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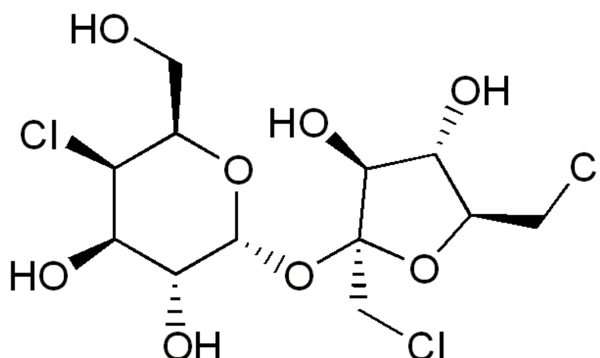
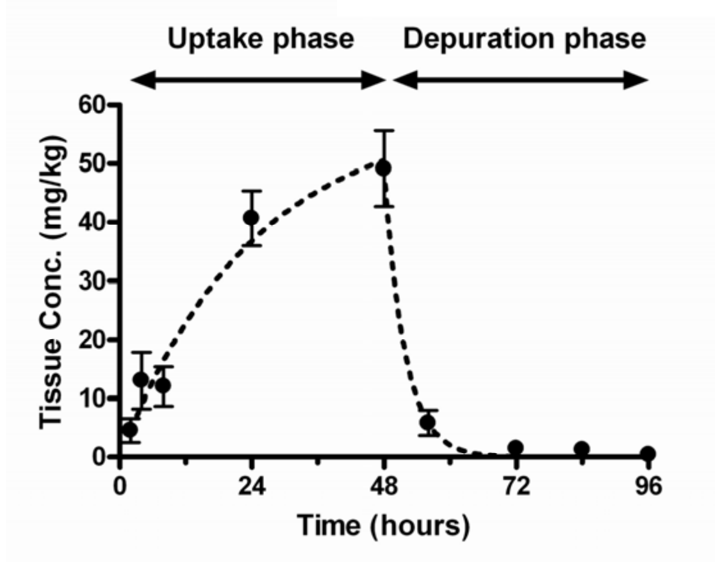
REPORT SNO 5865-2009

# Determination of the accumulation and elimination of sucralose in the zebrafish, *Danio rerio*



Norwegian Institute for Water Research
– an institute in the Environmental Research Alliance of Norway

# REPORT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Abstract

The bioaccumulation and subsequent elimination of sucralose in zebrafish (*Danio rerio*) was determined using a 48 hour semi static exposure system followed by 48 hours flow through of clean water for the depuration phase. Two exposure concentrations (10 and 100 mg/L) plus an appropriate control were used for the study. Fish were analysed on 5 occasions during the uptake phase and 4 times during the depuration phase using liquid chromatography – mass spectrometry (LC/MS). A steady state concentration in the fish was assumed after 24-48 hours and the bioconcentration factor (BCF) at steady state ($BCF_{ss}$) was calculated to be <1 for both concentrations tested. Furthermore, a kinetic BCF was calculated from the uptake rate constants and depuration rate constants to be <1 for both concentrations tested. This indicates that sucralose does not accumulate significantly in the tissues of zebrafish and the BCFss was considerably lower than the criteria set to identify persistent, bioaccumulative and toxic (PBT) substances (i.e. $\geq$ 2000).



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# Determination of the bioaccumulation and elimination of sucralose in the zebrafish, *Danio rerio*

# Preface

The study has been conducted by staff from the Section for Ecotoxicology and Risk Assessment, Norwegian Institute for Water Research (NIVA) in collaboration with the Aleström zebrafish lab, the Norwegian School of Veterinary Sciences. The authors acknowledge the contribution by Jan Roger Torp and Peter Aleström for supplying the zebrafish and Tor Fredrik Holth for support during the study.

Oslo, 04.11.2009

*Knut Erik Tollefsen*

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# 1 Abstract

The bioaccumulation and subsequent elimination of sucralose in zebrafish (*Danio rerio*) was determined using a 48 hour semi static exposure system followed by 48 hours flow through of clean water for the depuration phase. Two exposure concentrations (10 and 100 mg/L) plus an appropriate control were used for the study. Fish were analysed on 5 occasions during the uptake phase and 4 times during the depuration phase using liquid chromatography – mass spectrometry (LC/MS). A steady state concentration in the fish was assumed after 24-48 hours and the bioconcentration factor (BCF) at steady state ($BCF_{ss}$) was calculated to be <1 for both concentrations tested. Furthermore, a kinetic BCF was calculated from the uptake rate constants and depuration rate constants to be <1 for both concentrations tested. This indicates that sucralose does not accumulate significantly in the tissues of zebrafish and the $BCF_{ss}$ was considerably lower than the criteria set to identify persistent, bioaccumulative and toxic (PBT) substances (i.e. $\geq$ 2000).

# 2 Introduction

A zebrafish (*Danio rerio*) bioaccumulation study on sucralose was carried out at the Norwegian School of veterinary Sciences, Oslo; Norway at the request of Tate and Lyle (2200 East Eldorado, Decatur, 62521, Illinois, USA). The test was performed according to OECD test guideline 305. All chemical analyses was performed at NIVA Gaustadalléen 21, NO-0349, Oslo; Norway. The study number for the study was 28433-4 and the exposure dates were 20 April to 24 April 2009.

# 3 Materials and methods

## 3.1 Test substance

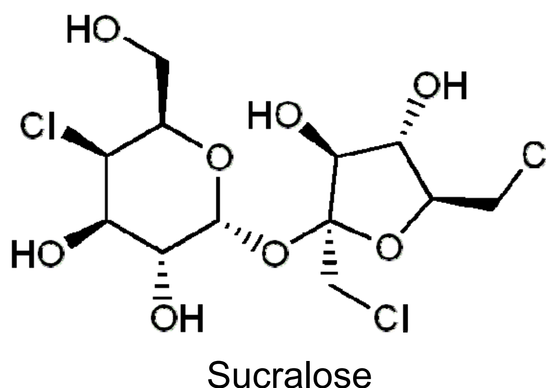
The test substance Sucralose (batch no. MM8G190A6M, manufactured date 07/2008) was supplied by Tate and Lyle (2200 East Eldorado, Decatur, 62521, Illinois, USA).

