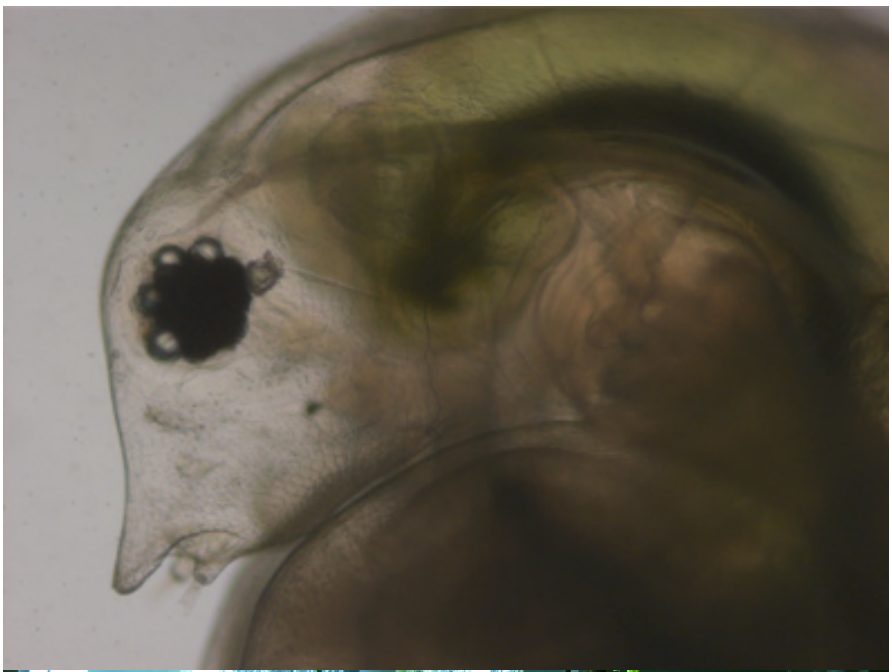
REPORT SNO 5866-2009

Determination of the bioconcentration factor of sucralose in the crustacean Daphnia magna



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REPORT

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Abstract

The bioaccumulation of sucralose in the crustacean *Daphnia magna* was determined using a 48 hour static exposure system. Two exposure concentrations (10 and 100 mg/L) plus an appropriate control were used for the study. Daphnids were analysed on 2 occasions during the exposure phase using liquid chromatography – mass spectrometry (LC/MS). A steady state concentration in the daphnids was achieved after 24 hours and the bioconcentration factor (BCF) at steady state (BCF_{ss}) was calculated to be 2.2 and 1.6 for the 10 and 100 mg/L test concentrations respectively. This indicates that sucralose does not accumulate significantly in the tissues of daphnids and the BCF_{ss} were considerably lower than the criteria set to identify persistent, bioaccumulative and toxic (PBT) substances (i.e. $\geq$2000).

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2. Sukralose	2. Sucralose
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Determination of the bioconcentration factor of sucralose in the crustacean *Daphnia magna*

Preface

This study has been conducted by staff from the Section for Ecotoxicology and Risk Assessment, Norwegian Institute for Water Research (NIVA). The authors acknowledge the contribution by Torsten Källqvist for support during the study.

Oslo, 04.11.2009

Knut Erik Tollefsen

Contents

1 Abstract

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2 Introduction

The bioaccumulation of sucralose in the crustacean *Daphnia magna* was carried out at NIVA Gaustadalléen 21, N-0349, Oslo, Norway at the request of Tate and Lyle (2200 East Eldorado, Decatur, 62521, Illinois, USA). The test was not performed according to any specific test guideline but adopted some of the principals of the OECD test guideline 305. All chemical analyses was performed at NIVA Gaustadalléen 21, N-0349, Oslo, Norway. The study number for the study was 28433-3 and the exposure dates were 19 to 21 May 2009.

