Organic and Biological Analytical Chemistry Group

REVISITED SAMPLE PREPARATION AND ANALYSES FOR DIOXIN MEASUREMENTS IN BIOLOGICAL MATRICES

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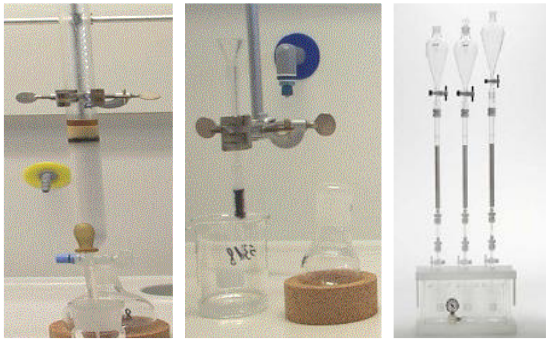
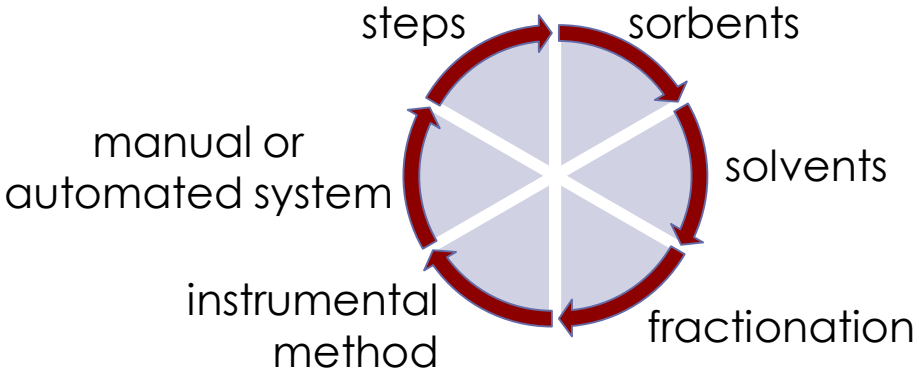
11th International Symposium
Recent Advances in POPs & Emerging contaminant Analysis
18th May 2017, Boston

Overview

- Need for fast and high throughput methods in PCDD/Fs and PCBs analysis.
- Sample preparation can be carried out in several ways





- GC-HRMS DFS Magnetic Sector, equipped with Dual Data Acquisition module, allows higher throughput

Dual Data valve

I. Sample Preparation

Extraction of Dioxins and PCBs

Food and feed samples:

ASE extraction (when needed)

Liq/liq extraction (milk)

Human serum:

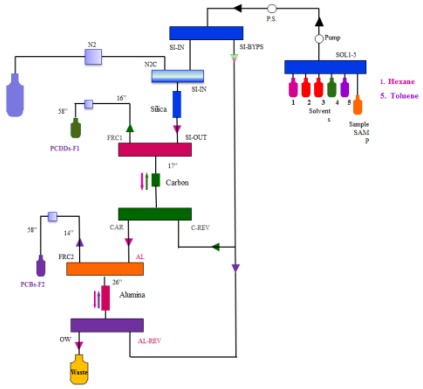
SPE extraction with manifold, 1g C18/10 mL cartridges

Automated SPE extraction with TurboTrace system
1g C18/15 mL cartidges (FMS)

Low Solvent, DCM Free Clean-up System

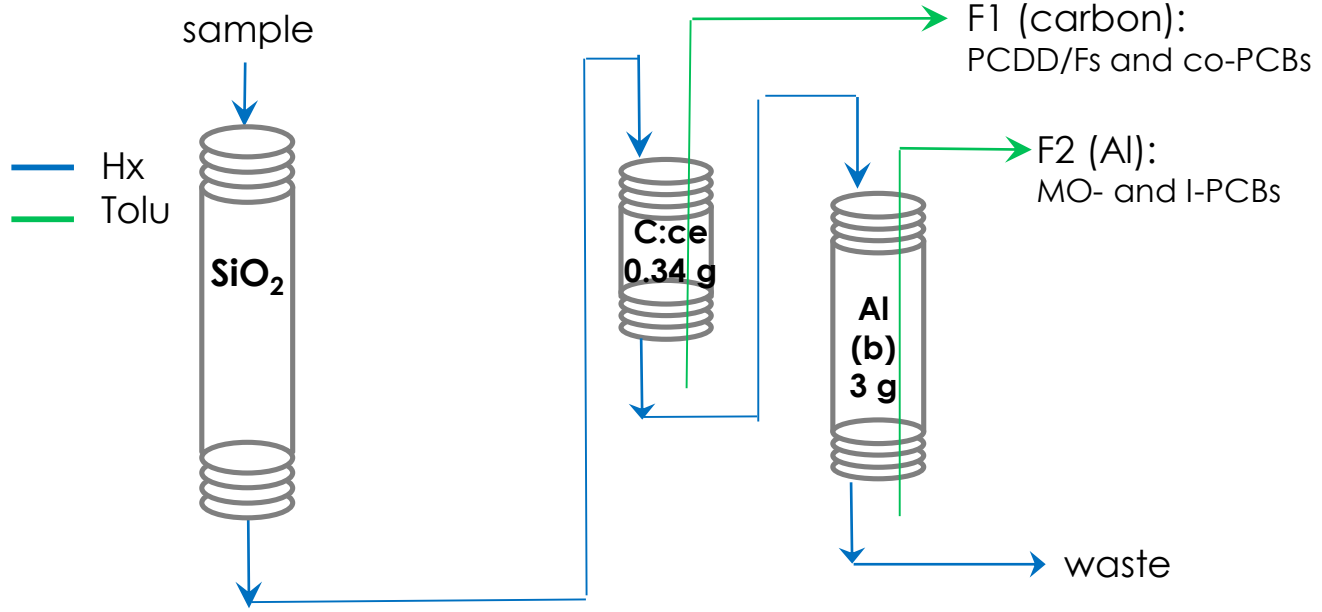
EconoPrep® clean-up system, new plumbing diagram: **Si -- C -- Al**

EconoPrep®



ABN Silica (x g*)
*variable size/fat capacity

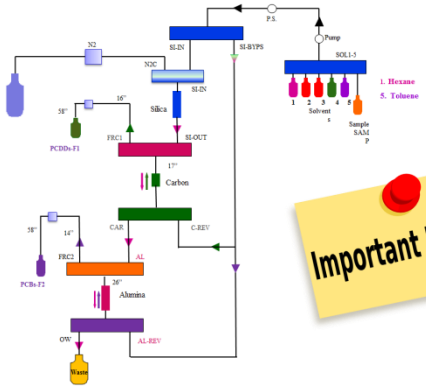
C-celite 0.34 g
Basic Al 3 g



Low Solvent, DCM Free Clean-up System

EconoPrep® clean-up system, new plumbing diagram: **Si -- C -- Al**

EconoPrep®



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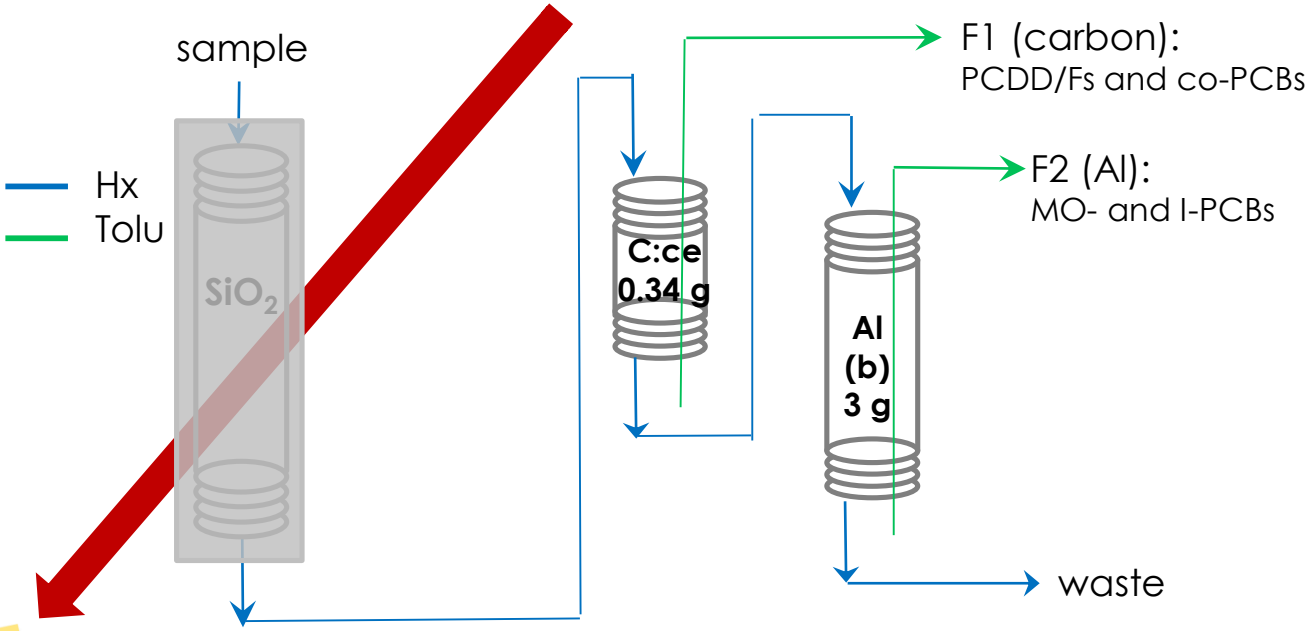


Important Notice

EconoPrep® is a **vey flexible** system suitable for the clean-up of all biological matrices, as Silica columns with different fat capacities can be connected

Sample Clean-up, Chemical Steps

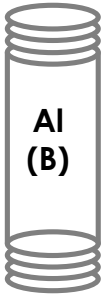
Sample clean-up consists of 2 main chemical steps:

1. **Fat digestion**, matrix degradation

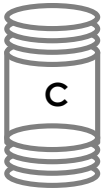
Acidic, basic and neutral (ABN) Silica gel

Variable size depending on fat amount to be processed

2. **Fractionation** of planar (PCDD/Fs and co-PCBs) and non-planar (MO- and I-PCBs) compounds for spectrometric quantification



Basic Alumina:
Polar and acid-base interactions with PCBs

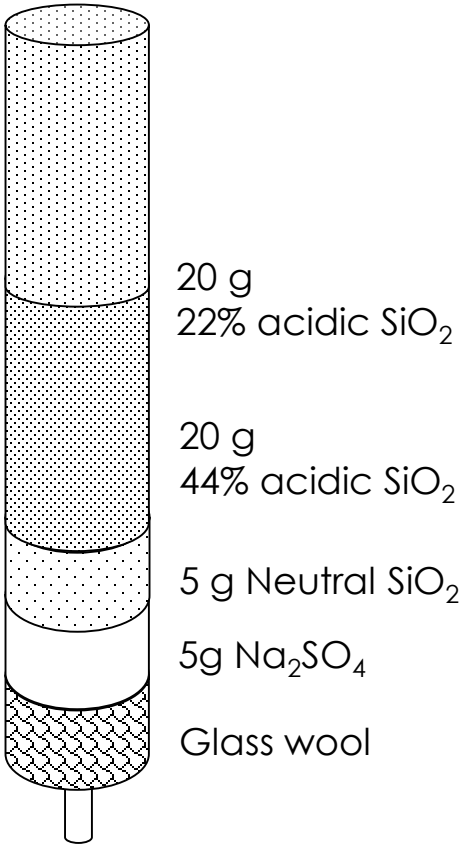


Carbon:
Geometric interactions with planar dioxins and coplanar PCBs

Silica Columns for Fat Digestion

Manually packed Silica column

Up to 7 g processed



Commercial Silica columns

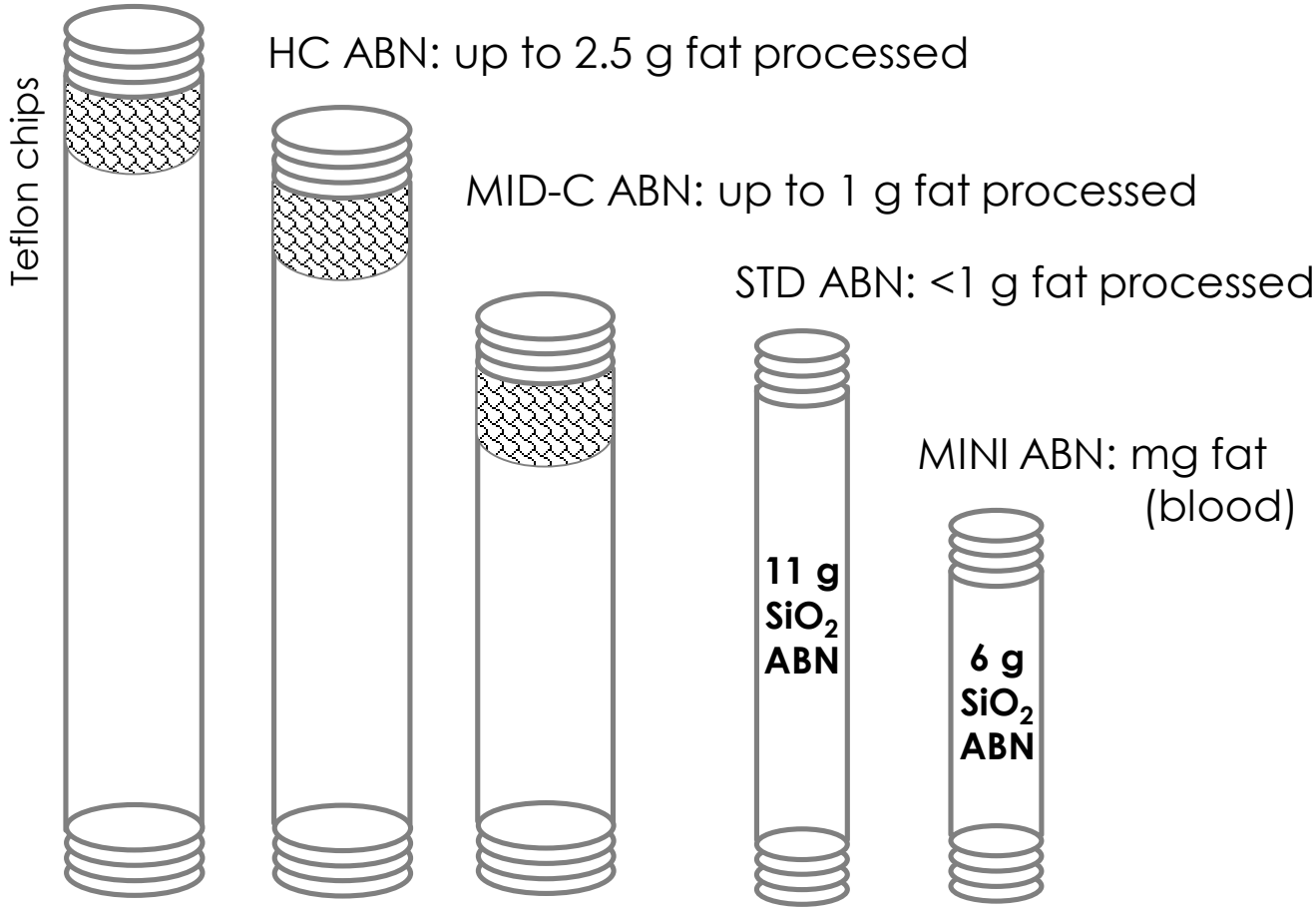
XL ABN: up to 5 g fat processed

HC ABN: up to 2.5 g fat processed

MID-C ABN: up to 1 g fat processed

STD ABN: <1 g fat processed

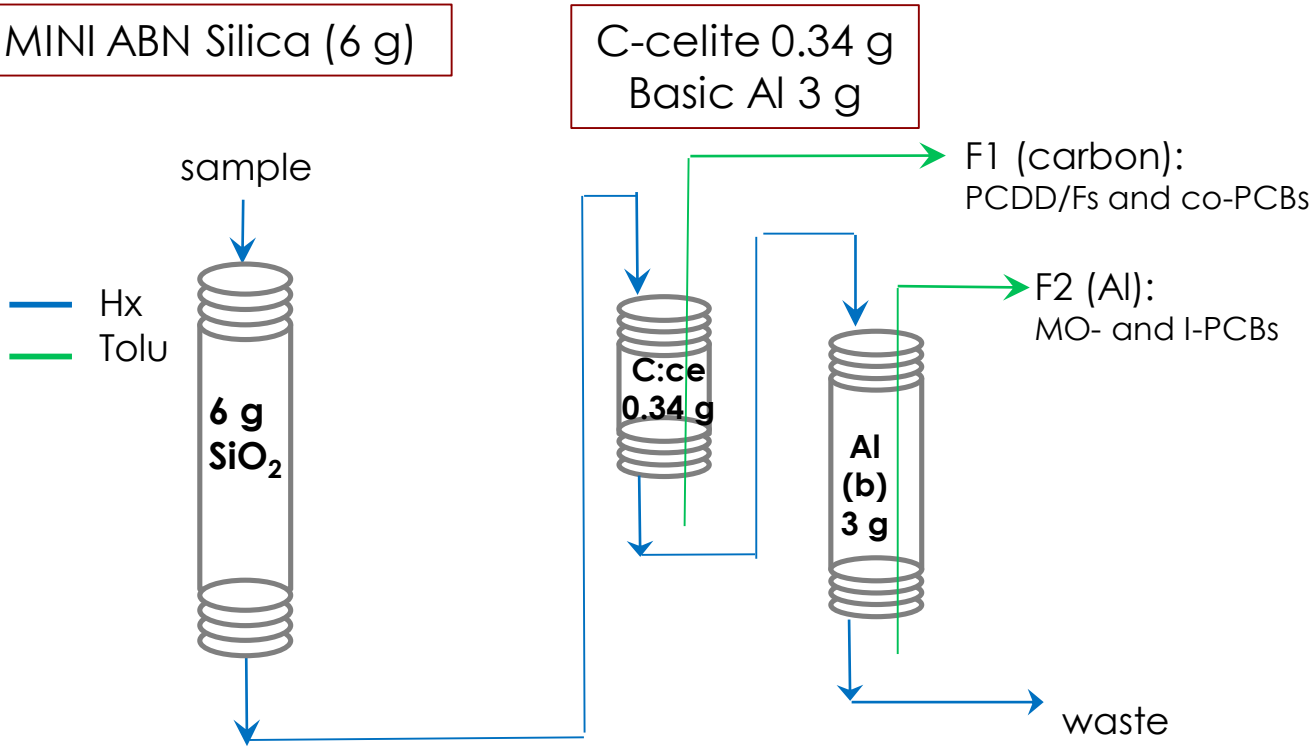
MINI ABN: mg fat (blood)



