Summer 2021

Determining Dynamic Population Trends with Biomarkers in Wastewater

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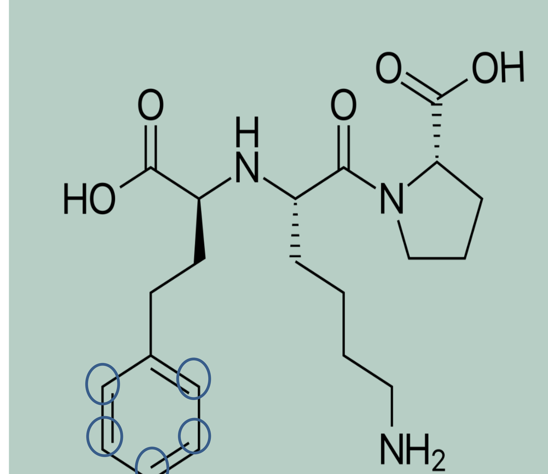
Introduction

- Small molecules in municipal wastewater can be analyzed and quantified to estimate drug consumption, environmental exposures, and viral loads of a population.
- Wastewater derived results are typically reported in grams/1000 people/day.
- The largest uncertainty in these estimates is the daily population.
- Wastewater may potentially contain quantifiable population biomarkers that could provide better population estimates.
- Potential population biomarkers have been evaluated with the following proposed: caffeine, gabapentin, hydrochlorothiazide, and acesulfame. Additionally, we are also evaluating lisinopril and sucralose.
- Mobile device data may also provide insight into dynamic populations which could be used to validate biomarker results.

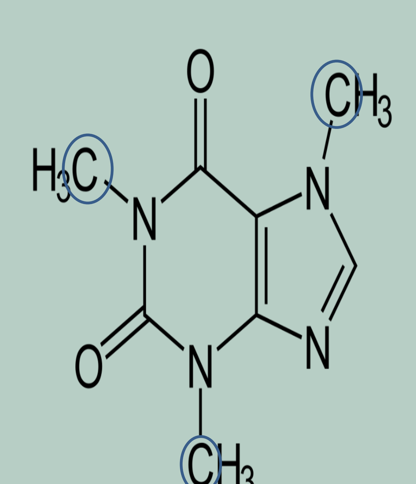
Project Goals

- Create shape files for cell phone data acquisition
- Validation of each compound in tap and wastewater
- Begin degradation study to determine how much each compound degrades in raw wastewater over time
- Begin quantifying samples from August 2020 and April 2021

