

# A Robust Screening Method for Dioxins and Furans by Ion Trap GC-MS/MS in a Variety of Matrices

## Key Words

- Polaris Q Ion Trap
- GC-MS/MS
- Dioxins
- Electron Impact
- Environmental
- Furans

## Overview

**Purpose:** Develop a methodology for part-per-trillion level screening of the 17 2,3,7,8-chlorinated dibenzo dioxins and furans with minimal sample cleanup utilizing a benchtop GC/MS.

**Method:** Soil, cow's milk, and coal fly ash extracts are injected into a GC-MS using the MS/MS mode of operation with minimal sample cleanup. MS/MS parameters are adjusted to give maximum sensitivity.

**Results:** Optimization of trap conditions allows detection down to the low part per trillion levels using the split injection technique.

## Introduction

The determination of the presence of chlorinated dioxins and furans in the environment has long been of great concern. Since there are no known safe levels of exposure to these substances, the only way to minimize risk is to minimize exposure. Therefore, regulatory levels set the allowable concentration at extremely low levels. These detection limits can be routinely achieved by using high-resolution magnetic sector instruments usually after a very thorough, costly, and time-consuming sample cleanup. The typical sample cleanup can require up to six days. Purchasing automated sample preparation and cleanup equipment costing several tens of thousands of dollars can considerably reduce cleanup time.

Ion trap mass spectrometers have long been known for their sensitivity and relatively low cost compared to other types of mass spectrometers. The limit of detection of the Polaris Q for this screening methodology is shown in Figure 4, a mass chromatogram of a 500 fg/μL injection of 2,3,7,8-TCDD. Applying ion trap MS/MS technology and split injections to the determination of dioxins and furans leads to a viable and robust analysis technique that can be used for screening, quantitation, and confirmation down to the low part-per-trillion range without extensive sample cleanup. Figure 1 demonstrates quite clearly the gain in signal to noise that MS/MS can provide over full scan or low-resolution SIM. Comparing the top two traces indicates roughly a 10-fold increase in sensitivity in the MS/MS mode over the full scan data when analyzing a pure standard. The lower two traces show the tremendous improvement that MS/MS provides when background matrix interferes with full scan or low resolution SIM.

The selectivity of MS/MS for this method is performed by monitoring the transition of the molecular ion of each of the PCDD's and PCDF's to the loss of COCl. Figure 2 shows a comparison of the high resolution, low resolution and MS/MS mass chromatograms. The use of the ion trap mass spectrometer and reduced sample cleanup for the screening of dioxins and furans is both an economical and time saving alternative prior to the extensive traditional high-resolution sector GC-MS sample cleanup methodology.

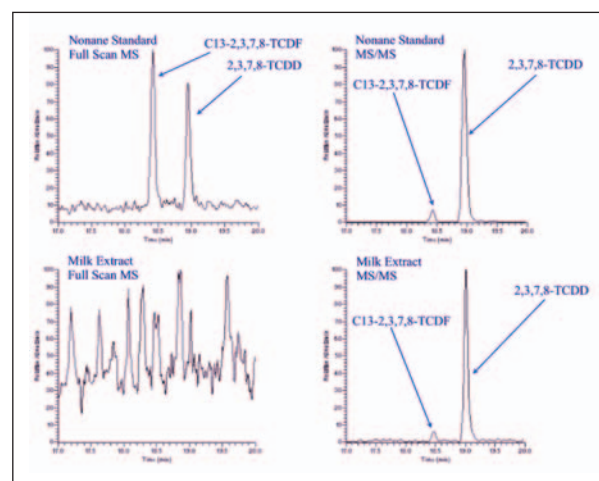


Figure 1: MS and MS/MS sensitivity comparison for the analysis of 2,3,7,8-TCDD in a standard and a milk extract

