

Project memo

Validation of d-EVA

Comparison of Multivap evaporation and d-EVA Vacuum Concentrator for PCDDs/Fs and PCBs in marine oils

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ABSTRACT

The Research group for Mass Spectrometry was considering purchasing a new evaporation unit LC-Tech d-EVA Vacuum Concentrator. Before deciding on purchasing, the unit was tested in-lab, and compared with the current Multivap evaporation unit.

Accepted performance in terms of recovery was equally good results from d-EVA as from the Multivap system, which was fulfilled. No significant difference was detected.

Differences for the measured concentration of the analytes evaporated on the two units should not exceed the precision of the methods. The acceptance criterion was fulfilled.

In addition, accuracy and precision were investigated where the results are as good for d-EVA as for Multivap, and there is nothing to suggest that the blank samples differ.

d-EVA meets all given requirements and can be used in routine analysis of PCBs and dioxins in marine oils.

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APPENDICES

1 Background

The Research group for Mass Spectrometry is considering purchasing a new evaporation unit LC-Tech D-Eva Vacuum Concentrator. Before deciding on purchasing, the unit was tested in-lab, and compared with the current Multivap evaporation unit for about 4 weeks. LCTech and Matriks was installing the unit in the lab the 19th November.

2 Performance requirements

Accepted performance in terms of recovery is that the results from D-Eva is as good or better than the results from the Multivap system. Differences for the measured concentration of the analytes evaporated on the two units should not exceed the precision of the methods. At the same time, there is an expectation of better usability, and that hands-on time use on D-Eva is significantly less than when using Multivap.

3 Validation setup and data processing

Comparison of the units was carried out using the following test samples:

- Three parallels of spiked samples in solvent at two different concentration levels (n=6)
- One blank sample (n=2)
- Two parallels of fish oil from proficiency testing program (n=2)
- Two parallels of a real sample (fish oil) (n=2)
- Two parallels of internal control sample (fish oil) (n=2)

The samples of blanks and fish oil was cleaned up by using DexTech-16 before concentration and evaporation. All samples were analysed using GC-MS/MS and validated methods for PCDD/F and PCB together with calibration standards. The validation results apply to marine oils, which also include marine oil extracted from marine tissues and organs, marine fluids and marine flour, and dry matter. Coverage of accredited matrix is considered complete.

Measured concentration (ng/ml) and recovery (%) from the two different evaporation methods are compared by using statistical tools like e.g. ANOVA and Bland Altman.

4 Results

All quantitative results are evaluated together without regard to sample type and where appropriate, the results are sorted by congener. Values below the LOD is not included in the data set.

4.1 Measured concentration

The concentration range of the samples studied varies with congeners, but the coverage is nevertheless preserved because of matrix spikes of Cal 4 and Cal 8 which are included in the assessment. Table 1 gives an overview about average measured concentrations and concentration ranges.

Statistical analysis (Bland Altman) reveals no significant differences related to measured concentrations from samples evaporated by using the two different units ($p > 0.05$, mean difference; 0.002 ng/ml, $n=162$), which indicates that the two units are complementary (Figure 1).

Table 1. Concentration coverage and test sample number used in the evaluation

Congener	n pr unit	Mean (ng/ml)	Conc. Range (ng/ml)
2378-TCDD	12	0.45	0.03 – 2.0
12378-PeCDD	12	0.56	0.05 – 2.0
123478-HxCDD	10	1.1	0.04 – 4.0
123678-HxCDD	10	1.2	0.15 – 4.0
123789-HxCDD	10	1.1	0.02 – 4.0
1234678-HpCDD	12	0.87	0.03 – 4.0
OCDD	10	2.7	0.05 - 10
2378-TCDF	12	1.4	0.10 – 2.6
12378-PeCDF	12	0.57	0.08 – 2.0
23478-PeCDF	12	1.1	0.10 – 2.3
123478-HxCDF	8	1.4	0.16 – 4.0
123678-HxCDF	12	0.89	0.02 – 4.0
123789-HxCDF	12	0.85	0.02 – 4.0
234678-HxCDF	12	0.92	0.03 – 4.0
1234678-HpCDF	10	1.1	0.02 – 4.0
1234789-HpCDF	8	1.4	0.02 – 4.0
OCDF	6	5.3	0.50 - 10
PCB-77	12	12	0.50 - 24
PCB-81	12	2.4	0.25 - 10
PCB-126	12	6.8	0.50 – 12
PCB-169	12	4.2	0.50 - 10
PCB-105	12	15	0.06 - 36
PCB-114	12	2.8	0.06 – 6.0
PCB-118	12	41	0.06 - 102
PCB-123	10	10	0.06 - 22
PCB-156	12	4.8	0.06 – 9.3
PCB-157	12	2.5	0.06 – 6.0
PCB-167	12	13	0.06 - 31
PCB-189	12	1.6	0.06 – 6.0
PCB-28	12	24	0.30 - 41
PCB-52	12	36	0.30 - 72
PCB-101	12	62	0.30 - 140
PCB-138	12	80	0.30 - 207
PCB-153	12	102	0.30 - 266
PCB-180	12	26	0.30 - 46