Fast Sample Clean-up for Serum Samples

EconoPrep® equipped with "MINI" column set for lipid content < 0.5%

FMS EconoPrep®



Total mL	**110 mL**
Total min	**20 min**

* Calaprice C., Focant J.-F., Organohalogen Compounds, Vol 78 (2016) – *pp. 773-776*

Fast Sample Clean-up for Serum Samples

- Serum "MINI-columns"optimized automated method

Description	Solvent	Flow mL/min	Volume mL	Path
		mg Fat		
Column condit.	Hx	10	20	Si – C - Al
Sample loading	Hx	5	(7)	Sample – Si – C- Al - W
Silica elution	Hx	5	40	Si – C – Al - W
Backflush C	Tolu	5	25	C back – F1
Backflush Al	Tolu	5	25	Al back – F2

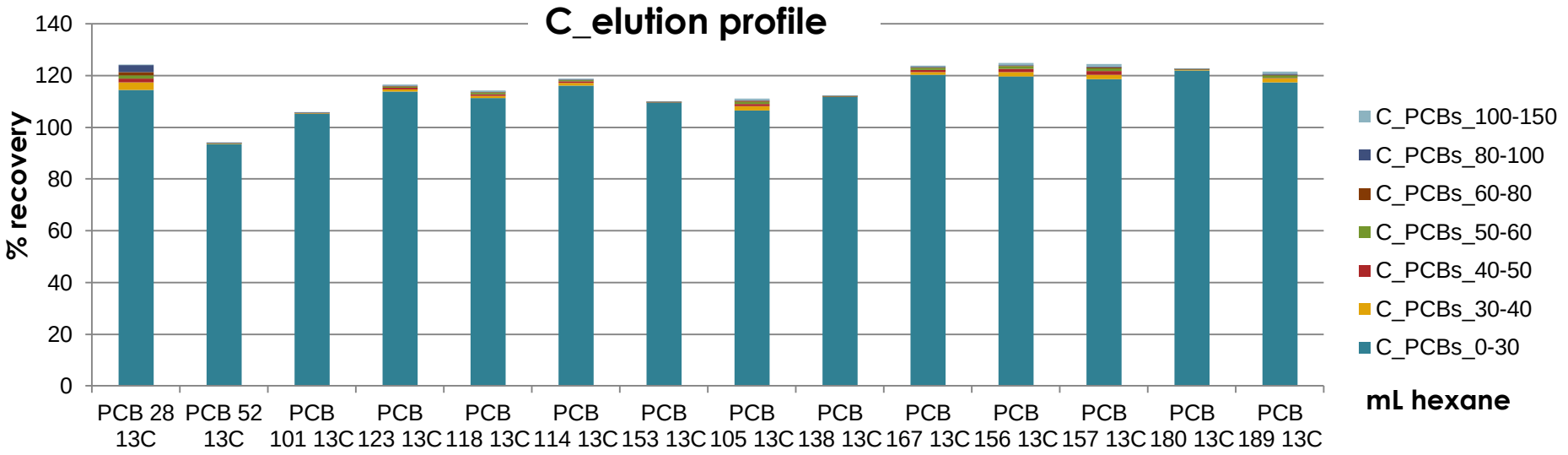
Total mL	**110 mL**
Total min	**20 min**

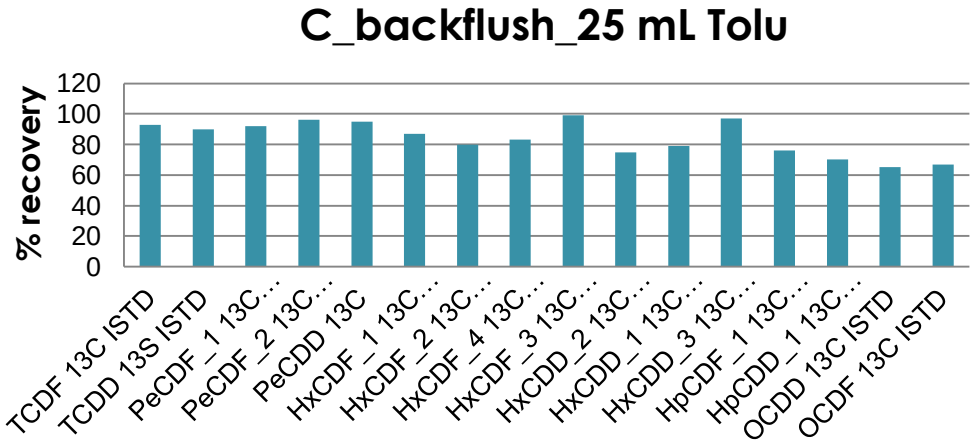
Comparison MINI vs Classical procedure*		Time min	Volume mL	Hx mL	DCM mL	Tolu mL
Method	Classical procedure	67.7	587	373	72	142
	Mini-columns procedure	20	110	60	0	50
Saving	Mini-columns procedure	**70%**	**81%**	**84%**	**100%**	**65%**
*in our routine laboratory						

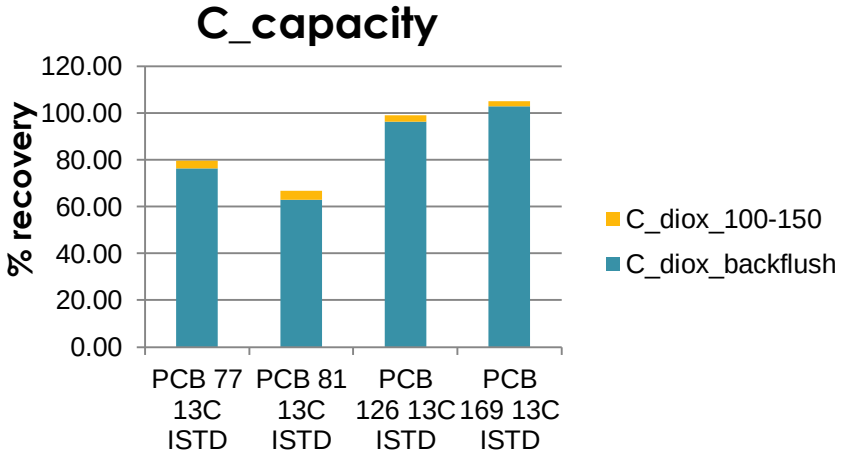
* Calaprice C., Focant J.-F., Organohalogen Compounds, Vol 78 (2016) – *pp. 773-776*

Fast Sample Clean-up for Serum Samples

- Silica, carbon and alumina column elution profiles have been studied



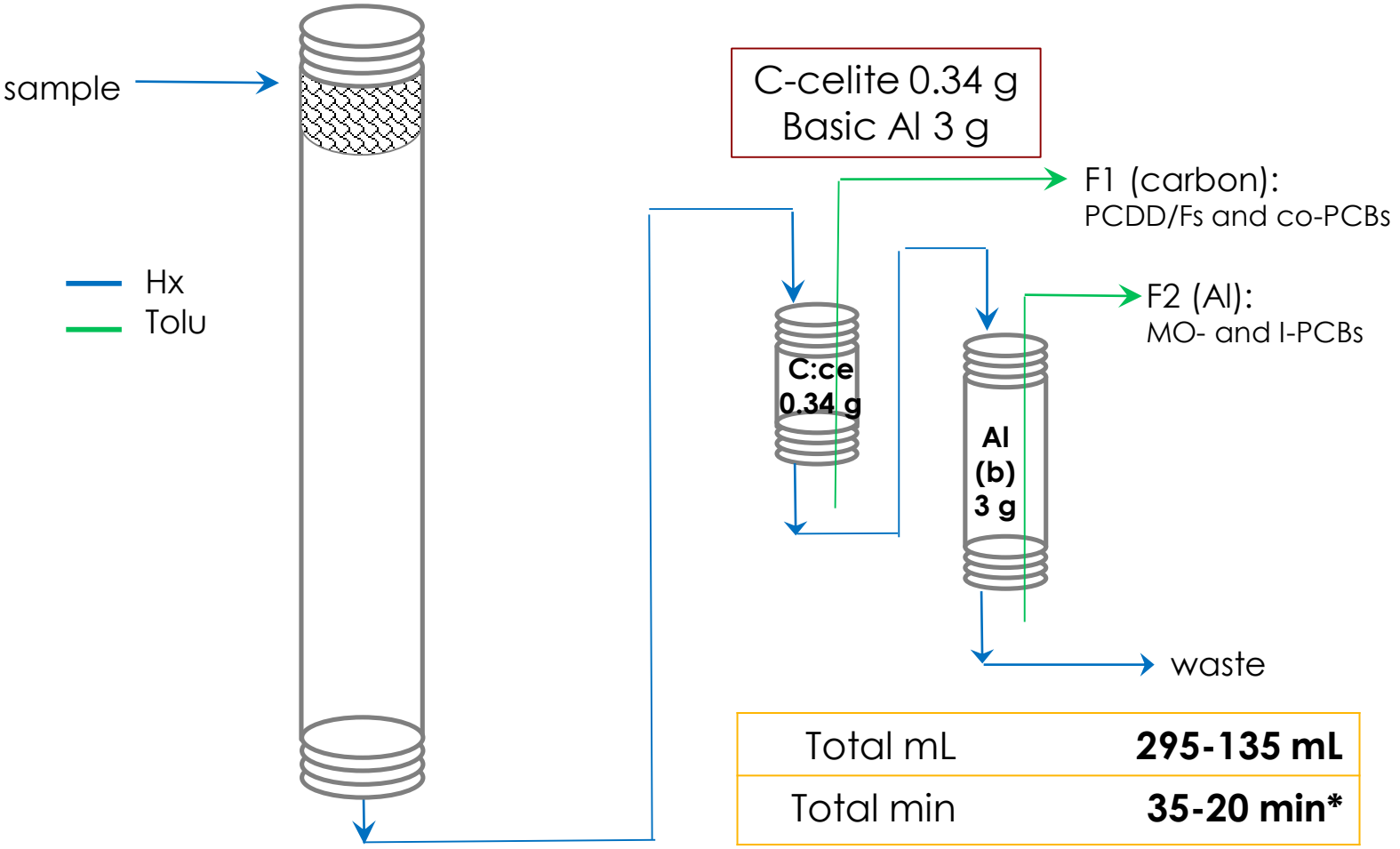




Fast Sample Clean-up for Food/Feed

EconoPrep® equipped with suitable Silica column, according to the amount of fat to be processed

XL ABN: up to 5 g fat processed (vegetable oil 4 g)



Total mL	**295-135 mL**
Total min	**35-20 min***

* Sample loading time NOT included

Fast Sample Clean-up for Food/Feed

- XL ABN column method

Description	Solvent	Flow mL/min	Volume mL	Path
Up to 5 g fat processed (4 g vegetable oil)				
Column condit.	Hx	10	65	Si – C - Al
Sample loading	Hx	5	(50)	Sample – Si – C- Al - W
Silica elution	Hx	10	180	Si – C – Al - W
Backflush C	Tolu	5	25	C back – F1
Backflush Al	Tolu	5	25	Al back – F2

Total mL	**295 mL**
Total min	**34.5 min***

* Sample loading time NOT included

Fast Sample Clean-up for Food/Feed

- XL ABN column method

- HC ABN column method

Description	Solvent	Flow mL/min	Volume mL	Path
Up to 2.5 g fat processed (2 g vegetable oil)				
Column condit.	Hx	10	55	Si – C - Al
Sample loading	Hx	5	(50)	Sample – Si – C- Al - W
Silica elution	Hx	10	110	Si – C – Al - W
Backflush C	Tolu	5	25	C back – F1
Backflush Al	Tolu	5	25	Al back – F2

Total mL	**245 mL**
Total min	**29.5* min**

* Sample loading time NOT included

Fast Sample Clean-up for Food/Feed

- XL ABN column method
- HC ABN column method
- MID-C column method*

Description	Solvent	Flow mL/min	Volume mL	Path
Up to 1 g fat processed				
Column condit.	Hx	10	20	Si – C - Al
Sample loading	Hx	5	(50)	Sample – Si – C- Al - W
Silica elution	Hx	5	80	Si – C – Al - W
Backflush C	Tolu	5	25	C back – F1
Backflush Al	Tolu	5	25	Al back – F2

Total mL	**150 mL**
Total min	**28** min**

* Food and Chemical Toxicology 100 (2017) *pp. 70-79*
** Sample loading time NOT included

Fast Sample Clean-up for Food/Feed

- XL ABN column method
- HC ABN column method
- MID-C column method
- STD ABN column method

Description	Solvent	Flow mL/min	Volume mL	Path
<1 g fat processed				
Column condit.	Hx	10	15	Si – C - Al
Sample loading	Hx	5	(50)	Sample – Si – C- Al - W
Silica elution	Hx	10	70	Si – C – Al - W
Backflush C	Tolu	5	25	C back – F1
Backflush Al	Tolu	5	25	Al back – F2

Total mL	**135 mL**
Total min	**18.5 min**

Clean-up for High Fat Content Matrices?

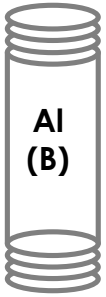
Sample clean-up consists of 2 main chemical steps:

1. **Fat digestion**, matrix degradation

Acidic, basic and neutral (ABN) Silica gel

Variable size depending on fat amount to be processed

2. **Fractionation** of planar (PCDD/Fs and co-PCBs) and non-planar (MO- and I-PCBs) compounds for spectrometric quantification



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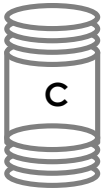
Polar and acid-base interactions with PCBs

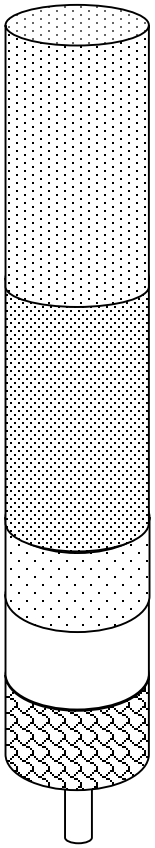


Carbon:
Geometric interactions with planar dioxins and coplanar PCBs

Sample Clean-up in our Routine Lab (up to 7 g)

