APPLICATION NOTE 22136

Resolving critical PCB isomers (28/31 and 128/167) using the Trace TR-PCB 8MS column

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Goal

Following Council Directive 96/59/EC, regulating the disposal of polychlorinated biphenyls (PCBs) and polychlorinated terphenyls (PCTs), Commission Regulation (EU) 2017/771 describes the analytical methods for the determination of the levels of dioxins and polychlorinated biphenyls. Although there are 209 known PCB congeners, only a few need to be individually quantified due to their toxicity to humans and their environmental impact. All 209 PCB masses are detectable at only 10 different characteristic masses, according to the number of chlorines present in their structure. Thus, it is necessary to discriminate each PCB by a specific retention time in order to separate those congeners of major concern.

Through a close collaboration with a well-known Contract Testing Lab (CTL), a rapid GC-MS/MS method has been developed for providing the appropriate chromatographic separation of targeted PCB congeners and interferent, especially most critical pairs, like PCB28/31 and PCB128/167. We demonstrate here the resolving power of the Thermo Scientific™ TRACE™ TR-PCB 8MS column, while the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system with advanced electron ionization (AEI) source enables achievement of the limit of quantitation set by the EU Regulation.

Introduction

PCBs released in the environment are very resistant to degradation. Because they persist in air, water, and soil, PCBs tend to spread through the food chain and accumulate in all living organisms. In humans, due to lipophilicity, they mostly accumulate in the fat tissue, blood, and milk.

According to WHO[1], the most toxic PCB congeners are 77, 81, 126, and 169 but these are rarely reported in

environmental samples. PCB 114, 123, 157, 167, and 189 have been rarely reported but have a moderately high toxicity. PCB 138, 153, and 180 are the most dominant. PCB 105, 118, 156, 179, and 180 make up about 20% of the total PCBs reported in animal tissue and persistent in humans, with PCB 153 prevalent in human milk.[2] PCB 28, 52, 101, 138, 153, and 180 are the "indicators" and represent 80% of the found PCBs because of their large contribution in Aroclor commercial mixtures.

The extraction technique to use depends on the matrix. Transformer oil or waste oil is diluted with solvent. Wastewater, sediment, or biological samples are solvent extracted, followed by a concentration step. SPE and SPME are sometimes used. Samples prepared by any approaches can be analyzed on the same gas chromatography (GC) column.

Regulation

Reporting Toxic Equivalency (TEQ)

Requirements of Commission Regulation (EU) 2017/771 are that the sum of PCB 28, 52, 101, 138, 153, and 180, referred to as non-dioxin-like PCB, does not exceed the maximum level laid down by Council Directive 2002/32/EC. The concentrations of the individual substances should be multiplied by their respective Toxic Equivalency Factor (TEF; see Table 1) and subsequently summed to give the total concentration of dioxin-like expressed as TEQ.

Table 1. Regulated and targeted PCBs

CAS	TEF	PCB ID	SRM transition (*m/z* → *m/z* @eV)	RT (min) multiresidue method	RT (min) TR-PCB 8MS column
37680-65-2		PCB 18	256 → 186 @22	7.49	6.77
15968-05-5		PCB 54	292 → 220 @26	7.75	7.15
16606-02-3		PCB 31	256 → 186 @22	7.87	7.42
7012-37-5		**PCB 28**	256 → 186 @22	7.87	7.49
35693-99-3		**PCB 52**	292 → 220 @26	8.2	7.84
37680-69-6		PCB 35	256 → 186 @22	8.36	8.29
41464-39-5		PCB 44	292 → 220 @26	8.39	8.19
37680-73-2		**PCB 101**	326 → 256 @26	9.13	9 .00
70362-50-4	0.0003	**PCB 81**	326 → 256 @26	9.42	9.37
32598-13-3	0.0001	**PCB 77**	292 → 220 @26	8.86	9.58
65510-44-3	0.00003	**PCB 123**	326 → 256 @26	9.88	9.97
38380-04-0		PCB 149	360 → 290 @26	9.88	9.77
31508-00-6	0.00003	**PCB 118**	326 → 256 @26	9.91	10.03
74472-37-0	0.00003	**PCB 114**	326 → 256 @26	10.05	10.17
35065-27-1		**PCB 153**	360 → 290 @26	10.22	10.21
32598-14-4	0.00003	**PCB 105**	326 → 256 @26	10.26	10.47
35065-28-2		**PCB 138**	360 → 290 @26	10.59	10.67
57465-28-8	0.1	**PCB 126**	326 → 256 @26	10.71	11.06
38380-07-3		PCB 128	360 → 290 @26	10.97	11.14
52663-72-6	0.00003	**PCB 167**	360 → 290 @26	11.00	11.23
38380-08-4	0.00003	**PCB 156**	360 → 290 @26	11.32	11.75
69782-90-7	0.00003	**PCB 157**	360 → 290 @26	11.41	11.86
35065-29-3	0.00001	**PCB 180**	394 → 324 @24	11.57	11.85
32774-16-6	0.03	**PCB 169**	360 → 290 @26	11.88	12.62
35065-30-6	0.0001	**PCB 170**	394 → 324 @24	12.00	12.51
39635-31-9	0.00003	**PCB 189**	394 → 324 @24	12.44	13.43
35694-08-7		PCB 194	430 → 359 @25	12.98	14.13
2051-24-3		PCB 209	498 → 428 @26	13.98	14.75

Most toxic congeners
Highly toxic congeners
Moderatly high toxicity
Indicators congeners

PCBs with same *m/z* and close RT

Required sensitivity

Commission Regulation (EU) 2017/771 specifies that for most PCBs, a limit of quantitation in the nanogram range (10^{-9} g) is already sufficient. For the measurement of the more toxic PCBs, the low picogram (10^{-12} g) should be reached.

