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Determination of the bioaccumulation factor of sucralose in the freshwater alga, Pseudokirchneriella subcapitata

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– an institute in the Environmental Research Alliance of Norway

REPORT

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Abstract

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Determination of the bioaccumulation factor of sucralose in the freshwater alga, *Pseudokirchneriella subcapitata*

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

A study to determine the bioaccumulation of sucralose in the freshwater alga, *Pseudokirchneriella subcapitata* 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 28433-2 and the exposure dates were 19 to 21 May 2009.

3 Materials and methods

3.1 Test substance

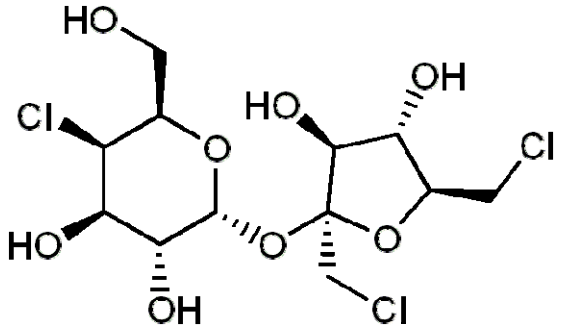
The test substance Sucralose (batch no. MM8G190A6M, manufactured date 07/2008) was supplied by Tate and Lyle (2200 East Eldorado, Decatur, 62521, Illinois, USA).

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 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

Algae used in the study were cultured from stocks maintained at the Norwegian Institute for Water Research (NIVA), Oslo, Norway. An optimum density of approximately 2000 x 10^6 cells/L of *P. subcapitata* was used. The cell density and average cell volume of the algal stock was measured prior to testing using a Coulter Multisizer M3 Miami, FL, USA. The culturing of the algae were performed according to methods described by Skulberg and Skulberg, 1990.

3.3 Dilution water

The dilution water used for the study was distilled water reconstituted with appropriate salts (Z8 media described by Skulberg and Skulberg, 1990). The temperature of the water was adjusted to approximately 23 ºC prior to preparing test solutions. The media 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 ± 2 ºC using a static test 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 continuous lighting. The test vessels used for the study were flat bottomed spherical vessels constructed from borosilicate glass with a working volume of approximately 3 L each fitted with loose fitting lids. Each vessel contained approx 2000 x 10^6 cells/L of *P. subcapitata*. The algae was exposed to two concentrations of sucralose 10 and 100 mg/L plus an appropriate control that were all treated in an identical way. Both algae and water were sampled simultaneously for quantification of sucralose.

The test was initiated by the addition of an appropriate amount of algae to the different test solutions of sucralose and the control in order to obtain the optimum density of algae (2000 x 10^6 cells/L) in each concentration. Both algae and water were sampled simultaneously for sucralose quantification at 0, 4, 8, 24, 30 and 48 hours exposure although only 0, 24 and 48 hour samples were analysed for quantification of sucralose. The algae was sampled for analysis by removing 100 ml from each of the test concentrations and passing the algae through a glass fibre filter using a vacuum pump to separate the test solution from the algal cells. The wet weight of the algae was recorded and the filter paper, containing the algae, was placed into an appropriate container and stored at -20 ºC until the analysis was performed. The remaining supernatant, following filtration, was retained for analysis of sucralose in the test solutions. On each sampling occasion, the algae and test solutions were sampled in triplicate. To ensure that the working volume did not decrease significantly during the study and to minimise the increase in cell density over the course of the study, an equal amount of fresh test solution was added to each vessel following sampling.

3.5 Physical parameters

The pH and temperature were measured on the freshly prepared test solutions at 0h (“on”) and at the end of the study at 48 h (“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 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. Sucralose dissolved in dilution water was then added to an appropriate amount of algae to obtain the optimum density of algae and made up to a final volume of 2 L of test solution. An additional stock solution was also prepared to replace the test solution when sampling for the algae and water.

3.7 Analysis of test substance in water

On each sample occasion, approximately 100 ml of the filtered test water was taken from each concentration in pre-cleaned 200 ml polypropylene bottles in conjunction with the algal 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 algae

Each sample of algae, taken for analysis, was briefly rinsed using 5 mL distilled water to remove any test solution from the outside of the cells.

The algae samples were extracted with the filter papers 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 algae extracted, a blank and a spiked control (100 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 112±10%.