## MS/MS Parameters for Dioxins

COMPOUND	PRECURSOR ION	WIDTH (AMU)	PRODUCT IONS SCAN RANGE	ISOLATION TIME (MS)	COLLISION ENERGY (V)	COLLISION TIME (MS)	Q	MICROSCANS / SCAN	MAXIMUM ION TIME (MS)
TCDD	322	5	253-263	16	3.5	30	0.45	1	100
PeCDD	356	5	288-298	16	3.5	30	0.45	1	100
HxCDD	390	5	322-332	16	3.5	30	0.45	1	100
HpCDD	426	5	357-367	16	4	30	0.45	1	100
OCDD	460	6	391-401	16	4	30	0.45	1	100

## MS/MS Parameters for Furans

COMPOUND	PRECURSOR ION	WIDTH (AMU)	PRODUCT IONS SCAN RANGE	ISOLATION TIME (MS)	COLLISION ENERGY (V)	COLLISION TIME (MS)	Q	MICROSCANS / SCAN	MAXIMUM ION TIME (MS)
TCDF	306	5	237-247	16	4	30	0.45	1	100
PeCDF	340	5	272-282	16	4	30	0.45	1	100
HxCDF	374	5	306-318	16	4.25	30	0.45	1	100
HpCDF	410	5	340-350	16	4.5	30	0.45	1	100
OCDF	444	5	375-385	16	5	30	0.45	1	100

Table 1 and 2: MS/MS parameters for the analysis of the dioxins and furans

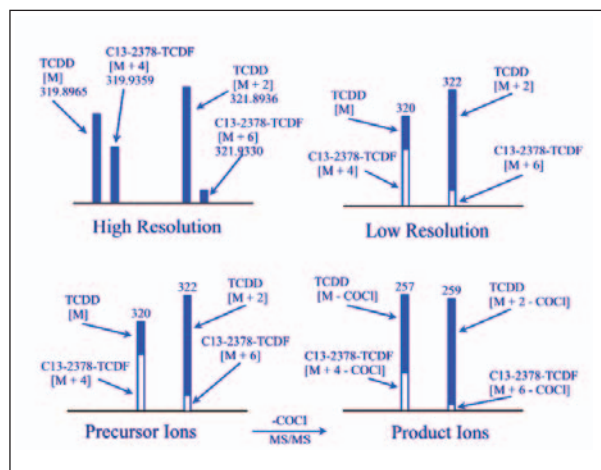


Figure 2: The "extra" peaks visible under all the TCDD mass chromatograms is an interference from the contribution of the [M + 4] and [M + 6] isotopes of the C13-2,3,6,7-TCDF. This phenomenon is visible for all of the PCDD's where there is a C13 furan internal standard. This is not an issue for magnetic sector instruments running at a resolution of 10,000, as the masses are separated.

## Instrument Parameters

### POLARISQ ION TRAP

Source Temperature:	250°C
Ionization Mode:	Electron Ionization
AGC:	50
Injection Waveforms:	On
Trap Flow:	1.8 mL/min
MS/MS Parameters:	See Table 1

### TRACE GC

Column:	BPX5, 0.15 mm x 25 m, 25 µm
Oven:	150°C, 1 min
Ramp One:	20°C/min to 200°C, hold 1.5 min
Ramp Two:	3°C/min to 300°C, hold 1.67 min
Split/Splitless Injector	
Injector Temperature:	250°C
Injection Mode:	Split
Split Ratio:	15:1
Constant Flow:	0.9 mL/min He

### AS2000

Injection Volume:	2 µL
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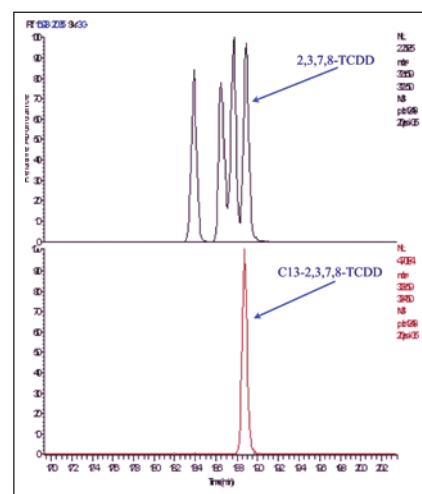


