

CAMMAG®



INSTALLATION GUIDE DBS-MS 500



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1 Introduction

The main function of the DBS-MS 500 is the fully automated solvent extraction of dried blood spot samples for detection by a mass spectrometer. The samples are delivered on cards, with up to four spots per card. To ensure reliable automated sample extraction and handling, these cards are stored in removable racks which hold them in exactly defined positions inside the instrument. The instruments handling module picks cards out of the rack and carries them to an image processing system and then to an optional station for the application of the internal standard before delivering the card to the extraction unit. Finally, the sampled cards are stored back in the rack position they were picked from. With the image processing system, multiple quality factors and identification features of each card will be measured and checked to ensure the correct spot is sampled and that this spot has not been incorrectly spotted, or previously sampled. The exact positioning of the card in the extraction unit will be made according to these values. The optional IS working solution will be applied at the internal standard station to only the sample to be extracted. At the extraction unit the analyte will be eluted out of the card using the preferred solvents (specific extraction solvent mixture). This extract will then be delivered to the HPLC-MS system for further separation and detection as required. Handling and extraction processes are to be designed for maximum robustness and minimum carryover.

1.1 Precaution



Before installing/using the instrument, check the section “Precaution” in the instruction manual

When working with analytical instrumentation, you must know the potential hazards of using chemical solvents. Many organic solvents, mobile phases and samples are harmful to health. Be sure that you know the toxic and infectious properties of all substances you are using.



This sign draws attention to the fact that failure to take note of the accompanying information (on instrument, in this manual and the respective instruction manual) may result in damage of the instrument



This sign draws attention to the potential risks due to pathogenic or genetically modified organisms and serves to reduce the dispersal and uncontrolled multiplication

of organisms and to protect people from unintentional infection. If handling with such substances be aware of the applicable guidelines.



Be aware of the special risks when opening the instrument

Use only original CAMAG spare parts

Switch off the instrument before you open the instrument

1.2 Parts supplied

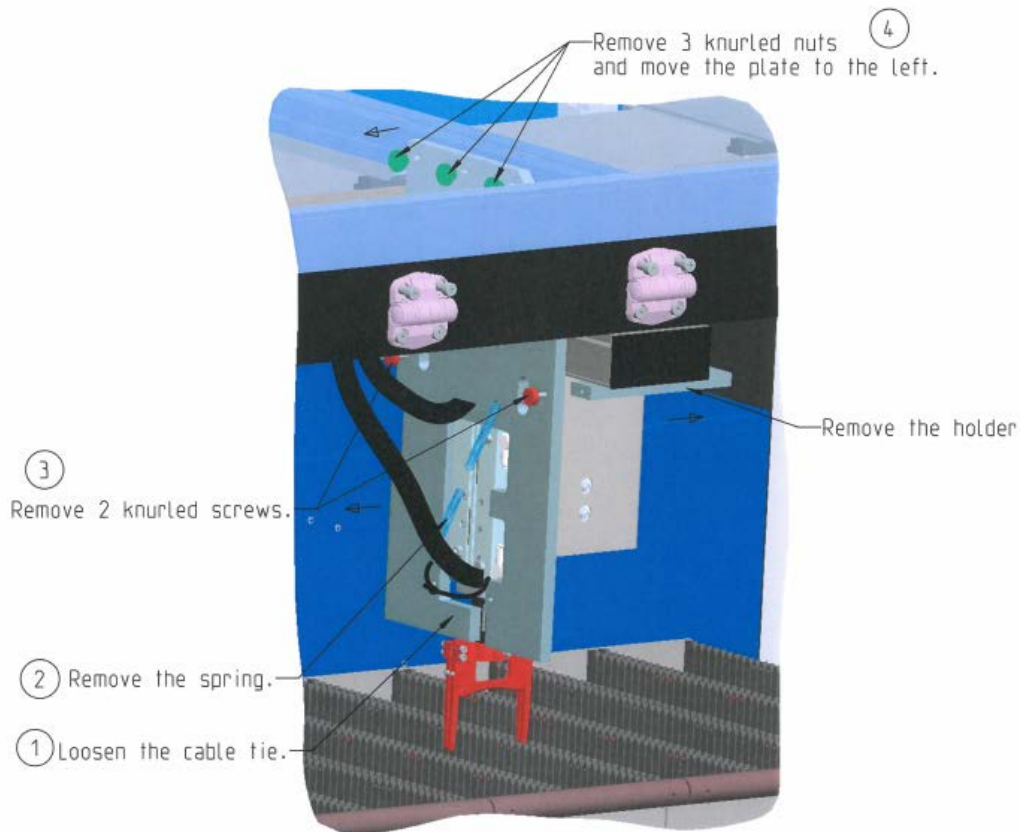
Check with reference to the parts supplied section in the instruction manual for complete shipment of instrument and accessories.

2 Unpacking/Installation

- Check the installation environment according the Environment Specification (CAMAG SOP 402)
- Lift the instrument with the necessary care (4 persons) out of its packaging.
- Place it according the Environment Specification

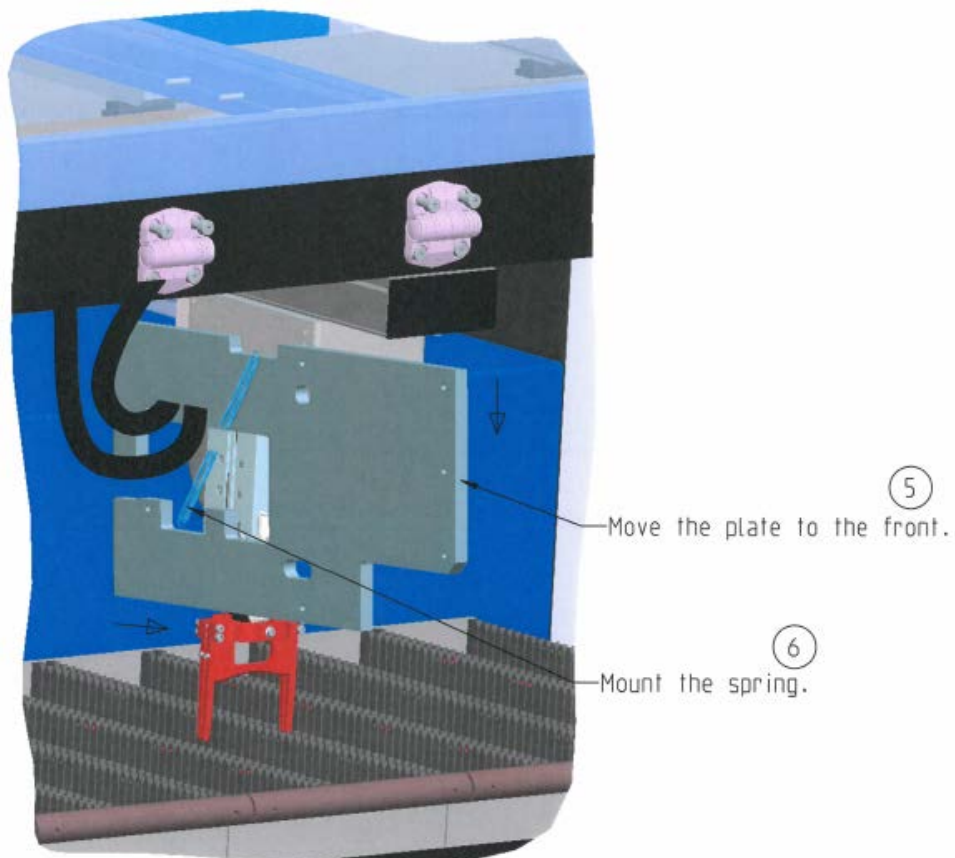
2.1 Removal of shipping protection

1. Loosen the cable tie
2. Remove the spring of the gripper arm
3. Remove the 2 knurled screws and the gripper arm holder
4. Remove the 3 knurled nuts and move the plate of the shipping protection to the left



5. Move the plate of the shipping protection to the front and

6. Mont the spring of the gripper arm



2.2 Installation of the instrument

- Mount the 5 racks (insert the backside of the racks first)

Fluidics



During usage, empty the waste canister on a regular base. If you refill the extraction/rinsing bottles check the solvent level of the waste canister.

Make sure all bottles are clearly and correctly labelled with their content.

CAMAG strongly recommends using degassed solvents only to reduce micro-bubbles in the system.

- Connect the waste canister according below figure



Fig. 1: Waste canister connection

- Fill and connect the solvent bottle 1 (500ml, tube “Elution 1” and “E1/R3”) with your appropriate elution solvent
- Fill and connect the solvent bottle 2 (500ml, tube “Elution 2” and “E1/R3”) with your appropriate elution solvent
- Fill and connect the rinsing liquid bottle 1 (1000ml, tube “Rinsing R1”) with your appropriate rinsing solvent
- Fill and connect the rinsing liquid bottle 2 (1000ml, tube “Rinsing R2”) with your appropriate rinsing solvent
- If applicable, fill and connect the internal standards (IS) bottles (25ml, IS1, 2, 3, 4) with your appropriate standard solution
- Fill and connect the IS rinsing bottle (250ml) with your appropriate solution
- Connect the external system pump with 10-32 fittings to Valve3, port 4; according the below shown figures
- Connect the MS with 10-32 fittings to Valve 2, port 7; according the below shown figures



CAMAG strongly recommends keeping the connection as short as possible (no longer then 2m) to maintain measurement accuracy and quality.

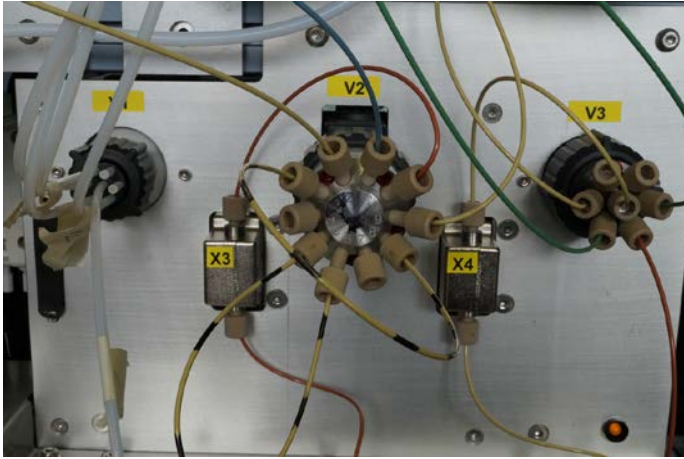
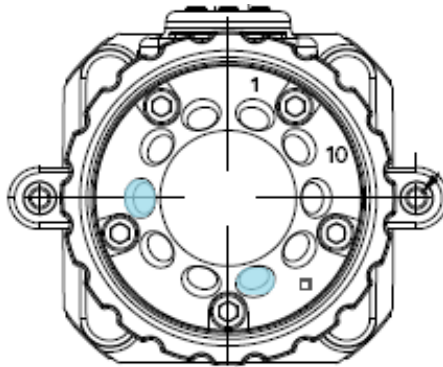


Fig. 2: Valves: Fluid Selection F-V1 / Loop F-V2/ CDM (Controlled Delivery Module) F-V3



