

How to Transfer a RapidTrace®+ Method to the Biotage® Extrahera™ HV-5000

This technical guide describes the considerations for transfer of a solid phase extraction (SPE) method from the RapidTrace®+ to the Biotage® Extrahera™ HV-5000 sample prep workstation.

For additional technical support, please contact your local Biotage representative.

A generic RapidTrace®+ method for extraction of basic drugs of abuse from a 2 mL urine sample or a 1 mL blood or plasma sample, using mixed-mode ISOLUTE® HCX 200 mg/3 mL SPE columns is used as an example procedure throughout.



Key Differences Between the RapidTrace® and Biotage® Extrahera HV-5000

Parameter	RapidTrace®+	Biotage® Extrahera™ HV-5000
Sample Batch Size (dependant on column size)	5–10 (depends on configuration)	12, 24, or 48
Column Compatibility	1, 3, and 6 mL	3, 6, 10, and 15 mL
Extraction Technique(s)	SPE only	SPE, SLE, PPT, Filtration, and DFE
Processing	Serial	Parallel
Flow rate	0.048–42 mL/min	0.1–5 Bar
Number of solvents	8	5 automatically + 5 manual
Collection/Fraction	Max 2 Fractions per sample	Max 4 Fractions per sample
Waste Outlets	4	3
Sample volume	Loads entire volume	Max 5 mL per aliquot
Solvent delivery	Direct Feed	Tips
Interface	External CPU Required	Integrated CPU (touchscreen)
Sample/Solvent Handling	Fixed Needle	Disposable 5000 µL Tips
Volume (sample and solvent)	mL	µL
Liquid sensing	Yes, must be selected	Yes. Intelligent replenishment of solvent reservoirs
Clog detection	No	Yes

Outline of SPE Method

Column:

ISOLUTE® HCX 200 mg/3 mL.

Sample:

Urine (2 mL) or blood / plasma (1 mL).

Pre-treatment:

Urine: Dilute urine (2 mL) with phosphate buffer (0.05-0.1 M, pH 6, 1 mL).

Blood/Plasma: Dilute blood or plasma (1 mL) with phosphate buffer (0.05-0.1 M, pH 6, 2 mL).

Condition:

Methanol (3 mL).

Equilibrate:

Water (3 mL) followed by phosphate buffer (0.05-0.1 M, pH 6, 3 mL).

Load:

Pre-treated sample (3 mL).

Wash 1

Water (2 mL).

Wash 2

HCl (0.1 M, 2 mL).

Wash 3

Methanol (2 mL).

Dry

5 mins.

Elute

Methylene chloride (DCM): isopropanol: ammonium hydroxide (78:20:2, v/v, 2 mL).

Evaporate and reconstitute appropriately before analysis.

RapidTrace®+ Method

Illustrated using screenshots

1. Reagent Setup

Reagent Number	Reagent Name	Reagent Abbreviation	Sip ml/sec
1	Water	Water	0.5
2	Methanol	MeOH	0.5
3	Phosphate b...	buffer	0.5
4			
5	Methylene C...	Elution	0.5
6	0.1 M Hydro...	0.1 M HCl	0.5
7			
8			

Waste Number	Waste Name	Waste Abbreviation
1	Organic	Organic
2	Aqueous	Aqueous
3	Biological	Bio

Column tubing volume: 0.40

Buttons: Close, Help, Default Entries, OK/Save as current

Note: Other unused solvents may be listed in positions 4, 7 and 8. The column tubing will either be 0.4 as above or 0.9 if a gas valve has been attached.

2. Method Set up

File Edit Reagents Setup Variables Password

Procedure Name

No	Step	Source	Output	Vol	ml/sec	Liquid Sense
1	Condition	Methanol	Organic Waste	3	0.25	No
2	Condition	Water	Aqueous Waste	3	0.25	No
3	Condition	buffer	Aqueous Waste	3	0.25	No
4	Load	Sample	Bio Waste	3	0.0167	No
5	Rinse	Water	Bio Waste	2	0.0333	No
6	Purge-Cannula	Water	Cannula	2	0.5	No
7	Rinse	0.1 M HCl	Bio Waste	2	0.0333	No
8	Rinse	Methanol	Bio Waste	2	0.0333	No
9	Dry	-----	Time =	5	min.	No
10	Collect	Eluent	Fract1	3	0.0167	No
11	Purge-Cannula	Methanol	Cannula	3	0.5	No
12	Purge-Cannula	Water	Cannula	3	0.5	No
13						No
14						No
15						No
16						No
17						No
18						No
19						No
20						No

Created

Last Modified

Run Time

Procedure Description

Items choosable for current grid selection

In the example window above, liquid sensing has been selected as 'No' for all steps.

