TN-2097

Robust Analysis of Dioxins in Fish Oil Samples by GC-MS/MS on a Zebron™ ZB-Dioxin GC Column

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Introduction

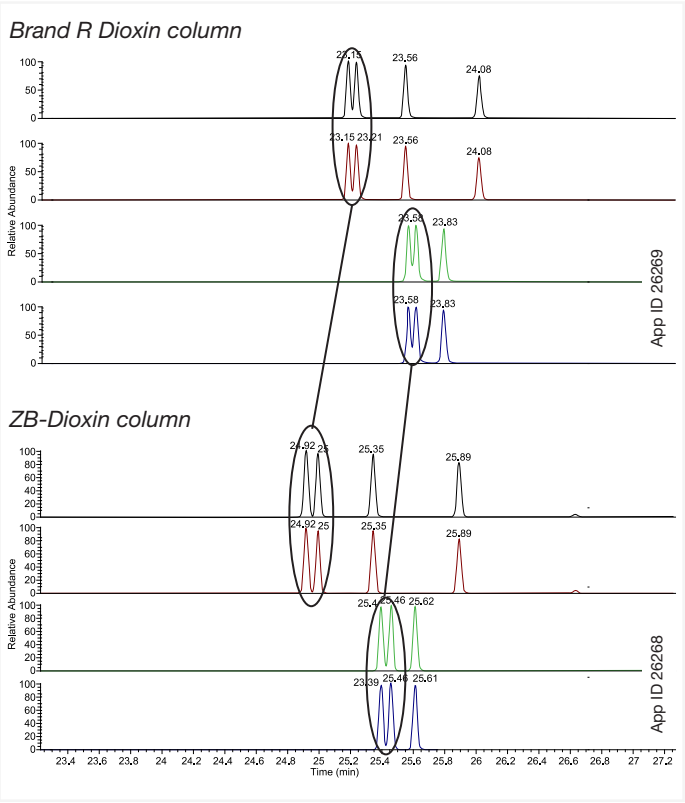
Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/F), along with polychlorinated biphenyls (PCBs), constitute a group of highly toxic organic compounds. While PCBs are no longer manufactured, both PCB and PCDD/F are often formed unintentionally as by-products of waste combustion and some industrial manufacturing processes (e.g. production of chlorinated pesticides). These pollutants fall into the category of persistent organic pollutants (POPs) which are compounds that are resistant to environmental degradation, bioaccumulate in the food chain and are toxic to humans and wildlife. PCBs and PCDD/F accumulate in the fatty tissue of animals and can be found in fatty foods such as meat, fish, eggs, and other dairy products. European guidelines have demanding regulatory limits for PCBs, and PCDD/F in food, feed, animal and fish products. Additionally, there has been an increased need for the testing of fish oil capsules to ensure safe levels of the aforementioned POPs in omega-3 and omega-6 supplements.

More than 90% of human exposure to dioxins and dioxin-like substances is through food. With progressively lower regulatory limits and decreasing PCDD/F levels in food, feed, and tissues, more demanding limits of detection, selectivity, and robustness are necessary in a high-throughput lab. The gold-standard for analysis of PCDD/F and PCB is GC/HRMS. Since 2014 (regulations (EU) No 589/2014 and (EU) No 709/2014), GC-MS/MS measurements can be used to confirm compliance with maximum levels. Analytical quality assurance measures are detailed in the regulations, stipulating that adequate blanks, spike recoveries, and reference materials are employed to ensure the accuracy of measurements. In the United States, the Food and Drug Administration (FDA) has been concerned about PCDD/F exposure and has been monitoring specific foods with the goal of identifying ways to reduce dietary exposure. Since 1995, the FDA has been monitoring dioxin levels in finfish, shellfish, and dairy products. In 1999, they initiated dioxin analysis of foods collected under its Total Diet Study (TDS) survey, which is an annual market basket survey covering 265 ready-to-eat core foods. The TDS survey is used to assess PCDD/F levels in various pesticide residues, nutrients and other contaminants. It is also used to estimate intakes of these substances in diets. Since 2002, the FDA has expanded its monitoring program to obtain more comprehensive data on background levels, as well as to identify opportunities to decrease human exposure to dioxins. Laboratories involved with dioxin analysis must often deal with a variety of sample matrices. A key factor for these laboratories is maintaining instrument uptime, column robustness and maintaining resolution of critical pairs. In this technical note we explore the robustness of the Zebron ZB-Dioxin column when analyzing dioxin presence in fish oil samples.

