Figure S1

Example MS/MS (MRM type) chromatograms from algae and wheat samples, QC samples, calibration points

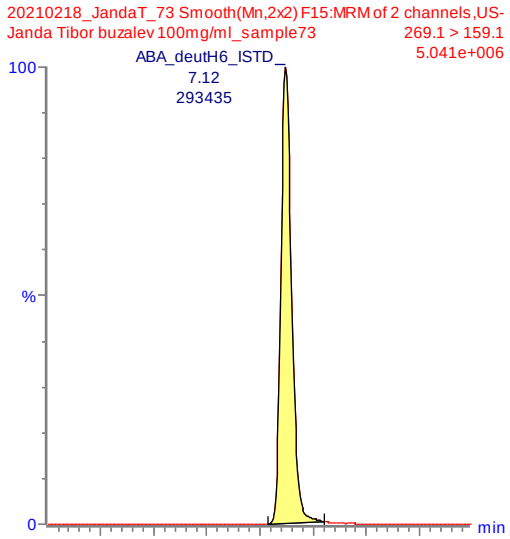
Target list of componensts:

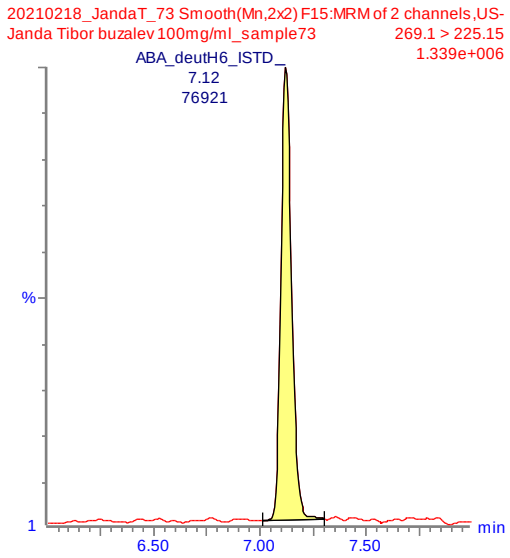
#	Name	Quant MS/MS transition m/z	Ionisation&polarity	RT (min)
1	t-Cinnamic ac	149 > 103	UniSpray positive	7.53
2	indole-3-acetic ac	176.1 > 130.1	UniSpray positive	5.98
3	Benzoic ac	121 > 77	UniSpray negative	5.44
4	Salicylic ac	137 > 93	UniSpray negative	5.8
5	p-Hydroxybenzoic ac	137 > 93.01	UniSpray negative	2.85
6	Acetylsalicylic ac	137 > 93.02	UniSpray negative	5.32
7	p-coumaric ac	163 > 93	UniSpray negative	4.05
8	o-coumaric ac	163 > 93	UniSpray negative	5.68
9	Jasmonic ac	209.12 > 58.9	UniSpray negative	8.28
10	Abscissic ac	263.1 > 153	UniSpray negative	7.16
11	ABA_dH6_ISTD	269.1 > 159.1	UniSpray negative	7.13
12	Naringenin	271 > 150.9	UniSpray negative	8.17
13	Phaseic ac	279.12 > 139	UniSpray negative	5.19
14	Dihydrophaseic ac	281.14 > 171.1	UniSpray negative	3.52
15	Kaempferol	285 > 92.9	UniSpray negative	8.44
16	DihydroKaempferol	287 > 259	UniSpray negative	6.07
17	Quercetin	301 > 150.95	UniSpray negative	7.38
18	Taxifolin	303 > 285	UniSpray negative	4.72
19	Myricetin	317 > 150.9	UniSpray negative	6.08
20	Jasmonic ac-ILe/Le	322.2 > 130.1	UniSpray negative	9.94
21	GA20	331.15 > 287.3	UniSpray negative	7.43
22	GA4	331.15 > 269.2	UniSpray negative	9.43
23	GibberellicAc_GA3	345 > 239.1	UniSpray negative	4.46
24	GA1	347.15 > 259.2	UniSpray negative	4.58
25	Chlorogenic ac+cryptochlorogenic ac	353 > 191	UniSpray negative	2.88
26	GA8	363.14 > 275.1	UniSpray negative	3.02
27	Rutin	609.1 > 300.1	UniSpray negative	4.23
28	Neochlorogenic ac	353 > 191	UniSpray negative	2.21

Pages 3-5: Sample chromatograms for compounds above limit of quatitation (LoQ) of wheat leaves extended with external solvent calibration points of abscisic acid (page 5.)

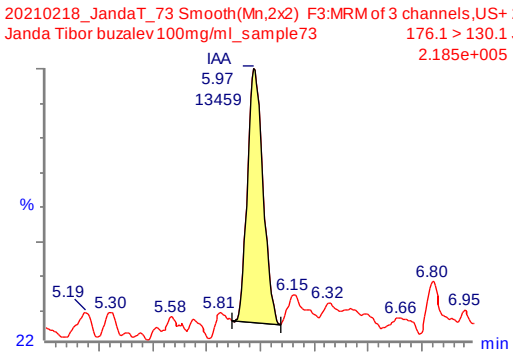
Example MRM type chromatograms for components found above LoQ in a wheat sample data file:20210212JandaT_73 , wheat leaf matrix 100mg/ml) – Slide 1 of 3

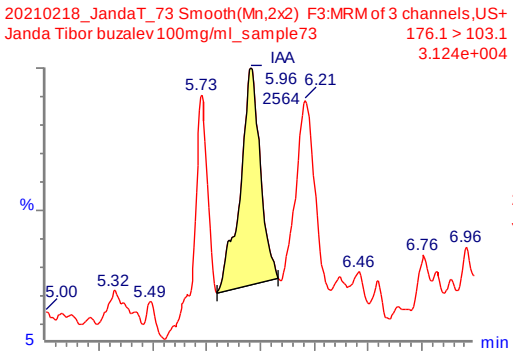
ABAd6 – isotope labelled internal standard

