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1260 Infinity III LC

# Delivery of Reactants From a Batch Reactor Using the Agilent InfinityLab Online LC Solutions

## Inclusion of third-party pumps by Agilent Online LC Monitoring Software

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### Introduction

For the online monitoring of homogenous chemical and biological transformations, the respective batch reactor must be connected to the Agilent InfinityLab Online LC Solutions by a pump or sample preparation and delivery device. This connected sampling device must be chosen according to the needs given by the chemical or biological conditions. Typically, the used sampling devices are individually customized to the needs by means of available parts. As a test case for the demonstration of the capability to deliver samples from the reactor in near real time to the Online LC System, an aldol reaction was chosen.<sup>1,2</sup> Four different types of sample delivery systems are described: an isocratic pump, a piston pump, a peristaltic pump, and a programmable syringe pump.

This technical overview demonstrates the capability of the Agilent Online LC Monitoring Software to trigger different third-party pumps and sampling devices in a generic manner. This way, a sample can be moved from a batch reactor to the external sampling interface of the Agilent 1260 Infinity II Online Sample Manager and then analyzed by the Online LC System. The necessary software settings and physical connections will be described. The sampling with third-party pumps and devices is completely orchestrated by the Online LC Monitoring Software. This enables easy use of third-party pumps and devices according to the user's needs, and facilitates the connection of reaction vessels to the Online LC System, controlled by Online LC Monitoring Software.

## Experimental

The instrumentation used in this study is detailed in Table 1, and the method parameters are outlined in Table 2.

**Table 1.** Instrumentation.

Product Type	Agilent Product Description
Instrument	<ul style="list-style-type: none"> <li>Agilent 1290 Infinity II High-Speed Pump (G7120A)</li> <li>Agilent 1260 Infinity II Online Sample Manager Set (G3167AA): <ul style="list-style-type: none"> <li>Agilent 1260 Infinity II Online Sample Manager (G3167A) clustered with external valve (part number 5067-6680) located at the Agilent 1290 Infinity Valve Drive (G1170A), and Agilent Online LC Monitoring Software</li> </ul> </li> <li>Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)</li> <li>Agilent 1290 Infinity II Diode Array Detector (G7117B) with Agilent InfinityLab Max-Light Cartridge Cell (10 mm, G4212-60008)</li> </ul>
Additional Pumps for Sample Delivery	<ul style="list-style-type: none"> <li>Agilent 1260 Infinity II Isocratic Pump (G7110B)</li> <li>Watson-Marlow 120U/DV peristaltic pump</li> <li>Masterflex Ismatec Reglo digital pump equipped with piston pump head</li> <li>New Era NE-1000 programmable syringe pump</li> </ul>
Column	<ul style="list-style-type: none"> <li>Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 30 mm, 1.9 µm (p/n 695775-302)</li> </ul>
Software	<ul style="list-style-type: none"> <li>Agilent OpenLab CDS, version 2.6</li> <li>Agilent Online LC Monitoring Software</li> </ul>

Please note that depending on the third-party device, specific ERI cables must be used to connect the device to the Agilent InfinityLab Online LC Solutions. The ERI cables used in this study, and their part numbers, are highlighted later and in the Appendix.

For a detailed description of sample delivery pump setup, see the Appendix.

**Table 2.** Method parameters.

Parameter	Value
<b>Analytical Method Conditions</b>	
Solvents	A) Water + 0.1% formic acid (FA) B) Acetonitrile (ACN) + 0.1% FA
Analytical Flow Rate	1.3 mL/min
Gradient	40% B to 90% B in 0.85 min, stop time: 1.0 min
Column Temperature	45 °C
Feed Speed	80% of analytical flow rate
Flush-Out Solvent	Water:ACN 9:1 + 0.1% FA (S2)
Flush-Out Volume	Automatic
Injection Volume	1 µL
Needle Wash	3 s, water:ACN 1:1 + 0.1% FA (S1)
Sampling	See sampling methods for sampling to vial
Diode Array Detector	290 ± 4 nm, reference: off, 40 Hz data rate

<b>Sampling (With Dilution)</b>	
Sampling	Sampling from reactor to deep-well plate sealed with silicon mats
Target Volume	600 µL
Dilution Factor	100
Sample Volume	6 µL
Draw Speed	Setting 2 – Draw speed: 100 µL/min – Wait time: 3.6 s – Dispense speed: 130 µL/min (Ejection of sample into well before dilution)
Dilution Solvent	S2
Dilution Eject Speed	10,000 µL/min (after sample ejection for mixing)
Schedule	Interval: 4 min Experiment run time: 120 min
<b>Reaction Conditions</b>	
Reactor	Mettler-Toledo EasyMax 102 equipped with 50 mL reaction vessel; temperature controlled by a connected JULABO thermostat
Solvent	50 mL acetone:water 2:1 (v/v)
Educt	p-Anisaldehyde, 0.5 mL
Stirring	At 25 °C
Reaction Start	Add 100 µL NaOH 50% in water (w/w)