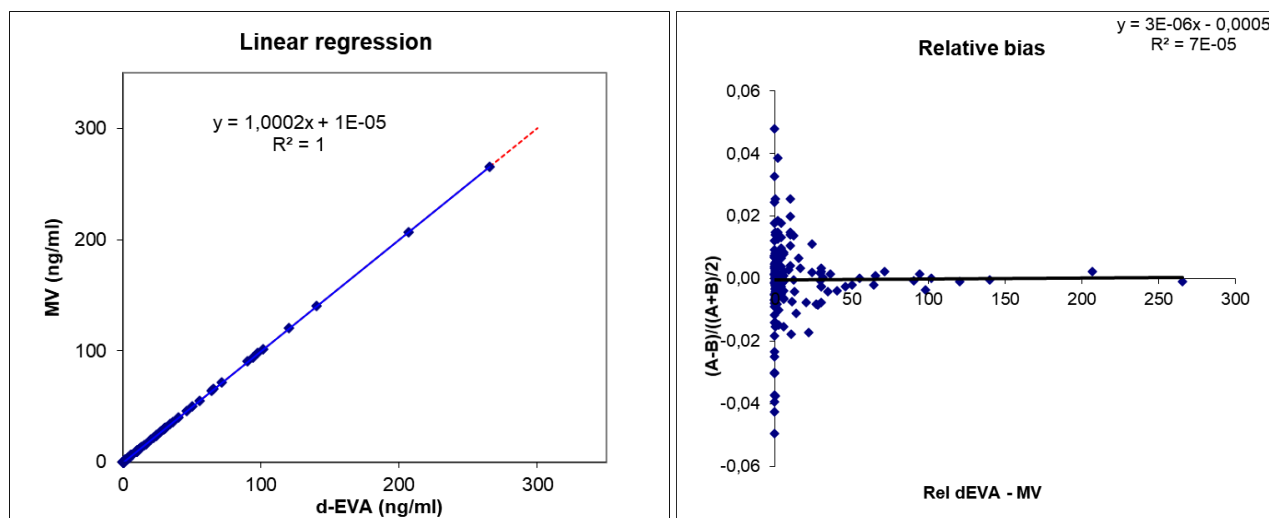


Figure 1. Linear regression and relative bias graph (Bland Altman) showing no significant differences between measured concentrations in test samples evaporated by Multivap (MV) and d-EVA (n=162)

4.2 Relative bias in measured concentrations

Relative bias in terms of differences between measured concentrations were calculated for five samples; SLP fish oil, matrix control, a real sample and two levels of spiked samples in fish oil (Cal 4 and Cal 8) with two or three replicates evaporated using Multivap and d-EVA, giving n= 12 on each unit. Relative differences (%) for all samples were compared to the method precision set during method validation (Table 2, Figure 2).

All differences regarding measured concentration for every congener is within the method precision, with exception of PCB-126 and 1234678-HpCDF where the latter contain concentration close the LOD. The case of PCB-126 is caused by matrix spike at 10 ng/ml (Cal 8) evaporated using Multivap, where repeatability is slightly increased due to a parallel that is measured a little low in relation to the expected value (RSD: 3.6%). By removing this input, the RSD decreases to 1.0%. Even so, the value is kept in the data set as the result is just outside the limit; 2.0% with limit 1.8%, because it was chosen to take into account the measurement uncertainty of the method (PCB-126; extended uncertainty 18%).

The performance criteria have been met in relation to concentration differences using the two different evaporation units as all relative bias is within the precision of the method.

Table 2. Relative bias in measured concentrations between Multivap and d-EVA compared to method precision

Congener	SLP Fish Oil	Matrix Ctr	Real sample	Cal 4 spike	Cal 8 spike	Method precision
2378-TCDD	2.6	3.0	3.0	1.2	1.5	4.5
12378-PeCDD	0.4	1.5	0.9	1.1	1.3	5.7
123478-HxCDD	0.8	1.8	—*	0.3	0.1	4.1
123678-HxCDD	0.8	0.7	—*	0.2	0.3	3.8
123789-HxCDD	3.7	4.8	—*	1.4	1.3	8.6
1234678-HpCDD	3.7	0.5	4.2	0.1	0.6	4.5
OCDD	2.5	1.8	—*	0.3	1.4	4.3
2378-TCDF	1.5	0.1	1.5	0.7	1.0	1.6
12378-PeCDF	0.2	0.4	0.2	0.7	0.5	2.9
23478-PeCDF	0.3	0.5	0.7	0.4	1.9	5.3
123478-HxCDF	0.8	—*	—*	0.3	0.9	3.1
123678-HxCDF	0.7	2.3	0.7	0.2	0.4	2.4
123789-HxCDF	1.4	0.1	2.5	0.1	1.0	2.5
234678-HxCDF	0.1	0.7	0.1	0.2	0.9	2.9
1234678-HpCDF	3.9	0.7	—*	1.4	1.8	2.7
1234789-HpCDF	—*	—*	—*	0.2	0.2	2.7
OCDF	—*	—*	—*	0.3	1.0	2.7
PCB-77	1.1	0.4	1.1	0.03	1.5	1.7
PCB-81	0.5	0.6	0.02	0.03	0.4	0.8
PCB-126	0.03	0.1	0.1	0.2	2.0	1.8
PCB-169	0.05	0.1	0.2	0.1	2.6	3.0
PCB-105	0.2	0.8	1.7	0.7	1.5	3.4
PCB-114	0.5	1.0	0.8	0.1	0.8	2.1
PCB-118	0.01	0.2	0.4	0.1	0.7	1.3
PCB-123	1.7	0.7	—*	0.5	0.6	1.7
PCB-156	0.3	0.1	0.3	0.9	0.8	1.9
PCB-157	0.1	0.1	0.03	0.3	0.2	2.3
PCB-167	0.7	0.7	1.4	0.1	0.4	1.9
PCB-189	0.1	0.04	0.1	0.5	0.1	2.5
PCB-28	0.4	0.04	0.3	0.1	0.3	2.8
PCB-52	0.2	0.05	0.2	0.6	0.2	2.4
PCB-101	0.03	0.1	0.2	0.3	0.3	2.4
PCB-138	0.2	0.4	0.1	0.8	0.2	2.4
PCB-153	0.1	0.1	0.1	1.5	0.2	1.9
PCB-180	0.3	0.8	0.8	0.5	0.1	2.3

