Investigating the Extraction Efficiency of Dioxins in Several Types of Aqueous Matrices by Automated Solid Phase Extraction Utilizing Disks

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Application Note Scope

The purpose of this application note is to outline an automated extraction method utilizing the Atlantic® DVB SPE Disk and the Biotage automated and manual SPE solutions for the extraction of dioxins and furans in several water matrices. The first section will highlight the use of the Biotage® Horizon 5000 fully automated extraction system and the method used for this application. Additionally, there will be an Application Modification section that will highlight the use of the Biotage® Horizon 4790 (with data and discussion) and Biotage® VacMaster™ Disk for this application.

Introduction

Dioxins are of great environmental concern due to their teratogenic, mutagenetic, and carcinogenic impact. These lipophilic compounds bioaccumulate in humans and wildlife and can have half-lives of up to 132 years. They are known to bind to sediment or suspended particles.

Extraction using a solid phase extraction (SPE) disk is advantageous for extraction sediment laden samples because traditional SPE using a cartridge requires two extraction processes; a filtration step to remove particulates before extraction of the water sample is required due to the small cross sectional area of the cartridge leading to clogging, then the filtered particulates endure a separate extraction step, such as soxhlet extraction. Not only that, if liquid-liquid extraction (LLE) is used, this can lead to formation of emulsions which can be difficult to break and may adversely affect the proper extraction of the particulate matter. The SPE disk extraction approach filters the particulates from the water sample on top of the disk while efficiently capturing the compounds of interest within the disk media. Prefilters are available but are not always required to filter the particulates from samples. The elution solvents delivered will effectively extract the dioxins and furans from the disk media while also extracting the compounds from the sediment on top of the disk.

Materials

- Biotage® Horizon® 5000
- 47 mm Atlantic® DVB Disks and pre-filters
- Sodium sulfate; combination clean-up column containing silica gel, alumina, and florisil
- Genevac; miVac Evaporator (or equivalent)
- High Resolution Gas Chromatography (HRGC)/ High Resolution Mass Spectroscopy (HRMS)

Method Summary

1. Clean all glassware and the extraction system using organic solvents and mild detergent to ensure the extractor's liquid flow path is free of contaminants between samples.
2. Prepare samples as per Table 1: spike each sample with 25 µL of a 20 pg/µL solution containing CDDs and CDFs and a 40 pg/µL for OCDD and OCDF and adjust the pH to 2.
   a. The samples represent a variety of matrices, including distilled water, very fine particulate laden river samples, river samples containing fine particles and sediment, and finally two wastewater influent samples.
   b. If larger sample volumes are required, the fast flow sediment disk holder with additional prefilters can be used, but since the disk holder is larger, solvent volumes and drain times would need to be increased accordingly to ensure recovery.
3. Let sit for 3 hours to equilibrate.
4. Extract samples with the automated Biotage® Horizon® 5000 extractor using Atlantic® DVB disks (47 mm), and pre-filters via the extraction method described in table 2.
5. Dry samples using sodium sulfate and then clean up using a coupling column, containing silica gel, alumina and florisil. Concentrate samples on a miVac Evaporator (Genevac).
6. Analyze by high resolution gas chromatography (HRGC)/ high resolution mass spectroscopy (HRMS) following the conditions outlined in table 3.

Table 1. Summary of samples prepared.

Step	Number of Samples	Sample Volume (mL)	Disk Type	pH	Prefilter	Large Particulates	Fine Particulates	Sediment
DI (LCS)	2	500	DVB	2	No	No	No	No
River Water	6	500	DVB	2	Yes	No	Yes	No
River Water	2	300	DVB	2	Yes	Yes	Yes	Yes
Wastewater	2	500	DVB	2	Yes	Yes	Yes	No

Table 2. Biotage® Horizon 5000 extraction method.

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/Elute (s)	Sample Delay (s)
Condition SPE Disk	Methylene Chloride	15	60	2	1	60	120	
Condition SPE Disk	Acetone	11	60	2	1	60	120	
Condition SPE Disk	Methanol	11	60	2	1	60	8	
Condition SPE Disk	Reagent water	15	60	2	1	5	4	
Condition SPE Disk	Reagent water	15	60	2	1	0	5	
Load Sample				2				45
Air Dry Disk				6			600	
Elute Sample Container	Acetone	8	15	2	1	90	60	
Elute Sample Container	Methylene Chloride	8	15	2	1	90	60	
Elute Sample Container	Methylene Chloride	8	15	2	1	90	60	
Elute Sample Container	Methylene Chloride	8	15	2	1	90	60	
Elute Sample Container	Methylene Chloride	8	15	6	1	90	120	

Table 3. Instrument Analysis Conditions: HRGC/HRMS.