Note: The Biotage® Extrahera™ HV-5000 monitors solvent reservoir levels, and automatically replenishes depleted solvent reservoirs S1-S5.

Programming the Biotage® Extrahera™ HV-5000

With the Biotage® Extrahera™ HV-5000 system, the extraction method is split into several tabs, reflecting each step of the extraction procedure. After the “General Parameters” tab there is a selectable tab for each method step.

General Parameters

Method Name

The Extrahera™ HV-5000 can save multiple methods. Use a suitable method name to identify your procedure. A larger number of characters can be entered than those visible in the window.

Sample Rack and Extraction Media

The sample rack and extraction media can either be selected from ready prepared lists or can be created manually. The sample rack and extraction media parameters instruct the instrument at which height to aspirate, mix, and dispense each solvent/sample.

The green buttons on the left allow the operator to select which stages of the method should be performed. If a stage is switched off the tab turns grey, and this step is omitted within the method.

Sample Type

The ‘sample type’ field is used to indicate the viscosity of the sample. Where the sample is diluted human urine, blood, or plasma, select ‘aqueous sample’.

Note: Unless the sample is particularly difficult to pipette e.g., undiluted animal urine or whole blood, then sample category “Aqueous sample” is the preferred default selection.

Two Solvent Tip Types?

For this procedure, select ‘No’ as the method does not require more tips.

Note: For most methods ‘2 tip types’ can be left as “No” and both the sample and solvent tip can be selected as “5000 µL Biotage tip”.

Starting Sample Volume

The starting sample volume shown below in the red circle, is a reminder to the operator of how much sample should be in the sample (position 4) when the instrument starts. This is particularly important if a pre-treatment step is performed. The entry on this page DOES NOT instruct the system how much sample to load. This is the volume of sample that will be diluted during pretreatment.

Note: It is recommended to have slightly more sample in the sample tube than is required to ensure the consumable is fully loaded with sample of interest.

Re-use Tips...

The box labelled “Re-use tips for sample pre-treatment, mix and sample load” is normally selected to ‘Yes’ to minimize tip wastage.

Method Comment

The method comment box is a free text box that can give the user any further information that may be necessary and will be archived in the instrument generated report. Information quoted here could include any ‘off-line’ pre-treatment details prior to running on the Biotage® Extrahera™ HV-5000.

An example of a completed General Parameters tab is shown below.

Sample Pretreatment Tab

In the case of our example RapidTrace®+ procedure, separate pretreatment procedures are used for urine and blood. One method would be designed for urine where 2 mL of sample is diluted with 1 mL of phosphate buffer.

A separate method is required for blood and plasma where 1 mL of sample is diluted with 2 mL of phosphate buffer.

An example of a correctly completed pretreatment tab for Basic Drugs from Urine ISOLUTE® HXC is shown below.

The screenshot shows the 'Edit SPE Method - Basic Drugs from Urine ISO...' interface. The 'Pretreatment' tab is selected, and the 'Number of steps' is set to 1. The solvent is '0.1M pH6 Phosphate b...'. The 'Load' step is configured with 'Pos 1' and a volume of '1250' µL. The 'Wash' and 'Elution' steps are also shown with their respective settings.

Solvent Tips

This tells the system which tips should be used to dispense solvent. Solvent tips are routinely used from Position 1 of the Biotage® Extrahera™ HV-5000 so this is always selected.

Volume

Our example method requires a 2 mL urine sample to be diluted with 1 mL of buffer. To achieve this for a sample starting volume of 2500 µL (2.5 mL) (as quoted on the front "General" page), a dilution buffer volume of 1250 µL (1.25 mL) is selected. This dilution leads to a final 'pre-treated sample volume' of 3.75 mL, which can comfortably accept the 3 mL load requirement.

