Comparison of Vegetation Extraction Techniques for Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans

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Introduction

The analysis of vegetation samples for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans is important for many studies dealing with the transport and fate of these compounds in the environment and levels present in food sources. It is necessary to refine methods used in analyzing these samples in order to achieve optimum precision and accuracy. Vegetation samples can present challenges particularly in sample preparation. Due to high water content and difficulty in homogenization and tissue breakdown, and the amount of co-extractable compounds, extractions of these samples can be inconsistent. Samples with very high water content (>80%) have been difficult to extract by Soxhlet because of the problems with dispersing the sample to allow solvent penetration. Techniques tried in the past such as filtering, oven or room temperature drying samples, and mixing the sample with sand or Na_2SO_4 have either been unsuccessful or impractical. It is also necessary to obtain a well homogenized sample and efficient tissue breakdown in order to ensure accurate native analyte concentrations. Unlike other biological matrices, vegetation samples have minimal lipid content; therefore, the extraction solvent does not further break down these tissues by dissolving lipid. These samples must be thoroughly broken down to increase surface area before or during extraction.

In light of these problems, a study of different extraction techniques was implemented. Three extraction methods were evaluated for their ability to extract dioxins and furans from vegetables. Carrots and cucumbers were chosen as matrices that particularly exhibit many of the problems mentioned above. A Soxhlet extraction method using toluene and two variations of a liquid/liquid extraction method previously used to extract blood and serum samples[1] were used. The samples were spiked with USEPA Method 23 field surrogates[2] before homogenization so recovery could be measured against internal quantitation standards (IQS) spiked before extraction. In this way, the recovery

through homogenization and tissue breakdown could be compared against the absolute recovery through the method.

Materials and Methods

Pre-extraction preparation: A sample, a matrix spike (MS), and a matrix spike duplicate (MSD) for each matrix were prepared for each extraction. Approximately 50-gram samples of carrots and cucumbers were cut from the whole vegetables and weighed. All samples were spiked with US EPA Method 23 field surrogate by spiking the solution into approximately 1 mL of acetone and injecting this solution in 50 µl aliquots throughout the whole vegetable. Samples were allowed to equilibrate in a refrigerator at least 12 hours before homogenization. They were homogenized by grinding in an electric blender or grating until the sample was fine and uniform. All equipment used to homogenize the sample was carefully cleaned and rinsed with acetone to prevent sample loss.

Soxhlet extraction: The homogenized samples were mixed with approximately 10 grams of cellulose. Samples were then transferred to a pre-extracted cellulose thimble. US EPA Method 23 Laboratory surrogate and native dioxin/furan solutions (for MS and MSD) were spiked into acetone and added to the sample. The thimbles were placed in Soxhlet extractors fitted with Dean-Stark apparati and allowed to equilibrate 1 hour prior to extraction with toluene for 21 hours. Water was drained from the Dean-Stark throughout extraction. Samples were then concentrated and solvent exchanged to hexane.

Liquid/liquid extraction: The homogenized samples were transferred to 250 mL Teflon centrifuge tubes. Laboratory surrogate and native solutions were spiked with acetone as in the Soxhlet extraction. After allowing the samples to equilibrate for 1 hour, 50 mL of each of the following was added: ethanol, saturated ammonium sulfate solution, and hexane. Samples were then extracted on a rotary extractor for 30 minutes, centrifuged and the hexane layer was pipetted off and put through Na_2SO_4. The extraction procedure was repeated twice more using 50 mL of fresh hexane.

Tissue miser®/liquid/liquid extraction: The homogenized samples were transferred to 250 mL Teflon centrifuge tubes. Then 50 mL each of ammonium sulfate and ethanol was added to the samples. A Tissue Miser® was inserted into the samples and run at hiqh speed until the sample was liquified. Lab surrogate and native solutions were spiked in acetone as in the preceding methods and allowed to equilibrate for 1 hour. Next 50 mL of hexane was added, and the above rotary extraction was performed.

