Clean Seas Environmental Monitoring Program (CSEMP)

Determination of OCPs, PAHs and PCBs in Mussel Tissue using the 7000 Series Triple Quadrupole GC-MS/MS system

Environment Agency

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Clean Seas Environmental Monitoring Program (CSEMP)

- Aims to detect long term trends in the quality of the UK's coastal and estuarine areas
- To fulfil the UK's commitment to EU directives and monitoring requirements under Oslo Paris Convention (OSPAR)
- Monitoring of selected Organo-chlorine Pesticides, Polyaromatic hydrocarbons, and Polychlorinated biphenyls in marine biota and sediments (plus Cd, Cu, Pb, Ni, Zn, As, Cr, Ag, Se)
- Required LODs : OCPs / PCBs 0.1 μg/kg, wet weight
  Napthalene & Phenanthrene 1 μg/kg, wet weight
  All other PAHs 0.5 μg/kg, wet weight

Clean Seas Environmental Monitoring Program (CSEMP)

- **Previous EA Sample Preparation Method:**

- ASE Extraction using 1:1 Hexane:Dichloromethane
- Concentrate the extract and clean up with GPC to remove lipids / sulphur or other co-extracts
- Further clean up using SPEs / Conc. Sulphuric acid

- Expensive Extraction and clean up equipment
- Use of large amount of solvents
- Time consuming
- Issues of sample carryover

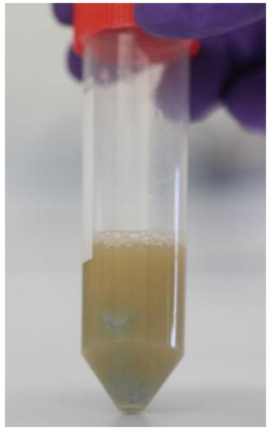
- 40 μL injection into GC/MSD, SIM mode

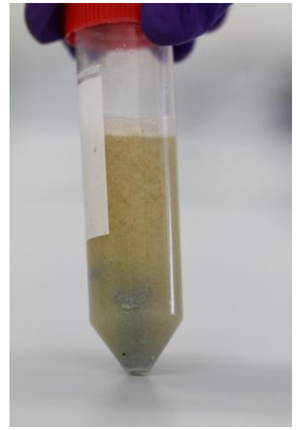
EA / Agilent Collaboration

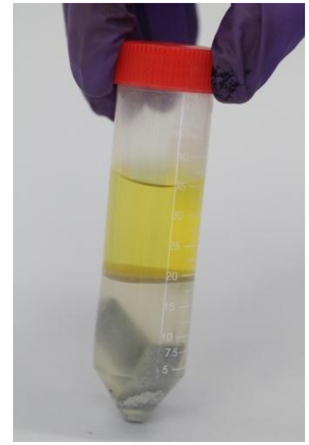
- ENVIRONMENT AGENCY **Sample preparation**
- Agilent Technologies **GC-MS/MS Method development**
  - Optimization of Large Volume Injection parameters
  - GC Method : Retention time locking, Back flush
  - Optimization of MRM transitions

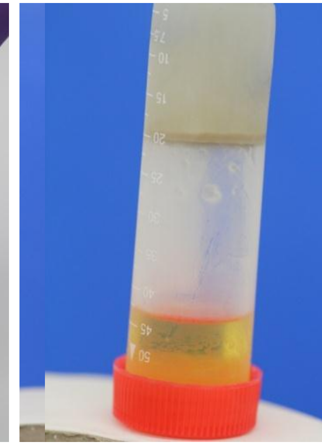
- ENVIRONMENT AGENCY **Method validation**

Mussel Tissue : Sample Prep (1) : Extraction









(1) 2g homogenized mussel tissue weighed into QuEChERS extraction tube.
(2) Add labelled ISTDs (prepared in acetone)

(3) Vortex mix sample for 30 seconds, add 13mL de-ionized water and 2 ceramic homogenizers

(4) Vortex mix sample for 1 minute. Add 15mL extraction solvent (1% acetic acid in acetonitrile), vortex mix 1 minute.

(5) Add QuEChERS AOAC salt mix (6 g $MgSO_4$, 1.5 g Na Acetate 5982-5755), shake tube by hand for 1 minute, vortex mix for 1 minute.

(6) Centrifuge tube for 5 minutes at 3900 rpm then cool tube in freezer at -20 °C for 30 minutes.

(7) Transfer acetonitrile layer to clean / dry centrifuge tube containing 1g anhydrous sodium sulphate.

(8) Shake by hand for 1 minute then place tube in freezer at -20 °C overnight.

QuEChERS
Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe

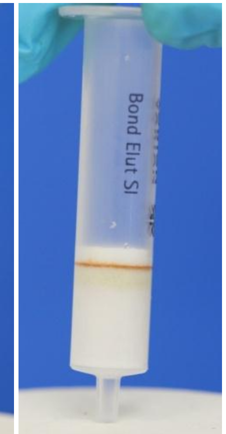
Mussel Tissue : Sample Prep (2) : Clean-up


(9) Pipette 10mL clear extract in to a clean turbovap tube, evaporate to 0.5 mL

(10) Add 20 mL dichloromethane (DCM) evaporate to 0.5mL, add 10 mL DCM. Evaporate to 0.5 mL.

(11) Add 10 mL Hexane, evaporate to 0.5 mL, add 10 mL Hexane and evaporate to 0.5 mL

(12) Activate silica at 180 °C overnight, add 1g to empty 15mL SPE tube

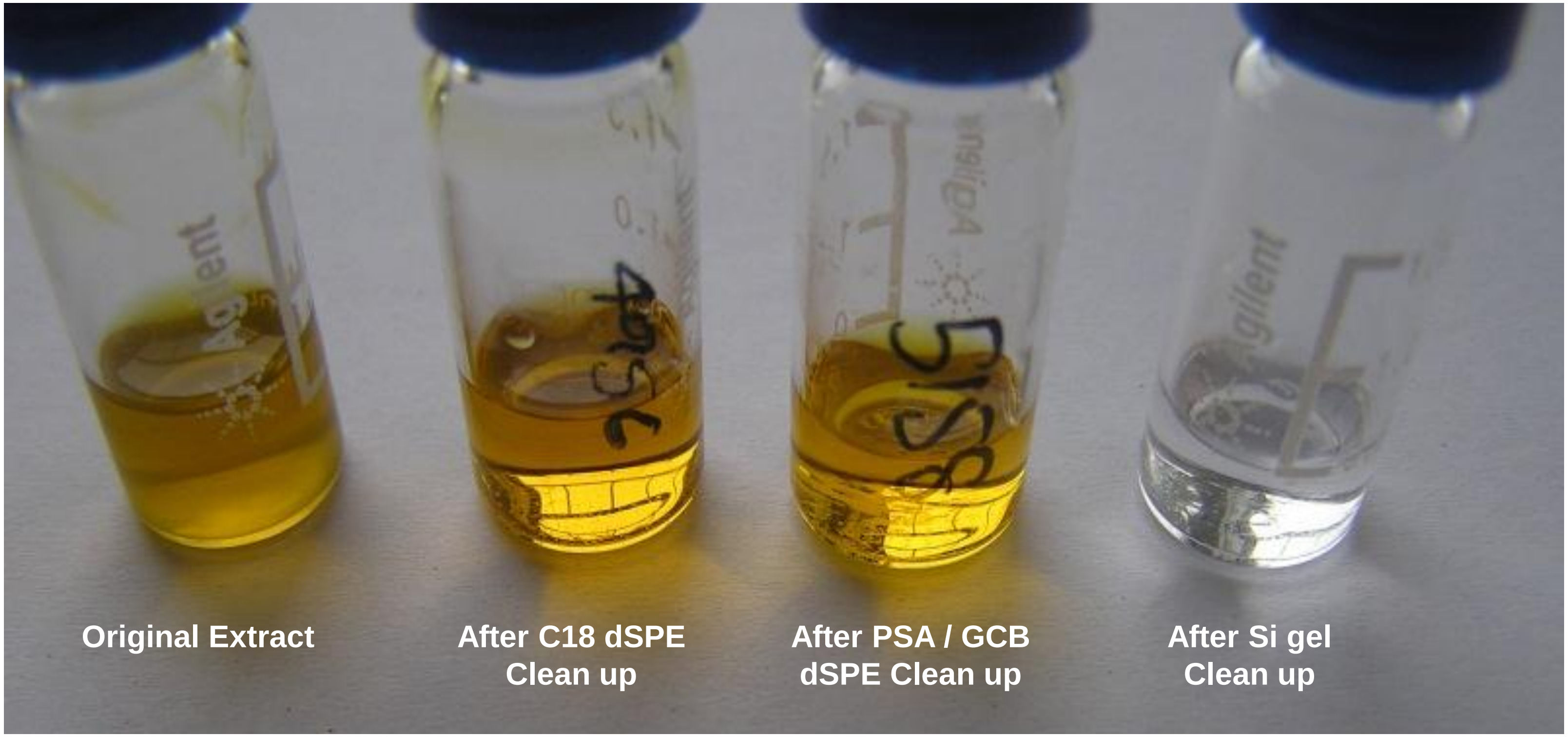
(13) Condition 1g Silica in SPE tube with 10 mL DCM and 20 mL hexane

(14) Apply concentrated extract to Silica SPE tube elute with 13 mL 40:60 DCM : hexane

(15) Evaporate extract to 0.5 mL, repeat steps (13) and (14)

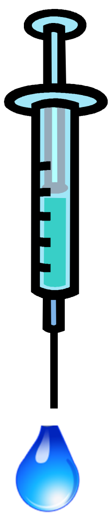
(16) Evaporate extract to 0.5 mL, transfer to 2 mL autosampler vial

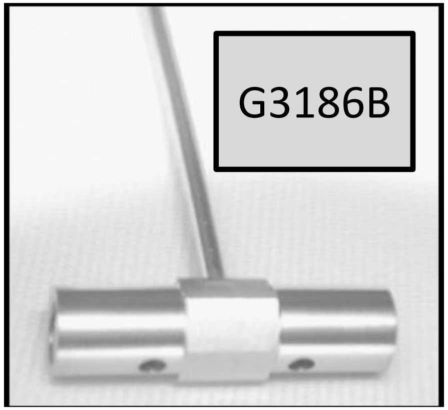
dSPE vs SPE

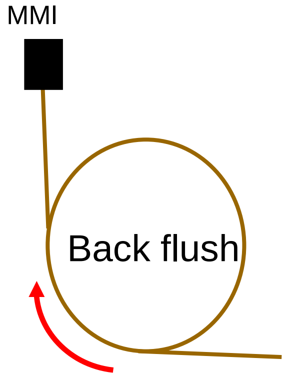

Original Extract
After C18 dSPE Clean up
After PSA / GCB dSPE Clean up
After Si gel Clean up

CSEMP – GC-MS/MS Method Development

- Cold splitless or Solvent Vent injection (LVI)
- CO2 cooled Multi-mode inlet with 2 mm ID dimpled deactivated liner (5190-2296)
- DB5-MS Ultra inert column
- Retention Time Locking (RTL)
- Pressure Controlled Tee (PCT) Capillary Flow Device (G3186B)
- Post column, post-run back flush

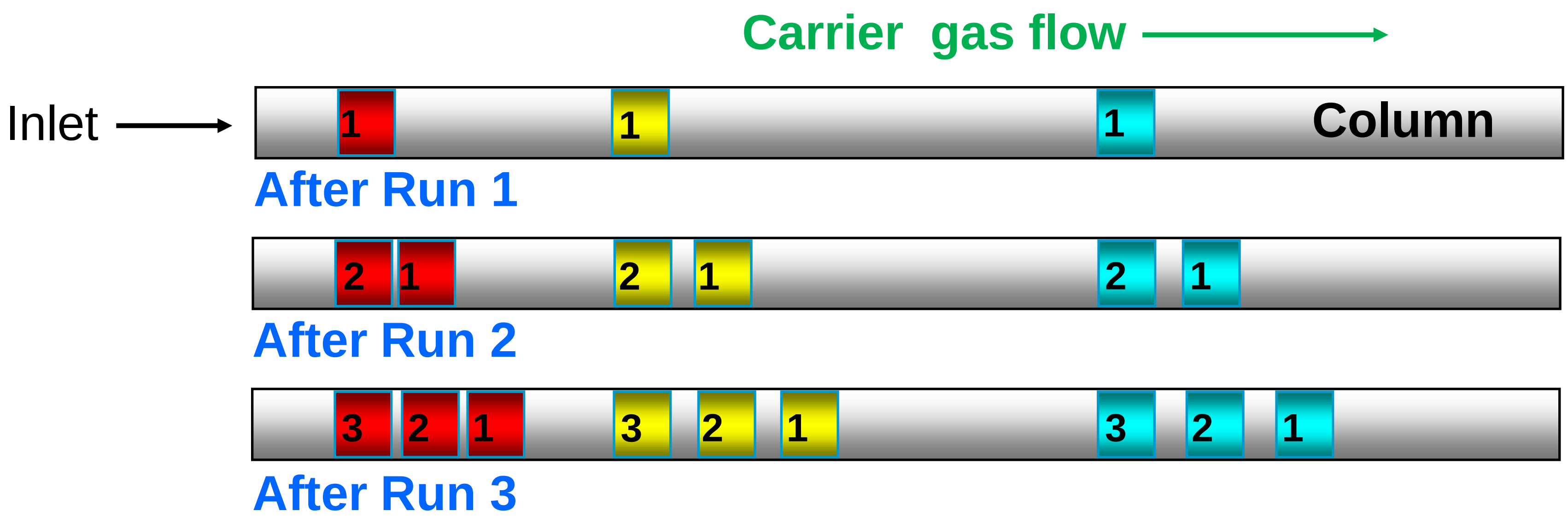




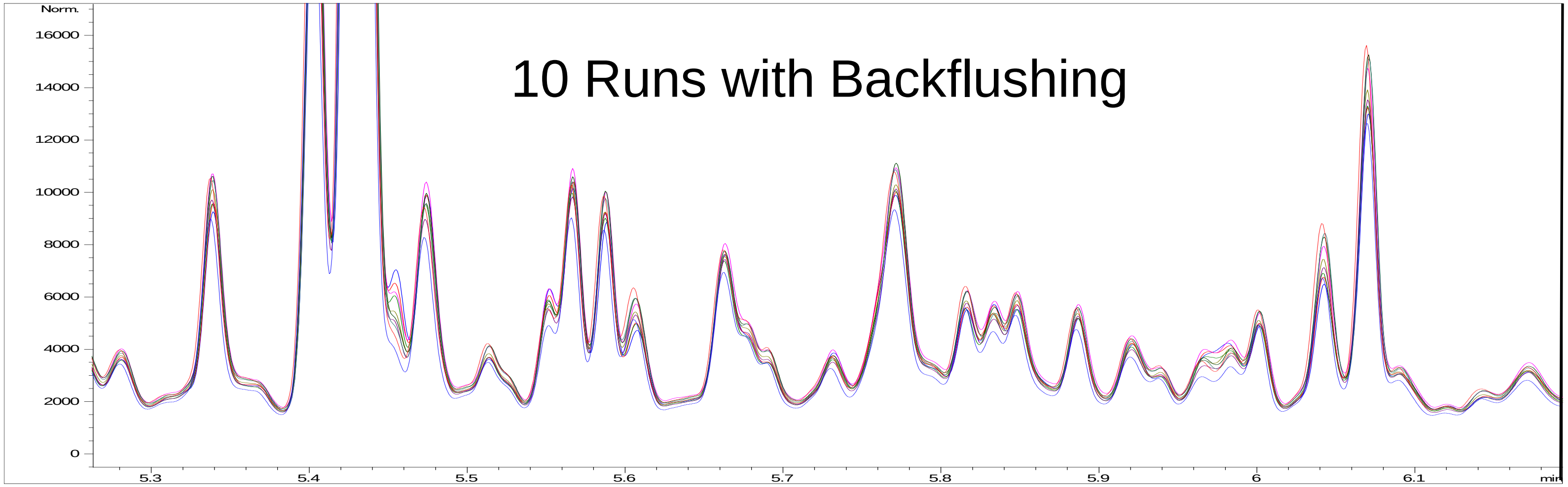
LVI **MMI** **RTL** **PCT**

Matrix Compounds may be left in the Column after each Injection

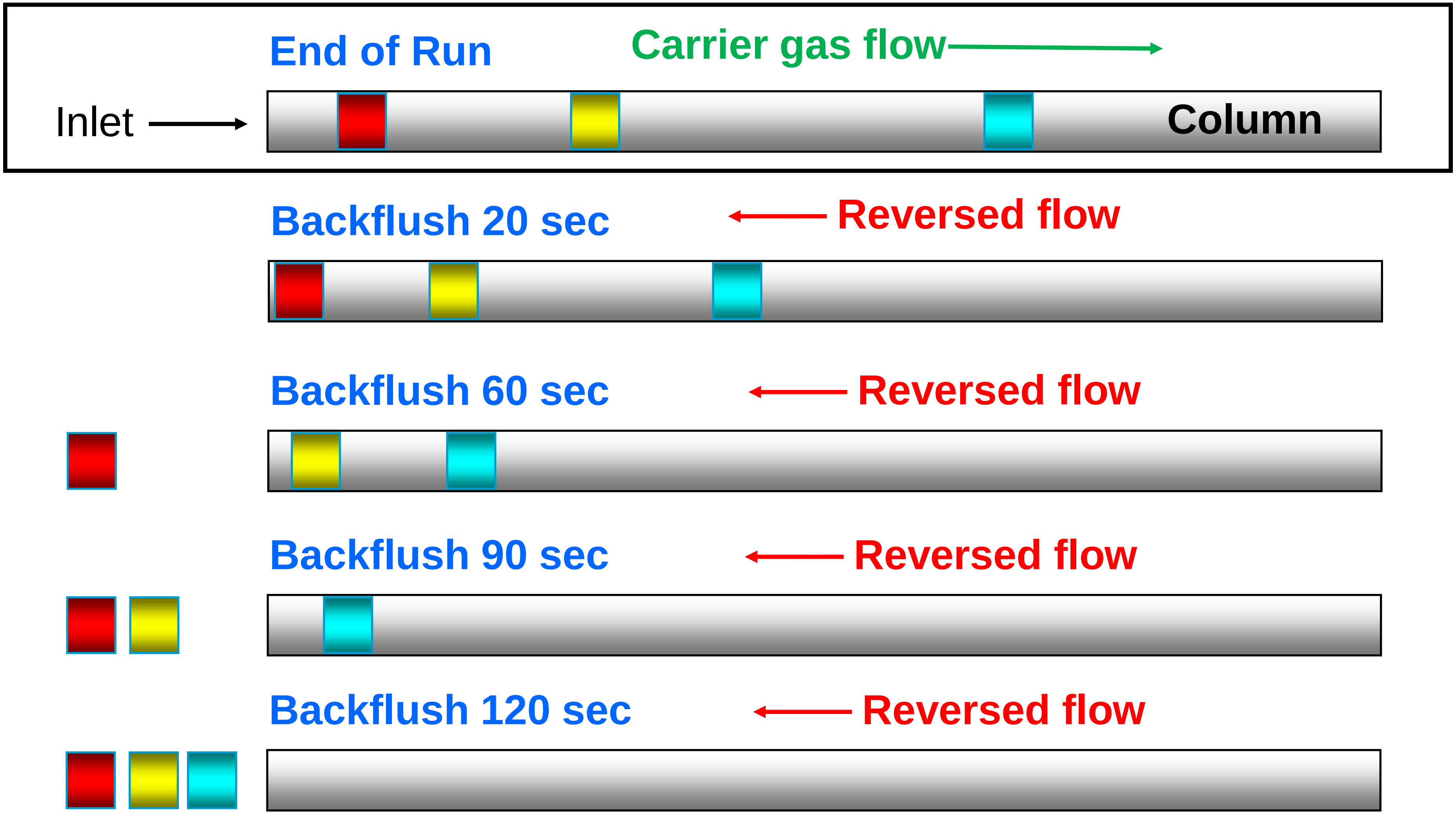


- These heavy matrix materials build up and travel further into the column with each injection
- This build-up of heavy materials can cause retention time shifts, peak distortion, higher bleed, dirty source and loss of sensitivity