Strategy

In high productivity laboratories, the habit is to monitor all contaminant classes through a multiresidue screening approach. Those analyses, often performed on a traditional "5% phenyl column" (5 MS type or equivalent), do not allow proper identification of specific pairs of PCB isomers (PCB 28/31 and PCB 128/167). For this experiment, the TRACE TR-PCB 8MS capillary GC column was used for this confirmation method of analytes of interest because it is specifically designed to give unique separation of PCB congeners. The run time of our multiresidue screening method, including PCBs, PAHs, and pesticides, has been established with a total run time of 16 minutes. The retention times of PCBs in the multiresidue method and in the PCB specific method are given in Table 1.

Only if a peak appears at the critical retention time with the same SRM transitions in the multiresidue method does the sample need to be re-analyzed on the TRACE TR-PCB 8MS column for confirmation. Appropriate chromatographic separation of critical congeners can be achieved with the last peak of PCB 209 eluted at 14.75 minutes. The resolutions and RTs of PCB 31/28 and PCB 128/167 are shown in Figures 1 and 2, respectively.

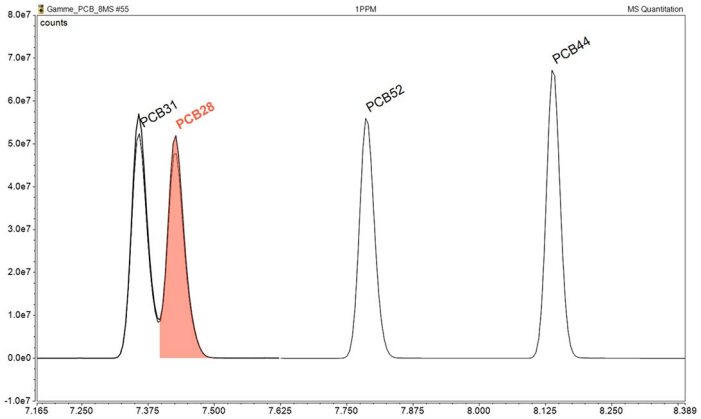

Figure 1. PCB 31 was eluted at 7.36 min; the chromatographic resolution using the EP formula is R=1.22; PCB 28 was eluted at 7.43 min.

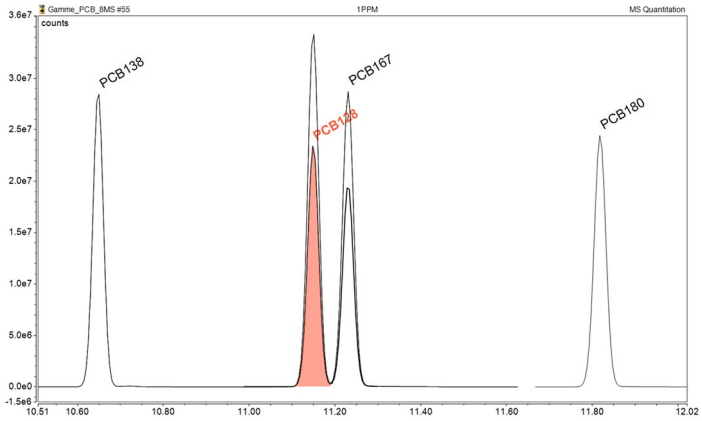

Figure 2. PCB 128 was eluted at 11.14 min; the chromatographic resolution using the EP formula is R=1.6; PCB 167 was eluted at 11.23 min.

Method

Gas chromatography method

- Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with SSL injector
- TRACE TR-PCB 8MS column, 50 m × 0.25 mm i.d. × 0.25 µm (P/N 26AJ148P)
- Thermo Scientific™ LinerGOLD™ Splitless liner, (P/N 453A1925-UI)

Parameter	Value
Injection volume	1 µL (Acetonitrile)
Injector temperature	300 °C
Splitless time	1 min
Column flow	2 mL/min (Helium)
GC oven program	150 °C, 1 min 20 °C/min to 250 °C, 5.71 °C/min to 268 °C, 30 °C/min to 310 °C, 4 min 15 °C/min to 350°C, 0.44 min (total run time 16 min)

Mass spectrometry method

- TSQ 9000 triple quadrupole with AEI source (300 °C)
- Electron ionization mode 70 eV
- SRM acquisition mode. The quantitation SRM transitions are given in Table 1.

