Analyzing Opioid Use and Disposal in Washington State Through Wastewater-Based-Epidemology

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Analyzing Opioid Use and Disposal in Washington State Through Wastewater Based Epidemiology
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Background

- Painkillers, such as Vicodin, are commonly over-prescribed
- Washington State’s Safe Medication Return Program began November 2020
- Take-Back program will allow a safer method of disposing unwanted drugs
- Wastewater-Based Epidemiology (WBE) is a new method that allows tracking of drug consumption and, we propose, can show changes in disposal by flushing

Objectives

- Develop a method to extract opioids and their metabolites from wastewater
- Use standards to create calibration curves for each compound and validate method
- Quantify desired compounds in wastewater
- Analyze opioid trends in participating cities

<table>
<thead>
<tr>
<th>Compound of Interest</th>
<th>Information about compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocodone</td>
<td>Common ingredient found in painkillers (Vicodin)</td>
</tr>
<tr>
<td>Nor-hydrocodone</td>
<td>Hydrocodone metabolic</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>Common ingredient found in painkillers (Oxycontin)</td>
</tr>
<tr>
<td>Nor-oxycodone</td>
<td>Oxycodone metabolic</td>
</tr>
<tr>
<td>Codeine</td>
<td>Common ingredient found in cough medicines</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Synthetic opioid prescribed for severe pain (Sublimaze)</td>
</tr>
<tr>
<td>Nor-fentanyl</td>
<td>Fentanyl metabolite</td>
</tr>
</tbody>
</table>

Figure 1. Structures of analytes. As shown above, the tertiary amine on hydrocodone and oxycodone reduces into a secondary amine once metabolized in the body, producing the other analytes of interest (norhydrocodone and noroxycodone).

Extraction Method

SOLID PHASE EXTRACTION

1. Condition Waters MCX Cartridge
2. Weigh out 25.00 g of wastewater
3. Spike 50 μL of 2 ppm internal standard into wastewater*
4. Add 2 drops of HCl
5. Centrifuge samples for 10 minutes
6. Load syringe with wastewater, push through 0.2 μm RC filter to MCX cartridge
7. Dry and wash MCX SPEs
   - i. Rinse with 1 mL of 85:15 Mixture (MeOH: ACN)
   - ii. Dry for 5 minutes
   - iii. Wash with 2 mL of LC-grade MeOH
8. Elute MCX cartridge with 3 mL Basic MeOH (5% NH₄OH in MeOH)
9. Evaporate solvent under stream of Nitrogen at 30-40°C
10. Reconstitute
   - i. Pipet 400 μL of 0.01% Formic Acid in 95% water/5% Methanol

*The use of internal standard allows for greater precision of quantitative analysis.
The internal standard contains deuterated version of the analyte, behaving like the original analyte but producing distinguishable signals in different between the two.

Table 1. R² value for both linear and quadratic calibration curve of each opioid. Favorable R² values are those close to 0.9999 with a target 0.9999

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear R² Value</th>
<th>Quadratic R² Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>0.9904</td>
<td>0.9996</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>0.8573</td>
<td>0.9989</td>
</tr>
<tr>
<td>Norhydrocodone</td>
<td>0.9885</td>
<td>0.9997</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>0.9647</td>
<td>0.9965</td>
</tr>
<tr>
<td>Noroxycodone</td>
<td>0.9015</td>
<td>0.9993</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.5214</td>
<td>0.9985</td>
</tr>
</tbody>
</table>

Results

Figure 2. Solid Phase Extraction (SPE) set up. The syringes are filled with wastewater that have been spiked with an internal standard.

Figure 3. Chromatogram of hydrocodone in wastewater. The top chromatogram represent the qualifier ion (109.07). The middle chromatogram represent the qualifier ion (171.08), and the bottom chromatogram represents the internal standard, Hydrocodeone-D₃. Each chromatogram provides the retention time, the area, and the SN, respectively.

Figure 4. Norfentanyl standard (A) and normorfentanyl standard (B). Due to low concentration of norfentanyl in wastewater it becomes difficult to measure/track.

Figure 5. Ratio comparison of Hydrocodone/Nor-hydrocodone concentration (right) and Oxycodone/Noroxycodone concentration (left). The first week of April 2020 (census data) was compared to the last week of April 2021 samples from all seven participating treatment plants. Smaller ratios indicate higher concentration of the metabolite, while larger ratio indicates higher concentration of the original compound. Smaller ratios could indicate fewer flushing of the parent drug.

Future Work

- Continue analyzing wastewater
- Analyze opioid trend
- Identify whether the Take-Back program is successful
- Optimize a method to measure Nor-Fentanyl

Conclusion

- Standards successfully created calibration curves
- Successful in narrowing compound of interest list to 6 compounds
- Results from April ‘20 and ‘21 showed ratios from before and during the Take-Back program
- Too early to determine effectiveness of program

Acknowledgement

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