REACH Devices

RDn: ...-...-...

n-Channel Preparative Chromatography Detector Family

User Manual

(for unit with the serial numbers 2500-0 and up)

Innovative Equipment Designed by and for Separation Scientists.

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1. Overview

RDn is a standalone detector family for preparative chromatography designed to operate with conventional glass chromatography columns, FPLC chromatography systems, or preparative HPLC columns.

Operation with analytical HPLC columns is not advised due to RDn's relatively large flow cell volume (150 uL). The detector employs standard 1/4” 28TPI connectors, and is compatible with virtually any organic or aqueous media, including 1M NaOH. This excludes hydrofluoric acid at any concentration. There are some further limitations (for exotic media, rarely encountered in practice) in chemical compatibility disclosed in Chapter 5.

When referring to the RD-model detectors, the number that follows refers to the number of individual data streams the device can measure and store in its nonvolatile memory. As an example, the RD1 will measure one type of data (typically UV absorption at a fixed wavelength), and the RD5 will measure five (typically UV at 250nm, UV at 280nm, Conductivity, RI and pH). In this respect, we will collectively refer to the RD product line as RDn where n is the number of measured data streams for a given device.

All models also measure and store elutant temperature.

The detector is designed to minimize resistance to solvent flow rates of up to 200 ml/min. Even simple gravity chromatography can be carried out with RDn (see Chapter 7.2 for details).

A unique feature of RDn design is that UV absorption data is simultaneously collected for optical path-lengths from 0.001 to 6.54 mm. For user's convenience, the data is presented as if the acquisitions occurred using a 10 mm optical path-length with 0.001 - 800 AU (Absorption Units) range. Unlike other UV monitors, the chromatogram collected by the RD4 detector does not plateau when the concentration of UV-absorbing compounds is high, allowing the user to see true analyte peaks as they leave the column. For more information see Appendix 1.

In most advanced configuration RDn (e.g. RD5) detector simultaneously records the following properties of the elutant:

1. Optical density at 250 nm (10 nm bandwidth) in 0.001-800 AU range for the theoretical 10 mm optical path length with 0.0001 AU resolution
2. Optical density at 280 nm (10 nm bandwidth) in 0.001-800 AU range for the theoretical 10 mm optical path length with 0.0001 AU resolution
3. Conductivity in 0.001- 40 S/m range (user calibration recommended)
4. Refractive Index at 280 nm, temperature compensated (user calibration required)
5. pH or ion-selective electrode signal (user calibration required)
6. Elutant temperature in ^{o}C, 0.01^{o}C resolution, 0^{o}C to 53^{o}C range

The measured data is recorded to non-volatile memory of the detector according to the acquisition rate setting. This rate parameter may be selected by user to take measurements within every 0.2 to 10 seconds, which corresponds to acquisition rates of 5 to 0.1 Hz. Note that the 5 Hz acquisition rate setting leads to a rapid (about 7 hours) filling of the detector memory, while the 0.1 Hz rate allows for 15 days of continuous data collection. Data acquisition can be stopped/resumed at any time, and as many times as desired, during the run. Unexpected power outage or powering down the detector does not corrupt, erase, or alter already-collected data in any way, and normal acquisition will be automatically resumed from the last point collected after the unit is powered on again. Collected data can only be erased by pressing the “**Run**” and then “**New**” soft keys in addition to some advanced functions in “**Settings**” menu.

The unit's color display shows data as it is collected in real time. Zoom can be set to automatically adjusted so that every newly acquired point will land within screen coordinates. The user may select up to four traces to be shown, and manually adjust graph zoom during data acquisition.

The collected data can be transferred onto a conventional flash drive. The file written is a space-delineated text file, which can be imported into Excel, MathCAD, KaleidaGraph, R, MATLAB, or any other software capable of building 2D graphs.

The RDn is equipped with a real time clock powered continuously by a 3V lithium battery (CR2430). The battery should last for at least 5 years after which it needs to be replaced. The battery may be accessed by counter-clockwise twisting of the battery cap on the bottom of the detector unit.

There is a communication interface built in RDn that is physically arranged as a female 8-pin socket on the back of the detector. This interface includes 4 analog outputs (a user would need to set the desired transfer functions, described in Chapter in order to use them), and a digital input which can count external electrical pulses (such as fraction change events in a fraction collector). There are also one generic TTL input and one output. These do not have any function by default, but can be programmed at user request by REACH Devices.

2. Powering unit on and power outages

RDn detector is not a battery-powered device, thus it cannot collect data without the line power.

The close proximity of conductive or flammable elutants to a source of electrical sparks or to power sockets is hazardous. Even though RDn in not an explosion-proof device, the RDn detector unit does not have a power switch to provide maximum safety:

- The detector is powered on from its keyboard by pressing two keys simultaneously as engraved on the detector housing.
- To switch the unit off, the operator needs to go to "**Off** " menu and press "**Turn Unit Off**" softkey.

Normally, it is a good practice to disconnect the RDn from line power if it is not in use for more than 24 hours. However, if the detector is permanently situated in a cold room (4 ^{o}C) with high humidity, it should remain connected to a power line to maintain detector's housing temperature slightly above 4 ^{o}C, thus preventing water vapor condensation inside the unit.

An unexpected power outage would not disrupt future detector operation or cause data corruption. If an outage happens during data collection, the detector will shut down but turn on again and smoothly resume data collection after the power is on. If the detector was powered off from the keyboard, it will remain off until manually powered on by the user.

In the unlikely event of a power outage during data transfer to a flash drive, the data on the flash drive may become corrupted. The flash drive may need formatting after the incident. Again, detector's internal data would remain intact, and data transfer could be reattempted at any time.

3. Typical list of supplied parts (may vary with the model, check packing slip)

1. RDn detector unit, 90-240 VAC
2. One power cord, country plug style specified at purchase
3. Three 1/16" tubing connectors with ferrules, material: Teflon
4. Three 1/8" tubing connectors with ferrules, material: Teflon
5. One Male Luer Lock to 1/4"-28TPI male thread connector, material: PEEK
6. One Female Luer Lock to 1/4"-28TPI male thread connector, material: PEEK
7. One universal connector to a glass column, PTFE/Nylon-12, with two sets of three Viton O-rings
8. 4 feet of 1/16" PTFE tubing (1/32" bore)
9. 4 feet of 1/8" PTFE tubing (1/16" bore)
10. Connector cable to the customer-specified fraction collector (if applicable)

4. Safety

RDn detector was designed for usage in synthetic organic chemistry labs, where corrosive atmospheres exist and solvent splashes happen. The unit case is made of anodized titanium (no peeling paint) and the LCD is protected with a glass sheet. However, the unit may not be used in an explosive/flammable/highly corrosive atmosphere or be submerged in any liquid. Standard safety practices, pertinent to the workplace, must be carried out at all times. REACH Devices shall NOT be liable for any personal injury or property damages resulting from the use or misuse of this device.

5. Flow cell chemical compatibility and care

The flow cell of the detector is compatible with almost all commonly used solvents and reagents. The list of wetted materials:

- Titanium alloy 2
- Fused silica
- Sapphire
- PTFE
- FFKM (Markez Z1028, superior to most of grades of Kalrez material)

In addition, any RDn model capable of conductivity measurement will also have additional wetted materials

- Compact (bright) platinum
- PEEK

The following chemicals will cause rapid deterioration of the unit:

- Aqueous and nonaqueous solutions containing free hydrofluoric acid (HF) cannot be allowed to make contact with the flow cell, even intermittently. Please understand that HF is a weak acid and will form in any acidic solution which contains fluoride anions (like NaF + HCl or even TBAF + Acetic Acid). Fluorinating agents like DAST (dimethylaminosulfur trifluoride) or SF_4 solutions, are all incompatible with the flow cell. Immediate, severe, and irreparable damage to the flow cell will result upon exposure to any of these chemicals.
- Concentrated (>10% vol) hydrogen peroxide, especially in the presence of strongly chelating compounds, such as EDTA or glycolic acid, slowly dissolves titanium.
- Strong hot aqueous alkaline solutions (like 40% NaOH at 50 ºC) will cause corrosion of quartz and titanium surfaces and thus are not recommended for use with the detector.
- Concentrated (>20%) H_2SO_4, HNO_3 and HBr will slowly etch titanium and PEEK.

After using the device, it is imperative to rinse the flow cell with a compatible solvent.

For example, if an aqueous phosphate buffer was inside, rinsing with, say, methanol, will precipitate salts from the buffer and clog the flow cell. Use water in this case. Likewise, if a solution of a hydrophobic organic compound in acetonitrile was inside of the flow cell, then rinse with acetonitrile, not water.

Avoid introduction of immiscible solvents into the flow cell. For example, if hexane needs to be replaced with water, rinse with acetone first (acetone is miscible with hexane and with water), then with straight water.

The detector must be disconnected from liquid lines if it is not in use for more than 24 hours.

6. Attaching RDn detector to the column

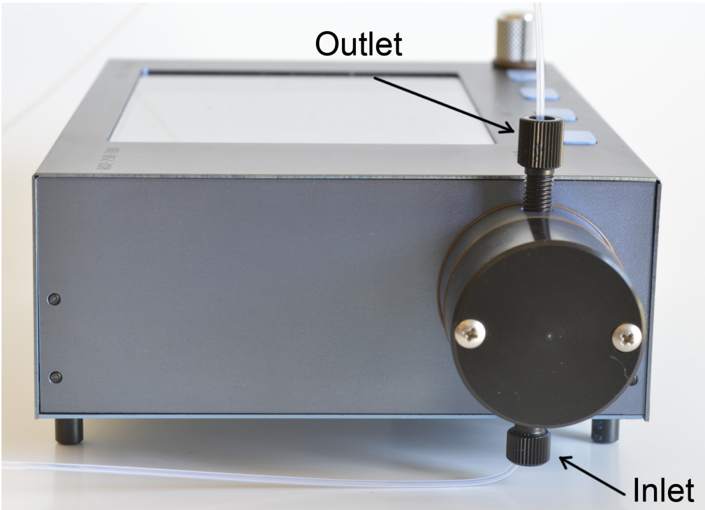
6.1. Column pressure

It is important to realize that the 2500 psi pressure limit does not preclude usage of RDn detector with an HPLC system where the elutant pressure on the top of the column may reach up to 4000 psi.