PCBs in Fish Oil. GC Analysis with ECD - Residual Sample Matrix Affects RTs

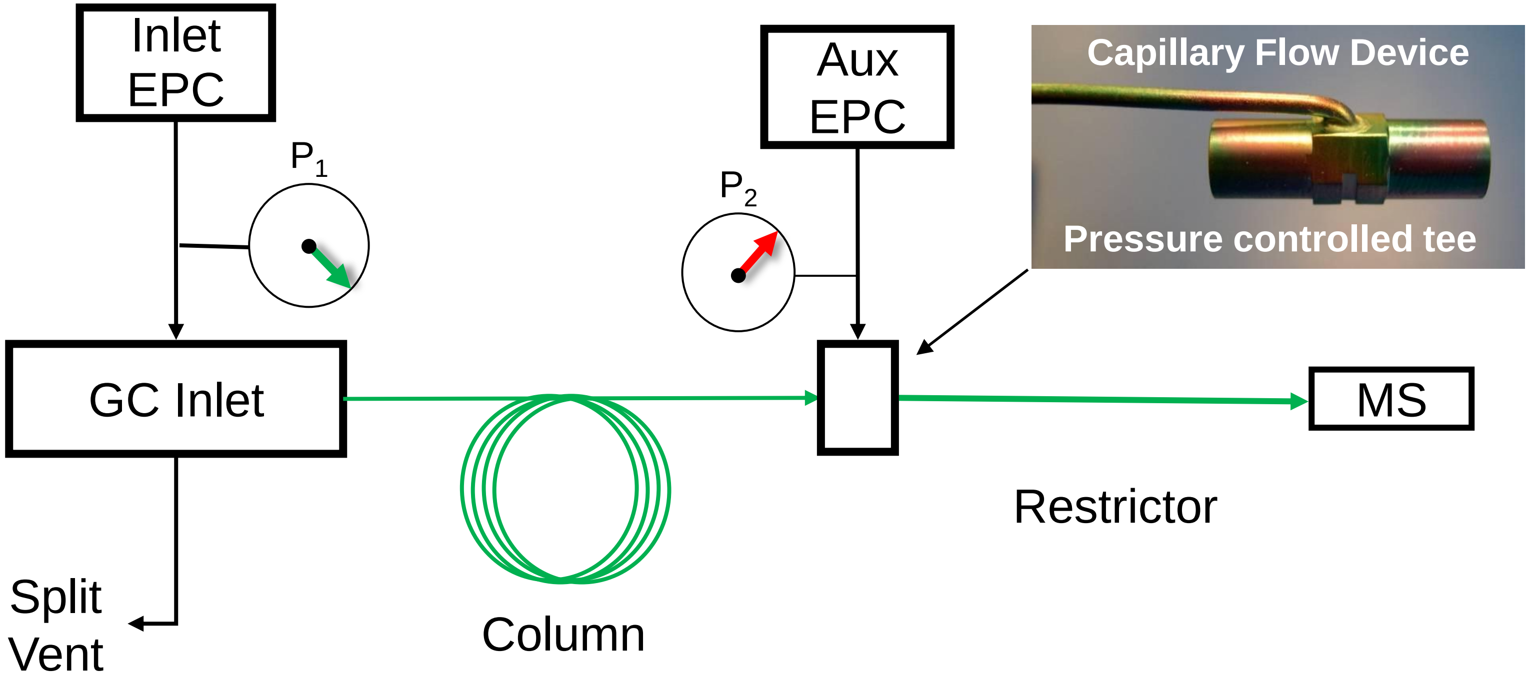


Back flushing After Each Injection



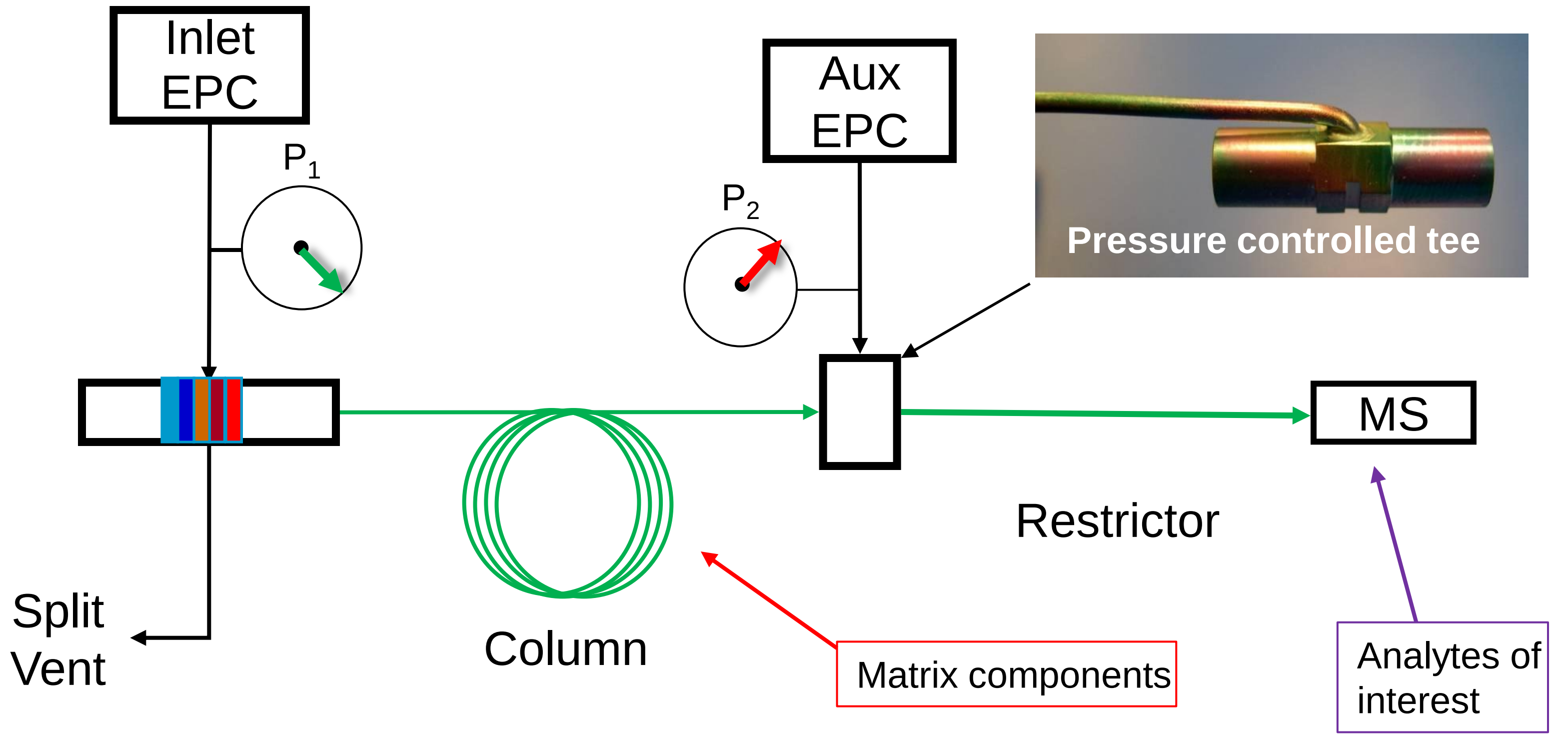
- Backflushing removes heavy materials after each injection, heaviest first

Post column, Post run back flush



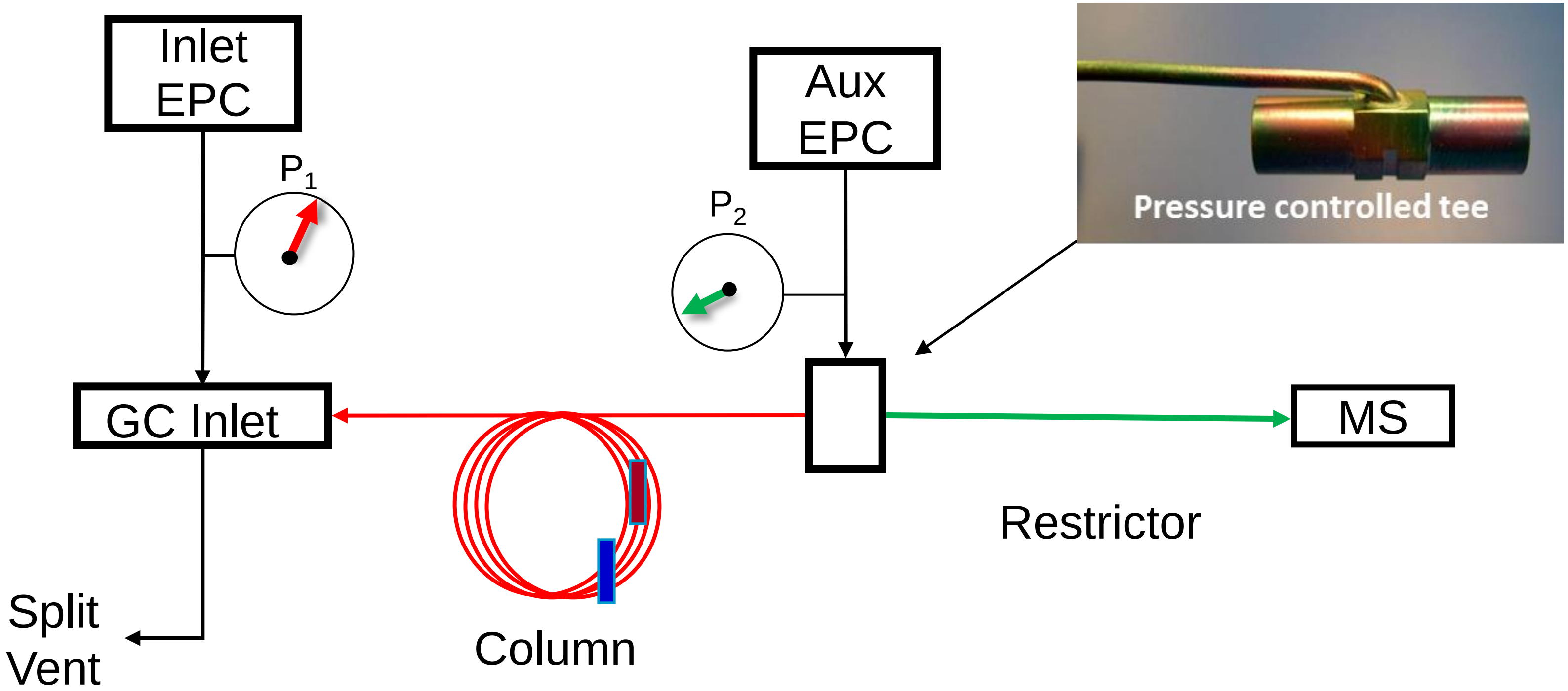
During the run, $P_1 > P_2$, Carrier gas flows towards MS

Post column, Post run back flush



During the run, $P_1 > P_2$, Carrier gas flows towards MS

Post column, Post run back flush

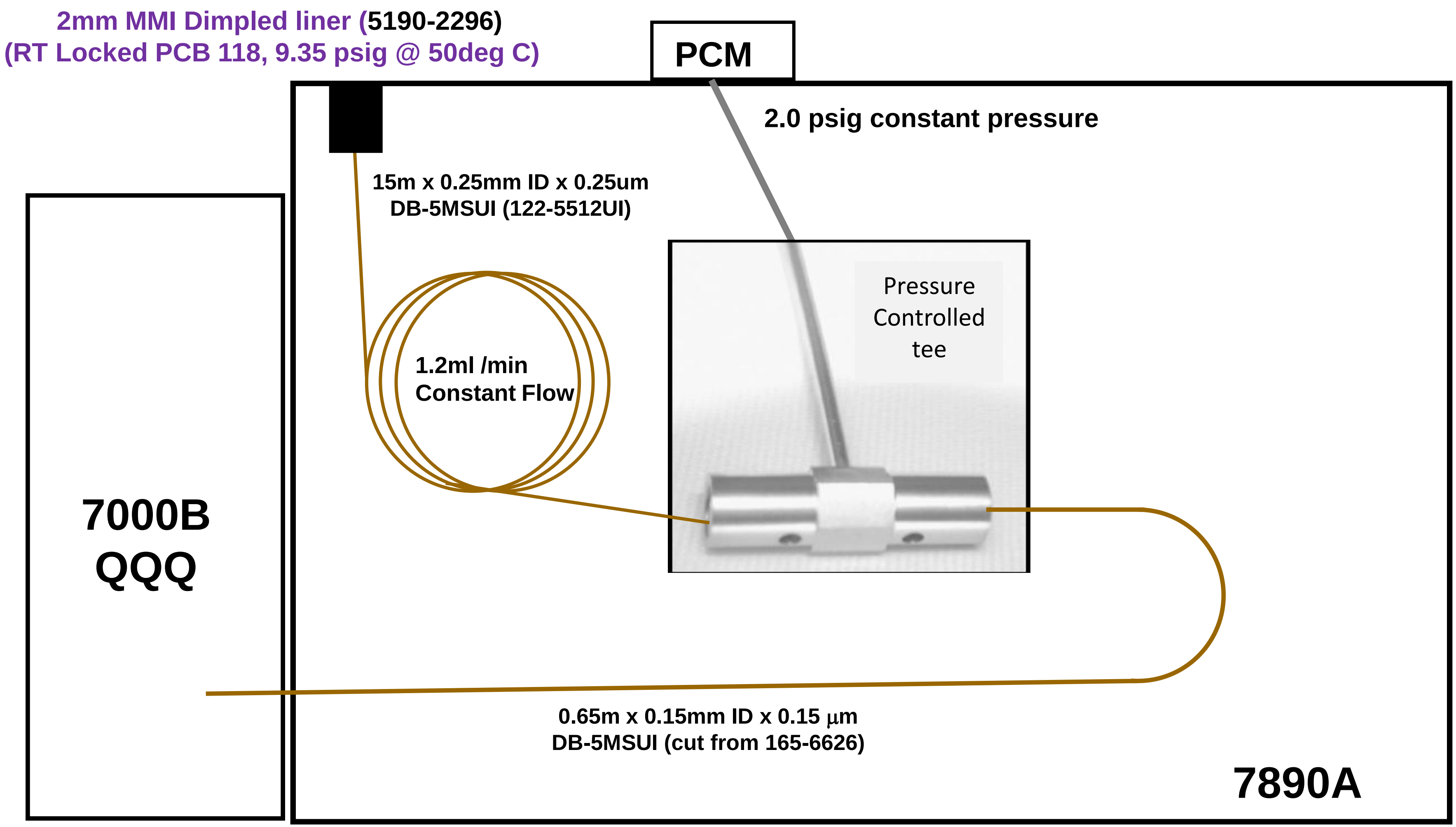


During post run backflush $P_1 < P_2$, Carrier flow reversed in column
Reversed carrier flow >>> analytical flow

Benefits of Back flush

- Consistent analyte retention times and responses
- Robust chromatography and consistent analyte chromatographic peak shapes
- Prevention of high boiling matrix from contaminating the MS ion source
- Extended column life-time and reduced cycle times by removing the need for high-temperature bake-out between runs

OCPs / PCBs / PAHs GC-MS/MS Method



OCPs / PCBs / PAHs: 15m x 0.25mm ID x 0.25um DB-5MSUI (122-5512UI) Constant flow + 0.65m x 0.15mm ID UDFS restrictor at 2.0 psig
Oven 50(1)-20-200(0)-10-300(1.5) ; Inlet 50 (0.05) – 600 deg C/min – 300 deg C
Post run back flush, 2.0 minutes, Inlet 1.0psig, PCM 60.0 psig

Scope of Method

Analyte	ISTD
HCBD	d3-135-TCB
a-HCH	d6-g-HCH
HCB	d6-a-HCH
b-HCH	PCB-155
g-HCH	d6-g-HCH
d-HCH	PCB-155
Aldrin	PCB-155
Isodrin	PCB-155
op-DDE	PCB-155
p,p-DDE	PCB-155
Dieldrin	C13-Dieldrin
op-DDD	PCB-155
Endrin	C13-Dieldrin
pp-DDD	C13-pp-DDT
o,p-DDT	C13-pp-DDT
p,p-DDT	C13-pp-DDT

Analyte	ISTD
Napthalene	d8-Napthalene
Acenapthylene	d8-Acenapthylene
Acenapthene	d10-Acenapthene
Fluorene	d10-Fluorene
Dibenzothiophene	d10-Fluorene
Phenanthrene	d10-Phenanthrene
Anthracene	d10-Phenanthrene
Fluoranthene	d10-Fluoranthene
Pyrene	d10-Pyrene
Benzo(a)anthracene	d12-Chrysene
Chrysene+Triphenylene	d12-Chrysene
benzo(b)fluoranthene	d12-Benzo(k)fluoranthene
Benzo(k)fluoranthene	d12-Benzo(k)fluoranthene
Benzo(e)pyrene	d12-Benzo(a)pyrene
Benzo(a)pyrene	d12-Benzo(a)pyrene
Perylene	d12-Benzo(a)pyrene
Indeno(123-cd)pyrene	d14-Dibenz(a,h)anthracene
Dibenz(a,h)anthracene	d14-Dibenz(a,h)anthracene
Benzo(g,h,i)perylene	d14-Dibenz(a,h)anthracene

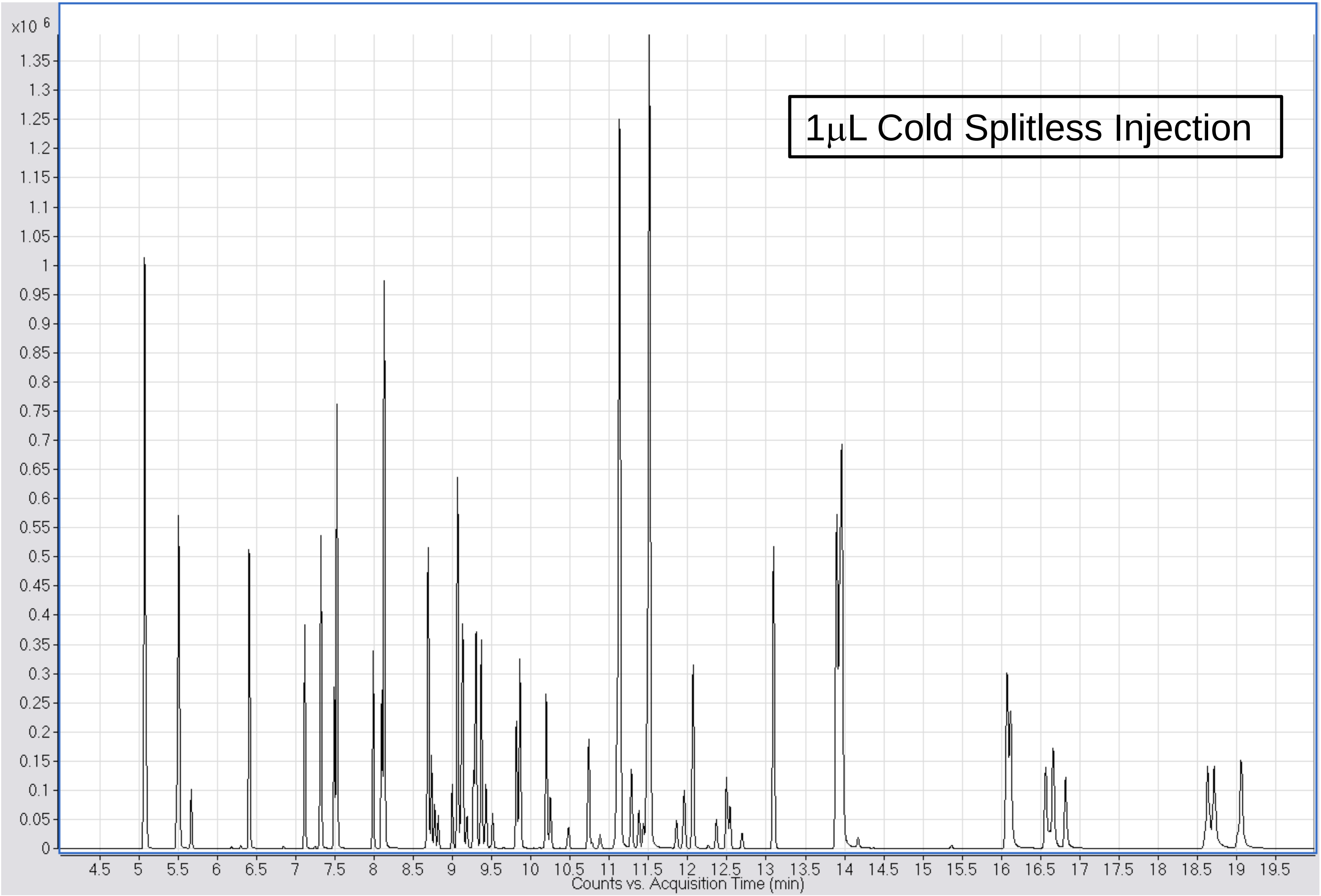
Analyte	ISTD
PCB 28	PCB-155
PCB 52	PCB-155
PCB 101	PCB-155
PCB 118	PCB-155
PCB 153	PCB-155
PCB 138	PCB-155
PCB 180	PCB-155

