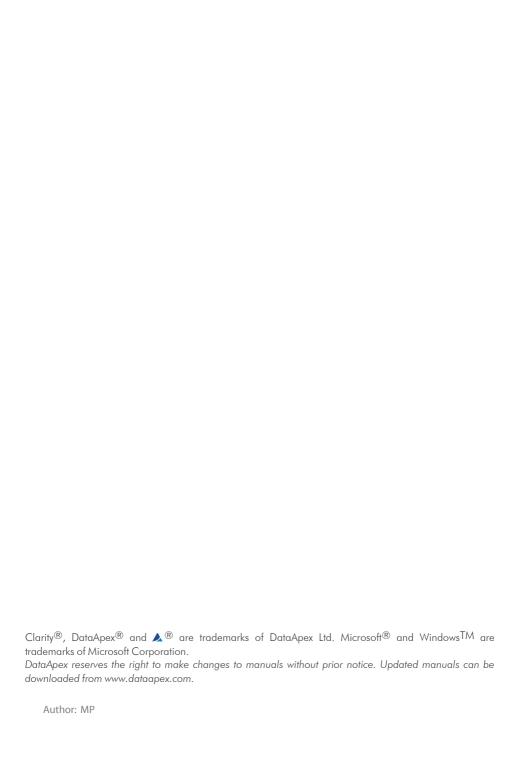


SPARK ALIAS

Clarity Control Module ENG

Code/Rev.: M102/90C Date: 2024-02-14

Phone: +420 251 013 400 clarity@dataapex.com www.dataapex.com DataApex Ltd.
Petrzilkova 2583/13
158 00 Prague 5
Czech Republic



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Spark Alias Table of Contents

To facilitate the orientation in the **Spark Alias** manual and **Clarity** chromatography station, different fonts are used throughout the manual. Meanings of these fonts are:

Open File (italics) describes the commands and names of fields in **Clarity**, parameters that can be entered into them or a window or dialog name.

WORK1 (capitals) indicates the name of the file and/or directory.

ACTIVE (capital italics) marks the state of the station or its part.

Chromatogram (blue underlined) marks clickable links referring to related chapters.

The bold text is sometimes also used for important parts of the text and the name of the **Clarity** station. Moreover, some sections are written in format other than normal text. These sections are formatted as follows:

Note: Notifies the reader of relevant information.

Caution: Warns the user of possibly dangerous or very important information.

Marks the problem statement or trouble question.

Description: Presents more detailed information on the problem, describes its causes,

etc

Solution: Marks the response to the question, presents a procedure how to remove it.

1 Spark Holland Alias control module

This manual describes the setting of the **Spark Holland Alias** autosampler. The control module enables direct control of the instrument over serial line.



Fig. 1: Spark Holland Alias

Direct control means that the autosampler can be completely controlled from the **Clarity** environment. Instrument method controlling the sample preparation conditions will be saved in the measured chromatograms.

Note:

It is recommended to check the user manual of the autosampler for its operating principles and restrictions.

Spark Alias 2 Requirements

2 Requirements

- Clarity Installation with AS Control module (p/n A26).
- Communication cable according to the type of communication used serial straight DB9F-DB9M cable (p/n SK02) in case of serial communication, LAN cross cable (p/n SK08) in case of LAN communication or USB A-B cable (p/n SK06) in case of USB communication.

Note: Cables are not part of Clarity Control Module. It is strongly recommended to order required cables together with the Control Module.

 Free communication port in the PC, based on the type of communication used - free serial COM port in case of serial communication, free ethernet port in case of LAN communication or free USB port in case of the USB communication.

Note: Modern computers usually have only 1 (if any) serial (COM) port installed. To use more devices requiring the port, the **MultiCOM** adapter (p/n MC01) is available.

Caution: Required autosampler firmware versions: 100, minumum revision 123 or version 117, minimum revision 100.

Spark Alias 3 Installation Procedure

3 Installation Procedure

3.1 Hardware - Wiring

Commands for the autosampler are communicated with **Clarity** through the communication cable given by the communication board installed in the **Spark Alias** autosampler. All cables that can be used (described in the chapter "**Requirements**" on pg. 2.) are common standard and can be acquired either from **DataApex** company or from local computer stores.

3.1.1 Connections of the autosampler and chromatographic system

The connection of the whole chromatography set is dependent on the many factors, such as control modules available for each particular part of the set. The common options for the **Spark Alias** autosampler will be either all modules controlled, or some of them not controlled. The typical wirings are shown on schemes below:

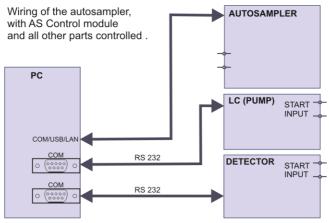


Fig. 2: Wiring of the autosampler - all parts of the set controlled

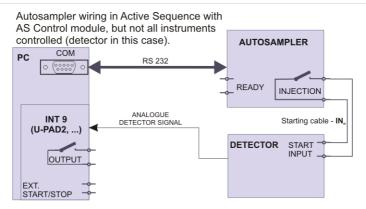


Fig. 3: Wiring of the autosampler - some parts of the set not controlled

Note: Other typical connections of a set with Autosampler can be found in the **Getting**Started manual (chapter **Device Setup and Wiring**).

Spark Alias 3 Installation Procedure

3.2 Spark Alias setup - communication

The **Spark Alias** autosampler can be controlled from **Clarity** via three different communication ways - serial, LAN or USB. The type of the communication used is governed by the communication board installed in the **Spark Alias** autosampler, only one communication board may be installed at the same time.

In addition, the *ID* of the **Spark Alias** autosampler must be the same that will be later set in **Clarity**. The *ID* is hard-set to the value of *61* for **Spark Alias** autosamplers.

LAN Communication settings

In case of LAN communication board, the **Spark Alias** autosampler may be switched to either *TCP/IP* or *UDP/IP* communication mode. **Clarity** prior to version 6.1 worked with UDP communication mode, from version 6.1 works with the TCP/IP instead. To set the communication mode in the sampler please use the **Alias Service Manager** (ASM) utility provided by **Spark**. The procedure to change the communication mode is as follows:

- · open the ASM
- use the Settings Communication menu command
- switch to the Ethernet tab
- Switch to the desired mode (TCP/IP)
- press the Refresh button and select the Spark Alias unit
- press the Select button to apply the change of the communication mode to the Spark Alias

Spark Alias 3 Installation Procedure

3.2.1 Digital Inputs and outputs

The digital inputs and outputs of the **Spark Alias** autosampler are both present on the back panel of the instrument and simulated over the communication line. In usual cases, the outputs do not have to be connected by wire with the autosampler as they are communicated to **Clarity** digitally.

