Detection of Pharmaceutical and Personal Care Products (PPCPs) in 3 Maine Lakes by Synchronous-Scan Fluorescence Spectroscopy (SFS)

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ABSTRACT

The occurrence of pharmaceutical and personal care products (PPCP's) and their byproducts in the environment is of growing concern due to their potential harmful effects on environmental and human health. Synchronous-scan fluorescence spectroscopy (SFS) was used to detect caffeine, 17α-ethynylestradiol, and the PPCP additive Triclosan levels in Pushaw Lake, Branch Lake, and Sebasticook Lake. These lakes are three heavily populated Maine lakes. Detection and quantification of a mixture of compounds in environmental samples using SFS is a novel and a relatively inexpensive method that could provide an approximate estimate of compound levels in water. The benefit of this method is that individual compounds can be detected rapidly in a mixture without prior separation. Samples were collected from all lake inlets, outlets, and deep holes and were filtered to remove particulate matter. To develop a quantitative method, standard dilutions of the three compounds were scanned to determine the peak position for each. This data generated a standard curve for each compound that related concentration to peak intensity. A filtered environmental sample from each lake was analyzed using SFS and a standard addition of a PPCP mixture to compensate for organic carbon fluorescence. A concentration of 5.53×10^{-8}M 17α-ethynylestradiol was found in a sample from Branch Lake.

KEYWORDS: synchronous-scan fluorescence spectroscopy, caffeine, Triclosan, 17α-ethynylestradiol, lake water, Maine.

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INTRODUCTION

Every year, thousands of tons of pharmaceuticals and personal care products (PPCPs) and their metabolites are released into the environment [3, 15]. These compounds reach receiving surface and ground waters through wastewater effluent discharges (urine, feces, improper disposal of expired medications), agricultural runoff (animal manure, sewage sludge), and industrial runoff [2, 3, 6, 10]. In addition, PPCPs are inadvertently released into the environment [5]. PPCPs can have a profound impact on aquatic organisms. Thus, synthetic estrogen at low environmental concentrations has been linked to the feminization of male fish [3, 15]. Some have suggested that chronic exposure of PPCPs to non-target organisms can cause metabolic changes, physical deformities, and sexual alteration [5].

PPCPs have been detected by various methods at low levels (μg/L-ng/L) in environmental samples that include those from drinking water sources [5]. These concentrations are far below normal therapeutic doses, causing some health officials to consider the concentrations safe [13]. However, the health effects of chronic low-dose exposure of individual PPCPs are largely unknown. Additionally, these compounds and their metabolites exist as mixtures in the environment and the human toxicology of these mixtures is largely unknown and very difficult to predict [1,4, 11]. One toxicological study showed that mixtures of therapeutic pharmaceuticals at environmental concentrations decreased *in vitro* cell proliferation in human embryonic cells by as much as 30% [10]. Due to potential and unknown environmental and human health risks, it is crucial to test water sources for PPCP presence.

Several methods have been used for detecting PPCPs in the environment. These include, Gas Chromatography-Mass Spectrometry (GC/MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS) [12]. These methods require costly equipment, highly skilled operators, and can take several weeks to produce results [7, 12]. Synchronous-Scan Fluorescence Spectroscopy (SFS) is an alternative method of PPCP detection and quantification. SFS is the simultaneous and synchronous scanning of excitation and emission spectra while changing the difference between the emission and excitation wavelengths ($\Delta\lambda$). A major benefit of using SFS is that PPCP mixtures can be detected without prior separation. SFS is a rapid, economical, and convenient method for PPCP detection and quantification in environmental samples [8, 12]. That allows for more frequent testing of water sources for PPCP contaminants. SFS is effective for analysis of a Polycyclic Aromatic Hydrocarbons (PAH's) mixtures [9] and has been used to identify individual aromatic compounds in soils contaminated with fuel oil [14]. In this

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study, a method was developed for using SFS to detect 3 PCPP's (Triclosan, 17α-ethynylestradiol, and caffeine) in natural water samples.

