Online HPLC for Biotechnology: Groton Biosystems Automated Reactor Sampling System and Agilent 1200 Series LC System

Technical Note

Introduction

Online analytical instrumentation facilitates productive, efficient monitoring of fermentation or cell culture processes in real time. The Groton Biosystems Automated Reactor Sampling system (ARS) in combination with the Agilent 1200 Series LC system introduces and complies with the goals of the FDA's Process Analytical Technology (PAT) initiative. Described here is a new technology which introduces automatic, hands-free, online sampling for HPLC analysis. This Technical Note demonstrates this instrumentation for amino acid analysis.

GROTON BIOSYSTEMS

Agilent Technologies

Automated Reactor Sampling

Amino acids are important analytes in the clinical, pharmaceutical, biotechnology, and food and beverages industries. It is important to diagnose, monitor processes, and assess the quality of the final products. HPLC is a robust, accepted analytical technique for the separation and identification of very low amino acids concentration. The technique offers both precision and accuracy and that has driven the growth of many applications. Groton Biosystems offers a solution – the Automated Reactor Sampling system (ARS) – to interface an HPLC system to process lines, purification columns, reactors and fermentors. The ARS communicates with an HPLC system via digital control and status signals to synchronize operations. Sample sequences and the timing of these sequences to acquire an online sample are scheduled to meet the requirements of the process (and the HPLC). The ARS also has an option to provide sample preparation. For instance, if amino acid assays from a bioreactor are required, the ARS sample preparation option separates the protein/peptide fraction from the desired low molecular weight fraction. The ARS system can interface to a variety of bioreactors, fermentors, and HPLC systems. The ARS technology assists companies to comply with the FDA PAT Initiative by providing an automated production tool. This will increase process knowledge and understanding, thus yielding more efficient and profitable operations. A closed loop "PAT" solution for cell culture and fermentation production is shown in figure 1.

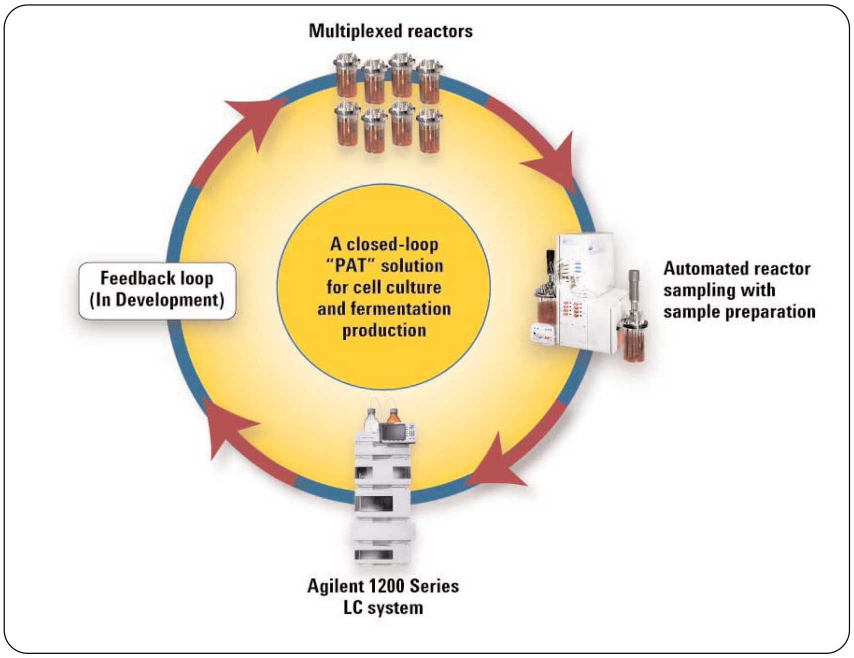

Figure 1
A closed loop "PAT" solution for cell culture and fermentation production.

Sterility

The proprietary Remote Interface Module has been proven to maintain sterility in bioreactors and fermentors using automatic internal Clean-in-Place (CIP) systems. Steam-in-Place (SIP) is available for large scale systems.