Column	DB5 (60m)
Flow (mL/min)	1.0
Sample Injection Volume (µL)	1.8
Initial Column Ramp	180 °C to 190°C at 2°C/min
Final Column Ramp	190 °C to 240°C at 5°C/min
Total Run Time (min)	52

Acknowledgements

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References

EPA Method 1613, Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B, October 1994.

ISO Method 18073:2004 Determination of Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/ HRMS, reviewed and confirmed in 2013.

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Application Modifications

Biotage® Horizon 4790 Method Summary

1. Clean all glassware and the extraction system using organic solvents and mild detergent to ensure the extractor's liquid flow path is free of contaminants between samples.
2. Prepare samples as per Table 1: spike each sample with 25 µL of a 20 pg/µL solution containing CDDs and CDFs and a 40 pg/µL for OCDD and OCDF and adjust the pH to 2.
   c. The samples represent a variety of matrices, including distilled water, very fine particulate laden river samples, river samples containing fine particles and sediment, and finally two wastewater influent samples.
   d. If larger sample volumes are required, the fast flow sediment disk holder with additional prefilters can be used, but since the disk holder is larger, solvent volumes and drain times would need to be increased accordingly to ensure recovery.
3. Let sit for 3 hours to equilibrate.
4. Extract samples with the automated Biotage® Horizon® 4790 extractor using Atlantic® DVB disks (47 mm), and pre-filters via the extraction method described in table 4.
5. Dry samples using sodium sulfate and then clean up using a coupling column, containing silica gel, alumina and florisil. Concentrate samples on a miVac Evaporator (Genevac).
6. Analyze by high resolution gas chromatography (HRGC)/ high resolution mass spectroscopy (HRMS) following the conditions outlined in table 3.

Results and Discussion

The tables below outline the results from each sample. Several different types of water samples were examined to see if significant differences in recovery were observed. Samples with suspended particles yielded higher recoveries (Table 6) than the LCS (Table 5) by a small amount. Table 7 shows results for samples with both particulate and sediment, providing an increasingly challenging matrix. The suspended particles and sediment in the bottle did lower the recoveries due to the compounds natural tendency to adhere to soil particles. Wastewater influent samples, also a challenging matrix, were analyzed and the results shown in (Table 8). Even in this matrix most of the compounds showed a reasonable recovery and good precision.

Table 4. Biotage® Horizon 4790 extraction method.

Step	Solvent	Soak Time (s)	Dry Time (s)
Prewet 1	Methylene Chloride	60	60
Prewet 2	Acetone	60	60
Prewet 3	Methanol	60	4
Prewet 4	Reagent water	5	2
Prewet 5	Reagent water	0	0
Sample Process			
Air Dry			300
Rinse 1	Acetone	90	30
Rinse 2	Methylene Chloride	90	30
Rinse 3	Methylene Chloride	90	30
Rinse 4	Methylene Chloride	90	30
Rinse 5	Methylene Chloride	90	60

Table 5. DI water LCS results.

Compounds	1 % Rec.	2 % Rec.	Avg.	SD
2,3,7,8-TCDF	74.2	91.7	82.95	12.4
1,2,3,7,8-PeCDF	78.6	87.6	83.1	6.36
2,3,4,7,8-PeCDF	70.4	85.0	77.7	10.3
1,2,3,4,7,8-HxCDF	85.2	76.4	80.8	6.22
1,2,3,6,7,8-HxCDF	85.3	76.2	80.75	6.43
2,3,4,6,7,8-HxCDF	72.6	82.9	77.75	7.28
1,2,3,7,8,9-HxCDF	74.6	83.0	78.8	5.94
1,2,3,4,6,7,8-HpCDF	70.1	52.4	61.25	12.5
1,2,3,4,7,8,9-HpCDF	65.5	56.0	60.75	6.72
OCDF	63.6	55.0	59.3	6.08
2,3,7,8-TCDD	70.9	82.8	76.85	8.41
1,2,3,7,8-PeCDD	98.6	85.0	91.8	9.62
1,2,3,4,7,8-HxCDD	75.4	79.4	77.4	2.83
1,2,3,6,7,8-HxCDD	70.5	87.3	78.9	11.9
1,2,3,4,6,7,8-HpCDD	70.0	53.3	61.65	11.8
OCDD	69.2	36.6	52.9	23.1

The use of an automated extraction system equipped with SPE disk technology yielded acceptable recoveries with particulate-laden water samples without the means of another extraction apparatus such as SDS (Soxhlet/Dean-Stark extractor). Automated SPE extractions provide a fast method of extracting tetra through octa- chlorinated dioxins and furans from water matrices with analytical sensitivities to ppt (ng/L) levels.

