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Using SPE and HPLC-MS to Quantify and Identify Pharmaceutical Compounds in St. John's University Wastewater

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Project Title: **Using SPE and HPLC-MS to quantify and identify pharmaceutical compounds in St. John's University wastewater**

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ABSTRACT

Pharmaceuticals in wastewater have become a concern of environmental toxicologists. An efficient method of discovering the concentrations of these pharmaceuticals in wastewater has not yet been produced. The method we developed includes an automated Solid Phase Extraction (SPE) procedure prior to injecting a sample of wastewater into the High Performance Liquid Chromatograph (HPLC) and Mass Spectrometer – Electrospray Ionization (MS-ESI). Three unknown peaks were identified on the HPLC and MS from wastewater obtained from the St. John's Wastewater Treatment Plant in June 2013. Methods of analysis including NMR, GC-MS and IR have been used to determine the composition of these compounds that are potentially in significant concentration in the wastewater.

INTRODUCTION

Until recently, the presence of pharmaceuticals in wastewater has not been a concern of environmental toxicologists. However, studies have shown that concentrations as low as 1 ppb (part per billion) and sometimes 1 ppt (part per trillion) can have adverse environmental effects.[1] Pharmaceuticals can easily be deposited into aquatic environments through effluents such as wastewater, and there is little known about their possible synergistic effects. Because of the potential for biological consequences in various communities, including CSB|SJU, it is critical to determine an efficient method of discovering the concentrations of these pharmaceuticals in wastewater. Current methods of evaluation include manual SPE coupled with LC/MS-ESI(+) and continuous liquid-liquid extraction (CLLE).[2]
The method we developed includes an automated, rather than manual, SPE procedure prior to injecting a pre-concentrated sample of wastewater into the HPLC/MS-ESI. This paper describes the research from June 2012 to April 2014 to determine the limit of detection (LOD) of this method starting with known amounts of antidepressants in E-pure water as well as the results of testing wastewater from St. John's University, Collegeville, MN.

METHODS

Part 1: Determining the LOD

Preparation of the antidepressant solutions

Six antidepressant drugs were dissolved in methanol in various ways to be prepared into approximately 10 mM solutions. Paroxetine HCl (Paxil) was weighed out as a pure substance and added to a 100 mL volumetric flask. Tablets of Sertraline HCl (Zoloft), Quetiapine Fumerate (Geodon), and Escitalopram Oxalate (Lexapro) were crushed with mortar and pistol, transferred into a 100 mL volumetric flask, sonicated for 15 minutes with a Branson 2510 Ultrasonic Cleaner, and filtered using 0.45um nylon filter paper. Similarly, Aripiprazole (Abilify) was crushed, transferred into a 50 mL volumetric flask because there was a limited supply of aripiprazole, sonicated, and filtered. Ziprasidone HCl came as a capsule, and the insides were transferred into the 100 mL volumetric flask, which was sonicated and filtered. Each solution was placed in a separate amber bottle to prevent possible photodecomposition.

Direct Injection HPLC

One hundred µL of each was placed separately into 6 small amber HPLC vials, and 1 mL of E-pure H_2O was added to each vial. Two direct injection HPLC methods were attempted with 1.00 µL of each of these six solutions that did not show any peaks, including a mobile phase of 50% methanol/50% pH 9

100 mM ethanolamine buffer and 90% methanol/10% pH 9 buffer, each run through a C18, 2.6µm Kinetex analytical column with the Thermoscientific Surveyor HPLC at 0.500 mL/min. Every HPLC method used a wavelength of 215 nm. An 80% methanol 20% pH 9 mobile phase run at 1.00 mL/min showed peaks, but they were very close together. At a flow rate of 0.500 mL/min, every peak showed up except for aripiprazole. A mobile phase of 70% methanol 30% pH 9 buffer at a flow rate of 0.500 mL/min showed consistent, distinct peaks for every compound. The LC/MS was added to give MS chromatograms of the data as well.

The six antidepressant solutions of about 10 mM were then added together by placing 10 mL of each into one amber bottle. When run under the 70% methanol/30% pH 9 buffer, 0.500 mL/min flow rate conditions, they showed distinct peaks. When precipitate was found in some of the sample vials containing quetiapine fumarate, escitalopram oxalate, and ziprasidone, these three solutions were re-made using acetonitrile as the solvent instead of methanol, but precipitate formed in the acetonitrile solutions as well. Equal amounts of the 10 mM solutions of paroxetine, sertraline, and aripiprazole were then combined in an amber bottle. The paroxetine, sertraline, aripiprazole (PSA) solution was analyzed under the 70% methanol/30% pH 9 buffer conditions, and then made more dilute until the peaks no longer showed in order to determine the LOD. This method was accomplished by placing 500, 300, 200, 100, 75, 50, 25, 15, 10, and 5 µL into separate amber vials, adding 1 mL of methanol to each, and running it through the 70% methanol/30% pH 9 buffer HPLC method.

Manual SPE/HPLC

The next step in our process was to make up solutions that were at even lower concentrations than the direct injection HPLC method could detect and do a Solid Phase Extraction (SPE) to concentrate the compound prior to HPLC injection. Doing this iteratively with smaller concentrations each time helped to determine how low of concentrations the HPLC can detect after using SPE.

The manual SPE experimental procedure is as follows: 1 L of E-pure water was placed in each of three 3 L amber bottles and then ethanolamine, acetic acid, and formic acid were weighed out so that each jug contained 10 mM of the respective buffer. The pH of each bottle was adjusted to pH 9, pH 5, and pH 3, respectively, with ammonium hydroxide (14.8 M) or HCl (6 M). The LOD of this method was determined by adding the paroxetine, sertraline, aripiprazole (PSA) solution to the adjusted buffer solutions in smaller and smaller amounts. These solutions were then extracted under vacuum at about 15 mmHg using Oasis HLB 6cc SPE columns at a flow rate of 2 mL/min. The columns were prepped with 5 mL 90% TBME/10% MeOH until dried, 5 mL 100% MeOH until dried, and 5 mL 100% E-pure H_2O until there remained a thin layer of water on the column. After the columns were loaded with the PSA/buffer solutions, a centrifuge tube was placed underneath the columns and 3-5 mL of 90%TBME/10% MeOH was used to extract the antidepressants from the column. The centrifuge tubes, now containing 3-5 mL of 90% TBME/10% MeOH and the antidepressant compounds that eluted, were then placed in a sand bath heated to about 45° C and put under a constant flow of nitrogen for about 3 hours to evaporate to dryness. Then 0.5 mL HPLC-grade methanol was added to each tube, which was vortexed until the compounds dissolved. Next, 0.25 mL of each solution was placed into a small 300 µL insert which was inside a regular sized amber vial. HPLC was run on each of the vials with the same conditions (70/30, 0.5 µL/min). This resulted in a concentration increase of 1000 times.

