Azaspiracids

Northwest Fisheries Science Center

HPLC parameters

Column: Phenomenex Luna C8 (2) reverse phase, 2.1 mm x 100 mm, 5 µm particles
Column temperature: 40°C
Sample temperature: 4°C
Injection volume: 10 µL
Mobile phase A: water, 2 mM ammonium formate, 50 mM formic acid
Mobile phase B: 95% acetonitrile/5% water, 2 mM ammonium formate, 50 mM formic acid
Flow rate: 0.4 mL/min
Run time: 17 min

Gradient:

Time (min)	%A	%B
0	70	30
8	10	90
11	10	90
11.5	70	30
17	70	30

MS/MS parameters

MS/MS system: ABSciex QTRAP 5500
Ion source: Turbo Spray
Ionization: electrospray ionization, positive ion mode
Scan type: multiple-reaction monitoring
Curtain gas flow rate: 20 (arbitrary units)
Collision-activated dissociation: medium
Source temperature: 650oC
Gas 1 flow rate: 40 (arbitrary units)
Gas 2 flow rate: 60 (arbitrary units)
Ion spray voltage: +4500 V
Exit potential: +6 V
Declustering potential: -+81 V
Entrance potential: +4.5 V
Resolution Q1: Unit
Resolution Q3: Unit
MRM Period 1: 0-9 min; 493 scans per period
Total scan time: 0.29 sec per scan

Acquisition parameters:

Analytes	Precursor ion (m/z)	Product ion 1 (m/z)[a]	Product ion 2 (m/z)[b]	dwell time (msec)	Collision energy (V)	Exit potential (V)
AZA3	828.2	810.4	792.5	35	+55	+6
AZA1	824.2	824.4	806.3	35	+55	+6
AZA2	856.2	838.3	820.2	35	+55	+6

[a] used as quantitation transition
[b] used as confirmation transition

Calibration method

A quadratic natural-log normalized calibration curve (curve type Wagner), containing a minimum of 6 points, is created for each target analyte. The equation used is as follows:

$$\ln y = a_2(\ln x)^2 + a_1(\ln x) + a_0$$

The concentrations of the analytes (ng/g) in the samples are calculated based on the LC-MS/MS calibration standards for response factor calibration curves.

MultiQuant 2.0.2

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Components & Groups

All Components

AZA3 1
AZA3 2
AZA1 1
AZA1 2
AZA2 1
AZA2 2

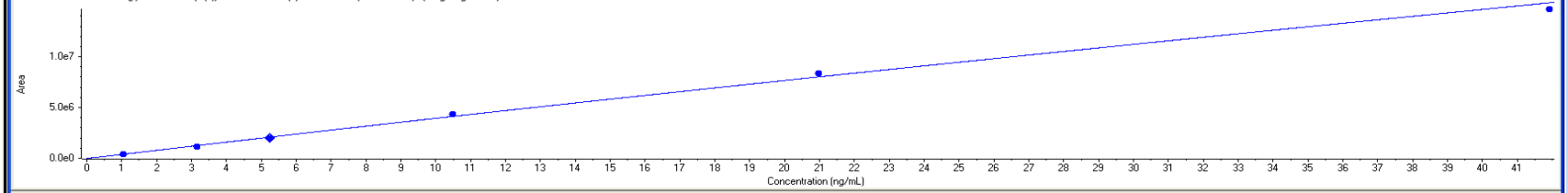
[MQ4] Results Table (20160114 MH3294 AZA.qsession)

