**Scope**

The scope of this document is to present an overview of the high performance liquid chromatography (HPLC) system Ultimate 3000 and to provide the reader with guidelines for standard operations, routine maintenance and simple trouble shooting. Detailed instrument description can be found in Dionex Reference Library (CD-ROM) which contains all relevant and specific manuals.

**Instrument overview**

The Dionex Ultimate 3000 system is a dual pump system with three channels on each pump. It is capable of operating two columns simultaneously via the “LEFT” pump and the “RIGHT” pump. In practice the common use will be one column at a time, and therefore regular changes between the two pumps will be necessary to prolong the lifetime of the system. The detection principle is based on UV-VIS (ultraviolet-visible light) detection and up to four different wavelengths can be detected and logged at the same time; ranging from 190 to 740 nm. Figure 1 shows an instrument overview.

_HPLC systems are delicate equipment and should be handled accordingly. Regularly check the status of the instrument, preferably on a daily basis and always be careful when operating. Eluents should ALWAYS be prepared from fresh top quality water (Milli-Q/18.2 MΩ·cm) and preferably sample preparation should also be made based on this water quality._

The chromatographic system is controlled by a computer installed with Chromeleon™ 7 software (hereafter CM) version 7.1.1.1127 for instrument control and data handling. All commands and instrument handlings goes through this software.
The system consists of 5 compartments/modules (from top):

1. Solvent rack (SRD-3600)
2. Dual pump (DGP-3600A)
3. Autosampler (Well Plate Sampler, WPS-3000SL)
4. Column compartment (Temperature Controlled Compartment, TCC 3200 2x2P-10P)
5. UV detector (Variable Wavelength Detector, VWD-3400)
Figure 2: Instrument Controller. Software unit that controls communication between computer and HPLC. Must be running at all times when system is operated.

Communication between the computer (CM) and the HPLC is controlled by the Instrument Controller Software found under the Start Menu/Programs/Chromeleon/Service Manager. Alternatively, double click the Chromeleon Icon in the Windows task bar to open the Instrument Controller (figure 2). When the HPLC is to be controlled/operated the Instrument Controller must be running. Starting and stopping is done by pressing the command “Start/Stop Instrument Controller” (figure 2). The window can be closed afterwards. The Instrument Controller does not start automatically after a computer restart and it should therefore be checked that it is running, before starting operation.

In the operating software (CM) each module has its own operational screen (except the solvent rack), which may be found as tabs in the top of the window (figure 3).
Operating the pump

The internals of the dual pump system is depicted in figure 4. The right hand side of the pump is operating the “RIGHT” system and the left hand side of the pump is operating the “LEFT” system. The pumps are operated at flow rates between 0 and 5 mL/min and max pressure of 400 bars. Each pump is connected to three solvent channels (A, B and C). A Rear Seal Wash system is installed to periodically flush the backside of the pump heads in order to avoid salt precipitations and corrosion. The wash is done automatically, but the rear seal wash solutions needs to be changed on a weekly basis and consists of 20% methanol/ethanol (figure 5). Note dates of change in the equipment logbook!
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Figure 4: Internals of Dual Pump

Figure 5: Rear seal wash solution bottle. Wash solution needs to be changed on a weekly basis and consists of 20% methanol or ethanol. The bottle is fitted in a metal holder and the entire holder is lifted off.

Changing eluents

When eluents are changed the relevant pump needs to be purged to remove air bubble formation and old eluent from the tubes. The system is sensitive to air and purging is therefore of outmost importance. Purging the eluent channels is done by applying high flow through the specified channels and will create high back pressure on the system, if the purge valve has not been opened. This is done by loosening the purge valve (screw) on the pump head (figure 6) by approx. half a turn. This will direct the flow path to a waste tube and relieve the pressure on the system (and the column in particular).
Figure 6: Pump head with purge valve and outlet to autosampler. Changing from one operating pump head to the other you simply move the yellow connection tube to the other pump head outlet.

Figure 7: Pump tab. Relevant functions marked for purging, flow settings etc. Equal functions for Right Pump found just below.
Once the purge valve has been opened choose the appropriate channels and flow distribution to purge in CM and activate the purge function (figure 7). It is possible to purge more than one channel at a time by setting the percentage distribution between the channels as desired (equal distribution, 50:50 when two channels and 33% when all three channels are to be purged). The system is set to purge via the sampler, meaning that the purge flow goes through the sampler needle. The sample needle will therefore change position when purging begins and ends. The software is preprogrammed to perform purging for 300 seconds at 3 mL/min. Repeat purging until air bubbles are no longer visible in the eluent channels from the bottles to the pump.

