EPA Method 8141B: Organophosphorous Pesticides Using Automated Solid Phase Extraction (SPE)

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Scope

This application note will outline appropriate methods for the extraction of organophosphorus pesticides from ground water as outlined in EPA method 8141B using the Atlantic® C18 SPE disk processed using Biotage automated or manual SPE solutions and DryVap® Concentrator System. 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 vacuum manifold for this application.

Introduction

EPA Method 8141B describes the performance-based procedure for determination of low ppb levels of organophosphorous (OP) pesticides in ground water. The primary extraction solvent is hexane which in turn eliminates the need to perform a solvent exchange.

Organophosphorus pesticides are the most widely used pesticides in the world. They are readily hydrolyzed and therefore do not persist in the environment for very long nor accumulate in the body fat of humans or other animals. The ubiquitous nature of the OP pesticides results in routine exposure for humans through the consumption of fresh and processed vegetables, contact with pesticide-contaminated surfaces, breathing air near pesticide applications, and drinking pesticide contaminated water. OP pesticides interfere with the nervous system of both insects and humans. In humans, these compounds block the production of cholinesterase. The main target organs in humans are the nervous system, respiratory tract, and cardiovascular system. The EPA classifies most OP compounds as toxicity class I (highly toxic) or toxicity class II (moderately toxic).

The OP pesticides are extracted using solid phase extraction (SPE) using Atlantic® C18 disks, based on the procedure outlined in Method 3535A for OP pesticides by Method 8141. The analysis is performed with capillary GC using an FPD (flame photometric detector) utilizing a splitless injection technique.

Instrumentation

- Biotage Instruments:
  - Biotage® Horizon 5000 Automated Extraction System
  - DryVap® Concentrator System
  - DryDisk® Separation Membranes
  - Atlantic™ C18 Disks (47 mm)
- Agilent 6890 GC with dual FPDs
- J&W Columns
  - Column A: DB-1701, 30 m x 0.320 mm x 0.25 µm
  - Column B: DB-5ms, 30 m x 0.250 mm x 0.5 µm

Method Summary

1. IPR samples: Four replicates are prepared using 500 mL of DI water at pH 5.0–9.0, spiked at 5.00 µg/L.
2. MDL samples: Seven replicates are prepared using 500 mL of DI water at pH 5.0–9.0 and spiked at 1.25 µg/L for each pesticide. (Must be performed over 3 days)
3. Place the sample bottle on the Biotage® Horizon 5000 Extraction System and place the Atlantic® C18 disk in the standard 47 mm disk holder. Attach collection vessels to the system
4. Extract the samples using the method in table 1 and collect the final sample extract.
5. Upon completion of the extraction, dry and concentrate the final sample extracts to 1.0 mL.
6. Extracts are analyzed by GC/dual FPD detectors and the conditions are listed in table 2.

Table 1. 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	Acetone	11	60	2	1	60	180	
Condition SPE Disk	Methanol	11	60	2	1	60	5	
Condition SPE Disk	Reagent water	15	60	2	1	60	5	
Load Sample				2				45
Air Dry Disk				6			360	
Elute Sample Container	Acetone	8	15	2	1	60	120	
Elute Sample Container	Hexane	8	15	2	1	60	120	
Elute Sample Container	Hexane	8	15	2	1	90	240	
Elute Sample Container	Hexane	8	15	6	1	90	240	

Table 2. GC Settings.

GC Conditions		Oven Temperature Program			
Inlet A Temperature	225 °C	**Initial Temperature**		80 °C	
Detector A Temperature	250 °C	**Initial Time**		0.00 min	
Detector B Temperature	250 °C	Level	Ramp (C/min)	Temp (C)	Final Time(min)
Constant Pressure	25.0 psi	1	20	170	0.00
Carrier Gas	He	2 (A)	10	280	5.53
Detector Gas	He, H, Air	3 (B)	0	0	0

Acknowledgements

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Application Modifications

Biotage® Horizon 4790 Method Summary

1. IPR samples: Four replicates are prepared using 500 mL of DI water at pH 5.0–9.0, spiked at 5.00 µg/L.
2. MDL samples: Seven replicates are prepared using 500 mL of DI water at pH 5.0–9.0 and spiked at 1.25 µg/L for each pesticide.
3. Biotage® Horizon 4790 Extraction Systems are purged in preparation for extraction.
4. The 47 mm Atlantic® C18 disks are placed in the disk holder assemblies and loaded onto the extraction systems.
5. Sample bottles are loaded onto the extraction systems and the collection vessels are attached.
6. IPR and MDL samples are extracted using the Horizon 4790, a 47 mm C18 disk, and the method shown in table 3.
7. At the end of the extraction the extracts were dried and concentrated to 1.0 mL.
8. Extracts were analyzed by GC/dual FPD detectors and the conditions are listed in table 2 in the previous section.

