DSP systems
The missing link in your laboratory!

Performance study of the Determination of DL-PCBs and NDL-PCBs in one single GC-HRMS measurement using a Miura GO-xHT for Sample Purification (Part I; Food and Feed)

Guillaume ten Dam[1], Jeroen Markesteijn[2], Chris van Wakeren[1] and Wim Traag[1]
[1]DSP-Systems, Food Valley BTA12, Ede, The Netherlands, [2]NOFAlab, Jan van Galenstraat 41, Schiedam, The Netherlands

Goal

1 To measure all DL- and NDL-PCBs (EN16125) in one GC-MS run by combining the carbon and alumina fraction of the GO-xHT sample clean-up system

2 To assess the performance of the measurement of standards and samples containing all DL- and NDL-PCBs in one GC-MS run

Abstract

The sample clean-up by Miura GO-xHT technology results in 2 fractions containing 1) dioxins and NO-PCBs, and 2) MO-PCBs and NDL-PCBs. As certain laboratories apply a different measurement approach, e.g. DL-PCBs and NDL-PCBs in one measurement, the simultaneous analysis of these compounds using the GO-xHT technology was investigated. The GO-xHT technology delivers two ultra-pure extracts and could therefore be combined for the simultaneous measurement of DL-PCBs and NDL-PCBs. The results, recovery and quality of the analysis complied with current regulations and were similar to the accredited analysis at NofaLab proving the suitability of the GO-xHT technology for the simultaneous analysis of DL-PCBs and NDL-PCBs.

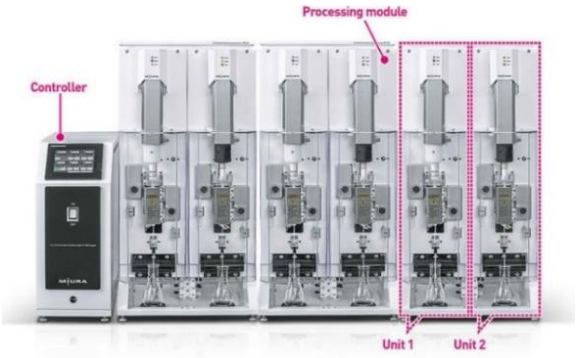

Figure 1 Miura GO-6HT sample clean-up system

Figure 2 DFS™ Magnetic Sector GC-HRMS System by ThermoFisher Scientific.

Introduction

In 2015 DSP-Systems launched in cooperation with Miura (Matsuyama, Japan) a new innovative line of sample clean-up systems (fig. 1) for the analysis of dioxins and PCBs[1]. In accordance with EN 16215[2] two fraction are obtained after clean-up, containing 1) dioxins and NO-PCBs, and 2) MO-PCBs and NDL-PCBs. The measurement as such however, is not routine practice in all laboratories as NO-PCBs and MO-PCBs are also frequently analysed in one single measurement. In cooperation with NofaLab, DSP-Systems has investigated the possibility to analyse DL-PCBs and NDL-PCBs in one measurement on a Thermo DFS™ Magnetic Sector GC-HRMS System (fig. 2) while using the Miura GO-xHT technology (fig. 1) for sample purification to respond to this routine practice.

熱・水・環境のベストパートナー MIURA
NofaLab laboratories
Cambridge Isotope Laboratories, Inc.
ThermoFisher SCIENTIFIC

Generally, PCBs are separated from the dioxin fraction to prevent interference of the PCBs on the dioxin measurement[3,4,5]. Contrary, the presence of dioxins in the measurement of PCBs does not interfere with the analysis. Moreover, methods for PCB analysis generally do not separate dioxins from PCBs. Considering this and the aforementioned purity of both fractions obtained from the GO-xHT technology, the simultaneous measurement of DL-PCBs and NDL-PCBs could be performed by combining both fractions obtained from the GO-xHT. This application was explored by analysing food and feed samples at NofaLab and the results are reported in this application note.

Samples and standards

Standards and standard mixtures of PCBs were obtained from Cambridge Isotope Laboratories (Andover, MA) and diluted to the appropriate levels:

- EC-5583, ^{13}C MO-PCB & NDL-PCB mix
- EDF-5581, ^{13}C PCDD/F & NO-PCB mix
- EC-5336, ^{13}C PCB159
- EC-5380, DL-PCB calibration solutions
- EC-5385, NDL-PCB calibration solutions
- EDF-9999-A, PCDD/F calibration solutions

Purified sample extracts containing 1) dioxins and NO-PCBs, and 2) MO-PCBs and NDL-PCBs from meat, cheese, fish, rice, fish oil, coconut oil, Tocobiol, palm fatty acids, animal feed and lecithin powder were obtained from NofaLab after official analysis and combined for the simultaneous analysis of DL-PCB and NDL-PCB.