Cleanup procedures: All samples were cleaned by acid/base partitioning and then put through a series of silica, alumina, and carbon columns. Samples were then spiked with a recovery standard and concentrated to a final volume of 20 µl.

Analysis: Samples were analyzed by HRGC/HRMS using a Hewlett Packard 5890 gas chromatograph with a DB-5ms chromatography column (60 meter, 0.25mm id, 0.25µm film thickness) and a VG70-250S in SIM mode operating at a resolving power of 10,000. USEPA Method 23[1] calculations were used with USEPA Method 1613[3] concentrations and calibrations for sample data, initial, and continuing calibration.

Results and Discussion

Average lab and field surrogate recoveries for both matrices are shown in tables 1 and 2. Field surrogate recoveries are calculated versus internal quantitation standards. IQS results show comparable results between all methods. MS and MSD recoveries and percent relative difference (%RPD) are shown in tables 3 and 4.

The Soxhlet and Tissue Miser® methods show the best field surrogate recoveries (62-101%) for both matrices. The rotary extraction without the tissue miser® step did not recover the field surrogate compounds as well (32-59%). The Soxhlet method showed no significant differences in field surrogate recoveries between matrices. However, the tissue miser® method shows slightly higher recoveries with the cucumber (tables 1,2).

The trends in field surrogate recovery (shown in table1) between the methods and matrices indicate tight precision for the liquified samples. The %RSD values for the Soxhlet method range from 10%-14% for carrots, and 13.22-16.83% for cucumbers, whereas the %RSD values for the tissue miser® method are lower (3.1-7.7% carrot, 2.5-6.5% cucumbers). A comparison of the data between matrices showed slightly better results for the Soxhlet extraction of carrots. The opposite was found with the tissue miser® method. This could be attributed to the fact that it is more difficult breaking down the carrot verses the cucumber using the tissue miser®. The cucumber has a higher water content compared to the carrot, making the Soxhlet extraction of the carrot more effective.

These results show that the rotary extraction is a precise and efficient method for extraction as long as the sample is broken down completely, as is accomplished during the tissue miser® step. This increases the surface area of the sample so that efficient solvent penetration and extraction of native compounds can be accomplished. Ammonium sulfate and ethanol alone are not sufficient. Mixing the sample with cellulose and Soxhlet extracting with toluene and a Dean-Stark apparatus is also an efficient extraction method although slightly less precise for these matrices.

Acknowledgement

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References

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3. USEPA Method 1613, Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B, Oct.1994.

Table 1. Comparison of Average IQS and field surrogate Recoveries and %RSD (Carrots)

	Soxhlet Carrots		Rotary/Tissue Miser Carrots		Rotary Alone Carrots	
	Ave. % Recovery	%RSD n=3	Ave. % Recovery	%RSD n=3	Ave. % Recovery	%RSD n=3
IQS						
13C2378TCDF	53	0.8	52	20	72	1.5
13C2378TCDD	58	1.0	54	21	73	9.9
13C12378PeCDF	75	1.4	70	23	108	5.2
13C12378PeCDD	59	0.7	54	22	79	11.3
13C123678HxCDF	72	4.2	64	22	107	0.0
13C123678HxCDD	75	6.3	70	24	92	0.7
13C1234678HpCDF	81	7.9	69	28	84	3.9
13C1234678HpCD	86	9.9	75	26	88	4.0
13C12OCDD	72	9.8	63	22	66	12.5
Field Surrogate						
37CL2378TCDD	93	10.7	87	4.5	59	12.3
13C23478PeCDF	65	10.3	62	6.6	38	14.5
13C123478HxCDF	101	9.0	89	3.6	46	11.5
13C123478HxCDD	99	14.0	86	3.1	52	16.2
13C1234789HpCDF	90	9.8	76	7.7	43	9.7

Table 2. Comparison of Average IQS and field surrogate Recoveries and %RSD (Cucumbers)