1. **Manual column fat digestion**

Fat capacity up to 7 g fat

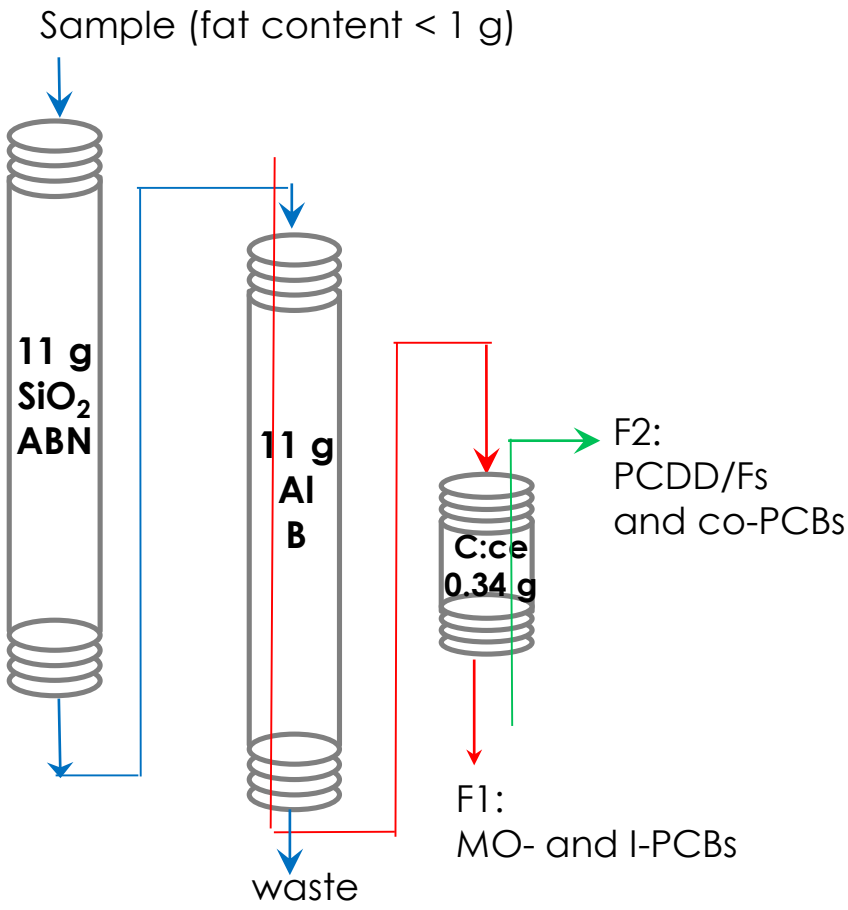


2. **Fractionation**

FMS PowerPrep®

- STD ABN Silica
- Al B 11g
- C PX-21 0.34 g

Hx
Hx:DCM
Tolu

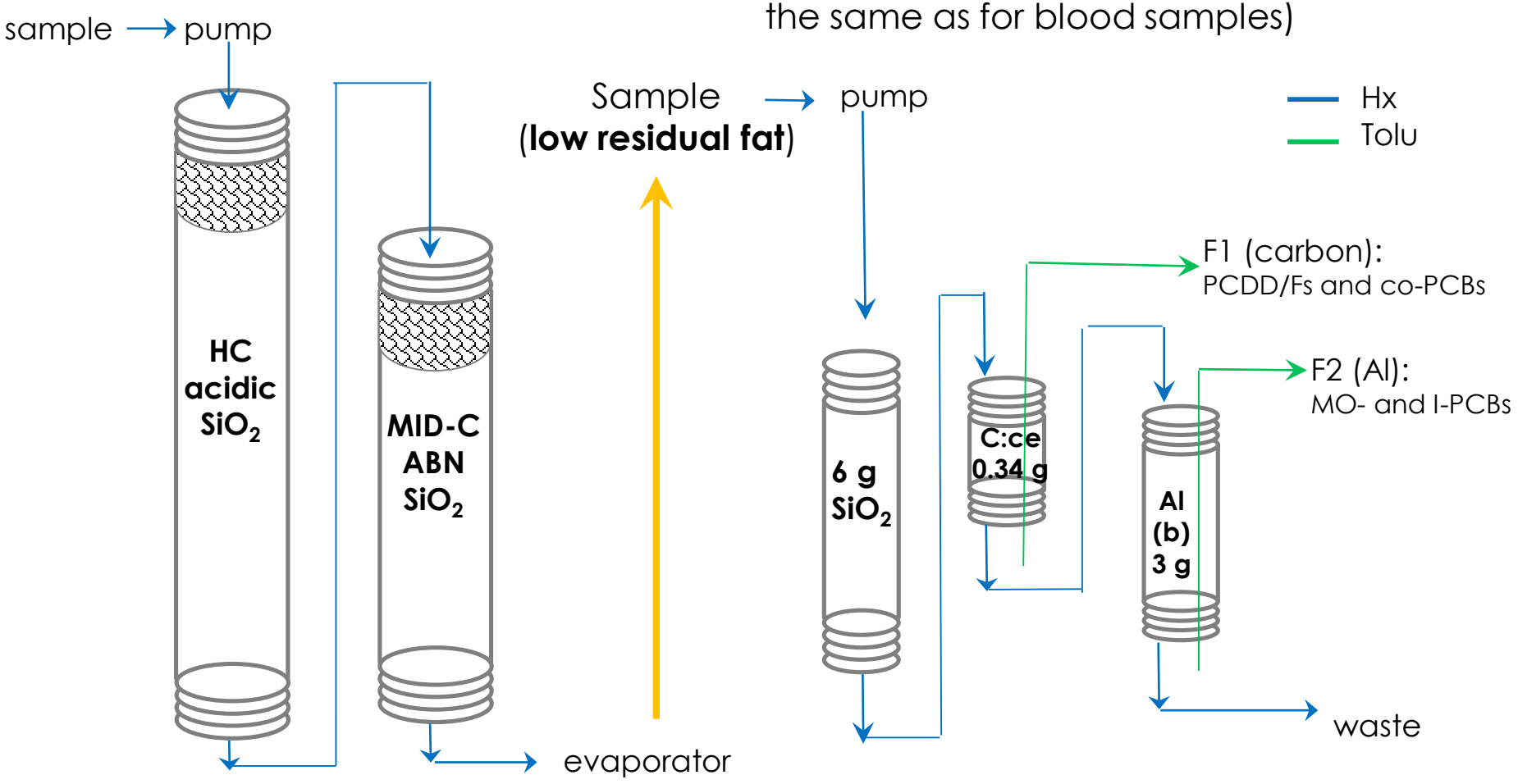


2-steps Automated clean-up for 7 g Fat

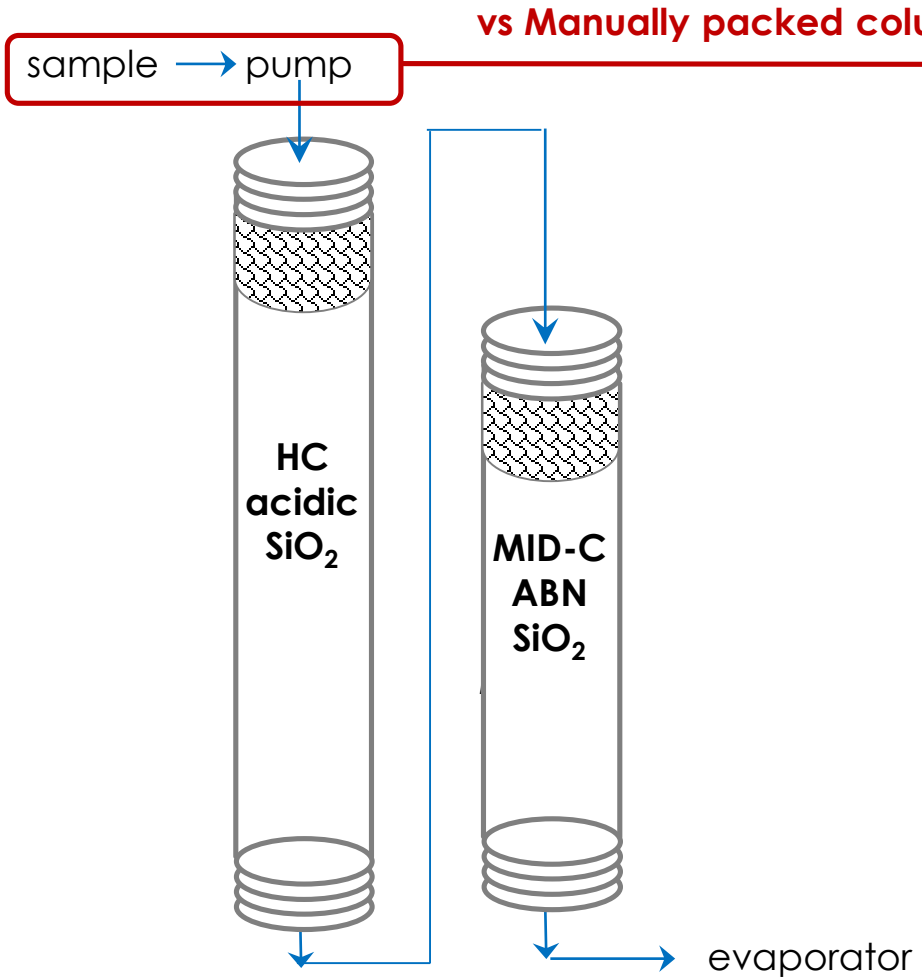
EconoPrep® was used to develop a **2 steps-automated method:**

1. **Automated fat digestion**

2. **Fractionation** with "MINI-column" fast, low solvent method
the same as for blood samples)



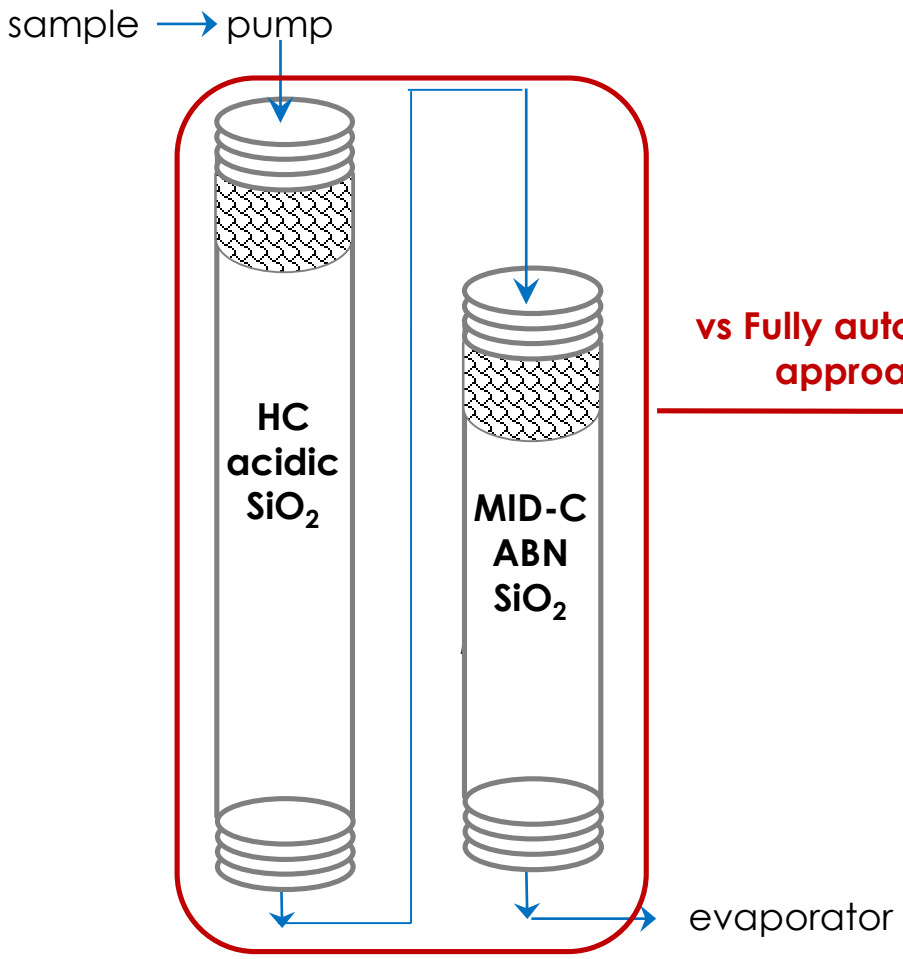
Advantages of the 2 Steps-automated approach



- No issue for high fat amount, viscous matrices, or matrices with non-soluble particles (feed, edible insects*).
- Faster because of the pump

* Food and Chemical Toxicology 100 (2017) *pp. 70-79*

Advantages of the 2 Steps-automated approach

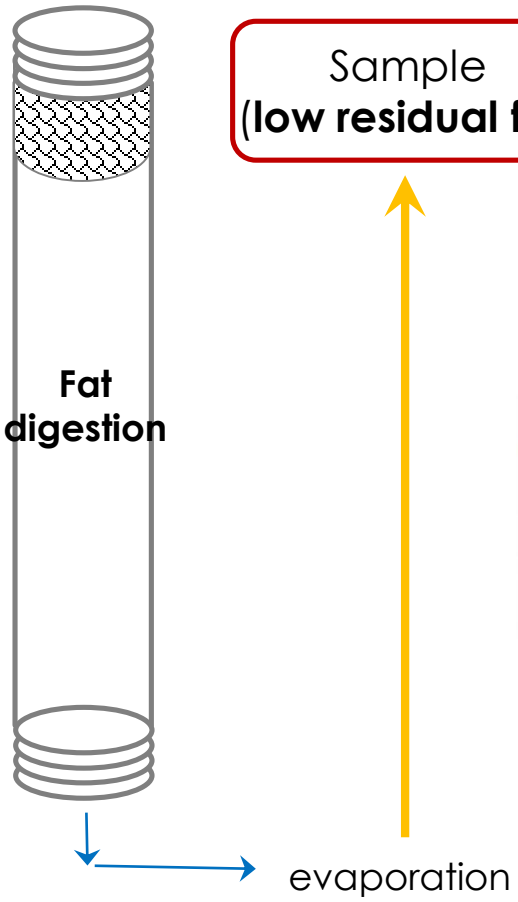


vs Fully automated approach

- Higher fat capacity (~7g).
- Further clean-up in the fractionation step
- Low **back-pressure** because of the direct collection of the sample (in the evaporator)
- Lower contamination risk

* Food and Chemical Toxicology 100 (2017) *pp. 70-79*

Advantages of the 2 Steps-automated approach



Fractionation with "MINI-column" method

- Clean-up procedure can be **standardized for all biological matrices**
- All biological matrices can be cleaned-up **simultaneously**
- More robust process, easier to **validate**
- Less risk of cross **contamination** and residual fat in final fractions

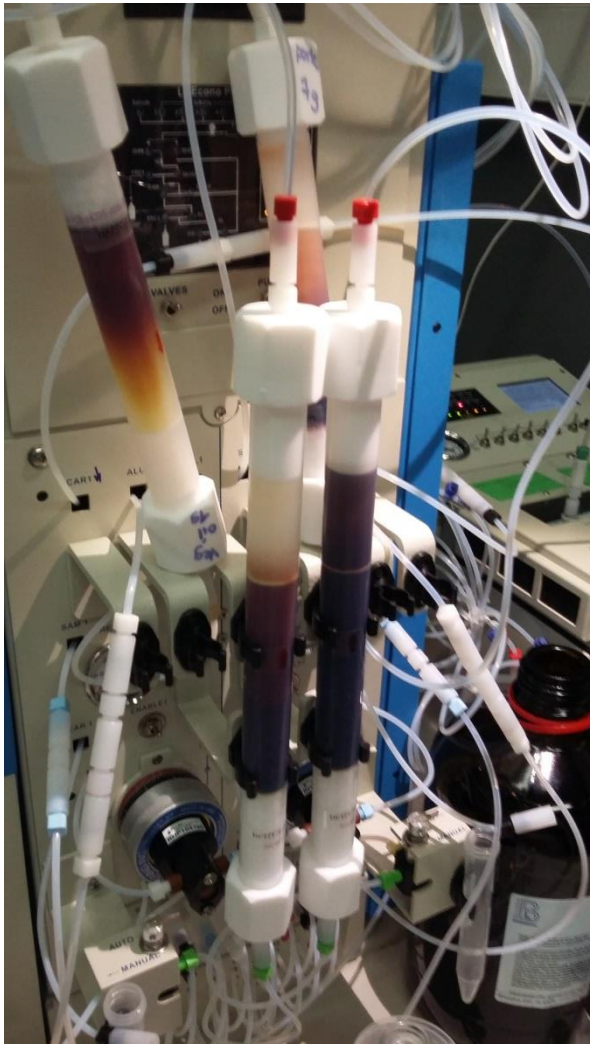
Features of the 2 Steps-Automated Approach

Description	Solvent	Flow mL/min	Volume mL	Path
Fat digestion, up to 7 g fat processed				
Condition Silica	Hx	10	60	Si – W
Sample loading	Hx	10	(50)	Sample – Si - Fr
Silica elution	Hx	10	180	Si - Fr
Fractionation				
Column condit.	Hx	10	20	Si – C - Al
Sample loading	Hx	5	(7)	Sample – Si – C- Al - W
Silica elution	Hx	5	40	Si – C – Al - W
Backflush C	Tolu	5	25	C back – F1
Backflush Al	Tolu	5	25	Al back – F2

Total mL	240 + 110 = **350* mL**
Total min	29 + 20 = **49* min**

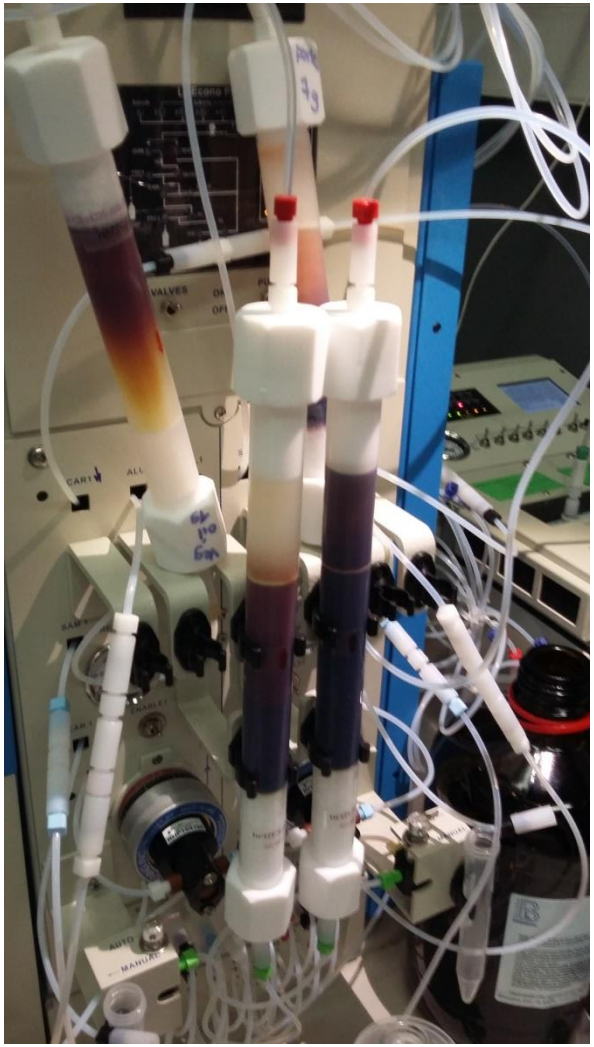
* Evaporation and sample loading time are NOT included

Real Samples: Fat Digestion



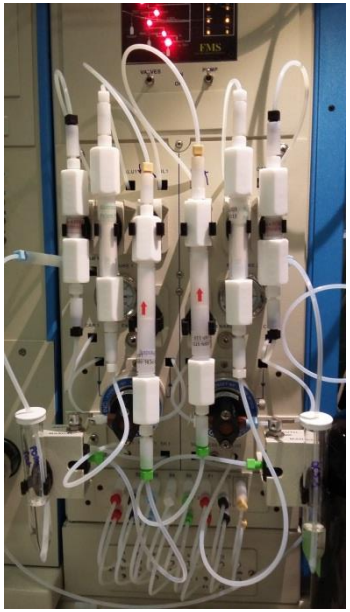
- 4 g of raw linseed vegetable oil (left)
- 7 g pork fat (right, QC in our laboratory)

Real Samples: Fat Digestion and Clean-up



- 4 g of raw linseed vegetable oil (left)
- 7 g pork fat (right, QC in our laboratory)