16 Organo-chlorine compounds

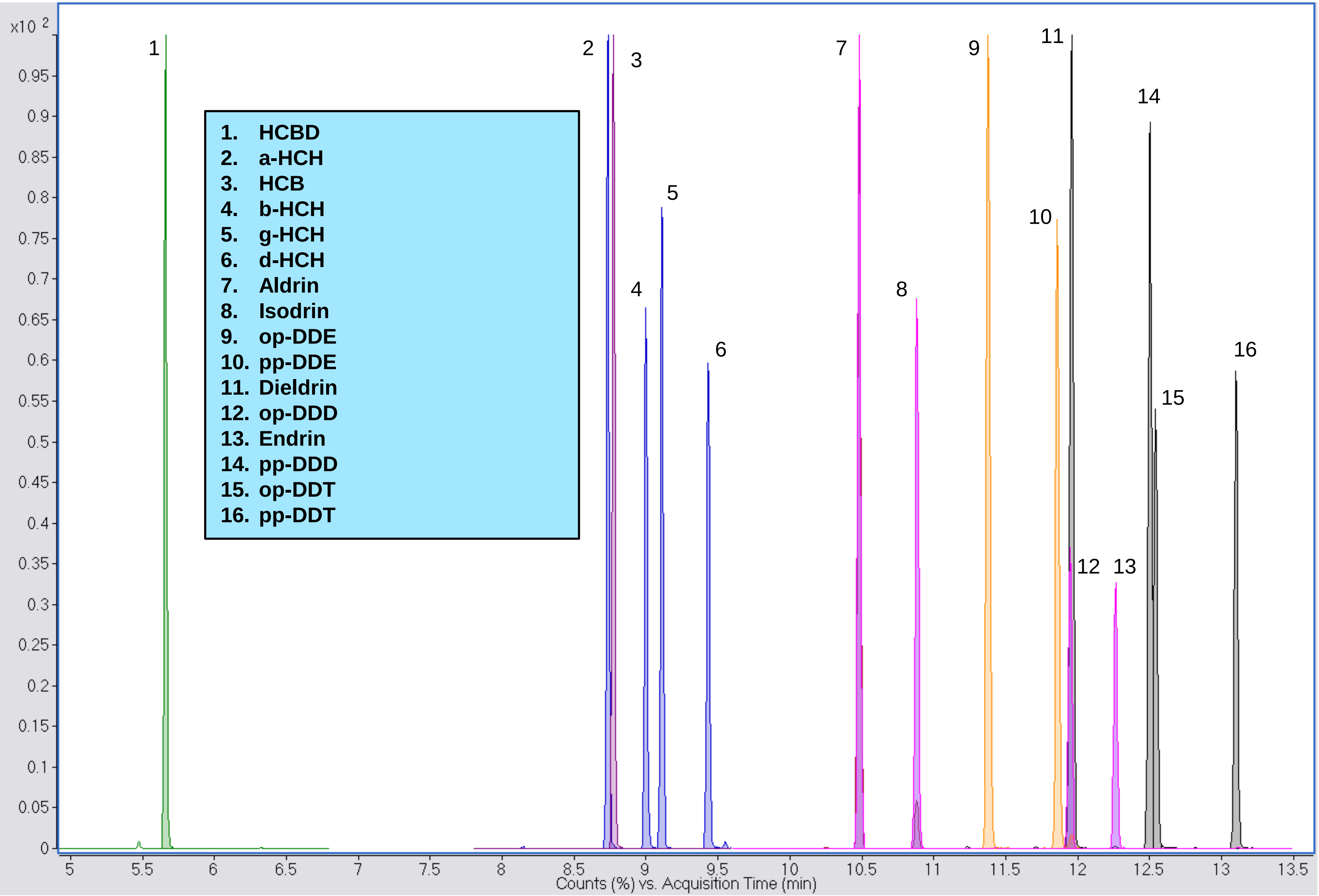
19 Poly Aromatic Hydrocarbons

7 PCB congeners

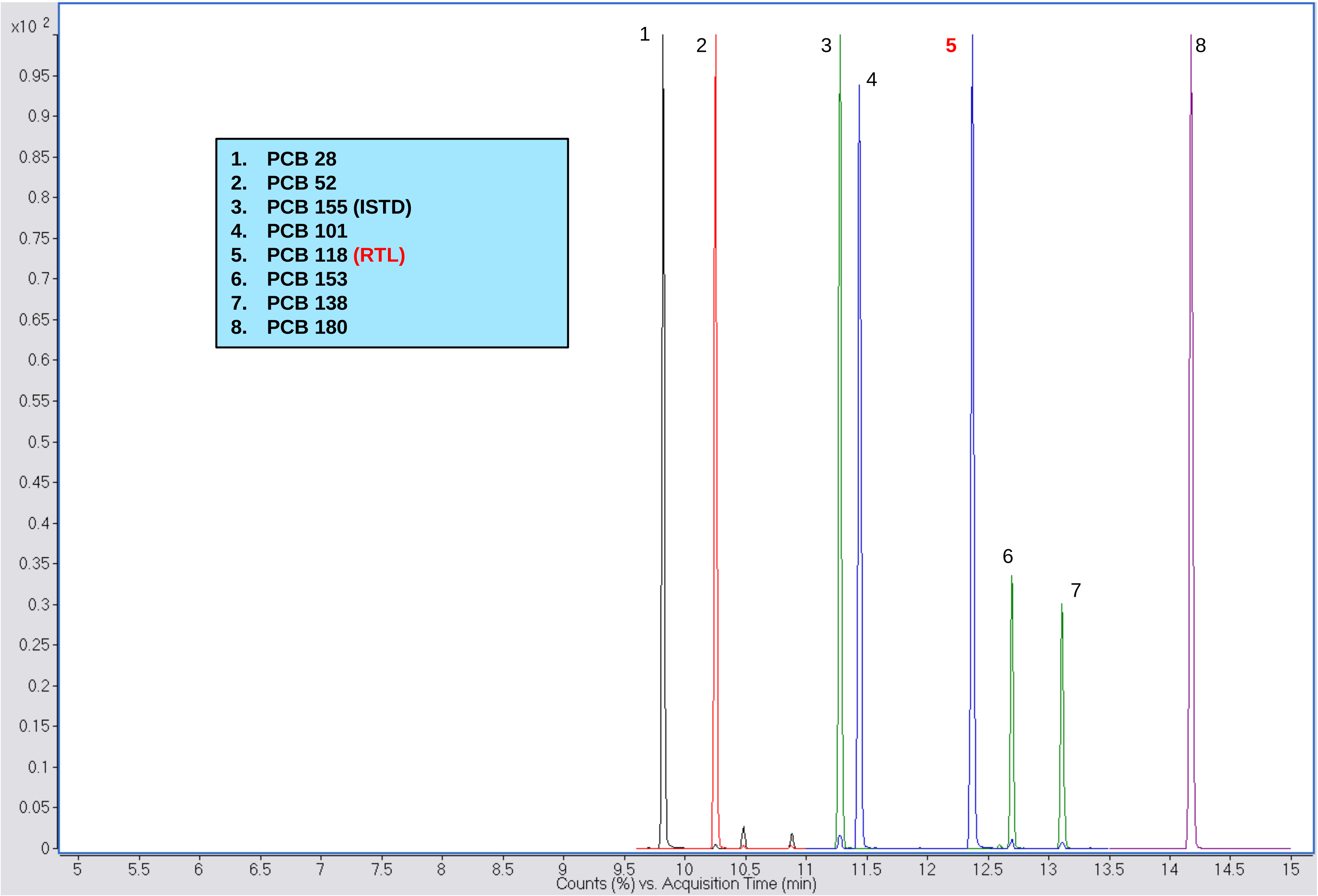
TIC MRM Chromatogram - Calibration Standard



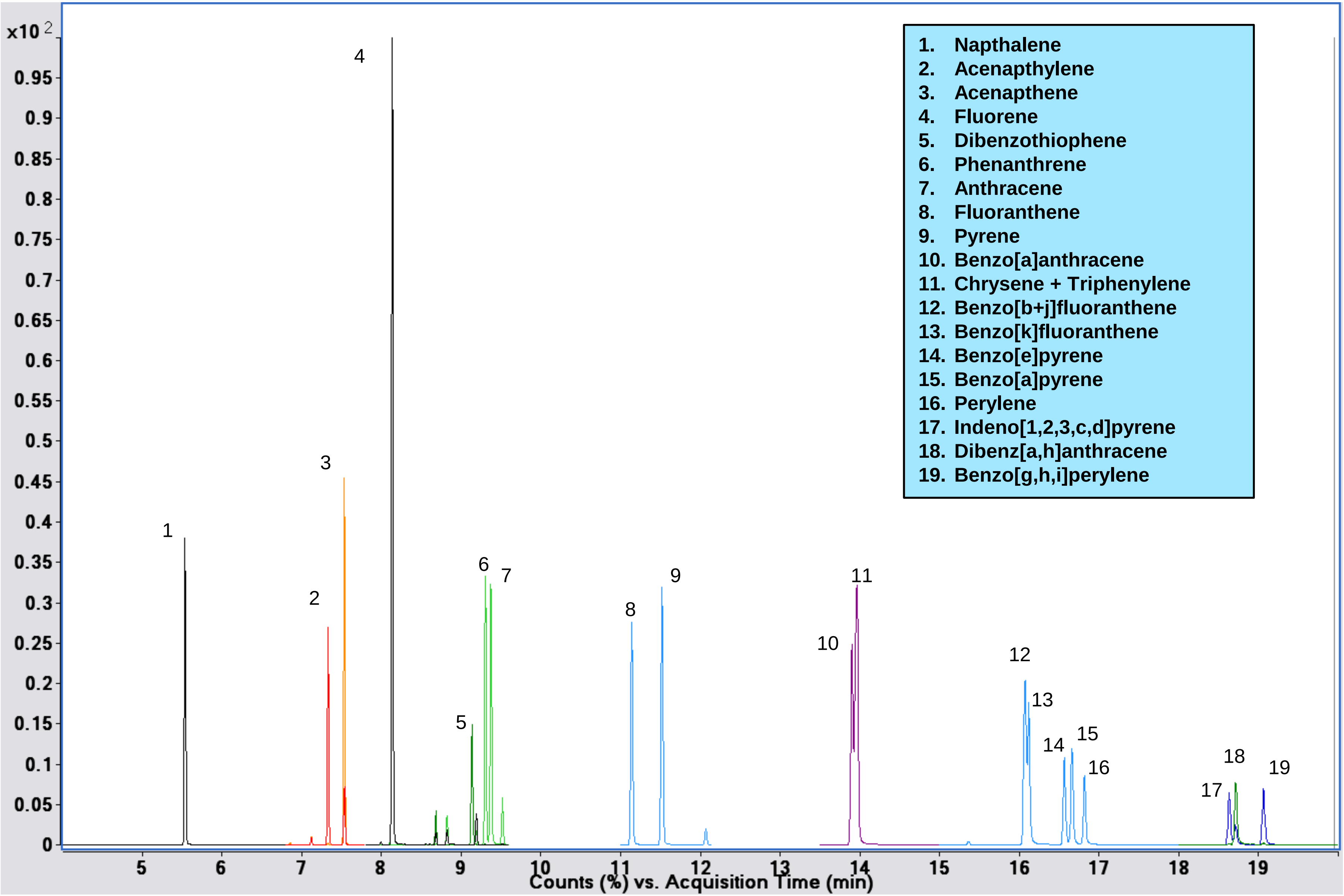
Organo-Chlorine Compounds



Polychlorinated Biphenyls

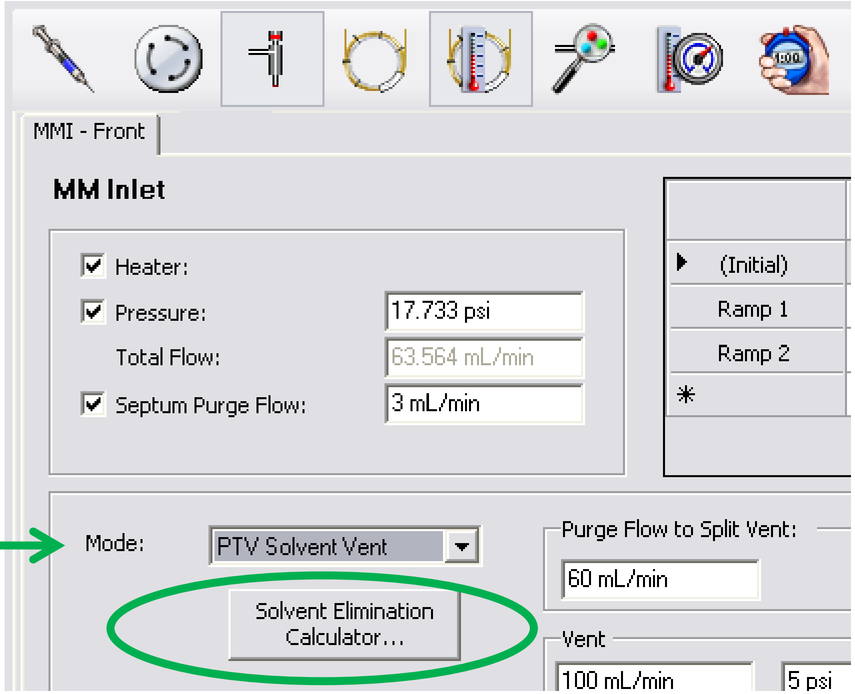


Poly Aromatic Hydrocarbons

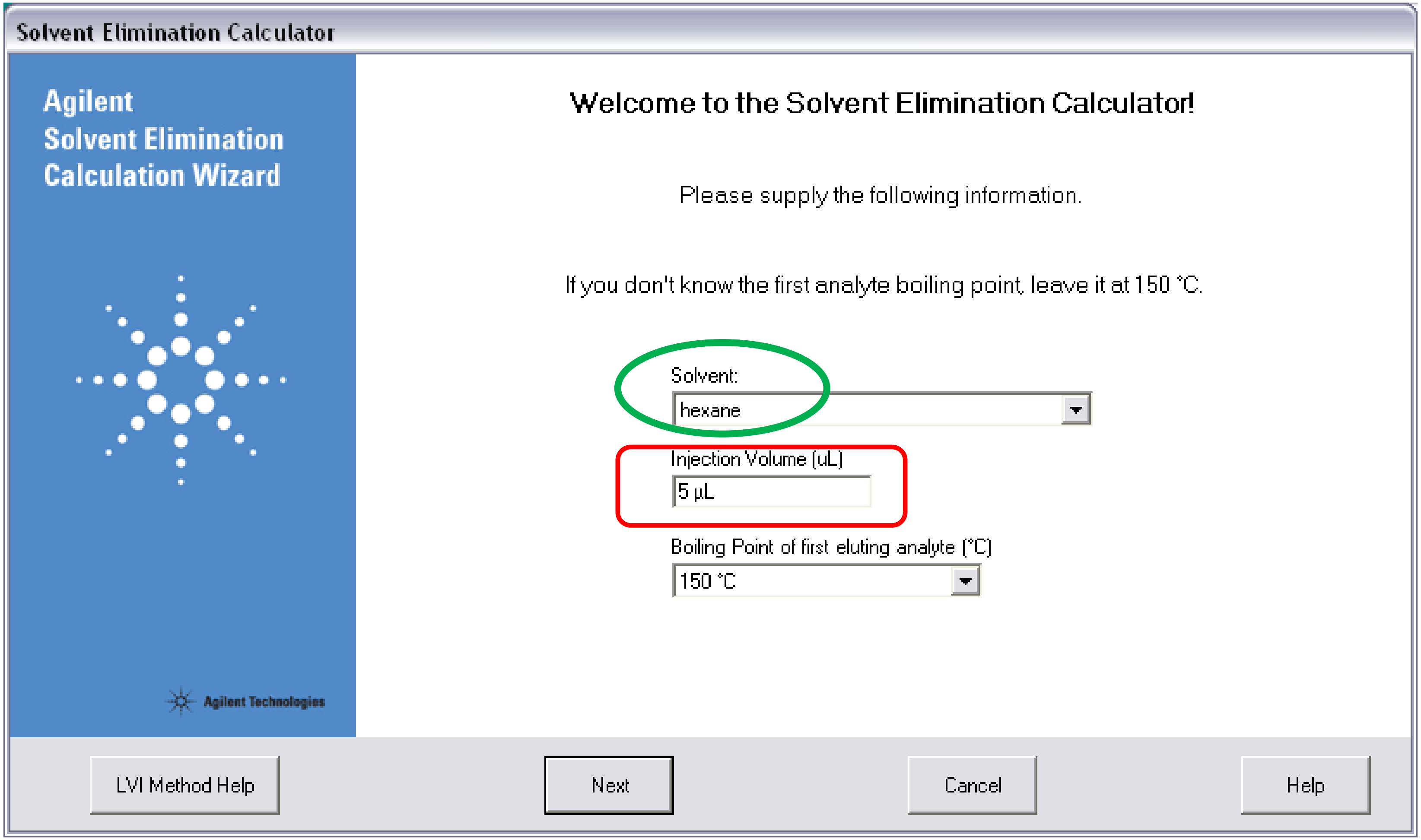


Scaling Injection volume from 1uL (Cold Splitless) to 5µL and 10µL (Solvent Vent) using the Multi-Mode Inlet and the Solvent Vent Calculator Wizard

