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APPLICATION NOTE

Analysis of Wastewater Samples using Disk Solid Phase Extraction (SPE) following US EPA Method 608.3

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Key Words

Wastewater, SPE, solid phase extraction, EPA Method 608.3

Introduction

Pesticides have gone far in increasing food supply, important in a world expecting to have 9.8 billion people by 2050. However, the mechanisms that make pesticides effective in eliminating pests can also cause harm to humans and animals. News stories continue to point to contamination through spills and exposure through the respiratory system. (1) Pesticides in water and food must be monitored to determine if they are compliant with levels of allowed pesticides or if they contain banned pesticides. Screening is often done with methods specifying gas chromatography mass spectrometry (GC/MS) for detection, evaluating a wide variety of compounds. Methods such as US EPA 525.2 and 625 fall into this category. Methods with specific detectors, such as electron capture or other halogen-specific detectors can detect pesticides very sensitively, often with better sensitivity than a quadrupole GC/MS.

SPE-DEX 5000 Automated Disk Extraction System

Method US EPA 608 is a dual column method specifying an electrochemical detector for gas chromatography (GC). The method was developed for organochlorine pesticides and PCBs in wastewater. The newest revision of the method, 608.3, specifically allows disk solid phase extraction to be used instead of liquid-liquid extraction, adding technology that was approved for this method through the Alternate Test Approval process in 1995.(2) Since 1995, laboratories have been able to submit data to the US EPA using SPE technology if they chose and now it is just more clearly accessible through language directly in the method. In all cases the quality control required by the method must be met.

This work demonstrates the use of SPE disks on wastewater samples using automation to provide control and consistency in all the steps in the extraction process, including conditioning the disk, loading the water sample, rinsing the sample bottle and eluting the sample in solvent. Since the use of a halogen-specific detector precludes use of a chlorinated solvent for the sample, the method specifies solvent exchange from methylene chloride into hexane before the analysis step. When SPE is used, the extraction and elution steps are separate, so extraction onto the SPE disk is one step and then elution can be considered separately. Since the final extract should be in hexane, it is possible to elute the disk directly into hexane, eliminating the elution with methylene chloride and subsequent solvent exchange step. Both elution with methylene chloride and elution with hexane will be demonstrated here.

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Experimental

Extraction of one-liter samples of reagent water, synthetic wastewater (ASTM D5905 (3)) and Publicly Owned Treatment Works (POTW) influent waters were extracted using the SPE-DEX® 5000, following the requirements of US EPA method 608.3.

The SPE-DEX 5000 (Horizon Technology) was used with Atlantic® C18 Disks. The Fast Flow disk holder (Horizon Technology) was used with 1 and 5 µm prefilters to hold any particulate matter above the SPE disk so flow through the disk is maintained but the particulate is included in the extraction and elution steps. A check standard containing the spiking material was prepared and sent with the samples for analysis and over the course of several sets of samples was recovered at about 90%.

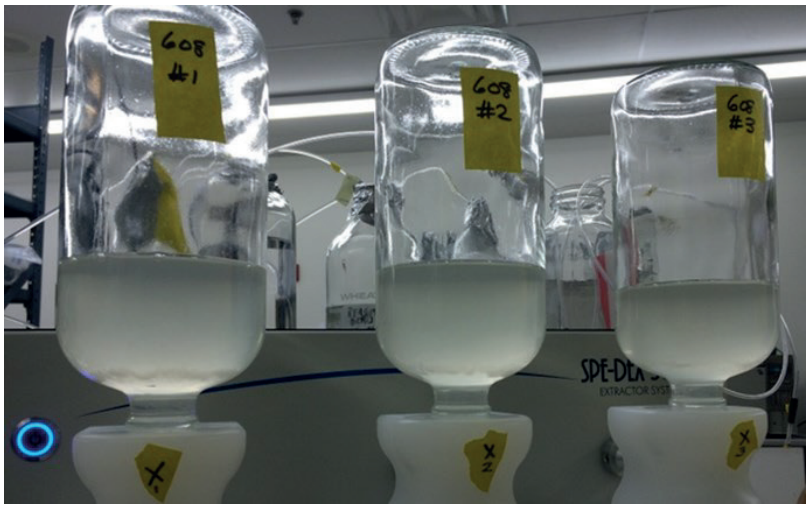

Figure 1. Shows synthetic wastewater being extracted with the SPE-DEX 5000. Note the particulate in each sample.