There are only two requirements that are mandatory for the safe usage of RDn detector with high-pressure systems:

1. The effluent flow may not be restricted downstream of the detector. This rule is especially pertinent to some fraction collectors that may indeed temporarily restrict flow downstream, for example, by closing a valve on the collector's intake. If solvent flow may potentially be interrupted in your chromatography set-up, a pressure-relief valve must be placed somewhere BEFORE the detector, or the solvent pump must be switched off during flow interruption.
2. The flow rates of 300 ml/min for 1/8” ID tubing, and 30 ml/min for 1/16” ID tubing may not be exceeded. This rule is only applicable for low-viscosity solutions, such as water, ethanol or acetonitrile; at higher viscosity (DMF or DMSO), even slower rates must be used.

6.2. Connectors



The liquid ports of RDn detector have 1/4”-28TPI threads for flangeless tubing connectors. Three Teflon 1/16” tubing connectors with ferrules and three Teflon 1/8” tubing connectors with ferrules are usually provided with the detector. Additionally, inlet/outlet ports of the detector are designed be compatible with most plastic fittings for FPLC systems, such as ferrule-based connectors, O-ring based connectors and flange fittings,. Therefore, the user’s own connectors that were not issued with the detector may also be used successfully. Metal fittings cannot be used with the RDn.

Only plastic tubing such as PTFE/Teflon, PFA, FEP, PEEK, polyethylene or polypropylene may be used with the detector. Metal tubing may not be used.

6.3. Note about flangeless fittings

Fittings that are issued with this detector are flangeless-fitting – no flanging/flaring of the tube is necessary to make a connection. Repairing a flanged tubing assembly usually requires only one new ferrule. These fittings were originally developed for DNA synthesizers, but later were borrowed by many manufacturers for various other uses, including FPLC. It is important to assemble the fitting correctly.

Unlike many compression fittings for metal tubes (such as Swagelok), the ferrule must be installed “backwards”. Before assembling the fitting, make sure that the tubing end is squarely cut by a new razor blade and is flush with the broader ferrule end.

A common assembly problem that often goes unnoticed occurs when the end of the tubing gets slightly pulled out of the ferrule prior to screw tightening. The subsequent tightening will crush the ferrule. Therefore, it is useful to slightly push the tubing into the screw nut during tightening. Do not use tools to tighten the connector body.

If a leak occurs, the following must be inspected before more torque is applied to the connector body:

1. There is no foreign material within the connector assembly.
2. The tubing end is not warped, cracked or deformed (this can easily be rectified by cutting off the damaged end with a razor blade).
3. The ferrule surface is smooth without dents, scratches or deformed areas. Replace ferrule if it is damaged.

6.4. Glass column adapter

The glass column adapter may be used to attach 1/8” or 1/16” tubing to a conventional glass column.

The adapter works by squeezing a flexible O-ring over a glass column outlet. Because there is no standard size for outlet diameters, three different sizes of O-rings are provided with the adapter. Only one most suitable O-ring should be used at a given time. The extra rings may be placed onto adapter outer surface for storage as shown.

By default, the provided O-rings are made of Viton – a brand of fluorocarbon elastomers. Viton O-rings are fairly stable to most solvents. However, prolonged exposure (days) to toluene, DMF, DMSO, and especially a pyridine / triethylamine mixture, will cause excessive, likely permanent swelling or disintegration. For applications involving extensive use of the above solvents, blue colored fluorosilicone O-rings are advised, and may be requested when ordering the adapter. It should be remembered that the fluorosilicone O-rings will swell extensively (but not dissolve or disintegrate) in acetone and to a lesser extent in ethylacetate.

To prolong the useful life span of the O-rings, the glass column adapter should not be stored attached to a column soaked with solvents. It is a good practice to rinse the adapter with an appropriate solvent and store it separately from a column in a dry place.

For most glass columns, 1/8” tubing is recommended to minimize the hydrodynamic resistance to liquid flow. 1/16” tubing should be used only for very small glass columns (less than 10 ml volume) to minimize the dead volume of the system.

7. Running columns with RDn detector

7.1. Air bubbles

RDn is a preparative detector, optimized for flow rates of at least 10 ml/min. At lower flow rates, an air (or other gas) bubble may be retained in UV/Conductivity flow cell, which would result in erratic readings.

If you intend to use the detector with aqueous solutions at < 10 ml/min flow rates, a following procedure is advised after detector unpacking.

1. Run 10% NaOH at 100 ml/min for one minute through the detector.
2. Run an aqueous surfactant (e,g. 1% SDS) at 100 ml/min for at least two minutes.
3. Purge RD4 for 15 seconds with the initial separation's solvent at 100 ml/min flow rate.

To use gravity to facilitate air bubble expulsion, the flow must be directed from the lower situated inlet to the higher situated outlet.

7.2. Gravity columns

RDn back-pressure is sufficiently low to allow running gravity columns in some cases. The maximum achieved flow rate will strongly depend on several things.

1. Difference in elevation between the level of elutant on the top of the column and the elutant exit point (usually end of the tubing or the level of the solution in the receiving vessel, whatever is higher)
2. Thickness and length of the connecting tubing
3. Column packing resistance to elutant flow.

In practice it is best to **locate the detector at the lowest point of the fluid path**. This will ensure slight positive pressure in the flow cell which would at least partially suppress outgassing.

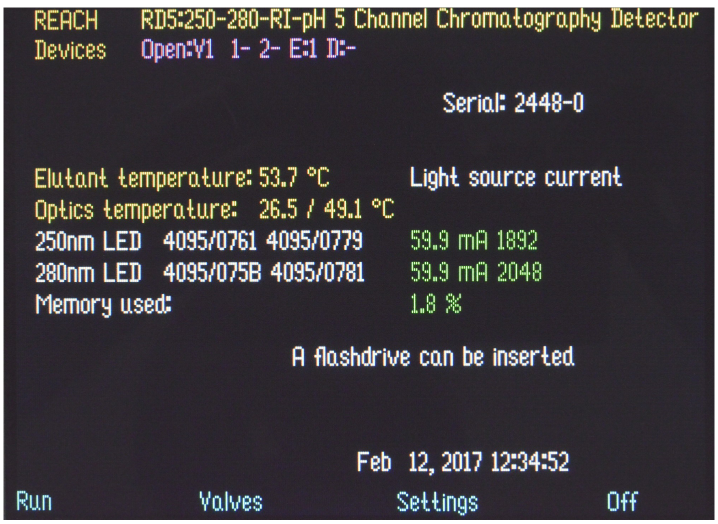
For example, in a gravity setup where the top of the elutant level was 18” (~46cm) above the collection vessel and supplied 1/8” tubing was used resulted is approximately 22mL/min flow rate of water at 25 °C. This value was obtained for an empty column. Column fill will further decrease the maximum flow rate.

7.3. Welcome Screen

After the detector is powered on, the Welcome Screen will display the following information:

1. **Device serial number**. This will also be the root directory name where detector will store the data files on a flash drive.
2. **Currents through the light sources**. The detector runs each UV light source (250 nm UV LED and 280 nm UV LED) so that the light output is constant. As the light source ages (or ambient temperature increases), more current is needed to maintain a set light intensity.
3. **Elutant temperature**, in ºC.
4. **Optical block / receiver photodiode temperatures**, in ºC.
5. **Memory usage**, %.
6. **Current date/time**. which should be set by the user from "Settings" menu. The correct date/time is essential, because is used to automatically create a correct directory tree on a flash drive without user intervention.
7. **Flash drive presence/absence**.
8. **Legends over four soft keys:**
9. If a flash drive is not inserted, the legends are: "**Run**" "**Valves**" "**Settings**" and "**Off**".
10. If a flash drive is inserted, the legends are: "**Run**" "**Valves**" "**Save data**" and "**Off**".

Pressing soft keys at the Welcome Screen menu will accomplish the following:



"**Run**" – on pressing this softkey, the detector switches to the acquisition mode, see Chapter 7.3 for details.

"**Valves**" – on pressing this softkey the detector will open the Event Editing screen, see Chapter 8 for details.

"**Settings**" – this function is available only when a flash drive is not inserted. See Chapter 7.4 for details

"**Save data**" – this function is available only when a flash drive is inserted.

The "**Save data**" softkey allows for transfer of the data accumulated within the non-volatile memory onto a flash drive. Pressing the "Save data" softkey will automatically create a directory tree:

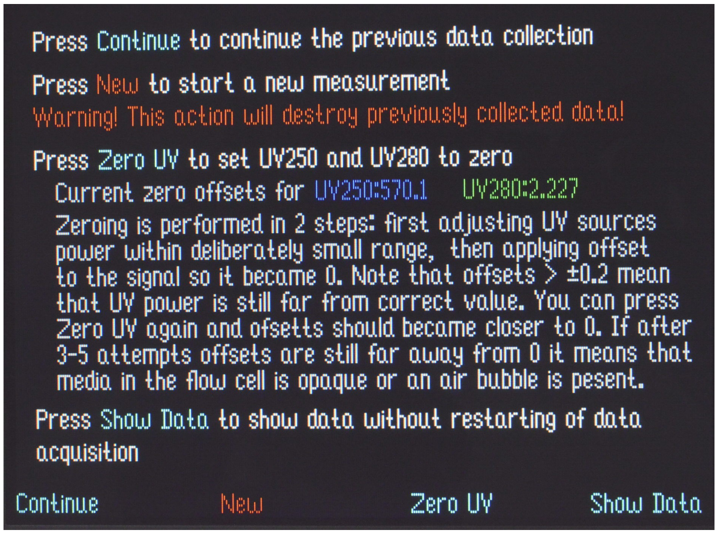
UnitSerialNumber/Year/Month/Day/ on the inserted flash drive. The directory's name reflects the date of the data transfer. The data file is saved to this directory with a name reflecting the time of the data transfer in the 24-hour notation: **XXhXXm.txt**.

