Identification and Fragmentation of Sucralose Using Accurate-Mass Q-TOF LC/MS and Molecular Structure Correlator Software

Application Note

Food and Environmental

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Abstract

The use of Accurate-Mass Q-TOF LC/MS and MS/MS, in both positive and negative electrospray ionization (ESI) modes, was evaluated for the identification of sucralose in water. Response and fragmentation pathways were investigated. Sucralose responded well using Q-TOF LC/MS in either the positive or negative ion ESI modes. The overall signal intensity obtained in positive ion mode was approximately twice that of negative ion mode.

In positive ion mode, sucralose was detected by its sodium adduct $[M+Na]^+$ at m/z 419.0038. Accurate mass MS/MS measurements provided structural confirmation of the sodiated fragments obtained (m/z 221.0187 and m/z 238.9848). In negative ion mode, the deprotonated molecule was observed ($[M-H]^-$ at m/z 395.0073). Fragmentation by MS/MS yielded one characteristic fragment ion (m/z 359.0306). Agilent MassHunter Molecular Structure Correlator (MSC) software was used to draw and investigate the fragmentation pathways for the negative and positive ion MS/MS analyses. The MSC software proved to be a useful tool in assisting with the characterization of the fragment ion structures.

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Introduction

Due to its intense sweetness, noncaloric properties, low bioaccumulation potential, low toxicity, and the dietary requirements of many consumers, sucralose has become one of the most popular artificial sweeteners used worldwide. Because the human body does not metabolize sucralose, it ends up in wastewater and surface water. Current waste-water treatment technologies do not address sucralose, so it is now ubiquitous in the environment. This is a point of significant concern. A recent study revealed the biological effects of sucralose in the aquatic environment, which may have important toxicological consequences [1]. For these reasons, there is growing interest in measuring sucralose in drinking water, groundwater, surface water, wastewater, and aquatic environments.

Due to its solubility, sucralose is readily analyzed by LC/MS. It contains three chlorine atoms, which produce a distinctive chlorine signature when analyzed by MS. Based on many papers published describing the analysis of sucralose, LC/MS/MS with multiple reaction monitoring (MRM) in negative ion mode is the most popular method. However, the MRM transitions used are not selective enough to identify sucralose in water with the same confidence as with accurate mass. The transitions are not discriminatory because they involve a chlorine loss, which can be present in many other common organic molecules.

This application note evaluates the use of an Agilent 6540 Accurate-Mass Q-TOF LC/MS system in both positive and negative ESI modes for the unequivocal identification of sucralose in water. Response and the usefulness of molecular structure correlation software were investigated. The complementary study of Analytical Methodologies for the Detection of Sucralose in Water in Analytical Chemistry [2] provides a detailed comparison of Q-TOF LC/MS and LC/MS/MS for the detection of sucralose in environmental water samples.

Experimental

A detailed description of the experimental procedures can be found in the complementary journal article published in Analytical Chemistry [2].

Standard preparation

Sucralose was purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of sucralose (1,000 µg/mL) was prepared in water and stored at −18 °C. From this solution, working standard solutions were prepared by dilution with methanol and water.

Instrumentation

The standard was analyzed using an Agilent 1290 Infinity Binary LC System coupled to an Agilent 6540 Accurate-Mass Q-TOF LC/MS system with Agilent Jet Stream technology for electrospray ionization.

The HPLC was equipped with a binary pump with an integrated vacuum degasser (G4220A) and an autosampler (G4226A). The HPLC parameters are shown in Table 1.

Table 1.   HPLC Parameters

Instrument	Agilent 1290 Infinity Binary LC System
Mobile phases	(A) acetonitrile (B) 0.1% formic acid in water
Gradient	Linear: Initial mobile phase composition was 10% A, held constant for 1.7 minutes, followed by a linear gradient to 100% A, for a total run time of 10 minutes.
Flow rate	0.4 mL/min
Column	Agilent ZORBAX Eclipse Plus reversed phase C18 analytical column, 50 × 2.1 mm, 1.8 µm particle size (p/n 959741-902)
Column temperature	25 °C
Injection volume	20 µL

Q-TOF MS accurate mass spectra were recorded across the range 30–1,000 *m/z* at 2 GHz. Polarity switching was not used; samples were injected twice, one under positive ion mode and the other under negative ion mode. MS/MS experiments were also carried out in both positive and negative ion modes. The Q-TOF MS and MS/MS parameters are shown in Table 2.

