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STANDARD OPERATING PROCEDURE
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HIGH-THROUGHPUT, ULTRA-TRACE ANALYSIS OF SUCRALOSE IN MULTI-MATRIX AQUEOUS SAMPLES BY ONLINE SPE HPLC-HIGH RESOLUTION MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

This document provides a procedure for high-throughput, quantitative determination of sucralose in aqueous samples. The method offers fast analysis and low costs due to the limited sample preparation steps, automated processing and minimum usage of consumables. The method is aimed to determine sucralose, a conservative water tracer of exclusive human origin, at trace concentrations (ppt to ppb) in environmentally relevant aqueous matrices. This protocol was developed to be included to typical water quality monitoring exercises that require assessing large number of samples in a relatively short period of time.

2.0 SUMMARY OF METHOD

This procedure is based on the combined performance of an Equan MAX Plus online Solid Phase Extraction (SPE) preconcentration system coupled to a high pressure liquid chromatography system equipped with high resolution mass spectrometry detection using a Q Exactive™ Orbitrap-quadrupole hybrid mass spectrometer (SPE-LC-HRMS). The method uses a heated electrospray ionization source (HESI) interface operating in negative mode to create $[M-H]^-$ ions of sucralose allowing full scan detection of the chlorine isotopic patterns $[M-H+2n]^-$. Samples (10 mL) are shaken, supplemented with the appropriate internal standards and automatically injected, pre-concentrated and cleaned in the online-SPE column before the HPLC-HRMS detection step.

Compound identification was confirmed positive when the following conditions were met: signals (S/N ratios >3 and retention times ±0.01 min of that of sucralose-D6, m/z 401.0449) were present for the three main sucralose anions in the isotopic pattern seen in figure 1 ($[M-H]^-$, m/z 395.0073; $[M-H+2]^-$, m/z 397.0043; $[M-H+4]^-$, m/z 399.0014) using a mass error window of 3 ppm. Additionally, the observed isotopic pattern for sucralose was monitored using the ratios of $[M-H+2]^-$ / $[M-H]^-$ and $[M-H+4]^-$ / $[M-H]^-$ which were required to fall within 10% of their natural abundance ratios.

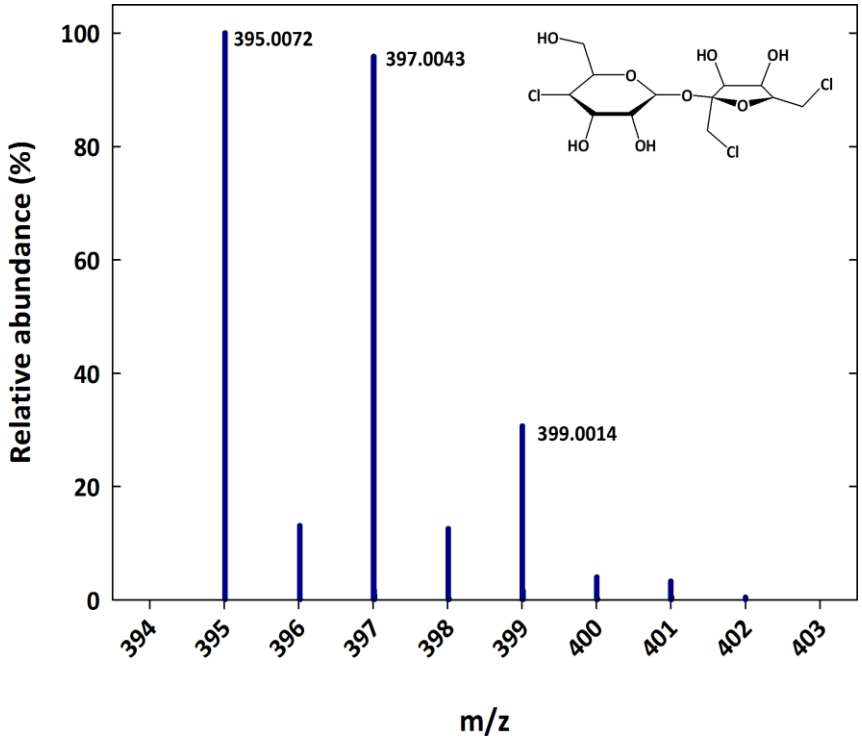

Figure 1. Isotopic pattern of sucralose upon negative mode HESI-HRMS detection[1,3]. Inset: Structure of sucralose.

3.0 REPORTING LIMITS

Method detection limits (MDLs) are determined according to USEPA guidelines, by measuring seven replicates of full strength samples from each matrix (fortified to 10 ng/L) and multiplying the obtained standard deviation by the Student t value ($t_{(7\text{-}1,\ 99)}$ = 3.143).

Method confirmation limits (MCLs) are determined similarly to MDLs but using the least sensitive confirmation signal ($[M\text{-}H+4]^-$,m/z 399.0014) to calibrate and quantitate. A summary of obtained MDLs/MCLs is shown in table 1. The obtained MCL for seawater confirm the ability of this methodology to quantify and confirm sucralose even in complex matrices such as seawaters at ultra-trace levels.