Chemical Name:

1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside

CAS RN: 56038-13-2

Molecular structure:



Sucralose

Octanol water partition coefficient: 0.32 (at 20C), log $P_{ow}$ = -0.51

The test substance was supplied as a white powder and was stored at ambient temperature in the container in which it was received until required for testing. A certificate of analysis supplied by the customer stated a purity of 100.4 ± 2 %.

## 3.2 Test organism

The test organism used for the test was the zebrafish (*Danio rerio*) and were obtained from the Norwegian School of Veterinary Sciences, Oslo; Norway where they were bred and held in stock tanks until the study commenced. The fish used for the study were approximately 10 months old and there had been no evidence of disease in the fish prior to the start of study. In addition, there were no mortalities observed in the 7 days prior to the start of the test. The fish used were Aleström Zebrafish lab strain (AZL wt) and only male fish were used for the experiment.

## 3.3 Dilution water

The dilution water used for the study was dechlorinated tap water. Salts were added as required to maintain a minimum hardness level and the temperature of the water was adjusted to approximately 25°C prior to supplying in the test laboratory as detailed in the appendices. The dilution water used for the study was analysed for metals and organic compounds as detailed in Table 3 of the appendices.

## 3.4 Test conditions and procedure

The test was performed at a temperature of 25ºC using a semi-static renewal system for the 48 hour uptake phase and a flow-through system for the 48 hour depuration phase. In both cases the test water was aerated continuously throughout the experiment. The photoperiod used for the study was 16:8 light:dark cycle. The test vessels used for the study were constructed from polyethylene (Exoterra, Malaysia) and had a working volume of 10L with loose fitting lids. Two replicate test vessels were used for each concentration to avoid over loading of fish, each tank containing the same number of fish. The loading of the fish (based on the weights of the fish analysed at 0hrs) at the start of the test was approximately 0.7 g of fish/L. During the depuration phase, the flow of dilution water into the tanks was calibrated by direct measurement of the flow using a 100 ml measuring cylinder. The flow rate was sufficient for approximately 5 tank replacements per day.

The test was initiated by the addition of 19 fish to each replicate vessel. Two exposure concentrations of sucralose 10 mg/L and 100 mg/L were used, in addition to a control group and 2 replicate test vessels were used per treatment (A and B). Both fish and water were sampled simultaneously for sucralose quantification although the water was only analysed at 0 and 24 hours (representing "on" and "off") and following replacement at 24 and 48 hours (representing "on" and "off"). Water was analysed once during the depuration phase to confirm absence of test material in the test system.

Fish were fed an appropriate commercially available diet (SDS 400, Special Diet Services, UK) *ad libitum* once a day and any excess food was removed from the test vessels within half an hour of feeding.

## 3.5 Physical parameters

Dissolved oxygen, pH and temperature were measured on the "on" and "off" solutions during the uptake phase. During the depuration phase, dissolved oxygen, pH and temperature was measured daily. The temperature was monitored continuously in one test tank throughout the study. In addition, samples of dilution water were taken for water quality analysis as detailed in the appendix.

## 3.6 Preparation of test solutions

The test solutions were prepared on the day of use, by direct addition of an appropriate amount of sucralose to each test vessel, dissolved in 10 L dilution water. After 24 hours exposure, fresh solutions were prepared in the test vessels, whilst the fish were temporarily held in a holding vessel. Following a further 24 hours exposure, the remaining fish were transferred to clean test vessels, containing only dilution water set up with a flow through system, for initiation of the depuration phase.

## 3.7 Analysis of test substance in water

Approximately 100 ml water samples were taken in pre-cleaned 200 ml poly propylene bottles at the same time as the fish samples. Once sampled the water bottle was frozen on dry ice and remained frozen until analysed.

Phenyl β-D-glucopyranoside (1 mg) was added to each sample as an internal standard. With each batch of water extracted, a blank and 2 spiked controls (10 mg and 100 mg sucralose) was prepared in ultrapure water and extracted for quality control purposes. The pH of each sample was adjusted to pH 3 with the addition of formic acid.

Solid phase extraction (SPE) using Oasis HLB (200 mg, 6 ml) was used to concentrate the samples. The cartridges were conditioned with 6 ml methanol followed by 6 ml ultrapure water and finally with 6 ml ultrapure water (acidified to pH3 with the addition of formic acid). The solid phase was not allowed to dry between solvent additions. After cartridge conditioning, the samples were applied under vacuum at a rate of approximately 2 ml/min and allowed to dry for 30 minutes under vacuum before sample elution.

Sucralose and phenyl β-D-glucopyranoside were eluted from the cartridge with 12 ml methanol/acetone (1:9) and the eluant evaporated under nitrogen to approximately 500 µl. The extract was diluted to approx 10 ml with ultrapure water in preparation for analysis by LC/MS. Method recoveries were 68±9%.

## 3.8 Analysis of test substance in fish

Fish were caught with a hand-held net and rinsed briefly with approximately 200 ml of system water and then anaesthetised for approximately 5 minutes using a 500 mg/L solution of MS222. Two fish were taken from each replicate test vessel at each time point. The fish were dried briefly on a paper towel before wet weight and standard length of each fish was measured. The fish were then killed humanely ensuring destruction of the brain, using a sharp instrument. The fish were placed individually in suitably sized bags and immediately frozen with dry ice. A minimum of 38 fish was used for each exposure concentration (19 per replicate test vessel). Four fish from each concentration were sampled at the following durations:

| | | |
|---|---|---|
| Uptake phase: | 2, 4, 8, 24, and 48 h. | Semi-static in duplicate 10L tank with aeration. |
| Depuration phase: | 8, 24, 32 and 48 h | Flow-through system in duplicate 10L tank with aeration. |

An additional 6 fish were sampled from the stock population at 0h and were retained for baseline analysis of sucralose.

The fish from each replicate were pooled and then homogenised. 1 µg phenyl β-D-glucopyranoside was added to each sample as an internal standard. With each batch of fish extracted, a blank and a spiked control (1 µg sucralose) was also extracted for quality control purposes.

The samples, blanks and control were double solvent extracted by sonication at 60 °C for 30 minutes. The first extraction was with 5 ml methanol (0.1% formic acid), followed by extraction with 5 ml methanol/acetone (1:9). The 2 extracts were combined and the acetone evaporated under nitrogen. The remaining methanol was diluted in ultrapure water to 150 ml in preparation for cleanup.

