



# Assessment of Sucralose, Caffeine and Acetaminophen as Anthropogenic Tracers in Aquatic Systems Across Florida

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## Abstract

Sucralose is one of the most popular artificial sweeteners worldwide. Due to its high stability, persistence and low removal efficiency in wastewater treatment plants, sucralose has been used as an indicator of wastewater intrusion into aquatic systems. However, its stability has also been a reason for discussion whether sucralose's presence in surface water could indicate a recent anthropogenic input. Caffeine and acetaminophen have been considered as tracers in human impacted aquatic ecosystems and potentially good indicators of recent anthropogenic inputs into the environment due to their short half-lives in water. Here, a novel, high throughput and sensitive method based on online SPE-LC-HRMS for the determination of caffeine, sucralose and acetaminophen was developed and validated for both fresh and seawater samples and applied to environmental water samples to evaluate the efficiency of these compounds as tracers of aquatic pollution. Caffeine and sucralose were detected in > 70% of samples, while acetaminophen was only detected in 3% of samples above the MDL, demonstrating its limited environmental applicability.

**Keywords** Anthropogenic tracers · HRMS · Surface water

Sucralose (Splenda) has gained popularity worldwide as an artificial sweetener and sugar substitute in low-calorie diets, being present in a variety of foods, drinks, pharmaceuticals and personal care products. Previous studies have found that sucralose is safe for human consumption, and due to its stability, persistence in water of up to several years and inefficient removal in wastewater treatment plants, sucralose has been widely used as a tracer of human impacts in aquatic ecosystems (Batchu et al. 2015; Cejas et al. 2010; Tollefsen et al. 2012). Sucralose has been selected as a tracer due to its ubiquitous occurrence in the environment, and because the majority of sucralose ingested is not adsorbed

or metabolized by humans. More recently, Schiffman and Rother found that sucralose produced multiple peaks in thin layer radiochromatographic profiles, showing the existence of at least some metabolites (Schiffman and Rother 2013). Unfortunately, the high stability of sucralose has also made it difficult to determine if its presence is due to recent contamination or if it has been in the environment for some amount of time. Mawhinney et al. previously studied the water intakes and outputs of 19 drinking water treatment plants across the U.S. They determined that the source water for 15 out of 19 drinking water treatment plants that they studied were contaminated with sucralose between 47 and 2900 ng/L. Thirteen of the plants studied contained sucralose in their finished water, and 8 plants demonstrated sucralose concentrations between 48 and 2400 ng/L in the water of their distribution systems (Mawhinney et al. 2011). This research was further supported by Batchu et al. (2013), who demonstrated sucralose's resistance to photodegradation in addition to detecting sucralose in > 80% of environmental samples analyzed. More recently a study by Cantwell et al. (2019) observed sucralose levels up to 3180 ng/L in Narragansett Bay, Rhode Island. Locally, in South Florida, Cejas et al. (2010) found concentrations of sucralose in the nearshore Florida Keys ranging up to 140 ng/L.

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Caffeine has also been shown to be a valuable tracer of human fecal contamination due to its use as a human dietary component, as it is found in items ranging from tea and coffee to chocolates and other assorted desserts (Cejas et al. 2010). Previous medical studies have shown that caffeine is metabolized by humans via both oxidation and N-acetylation metabolic processes (Rodríguez-Gil et al. 2018), although depending on population, anywhere from 0.5% to 10.0% of ingested caffeine is excreted without metabolic processing (Ferreira 2005; Rodríguez-Gil et al. 2018). Previous work published by Potera (2012) revealed that samples collected from stormwater pipes and discharge points in Montreal showed strong concentrations between caffeine and fecal coliform units (CFU). Caffeine concentrations above 400 ng/L is correlated strongly with samples showing at least 200 colony forming units (CFU) per 100 mL of water (Sauvé et al. 2012). Additionally, extremely high concentrations of caffeine in the range of 44–71 mg/L were shown to inhibit growth in both water flea (*Ceriodaphnia dubia*) and fish (*Pimephales promelas*), with *C. dubia* being the most sensitive species, demonstrating a 7-day LC<sub>50</sub> of 46 mg/L (Moore et al. 2008). A previous study conducted in Agrinio City, Greece detected caffeine at concentrations up to 6679 ng/L in wastewater treatment plant effluent, and up to 37.6 ng/L in the nearby Acheloos River (Stamatis and Konstantinou 2013). More recently a study by Cantwell et al. (2018) demonstrated levels of caffeine up to 2056.7 ng/L with a 100% detection rate in the Hudson River estuary. Locally in South Florida, caffeine concentrations have been determined by Gardinali et al. in the range of 4 to 41.2 ng/L in the Miami river (Gardinali and Zhao 2002).