*Conc < LOD

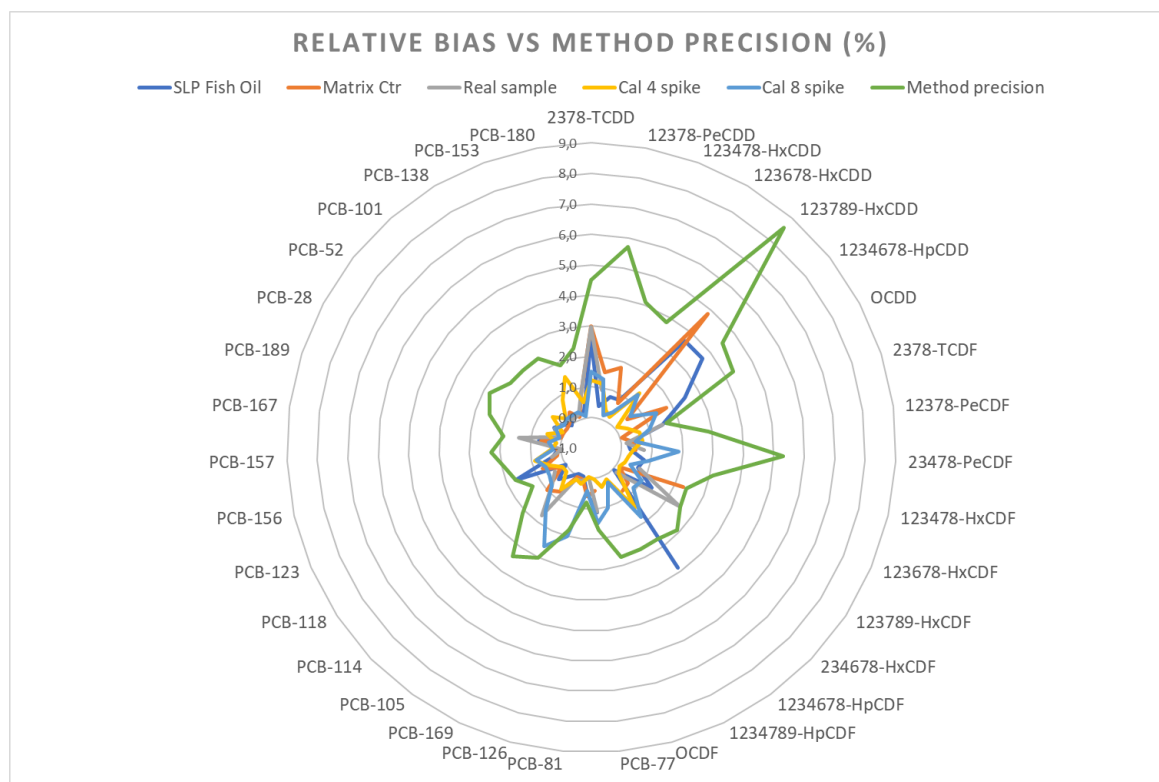


Figure 2. Illustration of relative bias in measured concentrations evaporated using Multivap and d-EVA compared to method precision where the green line is the acceptance limit (n for each congener as denoted in Table 1).

4.3 Accuracy

Accuracy in relation to the expected value was examined for the same samples in section 4.2. SLP fish oil was evaluated by using consensus mean reported in the proficiency program while the control sample was compared against mean value from internal control form. The real sample had no expected value so the values from the two units were only compared against each other. Spiked matrix samples, Cal 4 and Cal 8, were compared with theoretical value. Results are shown in Table 3 and Figure 3. Concentration below LOQ is not included.

Statistical analysis was performed on the data set (ANOVA, Bland Altman), showing no significant differences ($p > 0.05$) for calculated accuracy. d-EVA and Multivap shows equally good results in terms of accuracy with average values of 99.5 and 99.6%, respectively. All results regarding accuracy is within the accept limit of $\pm 20\%$.

Table 3. Accuracy (%) for each sample evaporated on Multivap (MV) and d-EVA, where n=2 for SLP fish oil, matrix control and real sample and n=3 for spiked matrix Cal 4 and Cal 8