Figure 3a

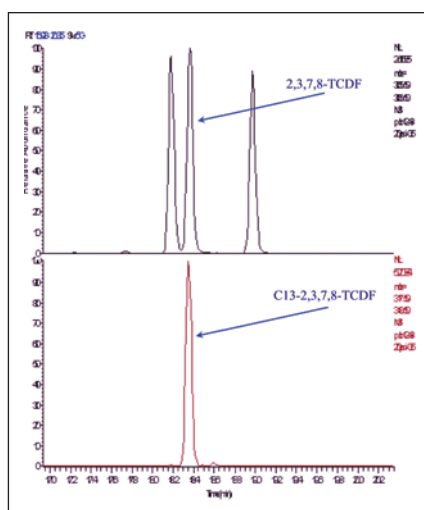


Figure 3b

Figure 3a and 3b: Demonstrates the ability to chromatographically separate the 2,3,7,8 substituted dioxins and furans from the other non-toxic dioxins and furans to eliminate false positives.

## Experimental

USEPA Method 1613 was used as a template to demonstrate the applicability of the Polaris<sup>Q</sup> for the determination of dioxins and furans. Standards were ordered from Cambridge Isotope Labs. Standards CS1 through CS5 were analyzed for calibration using the conditions described in Tables 1 and 2. The window defining mixture was used to determine the MS/MS segment time windows. The resolution test mixture was used to choose the correct column and chromatographic conditions for the proper separation of the tetra isomers. (see Figures 3a and 3b) The column used to produce the required separation in a single injection was the 25 meter BPX-5; 0.15 mm ID; 0.25 micron film thickness column from SGE. After establishing the proper chromatographic conditions, the window defining mixture was injected to determine the beginning and end of each isomer window. For the purpose of this application note, the five calibration standards were injected five times and the average response factor was used to determine the concentrations in each matrix. Three different matrices, soil, cow's milk, and coal fly ash were used for evaluation. A 25 gram aliquot of material was mixed with 50 grams of sodium sulfate and extracted with 50:50 methylene chloride hexane for 24 hours in a Soxhlet extractor. Next, the extracts were partitioned four times with 40 milliliter portions of concentrated sulfuric acid then partitioned once with a 40 milliliter portion of 5% sodium chloride solution. The matrix extracts were then evaporated to dryness under a stream of nitrogen and reconstituted with 100 microliters of a 10-fold dilution of USEPA method 1613 CS3 calibration standard. The final concentration of the extract is 2 pg/ $\mu$ L or 8 pg/g of the tetra substituted dioxins and furans. Twelve replicates of an analytical sequence of nonane standard, blank and each extracted matrix were run for a total of 60 injections.

This equals about 45 hours of continuous analysis. By injecting these extracts in split mode the chromatographic system is not overloaded with interfering matrix.

## Results and Discussion

The results of the column selection and chromatography can be seen in Figures 3a and 3b, Figure 3a showing the separation of the tetra-dioxin isomers and Figure 3b showing the separation of the tetra-furan isomers. The results of the five-point calibration can be seen in Tables 3 and 6 for the native dioxins and furans respectively. The reproducibility of the five replicates of the C13 labeled internal standards at each concentration is shown in Table 4 for the dioxins and Table 7 for the furans. In USEPA Method 1613 isotope ratios are used to confirm the presence of the chlorinated dioxins and furans. With this screening method the isotope ratios are calculated from the results of the isotope ratios of the calibration standards (see Tables 5 and 8). These calculated values are used to confirm the dioxins and furans in matrix samples. In all three matrices the isotope ratios are within the method designated 15% limit. (see Table 9) The results of the matrix spike of the tetra-dioxins and tetra-furans for all three matrices can be seen in Table 10. The calculated recovery and relative standard deviation of the replicate injections are all below 12%. The individual mass chromatograms of the dioxin and furan spike in the various matrices can be seen in Figures 5 - 10. All compounds are easily identifiable and have confirmatory isotope ratios (see Table 9).