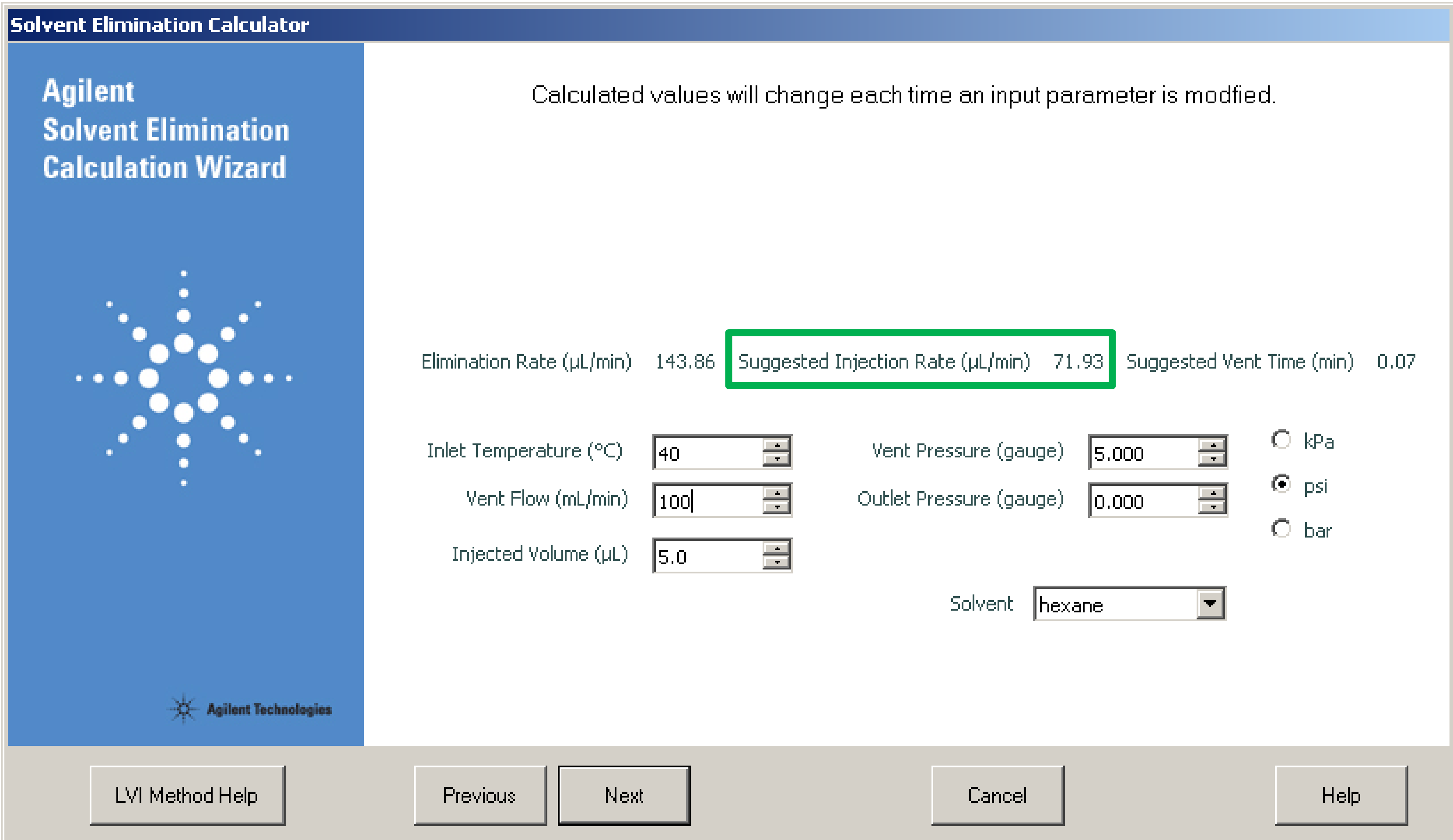
MMI Solvent Elimination Wizard for LVI



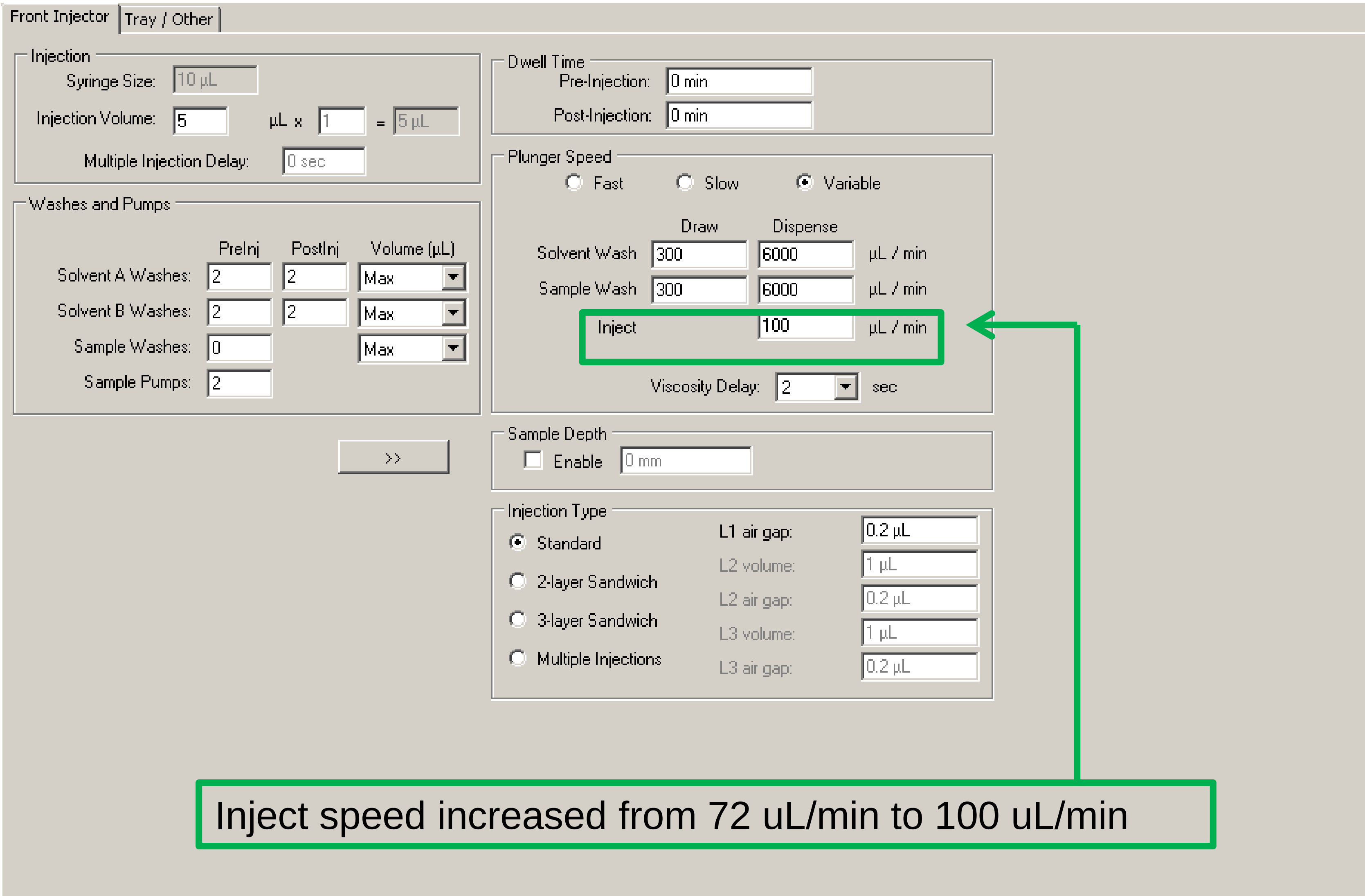
MMI Solvent Elimination Wizard for LVI



MMI Solvent Vent LVI Wizard – 5uL Hexane

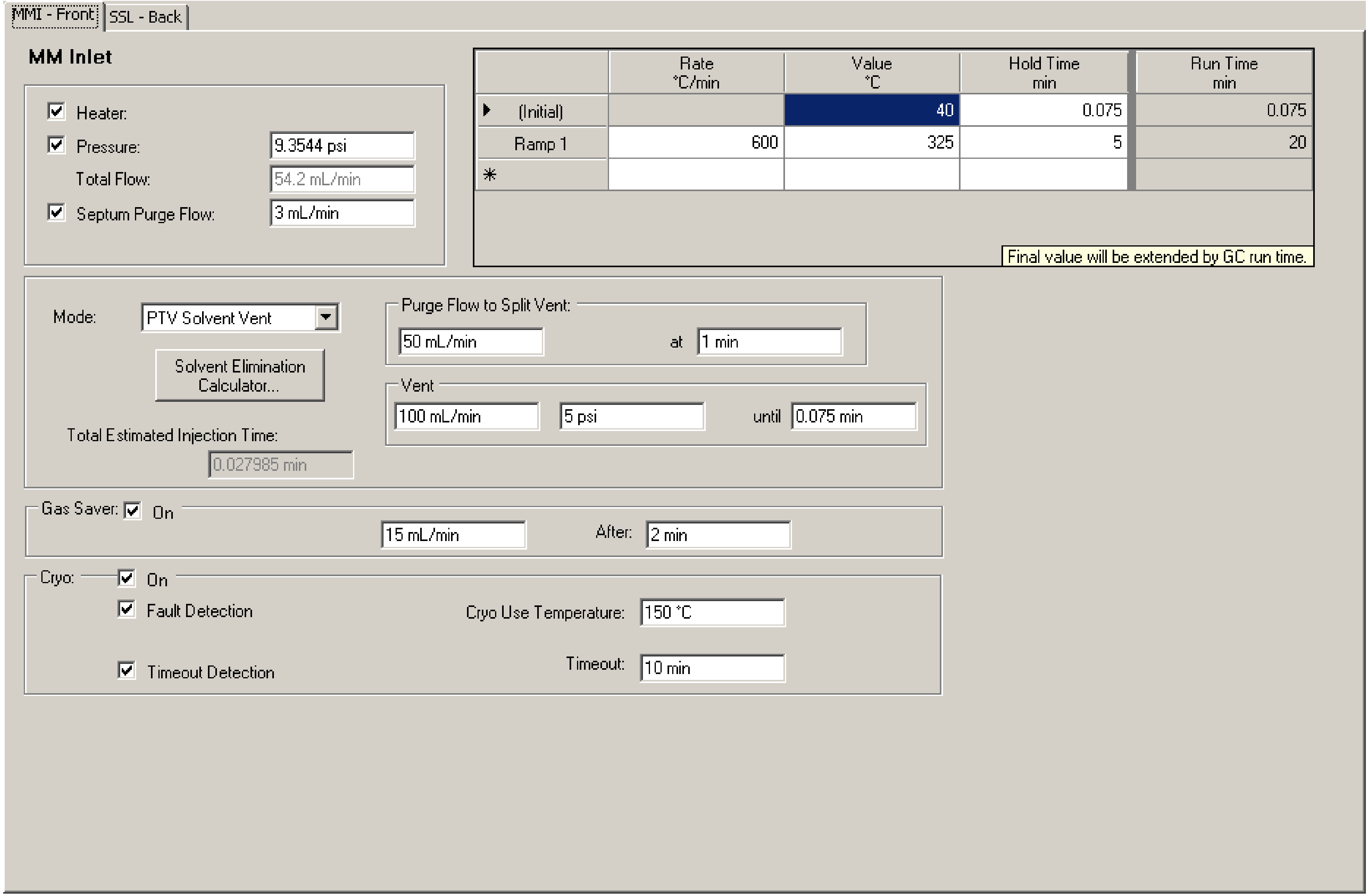


Syringe Parameters



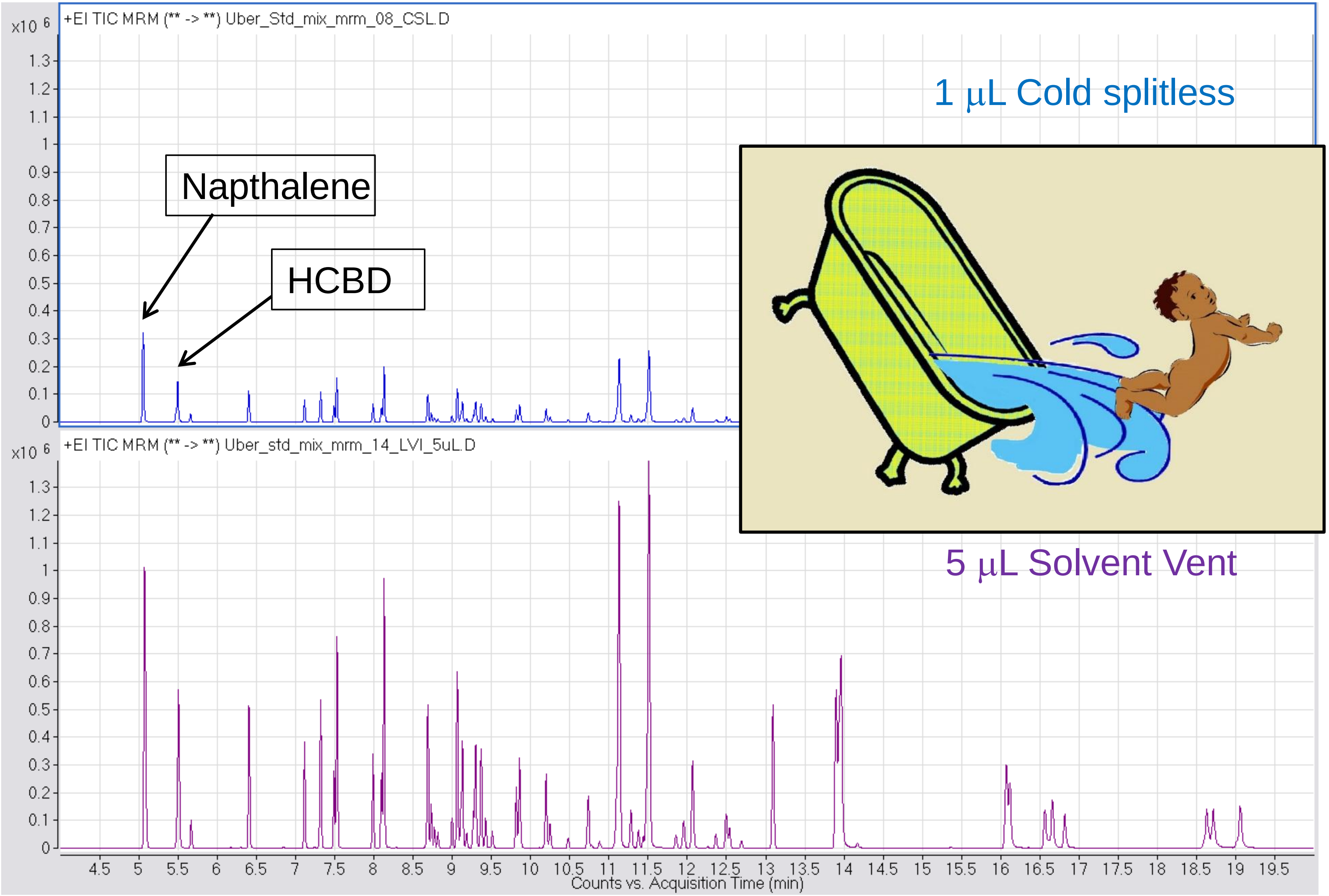
Inject speed increased from 72 uL/min to 100 uL/min

MMI Temperature and Gas Parameter Settings (5µL injection)

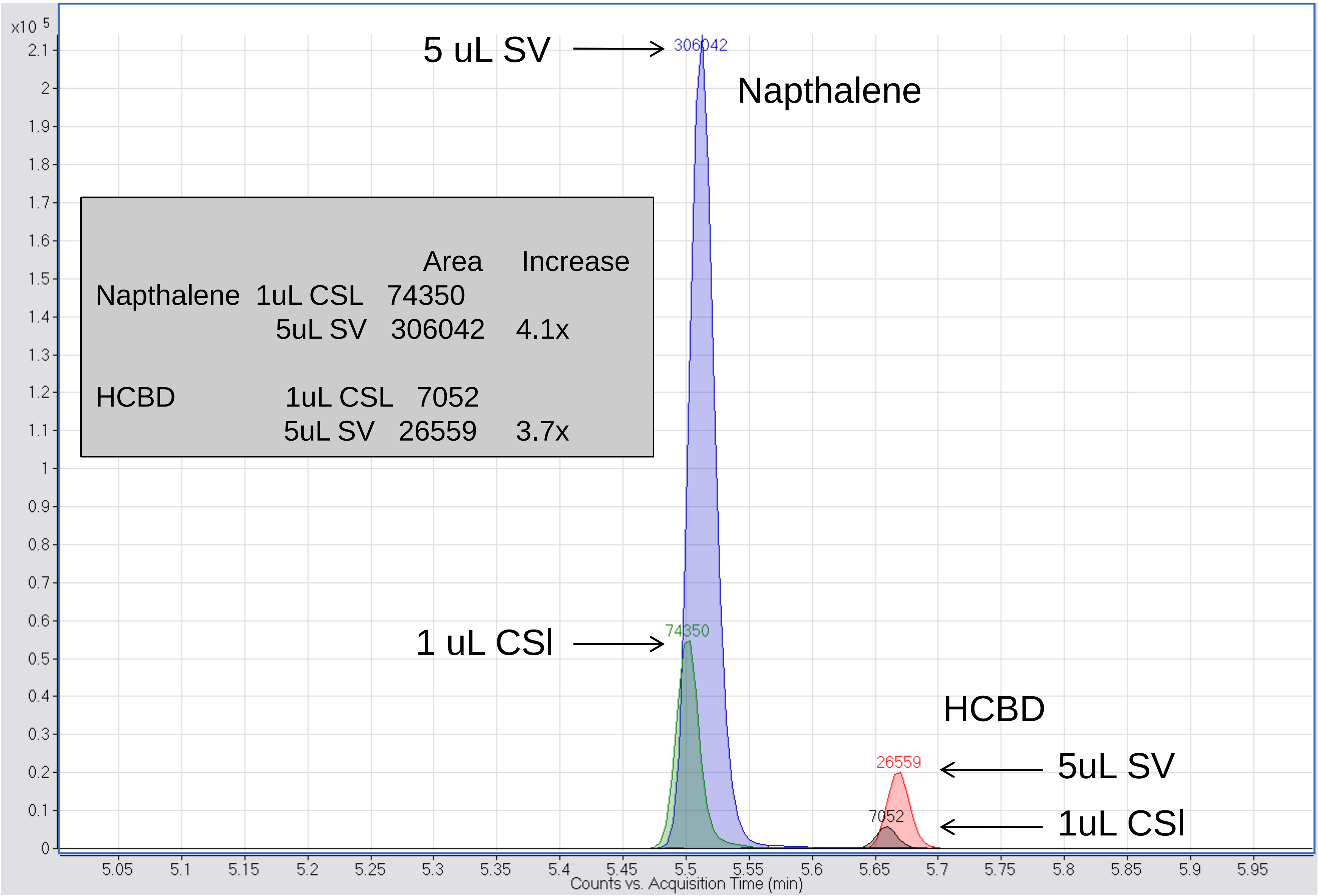


	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		40	0.075	0.075
Ramp 1	600	325	5	20
*				

1µL Cold Splitless vs 5µL Solvent Vent



Low boilers : 1uL CSl vs 5 uL SV Response

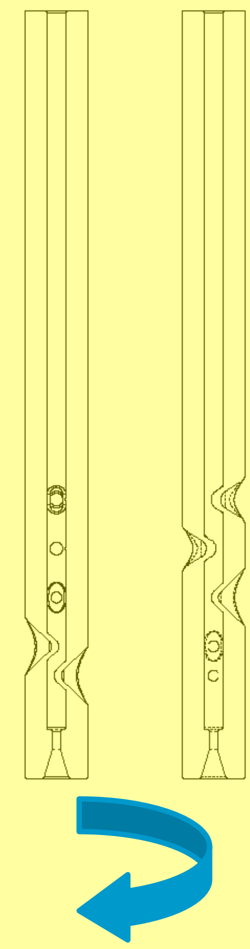


		Area	Increase
Napthalene	1uL CSL	74350	
	5uL SV	306042	4.1x
HCBD	1uL CSL	7052	
	5uL SV	26559	3.7x

Injection Parameters 5µL vs 10µL Solvent Vent with MMI

Sample injection volume (uL)	5		10
Syringe volume (uL)	10		25
Syringe injection speed (uL/min)	100		100
MMI Initial temperature (deg C)	40		20
MMI Initial time (min)	0.075		0.31
MMI Ramp rate (deg C/min)	600		600
MMI Final temp (deg C)	325		325
Vent pressure (psig)	5.0		5.0
Vent time (min)	0.075		0.31
Vent flow (ml/min)	100		100
Purge time (min)	1.0		1.0
Purge flow (ml/min)	50		50
Gas saver (ml/min)	20		20
Gas saver time (min)	2.0		2.0