Congener	SLP Fish Oil		Matrix Ctr		Real sample		Cal 4 spike		Cal 8 spike	
	MV	d-EVA	MV	d-EVA	MV	d-EVA	MV	d-EVA	MV	d-EVA
2378-TCDD	99	101	90	87	—*	—*	101	102	99	100
12378-PeCDD	105	105	101	99	105	106	101	100	98	99
123478-HxCDD	97	97	106	104	—*	—*	102	102	101	101
123678-HxCDD	100	101	96	95	—*	—*	100	100	100	99
123789-HxCDD	121	117	93	88	—*	—*	104	102	99	101
1234678-HpCDD	102	98	112	111	—*	—*	100	100	99	100
OCDD	101	104	103	105	—*	—*	101	100	101	103
2378-TCDF	100	99	102	102	99	101	100	101	98	102
12378-PeCDF	100	100	102	101	97	97	100	101	100	101
23478-PeCDF	101	101	100	100	112	113	101	101	96	98
123478-HxCDF	103	102	—*	—*	—*	—*	101	101	99	100
123678-HxCDF	97	97	106	104	80	80	101	101	100	101
123789-HxCDF	—*	—*	—*	—*	103	100	101	101	100	101
234678-HxCDF	109	109	100	99	—*	—*	101	101	100	101
1234678-HpCDF	104	100	—*	—*	—*	—*	100	102	99	100
1234789-HpCDF	—*	—*	—*	—*	—*	—*	103	103	101	101
OCDF	—*	—*	—*	—*	—*	—*	101	100	100	101
PCB-77	99	101	100	100	100	99	100	100	99	101
PCB-81	99	100	100	99	100	100	100	100	101	101
PCB-126	98	98	100	100	100	100	100	100	99	101
PCB-169	107	106	99	99	98	98	100	100	98	101
PCB-105	88	88	97	96	103	102	102	101	102	100
PCB-114	108	108	99	98	100	99	101	101	100	101
PCB-118	82	82	100	100	98	98	101	100	100	100
PCB-123	101	100	105	104	—*	—*	100	101	101	100
PCB-156	85	85	104	104	100	100	102	101	99	100
PCB-157	96	96	101	101	106	106	100	100	100	100
PCB-167	103	103	104	105	97	98	101	101	101	100
PCB-189	86	86	103	103	118	118	101	101	100	100
PCB-28	104	103	101	101	100	100	100	100	100	100
PCB-52	89	89	100	100	100	100	100	99	100	100
PCB-101	86	86	100	100	100	100	100	100	101	101
PCB-138	97	97	96	96	100	100	99	100	101	101
PCB-153	80	80	98	98	100	101	99	100	101	101
PCB-180	85	84	98	97	102	101	100	100	99	99

*Conc < LOQ

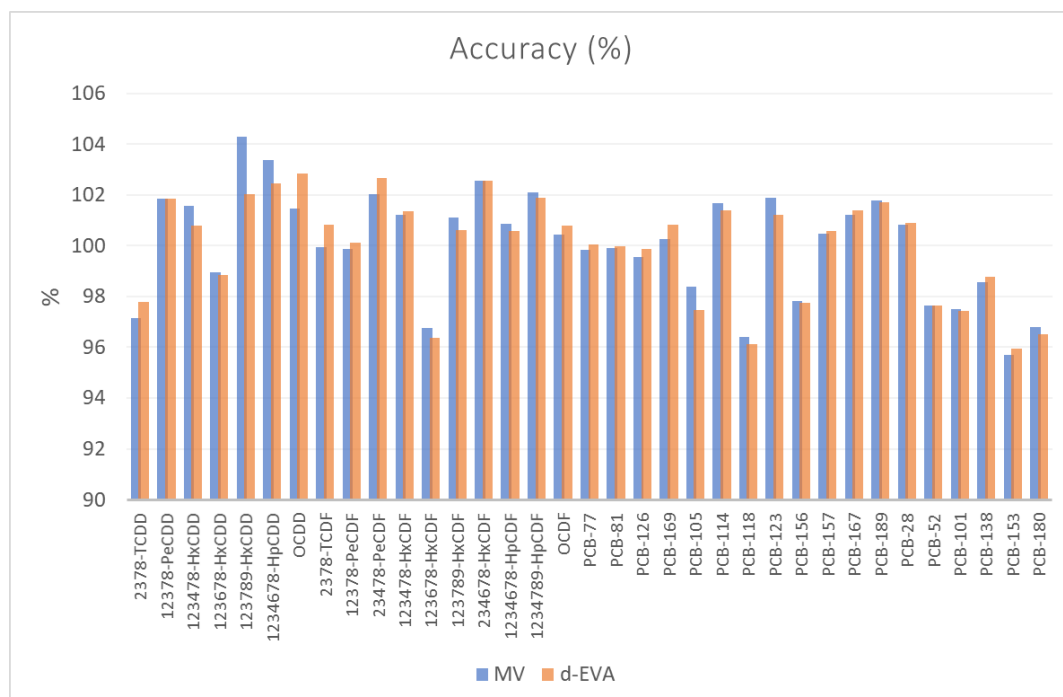


Figure 3. Average accuracy (%) of samples evaporated using Multivap (MV) and d-EVA, n for each congener as denoted in table 1.

4.4 Precision

Repeatability within the parallel samples was tested to ensure as good precision between d-EVA parallels as from Multivap (Table 4, Figure 4). Average precision is calculated to 1.35% and 1.39% for d-EVA and Multivap, respectively. No significant difference is detected (ANOVA, $p > 0.05$, $n = 162$), and all results are within the acceptance criteria of $\pm 10\%$.