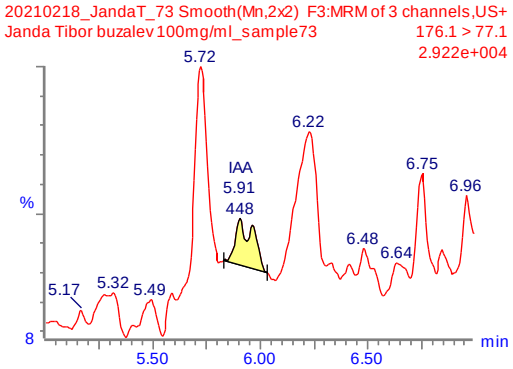




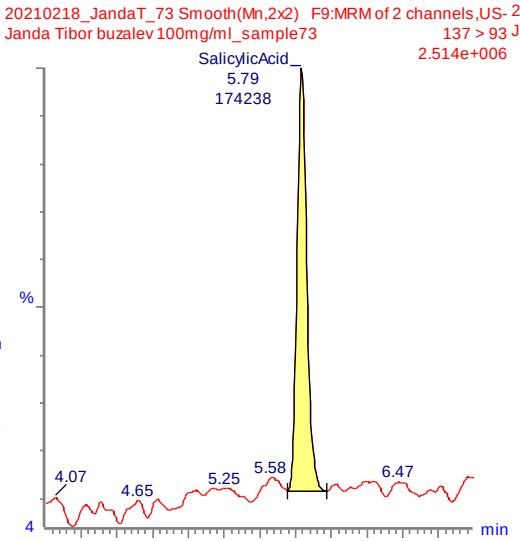
indole-3-acetic acid

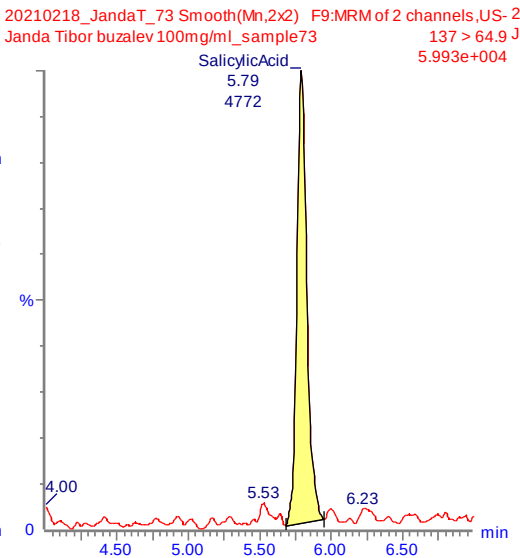




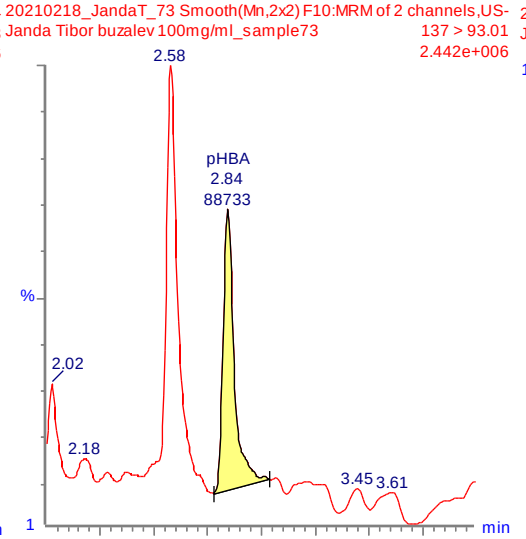


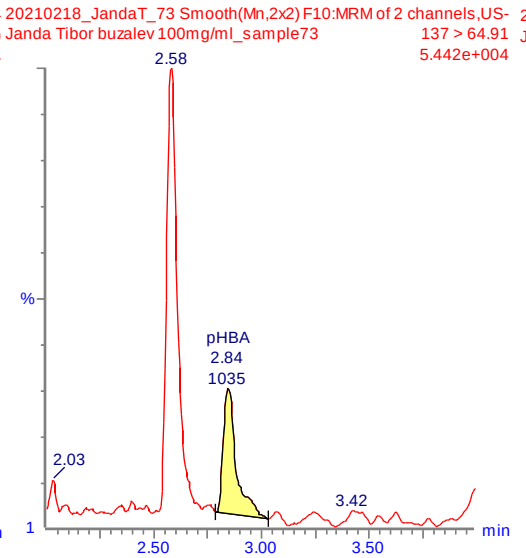
salicylic acid



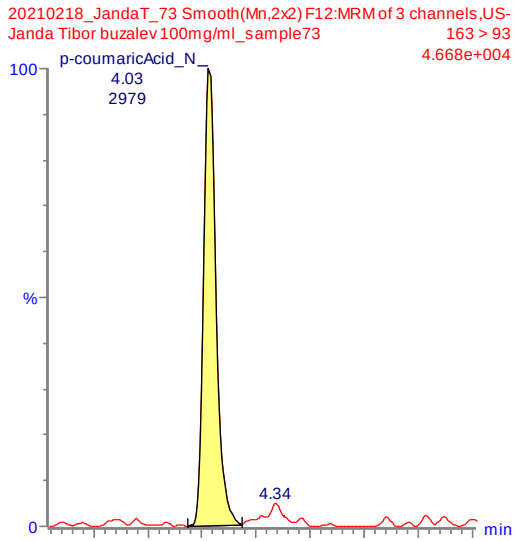


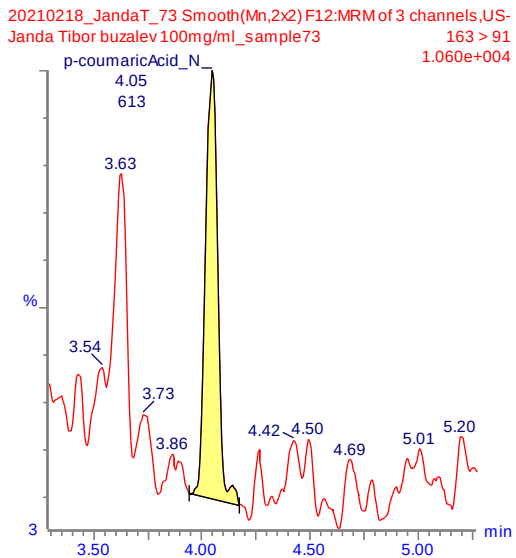
para-hydroxybenzoic acid





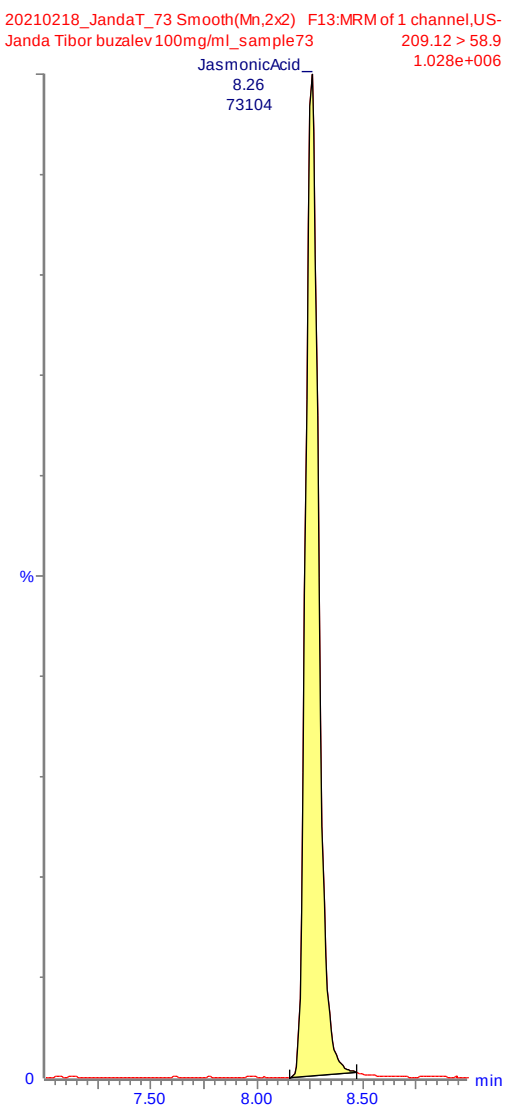
para-coumaric acid



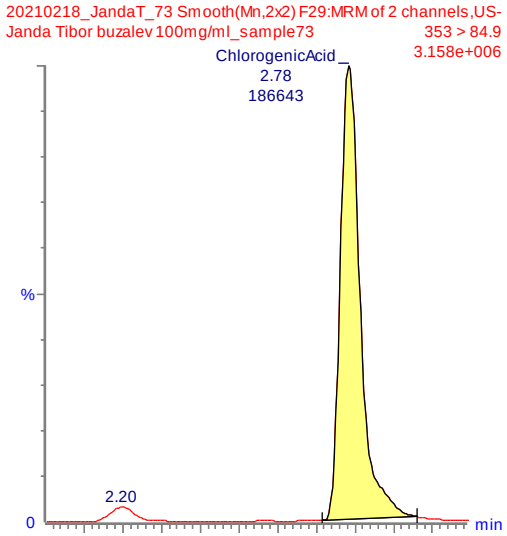


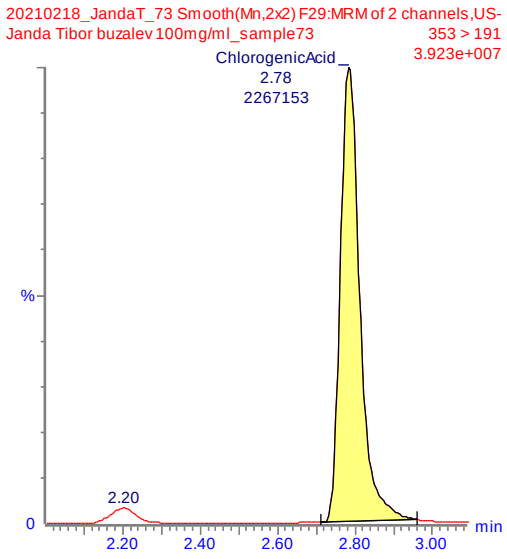
Example MRM type chromatograms for components found above LoQ in a wheat sample data file:20210212JandaT_73 , wheat leaf matrix 100mg/ml) – Slide 2 of 3

jasmonic acid

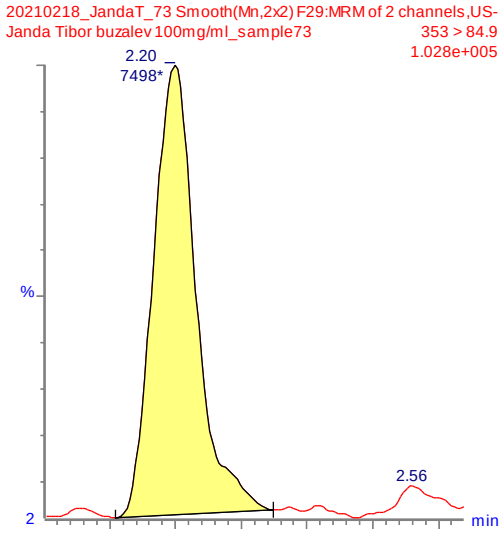


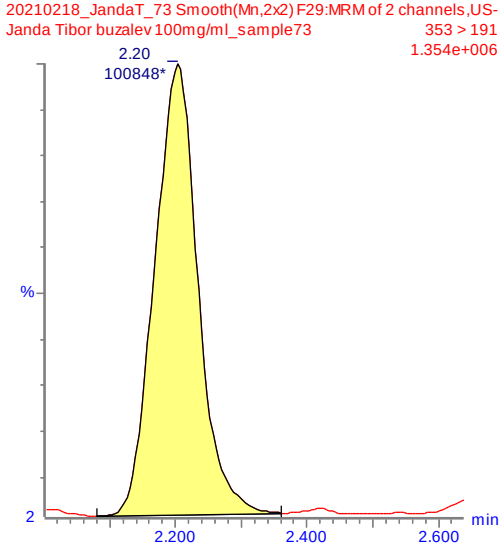
chlorogenic and cryptochlorogenic acid



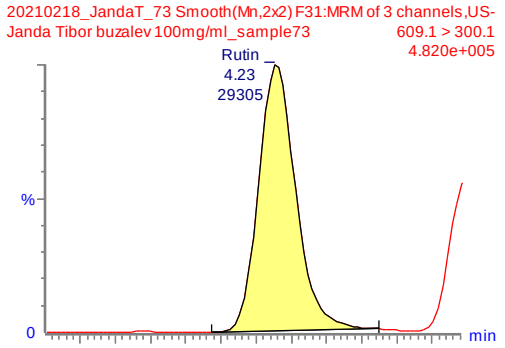


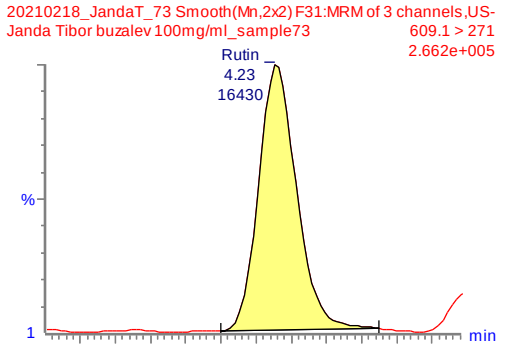
neochlorogenic acid

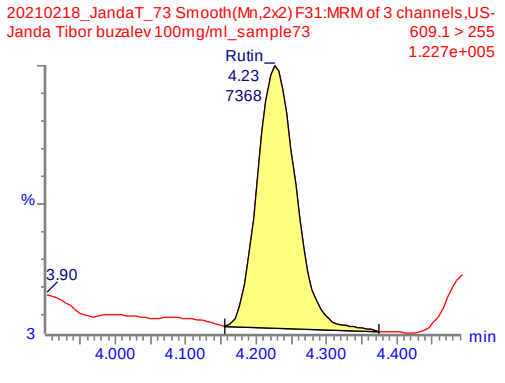




rutin

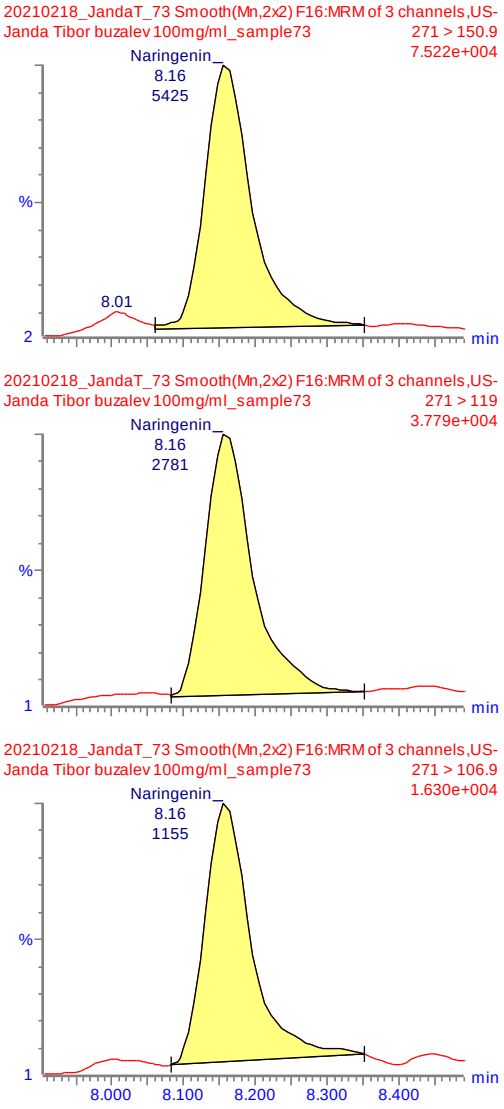




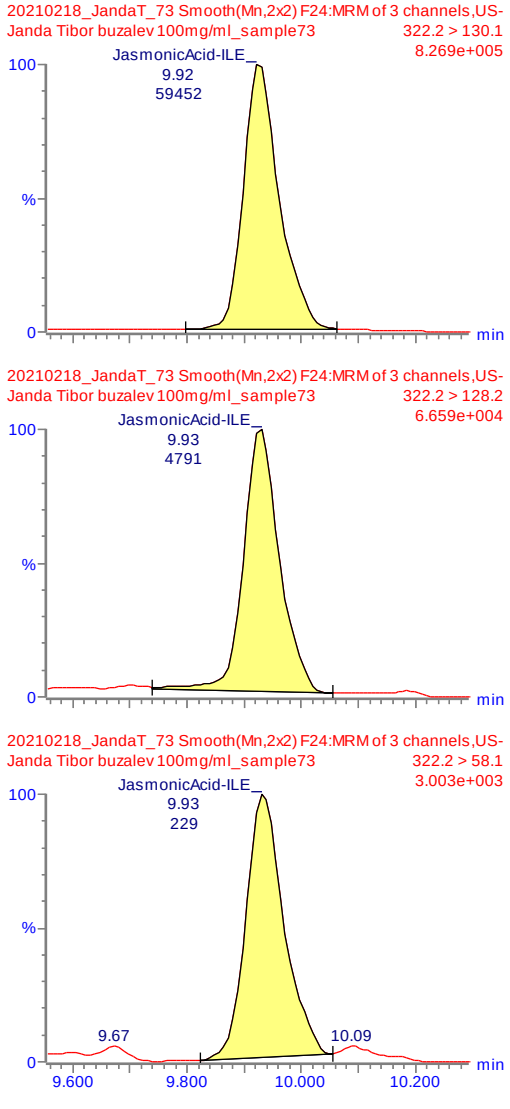


Example MRM type chromatograms for components found above LoQ data file:**20210212JandaT_73 , wheat leaf matrix 100mg/ml**) – Slide 3 of 3

