

**Acquity I-class TQ-XS Demo sample
analysis report**
-- *Group 2: Sweeteners*

Introduction

AVA has provided us with four groups of samples.

- Group -1 : Sudan Dyes
 - Group -2 : Sweeteners
 - Group -3 :Marine Biotoxins List A
 - Group -4 :Marine Biotoxins List B
-
- This system must be able to run a variety of applications, including a sensitive assay for all the group of compounds mentioned in the above samples.
 - This report demonstrates the potential for the I-Class FTN/XEVO TQ-S to provide a robust and sensitive results for the above mentioned compounds.
 -

- To detect composition of the sweetener standard solution
- To show linearity of the calibration curves in both food matrix and neat solvent
 - *Sample Blank – Z(Blank)*
 - *Spiked Extract 1 – Z(S1)*
 - *Spiked Extract 2 – Z(S2)*
 - *Sweetener standard standards – 100 ppb (Std Z) Solvent: 30% Acetonitrile in water*

Executive Summary

- Five sweeteners were successfully identified, they were Advantame, Acesulfame K, Saccharin, Aspartame, and Sucralose (Table 1)
- The SRM-derived quantitation plots for all the detected sweeteners were linear ($R^2 > 0.99$) in both matrix matched and neat solution standard curve (Table 2 & Table 3)
- Based on the matrix matched standard curves, the respective LLOQs for all the detected sweeteners except for Sucralose were 10 ppt
- The calculated instrument lower detection limits (LLOD) of all the detected sweeteners were in the range of 2 to 25 ppt in food matrix

Executive Summary (Continued)

- The fast polarity switching in Xevo® TQ-XS mass spectrometer allowed multi-analyte analysis based on very short UPLC gradient (8 min) without sacrificing MS duty cycle and data sampling points to achieve accurate quantification
- The ACQUITY UPLC system with small particle (sub-2 μm) column packing generated extremely narrow peaks (FWHM < 3 s), which allowed the MS system to generate enough data points (> 15) for accurate quantification
- MassLynx software provided integrated solution from method editing, data acquisition, to data processing and report generation

The background features a light green to yellow gradient. In the upper half, there are thin, light green circuit-like lines. The lower half is filled with a pattern of white and yellow dots of varying sizes, creating a halftone or dot-matrix effect. A large, semi-transparent white geometric shape, resembling a stylized 'A' or a large triangle, is centered behind the text.

Results

Identification & Quantification of Sweeteners in Spiked Extract 1 & 2

Table 1. The calculated concentration of detected sweeteners using matrix matched calibration curve

Sweetener	RT (min)	S1 (ppb)	S2 (ppb)
Advantame	1.54	1.04	0.49
Acesulfame K	1.03	0.93	0.43
Saccharin	1.09	1.18	0.46
Aspartame	1.15	1.00	0.45
Sucralose	1.21	9.37	5.29

Linearity of Calibration Curve & LLOQ / LLOD in Matrix

Table 2. The linearity of calibration curve for the detected sweeteners using matrix blank as diluent

Sweetener	Linearity (R^2) ¹	LLOQ (ppb)	S/N at LLOQ	Cal. LLOD (ppb) ²
Advantame	0.999	0.01	24	0.002
Acesulfame K	0.999	0.01	13.9	0.003
Saccharin	0.999	0.01	6.0	0.005
Aspartame	0.998	0.01	1.9	0.01
Sucralose*	0.999	0.5	63.5	0.025

Note: ¹ The calibration curve was ranging from 0.01 ppb to 10 ppb except for Sucralose, the calibration points were, 0.01, 0.05, 0.1, 0.5, 1, 5 and 10 ppb

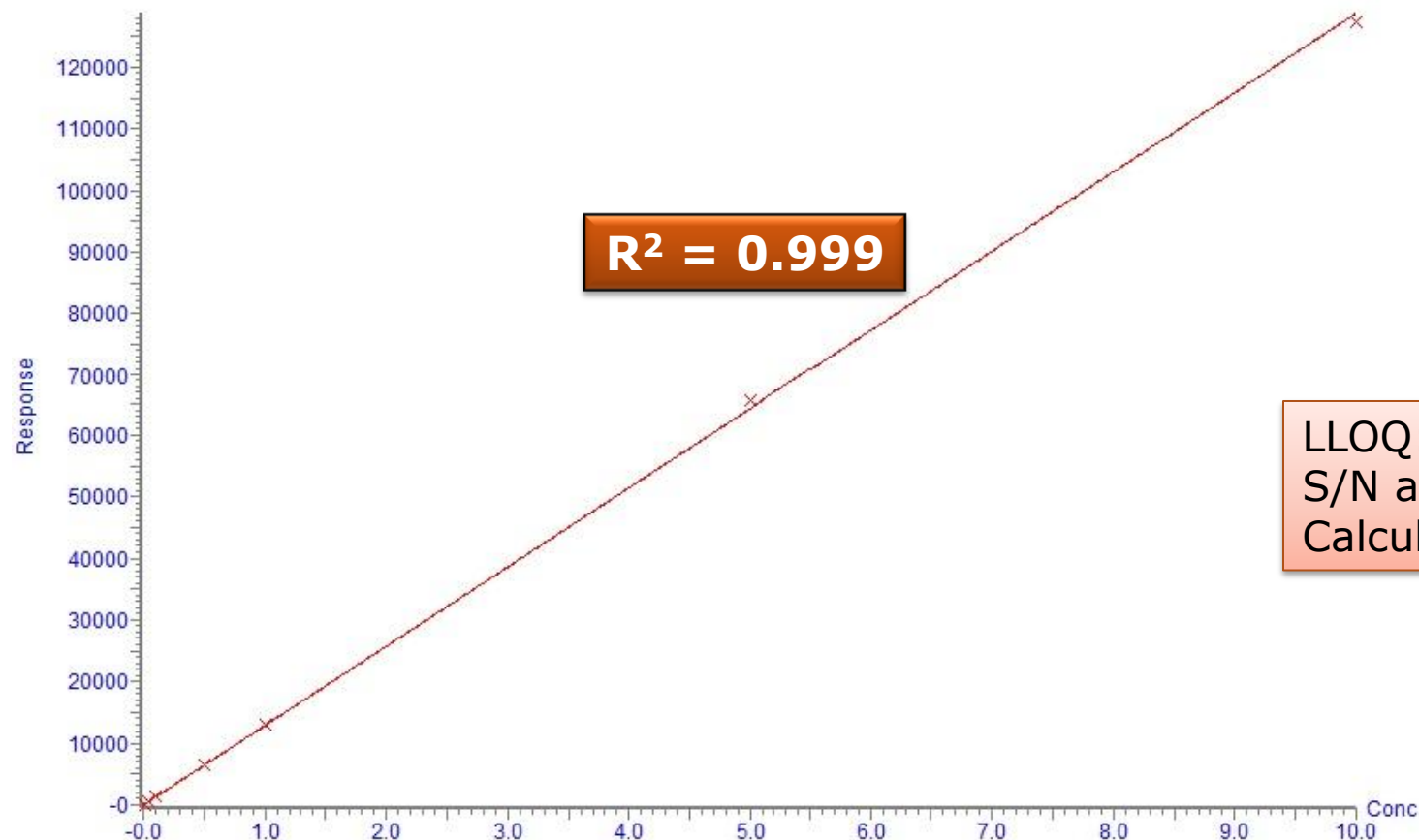
² Calculated LLOD based on the Peak signal-to-noise of LLOQ

