Effect of College Students on the Amount of Contraceptives in the Denton Waste Water Supply

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Abstract

This study examined the effects of college students on the concentration of a contraceptive (17α-Ethinylestradiol (EE2)). We tested the amount of 17α-Ethinylestradiol during the time that the University was in session and when the university was on break. We hypothesized that there would not be a significant change in the concentration of 17α-Ethinylestradiol in the wastewater supply, however we were wrong. As it turned out we were wrong. The difference was quite dramatic. It showed through all three sets of samples that, while the University was in session the concentration of 17α-Ethinylestradiol spiked considerably.
Introduction

17α-Ethinylestradiol (EE2) is a synthetic version of estrogen that is commonly used in many birth control medications. The main route by which EE2 is entering waterways is via post-human consumption (i.e. consumed and excreted) (Brooks et al., 2003; Hugget et al., 2003). Given that the presence of EE2 is likely correlated to the number of women using birth control medications, a better understanding of how population demographics influences EE2 water concentrations are needed. Our hypothesis was that there will be no difference in the concentration of EE2 in the Denton wastewater treatment plant effluent when college students are attending college and not attending college. The experiment was conducted to determine if the concentration of (EE2) in the Denton wastewater treatment plant (WWTP) effluent varies due to the presence of college students. Specifically, the concentration of EE2 will be measured in the WWTP effluent when students are in session (late August to beginning of May) and when they are on summer break (mid-May to mid-August).

Methods

Materials

- Masslynx Software, v. 4.1
- Micromass Quattro Ultima mass detector
- 1.5ml amber glass vials
- Waters 2695 separations module
- 100µL of methanol
- Waters 2998 UV/vis detector
- Waters Sunfire C18 Column

Procedures

1. A total of 1000 ml effluent samples were spiked with the internal standard d3-17b-estradiol, which was used to quantify EE2.
2. Once spiked, samples were liquid-liquid extracted twice using 500 ml of ethyl acetate.
3. The ethyl acetate layers were combined and dried under a gentle stream of nitrogen.
4. The resultant residues were reconstituted in 1 ml ethyl acetate and transferred to 1.5 ml amber glass vials (Fisher Scientific).
5. Reconstituted solvent was dried under nitrogen with residue re-suspended in 100 µl of methanol.
6. From this volume, a 50 µl aliquot was taken for estrogen quantifications using a dansyl chloride derivatization method detailed by Nelson, Grebe, O’Kane, and Singh (2004).
7. All Chromatographic separations were conducted using Waters™ 2605 separations module coupled to Waters™ 2998 UV/vis detector and Waters™ Sunfire C18 column (2.1 x 50 mm; 3.5 µm particle size.)
8. Electrospray ionization (positive ion mode) and mass-spectrometric analysis were conducted using a quadrupole-hexapole-quadrupole instrument (Micromass Quattro Ultima™ mass detector, Manchester, UK).

9. For all EE2, instrument determined (MassLynx software, version 4.1) criteria were used to set limits of detection.

Results

The level of EE2 fluctuates depending on the attendance of college students. EE2 was present in all of the samples taken during the month of April. EE2 was also present in all of the samples taken during the month of October. During the month of July, however, EE2 was below the level of analytical detection, which is 0.5 ng/L. Thus, the EE2 level decreased significantly while the college students were on summer break. The results of each sample during each month are shown in Figure 1 below. Based on these results, the hypothesis is rejected regarding the fluctuation of EE2.

Discussion
The data gathered over the course of the experiment does not support the hypothesis. The concentration of EE2 in the Denton wastewater treatment plant effluent was significantly higher during the months when college students were still attending college. The hypothesis stated that there would be no difference in the concentration of EE2 when college students are attending college and not attending college. However there was a significant difference in the two time periods while students were still attending college. The concentration of EE2 was higher during the spring semester, in April, as opposed to the fall semester in October. An error occurred during the first July trial, where the EE2 concentration was below the levels of analytical detection. This error occurred because there was not a large enough range in the criteria to detect EE2 concentration at a level as low as this one. To prevent this error from occurring, the range used for the criteria, which was used to set the limits of detection, could be increased. I feel that the methods used to conduct this experiment fully tested the hypothesis. A question to ask from the results of this experiment would be why is the concentration of EE2 typically higher in the spring semester as opposed to the fall. Although the results of the experiment did not reflect the hypothesis, we still received valuable data that can be used to aid other experiments.
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References