Linearity and sensitivity

1 µL of each calibrant of 0.1, 1, 10, 100, and 1000 pg/µL in ACN gave a linear response for all PCBs mentioned in Table 1. Linearity of external calibration of PCB 209 is given as an example in Figure 3.

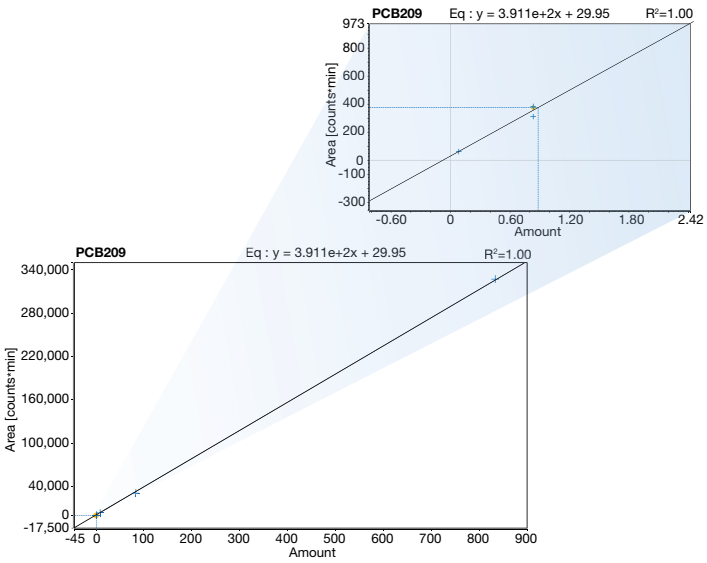

Figure 3. Five points calibration of PCB 209 from 0.1 to 1000 pg/µL with R^2=1.000

The detection limits of all PCBs were below 0.1 pg injected on column. This is comfortably in accordance to Commission Regulation (EU) 2017/771 guidelines of the low picogram (10^{-12} g) for the more toxic PCBs.

The peak area repeatability was determined by three replicate injections at the level of 1 pg on column. The results showed %RSD from 4.2% for PCB 28 to 11% for PCB 209. Examples of SRM chromatograms for PCB 209 (heaviest), PCB 167 (medium), and PCB 28 (light) are shown in Figures 4–6.

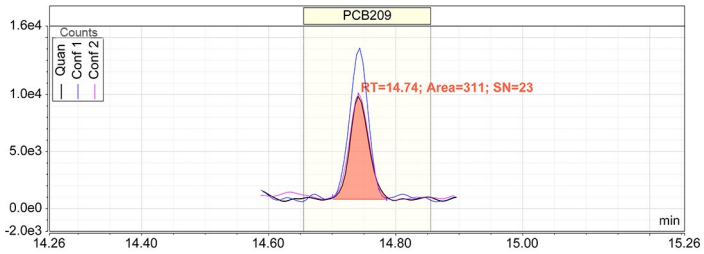

Figure 4. Overlap of three SRM transitions of PCB 209 at 1 pg on column, RSD 11%

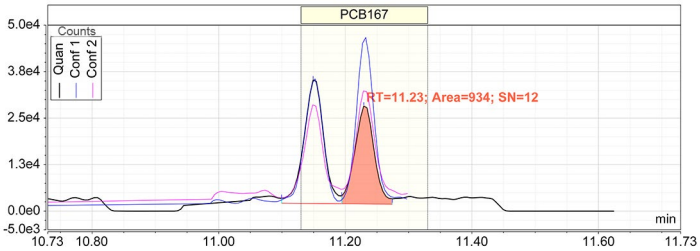

Figure 5. Overlap of three SRM transitions of PCB 167 at 1 pg on column, RSD 4.6%

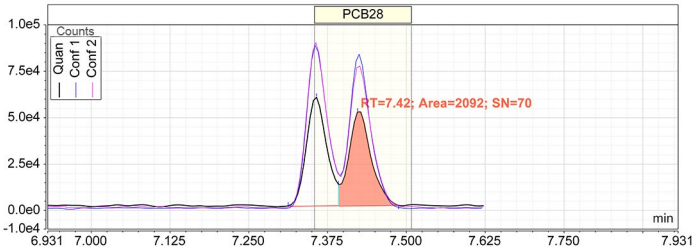

Figure 6. Overlap of three SRM transitions of PCB 28 at 1 pg on column, RSD 4.2%

Conclusions

Combining the advantages of the TRACE TR-PCB 8MS column to separate the congeners, particularly PCB 28 from PCB 31 and PCB 128 from PCB 167, which are not separated with the widely used 5MS-Type column, and the high sensitivity of the AEI source of the TSQ 9000 triple quadrupole GC-MS/MS, the 28 targeted PCB congeners at <0.1 pg on column can be determined in 16 min with excellent linearity range from 0.1 to 1000 pg/µL.

References

1. WHO, International Program on Chemical Safety, Dioxins and dioxin-like substances. https://www.who.int/ipcs/assessment/public_health/dioxins/en/
2. Humphrey, H.L. et al. PCB congener profile in the serum of humans consuming Great Lakes fish. *Environ. Health Perspect.* **2000,** *108,* 167–172. doi: 10.1289/ehp.00108167

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