Table 2.   Q-TOF MS and MS/MS Parameters

Instrument	Agilent 6540 Accurate-Mass Q-TOF LC/MS
Ionization mode	Positive and negative ESI with Agilent Jet Stream technology
Mass range	30–1,000 *m/z* at 2 GHz
Drying gas temperature	250 °C
Drying gas flow rate	10 L/min
Sheath gas temperature	350 °C
Sheath gas flow rate	11 L/min
Nebulizer gas	45 psi
Skimmer voltage	65 V
Octopole RF	750 V
Fragmentor	190 V
Capillary	3,500 V
Nozzle	1,500 V (negative mode) or 0 V (positive mode)
MS/MS parameters	
Targeted MS/MS	Precursors: sodiated (positive ion) and deprotonated molecule (negative ion)
Isolation width	Medium (~4 *m/z*)
Collision energies	10, 20, and 40 eV

A reference solution containing the internal reference masses (purine ($C_5H_4N_4$) at *m/z* 121.0509 and HP-921 [hexakis-(^{1}H,^{1}H,^{3}H-tetrafluoro-pentoxy)phosphazene] ($C_{18}H_{18}O_6N_3P_3F_{24}$) at *m/z* 922.0098 in positive ion mode, and *m/z* 119.0363 and 966.0007 (formate adduct) in negative ion mode), was delivered by an external quaternary pump.

Stability of mass accuracy was checked daily, and if values went above 2 ppm error, the instrument was recalibrated.

Data analysis

The accurate mass Q-TOF MS and MS/MS data was processed using Agilent MassHunter Workstation Software. Agilent MassHunter Molecular Structure Correlator (MSC) Software was used to draw and investigate the fragmentation pathways for the negative and positive ion MS/MS analyses.

Results and Discussion

Q-TOF LC/MS results

Figure 1 shows the positive and negative ion ESI mass spectra obtained for the sucralose standard using Q-TOF LC/MS. In negative ion mode (Figure 1A), the sucralose molecule lost a proton, forming the base peak with the exact mass of *m/z* 395.0073 (mass error 0.0 ppm). The ions at *m/z* 397.0045 and 399.0018 correspond to the Cl-37 isotopes of sucralose.

In positive ion mode (Figure 1B), the sucralose molecule adducted a sodium ion, forming the ion with a measured accurate mass of *m/z* 419.0040. The measured mass was within 0.5 ppm of the exact mass of *m/z* 419.0038. In this case, a protonated molecule was not formed. The two chlorine isotopes were found at *m/z* 421.0012 and 422.9988.
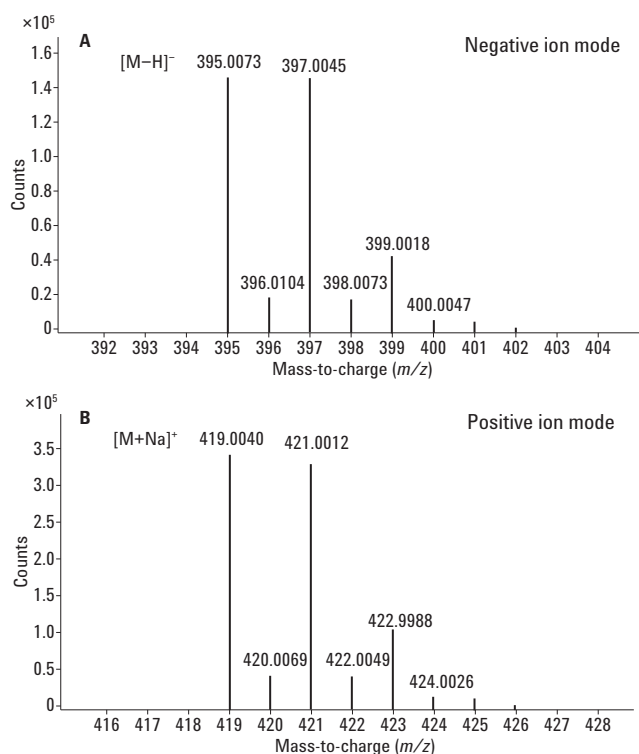

Figure 1.   Results from Q-TOF LC/MS analysis of sucralose in (A) negative ion mode and (B) positive ion mode, showing measured accurate masses. In positive ion mode, sucralose was detected as its sodium adduct [M+Na]⁺ at m/z 419.0040. In negative ion mode, the deprotonated molecule [M−H]⁻ was observed at m/z 395.0073.