A Remote Interface Module is located on, at or near every reactor sample port and provides the mechanism to clean the sample transfer lines and the valves in the module (which controls flow of sample from the reactor to the ARS). This interface provides complete isolation of the reactor or from the ARS and permits cleaning and flushing directly to the reactor port, protecting the reactor from contamination. The Remote Interface Module is equipped with multiple isolation valves in series between the reactor port and ARS, and has at least one drain valve. The ARS performs all functions of sample acquisition, transfer, assay and/or sample storage without contamination of the bioreactor or fermentor.

ARS- HPLC connection
The Groton Biosystems Automated Reactor Sampling system (ARS400) connects to an Agilent 1200 Series LC system via a 3-way valve and contact closure (figure 2).

Equipment and method
- ARS400
- ARS HPLC interface cable
- Interface valve
- ARS method for HPLC
- Agilent method
- Agilent 1200 Series LC system
- 3 groove rotor
- Contact closure board
- Agilent interface cables

The ARS provides a sterile and automatic connection between a bioreactor sample and an HPLC system. The sterility of the ARS system process for cell culture and/or fermentation media has been demonstrated in Groton Application Note MKT-010. The ARS instrument typically performs a sample prime, sample acquisition, and then system cleaning. The ARS is stored typically in WFI/DI/sterile water when a sample cycle is complete. During the next sampling sequence, the water is displaced by a prime volume and then a 1 mL sample is sent to the Agilent 1200 Series autosampler for sample analysis. The cleaning cycle for this study included a 0.05N NaOH rinse and then 2 successive water rinses. The total cycle time (time from 1 AA injection till the next one) was 30 minutes.

ARS- HPLC experimental operation
The ARS – HPLC experimental conditions are shown in table 1.

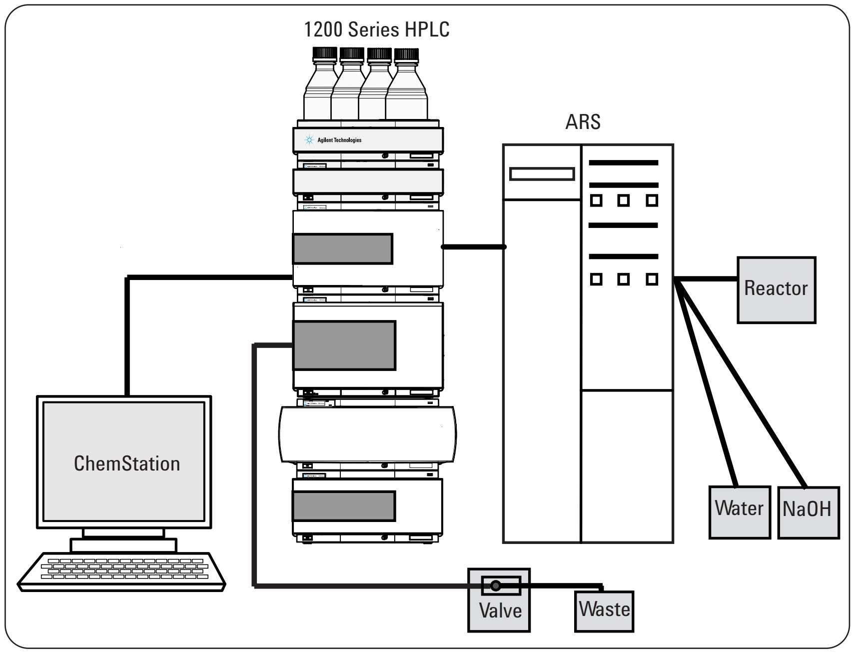

Figure 2
Equipment setup.