Due to its common use by humans, and rapid degradation time, the analgesic acetaminophen (Paracetamol) may have some potential as a secondary tracer. Acetaminophen is commonly used as an over the counter medication and is mostly excreted as metabolites by sulfate and glucuronide conjugation (Mazaleuskaya et al. 2015). Seven major metabolites of acetaminophen have been reported in total (Mrocheck et al. 1974), involving metabolism pathways that are not limited to humans, but in fact shared by many mammals (Jerzsele 2012). Stamatis and Konstantinou (2013) determined acetaminophen concentrations at levels up to 305 ng/L in the Acheloos River in Western Greece. Another study in China in 2015 detected acetaminophen at levels ranging from 44 to 134 ng/L (Sun et al. 2015). More recently the same study by Cantwell et al. mentioned above detected acetaminophen at concentrations up to 327.7 ng/L. As caffeine and acetaminophen are both considered ubiquitous components in aquatic ecosystems, and given their short half lives in the environment, they may be useful as secondary tracers to sucralose. As a first step, the present study aimed to develop and validate a new, high throughput and sensitive online solid phase extraction method for the

detection of caffeine, sucralose, and acetaminophen in sea-water samples at trace levels on the ng/L range by high resolution mass spectrometry. In the second step, the developed method was used to analyze surface and ground water samples from across South and Central Florida to evaluate the occurrence and spatial variability in concentrations of sucralose, caffeine, and acetaminophen. Water samples (n = 180) were collected or provided by interested parties from across six counties in South Florida, shown in Fig. 1. Out of 180 samples, 111 groundwater samples were provided by Collier County, FL, 42 samples were groundwater provided by Indian River County, FL, 13 surface water samples were collected from Biscayne Bay, FL, 7 samples from stormwater retention ponds were taken from Lee County, FL, and 5 groundwater samples were provided by St. Lucie County & Martin counties, FL. Finally, the goal of the present work is to evaluate the applicability of the selected compounds as anthropogenic tracers of aquatic pollution.

## Materials and Methods

Acetaminophen was purchased from Acros Organics (Geel, Belgium). Caffeine (1,3,7-trimethylxanthine) was purchased from Sigma Aldrich (St. Louis, MO, USA), and Sucralose standard was purchased from AK Scientific (Union City, CA, USA). Labeled standards acetaminophen-d<sub>4</sub>, and sucralose-d<sub>6</sub> were purchased from CDN Isotopes (Pointe-Claire,



**Fig. 1** Map of counties in South and Central Florida. Sample locations are located within highlighted counties

Quebec, Canada), and caffeine  $^{13}\text{C}_3$  was purchased from Cambridge Isotope Laboratories Inc (Tewksbury, MA, USA). A mixture of stock and internal standards solutions was prepared at concentrations of 100 mg/L in LC–MS grade water (Thermo Fischer, Fair Lawn, NJ, USA). Artificial seawater was prepared via the addition of Instant Ocean salt mix (Blacksburg, VA) to LC–MS grade water to a density of 1.023–1.025 g/cm<sup>3</sup> as measured by an optical refractometer.

A novel online solid phase extraction liquid chromatography-high resolution mass spectrometry (SPE-LC-HRMS) method was developed for the detection of caffeine, sucralose, and acetaminophen using a Thermo Accela Open AS autosampler. Two six-port switching valves were used in the method. The online SPE system with the valve switching setup is shown in Fig. 1S (Supp Info). The analytical separation was achieved using a Hypersil Gold aQ column (100 × 2.1 mm, 1.9 μm) and the SPE pre-concentration column used a Thermo Retain PEP column (3.0 × 0.5 mm). The procedure involved very little sample preparation; the samples were filtered (0.2 μm) and 200 μL of 22 μg/L internal standards mixture of the labeled standard analyte were added. Valve mechanism and steps used in the online solid phase extraction method is described in Fig. 1S.

