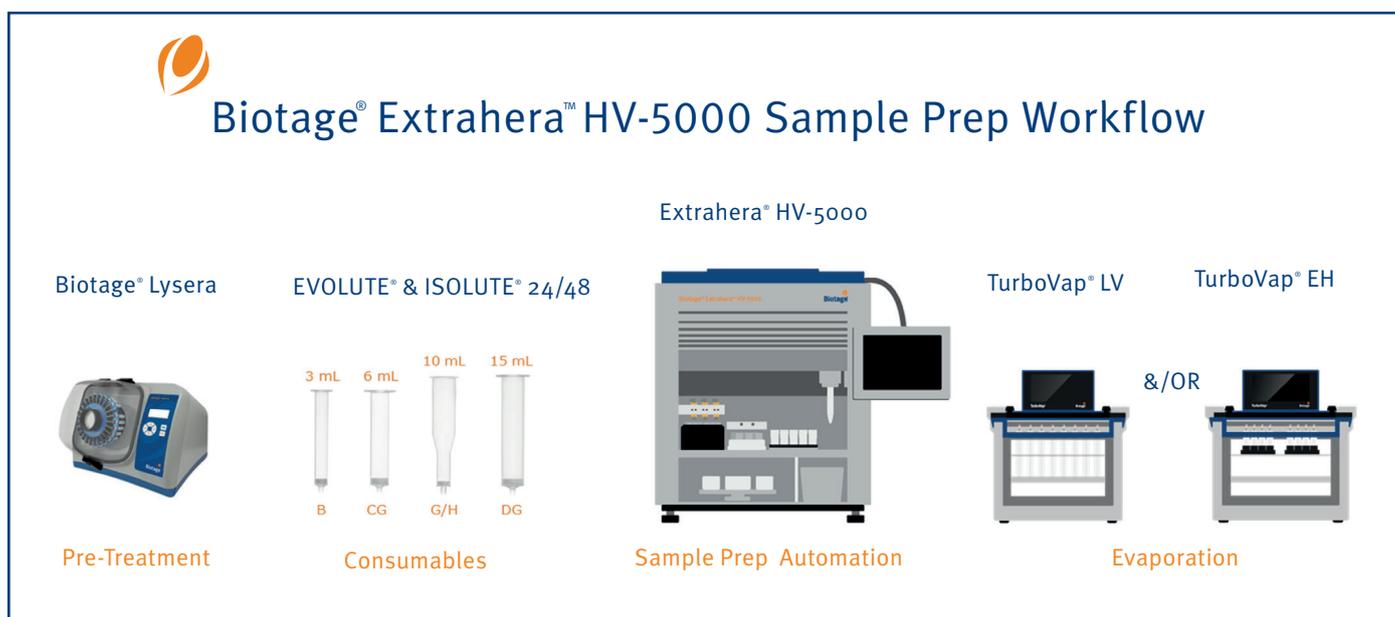


Guide to Environmental Applications

Using Biotage® Extrahera™ HV-5000



Guide to Environmental Applications Using Biotage® Extrahera™ HV-5000



CONTENTS

- 4** Introduction to The Biotage® Extrahera™ HV-5000 Automation System
- 7** EPA Method 3610B; Alumina Clean Up for Phthalate Esters
- 8** EPA Method 3620C; Florisil Clean Up for Phthalate Esters (where organochlorine pesticide residues are known to be present)
- 9** EPA Method 3620C; Florisil Clean Up for PCBs and Organochlorine Pesticides (combined fractions)
- 10** EPA Method 3620C; Florisil Clean Up for PCBs and Organochlorine Pesticides (separate fractions)
- 11** EPA Method 3620C; Florisil Clean Up for Chlorinated Hydrocarbons
- 12** EPA Method 3630; Silica Gel Clean Up for Derivatized Phenols
- 13** EPA Method 3630; Silica Gel Clean Up for Organochlorine Pesticides and PCBs
- 14** Clean up of EPH (Extractable Petroleum Hydrocarbons) from water or soil extracts using ISOLUTE® EPH SPE columns
- 15** Method Development Using the Biotage® Extrahera™ HV-5000
 - Impact of Column Format on Batch Size
 - Impact of Column Format on Extraction Time
 - Impact of Column Format on Performance
 - Choice of SPE column
 - Choice of sample and collection tube
- 18** Ordering information

Why do we need Sample Preparation?

There are very few techniques that can reliably analyze raw samples. Components in the sample matrix frequently interfere with analysis and mask analytes, especially in chromatographic analyses. The solution is to perform sample preparation that can bring significant benefits to the analytical laboratory:

- » More accurate results
- » Improved Quality Control charts
- » Lower detection limits
- » Less instrument downtime
- » Fewer reruns

Typical workflows in the environmental laboratory depend on the sample type.

Sample Pre-Treatment

For liquid samples, pre-treatment might include filtration to remove particulates or pH adjustment prior to extraction by liquid-liquid extraction or solid phase extraction (SPE).

For solid samples such as soil, analytes must be extracted into a solvent that is suitable for the chosen analysis. Efficient extraction relies on good sample-solvent interaction, which means that the sample might need to be homogenized (or ground up finely) before extraction. Biotage® Lysera is a bead mill homogenizer used to process solid or semi solid samples before extraction.

Homogenized samples are then often extracted using a suitable organic solvent.

Solid Phase Extraction Consumables for Automated Extract Clean up

Once extracted into a suitable solvent, further clean-up is often required. High quality SPE consumables in a range of formats facilitate automated clean up processes. This guide focuses on post extraction clean up techniques using ISOLUTE® SPE products.

Benefits of Automated Sample Preparation

Biotage® Extrahera™ automated sample preparation systems can handle a range sample volumes and SPE column sizes ideal for SPE based sample clean up. Automation of the clean-up process brings.

- » Improved throughput and efficiency
- » Improved data and error reduction
- » Reduced cost per sample

Evaporation Before Analysis

Cleaned up samples usually require a concentration step before analysis. Biotage TurboVap® blowdown evaporators use a patented vortex evaporation system that increases the speed of evaporation by a factor of 10 compared with other techniques.

A range of features are incorporated that ensure the safety of your samples, with efficient, high throughput evaporation that matches your lab's needs.

- » Faster Evaporation
- » Reduced RSD
- » Improved recovery

This user guide contains methodologies for processing environmental samples using Biotage® Extrahera™ HV-5000.

Selected procedures are based on EPA methods and have been adapted for use on an automated SPE system using pre-packed SPE columns, wherever allowed by the EPA method.

In addition, there is a guide to method optimization using the HV-5000, outlining the factors which should be considered when for optimizing a solid phase extraction method or transferring a method to the automation system.