Automated SPE/HPLC

An automated SPE was tested to compare to the manual SPE method. Using an Oasis HLB 3.9x20 mm, 15 µm, C18 guard column as the SPE pre-concentration medium, a similar procedure was attempted. The automation was accomplished through a computer program that communicated with a pressurized flow rate adjustor and solution valve selectors, which were hooked up to HPLC-grade TBME, HPLC-grade methanol, E-pure H_2O and the PSA/buffer solution. Two filter columns were placed in between the sample and the guard column in the HPLC set-up in order to increase the longevity of the guard column and the analytical column. The pre-concentration column, as in the Oasis HLB guard column mentioned earlier, was prepped by pumping 90% TBME/10% MeOH for 1 min at 10 mL/min, then 100% MeOH for 1 min at 10 mL/min, and finally 100% E-pure H_2O for 2 min at 10 mL/min. The 1 L PSA/buffer solution was then pumped through the column for 100 min at 10 mL/min. When this time lapsed, the HPLC method was initiated. The first attempt to separate the peaks included pumping 100% MeOH for 30 seconds immediately followed by 70% MeOH/30% pH 9 ethanolamine buffer for 30 minutes total. The method that gave consistent peaks for all three compounds was 70% MeOH/30% pH 9 buffer for the entire run, which was set to about 50 mins.

After the automated SPE process was used to determine the LOD with the PSA/buffer solution, wastewater was tested. This water, after being passed through a primary and secondary wastewater treatment to remove most of the organic and inorganic materials, then a sand filtration and finally, through a UV system to kill off any remaining bacteria, was obtained from the St. John's Wastewater Treatment Plant.

Part 2: Analysis of wastewater

Preparation of wastewater

Each liter of wastewater collected was filtered through a 0.8 µm nylon filter followed by a 0.45 µm nylon filter. The pH of the water was then changed to pH 9 by adding ethanolamine to a concentration of 10 mM and adjusting to pH 9 using 6 M HCl. This pH 9 water/buffer solution was then filtered through another 0.45 µm nylon filter in order to prevent large particles from entering and obstructing the HPLC columns. These solutions were then run through the automated SPE/HPLC/MS as described in the following paragraphs.

A solution of 3700 ppm (about 10 mM) paroxetine (active ingredient in the antidepressant Paxil) dissolved in MeOH was made. This paroxetine solution was pipetted into the 1 L sample of pH 9 wastewater to be at a concentration of 0.74 ppm.

Automated SPE/HPLC

The 1 L paroxetine/wastewater sample was pumped through a 15 µm Oasis HLB online pre-concentration column at 10 mL/min. When 100 minutes passed, the HPLC method was enacted: 30 seconds of 100% MeOH and then 70% MeOH/30% pH 9 ethanolamine buffer for 40 mins. This last step was changed to a 45%/55% method after a few runs to increase separation between the peaks. Between runs, the SPE column was automatically equilibrated by pumping 20 mL of 100% MeOH and 20 mL of E-pure H_2O. This SPE/HPLC method was followed as described above first with the paroxetine/wastewater sample, and the paroxetine peak was apparent in the chromatogram. The concentration of paroxetine after this SPE/HPLC method was comparable to the concentration following the E-pure PSA solution method based on the height and width of the peaks in each HPLC chromatogram. After discovering peaks in the chromatogram that did not pertain to paroxetine, the

wastewater was evaluated with HPLC-MS without the addition of the antidepressant in order to determine what compounds pertained to these peaks.

Separation was accomplished using the following conditions:

- 2.6 µm C18 Kinetex column
- Mobile phase of 45% MeOH/55% 100 mM pH 9 ethanolamine buffer
- 40 mins run time at 0.500 mL/min
- Thermoscientific Surveyor HPLC
- Advantage MS using an ESI source

Three distinct peaks consistently showed on the chromatograph. To identify which compounds pertained to each peak, fractions were collected during multiple HPLC runs. The three consistent peaks in the wastewater samples were analyzed three different ways in order to identify them:

1. MS
   - An ESI probe source was used and a mass range of 50-1000 m/z was set.
2. NMR
   - The collective fractions were vacuum dried under nitrogen, dissolved in deuterated chloroform and run through a ^{1}H-NMR at a 1024 scan rate and ^{13}C-NMR.
3. GC/MS
   - 1 µL of sample was injected and run at 300°C for 30 minutes with a split ratio of 10:1.

RESULTS

Part 1: Determining the LOD

The following table comprises the HPLC/MS data that was used to distinguish which peak belongs to which antidepressant compound.

Table 1: HPLC/MS data from all six antidepressants with a 70% MeOH/30% pH 9 buffer solution, 0.500 mL flow rate, 215 nm

Compound	Brand Name	Molec. Weight (g/mol)	LC - retention time (min)	MS - m/z
Escitalopram oxalate	Lexapro	414.43	5.97	325.15
Quetiapine fumerate	Seroquel	383.1	6.70	384.14
Paroxetine	Paxil	365.82	7.38	330.16
Ziprasidone	Geodon	467.4	11.89	413.17
Sertraline	Zoloft	342.69	19.08	305.94
Aripiprazole	Abilify	448.39	35.60	448.76

The following are results from each distinct method:

Direct Injection

- A mobile phase of 70% MeOH/30% pH 9 ethanolamine buffer created the greatest amount of separation and detection.
- A solution of about 13 ppm was the lowest detectable concentration of direct injection with the HPLC. This is the starting concentration of the manual SPE method.

Manual SPE

- The pH 5 solution gave the most quantifiable results with both the manual SPE and the automated SPE method.
- A 0.014 ppm solution was the lowest detectable concentration under the manual SPE method with the HPLC.
- Most of the compounds tended to elute off the column later when at lower concentrations.

Automated SPE

- A 0.0072 ppm solution was the lowest detectable concentration under the automated SPE method with HPLC.
- A 0.36 ppm solution was the lowest detectable concentration under the automated SPE method with the APCI MS so far. Future work will be done to determine if it can detect any lower.
- An APCI source has a lower detection limit than an ESI source for these compounds.

Part 2: Analysis of wastewater

- The pH 9 solution of wastewater showed three distinct peaks.
- These compounds eluted consistently around 6.75, 8.10, and 9.25 minutes under the stated conditions.
- These compounds were in a significant concentration in the tenths of ppm range.
- No peaks were apparent when a GC/MS method was enacted.
- A ^{1}H-NMR of each fraction did not show any significant peaks.

Table 2: Mass range based on elution time of the compound

Elution time (min)	Mass range (m/z)
6.75	281-282 & 477-478
8.10	259-260
9.25	513-515

DISCUSSION

Part 1: Determination of LOD

Figures 1-12 support the results displayed in Table 1. They include chromatograms of each antidepressant; all six antidepressants combined; a combination of the three antidepressants paroxetine, sertraline and aripiprazole at a high concentration as well as at the LOD. An explanation is included with each figure.