GC-MS/MS Conditions

Column: Zebron ZB-Dioxin
Dimensions: 60 meter x 0.25 mm x 0.20 µm
Part No.: 7KG-G045-10
Injection: Splitless @ 230 °C, 1 µL
Recommended Liner: Zebron PLUS Liner Compatible with Agilent & Thermo, 4mm ID Single Taper Wool on Bottom
Liner Part No.: AG2-0A11-05
Carrier Gas: Helium @ 1.3 mL/min (constant flow)
Oven Program: 90 °C for 1 min to 235 °C @ 40 °C/min to 240 °C @ 2 °C/ min to 246 °C @ 0.5 °C/min to 260 °C @ 6 °C/min to 330 °C @ 25 °C/min for 8 mins
Detector: GC-MS/MS
Transfer Line Temperature: 230 °C

Figure 1.
Improved Resolution of a Hexachloro Dibenzo Dioxin (1,2,3,7,8,9-HxCDD) standard on a Brand R Dioxin column versus the ZB-Dioxin.



Comparative separations may not be representative of all applications.

Table1.
SRM Transitions for Dioxins and Furans on ZB-Dioxin GC Column

Analyte	RT Start (min)	Ion Polarity	Window	Parent Ion Mass	Product ion Mass	Collision Energy (eV)	Dwell Time Priority
C13-TCDF	18.00	Positive	7	315.9	251.9	25	Normal
C13-1,2,3,4-TCDD	18.00	Positive	7	331.9	267.9	15	Normal
C13-2,3,7,8-TCDD	18.00	Positive	7	331.9	267.9	17	Normal
C13-1,2,3,4-TCDD	18.00	Positive	7	333.9	269.9	15	Normal
C13-2,3,7,8-TCDD	18.00	Positive	7	333.9	269.9	17	Normal
2,3,7,8-TCDF	18.01	Positive	7	303.9	240.9	23	High
2,3,7,8-TCDF	18.01	Positive	7	305.9	242.9	23	High
C13-TCDF	18.01	Positive	7	317.9	253.9	25	Normal
2378-TCDD	18.01	Positive	7	319.9	256.9	16	High
2378-TCDD	18.01	Positive	7	321.9	258.9	16	High
FC 43	20.00	Positive	20	414	264	10	Normal
C13-1,2,3,7,8-PeCDF	22.00	Positive	5	349.9	285.9	24	Normal
C13-2,3,4,7,8-PeCDF	22.00	Positive	5	349.9	285.9	24	Normal
C13-1,2,3,7,8-PeCDF	22.00	Positive	5	351.9	287.9	24	Normal
C13-2,3,4,7,8-PeCDF	22.00	Positive	5	351.9	287.9	24	Normal
C13-1,2,3,7,8-PeCDD	22.00	Positive	5	365.9	301.9	15	Normal
C13-1,2,3,7,8-PeCDD	22.00	Positive	5	367.9	303.9	15	Normal
1,2,3,7,8-PeCDF	22.01	Positive	5	339.9	276.9	23	High
2,3,4,7,8-PeCDF	22.01	Positive	5	339.9	276.9	25	High
1,2,3,7,8-PeCDF	22.01	Positive	5	341.9	278.9	23	High
2,3,4,7,8-PeCDF	22.01	Positive	5	341.9	278.9	25	High
1,2,3,7,8-PeCDD	22.01	Positive	5	353.9	290.9	17	High
1,2,3,7,8-PeCDD	22.01	Positive	5	355.9	292.9	17	High
C13-1,2,3,7,8,9-HxCDF	25.00	Positive	5	385.9	321.9	24	Normal
C13-1,2,3,7,8,9-HxCDF	25.00	Positive	5	387.9	323.9	24	Normal
C13-1,2,3,4,7,8-HxCDD	25.00	Positive	5	401.9	337.9	16	Normal
C13-1,2,3,7,8,9-HxCDD	25.00	Positive	5	401.9	337.9	16	Normal
C13-1,2,3,4,7,8-HxCDD	25.00	Positive	5	403.9	339.9	16	Normal
C13-1,2,3,7,8,9-HxCDD	25.00	Positive	5	403.9	339.9	16	Normal