However, when other instrumentation needs to be acknowledged of the analysis start etc. by wire, the I/O connector on the back panel of the autosampler may be used. The mapping of the pins on the connector is as follows:

Tab. 1: I/O connector pins mapping

Pin	Function
1	Output - Common
2	Output - Normally Open
3	Input 1
4	Input 2
5, 7, 8, 9	GND
6	Output - Normally Closed

3.3 Clarity Configuration

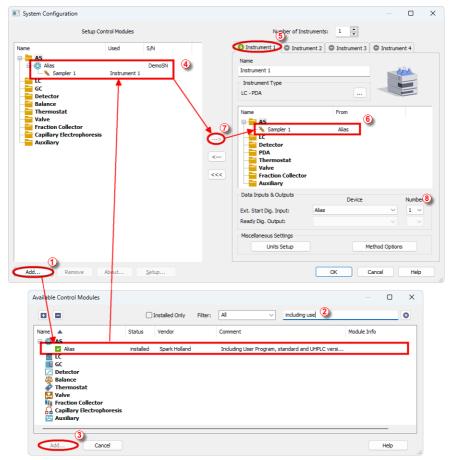


Fig. 4: System Configuration

- Start the Clarity station by clicking on the A icon on the desktop.
- Invoke the *System Configuration* dialog accessible from the *Clarity* window using the *System Configuration...* command.
- Press the Add button ((see Fig. 4 on pg. 7.) to invoke the Available
 Control Modules dialog.
- You can specify the searching filter ② to simplify the finding of the driver.
- Select the **Alias** sampler and press the *Add* ③ button.

The Spark Alias Setup dialog will appear.

Spark Alias 3 Installation Procedure

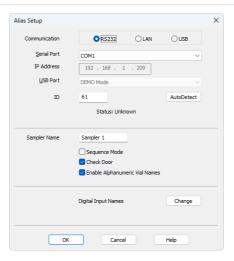


Fig. 5: Spark Alias Setup

Select the correct type of the Communication and fill in the appropriate Serial Port, IP Address or USB Port field. Also, fill in the correct ID for the autosampler. Then press the Autodetect button. If the communication is correct, the Connected inscription along with the firmware version and serial number of the Alias autosampler will be shown in the Status row. The ID must match the device identifier set previously in the autosampler setup, as described in the chapter "Spark Alias setup - communication".

Note: The Spark Alias Setup dialog is more closely described in the chapter "Spark Alias Setup" on pg. 34.

The **Alias** autosampler item will appear in the *Setup Control Modules* list of the *System Configuration* dialog.

- Drag and drop the Alias icon from the Setup Control Modules ① list on the left side of the System Configuration dialog to the desired Instrument ③ tab on the right side ⑥ (or use the button ⑦ to do so).
- Set the Ext. Start Dig. Input and Ready Dig. Output numbers ^(§) for your acquisition card according to the wires being used for synchronization. If you wish to synchronize the Clarity start with the autosampler over serial line, you can set the Alias in the Ext. Start Dig. Input drop-down menu, using the 1 as a input Number. In such case it is necessary to change the behavior on the Method Setup Measurement tab later from Down to Up, else the start signal will be delayed by pulse length (approximately 2 seconds).

3.4 Setting start synchronization

For synchronization there are several options. Either synchronization without *Sequence Mode* or with *Sequence Mode* can be used. When the *Sequence Mode* is selected, more options become available. The *Sequence Mode* can be set in the Spark Alias Setup dialog.

Note:

The <u>Sequence Mode</u> is more closely described in the chapter "Synchronization with Sequence Mode" on pg. 9.

3.4.1 Synchronization without Sequence Mode

Without sequence mode, Clarity sends instructions to the sampler for each injection separately, so the timings are completely controlled by Clarity and overlap timing is not possible.

The Spark Alias has to be set as Ext. Start Dig. Input in the <u>System Configuration</u> window.

3.4.2 Synchronization with Sequence Mode

The **Spark Alias** autosampler switched to the *Sequence Mode* operation may be synchronized with **Clarity** in two ways - either using the *Analysis Time* parameter from the <u>Method Setup - AS - Injection</u> tab or by using the autosampler's <u>Freeze input</u>.

Note:

When the autosampler is switched into the *Sequence Mode* and simultaneously uses the **User Program**, only the *Freeze input* synchronization is available.

3.4.2.1 Synchronization using the Analysis Time parameter

In Sequence mode, Clarity sends instructions for several injections to the sampler and then just waits for the start signals from the sampler, the timings are governed by the sampler. The Analysis Time in the Method Setup - AS - Injection must be set to ensure next injection will be performed when Clarity is ready for it.

Note:

When the autosampler uses the **User Program**, this type of synchronization is not available. Only the *Freeze input* synchronization is available.

Spark Alias 3 Installation Procedure

3.4.2.2 Synchronization using the freeze synchronization

Using the Freeze input, user should do several operations:

 Disable the standard Clarity ready-out synchronization. In the System Configuration dialog, set the Number field for the Ready Dig. Output to ---.



Fig. 6: System Configuration - Ready Out settings

Note: This step is probably optional, but cleans the station operation of unnecessary switching of the output at the wrong time.

- Enter the Method Setup AS dialog and set one of the Inputs as Freeze input
 as described in the chapter "Inputs & Outputs" on pg. 18.
- Enter the Method Setup Event Table dialog and insert the following rows there:

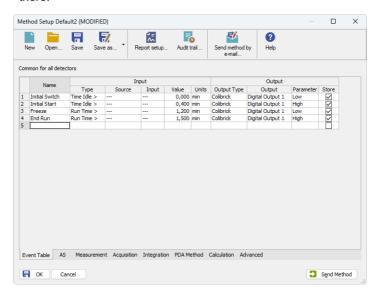


Fig. 7: Event Table - synchronization settings

Modify the times entered in the *Value* column to suit your analysis and the items entered in the *Output Type* and *Output* columns to match the digital output used for synchronization. The values should be defined as follows:

Spark Alias 3 Installation Procedure

Event called *Initial Switch* should always perform at the *Idle Time* = 0.000 minutes. It serves for initial switching of the output for the first sample in the sequence (or for the first sample in the pack of sequence rows performed as one block) regardless of Clarity initial output state settings.