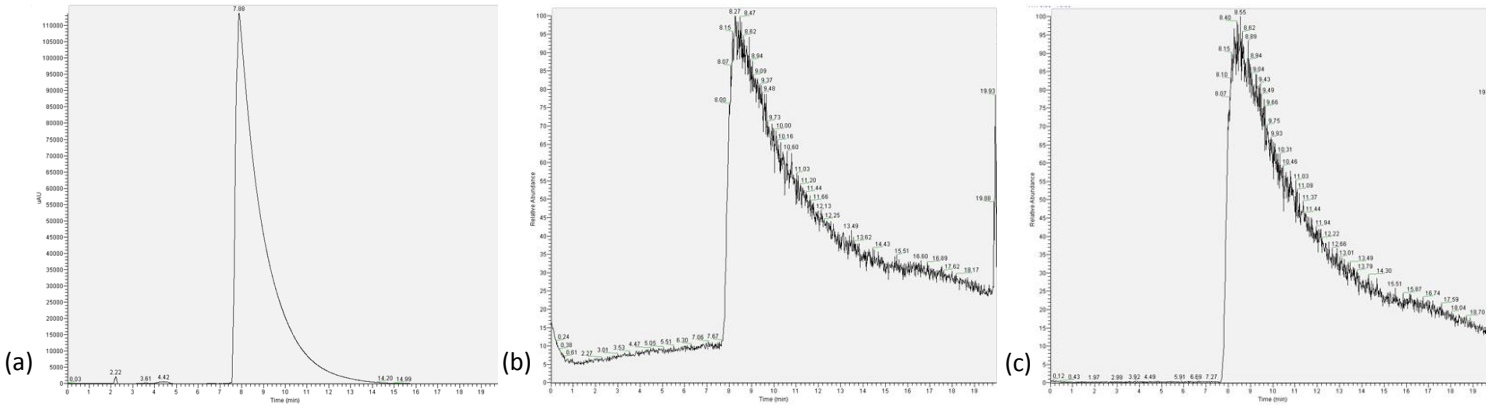


Figure 1: Chromatograms of paroxetine over a 20 minute elution (a) HPLC (b) MS (c) MS with mass range of 330-331 m/z

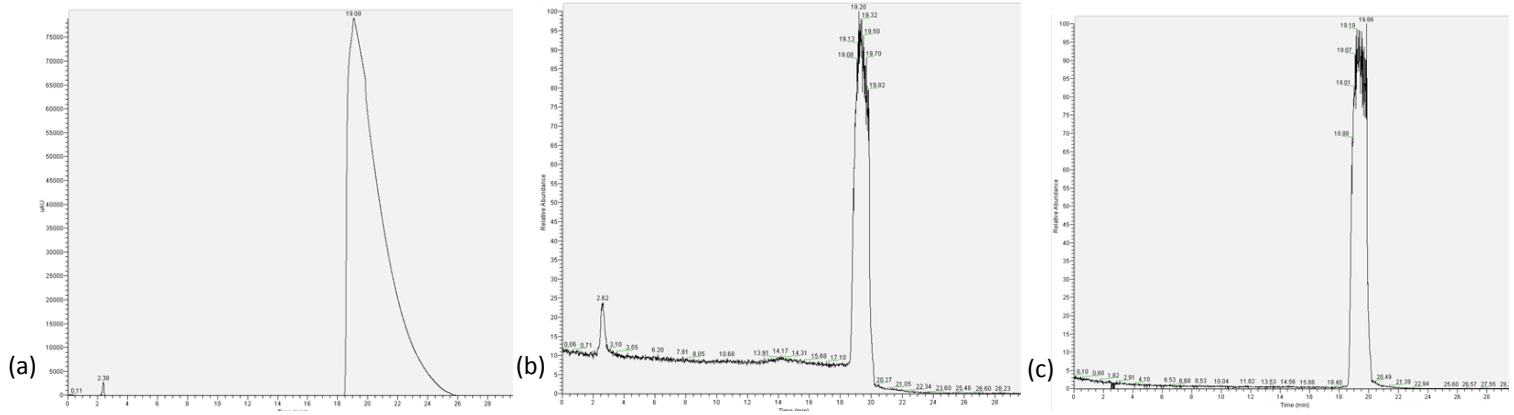


Figure 2: Chromatograms of sertraline over a 30 minute elution (a) HPLC (b) MS (c) MS with mass range of 305-306 m/z

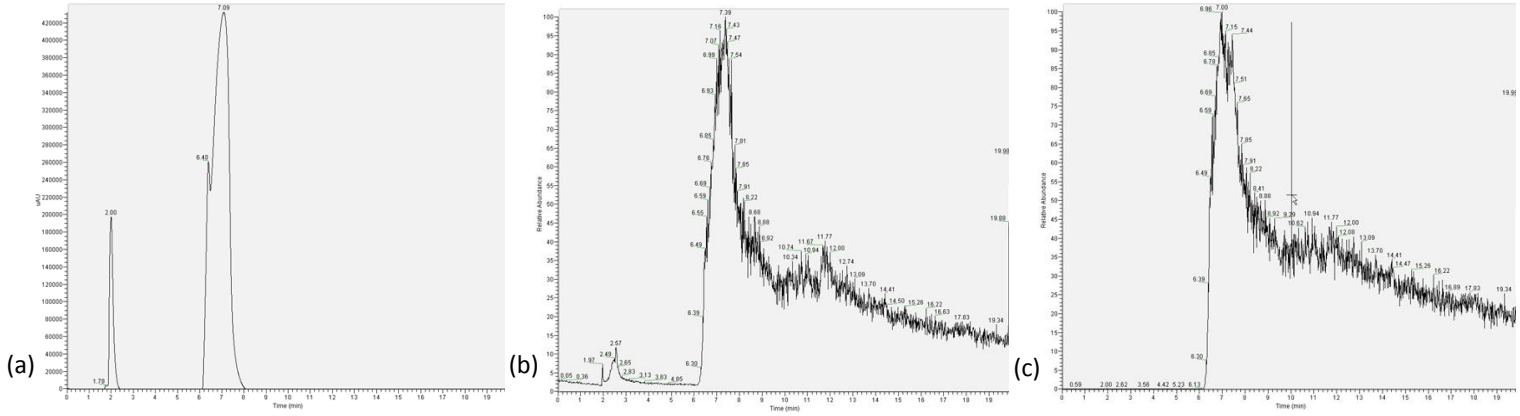


Figure 3: Chromatograms of quetiapine fumerate over a 20 minute elution (a) HPLC (b) MS (c) MS with mass range of 384-385 m/z