**IMPORTANT:** Before purging, the software will inform that this operation will generate a high flow through the system and that the purge valve should be open before executing (figure 6). Press OK, if the purge valve is open. If the system senses any pressure increase (even minor) during purging, the command will be terminated. Make sure that the valve has been sufficiently loosened and try again.

Once the purging is done, close the purge valve (figure 6) again tightly to redirect the flow path to the column. If the valve is not tightened sufficiently, the pressure and flow delivery during operation will be insufficient. No warnings about low pressure will occur, unless pressure is completely lost (less than 5 bars) so please do observe that correct pressure is obtained during start up. It is allowed for the pressure to show certain fluctuations during start up and running and these will be periodic. If fluctuations become too large, or if stable pressure cannot be obtained, this indicates air trapped in the system. Air is compressible in contrast to liquids and if air is in the system, periodic, frequent fluctuations in the pressure are observed. The size of these fluctuations should not exceed 2-4% of the current operating pressure and if so, purging must be repeated until stable pressure can be obtained.

**Changing between pumps**

Most often, only one pump is used at a time, and in order to optimize the lifetime of the pump module, the two pump heads needs to work approx. the same amount of time. Therefore, operation should be divided equally between the two pumps Therefore, once every month the pump setup should be switched between the pumps. This is done by moving the yellow tubing connecting the pump head in action with the autosampler to the other pump head. Figure 6 illustrates where the outlet is placed on the pump head. Once the connection is correct, eluents need to be changed, so that the active pump has the analytical eluents, whereas the resting pump channels are stored in 50% methanol. Hereafter
purge ALL channels (both Left and Right Pump) to accommodate the new eluents. Note in the equipment logbook the data for changing between pumps and be aware to choose the correct instrument method for operation from the correct pump.

**Operating the autosampler**

Samples are placed in the autosampler prior to operation. This is done by sliding the grey plastic lid aside and placing the vials in the appropriate racks. As a default setting the autosampler is set to 40 vial racks, but this may be changed to other formats ex. 96 well plates. Consult the equipment manual or superuser to do so. The sampler is divided into three compartments (R=Red, G=Green, B=Blue) and the carousel may be turned by hand or by the software to expose each vial rack to the lid opening (figure 8). The vial rack may be removed from the autosampler while filling. Naming the sample position is done by assigning a correct code in the sequence: ColorLetterRowLetterColumnNumber (i.e. RA2 is a vial placed in Red tray, position A2). When placing the vial rack in the carousel make sure that it fits correctly.

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**Figure 8: Upper: Autosampler - view from lid opening. Lower: Vial rack layout.**

Before initializing a new sequence wash the buffer loop to remove any residual sample or eluent from previous runs. This is done by pressing the command “Wash buffer loop” in CM (figure 9). The autosampler will automatically wash with 300 µl and stop. Furthermore, prime the sample syringe to remove air bubbles, which is also done from CM by pressing the command “Prime Syringe” (figure 9).
Operating the column compartment

The column compartment is the module where the column is connected. During operation the front cover of the column compartment should be closed, especially if temperature control is applied (NB! temperature often have a large effect on retention times).
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**Figure 10:** Internals of column compartment with two 10-port switch valves. Blue arrow indicates flow direction, which should fit with column flow directions.

When the system is operated with two different columns, the columns are typically connected via the 10-port switch valves (figure 11), but for most use when only one column is fitted, there is a direct connection from the pump to the autosampler and then to the column. The inlet of the column is connected to the tubing running from the autosampler. Be aware that the flow direction on the column is correct (arrows on the column indicate flow direction). The outlet of the column is connected directly to a tube to the detector.

**Figure 11:** 10-port switch valve with port numbers.

When a column is fitted and flow is applied, please ensure that no leaks can be observed. Leaks at the column inlet will result in pressure decrease, whereas leakages at the column outlet may not affect the...
pressure but will affect the detector signal and column compartment flooding!. If leaks are observed or registered by the system, further tighten the connections either by hand or by a wrench key (NB! Be careful). When applying low viscosity solvents it may be necessary to tighten the connections several times.

Column temperature is specified in CM (figure 12) and the desired temperature is written in the field for “Nominal Temperature”. Press Enter to execute the command. The color of the Actual Temperature field will turn red for “Not Ready” and will turn green when the temperature is within the specified range settings. The field “Temperature Ready Delta” will specify a threshold value with the acceptable margins for temperature deviations (please note that usually a ready temp delta is specified in each separate method. Your first sample may thus not start before the methods temperature setting has been reached.