For this analysis, method detection and confirmation limits in seawater are 1.4 ng/L and 5.7 ng/L respectively.

Table 1. Estimated method detection, confirmation limits[a] (ng/L) and recoveries for Sucralose.

Matrix	Spike level	MDL $[M-H]^-$	MCL $[M-H+4]^-$	Average Spike Recoveries (%) (n=7)
DIW	5.0	0.4	0.5	84
Seawater	10.0	1.4	5.7	109
Drinking water	100[b]	11	24	81

[a]All concentrations in ng/L. [b]Reverse MDLs/MCLs (using sucralose-D6 as analyte).

4.0 DYNAMIC RANGE

This method was tested with a calibration range from 0 to 2000 ng/L for 10 mL injections. This range can be modified provided that linearity is maintained and carryover control is demonstrated.

5.0 SAMPLE HOLDING TIME AND PRESERVATION

According to stability studies previously performed[1] Sucralose degradation is almost negligible under most conditions but depends on the type of matrix. There is no evidence that degradation of sucralose while in long-term storage (frozen at or below -20°C for up to 180 days) will exceed the QA/QC requirements of replicate analyses.

For the purpose of this specific SOP field samples should be transported to the laboratory on ice and stored in the dark at 4 °C. Upon arrival, samples should be checked for integrity and stored frozen, at or below -20°C until the time they are prepared for analysis. Given the results of the stability study performed in the laboratory[1] the maximum storage time is set to 180 days. This is defined as the Storage Holding Time. Once samples are scheduled for analysis and brough back to room temperature the analysis should be performed within 30 days of having brought the samples to room temperature (Preparation Holding Time).

6.0 ASSOCIATED SOPs

In some cases, projects require the analysis of the samples immediately upon collection. For those instances, field samples should be transported to the laboratory on ice and stored in the dark at 4 °C and immediately prepared for analysis without freezing. The only relevant holding time for such procedure is the 30-day Preparation Holding Time. These procedures are described in SOP-2025-O-138.

7.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in this EARL standard operating procedure have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Safety Data Sheets (SDS), which contain information regarding the safe handling of chemicals used at EARL's facilities. A reference file of SDS is available to all personnel involved with these materials.

All laboratory personnel should direct any questions regarding safety issues to their supervisors or the office of Environmental Health and Safety (305-348-2621). Personal protective equipment is required at all times.

8.0 INSTRUMENTS, CHEMICAL AND REAGENTS

8.1 Instrumentation

An Ultra High Performance Liquid Chromatography (UPLC) pump system equipped with an analytical HPLC column is coupled with a hybrid quadrupole-Orbitrap Mass Spectrometer (Thermo Q Exactive™), by means of a HESI interface, operated in negative mode.

For online solid phase extraction (SPE) preconcentration, a HPLC pump is used to pass a high volume of sample through a loading column, which is later connected to the main LC-HRMS system for separation and detection.

The system should include an autosampler capable of making large volume injections (equipped with a 10 mL sample loop and a 5 mL glass syringe, or larger). Detection was performed on a Q Exactive™ hybrid quadrupole-orbitrap mass spectrometer, equipped with an Ion Max API source with a heated electrospray (HESI) probe (Thermo Scientific). The analytical system was controlled using the Xcalibur 2.1 data acquisition software (Thermo Scientific).

8.2 LC Columns

A Hypersep Retain PEP® (20 mm × 2.1 mm, 12 µm) is used as the online SPE column. Analytical separations are performed using a Hypersil Gold® column (50 × 2.1 mm, 3 µm), protected by a Hypersil Gold® guard column (10 × 2.1 mm, 3 µm).

8.3 Reagents

The aqueous LC mobile phase consist in LC-MS grade (or better) water. The organic mobile phases consist in LC-MS grade (or better) methanol, ammonium formate and acetonitrile. Ammonium hydroxide (trace metal grade) is also used. The autosampler rinsing solvents are deionized water and Optima grade (or better) methanol.
Artificial saltwater (3.5% w/v) is prepared by dissolving Instant Ocean® (or equivalent product) in deionized water, to attain a concentration of about 30 PSU, with 0.2 µm filtration. If no artificial saltwater is available, natural saltwater (filtered through a 0.2 µm membrane) can be employed as a substitute. Natural saltwater employed for this purpose should be demonstrated to be free of sucralose prior to use.

9.0 STANDARDS

9.1 Standard solutions

Sucralose and sucralose-D6 certified standards are purchased from Accustandard (New Haven, CT, USA). Stock solutions were prepared in acetonitrile and stored at -18 °C until needed. Sucralose and sucralose-D6 aqueous working solutions are prepared each analysis day directly from stock solutions or certified standards.