Pork fat, vegetable oil and serum **simultaneous** clean-up

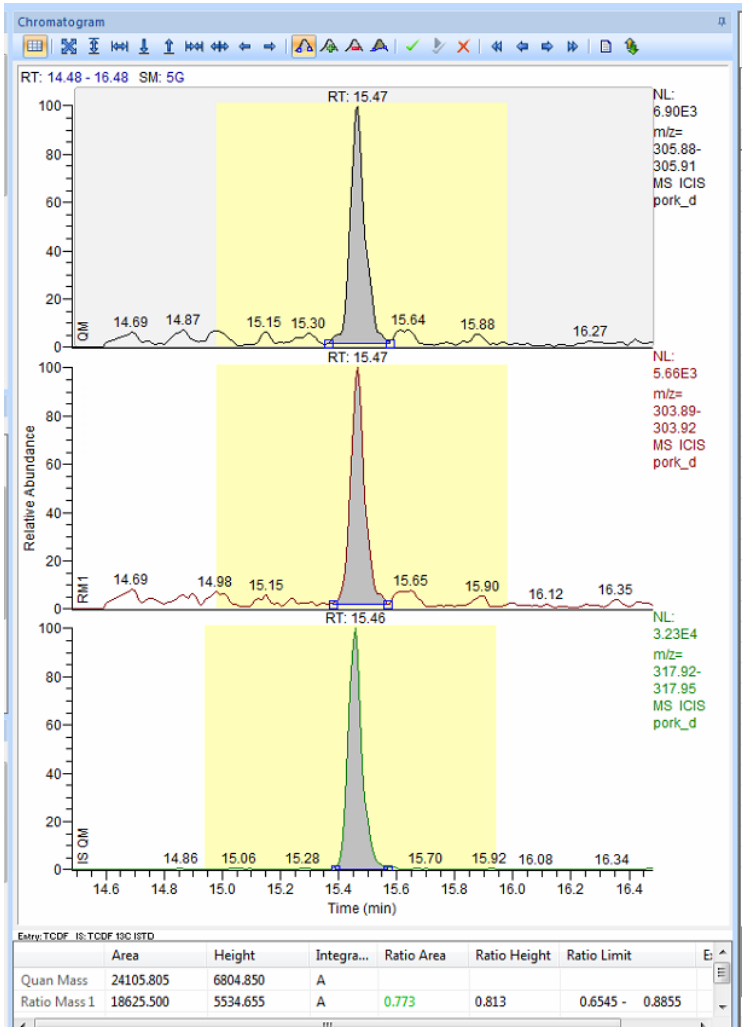


7g pork fat (left)
and serum (right)

4 g vegetable oil (left)
and serum (right)

Results for Pork Fat (7g)

- DFS Magnetic Sector equipped with 2 Trace 1300 GCs, each with SSL injector and **DualDataXL** module



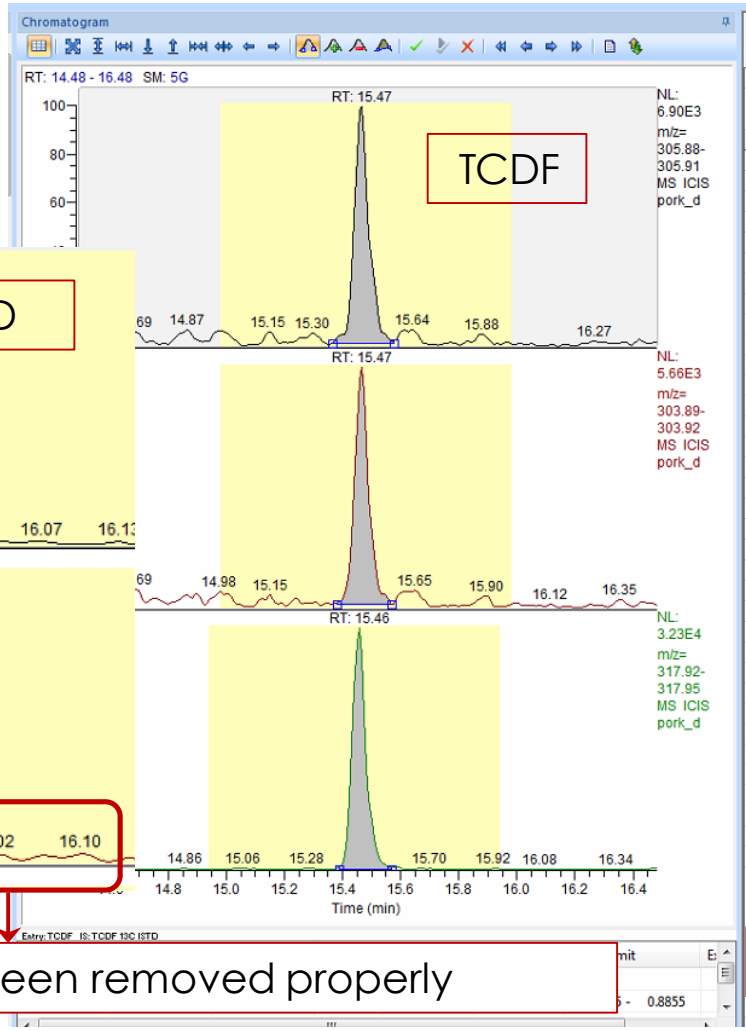
Entry Identifier: TCDF

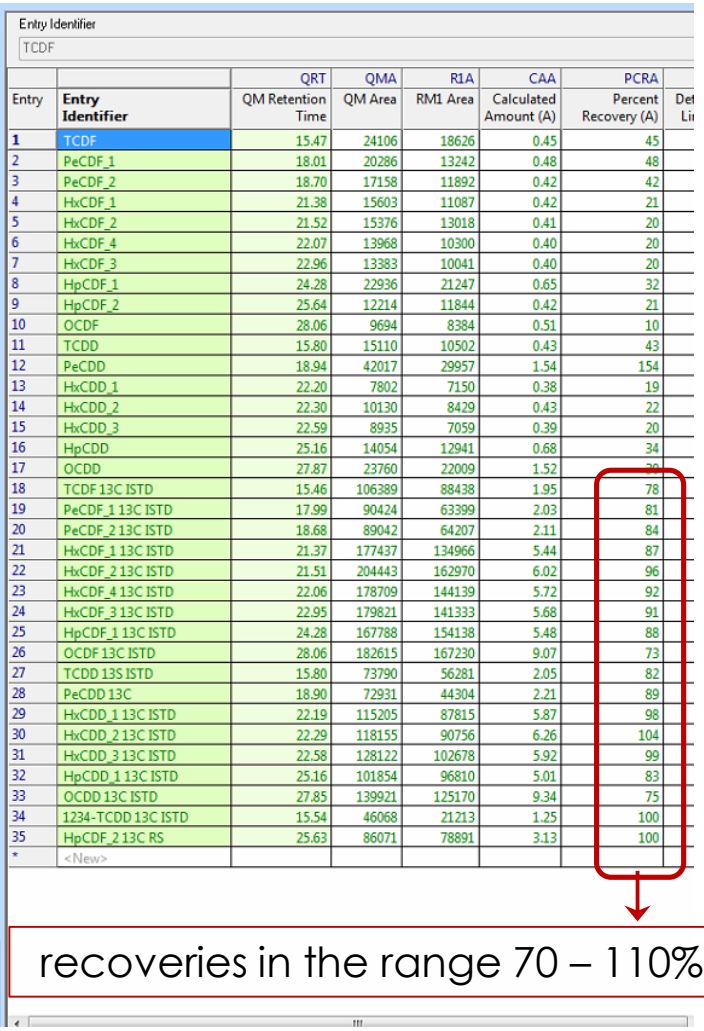
Entry	Entry Identifier	QRT QM Retention Time	QMA QM Area	R1A RM1 Area	CAA Calculated Amount (A)	PCRA Percent Recovery (A)
1	TCDF	15.47	24106	18626	0.45	45
2	PeCDF_1	18.01	20286	13242	0.48	48
3	PeCDF_2	18.70	17158	11892	0.42	42
4	HxCDF_1	21.38	15603	11087	0.42	21
5	HxCDF_2	21.52	15376	13018	0.41	20
6	HxCDF_4	22.07	13968	10300	0.40	20
7	HxCDF_3	22.96	13383	10041	0.40	20
8	HpCDF_1	24.28	22936	21247	0.65	32
9	HpCDF_2	25.64	12214	11844	0.42	21
10	OCDF	28.06	9694	8384	0.51	10
11	TCDD	15.80	15110	10502	0.43	43
12	PeCDD	18.94	42017	29957	1.54	154
13	HxCDD_1	22.20	7802	7150	0.38	19
14	HxCDD_2	22.30	10130	8429	0.43	22
15	HxCDD_3	22.59	8935	7059	0.39	20
16	HpCDD	25.16	14054	12941	0.68	34
17	OCDD	27.87	23760	22009	1.52	38
18	TCDF 13C ISTD	15.46	106389	88438	1.95	78
19	PeCDF_1 13C ISTD	17.99	90424	63399	2.03	81
20	PeCDF_2 13C ISTD	18.68	89042	64207	2.11	84
21	HxCDF_1 13C ISTD	21.37	177437	134966	5.44	87
22	HxCDF_2 13C ISTD	21.51	204443	162970	6.02	96
23	HxCDF_4 13C ISTD	22.06	178709	144139	5.72	92
24	HxCDF_3 13C ISTD	22.95	179821	141333	5.68	91
25	HpCDF_1 13C ISTD	24.28	167788	154138	5.48	88
26	OCDF 13C ISTD	28.06	182615	167230	9.07	73
27	TCDD 13S ISTD	15.80	73790	56281	2.05	82
28	PeCDD 13C	18.90	72931	44304	2.21	89
29	HxCDD_1 13C ISTD	22.19	115205	87815	5.87	98
30	HxCDD_2 13C ISTD	22.29	118155	90756	6.26	104
31	HxCDD_3 13C ISTD	22.58	128122	102678	5.92	99
32	HpCDD_1 13C ISTD	25.16	101854	96810	5.01	83
33	OCDD 13C ISTD	27.85	139921	125170	9.34	75
34	1234-TCDD 13C ISTD	15.54	46068	21213	1.25	100
35	HpCDF_2 13C RS	25.63	86071	78891	3.13	100
*	<New>					

recoveries in the range 70 – 110%

Results for Pork Fat (7g)

- DFS Magnetic Sector equipped with 2 Trace 1300 GCs, each with SSL injector and **DualDataXL** module





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4	HxCDF_1	21.38	15603	11087	0.42	21
5	HxCDF_2	21.52	15376	13018	0.41	20
6	HxCDF_4	22.07	13968	10300	0.40	20
7	HxCDF_3	22.96	13383	10041	0.40	20
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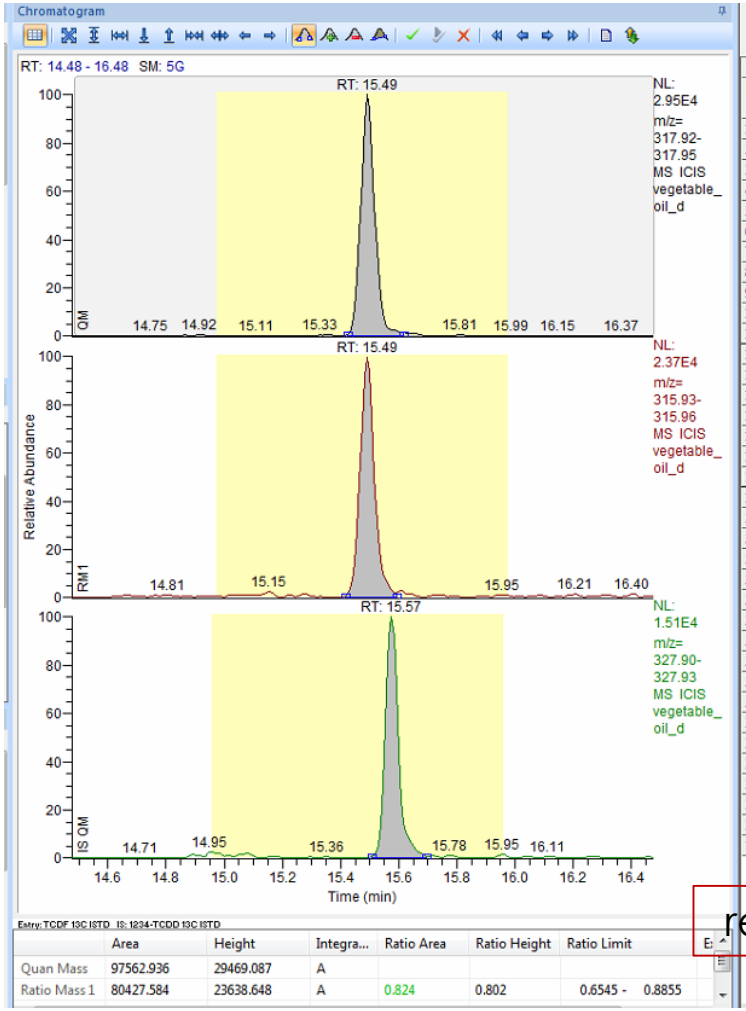
15	HxCDD_3	22.59	8935	7059	0.39	20
16	HpCDD	25.16	14054	12941	0.68	34
17	OCDD	27.87	23760	22009	1.52	78
18	TCDF 13C ISTD	15.46	106389	88438	1.95	81
19	PeCDF_1 13C ISTD	17.99	90424	63399	2.03	84
20	PeCDF_2 13C ISTD	18.68	89042	64207	2.11	87
21	HxCDF_1 13C ISTD	21.37	177437	134966	5.44	96
22	HxCDF_2 13C ISTD	21.51	204443	162970	6.02	92
23	HxCDF_4 13C ISTD	22.06	178709	144139	5.72	91
24	HxCDF_3 13C ISTD	22.95	179821	141333	5.68	88
25	HpCDF_1 13C ISTD	24.28	167788	154138	5.48	73
26	OCDF 13C ISTD	28.06	182615	167230	9.07	82
27	TCDD 13S ISTD	15.80	73790	56281	2.05	89
28	PeCDD 13C	18.90	72931	44304	2.21	98
29	HxCDD_1 13C ISTD	22.19	115205	87815	5.87	104
30	HxCDD_2 13C ISTD	22.29	118155	90756	6.26	99
31	HxCDD_3 13C ISTD	22.58	128122	102678	5.92	83
32	HpCDD_1 13C ISTD	25.16	101854	96810	5.01	75
33	OCDD 13C ISTD	27.85	139921	125170	9.34	100
34	1234-TCDD 13C ISTD	15.54	46068	21213	1.25	100
35	HpCDF_2 13C RS	25.63	86071	78891	3.13	
*	<New>					

Matrix effect has been removed properly

recoveries in the range 70 – 110%

Results for Vegetable Oil (4g)

- DFS Magnetic Sector equipped with 2 Trace 1300 GCs, each with SSL injector and **DualDataXL** module



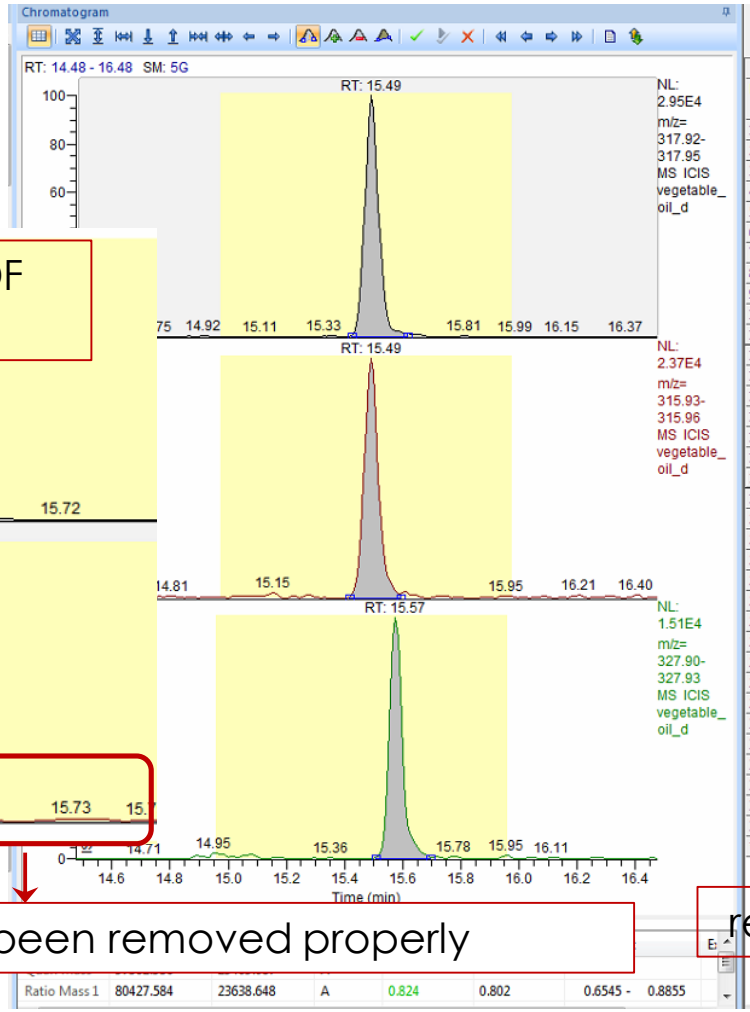
	Area	Height	Integra...	Ratio Area	Ratio Height	Ratio Limit	E
Quan Mass	97562.936	29469.087	A				
Ratio Mass 1	80427.584	23638.648	A	0.824	0.802	0.6545 - 0.8855	