Because the sucralose molecule is hydrogen-rich, allowing a proton shift as needed, the charged sodium can undergo a neutral loss. This mode of fragmentation can be called a sodium migration fragmentation, which is quite rare since sodium adducts are known to be quite difficult, if not impossible, to fragment to produce structurally significant ions [2]. This phenomenon is not commonly reported for ESI of sodium adducts, and has been underestimated for the detection of sucralose using positive ESI.

Figure 2 shows the difference in signal obtained from the sucralose standard using positive and negative ESI Q-TOF LC/MS. The signal intensity obtained in positive ion mode was approximately twice that of negative ion mode. Still, the ratio of signal-to-noise was slightly better in negative ion mode because background ions were less abundant.

Figure 3 shows the accurate mass spectrum obtained by applying Q-TOF MS/MS fragmentation to the sodium adduct of sucralose (positive ion mode). Two characteristic masses were obtained: m/z 221.0190 and 238.9853.

Proposed MS/MS fragmentation pathways

Figure 4 shows the proposed fragmentation pathways for negative and positive ion MS/MS. In positive ion mode, the sodium adducted sucralose ion splits into two saccharide fragments, each of which retains the sodium ion. The sodium ion migrates to either the glucose or fructose side of the molecule, giving rise to the ions at m/z 221.0187 and 238.9848. In negative ion analyses, sucralose fragments by the loss of HCl, which gives rise to the ion at m/z 359.0306. The chemical structures were easily confirmed by the Q-TOF accurate mass measurements with the help of the MSC software.
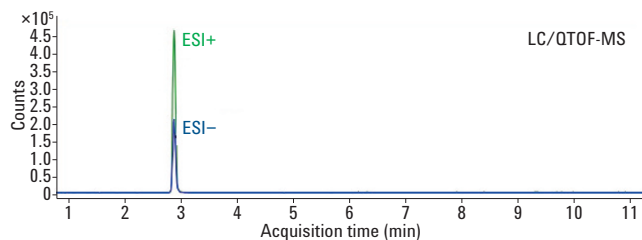

Figure 2. Q-TOF LC/MS analysis of the sucralose standard, comparing the signal intensity obtained in positive and negative ion modes.
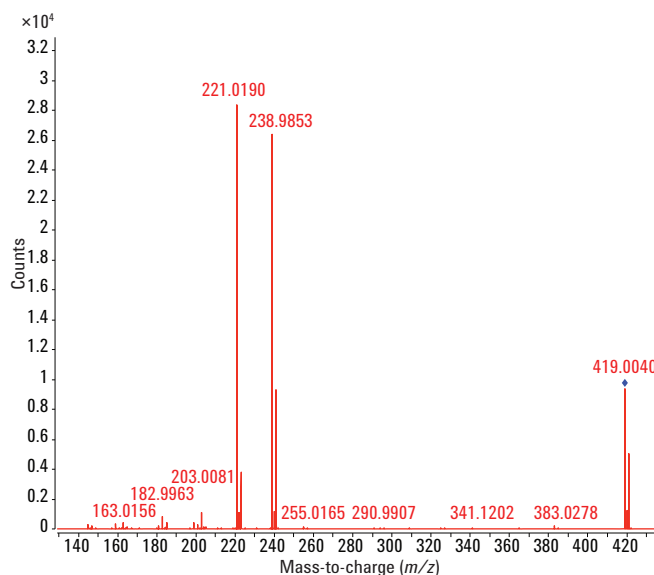

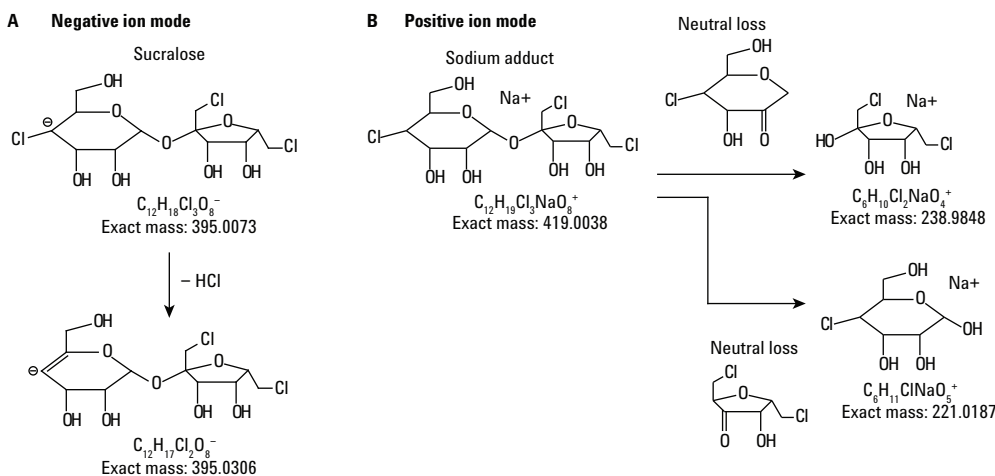Figure 3. Accurate mass MS-MS spectrum of the sodium adduct of sucralose (positive ion mode).

