APPLICATIONS

GC-MS/MS analysis of Tetra through Octa Dioxins and Furans on Zebron™ ZB-Dioxin GC column

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He has a PhD in Analytical Chemistry and over 15 years experience in chromatographic method development and troubleshooting. Ramkumar loves to write poems, read Shakespeare, and attend Shakespeare plays.

Introduction

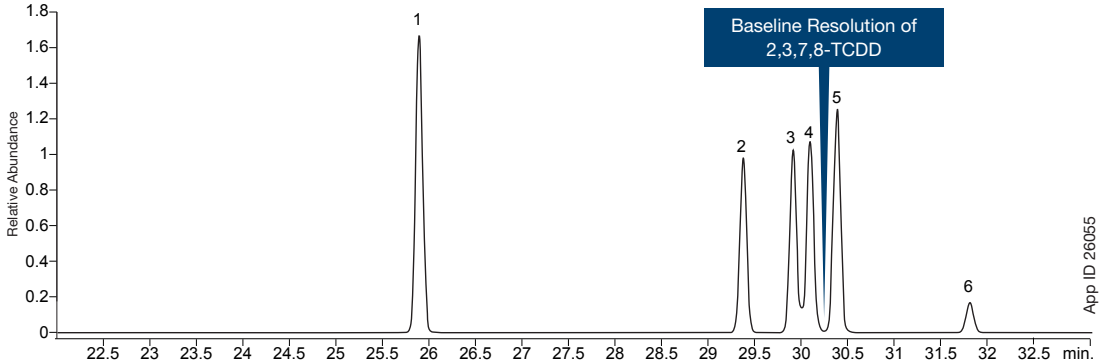
Dioxins are toxic compounds and exhibit stable structure due to the presence of electron withdrawing group Chlorine attached to aromatic ring. Due to the stability of the bond, these analytes remain stable in the environment and falls under Persistent Organic Pollutants (POPs) category. They are continuously monitored in environmental samples. In addition to environmental samples, dioxins also enter into food chain. So, there is a necessity for highly efficient method to monitor these toxic compounds and separate them from matrix. Common method of analysis utilizes a GC-HRMS or a GC-MS/MS to accurately quantitate and separate toxic Dioxins and furans. EPA-1613 and EPA-8290 recommends analysis by GC-HRMS on 2 different GC columns. Presented here is a GC-MS/MS method that utilizes single column to exceed EPA-1613 and EPA-8290 requirements. In addition, Food samples have a lot of interfering components that needs monitoring of parent to daughter ion transition in order to authentically quantitate toxic dioxins by GC-MS/MS.

The method presented utilizes specific isotopically labeled dioxin and furan compounds as internal standard to accurately quantify dioxins and furans. Detailed evaluation of analytical figure of merits for the GC-MS/MS method was performed. The selectivity of ZB-Dioxin GC column provides increased resolution for 2,3,7,8-TCDD and 2,3,7,8-TCDF from its isomer components, thereby making GC-MS/MS method optimization easier and as an alternative technique to traditional HRMS analysis.

GC-MS/MS Method Parameters for all chromatograms

Column: Zebron ZB-Dioxin
Dimensions: 60 meter x 0.25 mm x 0.20 µm with 5 meter Guardian™
Part No.: 7KG-G045-10-GGA
Injection: Pulse Splitless (1.0 min, 30 psi) @ 110 °C for 0.31 min to 330 °C at 600 °C/min
Recommended Liner: Zebron PLUS 2 mm ID Straight Wool in Middle
Liner Part No: AG2-0E00-05
Carrier Gas: Helium @ 1.056 mL/min (constant flow)
Oven Program: 100 °C for 0 min to 220 °C @ 30 °C/min for 15.5 min to 240 °C @ 2 °C/ min for 5 min to 270 °C @ 5 °C/min for 5.0 min to 330 °C @ 15 °C/ min for 7.0 min
Detector: GC-MS/MS
Transfer Line Temperature: 340 °C

Figure 1
GC-MS/MS Separation of 2,3,7,8-TCDD and its isomer on a 60 meter Zebron ZB-Dioxin GC Column with 5 meter Guardian.