Table 4. Repeatability (%) for each sample evaporated on Multivap (MV) and d-EVA, where n=2 for SLP fish oil, matrix control and real sample and n=3 for spiked matrix Cal 4 and Cal 8

Congener	SLP Fish Oil		Matrix Ctr		Real sample		Cal 4 spike		Cal 8 spike	
	MV	d-EVA	MV	d-EVA	MV	d-EVA	MV	d-EVA	MV	d-EVA
2378-TCDD	0.2	2.1	5.8	0.7	3.7	1.2	0.3	3.0	3.4	0.8
12378-PeCDD	1.5	2.3	3.4	0.02	0.1	0.1	0.9	3.1	3.5	0.5
123478-HxCDD	1.8	1.3	10	8.4	—*	—*	0.3	0.7	1.1	0.9
123678-HxCDD	0.3	0.8	1.0	2.8	—*	—*	0.8	0.5	1.2	0.9
123789-HxCDD	4.0	8.8	0.1	7.5	—*	—*	0.1	1.7	1.4	1.0
1234678-HpCDD	5.2	0.1	0.6	1.1	—*	—*	0.4	0.3	0.4	0.5
OCDD	3.2	0.2	2.1	1.4	—*	—*	0.2	0.1	1.8	2.1
2378-TCDF	0.8	0.3	3.1	0.2	0.8	1.0	0.9	2.7	3.2	1.0
12378-PeCDF	2.0	2.0	2.7	1.8	1.0	2.9	0.3	0.5	0.9	0.2
23478-PeCDF	0.2	0.3	1.5	0.5	1.8	0.8	0.3	2.4	3.4	2.7
123478-HxCDF	0.0	0.8	—*	—*	—*	—*	0.6	0.6	0.6	0.8
123678-HxCDF	0.7	2.6	4.2	3.1	0.2	1.6	0.7	0.1	1.1	0.8
123789-HxCDF	0.1	2.7	—*	—*	5.2	5.8	0.6	0.2	1.8	0.7
234678-HxCDF	1.5	2.0	3.8	2.7	8.1	8.4	0.5	0.2	0.4	1.2
1234678-HpCDF	7.7	2.7	2.4	4.0	—*	—*	0.3	1.2	1.1	0.2
1234789-HpCDF	—*	—*	—*	—*	—*	—*	1.0	0.3	0.2	1.1
OCDF	—*	—*	—*	—*	—*	—*	0.01	0.5	1.5	1.1
PCB-77	0.2	0.2	0.5	1.4	0.3	0.7	0.02	0.2	2.3	0.8
PCB-81	0.4	0.3	0.4	0.8	0.3	0.6	0.1	0.1	3.7	0.05
PCB-126	0.7	0.3	0.03	0.2	1.0	0.9	0.2	0.1	3.6	1.0
PCB-169	1.2	0.2	0.2	0.1	1.3	0.8	0.7	0.2	4.7	0.5
PCB-105	0.1	0.5	1.1	2.2	0.2	0.4	0.5	1.5	0.4	0.8
PCB-114	0.6	0.7	0.003	1.1	0.1	0.3	0.5	0.5	0.1	0.4
PCB-118	0.2	0.3	0.3	0.1	1.6	0.05	0.8	0.8	0.7	0.7
PCB-123	1.0	0.3	1.6	5.4	—*	—*	1.0	0.7	1.2	0.6
PCB-156	0.8	0.6	0.1	0.2	0.2	0.3	1.6	1.5	0.9	1.3
PCB-157	0.1	0.4	2.0	1.2	0.7	1.1	0.4	0.8	0.5	0.5
PCB-167	0.4	1.3	0.9	2.4	4.6	2.8	1.1	1.3	0.4	0.3
PCB-189	0.5	0.02	4.1	6.3	0.8	0.7	0.7	1.4	0.2	0.5
PCB-28	0.7	1.5	0.6	1.1	1.3	1.0	0.2	0.3	0.1	0.04
PCB-52	0.7	0.2	0.8	0.03	0.1	1.0	2.3	1.4	0.2	0.2
PCB-101	0.03	0.04	0.6	0.5	0.6	0.3	0.3	0.6	0.2	0.4
PCB-138	0.2	0.1	0.7	0.8	0.5	0.2	1.4	0.2	0.4	0.1
PCB-153	0.1	0.1	0.01	0.1	0.3	0.2	2.1	0.4	0.7	0.4
PCB-180	0.8	0.3	0.2	0.9	0.2	0.0002	0.7	0.2	0.5	0.7