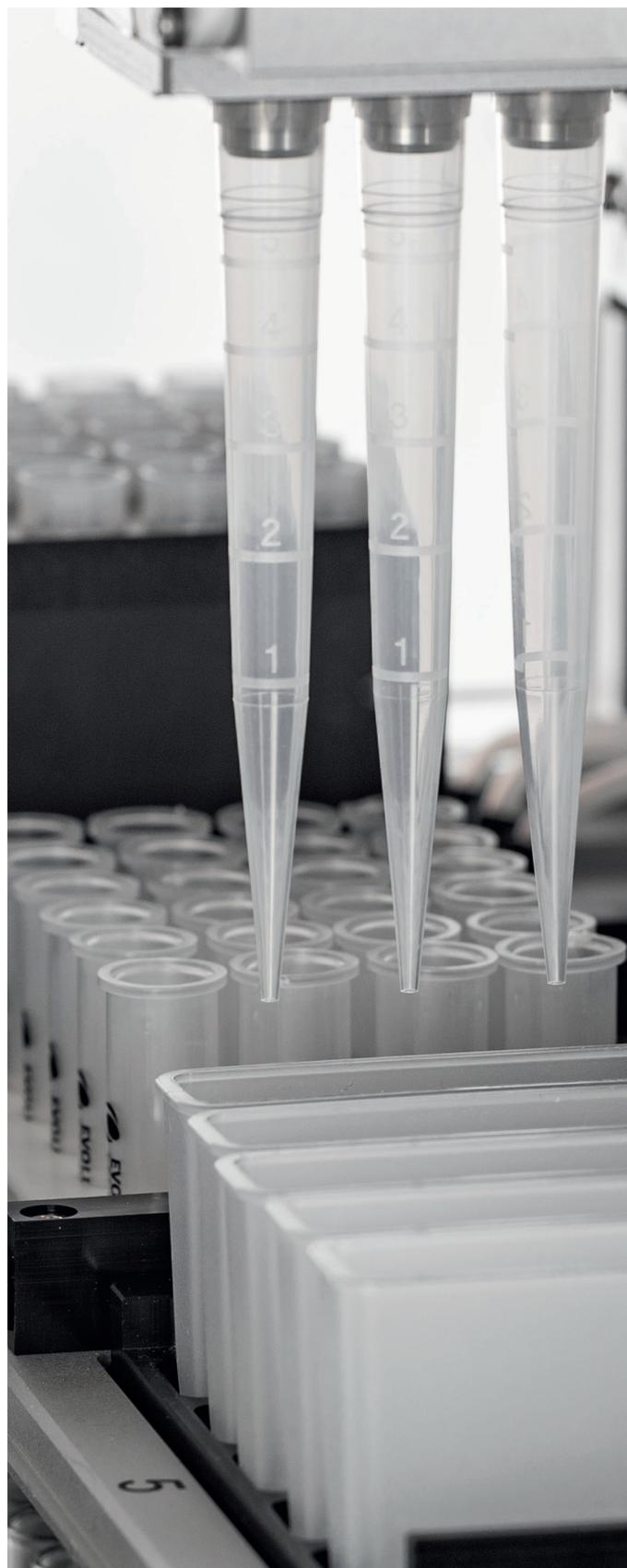
Additional Notes

If sample volume is limited or scarce, then the urine and dilution buffer volumes can be reduced to the minimum 2 mL and 1 mL respectively. A flush step can be used when loading the sample to achieve loading of the entire volume.

Where a pretreatment step is simply being used to dilute the sample then selecting a mix step on this screen is not normally necessary.

To keep processing times to a minimum, mixing (selected as conditioning) during the load step has the effect of mixing the sample AND conditioning the tip at the same time.

Where a sample pre-treatment step includes reagents that need to react with the sample components (for example hydrolysis enzyme) a mix step should be used.



Using Manual Procedure Mode to Optimize Flows

Special consideration should be given to conversion of processing conditions from ‘flow rates’ (as used on RapidTrace®+) to ‘pressure’ and ‘time’ based processing (as used on Biotage® Extrahera™ HV-5000).