### PCDD Natives

COMPOUND	% RSD
2,3,7,8-TCDD	10.8
1,2,3,7,8-PeCDD	6.4
1,2,3,4,7,8-HxCDD	6.9
1,2,3,6,7,8-HxCDD	9.1
1,2,3,7,8,9-HxCDD	8.7
1,2,3,4,6,7,8-HpCDD	4.9
OCDD	5.2

Table 3: The calibration curve data from 500 fg/ $\mu$ L to 200 pg/ $\mu$ L for TCDD and 2.5 pg/ $\mu$ L to 1000 pg/ $\mu$ L for PeCDD through HpCDD and 5 pg/ $\mu$ L to 2000 pg/ $\mu$ L for OCDD. The internal standards are at 100 pg/ $\mu$ L for each except C13-OCDD which is at 200 pg/ $\mu$ L.

### PCDD C13 Internal Standards

COMPOUND	% RSD
C13-2,3,7,8-TCDD	4.2
C13-1,2,3,7,8-PeCDD	5.6
C13-1,2,3,4,7,8-HxCDD	8.6
C13-1,2,3,6,7,8-HxCDD	6.9
C13-1,2,3,7,8,9-HxCDD	7
C13-1,2,3,4,6,7,8-HpCDD	8.9
C13-OCDD	9.2

Table 4: The RSD data from the internal standard areas of the CS1 through CS5 calibration curve. Each calibration level was injected five times.

## Calculated PCDD Isotope Ratios

COMPOUND	IONS	RATIO	% RSD
2,3,7,8-TCDD	259/257	0.97	22.7
1,2,3,7,8-PeCDD	293/295	0.83	10
1,2,3,4,7,8-HxCDD	325/327	0.69	20.1
1,2,3,6,7,8-HxCDD	325/327	0.66	13.2
1,2,3,7,8,9-HxCDD	325/327	0.69	14.4
1,2,3,4,6,7,8-HpCDD	361/363	1.35	9.5
OCDD	395/397	1.14	5.7

Table 5: The ion ratio stability of the MS/MS fragment of each level of chlorination. The large variability for the isotope ratios for the TCDD and HxCDD could be attributed the low level of detection and the number of MS/MS transitions during acquisition.

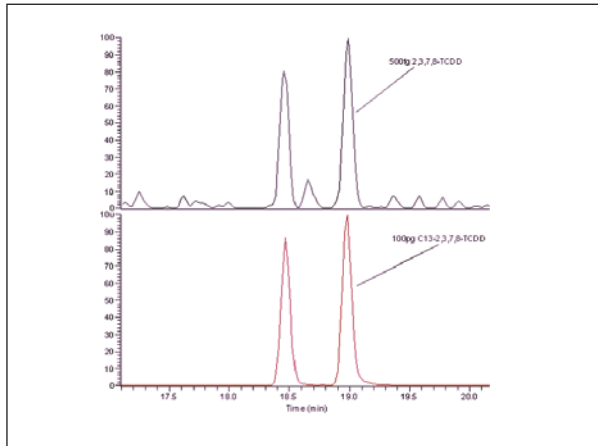


Figure 4: Typical 500 fg/μL injection. A typical 500 fg/μL injection of 2,3,7,8-TCDD in nonane using MS/MS. The large peak to the left of TCDD is the contribution from C13-2,3,7,8-TCDF internal standard.

