Determination of Monocrotophos, Diazinon, Malathion, EPN, and Methamidaphos from Aqueous Samples Using Atlantic HLB SPE Disks

Authors: Jim Fenster, Julie McGettrick, Biotage Inc., Salem, NH
Revision By: Matt Harden & Deanna Bissonnette

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This application note outlines the extraction of five organophosphate compounds monocrotophos, diazinon, malathion, EPN, and methamidophos using one solid phase extraction method with one pre-treatment step of sodium chloride (NaCl) 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 and Biotage® VacMaster™ Disk for this application.

Introduction

Monocrotophos, diazinon, malathion, EPN, and methamidophos are commonly used pesticides for the control of insects and aquatic pests in rice production, other agricultural production, and fish aquaculture in parts of the world. Methamidophos in particular is used in great quantities in rice fields in China where rice–fish culture is common as well as in many other rice-producing countries (e.g., Thailand, Malaysia, and the Philippines). Given their prevalent use throughout Asia, residues of monocrotophos, diazinon, malathion, EPN, and methamidophos show up in many food sources and are commonly monitored in wastewater and drinking water in these regions. As a result, many analytical methodologies have been created to monitor these compounds in the environment.

The traditional extraction methods employed use solid phase extraction (SPE) for monocrotophos, diazinon, malathion, EPN and a separate liquid-liquid extraction (LLE) method for methamidaophos. Methamidophos is problematic to extract in traditional SPE and LLE methodologies due to its extreme hydrophilic nature making exchange into a non-polar solvent or absorption onto a solid phase sorbent very difficult, resulting in extremely low recoveries of this compound. In the separate LLE method it is necessary to add a quantity of salt (NaCl) in order to decrease Metamidophos's affinity for the water phase making it partition more easily into the organic phase. This technique, Salting-out Liquid-Liquid Extraction (SALLE)[1], has been employed for many years when trying to extract extremely hydrophilic polar molecules from aqueous matrices. Extraction of all five of these compounds takes time as two separate extraction methodologies must be used.

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Instrumentation

- Biotage® Horizon 5000 Automated Extraction System with OnePass Kit
- Atlantic® HLB-H SPE Disk (47 mm)
- One-Pass Carbon Cartridge Max Detect, 20 cc
- DryVap® Concentrator System
- DryDisk® Solvent Drying System

Method Summary

HLB Fraction Elution

1. Adjust a 1 L aqueous sample to pH 2 with HCl and add 100 grams of NaCl (+ 80 mesh, reagent grade, less than or equal to 98% purity, Sigma-Aldrich), cap the bottle and mix.
2. Spike organophosphate pesticide (OPP) Standard mix (100 µg/mL in MeOH) into any control or matrix spike samples.
3. Screw sample bottle on to the water inlet valve and place on the Biotage® Horizon 5000.
4. Load the disk holder with the Atlantic® HLB-H 47 mm disk and connect the carbon cartridge to the OnePass sample lines, placing it in the perch.
5. Place a clean VOA vial or equivalent receiver onto the extractor.
6. Start the extraction method detailed in Table 1.
7. The software will pause and come up with a message alerting you that the HLB fraction is complete.
8. Collect the sample extract (approximately 30 mL).
9. Label and cap the flask to indicate that it contains the HLB fraction.

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)
Pause with Message	Part 1 of 2: Have the HLB-H disk in the 47 mm disk holder with any prefilters. Detach the waste lines from one another and attach the carbon cartridge in-line on the OnePass lines. Press "Continue" to begin part 1.							
Condition SPE Disk	Dichloromethane	15	60	2	1	30	30	
Condition SPE Disk	Acetone	11	60	2	1	30	30	
Condition SPE Disk	Reagent water	15	60	2	1	10	4	
Condition SPE Disk	Reagent water	15	60	2	1	10	4	
Load Sample				3				45
Air Dry Disk				6			60	
Elute Sample Container	Acetone	8	15	2	1	180	40	
Elute Sample Container	Dichloromethane	8	15	2	1	180	40	
Elute Sample Container	Dichloromethane	8	15	2	1	60	40	
Elute Sample Container	Dichloromethane	8	15	2	1	60	40	
Elute Sample Container	Dichloromethane	8	15	2	1	60	120	
Pause with Message	Part 2 of 2: Detach the carbon cartridge and reattach the OnePass lines together. Using the plunger, plunge once to remove the excess water and reseat the frit. Remove the disk holder from the platform and replace it with the cartridge and 20 cc funnel adapter. Press "Continue" to begin part 2.							
Air Dry Disk				6			600	
Elute Sample Container	Acetone	8	15	2	1	60	0	
Elute Sample Container	Acetone	8	15	2	1	60	120	
Elute Sample Container	Dichloromethane	8	15	2	1	60	40	
Elute Sample Container	Dichloromethane	8	15	2	1	180	40	
Elute Sample Container	Dichloromethane	8	15	2	1	60	40	
Elute Sample Container	Dichloromethane	8	15	6	1	60	120	