The flow rates delivered by the Extrahera™ HV-5000 can be varied by selecting higher or lower positive processing pressures. The most effective way to optimize a pressure is to perform initial experiments using the Manual Control operation. This process was used to determine the appropriate pressure settings on the method tabs for our example method.

Note: the Biotage® Extrahera™ HV-5000 will not apply pressure unless a time is stated.

For each stage of the extraction where a solvent or sample is passed through the column the following steps should be considered. These series of steps will allow you to visualize the rate of sample or solvent elution onto your consumable to determine the appropriate time and pressure required. Generally, it is desired to achieve flow rates of 1 mL/min; however, this is dependent upon the amount of sample and consumable sorbent.

1. Place columns in the column rack and secure the rack in position 3 of the Extrahera™ HV-5000 processing shelf. Ensure the vacuum pump is on to ensure the removal of any sample or solvent waste. Have the solvents to be added, a manual pipette and a stopwatch handy. Insert a collection rack with test tubes in position A within the carousel to collect your sample or solvent. You will use these to determine the volume collected.
2. Select ‘Maintenance’ screen on the Extrahera™ HV-5000 system. Switch on the lower light (optional) and then select Manual Control.
3. Add the required volume of your solvent or sample into your column using a manual pipette. Close the door and instruct the pressure head to engage the column rack in position 3 by selecting “Move Out” under “Pressure Unit.”
4. Under “Carousel and Lift” select “A” under the “Move plate or rack to collect.” This will rotate the carousel so position “A” is where you can collect any sample or solvent.
5. The solvent or sample may begin to flow under gravity. Start your timer and monitor the solvent level collected. Once the flow stops, record the time and measure the volume collected. This will provide an approximate flow rate in milliliters (solvent/sample) per minute (elapsed time).
6. If the sample or solvent is not flowing under gravity, under “Pressure Head - Processing” select the pressure (bar) and time (seconds) you wish to apply. Select “Start” to begin. Start your timer once you observe solvent or sample eluting. Monitor the solvent level collected. Once the flow stops, record the time and measure the volume collected. This will provide an approximate flow rate in milliliters (solvent/sample) per minute (elapsed time).
7. Repeat until you achieve the desired flow characteristics needed for your method to equal that of the RapidTrace®+.

Additional Notes

Generally, there may be a slight difference from one column to another add about 15–25% to the timings measured in manual mode when programming the actual method. For example, if it takes 100 seconds at 1.5 bar to push through 2 mL of water consider programming a time of 120 seconds.

The instrument has a plate dry function where the maximum flow (5 bar, 600 mL/min) possible using the Extrahera™ HV-5000 components is pushed through the columns. This is normally recommended for any steps where the sorbent needs to be as dry as possible. This option is typically employed in the last step prior to elution. For robust performance, add a dry step before and after the use of any water immiscible solvent wash steps.

Pressures can be applied using two approaches. In the first instance a single pressure can be used with or without a plate dry function at the end. Alternatively, advanced mode can be used. Advanced mode has three advantages.

1. A period of no pressure can be set if performing a method where the solvent flows rapidly through the cartridge under gravity alone.
2. Up to three different pressures can be set one after the other if there is uncertainty of ideal flows sorbent bed drying is not a concern.
3. The plate dry function (5 bar, 600 mL/min) can be used in combination with options 1 and 2.

Setting up New Solvents

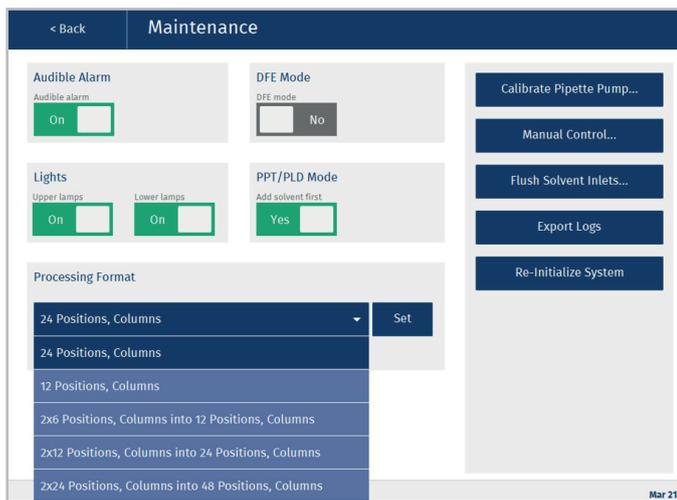
Solvents used in our example procedure or other methods may not be preinstalled on the Extrahera™ HV-5000. It is possible to add solvents by finding the closest solvent match (Data administration, manage solvents).