Extraction and Purification of samples

All analysis were performed in a routine setting. The samples were spiked with 100 pg ^{13}C DL-PCBs and 500 pg ^{13}C NDL-PCBs prior to extraction, or in case of oils and fats, prior to purification.

Samples fat and oil were liquified by heating the samples at 60°C for 10 minutes allowing direct application of 2.5 gram onto the column set.

Samples feed were extracted using a SER-158[6] with toluene:ethanol or hexane:dichloromethane as extraction solvent. The samples were evaporated till near dryness in a rotary evaporator. To achieve near dryness, a Teflon™ tube was attached onto the vent which stretched till the entrance of the sample flask. By venting (with indoor air), the flask volume was cleared from gaseous solvent preventing re-condensation of solvent into the flask. After evaporation the residues were reconstituted in 10 ml hexane.

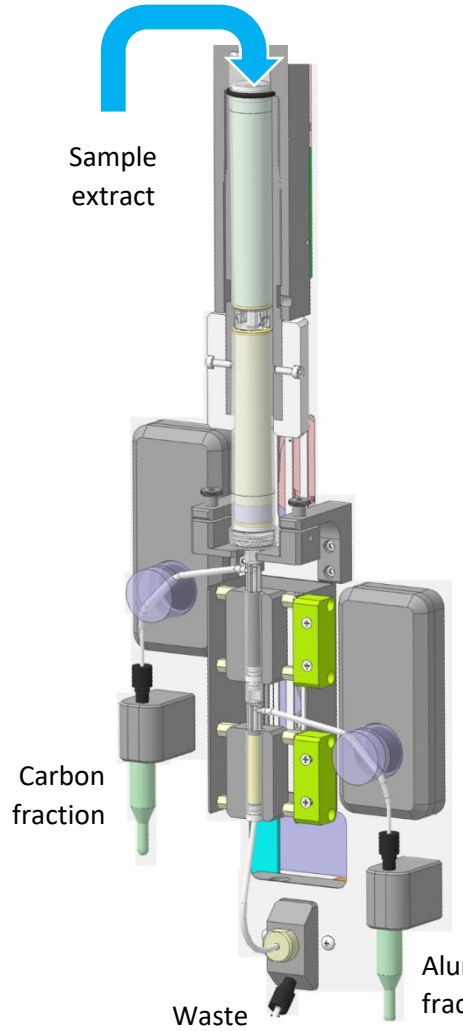

Figure 3 Schematic representation of a channel containing 4 columns for the purification of a sample.

For purification of samples and blanks an automated GO-6HT sample purification system (Miura) was used. For each sample a set of four columns was used, in-line: silica gel impregnated with silver nitrate (1st); silica gel impregnated with sulfuric acid (2nd); activated carbon (3rd) and alumina (4th) (fig. 3). Each extract was transferred on top of the first column and after complete adsorption, which took in general less than five minutes, the set of columns was placed in the GO-6HT system.

Next, the column set was eluted with 90 ml hexane at a flowrate of 2.5 ml/min. During this step the temperature of the two purification columns was maintained at 60°C. This elevated temperature weakens the adsorption with silica gel and as a result, the elution speed of dioxins and PCBs is enhanced. Also the chemical reaction rates (amongst others, oxidation with sulfuric acid) with sample matrices is accelerated. PCDD/Fs and the four NO-PCBs are trapped on the activated carbon column while the MO- and NDL-PCBs are trapped on the alumina column.

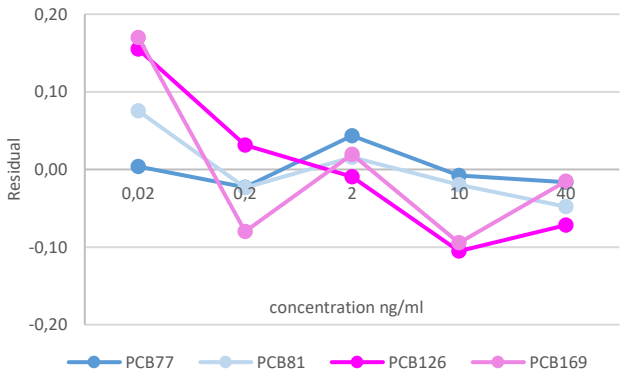

Figure 4 Residuals of a four point calibration curve (RF) of no-PCB in the combined analysis of DL-PCB and NDL-PCB.