### Chemicals

- p-Anisaldehyde
- Acetone
- NaOH
- Formic acid

### Additional materials

- Agilent 96-deep-well plates, 1 mL, polypropylene (part number 5043-9305)
- Agilent sealing mat, 96 wells, round, preslitted, silicone (part number 5043-9317)
- Agilent ERI cable ERI-ERI (part number 5188-8044)
- Agilent ERI cable APG-ERI (part number 5188-8045)
- Agilent ERI cable extension (part number 5188-8059)
- Agilent ERI cable with open end (part number 5188-8029)
- Agilent 0.8 mm id PTFE tubing (part number 5041-2191), ferrule (part number 5022-2154), PTFE nuts (part number 5022-2158), fittings, and ferrule (part number 5065-4454)

### Solvents and chemicals

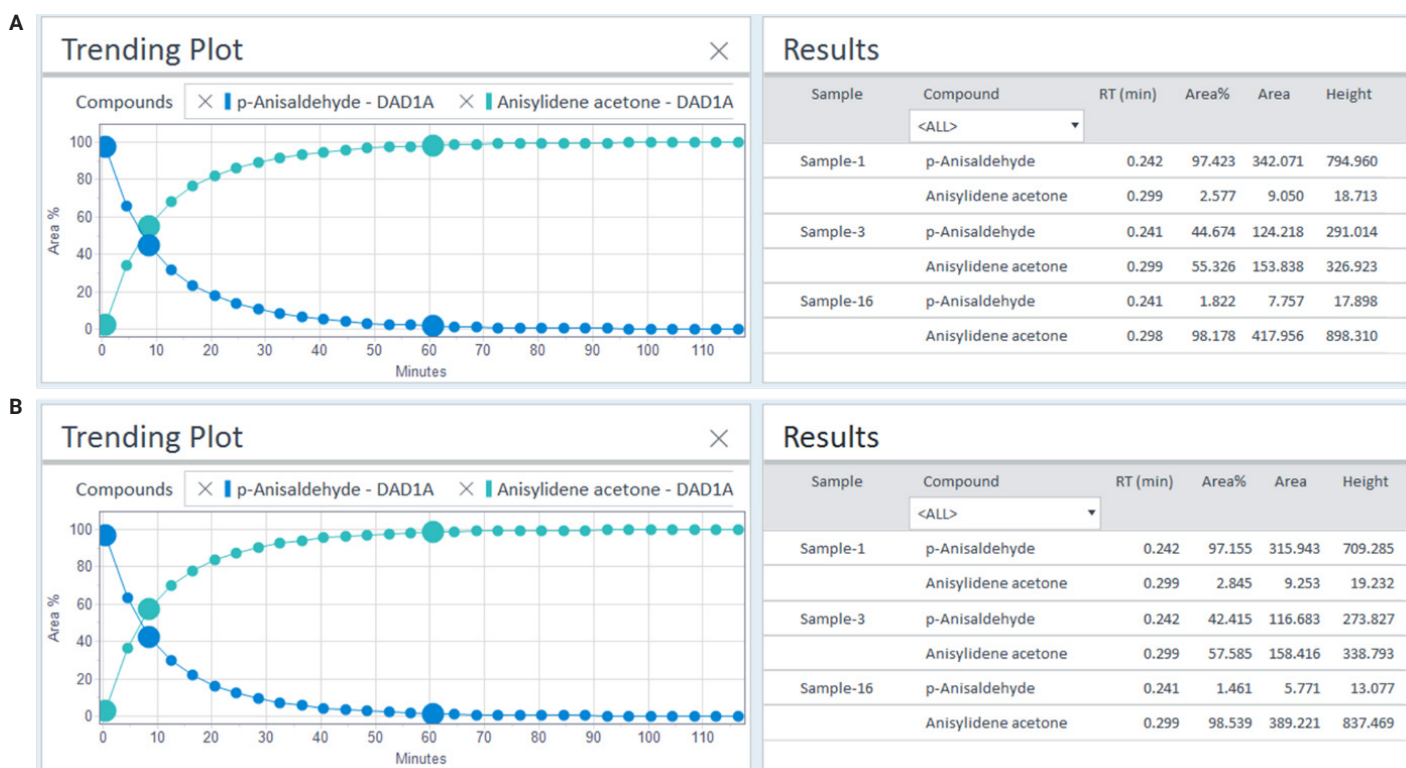
- All solvents were purchased from Merck, Germany.
- Chemicals were purchased from VWR, Germany.
- Fresh, ultrapure water was obtained from a Milli-Q integral system equipped with LC-Pak polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