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Carbon Cartridge Elution

1. Remove the 47 mm Disk Holder from the Biotage® Horizon 5000.
2. Disconnect the lines from the Carbon Cartridge and remove it from the perch.
3. Reconnect the lines removed from the Carbon Cartridge.
4. Using a 20 cc syringe, plunge the carbon cartridge with air through the cap adapter to reset the carbon bed on the frit.
5. Remove the cap from the Carbon Cartridge and install the funnel in its place.
6. Install the Carbon Cartridge / funnel assembly onto the Biotage® Horizon 5000.
7. Attach a 125 mL flask onto the extractor.
8. Selecting each individual station, resume the extraction method to begin the carbon elution phase.
9. Label the flask to indicate that it contains the carbon fraction. The extraction is now complete.

* Due to the high salt content, it is necessary to rinse the liquid flow path of the Biotage® Horizon 5000 system to remove the salt residue. This is accomplished by rinsing the flow path with a sample bottle of warm water placed on the Biotage® Horizon 5000 and running the sample drain method on the software, this is followed by running a purge method with reagent water, acetone, and methylene chloride.

Extract Concentration

1. Assemble the DryDisk® reservoir with a DryDisk® Separation Membrane.
2. Load the DryDisk reservoir onto the DryVap® and set the conditions as detailed in Table 2.

Table 2. DryVap Settings

PARAMETER	SETTING
Dry Volume	20
Heat Power	5
Auto Rinse Mode	OFF
Heat Timer	OFF
Nitrogen Sparge	20 psi
Vacuum	-7 in. Hg

3. Start the concentration process by adding the OPP HLB fraction into the DryDisk tube.
4. Allow the OPP HLB fraction extract to filter through the DryDisk into the concentration tube.
5. Manually rinse the OPP HLB fraction 40 mL VOA vial with methylene chloride adding this to the DryDisk reservoir. Allow the rinse solvent to process through the DryDisk. Do this three times.
6. Follow steps 4 and 5 for the carbon fraction. If DryVap transitions to heat stage press the stop button on the control panel and then press restart.
7. Once the OPP HLB fraction and carbon fractions filter through the DryDisk, manually rinse the DryDisk reservoir with methylene chloride.
8. Once the methylene chloride has filtered into the concentration tube, allow the station to transition to the heat stage.
9. Concentrate the extract to less than 1.0 mL. For enhanced recoveries of methamidaphos carry out the steps in the optional LLE extraction method following this section.
10. Rinse the sides and heater of the concentrator tube with methylene chloride and bring the extract up to a 1.0 mL final volume.
11. Transfer the extract to a GC vial.
12. Add 5 µg terphenyl-d14 as an internal standard.
13. Analyze by GC/MS using the conditions detailed in Table 3.

* Due to the high salt content, it is necessary to thoroughly rinse the sparge tube of the DryVap to remove all salt residues. Accomplish this by processing 100 mL of warm water followed by acetone and methylene chloride through the DryDisk tube into the evaporator tube.