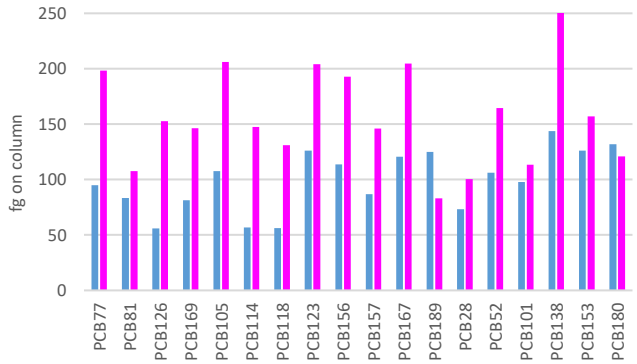

Figure 5 LODs in the combined analysis of DL-PCB and NDL-PCB calculated on RRF (blue) and response (pink).

Both the alumina and the carbon column were eluted in backflush using a small amount of toluene resulting in two fractions, each of 1.5 ml. During these elution steps the temperature of the carbon and alumina column was set at 90°C. At first only the alumina column was eluted with 1.5 ml toluene and the collected fraction contained the MO-PCBs and NDL-PCBs. Subsequently, the carbon column was also eluted with 1.5 ml toluene and this fraction contained all PCDD/Fs and NO-PCBs.

Both fractions were evaporated till near dryness using a CentriVap (Labconco) after which the carbon and alumina fractions were reconstituted in 20 µl dodecane containing 4 ng/ml $^{13}C_{12}$ PCB159 and ^{13}C 1,2,3,4-TCDD. For the analysis of 1) dioxins and NO-PCBs 5 µl and 2) MO-PCB and NDL-PCB 4 µl was injected on the GC-HRMS. Consequently, the alumina and carbon fractions were combined. Of the combined fractions 5 µl was injected on the GC-HRMS for measurement of DL-PCBs and NDL-PCBs.

GC-HRMS analysis

A GC-HRMS (DFS High Resolution Magnetic Sector MS - Thermo Scientific) was used for the analysis of 1) dioxins and NO-PCBs, 2) MO-PCBs and NDL-PCBs and 3) DL-PCBs and NDL-PCBs. The GC-HRMS was equipped with a PTV injector (Best P.T.V. injector) using a sintered glass liner (SGE) and a VF-5ms 60m x 0,25mm x 0.25µm + 5m EZ-guard (Varian). A flow of Helium was applied at 1 ml.min^{-1}. The mass spectrometer was operated in the electron ionization mode at a resolution of 10.000. The selected monitored ions, retention times and GC program are given in supplementary information 1 for the measurement of DL-PCBs and NDL-PCBs. The linear range was from 0.1 pg – 200 pg on-column.

Results

Results of samples were calculated using experimentally determined relative response factors (RRF). The RRFs were determined by measuring a standard at 2 ng/ml in duplicate, one before and one after measurement of the samples. The relative standard deviations (RSD) of the RRFs of the native PCBs were between 0.2 and 11% while they were between 0.8 and 16% for the ^{13}C labelled internal standards. The linearity was good over the whole range with residuals of <0.10 for over 85% of all observations between 0.2 and 40 ng/ml (fig. 4 displays residuals for NO-PCBs). At LOD level this was somewhat higher due to background levels in the system, yet nearly all residuals remained below 0.20.

In this study, the offset (uncorrected for recovery and sample intake) of the LOQ was determined by measuring 10 times a standard at the expected LOQ instead of procedural blanks as described by the EU guidance document[7]. The LOQ was defined as $LOQ_{S/N=3} = 6 \times \delta_{noise}$ for which δ_{noise} was considered the standard deviation over the 10 analysed standards. The LOQ was calculated using two different approaches, 1) SD over the RRF and 2) SD over the areas and subsequently quantified against the average area of the associated internal standards. The standard contained 0.02 ng/ml native PCBs and 2 ng/ml ^{13}C labelled PCBs.