3 Materials and methods

3.1 Test substance

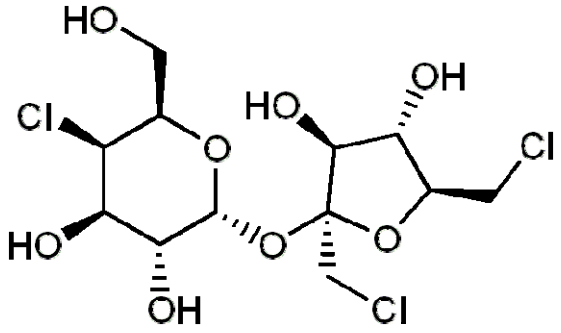
The test substance Sucralose (batch no. MM8G190A6M, manufactured date 07/2008) was supplied by Tate and Lyle (2200 East Eldorado, Decatur, 62521, Illinois, USA).

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 20º C), 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 crustacean *Daphnia magna* which were bred and maintained at NIVA Gaustadalléen 21, NO-0349, Oslo; Norway. The daphnids used for the study were approximately 7 days old and were obtained from a continuous culture. The culturing of the daphnids were performed according to methods described by Källqvist *et al*. (2005).

3.3 Dilution water

The dilution water used for the study was M7 media as described by Elendt (1990). 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 23 ºC using a static system with an exposure period of 48 hours for the uptake phase, there was no depuration phase included in this study. The photoperiod used for the study was 16:8 light:dark cycle. The test vessels used for the study were constructed from borosilicate glass and had a working volume of 3 L with loose fitting lids. Each beaker contained approximately 650 daphnids. The loading of the daphnids equated to approximately 325 daphnids/L.

The test was initiated by the addition of approx 650 daphnids to each test vessel. Two exposure concentrations of sucralose 10 mg/L and 100 mg/L were used, in addition to a control group. Both daphnids and water were sampled simultaneously for sucralose quantification at 0, 4, 8, 24, 30 and 48 hours exposure although only the 0, 24 and 48 hour samples were analysed.

The daphnids were not fed during the study.

3.5 Physical parameters

Dissolved oxygen, pH and temperature were measured on the freshly prepared test solutions at 0h ("on") and at the end of the study at 48 hours ("off") solutions during the uptake phase. In addition, samples of dilution water were taken for water quality analysis as detailed in Table 3 of the appendices.

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 2 L dilution water.

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 daphnid 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 pH3 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 daphnids

Triplicate samples of approximately 50 daphnids were caught with an adapted displacement pipette and rinsed briefly with approximately 50 ml of deionised water on a fine mesh. A piece of absorbent tissue was then placed under the mesh to draw as much excess water away from the daphnids as possible. The daphnids were then scraped from the mesh using a spatula and placed onto a pre-tarred weighing boat and weighed using a balance before being placed in a plastic sample container and immediately frozen on dry ice.

Triplicate samples of approximately 50 daphnids were also sampled from the stock population at 0 h and were retained for baseline analysis of sucralose.

The daphnid samples were extracted using double solvent extraction. 100 ng phenyl β-D-glucopyranoside was added to each sample tube to act as an internal standard followed by 5 ml methanol/acetone (1:9) extraction solvent. With each batch of daphnids extracted, a blank and a spiked control (200 ng sucralose) was also extracted for quality control purposes.

The samples were first sonicated at 60^{o}C for 30 minutes, and then centrifuged (2500 rpm, 10 minutes) before decanting off the solvent. The extraction was repeated and the solvent extracts combined. The acetone was evaporated under nitrogen and the remaining methanol transferred to a vial for LC/MS analysis. Extraction recoveries were 56±5%.

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 quadrapole mass spectrometer (Waters, Manchester, UK). 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^{o}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* 301.2 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:

$$\mathbf{BCF_{SS}=C_{daphnid}/C_{water}}$$

Where BCF_{SS} is the bioconcentration factor at equilibrium, $C_{daphnid}$ is the measured concentration of sucralose in the daphnids and C_{water} is the concentration in the water 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 parameter and water quality data for the study is shown in Table 2 and Table 3 of the appendices respectively. In summary, the pH of the study ranged between 7.3 and 7.7, the dissolved oxygen ranged between 6.4 and 7.7 mg/L and the temperature ranged between 23.2 and 23.5ºC. The water quality was within the range expected for the study and none of the analytes present would have affected the outcome of the study.