## PCDF Natives

COMPOUND	%RSD
2,3,7,8-TCDF	17.4
1,2,3,7,8-PeCDF	5.5
2,3,4,7,8-PeCDF	6.2
1,2,3,4,7,8-HxCDF	3.5
1,2,3,6,7,8-HxCDF	4
2,3,4,6,7,8-HxCDF	5.6
1,2,3,7,8,9-HxCDF	6.4
1,2,3,4,6,7,8-HpCDF	4.5
1,2,3,4,7,8,9-HpCDF	5.8
OCDF	5.4

Table 6: The calibration curve data from 500 fg/μL to 200 pg/μL for TCDF and 2.5 pg/μL to 1000 pg/ul for PeCDF through HpCDF and 5 pg/?L to 2000 pg/μL for OCDF. The internal standards are at 100 pg/μL for each except C13-OCDD which is at 200 pg/μL.

## PCDF C13 Internal Standards

COMPOUND	%RSD
C13-2,3,7,8-TCDF	4
C13-1,2,3,7,8-PeCDF	4.5
C13-2,3,4,7,8-PeCDF	4.7
C13-1,2,3,4,7,8-HxCDF	7.2
C13-1,2,3,6,7,8-HxCDF	5.5
C13-2,3,4,6,7,8-HxCDF	6.2
C13-1,2,3,7,8,9-HxCDF	7.7
C13-1,2,3,4,6,7,8-HpCDF	8.1
C13-1,2,3,4,7,8,9-HpCDF	9.4
C13-OCDD	9.2

Table 7: The RSD data from the internal standard areas of the calibration curve mentioned earlier

## Calculated PCDF Isotope Ratios

COMPOUND	IONS	RATIO	% RSD
2,3,7,8-TCDF	243/241	0.81	22.7
1,2,3,7,8-PeCDF	275/277	0.81	7.1
2,3,4,7,8-PeCDF	275/277	0.82	7
1,2,3,4,7,8-HxCDF	309/311	0.67	10.4
1,2,3,4,7,8-HxCDF	309/311	0.67	8.1
1,2,3,6,7,8-HxCDF	309/311	0.65	5.5
2,3,4,6,7,8-HxCDF	309/311	0.65	7.9
1,2,3,4,6,7,8-HpCDF	345/347	1.26	7.3
1,2,3,4,7,8,9-HpCDF	345/347	1.29	7
OCDF	379/381	1.14	5.6

Table 8: The ion ratio stability of the MS/MS fragment of each level of chlorination. The higher than proscribed RSD for the TCDF could be from the low level of compound being detected and the large number of MS/MS transitions acquired during this segment.

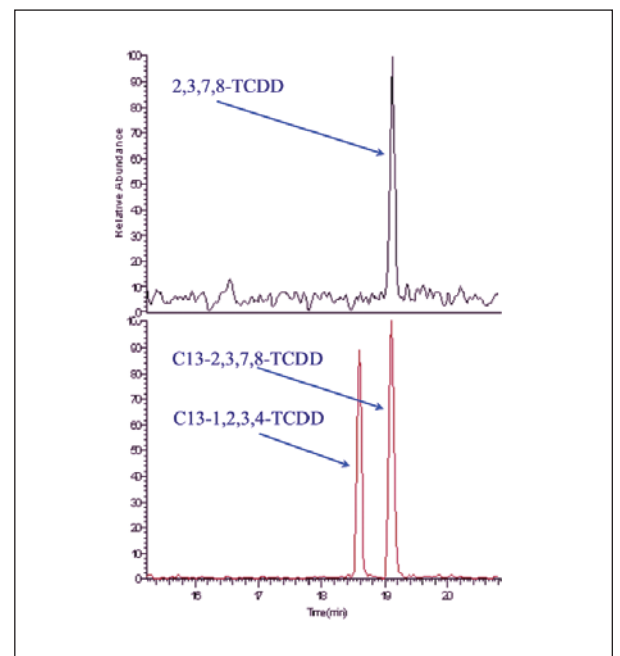


Figure 5: Mass chromatograms of the MS/MS analysis of 2,3,7,8-TCDD and internal standard from a spike of a 1/5 dilution of the CS3 calibration mixture into a 25 gram cow's milk extract. This is equivalent to 2 pg/μL injected or 8 (pg/g) parts per trillion in the milk.