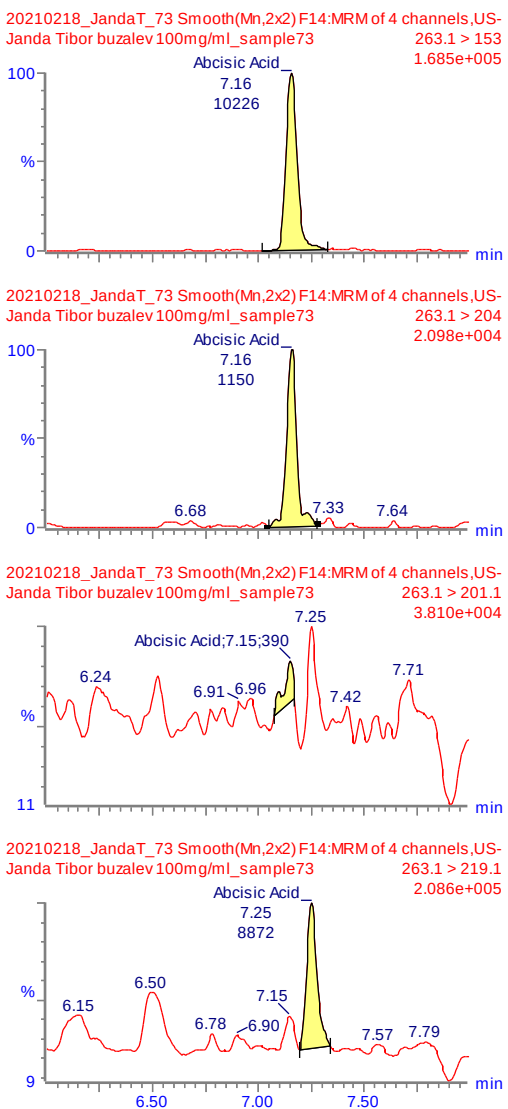
naringenin



jasmonic-acid-leucin/isoleucine conjugate



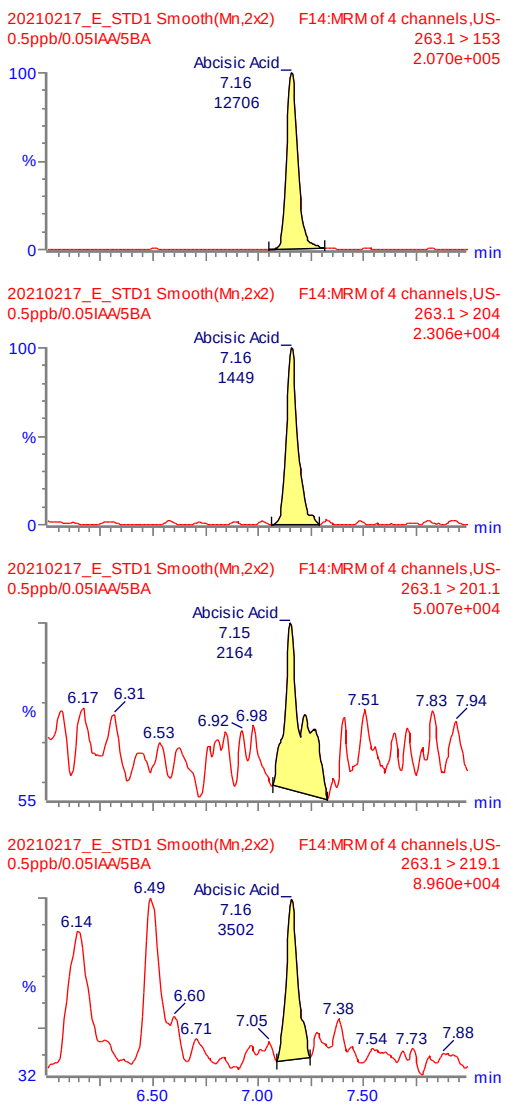
abscisic acid 4.1 ng/g FW. (0.41 ng/ml)



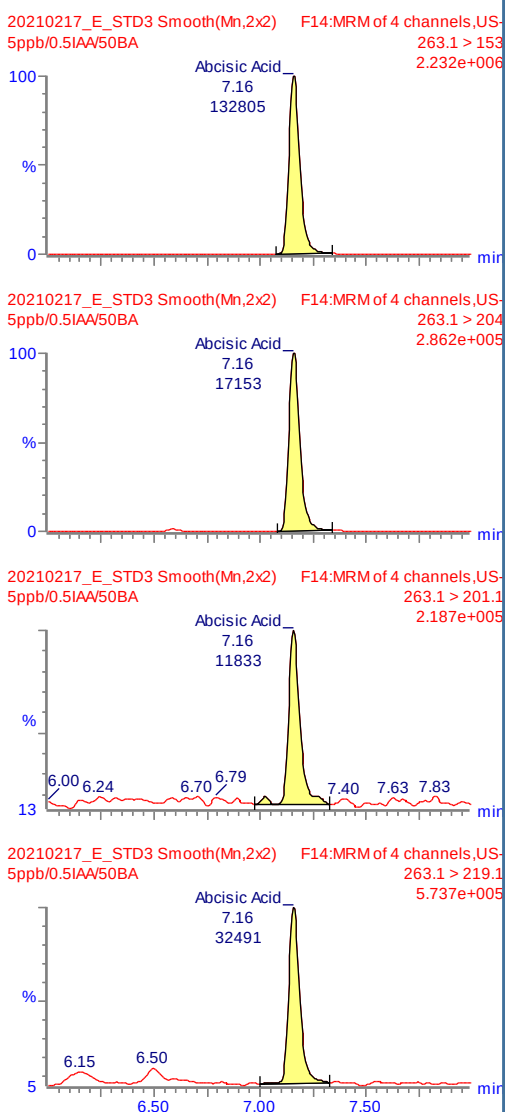
abscisic acid

External solvent calibration point of

0.5 ng/ml



5 ng/ml



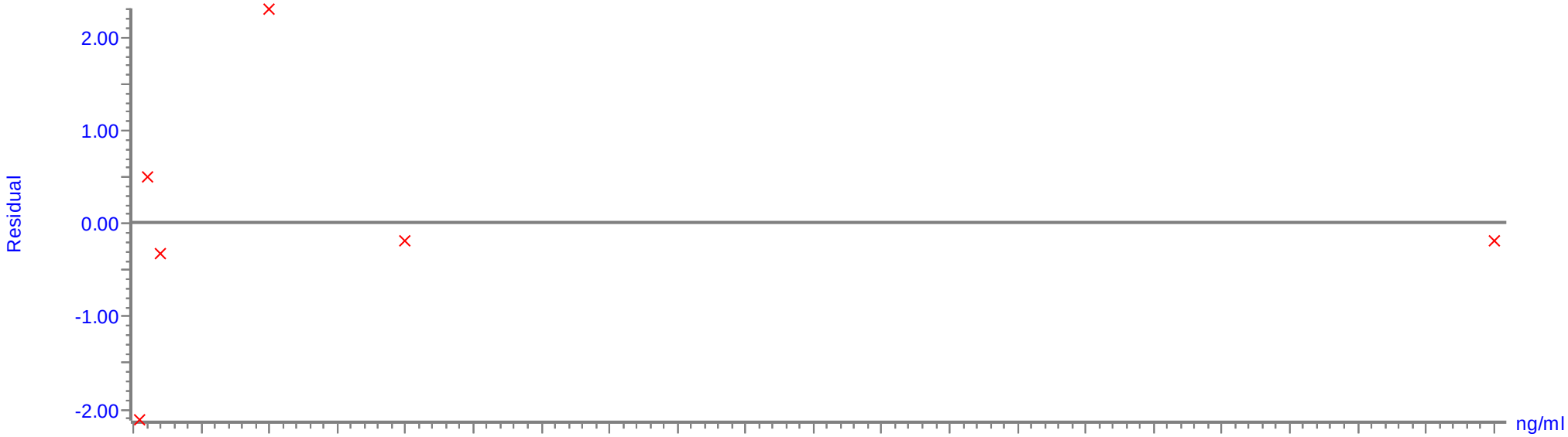
External solvent calibration points of abscisic acid (A) and the external calibration curve for this compound (B)

Compound name: Abcisic Acid
Correlation coefficient: r = 0.999976, r^2 = 0.999952
Calibration curve: 26035.4 * x + -376.299
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

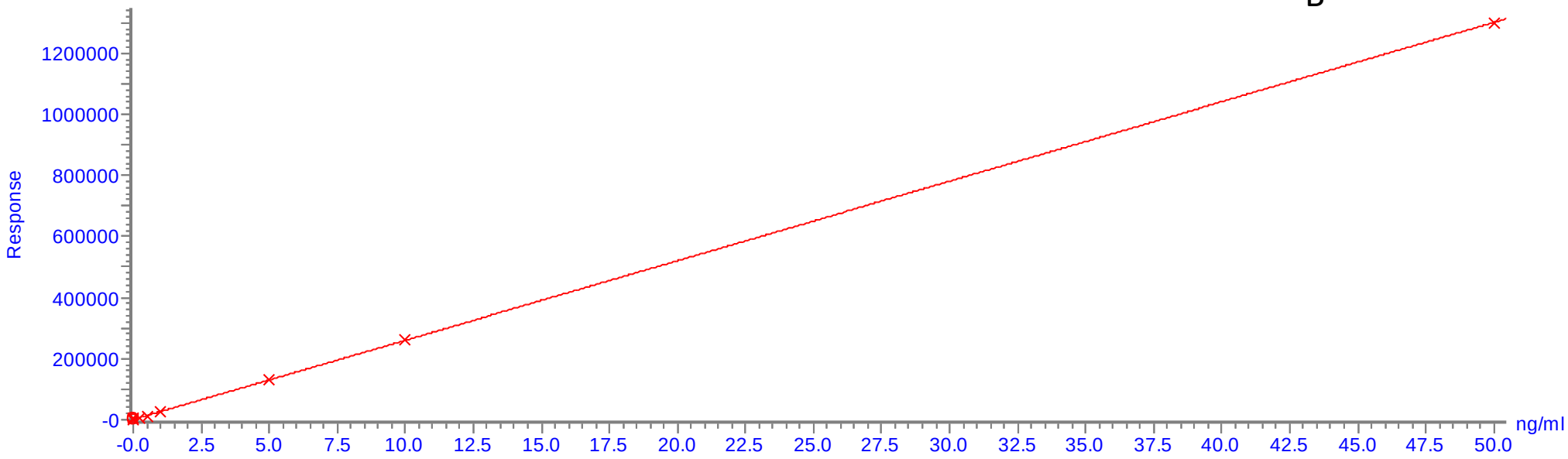
Example of external solvent calibration, compound abscissic acid, measured in US negative mode, quant transition 263>153

7 levels: 0.1 (or 0.25), 0.5, 1.0, 5.0, 10, 50, 100 ng/ml

A



B



Sample chromatograms of various compounds from four algae samples (922, 438, 430 and 612). <LoQ: below the limit of quantitation

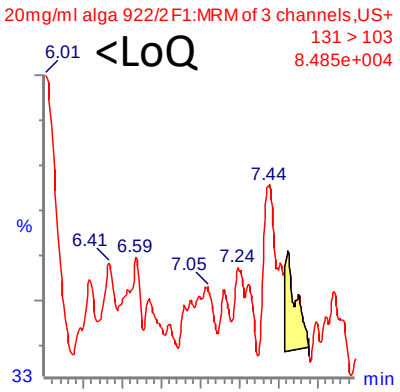
trans-Cinnamic acid

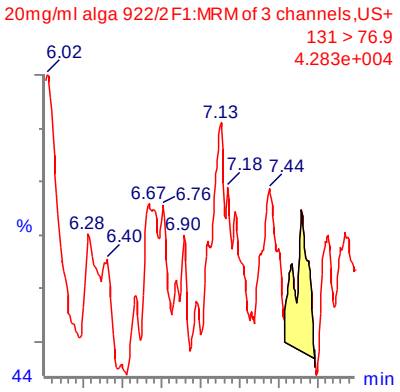
Four sample chromatograms, a spiked sample chromatogram and an external solvent calibration points of *trans*-cinnamic acid.