C13-1,2,3,4,7,8-HxCDF	25.50	Positive	5	385.9	321.9	25	Normal
C13-1,2,3,6,7,8-HxCDF	25.50	Positive	5	385.9	321.9	25	Normal
C13-2,3,4,6,7,8-HxCDF	25.50	Positive	5	385.9	321.9	25	Normal
C13-1,2,3,4,7,8-HxCDF	25.50	Positive	5	387.9	323.9	25	Normal
C13-1,2,3,6,7,8-HxCDF	25.50	Positive	5	387.9	323.9	25	Normal
C13-2,3,4,6,7,8-xCDF	25.50	Positive	5	387.9	323.9	25	Normal
C13-1,2,3,6,7,8-HxCDD	25.50	Positive	5	401.9	337.9	16	Normal
C13-1,2,3,6,7,8-HxCDD	25.50	Positive	5	403.9	339.9	16	Normal
1,2,3,4,7,8-HxCDF	25.51	Positive	5	373.9	310.9	24	Normal
1,2,3,6,7,8-HxCDF	25.51	Positive	5	373.9	310.9	24	Normal
1,2,3,7,8,9-HxCDF	25.51	Positive	5	373.9	310.9	24	Normal
2,3,4,6,7,8-HxCDF	25.51	Positive	5	373.9	310.9	24	Normal
1,2,3,4,7,8-HxCDF	25.51	Positive	5	375.9	312.9	24	Normal
1,2,3,6,7,8-HxCDF	25.51	Positive	5	375.9	312.9	24	Normal
1,2,3,7,8,9-HxCDF	25.51	Positive	5	375.9	312.9	24	Normal
2,3,4,6,7,8-HxCDF	25.51	Positive	5	375.9	312.9	24	Normal
1,2,3,4,7,8-HxCDD	25.51	Positive	5	389.8	326.9	17	Normal
1,2,3,6,7,8-HxCDD	25.51	Positive	5	389.8	326.9	17	Normal
1,2,3,7,8,9-HxCDD	25.51	Positive	5	389.8	326.9	16	Normal
1,2,3,4,7,8-HxCDD	25.51	Positive	5	391.9	328.9	17	Normal
1,2,3,6,7,8-HxCDD	25.51	Positive	5	391.9	328.9	17	Normal
1,2,3,7,8,9-HxCDD	25.51	Positive	5	391.9	328.9	16	Normal
C13-1,2,3,4,6,7,8-HpCDF	28.50	Positive	5	419.8	355.8	23	Normal
C13-1,2,3,4,7,8,9-HpCDF	28.50	Positive	5	419.8	355.8	23	Normal
C13-1,2,3,4,6,7,8-HpCDF	28.50	Positive	5	421.8	357.8	23	Normal
C13-1,2,3,4,7,8,9-HpCDF	28.50	Positive	5	421.8	357.8	23	Normal
C13-1,2,3,4,7,8,9-HpCDD	28.50	Positive	5	435.8	371.8	15	Normal

Table1.
SRM Transitions for Dioxins and Furans on ZB-Dioxin GC Column (cont'd)

Analyte	RT Start (min)	Ion Polarity	Window	Parent Ion Mass	Product ion Mass	Collision Energy (eV)	Dwell Time Priority
C13-1,2,3,4,7,8,9-HpCDD	28.50	Positive	5	437.8	373.8	15	Normal
1,2,3,4,6,7,8-HpCDF	28.51	Positive	5	407.8	344.8	24	Normal
1,2,3,4,7,8,9-HpCDF	28.51	Positive	5	407.8	344.8	22	Normal
1,2,3,4,6,7,8-HpCDF	28.51	Positive	5	409.8	346.8	24	Normal
1,2,3,4,7,8,9-HpCDF	28.51	Positive	5	409.8	346.8	22	Normal
1,2,3,4,6,7,8-HpCDD	28.51	Positive	5	423.8	360.8	15	Normal
1234678HpCDD	28.51	Positive	5	425.8	362.8	15	Normal
C13-OCDD	30.00	Positive	6	469.8	405.8	15	Normal
C13-OCDD	30.00	Positive	6	471.8	407.7	15	Normal
OCDF	30.01	Positive	6	441.75	378.8	23	Normal
OCDF	30.01	Positive	6	443.75	380.8	23	Normal
OCDD	30.01	Positive	6	457.75	394.8	14	Normal
OCDD	30.01	Positive	6	459.75	396.8	14	Normal