For example, if the detector's serial number is 1503-0-1234, and a data file is transferred to a flash drive at 10:43 a.m. on May 17, 2014 – a directory named "1503-0-1234/2014/MAY/17/" will be created on the flash drive, and a data file named "10h43m.txt" will be recorded to this directory. If another data file were to be saved to the same flash drive at 3:18 p.m on the same day – it will be named "15h18m.txt" and saved to the same directory.

Note that the time and date are those of the moment of the data transfer, not of the moment when the data collection had been started. This eliminates the possibility of data file overrides. Obviously, the real time clock must be set correctly. For more details about the data file see Chapter 9.

7.4. Detailed description of Acquisition Mode

7.4.1. Acquisition intermediate screen



Acquisition mode starts when the "**Run**" softkey is pressed, leading to the intermediate screen being opened. Legends change to: "**Continue**", "**New**" ,"**Zero UV**" and ,"**Show Data**" . Pressing soft keys will accomplish the following:

"**Continue**" – the detector switches to the acquisition mode. Any **previously-collected data is recalled** from non-volatile memory, all further collected information is added to the already-existing data set. This cannot be carried out if the non-volatile memory is full.

"**New**" – the detector will opens the Acquisition Screen and begin data recording. Any **previously-collected data is deleted** from the detector's non-volatile memory (but NOT from the flash drive). Data acquisition will continue until it is suspended by the user, or until the data memory is filled to maximum capacity (7 hours to 15 days depending on the acquisition rate setting). In the latter case, a message in red "Data memory is full" will appear, and the unit will not save any more data.

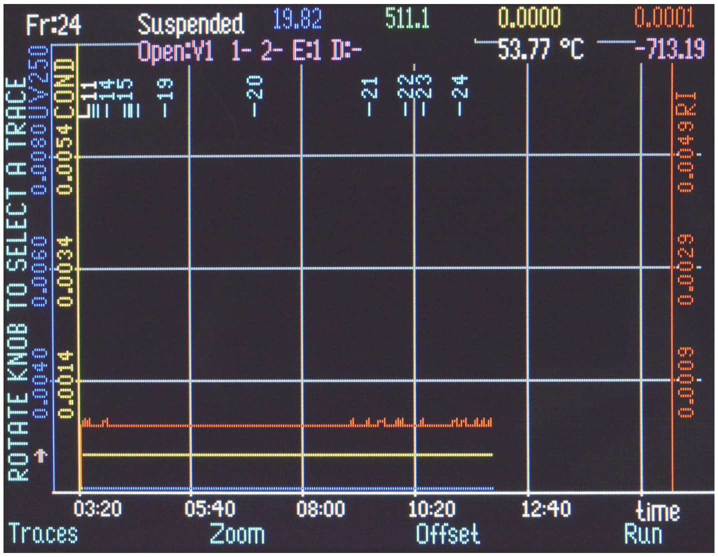
"**Zero UV**" – a UV zeroing process will start which firstly attempts to adjust UV power so that UV readings became close to zero (within ± 0.004 AU) and then calculate residual offsets to be added to raw reading so actual zero value is obtained. If the process was successful then residual offsets (which are shown on the screen) should be small (±0.02 AU or less). Larger values of the residual offsets means that RDn was not able to fully adjust UV power. Sometimes one would need to press "**Zero UV**" again, but in most cases **large residual offsets mean that an air bubble is stuck in the flow cell or the media is opaque for UV light**. One would need to clarify these conditions first before further pressing "**Zero UV**" for the third time. **Warning: persistent attempts to zero on an UV opaque media will cause UV LED currents to raise to unnecessarily high level, shortening their lifespan and eventually leading to the photolysis of the media. Depending on the media, photolysis may cause a permanent deposit of dark material on the windows. This may require sending the detector back for cleaning or even UV LED replacement.**

"**Show data**" – this function is similar to "**Continue**" but the unit will be in suspended mode (not collecting a new data). This allow a user to have a detailed look on collected data without continued data collection by the detector.

7.4.2. Acquisition screen

Acquisition Screen displays at least one and up to four traces with corresponding vertical axes in different colors. This screen also displays fraction collector (or valve) tick marks with fraction (or valve) numbers. The legends over the four soft keys are: "**Traces**" "**Zoom**" "**Offset**" and "**Suspend**". The acquisition can be suspended and resumed by pressing briefly on the rightmost softkey. The running state is conveyed at the top left corner of the screen by an advancing run-time clock while suspended sate is indicated by "Suspended" message instead of the running clock. Both of these messages are presented in white font.

The function of the turn knob is displayed in cyan colored font as a vertically oriented message on the far left of the screen. When no softkey is pressed the knob will move a red pointer (arrow) to select a value to be edited. The pointer can be set to five different values: up to 4 vertical axes and a common horizontal axis. The desired value can be edited by firstly moving a pointer to it, pressing "**Zoom**" or "**Offset**" softkey and further rotating the knob while keeping the softkey depressed until a desired change is effected. Please note that editing a vertical axis is only possible when the corresponding Auto scaling option is set to "off" (see below)



The color scheme is usually as follows:

UV 250 nm trace is presented in deep blue, the corresponding **Y-axis** is located on far left of the screen. Data is presented in Absorption units (AU) at 0.0001AU resolution.

UV 280 nm trace is presented in green, the corresponding **Y-axis** is located somewhere on the left of the screen. Data is presented in AU at 0.0001AU resolution.

E trace (Conductivity) is presented in brown color, the corresponding **Y axis** can be located on the left or on the right side of the screen. Data is presented in S/m at 0.0001 resolution.

RI trace (refractive index) is presented in red, the corresponding **Y-axis** can be located on the left or on the right side of the screen. Data is presented at 0.0001 resolution in user-defined units (See Chapter 7.4.2). Note that the refractive index reading is taken at 280 nm, not at 589 nm (sodium D-line), which is the standard for RI values.

pH trace is presented in magenta color. Data is presented in user-calibrated units (usually pH) at 0.01 resolution.

TEMP trace (elutant temperature) is presented in white color. Data is presented in ºC at 0.01 resolution.

In acquisition mode, up to eight current numerical values are displayed at the top of the screen, left to right:

- Fraction Number, received from a fraction collector (Value will be 0 if no fraction collector is used or no fraction change was detected)
- acquisition time (white font, XX hours XX minutes, XX seconds format)
- optical density at 250 nm (deep blue font, in AU)
- optical density at 280 nm (green font, in AU)
- conductivity (brown font, in S/m) and temperature right below it (white font, ºC)
- refractive index (red font, user units) and pH value right below it (Magenta font, user units)

Fraction collector/valve number tick mark are shown below these numerical values. All tick marks, except the first one, are in cyan color and indicate where the fraction change (or open valve change) have occurred. If screen space permits, a fraction number (or open valve number) is shown right above the tick mark.

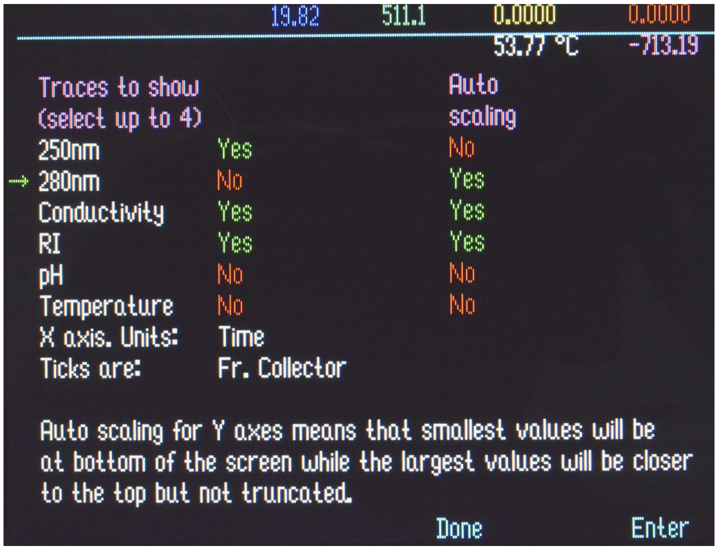
The first tick mark is in white color and always located on the left of the graphing area. The number above this tick mark shows which fraction number (or valve number) corresponds to the leftmost points of all graphs on the screen. Note, that if the X axis does not start from zero, the actual fraction change (valve change) event might have happened BEFORE this mark.

Pressing soft keys at the Acquisition Screen menu will accomplish the following:

"**Traces**" – this softkey opens Trace Interface Screen. Softkey legends change to "**Done**" and "**Enter**". Data acquisition and logging will continue (unless RDn was in "Suspended" mode")

By rotating a knob a trace visibility, auto scaling parameter, X axis units or type of tick marks can be selected. The selection is marked by a green pointer (arrow). Pressing "**Enter**" will toggle value between "Yes" or "No" (Except for X axis units, see below). Pressing "**Done**" will return to acquisition screen.

This menu will require that at least one but no more then four traces are selected. So it will be impossible to toggle the visibility of the last trace to "**No**" if all the others are set to "**No**". Similarly, it will be impossible to set trace visibility to "**Yes**" if already 4 traces are set to be visible. In this case, the user needs to turn off one trace before turning another trace on.



The auto scaling parameter has the following meaning:

"**Yes**" – the Y zoom and Y offset will be automatically adjusted so that all of the graph is in the viewing area

"**No**" – the user is in charge of vertical zoom and offset. New data points may land above or below of the viewing area and the graph will be truncated accordingly.

Note that X axis auto scaling cannot be turned off. If RDn is not suspended, the detector will always adjust X zoom so X coordinate of a new data point is within the viewing area.