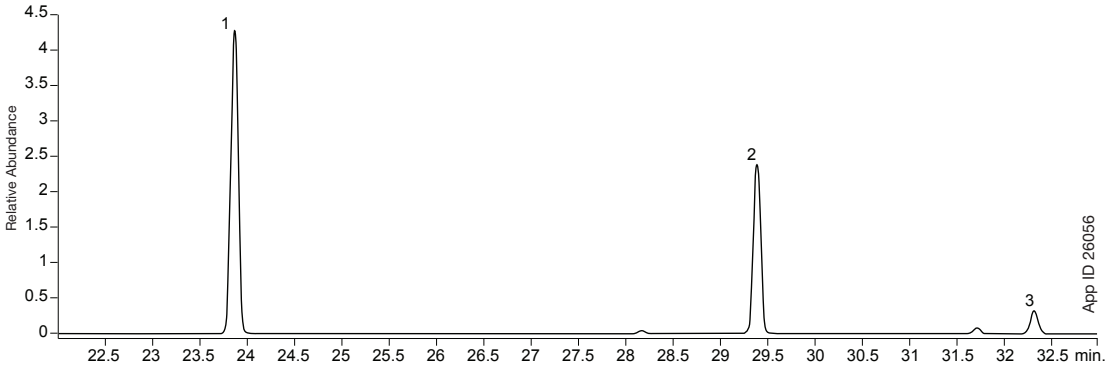


	Analyte Name	RT (min)
1	1,3,6,8-TCDD	25.56
2	1,2,3,4-TCDD	29.3
3	1,2,3,7-TCDD/ 1,2,3,8-TCDD	29.5
4	1,2,3,9-TCDD	30.3
5	2,3,7,8-TCDD	30.38
6	1,2,8,9-TCDD	31.70

Table1
MS/MS Parameters for Determination of Tetra Through Octa Dioxins and Furans.

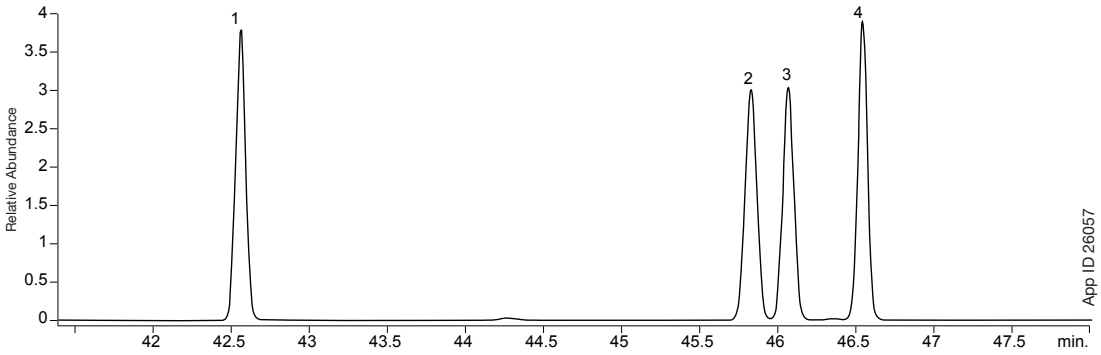
Analyte	Start Time (min)	Precursorion	Production	Dwell Time (ms)	CE (V)
13C-TCDD	22.0	333.9	269.9	50	26
		331.9	267.9		
37Cl-TCDD		328	263.5	10	26
		326	261.5		
TCDD		321.9	258.9	100	26
		319.9	256.9		
13C-TCDF		317.9	253.9	50	40
		315.9	251.9		
TCDF		305.9	242.9	100	40
		303.9	240.9		
13C-PeCDF	31.2	351.9	287.9	25	40
		349.9	285.9		
PeCDF		339.9	276.9	75	40
		337.9	274.9		
13C-TCDD		333.9	269.9	25	26
		331.9	267.9		
13C-TCDD		321.9	258.9	75	26
		319.9	256.9		
13C-TCDF		317.9	253.9	25	40
		315.9	251.9		
TCDF		305.9	242.9	75	40
		303.9	240.9		
13C-PeCDD	33.0	367.9	303.9	50	26
		365.9	301.9		
PeCDD		355.9	292.9	100	26
		353.9	290.9		
13C-PeCDF		351.9	287.9	50	40
		349.9	285.9		
PeCD		339.9	276.9	100	40
		337.9	274.9		
13C-HxCDDs	41.4	403.9	339.9	50	25
		401.9	337.9		
HxCDDs		391.8	328.8	100	25
		389.8	326.8		
13C-HxCDFs		387.9	323.9	50	40
		385.9	321.9		
HxCDFs		375.8	312.8	100	40
		373.8	310.8		
13C-HpCDD	48.0	437.8	373.8	50	24
		435.8	371.8		
HpCDD		425.8	362.8	100	24
		423.8	360.8		
13C-HpCDF		421.8	357.8	50	40
		419.8	355.8		
HpCDF		409.8	346.8	100	40
		407.8	344.8		
13C-OCDD	51.5	471.8	407.8	50	24
		469.8	405.8		
OCDD		459.7	396.7	100	24
		457.7	394.7		
13C-OCDF		455.8	391.8	50	40
		453.8	389.8		
OCDF		443.7	380.7	100	40
		441.7	378.7		