Although the ion ratio was in approximately half the observations not between -15 and 15%, the resulting LOQs from the RRF and responses were around the expected value of 100 fg on-column or 0.02 ng/ml extract (fig. 5 and S.I. 2). The advantage of isotope labelled internal standards are also demonstrated as the LOQ based on RRF was 50% lower compared to the LOQ based on the response itself (99 fg on column vs. 160 fg on column). Considering this LOQ, the LOQ based on 2.5 gram of a fat sample and 10 gram animal feed, the sample LOQs would be 100 / 2.5 = 40 fg/g fat and 100 / 10 = 10 fg /g containing 12% moisture animal feed assuming 100% recovery of the internal standards and GC syringe standard.

The data of the analysed samples were evaluated on 1) recovery of the internal standards, 2) accuracy/similarity of the results for the native compounds, 3) peak shape, separation of the congeners and conformity with legislation[6,7] and 4) suppression of the reference ions during the measurement.

1) Average recoveries of ^{13}C PCBs were well within 60-120% (fig. 6) and except for 3 congeners all recoveries including the standard deviation remained within this range for both approaches. On average, the recoveries in the official measurement was slightly higher, but then again, the standard deviation over the averages was higher compared to the combined measurement. Somewhat larger differences were observed for the NO-PCB, however, in the official measurements recoveries of ^{13}C NO-PCB were determined using ^{13}C 1,2,3,4-TCDD as a reference while in the combined measurement ^{13}C PCB159 was used. As of this, results might have differed slightly more for ^{13}C NO-PCBs.

2) Results for native compounds varied somewhat more than the internal standards due to the generally low levels in the samples and blank contribution of the system (fig. 7). At higher levels the similarity between the two approaches was better which is apparent in the

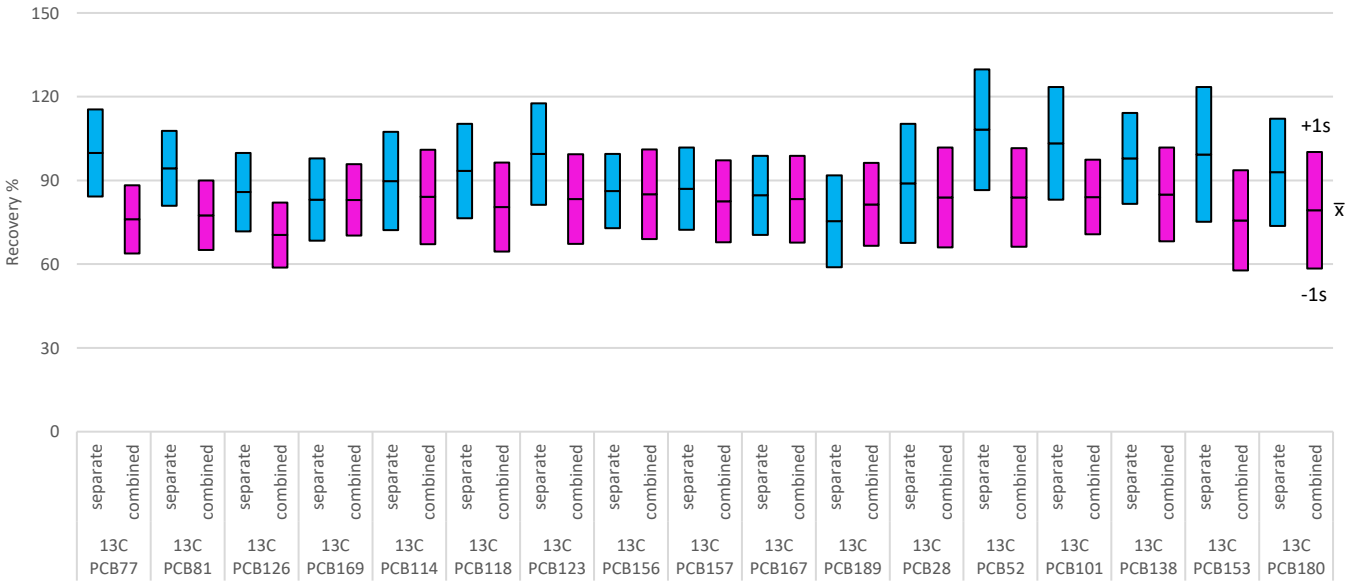

Figure 6 Average recovery and standard deviation for each internal standard in the separate measurement of 1) NO-PCB and 2) MO-PCB and NDL-PCB (blue), and the combined of DL-PCB and NDL-PCB (pink).

