

Automated SW solution for preparative HPLC:

Application examples of Clarity software for analytical and small scale preparative separation using multivendor analytical and preparative HPLC components.

Introduction

Besides its use in analytical GC, HPLC and CE, the Clarity chromatography software is well suited also for control of preparative chromatography systems. Using the FC-GP (General Purpose Fraction Collector) control module, many different fraction collectors could be controlled by the simple *Next* and *Collect/Waste* commands generated by the module and transferred to the devices either through the digital Outputs/Inputs or by suitable communication line (RS232, LAN, GSIOC). Not only dedicated fraction collectors, but also multi-position valves could be controlled. The fraction collection parameters (including time windows and signal level or slope triggered collection) are defined within the Clarity FC-GP method setup.

Two examples of automated systems used for isolation and purification of antibodies, based on common instrumentation and controlled by Clarity chromatography software are presented.

Automated Immunoaffinity Chromatography

This instrument setup is designed to isolate specific antibodies from clarified serum. The serum is obtained from an animal immunized with the antigen which we want to generate antibodies against. The media in the column has the same or a similar antigen covalently bound to it, in order to capture antibodies which are specific to that antigen.

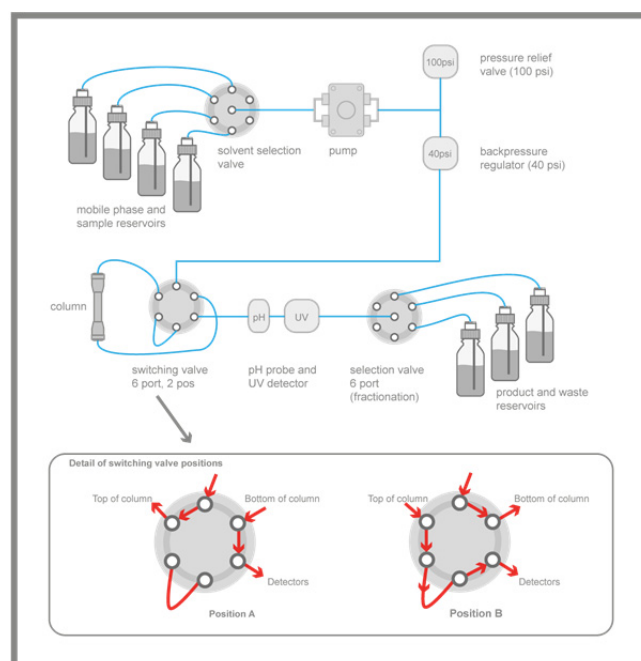


Fig. 1 – Diagram of the immunoaffinity separation system.

The instrument is configured around our existing lab infrastructure (benches, racks, shelves, rods for mounting columns, etc.). A 6 position selector valve allows selection of serum, a wash buffer, an elution buffer, or a column cleaning buffer. The unused positions are plugged so that they can be selected to put the instrument into a standby state which prevents any siphoning of the mobile phases due to gravity.

The output of the selector valve leads to the pump. Immediately after the pump is a backpressure regulator to ensure proper seating of the check valves and a second one, serving as a pressure relief valve, as the pump does not feature pressure monitoring.

The tubing is then plumbed into a 6 port, 2 position valve, used to allow the direction of flow through the column to be reversed (see plumbing diagram). This allows strongly retained antibodies at the top of the column to be eluted quickly, with minimal exposure to the harsh elution buffer. After flowing through the column, the mobile phase returns to the valve and is directed through pH and UV detectors to a second 6 position selector valve, where the output can be directed to reservoirs for waste, eluted material, or material to be reloaded for further processing.

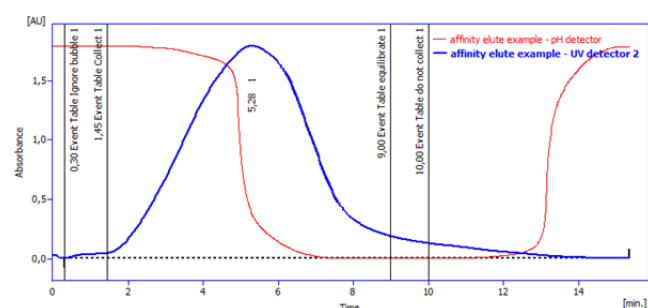


Fig. 2 - Example of the elution cycle in immunoaffinity separation

Currently we have 13 instruments in service that match this general description. Each instrument takes up roughly 50 cm x 50 cm of bench space, making use of shelving above the bench to hold large reservoirs. The “prototype” for this instrument design was assembled primarily from components we had in our lab that were not in use.

The full version of Clarity with the LC module is used on all instruments in order to allow for expansion. Most of the stations already have the maximum number of 4 instruments installed. Serial communication ports for the valves and pumps are provided by a DataApex Multicom device. All valves are mounted on Valco Microelectric Actuators with RS-232 communication. Analog signals from the pH probe and UV absorbance detector are acquired using either an INT9 A/D PCI card, a 4 channel Colibrick device, or a 2 channel UPAD, depending on the instrument. The PCs used are from various manufacturers, or are built to specifications in house.