To change X axis units, the user needs to set pointer onto "X axis units" by rotating the knob and then press "**Enter**". Softkey legend will change to "**Faster**" (this function is inactive in this case) and "**Accept**". Now rotating the knob will allow to choose between several options. The basic options are

- **time** – actual elution time in easily understandable format. For example, "04:33" label corresponds to 4 minutes and 33 seconds. The label "2h15:22" corresponds to 2 hours 15 minutes and 22 seconds.
- **volume** – effluent volume, calculated from the flow rate entered by the user on the Settings Screen and actual elution time

Some other options may be available in different RDn configurations. Pressing the "**Accept**" softkey will select the desired value and switch legends back to "**Done**" and "**Enter**". Again, pressing "**Done**" returns to the acquisition screen.

"**Zoom**" – this softkey allows for the change of the X or Y scale magnification and the position of traces. For vertical axes, it is only operational when the corresponding auto scaling parameter is set to "**No**" (see above). For the X axis, the user can adjust zoom within a certain range, as long as the next data point's X coordinate lands within viewing area.

If all auto scaling parameters are set to "**Yes**", RDn will "compact" the X and Y scales continuously so that ALL collected data is shown on the screen. After enough data is collected, it may seem that traces are "frozen" and not progressing any more. The same phenomenon occurs when someone is looking at hours hand of a clock – it seems to not be moving. This is a perfectly normal situation. However, if needed, one may have a better look at the current data behavior. Just select X axis by rotating the knob until the red arrow points to X the axis. Holding down the "**Offset**" softkey and rotating the knob counterclockwise will allow the user to shift all graphs to the left until only a small portion of them remains visible. Then, the user can zoom in by releasing the "**Offset**" softkey, and then pressing "**Zoom**" and again rotating the knob counterclockwise while "**Zoom**" is held down. This will expand the last portion of the X axis as needed.

Note that if acquisition was not suspended and a newly acquired point happens to be outside of the screen area, the detector will automatically readjust the X zoom in order to bring the new point back onto the screen area. This feature is specifically implemented to avoid a situation when the detector is running but the X zoom and/or offset are set by user in such a way that new data points would end up to the left or to the right of the screen area and could not be shown.

It should be remembered that, during acquisition, the data from all channels is continuously written to the non-volatile memory. Manipulating "**Traces**", "**Zoom**" or "**Offset**" functions has no effect on this process. So, for instance, if 280 nm trace is off, the unit still collects 280 nm data points. These data points may be observed if 280 nm trace is turned on again. Similarly, changing zoom does not change the unit sensitivity or affect acquisition in any way. Modifying zoom just changes how the graphs are presented to the user on the detector screen.

“**Suspend**” – this softkey allows temporary or permanent suspension of the acquisition. One **brief** press of this softkey will temporarily suspend the data collection; a second press will resume the acquisition. A **prolonged** pressing of “Suspend” softkey will stop the acquisition and switch the detector to the Welcome Screen (Chapter 7.2) from where data can be saved onto a flash drive, a new acquisition can be started or the previous acquisition can be resumed.

7.5. Detailed description of Settings Menu

When the “**Settings**” softkey is pressed from the Welcome Screen, several menu items appear, and the softkey's legends change to the following: ”**Done**” and “**Enter**”. The contents of this screen can vary greatly depending on RDn configuration. Regardless of the menu's contents, the navigation through it is fairly simple. First, select an item of interest by rotating a knob. The green arrow will indicate which item is selected. Then, pressing “**Enter**” will allow to interact with the chosen menu item. Pressing ”**Done**” will save changes, exit the menu, and return to the welcome screen.

After pressing “**Enter**”, a menu item can behave in several different ways:

1. by offering a list of fixed values. Please selected the desired one by rotating knob, then press “**Accept**”. This action will set the selected value and return to the menu screen. Example: “**Acquisition rate:**” will offer **0.2s**, **0.5s**, **1s**, **2s**, **5s** and **10s** values.
2. by offering an integer value confined to a range. Simple rotation of the knob will allow the user to change the value within the allowed limits. Again, press “**Accept**” to set the value and return to the menu
3. by offering a signed floating point value. To arrive to the desired value quicker, the user may need to hold down the “**Faster**” softkey while rotating the knob, which will increase the speed at which the knob rotation affects the floating point value. Please note, these values are not confined and so can be erroneously set to absurdly high or low values. Example: “**Flow rate, L/min:**” could be set to a negative value, which is not recommended
4. by opening its own unique screen with its own rules. Example “**Conductivity Calibration**” will open “Editing the calibration chart for conductivity” screen. Some of these entries are described in this manual, while some of them are mostly for debugging purposes and so should not to be changed.
5. by opening a nested menu (a menu within a menu). Example: “**Set Date/Time**” will open its own menu which contains “***Year:***”, “***Month:***”, “***Day:***”,”***Hour:***”,”***Minute:***” and ”***Second***” items all of which will behave as described in way 2. To avoid unnecessary complexity the nesting depth is limited to level 2 so the situation “a menu within a menu within a menu” (or deeper) will not happen.

7.5.1. Acquisition rate, data presentation lag and length of the run

Technically, accepted units for acquisition rate are Hertz, but in chromatography the X axis of a plot is typically presented in units of time. So it is convenient to set acquisition rate in seconds (which is reciprocal to Hertz). This way the acquisition rate simply means the distance on X axis between the adjacent datapoints collected by RDn.

Maximum Length of a Run vs Acquisition Rate

Acquisition rate in seconds	rate in Hz	Maximum length of a run	
		RD1 and RD2	RD4 and up
0.2	5	7 hours 16 min	10 hours 35min
0.5	2	18 hours 12 min	26 hours 28min
1	1	36 hours	52 hours
2	0.5	72 hours	4 days 9 hours
5	0.2	7 days 14 hours	11 days
10	0.1	15 days	22 days

RDn has an data presentation lag (delay) which is less or equal to the set acquisition rate. For example, if acquisition rate is set to 5 seconds then the sharp change in the elutant composition will be presented to the user after at most 5 seconds after it had happened (worst case scenario).

There are 3 things to consider when setting the acquisition rate:

1. Expected length of the run. Smaller value for the acquisition rate fills the memory faster. Some protein separations lasts days, so it is a good practice to have some healthy safety margin in terms of maximum length of a run (see table)
2. Sharpness of peaks. If one need to document rapidly changing elutant compositions, a smaller value for the acquisition rate will be needed. This is especially important in switched flow SMB applications
3. Signal to noise ratio. This is important only for RI measurements as RI will become less noisy with a larger value for the acquisition rate

7.5.2. Flow rate

The flow rate value in the "**Settings**" menu is just a number from which the X units will be calculated if user switches the X axis to "Volume" (See Chapter). It is an optional parameter which can be set by user to the actual value. RDn does not measure or automatically limit flow rate of elutant.

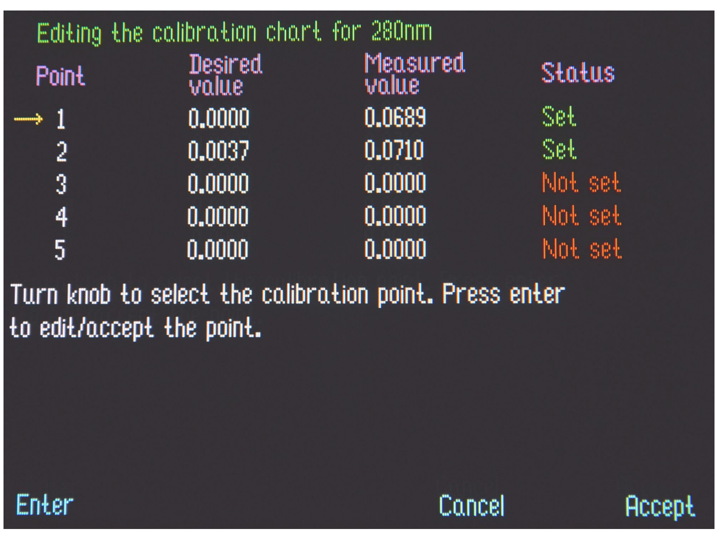
7.5.3. Pin1 is:

This menu item assigns which channel (one of the UV channels, Conductivity, Refractive Index or pH but NOT temperature) is linked to **Pin 1** of the interface connector socket. It is useful when a fraction collector or other external system to be connected to the detector has only one analog input. This way any of channels (except temperature) can be attached to the same input without a cable change.

7.5.4. 250nm or 280nm Calibration

These menu items open their own screens, where user can OPTIONALLY calibrate the presentation of the UV signals. Beer Law (see Appendix 1 for more details) manifests a linear relationship between the concentration of a UV absorbing compound and the absorption of the solution of this compound in a UV-transparent solvent. However, it is usually not applicable to concentrated (over 100 mM) solutions of UV-absorbing species. Therefore, absorption values observed by the RDn detector follow the following rules:

- for up to 0.1 M concentration of *any* UV absorbing compound in a UV-transparent solvent, the exact concentration of this compound can be calculated from the observed absorption value and the extinction coefficient of this compound.
- for over 0.1 M concentration of UV-absorbing species, increases in concentrations will correspond to increases in absorption, but linearity will not necessarily be preserved.



The hard-coded internal calibration table ensures that the detector has a linear response for up to 10 M acetone. If a linear response for high concentrations of another UV absorbing compound is needed, the detector may be re-calibrated using high-concentration standards of this compound as described below. Note that, outside of the user-created calibration table, the hard-coded internal calibration values will take precedence. The hard-coded table is never deleted, but simply bypassed. If the user wants to return to the factory calibration, he simply needs to delete his/her calibration values.

The UV "**280nm**" Chart Screen is shown on the left. "Measured values" correspond to the absorption values from **the hard-coded table**. "Desired values" are the absorption values that can be set by user.

It is important to understand that the “desired values” do not necessarily mean “correct” values.