* The calibration curve for Sucralose was ranging from 0.5 ppb to 10 ppb

Matrix Matched Standard Curve of *Advantame*

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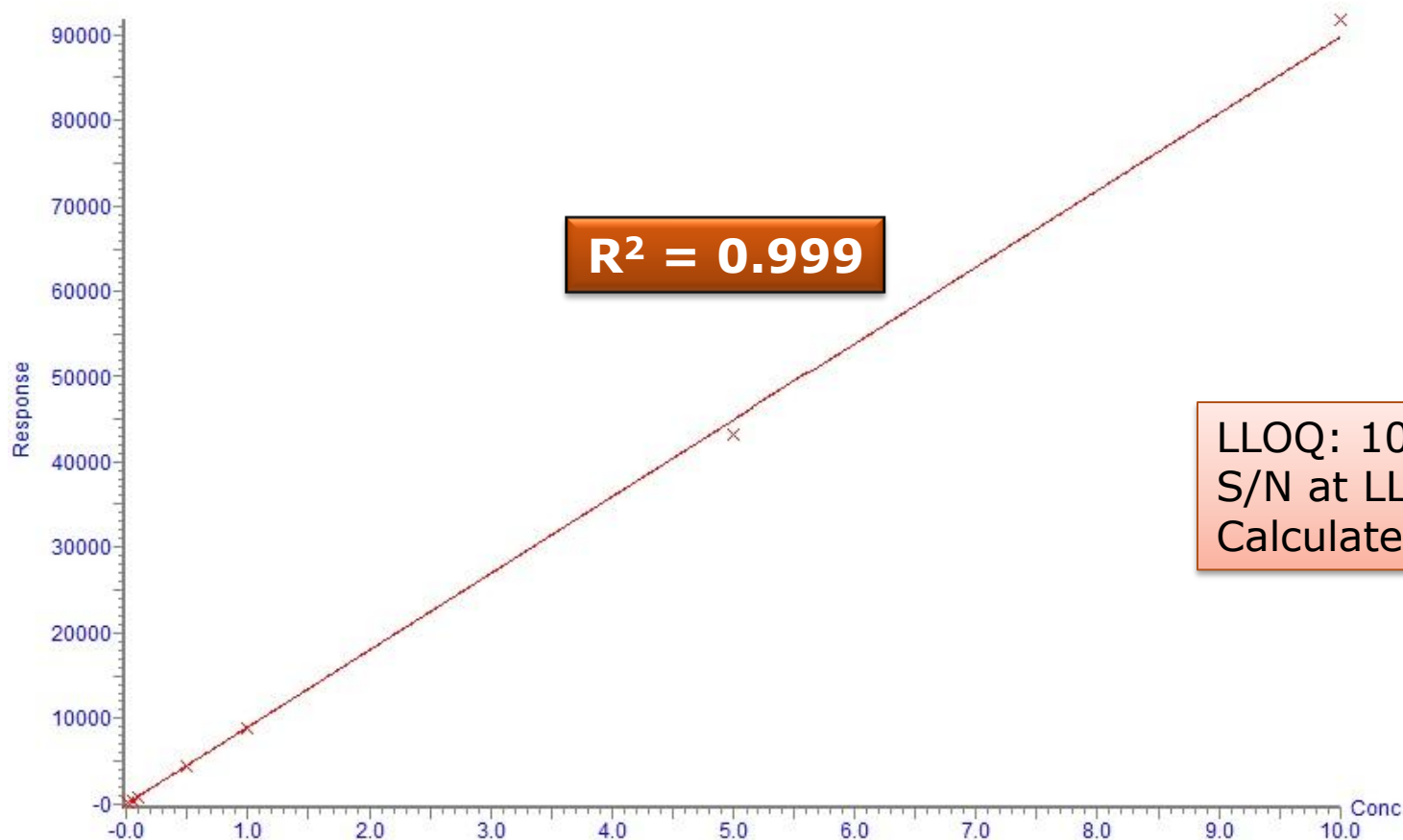
Compound name: ADVANTAME
Correlation coefficient: $r = 0.999896$, $r^2 = 0.999791$
Calibration curve: $12875 * x + -23.3868$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None



LLOQ: 10 ppt
S/N at LLOQ: 24
Calculated LLOD: 2 ppt

Matrix Matched Standard Curve of *Acesulfame K*

Compound name: ACESULFAME
Correlation coefficient: $r = 0.999577$, $r^2 = 0.999154$
Calibration curve: $8973.46 * x + 33.9084$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