Standards & QCs

Index	Sample Name	Sample ID	Sample Type	Component Name	Retention Time	Actual Concentration	Dilution Factor	Area	Modified	Used	Calculated Concentration	Accuracy
7	AZA Cal 3		Quality Control	AZA3 1	4.83	5.240	1	2008856	☑	☑	5.04	96
13	AZA Cal 3		Quality Control	AZA3 1	4.80	5.240	1	2047715	☐	☑	5.14	98
19	AZA Cal 3		Quality Control	AZA3 1	4.79	5.240	1	2069769	☐	☑	5.20	99
31	AZA Cal 1		Standard	AZA3 1	4.77	1.048	1	413986	☑	☑	1.08	103
37	AZA Cal 2		Standard	AZA3 1	4.77	3.144	1	1183040	☑	☑	2.98	95
43	AZA Cal 3		Standard	AZA3 1	4.76	5.240	1	2007486	☐	☑	5.04	96
49	AZA Cal 4		Standard	AZA3 1	4.76	10.480	1	4372753	☑	☑	11.14	106
55	AZA Cal 5		Standard	AZA3 1	4.75	20.960	1	8393709	☐	☑	22.02	105
61	AZA Cal 6		Standard	AZA3 1	4.74	41.920	1	14684980	☑	☑	40.08	96
73	AZA Cal 3		Quality Control	AZA3 1	4.74	5.240	1	1995699	☐	☑	5.01	96
127	AZA Cal 3		Quality Control	AZA3 1	4.72	5.240	1	2073716	☑	☑	5.21	99

Regression

Calibration for AZA3 1: ln(y) = -0.01755 (ln(x))^2 + 1.05299 ln(x) + 12.85503 (r = 0.99852) (weighting: None)

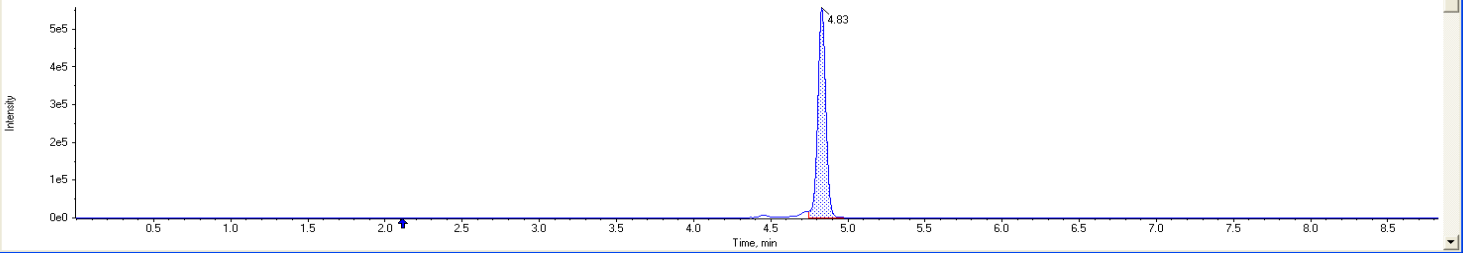


Apply

Manual Integration

Gaussian Smooth Width: 1.5 points
Expected RT: 2.11 min
RT Half Window: 5.0 sec
Update Expected RT: No
Report Largest Peak
Min. Peak Width: 3 points
Min. Peak Height: 0.00
Integration Parameters
Noise Percentage: 40.0 %
Baseline Sub. Window: 2.00 min
Peak Splitting 3 points

AZA Cal 3 - AZA3 1 (Quality Control) 828.2 / 810.4 - 20160114 - MH3294AZA.wiff (sample 2) - (1/14/2016 5:36:18 PM)
Area: 2008856, RT: 4.83 min -



start | ACQUITY UPLC Cons... | Analyst | MultiQuant 2.0.2 | 3:39 PM

MultiQuant 2.0.2

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DSPs

Components & Groups IS

All Components

AZA3 1
AZA3 2
AZA1 1
AZA1 2
AZA2 1
AZA2 2

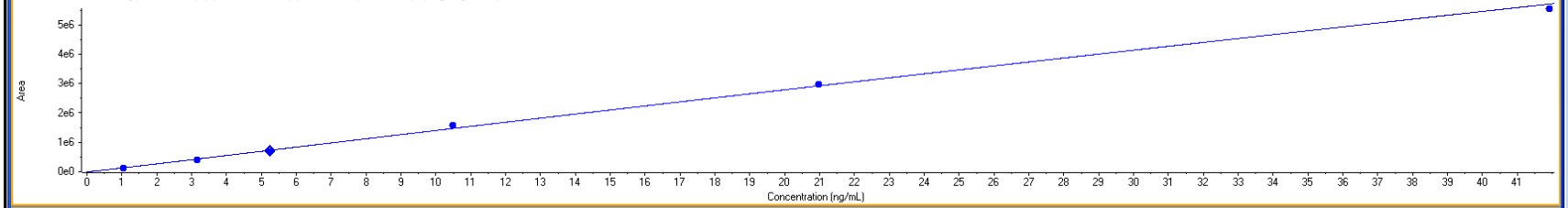
[MQ4] Calibration (20160114 MH3294 AZA.qsession)