The Biotage® Extrahera™ HV-5000 Automation System

The new Biotage® Extrahera™ HV-5000, is a GLP capable, **Automated Sample Prep Workstation** solution dedicated to column-based sample prep with support for classical SPE and SLE column consumable formats. Using its 4-channel pipette head, the HV-5000 will aspirate and dispense **High Volumes** from 250–5000 µL per channel. Combined with precision pipetting and positive pressure sample processing, the system caters to larger sample volumes compatible with 3-, 6-, 10- and 15-mL column consumables in 12, 24, and 48 position formats.



Configuration Kit

24 Position Configuration Kit 417610

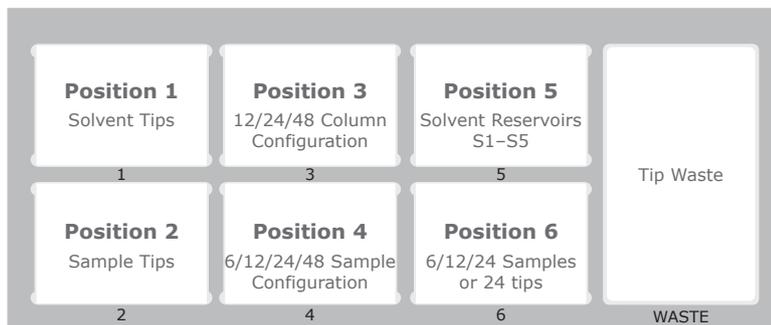


OR

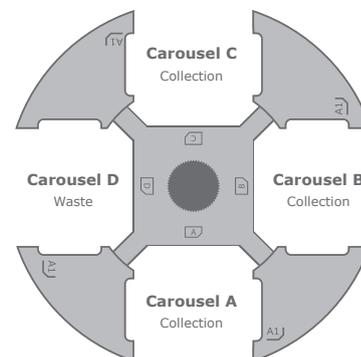
48 Position Configuration Kit 418374



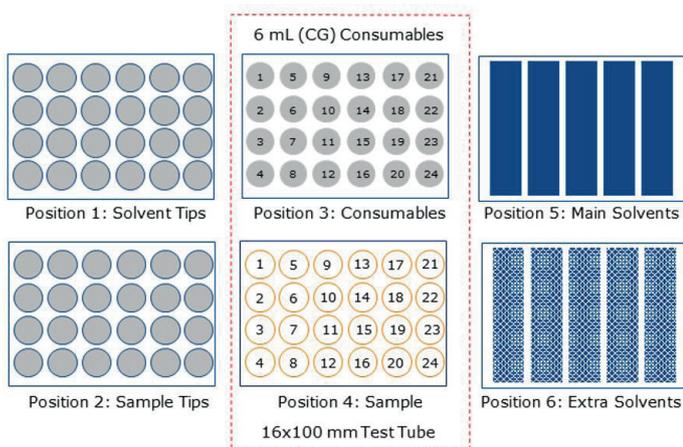
Processing Shelf



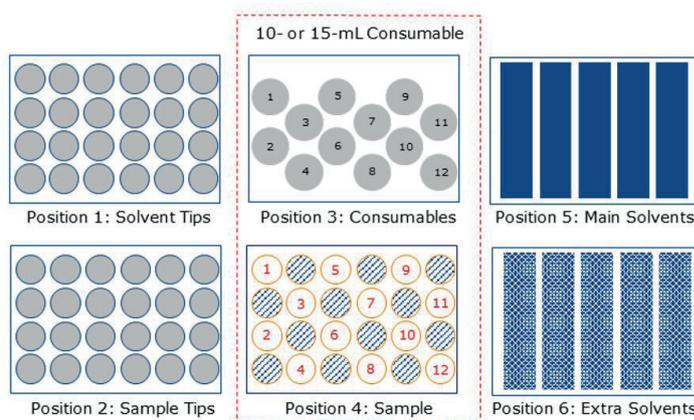
Carousel



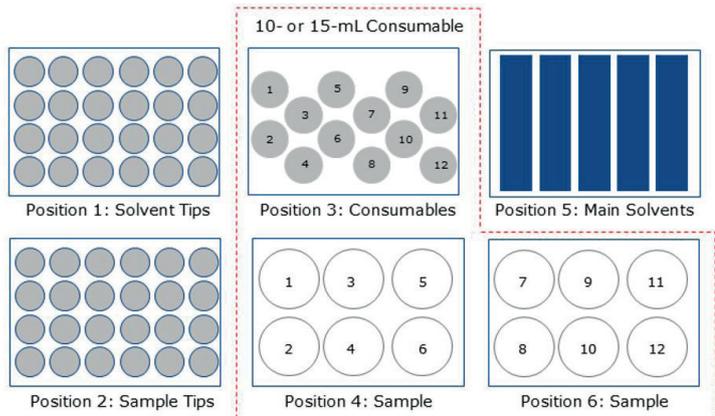
24 Position, Regular Format Sample Processing



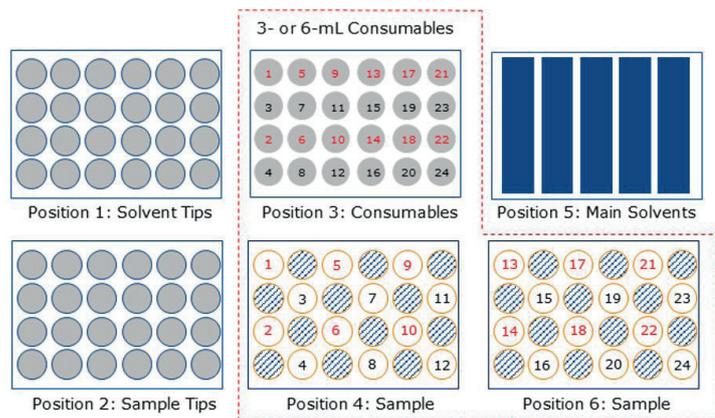
12 Position, Regular Format Sample Processing



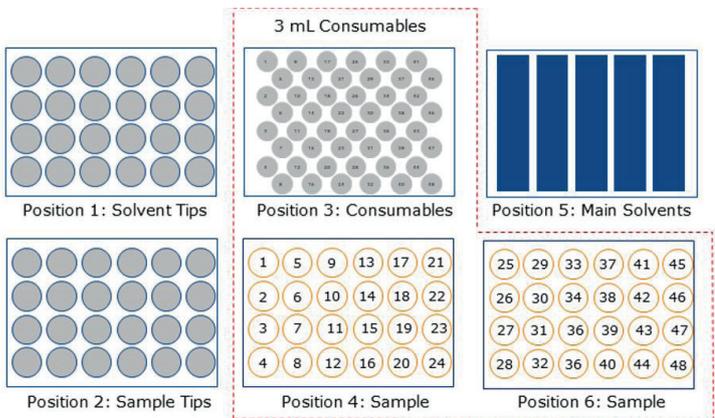
6+6 = 12 Mixed Format Sample Processing



12+12 = 24 Mixed Format Sample Processing



24+24 = 48 Mixed Format Sample Processing





Solid Phase Extraction Sorbents Used in this Guide

Alumina

Alumina is a highly porous form of alumina oxide, available in three pH ranges (acidic, basic neutral) for use in chromatographic clean up procedures.