9.2 Calibration solutions

At least seven calibration (0 to 2000 ng/L) standards should be freshly prepared for each analysis batch.

11 mL calibration solutions containing deionized water, working spiking solution and sucralose-D6 ISTD should be prepared directly into LC vials.

10.0 PROCEDURES

10.1 Sample collection

There is no indication that sucralose is affected by typical environmental processes such as volatilization, biodegradation, photodegradation, hydrolysis, sorption to particulate matter during the typical times required for analysis. However, because sucralose can be introduced by direct or indirect contact with sucralose-containing products during sampling, the general recommendation is to collect samples in new PET bottles without any further treatment. Sampling crews should be instructed to avoid contact with the water sampled and to wear protective equipment during collection. The same precautions used to collect samples for pharmaceutical and personal care products should be observed.

No studies on the effects of disinfectants to sucralose have been conducted. However, special precautions may be taken when collecting samples with free chlorine by adding sodium thiosulfate to a concentration of 50-100 mg/L.

In these cases, field and transit blanks could be used to identify potential interferences or to evaluate method limitations.

10.2 Sample Storage

Samples should be stored at or below 4°C from the time of collection. If needed, samples could be frozen and kept at or below -20°C if extended transit or analysis times are expected.

10.3 Sample Holding Times

Long-term frozen samples have a holding time of 6 months (the Storage Holding Time is defined as 180 days from collection date to sample preparation prior to analysis). Once samples are brought back to room temperature for preparation and analysis, samples must be analyzed within 30 days (this is defined as the Preparation Holding time). Combined, the SHT and PHT should not exceed 210 days. SOP-2025-O-138 describes the method to deal with samples that are never frozen. In that case, the 30-day Preparation Holding Time must be met.

If samples are held beyond any of the accepted holding times, sample results will be qualified with a “Q”.

10.4 Sample treatment

Prior to analysis, refrigerated or frozen samples are allowed to reach room temperature before sample filtration and analysis preparation. This gets recorded as Sample Preparation Time.

Prior to analysis, samples are vigorously shaken for at least 20 seconds and filtered through a PTFE certified filter. Sample aliquots (10 ± 0.1 mL) are transferred directly from the sampling containers into 11 mL LC vials, and sucralose-D6 internal standard is added to obtain a concentration of 140 ng/L. Solutions are thoroughly mixed and loaded into the online SPE system without any further treatment.

10.5 Online SPE-LC-HRMS Procedure

Analysis steps, determined by valve turning events, are graphically presented in figure 1. The samples, quality controls and calibration solutions are loaded onto the 10 mL stainless steel loop (rotary valve A, figure 2).

The SPE column is placed in a second rotary valve (valve B, figure 2), allowing connection with either the loading pump or the analytical pump. At step 1, valve A turns and connects the filled sample loop with the SPE column. 10 mL of sample are passed through the SPE column within 3.33 min, followed by a washing step with deionized water (2.6 mL in 0.87 min) to remove inorganic species. Additionally, during this step the analytical column is taken to initial gradient conditions. Valve B turns at 4.2 min and step 2 begins, connecting the SPE column with the analytical column starting the analyte transfer and elution using a ternary gradient with water, acetonitrile and a pH=9.5 ammonium/ammonia buffer.

At 5 min, valve A turns (step 3) and the sample loop is connected with the injection port. The autosampler sequentially injects 5 mL of methanol and 5 mL of water (to wash the sample loop) and two 5 mL portions of the next sample in the queue, while the chromatographic separation continues.
At 7 min valve B turns again (step 4) and connects the SPE LC pump with the SPE column which is washed using 90% acetonitrile (as a carryover prevention measure) and progressively taken to initial conditions (100% water). Gradient program is shown in table 2.

The system is now ready to start the cycle for the next sample that is being loaded in the sample loop (the loading time extends 5 min beyond the data acquisition time which is ended at 9 min in order to reduce output file size). These steps add to a total run time of 14 min per sample.