- Event called *Initial Start* should perform at the time reliably sufficient for the preparation of the sample before injection. You have to test that time for your analysis try to run a single-line analysis according to your method and measure the time needed from hitting the Run Sequence button to the injection, plus add a few seconds more. The value can be fine-tuned later, but it is imperative that the sampler will be in the *WAIT FOR INPUT* or *FREEZE ACTIVE* state before the event is triggered. The event is used for starting the first analysis in each block of rows solved together and is performed during the *Idle Time*.
- Event called Freeze should be set so that it occurs at the Run Time shortly after the internal autosampler analysis time counter ends its method. At that time, a new sample preparation is started and the output has to be switched to block the injection before it occurs. Ideal time of this event would be somewhere between Clarity analysis run time (as defined in the Method Setup Measurement tab under the parameter Run Time) minus the time defined for the Initial Start row, and the Clarity analysis run time (as defined in the Method Setup Measurement tab under the parameter Run Time).
- Event called End Run should be set so that it occurs at the end of the
 analysis. This time is set in the Method Setup Measurement tab under
 the parameter Run Time. It serves for triggering the injections of vials
 other than the first one in the group of the sequence rows sent together,
 and is performed in the Run Time.
- If you are not using the User Program, ensure that the End Time parameter
 on the Method Setup AS Inputs & Outputs tab is set correctly. It should be
 set so that it equals Clarity analysis run time (as defined in the Method
 Setup Measurement tab under the parameter Run Time) minus the time
 defined for the Initial Start row. At this time, the internal autosampler analysis
 time will elapse and a new injection preparation will be started.
- If you are using the User Program, ensure that the User Program method length equals Clarity analysis run time (as defined in the Method Setup -Measurement tab under the parameter Run Time) minus the time defined for the Initial Start row. At this time, the internal autosampler analysis time will elapse and a new injection preparation will be started.

4 Using the control module

New <u>Method Setup - AS</u> tab appears in the *Method Setup* dialog, enabling the setting of the **Spark Alias** autosampler control method.

4.1 Method Setup - AS

The Method Setup - AS dialog consists of six sub-tabs assigned for the various the Spark Alias autosampler method. These sub- tabs areInjection,Wash,Inputs & Outputs,Mix,User Program,System,Spec. Vials and Tray. Additional buttons allow to display the Hardware Configuration dialog of the Spark Alias autosampler or to read the instrument method from the Spark Alias autosampler. The method is sent to the autosampler every time the Send Method button is pressed. Other actions in different windows may also cause the sending of the instrument method to the controlled devices including the Spark Alias autosampler - most notable cases being pressing the Send Method button in the Single Run dialog or starting a new injection from the Sequence window (each injection is preceded with sending the instrument method).

To read the **Spark Alias** method from the autosampler it is necessary to use the *From AS* button available from all sub-tabs of the *Method Setup - AS* dialog. If the injection method is already established in the sampler, it is advisable to download it to **Clarity** using the *From AS* button and save it as a **Clarity** method.

4.1.1 Injection

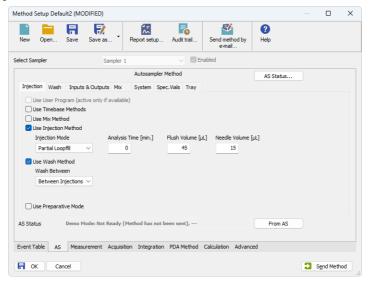


Fig. 8: Method Setup - AS - Injection

This is the main tab defining the AS control method. It defines which parts of the **Spark Alias** method will be performed and set some other parameters.

The options on this tab consist of two parts, which exclude themselves - if the *Use User Program* option is selected, no other options will be used. On the other hand, if the *Use User Program* checkbox is not checked, any combination of other method parts may be used.

Use User Program

When set, the whole injection part of the method will be governed by instructions set on the Method Setup - AS - User Program tab.

Use Timebase Methods

Sets whether the sampler method part used for outputting signals after injection will be used. This part of the method is then set on the Method Setup - AS - Inputs & Outputs tab.

Use Mix Method

Sets whether the sample pre-preparation part of the injection method will be used. If checked, the method defined on the Method Setup - AS - Mix tab will be performed.

Use Injection Method

This part of method contains the information on the injection routine, analysis time and flush volume.

Injection Mode

Allows to set the injection mode by selecting one of the possibilities:

Partial Loopfill - in this mode, only a part of the sample loop volume will be transferred into the column.

Full Loop - in this mode, the full sample loop will be transferred to the column. Only the value set in the Loop Volume field of the Method Setup - AS - System dialog is allowed for setting in the Inj. Vol. column in the Sequence Table in the Sequence window.

µl Pick up - defined volume of the sample will be injected into the column, preceded and followed by the transport liquid.

Analysis Time [min.]

Defines the time between switching the injection valve to the *INJECT* position and the start of next sample preparation. This parameter is critical for setting the analyses performed in the *Sequence Mode* without the synchronization wire.

Caution:

While using the synchronization wire for the **Spark Alias** autosampler, it is necessary to set the analysis time to 0.

Flush Volume [µl]

Defines the volume of the sample that will be used for flushing the sample loop and tubing before the sample is aspired. Possible values range from 0 to 9999 μ l, default value is 45 μ l. This option is only available for the *Partial Loopfill* and *Full Loop* injection modes.

Caution:

Setting the *Flush Volume* parameter to less than twice the volume of the needle and tubing will cause worse performance.

Needle Volume [µl]

Defines the volume of the sampler needle, including the tubing between the needle and the valve. Possible values range from 0 to 999 μ I, default value is 15μ I.

Use Wash Method

Defines the type of the washing method performed between injections or vials. The wash method itself is only defined on the Method Setup - AS - Wash tab.

Wash Between

Defines the time when the wash should be performed. Possible values are:

Between Vials - the wash will be performed after each sample measured. The option is only available if the Sequence Mode option is checked in the Spark Alias Setup dialog.

Between Injections - the wash will be performed after each injection.

Use Preparative Mode

Switches the method for the **Spark Alias** autosampler to the preparative mode. This means that the values for the *Injection Mode* on the *Method Setup - AS - Injection* tab, *Loop Volume* and *Syringe Volume* on the <u>Method Setup - AS - System</u> tab and the *Plate Types* on the <u>Method Setup - AS - Spec. Vials</u> tab are hard-set to those used by the **Spark Alias** autosampler with Prep mode option installed.

4.1.2 Wash

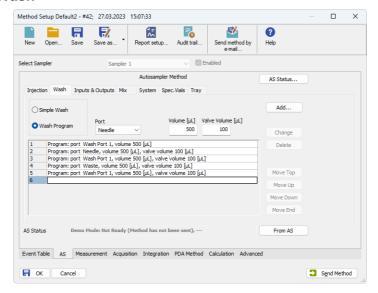


Fig. 9: Method Setup - AS - Wash

This tab defines the behavior of the **Spark Alias** autosampler when it comes to washing the sample loop. Two modes of washing are possible - the *Simple Wash* mode and the *Wash Program* mode. Moreover, the options available are influenced by the presence of optional **Spark Alias** autosampler parts - Wash Port 2 and Solvent Selection Valve (SSV).