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:

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

Where BCF_{SS} is the bioconcentration factor at equilibrium (steady state), C_{algae} is the measured concentration of sucralose in the algae 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 7.7 and 8.5, and the temperature ranged between 22.8 and 23.2°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 weights of the algae 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 4 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 10.0 and 107.1 mg/L respectively. 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 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 algae

The analytical chemistry data for the concentration of sucralose in the algae and the corresponding BCF data 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 Figure 2 of the appendices. The limit of detection for the analysis of sucralose in the algae was 5 ng/g.

4.4 Bioconcentration factors

It was considered that the steady state concentration in the algae 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 BCF_{SS} was 0.10 and 0.13 respectively. Table 4 in the appendices details the bioconcentration factors determined at the different sampling times.

5 Conclusions

The BCF values determined for the uptake of sucralose in this study were <1 for both the 10 and 100 mg/L test concentrations respectively. This indicates that sucralose does not accumulate significantly in *P. subcapitata* 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.

Skulberg, O.M. and Skulberg, R. (1990). Forskning med algekulturer-NIVAs kultursamling av alger (Research with algae culture-NIVAs culture collection of algae). NIVA-rapport ISBN:82-577-1743-6, 32 s.

Appendices

Table 2. Physical parameters of the test solutions

Exposure time	Test concentration (mg/L)	pH	Temp (^{o}C)
0 h	Control	7.7	23.0
	10	7.7	23.0
	100	7.7	22.8
48 h	Control	8.3	23.2
	10	8.5	23.2
	100	8.3	23.0
Overall	**Mean**	**8.0**	**23.0**
	min	**7.7**	**22.8**
	max	**8.5**	**23.2**

Table 3. Water quality analysis

Parameter	Unit	Detection limit	Dilution water used for exposure
pH	-	-	6.8
Conductivity	mS/m	0.05	8.48
Alkalinity	mmol/l	0.01	0.097
TOC	mg/l C	0.1	0.2
DOC	mg/l	0.1	0.24
Chlorine	mg/l	0.03	0.16
Sulphate	mg/l	0.04	0.95
Aluminium, reactive	µg/l	5	5
Aluminium, non reactive	µg/l	5	<5
Calcium	mg/l	0.02	1.07
Potassium	mg/l	0.02	1.49
Magnesium	mg/l	0.02	0.23
Sodium	mg/l	0.02	Nd
Suspended solids (v/860 nm)	FNU	0.05	0.4
Total PAH	ng/L	0.1-1*	<20
Total PCB	ng/L	2-10*	2.1

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

Table 4. 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	10.7
	24h	8.6
	48h	10.6
100 mg/L	0h	100.9
	24h	91.6
	48h	128.7
Mean Control		<LoD
Mean 10 mg/L		10.0
Mean 100 mg/L		107.1