Optional LLE Extraction for Methamidaphos

1. Transfer the retained water from the top of the DryDisk membrane in the previous concentration step to a 40 mL VOA vial (approximately 10 mL)
2. Add 2–2.5 grams of NaCl to each vial.
3. Add 20 mL of methylene chloride:acetone (80:20) to each vial, cap and shake vigorously.
4. Pour the contents into a DryDisk tube and press start on the DryVap control panel until all 20 mL of solvent have been pulled through the DryDisk. When all solvent is through and only residual water remains on the DryDisk press the stop bottom on the DryVap control panel.
5. Transfer the retained water to VOA vial and repeat steps 3, 4, and 5 two more times.
6. Manually rinse the DryDisk reservoir with methylene chloride
7. Once the methylene chloride has filtered into the concentration tube, allow the station to transition to the heat stage.
8. Concentrate the extract to less than 1.0 mL.
9. Rinse the sides and heater of the concentrator tube with methylene chloride and bring the extract up to a 1.0 mL final volume.
10. Transfer the extract to a GC vial.
11. Analyze by GC/MS using the conditions in Table 3.

* Due to the high salt content, it is necessary to thoroughly rinse the sparge tube of the DryVap to remove all salt residues. Accomplish this by processing 100 mL of warm water followed by acetone and methylene chloride through the DryDisk tube into the evaporator tube.

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Table 3. GC/MS Conditions

Parameter	Setting		
Oven			
Initial Temperature	60 °C		
Initial Time	2 minutes		
Ramps:	**Rate**	**Final Temp**	**Final Time**
	20.00	270 °C	0.00
	6.00	320 °C	2.00
Run Time	22.83 minutes		
Inlet			
Mode	Pulsed Splitless		
Initial Temperature	280 °C		
Pressure	8.24 psi		
Pulsed Pressure	25 psi		
Pulsed Time	1.00 minutes		
Purge Flow	50 mL/min		
Purge Time	2.00 minutes		
Mass Spectrometer			
Acquisition Mode	SIM		
Solvent Delay	5 minutes		
Group 1	5–9 minutes, ions 94 and 141		
Group 2	9–12 minutes, ions 127, 192, 179, 137, 173, 125		
Group 3	12 minutes–end, ions 244, 122, 157, 169		

Biotage® Horizon 5000 Automated Extraction System.

DryVap® Concentrator System.

Acknowledgements

1. Ronald E Majors LC GC North America , July 1 2009 Salting-out Liquid-Liquid Extraction (SALLE)

Application Modifications

Biotage® Horizon 4790 Method Summary

System Setup

1. Install the 8270 One Pass Hardware Kit:
    a. Mount the carbon cartridge perch to the side of the extractor shelf by tightening the thumbscrew.
    b. Disconnect the Water-to-Waste line on the back of the extractor.
    c. Connect one end of the yellow line to the extractor port labeled Water Waste.
    d. Connect one end of the green line to the Water-to-Waste line and the other end to the yellow line connected in step c.

Sample Processing and HLB Elution

1. Adjust a 1 L aqueous sample to pH 2 with HCl and add 100 grams of NaCl (+ 80 mesh, reagent grade, less than or equal to 98% purity, Sigma-Aldrich), cap the bottle and mix.
2. Spike OPP Standard mix (100 µg/mL in MeOH) into samples. Use 10 µL for a 10 µg spike. Place an EZ-Seal over the opening of the bottle and screw on the bottle cap adaptor.
3. Load the disk holder with the Atlantic® HLB-H 47 mm disk, and the perch with a 20 cc Carbon Cartridge on the side of disk holder.
4. Place a clean VOA vial or equivalent receiver onto the extractor.
5. Load the sample bottle onto the Biotage® Horizon 4790.
6. Start the OPP extraction method detailed in Table 4 and collect extract at a high vacuum of -25 in. Hg (15.5 in. Hg at the Solvent to Waste bottle) and 15.5 psi Solvent Bottle Pressure.
7. Collect the extract (approximately 30 mL).
8. Cap and label extract as OPP HLB fraction.

Carbon Cartridge Elution

1. Remove the 47 mm Disk Holder from the Biotage® Horizon 4790.
2. Disconnect the lines from the Carbon Cartridge and remove it from the perch.
3. Reconnect the lines removed from the Carbon Cartridge.
4. Remove the cap from the Carbon Cartridge and install the funnel in its place.
5. Install the Carbon Cartridge / funnel assembly onto the Biotage® Horizon 4790 for elution.
6. Attach a 125 mL flask onto the extractor.
7. Elute the Carbon Cartridge using the Carbon Elution Method detailed in Table 5 into the 125 mL flask (Collect approximately 50 to 60 mL of extract).
8. Label the flask to indicate that it contains the carbon fraction.