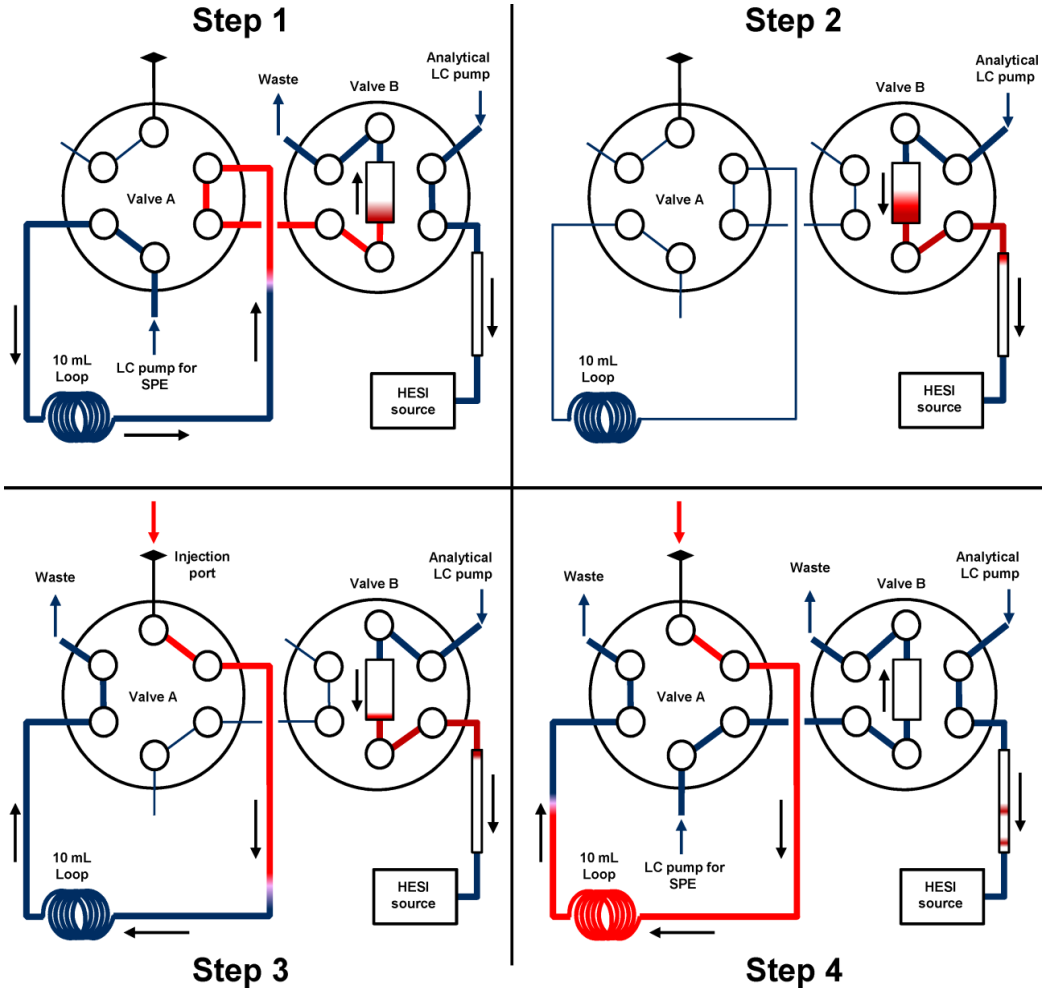

Figure 2. Online SPE system and automated analysis steps. Active flows are shown by arrows and thicker lines. Red: water sample. Blue: mobile phases. (Adapted from Ramirez et al., 2014).

SPE LC pump						Analytical LC pump					
Time (min)	A	B	C	Flow (mL/min)	Segment description	Time (min)	A	B	C	Flow (mL/min)	Segment description
0	100			0.200		0		90	10	0.250	

0.10	100		3.00						
	100		3.00	Solid phase extraction	2.00	80	10	10	0.250	
3.32	100		3.00							Analysis column reset
3.33	100		3.00	SPE column clean-up						
4.00	100		3.00		3.50	80	10	10	0.400	
4.20	100		3.00		4.20	80	10	10	0.400	
4.21		100	1.00	Waste	4.21	80	10	10	0.400	Elution
					5.00		90	10	0.400	
6.50		100	1.00							
7.00	10	90	1.00							
7.01	10	90	1.00	SPE column backwash						
8.00	10	90	2.00							
8.90	100		3.00	SPE column reset						
9.00	100		0.200		9.00		90	10	0.250	
9.01	100		0.200	Wait for AS	9.01		90	10	0.250	Wait for AS
14.0	100		0.200		14.0		90	10	0.250	

Table 2. Solvent delivery programs for both pumps. (Mobile phases are A: water; B: acetonitrile; C: ammonium/ammonia pH=9.5 buffer).

10.5.1 LC Interface parameters

Source housing type:	Thermo Ion Max
Probe Type:	Thermo HESI II
Gas type:	Nitrogen
Sheath gas:	25 arbitrary units
Auxiliary gas:	2 arbitrary units
Ion sweep gas:	0
Vaporizer temperature:	250 ºC
Capillary temperature:	350 ºC

10.5.2 HRMS detection parameters

Overall method settings

Global Settings

Use lock masses	best
Chrom. peak width (FWHM)	15 s
Time	
Method duration	9 min
Experiment	
Full MS — SIM	
General	
Runtime	0 to 9 min
Polarity	Negative
In-source CID	0.0 eV
Full MS — SIM	
Microscans	1
Resolution	140,000
AGC target	1e6
Maximum IT	100 ms
Number of scan ranges	1
Scan range	350-465 m/z
Spectrum data type	Profile
Contact Closure	
General	
Used	False
Start in Closed	True
Switch Count	0

10.6 Water Analysis procedure

10.6.1 Calibration for Water analysis

Calibration curves were obtained by plotting the peak area ratio of the [M-H]- anions of sucralose and sucralose-D6 (m/z 395.0073/401.0449) against concentrations in ng/L. Linearity was observed in the range used (R2 > 0.99; 0 to 2000 ng/L). Calibration stability was evaluated every 10 runs by injecting DI water fortified with sucralose to 100 ng/L. With every analysis batch, a negative (reagent and sampling) and a positive (fortified to 100 ng/L) blanks were also used. Additionally, one sample duplicate and one fortified

matrix sample was always analyzed per every 10 field samples. The system was continuously tested for carryover by injecting a reagent blank after the highest calibration standard and after every calibration verification standard.