MATERIALS AND METHODS

Sampling

Samples were taken from Pushaw Lake, Sebasticook Lake, and Branch Lake on Oct. 11th, 12th and 13th, Nov. 9th and Dec. 6th and 7th 2008. Each month, samples were collected from the surface waters of the inlets and outlets. In October only, deep holes were sampled. These three lakes were chosen because of the high population density around them and because of the presence of old shoreline camps. These features could contribute wastewater to the lakes through leaks from old septic systems. Branch Lake was specifically chosen because it is the water supply for the town of Ellsworth. All samples were collected in standard 1-L environmental sample bottles and were stored at 2-8°C in a refrigerator prior to analysis.

Filtering

In early January the samples were filtered using a 0.22µm filter and stored under refrigeration in glass bottles until analyzed. Between each sample, the filtration apparatus was cleaned several times with 5% soap solution, rinsed with HPLC grade methanol, and then with DI water.

Standard Dilutions

17α-ethynylestradiol, caffeine, and Triclosan (all 98% purity) were purchased from Sigma-Aldrich. Stock solutions of each were prepared in glassware that had be prewashed with methanol and rinsed with DI water. Even mixing and distribution of the compounds, was ensured by magnetic stirring. Because Triclosan and 17α-ethynylestradiol are sparingly soluble in water, they were first dissolved in HPLC grade methanol then diluted to volume with DI water. Dilutions ranged from 10^{-7}M to 10^{-12}M. All standards were prepared fresh daily and stored under refrigeration for no more than one day prior to analysis.

Creation of Standard Curves with SFS

All samples and dilutions were analyzed with a Jobin Yvon Fluorolog-3 spectrofluorimeter, which includes emission and excitation monochromators, a 400W Xenon lamp source, and a photomultiplier tube. The instrument also had excitation resolved correction which produced clear peaks at low wavelengths. Before each instrument session, the xenon lamp source calibration was checked and the position of the water Raman scattering peak was determined. Recalibration procedures were performed if either the lamp or water

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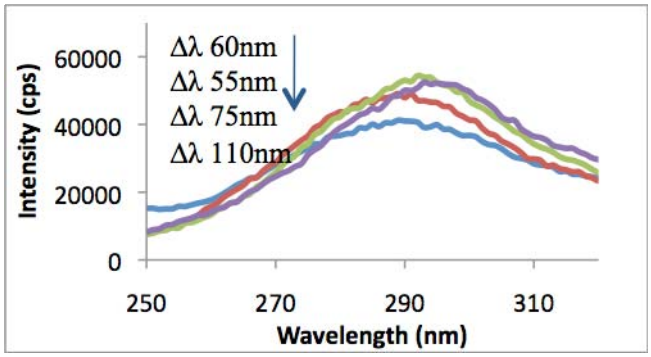

Fig.1. SFS of caffeine at various Δλ.

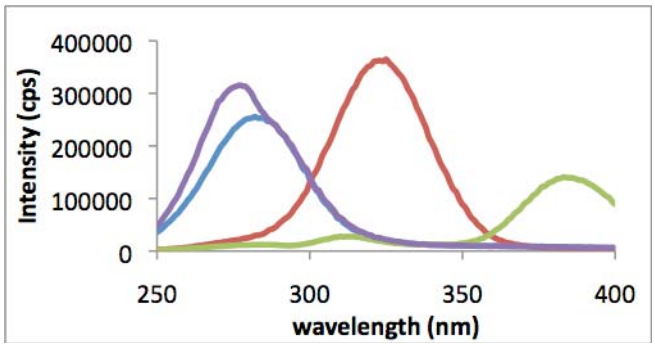

Fig.2. SFS of DI water at (L to R): Δλ 29, 40, 50, and 60nm

Raman peaks drifted out of the calibration position. Quartz cuvettes (5mm path length) were used to hold samples and were cleaned with DI water and cuvette cleaning solution between analyses.

Standard curves relating compound concentration to peak intensity was created by analyzing a series of standard dilutions for each compound. For each compound the lowest concentration was tested first to avoid contamination. For each compound, the emission and excitation peaks and their corresponding parameters were determined and recorded. The difference between the maximum emission and excitation wavelengths for a compound gave the Δλ which was used for synchronous scanning.