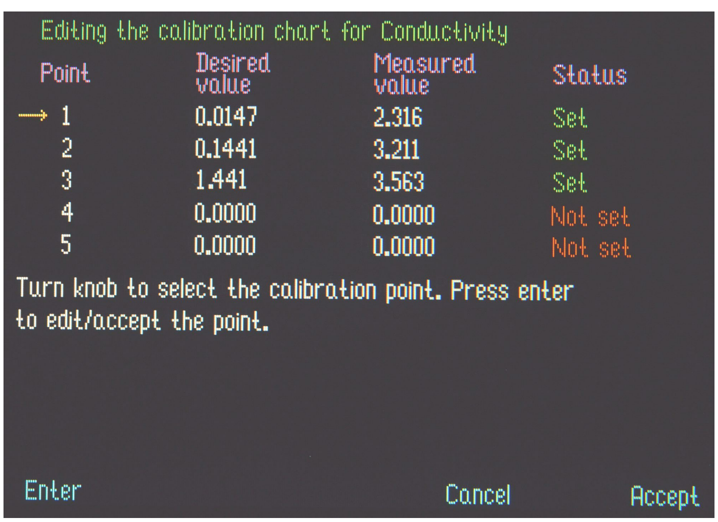
For example, imagine that your separation procedure calls for collection of four fractions of a UV absorbing product X: 10 to 50%, 50 to 70%; 70 to 50% and 50 to 35%.

For your convenience you may calibrate the detector using four solutions of X, and assign 10% of X a “desired” absorption of 1 AU, 50% of X a “desired” absorption of 5 AU, 70% of X a “desired” absorption of 7 AU, and 35% of X a “desired” absorption of 3.5 AU.

280nm Calibration procedure example:

1. Before any manipulations on the Chart Screen, at least two (maximum five) of **degassed** calibrating solutions of known UV absorptions must be purchased or prepared. The internal detector's calculations are done in a linear fashion, therefore at least two points need to be set to establish a meaningful outcome. However, if so desired, up to five calibration points can be set.
2. Press the “**280nm**” softkey from Chart Screen. This opens the UV 280nm Chart Screen, and the softkeys' legends change to: “**Enter**”, “**Cancel**” and “**Accept**”.
3. Select a calibration point by rotating the knob, which will move the yellow arrow up or down the calibration table. After the point is chosen, press the “**Enter**” softkey. The softkey's legends will change to “**Faster**”, “**Set**”, “**Cancel**” and “**Delete**”. Now, the chosen calibration point can be “set” (edited) or “deleted” (cleared).
4. Enter the “Desired value” of the calibration point – the known absorption value of the first calibration solution. This is accomplished by knob rotation. Faster knob rotation will cause exponentially faster value change. If this is still not fast enough, then the “Faster” softkey may be pressed simultaneously while the knob is rotated.
5. Run the calibration solution exhibiting the “Desired value” of absorption through the flow cell for at least 1 minute, at a flow rate of 20 ml/min or more. Afterwards, press the “**Set**” softkey. At this moment, a new “Measured value” corresponding to the previously entered “Desired value” will be recorded in the detector's memory, and will appear in the calibration table. The legends over the softkeys will change back to “**Enter**”, “**Cancel**” and “**Accept**”, thus allowing you to chose the next calibration point by pressing the “**Enter**” softkey.
6. Repeat the procedure for up to five **different** calibration solutions to establish up to five calibration points. The “set” calibration points do not need to be consecutive.
7. You **must** clear the unused calibration points. To do so, selecting each unused point with the yellow arrow, press “**Enter**”, then press “**Delete**”. The “Not set” message must appear next to these points.
8. To use the newly created calibration chart you **must** to press “**Accept**” softkey at the end of the procedure and follow the screen prompts.

7.5.5. Conductivity or RI calibration



The conductivity and RI channels of your detector have been factory-calibrated after its assembly. It is good practice to recalibrate these channels as needed to avoid accumulated errors due to electrode fouling, etc.

The aim of Conductivity and RI calibration is to transform raw numerical “Measured values” observed by detector's hardware into user-meaningful “Desired values” - for example numbers presented in S/m units for conductivity). The Conductivity Chart Screen presenting a typical factory calibrations of conductivity is shown below. Three solutions: 1, 10 and 100 mM aqueous KCl with known conductivities of 0.0147, 0.1441 and

1.441 S/m ("Desired values") were consequently run through the detector's flow cell. This resulted in raw numbers of 2.316, 3.211 and 3.536 (unitless "Measured values") were observed by detector's logarithmic amplifier. The calibration procedure relates the known conductivities to these raw numbers. Consequently, during a regular chromatographic run, this factory-recorded calibration table is used for calculating each conductivity value continuously presented on the detector's Acquisition Screen. Therefore, during a routine run, a user sees only the "Desired values".

It is important to understand that, as it has been described using an example of UV calibration, "desired values" do not necessarily mean "correct" values.

If desired, a user has the ability to re-calibrate the detector, however, a failure to accomplish the calibration procedure correctly will result in meaningless data collected by your detector during an actual run. To return to the previous calibration recorded in the detector's memory you may press "**Cancel**" soft key during any of the following steps.

Calibration procedure example: Conductivity

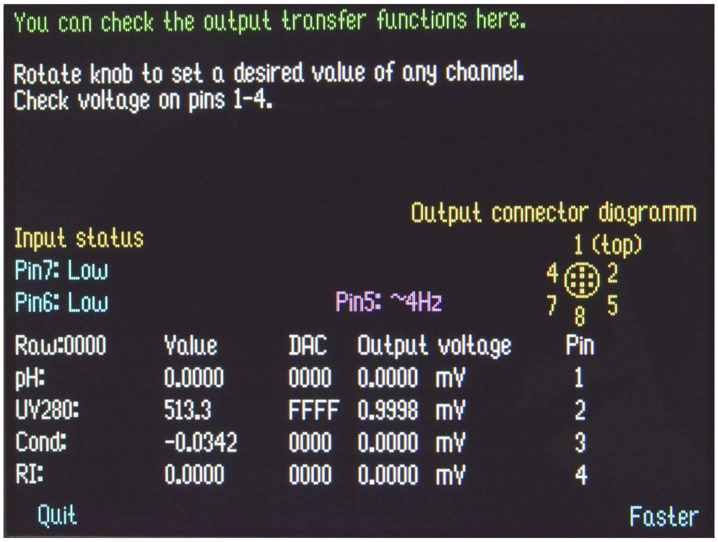
1. Before any manipulations on the Chart Screen, at least two (maximum five) of **degassed** calibrating solutions of known conductivities must be purchased or prepared. The internal detector's calculations are done in a linear fashion, therefore at least two points need to be set to establish a meaningful outcome. However, if so desired, up to five calibration points can be set.
2. Press the "**Conductivity**" softkey from Charts Screen. This opens the Conductivity Chart Screen, softkeys' legends change to: "**Enter**", "**Cancel**" and "**Accept**".
3. Select a calibration point by rotating the knob, which will move the yellow arrow up or down the calibration table. After the point is chosen, press the "**Enter**" softkey. The softkey's legends will change to "**Faster**", "**Set**", "**Cancel**" and "**Delete**". Now, the chosen calibration point can be "set" (edited) or "deleted" (cleared).
4. Enter the "Desired value" of the calibration point – the known conductivity value of the first calibration solution. This is accomplished by knob rotation. Faster knob rotation will cause exponentially faster value change. If this is still not fast enough, then the "Faster" softkey may be pressed simultaneously while the knob is rotated.
5. Run the calibration solution exhibiting the "Desired value" of conductivity through the flow cell for at least 1 minute, at a flow rate of 20 ml/min or more. Afterwards, press the "**Set**" softkey. At this moment, a new "Measured value" corresponding to the previously entered "Desired value" will be recorded in the detector's memory, and will appear in the calibration table. The legends over the softkeys will change back to "**Enter**", "**Cancel**" and "**Accept**", thus allowing you to chose the next calibration point by pressing the "**Enter**" softkey.
6. Repeat the procedure for up to five **different** calibration solutions to establish up to five calibration points. The "set" calibration points do not need to be consecutive.
7. You **must** clear the unused calibration points. To do so, selecting each unused point with the yellow arrow, press "**Enter**", then press "**Delete**". The "Not set" message must appear next to these points.
8. To use the newly created calibration chart you **must** to press "**Accept**" softkey at the end of the procedure and follow the screen prompts. After pressing "Accept" you will not be able to return to the previous calibration recorded in the detector's memory.

7.5.6. pH calibration

pH calibration must be done before each run. At least 2 buffers should be used. The pH electrode can be removed from the detector, so calibration can be done conveniently in small beakers. Temperature sensor is ignored during calibration (because it is located in the UV flow cell) so calibration should normally be conducted at 25^{o}C.

7.5.7. Output transfer functions

This menu item will open its own menu page where up to 5 pairs of floating point variables can be set. Each pair of these variables defines **linear relation** between observed value and physical voltage present in the output connector pins 1 to 4. For each channel desired to be utilized, the user can define the screen value which corresponds to 0V on a output pin and which screen value corresponds to 1V.



Analog outputs can only swing from 0V to +1V. The signals are generated by four 16-bit DACs so they have theoretically $1/(2^{16})V = 0.015mV$ resolution which in reality may be lower due to ambient electrical noise.

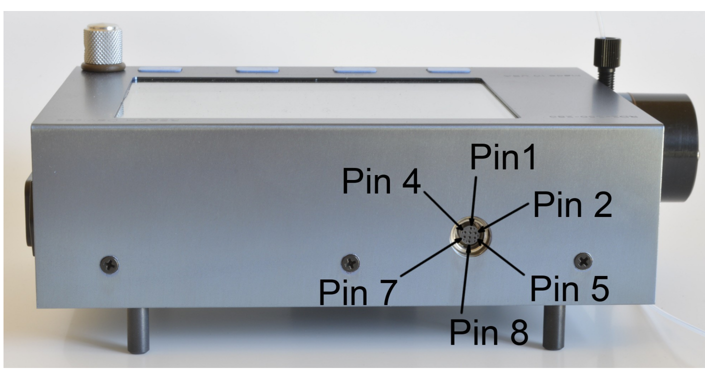
Analog outputs are galvanically isolated from the unit ground, which should eliminate the ground loop issue, but it is still impossible to transfer the entire range of the UV data without sacrificing resolution at lower AU, or vice-versa. For example, let's say that the user specifies that the value of 800 AU corresponds to 1V. Then, 0.0001 AU will correspond to 0.000125 mV. However, since the theoretical resolution of the analog output is 0.015 mV, the lowest AU range falls below this resolution power, and thus is lost, and the lowest resolved corresponding AU value will be 0.01AU. As a second, converse example, the user may set the 0.0001 AU value to correspond to the minimum resolved voltage of 0.015mV. However, then the largest transferrable value of 1V will correspond to a maximum of 6.7 AU.