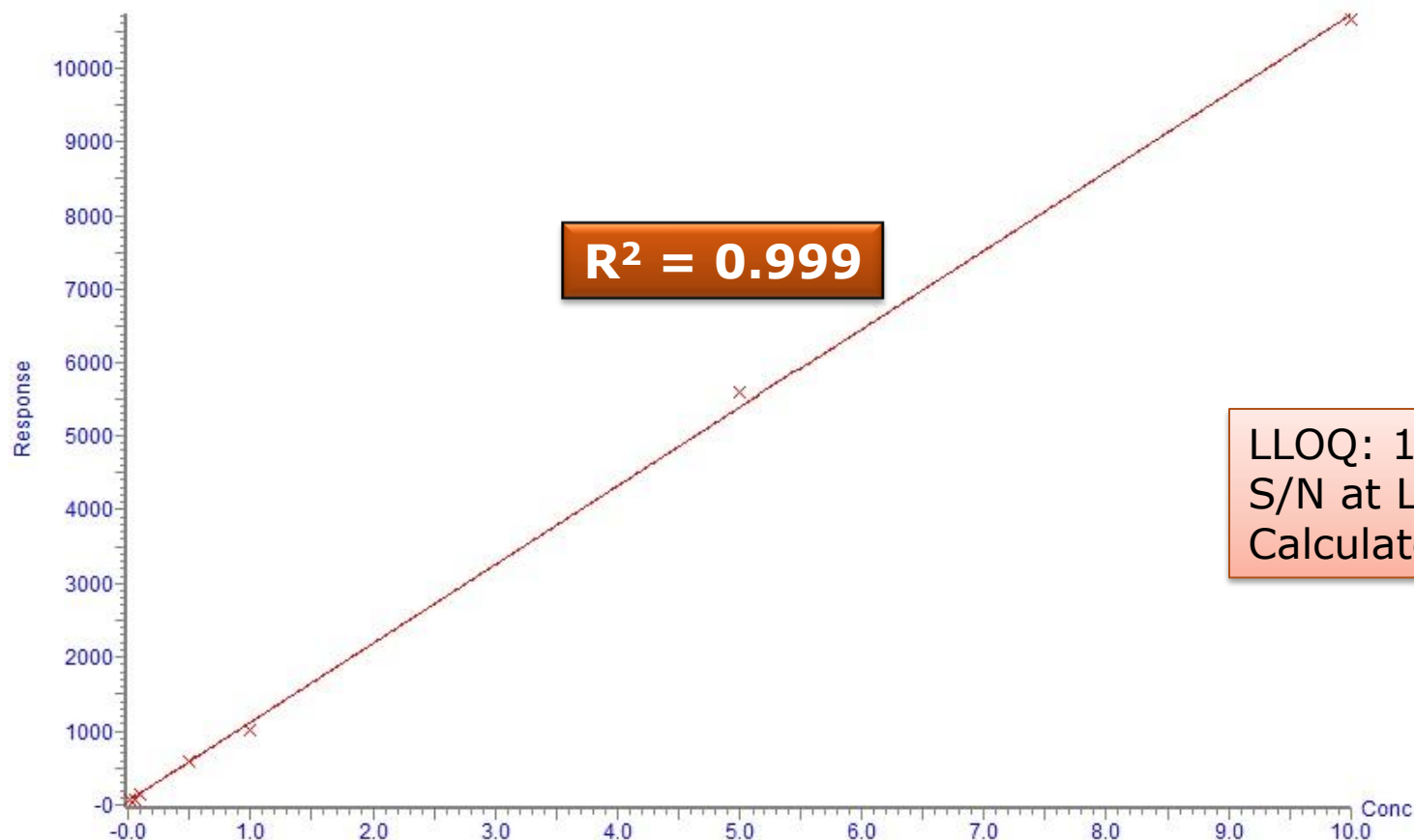


LLOQ: 10 ppt
S/N at LLOQ: 13.9
Calculated LLOD: 2.5 ppt

Matrix Matched Standard Curve of *Saccharin*

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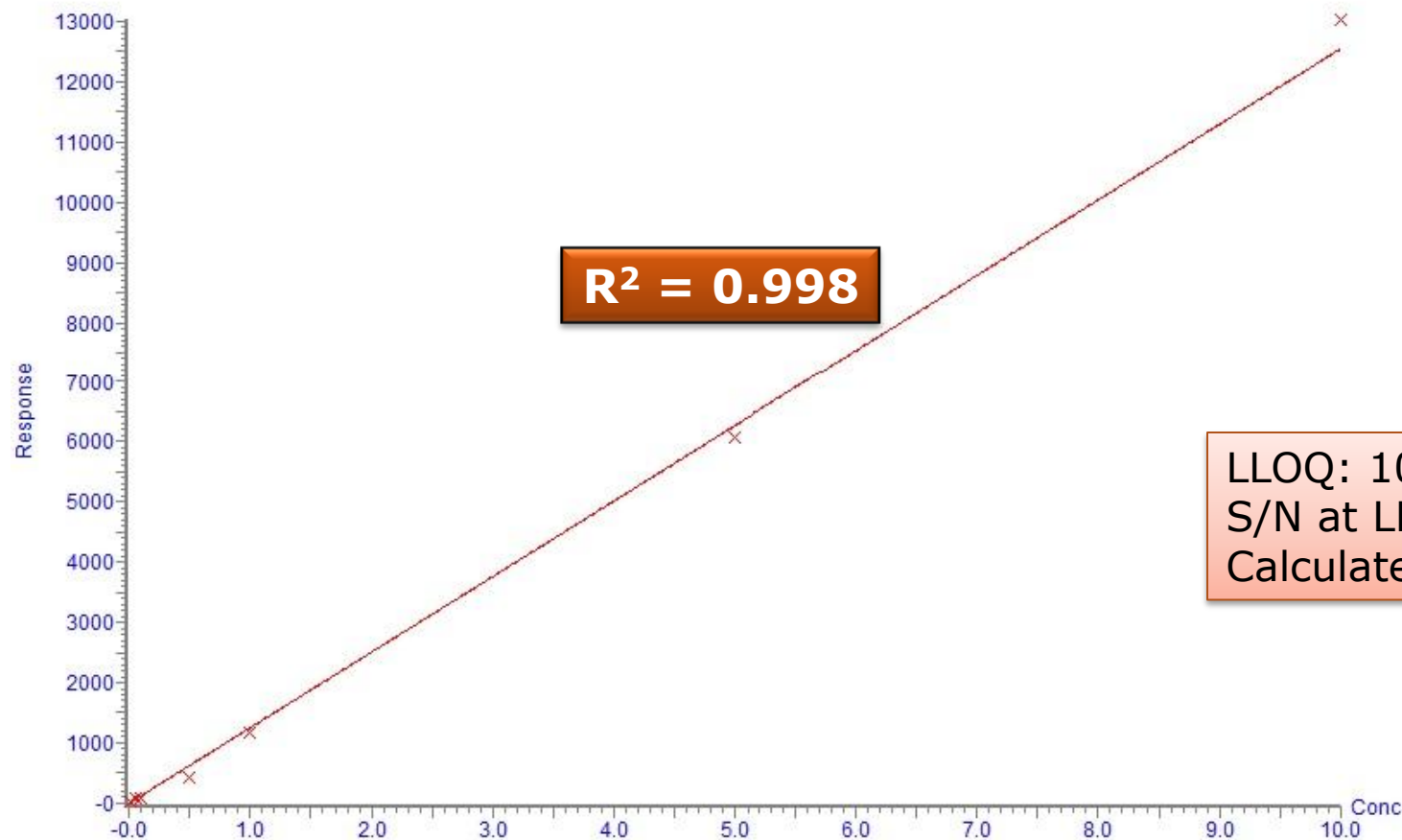
Compound name: SACCHARIN
Correlation coefficient: $r = 0.998954$, $r^2 = 0.997910$
Calibration curve: $1067.49 * x + 51.8519$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None



LLOQ: 10 ppt
S/N at LLOQ: 6
Calculated LLOD: 5 ppt

Matrix Matched Standard Curve of *Aspartame*

Compound name: ASPARTAME
Correlation coefficient: $r = 0.997599$, $r^2 = 0.995205$
Calibration curve: $1255.47 * x + -4.60137$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

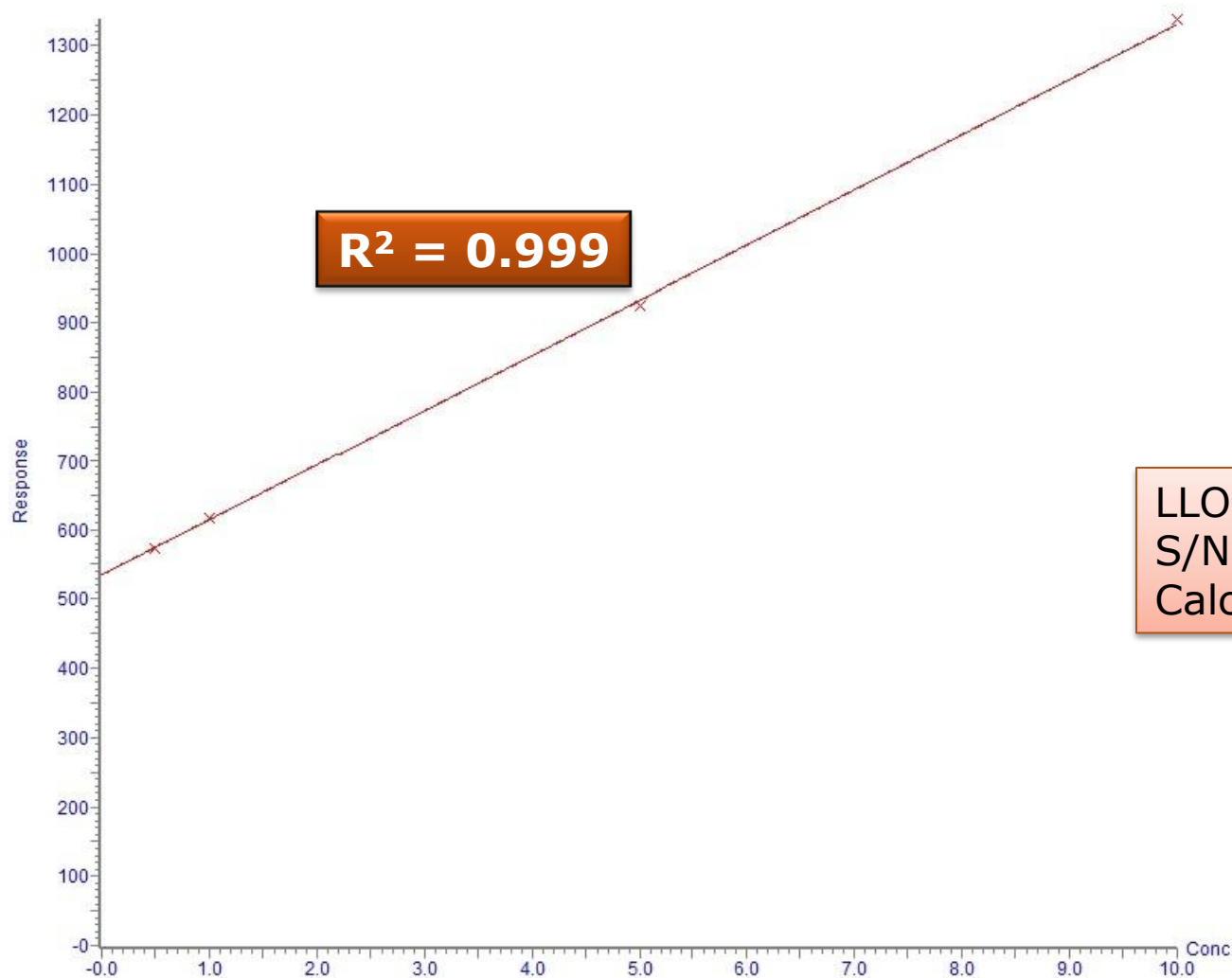


LLOQ: 10 ppt
S/N at LLOQ: 1.9
Calculated LLOD: 10 ppt

Matrix Matched Standard Curve of *Sucralose*

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Compound name: SUCRALOSE
Correlation coefficient: $r = 0.999793$, $r^2 = 0.999587$
Calibration curve: $79.6253 * x + 534.778$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None