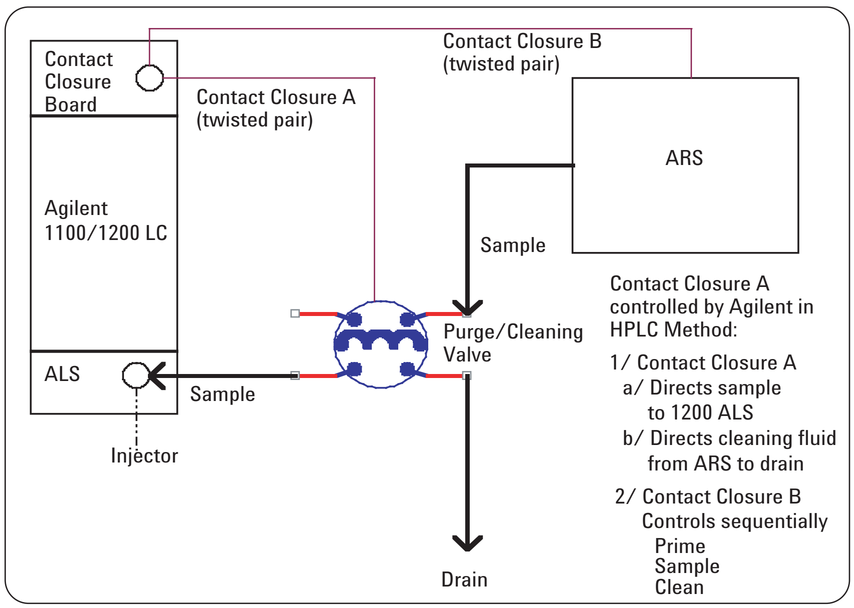

Figure 3
Instrument schematic

HPLC system:	Agilent 1200 Series
Detector:	Agilent 1200 Series diode array detector (G1315C)
Detector settings:	UV 338 nm 10 nm BW, ref. 390 nm BW 20 nm
Data collection:	Agilent ChemStation software
Sample:	9 nmol/ µL asparagine, 9 nmol/ µL glutamine, 5 nmol/ µL norvaline, 5 nmol/ µL tryptophan
Derivatization:	0.5 µL OPA (10 mg/mL in 0.4M Borate) + 0.5 µL AA + 2.5 µL 0.4M borate buffer
Injection volume:	5 µL
HPLC column:	ZORBAX Eclipse 4.6 X 50 mm 1.8 µm
Column temp:	40 °C
Flow rate:	2 mL/min
Mobile phase A:	10 mM Na_2HPO_4, 10M m Na2B4O7, 0.5 mM NaN_3
Mobile phase B:	Acetonitrile: Methanol: water 45:45:10
HPLC run time:	8 minutes
ARS system:	ARS400
ARS run time:	30 minutes

Table 1
ARS – HPLC experimental conditions.

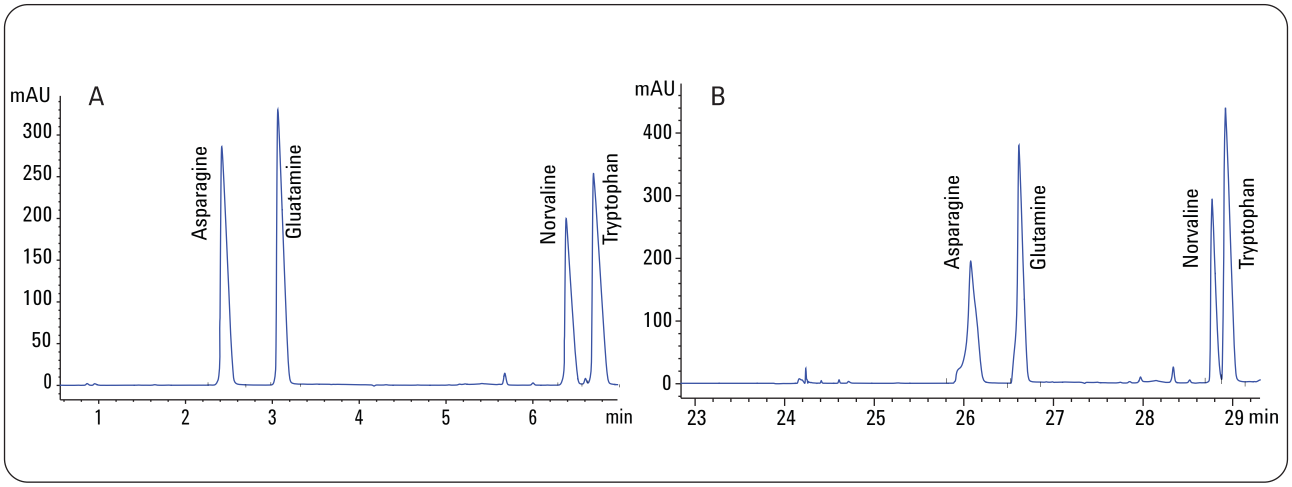

Figure 4
A) AA analysis when sample injected manually – Time from start of injection.
B) AA analysis when sample acquired by ARS400 – Time from start of acquisition of sample from reactor.

