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Alexa Mariah Fisher
Southern Adventist University, falexa@southern.edu

Benjamin J. Thornton
Southern Adventist University, benthornton@southern.edu

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Alexa Mariah Fisher[α*] and Benjamin J. Thornton[b]

Southern Adventist University

[α*] Department of Biology, Hickman Science Center, Southern Adventist University, Collegedale, TN 37315, USA; falexa@southern.edu
[b] Department of Biology and Allied Health, Hickman Science Center, Southern Adventist University, Collegedale, TN 37315, USA; 423-236-2859; benthornton@southern.edu

ABSTRACT

Studies of the effects of estrogen in aquatic ecosystems largely focus on fish. In fish, estrogen reduces fecundity in females, reduces testicular development and fertility in males, and alters vitellogenin production in both sexes. One way estrogens enter aquatic environments is via wastewater effluents. Effluent samples from the Moccasin Bend Wastewater Treatment Facility in Chattanooga, Tennessee were tested for the presence of 17-beta-estradiol (E2), using an Agilent 1260 Infinity LC (HPLC). We were not able to detect the presence of E2 in these samples with the methods described. However, when effluent samples were spiked with stock E2 (final concentration of 0.318 mM), consistent retention times with a corresponding peak were seen. If E2 is present, the absorbable readings could be below the minimum accurate detection limit of our machine (3-2,000 mAU).

INTRODUCTION

Environmental estrogens are considered endocrine disrupting chemicals (EDCs) which can affect the reproduction and development of organisms in freshwater ecosystems (Jobling et al., 2003; Ferguson, Iden, McElroy & Brownawell, 2001). With the addition of estrogen in the water, reproduction rates decrease, causing the population to decline and having possible deleterious effects on the community (Filby, Shears, Drage, Churchley & Tyler, 2010).

Reproductive effects of environmental estrogens have been documented in both fish and snails (Jobling et al., 2003; Rose et al., 2013). Estrogenic effects on reproduction in freshwater fish include reduced fertility (Filby, Shears, Drage, Churchley & Tyler, 2010), induced male feminization (Arnold et al., 2014; Tetreault et al., 2011), reduced sperm count (Kidd et al., 2007)

and increased numbers of intersex males (Jobling et a., 1998). Red-shiners, fathead minnows, and three-spined sticklebacks have all been found to be feminized after exposure to E2 (Vajda 2006; Vajda, et al. 2008; Wibe, et al., 2002a; Doyle and Lim, 2002). McGree, et al. (2010) documented the reproductive failure of red shiners after exposure to exogenous estrogen. Females deposited significantly fewer eggs when with males who were exposed to E2 compared to those with unexposed males. When males were exposed to E2, fertilization success was significantly lower. McGree speculated that the sperm may have been weakened or dead. Eggs fertilized by E2 exposed males failed to hatching. Exposed males displayed less dancing and courting behavior than those unexposed.

Freshwater snails experienced both increased and decreased egg production when exposed to environmental estrogens (Oehlmann, Schulte-Oehlmann, Tillmann & Markert, 2000) and was both time and dose-dependent. After seven days, snails exposed to lower concentrations of effluent (between 1 and 25 ng) experienced an increase in embryo production. However, after 21 days, those exposed to high concentrations (~100 ng) experienced an inhibitory effect (Jobling et al., 2003). Fish have been found to be more adversely affected than snails by environmental estrogens. Jobling et al. (2003, pg. 218), noted that fish experienced a negative effect after only three days, versus 14 days for the snails which could indicate a "slower effect of the effluent on embryo production in snails than on vitellogenin induction" in the fish. The effects of E2 can be significantly different depending on the fish species, the duration of exposure, concentration of the estrogen and developmental period. (Nash et al., 2004; Fenske et al., 2005; Schafers et al., 2007). Feminization could adversely affect fish populations by lowering genetic diversity (limited number of males capable of mating) or producing fewer viable offspring, (Filby, Shears, Drage, Churchley & Tyler, 2010).

Environmental estrogens commonly enter these aquatic ecosystems via sewage effluents (Sumpter & Jobling, 1995). Cumulatively, the world's human population discharges approximately 30,000 kg/yr of natural steroidal estrogens (E1, E2, E3) and an additional 700 kg/yr of synthetic estrogen (EE2) solely from birth control pills (Adeel, Song, Wang, Francis & Yang, 2016). Estrogenic compounds entering sewage systems are not completely removed by the wastewater facility, thus they are released in the effluents and can affect aquatic organisms (Ferguson, Iden, McElroy & Brownawell, 2001). In the current study we used HPLC in an attempt to detect E2 in the effluents released from the Moccasin Bend Wastewater Facility in Chattanooga, Tennessee.