Port 4: From LC Pump
Port 7: To LC/MS system

Fig. 3: Loop valve F-V2 connection scheme

Electrics

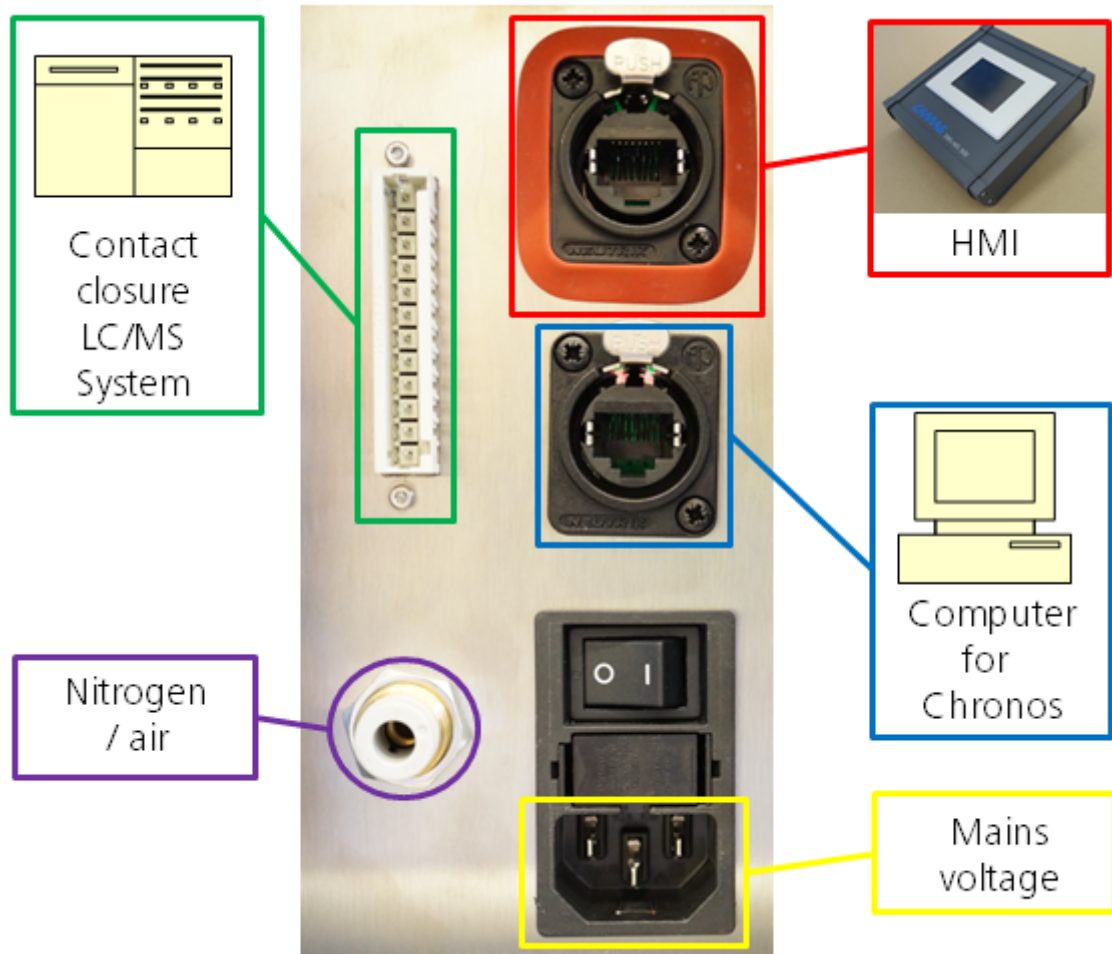


Fig 4. Electric connection panel

- Connect the HMI with the instrument (red)
- Connect the air or Nitrogen pressure (>5.5 bar up to 8 bar) with a 6mm outer diameter hose (purple). Make sure you keep the length of the hose as short and the diameter as big as possible
- Connect the required contact closure according table 1 (green, additional information about the contact closure you can find in chapter 3 Hardware Connections of this document) to your measurement system control
- Connect the power cord (use only the original delivered CAMAG power cord)
- Connect the LAN cable to the computer (for use of CHRONOS software)
- Switch on the unit

Software

Check the user manual of Chronos (by Axel Semrau GmbH & CO KG) for CAMAG for computer requirements and installation of the software.

Priming

Prior to first use or after a longer period of non-using the instrument, the fluidic has to be filled with the respective solvents (Priming).

Priming of extraction circuit

- Choose “Manual”
- Choose “Extractor”
- For the extraction pump (Prime EX):
If the system was completely empty, priming can take up to 50 cycles
- For the rinsing pump (Prime Ri): Activate the pump for about 60 sec.
Priming is finished when the pressure of extraction and rinsing pump is stable (extraction pump only during dosing). If the system was completely empty, priming can take up to 5 min.

Priming of IS circuit

- Choose “manual”
- Choose “Internal Standard”
- Choose the appropriate IS Bottle
- Set the no of Prime Cycles to 5
- Set the destination parameter to “to waste”
- Activate the priming
- Set the destination parameter to “to Needle”
- Activate the priming
- Repeat the steps above until no more air bubbles are visible in the IS syringe

Priming of IS cleaning circuit

- Choose “manual”
- Choose “Internal Standard”
- Set the time parameter to “5'000” (ms)
- Activate the priming by clicking the “cleaning” button
- Repeat the steps above until a nice and constant jet of IS cleaning solvent is visible from the IS needle



At the priming stage, watch out for any signs of leakage at the fluidic part of the instrument.

Your system is now ready to use!

3 Hardware Connections

Remote control allows easy connection between single instruments or systems to ensure coordinated analysis with simple coupling requirements.

CAMAG DBS-MS500 is simply to include into an LC/MS System similar to an Autosampler and uses a contact closure connector to communicate with other parts of the system. In this Attachment, you find some examples with the most common brands.

3.1 CAMAG DBS-MS500 contact closure

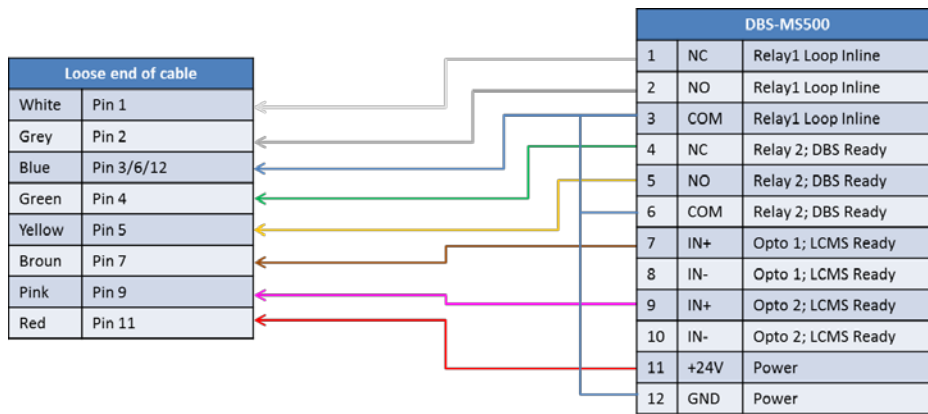
Pin	Signal	Description
1	NC	Relay 1; Loop Inline (Start signal to LC/MS)
2	NO	
3	COM	
4	NC	Relay 2; DBS Ready (DBS-MS500 is ready)
5	NO	
6	COM	
7	IN+	Opto 1; LCMS Ready (LC/MS is ready)
8	IN- (GND)	
9	IN+	Opto 2; Start NxtExtr (DBS-MS500 can start next extraction)
10	IN- (GND)	
11	+24V	Power
12	GND	Power

Contact closure connector

Legend: NC = normally closed
 NO = normally open
 IN+ = optical input positive (24V)
 IN- = optical input ground (GND)

The loose end cable delivered with the instrument has following connection scheme:

Hardware Connections

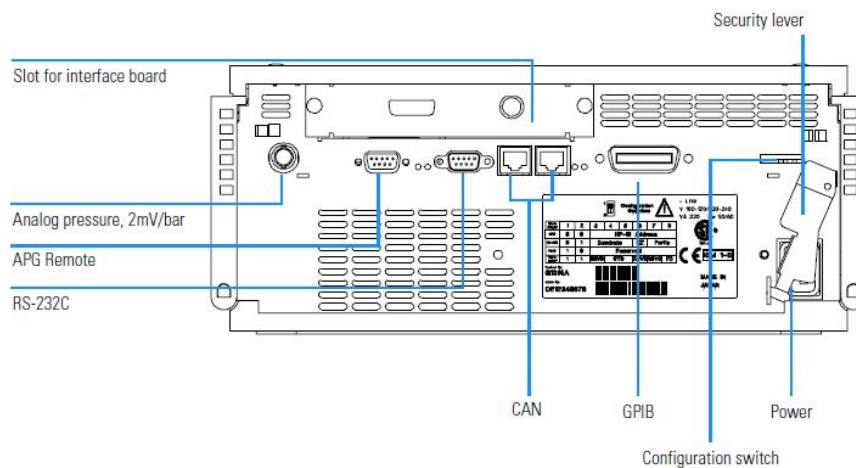


3.2 Connectors on measurement systems

Agilent APG remote interface

Agilent uses the APG Remote Interface; see following drawings and definitions:

Figure 4 Rear of Quaternary Pump



Remote Cables



One end of these cables provides a Agilent Technologies **APG** (Analytical Products Group) remote connector to be connected to Agilent 1100 Series modules. The other end depends on the instrument to be connected to.

- standard TTL levels (0 V is logic true, + 5.0 V is false),
- fan-out is 10,
- input load is 2.2 kOhm against + 5.0 V, and
- output are open collector type, inputs/outputs (wired- or technique).

Pin Agilent 1100	Signal Name	Active (TTL)	Type
1 - White	Digital ground		
2 - Brown	Prepare run	Low	Out
3 - Gray	Start	Low	In
4 - Blue	Shut down	Low	In/Out
5 - Pink	Not connected		
6 - Yellow	Power on	High	In/Out
7 - Red	Ready	High	Out
8 - Green	Stop	Low	In/Out
9 - Black	Start request	Low	Out

AB Sciex AUX/ I/O interface

AB Sciex uses the AUX I/O Interface (8); see following drawings and definitions:

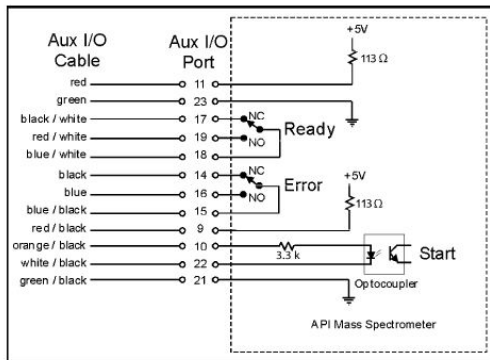
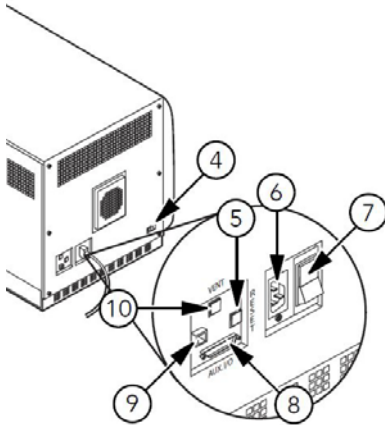


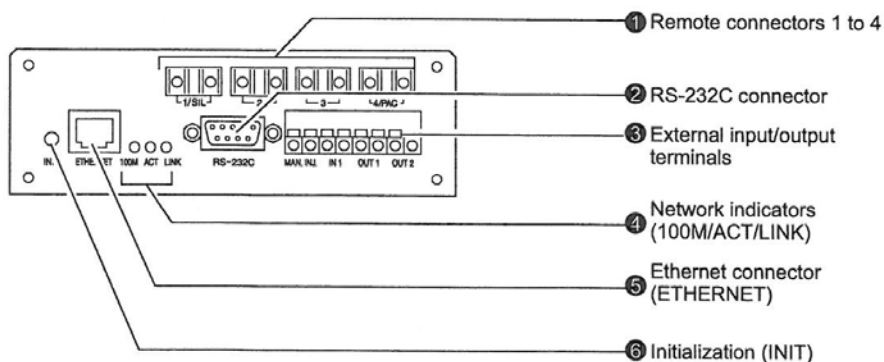
Figure A-2 Schematic of the AUX I/O interface and cable on the 5500 series of instruments.