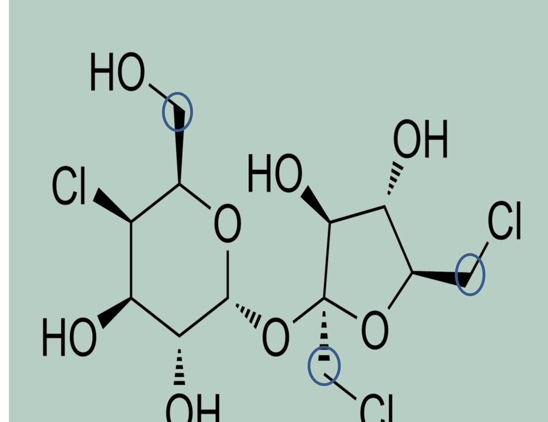
Positive Ion Mode



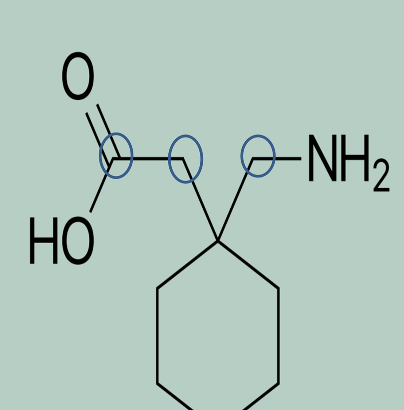
Lisinopril, 246.151 g/mol



Caffeine, 138.067 g/mol

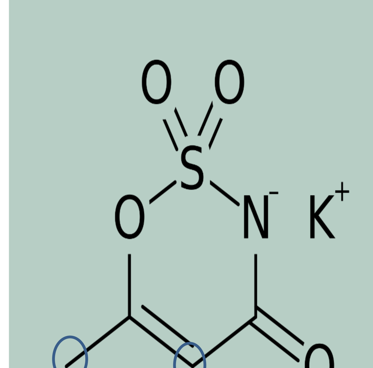


Sucralose, 221.021 g/mol

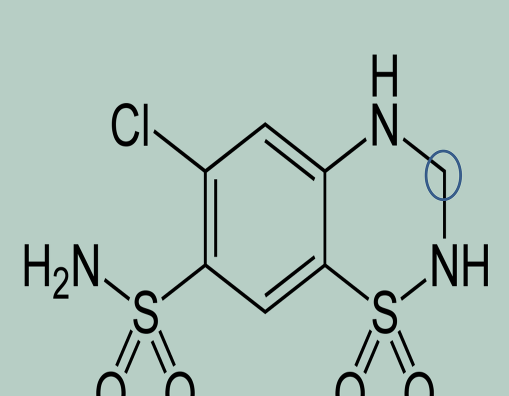


Gabapentin, 154.127 g/mol

Negative Ion Mode



Acesulfame, 82.031 g/mol



Hydrochlorothiazide, 268.949 g/mol

*Circles denote deuterium or 13C in isotopically labeled analogues for internal standards

Methods

1. Thaw samples in room-temperature water bath.
2. Filter wastewater into a small beaker using a 0.2 µm regenerated cellulose syringe filter.
3. Using a P1000 micropipette, add 950 uL of sample to autosampler vial
4. Add 50 uL of the internal standard (I.S.) solution into autosampler vial. (25 ng of each I.S.)
5. Cap vial and vortex samples.*
6. Analyze using LC-MS/MS in both positive and negative ion mode, along with a set of eleven calibrants and a blank analyte.
7. Convert concentrations into grams/day of compound using 24 h flow data given from treatment plants.

