likely to be exposed to these chemicals through wastewater effluent entering aquatic environments. The purpose of this study was to develop and validate an analytical method for quantifying TAM and metabolites in fish plasma. We tested and modified the published analytical methods of Zhu *et al*. (2008, J Pharmaceut Biomed, 46: 349-355), which use UV exposure to convert TAM and metabolites to fluorescent derivatives for HPLC detection. We evaluated mexiletine, propranol, methoxytamoxifen, clomiphene and tormifene for internal standards. To determine optimal extraction and analytical methods, plasma samples from untreated fish were spiked with TAM and metabolites. Different solvents for extraction were evaluated, including diethyl ether (DEE) and various hexane/butanol mixtures. We varied combinations of mobile phase to optimize the separation, settling on an 82:18, methanol: 1% aqueous triethylamine solution. The optimal time for photochemical conversion of TAM and metabolites to phenanthrene derivatives, using a 4-watt UV lamp, was assessed. Chromatographic separations were carried out on a Waters HPLC with a 2690 Alliance separation module, 996 photodiode array detector, and 474 scanning fluorescence detector. The column was an Agilent Zorbax Extend C_{18} maintained at 60°C preceded by a matching guard column. A flow rate of 0.75 ml/minute was found to maximize separation of all analytes. The method detection limit was 1 ng/mL. Cunner (*Tautogolabrus adspersus*) were given a single 25 mg/kg dose of TAM by oral gavage, then approximately 0.5 ml of plasma was drawn from a caudal vein 1, 4, 8, 16, 20, 24, 48 and 72 hours later. Three fish were sampled at each time point, and each fish was sampled twice during the study. The analytical method that we developed is being utilized to evaluate TAM and metabolites in the plasma of these fish.