Mass spectrometer AUX I/O cable		
	Pin 9 (power 5V)	Red with black stripes
	Pin 10 (anode)	Orange with black stripes
	Pin 22 (cathode)	White with black stripes
	Pin 21 (ground)	Green with black stripes

Shimadzu event interface

You have to connect a cable between the MAN. INJ. terminal and Dbs500 out signal "Loop is Inline"; see following pictures:

Back side of Shimadzu Controller CBM-20A/20Alite:



Hardware Connections



External input / output terminals for Contact-Closure controlling.

START Signal "Loop is Inline" from CC Connector of CAMAG DBS-MS500 is connected to signal MAN. INJ. In picture left there is connected an auto sampler to these terminals.

Pin	Signal	Description
(Gray)	1	NC
(Blue)	2	NO
	3	COM

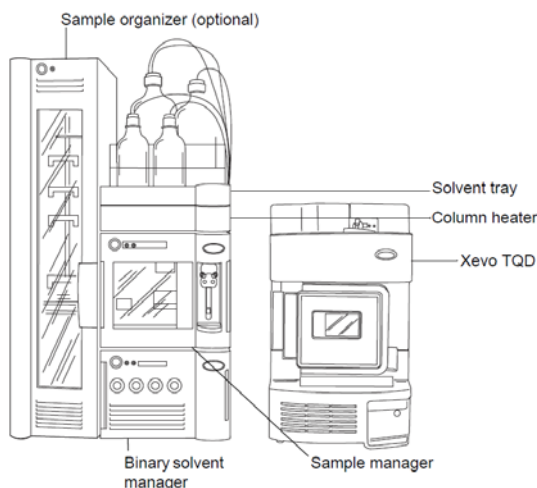
CAMAG DBS-MS 500 CC-Interface



Disconnect terminal 6 and 12 (GND) from CAMAG DBS-MS500 CC cable!

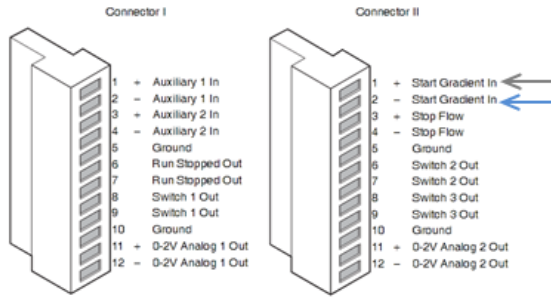
Waters Xevo TQD with AQUITY UPLC system

For remote control you have to use Connectors I/II of respectively used device (Solvent Manager or Xevo TQD Mass spectrometer), see following pictures:

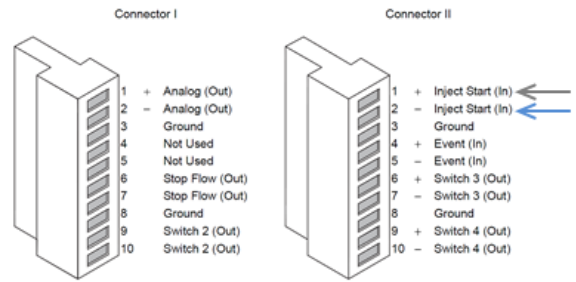


Hardware Connections

Waters Acquity Binary Solvent Manager

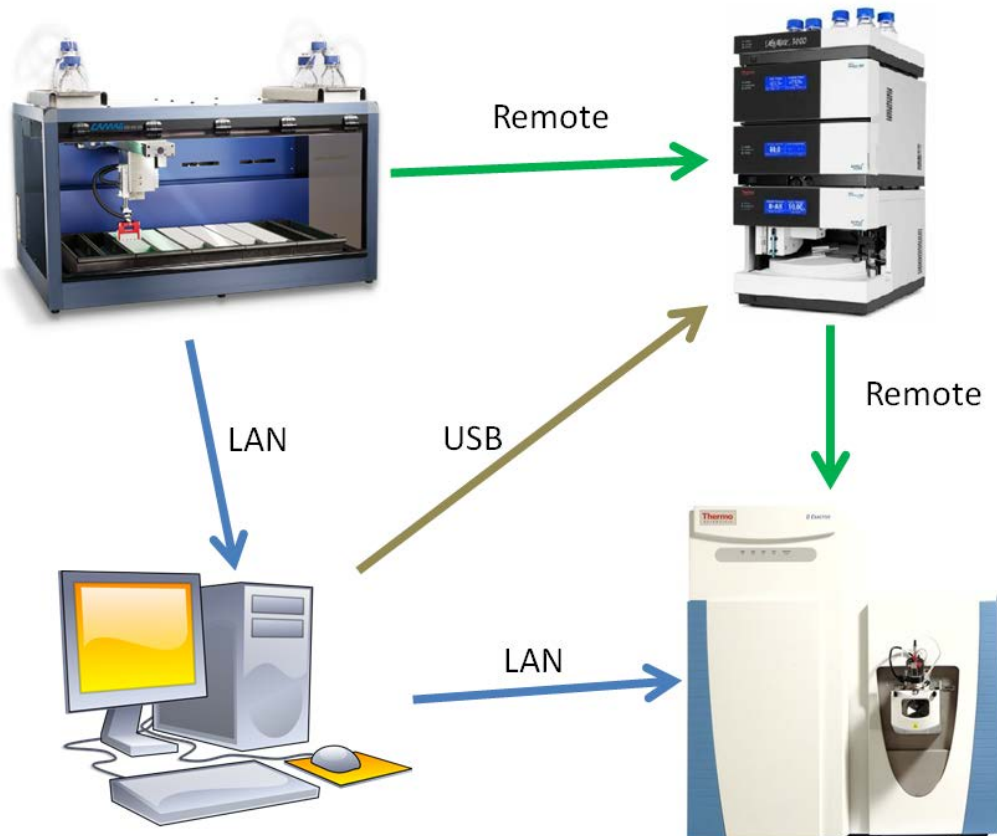


Waters Xevo TQD Mass Spectrometer



Output signal “Stop Flow” could be used as an “Error” signal to stop DBS-MS 500. Switch 2-4 are software configurable signals and could be used to send a “Start Next Sample” signal to the DBS-MS 500. Connect in minimum the DBS-MS 500 signal “Loop is Inline” to Waters connector II at Pin 1-2.

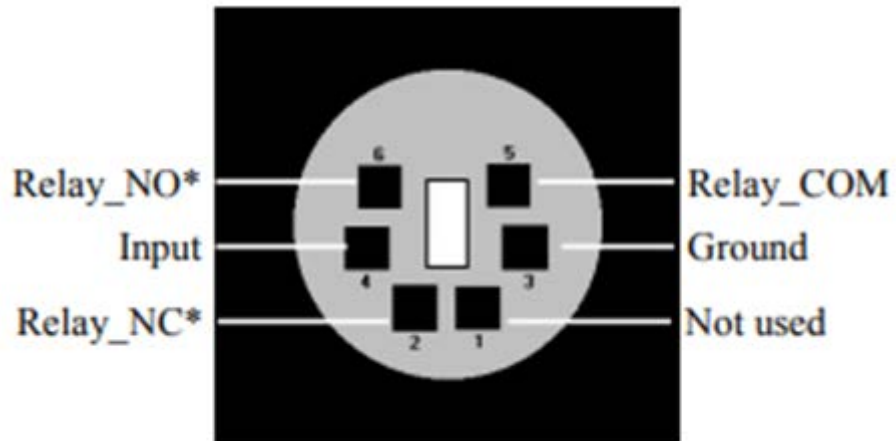
Thermo system under Xcalibur



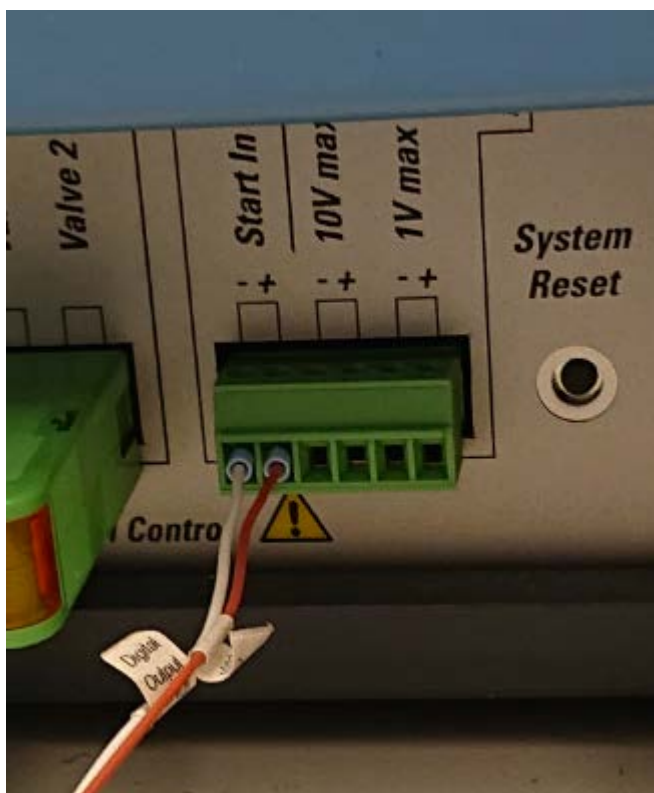
Remote connection between DBS-MS 500 and Thermo (use port 1 at the LC)



Thermo connector to the LC pump



Remote control from LC (output 2) to MS (start in)