Example of UV setting:
Pin1=0v UV250=0
Pin1=1v UV250=10

This transfer function will behave the following way

Current UV250 value	Pin1 voltage
<0	V=0
between 0 and 10	V=UV250 / 10
>10	V=1

7.5.8. Check Output transfer functions



This menu item opens its own unique screen. This advanced function should be of great help if troubleshooting of an attached device connection is needed.

On this screen one can find real time status of the Pin7 and Pin6. These are TTL-compatible inputs pulled up to +5V with 1k resistors (in the respect of Analog ground, Pin8).

A 4Hz square wave with $5V_{pp}$ amplitude will be present on Pin5.

Rotating knob will change the so-called “Raw” value. This is an artificial value, presented on the screen in hexadecimal format. It can be changed from 0000H to FFFFH (0 to 2^{16}) by rotation of a knob.

While is in this mode, RDn is not actually measuring anything real. Instead of real values collected by RDn from the elutant, the fake “raw” value become the “signal” being measured. All channels (except elutant temperature) receive the identical fake “raw” data.

In the case of any UV channel, this data will be transformed through a built-in UV linearization table, then through the user-defined UV calibration table (if defined). The value obtained is shown on the screen, and then further fed into the output transfer function. The produced results are shown on the screen as a hexadecimal value and a voltage value. This result is further fed into the output DAC, which causes the output voltage to appear on a corresponding pin.

In case of a non-UV channel the process is similar, except the UV linearization table is bypassed.

What is achieved is that one can scan through entire possible range of values just by rotating the knob and voltages on pins 1 to 4 will follow accordingly. This is a way to check an attached fraction collector's “peak detection” ability. By gently rotating knob back and forth while watching values on the screen one can trick a fraction collector that a peak is being detected.

7.6. Fraction collectors and other external equipment

7.6.1. General information and output transfer functions

The detector has an HR-25A 8-pin female socket (Hirose Electric Co Ltd, mating connector part number is HR25A-7P-8P), which provides the possibility to communicate with external hardware.

Pins 1-4 supply four galvanically isolated analog outputs (0 to 1V range, 10mA max) that are always active while the Acquisition Screen is displayed. See **Chapter 7.5.7**. above for output transfer functions.

Note that **Pin1** may be reconfigured in the Settings Screen (Chapter 7.5) to output any channel.The outputs are also active when “Suspended” message is shown on the left top corner of the Acquisition Screen and no data logging is occurring.

Pins 5 and 6 provide digital outputs that can be programmed by REACH Devices upon customer's request.

Pin 7 provides an input that has a 5K pull-up resistor to +5V. Open collector, open drain, TTL/CMOS (active low) or mechanical switch are all acceptable sources to be attached to **Pin7**. During data acquisition, a transition from logical 1 to 0 level (aka contact closure) is recorded to data memory as an external fraction number. This is implemented for faction collectors that produce an electrical pulse when they change the fraction. Note that only one transition per acquisition rate would be recorded. So, if the detector acquisition rate is set to 1 second (default), then fraction collector should not output more than 1 pulse per second.

Pin 8 is the detector ground.

7.6.2. Attaching fraction collectors

RDn can interface with virtually any model of a fraction collector. In general, depending on the collector's brand, two types of interface are possible.

1. **Event Marker / Tube change**: output on the faction collector, input on the detector. The detector 'listens' for a fraction change signal from the fraction collector, displays the actual number of the fraction on the X axis, and stores the fraction count in the output file. In this scenario, typically, the fraction collector fraction change event output should be connected to **Pin7** of the detector with a shielded cable.
2. **Analog output**: input on the faction collector, output on the detector. The detector supplies the fraction collector with an analog signal proportional to a channel output (UV250, UV280, Conductivity or RI) chosen by the user. The collector is programmed by the user to perform the tube change depending on the value of the data signal. In this scenario, typically, **Pin1** of the detector should be attached to the analog input of the fraction collector. It is very important to use a shielded cable.

Customers are encouraged to experiment with the actual connection pattern.

During the purchase, a customer may inform REACH Devices about the brand of the fraction collector he/she intends to be using with RDn. In this case, a connection cord with a connector fitting the particular fraction collector will be provided. If needed in the future, this cord can be cut and outfitted with a different connector to fit another fraction collector's brand. The wires are color coded as follow:

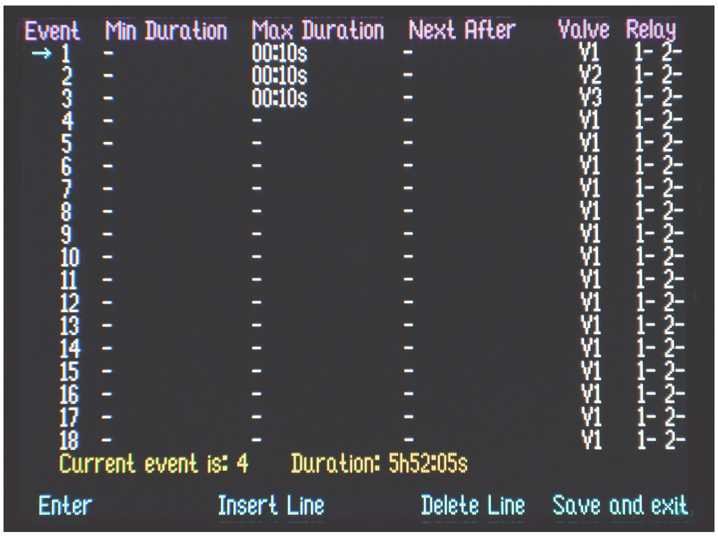
1	**Red** (Pin1 of the detector)	detector's analog output (Pin1), 0 to +1V range, may be assigned to any channel from the detector menu. *Maximum load:* +/- 20mA
2	**White** (Pin7 of the detector)	detector's digital input (Pin7). Pulled up to +5V via 5K resistor. Compatible with TTL, CMOS and mechanical switch. Active low.
3	**Black and / or bare wire**	Ground (Pin8)

After a fraction collector is attached, the connection can be easily tested from Calibration Screen as described in Chapter 7.4.2.1.

8. Setting up the valves

8.1. Overall description

Pressing "**Valves**" softkey from the Welcome Screen will open the Valves Menu.



The Valves Menu allows the user to set up a program controlling which valve is open on a given separation moment. Up to 16 valves can be requested during the detector's ordering.

The program consists of a sequence of up to fifty Events. Each Event is assigned by the user to a single valve being open. Therefore, each Event corresponds to a single valve, but several Events can be assigned to the same valve.

At the beginning of a new separation, the program will start from the Event 1. The Event 1 will expire once certain user-set conditions are met. On the expiration of the Event 1, the Event 2 will became active, and so on until an Empty Event (an Event with no conditions) or the Event 50 is reached. At this moment the program will stall indefinitely.

The user-set conditions may be of three types.

1. Time Conditions – user specifies the time for each valve to be opened.
2. Effluent Properties Conditions – user specifies an absorption, or conductivity, or RI value to be reached for the Event to expire.
3. Double-type Conditions – user specifies an Effluent Properties Condition, **but** a certain user-specified time **must** elapse before this conditions is checked by the detector's software.

Below are examples of valve programming.

Example One. Simple time-based collection (conditions type 1 only):

1. The effluent is routed to a waste vessel through the Valve 1 for the first **1 hour 05 minutes** after the beginning of the separation
2. For the next **14 minutes 30 seconds**, the first useful fraction is collected in a Vessel A through the Valve 2.
3. Afterward, the solution is again routed to the waste vessel through the Valve 1 for **18 minutes**.
4. For the following **7 minutes**, the second useful fraction is collected in a Vessel B through the Valve 3.
5. Afterward, the solution is again routed to the waste vessel through the Valve 1 indefinitely.

Event	Min Duration	Max Duration	Next After	Valve	Relay
1	-	1h05:00s	-	V1	1- 2-
2	-	14:30s	-	V2	1- 2-
3	-	18:00s	-	V1	1- 2-
4	-	07:00s	-	V3	1- 2-
5	-	-	-	V1	1- 2-

Example Two. Effluent properties and time-based collection (conditions type 1 and type 2 only):

1. The effluent is routed to a waste vessel through the Valve 1 until absorption at 280 nm rises to **3.9 AU**.
2. The first useful fraction is collected in a Vessel A through the Valve 2 until absorption at 280 nm drops below **0.54 AU**.
3. Afterward, an intermediate fraction is collected to a Vessel B through the Valve 3 for **8 minutes 40 seconds**.
4. Afterward, the solution is again routed to the waste vessel through Valve 1 indefinitely.

Event	Min Duration	Max Duration	Next After	Valve	Relay
1	-	-	UV280 < 3.900	V1	1- 2-
2	-	-	UV280 > 0.5400	V2	1- 2-
3	-	08:40s	-	V3	1- 2-
4	-	-	-	V1	1- 2-

Example Three. Double-type Conditions (conditions type 3 only):

1. Initially, the effluent is routed to a waste vessel through the Valve 1. The first fraction to be collected begins after absorption at 250 nm reaches **5 AU**. However, the user knows from previous experience that this can not happen sooner than about **10 minutes** after the beginning of the separation.
2. The user wants to keep collecting the product into a Vessel A through the Valve 2 until the absorption at 280 nm drops below **1.4 AU**. However, the collection can not last for more than **35 minutes**, because after 30 minutes the Vessel A will overflow.
3. Afterward, the solution is again routed to the waste vessel through Valve 1 indefinitely.