*repeat for every sample

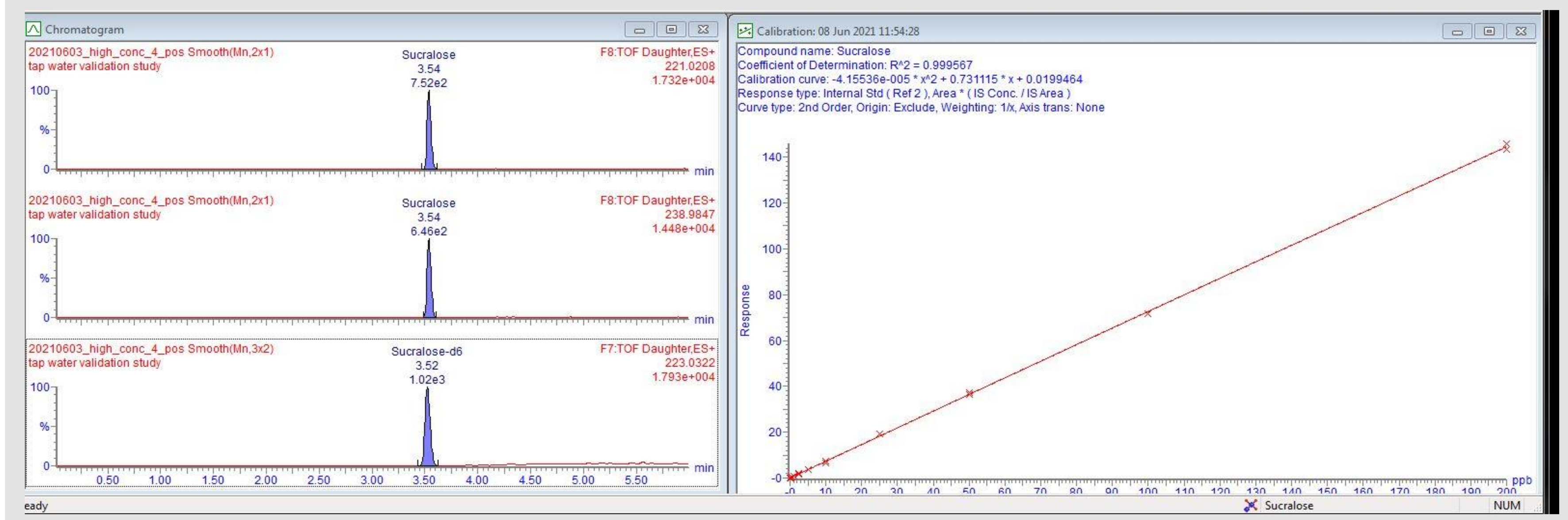
References

Castiglioni, S.; Bijlsma, L.; Covaci, A.; Emke, E.; Hernández, F.; Reid, M.; Ort, C.; Thomas, K; van Nuijs, A. L. N.; de Voogt, P.; et al. Evaluation of Uncertainties Associated with the Determination of Community Drug Use through the Measurement of Sewage Drug Biomarkers. *Environ. Sci. Technol.* **2012**. https://doi.org/10.1021/es302722f.

O'Brien, J. W.; Thai, P. K.; Eaglesham, G.; Ort, C.; Scheidegger, A.; Carter, S.; Lai, F. Y.; Mueller, J. F. A Model to Estimate the Population Contributing to the Wastewater Using Samples Collected on Census Day. Environmental Science & Technology 2013, 48 (1), 517–525.

Thomas, K. V.; Amador, A.; Baz-Lomba, J. A.; Reid, M. Use of Mobile Device Data To Better Estimate Dynamic Population Size for Wastewater-Based Epidemiology. Environmental Science & Technology 2017, 51 (19). (12).

Results



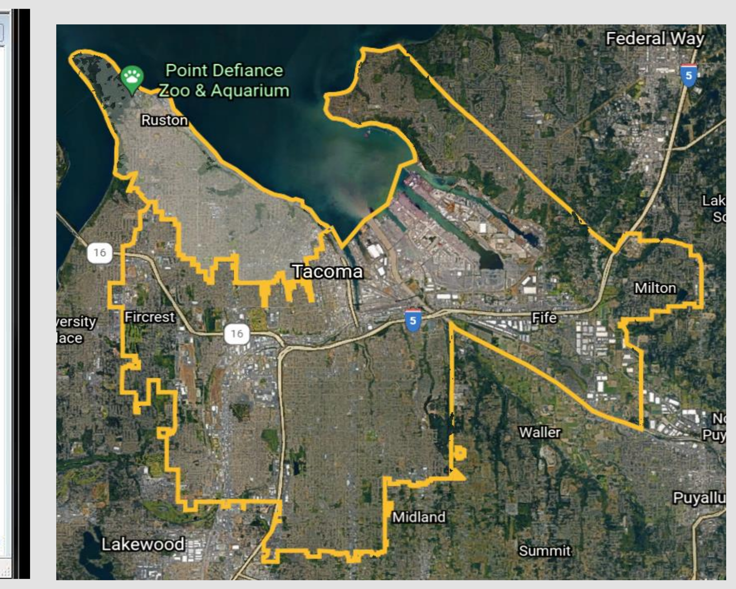
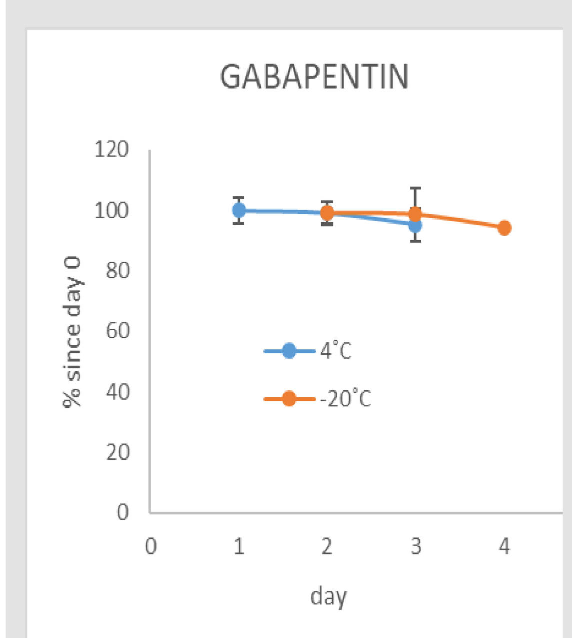
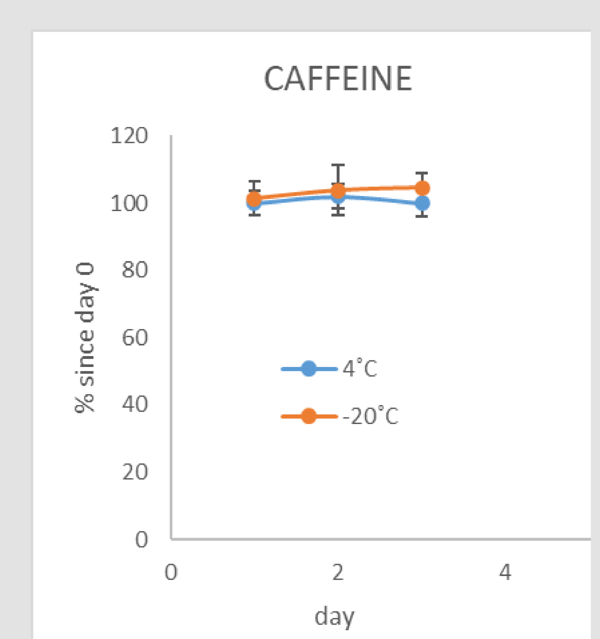