	Soxhlet Cucumbers		Rotary/Tissue Miser Cucumbers		Rotary Alone Cucumbers	
	Ave. % Recovery	%RSD n=3	Ave. % Recovery	%RSD n=3	Ave. % Recovery	%RSD n=3
Lab Surrogate						
13C2378TCDF	54	9.2	48	37.6	64	18.3
13C2378TCDD	57	9.5	49	34.9	63	23.2
13C12378PeCDF	79	16.6	65	38.0	83	22.7
13C12378PeCDD	62	11.2	53	33.1	63	21.6
13C123678HxCDF	54	16.1	61	27.3	69	18.8
13C123678HxCDD	56	15.6	65	26.2	79	23.9
13C1234678HpCDF	48	15.8	58	25.4	73	17.6
13C1234678HpCD	54	16.8	64	22.9	74	16.0
13C12OCDD	50	17.9	62	19.0	55	10.9
Field Surrogate						
37CL2378TCDD	90	13.8	92	2.5	38	12.6
13C23478PeCDF	66	16.8	72	6.5	26	13.9
13C123478HxCDF	98	14.6	100	3.3	37	10.6
13C123478HxCDD	99	13.2	97	3.6	32	11.5
13C1234789HpCDF	87	13.9	80	4.4	32	13.7

Table 3. MS/MSD %Recoveries and %RPD (Cucumbers)

	Soxhlet Cucumbers		Rotary/Tissue Miser Cucumbers		Rotary Alone Cucumbers	
	Ave. % Recovery	%RPD	Ave. % Recovery	%RPD	Ave. % Recovery	%RPD
Isomer						
2378TCDF	108	1.9	112	4.5	100	1.0
2378TCDD	111	0.0	112	0.9	100	0.0
12378PECDF	78	1.3	80	1.3	83	2.4
23478PECDF	68	11.8	72	11.1	74	5.4
12378PECDD	104	1.9	106	4.7	105	1.0
123478HXCDF	105	2.9	108	1.9	123	4.1
123678HXCDF	104	1.0	108	2.8	120	0.8
234678HXCDF	86	0.0	92	3.3	102	1.0
123789HXCDF	83	9.6	85	1.2	118	9.4
123478HXCDD	111	2.7	111	0.00	104	3.8
123678HXCDD	96	1.0	96	0.0	101	2.0
123789HXCDD	115	4.4	115	0.9	110	3.6
1234678HPCDF	100	0.0	103	3.9	104	0.0
1234789HPCDF	90	3.4	83	3.6	96	7.3
1234678HPCD	81	11.2	89	1.1	88	0.0
12346789OCDF	89	3.4	83	0.0	106	4.7
12346789OCD	98	1.0	105	3.81	103	1.0

Table 4. MS/MSD %Recoveries and %RPD (Carrots)

	Soxhlet Carrots		Rotary/Tissue Miser Carrots		Rotary Alone Carrots	
	Ave. % Recovery	%RPD	Ave. % Recovery	%RPD	Ave. % Recovery	%RPD
Isomer						
2378TCDF	103	1.0	101	4.0	105	2.9
2378TCDD	103	1.0	108	4.7	103	2.9
12378PECDF	83	0.0	81	1.2	84	3.6
23478PECDF	73	2.7	73	2.7	66	7.6
12378PECDD	104	2.9	108	2.8	101	1.0
123478HXCDF	122	1.6	127	7.1	94	1.1
123678HXCDF	121	5.8	118	3.4	115	0.9
234678HXCDF	102	2.0	104	5.8	77	0.0
123789HXCDF	111	19.0	108	5.6	75	6.7
123478HXCDD	119	3.4	119	7.6	109	7.3
123678HXCDD	103	1.9	103	2.9	95	0.0
123789HXCDD	125	0.8	118	9.4	113	1.8
1234678HPCDF	110	2.7	111	0.9	108	2.8
1234789HPCDF	100	2.0	102	1.0	88	1.1
1234678HPCD	90	2.2	92	3.3	88	2.3
12346789OCDF	99	12.1	99	6.1	100	15.1
12346789OCD	119	3.4	120	6.7	118	0.9