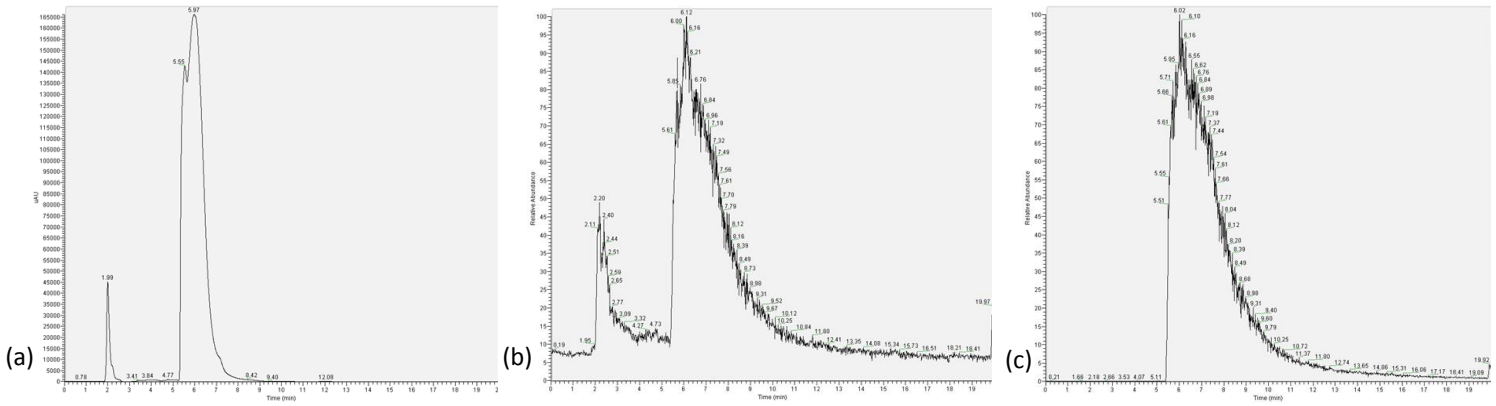


Figure 4: Chromatograms of escitalopram oxalate over a 20 minute elution (a) HPLC (b) MS (c) MS with mass range of 325-326 m/z

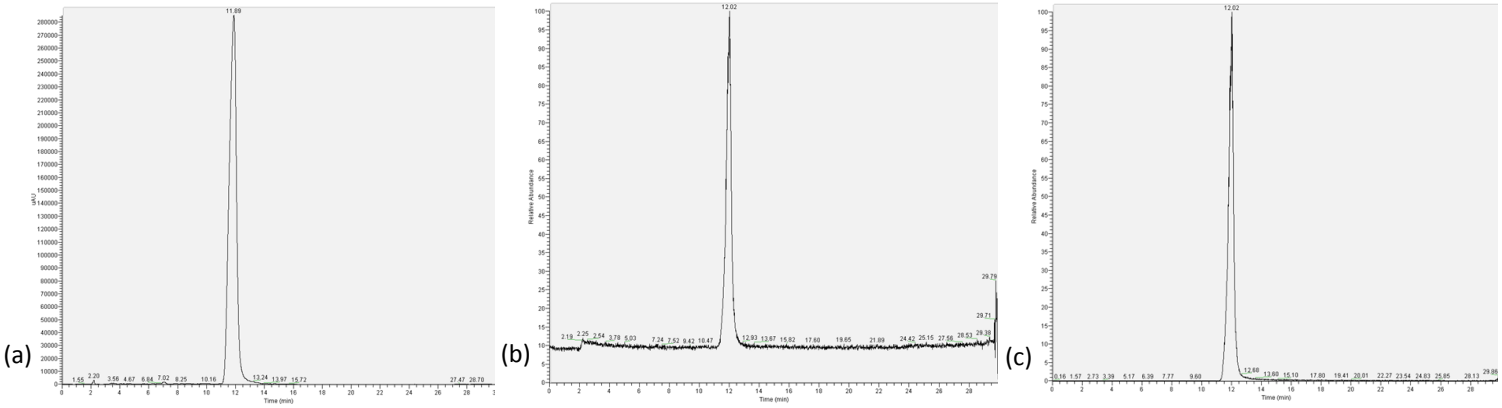


Figure 5: Chromatograms of ziprasidone over a 30 minute elution (a) HPLC (b) MS (c) MS with mass range of 413-414 m/z

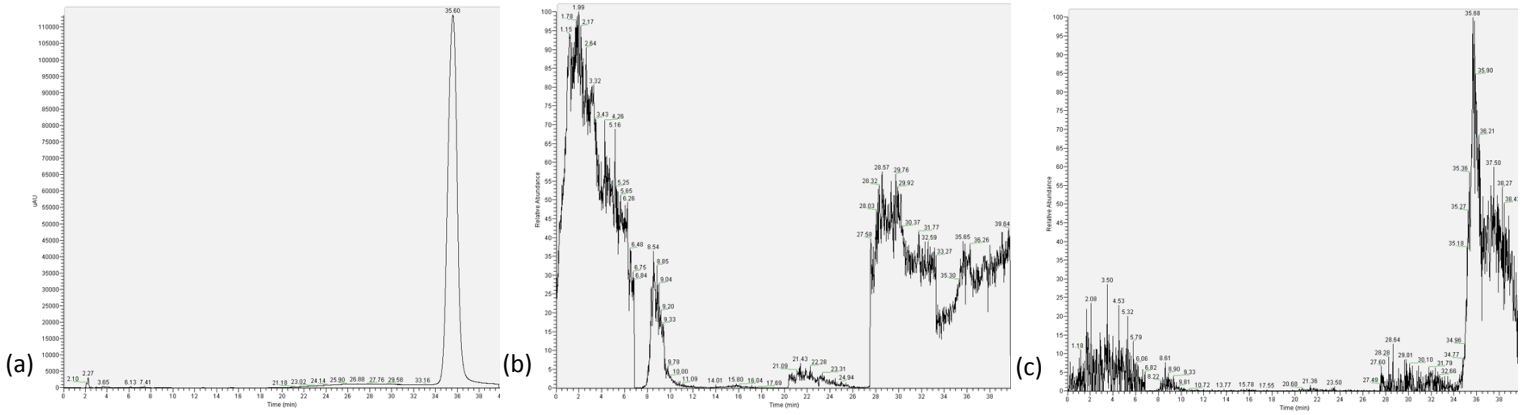


Figure 6: Chromatograms of aripiprazole over a 40 minute elution (a) HPLC (b) MS (c) MS with mass range of 448-449 m/z

Figures 1-6 show the separate times the six antidepressants eluted from the column and were detected by the HPLC, as well as their MS chromatograms. The peaks from the MS chromatograms become more defined when selected ion monitoring is used. The mass range is narrowed down to the smallest range containing the most common ion of the antidepressant.

Figure 7 shows the ability of the 70% MeOH/30% pH 9 buffer mobile phase to successfully separate the six antidepressant peaks when the antidepressants from Figures 1-6 are added together.