4.2 Biological data

The weights of the daphnids measured during the study are shown in Table 4 of the appendices.

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. The data indicated that the test concentrations did not vary considerably between the start and end of the study and the mean measured concentrations for the nominal 10 and

100 mg/L test concentrations were 10.1 and 108.0 mg/L respectively. A typical chromatogram for the analysis of sucralose in the test solutions is shown in Figure 2 of 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 Figure 1 of the appendices.

4.3.2 Analysis of sucralose in the daphnids

The analytical chemistry data for the concentration of sucralose in the daphnids 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 Figure 2 of the appendices. The limit of detection for the analysis of sucralose in the daphnids was 20 ng/g.

4.4 Bioconcentration factors

It was considered that the steady state concentration in the daphnids had been reached within 24 hours exposure. Therefore the BCF at steady (BCF_{SS}) was calculated as the mean of the 24 and 48 hours exposure data. For both the upper and lower concentrations (10 and 100 mg/L), the BCFss was 2.2 and 1.6 respectively. Table 6 in the appendices details the bioconcentration factors determined at the different sampling times.

5 Conclusion

The BCF values determined for the uptake of sucralose in this study were 2.2 and 1.6 for the 10 and 100 mg/L test concentrations, respectively. This indicates that sucralose does not accumulate significantly in the tissues of daphnids and the BCF_{SS} were considerably lower than the criteria set to identify persistent, bioaccumulative and toxic (PBT) substances (i.e. BCF$\geq$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.

Elendt BP (1990). Selenium deficiency in Crustacea; An ultrastructural approach to antennal damage in *Daphnia magna* Strauss. *Protoplasma*, **154**, 25-33.

Källqvist T, Grung M and Tollefsen KE (2005). Chronic toxicity of 2,4,29,49-tetrabromodiphenyl ether on the marine Alga *Skeletonema costatum* and the crustacean *Daphnia magna*. Environmental Toxicology and Chemistry, **25** (6) 1657–1662.

Appendices

Table 2. Physical parameters of the test solutions

Exposure time	Test concentration (mg/L)	Dissolved oxygen (mg/L)	pH	Temp (^{o}C)
0 hrs	Control	7.7	7.7	23.2
	10	7.7	7.7	23.2
	100	7.7	7.7	23.2
48 hrs	Control	6.4	7.3	23.5
	10	6.7	7.4	23.5
	100	6.8	7.4	23.3
Overall	**Mean**	**7.2**	**7.5**	**23.3**
	min	**6.4**	**7.3**	**23.2**
	max	**7.7**	**7.7**	**23.5**

Table 3. Water quality analysis

Parameter	Unit	Detection limit	Dilution water used for uptake phase
pH	-	-	7.44
Conductivity	mS/m	0.05	61.2
Alkalinity	mmol/L	0.01	0.719
Nitrogen	µg/L	10	Nd
TOC	mg/L	0.1	0.21
DOC	mg/L	0.1	0.24
Chlorine	mg/L	0.03	132
Sulphate	mg/L	0.04	40
Aluminium, reactive	µg/L	5	17
Aluminium, non reactive	µg/L	5	8
Calcium	mg/L	0.02	73.4
Potassium	mg/L	0.02	2.6
Magnesium	mg/L	0.02	10.4
Sodium	mg/L	0.02	17.7
Suspended solids (v/860 nm)	FNU	0.05	0.68
Total PAH	ng/L	0.1-1*	<2
Total PCB	ng/L	2-10*	<52

Nd Not determined
FNU Formazin Nephelometric Units
* Based on individual PAH and PCB analysis