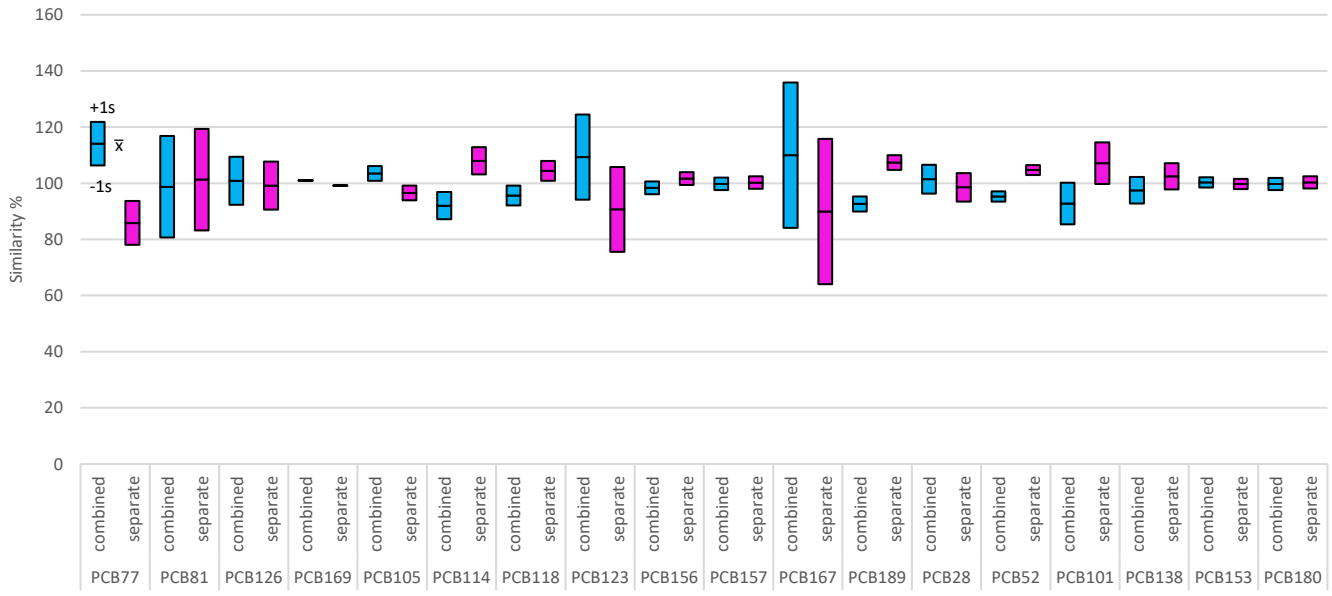

Figure 7 Average similarity and standard deviation for each individual congener in the separate measurement of 1) no-PCB and 2) mo-PCB and NDL-PCB (blue), and the combined of DL-PCB and NDL-PCB (pink). Similarity calculated as "result / average result of the two measurements x 100".

results of NDL-PCBs and in samples with higher amounts of NO-PCBs (93 ± 17%).

3) Each chromatogram was thoroughly controlled for appropriate gaussian peak shape and sufficient chromatographic resolution. The resulting data was consequently verified to conform to the relevant regulations[6,7], such as retention time and ion ratio. All chromatograms for NO-PCBs as well as MO-PCBs and NDL-PCBs in the combined measurement were similar to the chromatograms in the official analysis (fig. 8). Similarly, the retention times and ion ratios in the combined measurement met the criteria for all results at relevant levels.

4) The chromatograms of the reference mass were reviewed for suppressions and, as for the official analysis, no suppressions were observed (fig. 9).

Conclusion

The goal of his study was 1) to measure all DL- and NDL-PCBs in one GC-HRMS run by combining the carbon and alumina fraction of the GO-xHT sample clean-up and 2) to assess the performance of the measurement of standards and samples of all DL- and NDL-PCBs in one GC-HRMS run.

Average recoveries of internal standards in food and feed samples were 70 – 85% with standard deviations of 12 – 21%. Average results for native compounds were within 80 – 120% while the quality parameters were similar to the separate analysis of 1) dioxins and NO-PCBs and 2) MO-PCBs and NDL-PCBs.