For caffeine, the maximum excitation and emission wavelengths were in the range of 274-279nm (λ_{ex}) and 375-384nm (λ_{em}). Based on the peak positions in these ranges, synchronous scans were conducted from Δλ 55-110nm. The most intense was at a Δλ of60nm (see the green line in Fig1).This Δλ was selected for caffeine analysis. Using the same method, it was shown that 17α-ethynylestradiol had excitation and emission peaks ranging from 273-279nm and from 297-307nm respectively. A Δλ 29nm was the optimum synchronous scan peak but water Raman scattering appeared in the 17α-ethynylestradiol peak position when scanned at Δλ 29nm (Fig 2). A Δλ of 40nm was used. Triclosan had two SFS peaks (at Δλ 50 and at Δλ150). A Δλ of 50nm was selected because it gave the higher sensitivity.

The method for developing linear standard curves followed that of Liu *et al*. The curves were based on SFS peaks intensities at the Δλ values for pure caffeine, Triclosan, and 17α-ethynylestradiol at 60nm, 50nm, and 110nm respectively. The equations of each linear calibration were: 17-α ethynylestradiol: $y = 9 \times 10^{11}x + 96736$, caffeine: $y = 4 \times 10^{11}x + 14279$, and Triclosan: $y=4 \times 10^{11}x+ 13805$. SFS spectra and the standard curves can be found in annex I.

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Solid-State Fluorescence

Solid-state fluorescence experiments were conducted to explore the difference between the behavior of the compounds in DI water and in pure form. Excitation, emission, and synchronous scans were conducted on a PTI spectrofluorimeter equipped with excitation and emission monochromaters, a 200W xenon excitation lamp, and a PMT. This instrument did not have excitation resolved correction. Each of the three compounds was analyzed in their solid state to compare with their behavior in water. Caffeine was analyzed with λ_{ex}280nm, λ_{em}325-370nm, and SFS with Δλ 33-110nm (Fig. 3). Triclosan was analyzed with λ_{ex}270-307nm, λ_{em}314-390nm, and SFS with Δλ 31-150nm (Fig. 4). 17α-ethynylestradiol was analyzed with λ_{ex}270-305nm, λ_{em}305-335, and SFS with Δλ 28-33nm (Fig. 5).