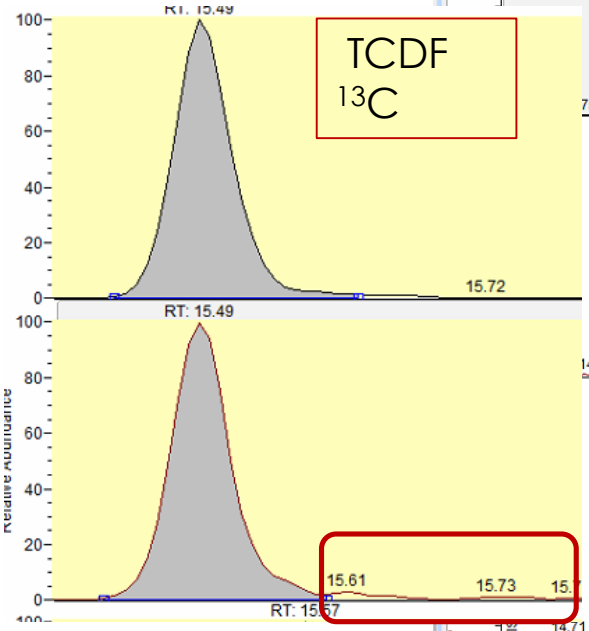
Entry	Entry Identifier	QRT QM Retention Time	QMA QM Area	R1A RM1 Area	CAA Calculated Amount (A)	PCRA Percent Recovery (A)
1	TCDF	15.50	1133	964	0.02	2
2	PeCDF_1	18.10	322	692	n.d. < 0.02	2
3	PeCDF_2	18.72	269	139	n.d. < 0.01	1
4	HxCDF_1	21.43	983	1092	n.d. < 0.03	2
5	HxCDF_2	21.56	78	117	n.d. < 0.00	0
6	HxCDF_4	22.13	329	105	n.d. < 0.01	0
7	HxCDF_3	23.17	99	55	n.d. < 0.00	0
8	HpCDF_1	24.29	8361	9321	0.29	14
9	HpCDF_2	25.66	1125	360	n.d. < 0.03	1
10	OCDF	28.09	3743	2811	0.19	4
11	TCDD	15.80	209	71	n.d. < 0.00	0
12	PeCDD	18.96	2293	267	n.d. < 0.06	6
13	HxCDD_1	22.22	35	222	n.d. < 0.01	0
14	HxCDD_2	22.32	364	352	n.d. < 0.02	1
15	HxCDD_3	22.58	42	110	n.d. < 0.00	0
16	HpCDD	25.19	2747	2725	0.15	7
17	OCDD	27.88	7946	4671	n.d. < 0.43	9
18	TCDF 13C ISTD	15.49	97563	80428	1.78	71
19	PeCDF_1 13C ISTD	18.02	84506	48966	1.77	71
20	PeCDF_2 13C ISTD	18.72	77239	50728	1.77	71
21	HxCDF_1 13C ISTD	21.41	165931	129818	4.86	78
22	HxCDF_2 13C ISTD	21.53	189846	149874	5.26	84
23	HxCDF_4 13C ISTD	22.09	164435	132086	4.96	79
24	HxCDF_3 13C ISTD	22.97	168264	125896	4.92	79
25	HpCDF_1 13C ISTD	24.28	150367	140062	4.66	75
26	OCDF 13C ISTD	28.07	184459	165441	8.57	69
27	TCDD 13S ISTD	15.83	65899	58001	1.96	78
28	PeCDD 13C	18.93	61976	40782	1.94	78
29	HxCDD_1 13C ISTD	22.22	103029	82170	5.05	84
30	HxCDD_2 13C ISTD	22.32	106199	87659	5.48	91
31	HxCDD_3 13C ISTD	22.61	121622	96306	5.28	88
32	HpCDD_1 13C ISTD	25.19	97570	86899	4.39	73
33	OCDD 13C ISTD	27.87	134387	120603	8.49	68
34	1234-TCDD 13C ISTD	15.57	47068	20055	1.25	100
35	HpCDF_2 13C RS	25.66	89387	85328	3.13	100
*	<New>					

recoveries in the range 70 – 110%

Results for Vegetable Oil (4g)

- DFS Magnetic Sector equipped with 2 Trace 1300 GCs, each with SSL injector and **DualDataXL** module





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7	HxCDF_3	23.17	99	55	n.d. < 0.00	0
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14	HxCDD_2	22.32	364	352	n.d. < 0.02	1
15	HxCDD_3	22.58	42	110	n.d. < 0.00	0
16	HpCDD	25.19	2747	2725	0.15	7
17	OCDD	27.88	7946	4671	n.d. < 0.43	9
18	TCDF 13C ISTD	15.49	97563	80428	1.78	71
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25	HpCDF_1 13C ISTD	24.28	150367	140062	4.66	75
26	OCDF 13C ISTD	28.07	184459	165441	8.57	69
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35	HpCDF_2 13C RS	25.66	89387	85328	3.13	100
*	<New>					

Matrix effect has been removed properly

recoveries in the range 70 – 110%

Take Home Message #1

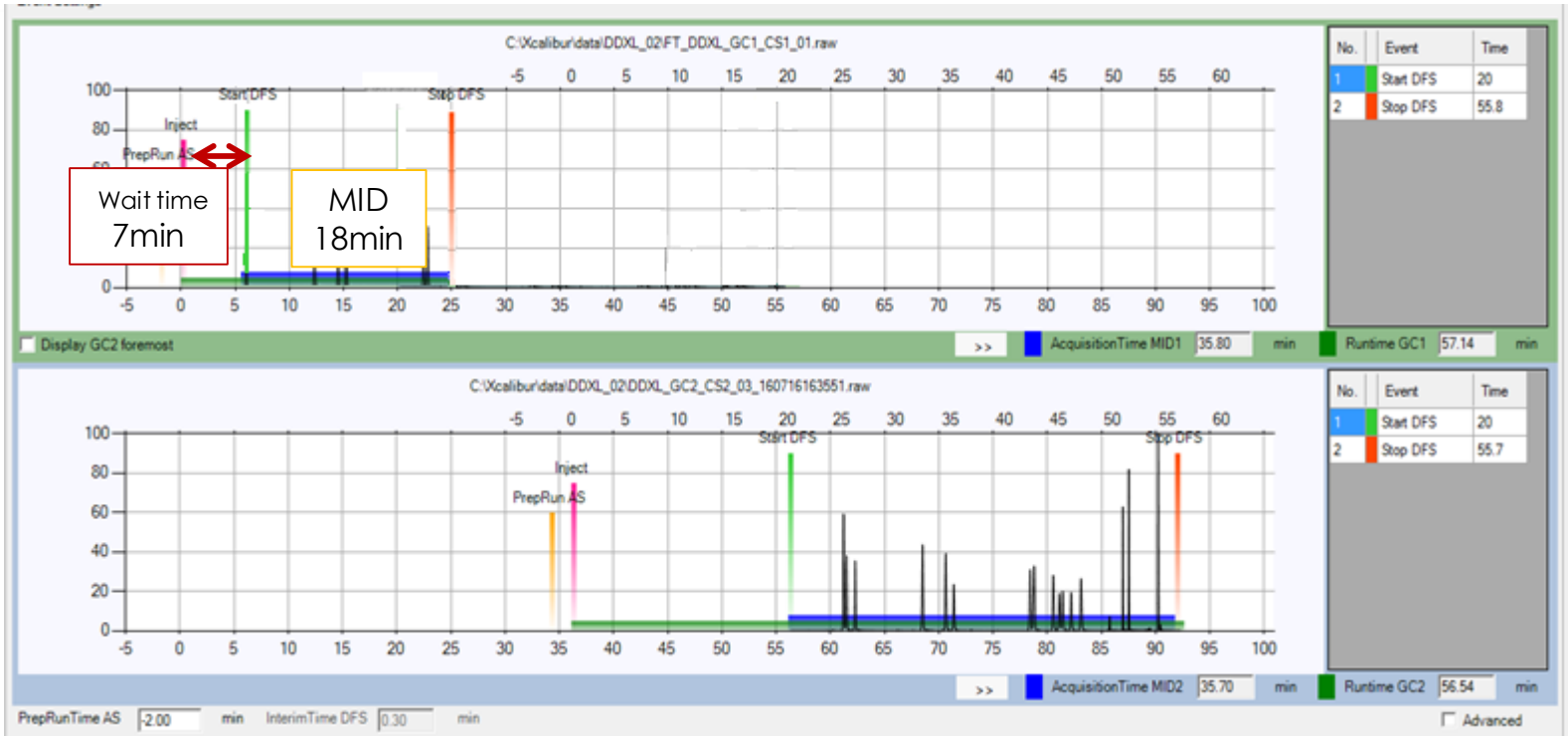
- Low(er) solvent consumption
- 110 mL / 20 min for low fat samples
- 350 mL / 49 min for high fat samples
- DCM-free sample preparation approach
- Universal mini-column set-up for all samples
- High quality extract standards maintained

II. GC-IDHRMS

Features of Our Routine Method: MO- and I-PCBs

Column: **HT-8** (**25 m** × 0.22 mm ID × 0.25 μm film thickness, SGE)

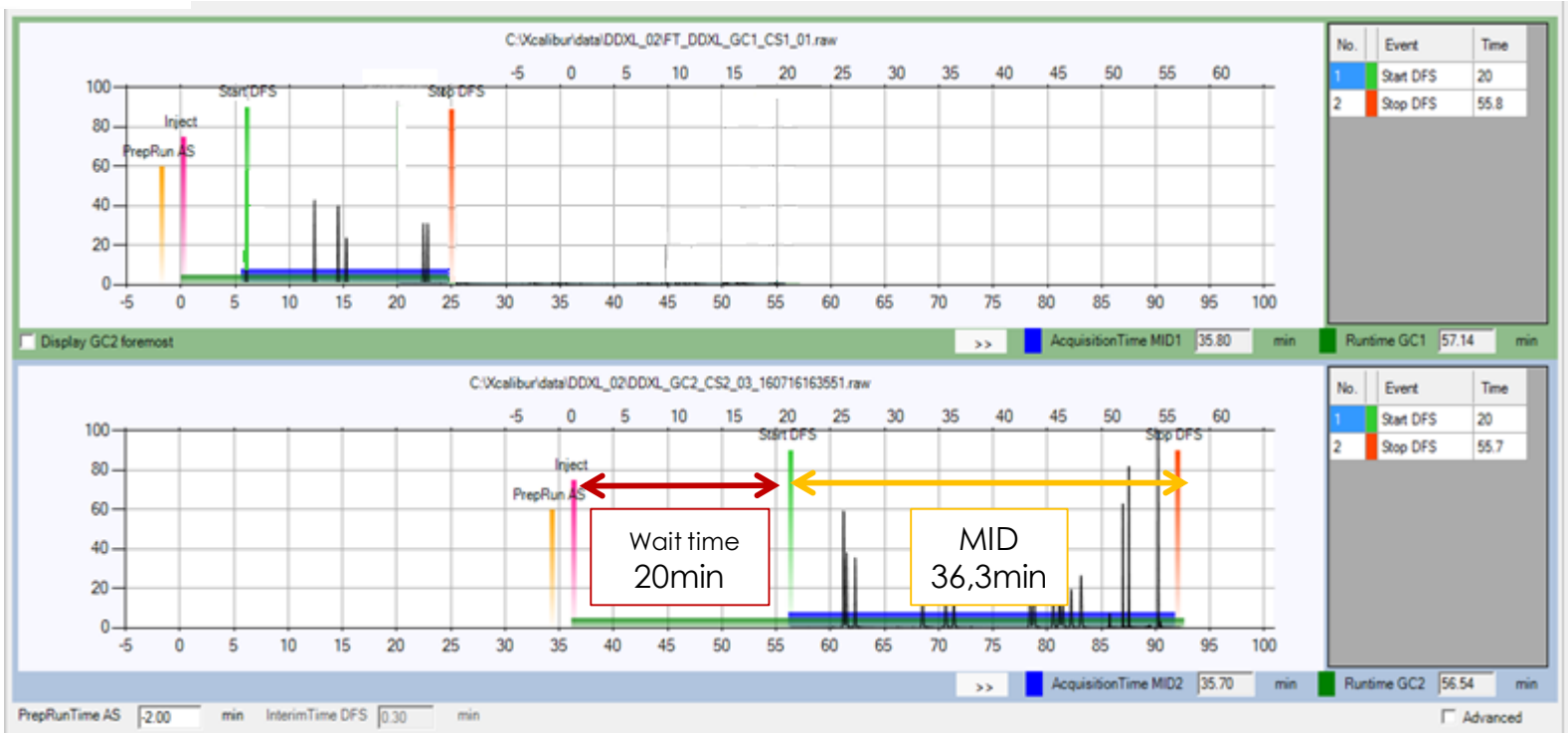
- Total Run Time: **25 min**
  Wait Time: 7 min
  Measuring Time: 18 min



Features of our Routine Method: PCDD/Fs and co-PCBs

Column: **DB-5ms** ultra inert (**60 m** x 0.25 mm x 0.25 µm, Agilent)

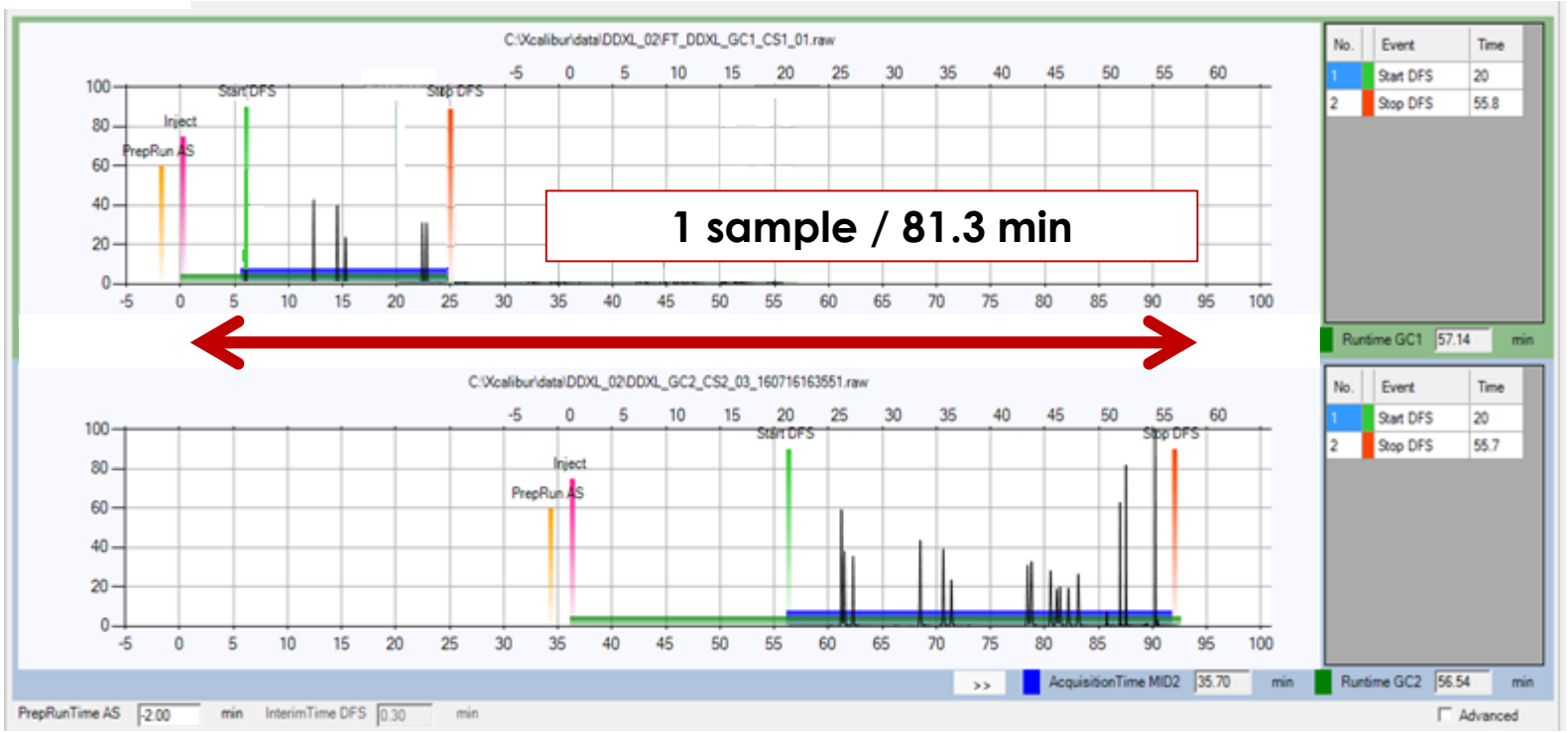
- Total Run Time: **56.3 min**
  Wait Time: 20.0 min
  Measuring Time: 36.3 min



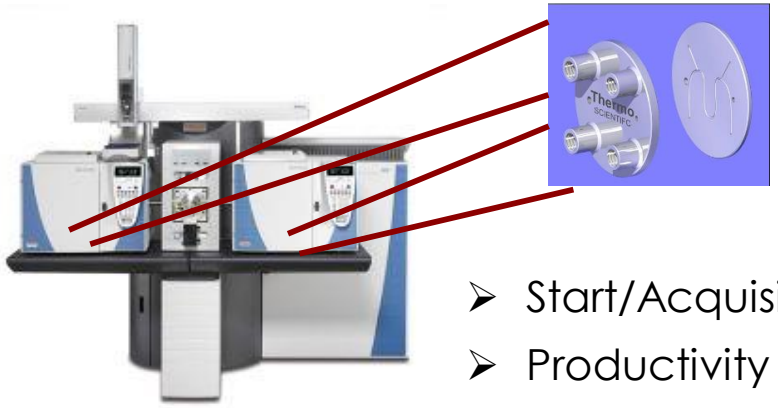
Features of our Routine Method: PCDD/Fs and all PCBs

1 complete sample, PCDD/Fs and co-, MO- and I-PCBs

- Total Run Time (56.3 + 25 min) = **81.3 min**



DualData XL Productivity Increase



DFS Magnetic Sector equipped with 2 Trace 1300 GCs, each with SSL injector and DualDataXL module