Initially, 10 mL of the samples were injected into a 10 mL stainless steel loop and then loaded onto the SPE Retain PEP column within 5.0 min, followed by a washing step with 0.1% formic acid (pH = 3) in LC–MS water and acetonitrile (98:2). After valve switching at 4.5 min, connecting the SPE column with the analytical column, the solvent flow through the SPE column is reversed, and the analytes were then back-flushed and eluted with a gradient of acetonitrile and 0.1% formic acid. At 9 min, the valve turned again connecting the SPE LC pump with the SPE column which was washed using 90% acetonitrile (as a carryover prevention measure) and progressively returned to the initial conditions (100% water). The samples were kept at 10°C in the autosampler. The total run time per sample was 13 min. Analytes were detected on a Q-Exactive Mass spectrometer equipped with a heated electrospray ionization source. The HESI temperature and capillary temperature were 250 and 350°C, respectively, with a discharge current of 5 kV and S-lens RF level of 80%. Sheath gas and auxiliary gas (N<sub>2</sub>) were used at a flow rate of 30 and 2 units, respectively. The mass spectrometer was set in parallel reaction monitoring (PRM) mode with a resolution of 35,000 in positive ionization mode to detect caffeine and acetaminophen at *m/z* of 195.0876 and 152.0706 respectively with confirmation ions at *m/z* 138.0662 for caffeine and 110.0600 for acetaminophen. Sucralose was determined separately in negative mode in full scan with 140,000 resolution scanning from 200–500 *m/z*. The exact masses of *m/z* 395.0073 and *m/z* 397.0044 were used for quantitation and confirmation purposes.

The method was validated in terms of linearity, precision, accuracy, matrix effect, and method detection limits. All calibration standards were prepared in LC–MS water. Analyte concentrations were calculated by dividing the peak area of the analyte compound by the area of the corresponding isotopically labelled internal standard compound. Linearity was determined via a 9-point calibration curve ranging from 10 to 5000 ng/L. Method detection limits (MDL's) were determined in LC–MS water and in artificial saltwater, by the analysis of five spiked replicates of 50 ng/L in LC–MS water, and of 100 ng/L in artificial saltwater. MDLs were estimated by the standard deviation of the replicates multiplied by the *t* value for 99% confidence (3.747). Intraday variability was assessed by analyzing nine replicate samples at three concentrations 50, 500, and 2000 ng/L, and determining the relative standard deviation (RSD). Similarly, interday variation was assessed by analyzing nine replicate samples at the same concentrations on five different days. Recoveries were calculated by subtracting out any analyte presence in the blank before dividing the measured analyte concentration by the expected concentration of the standards. Matrix effects were evaluated by spiking nine replicate solutions each of LC–MS water and artificial saltwater at 100 ng/L. Matrix effect was calculated by subtracting the ratio of concentrations in artificial saltwater and LC–MS water from 1, then multiplying by 100. Using this equation, negative values would indicate ion enhancement, while positive values would indicate suppression. Each batch of samples was processed with a laboratory reagent blank and laboratory fortified blanks prepared in LC–MS water, as well as duplicate and fortified matrix samples. Calibration quality was checked via a continuing calibration verification standard (CCV) at the concentration of 100 ng/L every 20 samples. All spiked quality control samples were required to show recoveries between 70% and 130%. Duplicate samples were required to vary by no more than 30%. Matrix spiked samples were spiked with an additional 100 ng/L of standard solutions and were required to exhibit recoveries within 30% of the expected value. All statistical measurements, including distribution tests and Spearman Rank Coefficient tests were undertaken using Sigmaplot v. 12.0 software.