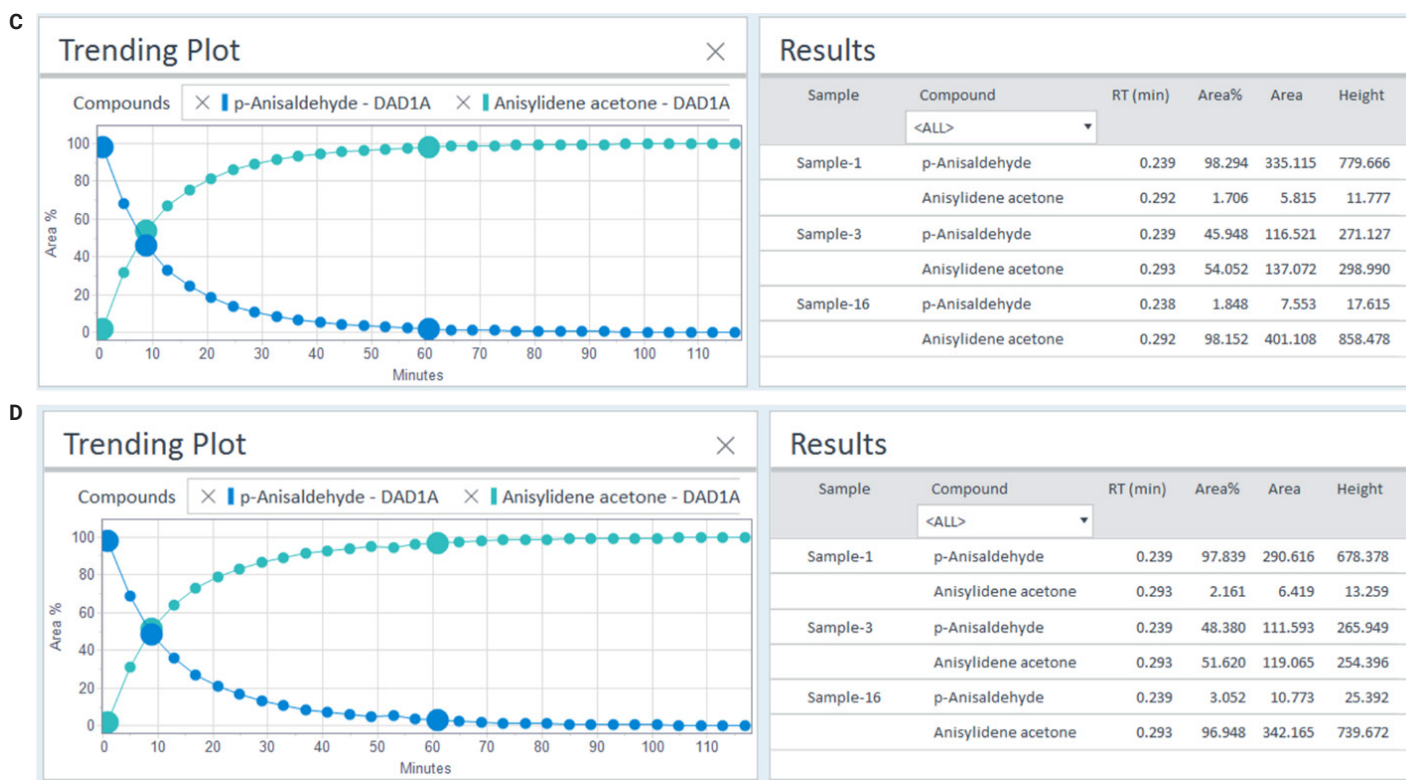
## Results and discussion

For the evaluation of different generic sample delivery pumps and systems, pumps with different working principles were randomly selected and used as examples: a piston pump, a peristaltic pump, and a syringe pump. As a reference pump, an Agilent 1260 Infinity II Isocratic Pump was used. Since HPLC pumps are high-performance pumps and are typically used for analytical workflows, a more economical piston pump can be utilized to transport the reactor solution to the Online LC system to draw a sample. As an example, the setup and use of the Masterflex Ismatec digital piston pump is described. A peristaltic pump can be utilized to deliver the sample from the chemical or biological reactor as a more economical alternative. The utilized tubing is made from chemically resistant material or specialized material for biological media. After use, the tubing can be discarded and replaced easily. As an example, the setup and use of the Watson-Marlow peristaltic pump is described. For extended sample handling, programmable sample delivery devices can be used. As an example, the setup and use of the New Era NE-1000 programmable syringe pump is described.