Figure 4. Proposed fragmentation pathways for negative and positive ion MS/MS of sucralose showing exact masses.

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As shown in Figures 5 and 6, the fragmentation pathways were investigated using the MSC software. The software correlates accurate mass MS/MS fragment ions of the compound of interest with one or more proposed molecular structures for that compound. MSC accomplishes this by trying to explain each observed fragment ion into the proposed structure using a "systematic bond-breaking" approach.

The input for the MSC software is an accurate mass MS/MS fragment spectrum, a molecular formula for the compound of interest, and candidate molecular structures. The user can input a molecular formula or structure manually, or select the most probable structure from the possible molecular formulas that the MSC calculates using the accurate mass MS and MS/MS information. The MSC then uses the selected formula, retrieves one or multiple possible structures from a .mol file, an .sdf file, a MassHunter compound database (PCD, PCDL), or ChemSpider (over the Internet), and scores how

well each candidate structure correlates with the MS/MS spectrum.

Because the MSC software does not currently handle sodiated ions, it was necessary to draw the structure of sucralose with the sodium on the glucose ring; the upper structure shown in the middle Figure 5. The fragment ion is shown on the far right. The row in the table on the far right, highlighted in blue, shows the measured mass of m/z 221.0189 that corresponded to the proposed structure for that fragment ion.

The structure of sucralose with sodium on the fructose ring was also drawn using the MSC software (Figure 6). The highlighted portion of the sucralose molecule in the far right box shows the only proposed structure of the fragment ion at m/z 238.9852 (the blue highlighted row in the table above the fragment structure.)

Figure 5.   MSC analysis showing the fragment of the sodiated molecule of sucralose with the sodium on the glucose ring.

Compound formula

[Add]

M = 417.9965; 6 formula candidates from MFG

ID	Formula	Isomers	Taut. Grps	dM(ppm)	IdM(ppm)I	Product
1	C12H18Cl3NaO8	0	0	-0.1	0.1	100
2	Cl2H21NaO19	0	0	-3.2	3.2	85
3	C18H13Cl2NaO6	0	0	5.2	5.2	100
4	C19H7NaO10	0	0	-6.8	6.8	80
5	C12H11NaO15	0	0	7.3	7.3	9
6	C25H9Cl2NaO	0	0	-8.9	8.9	34

Fragment formulas for C12H18Cl3NaO8

#	m/z	intensity	norm. intens.	formula	dM(ppm)
1	238.9851	50983.32	100.00	C6H10Cl2NaO4	-1.1
2	238.9851	50983.32	100.00	C8H9Cl2O4	9.0
3	238.9851	50983.32	100.00	Cl3H15NaO6	-10.3
4	221.0188	48926.17	95.97	C6H11ClNaO5	-0.4
5	221.0188	48926.17	95.97	Cl2H16NaO7	-10.3
6	221.0188	48926.17	95.97	C8H10ClO5	10.5
7	203.0082	3773.26	7.40	C6H9ClNaO4	-0.2
8	203.0082	3773.26	7.40	Cl2H14NaO6	-11.0
9	85.0288	1337.76	2.62	C4H5O2	-4.6
10	85.0288	1337.76	2.62	C2H6NaO2	-32.9
11	200.9898	823.28	1.61	Cl2H12NaO6	2.6
12	200.9898	823.28	1.61	C6H7ClNaO4	13.5
13	185.0421	821.51	1.61	C6H10NaO5	-0.3
14	185.0421	821.51	1.61	ClH15NaO7	-12.2
15	220.9744	751.14	1.47	C6H8Cl2NaO3	-0.6
16	220.9744	751.14	1.47	Cl3H13NaO5	-10.5
17	205.0059	700.03	1.37		
18	198.9918	644.53	1.26	C6H9Cl2O3	2.6
19	163.0149	635.19	1.25	C6H8ClO3	4.6
20	163.0149	635.19	1.25	C4H9ClNaO3	-10.2
21	145.0053	295.24	0.58	C6H6ClO2	-1.5
22	145.0053	295.24	0.58	C4H7ClNaO2	-18.1
23	121.0045	267.87	0.53	C4H6ClO2	4.8
24	182.9959	257.45	0.50	C6H9ClO2	8.3
25	180.9815	247.50	0.49	C6H7Cl2O2	1.4