It is used to separate analytes from interfering compounds of differing chemical polarity.

ISOLUTE® Alumina SPE sorbents (acidic, basic or neutral) are pre-packed into clean SPE cartridges for convenient use.

Florisil

Florisil®, a registered trade name of U. S. Silica Co., is a magnesium silicate with basic properties. It is used to separate analytes from interfering compounds prior to sample analysis by a chromatographic method

ISOLUTE® FL (Florisil PR grade) SPE sorbent is pre-packed into clean SPE cartridges for convenient use.

Silica Gel

Silica gel (silicic acid) is a regenerative adsorbent of silica with weakly acidic properties. It is used for the separation of analytes from interfering compounds of a different chemical polarity.

ISOLUTE® SI (silica gel) SPE sorbent is pre-packed into clean SPE cartridges for convenient use.

ISOLUTE® EPH

ISOLUTE EPH is a proprietary blend of media optimized for the fractionation of aliphatic and aromatic fractions of water or soil extracts.

Each batch of media is QC checked to ensure reproducible fractionation.

EPA Method 3610B; Alumina Clean Up for Phthalate Esters

This method utilizes ISOLUTE® AL-B (basic alumina) to clean up hexane extracts containing phthalate esters.

The methodology assumes that environmental samples have been previously prepared using an appropriate extraction method.

The cartridge clean up procedure uses solid phase extraction cartridges containing 40 µm particles of alumina (60 Å pores). Each cartridge is washed with hexane immediately prior to use. The sample extract (in hexane) is loaded onto the cartridge which is then eluted with a suitable solvent.

*Note: if organochlorine pesticides are known to be present, refer to method 3620.

Analytes

- » Bis(2-ethylhexyl) phthalate
- » Diethyl phthalate
- » Butyl benzyl phthalate
- » Dimethyl phthalate
- » Di-n-butyl phthalate
- » Di-n-octyl phthalate

Solid Phase Extraction Cartridge

ISOLUTE® AL-B, 1 g / 6 mL (tablets) (p/n 715-0100-CG).

Methodology

Condition	Hexane (5 mL).
Load	Sample extract in hexane (2 mL).
Rinse	Hexane (0.5 mL).
Elute	Acetone/hexane mix (20/80, v/v) (10 mL).
Post Clean Up	Prior to analysis, adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000

Batch Size	Time Taken (hh.mm.ss)*
24 samples (6 mL column)	00.37.08

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used.

Recommended Sample Rack and Collection Options

Batch Size	Sample Volume	Consumable Rack	Sample Tube and Rack Used	Elution Volume	Collection Tube and Rack Used
24	2 mL	Column Rack, 24 x 6 mL (tablets), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP	10 mL	18x75 mm collection rack, 415492



EPA Method 3620C; Florisil Clean Up for Phthalate Esters (where organochlorine pesticide residues are known to be present)

This method utilizes ISOLUTE® FL (Florisil, PR grade) to clean up hexane extracts containing phthalate esters.

The methodology assumes that environmental samples have been previously prepared using an appropriate extraction method.

The cartridge clean-up procedure uses solid-phase extraction cartridges containing 40 µm particles of Florisil (60 Å pores). Each cartridge is washed with hexane immediately prior to use. The sample extract (in hexane) is loaded onto the cartridge which is then eluted with a suitable solvent.

Analytes

- » Bis(2-ethylhexyl) phthalate
- » Diethyl phthalate
- » Butyl benzyl phthalate
- » Dimethyl phthalate
- » Di-n-butyl phthalate
- » Di-n-octyl phthalate

Solid Phase Extraction Cartridge

ISOLUTE® FL, 1 g / 3 mL (tabless) (p/n 712-0100-BG)
1 g / 6 mL (tabless) (p/n 712-0100-CG); 1 g/15 mL (tabless) (p/n 712-0100-DG).

Methodology

- Load:** Sample extract in hexane (1 mL).
- Rinse:** Hexane (0.5 mL).
- Elute: 1** DCM/hexane (20/80, v/v) (10 mL).
- Elute 2:** Acetone/Hexane mix (10/90, v/v) (10 mL).
- Post Clean Up:** Prior to analysis, adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000*

Batch Size	Time Taken (hh.mm.ss)
24 samples (3 mL cartridge)**	01:07:55
24 samples (6 mL cartridge)	00:40:37
12 samples (15 mL cartridge)	00:40:45

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used

** Elution volume > 5 mL means that multiple aliquots are required to meet the method requirement using the 3 mL cartridge size, increasing run time.

Recommended Sample Rack and Collection Options

Batch size	Sample volume	Consumable Rack	Sample Tube and Rack Used	Elution Volume	Collection Tube and Rack Used
24	1 mL	Column Rack, 24 x 6 mL (tabless), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP	2 x 10 mL	18x75 mm collection rack, 415492 x2
12	1 mL	Column Rack, 12 x 15 mL (tabless), 416848SP	16x100 mm or 15 mL centrifuge tube, 24 position sample rack, 414245SP, & key plate (417282SP)	2 x 10 mL	18x75 mm collection rack, 415492 x2

EPA Method 3620C; Florisil Clean Up for PCBs and Organochlorine Pesticides

This method utilizes ISOLUTE® FL (Florisil, PR grade) to clean up hexane extracts containing PCBs and organochlorine pesticides. Use this method if both analyte classes are to be collected and analysed together.

If PCBs and organochlorine pesticides are required in separate fractions, see method 3620 C 'separate fractions' on the next page.

The methodology assumes that environmental samples have been previously prepared using an appropriate extraction method.

The cartridge clean up procedure uses solid-phase extraction cartridges containing 40 µm particles of Florisil (60 Å pores). Each cartridge is washed with hexane immediately prior to use. The sample extract (in hexane) is loaded onto the cartridge which is then eluted with a suitable solvent.

Analytes

- » Organochlorine Pesticides
- » PCBs

Solid Phase Extraction Cartridge

ISOLUTE® FL, 1 g / 3 mL (tabless) (p/n 712-0100-BG) 1 g / 6 mL (tabless) (p/n 712-0100-CG).

Methodology

Condition: Hexane (4 mL).
Load: Sample extract in hexane (1 mL).
Rinse: Hexane (0.5 mL).
Elute: Acetone/hexane (10/90, v/v) (9 mL). This eluant will contain the combined organochlorine pesticides and PCBs.

Post Clean Up: Prior to analysis, adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000*

Batch Size	Time Taken (hh.mm.ss)
24 samples (3 mL cartridge)**	00:47:37
24 samples (6 mL cartridge)	00:25:05
12 samples (15 mL cartridge)	00:31:14

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used.

** Elution volume > 5 mL means that multiple aliquots are required to meet the method requirement using the 3 mL cartridge size, increasing run time.