The extract cleanup followed the same extraction protocol as the water extractions using Oasis HLB extraction cartridges. For the elution of sucralose and phenyl β-D-glucopyranoside, a mixed mode (Isolute, M-M, 100 mg, 3 ml) SPE cartridge was added in series for additional sample cleanup. Methanol/acetone (1:9) (12 ml) was passed through both cartridges and the eluant evaporated under nitrogen to approximately 500 µl. The extract was diluted to approx 1 ml with ultrapure water in preparation for analysis by LC/MS. Extraction recoveries were 114±8%.

## 3.9 LC/MS analysis

Liquid chromatography – mass spectrometry (LC/MS) analysis was used for quantification using a Waters Aquity UPLC coupled to a Waters Quattro Premier XE triple quadruple mass spectrometer. Analytes were separated on Waters Aquity BEH C18 1.7 µm column (2.1 x 50 mm) using water and methanol as mobile phases with a flow rate of 0.5 ml/min and a gradient elution program (Table 1).

The mass spectrometer was operated in negative ionisation mode with a source temperature of 120 °C, a cone voltage of 25 V and a gas flow of 50 L/hr. Single ion monitoring of *m/z* 395.3, 397.3 and 399.3 was used for qualification and quantification of sucralose and *m/z* 160.8 and 255.2 for the quantification of phenyl β-D-glucopyranoside.

**Table 1. Gradient elution program for the determination of sucralose.**

| Time (mins) | Water | Methanol |
|---|---|---|
| Initial | 95 | 5 |
| 2 | 95 | 5 |
| 8 | 0 | 100 |
| 10 | 0 | 100 |
| 11 | 95 | 5 |
| 13 | 95 | 5 |

## 3.10 Calculation of bioconcentration factors

The bioconcentration factor (BCF) of each of the two concentrations (10 and 100 mg/L) were determined on the basis of equilibrium (Steady state, SS) water and biota concentrations:

$$\mathbf{BCF_{SS}=C_{fish}/C_{water}} \quad (1)$$

Where $BCF_{SS}$ is the bioconcentration factor at equilibrium (steady state), $C_{fish}$ is measured concentration of sucralose in fish and $C_{water}$ is the concentration in the water phase.

The kinetic bioconcentration factor ($BCF_{(kinetic)}$) was determined on the basis of uptake kinetics and depuration kinetics according to the following equation:

$$\mathbf{BCF_{(kinetic)}=k_1/k_2} \qquad (2)$$

Where $BCF_{(kinetic)}$ is the kinetic bioconcentration factor, $k_1$ is the uptake rate constant and $k_2$ the depuration constant.

Uptake and depuration constants were calculated on basis of non-linear regression to experimental data using the following equations:

$$\textbf{Uptake phase: } C_{fish}=C_{water}\,(k_1/k_2)(1-e^{-k2t}) \qquad (3)$$

Where *t* is the time from the start of the exposure period,

$$\textbf{Depuration phase: } C_{fish}=C_{water}\,(k_1/k_2)(e^{-k2(t-td)}-e^{-k2t}) \qquad (4)$$

Where t*d* is the time after start of the depuration phase.

## 3.11 Raw data

All original data and a copy of the final report will be contained securely for a period of 5 years at NIVA. After this period, the sponsor's instructions will be sought.

# 4 Results

## 4.1 Physical parameters

The physical parameters and water quality data for the study are shown in Tables 2 and 3 of the appendices respectively. In summary, the pH of the study ranged between 7.3 and 7.6, the dissolved oxygen ranged between 6.6 and 7.6 mg/L and the temperature ranged between 23.0 and 27.0°C. The water quality was within the range expected and none of the analytes present would have affected the outcome of the study.

## 4.2 Biological data

The lengths and weights of the fish measured during the study are shown in Table 4 of the appendices. The fish were within the normal range expected for this study.

## 4.3 Chemical data

### 4.3.1 Analysis of sucralose in the test solutions

The analytical chemistry data for the concentration of sucralose in the test solutions are shown in Table 5 of the appendices. In addition, a typical chromatogram for the analysis of sucralose in the test solutions are also shown in the appendices. The limit of detection for the study was 15 ng/L. A 5 point calibration curve ($R^2$ value of >0.99) was determined for each batch of samples analysed. A typical calibration curve is included in the appendices.

The concentration of sucralose did not vary significantly between renewals of the test solutions. However, on one occasion the concentration of sucralose in the water sampled from the ON solution (24h B) was significantly lower than the nominal concentration. This value was not included in the calculation of the mean data as the OFF value from the same replicate indicated that the concentration of sucralose was actually nominal. This suggests that there was possibly an error during the sampling of the test solutions. With this taken into consideration, the calculation of the bioconcentration factors were based on mean measured concentrations.

The analysis of the water from the depuration tanks revealed that the concentration of sucralose was below the limit of detection for the 10 mg/L concentration and was measured to be 0.4 and 0.2 mg/L (A and B replicate tanks respectively) in the 100 mg/L test concentration. The presence of sucralose in the 100 mg/L depuration tanks was possibly either due to the elimination of sucralose from the fish into the water or carry over/contamination during the analysis.

## 4.3.2 Analysis of sucralose in the fish

The analytical chemistry data for the concentration of sucralose in the fish and the corresponding BCF data are shown in Table 6 of the appendices. In addition, a typical chromatogram for the analysis of sucralose in the test solutions are also shown in the appendices. The limit of detection for the analysis of sucralose in the fish was 7 ng/g. The mean concentration of sucralose measured in the fish for the duration of the study are presented in Figures 1 and 2.