Standards & QCs

Index	Sample Name	Sample ID	Sample Type	Component Name	Retention Time	Actual Concentration	Dilution Factor	Area	Modified	Used	Calculated Concentration	Accuracy
8	AZA Cal 3		Quality Control	AZA3 2	4.83	5.240	1	682503	☑	☑	4.92	94
14	AZA Cal 3		Quality Control	AZA3 2	4.80	5.240	1	699428	☐	☑	5.04	96
20	AZA Cal 3		Quality Control	AZA3 2	4.79	5.240	1	757065	☐	☑	5.44	104
32	AZA Cal 1		Standard	AZA3 2	4.77	1.048	1	137192	☐	☑	1.07	102
38	AZA Cal 2		Standard	AZA3 2	4.77	3.144	1	415502	☐	☑	3.04	97
44	AZA Cal 3		Standard	AZA3 2	4.76	5.240	1	695686	☐	☑	5.01	96
50	AZA Cal 4		Standard	AZA3 2	4.75	10.480	1	1567145	☑	☑	11.17	107
56	AZA Cal 5		Standard	AZA3 2	4.75	20.960	1	2988548	☐	☑	21.46	102
62	AZA Cal 6		Standard	AZA3 2	4.74	41.920	1	5549247	☑	☑	40.70	97
74	AZA Cal 3		Quality Control	AZA3 2	4.74	5.240	1	693842	☑	☑	5.00	95
128	AZA Cal 3		Quality Control	AZA3 2	4.72	5.240	1	753494	☑	☑	5.41	103

Regression

Calibration for AZA3 2: ln(y) = -0.01653 (ln(x))^2 + 1.07909 ln(x) + 11.75691 (r = 0.99934) (weighting: None)



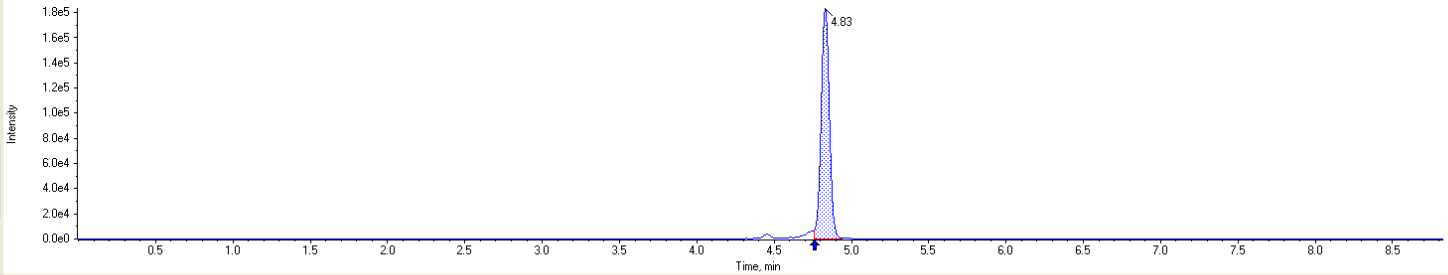
Apply

Manual Integration

Gaussian Smooth Width: 1.5 points
Expected RT: 4.76 min
RT Half Window: 5.0 sec
Update Expected RT: No
Report Largest Peak
Min. Peak Width: 3 points
Min. Peak Height: 0.00

Integration Parameters
Noise Percentage: 40.0 %
Baseline Sub. Window: 2.00 min
Peak Splitting 2 points

AZA Cal 3 - AZA3 2 (Quality Control) 828.2 / 792.5 - 20160114 - MH3294AZA.wiff (sample 2) - (1/14/2016 5:36:18 PM)
Area: 682503, RT: 4.83 min -



start | ACQUITY UPLC Cons... | Analyst | MultiQuant 2.0.2 | My Documents | MH3294 AZA3 (1) Cal... | 3:58 PM