Results and Discussion

Dioxin and furan separation is extremely challenging due to the number of isomeric compounds present. Therefore, it is essential to achieve optimal analyte selectivity. Although MS/MS transitions can provide separation of coeluting analytes, in dioxin analysis, there are multiple congeners that have similar fragmentation patterns and vapor pressures. This highlights the importance of chromatographic separation in the analysis of dioxins. Tetra through octa dioxins and furans were run on ZB-Dioxin GC column and on a commercially available Brand R dioxin column. A hexachlorodibenzo-p-dioxin (HxCDD) standard was run in this experiment. The ZB-Dioxin column provided improved resolution of critical peak 1,2,3,7,8,9-HxCDD from its neighboring hexa isomer (**Figure 1**). Due to the optimal selectivity of the ZB-Dioxin GC column, run times as short as 35 minutes were achievable without compromising the resolution of the critical pairs.

In order to confirm the robustness of the ZB-Dioxin column, 112 injections of fish oil samples were analyzed on the same column. The fish oil samples are a heavy matrix with a lot of high boiling contamination. Sample preparation was conducted on acid silica to collect the dioxin and furan isomers. Even after acid silica, the matrix is still considered a very strong source of interference. As such, this extract served as a high matrix interference sample to confirm the robustness of the GC separation of PCDD/F. Tetra through Octa dioxins and furans were spiked into the fish oil matrix to conduct this study. Three specific dioxins were chosen to evaluate the results of this study: early-eluting dioxin 2,3,7,8-TCDD, mid-eluting dioxin 1,2,3,7,8,9-HxCDD and late-eluting dioxin OCDD. They were evaluated for retention time and peak area consistency over 112 sample injections of fish oil extract samples.

ZB-Dioxin GC column provided reproducibility for retention times and consistency for peak area of the dioxin compounds. Evidence of reproducibility can be seen from the retention time data shown over the 112 injections (**Figure 2**). Interestingly, the individual peak areas of these three dioxins decreased with consecutive injections of fish oil matrix. This is an indication of mass spectrometer ion source contamination. Based on the experimental data, when the MS source was cleaned, the peak areas increased again (**Figure 3**). Although the absolute peak area changed between injections, there was negligible change in retention time over the course of the injections. **Figures 4**, **5** and **6** show the overlay of chromatograms for 2,3,7,8-TCDD, 1,2,3,7,8,9-HxCDD, and OCDD before and after source cleaning. While the three probe analytes are presented to simplify data analysis, all of the tetra through octa dioxins and furans tested showed the same pattern of peak area dropping after continuous injections and returning to normal after source cleaning (**Figure 7**). As seen previously, the retention times of each dioxin remained constant throughout all of the injections (**Figure 8**). Even though there was a drop in peak area with increasing number of injections, the percent recovery for almost all dioxins was close to 100 % or greater. This held true even after the source was cleaned (**Figure 9**). Such consistent and robust retention times are possible due to the intact polymer that is extensively crosslinked through Engineered Self Crosslinking™ (ESC™ in the ZB-Dioxin GC column. The proprietary stationary phase provides enhanced resolution for critical dioxin and furan isomers while the ESC provides intact stationary phase for reproducible dioxin analysis even with challenging matrices like fish oil.

Figure 2.
Retention times for 2,3,7,8-TCDD, 1,2,3,7,8,9-HxCDD and OCDD after 112 injections of fish oil samples on a Zebron ZB-Dioxin GC column.

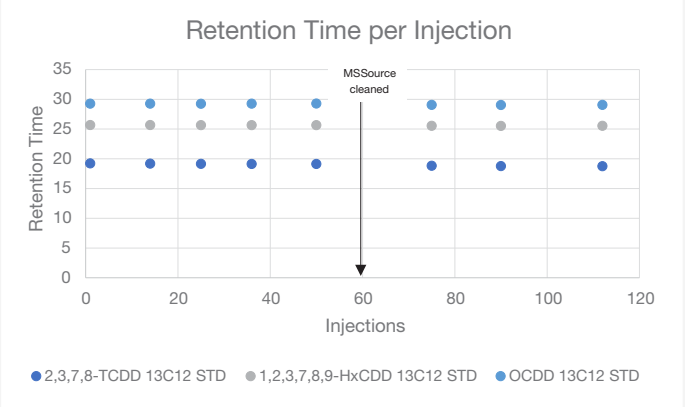


Figure 3.
Peak areas for 2,3,7,8-TCDD, 1,2,3,7,8,9-HxCDD and OCDD standards after 112 injections of fish oil samples on a Zebron ZB-Dioxin GC column.