The resulting extracts were dried using a DryDisk®-R (Horizon Technology) membrane drying technology on the DryVap® In-line Drying and Evaporation System (Horizon Technology) and concentrated to 1 mL. After solvent exchange to hexane the samples were concentrated to 10 mL and refrigerated until GC/ECD analysis. For the samples eluted directly in hexane a similar approach was employed and samples refrigerated until analysis. No further cleanup, such as the use of Florisil was done at Horizon Technology.

The SPE-DEX 5000 extraction program is shown in Table 1 for methylene chloride elution and Table 2 for hexane elution.

Table 1. Methylene Chloride Elution Extraction Program

Step	Operation	Solvent	Solvent Volume (mL)	Vent Purge Time (s)	Vacuum Pump Rate (s)	Satura-tion Time (s)	Soak Time (s)	Drain Time (s)	Done Load-ing Sample Delay	Dry Time (s)	N2 Blanket
1	Condition SPE Disk	Methylene chloride	40	60	6	3	60	120			
2	Condition SPE Disk	Acetone	20	60	3	3	60	30			
3	Condition SPE Disk	Methanol	40	60	3	3	30	30			
4	Condition SPE Disk	Reagent Water	20	60	3	3	15	30			
5	Load Sample				3				45		
6	Wash Sample Container	Reagent Water	20	30	6	3	20	30			OFF
7	Air Dry Disk Timer				6					360	OFF
8	Elute Sample Container	Acetone	40	20	3	3	60	90			OFF
9	Elute Sample Container	Methylene chloride	40	15	3	3	60	90			OFF
10	Elute Sample Container	Methylene chloride	40	15	3	3	60	90			OFF
11	Elute Sample Container	Methylene chloride	40	15	6	3	60	120			OFF

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Table 2. Hexane Elution Extraction Program

Step	Operation	Solvent	Solvent Volume (mL)	Vent Purge Time (s)	Vacuum Pump Rate	Satura-tion Time (s)	Soak Time (s)	Drain Time (s)	Done Load-ing Sample Delay (s)	Dry Time (s)	N2 Blanket
1	Condition SPE Disk	Hexane	40	60	6	3	60	120			
2	Condition SPE Disk	Acetone	20	60	3	3	60	30			
3	Condition SPE Disk	Methanol	40	60	3	3	30	30			
4	Condition SPE Disk	Reagent Water	20	60	3	3	15	30			
5	Load Sample				3				45		
6	Wash Sample Container	Reagent Water	20	30	6	3	20	30			OFF
7	Air Dry Disk Timer				6					360	OFF
8	Elute Sample Container	Acetone	40	20	3	3	60	90			OFF
9	Elute Sample Container	Hexane	40	15	3	3	60	90			OFF
10	Elute Sample Container	Hexane	40	15	3	3	60	90			OFF
11	Elute Sample Container	Hexane	40	15	6	3	60	120			OFF

The DryVap System was operated with the conditions shown in Table 3.

Analysis was done at Alpha Analytical Services, Westborough, MA, an accredited laboratory with environmental experience.

GC analysis was done with the conditions as shown in Table 4 on a 6890 GC with dual micro ECD (Agilent Technologies).

The calibration curve covered the range from 0.5-200 µg/L. Surrogate and organochlorine pesticide mix was supplied by Alpha Analytical. Samples were spiked with a full range of pesticides at 0.5 µg/L in each 1-L sample.

Figure 2 shows the software running the air dry and wash sample steps.

Table 3. DryVap Operating Conditions

Parameter	Setting
Dry Volume	200 mL
Heat Power	5
Heat Timer	OFF
Auto Rinse Mode	OFF
Nitrogen Sparge	20 psi
Vacuum	-8 in. Hg