MultiQuant 2.0.2

File Edit Process Audit Trail Window Help

Components & Groups

All Components

AZA3 1
AZA3 2
AZA1 1
AZA1 2
AZA2 1
AZA2 2

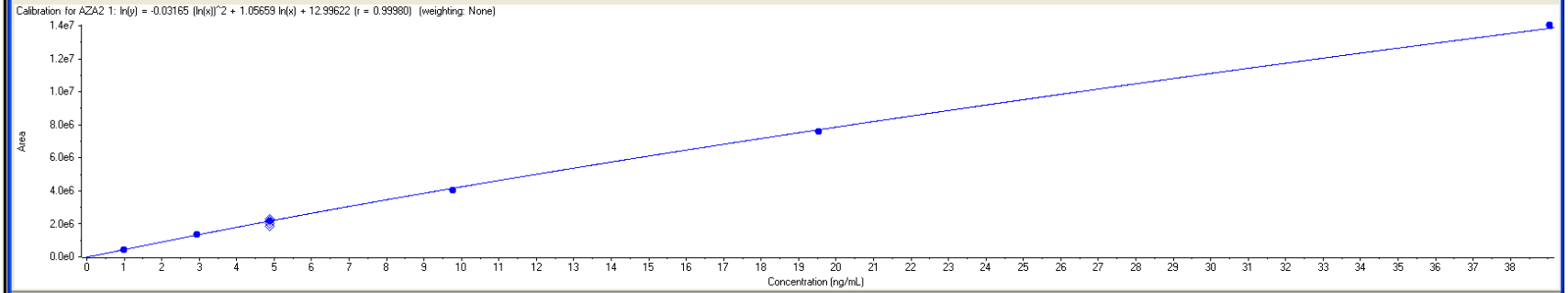
[MQ4] Results Table (20160115 MH3295 AZA.qsession)

Standards & QCs

Index	Sample Name	Sample ID	Sample Type	Dilution Factor	Component Name	Retention Time	Actual Concentration	Area	Height	Modified	Used	Calculated Concentration	Accuracy
11	AZA Cal 3		Quality Control	1.00	AZA2 1	5.40	4.880	2279833	604143	☐	☑	5.13	105
17	AZA Cal 3		Quality Control	1.00	AZA2 1	5.39	4.880	2181749	570413	☐	☑	4.90	100
23	AZA Cal 1		Standard	1.00	AZA2 1	5.39	0.976	423429	109029	☐	☑	0.96	99
29	AZA Cal 2		Standard	1.00	AZA2 1	5.39	2.928	1368308	347326	☐	☑	3.03	104
35	AZA Cal 3		Standard	1.00	AZA2 1	5.39	4.880	2187334	555270	☐	☑	4.91	101
41	AZA Cal 4		Standard	1.00	AZA2 1	5.38	9.760	4030608	1020554	☐	☑	9.45	97
47	AZA Cal 5		Standard	1.00	AZA2 1	5.38	19.520	7604862	1949474	☐	☑	19.25	99
53	AZA Cal 6		Standard	1.00	AZA2 1	5.38	39.040	14052539	3290105	☐	☑	39.77	102
65	AZA Cal 3		Quality Control	1.00	AZA2 1	5.39	4.880	2047126	506432	☐	☑	4.59	94
155	AZA Cal 3		Quality Control	1.00	AZA2 1	5.38	4.880	1843489	470606	☑	☑	4.11	84

Regression

Calibration for AZA2 1: ln(y) = -0.03165 (ln(x))^2 + 1.05659 ln(x) + 12.99622 (r = 0.99980) (weighting: None)