A Continuous Calibration Verification (CCV) solution should be injected every 10 or less injections. This CCV solution is prepared from the standard working solution. The measured concentration should not deviate more than 30% from the assigned value. In case of deviation, the instrument should be recalibrated before proceeding with the following injections.
A calibration logbook page should be created every day that the instrument is used. The logbook should contain the volumes used to prepare every solution and the serial numbers of the used standards.

10.6.2 Water analysis calculations

Sucralose measured concentrations in water samples are directly calculated by using the observed sucralose/ISTD peak area ratios and the calibration equation from the linear fit of the plot.

$Y = A*X + B, \quad Y = (Aa/Ais)$

Where:

A	=	Constant. Slope of the linear fit.
B	=	Constant. Intercept of the curve with the Y axis.
X	=	Sucralose concentration in the injection solution (ng/L).
Aa	=	Peak area for the Sucralose quantifier accurate mass.
Ais	=	Peak area for ISTD main accurate mass.

10.7 Data Review

All data generated by the analyst are subjected to supervisory review and authorization. The group leader or the QA/QC officer assess the validity of data generated by assuring that all QA/QC criteria are met by checking the review/ authorization criteria outlined in section 11.

11.0 QUALITY CONTROL

11.1 Sample batch and sequence sizes

A sample batch should contain 20 or fewer samples. One of each QC sample should be prepared and run per sample batch. Multiple sample batches can be analyzed within a single sequence, as long as the CCV keeps passing QC criteria showing instrument stability. The instrument has been shown to be stable for extended run periods by cleaning the ion transfer tube once a day, using abundant deionized water and methanol.

11.2 Laboratory reagent blank (LRB)

The LRB solution preparation is exactly the same as the calibration standard solutions, except no Sucralose is added. It is run in the instrument with every sample batch. An analogue solution is injected after every CCV to check for carryover. An acceptable LRB and carryover test analysis contains no sucralose at concentrations greater than the current MDL. If the LRB does not meet this criterion the analytical system is out of control. The source of the contamination must be documented and the analyst and QAQC officer will decide if the sample batch should be reanalyzed.

11.3 Laboratory Fortified Blank (LFB), Laboratory Fortified Blank Duplicate (LFB-D)

A Laboratory Fortified Blank (LFB) should be used to estimate analytical accuracy of the method. A Laboratory Fortified Blank Duplicate (LFB-D) is used to estimate both analytical accuracy and precision. Quality control samples are fortified with sucralose to 100 ng/L.
The acceptable LFB criteria are 70-130% recovery of sucralose and LFB duplicate analysis should not deviate in more than 30% RPD.
If the LFB recovery criterion is not met in one of both replicates, the LFB will be prepared again and injected in the LC-HRMS. If the re-injected LFB analysis meets the recovery criterion, then the reanalysis data is reported. If LFB recovery criterion fails in either LFB, or the %RPD, the analysis is terminated and the causes of the failure should be investigated and corrected.

11.4 Laboratory Fortified Matrix (LFM)

A Laboratory Fortified Matrix (LFM) is used to estimate analytical accuracy in the presence of a representative matrix. A sample is randomly chosen and fortified with the matrix fortification solution. The acceptable LFM criterion is 70-130% recovery of sucralose. If the LFM recovery criterion is not met, the LFM will be prepared again and injected in the LC-HRMS. If the re-injected LFM analysis meets the criterion, then the reanalysis data is reported. Otherwise, if sucralose is not present in the sample the violation is noted but no action is required. If sucralose is present in the sample in violation, the entire batch of samples is submitted for re-treatment, giving that sufficient sample is still available. If the sample was completely consumed, the data will be reported but designated as outside the QA criteria.

11.5 Duplicate Analysis (DUP)

A sample Duplicate (DUP) is used to demonstrate sample homogeneity and analytical precision in the presence of a representative matrix. A sample should be randomly chosen and split into subsamples, and both subsamples should be analyzed. Duplicate analysis should not deviate in more than 30% from the original sample (<30% RPD), for samples that contain sucralose at a concentration above the current MDL. If the RPD criterion is not met, the duplicate will be prepared again and injected in the LC-HRMS. If the re-injected duplicate analysis meets the criterion, then the reanalysis data is reported. If the re-injected sample still does not meet the RPD criterion, the data will be reported but designated as outside the QA criteria.