To compare pump performance, a small-molecule aldol condensation reaction was chosen as model reaction, with the expectation that all connected pumps provide an identical result in the Online LC Monitoring Software (Figure 1). As benchmark, the Agilent 1260 Infinity II Isocratic Pump was used. The trend plot achieved from the acquired data shows the crossing of the declining educt curve with the increasing product curve near to the drawn sample 3 at approximately 8.50 minutes (Figure 1A). Also highlighted are the sampling points at the beginning and at 60 minutes reaction time. The respective table in Figure 1A shows the area percentage value at these points. For instance, the first sampling shows an area percentage of 2.577% product, and the sampling point at the crossing of the curves displays 55.326% of product.

The results obtained for the other pumps are shown in Figure 1, B to D. A comparison of the results obtained for the different sampling points shows that they are very close to each other for the peak areas, heights, and area percentage at the respective sampling points. This clearly demonstrates that the used sample delivery pumps and devices with their different working principles provide similar results for a given reaction, and can be applied without significant differences.





**Figure 1.** Results of the model reaction for comparison of different sample delivery pumps and devices. (A) Agilent 1260 Infinity II Isocratic Pump (G7110B), (B) Watson-Marlow 120U/DV peristaltic pump, (C) Masterflex Ismatec digital pump equipped with piston pump head, (D) New Era NE-1000 programmable syringe pump.

## Conclusion

This technical overview demonstrates the use of generic sample delivery pumps and devices triggered by the Agilent Online LC Monitoring Software. These pumps and devices deliver the sample from a chemical or biological batch reactor to the external sampling interface valve of the Agilent Online LC System. The applied pumps and devices are based on different working principles and provide similar results. The use of different generic sampling pumps and devices enables the user to select the pumps and devices according to the need for the respective chemical or biological reaction medium.

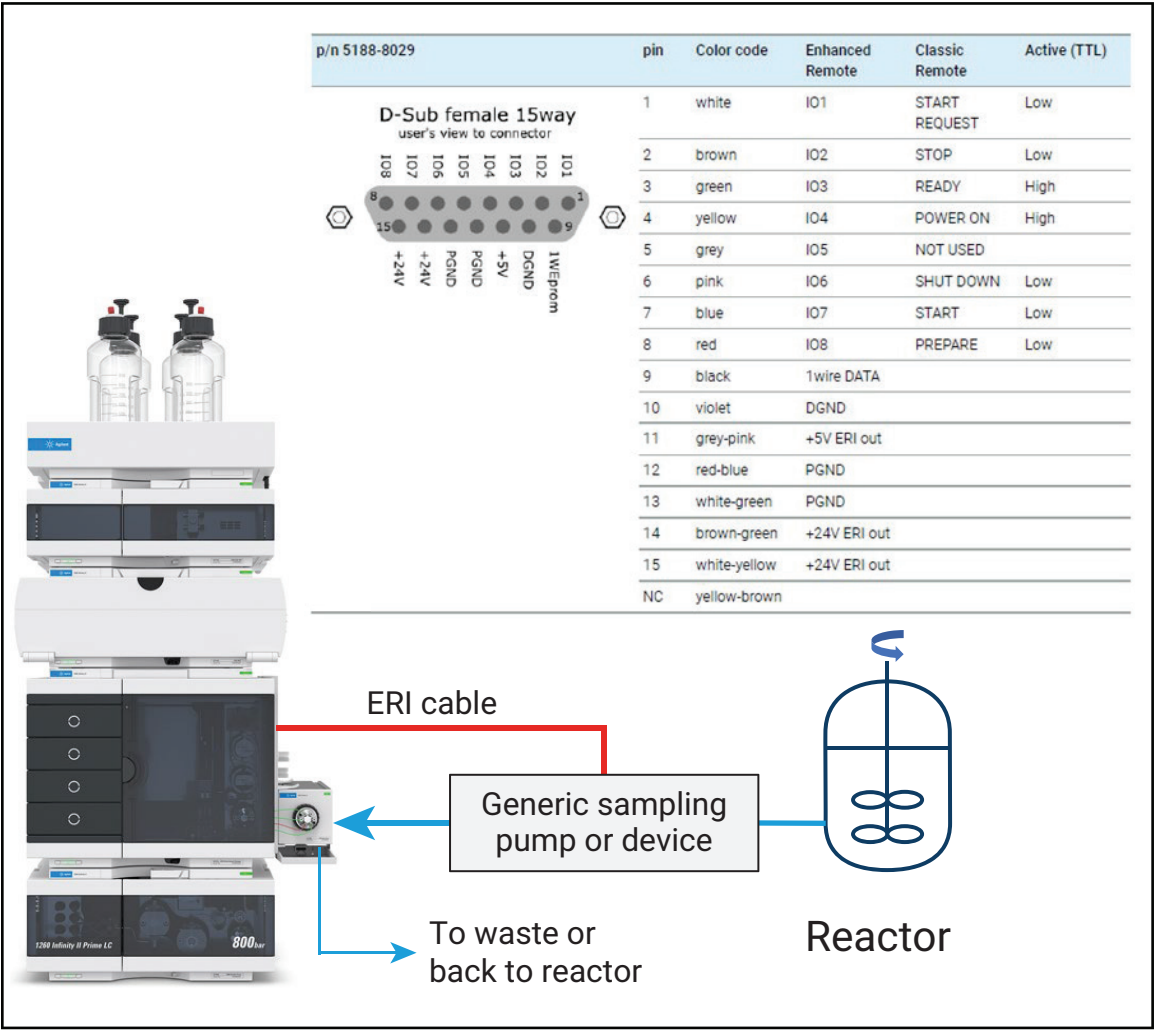
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Appendix