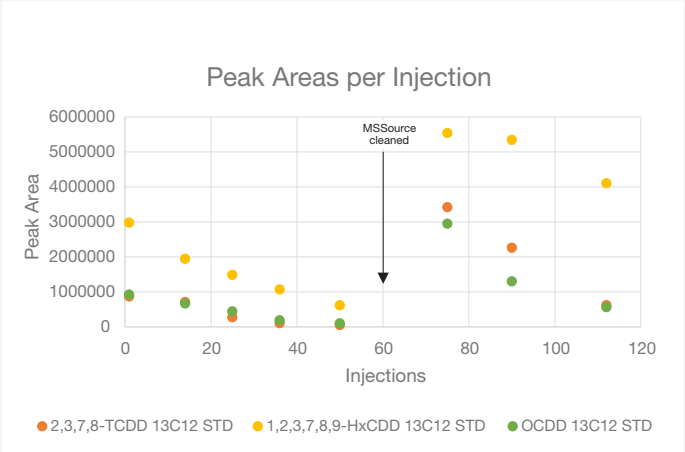


Figure 4. a, b
Peak overlays of 2,3,7,8-TCDD (a) before and (b) after source cleaning on a Zebron ZB-Dioxin GC column.

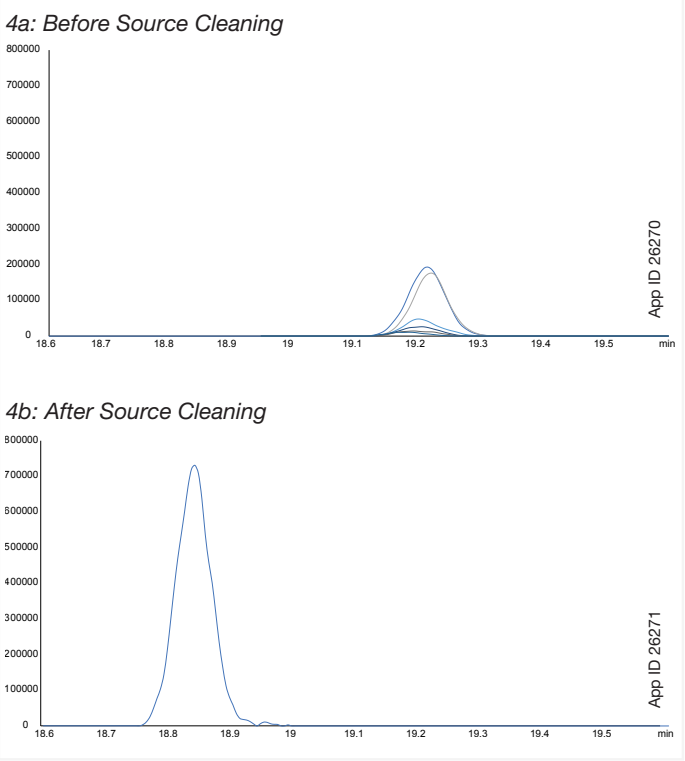


Figure 5: Peak overlays of 1,2,3,7,8,9-HxCDD (a) before and (b) after source cleaning on a Zebron ZB-Dioxin GC column