Figure 2. Software screen monitoring operation during sample analysis

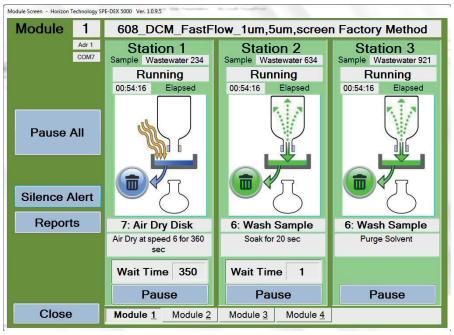

Table 4. GC Conditions

Columns	RTX-CLP 30 m x 0.32 mm fused silica capillary, 0.32 µm film thickness RTX-CLPII30 m x 0.32 mm fused silica capillary, 0.25 µm film thickness
Temperature Program	120°C for 0 min 45° C/min to 200°C, 0 min 15°C/min to 230°C, 0 min 30°C/min to 330°C, Hold for 2 min
Injection Volume	1 µL

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Table 6. Results from Reagent Water and Synthetic Wastewater Eluted Directly with Hexane

	Reagent Water % Recovery			Acceptable		RPD (%)	Synthetic Wastewater % Recovery			Acceptable		RPD (%)
	Blank	Spike	Spike Dup	% Recovery	RPD (%)	Limit	Blank	Spike	Spike Dup	% Recovery	RPD (%)	Limit
4,4'-DDD	ND	75.2	74.8	31-141	0.533	39	ND	68.2	69.6	31-141	2.03	39
4,4'-DDE	ND	72.6	70.8	30-145	2.51	35	ND	51.6	51.8	30-145	0.39	35
4,4'-DDT	ND	81.6	80.6	25-160	1.23	42	ND	79.0	81.6	25-160	3.24	42
Aldrin	ND	71.4	70.0	42-140	1.98	35	ND	74.0	73.6	42-140	0.54	35
Alpha-BHC	ND	87.8	85.0	37-140	3.24	36	ND	83.0	83.6	37-140	0.72	36
Beta-BHC	ND	82.0	74.4	17-147	9.72	44	ND	85.0	81.2	17-147	4.57	44
cis-Chlordane (alpha)	ND	78.4	75.6	45-140	3.64	35	ND	67.4	66.4	45-140	1.49	35
Delta-BHC	ND	88.8	85.8	19-140	3.44	52	ND	88.0	88.0	19-140	0.00	52
Dieldrin	ND	94.0	92.4	36-146	1.72	49	ND	83.2	84.4	36-146	1.43	49
Endosulfan I	ND	90.2	88.0	45-153	2.47	28	ND	78.0	78.0	45-153	0.00	28
Endosulfan II	ND	92.2	96	D-202	4.04	53	ND	92.8	96	D-202	3.18	53
Endosulfan sulfate	ND	90.8	90.6	26-144	0.221	38	ND	77.4	78.6	26-144	1.54	38
Endrin	ND	79.6	78.0	30-147	2.03	48	ND	69.8	70.4	30-147	0.86	48
Endrin aldehyde	ND	73.8	82.8		11.5		ND	66.4	70.0		5.3	
Endrin ketone	ND	89.4	89.0		0.448		ND	83.8	85.4		1.89	
Heptachlor	ND	86.2	83.4	34-140	3.30	43	ND	91.4	90.8	34-140	0.66	43
Heptachlor epoxide	ND	97.4	94.6	37-142	2.92	26	ND	103	90.2	37-142	13.3	26
Lindane	ND	87.8	81.6	32-140	7.32	39	ND	84.6	87.0	32-140	2.80	39
Methoxychlor	ND	79.2	79.4		0.25		ND	77.8	80.2		3.04	
trans-Chlordane (gamma)	ND	81.8	78.0	45-140	4.76	35	ND	65.2	65.6	45-140	0.61	35
Surrogate	**% Recovery**	**% Recovery**	**% Recovery**				**% Recovery**	**% Recovery**	**% Recovery**			
2,4,5,6-Tetrachloro-m-xylene	76.3	74.2	76.3				84.5	85.6	81.4			
Decachlorobiphenyl	13.2	17.1	15.8				93.4	90.8	90.8			