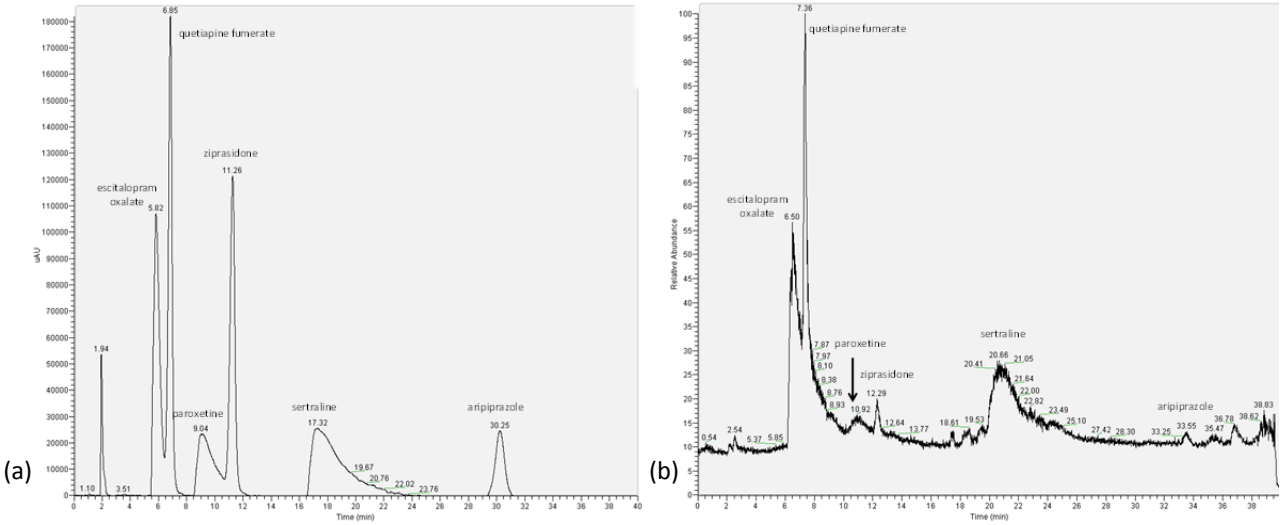


Figure 7: Chromatograms of six antidepressants at ~333 ppm using direct injection and a 40 min elution (a) HPLC (b) MS

Figure 8 was taken at both the highest concentration tested and the lowest concentration detectable with direct injection. The latter was obtained by injecting lower and lower concentrations until the peaks were no longer visible. Figure 8(b) therefore shows the concentration at which the LOD was reached under direct injection.

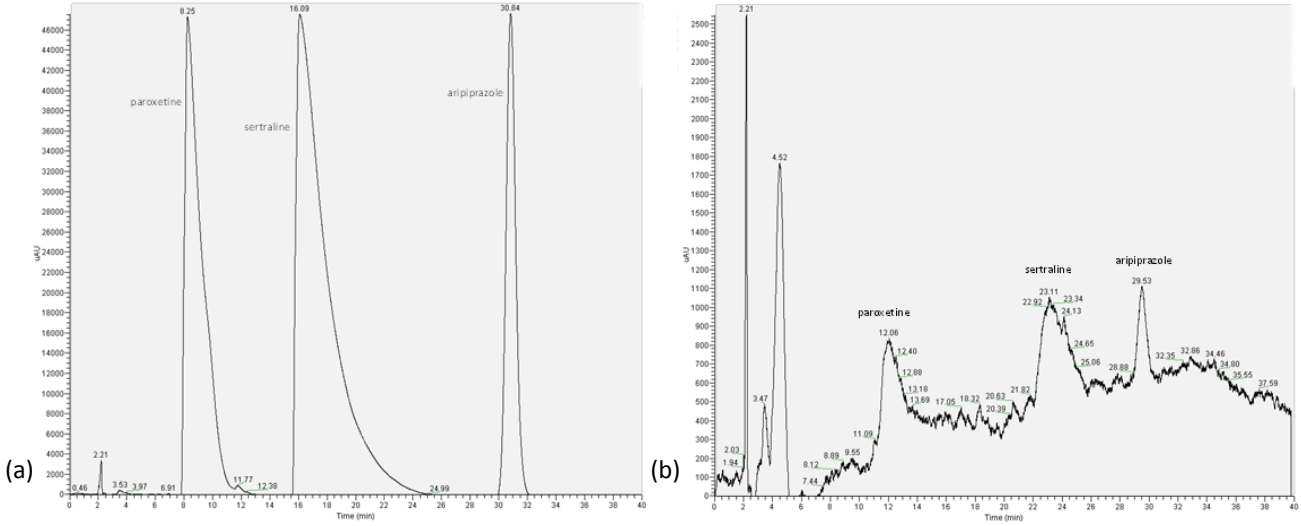


Figure 8: HPLC chromatograms of paroxetine, sertraline and aripiprazole using direct injection and a 40 minute elution (a) at ~1200 ppm (b) at ~12 ppm

Figure 9 shows the highest concentration tested for manual SPE, which was the same concentration as the lowest detectable by direct injection as well as the lowest concentration detectable after manual SPE, which was determined to be 0.014 ppm at pH 9.

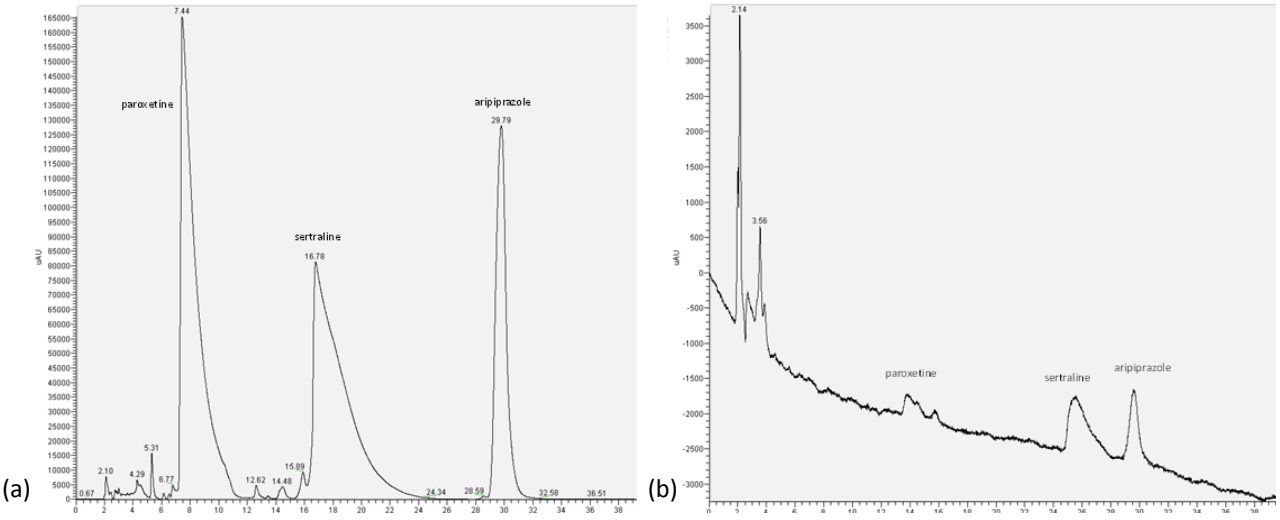


Figure 9: HPLC chromatograms of paroxetine, sertraline and aripiprazole after manual SPE and a 40 minute elution (a) at 13 ppm (b) at 0.014 ppm

Figure 10 shows 0.036 ppm pH 9 PSA solution chromatograms after automated SPE. The MS chromatogram was taken using ESI. No peaks were visible with ESI at this concentration.