Simple Wash

It is not possible to define the port from which the sample loop will be washed, the default wash liquid is used for that. The wash volume is hard-set to the syringe volume.

Wash Program

In this mode, it is possible to select the source of the wash liquid and volume of the wash liquids used. When the optional SSV is installed, the **Spark Alias** also allows to wash more than one times with the option to change the wash liquid between particular steps.

Times

This option, available only in the *Simple* mode, allows to set the number of times the sample loop will be washed by the wash liquid. The volume of each wash is dependent on the volume of the installed syringe - whole syringe will be used for each wash step.

Port

Sets the port from which the wash solvent will be taken. Only available in the *Program* mode. The options that can be set are *Wash Port 1*, *Needle* port, *Waste* port and *Wash Port 2* (optional).

SSV

Allows to set the SSV port for the selection of the wash solvent. Only available when the optional solvent selection valve is installed and when the *Port* field is set to the *Wash Port 2* option.

Volume [µl]

Defines the wash volume for the wash step used in the *Program* mode.

Valve Volume [µl]

Defines the valve volume for the pre-wash step, performed only when the SSV valve is switched to another position after previous step. The VALVE VOLUME parameter cannot be set for the first row of the wash *Program*.

Wash Table

Contains the instructions for performing the particular wash steps. In case of the *Simple Wash* mode or *Wash Program* mode performed without installed SSV valve, only one row is present in the **Wash Table**. On the other hand, *Wash Program* mode with the optional SSV valve and enabled Wash Port 2 option the table can contain up to 9 rows.

A new row can be added into the table by filling its parameters and pressing the *Add* button, or the existing row can be changed by selecting it, changing the necessary parameters and pressing the *Change* button. A row can be deleted using the *Delete* button.

When the row is selected, it can be moved up and down through the list by using the *Move Top, Move Up, Move Down* and *Move End* buttons.

4.1.3 Inputs & Outputs

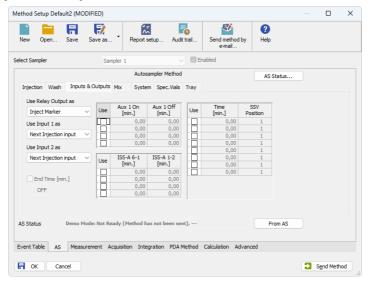


Fig. 10: Method Setup - AS - Inputs & Outputs

Tab defining the behavior of the **Spark Alias** autosampler while performing the Time Program. The tab governs the assignment of the inputs and output on the connector on the back side of the autosampler and allows to set the timetables for installed optional valves.

Use Relay Output as

Determines for which purpose the relay output contact on the back side of the **Spark Alias** will be used. The options are *Inject Marker*(sends a pulse signal at the moment the autosampler injects), *Auxiliary* (changes the output state on the set times based on the setting made in the **Aux table**) and *Alarm* (sends signal whenever the Spark Alias autosampler produces an error message).

Note:

The simulated start signal is sent to **Clarity** via RS-232 communication, it is thus not necessary to use the *Inject Marker* for starting the **Clarity** run. The length of the inject marker pulse is 1s, other values can only be set using the **Spark ASM** application.

Use Input 1 (2) as

Determines for which purpose will the particular input be used. The options are *Next Injection input* (when invoked, performs a new injection. Not functional with the **Clarity** control), *Freeze input* (when no *Analysis Time* is used in the **Sequence Mode**, blocks the injection until the analysis is ready) and *Stop input* (when the signal is received, the run of the autosampler is immediately aborted).

Caution:

When the *Freeze input* synchronization is used for the **Sequence Mode**, the Instrument will end in the *CONTROL* state when the last sample in the sequence is measured.

End Time

Sets the ending time of the Time Program. Without the value set, no options on this tab can be used or even set. The value of the *End Time* may be greater than the *Run Time* set on the *Method Setup - Measurement* tab to set signals or switch valves after analysis, the difference will be evaluated as a *CONTROL* time.

Aux table

Governs the switching of the digital output between *HIGH* and *LOW* states based on the time. The default state of the output depends on the connection to the Input/Output connector (for more details see the chapter **Spark Alias setup - communication** on pg. **5**). and it can be switched to *HIGH* (*Aux 1 On*) and back to *LOW* (*Aux 1 Off*) or vice versa up to four times. For the table to be available, the *Use Relay Output as* field must be set to *Auxiliary*.

ISS-A table

Governs the switching of the optional ISS-A valve from the *LOAD* (*ISS-A 6-1*) position to the *INJECT* (*ISS-A 1-2*) position.

Note:

Both the **Aux table** and **ISS-A** table allow to omit the setting of the *Aux 1 Off/ISS-A 1-2* on a row, in which case the auxiliary output/valve would not be switched back. However, when such setting is used, a new row cannot be added to the table

SSV table

Governs the switching of the optional solvent selection valve (present on the side of the **Spark Alias** autosampler) during the analysis run. Lines in the table can only be activated or deactivated successively and allow to define the *Time* and particular *SSV Position* to switch to. Only the checked rows will be performed.

4.1.4 Mix

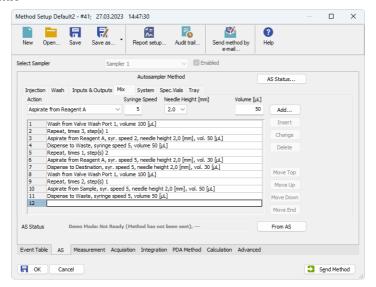


Fig. 11: Method Setup - AS - Mix

Mix part of the sampler method (defined on the *Method Setup - AS - Mix* sub-tab) governs the pre-preparation of sample before the injection. Several operations are possible with vials defined on the <u>Method Setup - AS - Spec. Vials</u> tab. The table fields are context-based, that is their content change according to the action defined in the first column on the actual row.

Note:

When any special vials (Reagent, Destination, Transport) should be used in the Mix method, check that there are these vials defined on the Method Setup - AS - Spec. Vials tab. If they are not defined, the default values will be used, which may invalidate given sample or other sample's analysis data.

A new row is added (or actual row is modified) using the *Action* drop-down list. When an action is selected, other parameters of the action emerge and can be modified. Using the *Add* or *Insert* button the action can be added as a new row into the table, either to the end of the list (*Add* button) or above the selected row (*Insert* button). The existing row can be changed by selecting it, changing the necessary parameters and pressing the *Change* button. A row can be deleted using the *Delete* button.

When the row is selected, it can be moved up and down through the list by using the *Move Top, Move Up, Move Down* and *Move End* buttons.

Note: Up to 15 lines can be programmed in the **Mix Table**.