11.6 Qualitative analysis

A chromatographic signal will be considered to be produced by sucralose when the following requirements are met:

- The signal is present in both sucralose accurate masses. This signal should have a minimum signal to noise ratio of 10.

- The ratio between accurate masses (%Ratio = $[M-H+i]^-/[M-H]^-$ * 100, where i = (2, 4) should be ±20% the natural abundance ratio.

Sample chromatograms presenting the used analytical signals for the quantification/confirmation of sucralose in a real field sample and its comparison to a calibration standard are presented in figure 3.

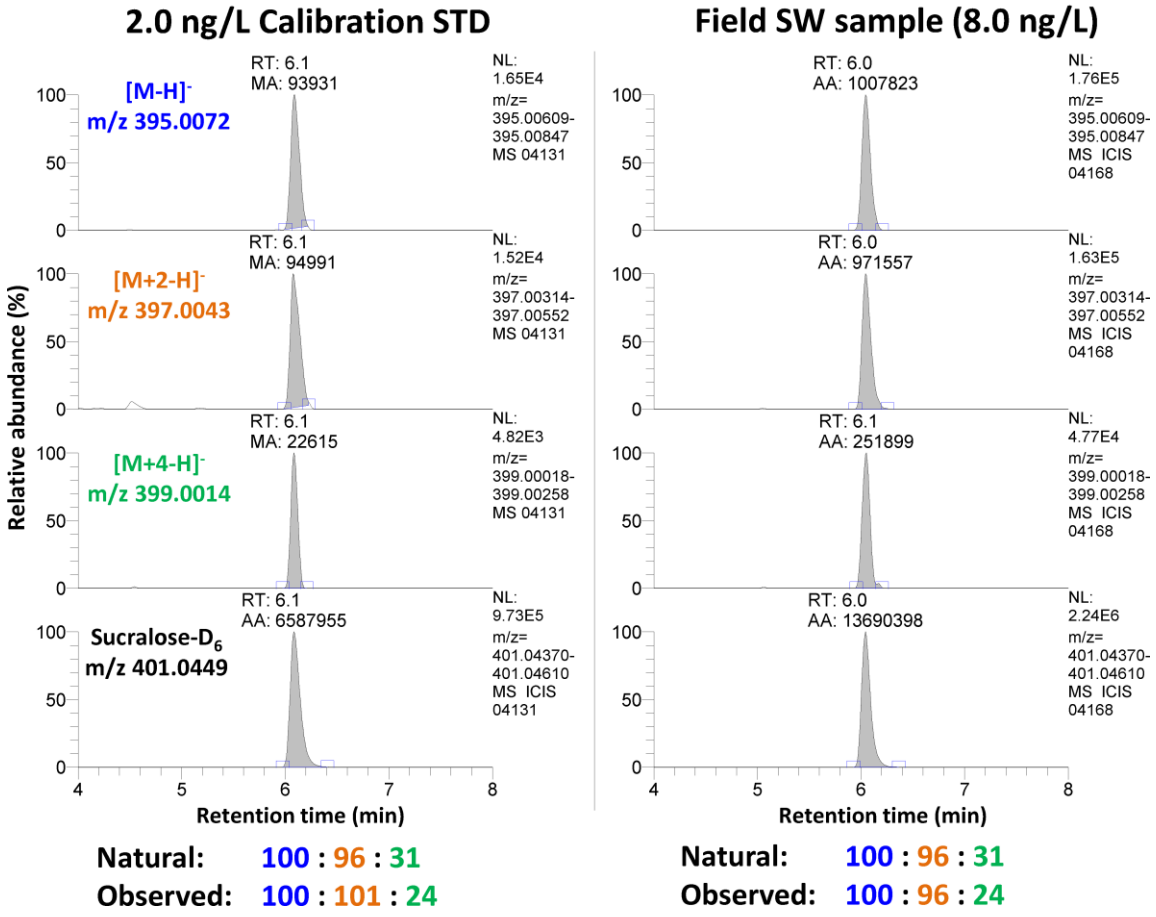

Figure 3. Real seawater chromatograms from a near-shore Atlantic Ocean site and its comparison to a calibration standard. Clean backgrounds and isotopic ratios corresponding to natural abundances are observed in both cases, along with retention time match to sucralose-D6 internal standard.

Summary of QC Requirements for Quantitative Analysis

Control	Control Limit Criteria	Frequency
Instrument Calibration	Minimum of 7 standards; correlation coefficient > 0.99	With each analytical batch
Lab. Reagent Blank	Sucralose background signal (if any) below MDL.	One per QC batch.
Duplicates	RPD ≤ 30% for all analytes when sucralose is present and >MDL.	Minimum one per QC batch.
Lab. Fortified Blank, Lab. Fortified Blank duplicate	% recovery within 70 to 130%. RPD for the spike recoveries should be < 30%.	One per QC batch.
Lab. Fortified Matrix	% recovery within 70 to 130%.	Minimum one per QC batch.