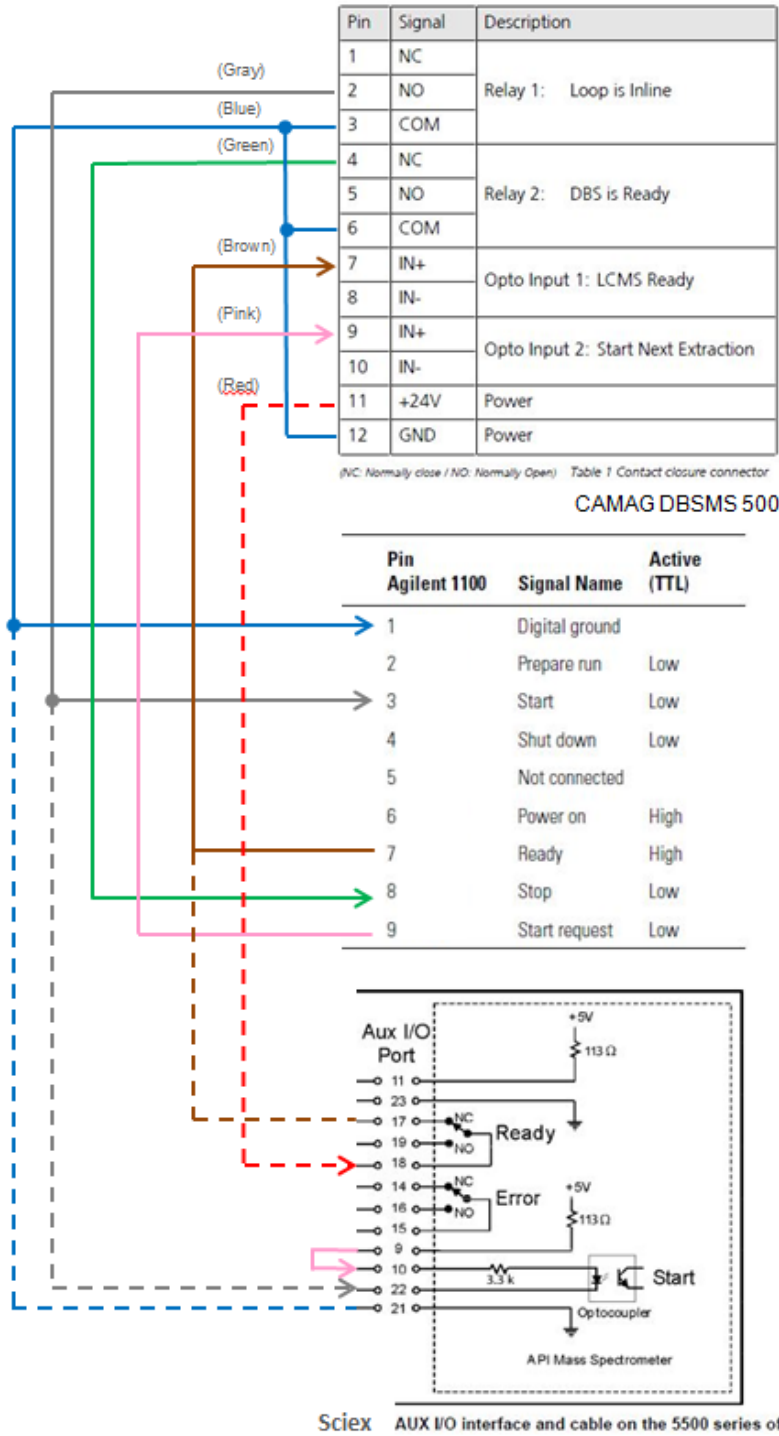
3.3 Connection examples

If you want to use input signals on DBS-MS500 you have to configure this in the service dialog of the DBS-MS500.

LC/MS system of Agilent and Sciex

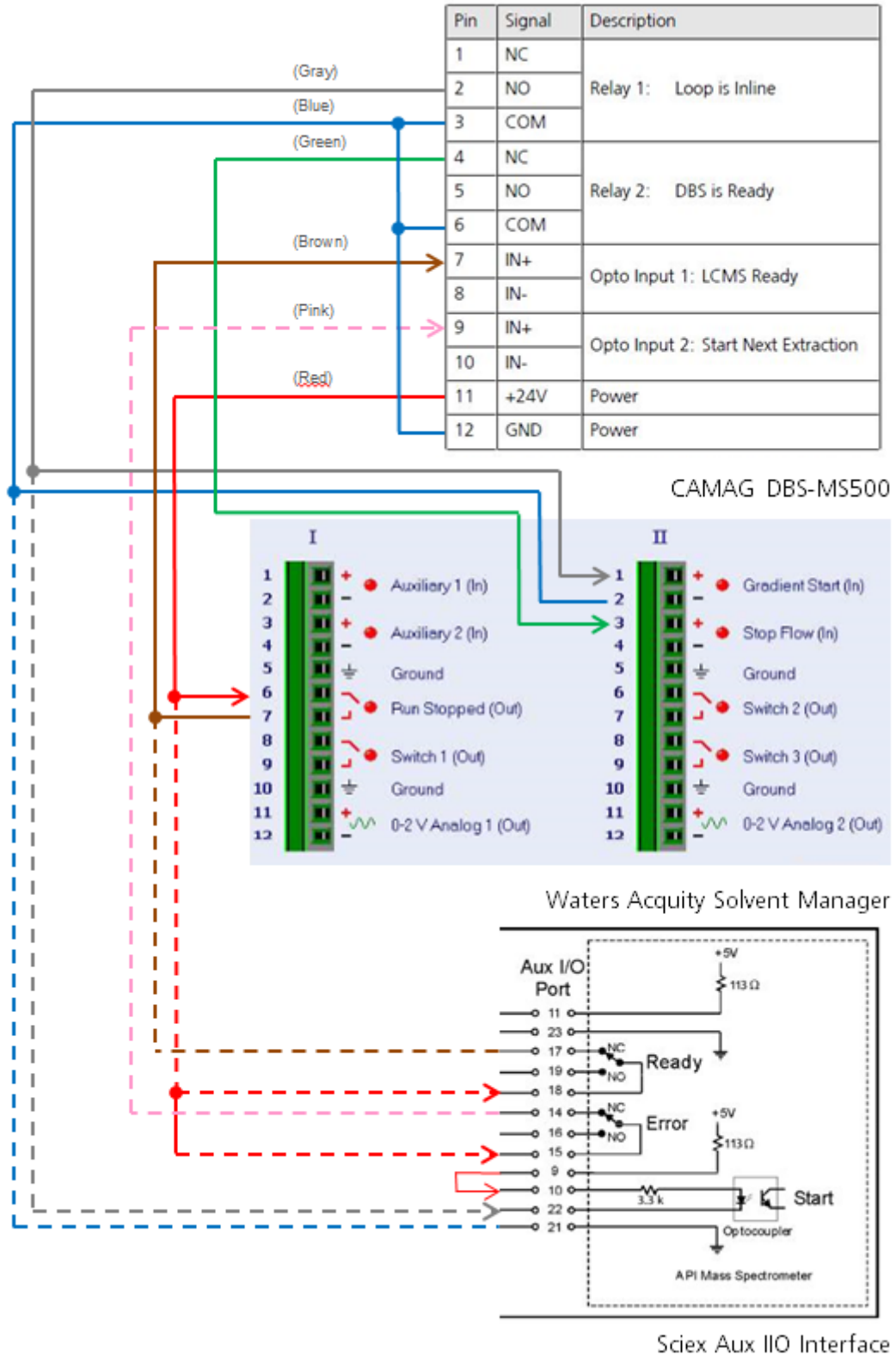
Near the main power socket the 12 Pin Contact/Closure Interface Connector of CAMAG DBS-MS500 can be found. For a minimum wiring you have to connect pin 2/3 of CAMAG connector to pin 1/3 of APG Remote Interface or to pin 21/22 (bridge between pin 9/10) of Sciex AUX I/O connector. Do not connect APG Remote Interface and Sciex AUX I/O in parallel. See following drawing:

Hardware Connections

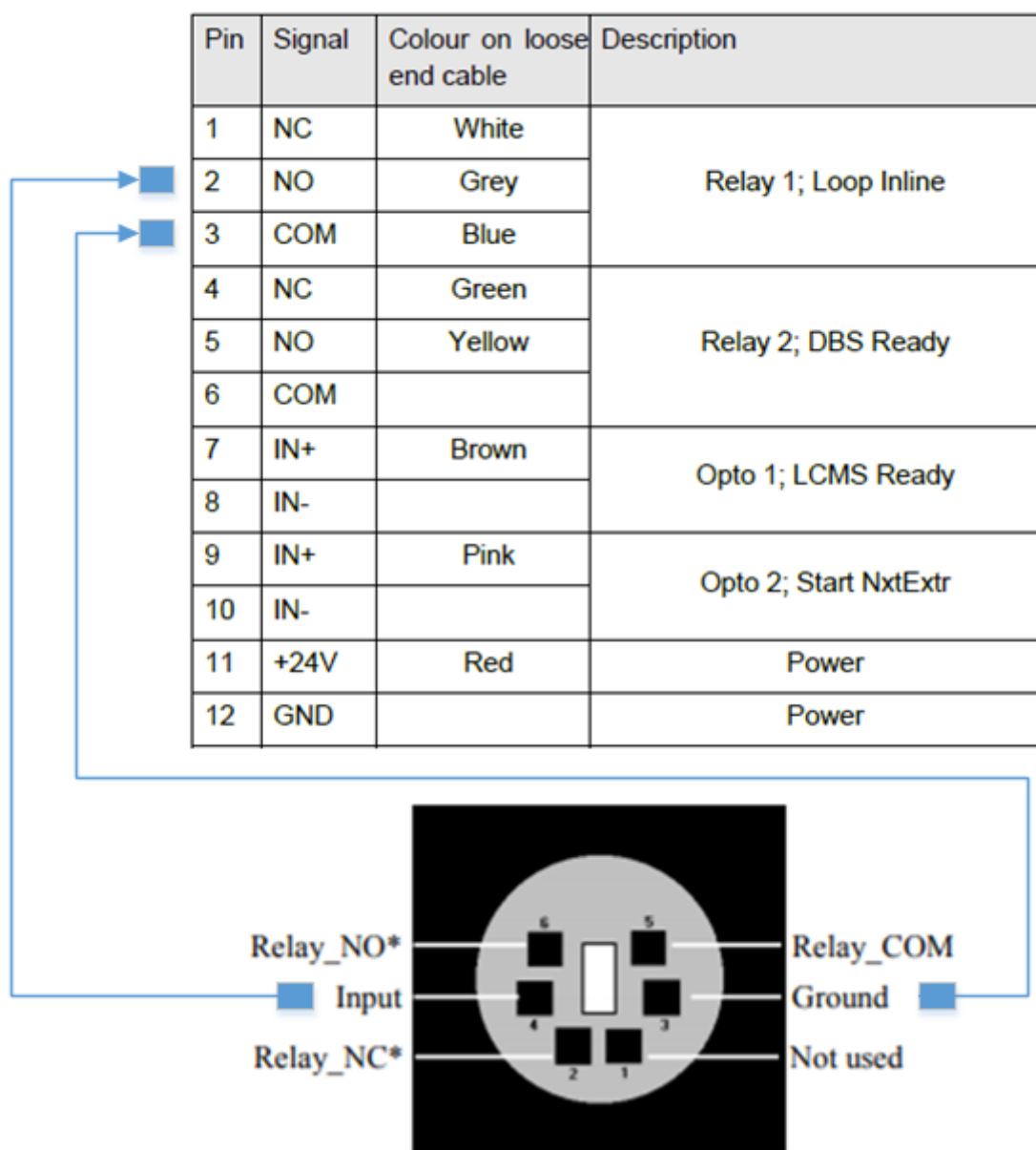


LC/MS system of Waters and Sciex

Near the main power socket, the 12 Pin Contact/Closure Interface Connector of CAMAG DBsMs500 can be found. For a minimum wiring you only have to connect wires on Pin 2/3. Do not connect Waters Interface and Sciex AUX I/O in parallel.