In the following sections, detailed technical information can be found on how to connect the devices mentioned in this technical overview to the Agilent Online LC System (Figure 2). In addition, this information can be used as an example of how to connect sampling devices from other manufacturers. Indeed, most devices capable of handling at least an external start signal are suitable. If the device is programmable and its external communication interface offers additional input/output controls, features like "Sample ready" or "Sampling done" can be used, as described for the syringe pump from New Era Systems. The communication via the ERI cable with the Agilent 1260 Infinity II Online Sample Manager

follows the TTL standard, using + 5 V for logic high level, and 0 V for logic low level. Pin 10 of the 1260 Infinity II Online Sample Manager is reserved for the digital ground reference. Due to the capability to select the desired pins and the desired polarities (= logic high and low level) in the configuration section of the Agilent Online LC Monitoring Software, control of these external sampling devices is straightforward. Information about which pin and polarity must be selected for a specific sampling device can usually be found in the manual of the respective device. The choice of the required ERI cable depends on the connector type of the sampling device, and on the compatibility of the location of the ground pins; in some cases a customized ERI cable might be required based on the Agilent ERI cable with open end (part number 5188-8029).



**Figure 2.** Schematic of the connection of a chemical or biological reactor to the Agilent 1260 Infinity II Prime Online LC System via a generic sampling pump or device, and connection with ERI cable for control by the Agilent Online LC Monitoring Software.



### Example 1: Generic sample delivery pump control

1260 Infinity II Isocratic Pump (G7110B)<sup>3</sup>

The Agilent 1260 Infinity II Isocratic Pump can be used in different ways to deliver a sample from a reactor to the interface valve of the Online LC System for sampling. This could be done by a complete standalone operation with control by the Agilent 1200 Infinity Instant Pilot controller, which is best suited for continuous running. Alternatively, it could be done by a CAN connection to the Agilent InfinityLab Online LC Solutions, enabling control of the isocratic pump by the Agilent OpenLab CDS acquisition software. In this case, the isocratic pump behaves like an LC pump incorporated in an analytical LC system with the respective programmability. The drawback of this approach is that no isolated prerun activity of the isocratic pump can be programmed. This could be overcome using a remote control (ERI-ERI remote cable, part number 5188-8044) of the isocratic pump by the Online LC Monitoring Software. The isocratic pump should be handled as a standalone instrument, not connected by CAN.

#### The following method can be programmed by the Instant Pilot controller:

- Flow rate: 0.00 mL/min
- Flow gradient: 0.00 min: 0.0 mL/min, 0.10 min: 5.0 mL/min
- Stop time: 0.5 minutes

In this example method, 5.0 mL/min corresponds to the flow rate used to pump the sample from the reactor to the Online LC System. The stop time of 0.5 minutes corresponds to the time that the isocratic pump is pumping the sample in the direction of the Online LC System. The required stop time depends on the tubing length and internal diameter, the flow rate, and the desired extra volume pumped, and must be determined individually.

#### Instrument setup and workflow:

1. Connect ERI cable (part number 5188-8044) to Online Sample Manager and isocratic LC pump. In case a longer cable is needed, use an extra extension cable (part number 5188-8059).
2. In the Configuration section of the Online LC Monitoring Software, configure an online monitoring system comprising a generic sample delivery pump and apply the settings shown in Figure 3.