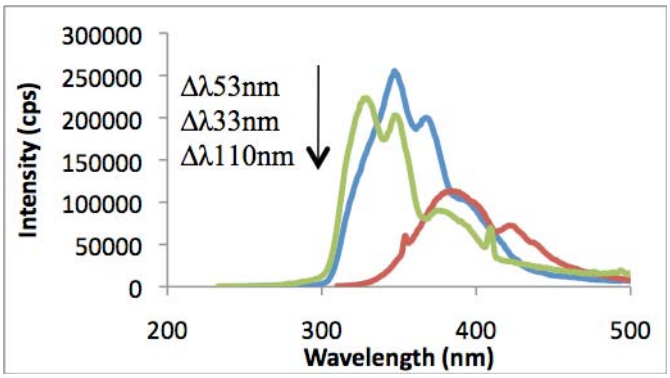

Fig.3. Solid SFS of caffeine at various Δλ

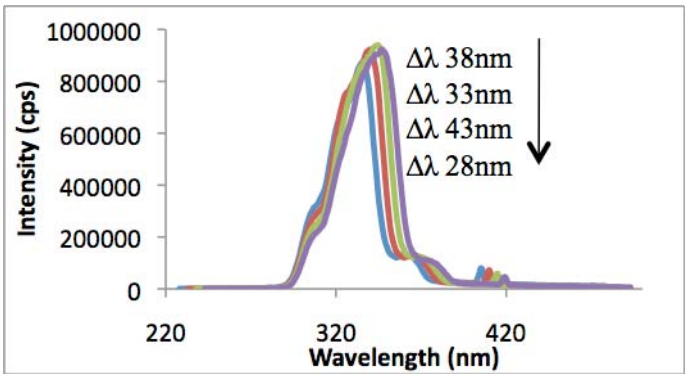

Fig. 4. Solid SFS of 17α-ethynylestradiol at various Δλ

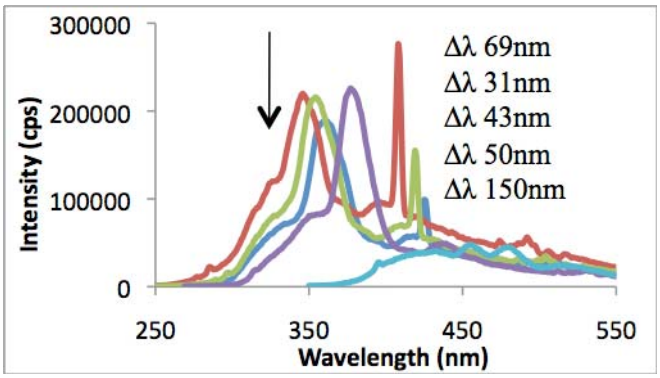

Fig.5. Solid SFS of Triclosan at various Δλ

Standard Additions

Standard Additions of 17α–ethynylestradiol(1.77×10^{-6}M) were prepared by the same method used for standard dilutions. The sites tested were Pushaw Stream (the major inlet to Pushaw Lake), Rocky Pond Stream (an inlet to Branch Lake), and Alder Stream (an inlet to Sebasticook Lake). These samples were collected on Nov. 9 and on Dec. 6 and 7, 2008. The caffeine, Triclosan, and 17α-ethynylestradiol were each added to the filtered lake water samples in a 2:1 ratio for each of the sites tested. Each filtered environmental sample was scanned using SFS with the following conditions: EM/EX slits: 5/5nm; range: 250-400nm; integration time: 0.1; increment: 0.25; Δλ: 40nm, 50nm, and 60nm. Δλ40nm is the difference for 17α-ethynylestradiol; 50nm and 60nm were used to compensate for water scattering.

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Further standard addition measurements were made for a mixture of 17α–ethynylestradiol, caffeine, and Triclosan with the water from the following sites: Pushaw Stream (the outlet from Pushaw Lake), Rocky Pond Stream, and Stetson Stream (an inlet from Sebasticook Lake). All samples were collected on Nov. 9. Equal volumes of the compound mixture and the filtered lake waters were mixed to give a concentration of 2.5 x 10^{-6}M of each compound in lake water. The conditions for SFS measurement were: EM/EX slits: 5/5nm; range: 250-400nm; integration time: 0.1; increment: 0.1. Preliminary mixture scans suggested that the Δλs values for caffeine, Triclosan, and 17α-ethynylestradiol were 110nm, 50nm, and 29nm respectively. The samples were scanned with these Δλ values.

RESULTS

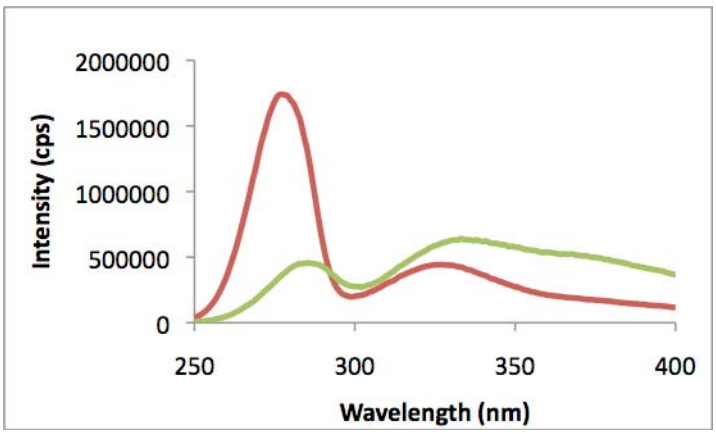

Fig. 6. The Rocky Pond November sample with and without added 17α-ethynylestradiol. The green line is the water sample before being spiked. The small peak on the left is water scattering. The red line is the sample after the standard addition.

