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, Clarity Chromatography software is also well suited for the control of preparative chromatography systems. Using the FC-GP (General Purpose Fraction Collector) control module, many different fraction collectors can 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 can 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 the 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 a clarified serum. The serum is obtained from an animal immunized with the antigen that we want to generate the antibodies against. The media in the column has the same or a similar antigen covalently bound to it in order to capture antibodies that are specific to that antigen.

The instrument is configured within our existing lab infrastructure (benches, racks, shelves, rods for mounting columns, etc.). A 6-position selector valve allows the 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 mobile phases due to gravity.

The output of the solvent selector valve leads to the pump. Two backpressure regulators are connected to the pump to ensure the proper setting of the check valves. One of the regulators serves as a pressure relief valve, as the pump does not feature pressure monitoring.

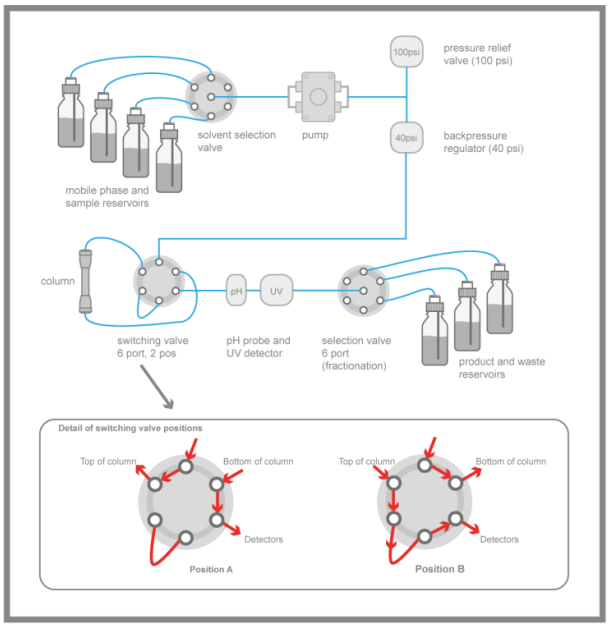

Fig. 1 – Diagram of the immunoaffinity separation system.

The tubing is then plumbed into a 6-port, 2-position valve, which allows the direction of the flow through the column to be reversed (see plumbing diagram). This allows the 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 another 6-position selector valve, where the output can be directed to reservoirs for waste, eluted material, or material to be reloaded for further processing.

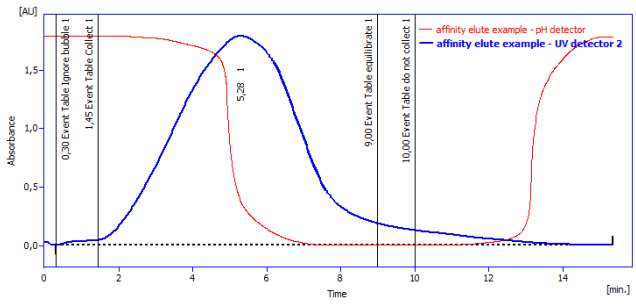

Fig. 2 - Example of the elution cycle in immunoaffinity separation

Currently, we have 13 instruments in service that match this general description. Each instrument takes up roughly 50 cm × 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 at the moment.

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 the 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 a 4-channel Colibrick device (connected to the PC via a USB cable).

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, providing backpressure for pump valves
5.	Backpressure regulator	100 psi, T-shape connection, serving as a pressure relief valve
6.	Switching valve	6-port, 2-position valve (Valco C22-6186EH)
7.	Column	5 cm diameter × 5 cm length
8.	pH detector	Flow cell (Sensorex, FC45C) with a flat pH electrode (Sensorex, S450CD)
9.	UV detector	UV absorbance detector (Bio-Rad, EM-1)
10.	Section valve	6-position selector valve (Valco, C25-6186EMH)
11.	Reservoirs	Waste, Pure Product, and 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 quantitatively. The Instrument is thus controlled via the Sequence table. Separate methods are used for the loading, washing, elution, and cleaning phases of the process. These methods are then repeated within 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.

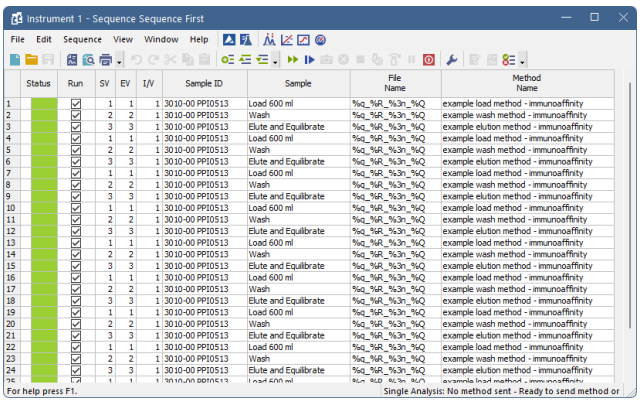

Fig. 3 - Example of sequence table for immunoaffinity separation

Preparative size exclusion chromatography with stacked injections using Clarity

This instrument setup is designed to facilitate the 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.