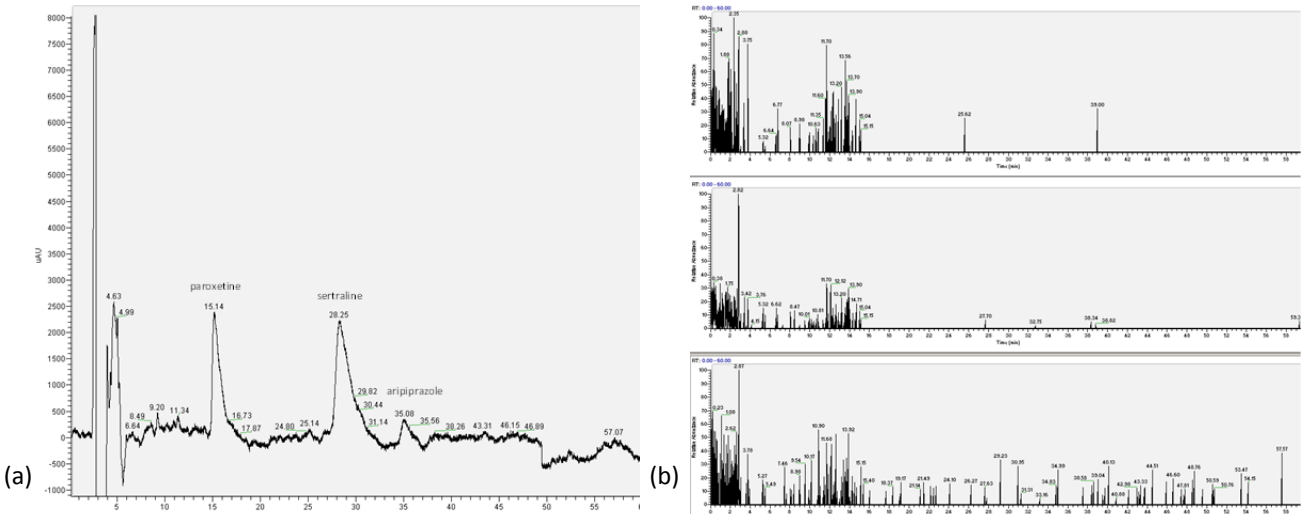
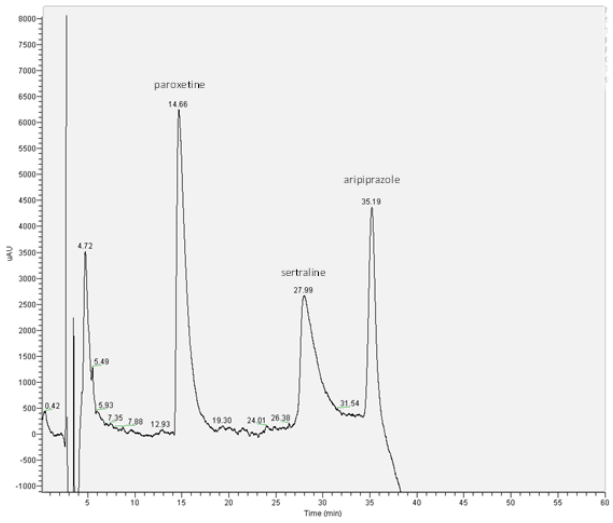


Figure 10: Chromatograms of paroxetine, sertraline and aripiprazole at 0.036 ppm after automated SPE and a 60 minute elution (a) HPLC (b) MS-ESI with mass ranges 330-331 m/z, 305-307 m/z and 448-449 m/z.

Figure 11 represents a 0.36 ppm pH 9 PSA solution after automated SPE with a MS chromatogram using Atmospheric Pressure Chemical Ionization (APCI) instead of ESI, and the peaks shown indicate that APCI source was able to detect all three compounds at this concentration.

(a)



(b)

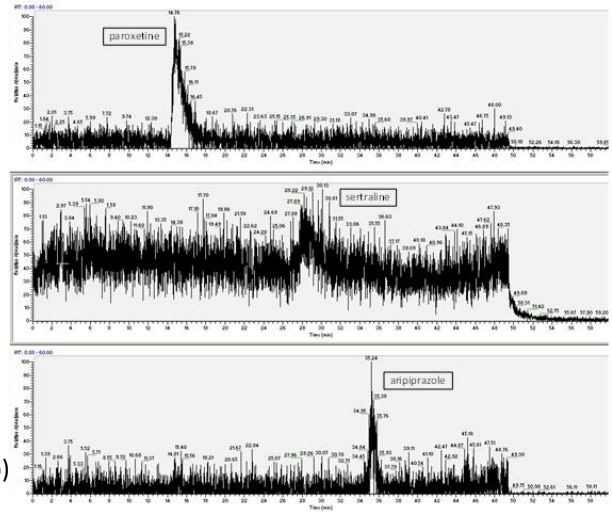


Figure 11: Chromatograms of paroxetine, sertraline and aripiprazole at 0.36 ppm after automated SPE and a 60 minute elution (a) HPLC (b) MS-APCI with mass ranges 330-331 m/z, 305-307 m/z and 448-449 m/z.

Figure 12 displays a 0.0072 ppm pH 9 PSA solution after automated SPE. This is the lowest concentration detectable by the HPLC after automated SPE. MS was employed because, as seen in figure 10(b), it was no longer useful at this concentration level.