No.	Name	Description
1.	Reservoirs	Sample, Wash, Eluent, Column Cleaning
2.	Selection valve	6 position selector valve, (Valco, C25-6186EMH)
3.	Pump	SSI/LabAlliance Series 1 Pump (Chromtech, P-040), with 40 ml/min heads
4.	Backpressure regulator	40 psi, to provide backpressure for pump valves
5.	Backpressure regulator	100 psi, connected by a tee, serving as pressure relief valve
6.	Switching valve	6 port, 2 position valve (Valco C22-6186EH)
7.	Column	5 cm diameter x 5 cm length
8.	pH detector	Flow cell (Sensorex, FC45C) with Flat pH electrode (Sensorex, S450CD)
9.	UV detector	UV absorbance detector (Bio-Rad, EM-1)
10.	Selection valve	6 position selector valve, (Valco, C25-6186EMH)
11.	Reservoirs	Waste, Pure Product, Impure Product for reloading
		1/8" ID Dupont PFA tubing is used throughout for connections.

Table 1- Immunoaffinity System Components

Multiple isolation cycles are needed to process the starting material quantity. The sequence table is thus used to control the instrument. Separate methods are written for the loading, washing, elution, and cleaning phases of the process. These methods are then repeated in the sequence to create cycles, which can be performed automatically until the desired amount of material has been processed. Sequences can also be run overnight, with the primary limitation being the size of the mobile phase and collection reservoirs.

Sts.	Run	SV	EV	I/V	Sample ID	Sample	File Name	Method Name
1	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
2	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
3	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
4	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
5	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
6	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
7	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
8	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
9	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
10	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
11	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
12	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
13	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
14	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
15	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
16	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
17	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
18	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
19	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
20	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
21	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
22	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
23	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
24	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity
25	✓	1	1	1	3010-00 PP10513	Load 600 ml	%q, %R, %N, %Q	example load method - immunoaffinity
26	✓	2	2	1	3010-00 PP10513	Wash	%q, %R, %N, %Q	example wash method - immunoaffinity
27	✓	3	3	1	3010-00 PP10513	Elute and Equilibrate	%q, %R, %N, %Q	example elution method - immunoaffinity

Fig. 3 - Example of sequence table for immunoaffinity separation

Preparative size exclusion chromatography with stacked injections using Clarity

This instrument setup is designed to facilitate removal of aggregates and other high molecular weight species from antibodies. Generally, the harshness of the elution buffer used in the affinity step leads to some degree of aggregation in the isolated antibodies.

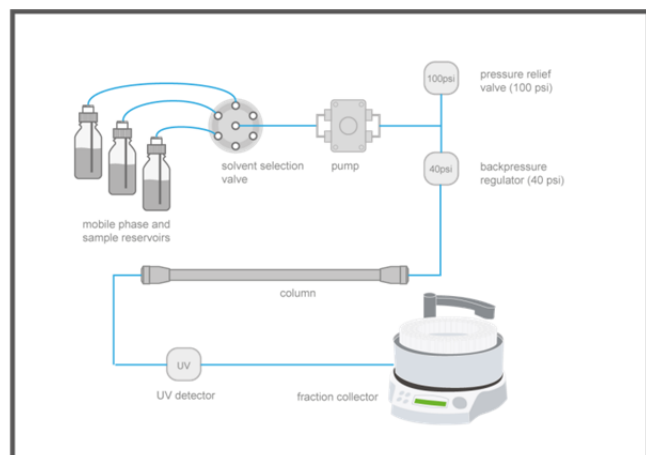


Fig. 4 - Diagram of the Stacked Injection SEC system

A 6 position selector valve is used to switch between a sample reservoir and a running buffer reservoir. This is connected to a pump, with backpressure regulator configured as a blow-off valve in order to protect the column from excessive pressure in the event of a clog. The outlet of the column is connected to a UV absorbance detector. Fractions are collected using a fraction collector.

No.	Name	Description
1.	Reservoirs	Sample, Eluent, Column Cleaning
2.	Selection valve	6 position selector valve, (Valco, C25-6186EMH)
3.	Pump	SSI/LabAlliance Series 1 Pump (Chromtech, P-040), with 40 ml/min heads
4.	Backpressure regulator	40 psi, to provide backpressure for pump valves
5.	Backpressure regulator	100 psi, connected by a tee, serving as pressure relief valve
6.	Column	5 cm x 100 cm (GE, XK-50/100) column packed with Superdex 200 Prep Grade.
7.	UV detector	UV absorbance detector (Bio-Rad, EM-1)
8.	Fraction collector	Isco Foxy 200 fraction collector
		1/8" ID Dupont PFA tubing is used throughout for connections.

Table 2 - Size Exclusion System Components

Again, multiple injections are needed to load all the sample to be processed. In order to reduce the time required to process large samples, a stacked injection technique is used. As a run begins, the solvent selection valve loads the first portion of the sample from the sample reservoir then switches back to the running buffer.