Recommended Sample Rack and Collection Options

Batch size	Sample volume	Consumable Rack	Sample Tube and Rack Used	Elution volume	Collection Tube and Rack Used
24	1 mL	Column Rack, 24 x 6 mL (tabless), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP	9 mL	18x75 mm collection rack, 415492
12	1 mL	Column Rack, 12 x 15 mL (tabless), 416848SP	16x100 mm or 15 mL centrifuge tube, 24 position sample rack, 414245SP, & key plate (417282SP)	9 mL	18x75 mm collection rack, 415492

EPA Method 3620C; Florisol Clean Up for PCBs and Organochlorine Pesticides

This method utilizes ISOLUTE® FL (Florisol, PR grade) to clean up hexane extracts containing PCBs and organochlorine pesticides. Use this method if the analyte classes are to be collected and analysed separately.

If PCBs and organochlorine pesticides are required in a single fraction, see method 3620 C ‘combined fractions’, on the previous page.

The methodology assumes that environmental samples have been previously prepared using an appropriate extraction method.

The cartridge clean up procedure uses solid-phase extraction cartridges containing 40 µm particles of Florisol (60 Å pores). Each cartridge is washed with hexane immediately prior to use. The sample extract (in hexane) is loaded onto the cartridge which is then eluted with a suitable solvent.

Analytes

- » Fraction 1: PCBs plus some Organochlorine Pesticides
- » Fraction 2: Most OC pesticides
- » Fraction 3: Remaining OC pesticides

Solid Phase Extraction Cartridge

ISOLUTE® FL, 1 g / 3 mL (tabless) (p/n 712-0100-BG) 1 g / 6 mL (tabless) (p/n 712-0100-CG).

Methodology

- Condition:** Hexane (4 mL).
- Load:** Sample extract in hexane (1 mL).
- Rinse:** Hexane (0.5 mL).
- Elute Fraction 1:** Hexane (3 mL) this fraction contains the PCBs and some pesticides.
- Elute Fraction 2:** Dichloromethane/hexane (26/74, v/v) (5 mL). This fraction contains most of the pesticides.
- Elute Fraction 3:** Acetone/hexane (10/90, v/v) (5 mL). This fraction contains the remaining pesticides.
- Post Clean Up:** Prior to analysis, adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000*

Batch size	Time taken (hh.mm.ss)
24 samples (3 mL cartridge)**	01:02:00
24 samples (6 mL cartridge)	00:48:02
12 samples (15 mL cartridge)	00:39:37

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used.

** Elution volume > 5 mL means that multiple aliquots are required to meet the method requirement using the 3 mL cartridge size, increasing run time.

The need for fractionation (3 separate fractions per sample) reduces the batch size that can be accommodated.

Recommended Sample Rack and Collection Options

Batch size	Sample volume	Consumable Rack	Sample Tube and Rack Used	Elution volume	Collection Tube and Rack Used
24	1 mL	Column Rack, 24 x 6 mL (tabless), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP	5 mL	16x75 mm collection rack, 415585
12	1 mL	Column Rack, 12 x 15 mL (tabless), 416848SP	16x100 mm or 15 mL centrifuge tube, 24 position sample rack, 414245SP, & key plate (417282SP)	5 mL	16x75 mm collection rack, 415585

EPA Method 3620C; Florisil Clean Up for Chlorinated Hydrocarbons

This method utilizes ISOLUTE® FL (Florisil, PR grade) to clean up hexane extracts containing chlorinated hydrocarbons.

The methodology assumes that environmental samples have been previously prepared using an appropriate extraction method.

The cartridge clean up procedure uses solid-phase extraction cartridges containing 40 µm particles of Florisil (60 Å pores). Each cartridge is washed immediately prior to use. The sample extract (in hexane) is loaded onto the cartridge which is then eluted with a suitable solvent.

Analytes

- » 2-Chloronaphthalene
- » Hexachlorobenzene
- » 1,2-Dichlorobenzene
- » Hexachlorobutadiene
- » 1,3-Dichlorobenzene
- » Hexachlorocyclopentadiene
- » 1,4-Dichlorobenzene
- » Hexachloroethane
- » 1,2,4-Trichlorobenzene

Solid Phase Extraction Cartridge

ISOLUTE® FL, 1 g / 3 mL (tablets) (p/n 712-0100-BG) ;
1 g / 6 mL (tablets) (p/n 712-0100-CG); 1 g/15 mL
(tablets) (p/n 712-0100-DG).

Recommended Sample Rack and Collection Options

Batch size	Sample volume	Consumable Rack	Sample Tube and Rack Used	Elution volume	Collection Tube and Rack Used
24	2 mL	Column Rack, 24 x 6 mL (tablets), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP	10 mL	18x75 mm collection rack, 415492
12	2 mL	Column Rack, 12 x 15 mL (tablets), 416848SP	16x100 mm or 15 mL centrifuge tube, 24 position sample rack, 414245SP, & key plate (417282SP)	10 mL	18x75 mm collection rack, 415492

Methodology

Condition: Acetone/hexane (10/90, v/v) (5 mL).

Load: Sample extract in hexane (2 mL).

Rinse: Acetone/hexane (10/90, v/v) (0.5 mL).

Elute: Acetone/hexane (10/90, v/v) (10 mL).

Post Clean Up: Prior to analysis, adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000*

Batch Size	Time Taken (hh.mm.ss)
24 samples (3 mL cartridge)**	00:53:03
24 samples (6 mL cartridge)	00:35:53
12 samples (15 mL cartridge)	00:31:20

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used.

** Elution volume > 5 mL means that multiple aliquots of elution solvent are required to meet the method requirement using the 3 mL cartridge size, increasing run time.

EPA Method 3630; Silica Gel Clean Up for Derivatized Phenols

This method utilizes ISOLUTE® SI (Silica) to clean up hexane extracts containing derivatized phenols.

The cartridge clean up protocol uses solid phase extraction cartridges packed with 2 g of silica gel adsorbent. Each cartridge is solvent washed immediately prior to use. Aliquots of sample extracts are loaded onto the cartridges, which are then eluted with suitable solvent(s).

This silica gel cleanup procedure is performed on sample extracts that have undergone pentafluorobenzyl bromide derivatization, as described in Method 8041. The sample extract must be in 2 mL of hexane at this point.

Analytes

- » Pentafluorobenzyl bromide derivatized phenols:
Phenol, 2-Methylphenol, 3-Methylphenol, 4-Methylphenol, 2,4-Dimethylphenol, 2-Chlorophenol, 2,6-Dichlorophenol, 4-Chloro-3-methylphenol, 2,4-Dichlorophenol, 2,4,6-Trichlorophenol, 2,3,6-Trichlorophenol, 2,4,5-Trichlorophenol, 2,3,5-Trichlorophenol, 2,3,5,6-Tetrachlorophenol, 2,3,4,6-Tetrachlorophenol, 2,3,4-Trichlorophenol, 2,3,4,5-Tetrachlorophenol, Pentachlorophenol

Solid Phase Extraction Cartridge

ISOLUTE® SI, 2 g / 6 mL (tabless) (p/n 460-0200-CG) ; 2 g/15 mL (tabless) (p/n 460-0200-DG).