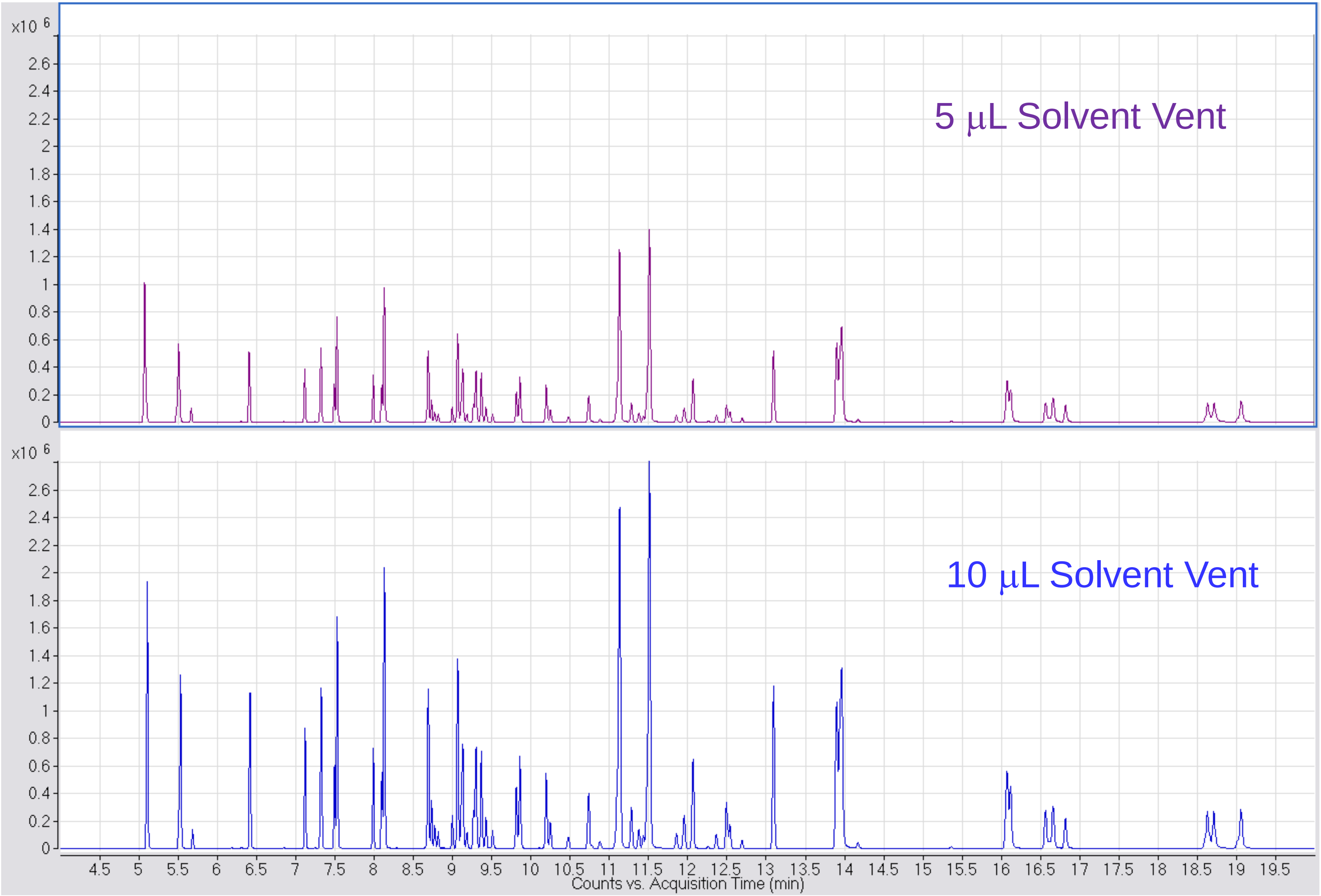
= Parameter change



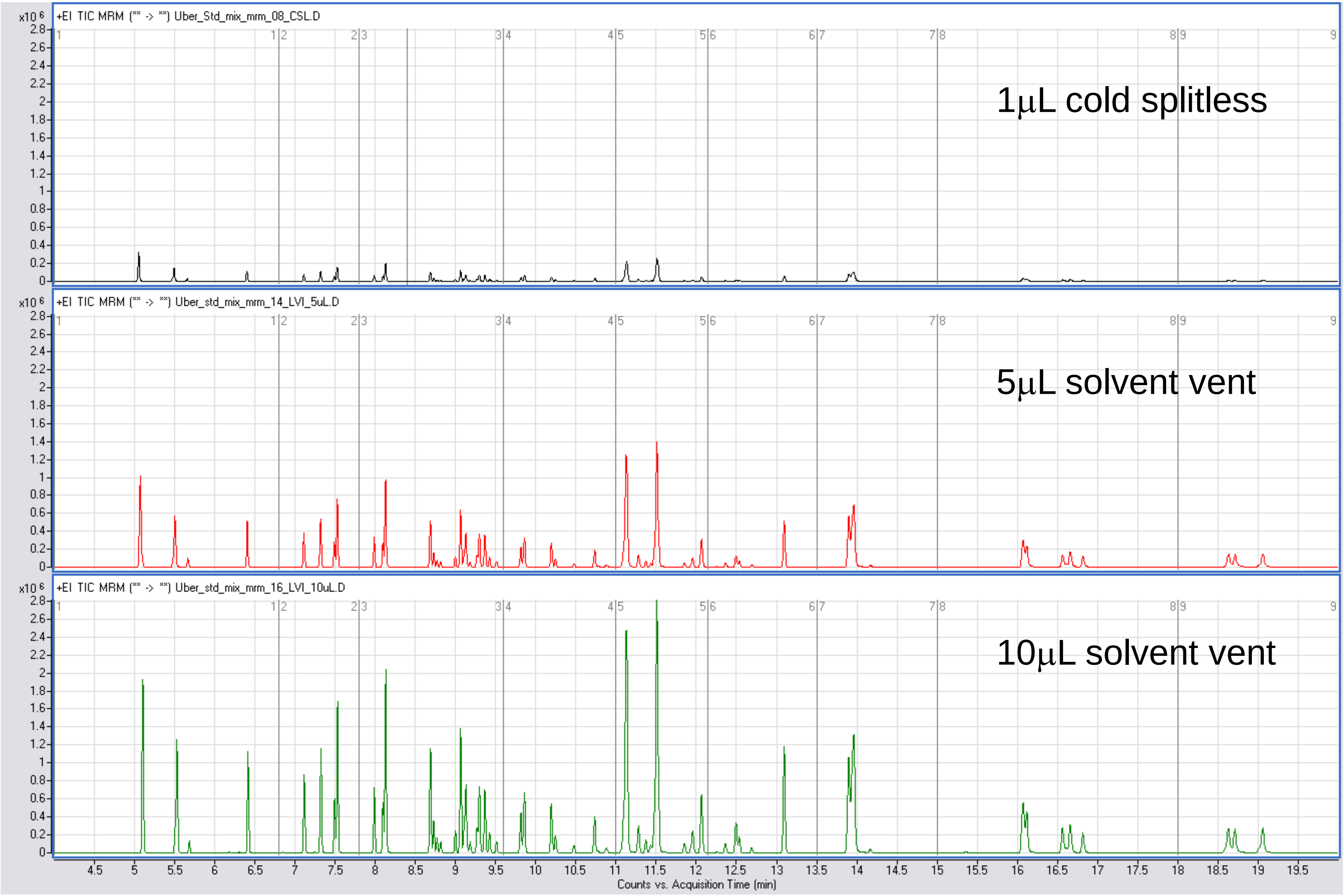
5190-2296

2mm ID dimpled deactivated liner

5μL vs 10μL Solvent Vent with MMI



1µL CSL vs 5µL SV vs 10µL SV



ENVIRONMENT AGENCY

Method Performance

Calibration Curve Fits

10μL Solvent vent injections, 5 point ISTD calibration, 0.4 – 200 pg/μL (equivalent to 0.1 - 50 μg/kg)

OCPs

Analyte	Curve fit	R^2
HCBD	Quadratic	0.9994
a-HCH	Linear	0.9996
HCB	Linear	0.9998
b-HCH	Linear	0.9995
g-HCH	Linear	0.9999
d-HCH	Linear	0.9991
Aldrin	Quadratic	0.9999
Isodrin	Quadratic	0.9999
op-DDE	Linear	0.9998
p,p-DDE	Linear	0.9993
Dieldrin	Quadratic	0.9992
op-DDD	Quadratic	0.9999
Endrin	Linear	0.9997
pp-DDD	Linear	0.9997
o,p-DDT	Linear	0.9992
p,p-DDT	Linear	0.9995

PAHs

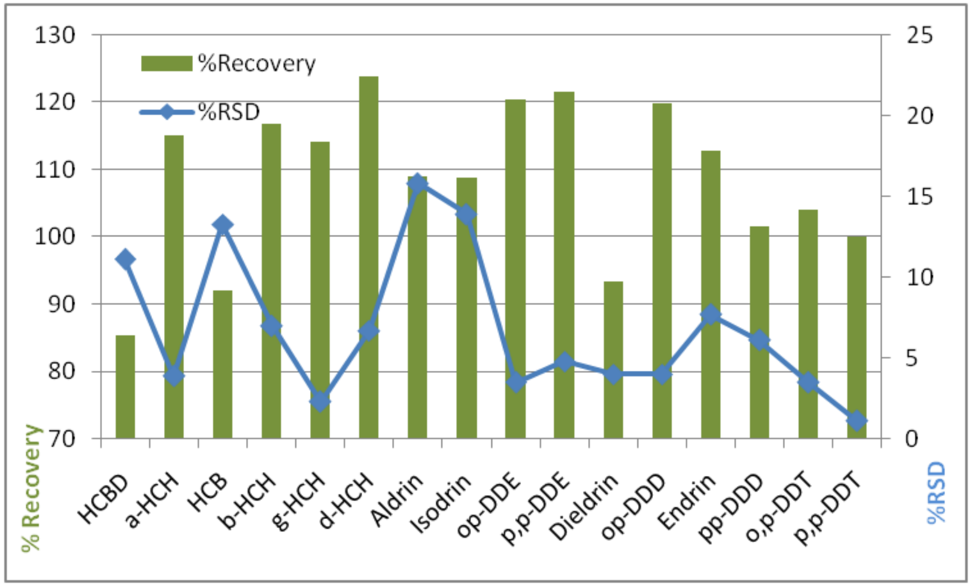
Analyte	Curve fit	R^2
Napthalene	Linear	0.9997
Acenapthylene	Linear	0.9997
Acenapthene	Linear	0.9999
Fluorene	Linear	0.9997
Dibenzothiophene	Quadratic	0.9999
Phenanthrene	Linear	0.9999
Anthracene	Linear	0.9997
Fluoranthene	Linear	0.9992
Pyrene	Linear	0.9996
Benzo(a)anthracene	Linear	0.9998
Chrysene+Triphenylene	Quadratic	0.9999
benzo(b)fluoranthene	Linear	0.9998
Benzo(k)fluoranthene	Quadratic	0.9997
Benzo(e)pyrene	Linear	0.9996
Benzo(a)pyrene	Linear	0.9998
Perylene	Linear	0.9999
Indeno(123-cd)pyrene	Quadratic	0.9996
Dibenz(a,h)anthracene	Quadratic	0.9999
Benzo(g,h,i)perylene	Quadratic	0.9997

PCBs

Analyte	Curve fit	R^2
PCB 28	Linear	0.9998
PCB 52	Linear	0.9998
PCB 101	Linear	0.9999
PCB 118	Linear	0.9996
PCB 153	Linear	0.9998
PCB 138	Linear	0.9998
PCB 180	Linear	0.9994

OCP Reproducibility / Recoveries (n=5)

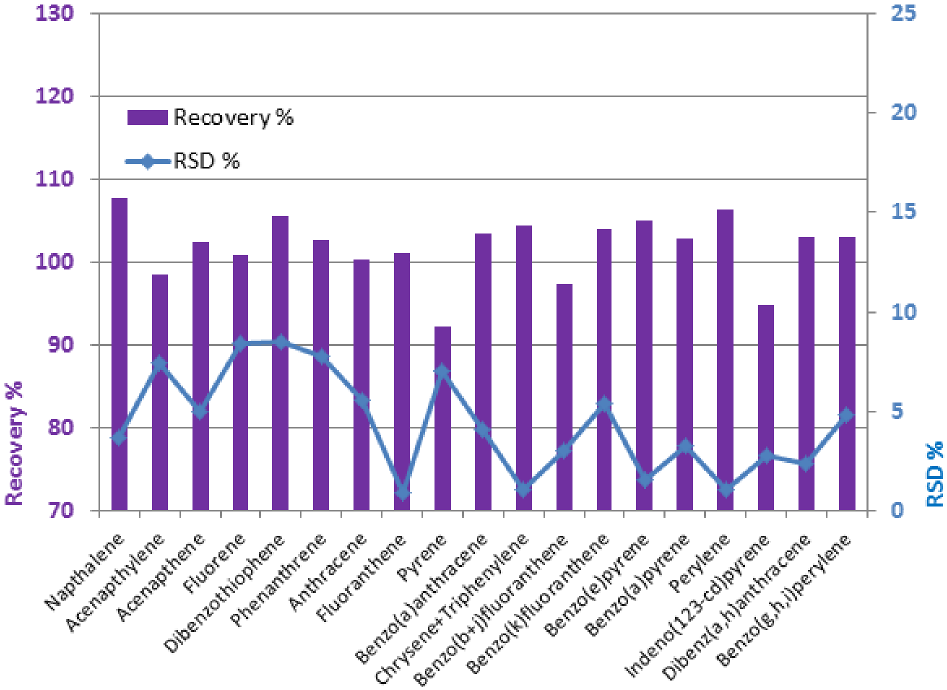
Analyte	ISTD	%RSD	%Recovery
HCBD	d3-135-TCB	11.1	85.4
a-HCH	d6-g-HCH	3.9	115.1
HCB	d6-a-HCH	13.3	92.0
b-HCH	PCB-155	7.0	116.8
g-HCH	d6-g-HCH	2.3	114.1
d-HCH	PCB-155	6.7	123.9
Aldrin	PCB-155	15.8	108.9
Isodrin	PCB-155	13.9	108.7
op-DDE	PCB-155	3.5	120.4
p,p-DDE	PCB-155	4.8	121.5
Dieldrin	C13-Dieldrin	4.0	93.4
op-DDD	PCB-155	4.0	119.9
Endrin	C13-Dieldrin	7.7	112.7
pp-DDD	C13-pp-DDT	6.1	101.6
o,p-DDT	C13-pp-DDT	3.5	104.1
p,p-DDT	C13-pp-DDT	1.1	100.0



Spike level = 4 μg/Kg

PAH Reproducibility / Recoveries (n=5)

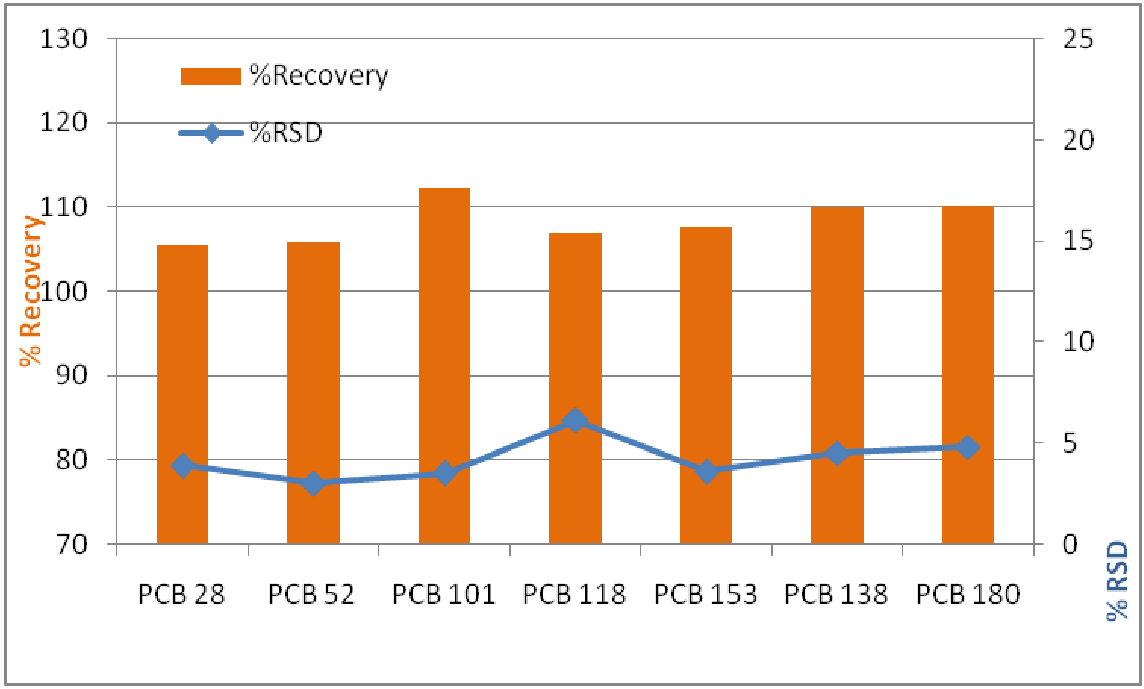
Analyte	ISTD	%RSD	%Recovery
Napthalene	d8-Napthalene	3.7	107.7
Acenapthylene	d8-Acenapthylene	7.4	98.5
Acenapthene	d10-Acenapthene	5.0	102.5
Fluorene	d10-Fluorene	8.4	100.9
Dibenzothiophene	d10-Fluorene	8.5	105.6
Phenanthrene	d10-Phenanthrene	7.8	102.6
Anthracene	d10-Phenanthrene	5.6	100.2
Fluoranthene	d10-Fluoranthene	0.9	101.0
Pyrene	d10-Pyrene	7.0	92.3
Benzo(a)anthracene	d12-Chrysene	4.1	103.5
Chrysene+Triphenylene	d12-Chrysene	1.1	104.5
benzo(b+j)fluoranthene	d12-Benzo(k)fluoranthene	3.1	97.3
Benzo(k)fluoranthene	d12-Benzo(k)fluoranthene	5.4	104.1
Benzo(e)pyrene	d12-Benzo(a)pyrene	1.6	105.0
Benzo(a)pyrene	d12-Benzo(a)pyrene	3.3	102.9
Perylene	d12-Benzo(a)pyrene	1.1	106.4
Indeno(123-cd)pyrene	d14-Dibenz(a,h)anthracene	2.8	94.9
Dibenz(a,h)anthracene	d14-Dibenz(a,h)anthracene	2.4	103.0
Benzo(g,h,i)perylene	d14-Dibenz(a,h)anthracene	4.8	103.1



Spike level = 4 µg/Kg

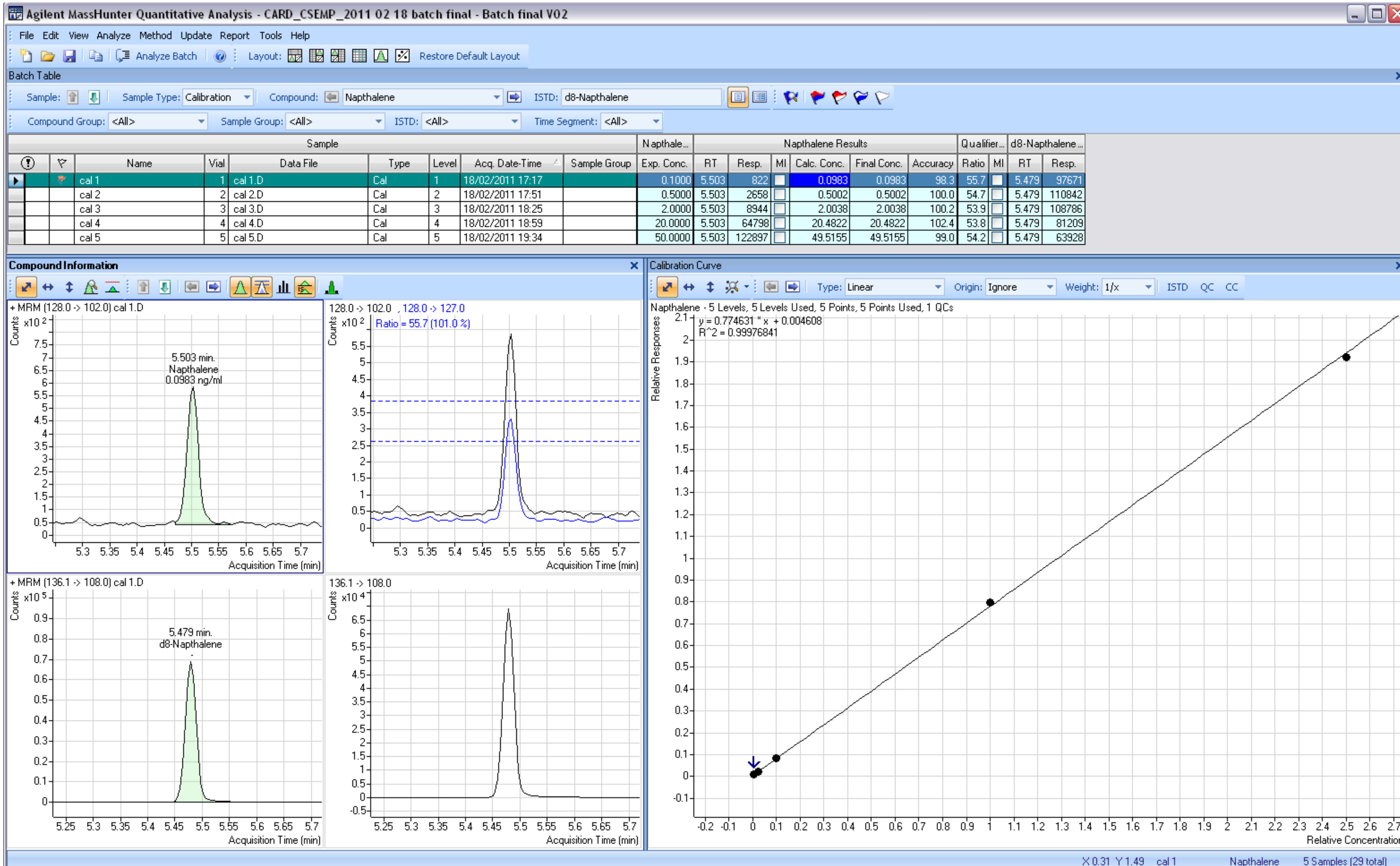
PCB Reproducibility / Recoveries (n=5)

Analyte	ISTD	%RSD	%Recovery
PCB 28	PCB-155	3.9	105.5
PCB 52	PCB-155	3.0	105.8
PCB 101	PCB-155	3.5	112.3
PCB 118	PCB-155	6.1	107.0
PCB 153	PCB-155	3.6	107.6
PCB 138	PCB-155	4.5	109.9
PCB 180	PCB-155	4.8	110.1



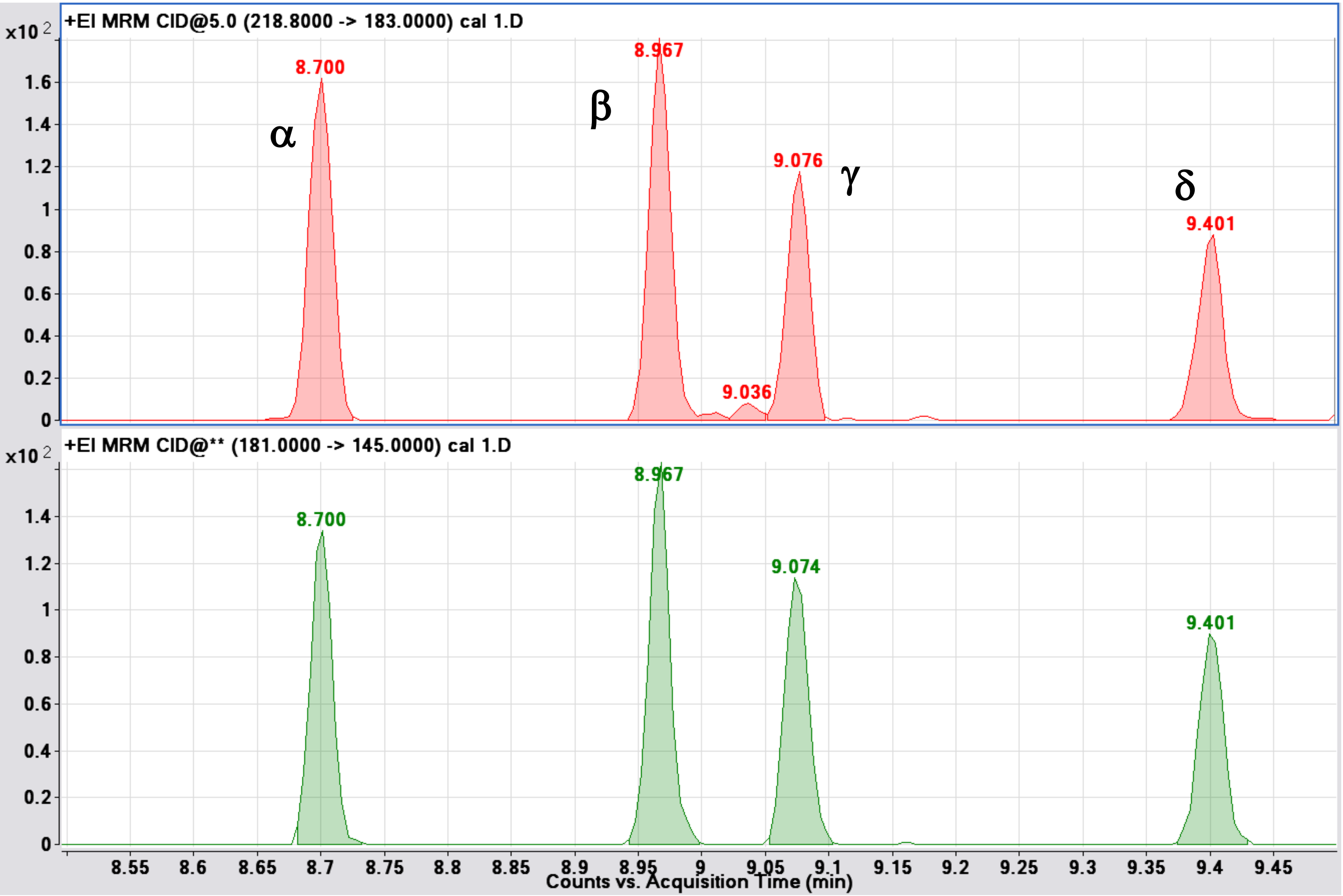
Spike level = 4 µg/Kg

Napthalene Cal 1 : 0.4 pg/uL (equiv. 0.1 µg/kg in mussel tussue)