Structure Search

[Parameters] Compatibles/Total: 0/0 [Add Structure]

ChemSpider (Web) ▼ [Go]   Sort by [Score] ▼ [Sort]

1
Standard InChIKey:
KJMZLKSPWHEFOI-LMUDQJKSSA-N
Score: 91.24
[MSC] [Save] [Delete]

2
Standard InChIKey:
GDXGWEFZTJCVAA-LMUDQJKSSA-N
Score: 94.70
[MSC] [Save] [Delete]

Fragments of structure #2 -- elucidated: 86.7% ions , 99.4% Weigh

#	Mass	Intensity	Weight(%)	No. of candid.	Best score
1	238.9851	50983.32	56.6	12	99.4
2	221.0188	48926.17	34.0	18	89.4
3	203.0082	3773.26	1.6	7	98.5
4	85.0288	1337.76	0.0	9	90.6
5	200.9898	823.28	0.3	5	74.9
6	185.0421	821.51	0.2	3	92.7
7	220.9744	751.14	0.5	4	98.5
8	205.0059	700.03	0.3	0	0.0
9	198.9918	644.53	0.2	7	97.7
10	163.0149	635.19	0.1	17	92.5
11	145.0053	295.24	0.0	10	87.3
12	121.0045	267.87	0.0	13	86.4
13	182.9959	257.45	0.1	3	91.5
14	180.9815	247.50	0.1	3	94.0

Penalty=1.0 dM=-1.1ppm Score=99.4
C6H9Cl2NaO4+H
1 ◇ Of 1

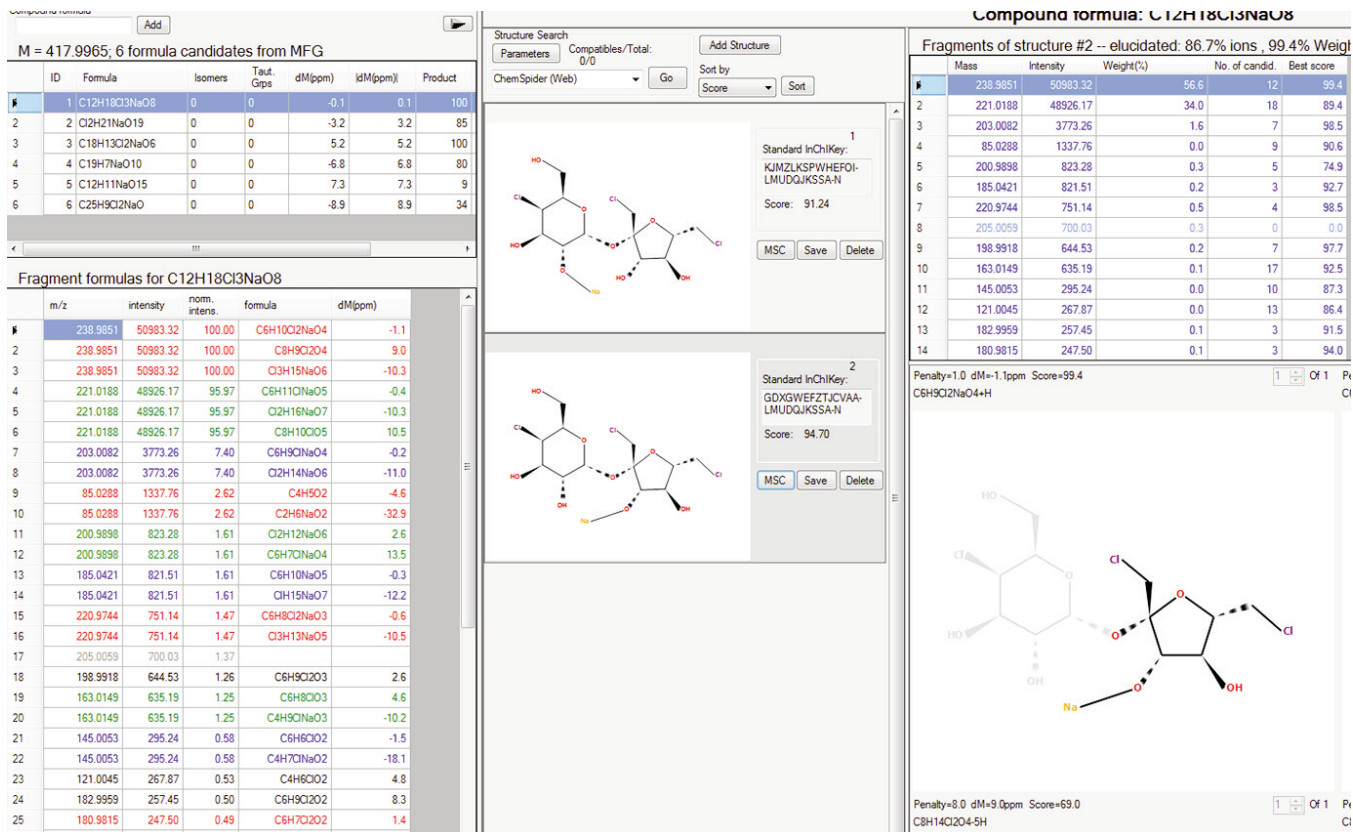Penalty=8.0 dM=9.0ppm Score=69.0
C8H14Cl2O4-5H
1 ◇ Of 1