LLOQ: 50 ppt
S/N at LLOQ: 63.5
Calculated LLOD: 2.5 ppt

Chromatogram of *Advantame* in Matrix

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0.1 ppb

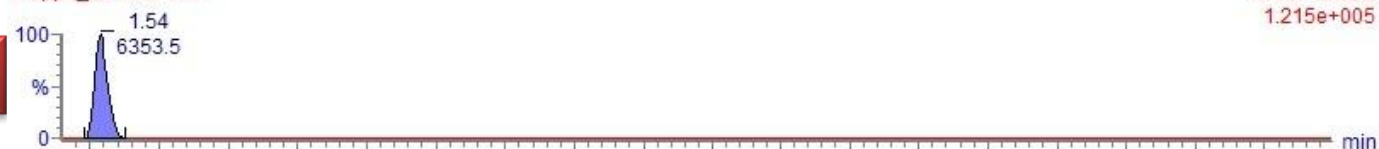
23102016_Sweetener1_Batch3_M1_20
0.1ppb_BZ STD 133



F1:MRM of 2 channels,ES+
459.2 > 252.2
2.444e+004

0.5 ppb

23102016_Sweetener1_Batch3_M1_21
0.5ppb_BZ STD 134



F1:MRM of 2 channels,ES+
459.2 > 252.2
1.215e+005

1 ppb

23102016_Sweetener1_Batch3_M1_22
1ppb_BZ STD 135



F1:MRM of 2 channels,ES+
459.2 > 252.2
2.481e+005

5 ppb

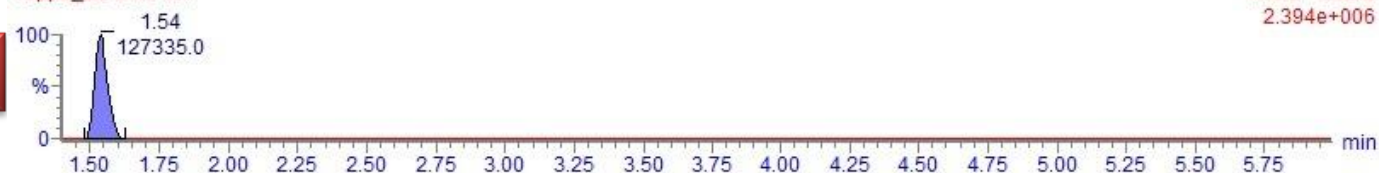
23102016_Sweetener1_Batch3_M1_23
5ppb_BZ STD 136



F1:MRM of 2 channels,ES+
459.2 > 252.2
1.265e+006

10 ppb

23102016_Sweetener1_Batch3_M1_24
10ppb_BZ STD 137



F1:MRM of 2 channels,ES+
459.2 > 252.2
2.394e+006

The retention time (RT) and peak response area are shown on peak top of the chromatogram

Chromatogram of *Acesulfame K* in Matrix

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0.1 ppb

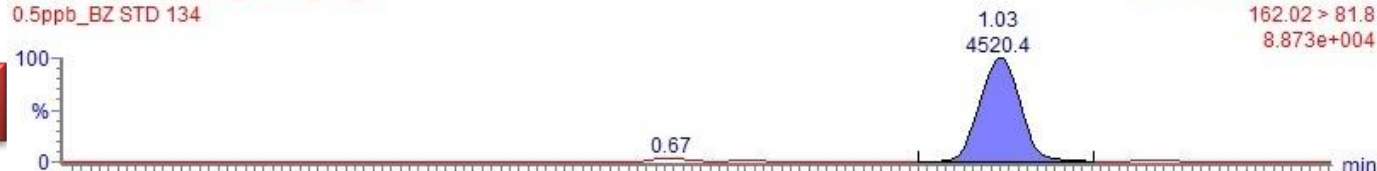
23102016_Sweetener1_Batch3_M1_20
0.1ppb_BZ STD 133



F2:MRM of 2 channels,ES-
162.02 > 81.8
1.501e+004

0.5 ppb

23102016_Sweetener1_Batch3_M1_21
0.5ppb_BZ STD 134



F2:MRM of 2 channels,ES-
162.02 > 81.8
8.873e+004

1 ppb

23102016_Sweetener1_Batch3_M1_22
1ppb_BZ STD 135



F2:MRM of 2 channels,ES-
162.02 > 81.8
1.645e+005

5 ppb

23102016_Sweetener1_Batch3_M1_23
5ppb_BZ STD 136



F2:MRM of 2 channels,ES-
162.02 > 81.8
7.743e+005

10 ppb

23102016_Sweetener1_Batch3_M1_24
10ppb_BZ STD 137



F2:MRM of 2 channels,ES-
162.02 > 81.8
1.663e+006

The retention time (RT) and peak response area are shown on peak top of the chromatogram

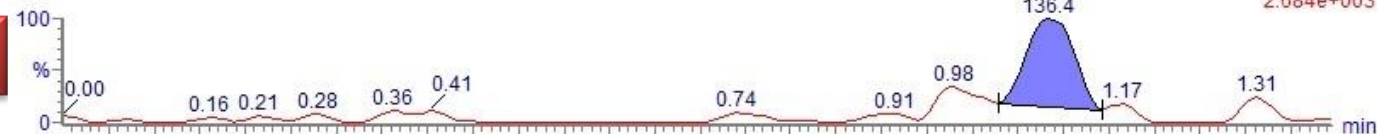
Chromatogram of *Saccharin* in Matrix

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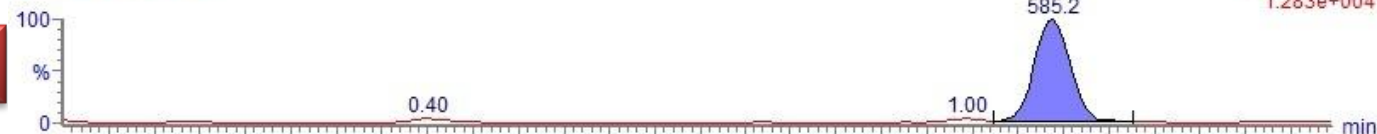
0.1 ppb

23102016_Sweetener1_Batch3_M1_20
0.1ppb_BZ STD 133



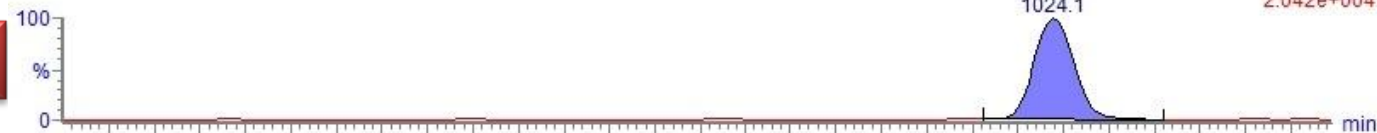
0.5 ppb

23102016_Sweetener1_Batch3_M1_21
0.5ppb_BZ STD 134



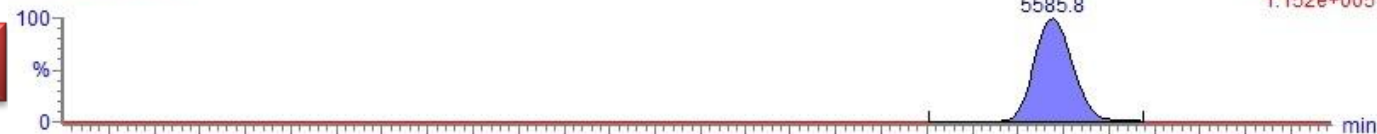
1 ppb

23102016_Sweetener1_Batch3_M1_22
1ppb_BZ STD 135



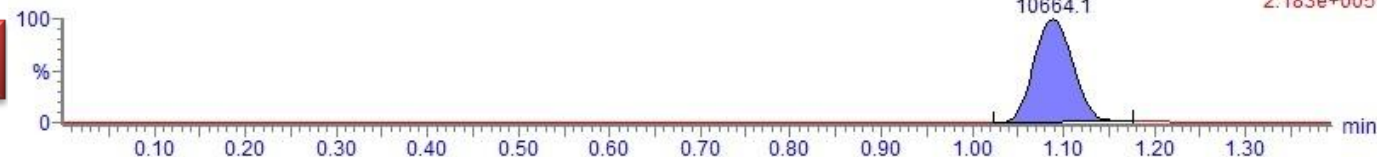
5 ppb

23102016_Sweetener1_Batch3_M1_23
5ppb_BZ STD 136



10 ppb

23102016_Sweetener1_Batch3_M1_24
10ppb_BZ STD 137



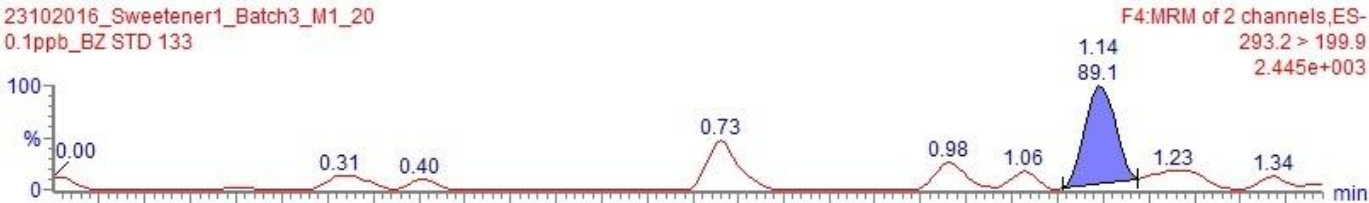
The retention time (RT) and peak response area are shown on peak top of the chromatogram