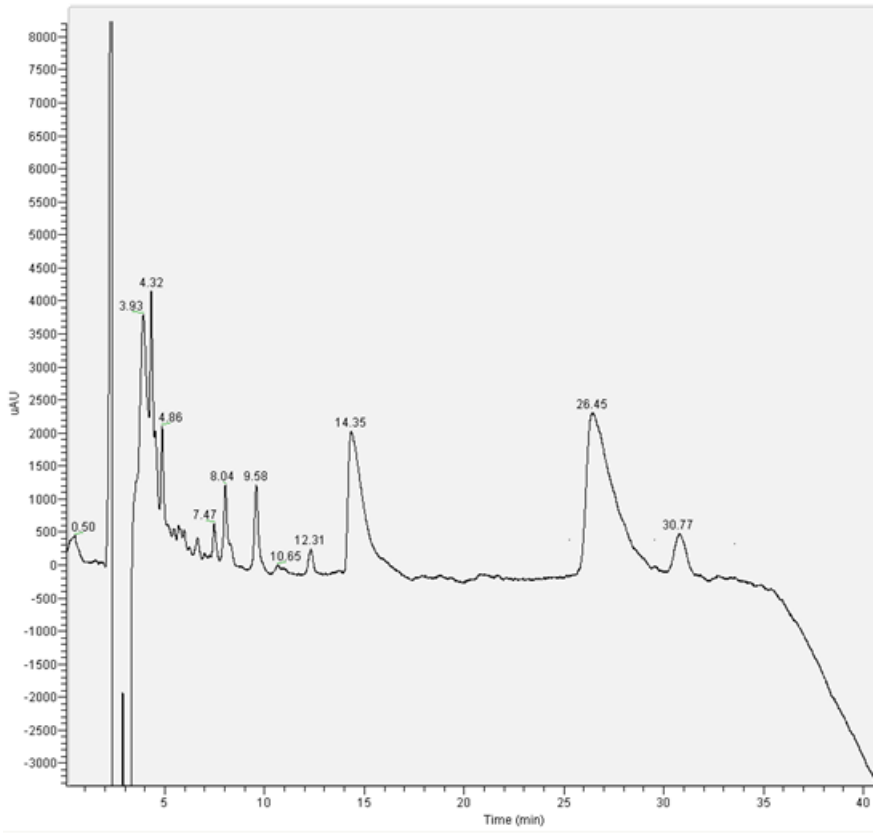


Figure 12: HPLC chromatogram of paroxetine, sertraline and aripiprazole at 0.0072 ppm after automated SPE and a 40 minute elution.

Part 2: Analysis of wastewater

Figure 13 and 14 are from the analysis of wastewater using SPE/HPLC-MS. While many similar chromatograms were compiled, these showed the data the most clearly. Figure 15 displays the ^{1}H-NMR of the third peak fraction collection as described in the methods section.

(a) (b)

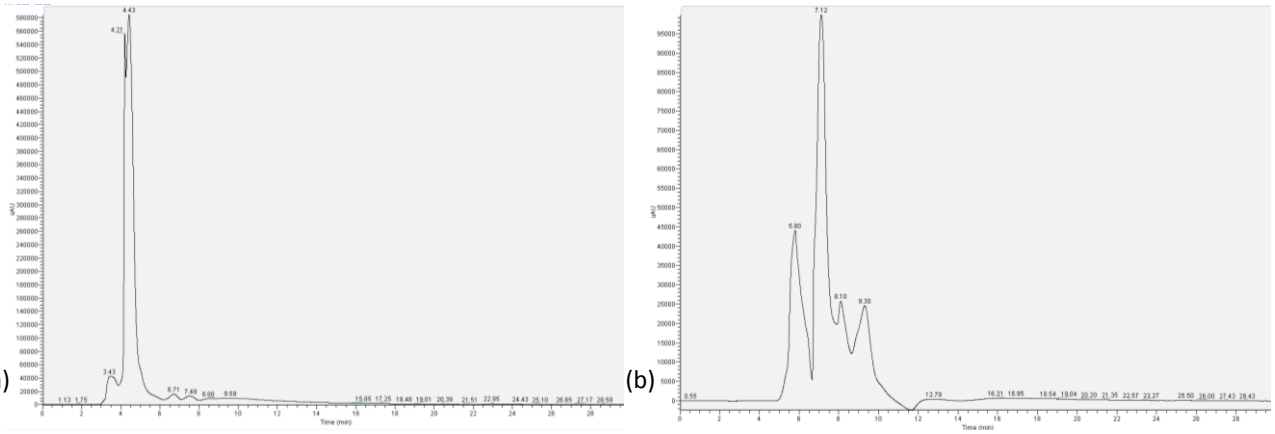


Figure 13: HPLC chromatograms of 30 minute elutions after automated SPE and a 30 minute elution (a) wastewater plus 0.74 ppm paroxetine (b) wastewater with three main unknown peaks at 5.80, 7.12 and 9.30 minutes.

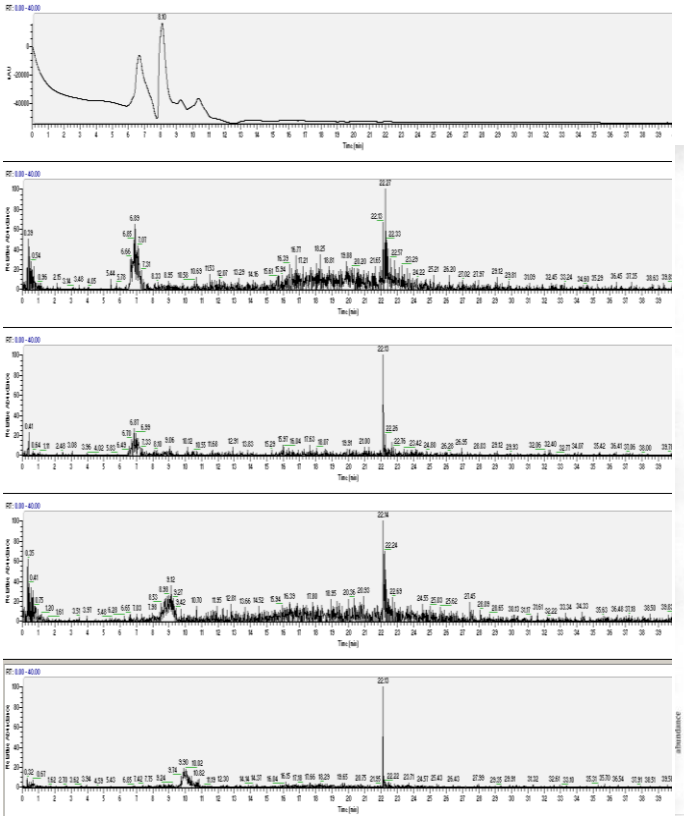


Figure 14: LC Chromatogram of 1 L of wastewater and Mass Spectra with mass ranges 281-282, 477-478, 259-260 and 513-515 m/z.