Conclusion

Future work on these compounds will involve the extraction of larger volumes of samples (1 L or greater) using the EZ Flow Disk Holder. These studies will enable the analysis of samples with heavy particulate matter to be processed in a timely and efficient manor while lowering detection limits to the range of ppq (pg/L).

Table 6. River water with fine particulates results.

Compounds	1 % Rec.	2 % Rec.	3 % Rec.	4 % Rec.	5 % Rec.	6 % Rec.	Avg.	SD
2,3,7,8-TCDF	94.0	91.8	115	85.1	91.4	86.9	94.1	10.9
1,2,3,7,8-PeCDF	94.2	92.7	100	84.2	81.2	78.8	88.6	8.46
2,3,4,7,8-PeCDF	101	96.0	105	85.8	87.3	83.3	93.0	8.82
1,2,3,4,7,8-HxCDF	80.5	81.5	111	80.0	84.0	86.5	87.3	12.0
1,2,3,6,7,8-HxCDF	79.6	80.4	108	78.1	81.8	82.7	85.1	11.3
2,3,4,6,7,8-HxCDF	82.7	85.3	115	81.3	88.1	90.6	90.5	12.4
1,2,3,7,8,9-HxCDF	83.5	83.1	101	83.0	89.4	94.7	89.2	7.55
1,2,3,4,6,7,8-HpCDF	71.9	70.0	97.3	76.7	75.5	76.1	77.9	9.85
1,2,3,4,7,8,9-HpCDF	83.5	83.3	113	84.7	91.0	90.5	91.0	11.3
OCDF	96.4	87.9	124	95.0	107	101	102	12.5
2,3,7,8-TCDD	81.4	79.9	111	75.2	89.9	85.1	87.0	12.6
1,2,3,7,8-PeCDD	99.7	91.8	104	77.6	88.2	82.9	90.7	10.0
1,2,3,4,7,8-HxCDD	79.6	81.8	118	81.2	86.7	87.1	89.0	14.3
1,2,3,6,7,8-HxCDD	79.9	84.0	114	79.4	85.1	85.0	88.0	13.2
1,2,3,4,6,7,8-HpCDD	78.7	71.3	103	73.8	76.7	81.5	80.8	11.3
OCDD	74.7	67.9	99.5	72.4	73.9	70.1	76.4	11.6

Table 7. River water with fine particulates and sediment laden results.

Compounds	1 % Rec.	2 % Rec.	Avg.	SD
2,3,7,8-TCDF	74.2	91.7	82.95	12.4
1,2,3,7,8-PeCDF	78.6	87.6	83.1	6.36
2,3,4,7,8-PeCDF	70.4	85.0	77.7	10.3
1,2,3,4,7,8-HxCDF	85.2	76.4	80.8	6.22
1,2,3,6,7,8-HxCDF	85.3	76.2	80.75	6.43
2,3,4,6,7,8-HxCDF	72.6	82.9	77.75	7.28
1,2,3,7,8,9-HxCDF	74.6	83.0	78.8	5.94
1,2,3,4,6,7,8-HpCDF	70.1	52.4	61.25	12.5
1,2,3,4,7,8,9-HpCDF	65.5	56.0	60.75	6.72
OCDF	63.6	55.0	59.3	6.08
2,3,7,8-TCDD	70.9	82.8	76.85	8.41
1,2,3,7,8-PeCDD	98.6	85.0	91.8	9.62
1,2,3,4,7,8-HxCDD	75.4	79.4	77.4	2.83
1,2,3,6,7,8-HxCDD	70.5	87.3	78.9	11.9
1,2,3,4,6,7,8-HpCDD	70.0	53.3	61.65	11.8
OCDD	69.2	36.6	52.9	23.1

Table 8. Wastewater influent results.

Compounds	1 % Rec.	2 % Rec.	Avg.	SD
2,3,7,8-TCDF	88.3	87.6	88.0	0.49
1,2,3,7,8-PeCDF	71.3	69.2	70.3	1.48
2,3,4,7,8-PeCDF	81.6	77.6	79.6	2.83
1,2,3,4,7,8-HxCDF	76.9	74.5	75.7	1.70
1,2,3,6,7,8-HxCDF	75.5	72.5	74.0	2.12
2,3,4,6,7,8-HxCDF	86.1	84.8	85.5	0.92
1,2,3,7,8,9-HxCDF	86.7	81.9	84.3	3.39
1,2,3,4,6,7,8-HpCDF	54.8	48.0	51.4	4.81
1,2,3,4,7,8,9-HpCDF	75.8	55.8	65.8	14.1
OCDF	79.3	28.1	53.7	36.2
2,3,7,8-TCDD	91.8	91.9	91.9	0.07
1,2,3,7,8-PeCDD	79.5	81.1	80.3	1.13
1,2,3,4,7,8-HxCDD	85.1	85.8	85.5	0.49
1,2,3,6,7,8-HxCDD	79.5	84.6	82.1	3.61
1,2,3,4,6,7,8-HpCDD	64.7	60.1	62.4	3.25
OCDD	61.2	54.9	58.1	4.45