Chromatogram of *Aspartame* in Matrix

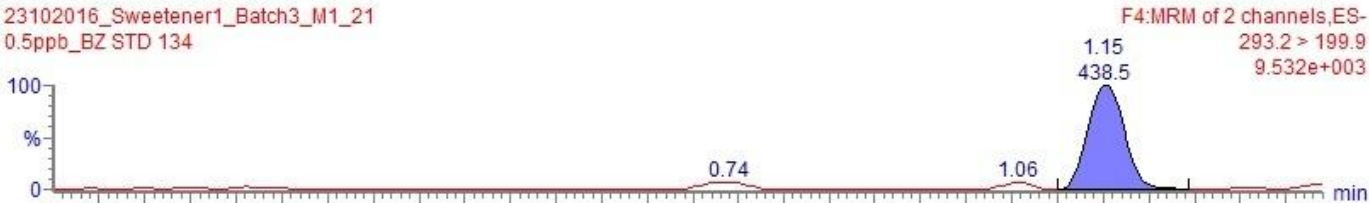
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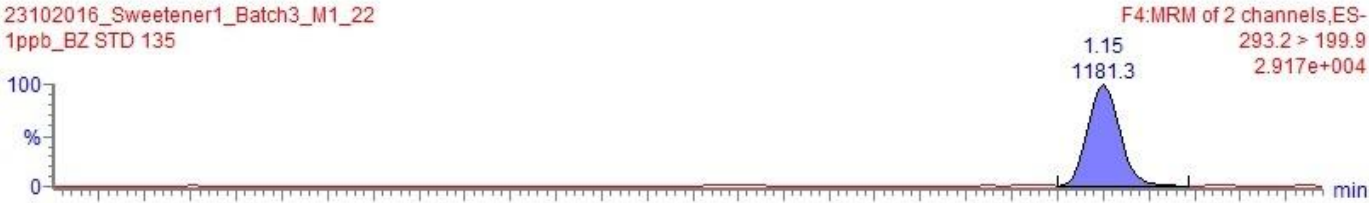
0.1 ppb



0.5 ppb



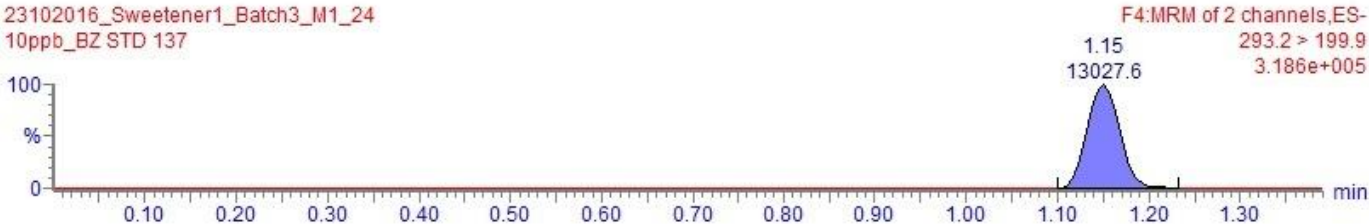
1 ppb



5 ppb



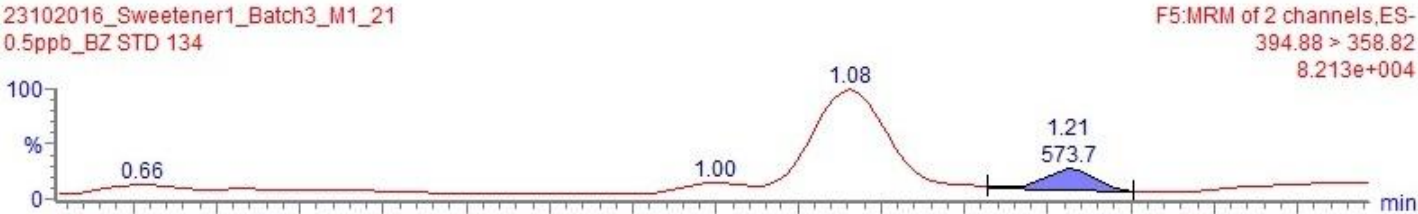
10 ppb



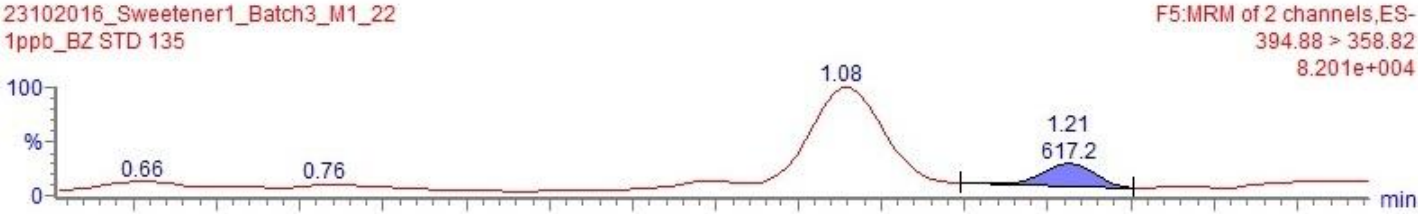
The retention time (RT) and peak response area are shown on peak top of the chromatogram

Chromatogram of *Sucralose* in Matrix

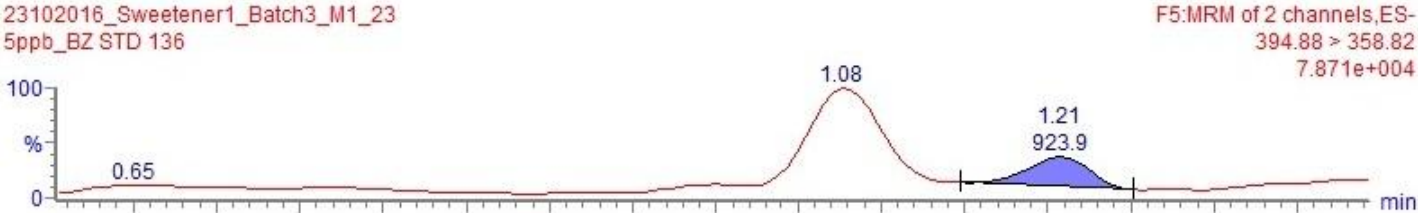
0.5 ppb



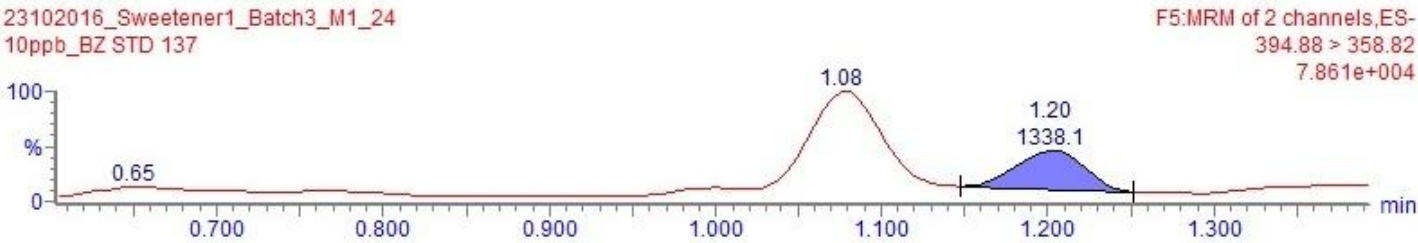
1 ppb



5 ppb



10 ppb



The retention time (RT) and peak response area are shown on peak top of the chromatogram