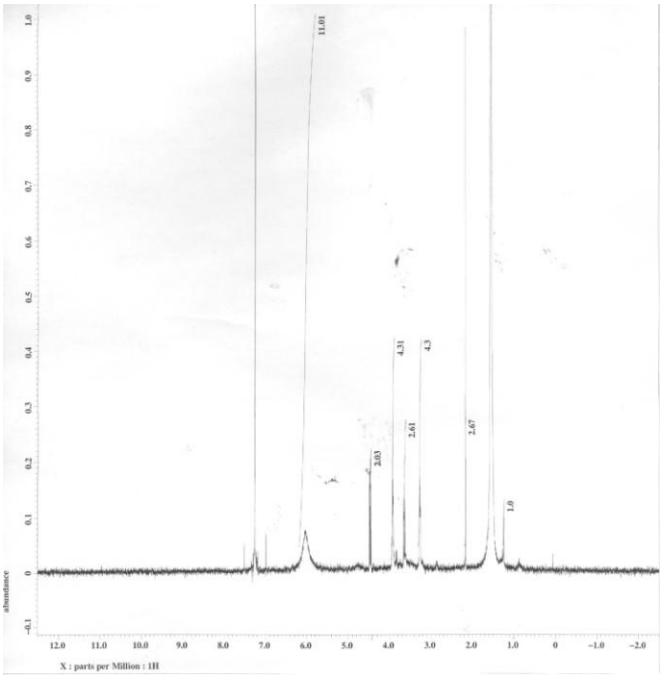


Figure 15: ^{1}H-NMR of the 3rd peak collected fraction. Water and deuterated chloroform are present at 1.5 and 7.2 ppm, respectively.

Figure 13(a) clearly shows there are some compounds in the wastewater which have concentrations that are not as great as 0.74 ppm like the paroxetine which was added, but greater than the 0.0072 ppm LOD of the automated method as discovered in Part 1. This can be concluded because the average absorbance of the LOD chromatogram was about 1500 and the average absorbance of the three peaks in the wastewater chromatogram was about 60,000, therefore much higher than the LOD.

In Figure 13(b), three distinct peaks can be seen which, after the procedure was replicated 20 times, were averaged to appear at 6.75, 8.10 and 9.25 minutes as shown in Table 2.

Figure 14 seemed to give an insight into what the compounds might be because of the mass range data that was recorded. The 6.25 minute peak pertained to mass ranges of 281-282 and 477-478 m/z, the 8.10 peak pertained to a mass range of 259-260 m/z and the 9.25 peak pertained to a mass range of 513-515 m/z. After doing some research on the internet, no common pharmaceuticals or compounds commonly found in wastewater were matched to this data. The fact that these compounds were able to be detected by the MS detector leads to the following conclusion: because the LOD of the LC-MS method in Part 1 was 0.14 ppm, the range of the concentrations of these three analytes can be approximated to be between 0.36 ppm and 0.74 ppm. This range is only speculation based on previous work, without knowledge of the actual compound and its ionization characteristics in the MS.

The ^{1}H-NMR of the third peak fraction as shown in Figure 15 was very similar to the ^{1}H-NMR of the first and second peaks in the chromatogram, and therefore was not useful in the identification in any of the compounds. Furthermore, the spectra were not matched to any known compound.

GC/MS chromatography was not useful in the identification of any of the peaks either, therefore a chromatogram is not included.

Based on the previous methods of analysis, this data is inconclusive in determining the identity of these analytes. In conclusion, the concentrations of the three unknown analytes in the wastewater were not significant enough to determine their composition with ^{1}H-NMR, GC-MS or LC-MS.

That being said, there are still compounds in a concentration significant enough to appear on the SPE/HPLC-MS method, therefore most likely above a concentration of the LOD, 0.0072 ppm. This concentration is of concern to the aquatic environment in Lake Gemini, into which the wastewater is discharged. Further work must be done by improving this method or using other methods of analysis to identify the compounds seen on the chromatograms.

Conclusion

The automated SPE method improved the LOD of the regular HPLC method by 1800 times. Using this highly improved and efficient method, three compounds were discovered in the wastewater from the St. John's Wastewater Treatment Plant, and identification is the subsequent step in the process.

ACKNOWLEDGEMENTS

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FUTURE RESEARCH

A continuation of this research would include identifying the three compounds found in significant concentration by collecting additional fractions and analyzing them with MS, NMR, and other methods. Once the compounds are identified, a further quantitative analysis would be performed. Pure samples would be tested in E-pure water in order to determine the initial concentration of each of these chemicals in the wastewater. These concentrations would be compared to known toxicity levels and determined to be toxic or non-toxic.

REFERENCES

1. Jones, O.A.H.; Lester, J.N.; Voulvoulis, N. "Potential Ecological and Human Health Risks Associated with the Presence of Pharmaceutically Active Compounds in the Aquatic Environment." *Critical Reviews in Toxicology*. 34(4): 335-350, 2004.
2. Kolpin, Dana; Furlong, Edward; Meyer, Michael; Thurman, E. Michael; Zaugg, Steven. "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams,1999-2000: A National Reconnaissance." *Earth Sciences Commons, University of Nebraska – Lincoln.* Paper 68. 2002.
3. Henry, Theodore B.; Kwon, Jeong-Wook; Armbrust, Kevin L.; Black, Marsha C. "Acute and Chronic Toxicity of Five Selective Serotonin Reuptake Inhibitors in Ceriodaphnia Dubia." *Environmental Toxicology and Chemistry*. 23(9): 2229-2233, 2004.
4. "Oasis on-line columns SPE columns for LC/MS/MS." Waters Corporation. July 2008.
5. Giddings, J.C. "Analytical Chemistry 2.0." *Unified Separation Science.* Wiley-Interscience: New York, 1991. pp: 783-884.
6. "Maximize your HPLC Throughput" Thermo Fischer Scientific Inc. 2010. Available online: http://apps.thermoscientific.com/media/flv/tbf2/index.php?id=3
7. Ban, Eunmi; Maeng, Jeong-Eun; Woo, Jong Soo; Kim, Chong-Kook. "Sensitive column-switching high performance liquid chromatography method for determination of propiverine in human plasma." *Journal of Chromatography B*. 831: 230-235, 2006.
8. Brazdova, Barbora; Kozak, Marta. "Screening and Quantification of Multiple Drugs in Urine Using Automated Online Sample Preparation and Tandem Mass Spectrometry." Thermo Fischer Scientific Inc. 2010.
9. "Measurement of Antibiotics in Environmental Waters using LC-QQQ and fully automated Online Enrichment." Agilent Technologies, Inc. April 2012.
10. "Analyze pharmaceutical compounds in surface water by direct injection." Agilent Technologies, Inc. April 2012.
11. Linfeng, Liu; Baltes, Andy. "HPLC Analysis of Pharmaceuticals in SJU Waste and Surface Water." College of St. Benedict Chemistry Department. Summer 2007.
12. Klobes, Michael Jeffrey. "Photodecomposition of s-Citalopram (Lexapro®)." College of St. Benedict Chemistry Department. Summer 2005.
13. Lee, Meghan. "The photodegradation of Zoloft®." College of St. Benedict Chemistry Department. 2004-2005.
14. Downing, Christopher. "Photolysis of Venlafaxine and Paroxetine in Aqueous solutions." College of St. Benedict Chemistry Department. 2003-2004.