17α-ethynylestradiol was detected (at 5.53 x 10^{-8}M) only in the November 9th, 2008 Rocky Pond Stream sample (see Fig 6 for SFS spectra). 17α-ethynylestradiol results for the other samples test were inconclusive, because the 17α-ethynylestradiol peak intensities of the spiked samples were lower than those of the 17α-ethynylestradiol added. The results for caffeine and Triclosan were also inconclusive because the peaks could not be seen after the standard addition.

The samples containing a mixture of the 3 compounds added were used for peak identification rather than quantification. Figure 7 shows the synchronous spectra of a controlled PPCP mixture run at the determined Δλ for each compound. The scan at Δλ50nm corresponds to 17α-ethynylestradiol and a clean peak is observed at 275nm. The scan at Δλ60nm corresponds to caffeine and the observed peak is in the

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same position as the 17α-ethynylestradiol peak, indicating the absence of caffeine peak intensity. The scan at Δλ110nm shows a small peak at 295nm that corresponds with Triclosan.

4. DISCUSSION

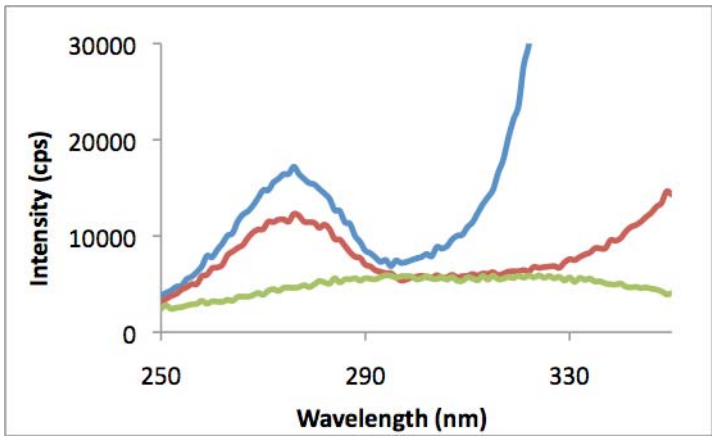

Fig. 7. A clean mixture of 17α-ethynylestradiol, caffeine, and Triclosan run at Δλ 50nm (blue), 60nm (red), and 110nm (green).

SFS scans of a PPCP mixture in filtered samples from Pushaw, Branch, and Sebasticook Lakes, demonstrated that SFS could detect PPCPs in environmental waters. Even though natural organic matter is highly fluorescent and can mask the SFS peaks of low-concentration. A standard addition method can be used to identify the peaks.

One of the two Rocky Pond Stream samples (collected in November), contained an estimated concentration of 17α-ethynylestradiol of 5.53×10^{-8}M. Even though additional testing of this sample would be required to verify this result, the presence of this estrogenic compound in Rocky Pond Stream is of concern. Low levels (µg/L-ng/L) of 17α-ethynylestradiol have been shown to affect the reproductive health of aquatic organisms (3,15),. In addition, the lake into which Rocky Pond Stream flow is the drinking water source for the city of Ellsworth, ME. Since 17α-ethynylestradiol is a synthetic hormone, its source in Rocky Pond Stream could be from a septic system leach field.

17α-ethynylestradiol was not detected in any of the Alder Stream and Pushaw Stream samples. These results could be further investigated for quenching of the 17α-ethynylestradiol signal by constituents of the dissolved organic carbon. A Stern-Volmner analysis, which determines the quenching rate for a system, should be conducted to investigate the interactions among the compounds and organic material. SFS results for the mixture of Triclosan, caffeine, and 17α-ethynylestradiol were not used for quantification, but rather to demonstrate peak position and identification of the compounds by changing the Δλ. At first, caffeine could not be detected in the mixture at a Δλ of 60nm. It is possible that caffeine is not as fluorescent as the other two compounds and that a higher concentration of caffeine is necessary. Another possibility is that the higher

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signal of 17α-ethynylestradiol (at a similar signal's Δλ and peak position) makes it difficult to see the caffeine in a mixture.