## Sample Delivery Device

Name	Generic Sample Delivery Pump		
Setup ERI Interface of Online Sample Manager			
	Pin	Polarity	Pump time [s]
Pump on (OUT)	7 ▾	Low ▾	30

**Figure 3.** The Agilent 1260 Infinity II Isocratic Pump expects a change of polarity from high to low on pin 7 to start the pumping process; this conforms to the settings shown in this figure. When connected to an Agilent Isocratic LC Pump, the pump time corresponds to the time the Agilent Online LC Sample Manager waits for the sample to arrive at the external sampling interface valve and is set to 30 seconds in this example. For best performance, we recommend setting this pump time 5 seconds longer than the stop time of the isocratic pump, hence making sure that the isocratic pump is already stopped at the time the Online LC System draws the sample from the external sampling interface valve. If it is desired to have the isocratic pump still running when the sample is drawn, use a pump time that is sufficiently shorter than the stop time.

3. In the Experiment Setup section of the Online LC Monitoring Software, create an experiment setup using the online monitoring system configured in step 2.
4. Using, for example, an Instant Pilot controller, apply a suitable method to the isocratic LC pump based on the example given previously, and switch it on (flow rate is 0 mL/min).
5. Start the experiment in the Online LC Monitoring Software. The isocratic pump will then automatically be triggered by the Online LC Monitoring Software to pump a sample from the reactor to the Online LC System when sampling is due.
6. Once the pump time is over, the Online LC System performs the injection/dilution, and so on, of the sample provided at the interface valve.

### Example 2: Generic sample delivery pump control

*Masterflex Ismatec digital pump equipped with piston pump head<sup>4</sup>*

This pump can be used to deliver a sample from a reactor to the external sampling interface of the Online Sample Manager for sampling.

#### Electrical connection:

For the electrical connection of the Ismatec pump to the Online Sample Manager, an ERI cable with open end is required (part number 5188-8029). In case a longer cable is needed, use an extra extension cable (part number 5188-8059). A suitable 25-pin connector needs to be connected to the open end of the ERI cable. There are connectors commercially available allowing the attachment of cables without soldering by using screw fittings. Connect red wire (pin 8 of Agilent ERI connector) to pin 15 of Ismatec 25-pin connector. Connect violet wire (pin 10 of Agilent ERI connector) to pin 17 of Ismatec 25-pin connector. All other wires are not needed, do not connect, and carefully isolate them to avoid any potential short circuits.

#### Settings on Masterflex Ismatec digital pump:

- Select **Time** for the mode
- Select the pump on time, e.g. 30 seconds
- Select the flow rate, e.g. 5 mL/min
- Select the pumping direction

In this example 5 mL/min corresponds to the flow rate used to pump the sample from the reactor to the Online LC System. The pump on time of 30 seconds corresponds to the time that the Ismatec pump is pumping the sample in the direction of the Online LC System. The required pump on time depends on the tubing length and internal diameter, the flow rate, and the desired extra volume pumped, and must be determined individually.

#### Instrument setup and workflow:

1. Connect the customized ERI cable to the Online Sample Manager (15-pin connector) and to the Ismatec pump (25-pin connector).
2. In the Configuration section of the Online LC Monitoring Software, configure an online monitoring system comprising a generic sample delivery pump and apply the settings shown in Figure 3, but select pin 8 instead of pin 7.
3. In the Experiment Setup section of the Online LC Monitoring Software, create an experiment setup using the online monitoring system configured in step 2.

4. Apply the settings to the Ismatec pump based on the example given previously.
5. Start the experiment in the Online LC Monitoring Software. The Ismatec pump will then automatically be triggered by the Online LC Monitoring Software to pump a sample from the reactor to the Online LC System when sampling is due.
6. Once the pump time is over, the Online LC System performs the injection/dilution, and so on, of the sample provided at the external sampling interface valve.

When connected to an Ismatec pump, the pump time in the Configuration section of the Online LC Monitoring Software corresponds to the time the Online LC Sample Manager waits for the sample to arrive at the external sampling interface valve of the Online LC System. For best performance, we recommend setting this pump time to be 5 seconds longer than the pump on time of the Ismatec pump, hence making sure that the Ismatec pump has already stopped at the time the Online LC System draws the sample from the external sampling interface valve. If it is desired to have the Ismatec pump still running when the sample is drawn, use a pump time that is sufficiently shorter than the pump on time of the Ismatec pump.