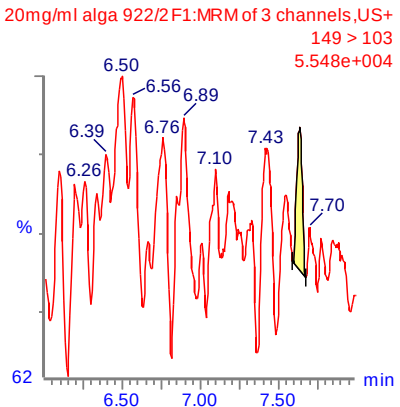
SAMPLES (20mg/ml):

trans-cinnamic acid MRM type MS/MS chromatograms, quantitative transition: US + 131>103

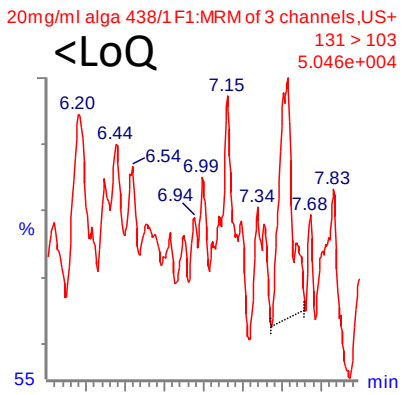
ALGAE 922/1

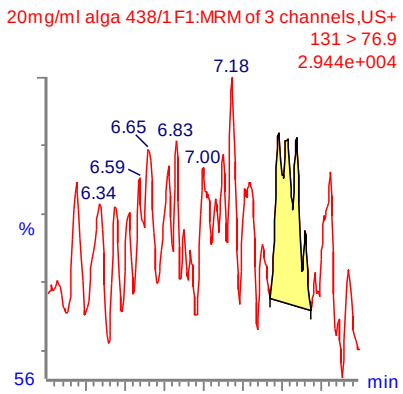


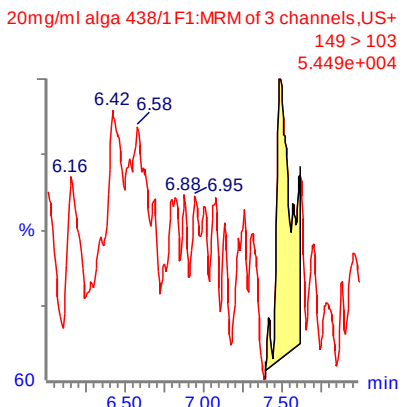




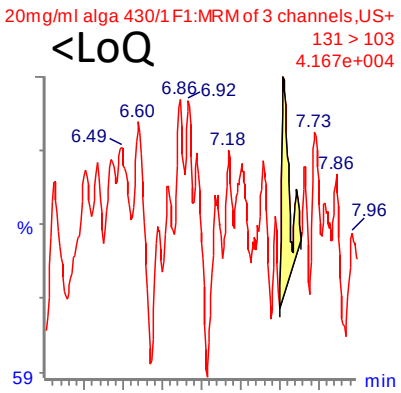
ALGAE 438/1

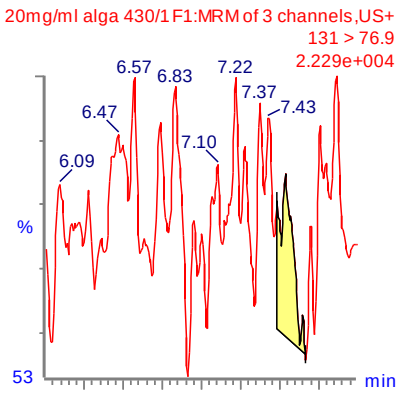


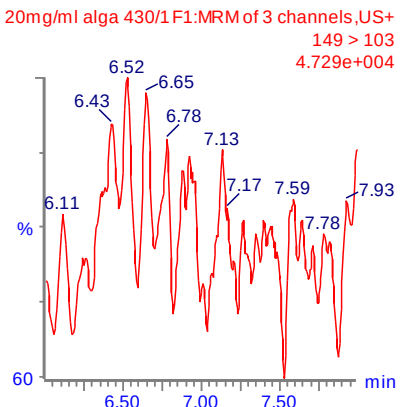




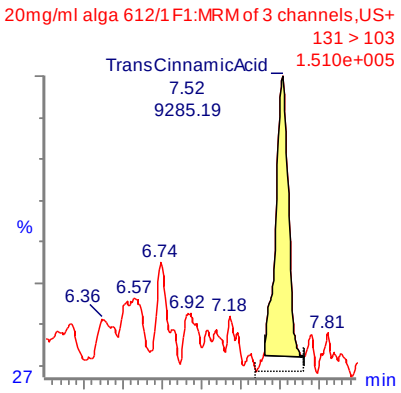
ALGAE 430/1

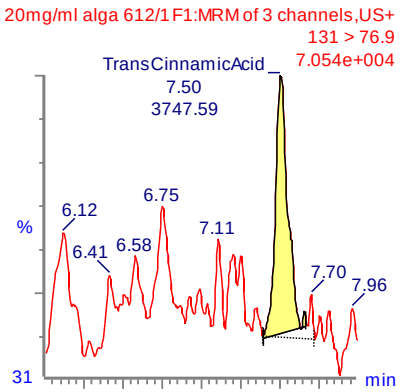


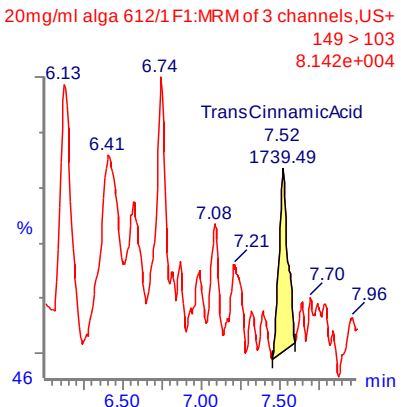




ALGAE 612/1

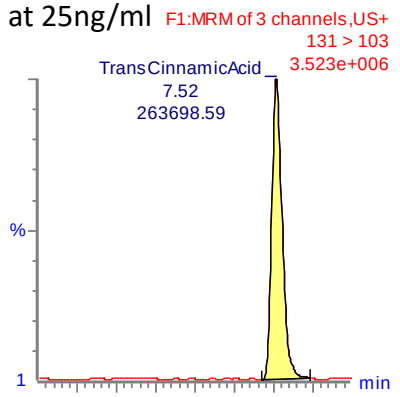


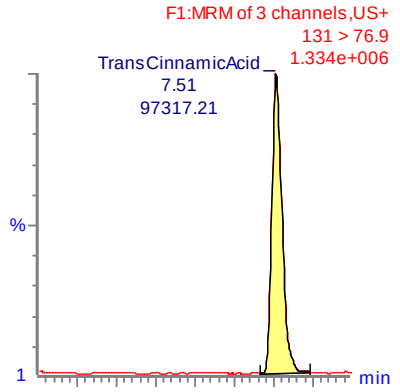


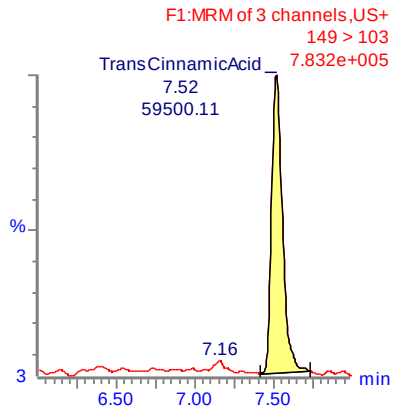


ALGAE 430/4S

spiked prior to extraction at 25ng/ml

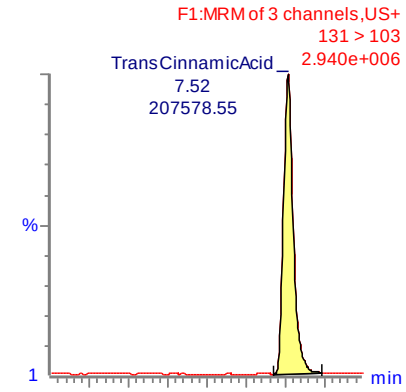


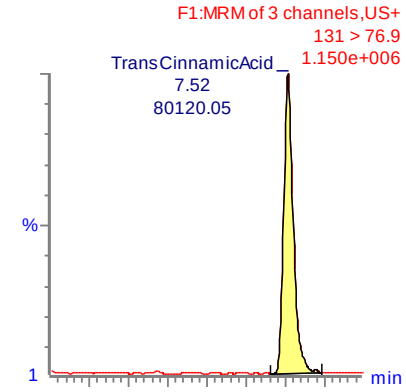


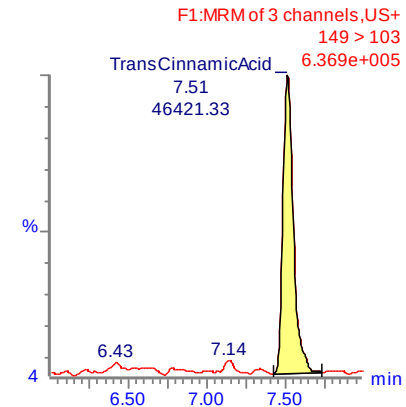


Procedural Solvent calibration point

prepared as samples at 25ng/ml final conc.







Indole-3-acetic acid (IAA)

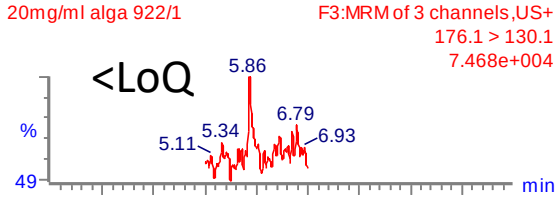
Page 12: Four sample chromatograms of IAA

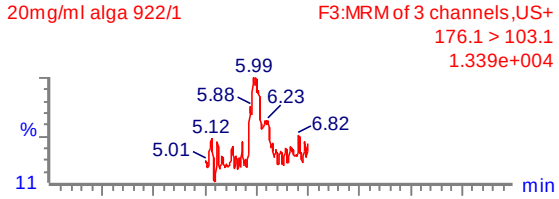
Page 13: Two sample chormatograms above and below LoQ, a spiked chromatogram of <LOQ sample and an external solvent calibration points of IAA.