12.0 CORRECTIVE ACTIONS

When data in the report are outside the established QA/QC criteria as shown by the appropriate qualifiers the analyst, the group leader, the lab manager, the QA/QC officer and or the lab director will evaluate the need of corrective actions. All corrective actions are documented in the sample folder checklist and the folder is returned to the analyst with a plan for implementation (i.e re-run, recalibrate instrument, etc)

13.0 POLLUTION PREVENTION

It is EARL's policy to comply with all safety, pollution prevention, and waste management policies dictated by FIU's Department of Environmental Health & Safety as described in their website https://ehs.fiu.edu/safety-programs/laboratory/index.html.

As such, the lab maintains a chemical inventory, segregates chemicals according to their reactivity and minimizes the on-hand inventory of toxic, flammable and or reactive substances.

14.0 WASTE MANAGEMENT

- All stock and working solutions are labeled and kept closed and sealed as appropriate.
- All solutions are to be discarded properly into the designated waste containers.
- All daily waste mobile phase accumulating containers have to be properly labeled (hplc waste/hazardous waste) and emptied at the end of the working day into the main hazardous waste accumulation containers.

15.0 SUPPLEMENTARY NOTES

Appendix 1: STABILITY AND PRESERVATION OF SUCRALOSE IN WATER SAMPLES

16.0 REFERENCES

(1) Batchu, S. R.; Quinete, N.; Panditi, V. R.; Gardinali, P. R. Chem. Cent. J. 2013, 7.

(2) Loos, R.; Gawlik, B. M.; Boettcher, K.; Locoro, G.; Contini, S.; Bidoglio, G. J. Chromatogr. A 2009, 1216, 1126–1131.

(3) Ferrer, I.; Zweigenbaum, J. a; Thurman, E. M. Anal. Chem. 2013, 85, 9581–9587.

(4) Ramirez, C. E.; Wang, C.; Gardinali, P. R. Anal. Bioanal. Chem. 2014, 406, 329–344.

(5) USEPA. CFR 40 title 40 part 163 Appendix B: Definition and Procedure for the Determination of the Method Detection Limit; 2010; pp. 353–356.

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Appendix 1

STABILITY AND PRESERVATION OF SUCRALOSE IN WATER SAMPLES

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September 2024

Introduction

Sucralose, a widely used artificial sweetener, is increasingly being detected in various aquatic environments due to its extensive consumption and resistance to degradation. Sucralose is exclusively consumed by humans thus its presence in environmental samples is linked to wastewater intrusions (treated or untreated). Accurate analysis of sucralose in environmental water samples is crucial for monitoring its presence as an effective wastewater indicator and understanding its transport in natural ecosystems. However, the stability of sucralose during sample storage and the effects of different preservation conditions on its integrity over time remain unknown but important factors that can influence analytical results.

Here we present a stability study designed to evaluate the effects of long-term storage and subsequent handling conditions on the quantification of sucralose in environmental surface water samples. Specifically, the study focuses on determining the stability of sucralose under frozen storage conditions and assessing the acceptable holding times after thawing, to ensure reliable analytical outcomes.

Objectives

The main objectives of the sucralose preservation study were to:

- Evaluate the long-term stability of sucralose in natural water samples that have been stored frozen for over 3 years.
- Determine acceptable holding times for thawed water samples by analyzing them at regular intervals over a period of 3 months.
- Compare the concentrations of sucralose obtained from the initial analysis (conducted 3.5 years ago) with those obtained after thawing and storing the samples at 4°C, to assess any degradation or loss of analyte.

Methods and Quality Assurance

Four water samples (3 brackish water and 1 freshwater) previously analyzed for sucralose content 3.5 years ago, with concentrations ranging between 521 and 1495 ng/L, were selected for this study. These samples were collected on 11/12/2020 in PET bottles and kept stored unfiltered and without any preservatives in a freezer at temperatures below -20°C since their initial analysis on 12/17/2020.

In May 2024, the frozen samples were thawed at room temperature and stored in a refrigerator at 4°C. Sucralose concentrations were measured immediately after thawing to establish a baseline. The samples were then reanalyzed at specific intervals (28, 55 and 78 days) over a 3-month period.

The samples were filtered and analyzed using online solid phase extraction high-performance liquid chromatography coupled to a Q-Exactive Orbitrap high resolution mass spectrometer (SPE-LC-HRMS), based on the method developed by Batchu et al. (2015). Quantification is based on an 8-point calibration curve (0-2000 ng/L) of the sucralose standard (99%, Fisher Scientific), and the addition of the internal standard sucralose-d6 (98.5%, LGC).