The items that can be selected for the particular row in a **Mix Table** are following:

Wait

Causes the sampler to wait for the specified time interval. The desired interval is entered into the *Time [min.]* field in minutes.

Repeat

Repeats the last one or more rows for the specified number of times. The *Times* field is used to set the number of repeats, the *Steps* field sets the number of **Mix Table** rows to be repeated.

Aspirate

Draws air or solution from sample, destination or specified reagent vial. The speed of the aspiration move can be changed in the *Syringe Speed* field, while the height of the needle tip above the vial bottom is governed by the value entered into the *Needle Height [mm]* field. The volume being aspirated is defined by the *Volume [µl]* field.

Dispense

Empties a given volume from the syringe into the specified vial or waste port. The height of the needle tip above the bottom of the vial tray and the speed of emptying is set in the *Needle Height* and *Syringe Speed* fields, respectively. The meaning of these fields is the same as in the *Aspirate* command, dispensing into the Waste port doesn't need to specify the *Needle Height* parameter. The volume being dispensed is defined by the *Volume [µl]* field.

Wash

Performs the standard wash operation from **Wash Port 1**. Specify the volume of the wash solvent in the *Volume* [µl] field.

Add - old version

Performs the sequence of steps involving aspiration from the vial (or port) defined in the *From* field and dispension into the vial defined in the *To* field. The volume that should be transported this way is defined in the *Volume [µl]* field.

Mix - old version

Mixes the liquid un the Destination vial by aspiring and dispensing the volume defined in the *Volume* [µI] field. The number of aspirations and dispensions is defined in the *Times* field.

Wait - old version

Causes the sampler to wait for the specified time interval. The desired interval is entered into the *Time [min.]* field in minutes.

Note:

The "old version" items are available due to the compatibility with the methods created before the firmware upgrade - formerly the **Spark Alias** autosampler was only able to process the "old version" actions.

4.1.5 User Program

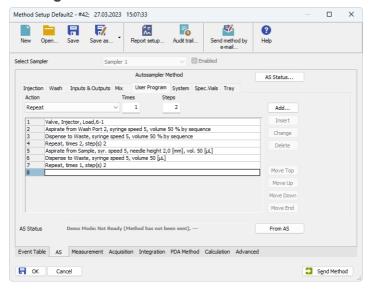


Fig. 12: Method Setup - AS - User Program

When user wants to use *User Program* and the **Spark Alias** autosampler is switched to the *Sequence Mode* Freeze synchronization has to be set.

The table on the *Method Setup - AS - User Program* tab enables to set all parts of the sampler method in one place, which means that no parameters on line-width:line-width. Outputs and Mix tabs will be necessary. *User Program* tab thus substitutes all tabs mentioned above and checking the checkbox on Method Setup - AS - Injection tab will disable all other checkboxes there. Several operations are possible with vials defined on the Method Setup - AS - Spec. Vials tab. The table fields are context-based, that is their content change according to the action defined in the first column on the actual row.

Note:

When any special vials (Reagent, Destination, Transport) should be used in the User Program method, check that there are these vials defined on the Method Setup - AS - Spec. Vials tab. If they are not defined, the default values will be used, which may invalidate given sample or other sample's analysis data.

A new row is added (or actual row is modified) using the *Action* drop-down list. When an action is selected, other parameters of the action emerge and can be modified. Using the *Add* or *Insert* button the action can be added as a new row into the table, either to the end of the list (*Add* button) or above the selected row (*Insert* button). The existing row can be changed by selecting it, changing the necessary

DeleteDelete button.

When the row is selected, it can be moved up and down through the list by using the *Move Top, Move Up, Move Down* and *Move End* buttons.

Note: Up to 239 lines can be programmed in the **User Program Table**.

The items that can be selected for the particular row in a **Mix Table** are following:

Wait

Causes the sampler to wait for the specified time interval. The desired interval is entered into the *Time [min.]* field in minutes.

Repeat

Repeats the last one or more rows for the specified number of times. The *Times* field is used to set the number of repeats, the *Steps* field sets the number of **User Program Table** rows to be repeated.

Aspirate

Draws air or solution from sample, destination, specified reagent vial or defined Wash port. For selected aspiration sources, the speed of the aspiration move can be changed in the *Syringe Speed* field, while the height of the needle tip above the vial bottom is governed by the value entered into the *Needle Height [mm]* field. The volume being aspirated is defined by the *Volume [µl]* field, which can be done either absolutely or relatively (in % of the injection volume as set in the **Sequence Table**) by checking the *By Sequence* checkbox.

Dispense

Empties a given volume from the syringe into the specified vial or waste port. The height of the needle tip above the bottom of the vial tray and the speed of emptying is set in the *Needle Height* and *Syringe Speed* fields, respectively. The meaning of these fields is the same as in the *Aspirate* command, dispensing into the Waste port doesn't need to specify the *Needle Height* and *Syringe Speed* parameters. The volume being dispensed is defined by the *Volume [µI]* field, which can be done either absolutely or relatively (in % of the injection volume as set in the **Sequence Table**) by checking the *By Sequence* checkbox.

Wash

Performs the standard wash operation from the specified port - Wash Port 1, Wash Port 2, Syringe Valve Needle Port or Syringe Valve Waste Port. Specify the volume of the wash solvent in the *Volume* [µl] field.

Note: Program a Dispense to Waste action prior to washing to prevent the cross-contamination from the buffer solution.

Needle Vertical

Plunges the needle to the actual position (vial, port) or withdraws it to the home position, according to the value set in the *Position* field. The depth to which the needle will be plunged is 2.0 mm above tray bottom by default and cannot be changed.

Needle Vertical Absolute

Plunges the needle to the depth specified in the *Position [mm]* field. The value of 0.00 mm is meaning the Home position (syringe is raised), the lowest position to which the needle can be plunged is 50.00 mm. Smallest step is 0.17 mm from the current position.

Needle Horizontal

Moves the needle over the position defined in the *Position* field. Available positions are *Home* (Waste) port, *Wash* port and *Transport* liquid port (if available).

Needle Horizontal Absolute

Moves the needle over the position defined in the *Position [mm]* field. The value of 0.00 mm is meaning the Home position (Waste port), the furthest position to which the needle can be moved is 180.00 mm. Smallest step is 0.05 mm from the current position.

Syringe Valve

Switches the syringe valve to one of its positions: Needle, Waste, wash Port 1 or wash Port 2.

Syringe Load

Forces the syringe to load the given *Volume [µl]* using the set *Syringe Speed*.

Syringe Unload

Forces the syringe to unload the given *Volume [µl]* using the set *Syringe Speed*.

Syringe Home

Forces the syringe to perform the home operation (the syringe will dispense itself into the last programmed position and will re-initialize).