Figure 6.   MSC analysis showing the fragment of the sodiated molecule of sucralose with the sodium on the fructose ring.

Figure 7 shows the Q-TOF MS/MS spectrum for sucralose in negative ion mode. The ion at m/z 359.0307 represents the loss of HCl, and is a major fragment ion for this compound. The ion at m/z 231.9874 is a complex re-arranged sucralose fragment.
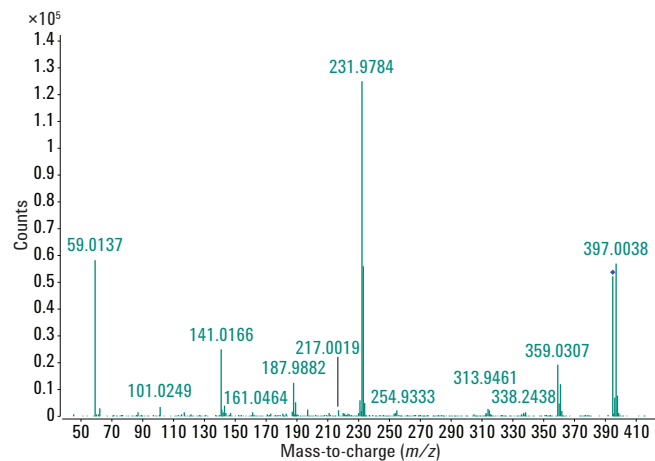

Figure 7.   MS/MS spectrum of sucralose (negative ion mode.)