Table 3. Biotage® Horizon 4790 extraction method.

Step	Solvent	Soak Time (s)	Dry Time (s)
Prewet 1	Acetone	60	90
Prewet 2	Methanol	60	0
Prewet 3	Reagent water	60	0
Sample Process			
Air Dry			180
Rinse 1	Acetone	60	60
Rinse 2	Hexane	60	60
Rinse 3	Hexane	90	120
Rinse 4	Hexane	90	120

Biotage® Horizon 4790 Results and Conclusions

Hexane was used as the extraction solvent to eliminate the solvent exchange step. Data using methylene chloride as an extraction solvent showed comparable results (not presented in this Application Note).

The Initial Precision and Recovery (IPR) data for the selected OP pesticides are shown in tables 4 and 5 for columns one and two, respectively. The RSD for the IPR data for all compounds falls below 4 % with recoveries between 61.0 and 120 %.

The Method Detection Limits (MDL) are shown in tables 6 and 7. Each pesticide was calculated by multiplying the standard deviation of seven runs from the replicate study by the student t-value of 3.143.

Acceptable recovery limits and MDL values for the compounds are specified in Method 1657A Section 9.2. Results presented in this paper from an independent laboratory indicate that automated SPE using the Biotage® Horizon 4790 provides accurate and precise results for the determination of organophosphorus pesticides in aqueous samples. Benefits of automated SPE include reduced solvent usage, elimination of emulsions, reduced exposure to solvents, improved recoveries and consistency of results, increased productivity, and reduction in labour costs.

Table 4. IPR for Column 1 (nominally 5 µg/L).

Compounds	IPR 1	IPR 2	IPR 3	IPR 4	Mean	Recovery	STDEV	RSD
Demeton-O & S	3.65	3.55	3.53	3.47	3.55	71.0	0.075	2.11
Diazinon	4.04	4.04	4.08	3.97	4.03	80.7	0.046	1.13
Disulfoton	3.06	3.06	3.04	3.03	3.05	61.0	0.015	0.49
Chlorpyrifos	3.99	4.00	4.07	3.96	4.01	80.1	0.047	1.16
Parathion, methyl	4.25	4.28	4.36	4.25	4.29	85.7	0.052	1.21
Malathion	4.33	4.33	4.44	4.30	4.35	87.0	0.062	1.42
Parathion, ethyl	4.11	4.10	4.21	4.14	4.14	82.8	0.050	1.2
Ethion	4.01	4.06	4.07	4.02	4.04	80.8	0.029	0.73
EPN	4.08	4.16	4.10	4.06	4.10	82.0	0.043	1.05
Azinphos-methyl	5.68	6.13	6.00	6.19	6.00	120.0	0.228	3.79

Table 5. IPR for Column 2 (nominally 5 µg/L).

Compounds	IPR 1	IPR 2	IPR 3	IPR 4	Mean	Recovery	STDEV	RSD
Demeton-O & S	3.65	3.55	3.53	3.47	3.55	71.0	0.075	2.11
Diazinon	4.04	4.04	4.08	3.97	4.03	80.7	0.046	1.13
Disulfoton	3.06	3.06	3.04	3.03	3.05	61.0	0.015	0.49
Chlorpyrifos	3.99	4.00	4.07	3.96	4.01	80.1	0.047	1.16
Parathion, methyl	4.25	4.28	4.36	4.25	4.29	85.7	0.052	1.21
Malathion	4.33	4.33	4.44	4.30	4.35	87.0	0.062	1.42
Parathion, ethyl	4.11	4.10	4.21	4.14	4.14	82.8	0.050	1.2
Ethion	4.01	4.06	4.07	4.02	4.04	80.8	0.029	0.73
EPN	4.08	4.16	4.10	4.06	4.10	82.0	0.043	1.05
Azinphos-methyl	5.68	6.13	6.00	6.19	6.00	120.0	0.228	3.79

Table 6. MDL for Column 1 (nominally 1.25 µg/L).