MATERIALS AND METHODS

17-beta- estradiol was purchased from Sigma-Aldrich (Madrid, Spain) and ultra pure water from Milli-Q system (Millipore, Bedord, MA, USA). A stock solution of 17-beta-estradiol was prepared in methanol (1000 ppm) and stored in an amber glass bottle at 4° C. HPLC- grade methanol and acetonitrile were obtained from Panreac Quimica, in Barcelona, Spain.

Effluents were collected at Moccasin Bend Wastewater Plant in Chattanooga, Tennessee on April 21, September 29, and October 6, 2017. Samples were purified by vacuum filtration with Sartolab Disposable Sterile filters containing 0.22 µm membranes, then stored in the dark at 4° C.

HPLC (Agilent 1260 Infinity LC) consisting of a dual pump, auto sampler, column oven, C18 column and UV-detector, was used to analyze the samples. The mobile phase consisted first, of a 50:50 solution of HPLC-grade water and acetonitrile (A) and second, of ultra-pure water (B). The flow rate was set at 1 mL/min, column oven at 30 ° C, and the UV detector at 280 nm.

Each effluent sample was divided into three aliquots. A 1,000 ppm standard stock solution of 17-beta-estradiol in methanol was run through HPLC to establish the approximate retention time. Retention times and absorbances were compared between each run.

RESULTS/DISCUSSION

A peak for E2 was not seen in the effluent runs (Figure 1). We ran a sample of stock E2 (3.18 mM) and found a consistent retention time in the range of 11-12 minutes, with an absorbance of 600 mAU (Figure 2). Because we were not able to detect E2 in the effluents, we spiked one sample of the effluents with E2 (final concentration of 0.318 mM) which resulted in a peak with a retention time between 11-12 minutes (Figure 3) similar to that of our stock. If E2 is present in the effluents, the absorbable readings could be below the minimum accurate detection limit of our machine (3-2,000 mAU).

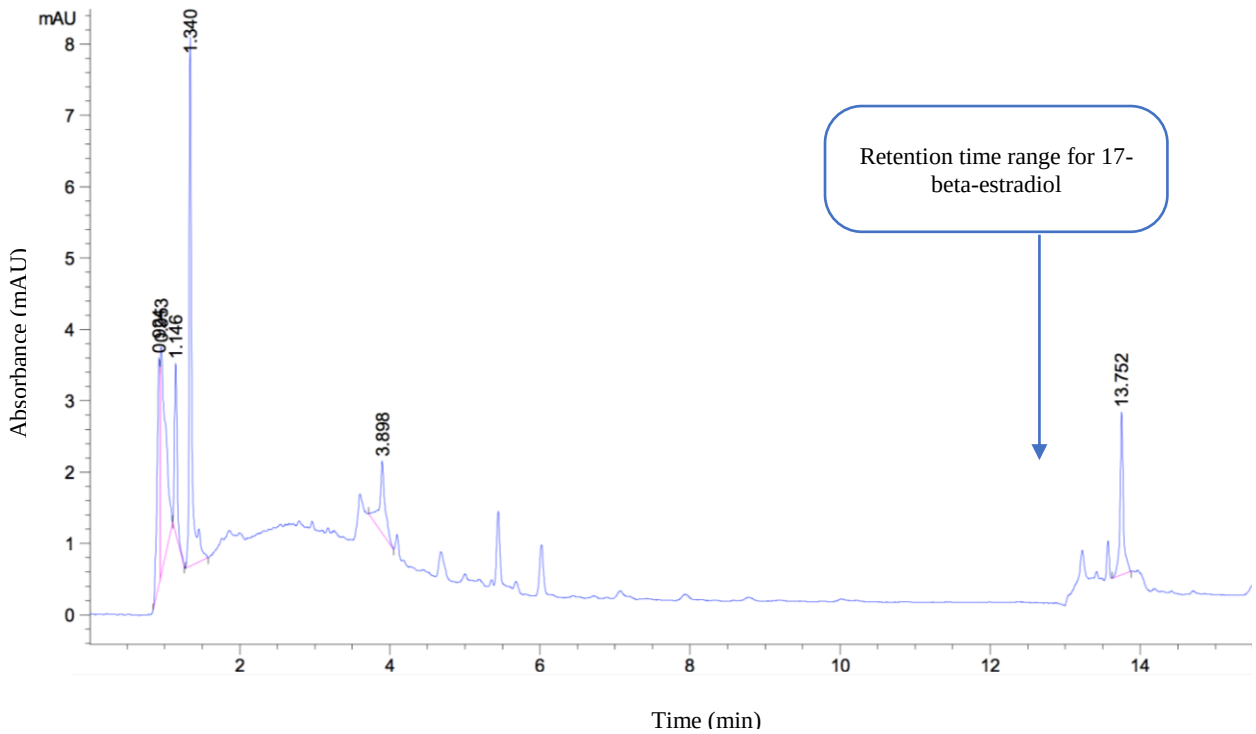

Figure 1. Representative HPLC chromatograph of the effluents taken from the Moccasin Bend Wastewater Treatment Facility in Chattanooga, Tennessee. UV detector was set at 280 nm and N=3. Samples collected on April 21, 2017, September 29, 2017, and October 6, 2017.