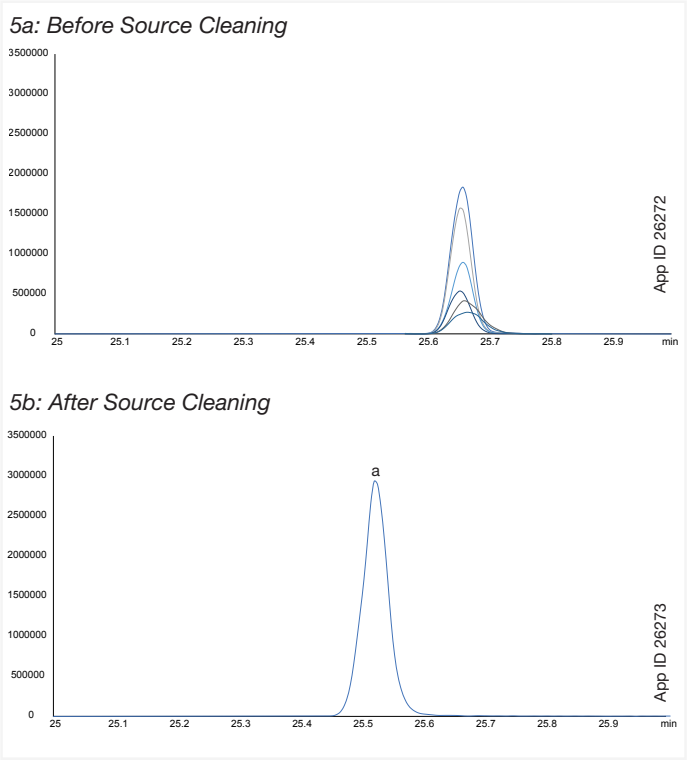
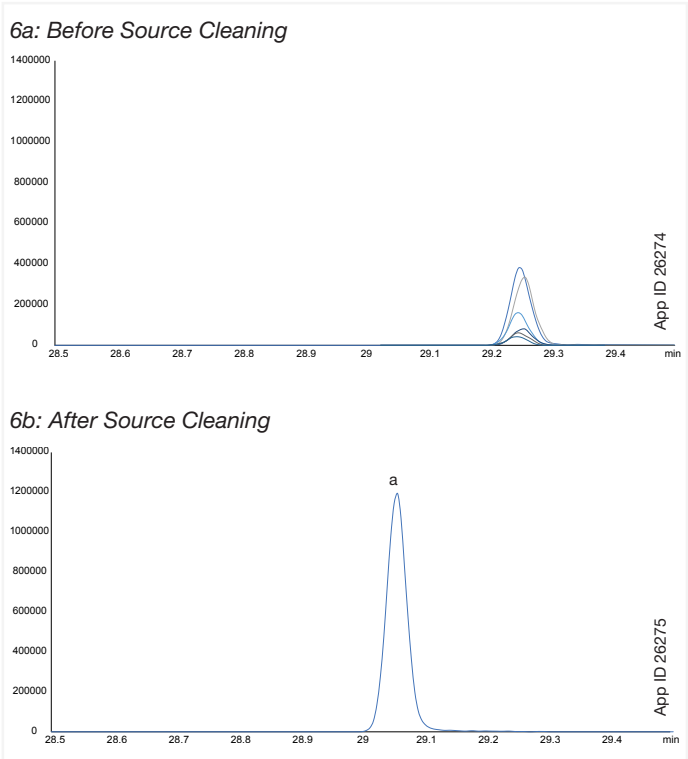


Figure 6: Peak overlays of OCDD (a) before and (b) after source cleaning on a Zebron ZB-Dioxin GC column



Data Courtesy of Pacific Rim Laboratories. Phenomenex is not affiliated with Pacific Rim Laboratories.

Figure 7.
Comparison of peak area of dioxins over multiple injections of spiked fish oil samples on a Zebron ZB-Dioxin GC column.

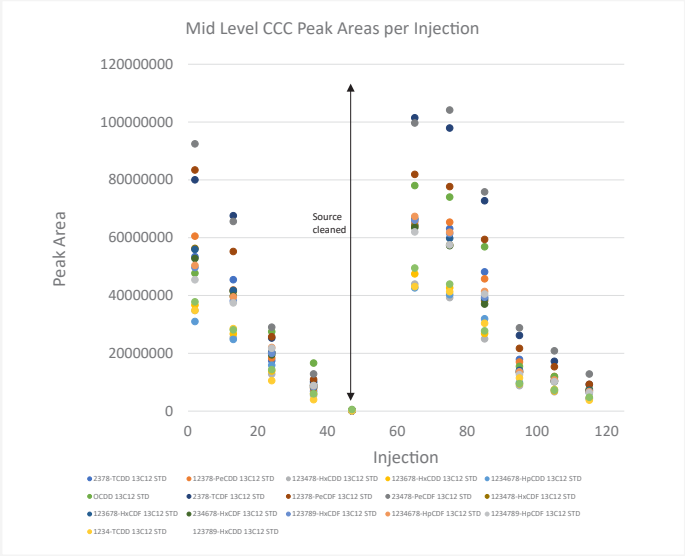


Figure 8.
Comparison of retention times of dioxins over multiple spiked fish oil injections on a Zebron ZB-Dioxin GC column.

