Determination of Trace Phthalate Ester Compounds for EPA Method 8061A

***Authors**: Michael Ebitson and Brett Holmes, Biotage, Salem, NH, USA.*
***Revision By:** Matt Harden and Deanna Bissonnette, Biotage, Salem, NH, USA.*
***Keywords:** Phthalates, phthalate esters, groundwater, SPE, solid phase extraction.*

Application Note Scope

This application note will outline optimized methods for the extraction of trace phthalate ester compounds from groundwater and other aqueous samples as outlined in EPA method 8061A using Atlantic® DVB solid phase extraction disks processed using Biotage automated or manual SPE solutions and DryVap® Sample 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

Phthalate esters are added mainly to plastics to improve characteristics such as flexibility, durability, transparency, or longevity of life. However, due to the lack of a covalent bond between the phthalate esters and the plastics they are mixed with, the phthalate esters are easily released. Studies have found that exposure to certain types of these phthalates can lead to detrimental health effects such as endocrine disruption or birth defects.

This study was conducted using the Biotage® Horizon 5000 Automated Extraction System with Atlantic® DVB (Divinyl Benzene) disks. The DryVap® Sample Concentrator System provided automatic sample drying to remove residual water with a patented DryDisk® membrane technology. The DryVap concentrator system automatically concentrates each dried extract by applying heat, vacuum, and sparge flow for up to six samples at one time. The solvent vapors from the DryVap system are then fed into the Biotage® Solvent Recovery SVOC System where they are condensed. These automated systems are specifically designed to streamline the sample handling required for the preparation and analysis of environmental samples. Method 8061A states gas chromatography/mass spectrometry (GC/MS) can be used as an alternative to GC-ECD for compound confirmation for phthalate esters.

- Biotage Instruments
  - Biotage® Horizon 5000 Automated Extraction System
  - Atlantic® DVB SPE Disk (47 mm)
  - DryVap® Sample Concentrator System
  - Biotage® Solvent Recovery SVOC System
- Agilent
  - 6890 GC
  - 5973 Mass Selective Detector
- Restek
  - 8061A Surrogate Standard (Cat #: 31848)
  - 8061A Phthalate Esters Mix (Cat #: 33227)
  - 8061A Benzyl Benzoate (is) (Cat #: 31847)
- Column: DB 5-ms 30 m X 0.25 mm ID X 0.25 µm

Method Summary

1. Purge the Biotage® Horizon 5000 extraction system twice to flush the system using the method in table 1.
2. Obtain 1 L bottles of DI water at neutral pH.
3. Spike 5 µg of 8061A surrogate and standard into any necessary spike samples, not spiking any intended method blank samples.
4. Place the sample bottle on the Biotage® Horizon 5000 extraction system and place the Atlantic® DVB disk in the standard 47 mm disk holder. Attach collection vessels to the system.
5. Start the extraction method shown in table 2 and collect the final sample extract.
6. Pour the extract into the DryDisk® reservoir and start the concentration process on the DryVap® Concentrator System using the conditions in table 3. Concentrate the extract to 0.9 mL.
7. When the sample concentration is complete, rinse the flat area of the DryVap concentrator tube with methylene chloride and bring final volume up to 1.0 mL.
8. Transfer 0.4 mL of the 1.0 mL to a GC vial and add 4 µL of 500 µg/mL 8061A internal standard.
9. Analyze by GC-MS using the conditions shown in table 4.

Table 1. Biotage® Horizon 5000 purge method.

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/Elute (s)	Sample Delay (s)
Elute Sample Container	Dichloromethane	8	15	2	1	0	60	
Elute Sample Container	Dichloromethane	8	15	2	1	0	60	
Elute Sample Container	Acetone	8	15	6	1	0	120	

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	Acetone	11	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				2				45
Air Dry Disk				6			60	
Elute Sample Container	Methanol	8	15	2	1	180	40	
Elute Sample Container	Methanol	8	15	2	1	180	40	
Elute Sample Container	Methanol	8	15	2	1	60	40	
Elute Sample Container	Methanol	8	15	2	1	60	40	
Elute Sample Container	Dichloromethane	8	15	6	1	60	120	

Table 3. DryVap® settings.

Parameter	Setting
Dry Volume	20
Heat Power	5
Auto Rinse Mode	OFF
Heat Timer	OFF

Table 4. GC Conditions.