Tray

Moves the tray to the position defined in the *Position* field.

Tray Absolute

Moves the tray to front or back, as defined in the *Position [mm]* field. The movement is defined by the distance from the home position (0.00 mm), where only moves differentiating by at least 0.05 mm from the current position will be performed.

Valve

Switches the injector valve or optional ISS-A valve to the desired position. The desired valve is set in the *Select* field, while the position is specified in the *Position* column. To be able to program ISS-A valve it is necessary to have it installed.

Compressor

Switches the compressor to put the air pressure on the sample *On* or *Off*, according to the *State* field. The compressor stays switched on until it is switched off (in some later step).

Wait for Input

Allows to set the input whose signal will temporarily override the Freeze function. In **User Program**, this is necessary to start the measurement in the *Sequence Mode* synchronized via the Freeze wiring. The input from which the signal is awaited is defined in the *Select* field, while the desired state unblocking the Freeze condition is

defined in the *State* field. Only available when the *Use Input 1 as* or *Use Input 2 as* field on the Method Setup - AS - Inputs & Outputs is set to the *Freeze input*.

Auxiliary 1

Switches the state of the auxiliary output *On* or *Off*, according to the *State* field. Only available when the *Use Relay Output as* field on the Method Setup - AS - Inputs & Outputs is set to the *Auxiliary*.

Output 1

Switches the output of the **Spark Alias** autosampler *On* or *Off*, depending on the value set in the *State* field.

SSV

Defines the optional SSV valve position in the range 1-6 specified in the *Position* field. To be able to program SSV valve it is necessary to have it installed.

Marker

Sets the marker type in the *Select* field. The options are *Inject Marker* and *Inj. Marker Status* - the difference is that the *Inj. Marker Status* works without the *Use Relay Output as* field on the <u>Method Setup - AS - Inputs & Outputs</u> tab set to the *Inject Marker* state, thus allowing the use of *Auxiliary* program. The real state of the relay is not changed this way.

Caution:

When setting the **User Program**, *Marker* has to be used to signal the injection. This operation is performed automatically in the Injection program, but has to be performed manually in the **User Program** as the user has full control of the injection valve settings.

Add - old version

Performs the sequence of steps involving aspiration from the vial (or port) defined in the *From* field and dispension into the vial defined in the *To* field. The volume that should be transported this way is defined in the *Volume* [µl] field.

Mix - old version

Mixes the liquid un the Destination vial by aspiring and dispensing the volume defined in the *Volume* [µI] field. The number of aspirations and dispensions is defined in the *Times* field.

Wait - old version

Causes the sampler to wait for the specified time interval. The desired interval is entered into the *Time [min.]* field in minutes.

Note:

The "old version" items are available due to the compatibility with the methods created before the firmware upgrade - formerly the **Spark Alias** autosampler was only able to process the "old version" actions.

4.1.6 System

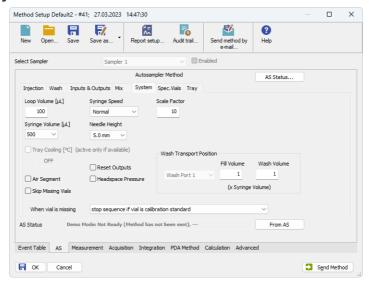


Fig. 13: Method Setup - AS - System

Tab defining various aspects of the **Spark Alias** operation.

Loop Volume [µl]

Defines the value of the sampling loop volume used for the injection (in μ l). Possible values range between 0.1 and 1000 μ l. When using Full Loop Injection Mode from the Method Setup - AS - Injection tab, only the Loop Volume value is allowed in the Sequence Table in the Sequence window.

Syringe Speed

Sets the syringe speed from the set of predefined values (Low, Normal, High).

Scale Factor

Sets the multiplier for the syringe speed. This variable can be set to values between 1 and 10, thus enhancing the *Syringe Speed* set from default speed to ten times the default speed. The default value is 10.

Syringe Volume [µl]

Defines the volume of the syringe installed in the autosampler. The value is dependent on the fact whether the *Preparative Mode* is switched on or off, varying between 500 μ l when not using the *Preparative Mode* and 2500 μ l in the *Preparative Mode*.

Needle Height

Sets the distance of the needle tip from the bottom of the tray during the aspiration. Possible values range from 2 to 6 mm, incremented after halves of millimeters.

Note:

When using trays with 12 or 48 vials, make sure that the *Needle Height* is set to more than 2 mm to prevent the needle touching the bottom of the vial.

Tray Cooling [°C]

Allows to enable the tray cooling function and set the temperature for the cooling (in the range 4 - 40 °C when cooler/heater is installed, 4 - 22 °C when only cooler is installed). This option is grayed-out if the **Spark Alias** sampler doesn't have the function built-in.

Air Segment

Sets whether the air segment should be used during the injection. The air segment normally separates the sample from the transport liquid.

Reset Outputs

While checked, forces the digital outputs to be reset after the sequence has ended.

Headspace Pressure

When checked, the sampler uses the pressure to facilitate the transfer of the sample into the sample loop. The pressure will only be used when the vials are airtight.

Wash Transport Position

This section governs the washing and filling of the Wash/Transport port of the **Spark Alias** autosampler.

Fill Port

Sets which port will be washed and filled. Under default conditions, only Wash Port 1 is available. Other options emerge only when the optional **SVW Port 2** valve is installed.

Fill Volume

Sets how many times the volume of the syringe will be used for filling the wash port. Possible values range from 1 to 9.

Wash Volume

Sets how many times the volume of the syringe will be used for washing the wash port before its filling. Possible values range from 1 to 9.

Skip Missing Vials

Defines the behavior of the autosampler when missing vial is detected. While unchecked, the autosampler will issue an error message and stop the processing of the sequence, but when checked, no error message is issued and the sequence behaves according to the settings in the *When vial is missing* field.

When vial is missing

Determines the behavior of **Clarity Sequence Table** when missing vial is detected by the autosampler and the *Skip Missing Vials* function is checked. The possible options are *stop sequence*, *stop sequence when vial is standard* or *continue sequence*.

Note:

Skip Missing Vial function is, due to the design of the Alias firmware, only designed for the Sequence Mode. It has not been thoughtfully tested.

4.1.7 Spec. Vials

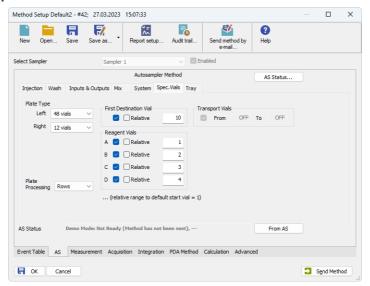


Fig. 14: Method Setup - AS - Spec. Vials

Governs the types of trays (or well-plates) used and allocation of special purpose vials (destination, transport and reagent vials) to particular vial well positions.