Apply

Manual Integration

Gaussian Smooth Width: 1.5 points

Expected RT: 5.46 min

RT Half Window: 5.0 sec

Update Expected RT: No

Report Largest Peak

Min. Peak Width: 3 points

Min. Peak Height: 0.00

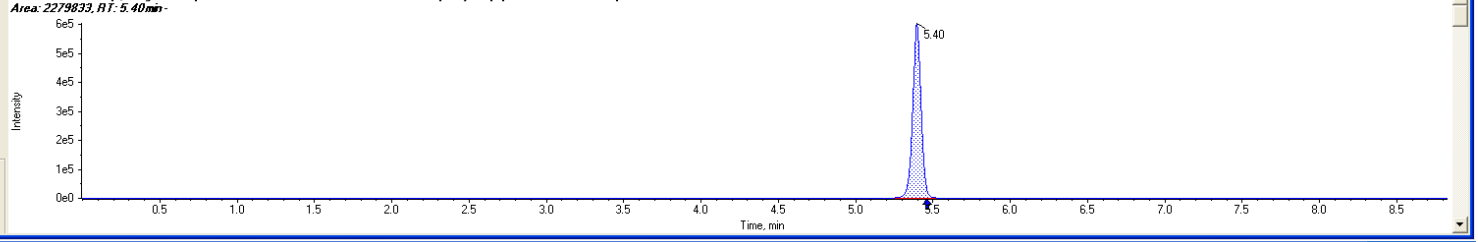
Integration Parameters

Noise Percentage: 40.0 %

Baseline Sub. Window: 2.00 min

Peak Splitting 3 points

AZA Cal 3 - AZA2 1 (Quality Control) 856.2 / 838.3 - 20160115 - MH3295AZA.wiff (sample 2) - (1/15/2016 3:55:06 PM)
Area: 2279833, RT: 5.40 min -



start | ACQUITY UPLC Cons... | Analyst | MultiQuant 2.0.2 | My Documents | MH3294 AZA3 (2) Cal... | 4:09 PM

Batch MH3294 AZAs

analyzed 1/14/2016

Measured concentrations

sample #	sample	AZA3 µg/100 g tissue	AZA1 µg/100 g tissue	AZA2 µg/100 g tissue
138-1	WDOH low mussel	<1.0	<1.00	<0.94
138-2	WDOH low mussel	<1.1	<1.00	<1.00
138-3	WDOH low mussel - post spiked	3.0	2.8	2.4
138-4	WDOH low mussel - post spiked	2.4	2.2	1.9
138-5	field sample	<1.00	<1.00	<1.00
138-6	field sample	<1.00	<1.00	<1.00

% recovery of spiked samples

sample #	sample	AZA3	AZA1	AZA2
138-3 PS	WDOH low mussel - post spiked	91%	84%	77%
138-4 PS	WDOH low mussel - post spiked	74%	69%	63%

Batch MH3295 AZAs

analyzed 1/15/2016

Measured concentrations

sample #	sample	AZA3 µg/100 g tissue	AZA1 µg/100 g tissue	AZA2 µg/100 g tissue
138-7	blank mussel	<1.0	<1.0	<0.95
138-8	blank mussel	<1.0	<1.0	<0.97
138-9	blank mussel	<1.1	<1.0	<0.97
138-10	spiked WDOH low mussel	2.6	2.3	1.8
138-11	spiked WDOH low mussel	2.5	2.2	1.7
138-12	spiked WDOH low mussel	2.2	2.0	1.7
138-13	solvent blank	<1.0	<1.0	<0.97
138-14	spiked solvent blank	2.3	2.2	1.9
138-7 PS	WDOH low mussel - post spiked	2.7	2.5	1.9
138-8 PS	WDOH low mussel - post spiked	2.7	2.4	2.0
138-9 PS	WDOH low mussel - post spiked	2.3	2.1	1.6
138-13 PS	solvent blank - post spiked	2.4	2.5	2.0

% recovery of spiked samples

sample #	sample	AZA3	AZA1	AZA2
138-10	spiked WDOH low mussel	76%	69%	57%
138-11	spiked WDOH low mussel	80%	72%	60%
138-12	spiked WDOH low mussel	77%	72%	64%
138-7 PS	WDOH low mussel - post spiked	89%	82%	67%
138-8 PS	WDOH low mussel - post spiked	86%	78%	68%
138-9 PS	WDOH low mussel - post spiked	75%	67%	56%
138-14	spiked solvent blank	75%	71%	66%
138-13 PS	solvent blank - post spiked	77%	80%	69%