*Conc < LOQ

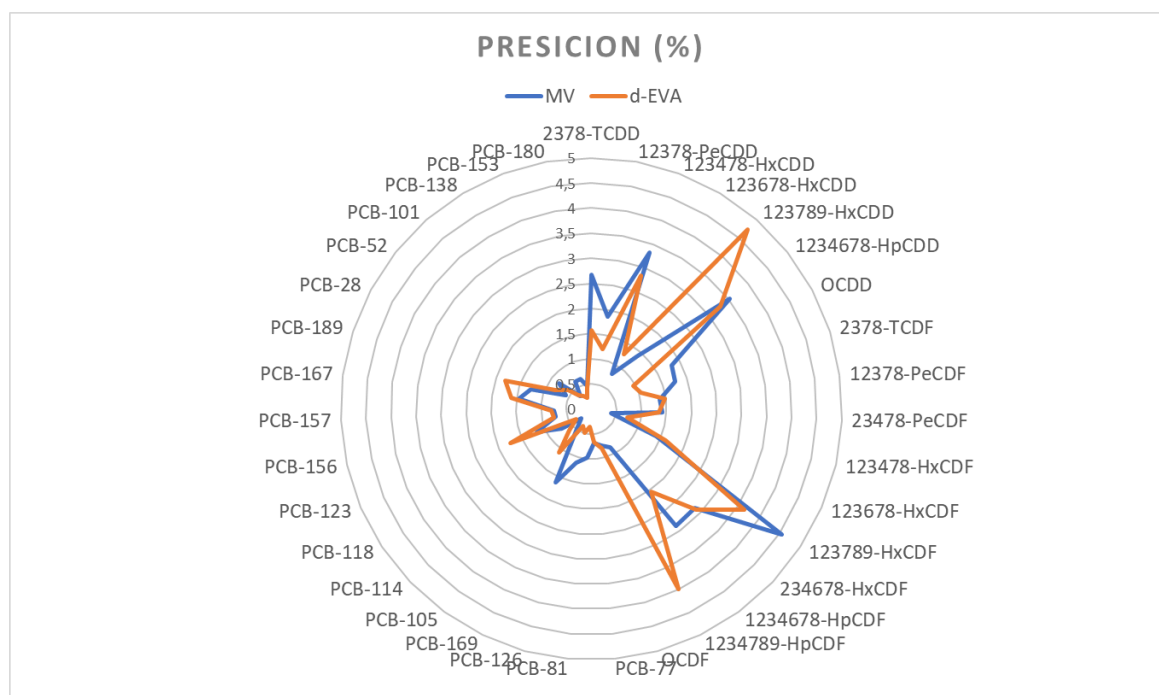


Figure 4. Average repeatability (%) of samples evaporated using Multivap (MV) and d-EVA, n for each congener as denoted in table 1.

4.5 Recovery

Accepted performance in terms of recovery is that the results from d-EVA is as good or better than the results from the Multivap system. Recovery is calculated directly in the MassHunter software by utilizing surrogate internal standards added during clean-up and extraction. Acceptable limits stipulated in the current legislation is 60 – 120%. Recovery data for the test samples are compared for evaporation using d-EVA vs Multivap (Table 5, Figure 5 and 6).

Statistical evaluation reveals no significant differences in relation to recovery of surrogate standard (ANOVA; $p > 0.05$. Bland Altman; mean difference 0.7%, $n = 210$). All recovery results fulfil the current limits of 60 – 120%.

Table 5. Recovery (%) for each sample evaporated on Multivap (MV) and d-EVA, where n=2 for SLP fish oil, matrix control and real sample and n=3 for spiked matrix Cal 4 and Cal 8

Congener	SLP Fish Oil		Matrix Ctr		Real sample		Cal 4 spike		Cal 8 spike		Blank	
	MV	d-EVA	MV	d-EVA	MV	d-EVA	MV	d-EVA	MV	d-EVA	MV	d-EVA
2378-TCDD	91	92	90	90	90	92	95	96	98	96	96	96
12378-PeCDD	95	95	101	99	99	99	105	103	108	108	107	107
123478-HxCDD	74	73	63	64	63	63	73	72	93	87	88	95
123678-HxCDD	73	76	69	69	68	67	75	76	96	100	91	97
123789-HxCDD	85	86	88	87	81	82	88	89	103	104	97	98
1234678-HpCDD	109	109	109	109	108	108	112	112	118	119	116	117
OCDD	98	98	118	118	112	111	105	104	111	109	108	110
2378-TCDF	86	85	80	78	83	81	90	89	98	94	97	95
12378-PeCDF	90	91	89	89	89	90	98	95	97	98	97	97
23478-PeCDF	93	94	96	94	94	93	99	98	100	99	101	101
123478-HxCDF	97	98	88	88	85	85	96	96	106	110	110	110
123678-HxCDF	101	102	93	92	92	92	98	99	107	107	107	106
123789-HxCDF	87	87	81	80	84	83	88	88	107	114	104	105
234678-HxCDF	64	64	61	61	69	61	75	75	79	79	77	82
1234678-HpCDF	100	99	92	93	95	95	99	99	107	116	104	106
1234789-HpCDF	106	106	107	107	107	106	104	103	115	114	112	109
OCDF	105	105	119	119	118	119	113	113	108	111	109	110
PCB-77	92	94	85	85	85	85	93	93	108	107	109	107
PCB-81	99	102	90	90	92	92	95	95	114	112	115	112
PCB-126	91	91	83	82	84	84	87	88	96	93	96	94
PCB-169	93	93	95	93	94	92	99	97	101	102	99	101
PCB-105	106	107	94	106	93	94	90	103	107	108	104	103
PCB-114	103	103	101	101	96	97	103	104	103	103	103	102
PCB-118	95	94	89	87	82	83	82	89	100	99	99	100
PCB-123	97	97	90	90	87	87	89	95	101	100	102	101
PCB-156	103	103	104	104	95	96	92	99	95	98	98	98
PCB-157	98	98	92	92	87	87	87	89	94	97	98	98
PCB-167	100	100	96	96	88	89	81	92	98	100	99	99
PCB-189	116	116	113	112	100	98	101	104	98	103	100	101
PCB-28	71	77	66	73	67	70	81	82	84	90	80	91
PCB-52	89	92	85	90	85	89	97	96	84	85	80	87
PCB-101	113	111	101	101	97	97	108	106	95	90	92	92
PCB-138	105	105	104	104	100	101	107	106	102	101	102	102
PCB-153	111	111	105	105	101	102	103	103	96	97	97	97
PCB-180	115	112	116	116	106	110	109	109	94	98	97	100