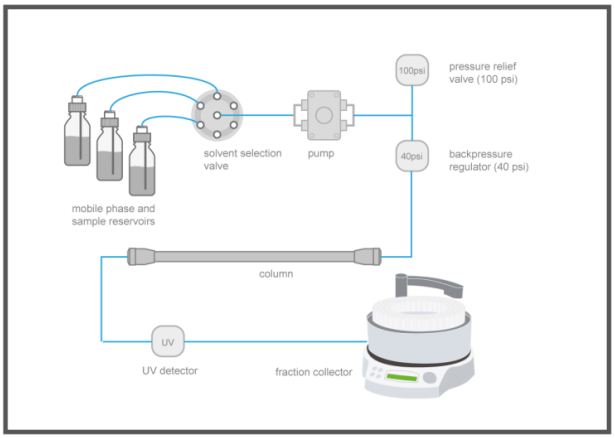

Fig. 4 - Diagram of the Stacked Injection SEC system

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 a backpressure regulator configured as a blow-off valve in order to protect the column from excessive pressure in the event of clogging. 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, providing backpressure for pump valves
5.	Backpressure regulator	100 psi, T-shape connection, serving as a pressure relief valve
6.	Column	5 cm diameter × 5 cm length
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 connection.

Table 2 - Size Exclusion System Components

Again, multiple injections are needed to load the whole 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 and then switches back to the running buffer.

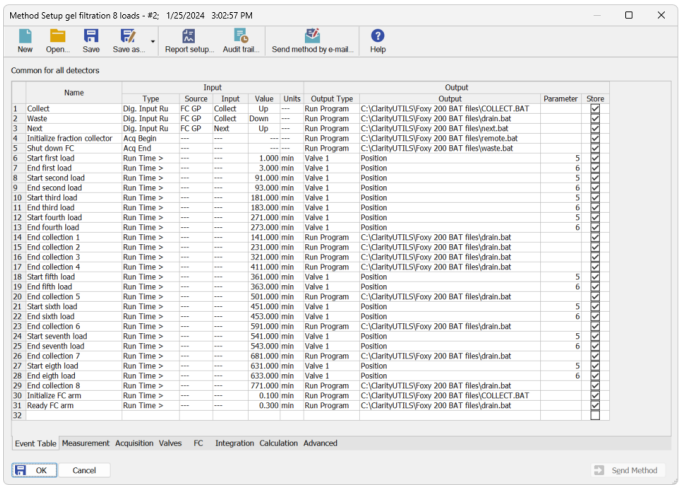

Fig. 5 - Example of Stacked Injection Method Event Table

After the first portion has migrated some distance down the column, the valve switches to the sample reservoir loading the second portion. After the second portion has migrated down the column, the third portion can be loaded, and so on. If the characteristics of the sample are known, spacing between samples can be reduced significantly (it is not necessary to load one sample and wait for it to elute completely). For 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.

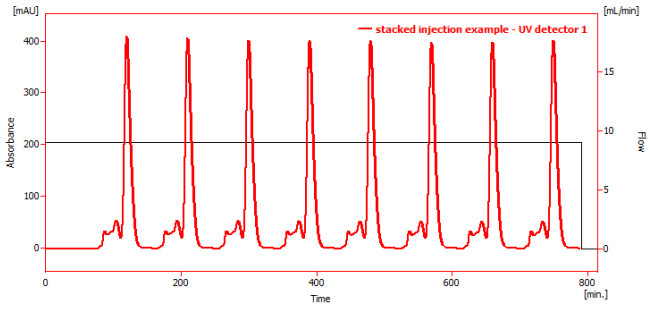

Fig. 6 - Example of stacked injection SEC separation

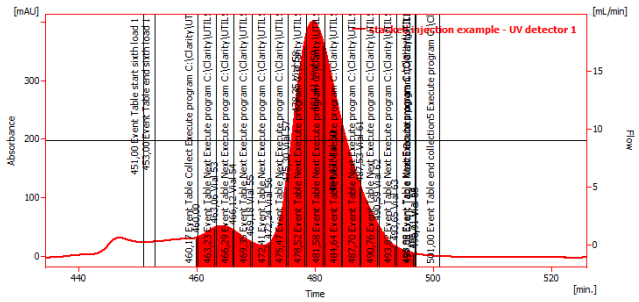

Fig. 7 - Detail of stacked injection SEC separation cycle

Conclusion

We have demonstrated the use of Clarity in the purification of antibodies using two specific setups: automated immunoaffinity chromatography and preparative size exclusion chromatography with stacked injections.

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

Featured Clarity products

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

Item	p/n
Clarity Single Instrument	C50
LC Control Module	A24
AD Converters, i.e. Colibrick, 4-channel	U34
Add-on Instruments (optional – to control more systems from one station)	C55

Table 3 - Clarity Software Components

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