ARS- HPLC amino acid analysis
Figure 4A shows a manually injected sample separation. Figure 4B shows the ARS delivering the amino acid sample to the HPLC system.

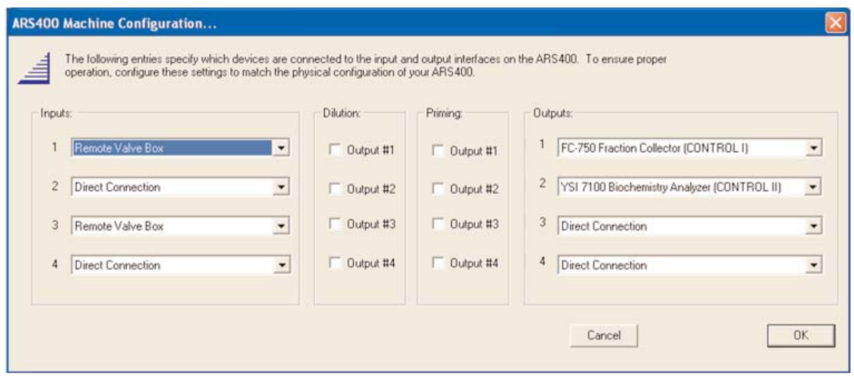

Figure 5
ARS Machine Configuration dialog box.

Using an HPLC system as an output device

Overview

ARS sampling systems can be configured to automatically inject samples to an HPLC instrument, either singly or in series. If this function is selected on the Machine Configuration dialog box (figure 5) the ARS will deliver the sample to the HPLC, clean its input and output lines and the HPLC injection loop, and then automatically get the next sample in line and repeat the process.

Communication between the HPLC and the ARS

The ARS is subordinate to the HPLC and the HPLC instrument control via the Agilent ChemStation. Once the system is properly configured and connected, the ARS will wait for a signal from the HPLC to inject the sample into the injector loop. It will then wait for a signal from the HPLC to go through a cleaning cycle. When the entire cycle is complete the ARS will wait for the next load command from the HPLC. The signal from the HPLC to the ARS is a simple contact closure through a cable supplied by Groton Bioystems. The cable connects to the ARS via a standard DIN connector and has flying leads to connect to the appropriate connector on the HPLC.

This mode of operation is shown in figure 6. The sample can be pulled directly from the reactor to the ARS or it could be supplied through an autosampler with the ARS controlling the autosampler.

Mode of operation

Sample input (from the reactor to the ARS) is programmed as described in section 4.6 of the operator's manual.

When *HPLC* is selected as the output device, the *Output dialog* box appears, as shown in figure 7 when the *Cycle Parameters* dialog box is opened. It will be necessary to edit each of the tabs as described below. The input parameters for the system are edited as described in the operator's manual.

The *Sample Prime* dialog (figure 7) is used to describe the sample priming step which fills the reactor inlet line with sample fluid before the sample is drawn. The *Sample Draw* dialog (figure 8) is used to indicate the number of times the sample loop should be filled.

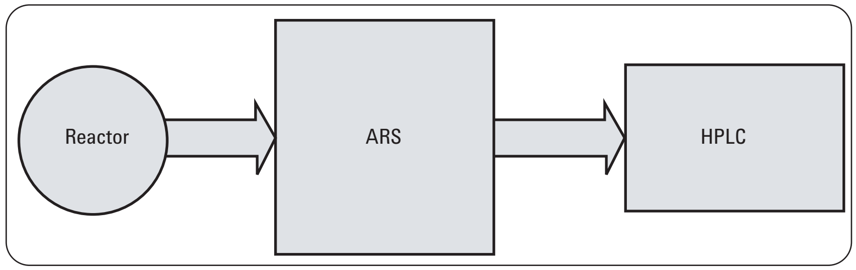

Figure 6
Mode of operation – ARS with HPLC system.