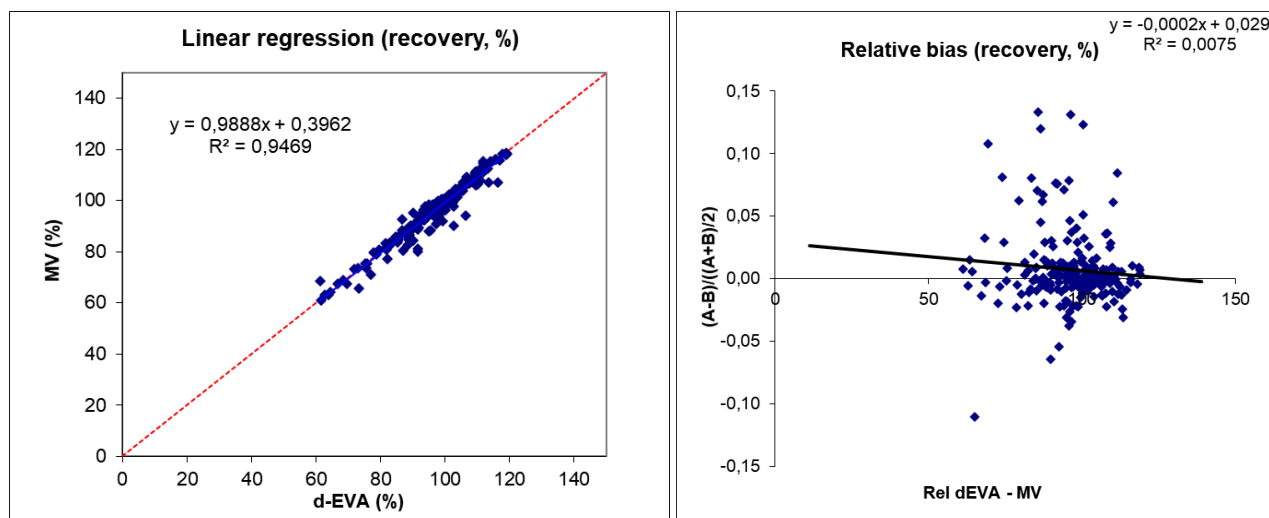


Figure 5. Linear regression and relative bias graph (Bland Altman) showing no significant differences between calculated recovery in test samples evaporated by Multivap (MV) and d-EVA (n=210)

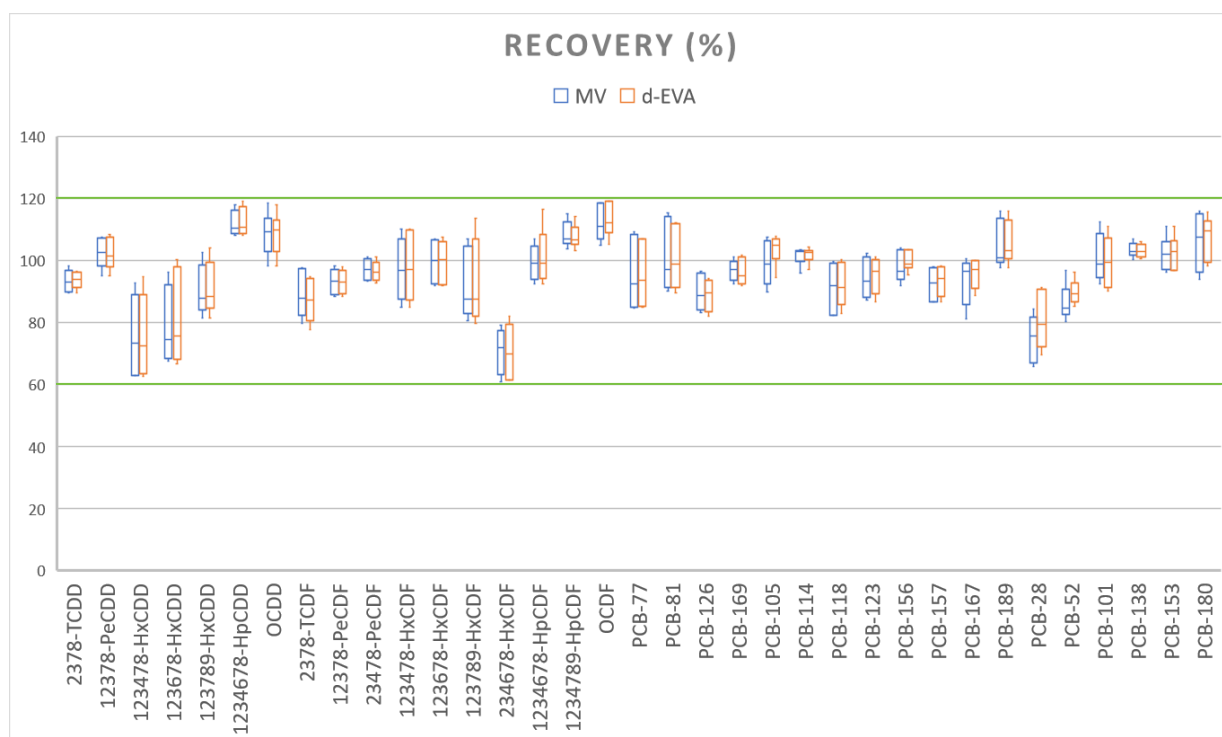


Figure 6. Average recovery for all congener inclusive variance due to the different sample types evaporated by Multivap (MV) and d-EVA (n=210). Acceptance limit shown as green lines.

4.6 Blank samples

Blank samples were following the DexTech procedure for clean-up an extraction whereas one parallel was evaporated using d-EVA while the other one by Multivap. MassHunter Qualitative Analysis was used for extraction of m/z of interest for each congener and overlay chromatograms for visual inspection of interfering peaks. The results from the PCB method show remarkable lower background disturbance in blank sample evaporated by d-EVA (Figure 7). Nevertheless, both blank samples are well below abundance for LOQ-levels. No distinct differences were observed in the blank samples analysed by the PCDD method (Figure 8).