Table 4. Daphnid weight data

Time (hours)	Weight of daphnids (g)		
	Control	10 (mg/L)	100 (mg/L)
4	0.023	0.035	0.042
	0.026	0.040	0.035
	0.039	0.046	0.031
8	0.035	0.032	0.028
	0.036	0.025	0.031
	0.027	0.026	0.021
24	0.024	0.022	0.031
	0.017	0.018	0.021
	0.020	0.012	0.022
30	0.023	0.034	0.029
	0.020	0.032	0.023
	0.033	0.018	0.005
48	0.019	0.014	0.011
	0.015	0.016	0.011
	0.011	0.021	-

- Not possible to obtain a sample

Table 5. Concentration of sucralose in the test waters

Test concentration (mg/L)	Time	Sucralose concentration (mg/L)
Control	0h	<LoD
	24h	<LoD
	48h	<LoD
10 mg/L	0h	10.1
	24h	9.6
	48h	10.7
100 mg/L	0h	80.2
	24h	121.0
	48h	122.8
Mean Control		<LoD
Mean 10 mg/L		10.1
Mean 100 mg/L		108.0

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

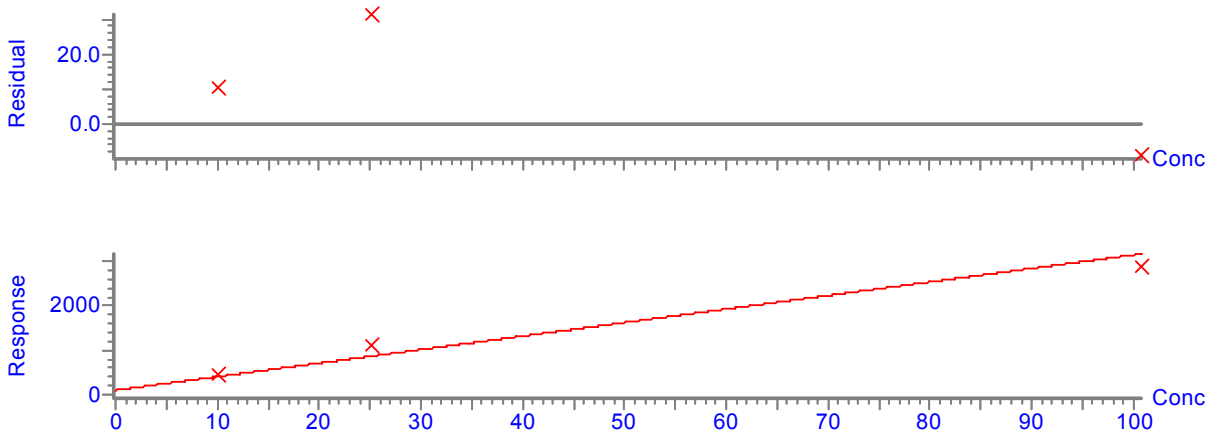
Table 6. Concentration of test substance in daphnids during the uptake phase

	Sample no.	Sucralose concentration (ng/sample)	Sucralose (µg/sample)	Weight of daphnids	Tissue conc (mg/kg)	Mean tissue conc (mg/kg)	BCF	**Mean BCF**
Base line*	1	<LoD	<LoD	-	-	-	-	**-**
	2	<LoD	<LoD	-	-	-	-	**-**
	3	<LoD	<LoD	-	-	-	-	**-**
Control 24 hr	22	<LoD	<LoD	0.024	<LoD	<LoD	-	**-**
	23	<LoD	<LoD	0.017	<LoD		-	
	24	<LoD	<LoD	0.020	<LoD		-	
10 (mg/L) 24 hr	25	589	0.589	0.022	26.8	26.7	2.65	**2.64**
	26	525	0.525	0.018	29.2		2.88	
	27	290	0.290	0.012	24.1		2.39	
100 (mg/L) 24 hr	28	8034	8.034	0.031	259	212	2.40	**1.96**
	29	3225	3.225	0.021	154		1.42	
	30	4900	4.900	0.022	223		2.06	
Control 48 hr	40	<LoD	<LoD	0.019	<LoD	<LoD	-	**-**
	41	<LoD	<LoD	0.015	<LoD		-	
	42	<LoD	<LoD	0.011	<LoD		-	
10 (mg/L) 48 hr	43	261	0.261	0.014	18.7	18.5	1.84	**1.83**
	44	332	0.332	0.016	20.7		2.05	
	45	339	0.339	0.021	16.1		1.59	
100 (mg/L) 48 hr	46	1465	1.465	0.011	133	137	1.23	**1.27**
	47	1547	1.547	0.011	141		1.30	