For example, when setting up the solvent “0.1M HCl” copy and rename any aqueous solution. The elution solvent (quoted in the example procedure as a combination of methylene chloride, isopropanol and ammonium hydroxide in the ratio 78:20:2 (v/v)) could be produced by copying Dichloromethane: Methanol : Ammonia (78:20:2) and renaming appropriately.



Check the Processing Format

The processing configuration of the Biotage® Extrahera™ HV-5000 is selected when choosing the Extraction media. The instrument can process 3 mL cartridges or 6 mL tabless cartridges using a 24-position column rack and the 24-position configuration kit. Additionally, the system processes 10 and 15 mL column consumables using a 12-position column rack with the 24-position configuration kit. Finally, the system can process 48 tabless, 3 mL columns using a 48 position rack with the 48-position configuration kit. To alternate between processing configurations, set the appropriate format the maintenance screen.



Using the example in this technical note, it is recommended that the following items are ordered.

1. 417610, Configuration kit, 24 positions Dual Flow – HV .
2. 414174SP, Column Rack 24 x 3 mL.
3. 902-0020-B, ISOLUTE® HCX 200 mg/3 mL SPE columns .
4. 414254SP, Sample Rack 16 x 100 mm, 24 positions .
5. 415491, Sample/Collection Rack 12 x 75 mm tubes, 24 positions.
6. C44651, 12 x 75 mm Test tubes for collection.

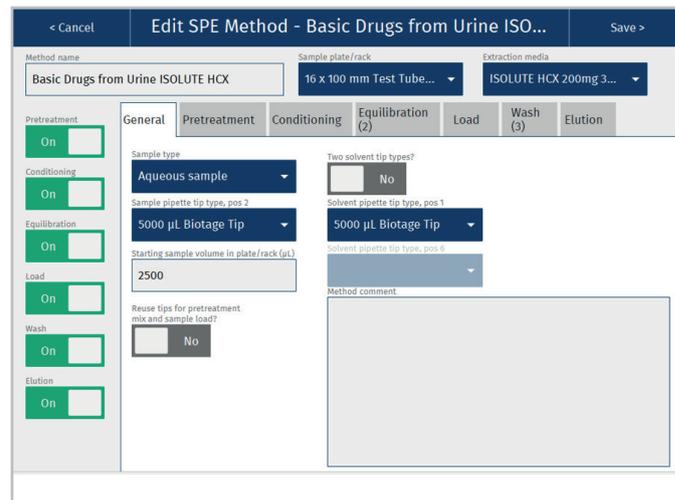
Should you wish to increase sample throughput of this method, the Extrahera™ HV-5000 can double its processing capacity from 24 to 48 samples using the following accessories.

1. 418374, Configuration kit, 48 positions Dual Flow - HV.
2. 415556SP, Column rack 48 x 3 mL (Tabless).
3. 902-0020-B, ISOLUTE® HCX 200 mg/3 mL SPE columns
4. 414254SP, Sample Rack 16 x 100 mm, 24 positions, (you will need two of these).
5. ISOLUTE® HCX 200 mg/3 mL SPE cartridges (902-0020-BG).
6. 415555SP, Sample/Collection Rack 12 x 75 mm, 48 positions.
7. C44651, 12 x 75 mm Test tubes for collection.