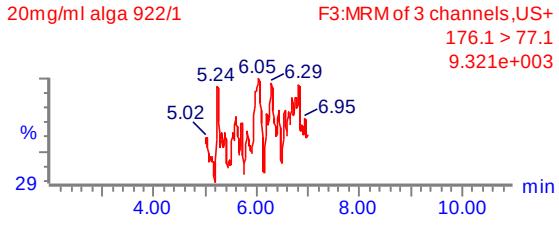
Indole-3-acetic acid (IAA) MRM type MS/MS chromatograms, slide 1 of 2
quantitative transition: US + 176.1>130.1

SAMPLES (20mg/ml):

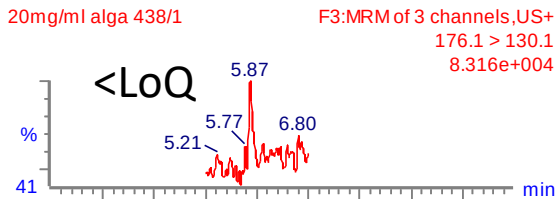
ALGAE 922/1

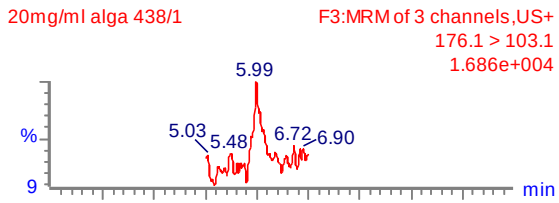


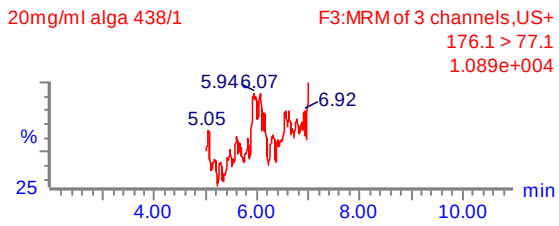




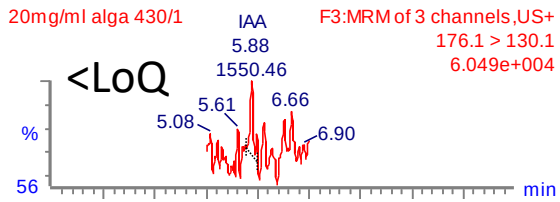
ALGAE 438/1

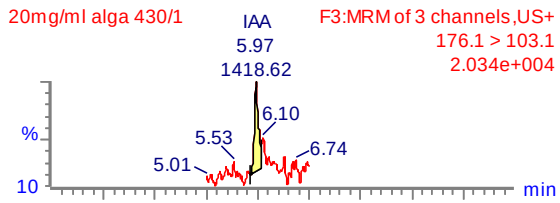


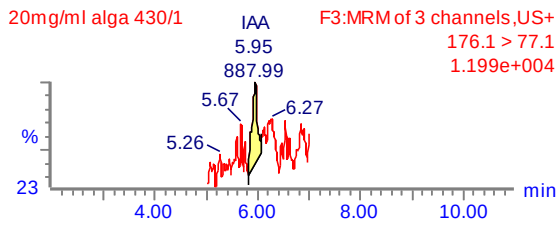




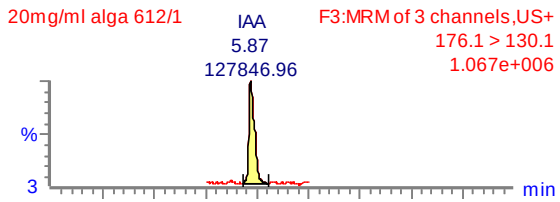
ALGAE 430/1

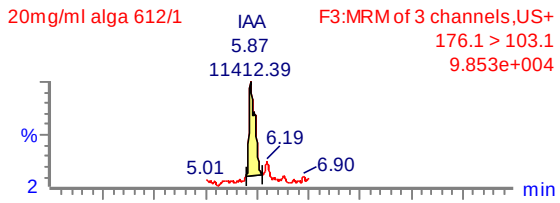


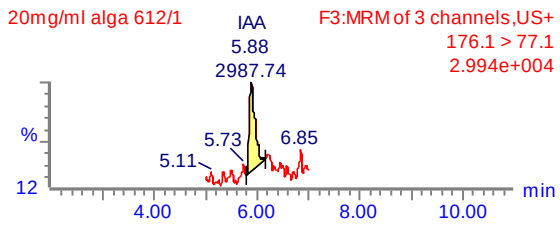




ALGAE 612/1



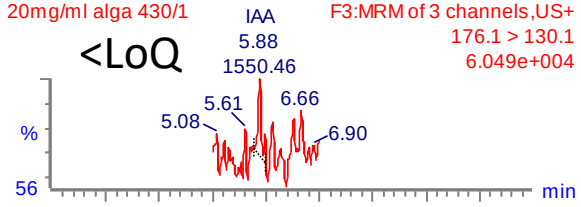


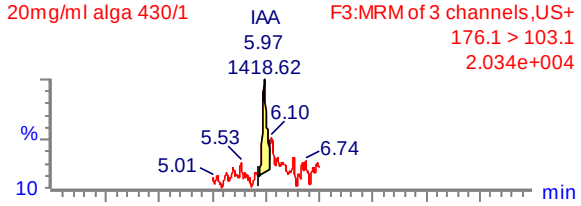


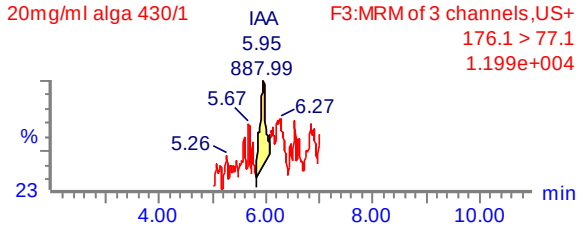
Indole-3-acetic acid (IAA) MRM type MS/MS chromatograms, slide 2 of 2
quantitative transition: US + 176.1>130.1

SAMPLES (20mg/ml):

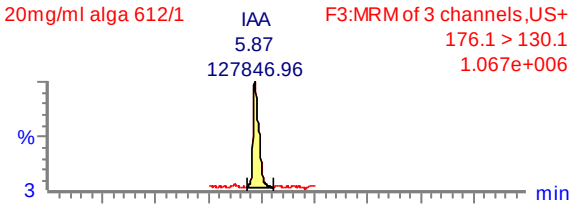
ALGAE 430/1

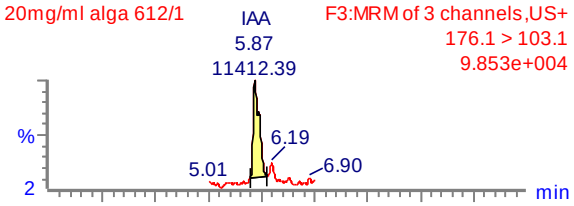


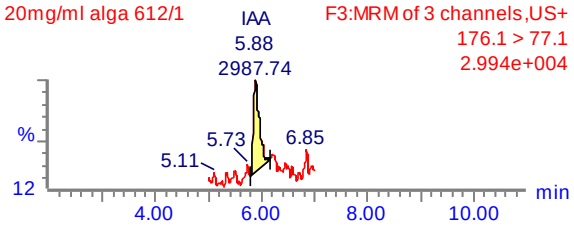




ALGAE 612/1

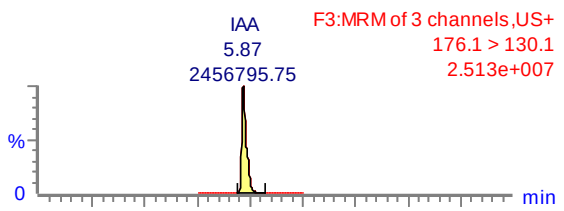


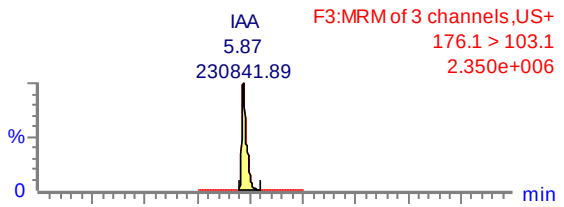


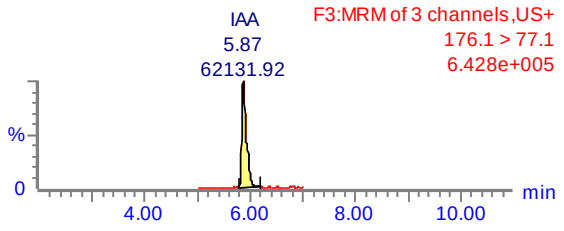


ALGAE 430/4S

spiked prior to extraction
at 25ng/ml

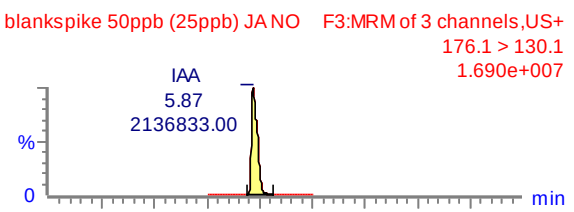


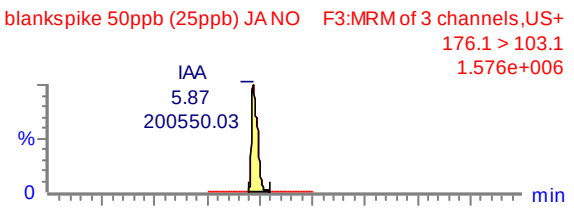


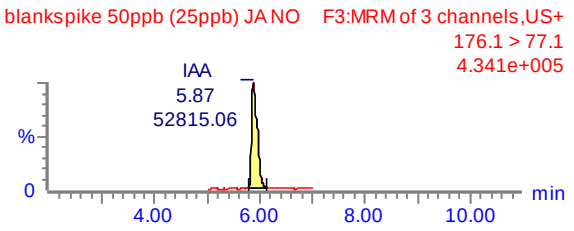


Procedural Solvent calibration point

prepared as samples at
25ng/ml final conc.







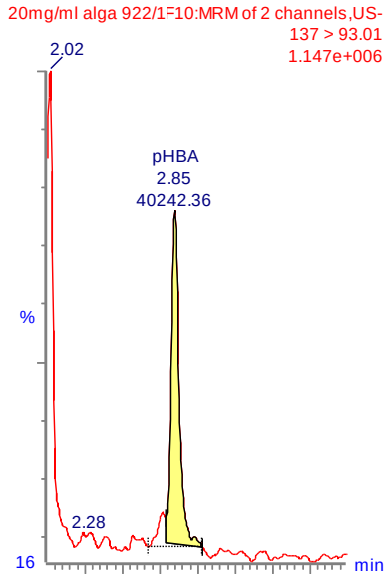
para-Hydroxibenzoic acid

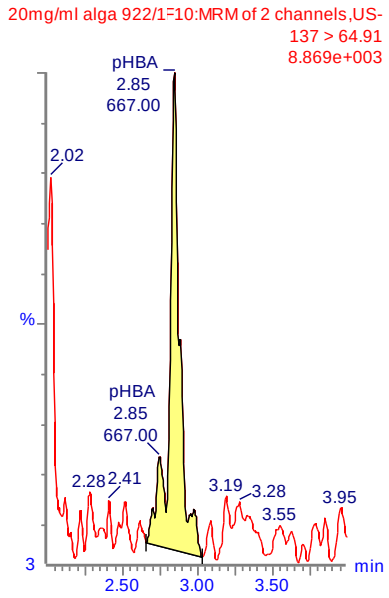
Four sample chromatograms, a spiked sample chromatogram and an external solvent calibration points of *para*-hydroxibenzoic acid.