## Results and Discussion

Calibration standards prepared in LC–MS water ranged from 10 ng/L to 5000 ng/L, and achieved  $r^2 > 0.99$ , showing good linearity for the studied concentration range. Calculated MDLs were 3.91 ng/L for sucralose, 9.39 ng/L for acetaminophen, and 8.72 ng/L for caffeine by analysis of spiked standards in LC–MS grade water, and 25.29 ng/L, 22.36 ng/L, and 35.05 ng/L in artificial saltwater, respectively. Recoveries of all analytes were determined to be

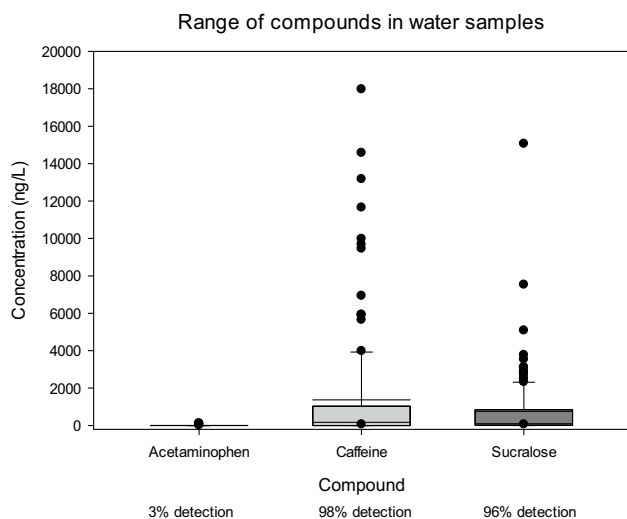
within 30% of the expected analyte concentration for all compounds, with average recoveries of  $110 \pm 0.16$  ng/L,  $91.9 \pm 0.14$  ng/L, and  $119 \pm 0.32$  ng/L for caffeine, acetaminophen, and sucralose respectively. Intraday variation was determined to be less than 7% for all compounds, and interday variation was less than 12% for all compounds. Duplicate samples exhibited variations within 30% of the expected value. Sucralose and acetaminophen demonstrated minimal matrix effect. Caffeine was the only analyte to exhibit a significant matrix effects in artificial saltwater, of up to 200% enhancement, and required dilution to maintain acceptable recovery. The method shows promise for detection of acetaminophen and sucralose at low ng/L levels in saltwater, which would allow the method to be used for further study along the Florida Coast, however the matrix effect of caffeine could prove problematic for detection at similar levels.

Over the course of 2017 and 2018, 181 samples were collected from South and Central Florida and analyzed with a novel online SPE LC-ESI-HRMS method to detect the concentrations of sucralose, caffeine, and acetaminophen in surface water samples. In the environmental samples, caffeine and sucralose were the most commonly detected compounds, present at levels above their respective MDL's in over 70% of samples, while acetaminophen was detected in only 3% of samples analyzed. Figure 2 shows the concentrations of analyte compounds within the sample set.

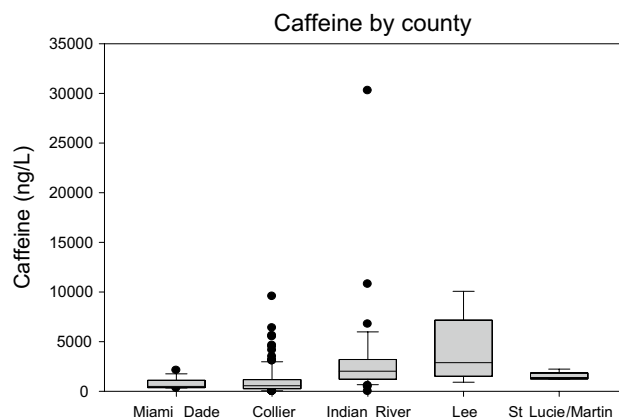
Caffeine ranged from 12.2 to 30,293 ng/L with a median concentration of  $956.9 \pm 2799$  ng/L. 127 of the 180 samples showed caffeine concentrations greater than 400 ng/L, which was previously determined to correlate with fecal coliform contamination. These concentrations are far greater than those seen previously in both wastewater effluent in Greece