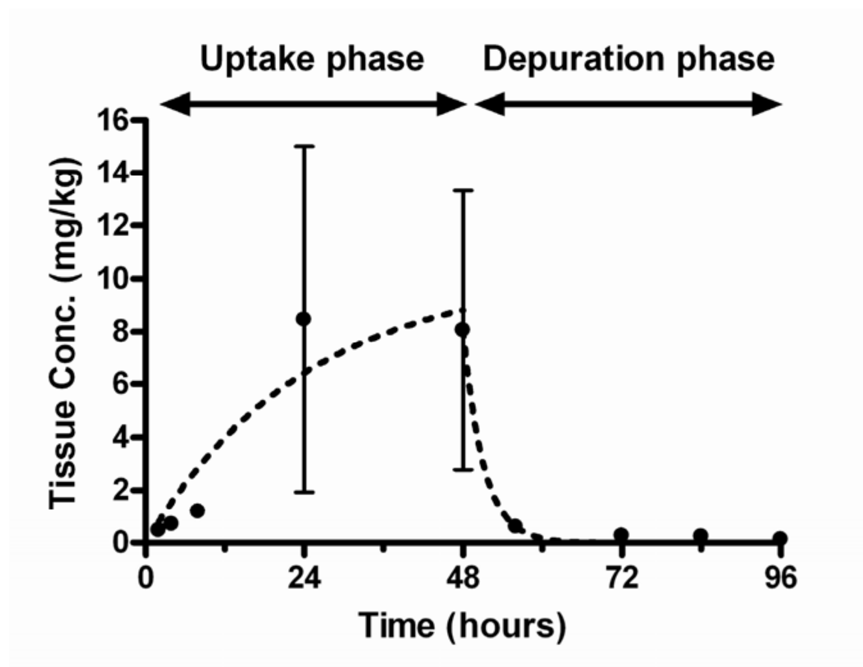



**Figure 1. Mean measured (±SD) concentrations of sucralose in fish exposed to 10 mg/L sucralose for 48 hours followed by a 48h depuration phase in clean water.**

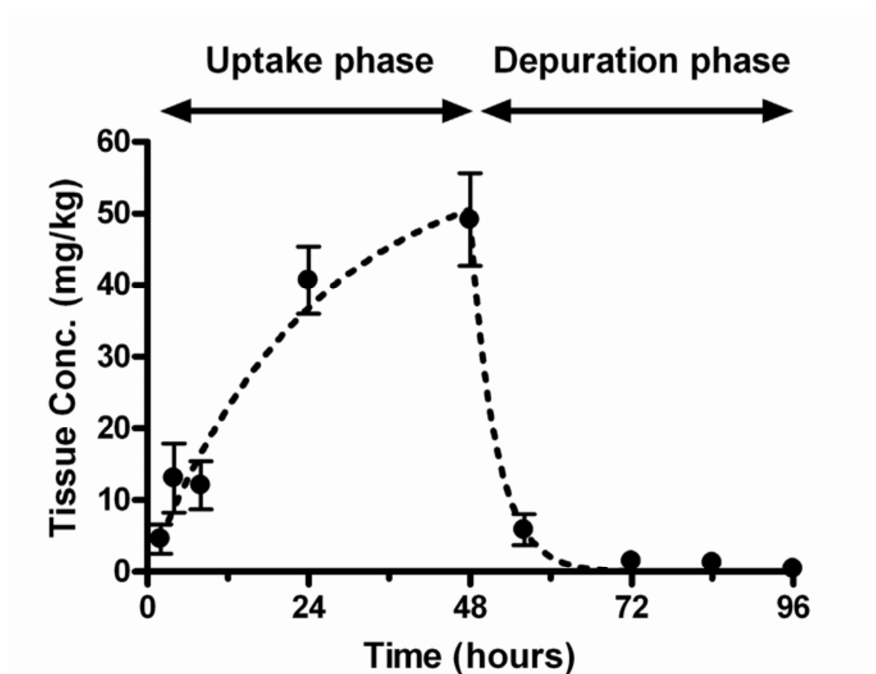



**Figure 2. Mean measured (±SD) concentrations of sucralose in fish exposed to 100 mg/L sucralose for 48 hours followed by a 48h depuration phase in clean water.**

## 4.4 Bioconcentration factors

It was considered that the steady state concentration in the fish had been reached within 24-48 hours exposure. Therefore the BCF at steady ($BCF_{ss}$) was calculated as the mean of the 24 and 48 hours exposure data. For the lower concentration (10 mg/L) the $BCF_{ss}$ was 0.72 and at the higher concentration the $BCF_{SS}$ was 0.47. Table 6 details the bioconcentration factors determined at the different sampling times.

## 4.5 Kinetic bioconcentration factors

Using the formulas detailed in section 2.10, the $BCF_{(kinetic)}$ for the 10 and 100 mg/L test concentrations were both calculated to be 0.46 and 0.35 respectively (10 mg/L $k_1$ = 0.1494 and $k_2$= 0.3228, 100 mg/L $k_1$ = 0.0196 and $k_2$ = 0.2641).

# 5 Conclusions

The BCF values determined for the uptake of sucralose in this study were <1 for both concentrations tested. This indicates that sucralose does not accumulate significantly in the tissues of zebrafish and the $BCF_{ss}$ was considerably lower than the criteria set to identify persistent, bioaccumulative and toxic (PBT) substances(i.e. BCF≥ 2000).

# 6 References

OECD (1996). OECD Guidelines for Testing of Chemicals 305: Bioconcentration, Flow through fish test. Organization for Economic Cooperation and Development (OECD), Paris.

# Appendices

**Table 2. Physical parameters of the test solutions**

| Exposure time | Nominal Conc. (mg/L) | ON solution | | | | | | OFF solution | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dissolved oxygen (mg/L) | | pH | | Temp (°C) | | Dissolved oxygen (mg/L) | | pH | | Temp (°C) | |
| | | A | B | A | B | A | B | A | B | A | B | A | B |
| 0-24 hrs | DWC | 7.6 | 7.6 | 7.6 | 7.6 | 27.0 | 27.0 | 6.8 | 7.2 | 7.4 | 7.5 | 23.1 | 23.3 |
| | 10 | 7.5 | 7.5 | 7.6 | 7.6 | 26.2 | 26.6 | 6.6 | 7.1 | 7.4 | 7.5 | 23.0 | 23.0 |
| | 100 | 7.6 | 7.6 | 7.6 | 7.6 | 26.2 | 26.4 | 7.1 | 7.1 | 7.6 | 7.5 | 23.3 | 23.1 |
| 24-48 hrs | DWC | 7.2 | 7.1 | 7.5 | 7.5 | 24.4 | 24.4 | 6.9 | 7.0 | 7.5 | 7.5 | 23.7 | 23.5 |
| | 10 | 7.2 | 7.2 | 7.6 | 7.5 | 24.3 | 24.3 | 6.6 | 7.1 | 7.3 | 7.5 | 23.5 | 23.4 |
| | 100 | 7.2 | 7.2 | 7.5 | 7.5 | 24.5 | 24.4 | 6.9 | 7.0 | 7.5 | 7.5 | 23.6 | 23.5 |
| 48 hrs flow through | DWC | 7.0 | 7.1 | 7.4 | 7.4 | 24.6 | 24.5 | - | - | - | - | - | - |
| | 10 | 7.1 | 7.2 | 7.4 | 7.4 | 24.4 | 24.3 | - | - | - | - | - | - |
| | 100 | 7.0 | 7.1 | 7.4 | 7.4 | 24.4 | 24.5 | - | - | - | - | - | - |
| 72 hrs flow through | DWC | 6.9 | 6.8 | 7.5 | 7.5 | 24.5 | 24.5 | - | - | - | - | - | - |
| | 10 | 6.7 | 6.9 | 7.5 | 7.6 | 24.4 | 24.2 | - | - | - | - | - | - |
| | 100 | 6.7 | 6.7 | 7.6 | 7.6 | 24.3 | 24.2 | - | - | - | - | - | - |
| 96 hrs flow through | DWC | 6.9 | 7.0 | 7.6 | 7.6 | 24.0 | 24.2 | - | - | - | - | - | - |
| | 10 | 6.9 | 6.9 | 7.6 | 7.6 | 24.0 | 24.0 | - | - | - | - | - | - |
| | 100 | 6.8 | 7.0 | 7.6 | 7.6 | 24.0 | 24.0 | - | - | - | - | - | - |