The Complete Procedure Converted for use on Biotage® Extrahera™ HV-5000

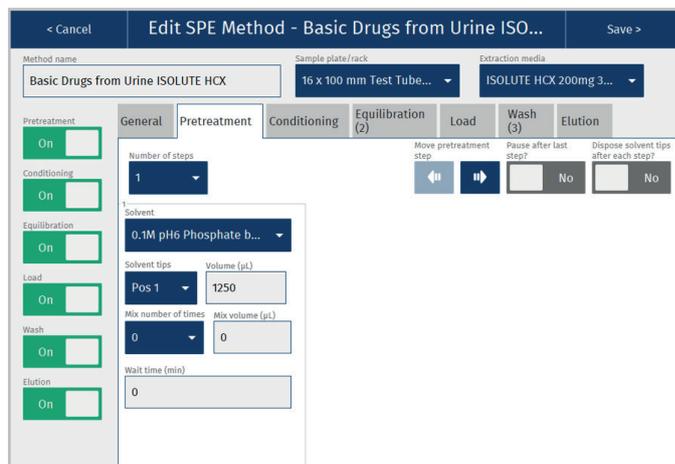
1. The method detailed below is set up for 24 tabbed or tabless 3 mL column use.
2. **All pressure settings quoted in these pages are approximate estimates and should be optimized before use.**
3. **Note:** all volumes on Extrahera™ HV-5000 are quoted in μL .

General Tab



This tab reflects the conditions discussed previously in this guide. It assumes a sample volume of 2500 μL (2.5 mL) is available in each position of the sample rack.

Pretreatment Tab



This tab reflects the conditions discussed previously in this guide. An aliquot of 1250 μL (1.25 mL) of 0.1 M phosphate buffer is added to 2500 μL (2.5 mL) of sample. No mixing is performed.

Conditioning Tab

A single aliquot of methanol (3000 µL / 3 mL) is loaded with pressure of 1.2 bar, for a time of 120 seconds. It is directed to waste (position D).

Load Tab

A pretreated (diluted) sample volume of 3000 µL / 3 mL is loaded at a pressure of 1.4 bar, for time of 180 seconds. It is directed to waste (position D).

Equilibration Tab

For the 2-stage equilibration process.

1. An aliquot of water (3000 µL / 3 mL) is loaded with pressure of 1.6 bar, for a time of 120 seconds.
2. An aliquot of 0.1M phosphate buffer (3000 µL / 3 mL) loaded with pressure of 1.6 bar, for a time of 120 seconds.

They are directed to waste (position D).

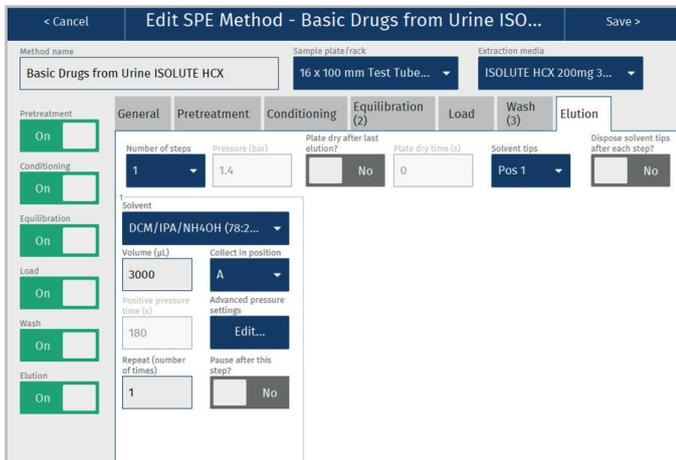
Wash Tab

For the 3-stage interference wash process:

1. An aliquot of water (2000 µL / 2 mL) is loaded at a pressure of 2.0 bar, for a time of 100 seconds, and directed to waste (position D).
2. An aliquot of 0.1M HCl (2000 µL / 2 mL) is loaded at a pressure of 2.0 bar, for a time of 100 seconds, and directed to waste (position D).
3. An aliquot of methanol (2000 µL / 2 mL) is loaded at a pressure of 2.0 bar, for a time of 80 seconds, and directed to waste (position D).

A dry step (300 seconds) is then performed prior to analyte elution.

Elution Tab



Selecting “Advanced pressure settings” on elution tab



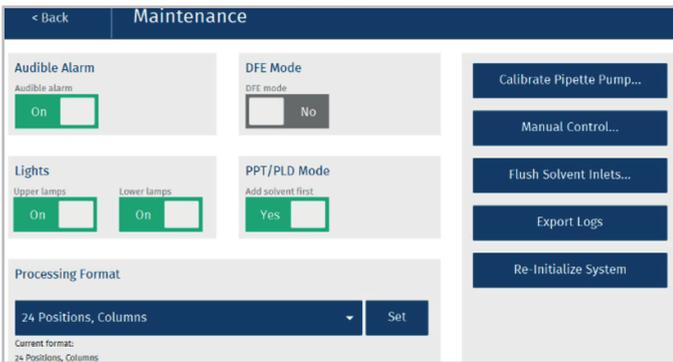
To ensure complete recovery of the elution solvent, a final pulse of pressure (3 bar for 15 seconds) is delivered. This is included to improve analyte recovery and method reproducibility.