Figure 8 shows the MSC analysis of the fragment ion at
m/z 359.0307. Both structures are compatible with the loss of
HCl. Many of the structures in ChemSpider match that of
sucralose; however, for sucralose to be the number one hit in
the MSC software, the structures need to be sorted by
number of literature references, not the compatibility score
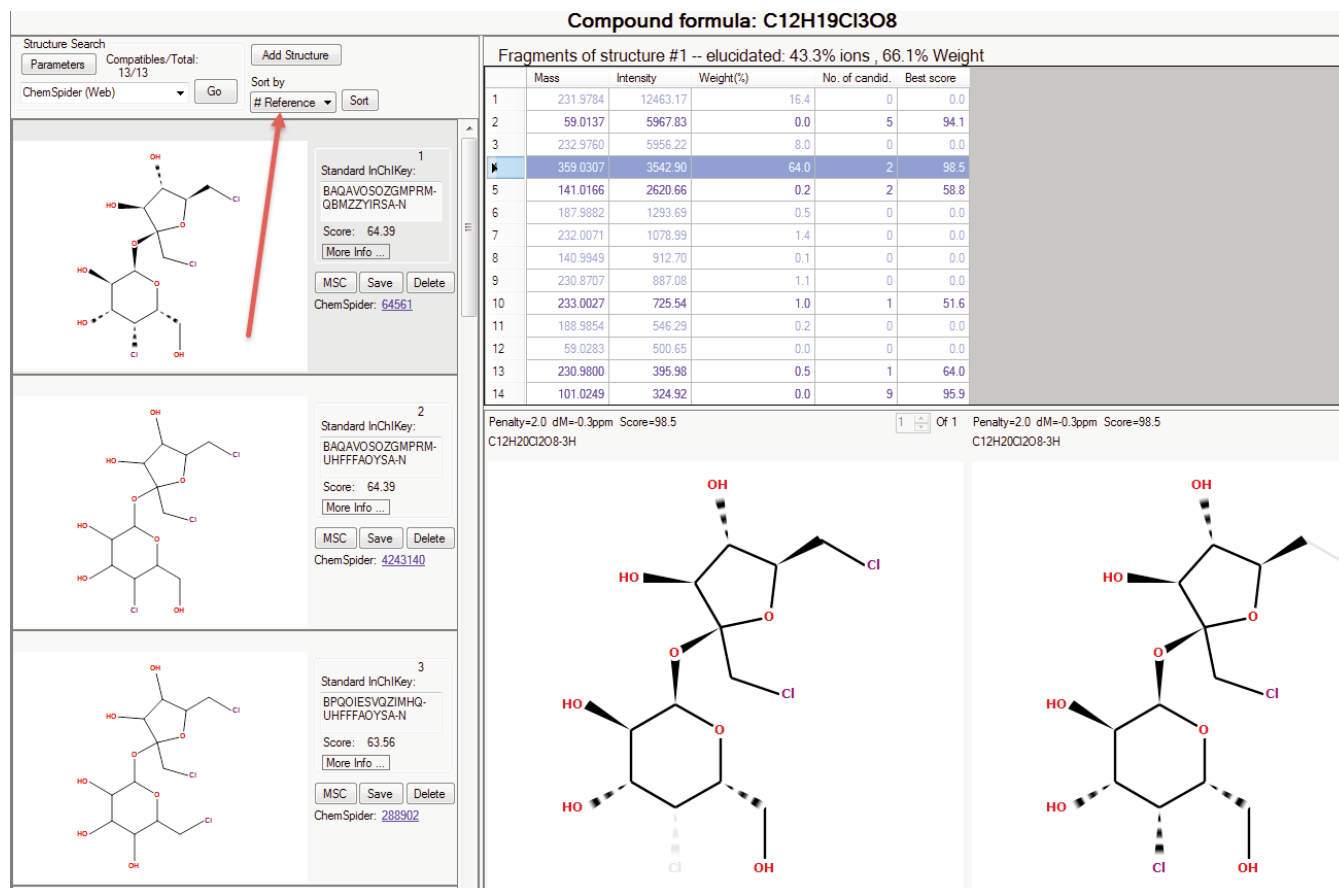(red arrow in Figure 8.)

Figure 8.   MSC analysis of the Q-TOF MS/MS spectrum obtained in negative ion mode. When searching ChemSpider, all structures have same score. Sorting by
number of literature references (# references) raises sucralose to the first (top) position (see red arrow).

Conclusions

Sucralose responded well using Q-TOF LC/MS when operated in either the positive or negative ESI mode. Sucralose formed a strong sodium adduct in positive ion mode and readily lost a proton in negative ion mode. The overall signal intensity obtained in positive ion mode was approximately twice that of negative ion mode. As demonstrated in the complementary study of detection methodologies for sucralose in water, for the triple quadrupole LC/MS MRM method, sensitivity was higher in the positive ion mode (using the two transitions shown in this application note), than in the negative ion mode [2].

Contrary to what is commonly reported for ESI analyses of sodium adducts, the strong sodium adduct formed in the positive ion mode was easily fragmented by MS/MS. The two characteristic accurate mass fragments produced can be used to identify sucralose unequivocally. The MSC software is a useful tool to assist with the characterization of fragment ion structures.

References

1. A.K.E Wiklund, M. Breitholtz, B.E. Bengtsson, and M. Adolfsson-Erici "Sucralose – An ecotoxicological challenger?" *Chemosphere* 2012, **86**, 50–55.

2. I. Ferrer, J.A. Zweigenbaum, and E.M. Thurman "Analytical Methodologies for the Detection of Sucralose in Water" *Analytical Chemistry* 2013, **85**, 9581-9587.

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