Figure 2
GC-MS/MS Separation of 2,3,7,8-TCDF and its isomer on a 60 meter Zebron™ ZB-Dioxin GC Column with 5 meter Guardian™.



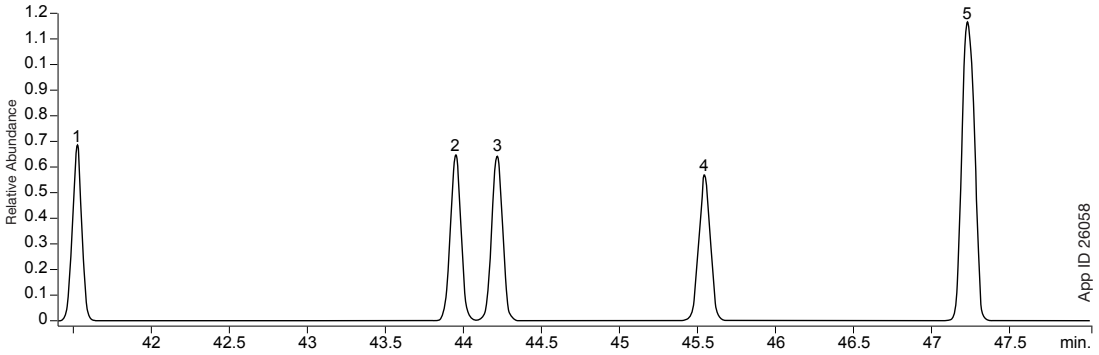
	Analyte Name	RT (min)
1	1,3,6,8-TCDF	23.7
2	2,3,7,8-TCDF	29.4
3	1,2,8,9-TCDF	32.4

Figure 3
GC-MS/MS Separation of HxCDD and its isomer on a 60 meter Zebron ZB-Dioxin GC Column with 5 meter Guardian.



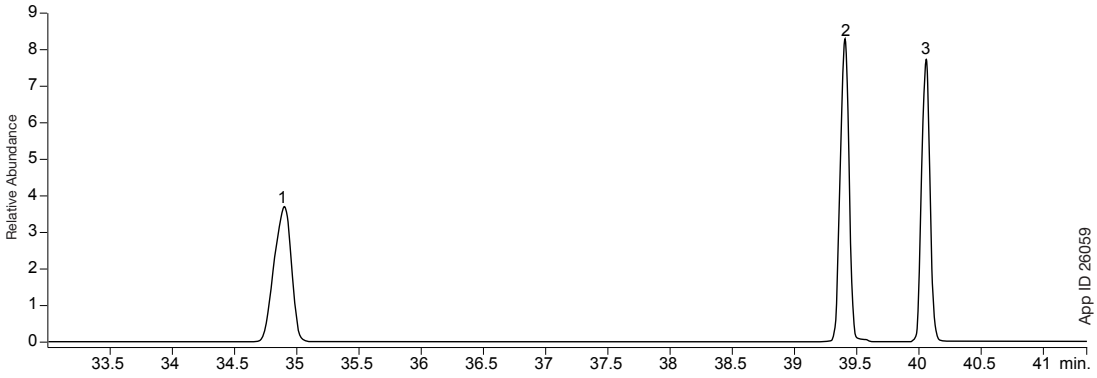
	Analyte Name	RT (min)
1	1,2,4,6,7,9-HxCDD	42.5
2	1,2,3,4,7,8-HxCDD	45.8
3	1,2,6,7,8-HxCDD	46.2
4	1,2,3,7,8,9-HxCDD	45.6