Further analysis of caffeine, Triclosan, and 17α-ethynlyestradiol in water is needed to better define the difference between the fluorescence of these compounds in their solid-states and in an aqueous environment. A slight peak shift and some broadening were observed in some instances. The peak position in water may be more difficult to determine since the solvent molecules, not present in the solid state, may affect energy transfer.

This research will continue with the sampling and analysis by SFS and standard addition of the inlets, outlets, and deep holes of Pushaw Lake, Sebasticook Lake, and Branch Lake. In addition, treated wastewater effluents will be collected from the Bangor Water Treatment Plant. If any more PPCPs are found in any of the collected samples, it is crucial to collect further samples and see if it is a persistent problem, or an isolated incident.

5. CONCLUSIONS

1. SFS, with standard addition, was found to hold promise for being a sensitive and economical method for the analysis of caffeine, Triclosan, and 17α-ethynylestrdiol in water.
2. Standard addition was effective in compensating for high dissolved organic carbon fluorescence in the SFS analysis of environmental water samples.
3. SFS was used to analyze Maine stream and lake waters. 17α-ethynylestradiol was detected in one stream sample feeding a lake whose water is used for public supply.

6. ABBRIEVIATIONS AND ACRONYMS

SFS: synchronous-scan fluorescence spectroscopy

PPCPs: pharmaceuticals and personal care products

7. ACKNOWLEDGMENTS

Credits

All research was conducted at the University of Maine under Dr. Howard Patterson and his research group from June 2008 and is ongoing. I would like to thank Jim Killarney for helping me every step of the way, and teaching me all about Fluorescence Spectroscopy. I would also like to thank Mr. Cary James, my mentor, for reading and editing my paper, giving me plenty of advice, and allowing me to work in his

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Author

I am a senior at Bangor High School in Bangor, ME. In the summer of 2008, I participated in an internship in the Department of Chemistry at the University of Maine, through the Maine Space Grant Consortium MERITS program. I was encouraged by my Chemistry teacher to continue my research throughout the school year. In addition to science, I enjoy playing the piano, the drums, singing, and reading. In high school, I was also a member of NHS and my school newspaper. I plan to major in Environmental Studies at The George Washington University in the fall.

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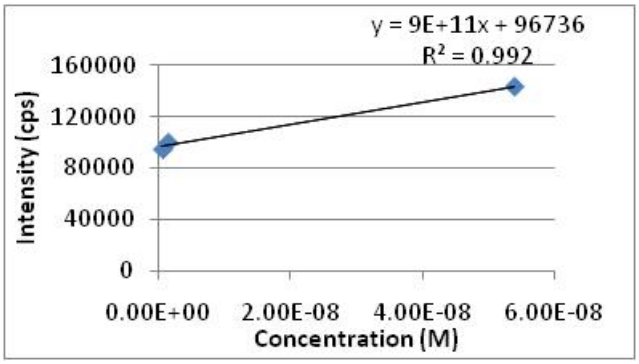
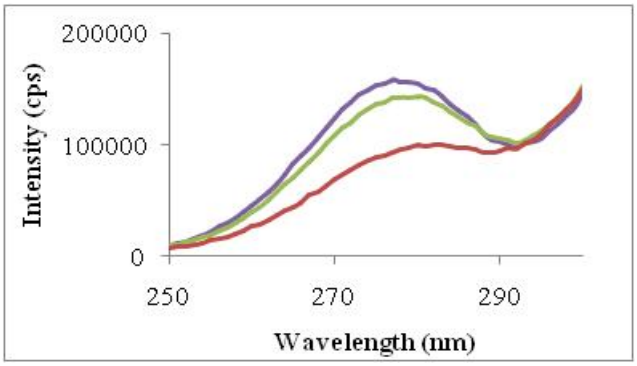
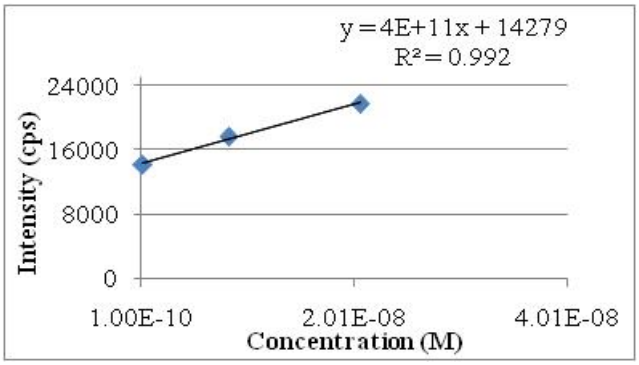
9. ANNEX 1: standard curves