**Table 3. Water quality analysis**

| Parameter | Unit | Detection limit | Dilution water used for uptake phase |
|---|---|---|---|
| pH | - | - | 7.8 |
| Conductivity | mS/m | 0.05 | 43.3 |
| Alkalinity | mmol/L | 0.01 | 0.692 |
| Nitrogen | µg/L | 10 | 40 |
| Nitrate | µg/L | 1 | 3 |
| DOC | mg/L | 0.1 | 0.11 |
| Chlorine | mg/L | 0.03 | 100 |
| Sulphate | mg/L | 0.04 | 9.07 |
| Calcium | mg/L | 0.02 | 18.9 |
| Iron | mg/L | 0.001 | <0.001 |
| Potassium | mg/L | 0.02 | 1.5 |
| Magnesium | mg/L | 0.02 | 6.2 |
| Sodium | Mg/L | 0.02 | 58.1 |
| Suspended solids (v/860 nm) | FNU | 0.05 | 0.42 |
| Total PAH | ng/L | 0.1-1* | <38.3 |
| Total PCB | ng/L | 2-10* | <2 |

* Based on individual PAH and PCB analysis
FNU Formazin Nephelometric Units

**Table 4. Fish weight and length data**

| Exposure time | Dilution water control | | | 10 mg/L | | | 100 mg/L | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fish number | Weight (g) | Length (mm) | Fish number | Weight (g) | Length (mm) | Fish number | Weight (g) | Length (mm) |
| 2 hrs | 7 | 0.35 | 29 | 11 | 0.27 | 26 | 15 | 0.39 | 30 |
| | 8 | 0.24 | 30 | 12 | 0.39 | 30 | 16 | 0.29 | 27 |
| | 9 | 0.39 | 29 | 13 | 0.27 | 27 | 17 | 0.29 | 28 |
| | 10 | 0.32 | 28 | 14 | 0.37 | 29 | 18 | 0.30 | 28 |
| | **mean** | **0.33** | **29** | **mean** | **0.33** | **28** | **mean** | **0.32** | **28** |
| 4 hrs | 19 | 0.39 | 29 | 23 | 0.43 | 30 | 27 | 0.29 | 27 |
| | 20 | 0.27 | 26 | 24 | 0.35 | 29 | 28 | 0.45 | 31 |
| | 21 | 0.35 | 28 | 25 | 0.28 | 28 | 29 | 0.26 | 28 |
| | 22 | 0.37 | 28 | 26 | 0.30 | 26 | 30 | 0.42 | 30 |
| | **mean** | **0.35** | **28** | **mean** | **0.34** | **28** | **mean** | **0.36** | **29** |
| 8 hrs | 31 | 0.41 | 30 | 35 | 0.42 | 29 | 39 | 0.36 | 27 |
| | 32 | 0.33 | 28 | 36 | 0.15 | 27 | 40 | 0.32 | 28 |
| | 33 | 0.35 | 30 | 37 | 0.34 | 28 | 41 | 0.44 | 30 |
| | 34 | 0.52 | 32 | 38 | 0.30 | 28 | 42 | 0.29 | 27 |
| | **mean** | **0.40** | **30** | **mean** | **0.30** | **28** | **mean** | **0.35** | **28** |
| 24 hrs | 43 | 0.35 | 29 | 47 | 0.37 | 28 | 51 | 0.49 | 31 |
| | 44 | 0.29 | 28 | 48 | 0.36 | 27 | 52 | 0.34 | 29 |
| | 45 | 0.52 | 31 | 49 | 0.31 | 28 | 53 | 0.45 | 29 |
| | 46 | 0.36 | 29 | 50 | 0.37 | 29 | 54 | 0.39 | 29 |
| | **mean** | **0.38** | **29** | **mean** | **0.35** | **28** | **mean** | **0.42** | **30** |
| 48 hrs | 55 | 0.27 | 28 | 59 | 0.47 | 30 | 63 | 0.39 | 30 |
| | 56 | 0.36 | 28 | 60 | 0.39 | 29 | 64 | 0.32 | 28 |
| | 57 | 0.34 | 27 | 61 | 0.45 | 30 | 65 | 0.35 | 28 |
| | 58 | 0.28 | 28 | 62 | 0.38 | 29 | 66 | 0.34 | 28 |
| | **mean** | **0.31** | **28** | **mean** | **0.42** | **30** | **mean** | **0.35** | **29** |
| 8 hrs depuration | 67 | 0.39 | 29 | 71 | 0.32 | 27 | 75 | 0.35 | 28 |
| | 68 | 0.35 | 29 | 72 | 0.31 | 28 | 76 | 0.35 | 28 |
| | 69 | 0.40 | 30 | 73 | 0.39 | 28 | 77 | 0.30 | 27 |
| | 70 | 0.37 | 28 | 74 | 0.36 | 28 | 78 | 0.33 | 27 |
| | **mean** | **0.38** | **29** | **mean** | **0.35** | **28** | **mean** | **0.33** | **28** |
| 24 hrs depuration | 79 | 0.30 | 27 | 83 | 0.29 | 27 | 87 | 0.41 | 30 |
| | 80 | 0.29 | 27 | 84 | 0.42 | 29 | 88 | 0.28 | 30 |
| | 81 | 0.35 | 29 | 85 | 0.41 | 30 | 89 | 0.43 | 30 |
| | 82 | 0.41 | 30 | 86 | 0.31 | 27 | 90 | 0.31 | 28 |
| | **mean** | **0.34** | **28** | **mean** | **0.36** | **28** | **mean** | **0.36** | **30** |
| 36 hrs depuration | 91 | 0.33 | 28 | 95 | 0.55 | 32 | 99 | 0.32 | 27 |
| | 92 | 0.41 | 31 | 96 | 0.39 | 29 | 100 | 0.43 | 30 |
| | 93 | 0.45 | 30 | 97 | 0.37 | 28 | 101 | 0.41 | 30 |
| | 94 | 0.36 | 28 | 98 | 0.40 | 30 | 102 | 0.45 | 29 |
| | **mean** | **0.39** | **29** | **mean** | **0.43** | **30** | **mean** | **0.40** | **29** |
| 48 hrs depuration | 103 | 0.50 | 31 | 107 | 0.35 | 29 | 111 | 0.36 | 28 |
| | 104 | 0.30 | 27 | 108 | 0.40 | 30 | 112 | 0.39 | 30 |
| | 105 | 0.36 | 29 | 109 | 0.36 | 28 | 113 | 0.28 | 26 |
| | 106 | 0.37 | 29 | 110 | 0.33 | 29 | 114 | 0.44 | 30 |
| | **mean** | **0.38** | **29** | **mean** | **0.36** | **29** | **mean** | **0.37** | **29** |