and the highest samples collected previously in South Florida by Gardinali et al. in 2002 (6679 and 41.2 ng/L, respectively), as well as those presented by Cantwell et al. (2018) of 2056 ng/L. Eleven samples in our study showed concentrations that were greater than 10% of the  $LC_{50}$  for *C. Daphnia*, suggesting that the levels observed could represent environmental health risks to aquatic organisms. Figure 3 shows the concentrations of caffeine across the studied counties. Indian River County contained the highest single measurement of caffeine recorded; however, Lee County demonstrated the most consistently high levels, with Miami Dade, Collier, and St. Lucie & Martin Counties showing similar average concentrations. The data recorded by this project was also compared to a database maintained by the Florida Department of Environmental Protection (FDEP). The database contained only 16 analyses for caffeine in local South Florida waters between December 2009 and 2019, all of which contained caffeine between 50 and 76 ng/L (FDEP 2019). The difference between the database and the data presented here is likely due to the samples in the FDEP database originating in northern Florida, which has a far lower population density than the studied area.

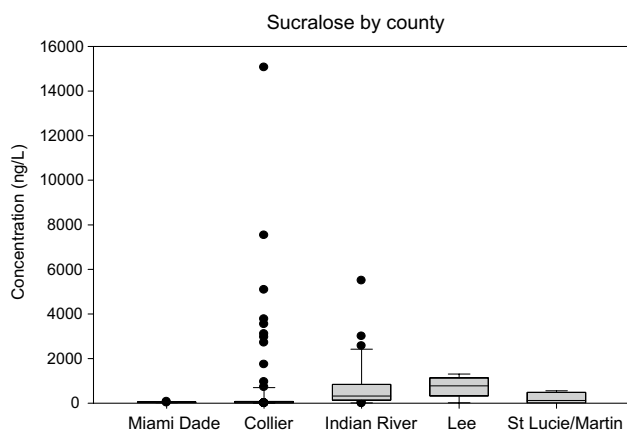
Sucralose analysis yielded a median of concentration of  $44.51 \pm 3079$  ng/L, and a range of 1.95 to 36,666 ng/L. Although it was generally detected at lower levels than caffeine, it was as frequently detected in 94% of samples. Figure 4 shows the distribution of sucralose within each county. Figure 4 does exclude one sample at very high level (36,666 ng/L of sucralose) to better display the rest of the samples. Lee county samples were consistently higher in sucralose than the other counties, however collier contained the three highest levels of 36,666 ng/L, 15,057 ng/L, and 7527 ng/L of sucralose. Indian River, St. Lucie and Martin counties contained similar levels of sucralose. The levels



**Fig. 2** Concentration ranges of the studied compounds in surface water, showing the median, first and third quartile concentrations for each analyte. ( $n = 180$ )



**Fig. 3** Distribution of caffeine within each county showing the median, first and third quartile concentrations for each analyte.  $n_{total} = 180$ ,  $n = 13$  for Miami Dade County, 111 Collier County, 42 Indian River County, 7 Lee County, and 5 St Lucie/Martin County



**Fig. 4** Sucralose distribution by county showing the median, first and third quartile concentrations for each analyte.  $n_{\text{total}}=180$ ,  $n=13$  for Miami Dade County, 111 Collier County, 42 Indian River County, 7 Lee County, and 5 St Lucie/Martin County

in 62 of these samples far exceed the 140 ng/L previously measured in the Florida Keys by Cejas et al. Similar to caffeine, sucralose results were compared to those found in the FDEP database. The database contained 4602 entries for sucralose analyses ranging from nondetectable to 910 ng/L, 2728 (59.7%) of which tested positive for sucralose with a significantly lower detection rate than that shown here (FDEP 2019). Much like caffeine the data in the FDEP database largely originates in the less densely populated northern regions of Florida, possibly accounting for the lower levels.

Acetaminophen was detected only in five samples from Indian River County, with the highest detected level being 378 ng/L. These samples contained both sucralose and caffeine in the ranges of 1824–4533 ng/L and 298–2998 ng/L respectively, indicating that they were highly contaminated. The detected levels are concurrent with those previously reported by Stamatis and Konstantinou in Greece, as well as several other studies which are summarized in Table 1S.