*Due to the high salt content, it is necessary to rinse the liquid flow path of the Biotage® Horizon 4790 systems to remove the salt residue. This is accomplished by rinsing the flow path with a sample bottle of warm water placed on the Horizon 4790 and running the sample drain method on the envision controller, this is followed by running a purge method with reagent water, acetone, and methylene chloride

Extract Concentration

1. Assemble the DryDisk® reservoir with a DryDisk® Separation Membrane.
2. Load the DryDisk reservoir onto the DryVap® and set the conditions as shown in Table 1 above.
3. Start the concentration process by adding the OPP HLB fraction into the DryDisk tube.
4. Allow the OPP HLB fraction extract to filter through the DryDisk into the Concentrator tube.
5. Manually rinse the OPP HLB fraction 40 mL VOA vial with methylene chloride adding this to the DryDisk reservoir. Allow the rinse solvent to process through the DryDisk. Do this three times.
6. Follow steps 4 and 5 for the carbon fraction. If DryVap transitions to heat stage press the stop button on the control panel and then press restart.
7. Once the OPP HLB fraction and carbon fractions filter through the DryDisk, manually rinse the DryDisk reservoir with methylene chloride.
8. Once the methylene chloride has filtered into the concentration tube, allow the station to transition to the heat stage.
9. Concentrate the extract to less than 1.0 mL. For enhanced recoveries of methamidaphos carry out the steps in the optional LLE extraction method following this section.
10. Rinse the sides and heater of the concentrator tube with methylene chloride and bring the extract up to a 1.0 mL final volume.
11. Transfer the extract to a GC vial.
12. Add 5 µg terphenyl-d14 as an internal standard.
13. Analyze by GC/MS using the conditions shown in Table 3 above.

*Due to the high salt content, it is necessary to thoroughly rinse the sparge tube of the DryVap to remove all salt residues. Accomplish this by processing 100 mL of warm water followed by acetone and methylene chloride through the DryDisk tube into the evaporator tube.

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Optional LLE extraction method for enhanced recovery of Methamidaphos

1. Transfer the retained water from the top of the DryDisk membrane in the previous concentration step to a 40 mL VOA vial (approximately 10 mL)
2. Add 2 – 2.5 grams of NaCl to each vial.
3. Add 20 mL of methylene chloride: acetone (80:20, v/v) to each vial, cap and shake vigorously.
4. Pour the contents into a DryDisk tube and press start on the DryVap control panel until all 20 mL of solvent have been pulled through the DryDisk. When all solvent is through and only residual water remains on the DryDisk press the stop bottom on the DryVap control panel.
5. Transfer the retained water to VOA vial and repeat steps 3, 4, and 5 two more times.
6. Manually rinse the DryDisk reservoir with methylene chloride
7. Once the methylene chloride has filtered into the concentration tube, allow the station to transition to the heat stage.
8. Concentrate the extract to less than 1.0 mL.
9. Rinse the sides and heater of the concentrator tube with methylene chloride and bring the extract up to a 1.0 mL final volume.
10. Transfer the extract to a GC vial.
11. Analyze by GC/MS using the conditions shown in Table 3 above.

*Due to the high salt content, it is necessary to thoroughly rinse the sparge tube of the DryVap to remove all salt residues. Accomplish this by processing 100 mL of warm water followed by acetone and methylene chloride through the DryDisk tube into the evaporator tube.

Table 4. Biotage® Horizon 4790 HLB Elution Method

Step	Solvent	Soak Time (s)	Dry Time (s)
Prewet 1	Dichloromethane	30	15
Prewet 2	Acetone	30	15
Prewet 3	Reagent Water	10	2
Prewet 4	Reagent Water	10	2
Sample Process			
Air Dry			30
Rinse 1	Acetone	180	20
Rinse 2	Dichloromethane	180	20
Rinse 3	Dichloromethane	60	20
Rinse 4	Dichloromethane	60	20
Rinse 5	Dichloromethane	60	60