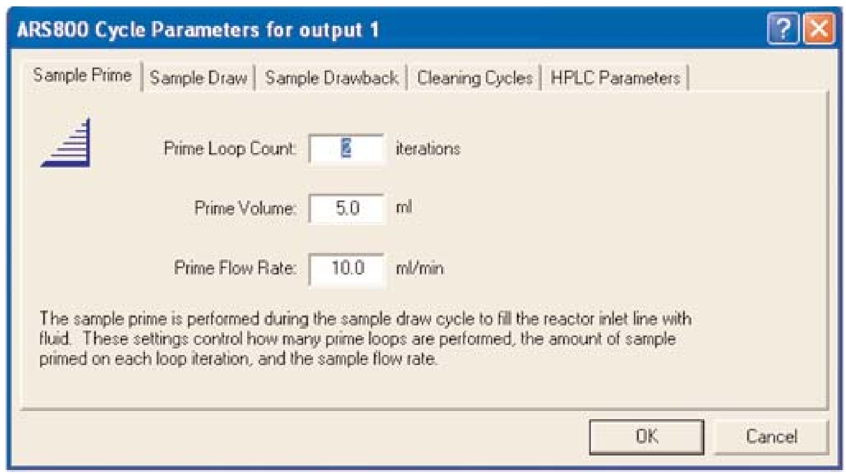

Figure 7
***Cycle prime* tab.**

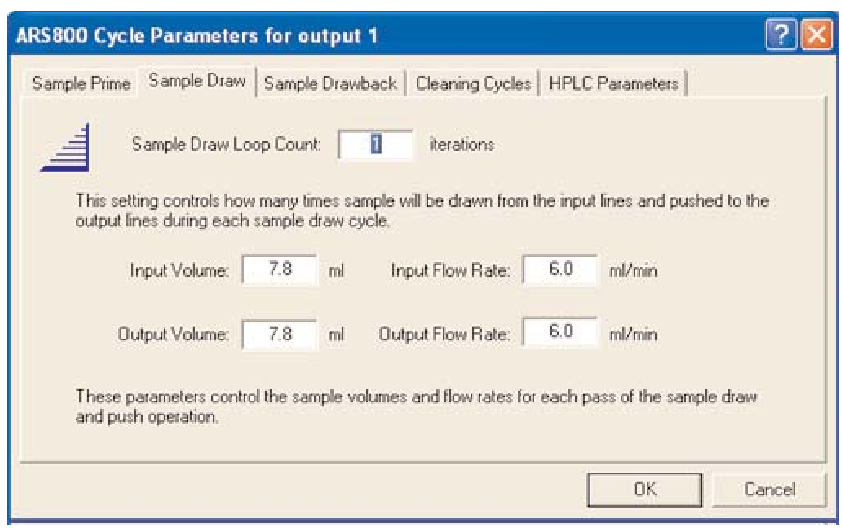

Figure 8
***Sample Draw* tab.**

The *Sample Drawback* dialog (figure 9) is used to indicate if drawback of the sample is employed to remove sample from the lines after the sample draw-back/push is completed. This is used to reduce sample carryover. If this option is used, the *Volume* and *Flow Rate* should be indicated.

The *Cleaning Cycles* dialog (figure 10) is used to set the various processes in the cleaning cycle.

The *HPLC Parameters* dialog (figure 11) is used to set flows through the HPLC interface for cleaning and flushing.

The cleaning cycles and reagents are determined based on the sample type being analyzed and actual results obtained during the installation process. Some experimentation may be required. The ARS uses standard 1 mm ID tubing for connections on both the inputs and outputs. The system is supplied with sufficient lengths of this tubing and connector types to allow the operator to handle most interface situations. Connections must be made to the appropriate input and output ports on the ARS and the HPLC. Sample can also be supplied to the ARS manually.