Compounds	MDL 1	MDL 2	MDL 3	MDL 4	MDL 5	MDL 6	MDL 7	Mean	STDEV	MDL
Demeton-O & S	0.88	0.89	0.90	0.87	0.90	0.89	0.91	0.89	0.013	0.04
Diazinon	0.95	0.96	0.96	0.96	0.98	0.94	0.94	0.96	0.014	0.04
Disulfoton	0.54	0.55	0.55	0.54	0.56	0.54	0.53	0.54	0.010	0.03
Chlorpyrifos	0.96	0.97	0.97	0.98	0.99	0.96	0.96	0.97	0.012	0.04
Parathion, methyl	1.02	1.08	1.07	1.08	1.10	1.05	1.06	1.07	0.026	0.08
Malathion	1.11	1.12	1.11	1.12	1.14	1.09	1.10	1.11	0.016	0.05
Parathion, ethyl	1.04	1.05	1.04	1.03	1.09	0.99	1.00	1.03	0.033	0.10
Ethion	1.01	1.01	1.03	1.04	1.06	0.98	1.01	1.02	0.026	0.08
EPN	1.12	1.14	1.14	1.12	1.18	1.12	1.12	1.13	0.022	0.07
Azinphos-methyl	1.82	1.84	1.87	1.85	1.97	1.92	1.88	1.88	0.051	0.16

Table 7. MDL for Column 2 (nominally 1.25 µg/L)

Compounds	MDL 1	MDL 2	MDL 3	MDL 4	MDL 5	MDL 6	MDL 7	Mean	STDEV	MDL
Demeton-O & S	0.56	0.55	0.55	0.55	0.54	0.51	0.57	0.55	0.019	0.06
Diazinon	1.00	1.00	0.99	1.02	1.01	0.99	1.01	1.00	0.011	0.03
Disulfoton	0.54	0.54	0.54	0.55	0.57	0.54	0.53	0.54	0.013	0.04
Chlorpyrifos	0.98	0.99	0.98	1.00	1.00	0.96	0.97	0.98	0.015	0.05
Parathion, methyl	0.98	0.98	0.98	1.01	1.03	0.95	0.98	0.99	0.026	0.08
Malathion	1.02	1.02	1.03	1.05	1.04	1.02	1.01	1.03	0.014	0.04
Parathion, ethyl	0.96	0.95	0.97	0.97	0.99	0.96	0.95	0.96	0.014	0.04
Ethion	1.04	1.03	1.04	1.05	1.08	1.02	1.03	1.04	0.020	0.06
EPN	1.17	1.14	1.15	1.14	1.18	1.12	1.14	1.15	0.020	0.06
Azinphos-methyl	1.29	1.21	1.17	1.23	1.31	1.18	1.21	1.23	0.053	0.17

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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® C18 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 manifold 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 8 below:
      I. Measure the appropriate VOLUME of SOLVENT 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 indicated (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 time indicated (in SECONDS).
      V. DRAIN to the appropriate waste destination for the time indicated (in SECONDS). Switch to the “OFF” position.

Table 8. Disk Conditioning.

Solvent	Vol. (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Drain (sec.)
Acetone	11	1	60	Organic	180
MeOH	11	1	60	Organic	5
Reagent Water	15	1	60	Organic	5

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 360 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 solvents will go into the collection flask inside the chamber, not to waste containers).
   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 9 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 indicated (in SECONDS) to “ORGANIC”.
      IV. SOAK the disk for the time indicated (in SECONDS).
      V. DRAIN to “ORGANIC” for the time indicated (in SECONDS). Switch to the “OFF” position.
      VI. Remove the chamber lid to release the vacuum from inside the chamber.

Table 7. Disk Elution.

Solvent	Vol. (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Elute (sec.)
Acetone	8	1	60	Organic	120
Hexane	8	1	60	Organic	120
Hexane	8	1	90	Organic	240
Hexane	8	1	90	Organic	240

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