**Table 5. Concentration of sucralose in the test waters**

| Exposure time | Tank rep | Dilution water control | 10 mg/L | 100 mg/L |
|---|---|---|---|---|
| 0 hrs ON | A | <LoD | 8 | 101 |
| | B | <LoD | 11 | 103 |
| | **Mean** | **<LoD** | **10** | **102** |
| 24 hrs OFF | A | <LoD | 10 | 117 |
| | B | <LoD | 11 | 86 |
| | **Mean** | **<LoD** | **10** | **102** |
| 24 hrs ON | A | <LoD | 13 | 103 |
| | B | <LoD | 3.0* | 74 |
| | **Mean** | **<LoD** | **13** | **89** |
| 48 hrs OFF | A | <LoD | 14 | 77 |
| | B | <LoD | 12 | 96 |
| | **Mean** | **<LoD** | **13** | **87** |
| overall mean | | - | 11 | 95 |
| stdev | | - | 2 | 15 |
| min | | - | 8 | 74 |
| max | | - | 14 | 117 |

*Result omitted from the summary statistics and the calculation of the BCF, as it was considered that this data was lower than expected due to either a problem with the analysis or the sampling procedure since the 48 hrs OFF sample was within the nominal range expected.

<LoD Limit of detection for the study was 15 ng/L

- Not calculated

**Table 6 Concentration of test substance in fish during the uptake phase**

| Exposure time | 10 mg/L | | | | | 100 mg/L | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fish no. | Mean fish weight (g) | Quantity of sucralose (µg) | tissue conc (mg/kg) | BCF | Fish no. | Mean fish weight (g) | Quantity of sucralose (µg) | tissue conc (mg/kg) | BCF |
| 2hrs | 11 | 0.33 | 0.22 | 0.67 | 0.06 | 15 | 0.34 | 2.26 | 6.65 | 0.07 |
| | 12 | | | | | 16 | | | | |
| | 13 | 0.32 | 0.09 | 0.28 | 0.02 | 17 | 0.30 | 0.74 | 2.51 | 0.03 |
| | 14 | | | | | 18 | | | | |
| | **mean** | **0.33** | **0.16** | **0.47** | **0.04** | **mean** | **0.32** | **1.50** | **4.58** | **0.05** |
| 4hrs | 23 | 0.39 | 0.23 | 0.59 | 0.05 | 27 | 0.37 | 3.06 | 8.27 | 0.09 |
| | 24 | | | | | 28 | | | | |
| | 25 | 0.29 | 0.24 | 0.83 | 0.07 | 29 | 0.34 | 6.08 | 17.88 | 0.19 |
| | 26 | | | | | 30 | | | | |
| | **mean** | **0.34** | **0.24** | **0.71** | **0.06** | **mean** | **0.36** | **4.57** | **13.08** | **0.14** |
| 8hrs | 35 | 0.29 | 0.33 | 1.16 | 0.10 | 39 | 0.34 | 2.97 | 8.74 | 0.09 |
| | 36 | | | | | 40 | | | | |
| | 37 | 0.32 | 0.39 | 1.22 | 0.10 | 41 | 0.37 | 5.64 | 15.45 | 0.16 |
| | 38 | | | | | 42 | | | | |
| | **mean** | **0.30** | **0.36** | **1.19** | **0.10** | **mean** | **0.35** | **4.31** | **12.09** | **0.13** |
| 24hrs | 47 | 0.37 | 0.70 | 1.92 | 0.16 | 51 | 0.42 | 18.83 | 45.37 | 0.48 |
| | 48 | | | | | 52 | | | | |
| | 49 | 0.34 | 5.10 | 15.00 | 1.28 | 53 | 0.42 | 15.12 | 36.00 | 0.38 |
| | 50 | | | | | 54 | | | | |
| | **mean** | **0.35** | **2.90** | **8.46** | **0.72** | **mean** | **0.42** | **16.98** | **40.69** | **0.43** |
| 48hrs | 59 | 0.43 | 5.73 | 13.33 | 1.14 | 63 | 0.36 | 15.14 | 42.65 | 0.45 |
| | 60 | | | | | 64 | | | | |
| | 61 | 0.42 | 1.16 | 2.80 | 0.24 | 65 | 0.35 | 19.19 | 55.62 | 0.59 |
| | 62 | | | | | 66 | | | | |
| | **mean** | **0.42** | **3.45** | **8.06** | **0.69** | **mean** | **0.35** | **17.17** | **49.14** | **0.52** |