Table 7. Results from POTW Influent

	Influent Wastewater % Recovery			Acceptable		RPD (%)
	Blank	Spike	Spike Dup	% Recovery	RPD (%)	Limit
4,4'-DDD	ND	96.4	86.6	31-141	10.7	39
4,4'-DDE	ND	83.6	89.6	30-145	6.93	35
4,4'-DDT	ND	84	75.2	25-160	11.1	42
Aldrin	ND	82.2	85.6	42-140	4.05	35
Alpha-BHC	ND	100	119	37-140	18.1	36
Beta-BHC	ND	100	92.2	17-147	8.32	44
cis-Chlordane (alpha)	ND	105	98	45-140	6.31	35
Delta-BHC	ND	104	106	19-140	1.14	52
Dieldrin	ND	117	115	36-146	2.41	49
Endosulfan I	ND	117	120	45-153	2.86	28
Endosulfan II	ND	112	102	D-202	9.70	53
Endosulfan sulfate	ND	122	105	26-144	14.7	38
Endrin	ND	100	92	30-147	7.90	48
Endrin aldehyde	ND	89.6	76.2		16.2	
Endrin ketone	ND	121	104		15.2	
Heptachlor	ND	107	118	34-140	9.96	43
Heptachlor epoxide	ND	119	116	37-142	1.87	26
Lindane	ND	109	106	32-140	2.60	39
Methoxychlor	ND	96.0	90.0		6.5	
trans-Chlordane (gamma)	ND	65.8	107	45-140	48.0	35
Surrogate	**% Recovery**	**% Recovery**	**% Recovery**			
2,4,5,6-Tetrachloro-m-xylene	110.3	111.3	108.2			
Decachlorobiphenyl	81.6	65.8	81.6			

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Results and Discussion

Method 608.3 consolidates the QC requirements for the method in a table (Table 4) by analyte. The reporting limit provided by Alpha Analytical for this method, prepared as described, is listed in Table 5 to help in defining the lower level that is reliably measured in the blank. The colors have been added to the table to make it easier to quickly see the agreement with the desired 100% recovery. Dark green is the closest to 100% and then it changes from dark green the further away the value is from 100%, lighter green and orange below 100% and more blue above 100%.
The recoveries of the spike and spike duplicate for reagent water and synthetic wastewater are excellent and well within the range specified in Table 4 of method 608.3. The relative percent difference indicates the quality of precision and is excellent. In many cases less than 1% RPD indicates the agreement between the spike and spike duplicate preparations is excellent.

Table 6 shows the same type of comparison to 608.3 Table 4 criteria for reagent water and synthetic wastewater.

The results again meet the criteria specified in Table 4 of method 608.3. It is interesting that in both cases the recovery of the surrogate decachlorobiphenyl is poor in reagent water, but acceptable in more complex matrices. Horizon Technology will do more work to understand this issue, but it may be the hydrophobic nature of the compound interacting with water-coated disk particles.

Table 7 shows the results from an influent wastewater from a medium-sized POTW facility.

Table 5. Results for Hexane Extract obtained through Solvent Exchange

		Reagent Water % Recovery			Acceptable		RPD (%)	Synthetic Wastewater % Recovery			Acceptable		RPD (%)
Analyte	RL (µg/L)	Blank	Spike	Spike Dup	% Recovery	RPD (%)	Limit	Blank	Spike	Spike Dup	% Recovery	RPD (%)	Limit
4,4'-DDD	0.04	ND	81.4	80.8	31-141	0.740	39	ND	72.6	69.8	31-141	3.93	39
4,4'-DDE	0.04	ND	78.4	78.2	30-145	0.255	35	ND	72.2	73.8	30-145	2.19	35
4,4'-DDT	0.04	ND	79.4	79.4	25-160	0.000	42	ND	70.8	71.6	25-160	1.12	42
Aldrin	0.02	ND	71.2	71.4	42-140	0.281	35	ND	69.8	71.4	42-140	2.27	35
Alpha-BHC	0.02	ND	80.4	79.8	37-140	0.749	36	ND	79.2	80.0	37-140	1.01	36
Beta-BHC	0.02	ND	84.0	84.4	17-147	0.475	44	ND	81.8	78.4	17-147	4.24	44
cis-Chlordane (alpha)	0.02	ND	79.4	79.0	45-140	0.505	35	ND	71.2	71.4	45-140	0.281	35
Delta-BHC	0.02	ND	83.8	84.4	19-140	0.713	52	ND	78.0	82.0	19-140	5.00	52
Dieldrin	0.04	ND	87.0	86.6	36-146	0.461	49	ND	80.6	79.2	36-146	1.75	49
Endosulfan I	0.02	ND	83.0	82.6	45-153	0.483	28	ND	75.8	76.4	45-153	0.788	28
Endosulfan II	0.04	ND	76.6	75.2	D-202	1.84	53	ND	72.2	71.6	D-202	0.83	53
Endosulfan sulfate	0.04	ND	87.8	87.2	26-144	0.686	38	ND	79.6	77.2	26-144	3.06	38
Endrin	0.04	ND	84.0	83.6	30-147	0.477	48	ND	91.2	101.2	30-147	10.4	48
Endrin aldehyde	0.04	ND	76.0	74.4		2.13		ND	67.2	65.8		2.11	
Endrin ketone	0.04	ND	87.6	87.4		0.229		ND	82.6	82.4		0.242	
Heptachlor	0.02	ND	75.2	75.0	34-140	0.266	43	ND	70.0	72.6	34-140	3.65	43
Heptachlor epoxide	0.02	ND	88.0	87.2	37-142	0.913	26	ND	73.2	73.4	37-142	0.273	26
Lindane	0.02	ND	86.6	86.8	32-140	0.231	39	ND	82.4	82.6	32-140	0.242	39
Methoxychlor	0.1	ND	95.4	93.0		2.55		ND	79.4	90.8		13.4	
trans-Chlordane (gamma)	0.02	ND	81.6	79.8	45-140	2.23	35	ND	71.8	72.4	45-140	0.83	35
Surrogate		% Recovery	% Recovery	% Recovery				% Recovery	% Recovery	% Recovery			
2,4,5,6-Tetrachloro-m-xylene		64	65	59				70	73	72			
Decachlorobiphenyl		11	12	13				70	57	71			