Table 5. Biotage® Horizon 4790 Carbon Elution Method

Step	Solvent	Soak Time (s)	Dry Time (s)
Air Dry			300
Rinse 1	Acetone	60	0
Rinse 2	Acetone	60	60
Rinse 3	Dichloromethane	60	3
Rinse 4	Dichloromethane	60	3
Rinse 5	Dichloromethane	60	3
Rinse 6	Dichloromethane	180	3
Rinse 7	Dichloromethane	60	3
Rinse 8	Dichloromethane	60	3
Rinse 9	Dichloromethane	60	3
Rinse 10	Dichloromethane	60	60

Biotage® Horizon 4790 Results and Conclusions

In order to track the extraction efficiency and mobility of the various analytes, extractions of aqueous samples were carried out using a 47 mm Atlantic® HLB-H extraction disk and a 20 cc carbon cartridge arranged on the instrument so that water sample flowed through the HLB disk and carbon cartridge in series. The HLB extraction disk and the carbon cartridge were extracted and analyzed separately and results of this series of extractions are shown in Table 6. Extraction results for HLB Disks in Table 6a) showed very good recoveries for monocrotophos, diazinon, malathion, and EPN with a low of 73% and a high of 91% and poor recovery of 5% for methamidaphos. The data in Table 6b) shows the results of the carbon cartridge fractions and average recoveries of methamidaphos were 52% indicating that methamidaphos is being retained on the carbon cartridge. Data in Table 6c) shows the recoveries for monocrotophos, diazinon, malathion, EPN, and methamidaphos for HLB and Carbon Cartridge combined with a low of 56% for methamidaphos and a high of 98% for monocrotophos. This data also demonstrates excellent reproducibility with the RSDs lying between 4.2% and 9.1% for all fractions.

Due to the extreme hydrophilic properties of methamidaphos, it tends to repartition back into the residual water and not transfer with the solvent when extracts are dried using the DryDisk. If higher recoveries of methamidaphos are desired, an additional extraction of this residual water fraction is required. Data for this additional extraction is shown in Tables 7 and 8. Data in Table 6c) indicate excellent recoveries of all five compounds being studied with a low of 83% for diazinon and a high of 103% for monocrotophos. The data in Table 8 show the average recoveries and statistics for this additional procedure plus Atlantic® HLB and carbon cartridge fractions combined. Recoveries were excellent for monocrotophos, diazinon, malathion, EPN, and methamidaphos with very good reproducibility ranging between 2.0% and 13.8% for all compounds.

Table 6. Recovery Data from the Extraction

Compound	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	Average (%)	STDEV	RSD (%)
a) HLB Portion of the Extraction								
Methamidophos	4	5	4	5	5	5	0.24	5.28
Monocrotophos	83	88	89	94	101	91	7.10	7.81
Diazinon	69	74	74	71	77	73	3.09	4.24
Malathion	79	81	88	86	93	85	5.58	6.53
EPN	80	80	92	85	92	86	5.87	6.85
b) Carbon Cartridge Portion of the Extraction								
Methamidophos	53	58	55	50	44	52	5.26	10.13
Monocrotophos	7	8	7	8	7	8	0.40	5.27
Diazinon	0	0	0	0	0	0	0.00	0.00
Malathion	0	0	0	0	0	0	0.00	0.00
EPN	6	6	6	6	6	6	0.11	1.83
c) Total HLB and Carbon Cartridge Extraction								
Methamidophos	57	63	59	54	49	56	5.16	9.13
Monocrotophos	90	96	96	101	109	98	6.99	7.10
Diazinon	69	74	74	71	77	73	3.09	4.24
Malathion	79	81	88	86	93	85	5.58	6.53
EPN	87	86	98	91	98	92	5.86	6.36

Table 7. Recovery Data from Total HLB and Carbon Cartridge Extraction

Compound	HLB-H 1	CC 1	LLE 1	Total 1	HLB-H 2	CC 2	LLE 2	Total 2	HLB-H 3	CC 3	LLE 3	Total 3
Methamidophos	5	61	35	101	5	56	15	77	7	55	30	93
Monocrotophos	96	0	0	96	104	0	0	104	109	0	0	109
Diazinon	83	0	0	83	82	0	0	82	85	0	0	85
Malathion	94	0	0	94	96	0	0	96	101	0	0	101
EPN	91	0	0	91	93	0	0	93	97	0	0	97