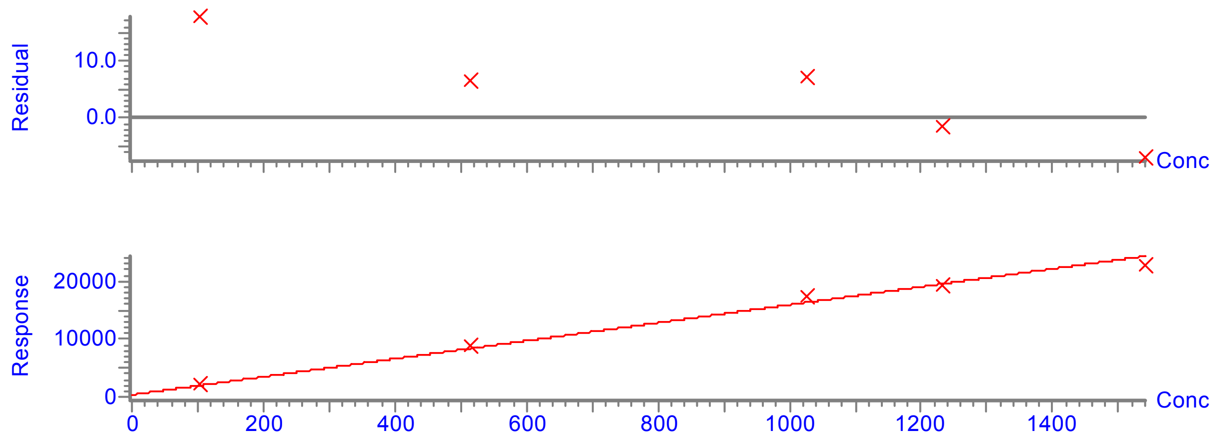
**Table 6 Concentration of test substance in fish during the depuration phase**

| Exposure time | 10 mg/L | | | | | 100 mg/L | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fish no. | Mean fish weight (g) | Quantity of sucralose (µg) | tissue conc. (mg/kg) | % dep* | Fish no. | Mean fish weight (g) | Quantity of sucralose (µg) | tissue conc. (mg/kg) | % dep* |
| 8hrs dep | 71 | 0.32 | 0.27 | 0.86 | 10.38 | 75 | 0.35 | 1.30 | 3.71 | 8.27 |
| | 72 | | | | | 76 | | | | |
| | 73 | 0.38 | 0.13 | 0.35 | 4.20 | 77 | 0.32 | 2.53 | 8.03 | 17.88 |
| | 74 | | | | | 78 | | | | |
| | **mean** | **0.35** | **0.20** | **0.60** | **7.29** | **mean** | **0.33** | **1.92** | **5.87** | **13.08** |
| 24hrs dep | 83 | 0.36 | 0.08 | 0.23 | 2.73 | 87 | 0.35 | 0.66 | 1.91 | 4.26 |
| | 84 | | | | | 88 | | | | |
| | 85 | 0.36 | 0.10 | 0.28 | 3.36 | 89 | 0.37 | 0.37 | 1.00 | 2.23 |
| | 86 | | | | | 90 | | | | |
| | **mean** | **0.36** | **0.09** | **0.25** | **3.05** | **mean** | **0.36** | **0.52** | **1.46** | **3.24** |
| 36hrs dep | 95 | 0.47 | 0.17 | 0.36 | 4.38 | 99 | 0.38 | 0.25 | 0.67 | 1.48 |
| | 96 | | | | | 100 | | | | |
| | 97 | 0.39 | 0.04 | 0.10 | 1.26 | 101 | 0.43 | 0.82 | 1.91 | 4.25 |
| | 98 | | | | | 102 | | | | |
| | **mean** | **0.43** | **0.11** | **0.23** | **2.82** | **mean** | **0.40** | **0.54** | **1.29** | **2.87** |
| 48hrs dep | 107 | 0.38 | 0.06 | 0.16 | 1.94 | 111 | 0.38 | 0.21 | 0.56 | 1.25 |
| | 108 | | | | | 112 | | | | |
| | 109 | 0.35 | 0.03 | 0.09 | 1.05 | 113 | 0.36 | 0.12 | 0.33 | 0.74 |
| | 110 | | | | | 114 | | | | |
| | **mean** | **0.36** | **0.05** | **0.12** | **1.49** | **mean** | **0.37** | **0.17** | **0.45** | **0.99** |

* % depuration, calculated as the ratio of the loss of substance to the mean $BCF_{ss}$

**Figure 1. Calibration curves for sucralose and phenyl β-D-glucopyranoside.**

Compound name: Sucralose
Correlation coefficient: r = 0.996479, r^2 = 0.992970
Calibration curve: 15.5483 * x + 368.502
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None




Compound name: Int Std
Response Factor: 10916.8
RRF SD: 1515.31,  % Relative SD: 13.8806
Response type: External Std, Area
Curve type: RF