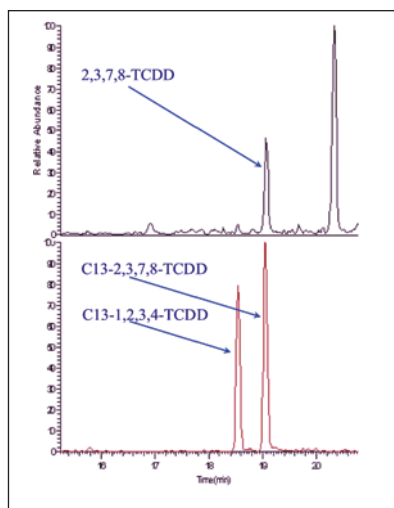


Figure 6: Mass chromatograms of the MS/MS analysis of 2,3,7,8-TCDD and internal standard from a spike of a 1/5 dilution of the CS3 calibration mixture into a 25 gram soil extract. This is equivalent to 2 pg/ $\mu$ L injected or 8 (pg/g) parts per trillion in the soil

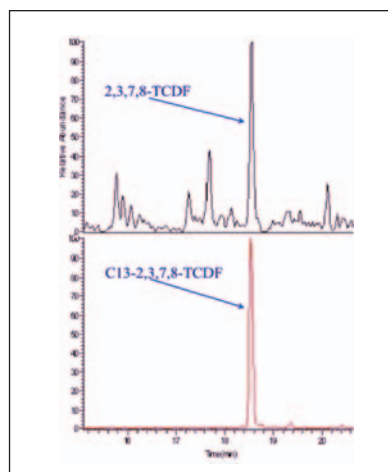


Figure 9: Mass chromatograms of the MS/MS analysis of 2,3,7,8-TCDF and internal standard from a spike of a 1/5 dilution of the CS3 calibration mixture into a 25 gram soil extract. This is equivalent to 2 pg/ $\mu$ L injected or 8 (pg/g) parts per trillion in the soil.

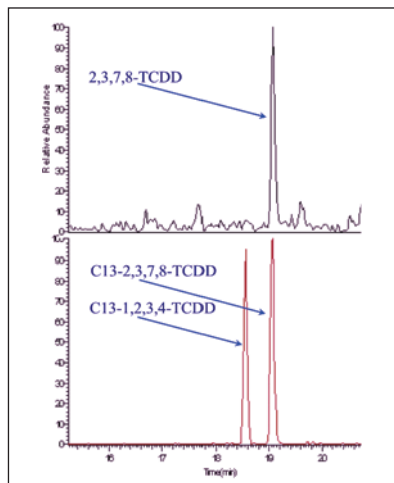


Figure 7: Mass chromatograms of the MS/MS analysis of 2,3,7,8-TCDD and internal standard from a spike of a 1/5 dilution of the CS3 calibration mixture into a 25 gram coal fly ash extract. This is equivalent to 2 pg/ $\mu$ L injected or 8 (pg/g) parts per trillion in the coal fly ash

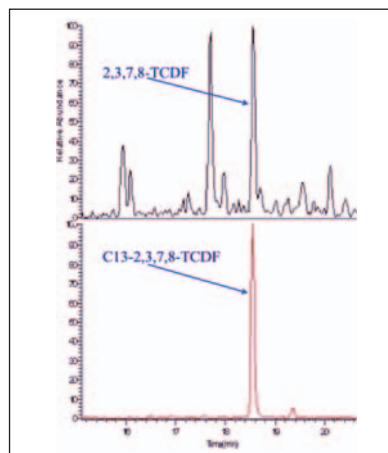


Figure 10: Mass chromatograms of the MS/MS analysis of 2,3,7,8-TCDF and internal standard from a spike of a 1/5 dilution of the CS3 calibration mixture into a 25 gram coal fly ash extract. This is equivalent to 2 pg/ $\mu$ L injected or 8 (pg/g) parts per trillion in the coal fly ash