Methodology

- Condition:** Hexane (4 mL).
- Load:** Sample extract in hexane (2 mL).
- Rinse:** Hexane (0.5 mL).
- Wash:** Hexane (5 mL). Discard.
- Elute:** Toluene/hexane (25/75, v/v) (5 mL)

Post Clean Up: Prior to analysis, adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000*

Batch size	Time taken (hh.mm.ss)
24 samples (6 mL cartridge)	00:48:52
12 samples (15 mL cartridge)	00:43:26

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used.

Recommended Sample Rack and Collection Options

Batch size	Sample volume	Consumable Rack	Sample Tube and Rack Used	Elution volume	Collection Tube and Rack Used
24	2 mL	Column Rack, 24 x 6 mL (tabless), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP	5 mL	16x75 mm collection rack, 415585
12	2 mL	Column Rack, 12 x 15 mL (tabless), 416848SP	16x100 mm or 15 mL centrifuge tube, 24 position sample rack, 414245SP, & key plate (417282SP)	5 mL	16x75 mm collection rack, 415585

EPA Method 3630; Silica Gel Clean Up for Organochlorine Pesticides and PCBs (where PCBs are known to be present)

This method utilizes ISOLUTE® SI (Silica) to clean up hexane extracts containing organochlorine pesticides and PCBs (where PCBs are known to be present).

The cartridge clean up protocol uses solid phase extraction cartridges packed with 1 g of silica gel adsorbent. Each cartridge is solvent washed immediately prior to use. Aliquots of sample extracts are loaded onto the cartridges, which are then eluted with suitable solvent(s).

Analytes

- » **Fraction 1:** Heptachlor, Aldrin, 4,4'-DDE, 4,4'-DDT, Aroclor 1016, Aroclor 1221, Aroclor 1232, Aroclor 1242, Aroclor 1248, Aroclor 1254, Aroclor 1264.
- » **Fraction 2:** alpha-BHC, gamma-BHC, beta-BHC, delta-BHC, Heptachlor epoxide, Endosulfan I, Dieldrin, Endrin, 4,4'-DDD, Endosulfan II, Endrin aldehyde, Endosulfan sulfate, 4,4'-Methoxychlor

Solid Phase Extraction Cartridge

ISOLUTE® SI, 1 g/3 mL (tablets) (p/n 460-0100-BG);
1 g /6 mL (tablets) (p/n 460-0100-CG) ; 1 g/15 mL (tablets)
(p/n 460-0100-DG).

Methodology

Condition: Hexane (4 mL).

Load: Sample extract in hexane (2 mL).

Rinse: Hexane (0.5 mL).

Elute Fraction 1: Hexane (5 mL)

Elute Fraction 2: Diethyl ether/hexane (50/50, v/v)(5 mL)

Combine the fractions if analyte fractionation is not required

Post Clean Up: Prior to analysis, adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000*

Batch Size	Time Taken (hh.mm.ss)
24 samples (3 mL cartridge)**	01:01:26
24 samples (6 mL cartridge)	00:51:11
12 samples (15 mL cartridge)	00:37:45

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used.

**Elution volume > 5 mL means that multiple aliquots of elution solvent are required to meet the method requirement using the 3 mL cartridge size, increasing run time.

Recommended Sample Rack and Collection Options

Batch size	Sample volume	Consumable Rack	Sample Tube and Rack Used	Elution volume	Collection Tube and Rack Used
24	2 mL	Column Rack, 24 x 6 mL (tablets), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP	2 x 5 mL	16x75 mm collection rack, 415585, x2
12	2 mL	Column Rack, 12 x 15 mL (tablets), 416848SP	16x100 mm or 15 mL centrifuge tube, 24 position sample rack, 414245SP, & key plate (417282SP)	2 x 5 mL	16x75 mm collection rack, 415585, x2

Clean Up of EPH (Extractable Petroleum Hydrocarbons) from Water or Soil Extracts Using ISOLUTE® EPH SPE Columns

This method utilizes ISOLUTE® EPH to clean up hexane extracts containing of extractable petroleum hydrocarbons from soil or water extracts. The method cleanly separates the aliphatic fraction from the aromatic fraction for GC analysis.

The procedure assumes samples have been extracted into hexane using an appropriate sample pre-treatment method.

Analytes

- » **Fraction 1:** Aliphatic hydrocarbons
- » **Fraction 2:** Aromatic hydrocarbons

Solid Phase Extraction Cartridge

ISOLUTE® EPH, 1.45 g/3 mL (tablets) (p/n 928-0145-BG).

Methodology

- Condition:** Hexane (6 mL).
- Load:** Sample extract in hexane (2 mL).
- Fraction 1:** Hexane (1.5 mL). This contains the aliphatic fraction.
- Fraction 2:** Dichloromethane (4.5 mL). This contains the aromatic fraction.
- Post Extraction:** Evaporate and reconstitute in a solvent suitable for the analytical method. See Application note AN877 for additional details.

Time Taken for Procedure Using Biotage® Extrahera™ HV-5000*

Batch size	Time taken (hh.mm.ss)
24 samples (3 mL cartridge)	00:48:48
48 samples (3 mL cartridge)	01:06:14

*Timing derived by programming the HV-5000 system to perform the methodology assuming appropriate consumables and solvent volumes per batch size are used.

Recommended Sample Rack and Collection Options

Batch size	Sample volume	Consumable Rack	Sample Tube and Rack Used	Elution volume	Collection Tube and Rack Used
24	1 mL	Column Rack, 24 x 6 mL (tablets), 413640SP	16x100 mm test tube, 24 position sample rack, 414245SP, x1	1 x 1.5 mL 1 x 4.5 mL	16x75 mm collection rack, 415585, x2
48	1 mL	Column Rack, 48 x 3 mL (tablets), 415556SP, x1	16x100 mm test tube, 24 position sample rack, 414245SP, x2	1 x 1.5 mL 1 x 4.5 mL	12x75 mm collection rack, 415555SP, x2

Method Development Using the Biotage® Extrahera™ HV-5000

Guide to Sample, SPE Column and Collection Tube Options

For optimum throughput for your method, you should consider the following factors:

- » Batch size
- » Sample volume
- » Collection volume

Impact of Column Format on Batch Size

The maximum number of samples that can be extracted using the HV-5000 is dependent on column format.

Format	Maximum Batch Size
3 mL	48
6 mL	24
15 mL	12

As can be seen from the examples in the application section, moving to a larger format column (for example from a 1 g/3 mL format to a 1 g/6 mL or 1 g/12 mL format, see image) can adversely impact the number of samples that can be run in a single batch, but may allow for fewer solvent dispense steps, offsetting the apparent reduced throughput.