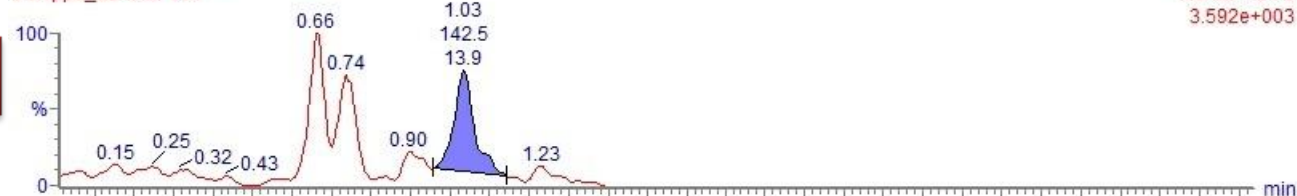
Peak Signal-to-Noise of Detected Sweeteners at LOQ Level (0.01 ppb)

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Acesulfame K

23102016_Sweetener1_Batch3_M1_18
0.01ppb_BZ STD 131

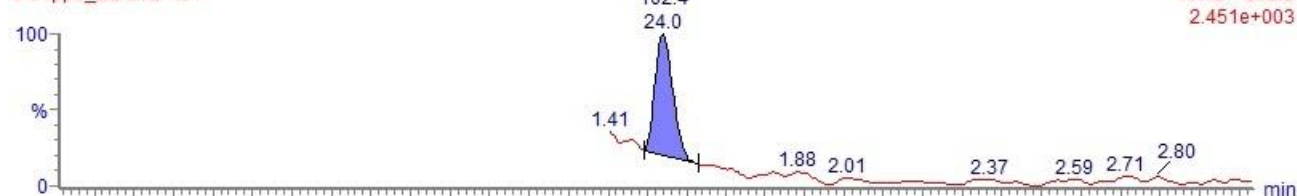
F2:MRM of 2 channels,ES-
162.02 > 81.8
3.592e+003



Advantame

23102016_Sweetener1_Batch3_M1_18
0.01ppb_BZ STD 131

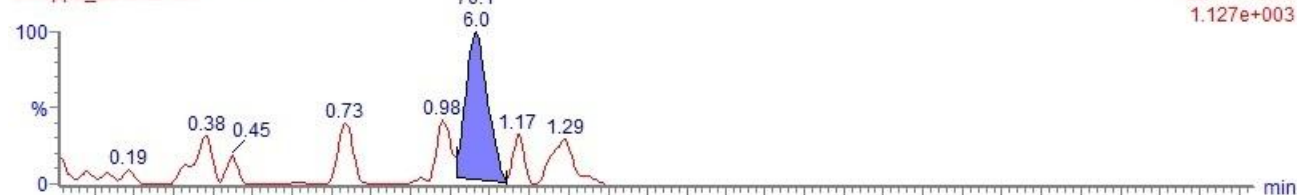
F1:MRM of 2 channels,ES+
459.2 > 252.2
2.451e+003



Saccharin

23102016_Sweetener1_Batch3_M1_18
0.01ppb_BZ STD 131

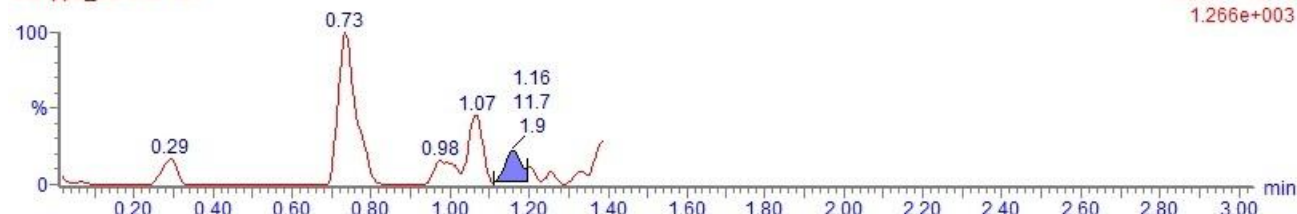
F3:MRM of 2 channels,ES-
182.1 > 105.3
1.127e+003



Aspartame

23102016_Sweetener1_Batch3_M1_18
0.01ppb_BZ STD 131

F4:MRM of 2 channels,ES-
293.2 > 199.9
1.266e+003

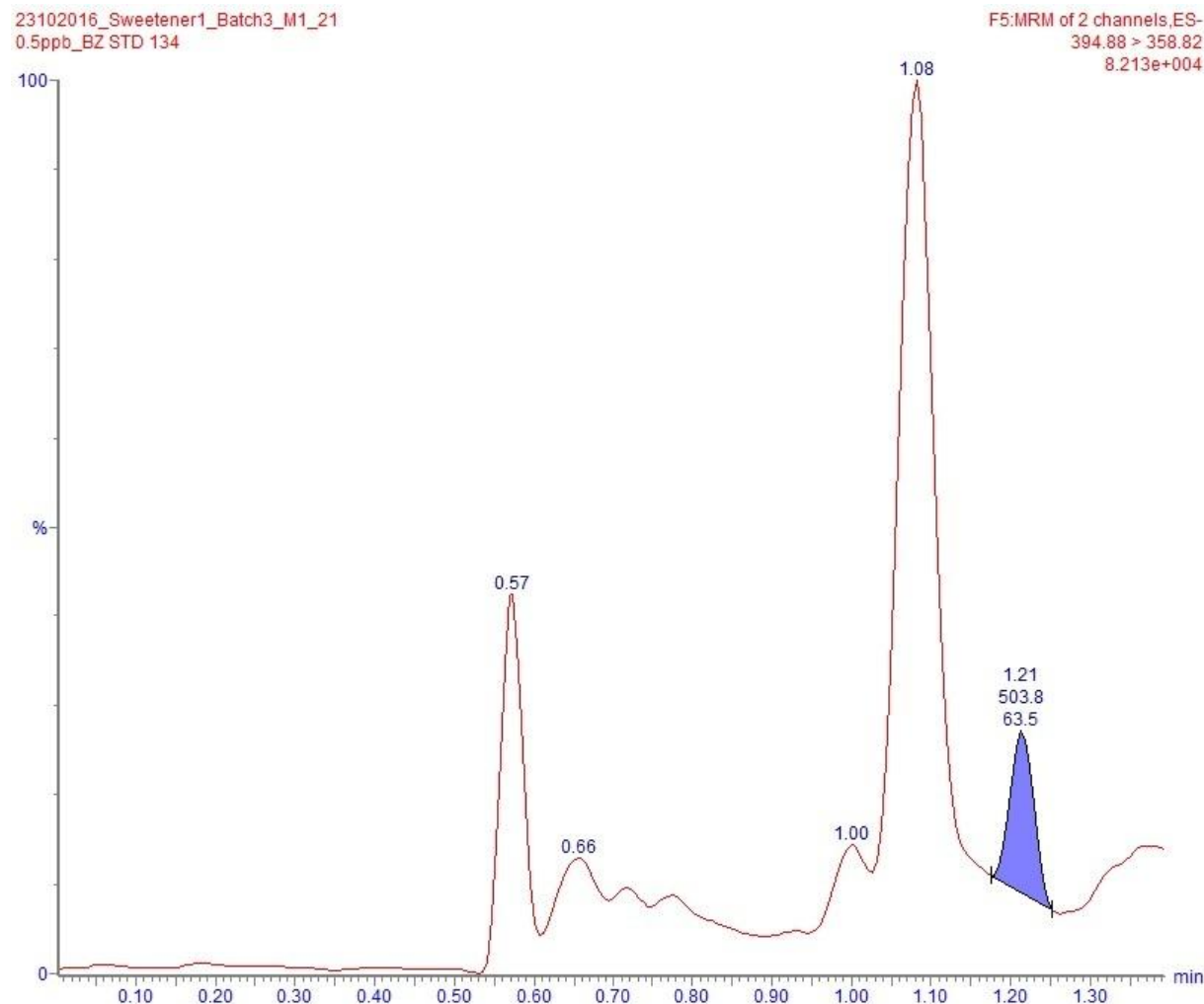


The retention time (RT), peak response area and peak signal-to-noise (S/N) are shown on peak top of the chromatogram