These studies generally show concentrations consistent with those reported here, however with much higher detection rates, although Vulliet and Cren-Olivé (2011) reported that based on location their detection frequency ranged from 15% to 24% with a detection rate of 3.3% in surface and subsurface samples in USA in the range 2.1–12.3 ng/L. Like sucralose and caffeine, acetaminophen data was also compared to entries recorded in the FDEP database. The database stored 2401 analyses of samples for acetaminophen between 2009 and 2019 with only 116 detections (4.7%), which is a detection rate far more comparable to the data discussed here (FDEP 2019). These low detection rates are in direct contrast to values reported for septic system wastewater by James et al. (2016), who reported a detection rate for acetaminophen of 53%. The difference in these detection values likely occur due to a combination of the high removal

efficiency of wastewater treatment plants for acetaminophen which has been reported as being as high as  $100\% \pm 0.2\%$  (Du et al. 2014) and the high lability of acetaminophen in the environment. Supporting this, a recent study of the Hudson River estuary by Cantwell et al. (2018) observed a low detection rate of acetaminophen except in sample sites nearest wastewater treatment plants. Additionally, those detections were generally at extremely low levels ( $< 18$  ng/L), which would dilute rapidly to below detection limits, although the maximum observed level is similar to what is reported here. The combination of these factors demonstrates that acetaminophen is likely a poor tracer for anthropogenic impacts in surface waters, especially in areas where septic systems are not prevalent. These detection frequencies are still far lower than those reported for caffeine and sucralose, therefore, indicating that those are still likely better candidates for human waste tracers in surface waters.

Statistical analysis demonstrates that acetaminophen is not significantly correlated to either analyte due to its extremely low detection rate as demonstrated in Fig. 2S. A spearman rank order test demonstrated a small but statistically significant correlation between caffeine and sucralose (correlation coefficient of 0.188,  $p=0.0118$ ,  $n=180$ ). Caffeine was frequently detected at levels greater than sucralose in most of the samples, indicating to be as ubiquitous as sucralose. This shows that despite its easily degradable nature, caffeine is still an effective tracer of human impacts, especially given its established link to fecal coliform bacteria. It is possible that the 19 samples where sucralose was higher than caffeine were examples of sites where caffeine had already been degraded, therefore suggesting that these locations had not been recently impacted by wastewater intrusion, having an older anthropogenic input. Alternately these sites could exhibit contamination from a different source than the rest of the sites as suggested by Cantwell et al. (2019). Acetaminophen was present only in samples that were already shown to be highly contaminated by caffeine (1824–4533 ng/L) and were also shown to have high levels of sucralose. Based on this method acetaminophen was mostly detected in concentrations below the MDL, which corroborate for its limited environmental applicability, resulting in poor performance as a tracer for aquatic pollution.

A method for the detection of acetaminophen, caffeine, and sucralose by online SPE coupled to LC-ESI-HRMS was successfully developed and validated. Surface water samples ( $n=180$ ) from five counties across South and Central Florida were analyzed using this method. Sucralose was detected at measurable concentrations in 95% of samples, and 69% of samples were determined to have caffeine at levels exceeding 400 ng/L, which have been linked to contamination with fecal coliform (Sauvé et al 2012; Sidhu et al. 2013). Sucralose's high detection rate and correlation with



caffeine show that it is still an effective human impact tracer. The similar prevalence of caffeine and sucralose in surface waters, in addition to the high levels observed and its link to fecal coliform support previous works that demonstrated caffeine's effectiveness as a wastewater intrusion tracer (Potera 2012; Sauv e et al 2012; Stamatis and Konstantinou 2013). Acetaminophen in contrast, demonstrated a detection rate of only 3% in the samples, all of which were shown to also be heavily contaminated by caffeine and sucralose, and 4.7% in a locally maintained database (FDEP 2019). This in combination with its short environmental half-life of as low as one day in soils and groundwater (Kibuye et al. 2019) and the fact that acetaminophen is primarily excreted as sulfate and glucuronide conjugates demonstrates that acetaminophen is likely not a good tracer of human waste impact in surface waters at the current detection limits.

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