Considering the results of the experiments performed in this study the applicability of the Miura GO-xHT technology for the simultaneous measurement of DL-PCBs and NDL-PCBs on a Thermo DFS™ Magnetic Sector GC-HRMS System was demonstrated.

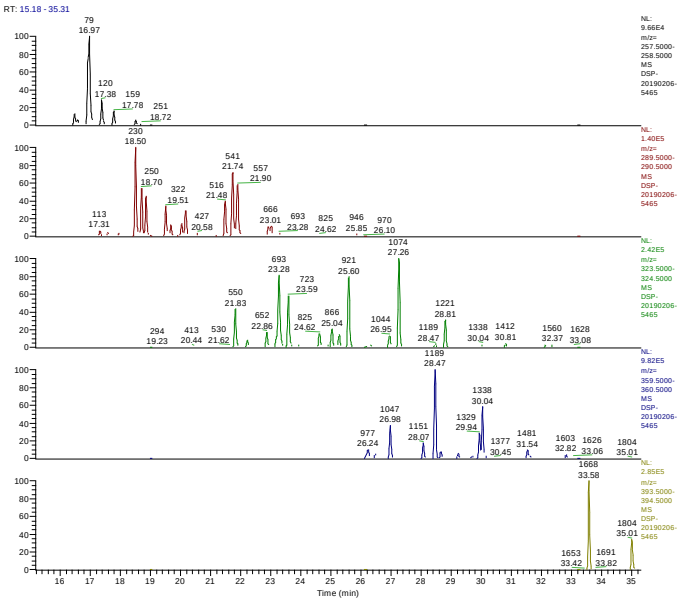

Figure 8 Chromatograms of tri- till hepta-PCB in a sample fish.

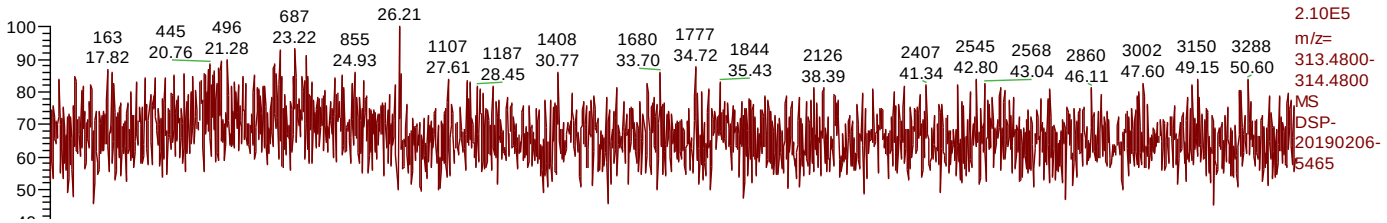

Figure 9 Chromatogram a reference mass in a sample lecithin powder.

Literature

1) DSP-Systems, Application note; Automated analysis of pcdd/fs, dioxin-like pcbs, non-dioxin-like pcbs and polybrominated diphenyl ethers in food and feed

2) NEN-EN 16215, Animal feeding stuffs - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

3) EPA Method 1613, Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

4) Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs

5) Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

6) DSP-Systems, Application note; Automated analysis of pcdd/fs, dioxin-like pcbs, non-dioxin-like pcbs and polybrominated diphenyl ethers in food and feed

7) Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food

Supplementary information

S.I. 1A Injector and GC program for the analysis of DL-PCB and NDL-PCB at a gas flow of 1.0 ml.min^{-1}

GC Program		
Rate	Temp	hold
Initial	140	1.50
10.0	220	5.20
4.50	235	4.00
5.40	310	13.08

Injector Program		
Rate	Temp	hold
Initial	105	0.10
14.5	310	3.00
14.5	310	30.0

S.I. 1B Monitored ions and retention times for the analysis of DL-PCBs and NDL-PCBs