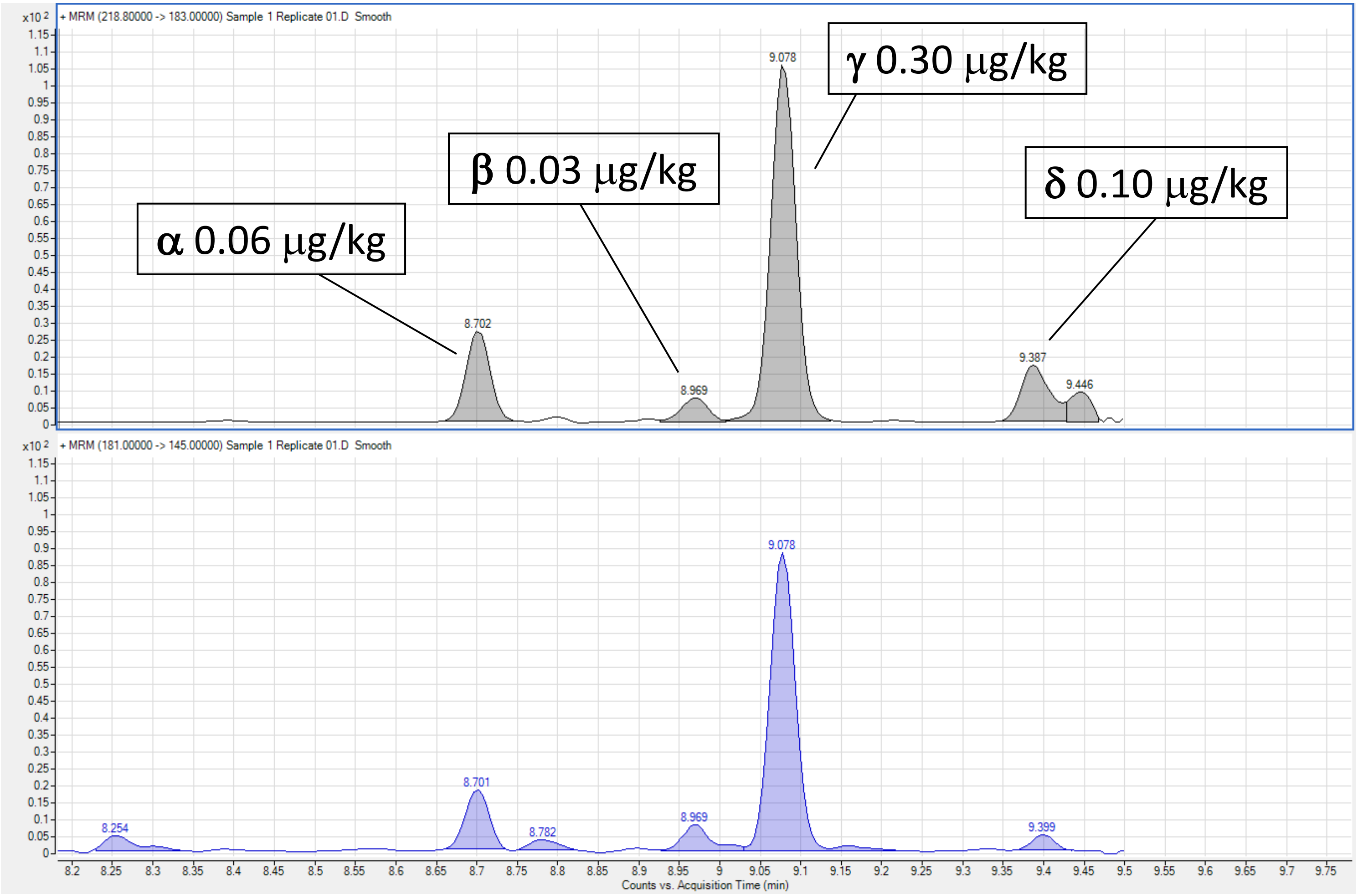


Name	Vial	Data File	Type	Level	Acq. Date-Time	Sample Group	Napthalene Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Qualifier Ratio	MI	d8-Napthalene RT	d8-Napthalene Resp.
cal 1	1	cal 1.D	Cal	1	18/02/2011 17:17		0.1000	5.503	822		0.0983	0.0983	98.3	55.7		5.479	97671
cal 2	2	cal 2.D	Cal	2	18/02/2011 17:51		0.5000	5.503	2658		0.5002	0.5002	100.0	54.7		5.479	110842
cal 3	3	cal 3.D	Cal	3	18/02/2011 18:25		2.0000	5.503	8944		2.0038	2.0038	100.2	53.9		5.479	108786
cal 4	4	cal 4.D	Cal	4	18/02/2011 18:59		20.0000	5.503	64798		20.4822	20.4822	102.4	53.8		5.479	81209
cal 5	5	cal 5.D	Cal	5	18/02/2011 19:34		50.0000	5.503	122897		49.5155	49.5155	99.0	54.2		5.479	63928

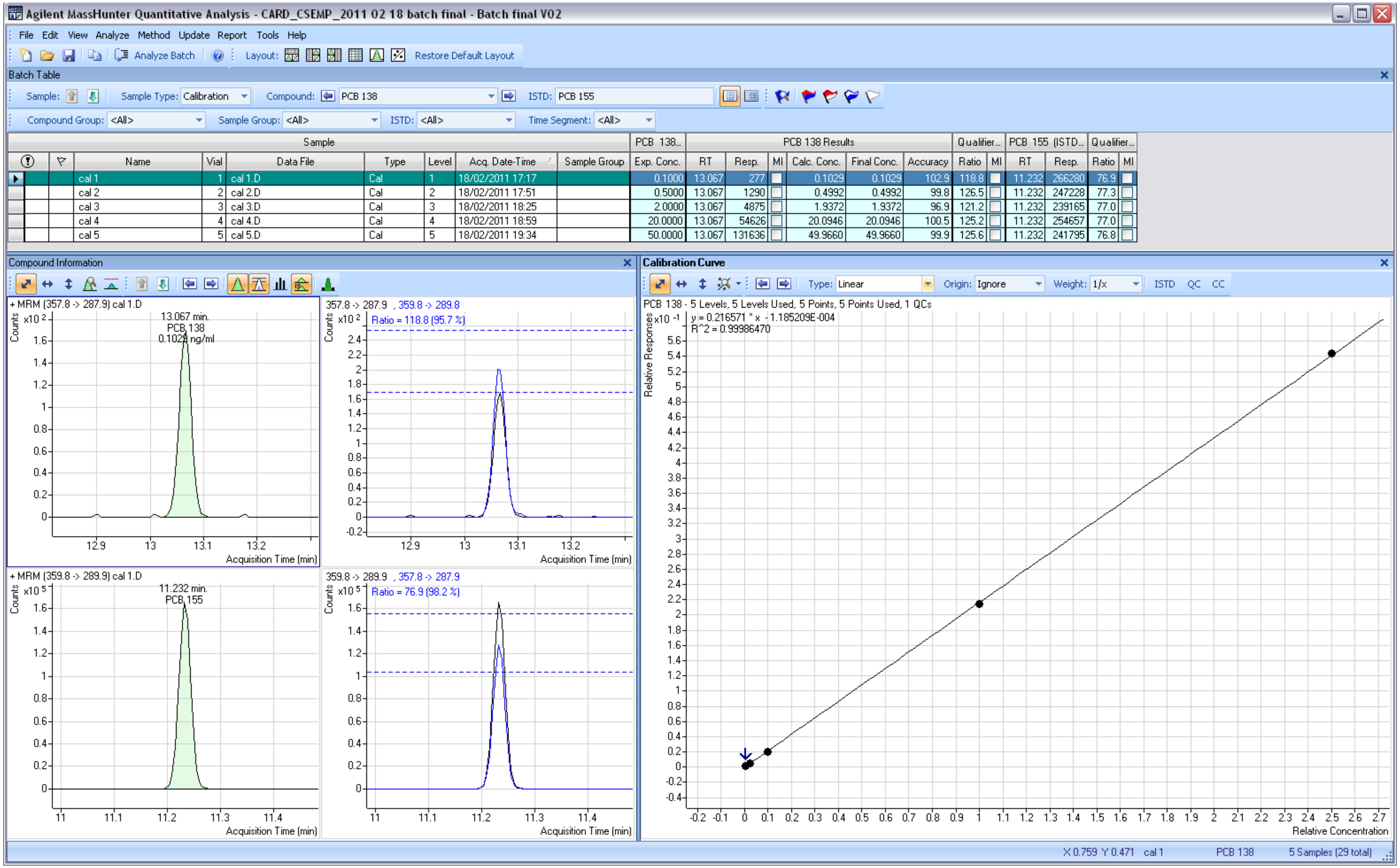
HCHs, Cal 1 : 0.4 pg/uL (equiv. 0.1 µg/kg in mussel tissue)



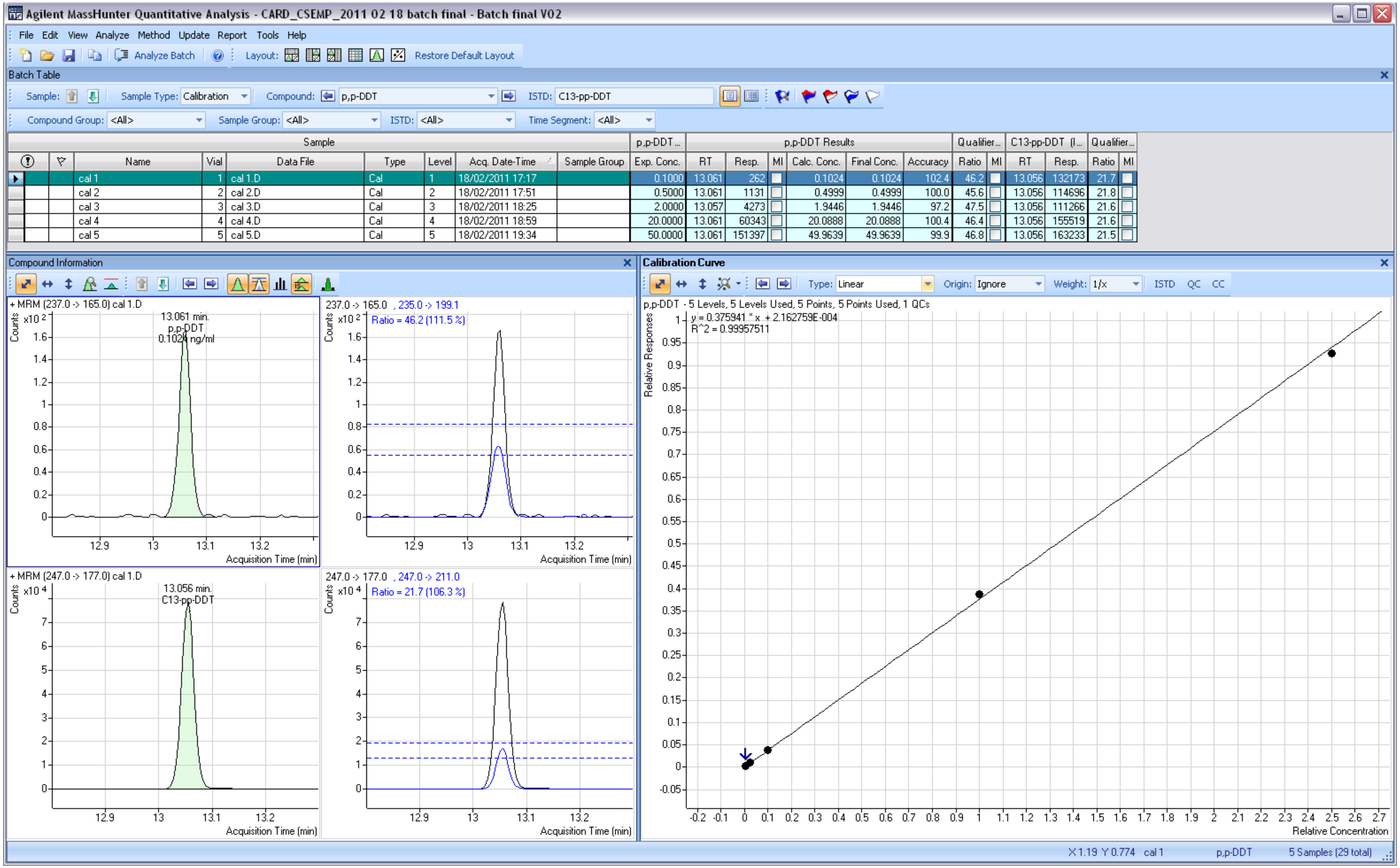
Incurred HCH Isomers in Mussel Sample



PCB 138 Cal 1 : 0.4 pg/uL (equiv. 0.1 µg/kg in mussel tissue)

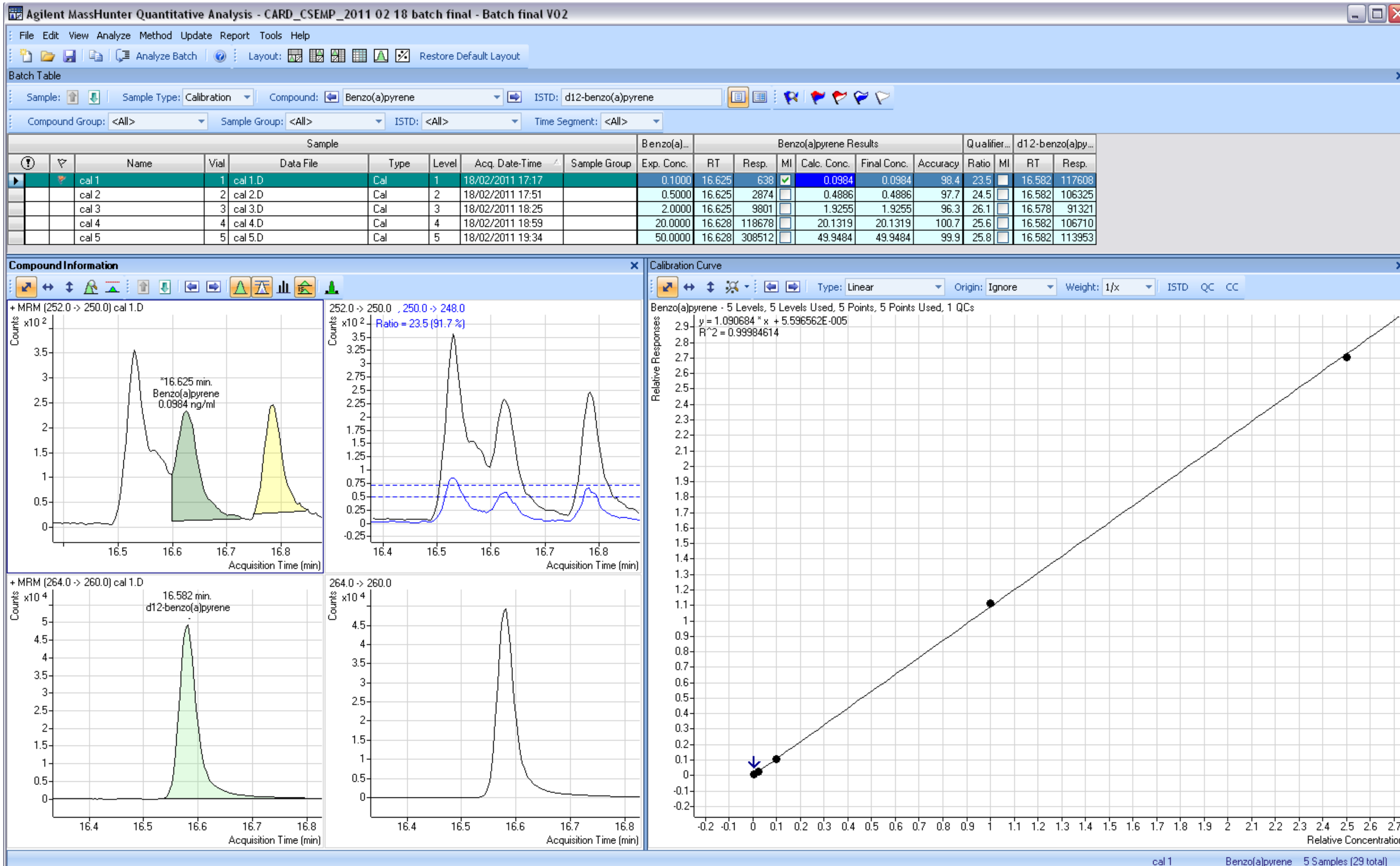


pp’-DDT Cal 1 : 0.4 pg/uL (equiv. 0.1 µg/kg in mussel tissue)

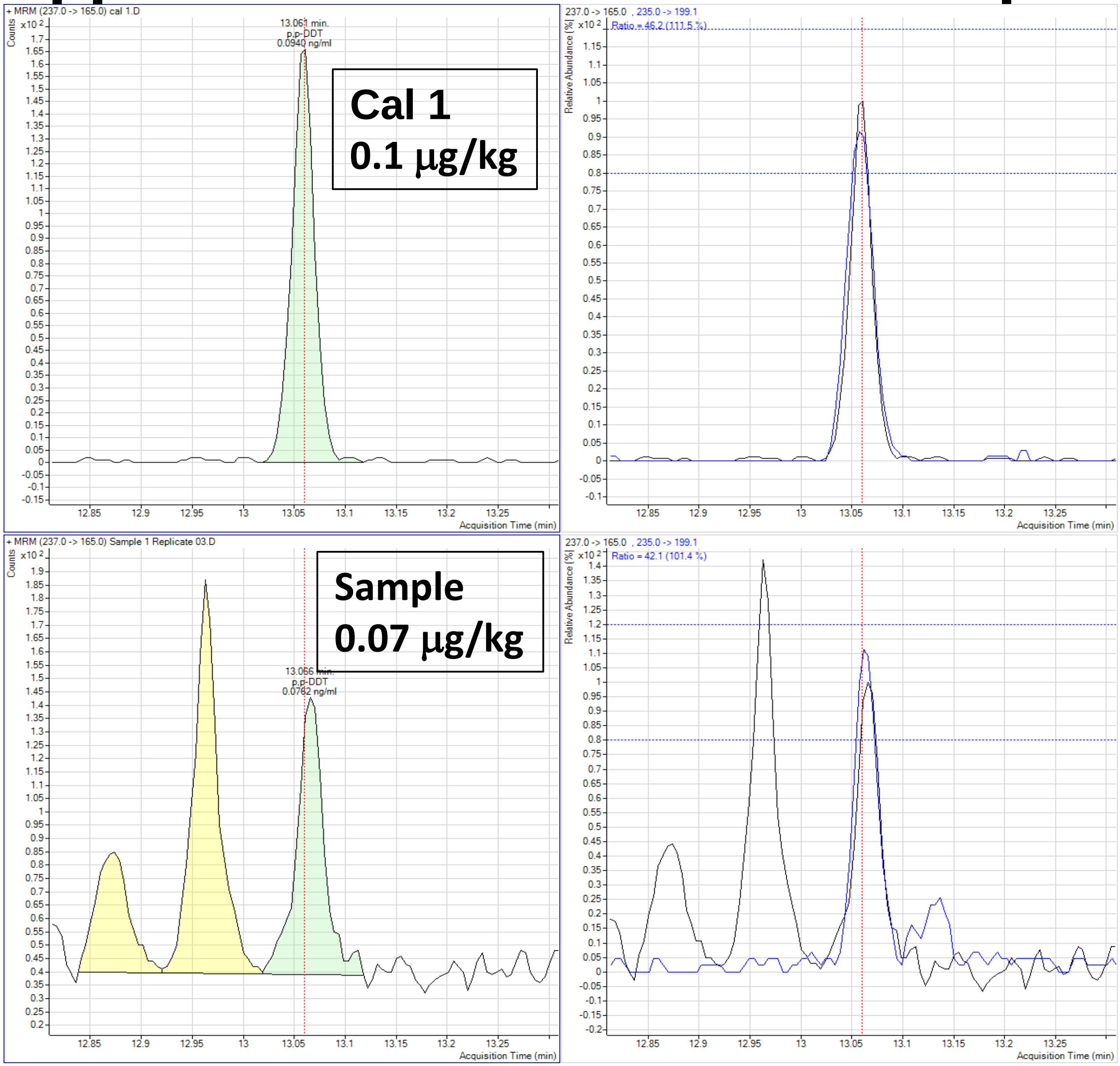


Name	Vial	Data File	Type	Level	Acq. Date-Time	Sample Group	p,p-DDT Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Qualifier Ratio	MI	C13-pp-DDT RT	Resp.	Qualifier Ratio	MI
cal 1	1	cal 1.D	Cal	1	18/02/2011 17:17		0.1000	13.061	262		0.1024	0.1024	102.4	46.2		13.056	132173	21.7	
cal 2	2	cal 2.D	Cal	2	18/02/2011 17:51		0.5000	13.061	1131		0.4999	0.4999	100.0	45.6		13.056	114696	21.8	
cal 3	3	cal 3.D	Cal	3	18/02/2011 18:25		2.0000	13.057	4273		1.9446	1.9446	97.2	47.5		13.056	111266	21.6	
cal 4	4	cal 4.D	Cal	4	18/02/2011 18:59		20.0000	13.061	60343		20.0888	20.0888	100.4	46.4		13.056	155519	21.6	
cal 5	5	cal 5.D	Cal	5	18/02/2011 19:34		50.0000	13.061	151397		49.9639	49.9639	99.9	46.8		13.056	163233	21.5	