### Example: Generic sample delivery pump control

*Watson-Marlow 120U/DV peristaltic pump<sup>5</sup>*

This pump can be used to deliver a sample from a reactor to the external sampling interface valve of the Online Sample Manager for sampling. When powering on the entire system, take care to first switch on the Online LC System before the Watson-Marlow pump.

#### Electrical connection:

For the electrical connection of the Watson-Marlow pump to the Online Sample Manager, an ERI cable with open end is required (part number 5188-8029). In case a longer cable is needed, use an extra extension cable (part number 5188-8059). A suitable 15-pin connector needs to be connected to the open end of the ERI cable. Either the connector provided with the pump is used (requires soldering), or a commercially available connector allowing the attachment of cables using screw fittings. Connect red wire (pin 8 of Agilent ERI connector) to pin 8 of Watson-Marlow 15-pin connector. Connect violet wire (pin 10 of Agilent ERI connector) to pin 12 of Watson-Marlow 15-pin connector. All other wires are not needed; do not connect, and carefully isolate them to avoid any potential short circuits. Carefully label both connectors to make sure the connectors meant for the Watson-Marlow pump and the Agilent Online LC Sample Manager are not mixed up.

If you desire to use an Agilent ERI cable comprising two 15-pin connectors (part number 5188-8044) instead of the Agilent ERI cable with open end, do not use as it is; the wires must be modified by soldering to match the configuration described above. Pay attention to the color coding of the wires, they do not match the color coding of the ERI cable with open end.

#### **Settings on the Watson-Marlow pump:**

- Use the arrow keys to select the pump speed (85 rpm is approximately 5 mL/min)
- Click **Auto Start** to activate
- Select the pump direction
- Click **Start** to enable remote start/stop.

In this example, 5 mL/min corresponds to the flow rate used to pump the sample from the reactor to the Online LC System.

#### **Instrument setup and workflow:**

1. Connect the customized ERI cable to the Online Sample Manager and to the Watson-Marlow pump. Take care to connect it in the correct direction as both connectors are identical.
2. In the Configuration section of the Online LC Monitoring Software, configure an online monitoring system comprising a generic sample delivery pump and apply the settings shown in Figure 3, but select pin 8 instead of pin 7.
3. In the Experiment Setup section of the Online LC Monitoring Software, create an experiment setup using the online monitoring system configured in step 2.
4. Apply the settings to the Watson-Marlow pump based on the example given above.
5. Start the experiment in the Online LC Monitoring Software. The Watson-Marlow pump will then automatically be triggered by the Online LC Monitoring Software to pump sample from the reactor to the Online LC System when sampling is due.
6. Once the pump time is over, the Online LC System performs the injection/dilution, and so on, of the sample provided at the interface valve.

When connected to a Watson-Marlow pump, the pump time in the Configuration section of the Online LC Monitoring Software corresponds to the time that the Watson-Marlow pump is running; once this time is over, the Watson-Marlow pump automatically stops.

The required pump time depends on the tubing length and internal diameter, the flow rate, and the desired extra volume pumped, and must be determined individually.

#### **Example 4: Generic sample delivery device control**

##### *New Era NE-1000 syringe pump<sup>6</sup>*

In our example, the syringe pump was equipped with a 1 mL glass syringe and connected to port 1 of the reactor interface valve of the Online LC System by PTFE tubing. Port 6 of the valve of the external sampling interface was connected to the reaction vessel by PTFE tubing. Other syringe sizes and tubing materials may also be used as needed.

#### **Electrical connection:**

The syringe pump is connected to the Online Sample Manager by an Agilent ERI cable with open end (part number 5188-8029): The 15-pin connector is connected to the Agilent Online LC Sample Manager, and a 9-pin connector is fitted to the open end as described later, and then connected to the New Era Systems programmable syringe pump. In case a longer cable is needed, use an additional extension cable (part number 5188-8059).

Connect a suitable 9-pin connector to the open end of the ERI cable as follows: Connect violet wire (pin 10 of the Agilent ERI connector) to pin 9 of the 9-pin connector, corresponding to the "ground" of the syringe pump. Connect yellow wire (pin 4 of ERI connector) to pin 5 of the 9-pin connector, corresponding to the "Output Pin" of the syringe pump. Connect red wire (pin 8 of ERI connector) to pin 2 of the 9-pin connector, corresponding to the "Operational Trigger" of the syringe pump. All other wires are not needed; do not connect, and carefully isolate them to avoid any potential short circuits.

If you desire to use the Agilent ERI/APG cable (part number 5188-8045) comprising a 15-pin connector and a 9-pin connector instead of the ERI cable with open end, do not use as is—the wires must be modified by soldering to match the configuration described above. Pay attention to the color coding of the wires, they do not match the color coding of the ERI cable with open end.