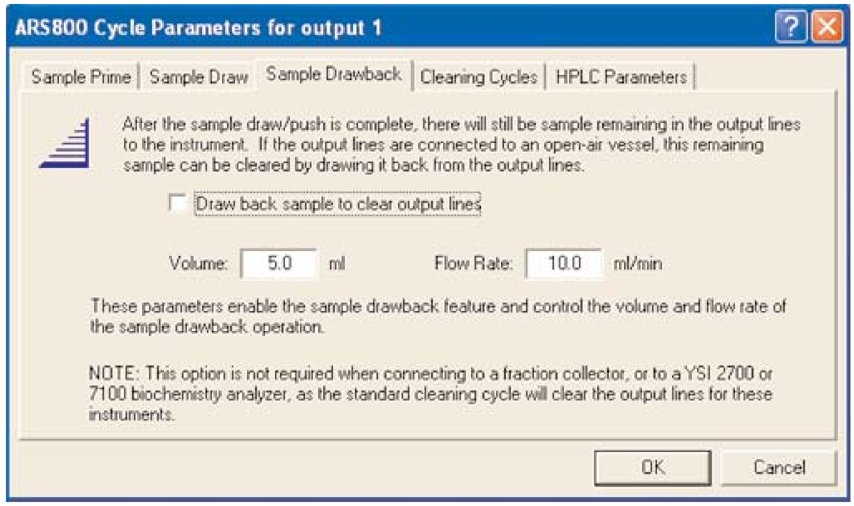

Figure 9
***Sample Drawback* tab.**

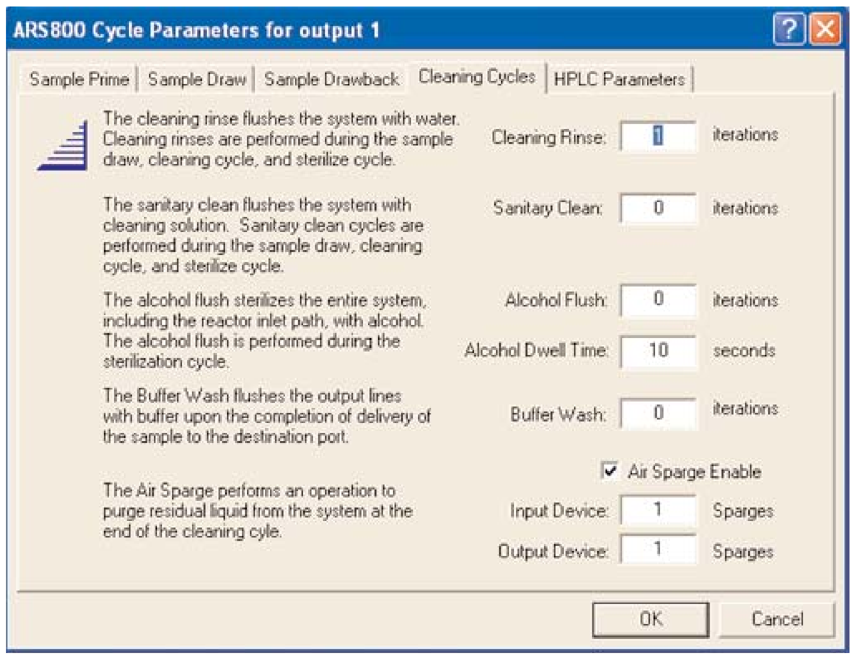

Figure 10
***Cleaning Cycles* tab.**

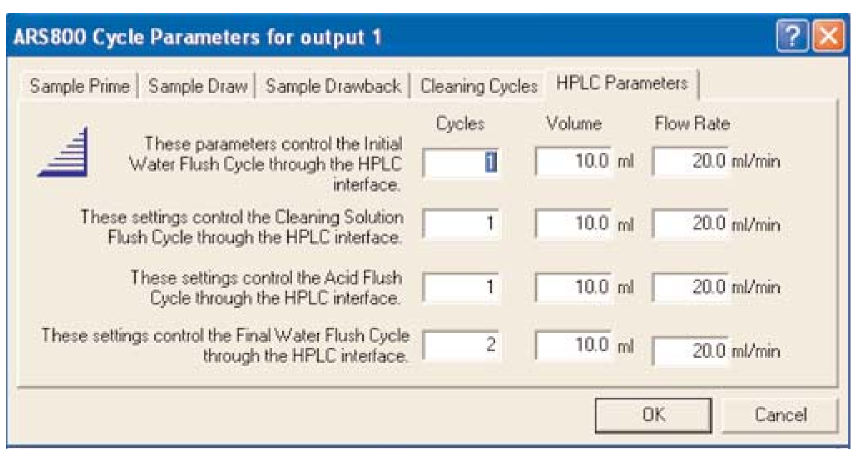

Figure 11
***HPLC Parameters* tab.**

Initial operation

Once the ARS is programmed and connections are made there are several parameters that must be determined before the combined set up is ready for ongoing operations. The ARS needs to run one sample draw. The ARS will wait until it receives a contact closure signal from the HPLC. Therefore, the HPLC must be programmed to perform a contact closure once the injection loop is in the load position. This contact closure should be held for a period of approximately 5 seconds and then opened. Once the ARS senses closure, it will start its sample draw cycle. This will consist of a sample prime draw and then an actual sample to be delivered to the HPLC injection loop. The ARS will deliver more than the typical 20 µL that is required. The excess must be allowed to flow to waste in the HPLC by plumbing the injector valve with a waste line at port 4. After the ARS has completed its sample process it will then wait for a second contact closure to begin its cleaning cycle. At this point the HPLC is also programmed to inject sample from the loop onto the column and then return the injection loop to the load position and the HPLC should provide the second contact closure to the ARS to start cleaning. This contact closure should also last approximately 5 seconds.

The key elements are related to timing within the HPLC protocol. The initial contact closure should not be made for approximately 10 seconds after starting the ARS sample draw. It must be opened again, preferably within a short time after initial closure but allow approximately 5 seconds for the ARS to recognize the signal.