Figure 4
GC-MS/MS Separation of HxCDF and its isomer on a 60 meter Zebron ZB-Dioxin GC Column with 5 meter Guardian.



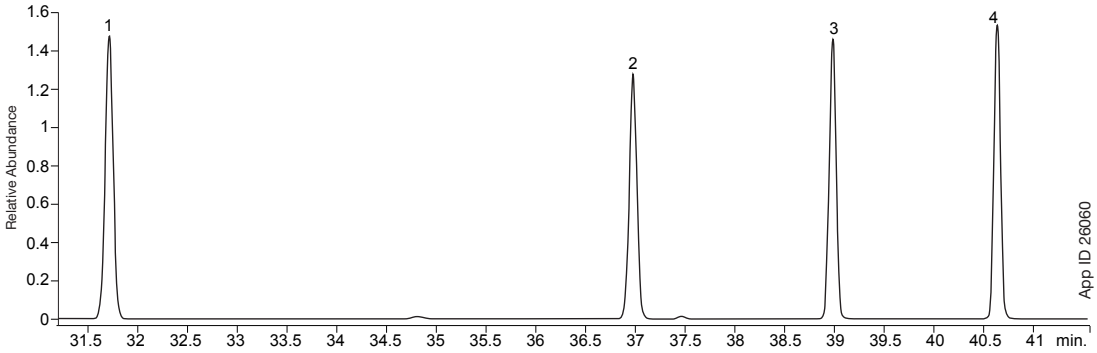
	Analyte Name	RT (min)
1	1,2,3,4,7,8-HxCDF	41.1
2	1,2,3,4,6,8-HxCDF	43.9
3	1,2,3,6,7,8-HxCDF	44.3
4	2,3,4,6,7,8-HxCDF	45.6
5	1,2,3,7,8,9-HxCDF	47.2

Figure 5
GC-MS/MS Separation of PeCDD and its isomer on a 60 meter Zebron™ ZB-Dioxin GC Column with 5 meter Guardian™.



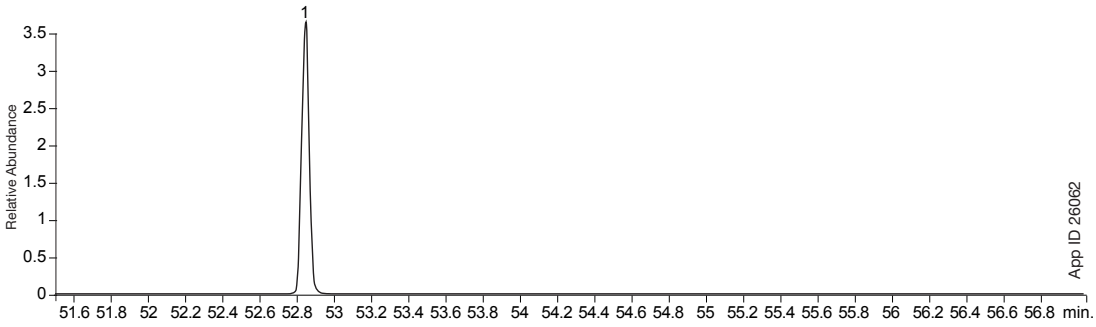
	Analyte Name	RT (min)
1	1,2,4,7,9-PeCDD	34.8
2	1,2,3,7,8-PeCDD	38.9
3	1,2,3,8,9-PeCDD	40.1

Figure 6
GC-MS/MS Separation of PeCDF and its isomer on a 60 meter Zebron ZB-Dioxin GC Column with 5 meter Guardian.