- Start/Acquisition time are synchronized so that **wait time** is saved
- Productivity increase depends on the methods (**30%** in our case)

III. ‘Fast’ GC-IDHRMS

Fast GC Alternative Approach

DFS Magnetic Sector, 2 Trace 1300 GCs, each with SSL injector and shorter columns, smaller ID and film thickness

- **NO DualDataXL** module but it might be used (better peak shape without)

PCDD/Fs and co-PCB fraction

Column: Rtx-5 (20 m x 0.18 mm x 0.20 µm, Restek)

Flow 1 mL/min

Injection volume 0.70 mL

- Total Run Time: **17 min**

70% time saving

	Rate (°C/min)	Temp (°C)	Hold time (min)
Initial		120	1.3
1	60	225	2.8
2	20	232	1.6
3	30	245	0.8
4	2.5	253	0
5	20	283	2.5
6	80	310	0.5

MO- and I-PCB fraction

Column: HT8 (10 m x 0.10 mm x 0.10 µm, SGE)

Flow 0.45 mL/min

Injection volume 0.4 mL

- Total Run Time: **11.5 min**

54% time saving

	Rate (°C/min)	Temp (°C)	Hold time (min)
Initial		60	0.35
1	45	200	0
2	5	215	0
3	7	235	0
4	20	255	0
5	40	300	0

Fast GC – Ms Parameters

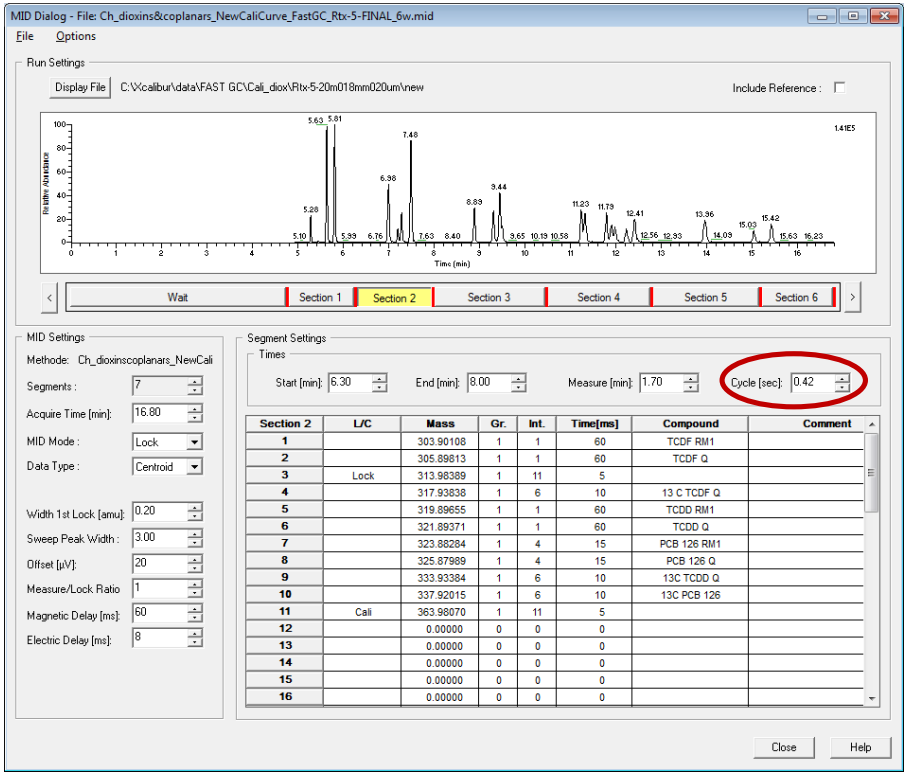
- **High acquisition frequency** to have min 10 scans/chromatographic peak

PCDD/Fs and co-PCBs - Tetra-congeners window

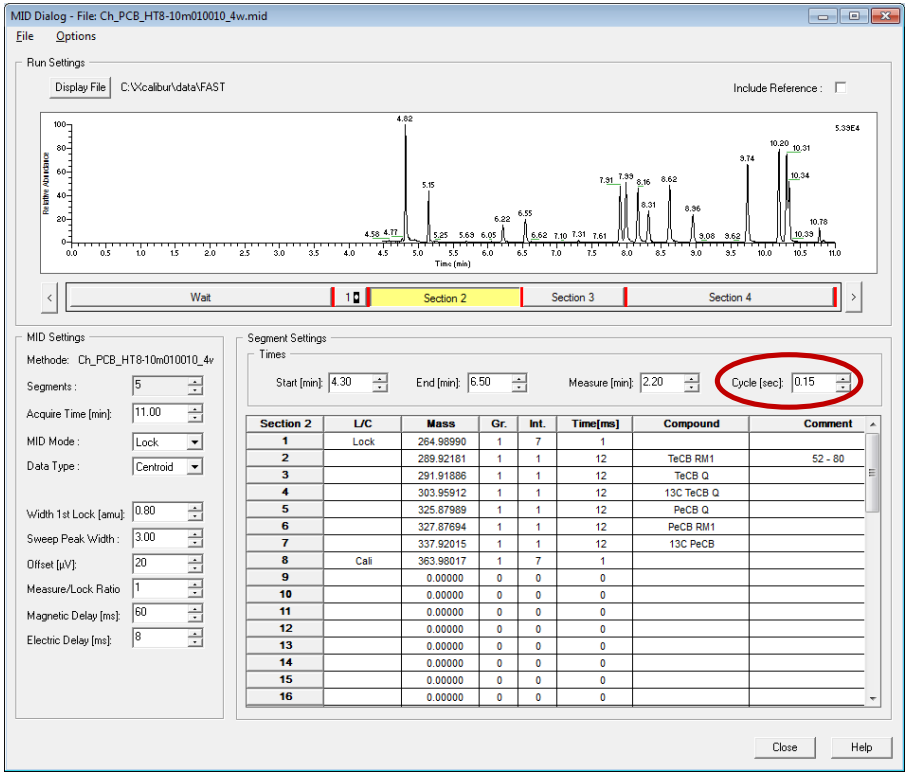
- Cycle = 0.42 sec
- TCDD/TCDF (Quant/Qual) dwell time = 60 msec
- PCB 126 (Quant/Qual) dwell time = 15 msec*
- ^{13}C labelled ISTDs (Quant)dwell time = 10 msec**

MO- and I-PCBs - Tetra-congeners window

- Cycle = 0.15 sec
- Dwell time (natives and labelled) = 12 msec



Section 2	L/C	Mass	Gr.	Int.	Time[ms]	Compound	Comment
1		303.90108	1	1	60	TCDF RM1	
2		305.89813	1	1	60	TCDF Q	
3	Lock	313.98389	1	11	5		
4		317.93838	1	6	10	13 C TCDF Q	
5		319.89655	1	1	60	TCDD RM1	
6		321.89371	1	1	60	TCDD Q	
7		323.88284	1	4	15	PCB 126 RM1	
8		325.87989	1	4	15	PCB 126 Q	
9		333.93384	1	6	10	13C TCDD Q	
10		337.92015	1	6	10	13C PCB 126	
11	Cali	363.98070	1	11	5		
12		0.00000	0	0	0		
13		0.00000	0	0	0		
14		0.00000	0	0	0		
15		0.00000	0	0	0		
16		0.00000	0	0	0		



Section 2	L/C	Mass	Gr.	Int.	Time[ms]	Compound	Comment
1	Lock	264.98990	1	7	1		
2		289.92181	1	1	12	TeCB RM1	52 - 80
3		291.91886	1	1	12	TeCB Q	
4		303.95912	1	1	12	13C TeCB Q	
5		325.87989	1	1	12	PeCB Q	
6		327.87694	1	1	12	PeCB RM1	
7		337.92015	1	1	12	13C PeCB	
8	Cali	363.98017	1	7	1		
9		0.00000	0	0	0		
10		0.00000	0	0	0		
11		0.00000	0	0	0		
12		0.00000	0	0	0		
13		0.00000	0	0	0		
14		0.00000	0	0	0		
15		0.00000	0	0	0		
16		0.00000	0	0	0		

* Concentration 10 times higher than TCDD in our lowest cali point

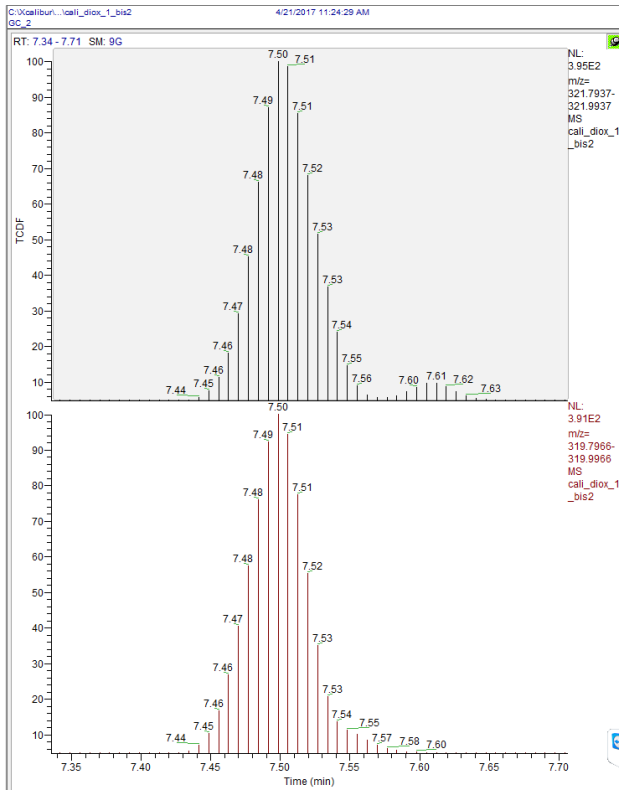
** No Qual ion acquired, concentration 50 times higher than TCDD in our lowest cali point

Fast GC – MS Parameters

- **High acquisition frequency** to have min 10 scans/chromatographic peak

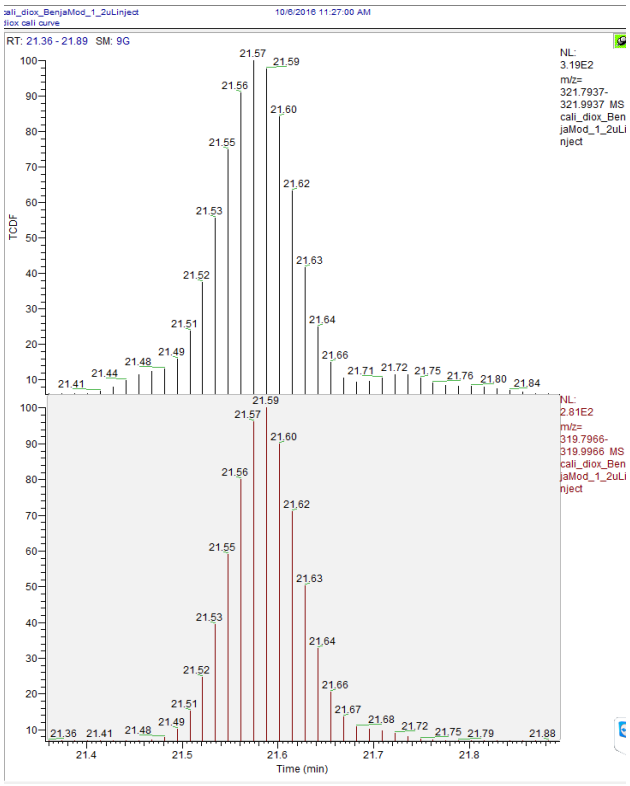
FAST GC - TCDD

- Cycle = 0.42 sec
- Dwell time = 60 msec
- Peak width (base) = 7 sec



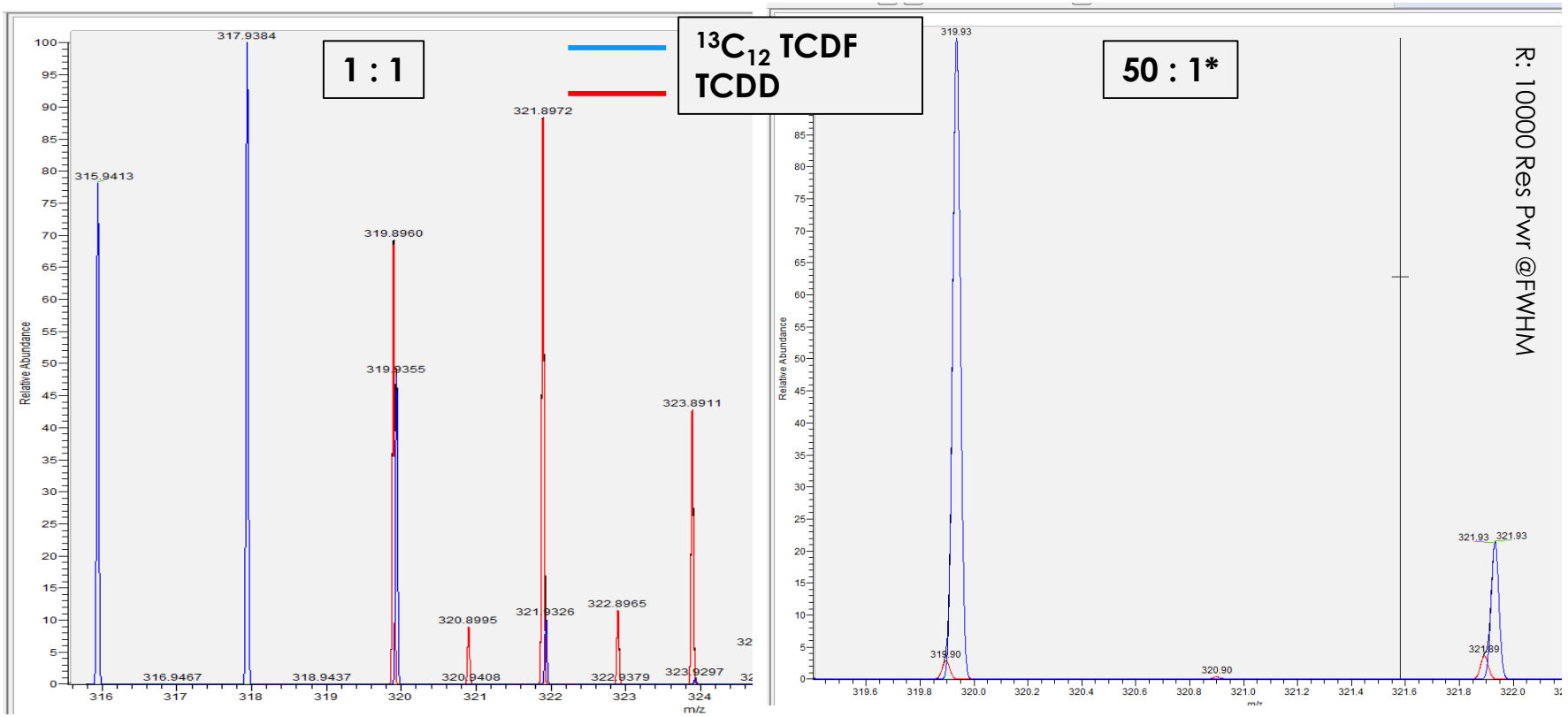
REGULAR GC - TCDD

- Cycle = 0.80 sec
- Dwell time = 75 msec
- Peak width (base) = 14 sec



Fast GC – Target for Chromatographic Resolution

- **PCDD/Fs and co-PCB fraction possible interferences:**
  1. $^{13}C_{12}$ Furans labelled ISTD & native Dioxins