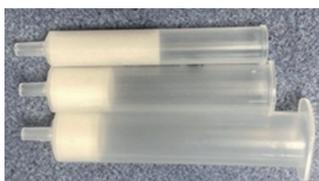


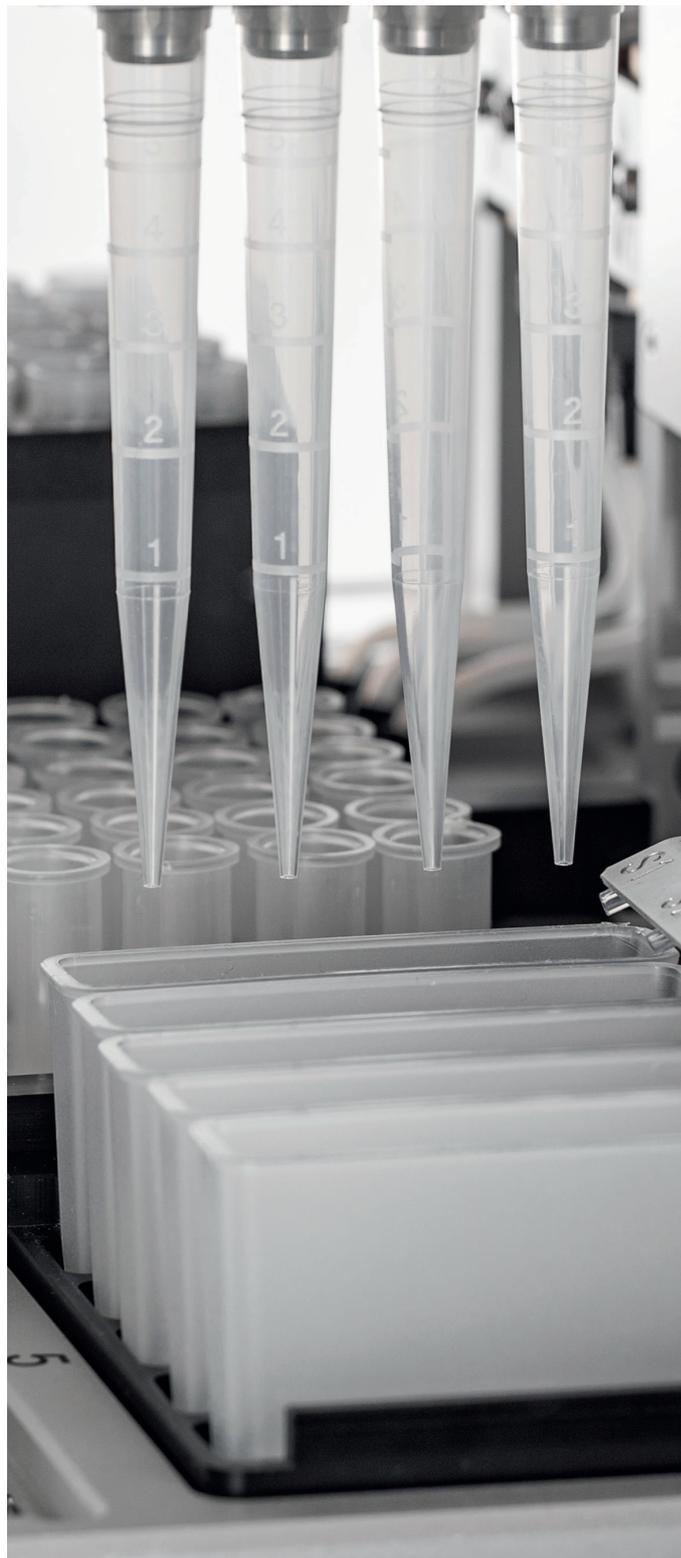
Figure 1.
Top = 1 g/3 mL,
Middle = 1 g/6 mL,
Bottom = 1 g/12 mL.

Impact of Column Format on Extraction Time

If the method requires a 5 mL elution volume, then the sample can be added in one step for the D column format, so the positive pressure settings only need to be applied once. Since the 5 mL elution volume is larger than the column format capacity it needs to be added in two parts meaning that the positive pressure settings need to be applied twice to load the entire sample. As the 5 mL elution volume is considerably larger than the 3 mL column format capacity the procedure needs to perform 4 times leading to a considerably longer run time.

For the columns shown in Figure 1 the maximum solvent capacity is as follows:

Column Format	Maximum Reservoir Capacity (with sorbent)	# Dispense Steps Required for 5 mL Elution
1 g/3 mL (B)	1.5 mL	4
1 g/6 mL (C)	3 mL	2
1 g/15 mL (D)	12 mL	1



Impact of Column Format on Performance

In theory, a taller, narrower SPE column can provide more effective clean up (better interference removal) than a shorter, wider column packed with the same weight of sorbent.

In practice however, this is highly dependent on the solvent used, and the characteristics of the interferences being removed. Flow rates through shorter, wider columns are usually faster than taller, narrow equivalents, so if performance is not impacted, overall time for the extraction may be reduced with larger format columns.

For this reason, the choice of a 10 mL XL format column may be beneficial for some methods, as it provides increased reservoir volume with the same tall, narrow sorbent bed dimensions as a B (3 mL) format column.

Choice of SPE Column:

In addition to throughput considerations, to develop and optimize a method on the Extrahera™ HV-5000, you must determine the mass of SPE sorbent required to ensure enough chemical capacity for the analytes of interest. In general, a good place to start is to assume ~5% of the SPE bed mass is equivalent to the maximum chemical loading capacity. For example, if you have 500 mg of SPE sorbent, then your estimated total analyte loading capacity will be roughly 25 mg.

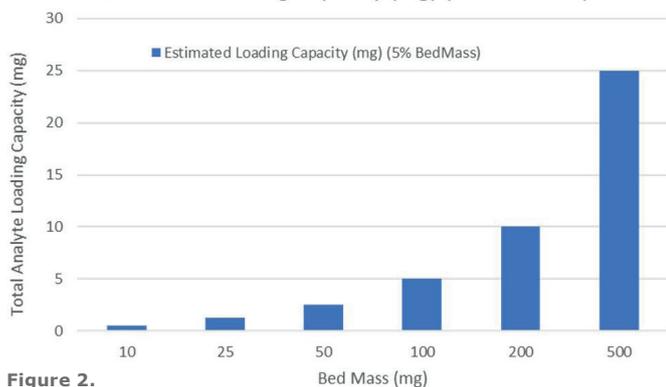


Figure 2.

Next you must determine the number of analytes, surrogates, and internal standards that you are screening for and divide the total loading capacity by the number of analytes. For example, if your total analyte loading capacity is 25 mg and you are screening for 25 total compounds, your maximum spike level for a single analyte is 1 mg.