Event	Min Duration	Max Duration	Next After	Valve	Relay
1	10:00s	-	UV250 < 5.000	V1	1- 2-
2	-	35:00s	UV280 > 1.400	V2	1- 2-
3	-	-	-	V1	1- 2-

In this case, setting of Double-type Conditions prevents the situation when a spurious air bubble, or a solid particle, or a line power glitch would cause a short (a second or two) spike of absorption over 5 AU, and the program advances to the next event collecting the waste solution into Vessel A.

8.2. Editing the Valve Table

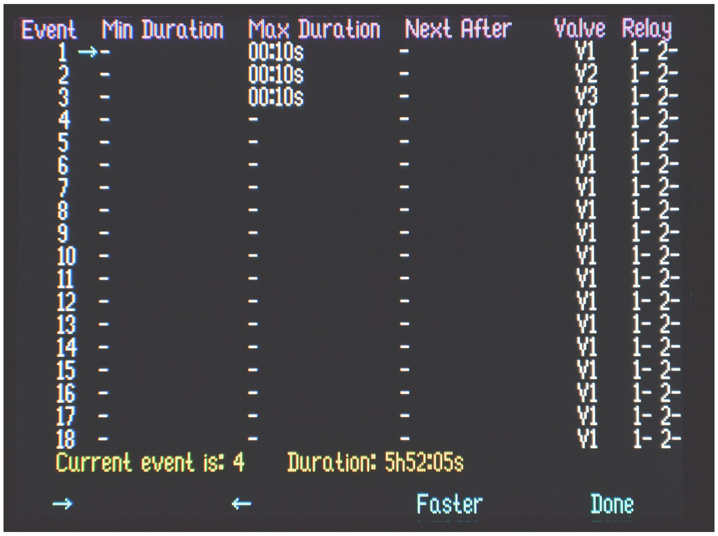
Once in the Valve Menu, select an Event by rotating the knob, which will move the yellow arrow up or down the Event Table. Moving the pointer beyond the bottom of the screen will scroll up revealing more Events.

When the arrow points to the white-font Event row, the softkeys have the following meanings:

"**Insert Line**" – pressing this softkey will insert a new blank Event line at the pointer, while the very last (fiftieth) line will be deleted, line numbering will be kept consecutive.

"**Delete Line**" – pressing this softkey will remove the line at the pointer, a blank fiftieth line will be added at the very end on the table.

"**Save and exit**" – pressing this softkey will save the Event Table to the detector's permanent memory and exit to the Welcome Screen.



"**Enter**" – pressing this softkey will change the softkey's legends to "→", "←", "**Faster**" and "**Done**". The pointer will jump in to the next column (Min Duration) of the table. Now, Min Duration can be edited by rotating the knob. Faster rotation will cause faster value change. Holding down the "**Faster**" softkey while rotating the knob, will change the value even more rapidly. Pressing "→" or "←"allows the user to move horizontally along the Event line. Once the line is edited, pressing "**Done**" will return the user to the Valves Menu, where the next Event may be chosen for editing.

When the arrow points to the yellow font special row, only the "**Enter**" softkey may be pressed.

While the Event Table is edited by the user before the beginning of the separation, the special yellow row will read "**E Current event is: 1 Duration 00:00s**". Pressing the "**Enter**" softkey at this time is meaningless.

Only after the actual separation is started, and the automatic events count is underway, the special line may be used.

While the separation is running, a magenta line "**Valve:X Event:Y Duration mm:ss**" is always shown on the Acquisition Screen, informing the user about the Event Table progress. The user can skip an Event(s) or return to a previous Event(s) to be repeated. To do so, the user needs to execute the following:

- suspend the acquisition
- go to Valves Menu, navigate to the special yellow line and press the "**Enter**" softkey
- change the current event number by rotating the knob. The **Duration** will be automatically set to 00:00. Optionally, the user may now edit the Event Table.
- press "**Done**" to return to the Valves Menu
- press "**Save and exit**" to save the edited Event Table and to exit to the Welcome Screen
- press "**Run**", then press "**Continue**" to continue the acquisition.

8.3. Optional relays

Upon user request, optional relays 250 VAC, 4 A can be installed. If the relays are installed, an operator "GO TO EVENT #N will be available to allow for process control loops. The relays are controlled by the last column in the Valve Menu. Notations are as follows.
Both relays are OFF: 1- 2- ; Relay 1 is ON, Relay 2 is OFF: 1+ 2- .

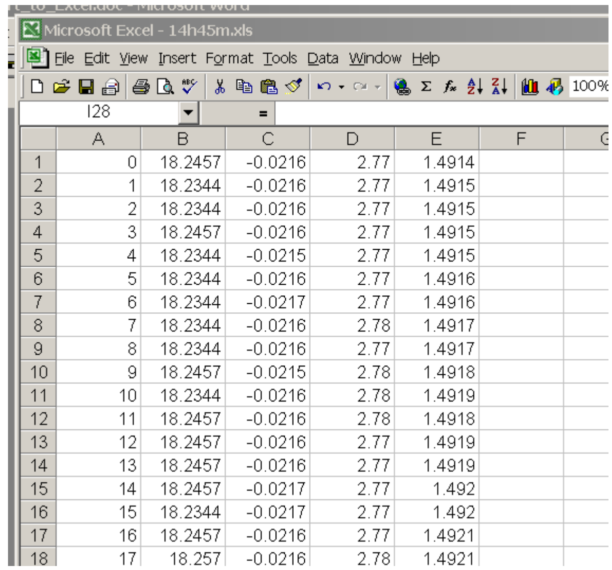
9. Import of data

The collected data can be recorded onto a flash drive as a text data file consisting of multiple strings of six space-separated numbers. A carriage return sequence (0DH, 0AH Microsoft style) concludes each string of data.

1. The first number depends on X axis units that are currently selected (Chapter 7.3). If X units are set to **time** then the first number represents amount of seconds passed since the acquisition started. If X units are set to **mL** then the first number is the volume of elutant calculated from Flow rate (Settings/Parameters menu) and actual elution time. Finally, if X units are set to **N** then the first number is meaningless.
2. The second number is the optical density at 250 nm in Absorption Units (AU), -2.0000 to 120.0 range.
3. The third number is the optical density at 280 nm in Absorption Units (AU), -2.0000 to 120.0 range,

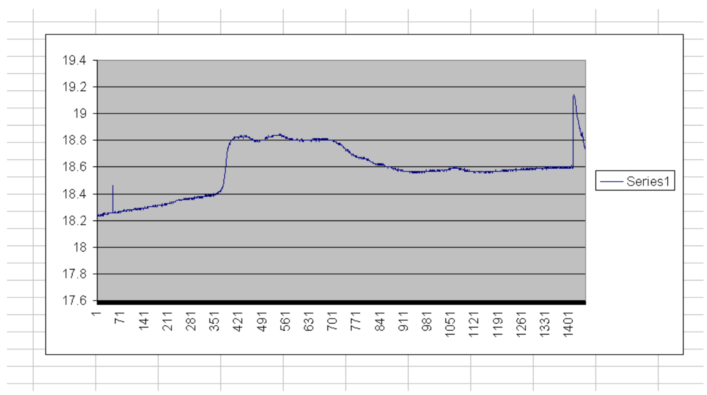
4. The forth number is the Refractive Index at 280 nm, unitless, 1.0000 to 3.0000 range.
5. The fifth number is the Conductivity in S/m, 0.0000 to 50.00 range.
6. The last number is the external fraction number, an integer in 0 to 65535 range. This value is only meaningful if a fraction collector was attached during the run.
7. The last number is the valve being open during this time.

9.1. Import to Excel



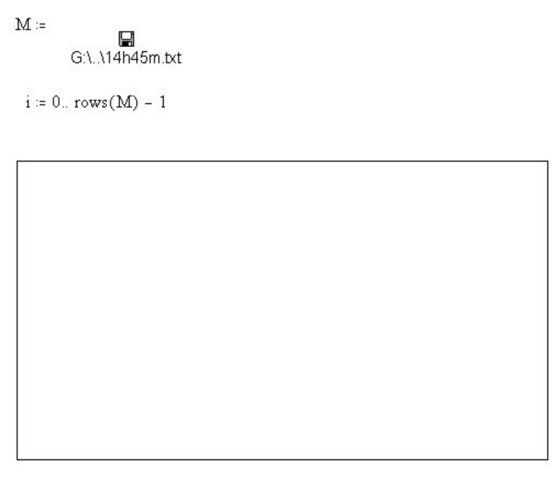
	A	B	C	D	E	F
1	0	18.2457	-0.0216	2.77	1.4914	
2	1	18.2344	-0.0216	2.77	1.4915	
3	2	18.2344	-0.0216	2.77	1.4915	
4	3	18.2457	-0.0216	2.77	1.4915	
5	4	18.2344	-0.0215	2.77	1.4915	
6	5	18.2344	-0.0216	2.77	1.4916	
7	6	18.2344	-0.0217	2.77	1.4916	
8	7	18.2344	-0.0216	2.78	1.4917	
9	8	18.2344	-0.0216	2.77	1.4917	
10	9	18.2457	-0.0215	2.78	1.4918	
11	10	18.2344	-0.0216	2.78	1.4919	
12	11	18.2457	-0.0216	2.78	1.4918	
13	12	18.2457	-0.0216	2.77	1.4919	
14	13	18.2457	-0.0216	2.77	1.4919	
15	14	18.2457	-0.0217	2.77	1.492	
16	15	18.2344	-0.0217	2.77	1.492	
17	16	18.2457	-0.0216	2.77	1.4921	
18	17	18.257	-0.0216	2.78	1.4921	

1. In Excel, go to **File** → **Open** in the top menu (alternatively, press Ctrl+O).
2. Select **Text files** (*.prn *.txt *.csv) file type from the file type sub-menu.
3. In the file list, select the file you wish to process, and click "**Open**".
4. On next screen chose "**Delimited** – Characters such as comma or tabs separates each field".
5. On the next screen, make sure "**Space**" as delimiter character is selected (box is checked), and click "**Finish**" . Now the file is imported to Excel and should look like this:
6. Select a column (column **A** is time in seconds, column **B** is absorption at 250 nm, **C** is absorption at 280 nm, **D** conductivity **E** is RI and **F** is open valve number) by clicking the column's letter at the top of the column.
7. Click: **Insert** → **Chart** → **Line** and click through subsequent screens (if any) to get the graph printed. In the later versions of excel, click "**Insert**" and select the chart type (we recommend **Plain line** charts). The default result looks like this (column **B** was selected):



Of course, the user should adjust axes, legends, color, etc. as usual.