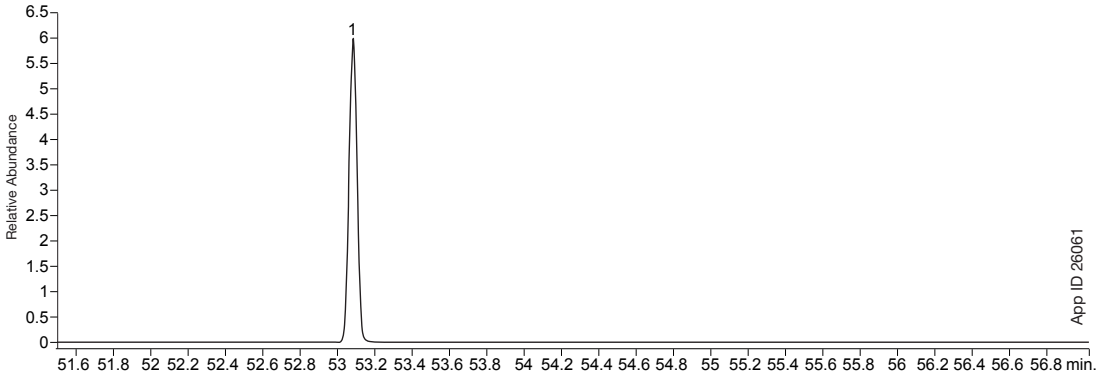
	Analyte Name	RT (min)
1	1,3,4,6,8-PeCDF	31.6
2	1,2,3,7,8-PeCDF	37.0
3	2,3,4,7,8-PeCDF	39.0
4	1,2,3,8,9-PeCDF	40.6

Figure 7
GC-MS/MS Separation of OCDD on a 60 meter Zebron ZB-Dioxin GC Column with 5 meter Guardian.



	Analyte Name	RT (min)
1	OCDD	52.8

Figure 8
GC-MS/MS Separation of OCDF on a 60 meter Zebron™ ZB-Dioxin GC Column with 5 meter Guardian™.



	Analyte Name	RT (min)
1	OCDF	52.8

Figure 9
Zebron ZB-Dioxin GC column with integrated Guardian

The ZB-Dioxin column with integrated Guardian, provides the following benefits:

- Protects against non-volatile contamination by acting as a guard
- Prevents excessive trimming of analytical column thereby by extending the resolution of critical pairs
- Provides solvent focusing by acting as a retention gap for large volume samples and for low boiling solvent when used as the extracting solvent
- Seamless union of analytical and guard column leak-free connection that reduces Mass spec repair and downtime

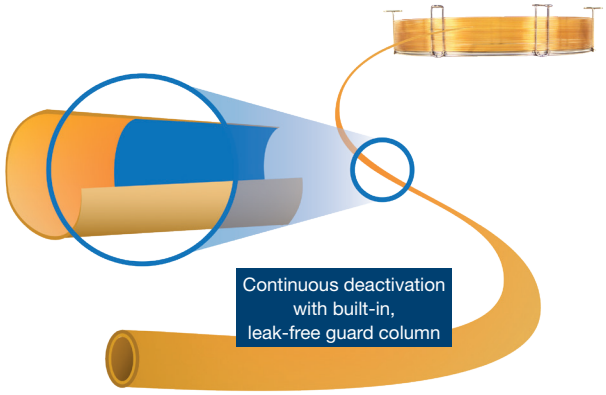


Table 2
Linearity and Accuracy of Tetra through Octa Dioxins and Furans on ZB-Dioxin by GC-MS/MS.