<LoD Limit of detection for the study was 20 ng/g
* Baseline analysis used to confirm absence of test substance in the algal culture prior to testing
- Not calculable

Figure 1. Calibration curves for sucralose and phenyl β-D-glucopyranoside internal standard quantification

Compound name: Sucralose
Correlation coefficient: r = 0.978266, r^2 = 0.957005
Calibration curve: 30.4923 * x + 101.225
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None



Compound name: Int Std
Correlation coefficient: r = 0.994346, r^2 = 0.988724
Calibration curve: 8.36404 * x + -22.7596
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None

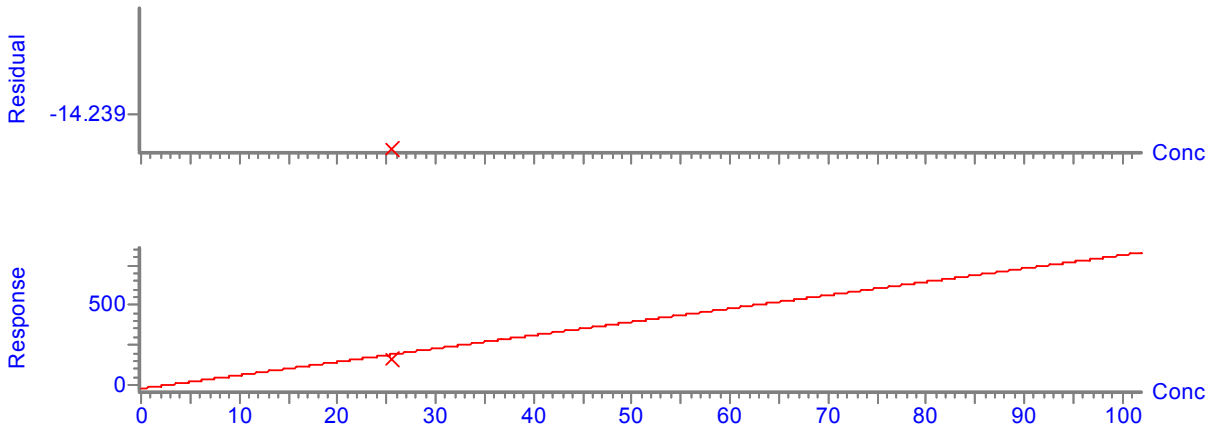


Figure 2. Extracted ion chromatogram of A. daphnid (10 mg/L 24 hr) and B. exposure water (10 mg/L 24 hr)