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

Table 5. Concentration of sucralose in the algae and associated BCF values

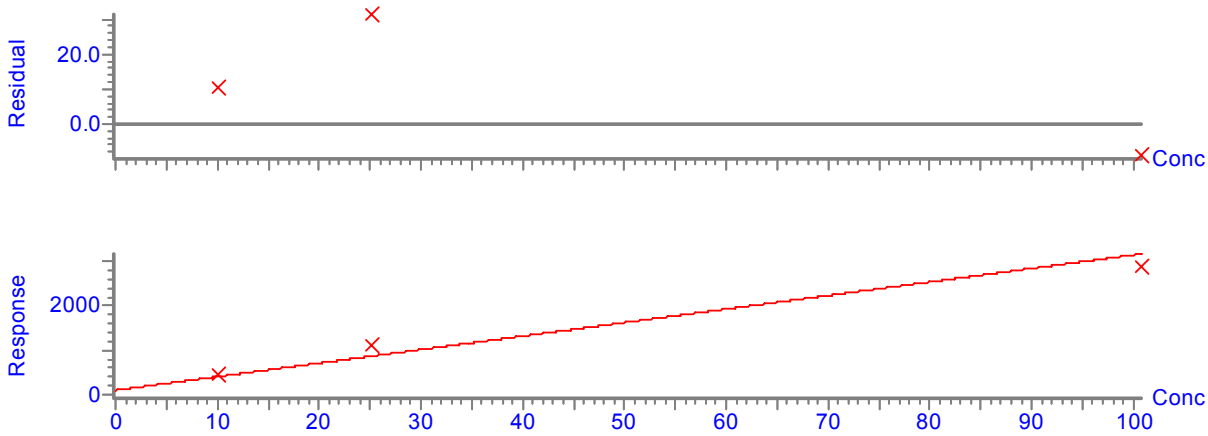
	Sample no.	Sucralose (ng/ sample)	Sucralose (ug/ sample)	Wet weight of algae		Tissue conc (mg/kg)	Mean tissue conc (mg/kg)	BCF	**Average BCF**
				Filter (g)	Algae-filter paper (g)				
Baseline*	2	<LoD	<LoD	-	-	-	-	-	**-**
	3	<LoD	<LoD	-	-	-	-	-	**-**
control 24h	22	<LoD	<LoD	1.155	0.895	<LoD	<LoD	-	**-**
	23	<LoD	<LoD	1.207	0.947	<LoD		-	
	24	<LoD	<LoD	1.277	1.017	<LoD		-	
10 mg/L 24h	25	1521	1.521	1.172	0.912	1.668	1.040	0.167	**0.104**
	26	893	0.893	1.201	0.941	0.949		0.095	
	27	515	0.515	1.285	1.025	0.502		0.050	
100 mg/L 24h	28	13153	13.15	1.218	0.958	13.730	19.890	0.128	**0.186**
	29	40928	40.93	1.250	0.990	41.341		0.386	
	30	4272	4.27	1.189	0.929	4.598		0.043	
control 48h	40	<LoD	<LoD	1.220	0.960	-	-	-	**-**
	41	<LoD	<LoD	1.266	1.006	-		-	
	42	<LoD	<LoD	1.302	1.042	-		-	
10 mg/L 48h	43	760	0.760	1.229	0.969	0.785	0.889	0.079	**0.089**
	44	1014	1.014	1.279	1.019	0.995		0.100	
	45	1011	1.011	1.400	1.140	0.886		0.089	
100 mg/L 48h	46	9929	9.93	1.234	0.974	10.194	7.266	0.095	**0.068**
	47	7231	7.23	1.344	1.084	6.671		0.062	
	48	5490	5.49	1.373	1.113	4.933		0.046	

* Baseline analysis used to confirm absence of test substance in the algal culture prior to testing

<LoD Limit of detection for the study was 5 ng/g
\- 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

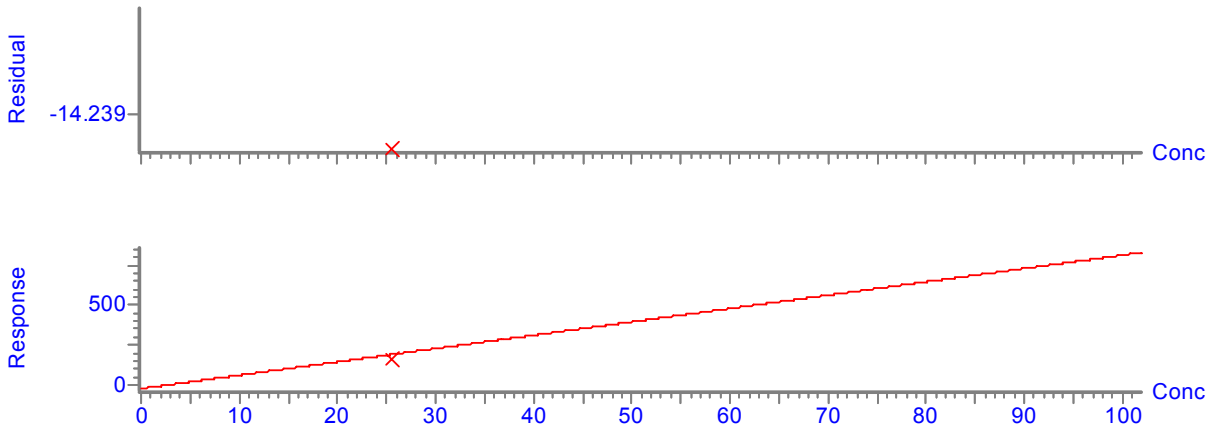
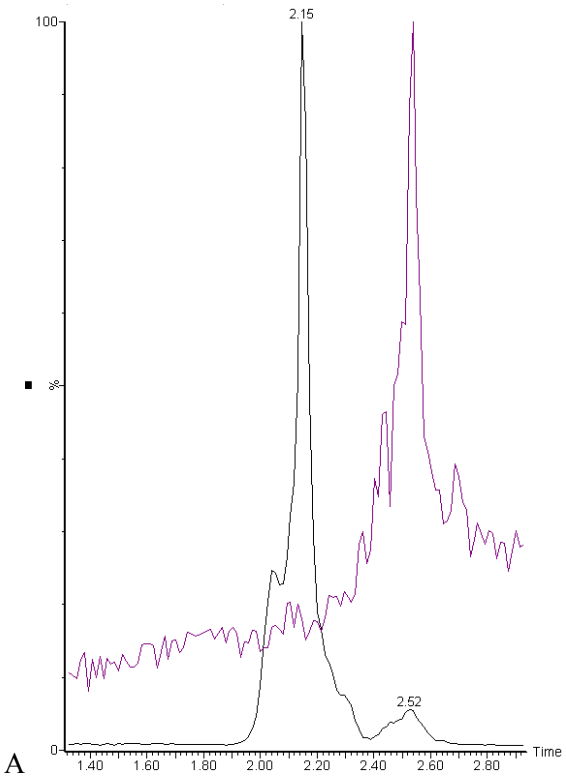