Once the HPLC timing is established, the ARS sample draw cycle must be established. The HPLC protocol must allow for this time period adding an additional minute to each method step for margin before closing the injection loop and injecting the sample into the column. The loop should then be returned to the load position and then the second contact closure needs to be sent to the ARS for cleaning. The cleaning cycle must also be timed so that the HPLC protocol does not request another sample draw from the ARS. This is critical when running multiple sequential sample draws. A typical timeline is shown in figure 12.

Note: If any of the ARS set up parameters are changed, it will be necessary to run a new sample cycle to verify the timing required for the HPLC protocol. Set-up changes to the ARS will affect the sample draw and cleaning cycle times.

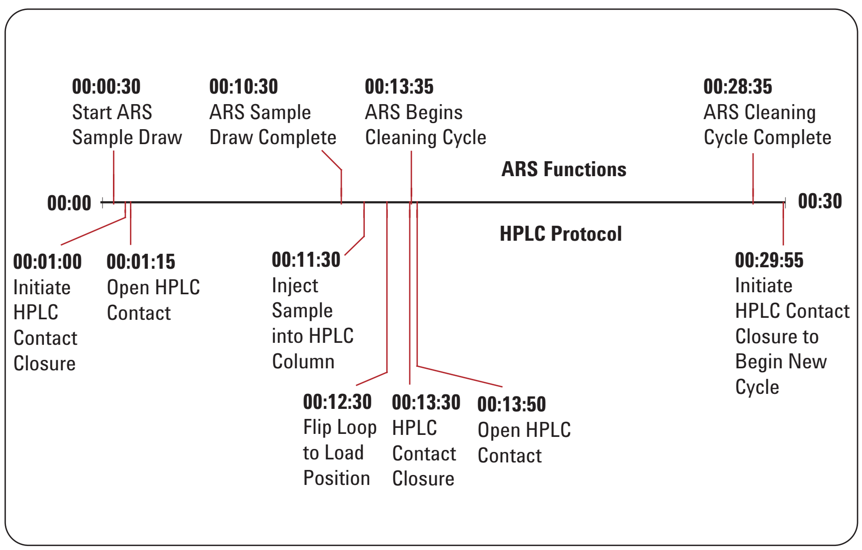

Figure 12
Timing example – ARS and HPLC.

Conclusion

This Technical Note describes a completely automatic, hands-free HPLC system for online analysis. The hyphenated system combines the automatic sampling and reactor sterility protection of the Groton ARS with a high performance amino acid separation system provided by the Agilent 1200 Series LC system. This system is invaluable in applications requiring the combination of sterile sampling and accurate, precise separation and detection of analytes typically found in bioprocesses.

Literature

1.
MKT-032 *Product Bulletin / Agilent Publication Number 5989-6156EN*, **2007.**

2.
"Increase Efficiency with a Powerful Process Analytical Technology Solution for the Biopharmaceutical Industry", *Agilent in Pharmaceutical Analysis Newsletter – Number 18*, **2007.**

www.grotonbiosystems.com
www.agilent.com/chem/1200

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