AZA LC/MSMS Matrix Effects Test 1

Analyzed 1/22/2016

Sample	Type	% extract by volume	AZA 3 actual ng/mL	AZA 3 % Accuracy	AZA1 actual ng/mL	AZA1 % Accuracy	AZA 2 actual ng/mL	AZA 2 % Accuracy
AZA T-1	blank mussel - post spiked	94% extract	3.144	78	3.12	73	3.12	70
AZA T-2	blank mussel - post spiked	47% extract	3.144	103	3.12	98	3.12	94
AZA T-3	blank mussel - post spiked	23.5% extract	3.144	109	3.12	98	3.12	94
AZA T-4	blank mussel - post spiked	80% extract	10.48	96	10.4	90	10.4	78
AZA T-5	blank mussel - post spiked	40% extract	10.48	109	10.4	98	10.4	89
AZA T-6	blank mussel - post spiked	20% extract	10.48	109	10.4	104	10.4	95
AZA T-7	blank mussel - post spiked	40% extract	5.24	113	5.2	102	5.2	93
AZA T-8	blank mussel - post spiked	20% extract	2.62	99	2.6	99	2.6	95
AZA T-9	spiked methanol	0% extract	3.14	116	3.12	108	3.12	103
AZA T-10	spiked methanol	0% extract	10.48	111	10.4	104	10.4	100
AZA T-11	spiked methanol	0% extract	5.24	114	5.2	112	5.2	108

Batch MH3309 AZAs

analyzed 3/3/2016

Measured concentrations

sample #	sample	AZA3 µg/100 g tissue	AZA1 µg/100 g tissue	AZA2 µg/100 g tissue
138-15	solvent blank	<0.16	<0.15	<0.14
138-16	solvent blank	<0.16	<0.15	<0.14
138-17	Zero Mussel CRM	<0.16	<0.16	<0.15
138-18	spiked Zero Mussel CRM	0.26	0.16	0.18
138-19	Zero Mussel CRM	<0.16	<0.15	<0.14
138-20	spiked Zero Mussel CRM	0.28	0.17	0.20
138-21	WDOH low mussel	<0.15	<0.15	<0.14
138-22	WDOH low mussel	<0.16	<0.16	<0.15
138-23	Sequim Bay mussel 8/12/14	<0.16	<0.16	<0.15
138-24	Sequim Bay mussel 8/19/14	<0.15	<0.15	<0.14
138-25	spiked WDOH low mussel	0.30	0.26	0.20
138-26	spiked WDOH low mussel	0.30	0.27	0.20
138-15 PS	solvent blank - post spiked	1.0	1.1	1.0
138-16 PS	solvent blank - post spiked	1.1	0.95	0.91
138-17 PS	Zero Mussel CRM - post spiked	0.92	0.49	0.54
138-19 PS	Zero Mussel CRM - post spiked	0.71	0.49	0.55
138-21 PS	WDOH low mussel - post spiked	0.76	0.64	0.53
138-22 PS	WDOH low mussel - post spiked	0.77	0.69	0.53

Batch MH3309 AZAs

analyzed 3/3/2016

% recovery of spiked samples

sample #	sample	AZA3	AZA1	AZA2
138-18	spiked Zero Mussel CRM	83%	51%	61%
138-20	spiked Zero Mussel CRM	89%	54%	68%
138-25	spiked WDOH low mussel	95%	83%	72%
138-26	spiked WDOH low mussel	99%	90%	72%
138-18 0.5	spiked Zero Mussel CRM	108%	77%	82%
138-20 0.5	spiked Zero Mussel CRM	108%	77%	82%
138-25 0.5	spiked WDOH low mussel	115%	103%	89%
138-26 0.5	spiked WDOH low mussel	102%	103%	89%
138-15 PS	solvent blank - post spiked	100%	103%	103%
138-16 PS	solvent blank - post spiked	102%	92%	94%
138-17 PS	Zero Mussel CRM - post spiked	88%	47%	55%
138-19 PS	Zero Mussel CRM - post spiked	69%	48%	57%
138-21 PS	WDOH low mussel - post spiked	74%	63%	55%
138-22 PS	WDOH low mussel - post spiked	73%	66%	54%