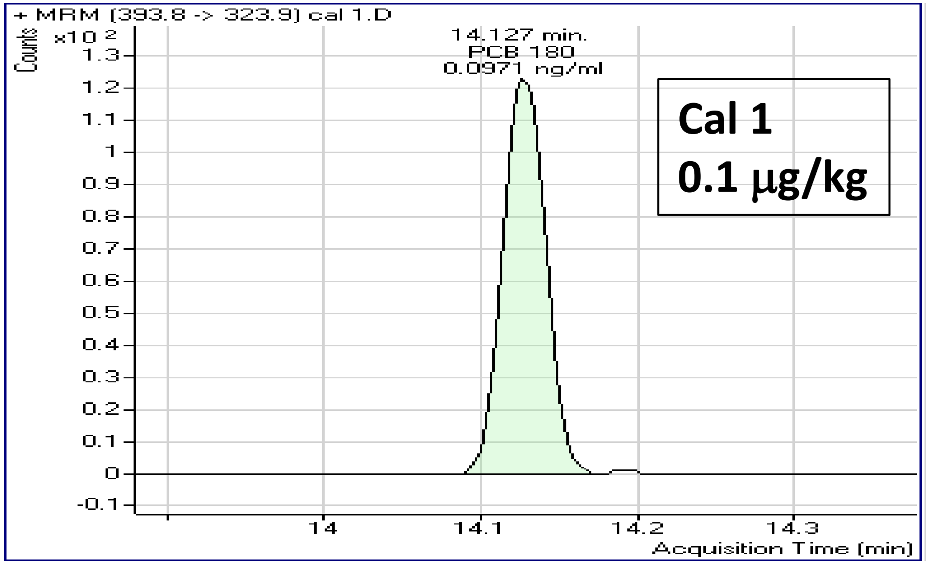
Benzo(a)pyrene Cal 1 : 0.4 pg/uL (equiv. 0.1 µg/kg in mussel tissue)

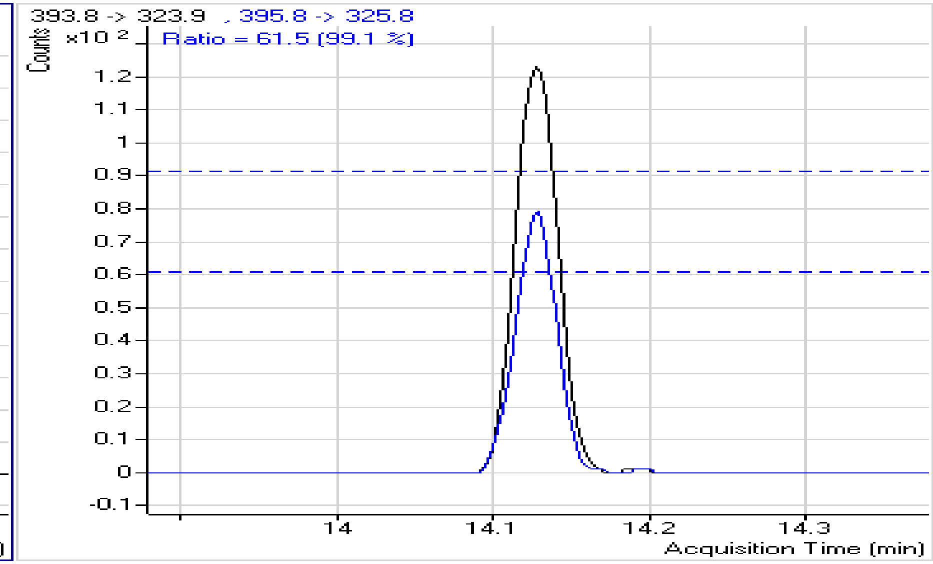


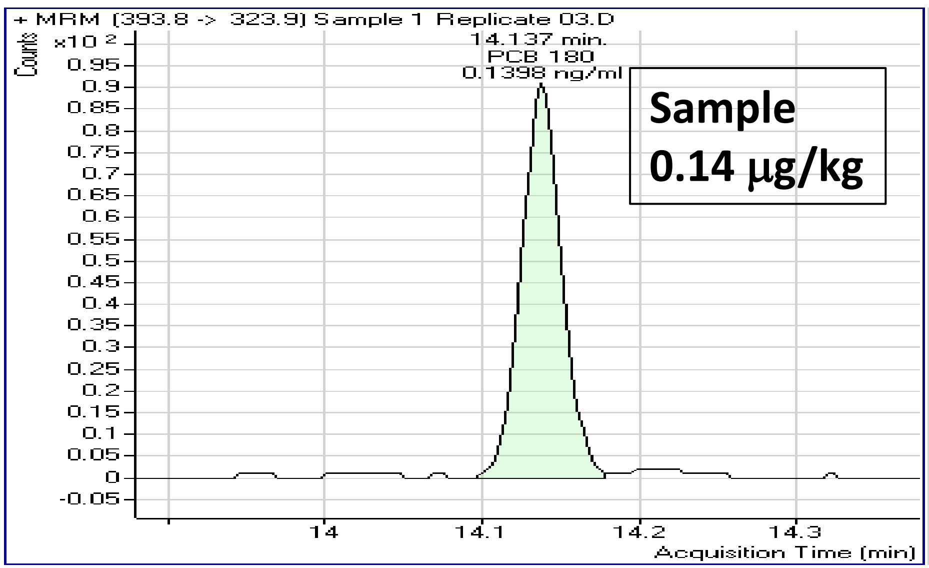
pp'-DDT Cal 1 and Mussel Sample

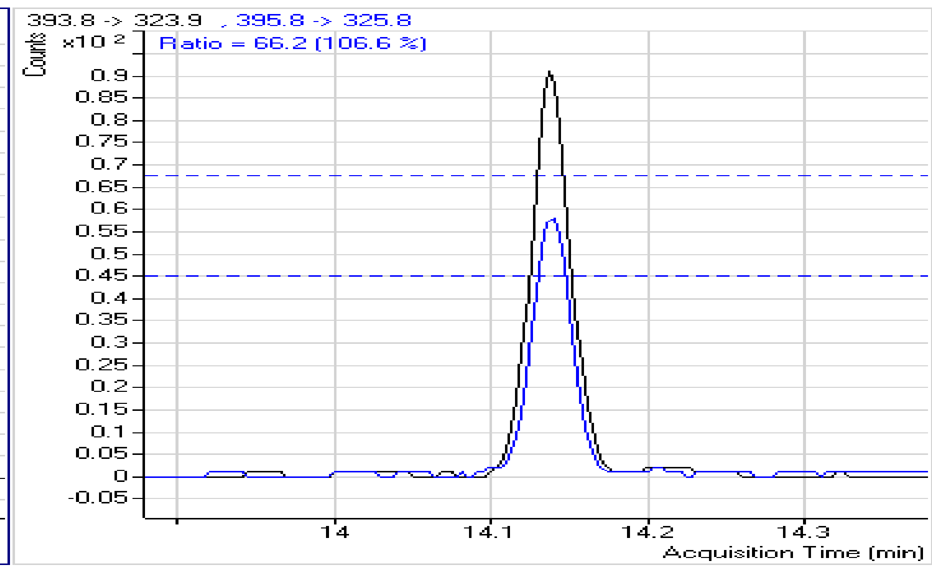


PCB 180 Cal 1 and Mussel Sample

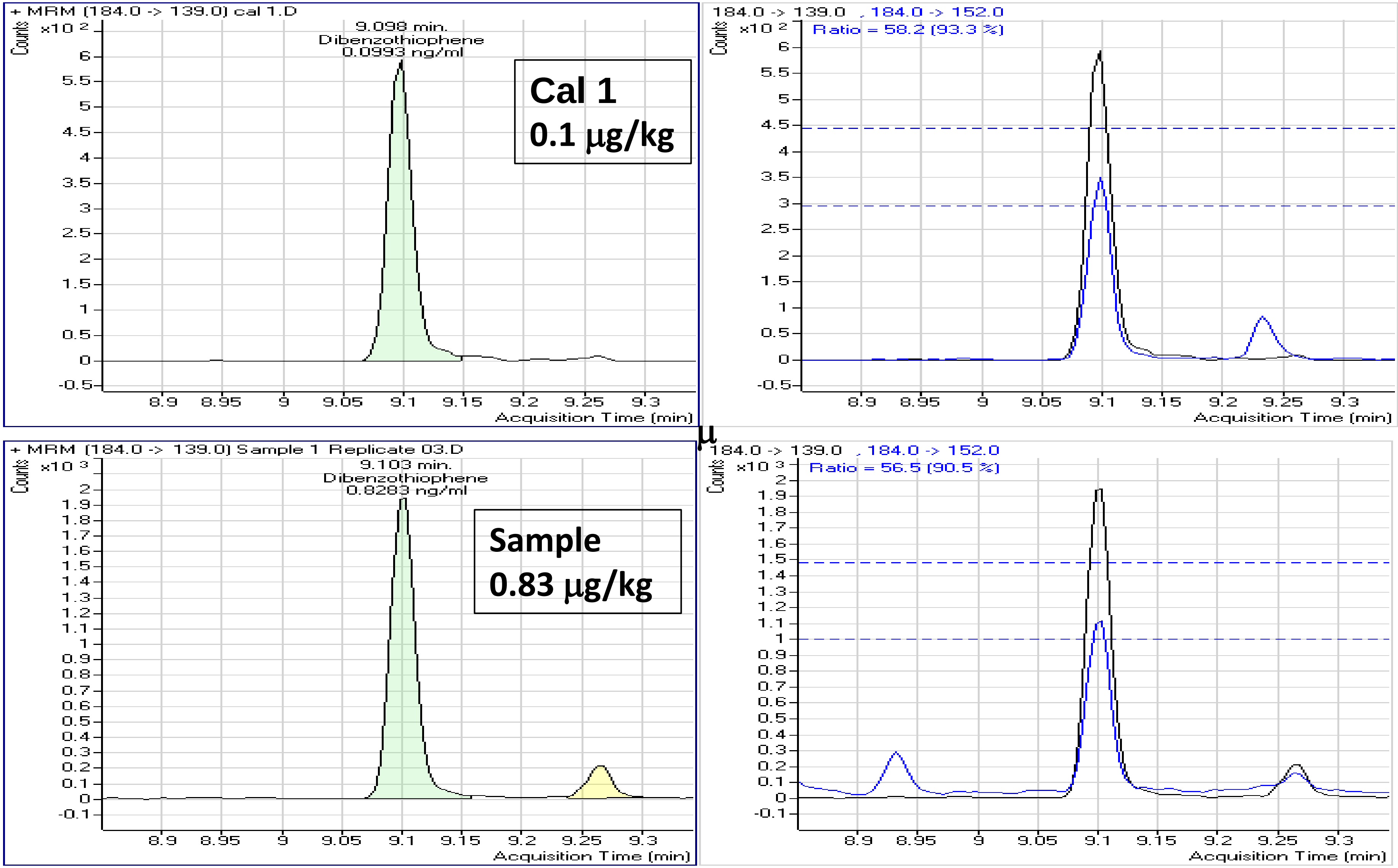








Dibenzothiophene Cal 1 and Mussel Sample



Application Note 5990-7714EN

Determination of Chemical Contaminants in Marine Shellfish using the Agilent 7000 Triple Quadrupole GC/MS System

Application note

Food Safety

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Abstract

A sample preparation method based on a modified QuEChERS extraction has been developed along with a GC/MS/MS method for the determination of selected Organo-chlorine pesticides, Polyaromatic hydrocarbons and Polychlorinated biphenyl congeners. The analytical method meets the detection limit requirements for the organic chemical contaminants in marine shellfish tissue (mussel) stipulated in the Clean Seas Environmental Monitoring Program.

Agilent Technologies

Summary

- A sample extraction / clean-up method based on a modified QuEChERS technique has been developed for a set of organic chemical contaminants in marine mussel tissue
- A large volume injection GC-MS/MS method has been developed for 16 OCPs, 19 PAHs and 7 PCB congeners
- The GC-MS/MS method includes LVI, RTL and post-column, post-run back flush
- The analytical method gives reproducible and sensitive detection of the analytes down to the required LODs given by the CSEMP legislation

ENVIRONMENT AGENCY

Agilent Technologies

Acknowledgements

Praveen Kutty, Kathleen Thomson, Anthony Gravell

Natural Resources Wales

(*Formerly* Centre for Analytical Research & Development

National Laboratory Service Innovation

Environment Agency, Llanelli, Wales)

Joan Stevens

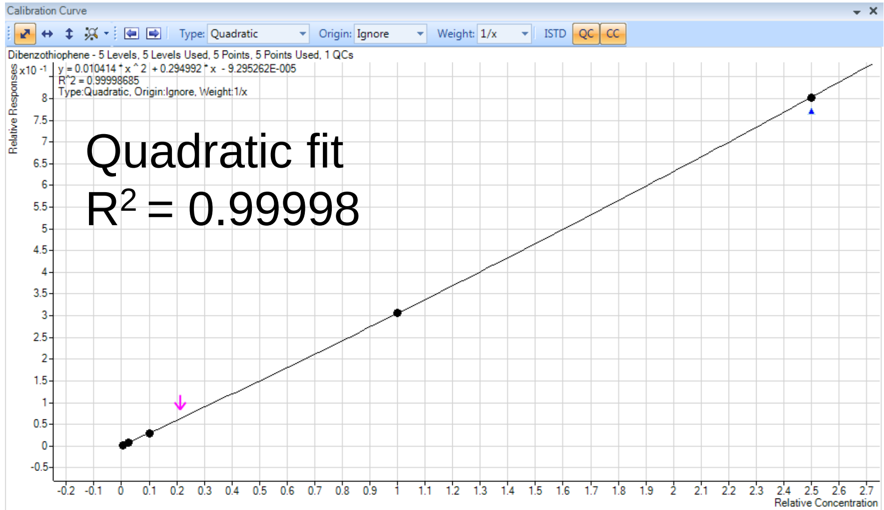
Sample Prep Specialist

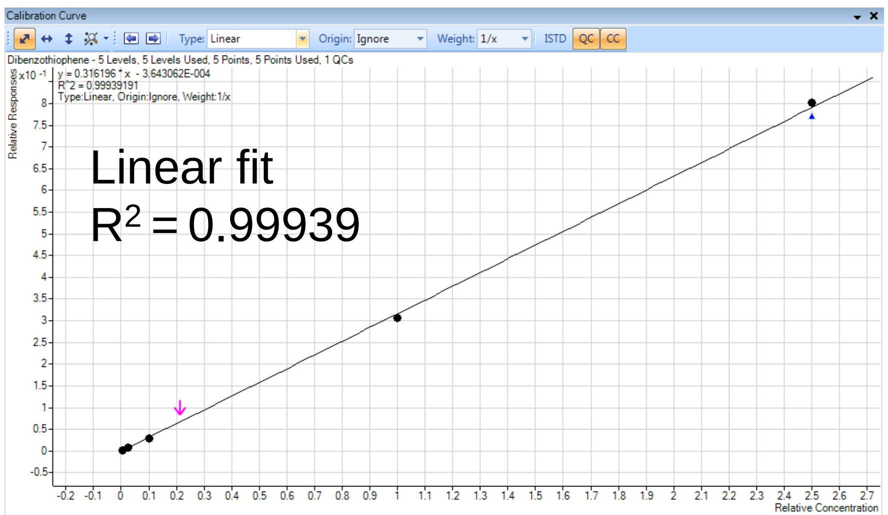
Agilent Technologies Inc, USA

RTL GC Analysis Conditions for 1uL Cold Splitless Injection

Gas Chromatograph	Agilent 7890A
Injection port	Agilent CO2 cooled Multi Mode Inlet
Injection mode	1uL Cold Pulsed Splitless Inlet Temp : 50 (0.05) – 600 deg C/min – 300 deg C Inlet 13.0 psig for 0.75 minutes, Purge 50 ml/min at 1.0 minute
Injection port liner	MMI 2mm ID dimpled deactivated liner (5190-2296)
Columns	(1) 15.0m x 0.25mm ID x 0.25um DB5-MS UI (122-5512) (2) 0.65m x 0.15mm ID x 0.15 μm DB-5MSUI (cut from 165-6626) Joined by G3186B capillary flow pressure controlled tee
Carrier gas	Helium
Carrier gas mode	Column 1: Constant Flow 1.20 ml/min (Inlet) Column 2 : Constant pressure 2.0 psig (PCM)
Oven temp program	50 (1) – 20 – 200 (0) – 10 – 300 (1.5)
RTL Locking compound	PCB 118, Locked to 12.37 minutes (9.35psig @ 50 deg C)
Post run Back flush	2.0 minutes, Col 1 -8.16 ml/min, PCM Pressure 60 psig, Inlet pressure 1psig
Oven temp program	50 (1) – 20 – 200 (0) – 10 – 300 (1.5)
Mass Spectrometer	Agilent 7000B QQQ
MS Interface	320 deg C
MS Source	300 deg C
MS Quad 1	150 deg C
MS Quad 2	150 deg C
Collision cell gases	Nitrogen 1.5 ml/min, Helium 2.25 ml/min
Detection mode	MRM
Tune	Gain normalized EI Autotune
Detector Gain	5

Dibenzothiophene





Analyte RTs, MRMs, CEs

1	4.00	d3-135-TCB	5.050	182.9	147.9	35	182.9	110.9	35
		135-TCB	5.068	179.9	144.9	35	179.9	108.9	35
		d8-Napthalene	5.479	136	108	25			
		Naphthalene	5.504	128	102	22	128	127	20
		HCBD	5.658	224.9	189.9	22	224.9	187.9	22
		2-methylnaphthalene	6.295	142	141	30	142	115	30
		1-methylnapthalene	6.407	142	141	30	142	115	30
2	6.80	1,3-Dimethylnapthalene	7.119	156	115	30	156	141	30
		d8-Acenapthylene	7.308	160	132	30	160	108	30
		Acenaphthylene	7.321	152	151	40	152	150	40
		d10-Acenapthene	7.494	164	162	30	164	160	30
		Acenaphthene	7.525	154	152	40	153	152	40
3	7.80	2,3,5-Trimethylnapthalene	7.992	170	155	25	170	153	25
		d10-Fluorene	8.099	176	174	30			
		Fluorene	8.131	166	165	30			
		d6-HCH - alpha	8.699	224	187	15	224	150	15
		HCH - alpha	8.730	181.0	145.0	15	181.0	109.0	30
		HCB	8.770	283.9	248.8	25	283.9	213.9	35
		HCH- beta	8.990	181.0	145.0	15	181.0	109.0	30
		d6-HCH- gamma	9.077	224	187	15	224	150	15
		HCH - gamma	9.107	218.8	183.0	5	181.0	109.0	30
		Dibenzothiophene	9.110	184	152	40	184	139	40
		d10-Phenanthrene	9.274	188.0	184.0	40	188.0	160.0	40
		Phenanthrene	9.299	178	176	34			
		Anthracene	9.367	178	176	34			
		HCH - delta	9.428	181.0	145.0	15	181.0	109.0	30
4	9.60	PCB 28	9.820	256	186	26	258	186	26
		2-Methyldibenzothiophene	9.861	198	197	30	198	165	30
		1-Methylphenanthrene	10.195	192	191	30	192	165	30
		PCB 52	10.250	289.9	220	28	291.9	222	28
		Aldrin	10.480	298.0	263.0	8	263.0	191.0	30
		3,6-Dimethylphenanthrene	10.733	206	190	30	206	205	30
		1,2-Dimethyldibenzothiophene	10.788	212	211	30	212	197	25
		Isodrin	10.880	262.9	193.0	35	262.9	191.0	35

