**HPLC Analysis of Pharmaceuticals in SJU Waste and Surface Water**

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Research this summer fit into a larger project about the photodecomposition of various anti-depressant pharmaceuticals. This was the first summer where analysis of actual waste and surface water was begun, and as such minimal results were achieved. A successful method was devised, but no pharmaceutical compounds were identified.

**Experimental Procedure: Sample Preparation**

This summer, water samples from three different points were analyzed. We looked at SJU waste water treatment plant effluent before and after UV treatment (in order to determine the effects of the organism eradicating lights on the compounds of interest), as well as water directly from East Gemini Lake (EGL), where waste water is deposited. Samples were taken three times from each of the points over the course of the summer.

After collection, 6 L samples were measured. Because of the amount of macromolecules present in the EGL water, it was first filtered with regular Whatman number 1 filter paper, then with a Whatman Polycap 75 AS 0.45 µm filter capsule using a carboy and gravity. Two to four ENVI-18 SPE disks were conditioned with methanol then water on the vacuum filtration device (we discovered that filtering went much faster if the disk was never allowed to come in contact with air.) The 6L samples filtered in anywhere between 1 and 9 hours. After drying the disk, the organic material was extracted using 10x 2mL methanol (or acetonitrile for the last sample.) The solvent was mostly evaporated using N2 and solution then reconstituted to 10mL, after which HPLC samples were made using a 0.45 µm syringe filter.

Samples with variable ion concentration and pH were also made. In the first sample of Pre-UV, second filter, 87.663 g NaCl was added to the 6L quantity to make a 0.25 M solution. In the 2nd samples, 2nd filters of pre-UV and EGL, formic acid was added to the 6L quantity to make a pH 3 solution. Likewise, in the 2nd sample, 2nd filter of Post-UV, acetic acid was added to make a pH 5 solution.

**Experimental Procedure: HPLC Method Development**

In order to develop an HPLC method for analysis, solutions of each of the following compounds were used: bupropion, fluoxetine, paroxetine, sertraline, and caffeine (the first 4 are anti-depressants.) A method was eventually developed on the HPLC-UV using a Luna 5u C-18 50x3.00mm column at 0.5 mL/min. Ethanolamine buffer with 10% acetonitrile (pH9) and methanol were used in the following gradient: 100:0 to 1.5 min., down to 30:70 at 2 min. until 7 min., up to 0:100 at 7.5 min. However, this gradient is not completely satisfactory, likely due to the shortness of the column. For most data analysis on the HPLC-MS, an Ascentis Express C-18 10cm x 2.11mm column at 0.1 mL/min was used. Water and methanol were used in the following gradient: 75:25 to 5 min., up to 0:100 at 15 min.

**Results**

The first step in this experiment was to test the reproducibility of both the filtration method and of the actual water samples from day to day. Using our HPLC methods, both seem to be fairly good.

In the first water samples, each sample was filtered and analyzed twice over a period of 7 days in order to determine reproducibility of the method. The chromatograms for the two filtrations of Post-UV appear to be almost exactly the same. The chromatograms for the two filtrations of EGL appear to be quite different. The first filtration gives higher peaks with much better resolution. The reasons are unknown.

Each of the samples were collected three times over the course of the summer in order to show reproducibility within the collection points themselves. The third samples are as of yet to be completely analyzed, though, due to an error with the HPLC-MS. The first and second samples of Pre-UV appear to be fairly similar. There are differences though, especially in the MS data. Some compounds that are in one are missing in the other. Almost the exact same thing is true for the Post-UV samples. Data for the second sample of EGL is incomplete due to the HPLC-MS error.

Next, different variables in the sample extraction process were tested. These included the addition of NaCl, lowering of pH, and extraction from the SPE disk with acetonitrile instead of methanol.

In the 1st sample, 2nd filter of Pre-UV, where NaCl was added, an addition peak at about 3.7 min RT appears. The chromatogram is also higher with slightly poorer resolution. Because the improvement was only very small, or non-existent depending on what was desired, and because adding NaCl made filtration go much slower, it was decided not to employ it.

The solutions of differing pH are as of yet incompletely analyzed due to the HPLC-MS error. However, preliminary data from the HPLC-UV shows that a decrease in pH gives higher peaks for one portion of the chromatogram between about 0 and 2 minutes. The rest of the chromatogram remained unchanged.

Each of the third samples was treated in the standard way except that extraction from the SPE disk was done with acetonitrile instead of methanol. Preliminary data from the HPLC-UV shows that the third samples are a little different from previous samples. The first set of peaks on the chromatogram appear at two distinct peaks with acetonitrile, as opposed to one peak with shoulders with methanol. The second set of peaks are the same but lower with acetonitrile. In addition, the peaks became lower from day to day much faster when using acetonitrile. Because of this, it appears methanol is a better choice for extraction from the SPE disk.

**Conclusion**

It seems as though the filtration method and samples were fairly reproducible and this experiment will be able to move forward into the compound identification and possibly quantification stage. 6L samples appeared to be adequate for analysis. Treatment to lower the pH may be the only variable that could be changed to give reasonably better results.

Further work might include using another HPLC column, using HPLC-MS/MS, and using NMR and IR on sample fractions for compound identification.