Analyte	Retention Time (min)	Internal Standard	Range (ppb)	R^2
13C-1,2,3,4-TCDD	29.41	13C-2,3,7,8-TCDD	100	-
2,3,7,8-TCDF	29.37	13C-2,3,7,8-TCD	0.05-219	0.98996
37Cl-2,3,7,8-TCDD	30.364	13C-2,3,7,8-TCDD	0.2-222	0.98562
2,3,7,8-TCDD	30.38	13C-TCD	0.05-224	0.98996
1,2,3,7,8-PeCDF	36.95	13C-1,2,3,7,8-PeCDF	0.3-1045	0.99778
2,3,4,7,8-PeCDF	38.97	13C-2,3,4,7,8-PeCDF	0.3-1042	0.99789
1,2,3,7,8-PeCDD	39.39	13C-1,2,3,7,8-PeCDD	0.2-1055	0.99638
1,2,3,4,7,8-HxCDF	43.93	13C-1,2,3,4,7,8-HxCD	0.2–1109	0.98727
1,2,3,6,7,8-HxCDF	44.20	13C-1,2,3,6,7,8-HxCDF	0.25-1073	0.99391
2,3,4,6,7,8--HxCDF	45.53	13C-2,3,4,6,7,8-HxCDF	0.22-1121	0.98465
2,3,4,6,7,8-HxCDD	45.81	13C-1,2,3,4,7,8-HxCDD	0.24-1114	0.98631
1,2,3,7,8,9-HxCDD	46.53	13C-1,2,3,7,8,9-HxCD	0.22-1120	0.98552
1,2,3,4,6,7,8-HpCDF	48.96	13C-1,2,3,4,6,7,8-HpCDF	0.26-1003	0.99995
1,2,3,4,6,7,8-HpCDD	50.11	13C-1,2,3,4,6,7,8-HpCDD	0.25-1021	0.99998
1,2,3,4,7,8,9-HpCDF	50.66	13C-1,2,3,4,7,8,9-HpCDF	0.25-1021	0.99942
OCDD	52.83	13C-OCDD	0.6-1950	0.99917
OCDF	53.08	13C-OCDF	0.5 to 2043	0.99941

Table 3
Detection Limit and Quantitation Limit Calculation for 2,3,7,8-TCDF from Linearity.

Concentration in ppb	Relative Response
0.05	0.0005
0.45	0.005
1.90	0.0212
10.26	0.1145
42.45	0.4745
219.21	2.4468
Slope	0.01
Residual Standard Deviation (RSD)	0.000316
Detection Limit for 2,3,7,8-TCDF	**3.3*RSD/Slope**
	0.09 ppb
Quantitation Limit for 2,3,7,8-TCDD	**10*RSD/Slope**
	0.28 ppb

Results and Discussion

Dioxin analysis is complex due to numerous dioxin and furan compounds that are close in structure. Under EI mass spec source, the fragmentation in the source are similar for closely resembling compounds. There is a necessity for GC stationary phase to resolve critical pairs chromatographically, irrespective of high end detectors like GC-HRMS or GC-MS/MS. While a GC-MS/MS method can certainly resolve coelutions spectrally in many case, however with enhanced chromatographic resolution, optimization of a GC-MS/MS method becomes easier. Presented in **Figure 1** is the separation of toxic dioxin isomer 2,3,7,8-TCDD from its isomer. Regulated methods like EPA-1613 mandate that the resolution is less than 25% valley for these critical pairs. With Zebron™ ZB-Dioxin GC column, a baseline resolution of 2,3,7,8-TCDD from its neighboring isomer 1,2,3,9-TCDD is achieved. **Figure 1-8** shows separation of Tetra through Octa Dioxins and Furans on a single method. Traditionally, a confirmation column like a 225 phase would be used to resolve coelution of TCDF isomers. With Zebron ZB-Dioxin GC column, the selectivity of the stationary phase allows for the complete resolution of 2,3,7,8-TCDF from its isomers, thereby cancelling out the necessity for a secondary confirmation column.