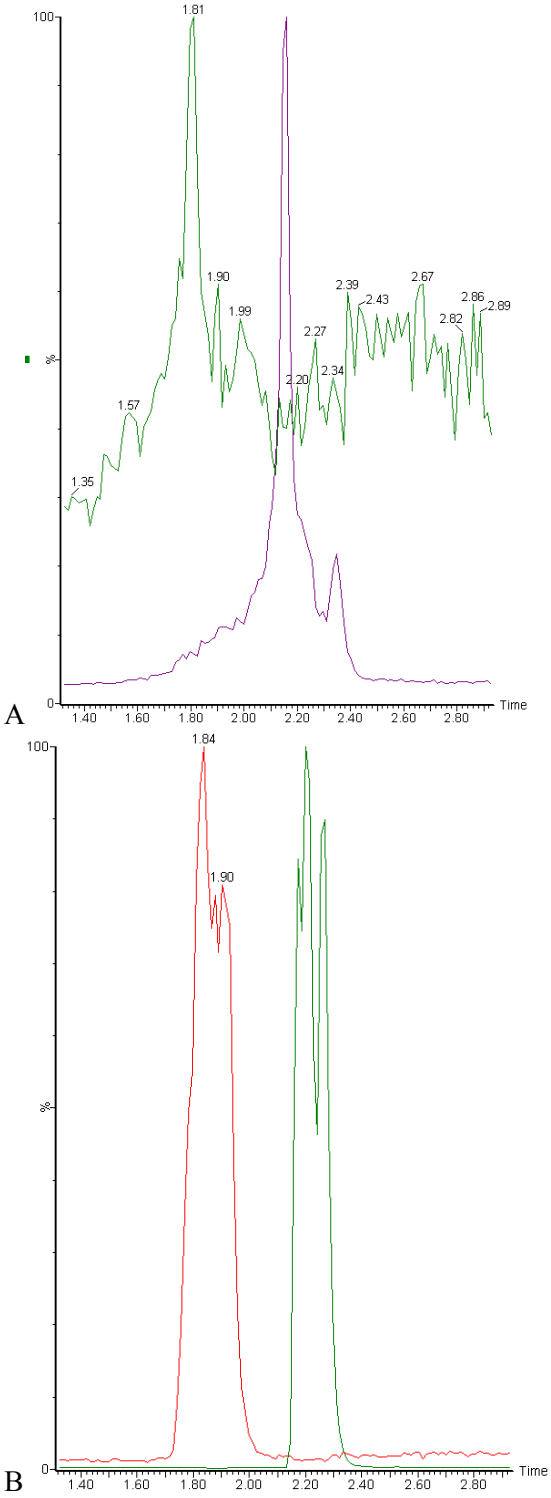
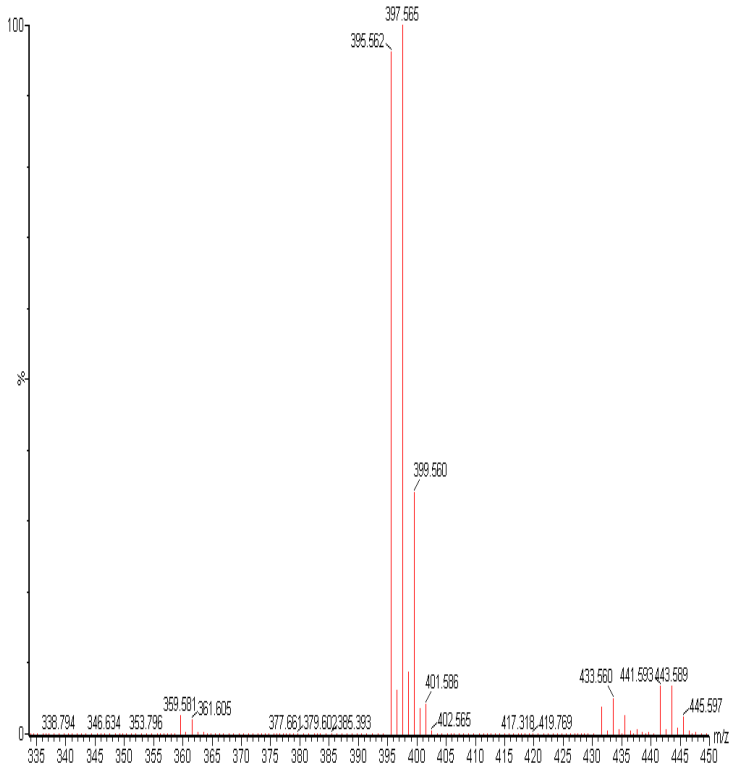


Figure 3. Mass spectrum of sucralose



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