Figure 1. Sucralose quantifier ion (top), qualifier ion (mid), and I.S. quantifier (bottom) with a calibration curve of sucralose. This is an example of the six biomarkers of interest

	CAFFEINE													
	cell data	g/day	cell data	g/day	cell data	g/day	cell data	g/day	cell data	g/day	cell data	g/day	cell data	g/day
Day	WW		NTP		YAK		LOTT		SPOK		CTP		SEA	
1-Apr-20	1757	122.3234	3103	149.8694	5075	348.6133	10529	438.0418	15602	891.505	18864	526.21	33458	2795.67
2-Apr-20	1716	231.7896	3108	149.189	4976	351.5022	10188	498.797	15270	826	18461	576.3	32660	2802.13
3-Apr-20	1622	301.7555	3184	159.5084	5114	357.1941	10351	485.7569	15274	752.76	19223	560.18	32018	2465.03
4-Apr-20	1709	323.7147	3034	171.5062	4914	371.013	10209	410.3317	14687	814.73	17230	543.04	30709	2588.83
5-Apr-20	1586	310.9966	2846	150.4894	4639	331.1666	9515	417.9831	13941	760.14	15421	680.47	28443	2505.25
6-Apr-20	1595	125.7209	3071	155.3845	4904	359.8106	9755	357.2651	14596	790.65	18438	639.3	30491	2653.09
7-Apr-20	1601	291.1526	3095	180.8541	5006	333.3166	9902	520.5943	15267	845.45	19299	647.04	31342	3001.75

Table 2. The beginnings of cellular data comparison to grams per day of Caffeine in wastewater during US Census week in April 2021. Each set of points convey one of seven treatment plants. In the left columns, cellular device data for each city is shown, while in the right columns is the amount of each compound per treatment plant in grams per day.





Figures 3 and 4. Plots showing the degradation of Gabapentin and Caffeine over the span of three days, starting on the day fresh wastewater was acquired. These plots show the compounds of interest are stable during storage.

Sucralose	
Sample	HIGH CONC (ppb)
1	76.7
2	67.6
3	65.4
4	63.6
5	70.6
average	68.8
% recovery	74.1

Table 1. Validation of Sucralose at a high spiked concentration in wastewater with the percent recovery of the compound after analysis. Tap water studies showed a **104% recovery.** Similar tables were made for all six compounds regarding both studies.

Conclusions

- Developed a method to process, analyze, and quantify six population biomarkers
- Validated method with tap and wastewater studies with 90% recovery for most compounds at high spike levels
- Began degradation study that appears to show that all compounds are stable in frozen samples
- Made shape files for seven wastewater treatment plants that are currently being. used by Echo Analytics to obtain historical, current, and future mobile device data for eight weeks of sampling.

Future Work

- Finish processing samples from August 2020 and April 2021 and begin August 2021
- Concentrations into grams/day of compound.
- Continue degradation study for months three (Oct) and six (Jan).
- Compare April 2020 data to U.S. Census data to estimate how the compounds accurately predict population.
- Compare wastewater results to mobile device results to estimate how the compounds precisely predict the trends in population.

Acknowledgements

This research is funded by NIH-NIDA 1R15DA051792-01