IMPORTANT: When warming up and cooling down a column, KEEP a certain flow! Otherwise liquid inside the column may release air bubbles that can ruin the packing material in the column. When temperature control is applied make sure to keep the compartment door closed.
Operating the detector

A UV-Vis detector consists of a flow cell where the flow path is directed through, two lamps (the UV lamp and the Deuterium lamp) and a number of mirrors, reflectants and slits. The column outlet is connected to the detector/flow cell inlet via appropriate tubing and the flow cell outlet is connected to waste.

The UV and visible light lamps are turned on and off in CM by ticking off either of the tick boxes (figure 13). It will take a minute before the tick mark appears so ONLY press it once. The lamps need to warm up before they are operational and should therefore be turned on at least 60 min before they are expected to perform. Wavelengths are set in each of the four channels by writing in each field. An empty field means that no data collections occur at that specific channel. Please note that UV lamps have about 1000 operational hours (lamps are expensive and on/off switching reduce operation time). If the system is to be operated day after day it may therefore be beneficial for the lamp lifetime not to turn it off after each run. Keeping lamps on should, however, only be done when you know you will use the system the following day!
Waste collection

From the detector the flow stream is passed to a waste container. This waste container needs to be checked regularly and emptied. **This means before you start your analysis!!!!** Be aware what waste category the outlet flow complies with, which will depend on the content of organic phase, acids, halogens or other modifiers.

Starting up for operation

1. Make sure that the correct eluents are present at the correct channels and that the volume of eluents is sufficient for the entire planned run. **NEVER let the HPLC run out of eluent!**
2. Check that the column is securely fitted.
3. Start the flow at a low flow rate, for instance 25% of what the end flow should be. For flow rates at 0.4 mL/min start the instrument at 0.1 mL/min (this is to prevent a sudden pressure increase, which may be detrimental to columns).
4. If column temperature control is applied, set the desired column temperature.
5. Turn on the UV lamp. It needs approx. 60 min to warm up and yield reproducible intensities.
6. Start “Baseline Monitoring” (see figure 14) and watch the pressure stabilize.
7. When pressure has stabilized, increase the flow rate in increments of 0.1-0.2 mL/min until the desired flow rate has been reached. Let the pressure stabilize between each increment.
8. Wash the autosamplers’ buffer loop and prime syringe.
9. Place the samples in the autosampler and prepare the sequence (see section below). Always include at least 1-2 blank runs (samples without injection, running the methods gradient only) in the sequence before injection of the first real sample/standard in order to condition the column properly.
10. Stop baseline monitoring.
11. Start the run either by starting the sequence directly or by queuing the sequence (add to queue in the “Queue” tab (figure 14)). Press “Ready Check” to get an estimate of eluent use during the entire queue. And most importantly that there are no errors in your sequence!
Figure 14: Top screen dump: Queue tab. Here sequences can be queued for automatic start and general checking of system status (ready check) is performed. Lower screen dump: Ready Check with estimated eluent consumption.

Successful ready check

Adding a sequence to the queue.

Queue tab. For queuing several sequences

Monitoring baseline. Monitor pump pressure and detector signal

Ready check. Testing if the system is ready for operation. Gives an estimate of eluent use.

Adding a sequence to the queue.

Queue tab. For queuing several sequences

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Successful ready check

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Successful ready check

Ready check. Testing if the system is ready for operation. Gives an estimate of eluent use.
Shutting down after operation

After a finished sequence the instrument needs to be shut down in a proper manner like the following:

1. If temperature has been applied during sample analysis, set the temperature to 30 °C and allow the column to cool down WITH flow on. BEWARE if the pressure will increase unacceptably during cooling, and in such a case, reduce the flow rate to half. Depending on the column temperature, this cool down will take from 20-40 minutes.
2. Fill the column with the appropriate storage solution (typically high organic solvent content and replacement of aqueous buffers with water). This is done by setting the solvent distribution to the desired concentrations and turning on baseline monitoring. Continue the flow rate settings from step 1. The pressure will decrease and stabilize when the column volume has been displaced with the storage solvents.
3. Turn off the flow rate.
4. Turn off the UV/VIS lamps. NOTE: If the system (and the lamps) is to be used again within 12 hours leave them on! This will prolong the lamps lifetime and save the next user for warm up time.