Type	DL&NDL-PCB	Segment	RT	Cl	^{12}C m/z 1	^{12}C m/z 2	^{13}C m/z 1	^{13}C m/z 2
NDL	PCB 28	1	16.80	3	257.9578	259.9550	269.9981	271.9952
NDL	PCB 52	1	18.40	4	289.9223	291.9194	301.9626	303.9596
	PCB 70*	1	21.58	4	289.9223	291.9194	301.9626	303.9596
	PCB 60*	1	22.85	4	289.9223	291.9194	301.9626	303.9596
NDL	PCB 101	2	23.12	5	323.8834	325.8804	335.9236	337.9207
NO	PCB 81	2	25.04	4	289.9223	291.9194	301.9626	303.9596
	PCB 111*	2	24.67	5	323.8834	325.8804	335.9236	337.9207
NO	PCB 77	2	25.69	4	289.9223	291.9194	301.9626	303.9596
MO	PCB 123	3	26.90	5	323.8834	325.8804	335.9236	337.9207
MO	PCB 118	3	27.09	5	323.8834	325.8804	335.9236	337.9207
MO	PCB 114	3	27.78	5	323.8834	325.8804	335.9236	337.9207
NDL	PCB 153	3	28.32	6	359.8415	361.8385	371.8817	373.8788
	PCB 127*	3	28.67	5	359.8415	361.8385	371.8817	373.8788
MO	PCB 105	3	28.67	5	323.8834	325.8804	335.9236	337.9207
NDL	PCB 138	3	29.90	6	359.8415	361.8385	371.8817	373.8788
NO	PCB 126	3	30.54	5	323.8834	325.8804	335.9236	337.9207
IS_{Syr}	PCB 159	3	30.84	6			371.8817	373.8788
MO	PCB 167	3	31.48	6	359.8415	361.8385	371.8817	373.8788
MO	PCB 156	4	32.69	6	359.8415	361.8385	371.8817	373.8788
MO	PCB 157	4	32.93	6	359.8415	361.8385	371.8817	373.8788
NDL	PCB 180	4	33.46	7	393.8025	395.7995	405.8427	407.8398
NO	PCB 169	4	34.60	6	359.8415	361.8385	371.8817	373.8788
	PCB 170*	4	34.89	7	359.8415	361.8385	371.8817	373.8788
MO	PCB 189	4	36.29	7	393.8025	395.7995	405.8427	407.8398

* Only in calibration standards

S.I. 2a RRF of multiple injections of a standard of 0.02 ng.ml^{-1} native PCBs, standard deviation and LOQ

PCB	1	2	3	4	5	6	7	8	9	10	$\bar{x}$	sd	RSD %	LOQ fg
77	1.28	1.09	1.36	0.80	0.89	1.11	1.21	1.25	1.16	1.29	1.14	0.18	16	95
81	1.10	1.07	1.32	1.65	1.22	1.30	1.21	1.55	1.27	1.33	1.30	0.18	14	83
126	1.18	1.09	1.14	1.37	1.14	1.08	1.33	1.11	1.20	1.03	1.17	0.11	9	56
169	1.21	1.08	1.64	1.37	1.35	1.31	1.01	1.32	1.25	1.21	1.28	0.17	14	81
105	1.21	1.51	1.02	0.75	1.09	1.02	1.24	1.08	1.08	1.00	1.10	0.20	18	108
114	1.13	1.20	1.08	1.36	1.33	1.15	1.04	1.14	1.12	1.05	1.16	0.11	9	57
118	1.09	1.26	1.15	1.36	1.18	1.16	0.97	1.06	1.16	1.23	1.16	0.11	9	56
123	1.36	1.05	1.70	1.49	1.34	1.24	1.16	1.33	1.21	0.71	1.26	0.26	21	126
156	1.43	1.41	0.85	1.46	1.14	1.27	1.05	0.99	1.10	0.93	1.16	0.22	19	114
157	1.01	0.78	1.06	1.32	1.08	1.19	1.23	1.28	1.00	1.09	1.10	0.16	14	87
167	1.24	1.36	1.42	0.74	1.26	1.01	1.30	1.19	1.24	0.79	1.16	0.23	20	120
189	1.04	0.91	1.19	1.77	1.14	0.94	1.32	1.32	1.09	1.34	1.21	0.25	21	125
28	1.47	1.76	1.44	1.88	1.82	1.50	1.24	1.69	1.67	1.58	1.61	0.20	12	73
52	1.34	1.18	1.06	0.90	1.34	1.19	0.85	1.13	1.50	1.01	1.15	0.20	18	106
101	1.13	1.35	1.17	1.67	1.45	1.28	1.47	1.00	1.67	1.32	1.35	0.22	16	98
138	1.86	1.24	1.16	1.26	1.51	0.92	0.93	1.06	1.03	1.10	1.21	0.29	24	144
153	1.26	1.26	1.53	1.54	1.20	0.79	1.03	0.89	1.43	1.38	1.23	0.26	21	126
180	1.50	1.46	1.65	1.91	1.04	0.99	1.50	1.18	1.61	1.07	1.39	0.31	22	132