Plate Type

Defines the plate type inserted into the *Left* and *Right* tray position. The plate types may differ between positions, but if the *84+3* tray is used, it fills both positions. The visualization of the trays is displayed on the Method Setup - AS - Tray tab.

Plate Processing

Defines the numbering of vials (or wells) in the selected trays. The vials will be either counted in *Rows* or in *Columns*. For the 84+3 tray type only the *Rows* option is available. Actual numbers of particular vials (wells) are displayed on the <u>Method Setup - AS - Tray</u> tab.

First Destination Vial

Defines the position of destination vial(s) used in the *Mix* method and *User Program*. The most common usage of the destination vials is with the *Relative* checkbox checked, as it gives each vial in the sequence it's own destination vial.

Note: When using destination vials and mix method in general, do not use more than one injection per vial.

Reagent Vials

Defines the position of Reagent vial(s) used in the *Mix* method and *User Program*. Up to four different Reagent vials are available, each of them can have a fixed position or *Relative* position given to the sample vial. If the *84+3* tray is used, only

two reagent vials can be programmed and they can only be mapped to the large volume vials (85-87).

Transport Vials

Defines the position of vials containing the transport liquid (used with μl *Pick up* injection mode and together with the *84+3* plate type). The algorithm defining from which vial will the transport liquid be actually aspired is stored in the **Spark Alias** sampler. If the transport vials are used, they must form an uninterrupted row beginning in the well number defined in the first field and ending by the vial defined in the second field.

4.1.8 Tray



Fig. 15: Method Setup - AS - Tray

Shows the visualization of the **Spark Alias** autosampler tray(s), along with the mapping of the vials as performed on the Method Setup - AS - Spec. Vials tab.

Temp. Start Vial

Allows to set the temporary position of the first sample vial that will be used in the measurement to assess the positions of other special vials. The position of this temporary starting vial will be reset whenever the *Method Setup* dialog is closed.

4.2 Hardware Configuration



Fig. 16: Hardware Configuration

The AS Status button in the Method Setup - AS displays the Hardware Configuration dialog. In the full version, this dialog displays autosampler model, automatically detected communication parameters, firmware revision and the presence of several optional parts of the **Spark Alias** autosampler.

In the demo version the presence of the particular options can be set to demonstrate the possibilities of the sampler with selected configuration.

Type of Sampler

Shows the **Spark Alias** autosampler type as automatically detected by the control module.

Connection

Shows the communication parameters as detected from the sampler. The value shown is dependent on the type of communication used - either it is a com port, IP address or USB port.

Firmware Revision

Shows the firmware revision loaded into the **Spark Alias** autosampler.

Tray Cooling

Shows whether the **Spark Alias** sampler is equipped with the tray cooling/heating option.

SSV

Shows whether the **Spark Alias** sampler is equipped with the solvent selection valve (SSV).

SVW Port 2

Shows whether the Spark Alias sampler is equipped with the optional syringe valve wash port 2 (SVT).

ISS-A

Shows whether the **Spark Alias** sampler is equipped with the optional integrated stream-switching (ISS) valve.

1 out 6 Valve

Shows whether the **Spark Alias** sampler is equipped with the 1 out 6 column selection valve.

Mix and Dilute

Lists whether the firmware revision of the **Spark Alias** supports the *Mix* program.

User Program

Lists whether the firmware revision of the **Spark Alias** supports the *User Program*.

4.3 Spark Alias Setup

Spark Alias Setup dialog (accessible through the System Configuration dialog) allows to manually set the parameters needed for the communication with the Spark Alias autosampler.

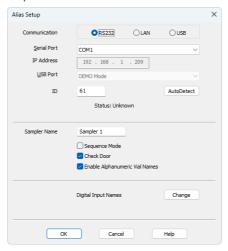


Fig. 17: Spark Alias Setup

Communication

Sets the type of the communication for the **Spark Alias** autosampler. The possible options are *RS232*, *LAN* or *USB*. The selection dependent on the communication card set in the autosampler then influences the fields available in the *Spark Alias Setup* dialog.

Serial Port

In case of serial (RS232) communication sets the COM Port used for the communication between the **Spark Alias** autosampler and **Clarity**.

IP Address

In case of LAN communication sets the IP address of the **Spark Alias** autosampler connected to the computer via network.

USB Port

In case of the USB communication sets the particular USB port used to communicate with the **Spark Alias** autosampler.

ID

Shows the device identifier of the **Spark Alias** autosampler. The number listed here is hard-set for **Spark Alias** autosamplers to the value *61*. For more details see the chapter **Spark Alias setup - communication** on pg. **5**.

Autodetect

When pressed, checks whether there is the **Spark Alias** autosampler present using the selected *Communication* option, with the given *Serial Port*, *IP Address* or *USB Port*. The ID is also checked for the successful communication attempt. The result of the autodetection is then displayed in the *Status* row.

Status

Shows the status of the communication with the **Spark Alias** autosampler after the *Autodetect* button has been used. The displayed information, in case of the successful communication attempt, includes the version of the firmware in the autosampler and the sampler's serial number.

Sampler Name

Allows to set the custom name of the **Spark Alias** autosampler, which will be then shown in the *Device Monitor* window, in the reports and on other places in **Clarity**.

Sequence Mode

This option governs the mode of the operation with the **Spark Alias** autosampler control module. While checked, as much of the sequence table as possible is sent to the autosampler in one package (no method sending prior each injection will be performed for the injections package). The sequence should be written in the manner where following lines are analogical (e.g. same number of injections from each row, but the injection vial is incremented between rows etc.). More information on the *Sequence Mode* synchronization can be found out in the chapter **"Setting start synchronization"** on pg. **9**.

While not checked, the operation will be governed by **Clarity** - the method will be sent to the autosampler prior each injection and each injection will thus be considered a single one-injection series by the autosampler. This prevents the use of washing between samples and few other features.

Check Door

Sets whether the check for the opened autosampler cover door will be performed or not for the autosampler to operate. If possible, it is recommended to leave this option checked to avoid injury from the fast-moving **Spark Alias** compartments.

Enable Alphanumeric Vial Names

Sets whether the vial positions on the **Spark Alias** vial tray or well plate will be referenced from the **Clarity Sequence** window using the alphanumeric positioning (rows and columns, one using alphabet, second using numbers) or strictly using the numbers.

Digital Input Names

Pressing the *Change* button opens the *Digital Input Names* dialog which allows to set the custom name for the virtual **Spark Alias** digital inputs. These inputs allow to start **Clarity** run by the signal of the **Spark Alias** autosampler simulated over the communication line instead of the usual synchronization wiring, send warnings on autosampler errors or govern auxiliary devices.