An aliquot of DCM/IPA/NH₄OH (78/20/2, v/v) (2000 µL/2 mL) is loaded with a pressure of 1.4 bar, for a time of 180 seconds. It is collected in collection tubes, located in position A.

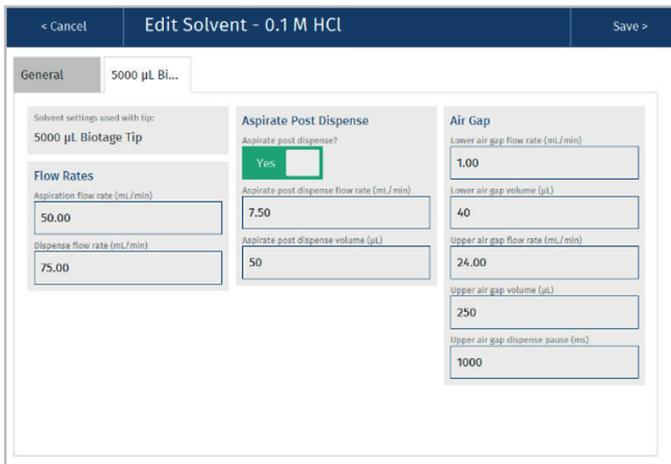
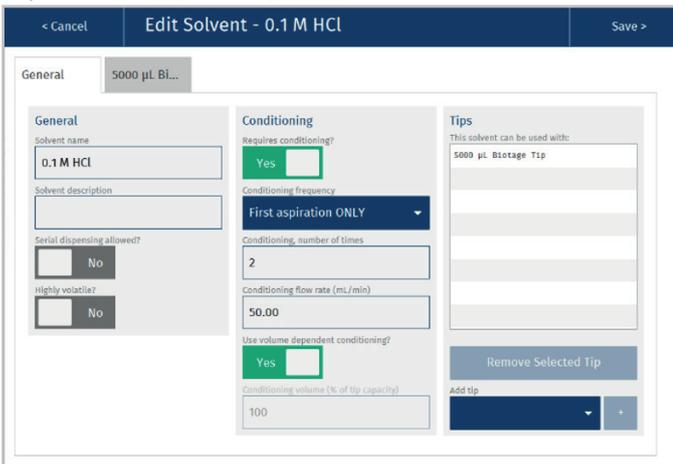


Other Selected Screens

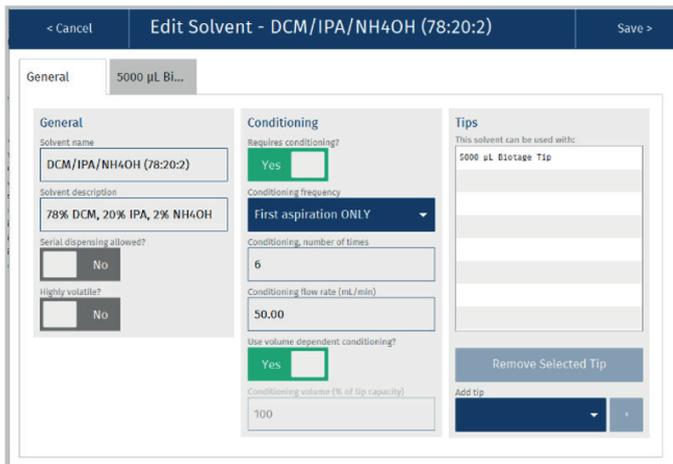
Maintenance screen showing “24 positions, columns” mode



Liquid handling settings for 0.1M HCl



Liquid handling settings for elution solvent DCM (Methylene Chloride): IPA: Ammonia solution (78:20:2 v/v/v)



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

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

Part Number: UI497

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