SAMPLES (20mg/ml):

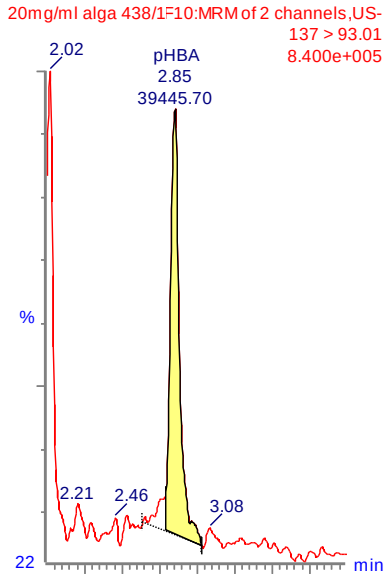
Para-hydroxybenzoic acid MRM type MS/MS chromatograms, quantitative transition: US - 137>93

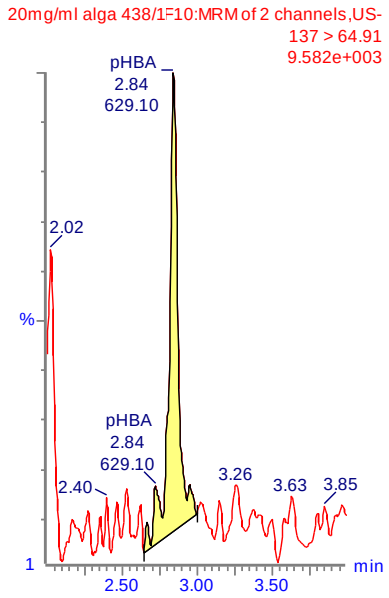
ALGAE 922/1



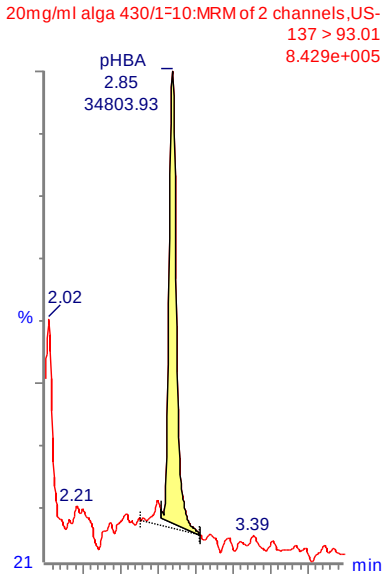


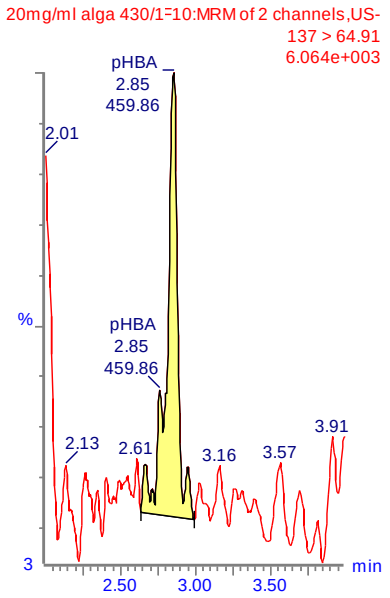
ALGAE 438/1



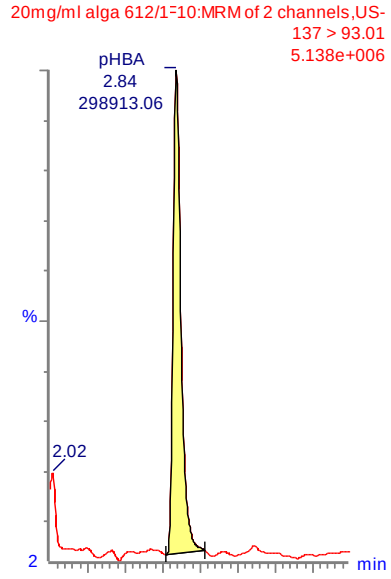


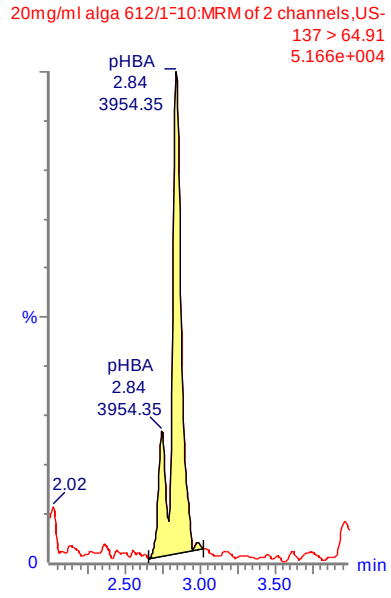
ALGAE 430/1





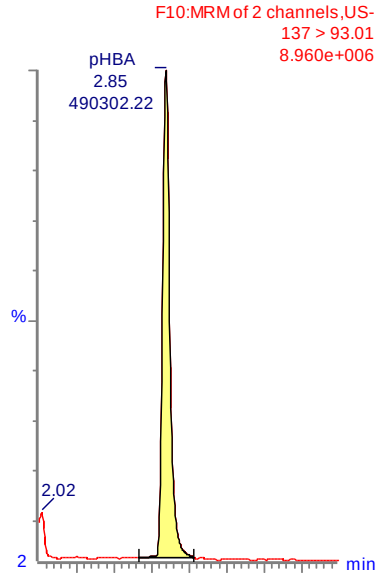
ALGAE 612/1

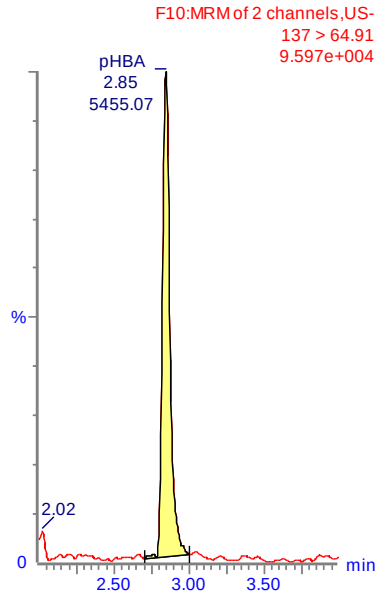




ALGAE 430/4S

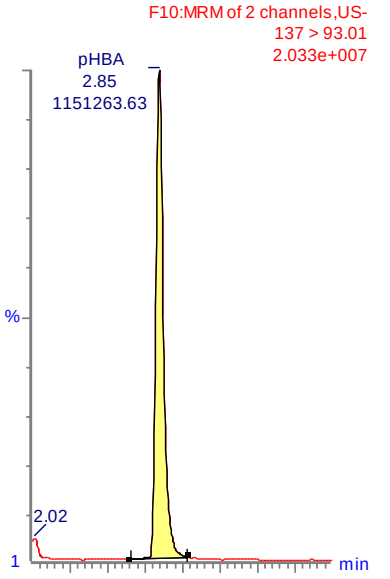
spiked prior to extraction at 25ng/ml

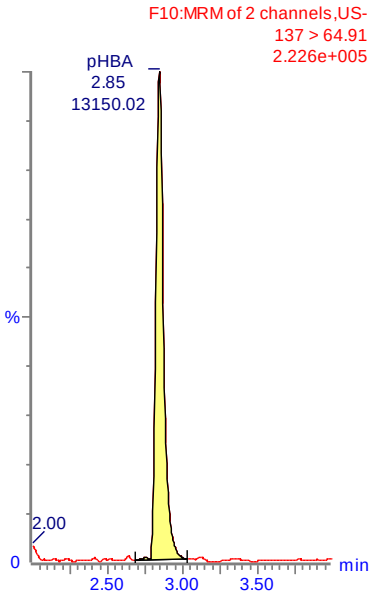




Procedural Solvent calibration point

prepared as samples at 25ng/ml final conc.





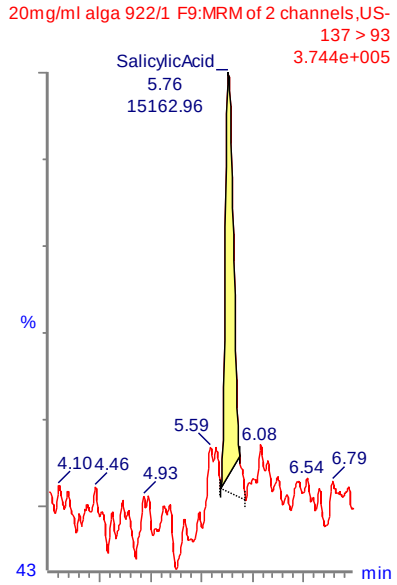
Salycilic acid

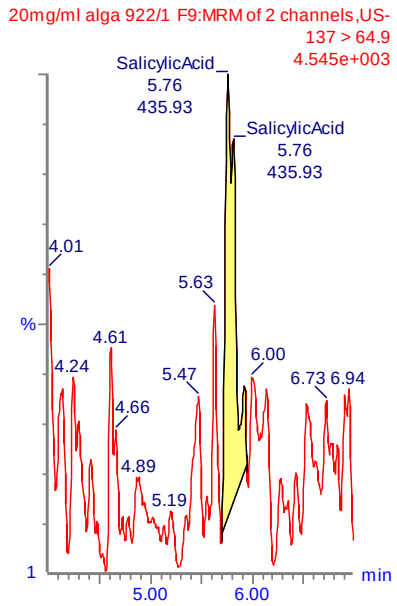
Four sample chromatograms, a spiked sample chromatogram and an external solvent calibration points of *s*alycilic acid.

SAMPLES (20mg/ml):

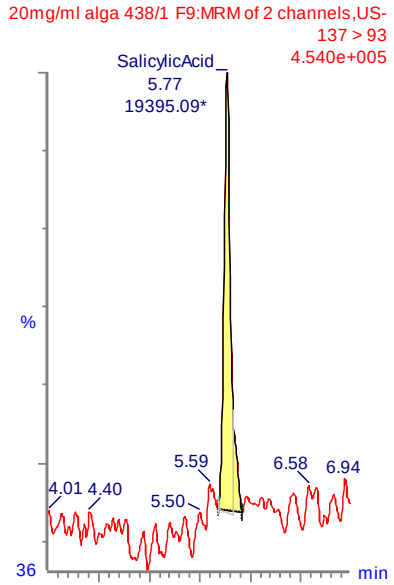
Salicylic acid MRM type MS/MS chromatograms, quantitative transition: US - 137>93