Name	Type	Source	Input	Value	Units	Output Type	Output	Parameters	Status
1. Collect	Op. Input Run	PC GP	1	---	---	Run Program	C:\Clarity\Utils\Every 200 BAF\Res\Collect.BAT		OK
2. Waste	Op. Input Run	PC GP	1	Down	---	Run Program	C:\Clarity\Utils\Every 200 BAF\Res\Waste.BAT		OK
3. Wait	Op. Input Run	PC GP	2	---	---	Run Program	C:\Clarity\Utils\Every 200 BAF\Res\Wait.BAT		OK
4. Transfer fraction collector	Acq Begin	---	---	---	---	Run Program	C:\Clarity\Utils\Every 200 BAF\Res\Transfer.BAT		OK
5. Shut down PC	Acq End	---	---	---	---	Run Program	C:\Clarity\Utils\Every 200 BAF\Res\Shutdown.BAT		OK
6. start first load	Run Time >	---	---	1,000	min	Get Filtration Valve	Position		5
7. end first load	Run Time >	---	---	3,000	min	Get Filtration Valve	Position		6
8. start second load	Run Time >	---	---	90,000	min	Get Filtration Valve	Position		6
9. end second load	Run Time >	---	---	90,000	min	Get Filtration Valve	Position		6
10. start third load	Run Time >	---	---	180,000	min	Get Filtration Valve	Position		6
11. end third load	Run Time >	---	---	180,000	min	Get Filtration Valve	Position		6
12. start fourth load	Run Time >	---	---	270,000	min	Get Filtration Valve	Position		5
13. end fourth load	Run Time >	---	---	270,000	min	Get Filtration Valve	Position		6
14. start fifth load	Run Time >	---	---	360,000	min	Get Filtration Valve	Position		5
15. end fifth load	Run Time >	---	---	360,000	min	Get Filtration Valve	Position		6
16. start sixth load	Run Time >	---	---	450,000	min	Get Filtration Valve	Position		5
17. end sixth load	Run Time >	---	---	450,000	min	Get Filtration Valve	Position		6
18. start seventh load	Run Time >	---	---	540,000	min	Get Filtration Valve	Position		5
19. end seventh load	Run Time >	---	---	540,000	min	Get Filtration Valve	Position		6
20. start eighth load	Run Time >	---	---	630,000	min	Get Filtration Valve	Position		5
21. end eighth load	Run Time >	---	---	630,000	min	Get Filtration Valve	Position		6
22. start ninth load	Run Time >	---	---	720,000	min	Get Filtration Valve	Position		5
23. end ninth load	Run Time >	---	---	720,000	min	Get Filtration Valve	Position		6
24. ready to arm	Run Time >	---	---	0,300	min	Run Program	C:\Clarity\Utils\Every 200 BAF\Res\Collect.BAT		OK
25. ready to arm	Run Time >	---	---	0,300	min	Run Program	C:\Clarity\Utils\Every 200 BAF\Res\Collect.BAT		OK

Fig. 5 - Example of Stacked Injection Method Event Table

After the first portion has migrated some distance down the column, the valve switches to the sample reservoir loading a second portion. After the second portion has migrated down the column a third can be loaded, and so on. If the characteristics of the sample are known, spacing between samples can be reduced significantly versus loading one sample and waiting for it to elute completely. In one example, a single sample takes 165 minutes to elute completely, however 8 samples can be processed in 790 minutes, saving almost 9 hours by using stacked injections. Multi-gram quantities of material can be processed per day using this technique.

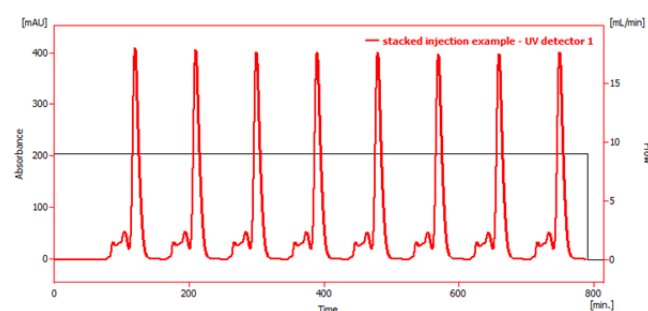
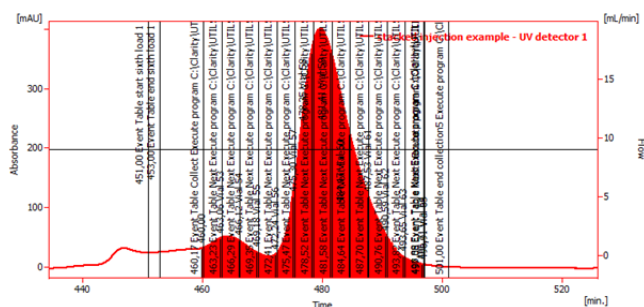


Fig. 6 - Example of stacked injection SEC separation



Conclusion

Similar setups can be used with large range of fraction collectors and multi-position valves. The description can also help with setting up systems for other preparative applications.

In both applications the same Clarity Chromatography Software system has been used:

Table 3 - Clarity Software Components

Authors