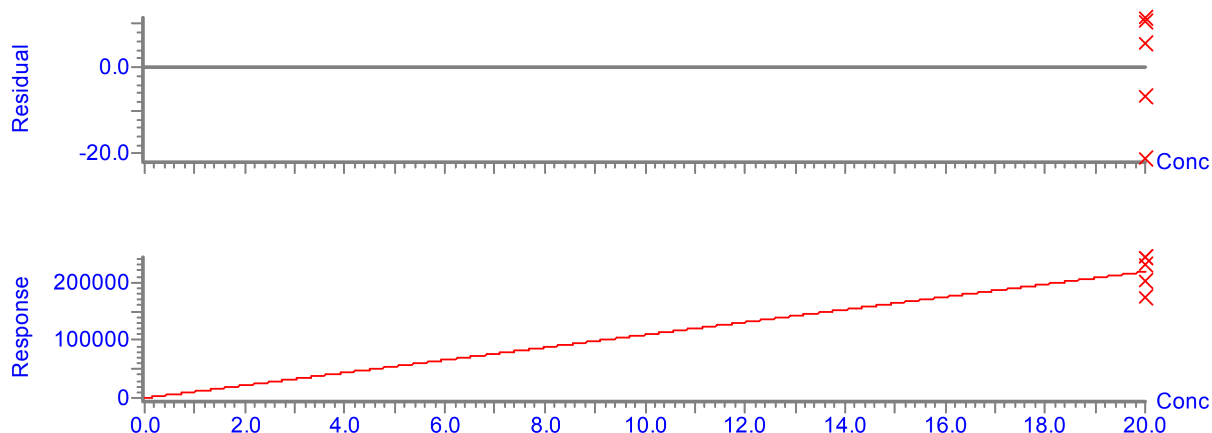
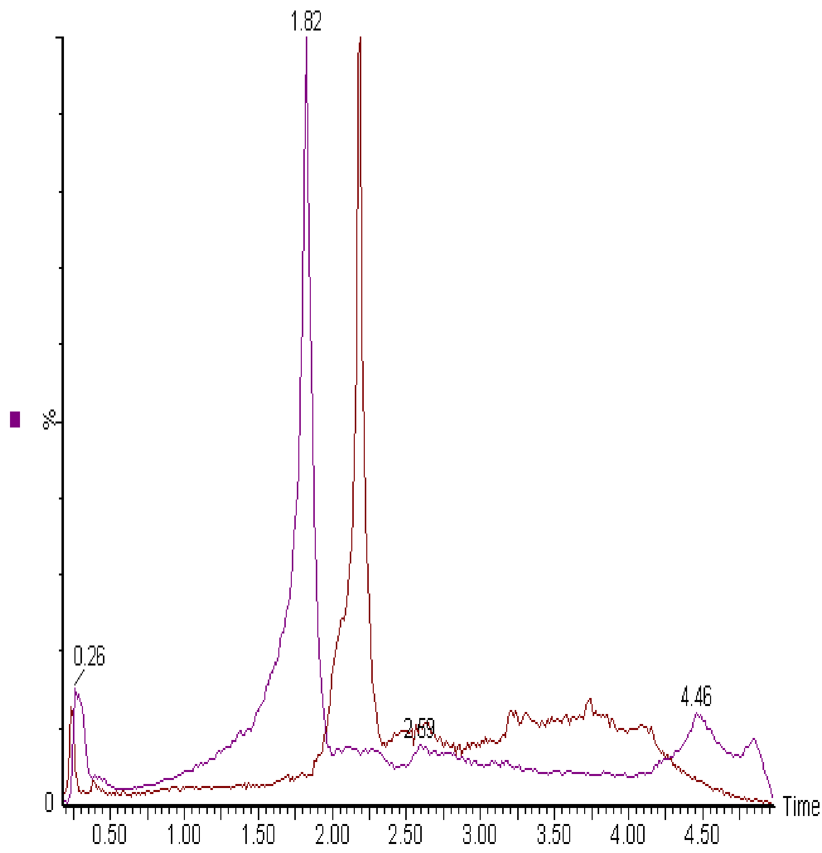


**Figure 2. LC/MS extracted ion chromatogram for (A) 10 mg/L 8 hr depuration zebra fish sample and (B) 10 mgL A 24 hr on water sample**

A

10 mgL 71/72




B

10 mgL A 24hr on

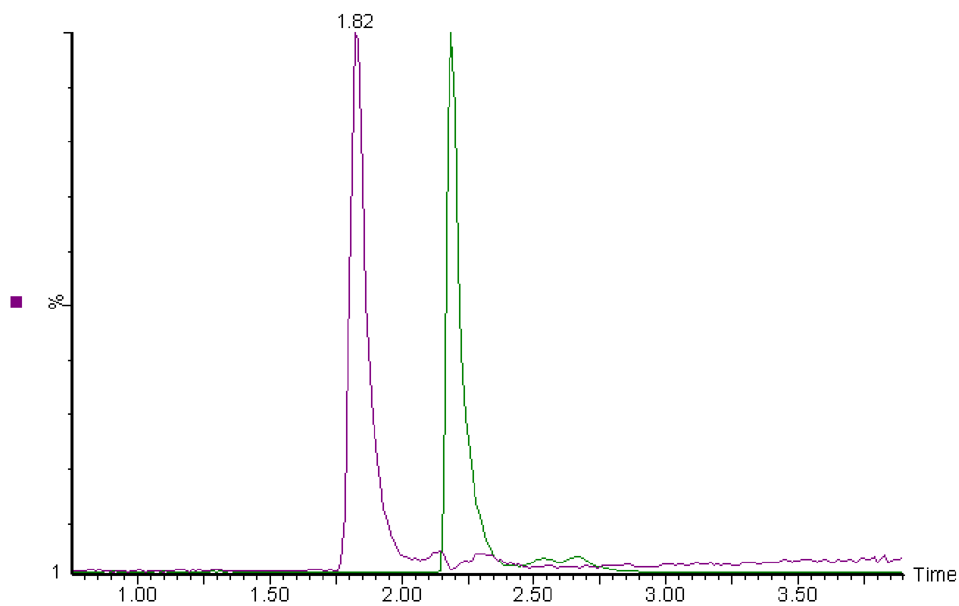
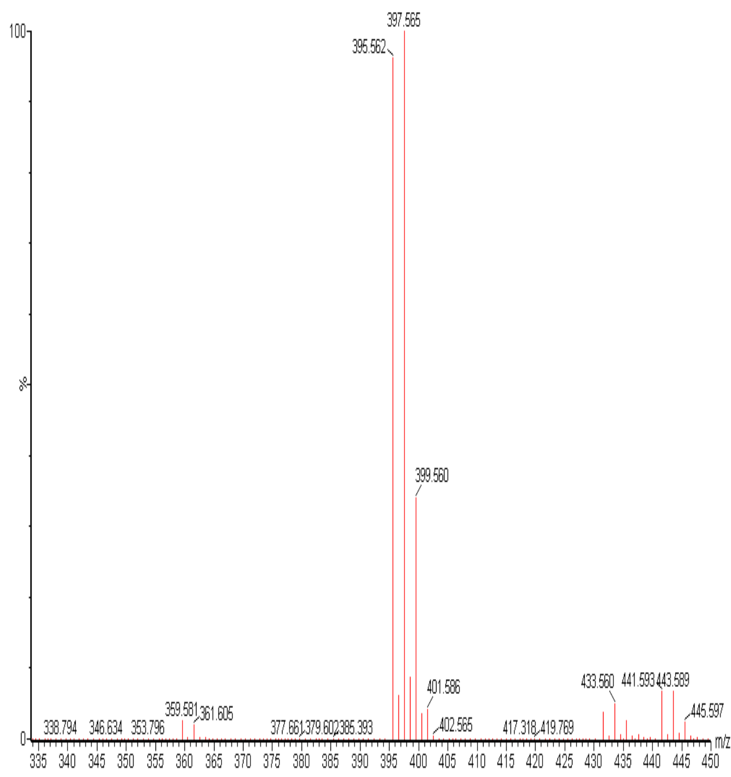


**Figure 3. Mass spectrum of sucralose**



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