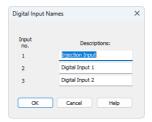


Fig. 18: Digital Input Names

4.4 Device Monitor



Fig. 19: Spark Alias Device Monitor

The Device Monitor window for the **Spark Alias** autosampler enables to control some of the actions of the **Spark Alias** autosampler.

Initial Wash - Start

When invoked, performs the Initial Wash operation to rinse all tubing.

Reset Error

When invoked, resets the Error state that occurred on the sampler.

AS Status

Opens the <u>Hardware Configuration</u> dialog described in the chapter "Hardware Configuration" on pg. 32.

Switch Tray Cooling/Heater On/Off

Switches the tray cooling and/or heater on or off. Sending a method will reset the cooler/heater to the state defined in the method.

Service

Displays the <u>Service</u> dialog for controlling the **Spark Alias** using other service commands.

Tray Move Front

Moves the tray from the home position (further from the door) to the front.

Tray Move Home

Moves the tray from the front position (near the door) back to the home position.

Valve Position change - Load/Inject

Changes the position of the valve from *LOAD* to *INJECT* position or the other way around. This only works when the autosampler is not working.

SSV Position

This drop-down menu shows and allows to switch the status of the optional SSV valve, if it is installed in the **Spark Alias** autosampler. This only works when the autosampler is not working.

ISS Valve 1/2

Allows to switch the solvent selection valve installed.

4.4.1 Service



Fig. 20: Service dialog of the Spark Alias

This dialog is designed for controlling the **Spark Alias** using service commands.

Reset Error

When invoked, resets the Error state that occurred on the sampler.

Initial Wash - Start

When invoked, performs the Initial Wash operation to rinse all tubing.

Tray Move Front

Moves the tray from the home position (further from the door) to the front.

Trav Move Home

Moves the tray from the front position (near the door) back to the home position.

Valve Position change - Load/Inject

Changes the position of the valve from Load to Inject position or the other way around

Fill Transport Reservoir

By setting the required *Fill Times* and *Fill Port* it is possible to fill up the transport reservoir.

Exchange Needle

Allows to exchange the sampling needle. Follow the instructions on screen to perform the exchange.

Exchange Syringe

Allows to exchange the syringe. Follow the instructions on screen to perform the exchange.

4.5 Select Vial

This dialog serves for entering the alphanumerical numbering of the vials. It is accessed by clicking on the small arrow in the *Start Vial* or *End Vial* column in the *Sequence* window, when the alphanumerical numbering of vials was enabled in the *Spark Alias Setup* dialog by using the *Enable Alphanumeric Vial Names* checkbox.

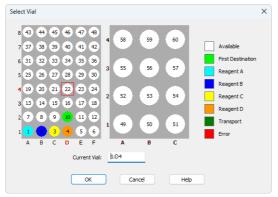


Fig. 21: Select Vial

The functionality of the dialog is simple - either add the number of the vial needed into the *Current Vial* field, or click on the position in the graphical view of the autosampler trays. The actual position is highlighted by red square, and the letter and number of the row and column are also highlighted by red colour.

Spark Alias 5 Report Setup

5 Report Setup

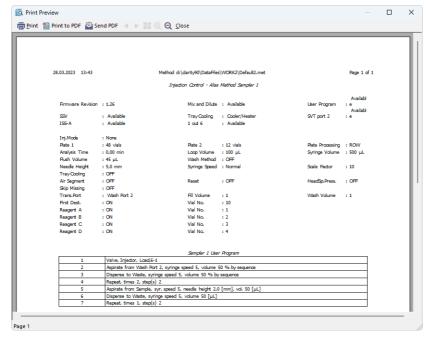


Fig. 22: Spark Alias report preview

All autosampler-specific settings (that means the data from all sub-tabs of the Method Setup - AS tab) are reported as a part of the data displayed by the use of Injection Control checkbox of the Report Setup - Method dialog.

Spark Alias 6 Troubleshooting

6 Troubleshooting

When the remedy for some problem cannot be discovered easily, the recording of communication between **Clarity** and the autosampler can significantly help the **DataApex** support to discover the cause of the problem.

The recording can be enabled by adding or amending the COMMDRV.INI file in the **Clarity** installation directory (C:\CLARITY\CFG by default). The file can be edited in any text editor (e.g. Notepad). Following section should be edited or added:

[COM1] echo=on textmode=on filename=SparkAlias_%D.txt reset=off

Note:

Instead of COM1 type the correct port (or IP address) used to communicate with the **Spark Alias** autosampler. This information is displayed when the *AS Status* button in the <u>Method Setup - AS</u> dialog is invoked or in the <u>Spark Alias Setup</u> dialog. The correct settings should have a format similar to the ones shown in the table for different types of the communication:

Note:

%D (or %d) in the filename parameter means that the log will be created separately for each day. The *reset=off* parameter disables deleting the content of the log each time the station is started during the same day.

Tab. 2: Format of the port settings in COMMDRV.INI

Communication	Format syntax
Serial communication	COMx (where x is the number of the COM port)
LAN communication	UDP x.x.x.x:2101 TCP_IP x.x.x.x:2101 - since version 6.1 (where x.x.x.x is the IP address)
USB communication	FTD2XX SNx (where x is the serial number of the sampler)

The created *.TXT files will greatly help in diagnosis of unrecognized errors and problems in communication. Note that the file size may be quite significant, so in case the error occurs on a regular basis, it might be better to set the Reset=on, start Clarity, invoke the error, close Clarity and send the diagnostics file (the file will be once more reset during the next start of Clarity).

Spark Alias 6 Troubleshooting

6.1 Specific Problems

An error message "Cannot establish communication with ..." appears when opening Clarity Instrument.

Solution:

Check the power cable (**Spark Alias** sampler must be switched on), communication cable and communication settings in the **Spark Alias Setup** dialog.

An error message "AS Error" appears during the Clarity operation.

Solution:

The communication has been interrupted. Check the communication cable as it is most probably disconnected. This message may also occasionally appear after aborting the *ACTIVE* Sequence.

Injection volume set in the Sequence window is not accepted.

Solution:

Either you are using the *Full Loop* option and the injection volume doesn't match the one of the installed injection loop, or you are trying to enter the volume that is greater than the half of the installed *Loop Volume* in the *Partial Loopfill* injection mode.

The Spark Alias autosampler does not start with the injection.

Solution:

Check the Freeze input settings on the <u>Method Setup - AS - Inputs & Outputs</u> tab and used wiring. When the Freeze input is used for synchronization, the injection will not start until the respective input is closed (or opened - the logic is set in the ASM).