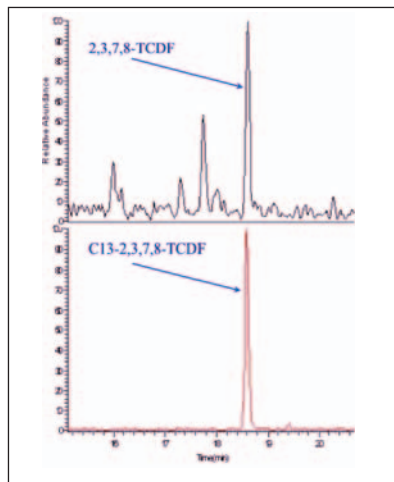


Figure 8: Mass chromatograms of the MS/MS analysis of 2,3,7,8-TCDF and internal standard from a spike of a 1/5 dilution of the CS3 calibration mixture into a 25 gram cow's milk extract. This is equivalent to 2 pg/ $\mu$ L injected or 8 (pg/g) parts per trillion in the milk.

## Calculated Isotope Ratios in Matrix

COMPOUND	M-COCL IONS	CALCULATED RATIO	2 PG/ $\mu$ L RATIO IN NONANE	2 PG/ $\mu$ L RATIO IN MILK	2 PG/ $\mu$ L RATIO IN SOIL	2 PG/ $\mu$ L RATIO IN COAL FLY ASH
TCDD	257/259	0.97	0.93	1.06	0.83	0.99
TCDF	243/241	0.81	0.81	0.87	0.82	0.89

## Recovery of 2 pg/ $\mu$ L Spike in Various Matrices

COMPOUND	QUAN. IONS	SPIKE AMOUNT	NONANE	MILK	SOIL	COAL FLY ASH
TCDD	257 + 259	Recovery RSD	2.18 pg/ $\mu$ L10.2 %	2.28 pg/ $\mu$ L7.6 %	1.91 pg/ $\mu$ L9.7 %	2.05 pg/ $\mu$ L10.2 %
TCDF	241 + 243	Recovery RSD	1.84 pg/ $\mu$ L7.8 %	1.92 pg/ $\mu$ L11.5 %	1.90 pg/ $\mu$ L11.5 %	1.95 pg/ $\mu$ L5.6 %

Table 9 and 10: Isotope ratios, spike recovery amounts, and relative standard deviations obtained from a series of injections of nonane standards, milk, soil, and coal fly ash matrix. The total number of injections was 60 with each type of matrix injected twelve times.

## Conclusion

The PolarisQ has been demonstrated to be well suited for the part-per-trillion level screening of dioxin and furans in a variety of matrices without extensive cleanup. This is accomplished by incorporating the split injection technique for chromatographic robustness and MS/MS for selectivity. Significant savings in time and money can be realized by employing the reduced cleanup procedure and the GC-MS/MS analytical technique. With additional cleanup and larger sample sizes the instrument and methodology could be used to detect even lower levels. The PolarisQ is a perfect fit for laboratories that require part-per-trillion detection limits for the purposes of screening the 2,3,7,8-substituted dioxins and furans in areas where a high resolution instrument is considered an economic burden. In addition the PolarisQ can also be used to relieve the sample load and reduce the risk of contaminating the high-resolution instrument from the occasional “high” level sample.

## References

1. Grabic R., Novak J., Pacakova V.; “Optimization of a GC-MS/MS Method for the Analysis of PCDDs and PCDFs in Human and Fish Tissue”; J. High Resol. Chromatogr. 23, 595-599 No. 10, 2000)
2. Method 1613, Tetra- through Octa-chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, US EPA, November 1994.

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