Presented in **Table 2** are the analytical figures of merits for the developed GC-MS/MS method on Zebron ZB-Dioxin GC column. The method provided a wide linear range from low to high parts per billion for various analytes. The exact range for individual dioxin is listed in the same table. In order to eliminate discrepancy in accuracy due to difference in physico-chemical properties of the analytes, it is essential to use surrogates and internal standards that are close in structure to the analyte of interest. For the same reason, multiple isotopically labeled dioxins and furans with 13C label and 37Cl were chosen. A six point calibration curve was plotted for individual dioxins and furans to establish the linearity and range of the method. The R^2 values are displayed in the table demonstrating linearity of the developed method. Since analysis of samples by GC-MS/MS eliminates the noise level by being selective in the first and third quadrupole, it is prudent to consider an alternate technique to determine the detection and quantitation limit of analytes instead of adopting traditional add hyphens signal-to-noise method. Presented in **Table 3** is the detection limit and quantitation limit calculation for 2,3,7,8-TCDF calculated using linearity data. This method is instrument independent and method specific and considers the error in the regression line to calculate accurate detection and quantification limits.

This method can be considered for both environmental and food based samples. Specifically, when the samples are expected to have non-volatile contaminants, it is very essential to use a guard column to trap non-volatile matrix contaminants. The chosen column dimension for this method utilizes a 5 meter integrated Guardian™, which is a seamless union of guard column to the analytical column (**Figure 9**). This offers leak-free connection which is extremely useful for MS based detection. With traditional guard columns, there is a high probability of leaks from the connections that can lead to expensive Mass spec repair and maintenance.

The Zebron ZB-Dioxin column with integrated Guardian, provides the following benefits:

- Protects against non-volatile contamination by acting as a guard
- Prevents excessive trimming of analytical column thereby extending the resolution of critical pairs
- Provides solvent focusing by acting as a retention gap for large volume samples and for low boiling solvent when used as the extracting solvent
- Seamless union of analytical and guard column provides leak-free connection that reduces Mass spec repair and downtime

These proven benefits of Guardian ensures less frequent column change, reduces instrument downtime and improves lab productivity.

In addition to providing enhanced resolution of TCDD and TCDF isomers, Zebron ZB-Dioxin stationary phase undergoes extensive cross-linking through Engineered Self Cross-Linking™ (ESC™) process to provide mass spec compatible low bleed column. This reduces potential column bleed to reduce instrument downtime and maintenance. Thus, the proprietary selectivity, enhanced resolution of TCDD and TCDF, ESC, integrated Guardian protection and high efficiency makes ZB-Dioxin, the ideal choice for the separation of dioxin and furan compounds by GC-MS/MS.

Conclusion

The Zebron ZB-Dioxin GC column provides the highest resolution for 2,3,7,8-TCDD and 2,3,7,8-TCDF from its isomer compounds and serves as a single GC column solution for dioxin separation by GC-MS/MS. The integrated Guardian option prevents excessive trimming of analytical column thereby increasing lab throughput.

Acknowledgement

This study was performed at Weck Laboratories, Inc. and we thank them for their collaboration on this project. Phenomenex is not affiliated with Weck Laboratories, Inc.

Ordering Information

Zebron™ ZB-Dioxin GC Column			
ID(mm)	df(µm)	Temp. Limits °C	Part No.
60-Meter			
0.25	0.2	40 to 320/340	7KG-G045-10
60-Meter with 5-Meter Guardian™			
0.25	0.2	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
For Agilent® and Thermo®					
Single taper	S/SL	4 x 78.5	PLUS Inert	AG2-0A10-05	5/pk
For Agilent and Thermo					
Single taper	S/SL	2 x 78.5	PLUS Inert	AG2-0E00-05	5/pk

Standard Z-Guard™ Columns and Kits			
ID (mm)	Description	Part No. 5-Meter	Part No. 10-Meter
0.25	Guard Column	7AG-G000-00-GZ0	7CG-G000-00-GZ0
	Guard Column Kit	7AG-G000-00-GZK	7CG-G000-00-GZK

Easy Seals™ Inlet Base Seals				10/pk
Description	Injection Type	Groove Style	Inlet Hole Diameter (mm)	Part No.
Easy Seals Gold Inlet Seal	Splitless	Single	0.8	AG0-8620

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