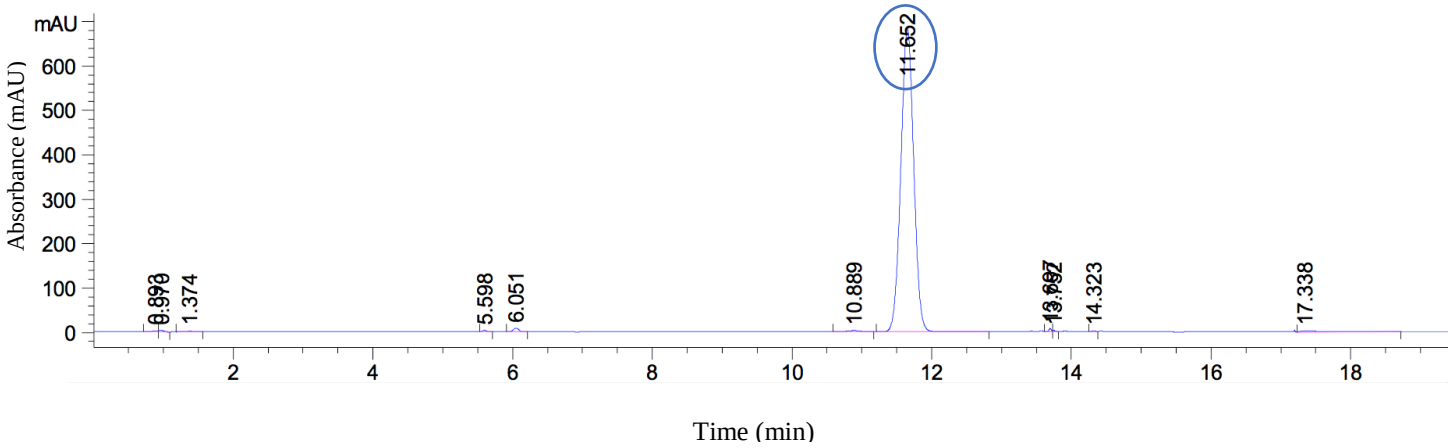

Figure 2. HPLC chromatograph of 17-beta-estradiol (3.18 mM in methanol) had a consistent retention time in the range of 11-12 minutes and with an absorbance of approximately 600 mAU. UV detector was set a 280 nm.

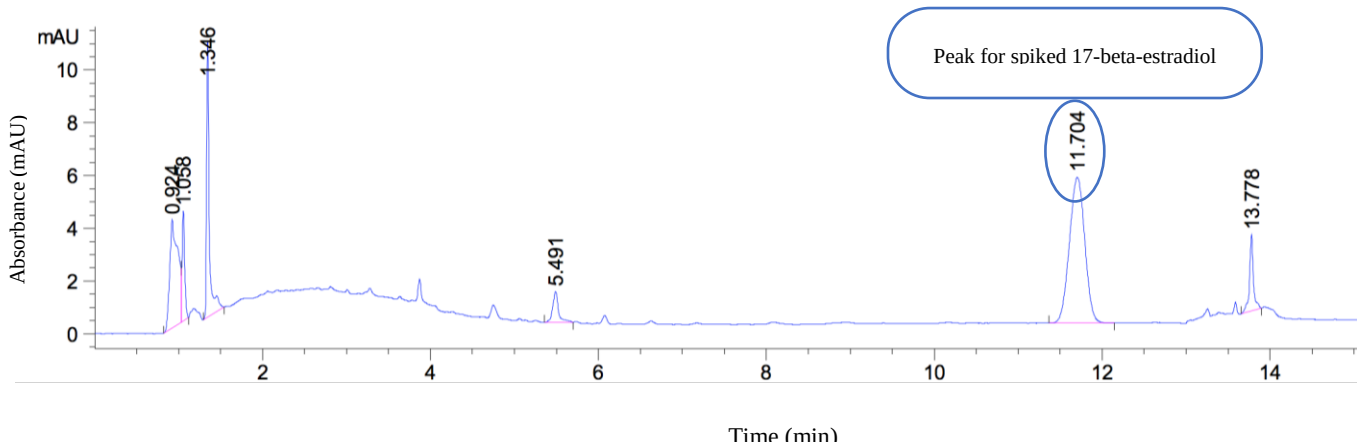

Figure 3. HPLC chromatograph of 17-beta-estradiol (0.318 mM) spiked effluent (concentration of 1mg/1ml) taken from the Moccasin Bend Wastewater Treatment Facility. Peak for 17-beta-estradiol appears at 11.704 minutes as in Fig. 2. UV detector was set at 280 nm.

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