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HPLC Detection of the Possible Presence of 17-a-ethindyl estradiol in Treated Effluents Released from the Chattanooga Water Treatment Plant

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Abstract - Research has confirmed that elevated synthetic estrogen in surface waters can lead to intersex characteristics in aquatic vertebrates. Unmetabolized antibiotics, hormones from animal wastes, including humans, and discarded pharmaceuticals are some ways synthetic estrogen enter aquatic ecosystems through the release of contaminated effluents. In this investigation, the Agilent 1260 Infinity HPLC was used to detect the possible presence of 17-α-ethindyl estradiol in effluents released from the Chattanooga Sewer Treatment Plant. Results were analyzed by comparing HPLC chromatograms from effluent and spiked samples. We detected a possible peak of synthetic estrogen in the effluent samples with a retention rate of 6 minutes detected at 280 nm. This method could be used to determine if synthetic estrogen is present, within the mdl, in the Tennessee River at various distances downstream from the Chattanooga Sewer Treatment Plant and to compare these levels with those documented as being able to feminize male fish.

Introduction

Daughton (2004) detected elevated levels of synthetic estrogen (17-α-ethinyl estradiol; EE2) in surface and groundwater downstream of farms and agricultural land. Wier (2003) estimated that 26.6 million pounds of antibiotics were fed to livestock to promote growth and fertility. Unmetabolized antibiotics and hormones, present in the animal wastes, are washed from rearing facilities into aquatic ecosystems. This can contribute to the presence of synthetic estrogen in rivers.

Human excretion and discarded pharmaceuticals have also contributed to the increased levels of synthetic estrogen in effluents released from water treatment plants. In 2014, over 10 million women across the United States took oral contraceptives (Brooks et al. 2014); human excretion of estrogen from birth control pills is a primary route through which synthetic estrogen becomes available to organisms in aquatic habitats. The estrogen found in oral contraceptives is more stable than endogenous estrogen and remains in the body longer because they are resistant to degradation and can be stored in fatty tissue. As a result, 40% of ingested estrogen reaches sewage treatment plants and is released in effluents (Brownawell 2006).

Synthetic estrogens have negative effects on the fertility of non-target species in aquatic ecosystems. Male fish living downstream of wastewater treatment plants can be exposed to exogenous estrogen resulting in feminization. In 2004, male bass in the Potomac River were discovered displaying inter-sex characteristics. Over half of the male bass collected downstream from a sewage plant tested positive for the protein vitellogenin which is involved in egg production. McAvoy et al. (2008) demonstrated that when adult male bass are exposed to acute low concentrations of estrogen (3-5 ng/L), reproductive rates can drop by 50%.

Synthetic estrogen exposure can also affect behavior. Juvenile black striped pipefish, exposed to synthetic estrogen, display altered swimming patterns and defensive responses to mosquitofish (a potential predator; Rose et al. 2013). Rose et al. (2013) added synthetic estrogen to a lake in Ontario, Canada, at the beginning of the summer, over the course of three years. After the first summer, male fat-head minnows were producing vitellogenin; after the second summer, they had undeveloped sperm cells and were producing eggs. They found a sharp drop in the minnow population because of the lack of reproduction after the first exposure to synthetic estrogen (5-6 ng/L).

In medaka fish, goldfish, and zebrafish, exposure to estrogen altered courtship displays and aggression levels (Oshima et al., 2003; Bjerselius et al., 2001; Coleman et al., 2009). Males failed to court females and did not defend mating territories. Additionally, male sand gobies exposed to synthetic estrogen experienced difficulty defending their nests, causing females to prefer unaffected (Saaristo et al. 2009). Male gulf pipefish, exposed to 5 ng/L of estrogen, expressed female-like secondary traits thus changing how females responded to them. Reproductive success diminished resulting in a complete reproductive failure in these fish (Jones et al. 1997).

Detection of synthetic estrogen in aquatic ecosystems can be challenging. However, with all the possible effects as stated previously, it would be important to create a method able to detect synthetic estrogen in effluents. HPLC is one of the most widely used methods to detect chemicals or pharmaceuticals because it is highly specific, sensitive, reproducible, and has sufficient precision.

Desbrow et al. (1998) identified synthetic estrogen in the effluents of seven sewage-treatment facilities, discharging into rivers. HPLC was used due to its sensitivity and column

structure for each collected sample; they determined the concentration of 17-α-ethinyl estradiol was generally below the limit of detection but was positively identified in three of the effluent samples at concentrations ranging from 0.2 to 7.0 ng/L.

In another study, Wang et al. (2006) proposed a method for HPLC to detect trace amounts of estrogen in wastewater treatment plant effluents. The method resulted in consistent and replicable results because of HPLC separation efficiency. This method was also tested with spiked samples and obtained good recoveries of around 99.5%.