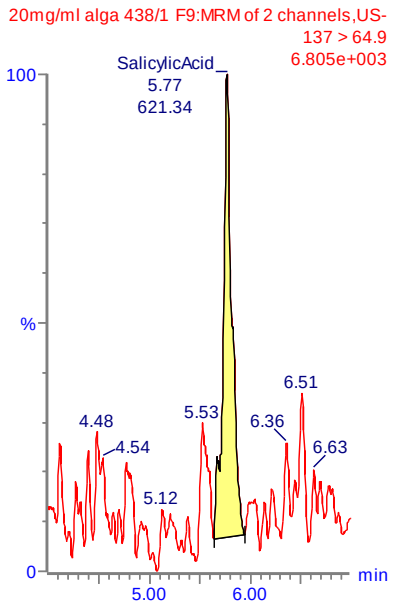
ALGAE 922/1



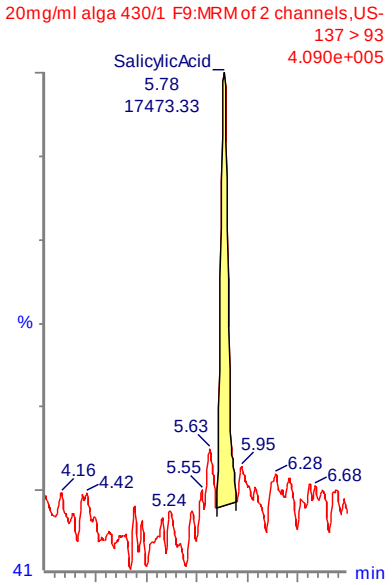


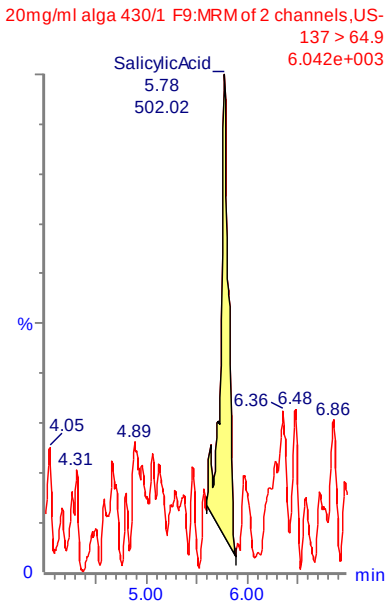
ALGAE 438/1



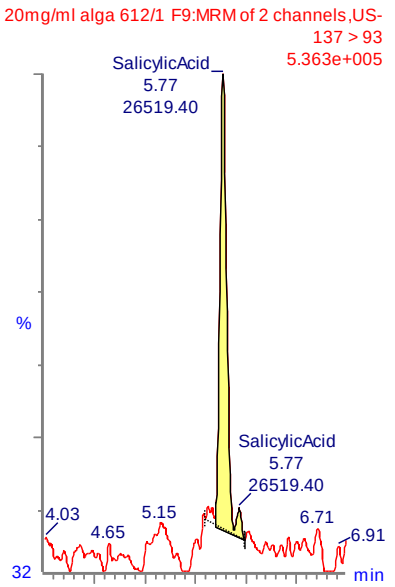


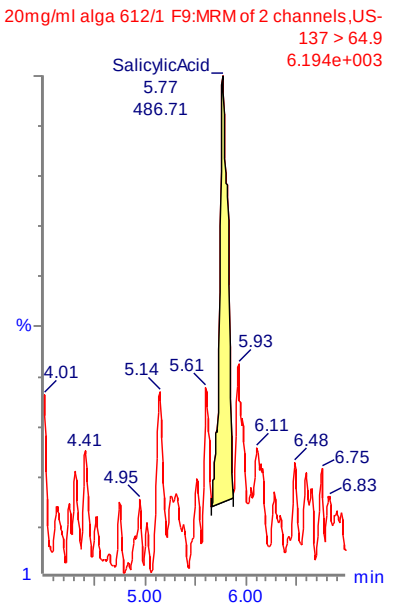
ALGAE 430/1





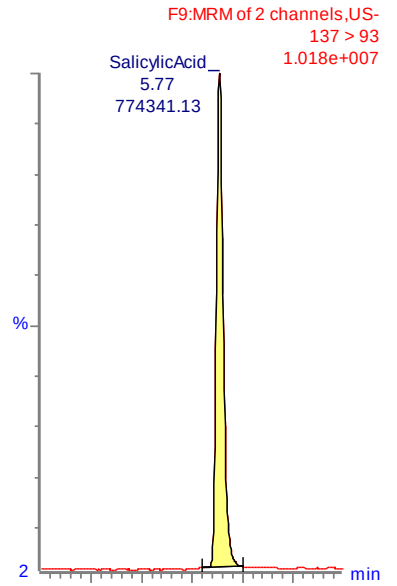
ALGAE 612/1

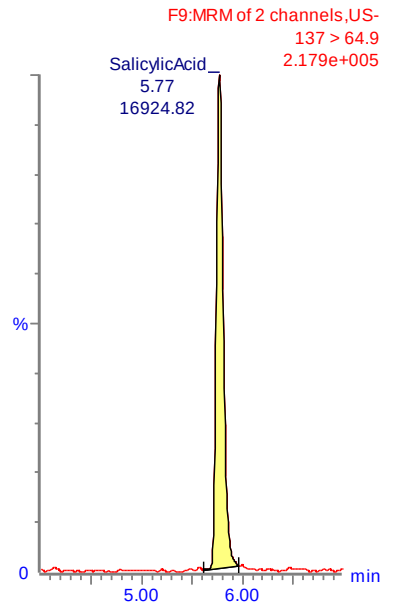




ALGAE 430/4S

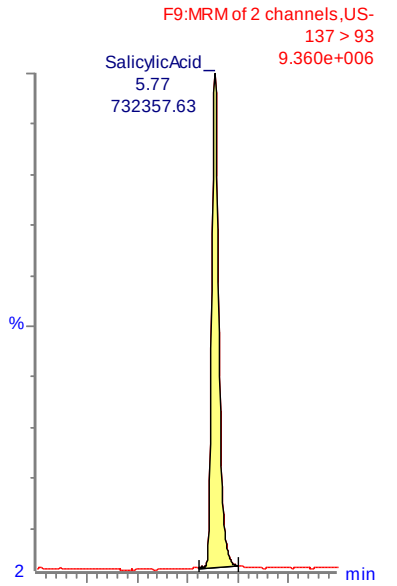
spiked prior to extraction at 25ng/ml

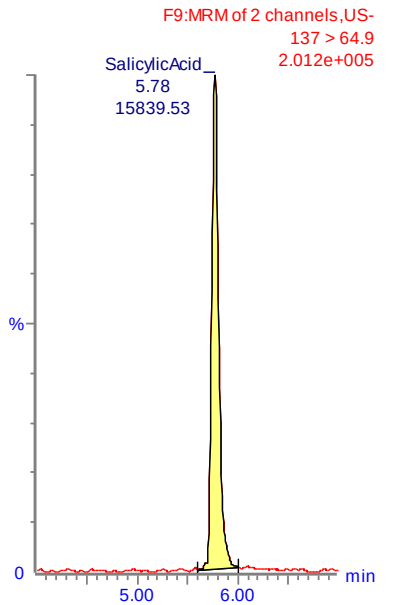




Procedural Solvent calibration point

prepared as samples at 25ng/ml final conc.





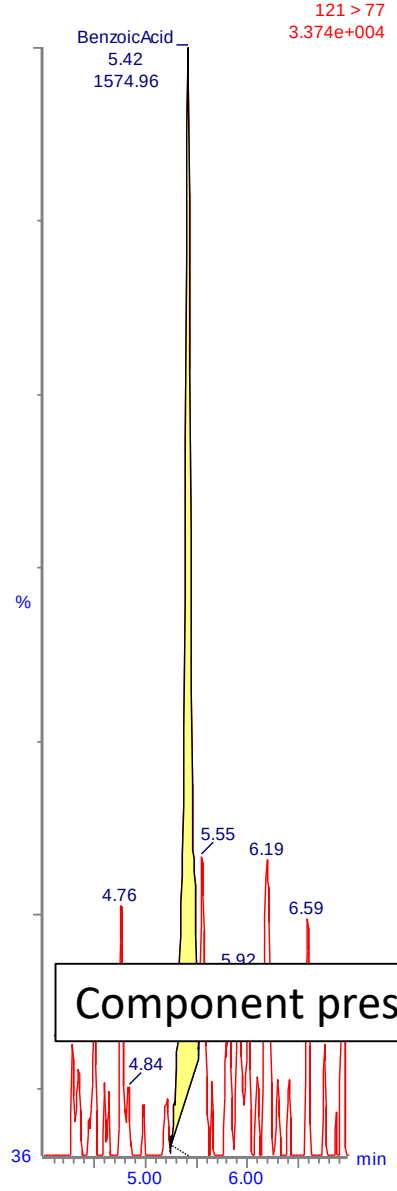
Benzoic acid

Four sample chromatograms, a spiked sample chromatogram and an external solvent calibration points of benzoic acid.

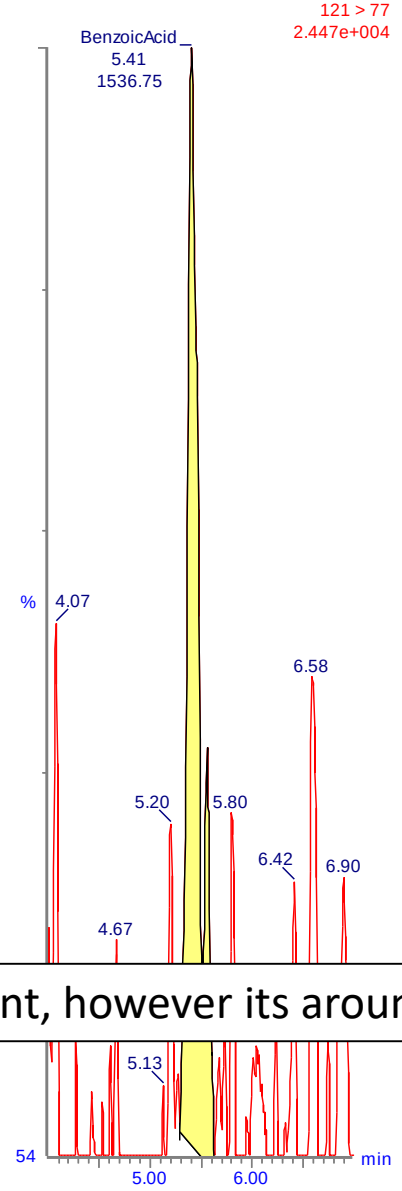
SAMPLES (20mg/ml):