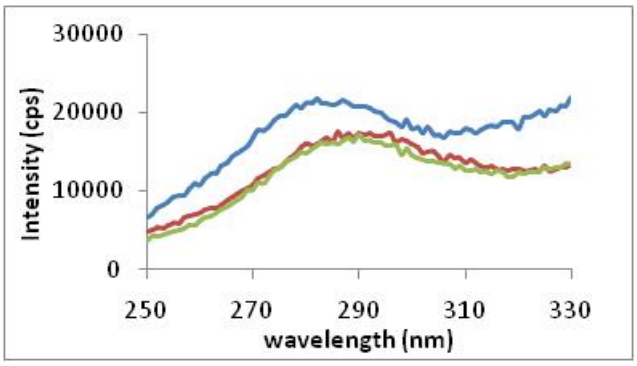
Standard curve for 17α-ethynylestradiol



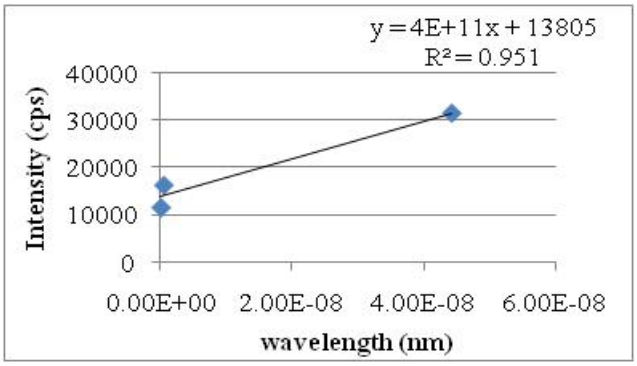
SFS spectra of 17α-ethynylestradiol (top to bottom) 5.04x10^{-8}M, 1.37x10^{-9}M, 5.04x10^{-10}M



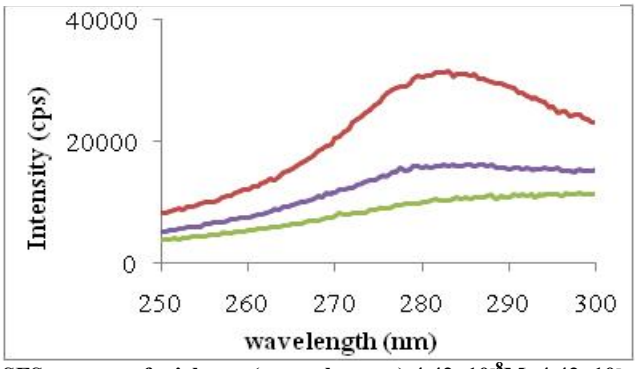
Standard curve for caffeine



SFS spectra of caffeine (top to bottom) 2.06x10^{-8}M, 8.24x10^{-9}M, 2.06x10^{-10}nm



Standard Curve for triclosan



SFS spectra of triclosan (top to bottom) 4.42x10^{-8}M, 4.42x10^{-10}M, 4.42x10^{-11}M

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ANNEX II: Sampling Chart

The first bullet for each stream is the November sample, and the second one is the December sample. The negative values show the amount that was unrecovered from the spiked samples, and the positive values show the amount present in the samples.

Pushaw Stream	• -3.82E-7M • -3.33E-7M
Alder Stream	• -1.07E-7M • -1.06E-7M
Rocky Pond	• 5.53E-8M • -3.12E-12M

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