A successful HPLC method was applied to the detection of synthetic estrogens in the Tiber River near the city of Rome. The development of accurate and sensitive methods was necessary for their detection in aquatic ecosystems, and the study validated a simple and reliable analytical procedure for determining selected estrogens in waste and surface waters using HPLC. This method was useful and affordable when more sophisticated techniques were not available (Patrolecco et al. 2013).

In this investigation, HPLC was used to detect the possible presence of 17-α-ethinyl estradiol in treated effluents released from the Chattanooga Sewer Treatment Plant.

Methods

Treated effluents released from the Chattanooga Sewage Treatment Plant were collected on April 21, September 22, and September 25, 2017, in 60 mL in Amber I-Chem Septa vials, and each vial was placed directly below the effluent pipe until the desired volume was reached. After the collection, the vials were sealed, wrapped in aluminum foil, and transported at room temperature. Each sample was then filtered through a Buchi/Sartorius vacuum (product number: 180C1E; 0.22 µm polyethersulfone; 0.45 µm surfactant-free cellulose acetate) and refrigerated at

4°C until subsequent analysis, usually 24 hours. Filtered effluents were analyzed using HPLC: Agilent 1260 Infinity HPLC; Zorbax Eclipse PLUS-C18 column (product number: 959961-902, 5 µm, 4.6 x 150 mm). The mobile phase was made of a mixture of acetonitrile and water. We created a linear gradient from 0-4 minutes with a 46:54 ratio followed by an isocratic gradient from 4-12 minutes at a 50:50 ratio and stepped it up to an 100:0 ratio with a flow rate of 1 ml/min. Column temperature was maintained at 40°C, and initial injection volume was 20 µl. We detected at 280 nm and also collected spectra from 190-400 in 2 nm increments. We performed a serial dilution to determine HPLC detection limits of the synthetic estrogen. We also performed a 1 ppm stock solution of 17-α-ethinyl estradiol and a treated effluents sample spiked with 1 ppm stock solution of 17-α-ethinyl estradiol were made and run on the HPLC to use as reference chromatograms. Results were analyzed by comparing HPLC chromatograms and detecting possible peaks for 17-α-ethinyl estradiol.

Results and Discussion

By performing a serial dilution of the synthetic estrogen, we were able to determine the HPLC detection limits (Figure 1). In order to detect an accurate retention time for 17-α-ethindyl estradiol, a 1 ppm standard stock solution containing 17-α-ethindyl estradiol in methanol was run through HPLC (Figure 2). A consistent retention time was obtained at 6.05 minutes with 1800 mAU. As shown in the treated effluent sample (Figure 3.A), the peak of interest had a very similar retention time at around ~6 minutes detected at 280 nm, but the intensity of the peak was low, around ~1 mAU. In order to confirm the possibility of a synthetic estrogen peak in the effluent sample, the retention time and absorbance was compared, ~6 minutes and ~150 mAU, to the effluent sample spiked with 17-α-ethindyl estradiol (Figure 3.B). The differences in

intensity could be from the HPLC's detection limits; anything below 2 mAU decreases the accuracy of the machine and causes fluctuations in results, specifically absorbance readings. However, the results mirrored a similar study conducted in various Brno reservoir sites, Svratka, and Svitava rivers located in the Czech Republic where researchers obtained a retention time of ~5.9 minutes and 0.40 mAU for 17-α-ethindyl estradiol (Nekvapil et al. 2008). Therefore, the results of the current study suggest that HPLC is an effective technique for detecting 17- α-ethindyl estradiol in effluents and possibly aquatic ecosystems and effluents.