Table 8. Total Recovery Data HLB Disk, Carbon Cartridge and LLE of Residual Water

Compound	1 (%)	2 (%)	3 (%)	Average (%)	STDEV	RSD (%)
Methamidophos	101	77	93	90	12.45	13.83
Monocrotophos	96	104	109	103	6.72	6.50
Diazinon	83	82	85	83	1.67	2.00
Malathion	94	96	101	97	3.52	3.65
EPN	91	93	97	94	3.12	3.33

This application note demonstrates an efficient SPE extraction scheme for monocrotophos, diazinon, malathion, EPN, and methamidaphos. The method demonstrated excellent recoveries for an extraction scheme which uses only SPE as the extraction mechanism. It also demonstrated how, with an additional step, these recoveries can be augmented further. This efficient extraction scheme utilizes Biotage's Atlantic® HLB SPE disks and Carbon Cartridges, working on the Biotage® Horizon 4790 automated extraction platform. For the extraction of these five pesticides, this method allows for fast extraction times while yielding excellent recoveries of all five organophosphate pesticides of interest in this study. This method employing a combination of salt addition to the sample matrices as a pre-step followed by SPE extraction can also serve as a model extraction method for other extremely hydrophilic compounds that normally would be difficult to get good extraction recoveries.

Literature Number: AN078-HOR.v1

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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® HLB-H 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. Detach the water waste one-pass lines from each other. Attach the carbon cartridge cap to the Carbon Cartridge Max Detect, put the carbon cartridge in-line with the water waste line using the one-pass kit lines, and place the cartridge upright to prevent channeling in the carbon media. (Please note: the sample will flow from the bottom of the cartridge to the top of the cartridge and to waste).
5. If using the multifunnel, place onto the disk holder assembly. If not using the multifunnel, omit those directions throughout the method.
6. Condition the SPE Disk.
   a. Guide for each conditioning step in Table 9 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.
7. 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.
8. 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 60 SECONDS. Switch to the "OFF" position.
   b. Remove the sample bottle from the multifunnel if it was used.
9. Elute the SPE Disk: (Please note: the elution solvent 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 Biotage® 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. Disk Elution" on page 9:
      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 9. Disk Conditioning

Solvent	Volume (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Drain (sec.)
Methylene Chloride	15	1	30	Organic	30
Acetone	11	1	30	Organic	30
Reagent Water	15	1	10	Organic	4
Reagent Water	15	1	10	Organic	4

Table 10. Disk Elution

Solvent	Volume (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Elute (sec.)
Acetone	8	1	180	Organic	40
Methylene Chloride	8	1	180	Organic	40
Methylene Chloride	8	1	60	Organic	40
Methylene Chloride	8	1	60	Organic	40
Methylene Chloride	8	1	60	Organic	120

Remove the 125 mL collection flask, label it, and cap it. Set aside. Using the syringe, plunge the cartridge with air through the cap adapter to reseat the carbon bed. Remove the cap adapter and replace it with the cartridge adapter funnel. Reconnect the water waste one-pass lines back together. Remove the chamber and put the cartridge directly on the VacMaster Disk manifold and continue to the next step.

10. Air Dry the SPE Cartridge:
    a. Air dry the cartridge to “AQUEOUS” waste for an additional 600 SECONDS. Switch to the “OFF” position.
11. Complete the elution following the guidelines from step 9 and elution procedure shown in Table 11 for the cartridge and cartridge adapter funnel.

Table 11. Cartridge Elution

Solvent	Volume (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Elute (sec.)
Acetone	8	3	60	Organic	0
Acetone	8	3	60	Organic	120
Methylene Chloride	8	3	60	Organic	40
Methylene Chloride	8	3	180	Organic	40
Methylene Chloride	8	3	60	Organic	40
Methylene Chloride	8	3	60	Organic	120

EUROPE
Main Office: +46 18 565900
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 800 446 4752
Order Fax: +1 704 654 4917
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 68162810
Fax: +86 21 68162829
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: +82 31 706 8500
Fax: +82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

INDIA
Tel: +91 11 45653772
india@biotage.com

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