Peak Signal-to-Noise of Sucralose at LOQ Level (0.5 ppb)

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Sucralose



The retention time (RT), peak response area and peak signal-to-noise (S/N) are shown on peak top of the chromatogram

Linearity of Calibration Curve and S/N at 0.01 ppb of Detected Sweeteners in 30% Acetonitrile

Table 3. The linearity of calibration curve for the detected sweeteners using solvent blank as diluent

Sweetener	Linearity (R^2)	S/N at 0.01 ppb
Advantame	0.999	4.1
Acesulfame K	0.999	10.3
Saccharin	0.998	15.5
Aspartame	0.999	9.7
Sucralose	0.996	4.4

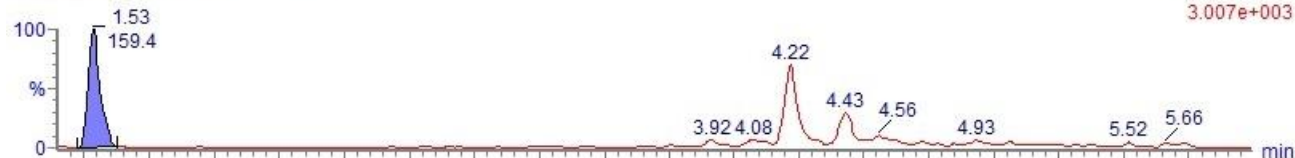
Chromatogram of *Advantame* in 30% Acetonitrile

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0.1 ppb

23102016_Sweetener1_Batch3_M1_09
0.1ppb_30ACN STD 122



F1:MRM of 2 channels,ES+
459.2 > 252.2
3.007e+003

0.5 ppb

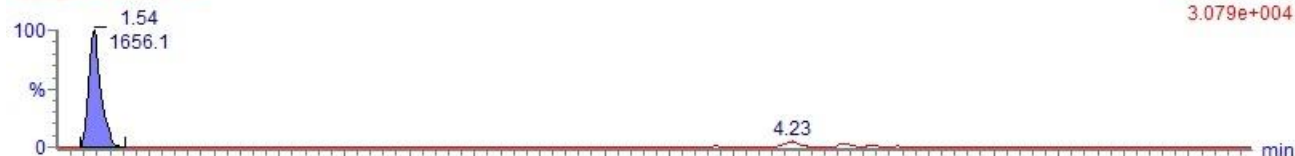
23102016_Sweetener1_Batch3_M1_10
0.5ppb_30ACN STD 123



F1:MRM of 2 channels,ES+
459.2 > 252.2
1.505e+004

1 ppb

23102016_Sweetener1_Batch3_M1_11
1ppb_30ACN STD 124



F1:MRM of 2 channels,ES+
459.2 > 252.2
3.079e+004

5 ppb

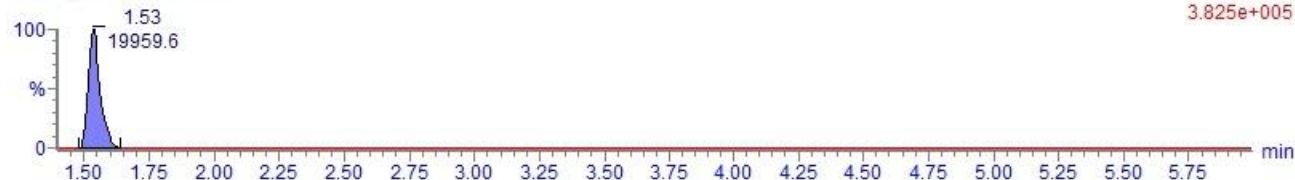
23102016_Sweetener1_Batch3_M1_12
5ppb_30ACN STD 125



F1:MRM of 2 channels,ES+
459.2 > 252.2
1.758e+005

10 ppb

23102016_Sweetener1_Batch3_M1_13
10ppb_30ACN STD 126



F1:MRM of 2 channels,ES+
459.2 > 252.2
3.825e+005

The retention time (RT) and peak response area are shown on peak top of the chromatogram

Chromatogram of *Acesulfame K* in 30% Acetonitrile

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0.1 ppb

23102016_Sweetener1_Batch3_M1_09
0.1ppb_30ACN STD 122



0.5 ppb

23102016_Sweetener1_Batch3_M1_10
0.5ppb_30ACN STD 123



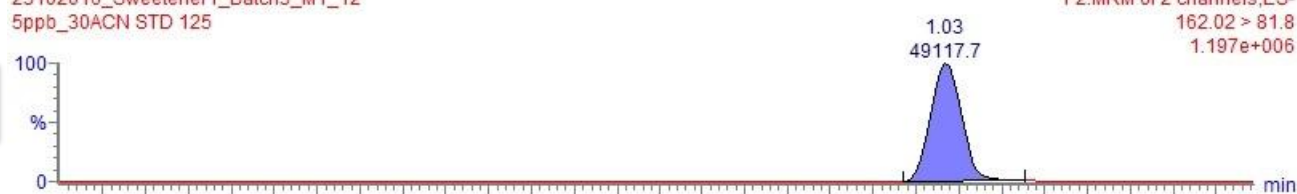
1 ppb

23102016_Sweetener1_Batch3_M1_11
1ppb_30ACN STD 124



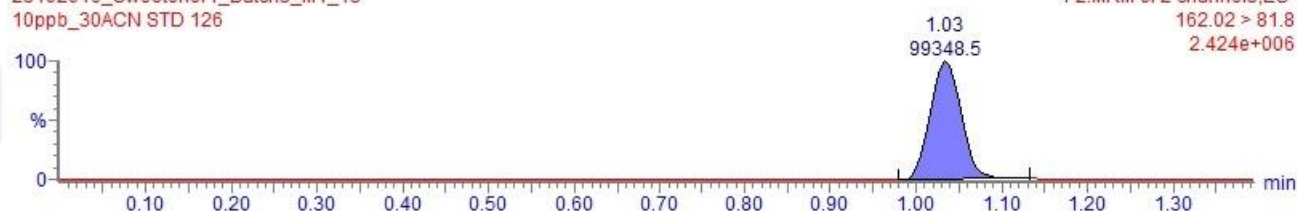
5 ppb

23102016_Sweetener1_Batch3_M1_12
5ppb_30ACN STD 125



10 ppb

23102016_Sweetener1_Batch3_M1_13
10ppb_30ACN STD 126



The retention time (RT) and peak response area are shown on peak top of the chromatogram

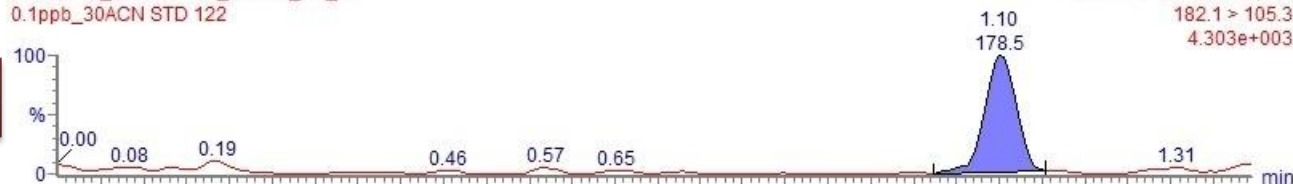
Chromatogram of *Saccharin* in 30% Acetonitrile