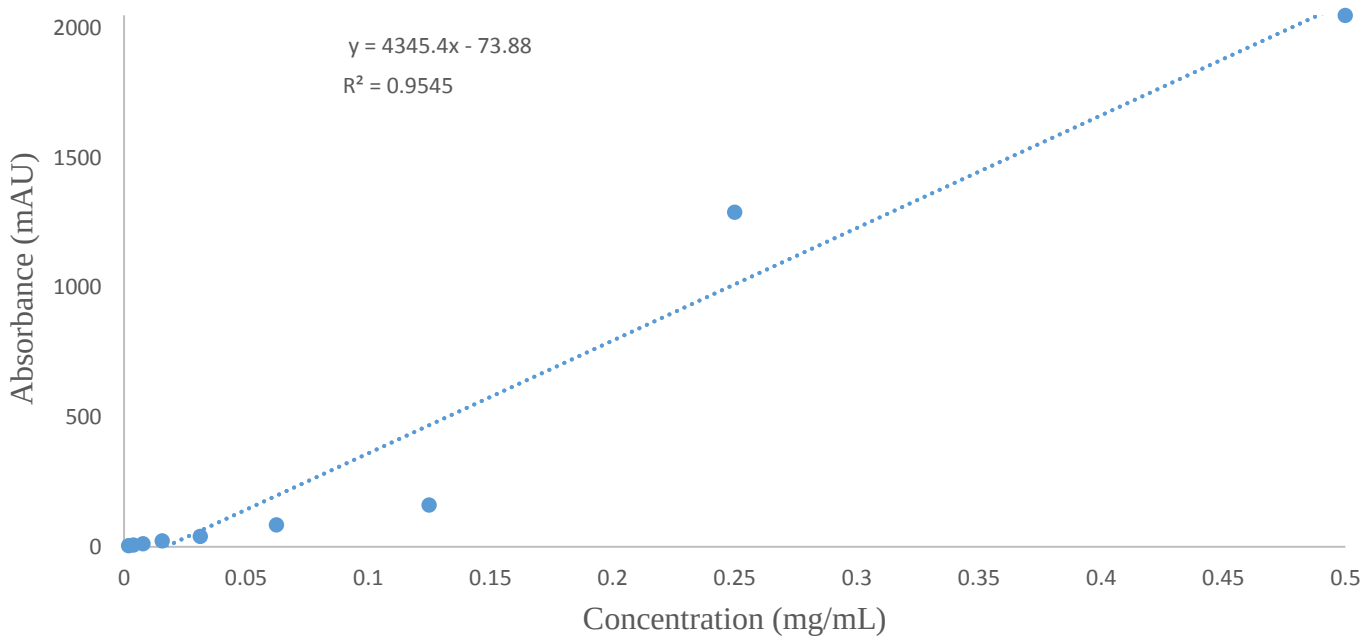

Figure 1. Serial dilution of synthetic estrogen to measure HPLC's minimum detection limits. A regression equation and R^2 value was calculated for the data.

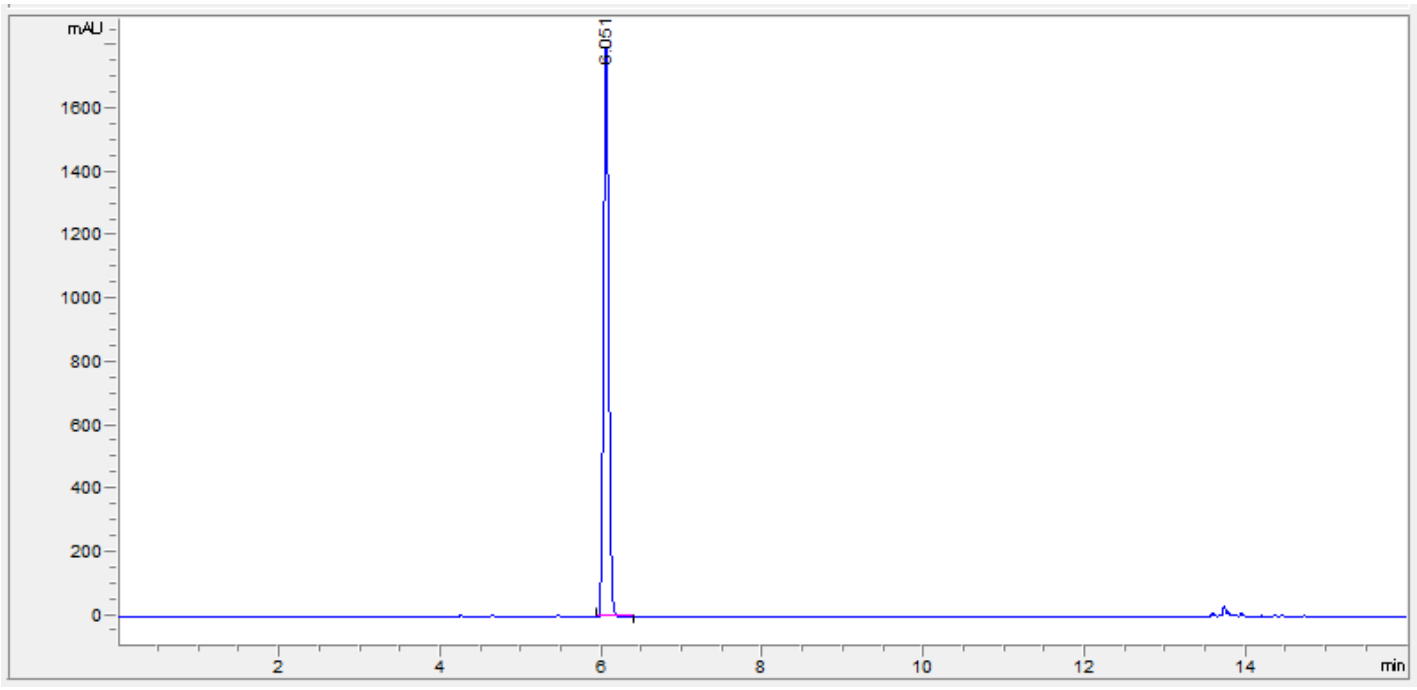

Figure 2. One ppm standard stock solution of 17-α-ethindyl estradiol in methanol. Column Eclipse PLUS-C18; 5 µm; 4.6 x 150 mm. Mobile phase acetonitrile/water 50:50, flow 1 ml/min. Column temperature 40ºC, the injection volume was 35 µl. Detection UV at 280 nm with retention time of 6.05 minutes.