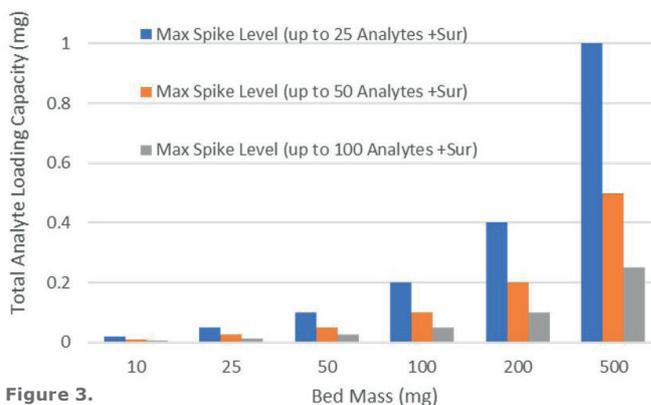


Figure 3.

Based on the bed mass utilized for the extraction method you can then estimate the maximum and minimum elution volumes required for the extraction method. Estimations for these volumes can be noted in the chart below.

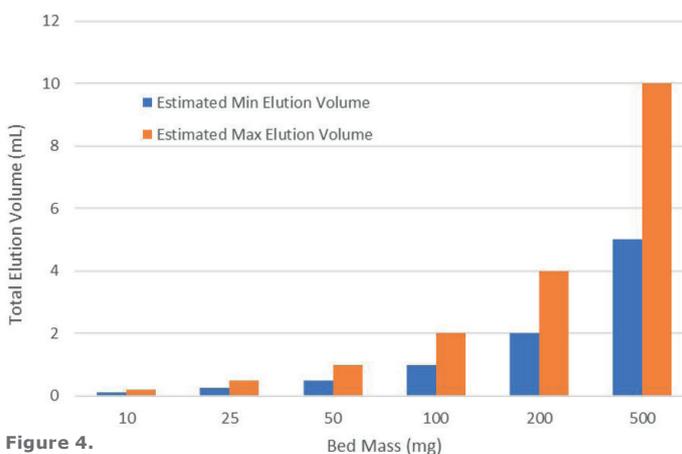


Figure 4.

This information can be used as a starting point for method optimization. For example, a method which screens for 25 compounds at a maximum spike level of 100 ug (i.e., 0.1 mg) per analyte would likely require approximately 100 mg of SPE sorbent.

Choice of Sample and Collection Tube

A wide variety of tubes and containers can be used. The table below summarizes the options available.

POS 3 (SPE consumable)	POS 4 (sample)	POS 6 (sample)	Collection (A-D)
48 x 3 mL (B tube, Diag.)	24, 16x100 mm	24, 16x100 mm	48, 12x75mm 48, 5 mL well plate
24 x 3 mL (B tube)	24, 16x100 mm	N/A	24, 12x75mm, 16x75mm, or 18x75mm
24 x 3 mL (B tube)	12, 15 mL Centrifuge Tube	12, 15 mL Centrifuge Tube	24, 12x75mm, 16x75mm, or 18x75mm 24, 10 mL well plate
24 x 6 mL (DC tube)	24, 16x100 mm	N/A	24, 12x75mm, 16x75mm, or 18x75mm
24 x 6 mL (DC tube)	12, 15 mL Centrifuge Tube	12, 15 mL Centrifuge Tube	24, 12x75mm, 16x75mm, or 18x75mm 24, 10 mL well plate
12 x 15 mL (DG Tube)	12, 15 mL Centrifuge Tube	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 15 mL (DG Tube)	12, 16x75 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 15 mL (DG Tube)	12, 18x75 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 15 mL (DG Tube)	12, 16x100 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 15 mL (DG Tube)	6, 50 mL Centrifuge Tube	6, 50 mL Centrifuge Tube	12, 12x75mm, 16x75mm, or 18x75mm
12 x 15 mL (DG Tube)	6, 40 mL VOA Vial	6, 40 mL VOA Vial	12, 12x75mm, 16x75mm, or 18x75mm
12 x 15 mL (DG Tube)	6, 20 mL VOA Vial	6, 20 mL VOA Vial	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (G Tube)	12, 15 mL Centrifuge Tube	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (G Tube)	12, 16x75 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (G Tube)	12, 18x75 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (G Tube)	12, 16x100 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (G Tube)	6, 50 mL Centrifuge Tube	6, 50 mL Centrifuge Tube	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (G Tube)	6, 40 mL VOA Vial	6, 40 mL VOA Vial	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (G Tube)	6, 20 mL VOA Vial	6, 20 mL VOA Vial	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (H Tube)	12, 15 mL Centrifuge Tube	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (H Tube)	12, 16x75 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (H Tube)	12, 18x75 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (H Tube)	12, 16x100 mm	N/A	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (H Tube)	6, 50 mL Centrifuge Tube	6, 50 mL Centrifuge Tube	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (H Tube)	6, 40 mL VOA Vial	6, 40 mL VOA Vial	12, 12x75mm, 16x75mm, or 18x75mm
12 x 10 mL (H Tube)	6, 20 mL VOA Vial	6, 20 mL VOA Vial	12, 12x75mm, 16x75mm, or 18x75mm

Practical Considerations

The width of the 5 mL tips means the instrument can reach the bottom of some of these tubes and not others. Some of the tubes and their features are detailed below.

For each tube there is a practical and theoretical volume maximum. The theoretical maximum gives a volume to the very top of the tube that may not allow the tube to be rotated on the turntable or allow for minimal immersion of the Extrahera™ HV-5000 tips. In these cases, the practical volume maximum should be used instead.