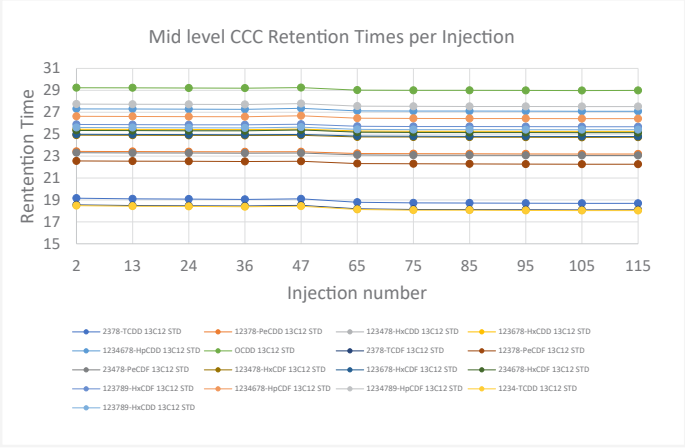
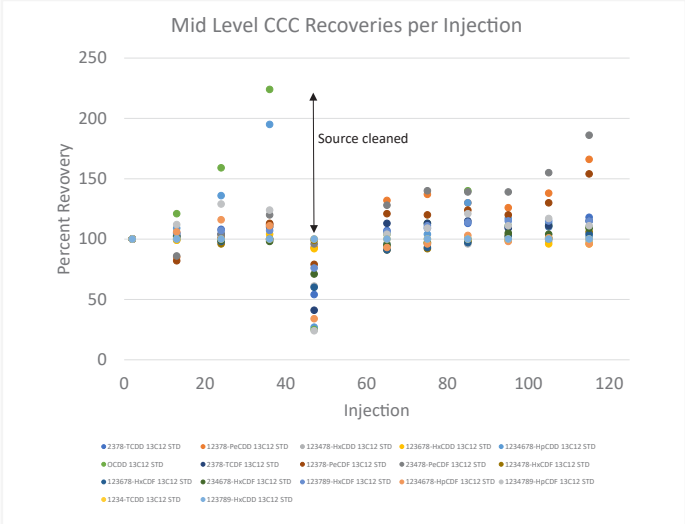


Figure 9.
Comparison of recovery of dioxins per injection of spiked fish oil on a Zebron ZB-Dioxin GC column.



Conclusion

Complex applications like dioxin analysis require enhanced resolution of critical pairs. Achieving a consistent chromatographic profile and reproducible retention times are key aspects of the separation. This study has demonstrated that ZB-Dioxin GC columns provide improved resolution of critical pairs compared to a Brand R Dioxin column. Although there was a loss of peak area after multiple fish oil injections, due to MS source contamination, there was minimal change in retention times for dioxins and furans. Such robust analysis is the result of an intact stationary phase in the ZB-Dioxin GC column and demonstrates that the column maintained robustness in spite of a challenging matrix like fish oil.

Ordering Information

Zebron™ ZB-Dioxin GC Column			
ID(mm)	df(µm)	Temp. Limits °C	Part No.
60-Meter			
0.25	0.20	40 to 320/340	7KG-G045-10
60-Meter with 5-Meter Guardian™			
0.25	0.20	40 to 320/340	7KG-G045-10-GGA
40-Meter			
0.18	0.14	40 to 320/340	7PD-G045-47

Zebron PLUS Liners					
Description	Inlet Style	Dimensions ID x L (mm)	Deactivation	Part No.	Unit
Compatible with Agilent® 5890, 6890 and 7890 Models					
Single taper	S/SL	4 x 78.5	PLUS Inert	AG2-0A10-05	5/pk
Compatible with Agilent® and Thermo Scientific® Models					
Single taper	S/SL	2 x 78.5	PLUS Inert	AG2-0E00-05	5/pk

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