#### **Settings on the New Era programmable syringe pump:**

The programming of the syringe pump can be done manually according to the user manual<sup>6</sup> or in a convenient way by a Microsoft Excel file provided by the pump manufacturer. This allows the sending of a pumping program directly to the syringe pump using an Excel macro and a corresponding special communication cable. The used pumping program is shown in Figure 4.



Phase #	Program Function	Pumping Rate, Value, Phase # or Label	Pumping Rate Units	Volume to be Dispensed (mL). 0 = Continuous Pumping	Time (minutes : seconds)	Pumping Direction	Program Function TTL Trigger Select
1	TTL Pin 2 Trigger Configuration						Start on Low Level
2	Set TTL Pin 5 Output	1	----				
3	Pumping Rate	1	mL/min	0.500		Withdraw	
4	Set TTL Pin 5 Output	0	----				
5	Pause (seconds)	0	----				
6	Pumping Rate	1	mL/min	0.500		Infuse	
7	Stop Pump		----				

**Figure 4.** Screenshot from the Excel file used for the programming of the New Era syringe pump downloaded from the manufacturer's website. In phase 1, the required TTL trigger is set; in phase 2, the output pin is set to polarity "high"; in phase 3, the pumping rate used to draw the sample from the reactor and into the external sampling interface valve of the Online LC System is defined, as well as the drawn volume. The volume to be drawn depends on the tubing length and internal diameter, as well as the desired extra volume pumped, and must be determined individually. In phase 4, the output pin is set to polarity low, telling the Online LC System that the pumping process is finished; in phase 5, the syringe pump pauses until the Online LC System sends a ready signal to the syringe pump; in phase 6, the syringe pump pushes back the remaining sample into the reactor. The volume should be set to the same value as in phase 3 to make sure that the syringe is empty at the end of each sampling process.

Detailed information on how to use this Excel file is available in the manual included with the communication cable from New Era Systems. The procedure requires the software programs Pumpterm and DOSBox—both available to download free of charge from the syringe pump manufacturer. If you encounter issues running the procedure, you might change the file properties of Pumpterm\_shortcut.bat to be executed, and avoid blocking by the firewall. The same file might also be edited to reflect the used COM port; see the Device Manager of Microsoft Windows. In case a USB-serial converter is used, take care to install the corresponding driver.

#### Instrument setup and workflow:

1. Connect the customized ERI cable to the Online Sample Manager and to the New Era programmable syringe pump.
2. In the Configuration section of the Online LC Monitoring Software, configure an online monitoring system comprising a generic sample delivery device and apply the settings shown in Figure 5.
3. In the Experiment Setup section of the Online LC Monitoring Software, create an experiment setup using the online monitoring system configured in step 2.

## Sample Delivery Device

Name	Generic Sample Delivery Device			
Setup ERI Interface of Online Sample Manager				
	Pin	Polarity	Pulse [s]	Timeout [min]
Sample request (OUT)	8	Low	1	
Sample ready (IN)	4	Low		10
Sampling done (OUT)	8	Low	1	

**Figure 5.** Configuration setup of a generic sample delivery device in the Online LC Monitoring Software. When the New Era syringe pump is programmed and connected as described previously, it starts the pumping process upon a pulse on pin 8 with low polarity and a pulse duration of 1 second. The Online LC Sample Manager then waits for a "ready" signal from the syringe pump in the form of a pulse on pin 4. If no pulse is received on pin 4 within a timeout of 10 minutes, the Online LC Monitoring Software then annotates the corresponding sample with "failed" and proceeds with the next scheduled sample. Once the injection/dilution, and so on, process is finished, the Online LC System sends a "ready" signal to the syringe pump in form of a pulse on pin 8 with a pulse duration of 1 second. This signal allows the syringe pump to push the remaining sample back into the reactor.

4. Apply the settings to the New Era programmable syringe pump based on the example given previously.
5. Start the experiment in the Online LC Monitoring Software. The syringe pump will then automatically be triggered by the Online LC Monitoring Software to pump sample from the reactor to the Online LC System when a sampling is due, "Sample Request (Out)."
6. Once the syringe pump has finished pumping the sample to the interface valve of the Online LC System, the syringe pump sends a corresponding "Sample ready (In)" signal to the Online LC System.
7. The Online LC System then performs the injection/dilution, and so on, of the sample provided at the external sampling interface valve. Once this process is finished, the Online LC System sends a "Sample Done (Out)" signal to the syringe pump. Then the syringe pump pushes back the remaining sample into the reactor until the syringe is empty.

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