Simulated mass spectra at R: 10000 Res Pwr @FWHM

* ratio $^{13}C_{12}$ TCDF : TCDD in our first calibration point

Fast GC – Target for Chromatographic Resolution

- **PCDD/Fs and co-PCB fraction possible interferences:**
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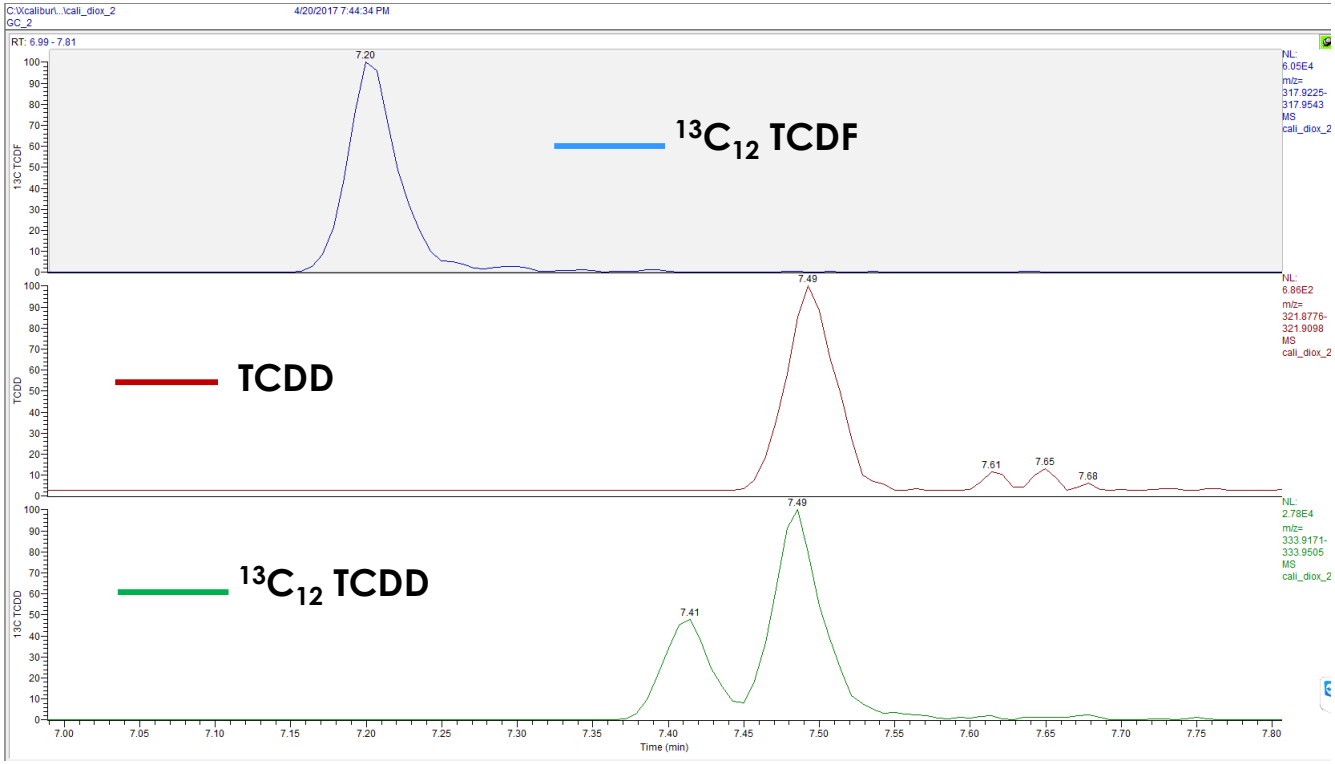
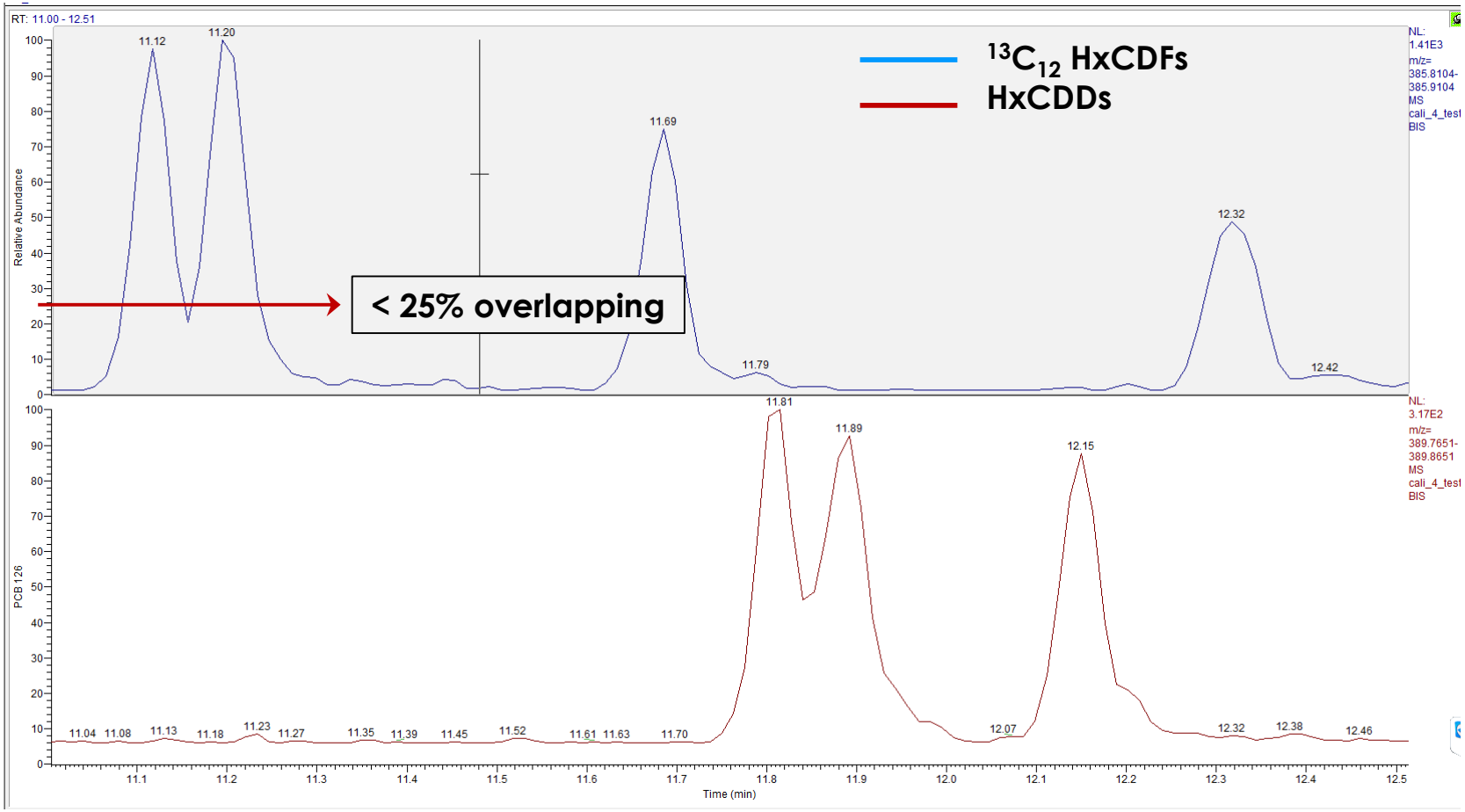
Chromatograms



* ratio $^{13}C_{12}$ TCDF : TCDD in our first calibration point

Fast GC – Target for Chromatographic Resolution

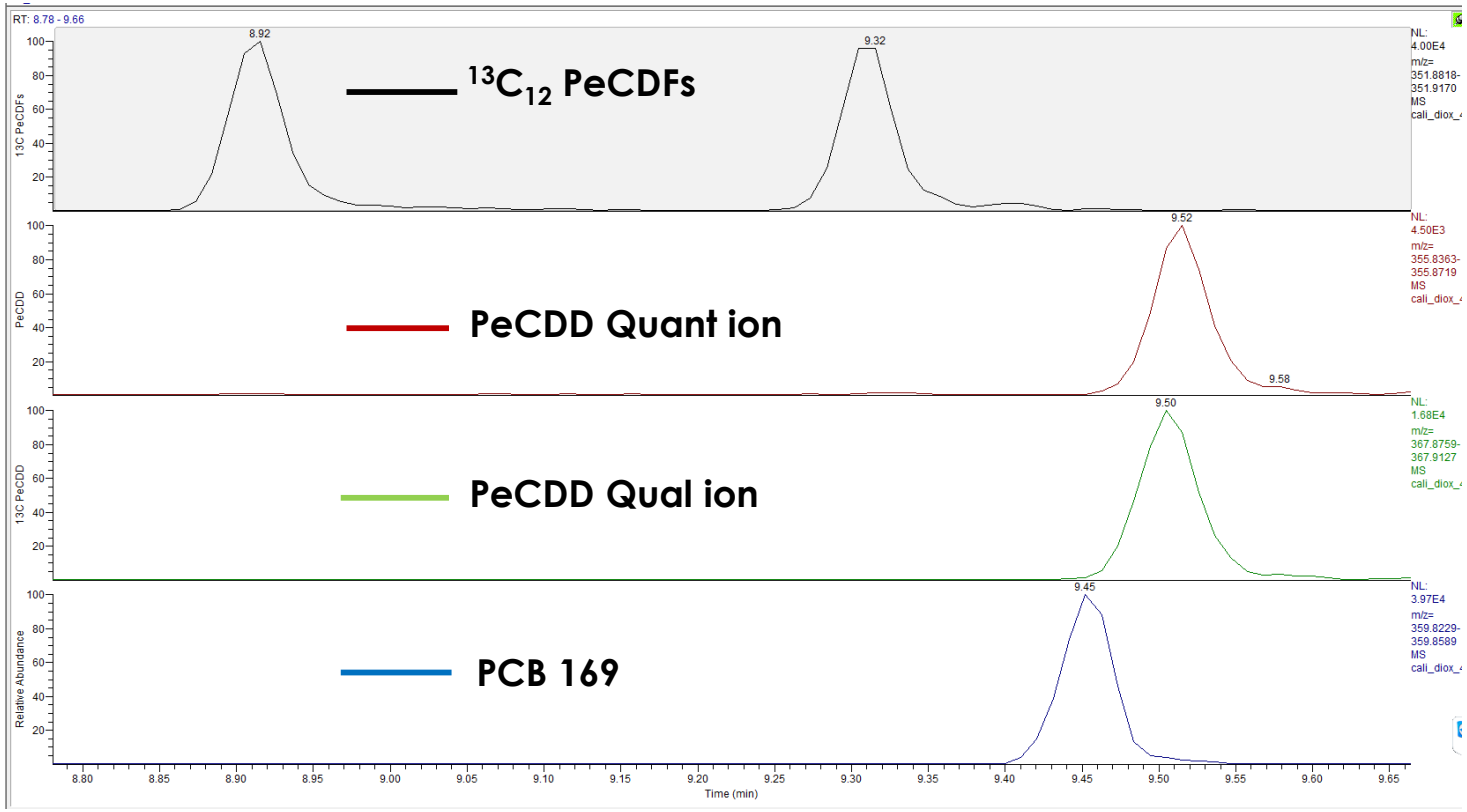
- **PCDD/Fs and co-PCB fraction possible interferences:**
  1. $^{13}C_{12}$ Furans labelled ISTD & native Dioxins
  2. < 25% overlapping between Hexa Furans*



* Commission Regulation (EU) No 589/2014

Fast GC – Target for Chromatographic Resolution

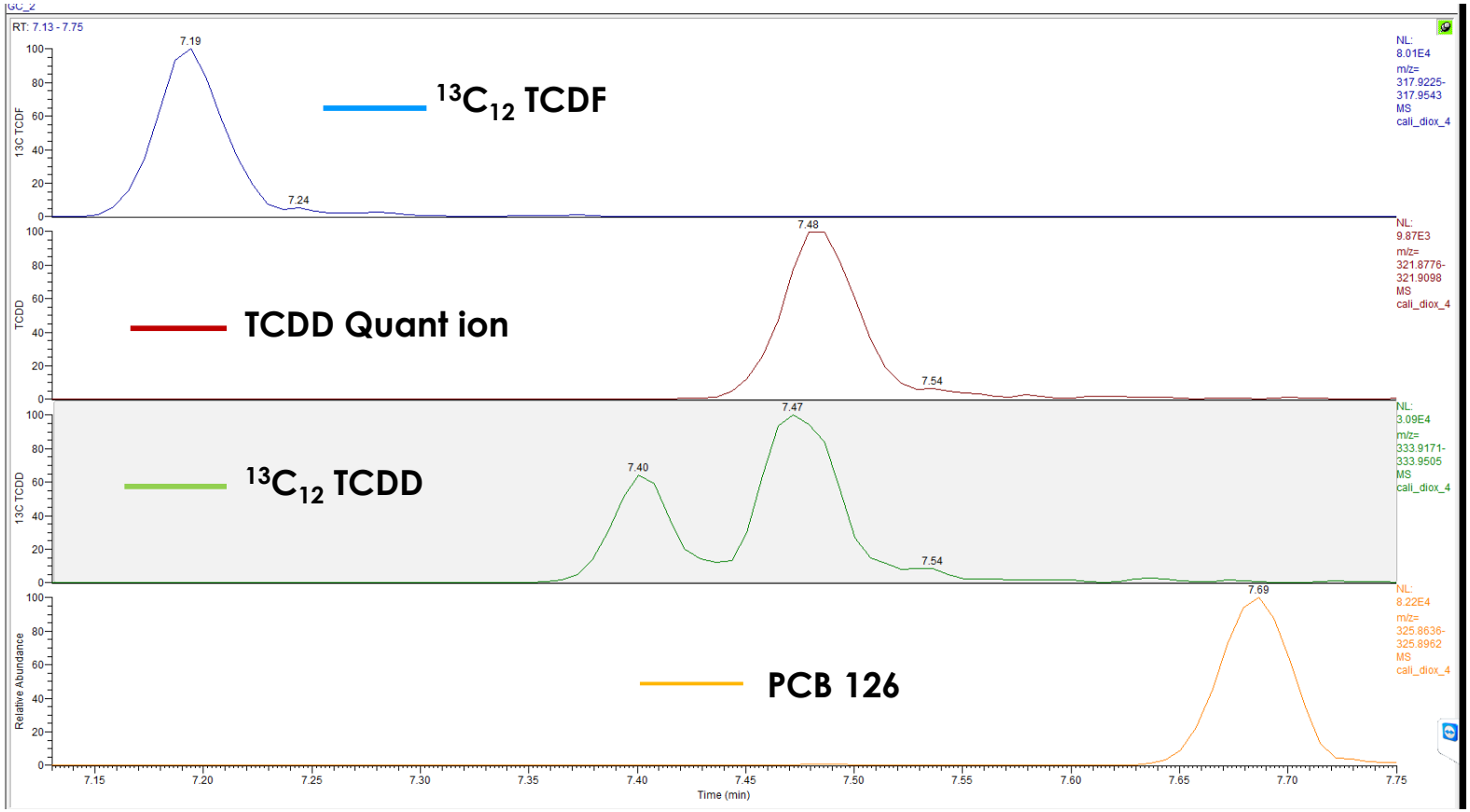
- **PCDD/Fs and co-PCB fraction possible interferences:**
  1. $^{13}C_{12}$ Furans labelled ISTD & native Dioxins
  2. < 25% overlapping between Hexa Furans*
  3. PCDD/Fs and co-PCBs



* Commission Regulation (EU) No 589/2014

Fast GC – Target for Chromatographic Resolution

- **PCDD/Fs and co-PCB fraction possible interferences:**
  1. $^{13}C_{12}$ Furans labelled ISTD & native Dioxins
  2. 25 % overlapping between Hexa Furans (point 6.3 REGULATION (EU) No 589/2014)
  3. PCDD/Fs and co-PCBs

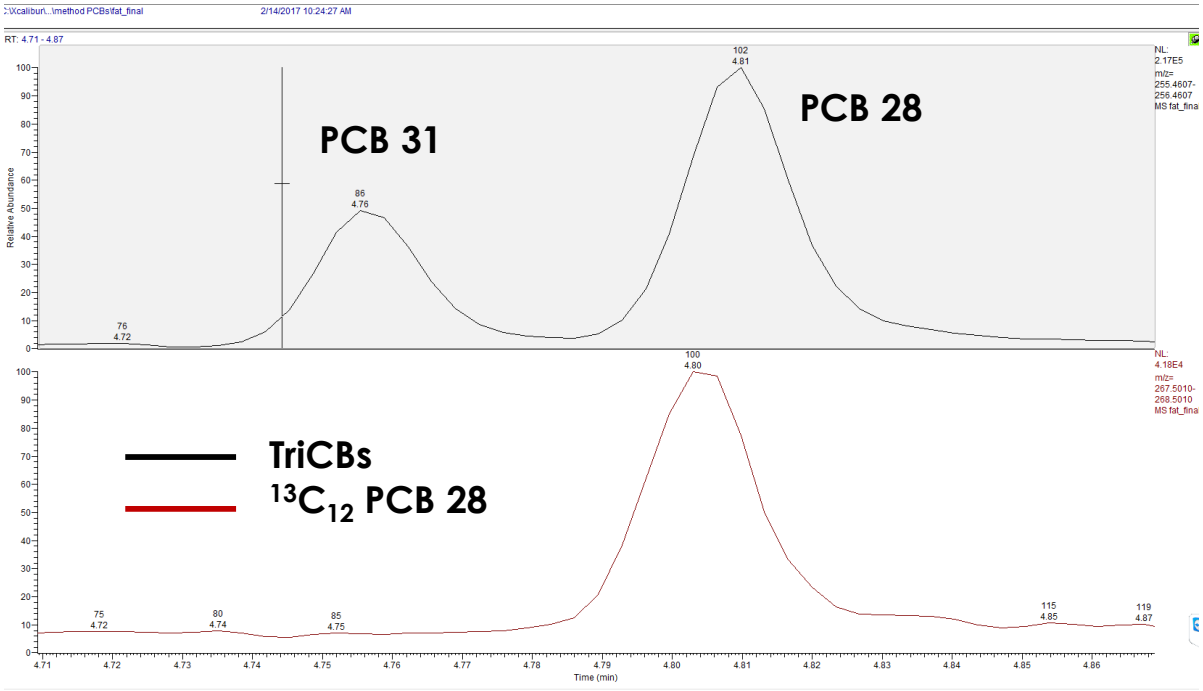


* ratio $^{13}C_{12}$ TCDF : TCDD in our first calibration point

Fast GC – Target for Chromatographic Resolution

- **MO- and I-PCBs fraction possible interferences*:**

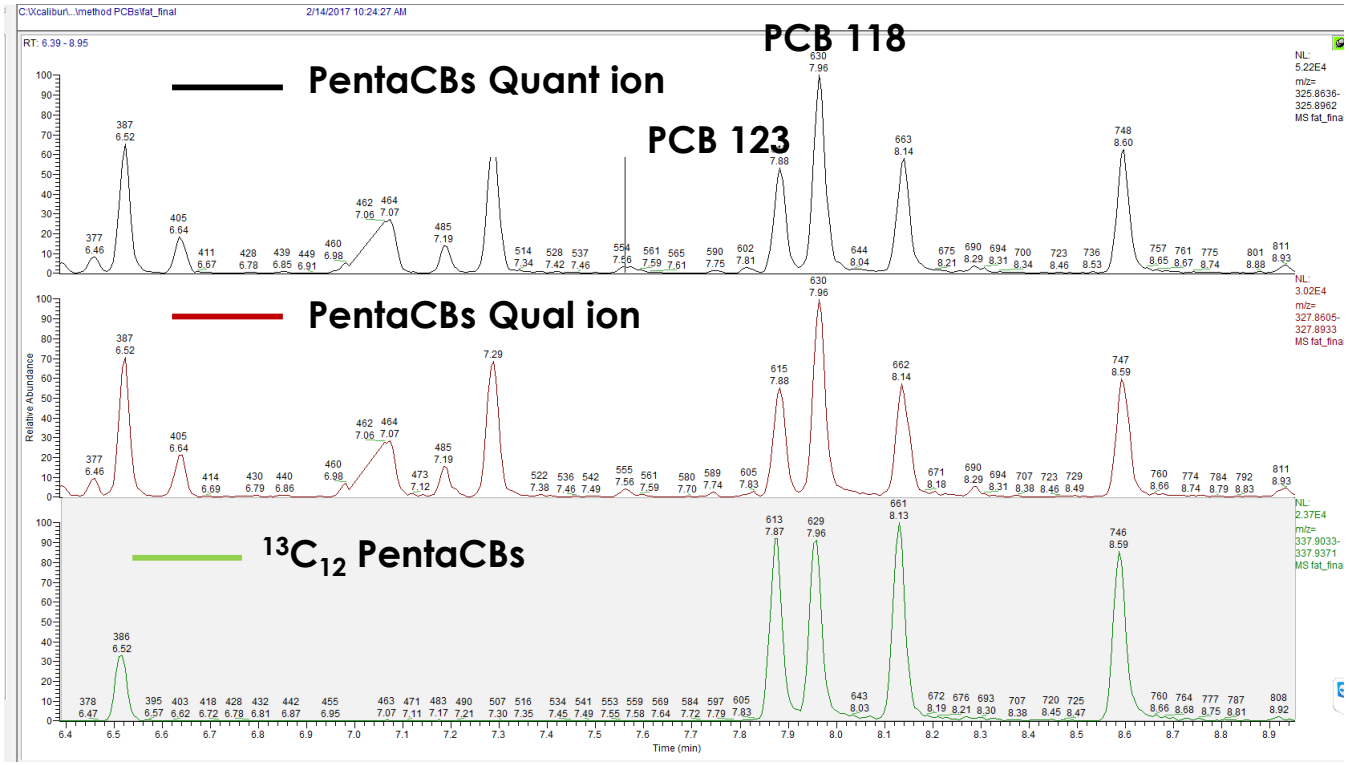
  1. PCB 31 and 28



* Real sample of butter, processed in our routine lab

Fast GC – Target for Chromatographic Resolution

- **MO- and I-PCBs fraction possible interferences*:**
  1. PCB 31 and 28
  2. PCB 123 and 118