Setting up a sequence in CM

The data structure in CM is shown in figure 15, which is initially divided between Left and Right instrument. Hereafter, a general data folder contains a folder for each user, specified by name. Maintain this order of data structure and save your sequence under your own name.
Figure 15: Data structure in CM. Note the switch between Instrument and Data panel.

When making a sequence, choose “Create” from the top menu and choose “Sequence”. Hereafter a wizard will start, that will guide you through setting up a sequence:

1. Choose system: HPLC
2. Write a recognition name that it will be copied to for each sample (may be edited later), choose number of samples, number of injections pr. sample (most often 1), position of 1st vial and injection volume (uL)
3. Choose Instrument Method (analytical run method) by accepting the lastly used method or by browsing through folders. Choose Processing Method if applicable. Can also be added later. Set default channel to UV_VIS_1.
4. Write a comment describing the particular samples (not compulsory).
5. Press finish and save the sequence to your folder under an appropriate name starting with the following date format: YYMMDD_ (this is important because of data backup issues which are periodically taken care of by super users)

Figure 16 shows an example of a prepared, running sequence. Most fields in the sequence can be edited before, during and after the run. This includes: Name, Type (blank, unknown, standard), Level (only relevant for quantification), Position (vial position in the autosampler), Volume (injection volume)
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volume), Instrument Method (analytical run method) and Processing Method (qualitative/quantitative method). Some fields have scroll down menus, others are text fields. Always press “Save” after editing any fields. A shortcut called “Fill Down” is made for fast assigning sample position in the autosampler. Mark the “Position” field from the position of the first vial, press F9 and choose “Renumber”. Press enter. Alternatively, select the Fill Down menu (figure 17), choose Renumber and write the name of the first sample position. Press OK.

**IMPORTANT:** Changing any of these fields mentioned above will not affect the results, but changing the field “Status” will erase data behind, if the samples have been run; “Idle” indicates a sample not yet analyzed, “Finished” indicates a sample analyzed and therefore a file containing data. “Interrupted” is a sample stopped during analysis due to some sort of error message, may also contain data, but sometimes not useful data.

![Image of a computer interface showing a labeled table with columns for Sample Name, Volume, etc., with various options like 'Delete', 'New Prod. Method', 'Import', 'Export', 'Paste', 'Sort', 'Group', 'Arrange', and 'Sequence'.]

**Figure 16: Prepared, running sequence.**
Figure 17: Fill Down menu for fast assignment of sample position. Please mark the field of the first sample in the order.
**Alarms and warming**

**Pressure alarm**

If the pressure falls below or reaches the upper limits of the settings (which are mainly set to protect the column) a pressure sound alarm will be initiated. At the same time, all flow will be stopped. High pressure may occur if the samples contain insoluble particulate material, if the solvent concentration suddenly changes, temperature control fails or if the column is overloaded with sample due to many injections without regeneration. Loss of pressure may occur if the eluent bottles are empty, air is introduced in the system or if leaks happen on the “upstream”/pressure side of the column. If leakage is the cause, the system will detect the compartment where this is occurring via leak detectors and the audit trail will inform about this.

The sound alarm is turned off by using the blue touch pen sitting in the autosampler. Press it against the little dot underneath the lighted panel on the pump. If any other alarms are active in any other of the compartments, the same procedure will silence those (pressing the blue pen against the dot underneath the lighted panel on each module).

Identify the source of the pressure violations, take proper action and restart the system.

**Leak/humidity alarm**

Identify the reason for leakage (which compartment) and correct if possible. Dry up any spillage in the compartments with soft tissue paper. Silence the alarm by using the blue pen (see above).

**No pressure?**

Check that the purge valve is properly closed. Check that all fittings are securely tightened. If this is not enough purge the channels as air may have been trapped in the pump.

**No connection to modules?**

Check that the Instrument Controller is running (figure 2). When this is positively running, reconnect the non-connected modules by pressing “Module connect” in each module tab (see figure 7 or 9). If there is still no connection restart the computer and start the Instrument Controller again. Still no connection – call security.

**Software error messages**

Close all active windows and restart CM. Still software errors, restart computer and start the Instrument Controller again. Still errors – call security.
**Autosampler “Not Ready”**

Press “Wash buffer loop” to reset autosampler. Wait for the sampler to finish the command and see ready status. Still not ready, close all active windows, stop and start the Instrument Controller. Restart CM and check ready status for autosampler. If not ready – call security.

**Call security**

For more advanced guidance either contact Superusers or consult the general equipment manuals found on the CD-rom: “Reference Library” provided by Dionex.

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**Editorial log**

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