Thermo system under Xcalibur



Note that DBS-MS 500 port 3, 6 and 12 are connected internally within the plug.

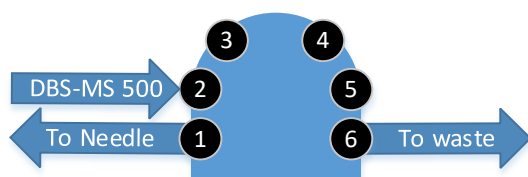
3.4 Coupling a PAL fraction collector

Installation of a PAL fraction collector on the DBS-MS500

- Unpacking and installing the hardware
- Unpack the PAL and place it as close as possible to the DBS-MS 500
- Mount the tools on the PAL and connect them accordingly.
 - Ethernet connection directly into the SWITCH box of the DBS-MS500
 - Power and connection cables according to the “PAL RSI User Manual”

Hardware Connections

- Fluidic capillaries (setup can be changed by adapting the PAL method in the script editor, not recommended):



- Insert a 100 µl syringe into the dilutor and prime it
- Connect the interface cable (remote cable) with the DBS-MS500

Pin PAL	Description	Wire color	Pin DBS-MS500	Description
7	TTL-In 1 Start	Yellow	1	Loop inline (NC)
8	Gnd	Green	3	Loop inline (NC)
3	SW-Out1 (NC)	White	9	+24V
4	SW-Out2 (NC)	Brown	11	Next Sample

- Configure the PAL according the “PAL RSI User Manual”
 - IP = 172.20.62.35

1. Teach all modules

Menu I/O

SWOut 1

Signal On

Low Active

TTL In 1

Signal On

Debug Time 20 ms

Low Active

Menu, Input Signal 1

Signal On x

Time 0 ms

TTL In 1

Menu, Output Signal 1

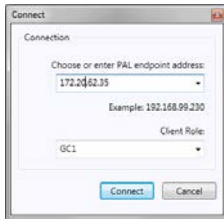
Signal On

SW Out 1

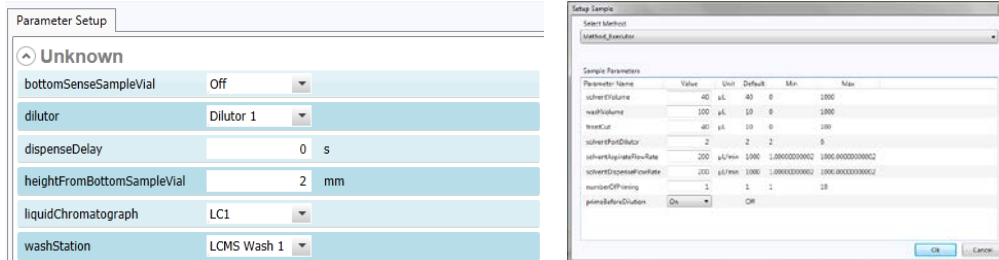
Dest Aux None

Pulse Duration 2000 ms

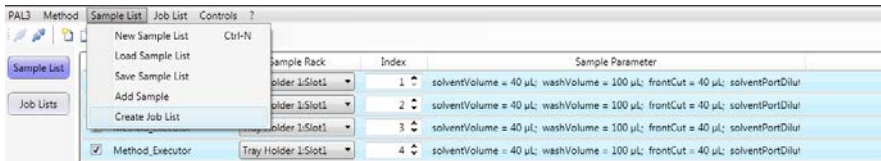
- Open the PAL executor (program on “PAL data for installation and analysis (copy to stick)”)
 - Enter the IP address



- Load “PAL_method.xml” and define all parameters;



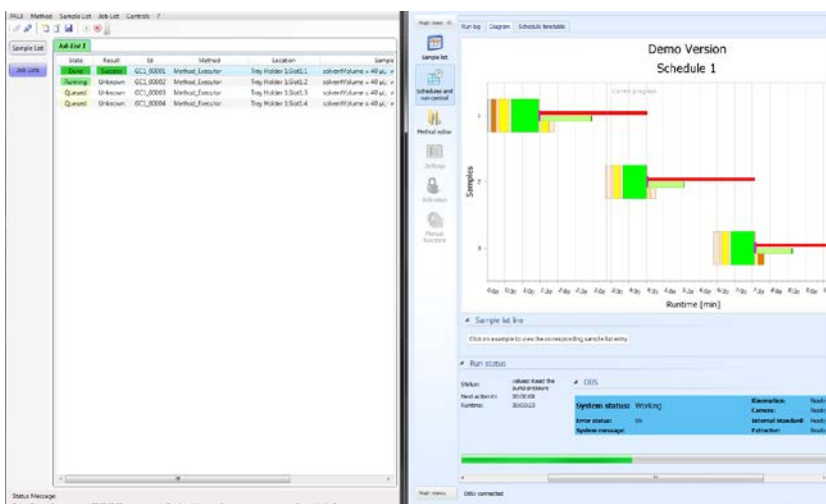
- Create or load a sample list and convert it to a job list



- Load Chronos and create a sample list with the method “Method_Chronos.cam”

Analysis Method	Rack No	Card	Spot	Card Type
1 C:\Users\Administrator\Documents\CAMAG\Using the PAL Method_Chronos.cam	Rack1	1	1	Camag2...
2 C:\Users\Administrator\Documents\CAMAG\Using the PAL Method_Chronos.cam	Rack1	1	2	Camag2...
3 C:\Users\Administrator\Documents\CAMAG\Using the PAL Method_Chronos.cam	Rack1	1	3	Camag2...

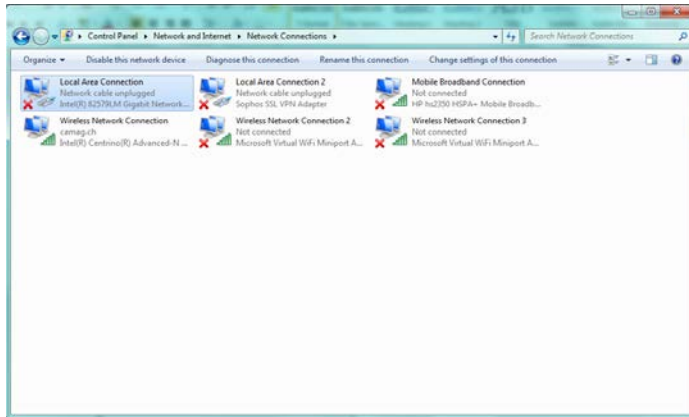
- Start both runs



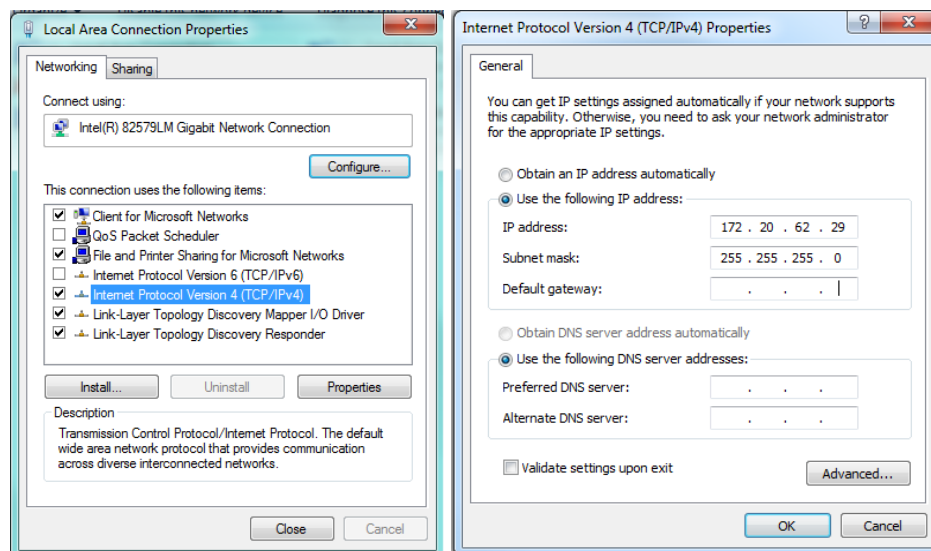
4 Software coupling

4.1 Preparation of the network configuration

- Connecting the DBS to a computer which **is not** on a network
 - Disable wireless



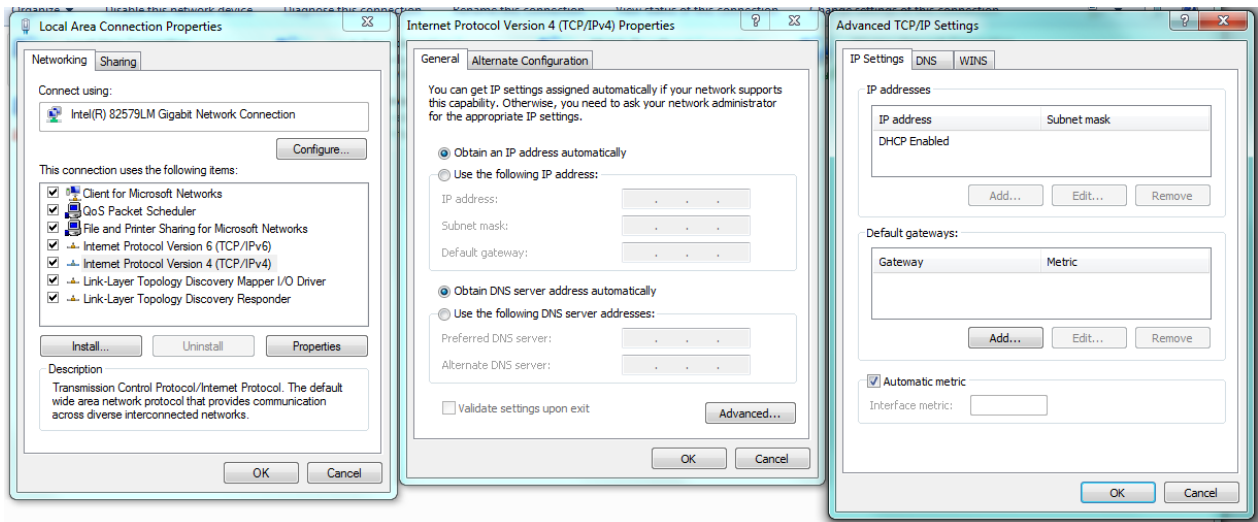
- Enter an IP address in the range of the DBS-MS 500 IP address at the local area connection properties of TCP/IPv4.