S.I. 2b Area of multiple injections of a standard of 0.02 ng.ml^{-1} native PCBs, standard deviation and LOQ (fg)

PCB	1	2	3	4	5	6	7	8	9	10	$\bar{x}$	sd	RSD %	LOQ fg
77	8.53E+3	4.47E+3	5.22E+3	2.12E+3	3.90E+3	4.67E+3	5.01E+3	5.33E+3	4.43E+3	4.46E+3	4.81E+3	1.59E+3	33	198
81	7.06E+3	4.28E+3	4.99E+3	4.09E+3	5.31E+3	5.33E+3	4.75E+3	6.19E+3	4.65E+3	4.46E+3	5.11E+3	9.17E+2	18	108
126	7.56E+3	4.48E+3	4.30E+3	3.29E+3	5.11E+3	4.37E+3	5.33E+3	4.56E+3	4.32E+3	3.46E+3	4.68E+3	1.19E+3	25	153
169	6.72E+3	4.71E+3	6.95E+3	3.21E+3	6.35E+3	5.95E+3	4.45E+3	5.54E+3	4.40E+3	3.87E+3	5.22E+3	1.27E+3	24	146
105	7.18E+3	5.31E+3	3.67E+3	1.68E+3	4.30E+3	3.73E+3	4.44E+3	4.40E+3	3.75E+3	3.14E+3	4.16E+3	1.43E+3	34	206
114	6.71E+3	4.22E+3	3.88E+3	3.08E+3	5.25E+3	4.19E+3	3.75E+3	4.67E+3	3.90E+3	3.28E+3	4.29E+3	1.06E+3	25	148
118	4.10E+4	2.80E+4	2.63E+4	1.94E+4	2.98E+4	2.68E+4	2.20E+4	2.55E+4	2.49E+4	2.36E+4	2.67E+4	5.83E+3	22	131
123	7.62E+3	3.70E+3	5.89E+3	3.19E+3	5.23E+3	4.43E+3	4.18E+3	5.02E+3	3.97E+3	2.03E+3	4.53E+3	1.54E+3	34	204
156	8.20E+3	5.74E+3	3.62E+3	3.43E+3	5.48E+3	5.76E+3	4.53E+3	4.28E+3	3.90E+3	3.02E+3	4.80E+3	1.54E+3	32	193
157	5.79E+3	3.23E+3	4.50E+3	3.02E+3	5.32E+3	5.35E+3	5.47E+3	5.50E+3	3.45E+3	3.52E+3	4.52E+3	1.10E+3	24	146
167	7.44E+3	5.93E+3	5.84E+3	1.80E+3	5.92E+3	4.46E+3	5.51E+3	5.11E+3	4.43E+3	2.60E+3	4.90E+3	1.67E+3	34	205
189	4.62E+3	3.69E+3	4.61E+3	3.45E+3	4.67E+3	3.95E+3	5.10E+3	4.79E+3	3.54E+3	3.95E+3	4.24E+3	5.87E+2	14	83
28	6.00E+3	5.25E+3	4.20E+3	3.97E+3	5.63E+3	4.85E+3	3.77E+3	5.13E+3	4.40E+3	3.82E+3	4.70E+3	7.88E+2	17	101
52	5.58E+3	3.92E+3	3.65E+3	2.00E+3	4.61E+3	4.17E+3	3.05E+3	3.56E+3	4.46E+3	2.65E+3	3.76E+3	1.03E+3	27	165
101	5.17E+3	3.83E+3	3.60E+3	3.06E+3	4.51E+3	3.92E+3	4.47E+3	2.90E+3	4.47E+3	3.21E+3	3.91E+3	7.39E+2	19	113
138	8.53E+3	3.87E+3	3.83E+3	2.27E+3	4.95E+3	2.97E+3	3.20E+3	3.59E+3	3.25E+3	3.33E+3	3.98E+3	1.74E+3	44	263
153	5.82E+3	4.09E+3	5.30E+3	3.13E+3	4.06E+3	2.63E+3	3.33E+3	2.88E+3	4.22E+3	3.70E+3	3.92E+3	1.02E+3	26	157
180	4.96E+3	4.25E+3	4.61E+3	3.09E+3	3.62E+3	3.22E+3	4.50E+3	3.71E+3	3.99E+3	2.46E+3	3.84E+3	7.74E+2	20	121