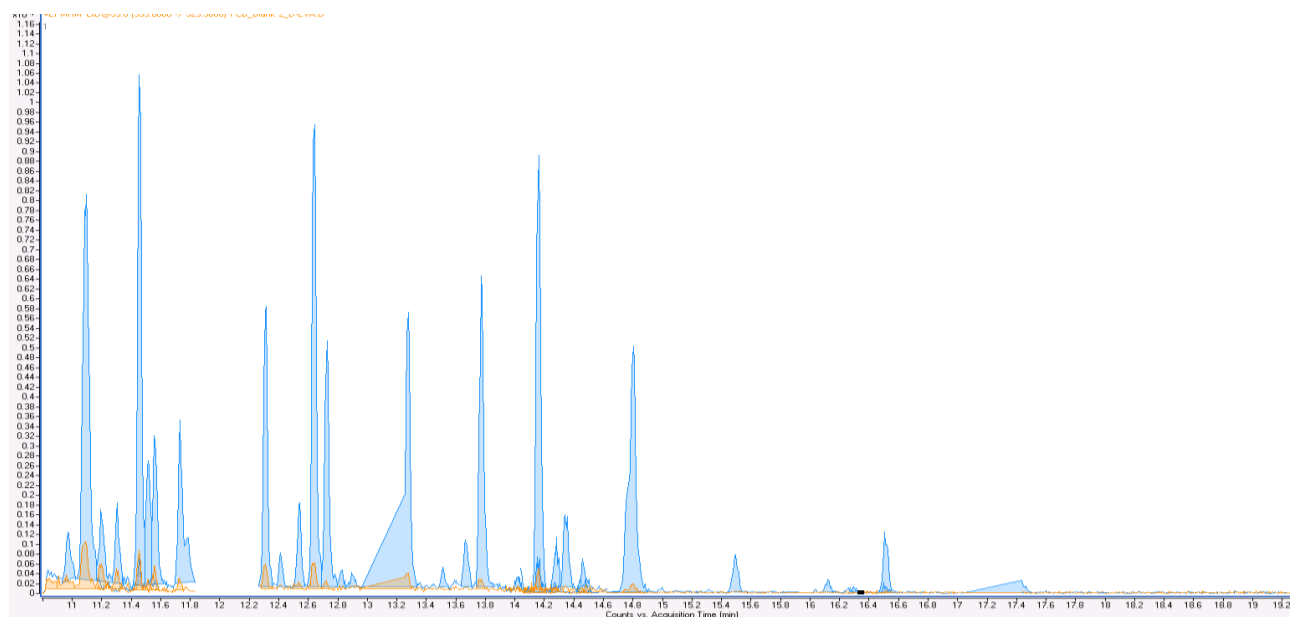


Figure 7. Overlay chromatogram of blank samples analysed using PCB method, evaporated by Multivap (blue peaks) and d-EVA (orange peaks).

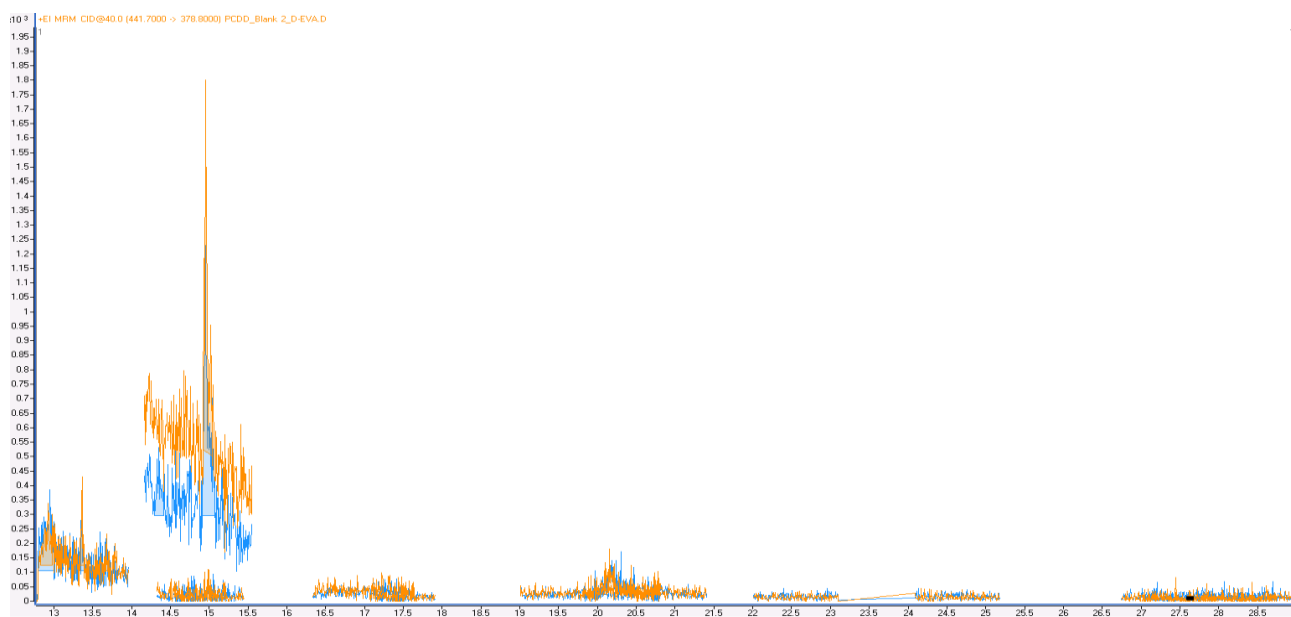


Figure 8. Overlay chromatogram of blank samples analysed using PCDD/F method, evaporated by Multivap (blue peaks) and d-EVA (orange peaks).



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