- Enable and disable the LAN connection
- Connecting the DBS to a computer which **is** on a network
 - Add a network port to the PC and install the driver
 - Follow steps of "Connecting the DBS to a computer which **is not** on a network"
- Connecting the DBS to a computer which is on a network (2)
 - Plug the DBS-MS 500 into a port of the MS or vice versa
 - Disable wireless

Software coupling

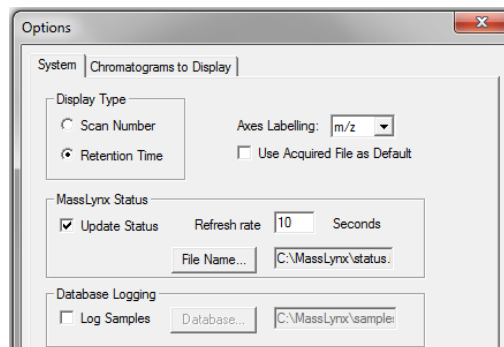
- Enter a IP address in the range of the IP address of the DBS-MS 500 at the local area connection properties of TCP/IPv4 (shown under “Connecting the DBS to a computer which is not an a network”).
- Add the IP address of the MS at Advanced TCP/IP settings



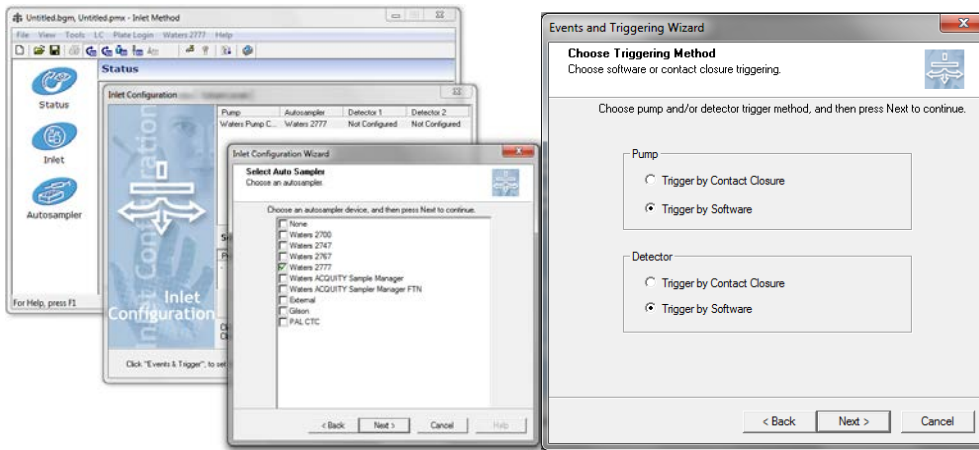
4.2 Masslynx coupling

There are additional steps for the installation of Chronos in combination with Waters devices.

- Check the “Update status” (Masslynx/ Tools/ Options/ Update Status)



- The Folder Masslynx/ Failed needs to be empty
- Masslynx/ Inlet method/ Configuration
 - Signal to External (instead of pump xxx)
 - Trigger via Contact-Closure

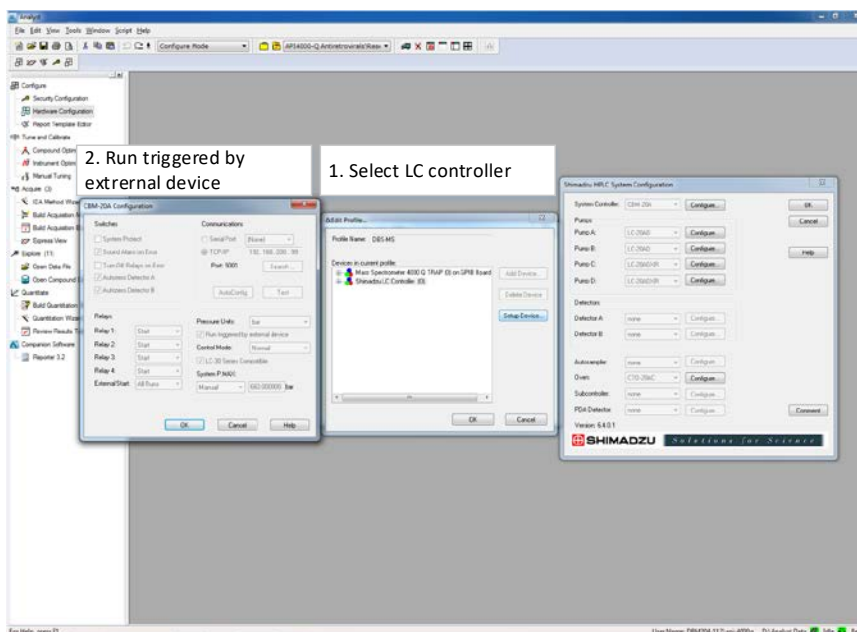
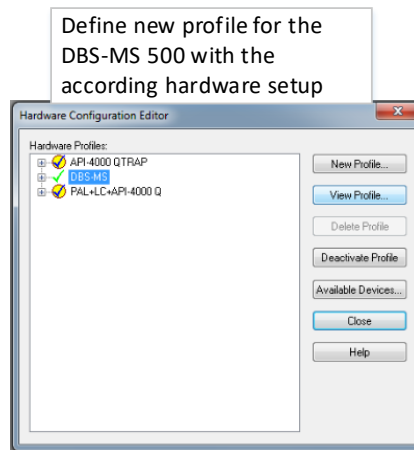


- The Chronos files send by acquisition tasks should have the ending .raw

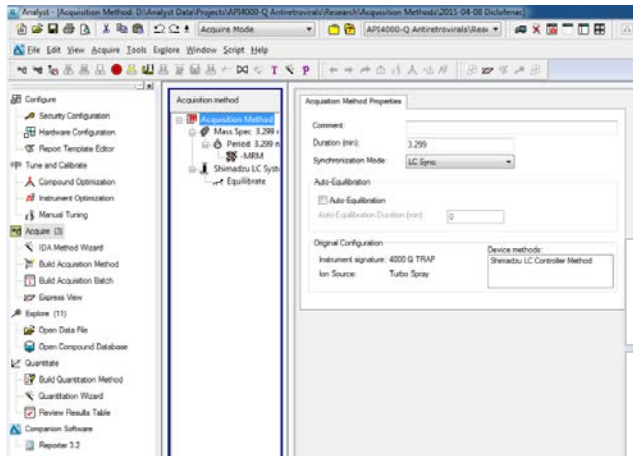
4.3 Analyst coupling

There are additional steps for the installation of Chronos in combination with Sciex devices.

- Hardware configuration in Analyst



- LCMS Method programming



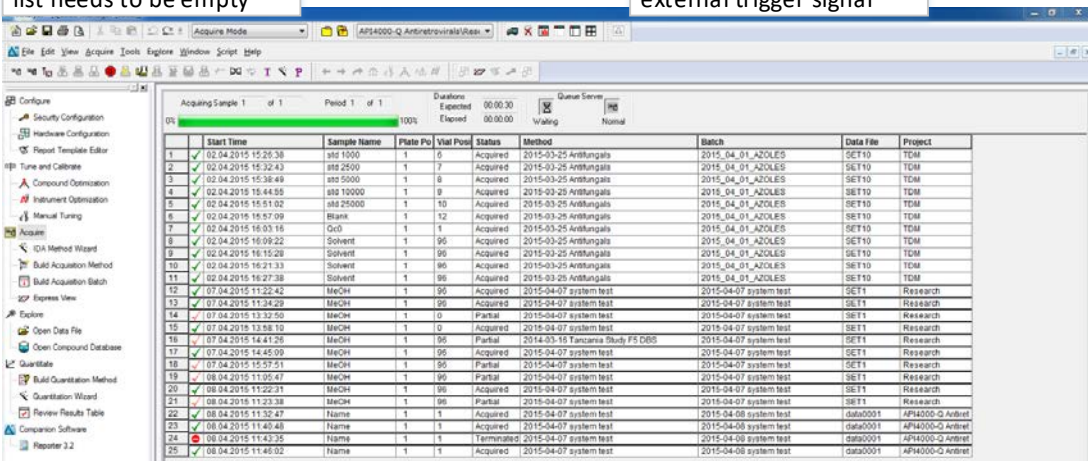
Define the LC-synchronization in the LCMS method

Uncheck auto-equilibration

- Starting a run

1. Start a run here, job list needs to be empty

2. System is waiting for external trigger signal



2.1 LCMS is ready

- The Chronos data file names should have the ending .wiff
- The Method, Output directory and batch have the same name/ directory in Chronos like as in the loaded method of Analyst

MS Method	%METHOD%	String	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	D:\Analyst Data\Projects\API4000-Q Antiretrovirals\Research\Acquisition Methods\2015-04-08 Dicofenac.dam
Output Dir	%DIR%	String	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	D:\Analyst Data\Projects\API4000-Q Antiretrovirals\Research\Data\2015-04-08 dicofenac
Batch	%BATCH%	String	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	D:\Analyst Data\Projects\API4000-Q Antiretrovirals\Research\Batch\2015-04-08 dicofenac.dab

The batch token was added at extended properties.

- Sample position

Analyst uses a different format for the DBS location:

Properties	
Name	Value
Enabled	True
Runtime [s]	1
RespectRuntime	False
ScheduledAfter	-1
AcqService	Analyst
Instrument	
StartDeviceIndex	0
SoftHandshake	True
SequenceType	
SampleType	%SAMPTYPE%
Path	%DIR%
FileName	%FILE%
SampleName	%NAME%
SampleVolume	0
SampleWeight	0
InjectionVolume	0
Position	%RACK%,%CARD%,%SPOT%,1,1
InstrumentMeth	%METHOD%
ProcessingMeth	D:\Analyst Data\Projects\API4000-Q Antiretrovirals\Research\Batch\2015-04-07...

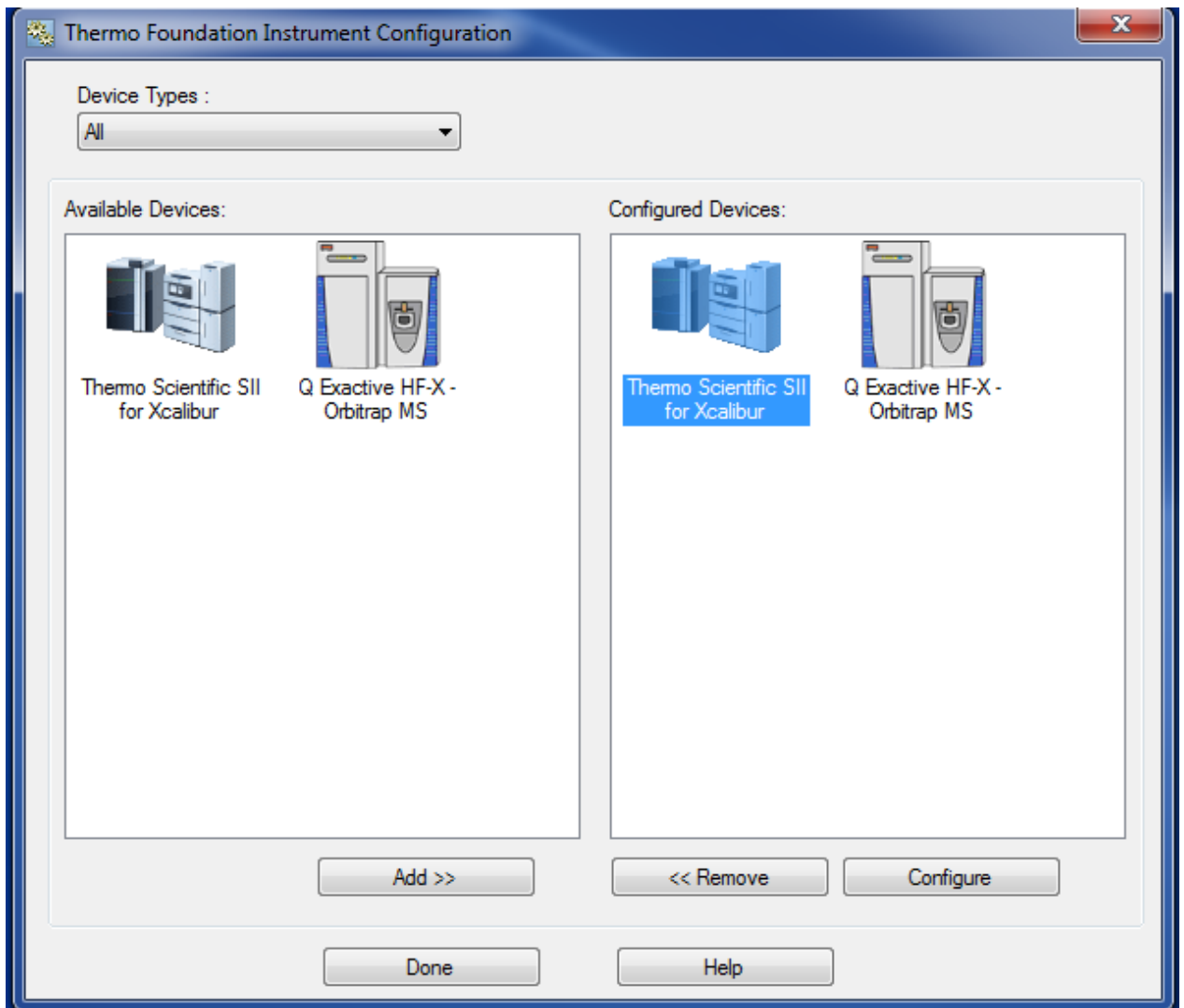
- Check Chronos if the acquisition service was integrated (settings/ instrumental)