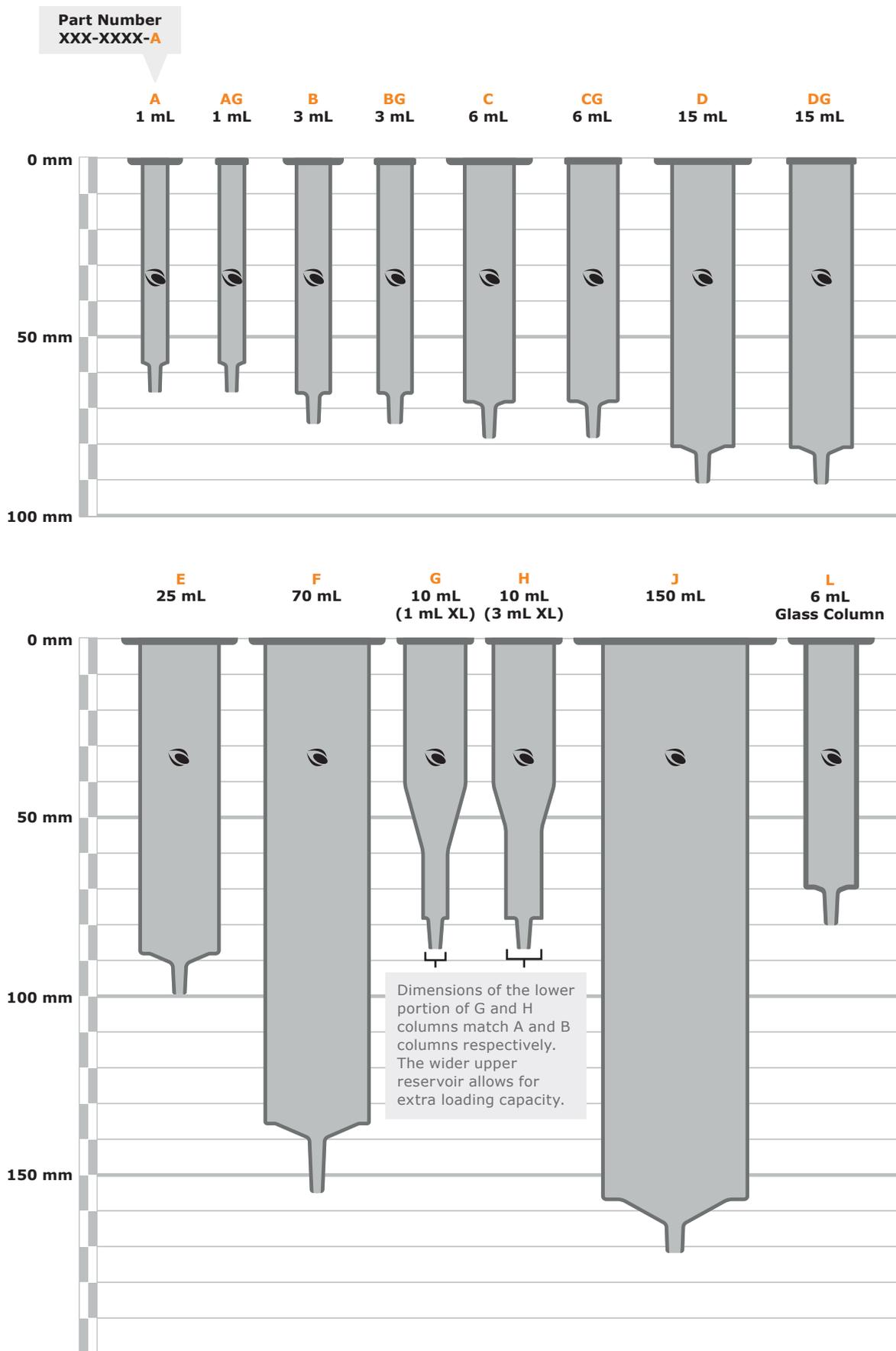
Where the tip can't reach the bottom of the tube the approximate dead volume is also included measuring the volume of sample in the bottom of the well that can't be reached by the HV pipette tip even at its lowest setting.

Tube Description	Volume (mL) Theoretical	Volume (mL) Practical	Appropriate for Sample	Appropriate for Elution	Can Tip Reach Bottom?
10 x 75	4.0	4.0	No	No	No
12 x 75	6.0	5.5	No	Yes	No (1.6 mL dead vol.)
13 x 100	10.0	9.5	No	No	No (2.6 mL dead vol.)
16 x 100	17.0	15.0	Yes	No	Yes
16 x 75	12.0	10.0	Yes	Yes	Yes
18 x 75	13.5	13.0	Yes	Yes	Yes
Autosampler vial	2.0	1.5	No	Yes	No (0.8 mL dead vol.)
20 mL VOA	20	18	Yes	No	Yes
40 mL VOA	40	38	Yes	No	Yes
15 mL Centrifuge Tube	15	15	Yes	No	Yes
50 mL Centrifuge Tube	50	50	Yes	No	Yes
Biotage 24 Position Rack	10	9.0	Yes	Yes	Yes

Ordering Information

Product	Part Number	Qty.	Product	Part Number	Qty.
System			HV-5000 System Accessories for Positions 6		
Biotage® Extrahera™ HV-5000 System	417002	1	These available accessories allow customers additional flexibility for their workflows.		
Configuration Kits – you must pick at least one (or both)			Reservoirs		
24 Positions Dual Flow - HV This kit also runs 12-position configurations	417610	1	Solvent Rack, 25 mL	413991SP	1
48 Positions Dual Flow - HV	418374	1	Solvent Reservoirs, 25 mL	414045SP	5
HV-5000 System Accessories for Positions 1 & 2			Solvent Rack 40 mL	417315SP	1
These are the available accessories for station 1 & 2 of the HV-5000 processing shelf.			Solvent Reservoirs, 40 mL	417324SP	25
Sample and Solvent Tips			Solvent Rack, 100 mL	415560SP	1
Pipette Tips, Clear, 5000 mL	417007	1000	Solvent Reservoirs, 100 mL	414214SP	5
SP Retaining Clip for Tip Holder	414307	1	Tip Holder, 5000 mL	416845SP	1
HV-5000 System Accessories for Position 3			HV-5000 System Accessories for Carousel		
These are the available accessories for station 3 of the HV-5000 processing shelf.			These available accessories allow customers additional flexibility for their workflows.		
Column Racks			Collection Racks		
Column Rack, 48x3 mL, (Tabless),	415556SP	1	Sample/Collection Rack, 24 Positions, 12x75 mm	415491	1
Column Rack, 24x3 mL	414174SP	1	Sample/Collection Rack, 48 Positions, 12x75 mm	415555SP	1
Column Rack, 24x3 mL, Bond Elut	416114SP	1	Sample/Collection Rack, 24 Positions, 16x75 mm	415585	1
Column Rack, 24x6 mL, (Tabless)	413640SP	1	Sample/Collection Rack, 24 Positions, 18x75 mm	415492	1
Column Rack, 12x10 & 15 mL	416848SP	1	Collection Rack, 24 Positions, 12x75 mm	414511SP	1
Retaining Clip Kit, 4 weak and 4 strong	416128SP	1	Collection Rack, 24 Positions, 18x75 mm	414512SP	1
Positive Pressure Head			Inserts for 12x32 mm vial, for 414511SP rack	414578SP	1
24 Pressure Head Seal, Self-Adhesive	415603SP	1	Collection Tubes & Plates		
96 Pressure Head Seal, Self-Adhesive	415604SP	1	12x75 mm Test tube	C44651	1000
HV-5000 System Accessories for Positions 4			16x75 mm Test tube	413282	1000
Sample Racks			18x75 mm Test tube	414574	304
Sample Rack, 6 Positions	417283SP	1	Collection Plate, 24 Positions, 10 mL	121-5208	25
Sample Rack, 24 Positions, 16x100 mm	414245SP	1	Flow Through and Spacer		
12 Position Key Plate for 414245SP rack	417282SP	1	DFE Carousel Riser	416868SP	1
Sample Tubes			Flow-Through Plate, 24 Positions	414203SP	1
20 mL VOA Vial	160-0008-02	72	Flow-Through Plate, 48 Positions	414516SP	1
40 mL VOA Vial	160-0008	72			
15 mL Polypropylene Conical Tube	04-2002	50			
50 mL Polypropylene Conical Tube	04-2001	25			

SPE Column Sizes from Biotage



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