The analyses followed the protocol and quality control described in EARL SOP-2014-O-130.2, ensuring consistency with the initial analysis performed in 2020. Laboratory reagent blanks (LRB), laboratory fortified blanks (LFB), laboratory fortified matrix (LFM), LFB duplicates, and sample duplicates were included once for every batch. Additionally, a blank (analogue to the LRB) and a continuing calibration verification (CCV, analogue to the LFB) were injected after every 6 samples to check for instrument carryover and analytical accuracy/precision, respectively. An acceptable LRB or blank contains no compounds at concentrations greater than the current MDL (11.0 ng/L). The acceptable LFB and CCV criteria are 70-130% recovery of all the analyzed compounds and the duplicates should not deviate in more than 30% RPD.

Results

The sucralose results for the stability test are presented in Table 1. The samples were initially analyzed in December 2020, and then again at four subsequent time points after being thawed and stored at 4°C, with analyses conducted in May, June, and July 2024. Table 2 shows the statistical analyses of the experiment.

Table 1: Results obtained for the sucralose preservation test (RPD = Relative Percent Difference).

FIU ID	Sample Name	Sucralose (ng/L)	Analysis Date	Analysis Holding Time (Days)	RPD (%) to 2020
20-00992	CG8-Surface	1175	12/17/2020	35	-
20-00992	CG8-Surface (rep 1)	1311	05/01/2024	1266	11.6
20-00992	CG8-Surface (rep 2)	1477	05/29/2024	1294	25.7
20-00992	CG8-Surface (rep 3)	1590	06/25/2024	1321	35.3
20-00992	CG8-Surface (rep 4)	1473	07/18/2024	1344	25.4
20-00999	CG9-Surface	521	12/17/2020	35	-
20-00999	CG9-Surface (rep 1)	542	05/01/2024	1266	4.11
20-00999	CG9-Surface (rep 2)	601	05/29/2024	1294	15.3
20-00999	CG9-Surface (rep 3)	520	06/25/2024	1321	-0.288
20-00999	CG9-Surface (rep 4)	445	07/18/2024	1344	-14.6
20-01001	CG10-Surface	1365	12/17/2020	35	-

20-01001	CG10-Surface (rep 1)	1519	05/01/2024	1266	11.3
20-01001	CG10-Surface (rep 2)	1549	05/29/2024	1294	13.5
20-01001	CG10-Surface (rep 3)	1661	06/25/2024	1321	21.7
20-01001	CG10-Surface (rep 4)	1628	07/18/2024	1344	19.3
20-01003	CG11-Surface	1495	12/17/2020	35	-
20-01003	CG11-Surface (rep 1)	1307	05/01/2024	1266	-12.6
20-01003	CG11-Surface (rep 2)	1418	05/29/2024	1294	-5.12
20-01003	CG11-Surface (rep 3)	1557	06/25/2024	1321	4.12
20-01003	CG11-Surface (rep 4)	1494	07/18/2024	1344	-0.0578

Table 2: Basic statistical analysis of sucralose preservation study.

Sample name	Average (ng/L)	STD (ng/L)	RSD (%)	Average Absolute RPD (%) to 2020
All Runs (December 2020 - July 2024)				
CG8-Surface	1405	162	9.25	24.5
CG9-Surface	526	55.9	8.51	8.58
CG10-Surface	1544	116	5.99	16.4
CG11-Surface	1454	95.7	5.26	5.47
Current Runs (May - July 2024)				
CG8-Surface	1463	114	7.82	-
CG9-Surface	527	64.5	12.2	-
CG10-Surface	1589	66.3	4.17	-
CG11-Surface	1444	107	7.43	-

Sucralose concentrations where slightly higher for samples CG8 and CG10 in 2024 when compared to the initial 2020 values, with average RPDs of 24.5 % and 16.4 %, respectively. For samples CG9 and CG11, concentrations fluctuated between higher and lower values, showing low average RPDs of 8.58 % and 5.47 %, respectively. The only RPD found higher than 30% was for the June 2024 analysis of sample CG8, where concentration was 1590 ng/L against the initial concentration of 1175 ng/L (RPD: 35.3%). The lowest RPD was found for sample CG11, with the final concentration in July 2024 (1495 ng/L) being almost identical to the initial concentration (1494 ng/L, RPD: 0.0578%). Overall, the concentrations remained relatively consistent over the testing period.

Conclusions

The sucralose concentrations from each monthly analysis were compared with the initial concentrations to assess the long-term stability of sucralose (during storage) and to determine the acceptable holding times for thawed water samples (sample preparation step). The sucralose concentrations in the samples generally showed good stability over the 3-month period, with RPD values indicating minimal degradation or loss of analyte when compared to the initial 2020

analysis (could be consider replicates based on the SOP). CG8 and CG10 exhibited an increase in sucralose concentrations over time, possibly due to analytical variability, volume changes during storage or matrix effects. Sample CG11 demonstrated the most consistent results, with minimal variation between the initial and subsequent analyses. These results suggest that sucralose remains relatively stable in frozen water samples for 3.5 years, and acceptable holding times for thawed samples can extend up to 3 months with minimal analyte loss.

References

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