Flow Conditions		
Gas: Helium		
Pressure: 9 psi		
Flow Type: Constant		
Injection Conditions		
Ramp (°C/min.)	**Temp. (°C)**	**Hold (min.)**
0	280	0
Type	1 µL Split Injection	

Oven Program			
Initial Temp: 120 °C			
Initial Time: 1.0 min			
Level	**Ramp (C/min.)**	**Temp. (°C)**	**Hold (min)**
1	0	45	1.0
2	15	270	0.0
3	6	320	0.0

Application Modifications

Biotage®Horizon 4790 Method Summary

1. Purge the Biotage® Horizon 4790 extraction system twice to flush the system using the method in table 5.
2. Obtain six, 1 L bottles of DI water and leave at neutral pH.
3. Spike 5 µg of 8061A surrogate and standard into 5 samples and nothing in the sixth (it will serve as a blank).
4. Set the water to waste vacuum and the solvent waste vacuum at -15 in. Hg.
5. Place the sample bottle on the Biotage® Horizon 4790 Extraction System and place the Atlantic® DVB disk in the standard 47 mm disk holder. Attach collection vessels to the system.
6. Start the extraction method shown in table 6 and collect the final sample extract.
7. Pour the extract into the DryDisk® reservoir and start the concentration process on the DryVap® concentrator system using the conditions in table 3 above. Concentrate the extract to 0.9 mL.
8. When the sample concentration is complete, rinse the flat area of the DryVap® concentrator tube with methylene chloride and bring final volume up to 1.0 mL.
9. Transfer 0.4 mL of the 1.0 mL to a GC vial and add 4 µL of 500 µg/mL 8061A internal standard.
10. Analyze by GC-MS using the conditions shown in table 4.

Table 5. Biotage® Horizon 4790 purge method.

Step	Solvent	Soak Time (s)	Dry Time (s)
Rinse 1	Dichloromethane	0	30
Rinse 2	Dichloromethane	0	30
Rinse 3	Acetone	0	60

Table 6. Biotage® Horizon 4790 extraction method.

Step	Solvent	Soak Time (s)	Dry Time (s)
Prewet 1	Acetone	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	Methanol	180	20
Rinse 2	Methanol	180	20
Rinse 3	Methanol	60	20
Rinse 4	Methanol	60	20
Rinse 5	Dichloromethane	60	60

Biotage® Horizon 4790 Results and Conclusions

The recoveries for this method are shown in table 7. The replicate samples were spiked with 5 µg/mL of 8061A standard. The recoveries ranged from 78.6–85.0 %. The relative standard deviation of the spikes also demonstrated excellent results, ranging from 5.32% to 8.79%. The data in Table 7 meets the criteria for EPA Method 8061A and demonstrates that Biotage extraction systems are capable of fully automating EPA Method 8061A resulting in data that is both accurate and precise.

In this study, the coupling of the Atlantic® DVB Disk and Biotage® Horizon 4790 Automated Extraction System has proven to be a better alternative to traditional methods used for EPA Method 8061A extraction. The Biotage® Horizon 4790 Automated Extraction System eliminates human error, decreases solvent use, and has proven to deliver high, reproducible recoveries. Extraction times were typically 20 to 22 minutes and drying and concentrating times were approximately 25 minutes. The Biotage® Horizon 4790 Automated Extraction System with Envision Platform, DryVap® Concentrator System, and Biotage® Solvent Recovery SVOC System provide a complete "turnkey" solution that reduces analyst labor, solvent usage, turnaround time, and improves accuracy and precision.

Table 7. Analyte recoveries for Biotage®Horizon 4790 (* indicates the compound is a surrogate).

Target Compounds	Blank (µg/L)	#1 (µg/L)	#2 (µg/L)	#3 (µg/L)	#4 (µg/L)	#5 (µg/L)	Avg. (%)	RSD (%)
Dimethyl phthalate (DMP)	ND	4.41	4.13	3.75	3.71	3.64	78.6	6.60
Diethyl phthalate (DEP)	0.16	4.74	4.33	3.91	3.95	3.88	83.2	7.42
Di-n-butyl phthalate (DBP)	0.21	4.69	4.41	4.01	4.02	3.93	84.2	6.52
Butyl benzyl phthalate	0.14	4.63	4.37	4.02	3.94	3.9	83.4	6.32
Bis(2-ethylhexyl)phthalate (DEHP)	ND	4.45	4.21	3.88	3.78	3.72	80.2	6.22
Di-n-octyl phthalate (DOP)	0.22	4.39	4.14	3.76	3.76	3.67	78.9	6.17
* Diphenyl phthalate (DPP)	ND	4.89	4.49	3.98	4.03	3.82	84.8	8.79
* Diphenylisophthalate (DPIP)	ND	5.31	4.79	4.41	4.64	4.32	93.9	7.82
* Dibenzyl phthalate (DBZP)	ND	4.83	4.47	4.14	4.01	3.71	84.6	8.63

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 (47 mm): 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 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.
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 adjusted to a lower setting). 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 60 SECONDS. Switch to the "OFF" position.
   b. Remove the sample bottle from the multifunnel if it was used.
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 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 time indicated (in SECONDS). Switch to the "OFF" position.
      vi. Remove the chamber lid to release the vacuum from inside the chamber.

Table 8. Disk conditioning.

Solvent	Volume (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Drain (sec.)
Acetone	11	1	30	Organic	30
Acetone	11	1	30	Organic	30
Reagent Water	15	1	10	Organic	5
Reagent Water	15	1	10	Aqueous	5

Table 9. Disk elution.

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

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
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: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
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

Distributors in other regions are listed on www.biotage.com

Literature Number: AN058-HOR-V.1

Biotage®