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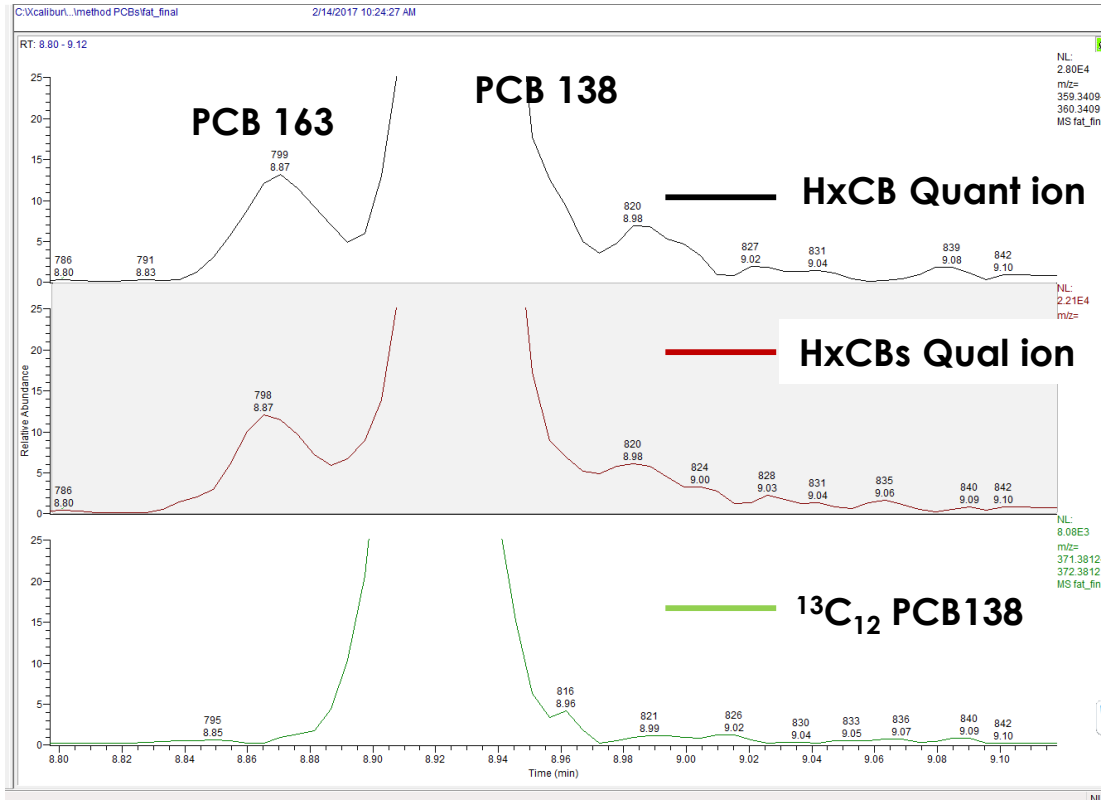
- **MO- and I-PCBs fraction possible interferences*:**
  1. PCB 31 and 28
  2. PCB 123 and 118
  3. PCB 163 and 138, PCB 156 and 157



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- **MO- and I-PCBs fraction possible interferences*:**
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  2. PCB 123 and 118
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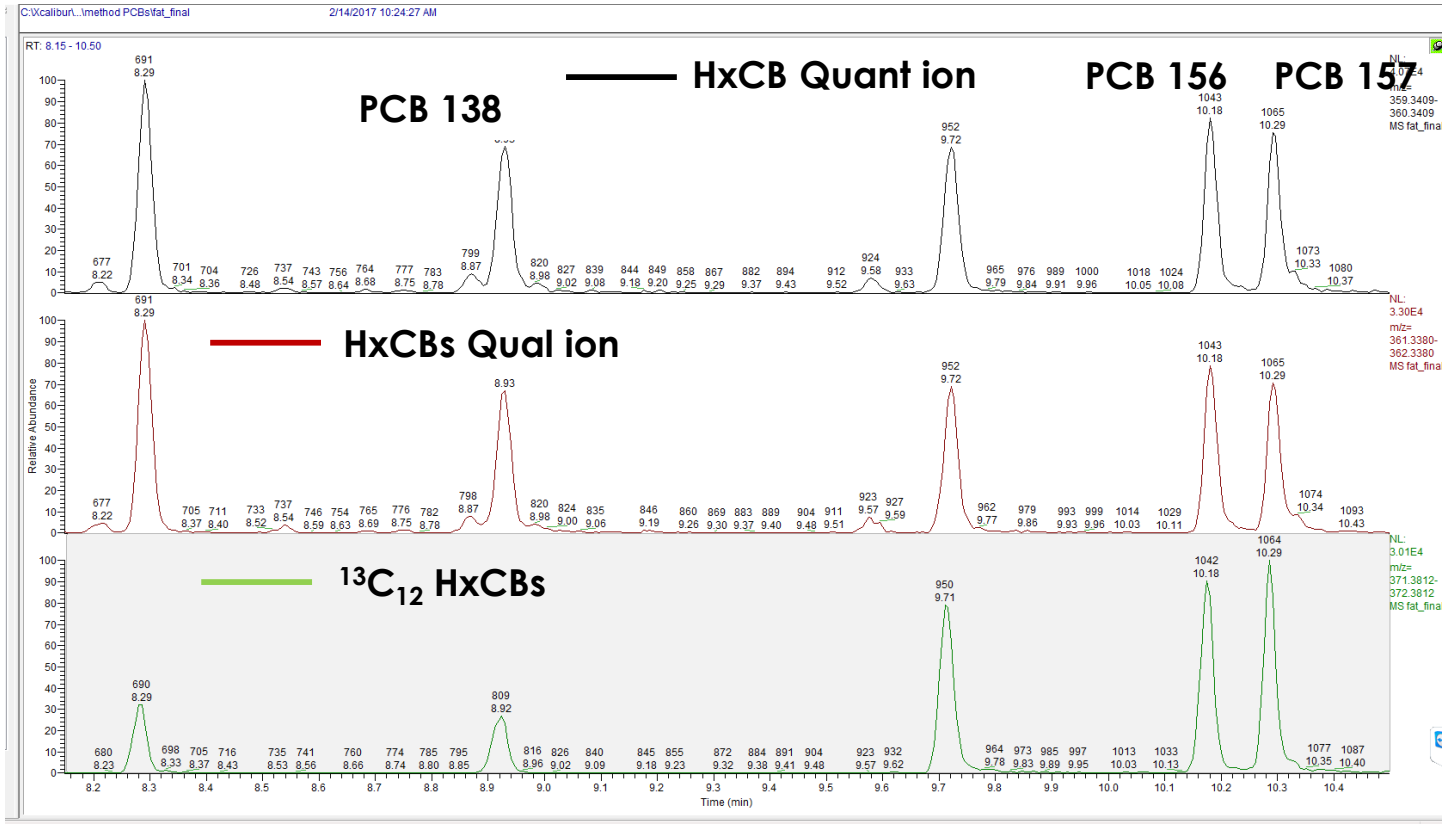


* Real sample of butter, processed in our routine lab

Fast GC – Sensitivity Increase

- Peak squeezing leads to significant sensitivity increase **(~70%)**

FAST GC

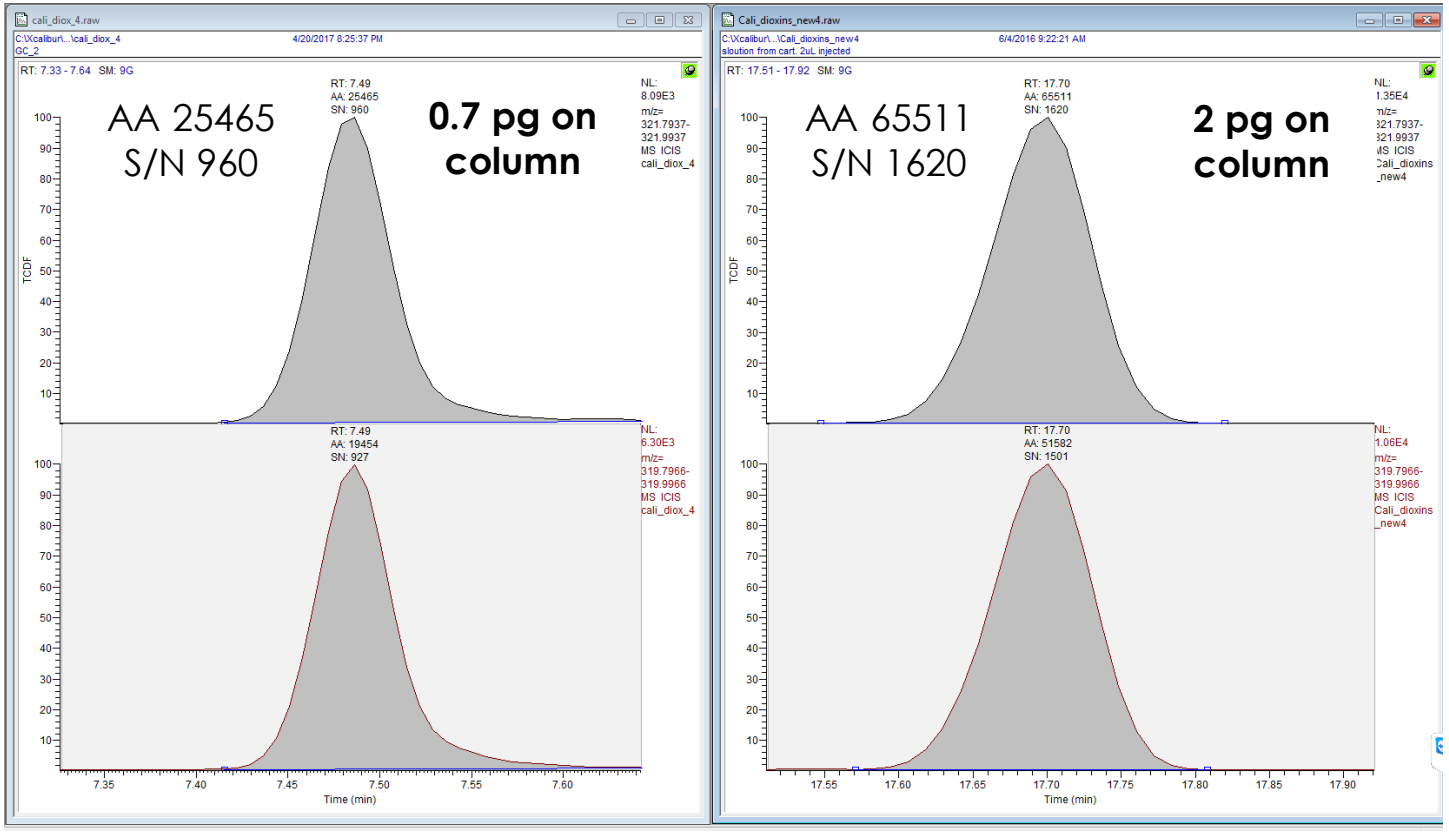
S/N = 1371/1 pg on column

REGULAR GC

S/N = 810/1 pg on column

TCDD Quant ion

TCDD Qual ion



Fast GC – Sensitivity Increase

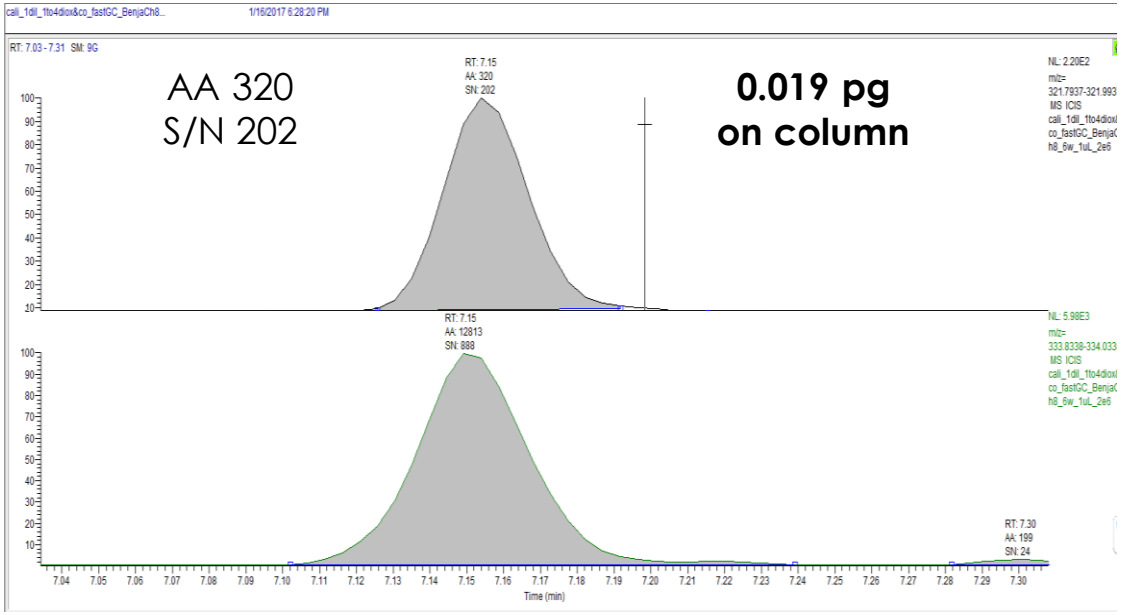
- Peak squeezing leads to significant sensitivity increase **(~70%)**

Our first calibration point (0.05 pg/µL TCDD) diluted 1 to 4 → **0.0125 pg/µL TCDD**

Fast GC analysis, 1.5 µL injected

TCDD Quant ion

$^{13}C_{12}$ TCDD



Fast GC – Method Validation (ongoing…)

- 6 points calibration curve has been injected and RRF RSD is < 15% for all the PCDD/Fs and PCBs.

	Cmp name	RRF average	STD	RSD %
1	2378-TCDF	1.23	0.04	3.26
2	12378-PeCDF	1.23	0.08	6.57
3	23478-PeCDF	1.25	0.06	4.85
4	123478-HxCDF	1.22	0.04	3.3
5	123678-HxCDF	1.24	0.06	4.48
6	234678-HxCDF	1.24	0.06	4.74
7	123789-HxCDF	1.16	0.04	3.59
8	1234678-HpCDF	1.07	0.05	4.51
9	1234789-HpCDF	1.09	0.05	5.02
10	OCDF	1.07	0.04	3.91
11	2378-TCDD	1.37	0.1	7.5
12	12378-PeCDD	1.38	0.08	5.74
13	123478-HxCDD	1.24	0.08	6.63
14	123678-HxCDD	1.23	0.09	7.57
15	123789-HxCDD	1.2	0.12	9.8
16	1234678-HpCDD	1.29	0.13	10.42
17	OCDD	1.36	0.09	6.72

	Cmp name	RRF average	STD	RSD %
1	PCB 28	1.01	0.02	1.61
2	PCB 52	1.33	0.09	6.5
3	PCB 101	1.23	0.07	5.51
4	PCB 123	1.13	0.03	2.71
5	PCB 118	1.19	0.02	1.75
6	PCB 114	1.18	0.03	2.3
7	PCB 153	1.3	0.02	1.25
8	PCB 105	1.14	0.02	1.79
9	PCB 138	1.18	0.03	2.84
10	PCB 167	1.19	0.02	1.75
11	PCB 156	1.15	0.02	1.9
12	PCB 157	1.1	0.02	1.99
13	PCB 180	1.11	0.05	4.53
14	PCB 189	1.18	0.04	3.59

Take Home Message #2

- 70% time saving on PCDD/F measurements (17 min)
- 54% time saving on PCB measurements (11,5 min)
- Dual-Data possibly to be added…
- Major co-elution concerns under control
- To be fully validated...