Biotage® VacMaster™ Disk Method Summary

1. Repeat the following steps for each active Biotage® VacMaster Disk station.
2. Setup the VacMaster Disk manifolds ensuring all waste lines and vacuum lines are attached. Set the vacuum pump to -24"Hg.
3. Prepare the disk holder assembly (47mm): ensure the support screen is flat in the center of the disk holder. Place the Atlantic® DVB Disk on top of the support screen with the ripples of the disk on top and add any prefilters on top of the disk. Place the disk holder assembly on the VacMaster Disk ensuring there is a tight seal with the luer fitting.
4. If using the multifunnel, place onto the disk holder assembly. If not using the multifunnel, omit those directions throughout the method.
5. Condition the SPE Disk:
   a. Guide for each conditioning step in Table 9 below:
      i. Measure the appropriate VOLUME of SOVENT into a graduated cylinder and pour into the disk holder assembly.
      ii. Using a Nalgene Wash Bottle (phthalate free), rinse the multifunnel and disk holder in a circle for about 3 seconds using the same SOLVENT (approximately 5 additional mL).
      iii. SATURATE the disk for the time shown (IN SECONDS). (Saturate means: quickly turn the knob to the appropriate waste destination and back to the "OFF" position. This brings the solvent into the disk media bed).
      iv. SOAK the disk for the the time shown (IN SECONDS).
      v. DRAIN to the appropriate waste destination for the time shown (IN SECONDS). Switch to the "OFF" position.

Table 9. Disk conditioning.

Solvent	Volume (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Drain (sec.)
Methylene Chloride	15	1	60	Organic	120
Acetone	11	1	60	Organic	120
Methanol	11	1	60	Organic	8
Reagent Water	15	1	5	Organic	4
Reagent Water	15	1	0	Aqueous	0

6. Load the Sample:
   a. For multifunnel: quickly and efficiently angle the bottle to rest on the multifunnel upside-down.
   b. For no multifunnel: pour a portion of the sample into the disk holder.
   c. Adjust the vacuum between -10"Hg and -15"Hg for sample load (please note, if the sample is flowing too slowly, the vacuum can be increased). Drain the sample to "AQUEOUS" waste. Continue to pour the sample into the disk holder ensuring the disk does not go dry or overflow for the duration of sample load.
7. Air Dry the SPE Disk:
   a. Return the vacuum to -24"Hg and continue to air dry the SPE disk to "AQUEOUS" waste for an additional 600 SECONDS. Switch to the "OFF" position.
   b. Remove the sample bottle from the multifunnel if it was used.

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8. Elute the SPE Disk: (Please note: the elution solvent will go into the collection flask inside the chamber, not to waste containers; omit multifunnel steps if not using)
   a. Place a clean 125 mL 24/40 tapered Erlenmeyer flask into the VacMaster Disk collection chamber. Place the cover on the chamber. Remove the disk holder assembly and place the disk holder assembly into the luer fitting on top of the collection chamber. Attach the luer fitting of the collection chamber assembly onto the manifold.
   b. Guide for each elution step in Table 10 below:
      i. Measure the appropriate VOLUME of SOLVENT into a graduated cylinder, pour into the sample bottle, and swirl around. Pour the solvent in the sample bottle into the disk holder assembly.
      ii. Using a Nalgene Wash Bottle (phthalate free), rinse the multifunnel and disk holder in a circle for about 3 seconds using the same SOLVENT (approximately 5 additional mL).
      iii. SATURATE the disk for the time shown (IN SECONDS) to “ORGANIC”.
      iv. SOAK the disk for the the time shown (IN SECONDS).
      v. DRAIN to “ORGANIC” for the time shown (in SECONDS). Switch to the “OFF” position.
      vi. Remove the chamber lid to release the vacuum from inside the chamber.

Table 10. Disk elution.

Solvent	Volume (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Elute (sec.)
Acetone	8	1	90	Organic	60
Methylene Chloride	8	1	90	Organic	60
Methylene Chloride	8	1	90	Organic	60
Methylene Chloride	8	1	90	Organic	60
Methylene Chloride	8	1	90	Organic	120

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