4.4 Xcalibur coupling

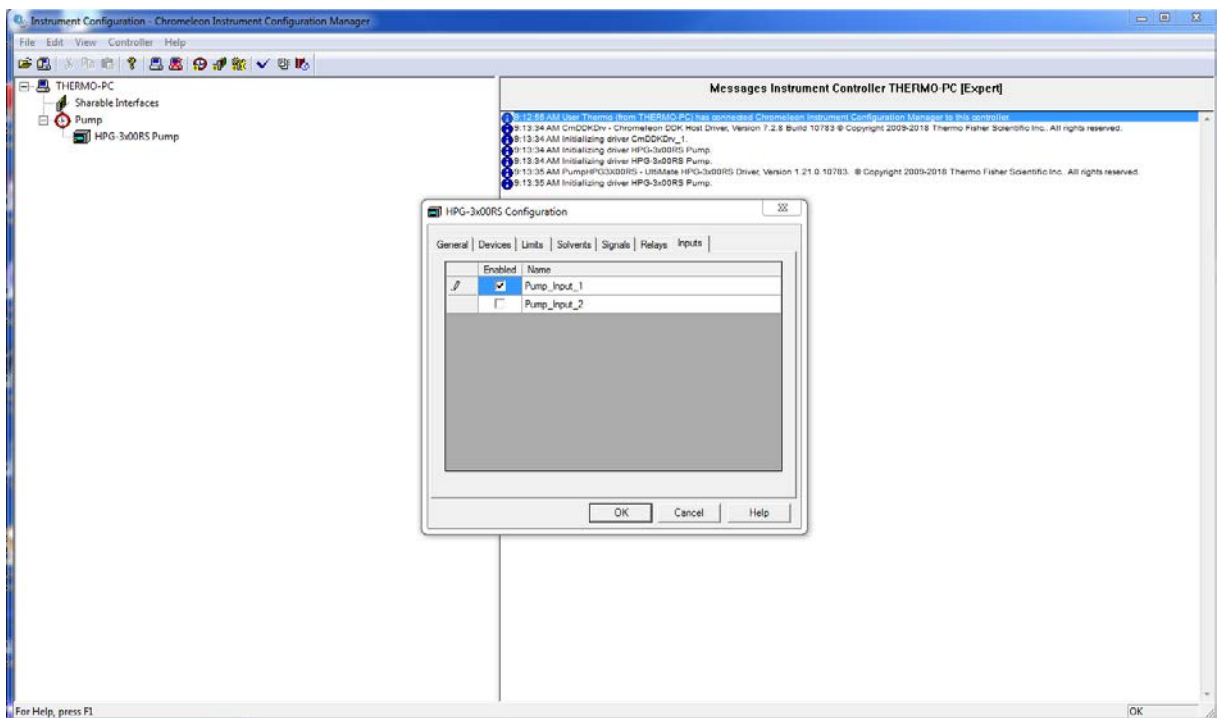
Go to instrument configuration



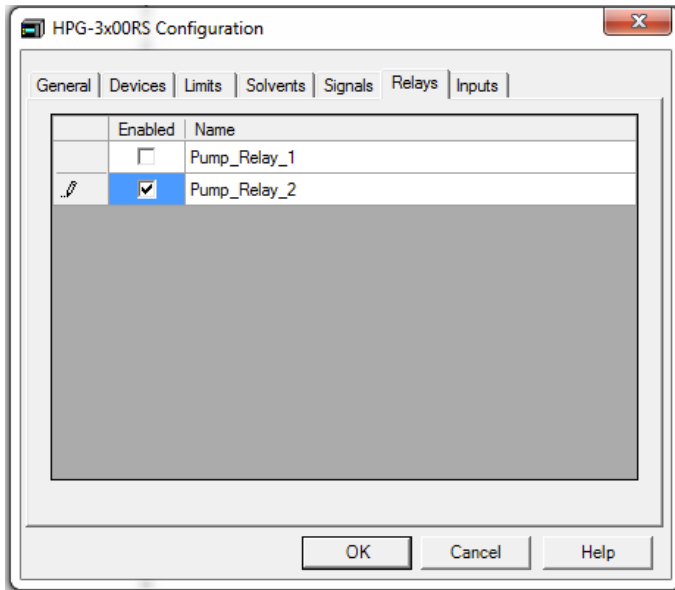
Configure the LC



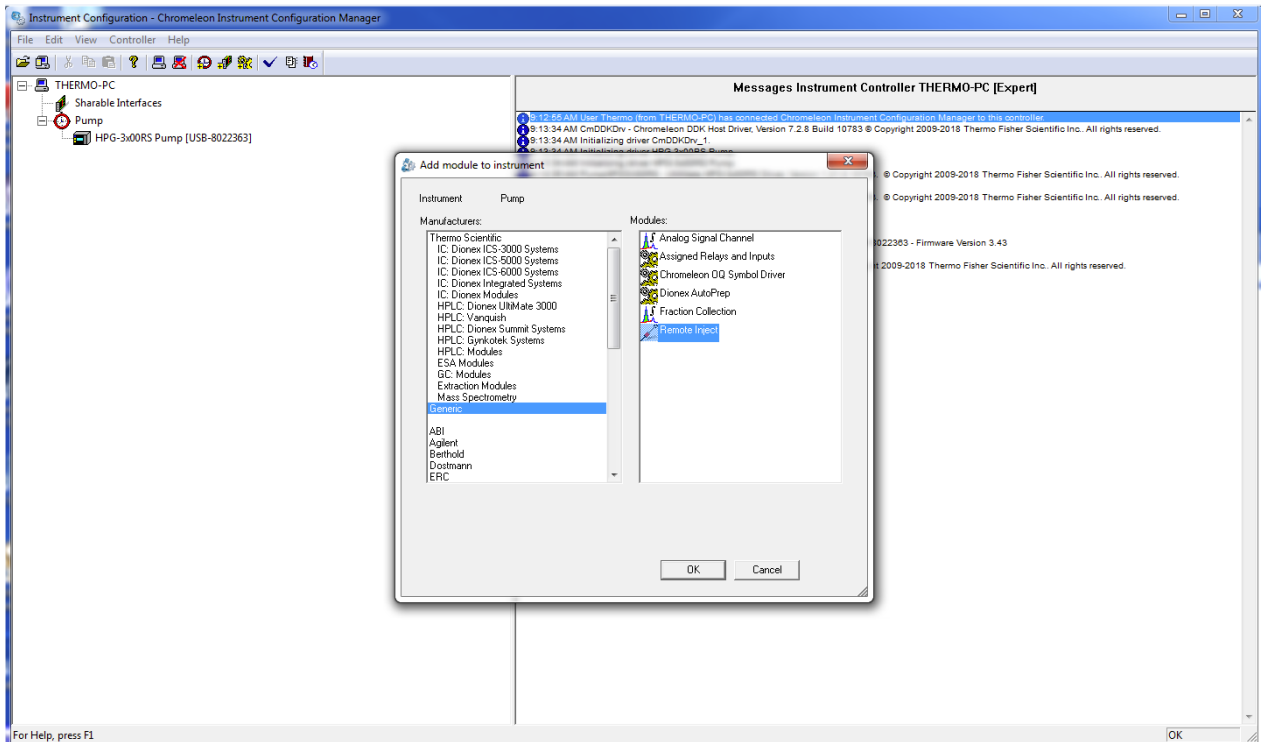
Add the pump:



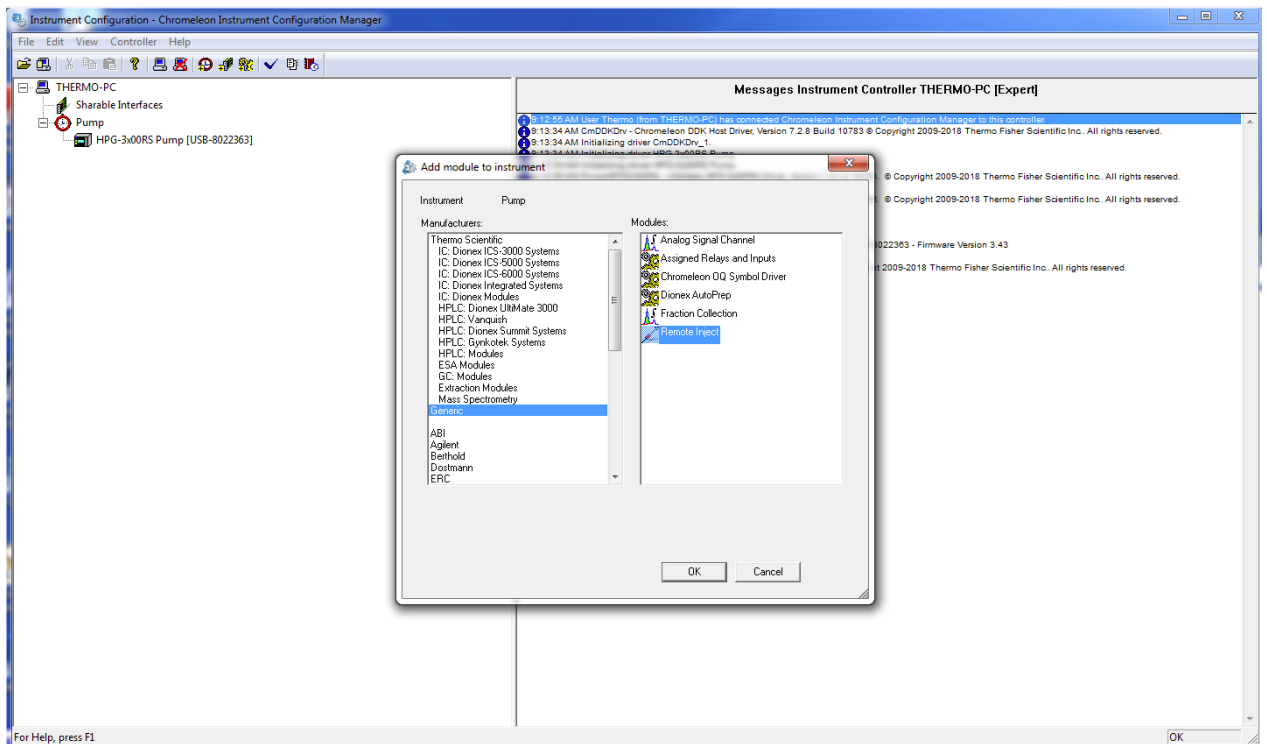
Within the pump setup, select input 1 and Relay 2