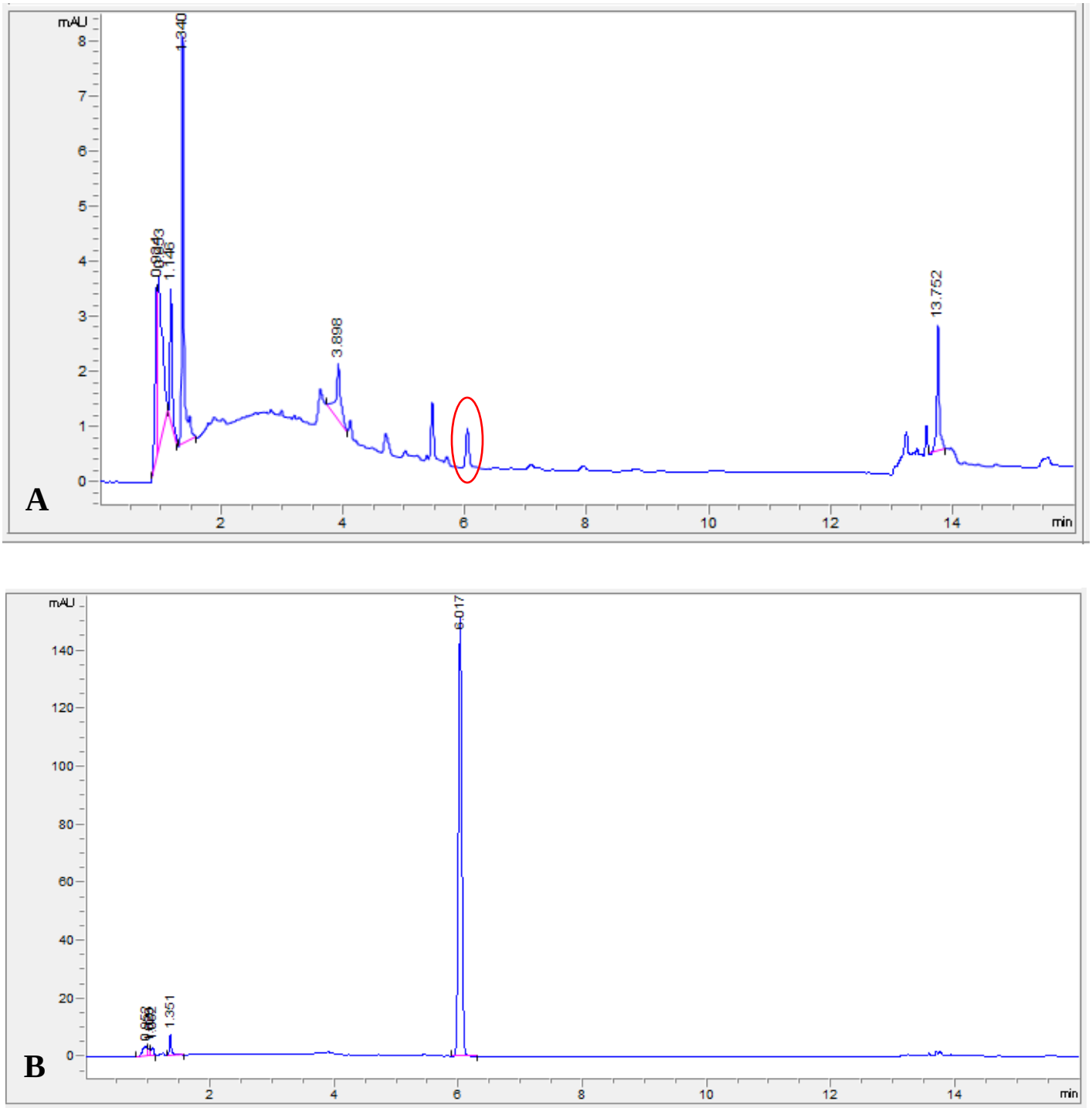

Figure 3. Chromatogram A- representative chromatogram of treated effluents released from the CSTP (Chattanooga Sewer Treatment Plant) only (n=3). The peak of interest is circled in red with a retention time of ~6 minutes detected at 280 nm. Chromatograph B- a sample of treated effluents spiked with 1 ppm of 17 α-ethindyl estradiol stock solution.

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