5	11.00	d10-Fluoranthene	11.103	212.0	210.0	45	212.0	208.0	45
		Fluoranthene	11.128	202	201	30	202	200	50
		PCB 155	11.280	357.8	287.9	28	359.8	289.9	28
		2,4,7-Trimethyldibenzothiophene	11.283	226	178	40	226	165	40
		op-DDE	11.375	248.0	176.0	30	246.0	211.0	20
		PCB 101	11.437	323.9	253.9	28	325.9	255.9	28
		d10-Pyrene	11.486	212.0	210.0	45	212.0	208.0	45
		Pyrene	11.512	202	201	30	202	200	45
		p p-DDE	11.857	248.0	176.0	30	246.0	211.0	20
		C13-Dieldrin	11.933	269.8	200	40	269.8	198	40
		Dieldrin	11.940	262.8	193.0	30	262.8	191.0	30
		op-DDD	11.956	237.0	165.0	20	235.0	200.0	8
		1,2,6, Trimethylphenanthrene	12.071	220.0	205.0	23	220.0	190.0	28
6	12.15	Endrin	12.265	281.0	245.0	20	263.0	193.0	35
		PCB 118 (RTL)	12.370	323.9	253.9	28	325.9	255.9	28
		pp-DDD	12.500	237.0	165.0	20	235.0	199.1	8
		op-DDT	12.543	237.0	165.0	20	235.0	199.1	20
		PCB 153	12.698	357.8	287.9	28	359.8	289.9	28
		C13-pp-DDT	13.091	247	177	20	247	211	20
		pp-DDT	13.099	237.0	165.0	20	235.0	199.1	20
		PCB 138	13.112	357.8	287.9	28	359.8	289.9	28
7	13.50	Benzo[a]anthracene	13.897	228	226	38			
		d12-Chrysene	13.915	240	236	35			
		Chrysene / Triphenylene	13.965	228	226	38			
		PCB 180	14.175	393.8	323.9	30	395.8	325.9	30
8	15.00	Benzo(b)fluoranthene	16.060	252	250	42	250	248	40
		d12-Benzo(k)fluoranthene	16.084	264	260	40			
		Benzo(k)fluoranthene	16.116	252	250	42	250	248	40
		Benzo[e]pyrene	16.561	252	250	42	250	248	40
		d12-Benzo(a)pyrene	16.616	264	260	40			
		Benzo[a]pyrene	16.654	252	250	42	250	248	40
		Perylene	16.814	252	250	42	250	248	40
9	18.00	d12-Indeno(123-cd)pyrene	18.600	288	284	50			
		Indeno{123-cd]pyrene	18.631	276	274	42			
		d14-Dibenz(a,h)anthracene	18.662	292	288	50			
		Dibenz[a,h]anthracene	18.712	278	276	38			
		d12-Benzo(g,h,i)perylene	19.020	288	284	45			
		Benzo[ghi]perylene	19.064	276	274	38			

16 Organo-chlorine compounds

7 PCBs

28 Poly aromatic hydrocarbons

19 ISTDS

Mussel Tissue : Sample Extraction / Clean-up

(1) 2g homogenized mussel tissue weighed into QuEChERS extraction tube

(2) Add labelled ISTDs (prepared in acetone)

(3) Vortex mix sample for 30 seconds, add 13mL de-ionized water and 2 ceramic homogenizers

(4) Vortex mix sample for 1 minute. Add 15mL extraction solvent (1% acetic acid in acetonitrile), vortex mix 1 minute

(5) Add QuEChERS AOAC salt mix (5982-5755), shake tube by hand for 1 minute, vortex mix for 1 minute

(6) Centrifuge tube for 5 minutes at 3900 rpm then cool tube in freezer at -20 °C for 30 minutes

(7) Transfer acetonitrile layer to clean / dry centrifuge tube containing 1g anhydrous sodium sulphate

(8) Shake by hand for 1 minute then place tube in freezer at -20 °C overnight

(9) Pipette 10mL clear extract in to a clean turbovap tube, evaporate to 0.5 mL

(10) Add 20 mL dichloromethane (DCM) evaporate to 0.5mL, add 10 mL DCM. Evaporate to 0.5 mL.

(11) Add 10 mL Hexane, evaporate to 0.5 mL, add 10 mL Hexane and evaporate to 0.5 mL

(12) Activate silica at 180 °C overnight, add 1g to empty 15mL SPE tube

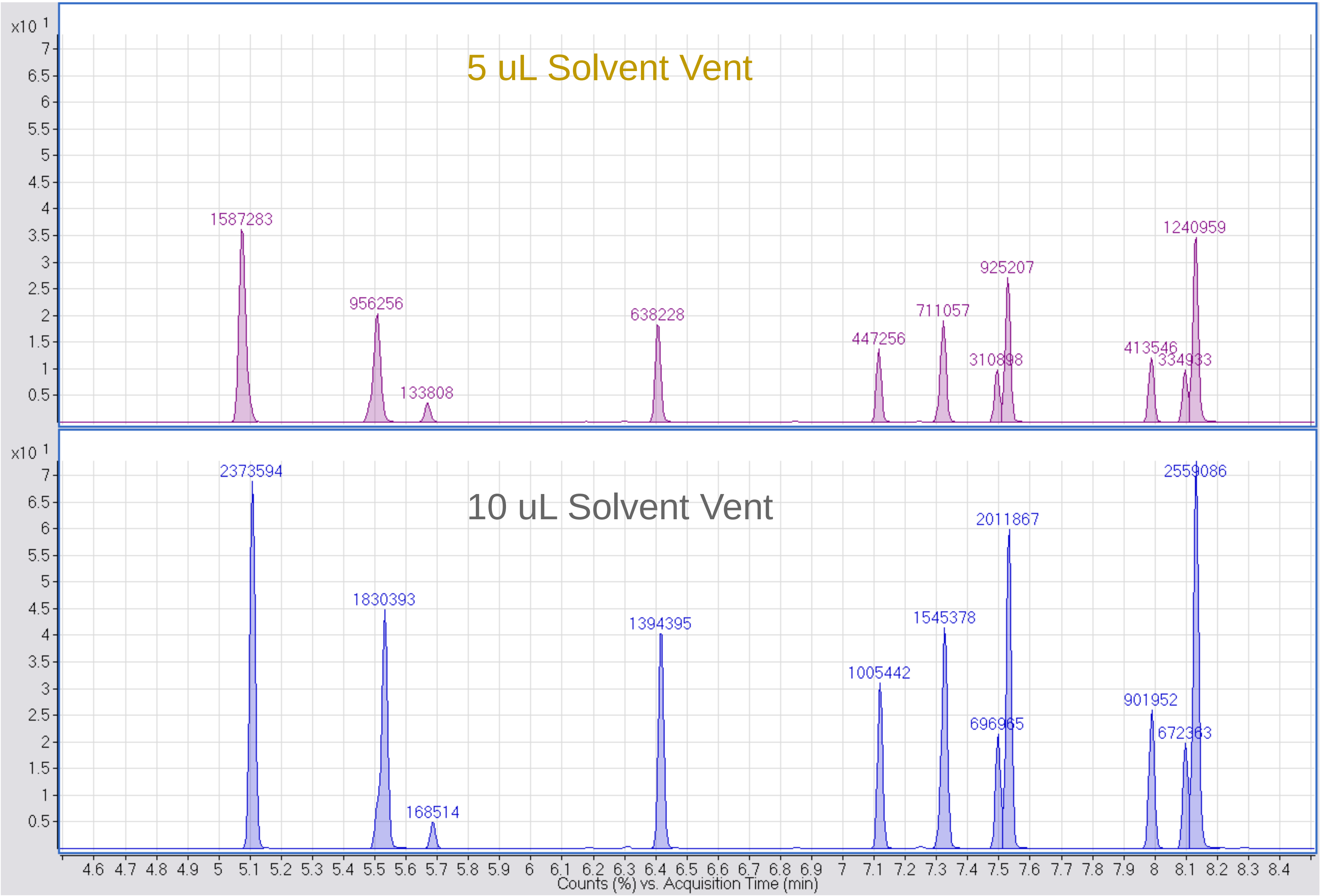
(13) Condition 1g Silica in SPE tube with 10 mL DCM and 20 mL hexane

(14) Apply concentrated extract to Silica SPE tube elute with 13 mL 40:60 DCM : hexane

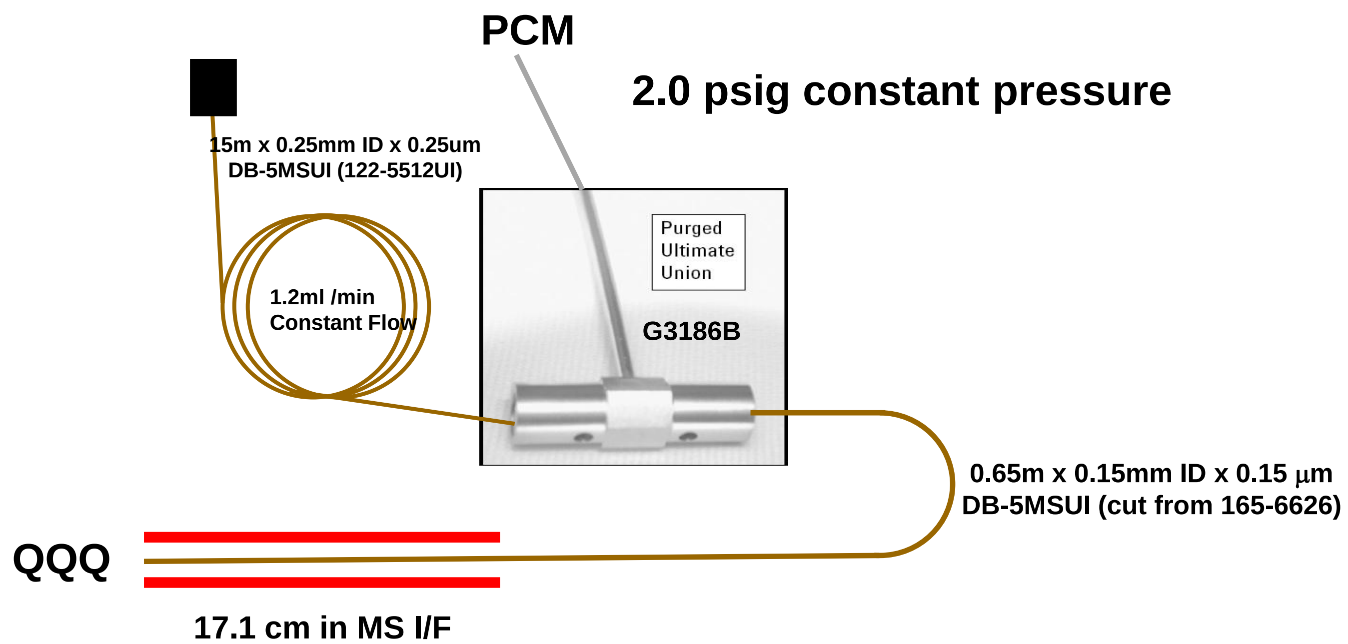
(15) Evaporate extract to 0.5 mL, repeat steps (13) and (14)

(16) Evaporate extract to 0.5 mL, transfer to 2 mL autosampler vial

Peak Areas of Early Eluters



Flow rate to QQQ



Oven Temperature program : 50 (1) – 20 – 200 (0) – 10 – 300 deg C
Column Constant flow 1.2 ml/min
MS I/F Temperature 325 deg C   Oven @ 50 deg C, Restrictor flow = 2.23ml/min
Oven @ 300 deg C, Restrictor flow = 1.23 ml/min

- Across entire GC run, Analytical flow < flow to QQQ
- Higher starting flow to QQQ allows for pulsed splitless injection (13.0 psig for 0.75 mins, = 1.95 ml/min) without disturbing PCT pressure set-point

Post column MS Restrictor Flow Rate Calculator

Quimby_Oven_Xfer_FlowCalc.xls [Compatibility Mode] - Microsoft Excel

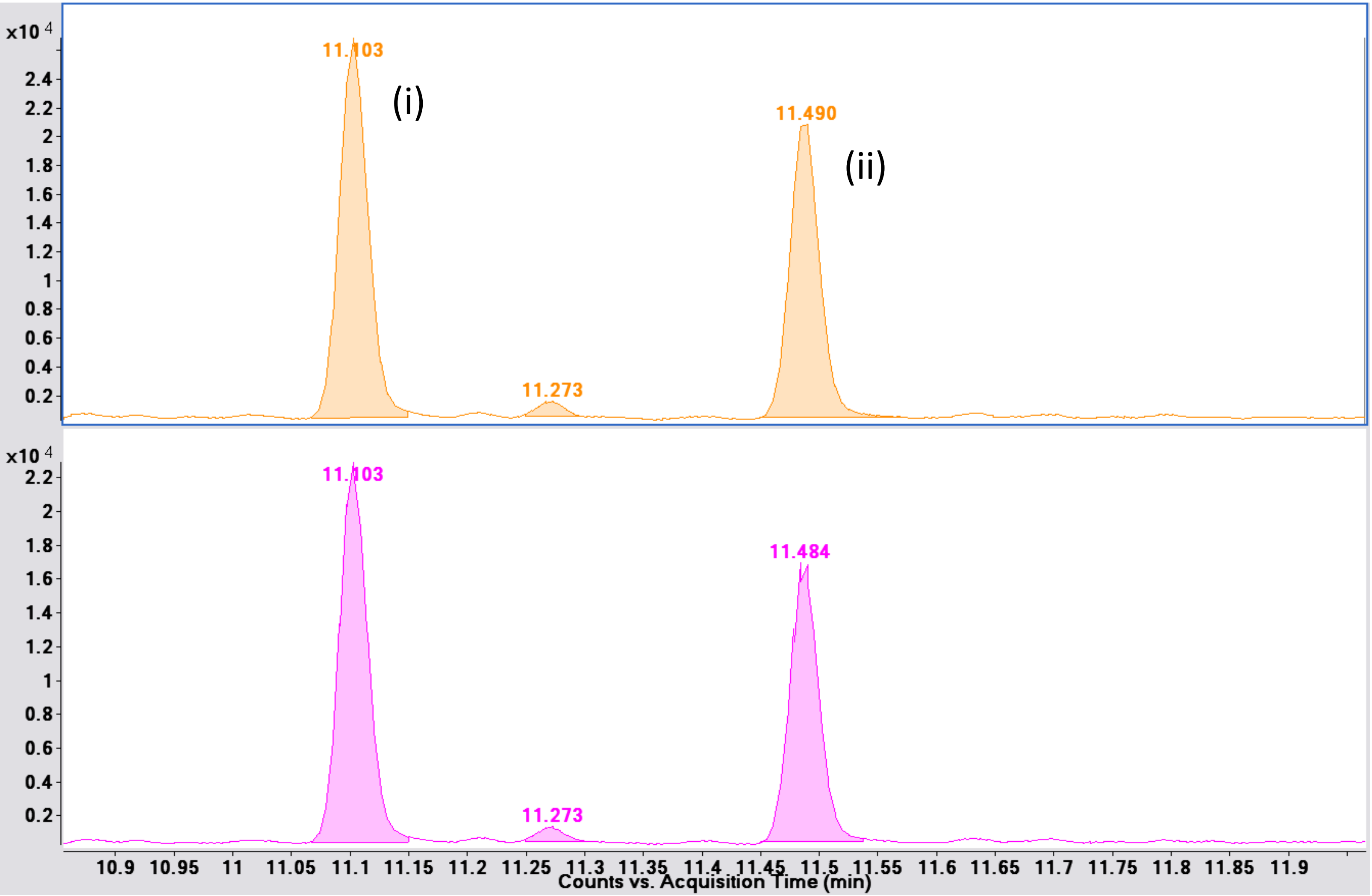
Carrier Gas (Helium,Hydrogen,Nitrogen,Argon)	Helium
Length of tube in oven (cm)	47.9
Diameter of tube in oven (mm)	0.15
Length of tube in transferline (cm)	17.1
Diameter of tube in transferline (mm)	0.15
Pressure in Purged Union (psi gauge)	2
Outlet pressure of transferline (psi absolute)	0

Note: All fields in blue font are INPUTS

Flow	Oven Temp °C	Xfer Line Temp °C
2.2345	50	325
2.1773	60	325
2.1218	70	325
1.9656	100	325
1.9167	110	325
1.7793	140	325
1.7364	150	325
1.6157	180	325
1.5414	200	325
1.4718	220	325
1.4065	240	325
1.3452	260	325
1.2876	280	325
1.2334	300	325
1.1825	320	325
1.1345	340	325
1.0893	360	325
1.0467	380	325
1.0065	400	325
0.9686	420	325
0.9327	440	325
0.8987	460	325
0.8666	480	325
0.8361	500	325
0.8073	520	325

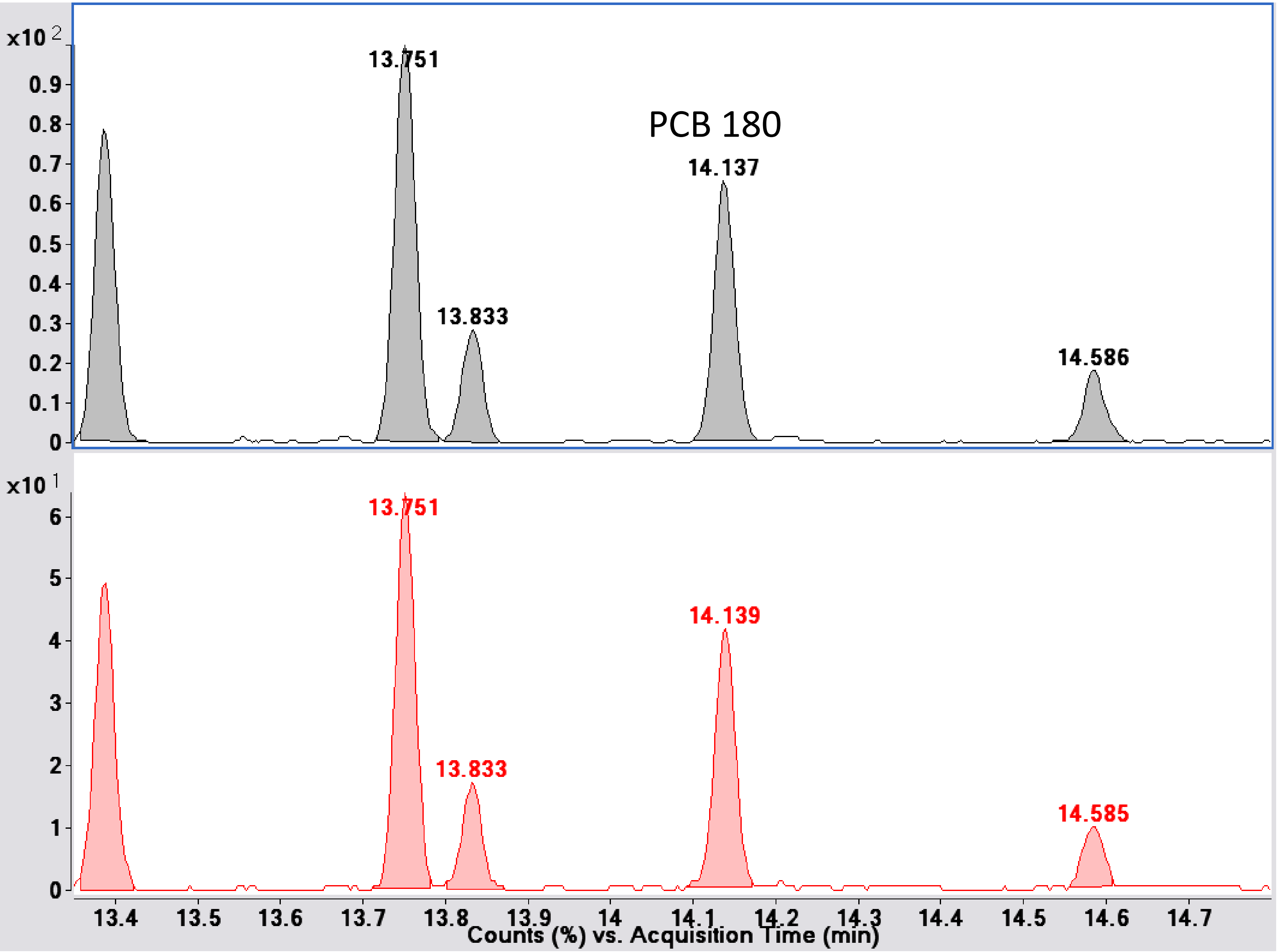
Oven_Xfer_FlowCalc

Incurred Fluoranthene and Pyrene in Mussel Sample



MRM Chromatograms for incurred Fluoranthene (i) and incurred Pyrene (ii) in mussel sample, Concentrations 8.64 and 5.83 μg/Kg, respectively.

Incurred PCB 180 in Mussel Sample



MRM Chromatograms for incurred PCB 180 in mussel sample, Concentration 0.14 μg/Kg.