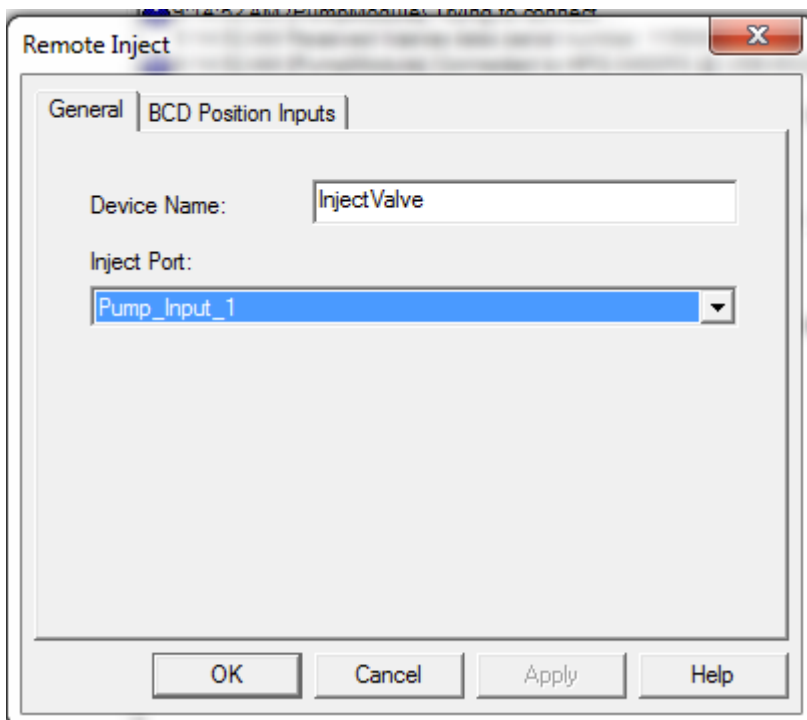
Add the remote inject task



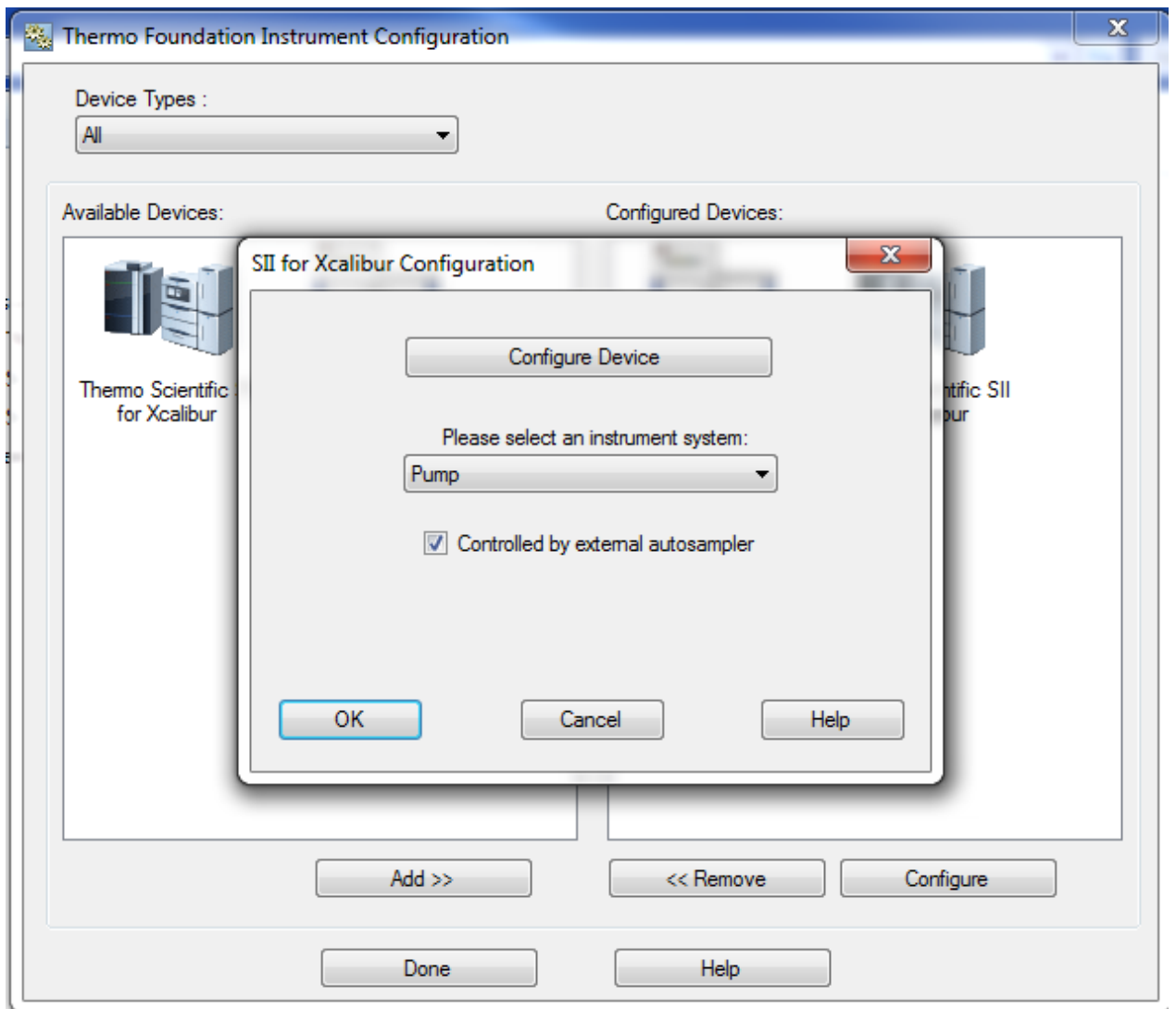
With inject port 1



With inject port 1



Exit the setup and select controlled by external autosampler



Open Xcalibur and program a LC method with inject and a relay tasks:

	Time	Command	Value	Comment
0	{Initial Time}	Instrument Setup		
1		PumpModule.Pump.%A.Equate	"%A"	
2		PumpModule.Pump.%B.Equate	"%B"	
3		PumpModule.Pump.%C.Equate	"%C"	
4		PumpModule.Pump.%D.Equate	"%D"	
5		PumpModule.Pump.Pressure.LowerLimit	0 [bar]	
6		PumpModule.Pump.Pressure.UpperLimit	800 [bar]	
7		PumpModule.Pump.MaximumFlowRampUp	0.101 [ml/min ²]	
8		PumpModule.Pump.MaximumFlowRampDown	9.998 [ml/min ²]	
9	0.000	Inject Preparation		
10		Wait	PumpModule.Pump.Ready	
11	0.000	Inject		
12		InjectValve.Inject		
13	0.000	Start Run		
14		PumpModule.Pump.Relay_2On	Duration=10	
15		PumpModule.Pump.Pump_PressureAcqOn		
16	0.000	Run		
17		PumpModule.Pump.Flow.Nominal	0.200 [ml/min]	
18		PumpModule.Pump.%B.Value	0.0 [%]	
19		PumpModule.Pump.%C.Value	0.0 [%]	
20		PumpModule.Pump.%D.Value	0.0 [%]	
21		PumpModule.Pump.Curve	5	
22	0.500			
23		PumpModule.Pump.Flow.Nominal	0.200 [ml/min]	
24		PumpModule.Pump.%B.Value	0.0 [%]	
25		PumpModule.Pump.%C.Value	0.0 [%]	
26		PumpModule.Pump.%D.Value	0.0 [%]	
27		PumpModule.Pump.Curve	5	
28	0.500	Stop Run		
29		PumpModule.Pump.Pump_PressureAcqOff		
30		End		

- Put the LCMS in a “ready” state:

Status Acquisition Queue

- Run Manager
 - Ready to Download
 - Sequence:
 - Sample Name:
 - Working On:
 - Position:
 - Raw File:
 - Inst. Method:
- Thermo Scientific SII for Xcalibur
 - Ready to Download
- Q Exactive HF-X - Orbitrap MS
 - Ready to Download

4) Simulate start signal

- (DBS service tool) Go to Service-> I/O signals-> fluidic-> give signals by activating “Lp Inline”
- If the run starts, be happy – if not, try other settings in Xcalibur like:
 - High-to-low edge or Low-to-high edge
 - Instrument configuration
 - Contact Thermo service engineer

After this point, you can start working with the instrument by programming independent sample lists in Chronos and in Xcalibur. For full Chronos integration continue further in this guideline.

5) Chronos integration

Use a template method from CAMAG when provided, otherwise:

- Isolate the acquisition task in Chronos method editor (see attached method)
- Select LCMS system and service, Start index must be 1 (due to start instrument information)

Properties		
Name	Value	Visible
Enabled	True	<input checked="" type="checkbox"/>
Runtime [s]	30	<input type="checkbox"/>
RespectRuntime	True	<input type="checkbox"/>
ScheduledAfter		<input type="checkbox"/>
AcqService	Xcalibur	<input checked="" type="checkbox"/>
Instrument		<input checked="" type="checkbox"/>
StartDeviceIndex	1	<input checked="" type="checkbox"/>
SoftHandshake	False	<input checked="" type="checkbox"/>
SequenceType		<input checked="" type="checkbox"/>
SampleType	Unknown	<input checked="" type="checkbox"/>
Path	%DIR%	<input checked="" type="checkbox"/>
FileName	%filename%	<input checked="" type="checkbox"/>
SampleName	%FILE%	<input checked="" type="checkbox"/>
SampleVolume	10	<input checked="" type="checkbox"/>
SampleWeight	0	<input checked="" type="checkbox"/>
InjectionVolume	10	<input checked="" type="checkbox"/>
Position	1	<input checked="" type="checkbox"/>
InstrumentMeth	%METHOD%	<input checked="" type="checkbox"/>
ProcessingMeth		<input checked="" type="checkbox"/>
CalibrationFile		<input checked="" type="checkbox"/>
Level	1	<input checked="" type="checkbox"/>
Multiplier	1	<input checked="" type="checkbox"/>
DilutionFactor	1	<input checked="" type="checkbox"/>
ISTDAmount	0	<input checked="" type="checkbox"/>
UpdateRF		<input checked="" type="checkbox"/>
UpdateRT		<input checked="" type="checkbox"/>
UpdateQI		<input checked="" type="checkbox"/>
UpdateMZ		<input checked="" type="checkbox"/>
Comment		<input checked="" type="checkbox"/>
UserName	Thermo	<input checked="" type="checkbox"/>
Password		<input checked="" type="checkbox"/>
Project		<input checked="" type="checkbox"/>

- The filename should have the ending .raw and the method file .meth
- If you manage to send the signal correctly to Xcalibur, import the working acquisition task into a Chronos standard method

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