Figure 2. Extracted ion chromatogram for A. algae (10 mg/L 24 hr) and B. exposure water (10 mg/L 24 hr)



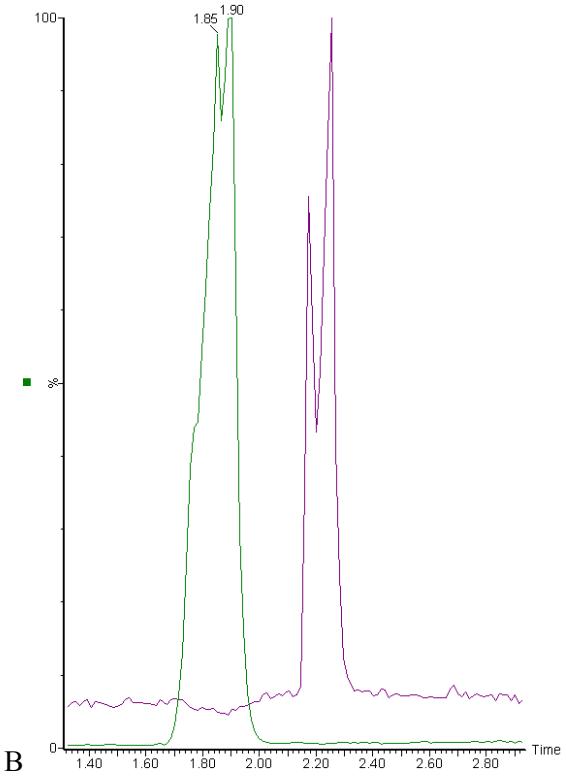
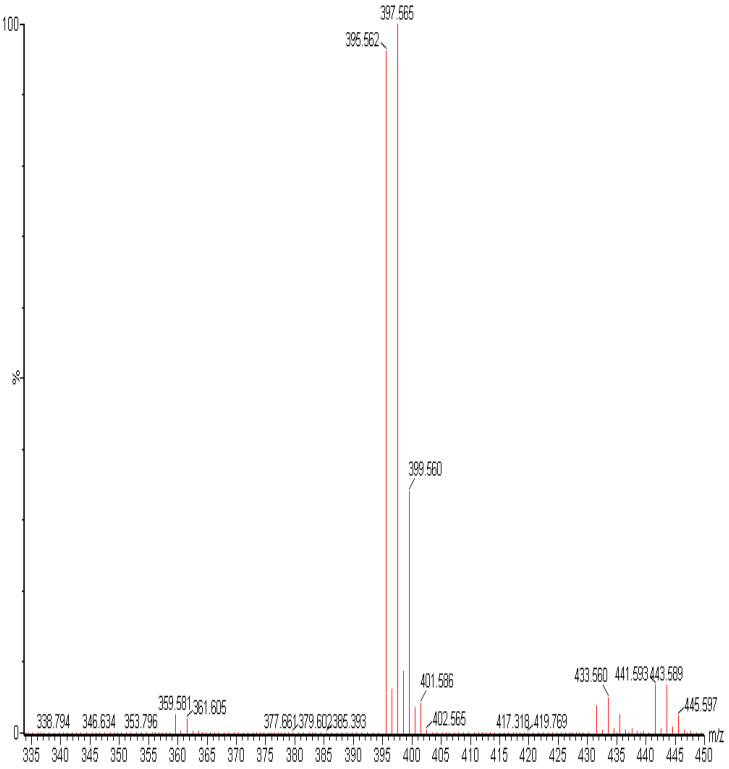


Figure 3. Mass spectrum for sucralose



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