9.2. Import to MathCAD



In MathCAD, start a new file first. Then go to

Insert → Component → File read or write → Read from a data source

Choose **File format** as **Text files** and then locate and click on the desired data file.A placeholder, similar to the one shown om the right, will appear in MathCAD window.

The user needs to type a matrix name (say letter M) instead of the red rectangle.

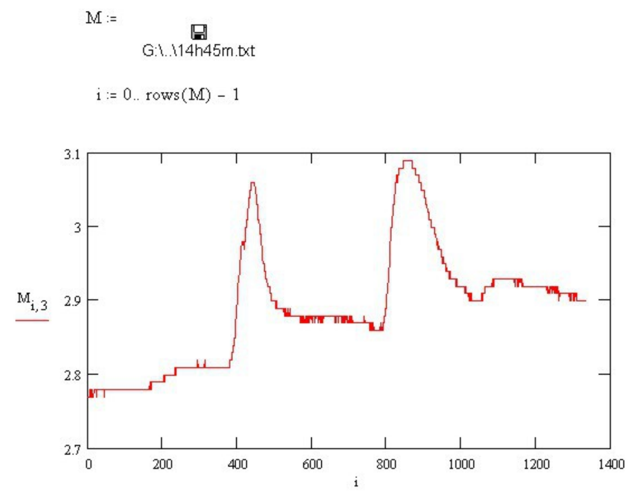
Below the matrix name, a component range expression should be placed by typing: **i:0;rows(M)-1**

Below the range expression, a 2D graph should be started by typing Shift+@ combination.

The content that should appear in MathCAD window is shown on the right.

The graph can be recalled by typing **i** instead of lower black rectangle and **M[i,1** (for 250nm graph; **M[i,2** for 280nm graph, **M[i,3** for conductivity graph, **M[i,4** for the RI graph and **M[i,5** for valve graph) instead of left black rectangle.

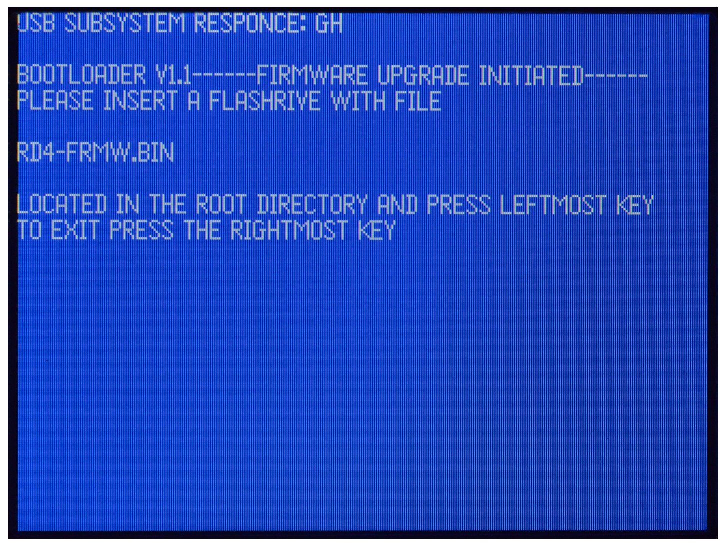
If automatic calculation is not enabled then **F9** key (or **Math → Calculate workshee**t menu) should be pressed, which will populate the graph area as is shown on the right image above. Again, the user can adjust axes, legends, color, etc. as usual.



10. Firmware download

If necessary, the firmware of RDn may be updated by the user in the field. To obtain a new firmware file RD4-FRMW.BIN, please contact REACH support by E-Mail. The file can then be downloaded to the detector from a flash drive. The following rules must be observed:

- the flash drive is FAT32 formatted, is not in 'write protect' mode and has at least 300Kb of free space
- a file RD4-FRMW.BIN is present in the root directory
- a file RD4-FRMW.OLD is not present in the root directory



To prevent any incompatibility issues, old firmware will be automatically uploaded to the flash drive, so the user can revert back if needed.

Firmware upload procedure:

1. Turn on the detector, the Welcome Screen will be displayed.
2. Unplug the detector from a power outlet while the Welcome Screen is shown.
3. Press and hold the two middle softkeys until step 5.
4. Plug the detector back to the power outlet. The blue screen of bootloader program will appear:
5. You may now release the middle two softkeys. Insert a flash drive with the new firmware.
6. Press the leftmost key to proceed with the download or rightmost key to exit the bootloader.

After the new firmware is downloaded, the file RD4-FRMW.OLD will be created in the root directory. This is the old firmware. This file should be saved in a safe place, **not** in the root directory of the flash drive. To use this file (and thus revert to the old firmware), it should be manually renamed as RD4-FRMW.BIN. Now, the firmware download procedure can be repeated using the renamed file placed in the root directory of a flash drive.

11. Appendix 1: Optical path-length

In any UV detector, a beam of light passes through a certain width of liquid to be analyzed. The detector measures absorbance A (expressed in Absorption Units, AU) of the sample, that is determined as follows:

A = - log(intensity of light emerging from the sample cell / Intensity of light directed onto the sample cell)

where log(x) is the base-ten logarithm of x.

for A = 1.00: 90% of the photons are absorbed, 10% reach the detector;
for A = 2.00: 99% of the photons are absorbed, 1% reach the detector;
for A = 5.00: 99.999% of the photons are absorbed, 0.001% reach the detector.

Contemporary UV detectors employ very low intensity of the irradiating UV light. Higher intensities are detrimental for the analyzed samples, and they would make the detectors dramatically larger, more expensive and require an extended maintenance. Reliable measurements of less then 0.001% of this low-intensity light is, at the moment, not viable for commercial instruments. Therefore, NO available detector can **directly** measure absorption exceeding 5 AU.

HOW IS THE ABSORPTION UP TO 100 AU RECORDED BY RDn?

According to the Beer-Lambert law:

A = E * b * C, where:

- **A** is the measured absorbance of a sample, expressed in Absorption Units, AU;
- **E** is molar absorption coefficient (at a particular wavelength λ) of the compound that is analyzed, expressed in l/(mol * cm);
- **b** is optical path length – the width of the analyzed liquid layer , which is equal to the distance between the inner faces of the sample cell, expressed in cm;
- **C** is the concentration of the compound in solution, expressed in mol/l.

With the exception of our detectors, all other available instruments have flow cells with a fixed **b**.

The illustrating table presents absorbances A of methyl benzoate and benzyl alcohol calculated for various optical path-lengths **b**, at typical for silica-based preparative chromatography purification concentrations C = 0.15 M. Visualization of a strongly absorbing product (methyl benzoate) requires b = 0.01 mm optical cells. However, such a small optical path-length will "miss" weakly absorbing admixtures (benzyl alcohol).

Optical path **b**, mm	Absorbance, Absorption Units	
	Methyl benzoate, 0.15M E = 14400 l/(mol cm) at λ= 242nm	Benzyl alcohol, 0.015M E=12 l/(mol cm) at λ= 250 nm
10	2200 AU	0.18 AU
5	1100 AU	0.09 AU
2	440 AU	0.036 AU
1	220 AU	0.018 AU
0.1	22 AU	0.0018 AU
0.01	2.2 AU	0.00018 AU

A unique feature of RDn is that UV absorption data is simultaneously collected for optical path-lengths from 0.001 to 6.54 mm. For user's convenience, the data is presented **as if** the acquisitions occurred using a 10 mm optical path-length with up to 800 AU range. The **apparent** 10 mm optical path-length can be changed by user at any time by simply pressing the detector's softkey. The chromatogram collected by the RDn detector does not plateau when the concentration of UV-absorbing compounds is high, allowing the user to reliably visualize both strongly and weakly absorbing analytes at essentially any concentration.

12. Appendix 2: Legal disclaimer

Installation and Use: Customer shall install and use the Products in accordance with instructions provided by REACH. REACH will not be responsible for any damage arising out of direct exposure to fire, flooding or severe mechanical impact, improper or unauthorized installation, opening or altering of the unit in which the Products are encased, negligence, neglect, abuse, misuse, or improper maintenance by Customer and any such acts will invalidate the warranty. Further, if the flow cell is destroyed by hydrofluoric acid, concentrated hydrogen peroxide, strong hot aqueous alkaline solutions or fuming nitric acid; or damaged by excessive pressure and/or if the flow cell is permanently clogged with solid material, those acts will invalidate the warranty. A fee will be charged for the flow cell replacement where it is destroyed, damaged or clogged.

Prohibited Uses: Customer shall not and shall not permit others to use the products for separations of mixtures containing hydrofluoric acid, concentrated hydrogen peroxide, strong hot aqueous alkaline solutions or fuming nitric acid. The detector cannot be used under pressures exceeding 15 psi. The detector cannot be used for separation of solutions containing suspended solid particles. Standard safety practices, pertinent to the workplace, must be maintained at all times.

Limited Warranty: REACH warrants all of its products to be free from defects in material and workmanship under normal use and service for a period of three years from the date of shipment. REACH's sole obligation under this warranty shall be limited either to replace or repair defective products or to refund the purchase price, at REACH's option, after inspection at REACH's facility verifies the claim. THERE ARE NO OTHER WARRANTIES WHICH EXTEND BEYOND THE DESCRIPTION OF THIS LIMITED WARRANTY, AND TO THE FULL EXTENT PERMITTED BY LAW, ANY AND ALL IMPLIED WARRANTIES, INCLUDING IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR

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