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0.1 ppb

23102016_Sweetener1_Batch3_M1_09
0.1ppb_30ACN STD 122



F3:MRM of 2 channels,ES-
182.1 > 105.3
4.303e+003

0.5 ppb

23102016_Sweetener1_Batch3_M1_10
0.5ppb_30ACN STD 123



F3:MRM of 2 channels,ES-
182.1 > 105.3
1.972e+004

1 ppb

23102016_Sweetener1_Batch3_M1_11
1ppb_30ACN STD 124



F3:MRM of 2 channels,ES-
182.1 > 105.3
4.204e+004

5 ppb

23102016_Sweetener1_Batch3_M1_12
5ppb_30ACN STD 125



F3:MRM of 2 channels,ES-
182.1 > 105.3
2.247e+005

10 ppb

23102016_Sweetener1_Batch3_M1_13
10ppb_30ACN STD 126



F3:MRM of 2 channels,ES-
182.1 > 105.3
4.177e+005

The retention time (RT) and peak response area are shown on peak top of the chromatogram

Chromatogram of *Aspartame* in 30% Acetonitrile

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0.1 ppb

23102016_Sweetener1_Batch3_M1_09
0.1ppb_30ACN STD 122



0.5 ppb

23102016_Sweetener1_Batch3_M1_10
0.5ppb_30ACN STD 123



1 ppb

23102016_Sweetener1_Batch3_M1_11
1ppb_30ACN STD 124



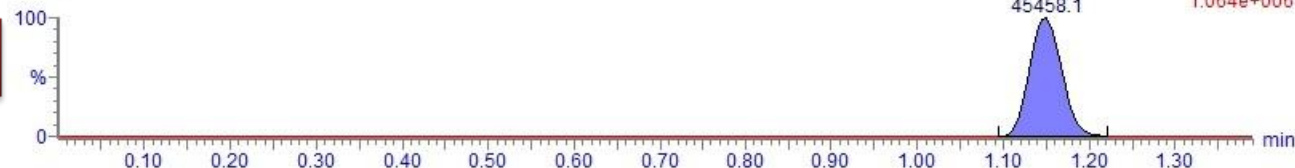
5 ppb

23102016_Sweetener1_Batch3_M1_12
5ppb_30ACN STD 125



10 ppb

23102016_Sweetener1_Batch3_M1_13
10ppb_30ACN STD 126



The retention time (RT) and peak response area are shown on peak top of the chromatogram

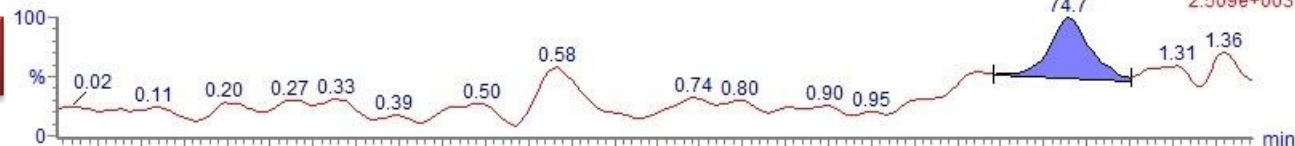
Chromatogram of *Sucralose* in 30% Acetonitrile

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0.1 ppb

23102016_Sweetener1_Batch3_M1_09
0.1ppb_30ACN STD 122



0.5 ppb

23102016_Sweetener1_Batch3_M1_10
0.5ppb_30ACN STD 123



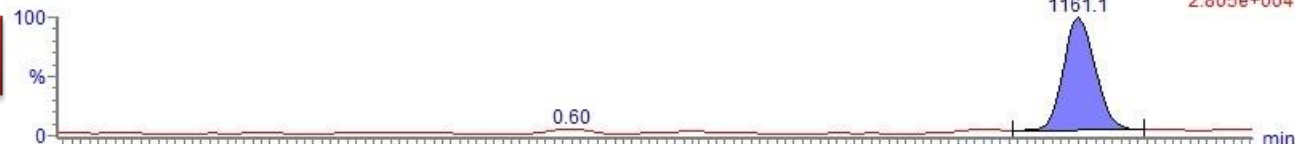
1 ppb

23102016_Sweetener1_Batch3_M1_11
1ppb_30ACN STD 124



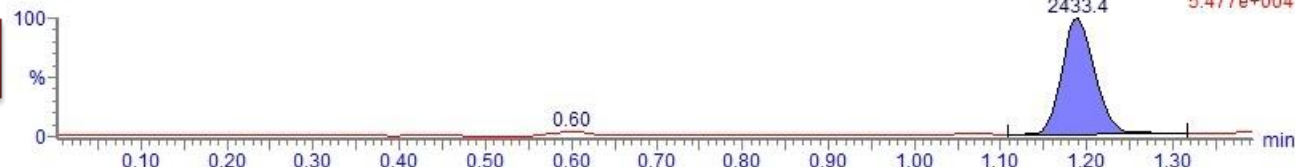
5 ppb

23102016_Sweetener1_Batch3_M1_12
5ppb_30ACN STD 125



10 ppb

23102016_Sweetener1_Batch3_M1_13
10ppb_30ACN STD 126



The retention time (RT) and peak response area are shown on peak top of the chromatogram

Conclusion

- The SRM quantification for sweeteners in food matrix was based on the most intense product ion for each analyte
- The demonstrated timed-SRM technique based on the ACQUITY UPLC coupled to Xevo TQ-XS showed excellent LLOQ/LLOD in food matrix
- The ease of use and flexibility for the MassLynx software allows the customer to focus on the research rather than method development, optimization, and report customization



Method

UPLC Conditions: I-Class

System	ACQUITY UPLC™ I-Class
Column	ACQUITY HSS T3 100 mm x 2.1 mm, 1.8 µm
Column temperature	40°C
Flow	0.4 mL/min
Mobile phase	(A) Water with 0.1% Formic Acid
	(B) Acetonitrile with 0.1% Formic Acid
Injection Vol (FTN)	10 µl



Time (min)	% A	% B
0.00	95	5
1.60	60	40
3.50	5	95
5.00	5	95
5.10	95	5
8.10	95	5

MS Conditions: Xevo TQ-XS

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Ionization Mode	ESI+, ESI-
Capillary voltage	1.5 kV (ESI+) & 1.0 kV (ESI-)
Source Temperature	150 °C
Desolvation Temperature	600 °C
Desolvation Gas Flow (L/Hr)	6 V



Xevo TQ-XS

MRM Transitions

Analyte	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone Voltage (V)	Collision energy (eV)	Mode of Ionization
Acesulfame K	162.02	77.8	0.01	10	24	ES-
Acesulfame K	162.02	81.8	0.01	10	14	ES-
Advantame	459.2	102.1	0.01	10	20	ES-
Advantame	459.2	252.2	0.01	10	12	ES-
Aspartame	293.2	199.9	0.01	10	14	ES-
Aspartame	293.2	261	0.01	10	12	ES-
Neotame	379.0	172.2	0.01	10	15	ES+
Neotame	379.0	85.2	0.01	10	24	ES+
Saccharin	182.1	105.3	0.01	10	20	ES-
Saccharin	182.1	41.0	0.01	10	20	ES-

MRM Transitions (Continued)

Analyte	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone Voltage (V)	Collision energy (eV)	Mode of Ionization
Sodium Cyclamate	178.1	79.8	0.01	50	20	ES-
Sodium Cyclamate	178.1	95.0	0.01	50	17	ES-
Sucralose	394.9	358.8	0.01	15	12	ES-
Sucralose	396.9	360.9	0.01	15	12	ES-
Alitame	330.1	311.9	0.01	10	12	ES-
Alitame	330.1	294.8	0.01	10	12	ES-
Stevioside	803.3	641.3	0.01	10	15	ES-
Stevioside	803.3	317.2	0.01	10	20	ES-
Rebaudioside A	965.4	803.4	0.01	20	20	ES-
Rebaudioside A	965.4	726.3	0.01	20	22	ES-

References for the MRM Transitions of Sweeteners

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2. Chui-Shiang Chang, Tai Sheng Yeh (2014) Detection of 10 sweeteners in various foods by liquid chromatography/tandem mass spectrometry, Journal of Food and Drug Analysis, 22(3): 318–328
3. Paweł Kubica & Jacek Namieśnik & Andrzej Wasik (2015) Determination of eight artificial sweeteners and common *Stevia rebaudiana* glycosides in non-alcoholic and alcoholic beverages by reversed-phase liquid chromatography coupled with tandem mass spectrometry, Anal Bioanal Chem, 407:1505–1512
4. <http://www.mn-net.com/tabid/12671/default.aspx>

THANK YOU

**For further details, please contact
Han_Wang@waters.com**

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