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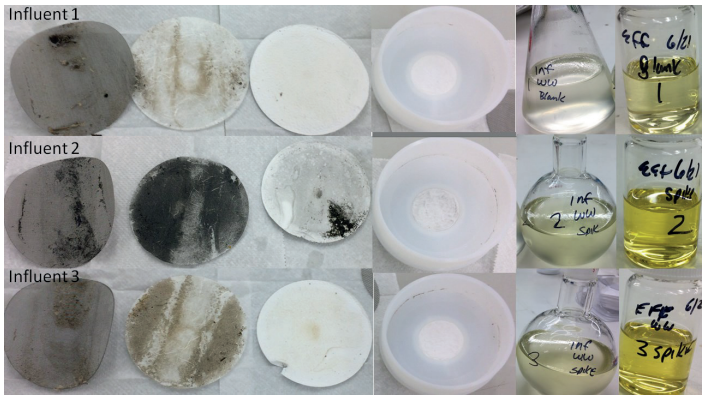
The results are very good for a complex matrix, meeting the goals in all cases for spike recovery. In the case of trans-chlordane, however, the agreement between the spike and spike duplicate are outside the acceptable range. There was some interference with the chromatography and the spike was cleaned with copper. The spike duplicate appeared cleaner and was not treated with copper. This issue was not observed with synthetic wastewater and was unexpected. As shown in Figure 3, the particulate matter in the spike and spike duplicate (influent 2 and 3) were different in amount and color. This could have contributed to a matrix effect which influenced the recovery of compounds more in one sample than the other, in spite of the copper treatment.

Figure 3. Wastewater Influent samples showing the screen, 5µm prefilter, 1 µm prefilter, and SPE disk in the Fast Flow Disk Holder, along with extracts and final dried and evaporated samples



Conclusion

The analysis of low concentrations of pesticides in wastewater is important to ensure the wastewater is clean before release into the environment. In addition, wastewater can make its way into drinking water, either directly or indirectly, carrying potential contamination along and increasing exposure. This work demonstrated automated extraction using solid phase extraction disks as an alternative to liquid-liquid extraction. Both reagent water and synthetic wastewater showed excellent results following method 608.3 for extraction and analysis with GC-ECD. Additionally, direct elution of the solid phase disk with hexane, rather than elution with methylene chloride and a solvent exchange step to hexane showed excellent results for reagent water and synthetic wastewater spikes. Elution with hexane can save time and effort required in solvent exchange required for an ECD detector, improving the workflow in a busy laboratory. Analysis of a medium-sized wastewater treatment plant influent wastewater showed good results using the hexane elution procedure, further demonstrating the ability of automated disk SPE to improve the laboratory workflow while maintaining method performance.

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