Benzoic acid MRM type MS/MS chromatograms, quantitative transition: US - 121>77

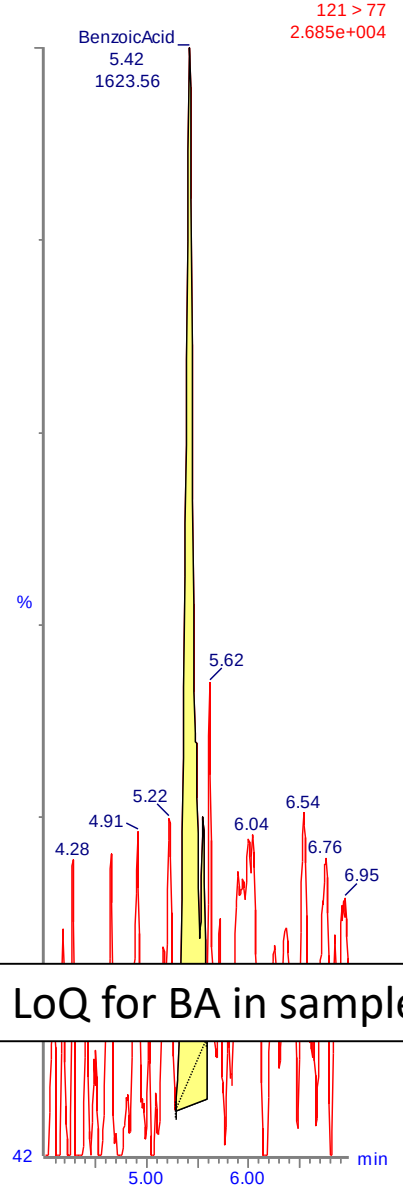
ALGAE 922/1



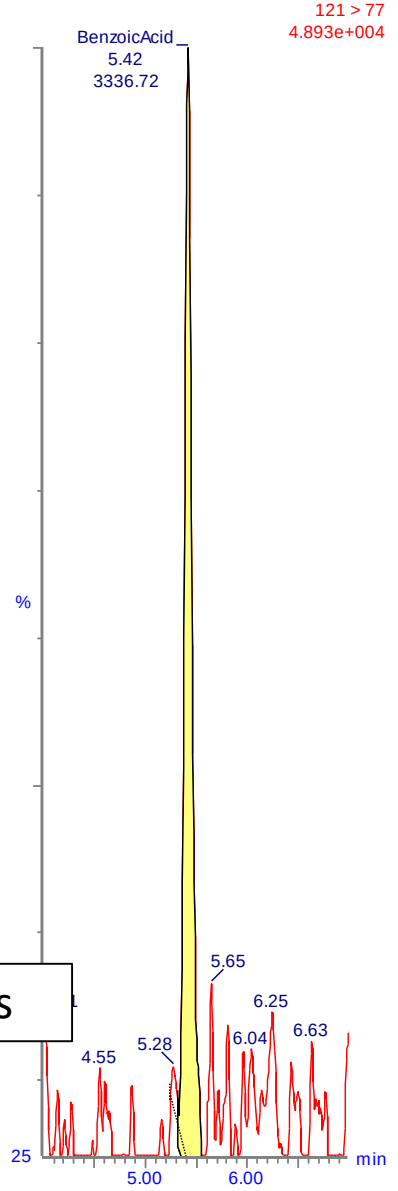
ALGAE 438/1



ALGAE 430/1

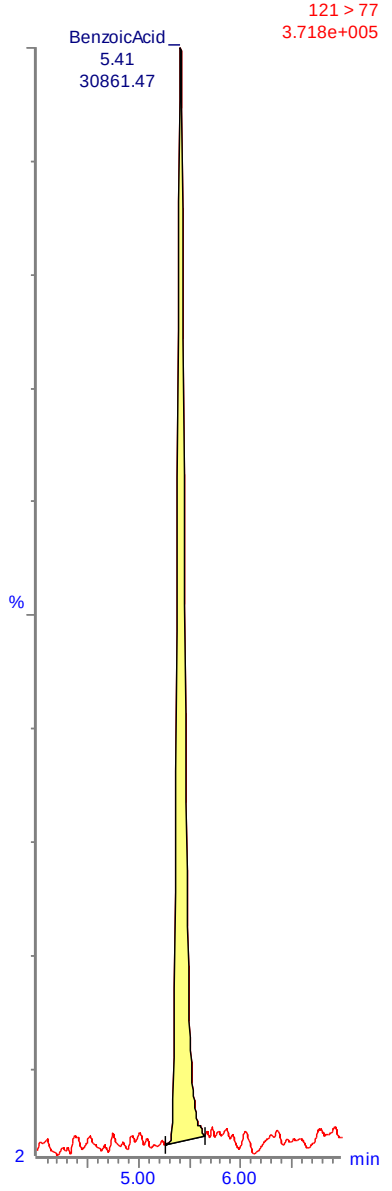


ALGAE 612/1



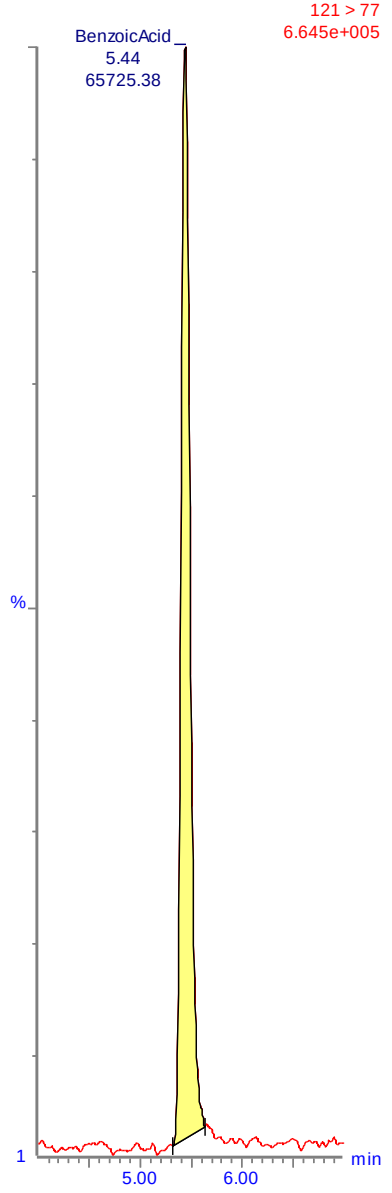
ALGAE 430/4S

spiked prior to extraction at 25ng/ml



Procedural Solvent calibration point

prepared as samples at 25ng/ml final conc.



Component present, however its around LoQ for BA in samples

Gibberellic acid (GA3)

Page 21: A sample chormatogram below LoQ, a spiked chromatogram of the same sample and two lower external solvent calibration points of GA3.

Page 22: Four sample chromatograms of GA3.

Example for a lower abundant plant hormone compound, that was <LoQ for all samples slide 1 of 2
Gibberellic acid (GA3) MRM type MS/MS chromatograms,
quantitative transition: US – 345>239

ALGAE 430/1

<LoQ

ALGAE 430/4S

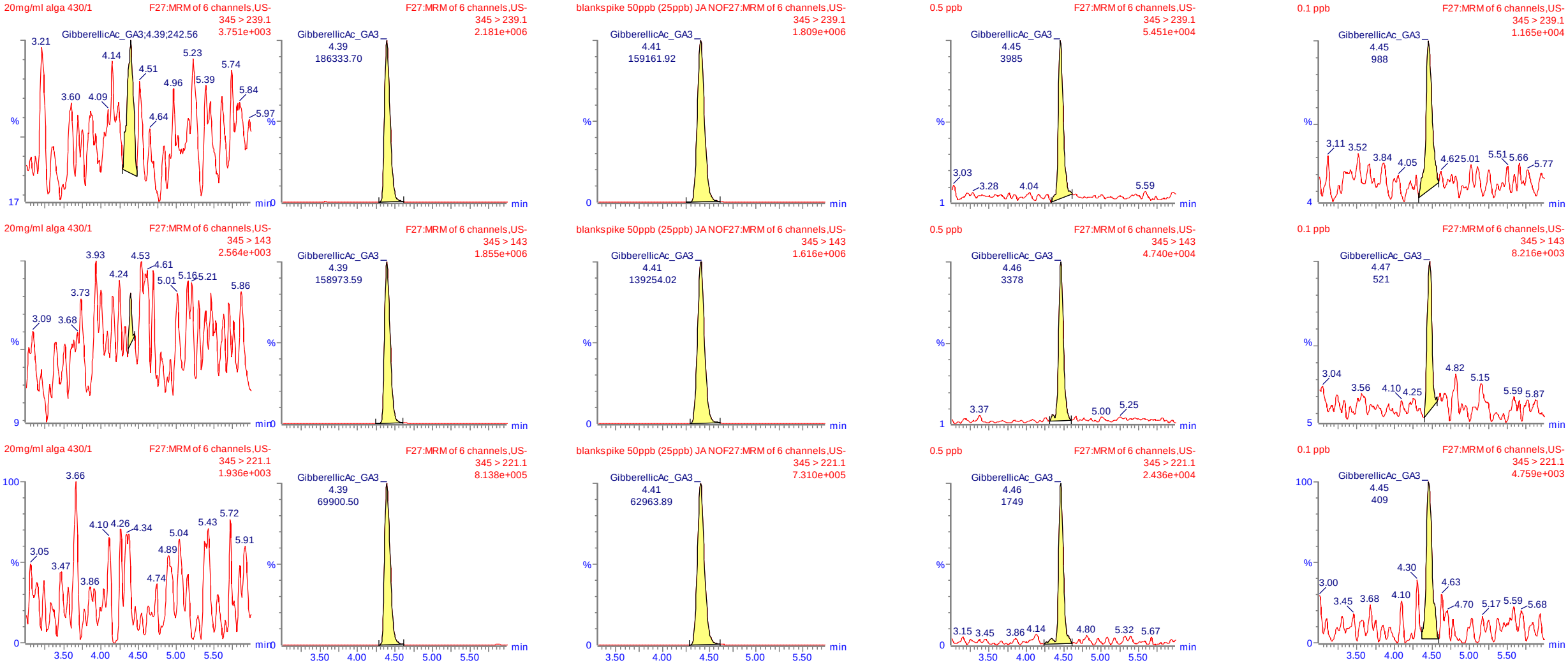
spiked prior to extraction at 25ng/ml

Procedural solvent calibration point

prepared as samples at 25ng/ml final conc.

Lower external solvent calibration points

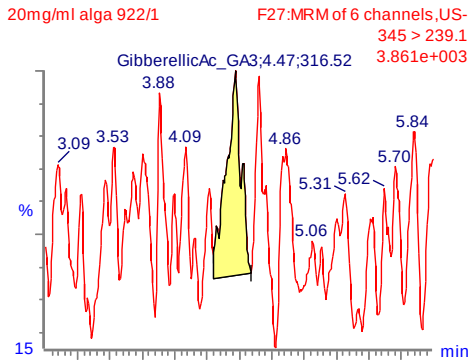
at 0.5 ng/ml and 0.1 ng/ml

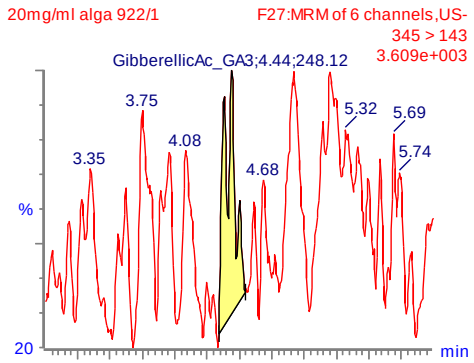


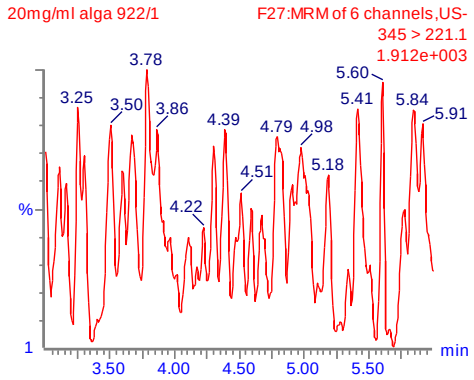
Example for a lower abundant plant hormone compound, that was <LoQ for all samples slide 2 of 2
Gibberellic acid (GA3) MRM type MS/MS chromatograms,
quantitative transition: US – 345>239

SAMPLES (20mg/ml):

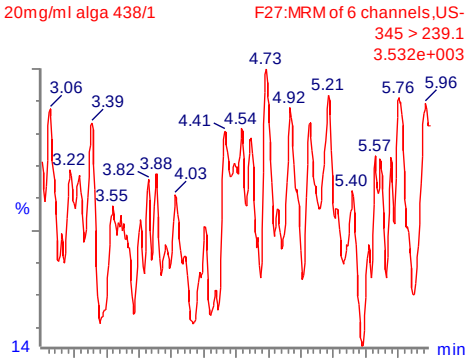
ALGAE 922/1 <LoQ

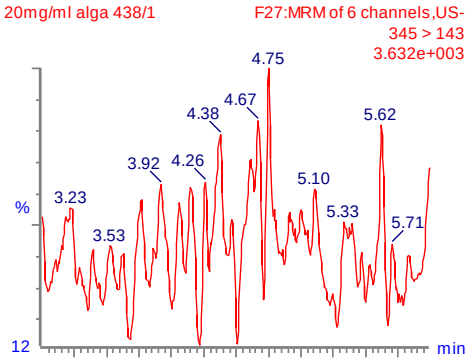


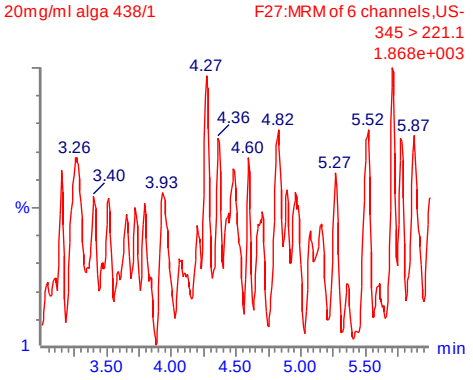




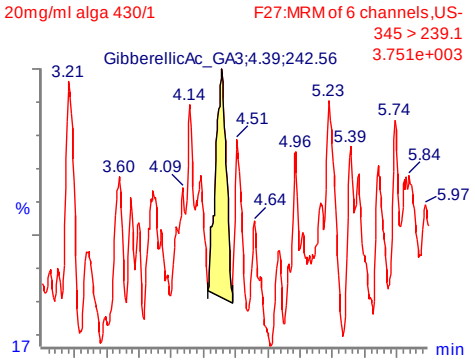
ALGAE 438/1 <LoQ

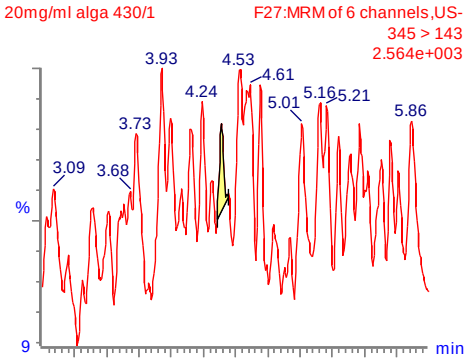


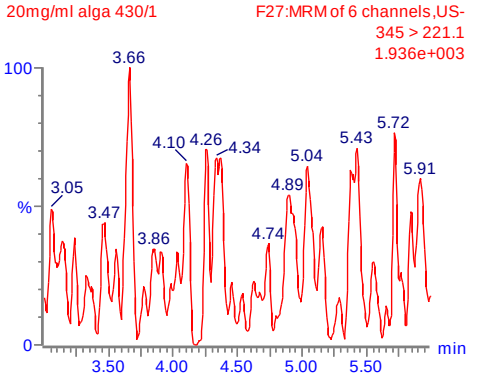




ALGAE 430/1 <LoQ







ALGAE 612/1 <LoQ