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.

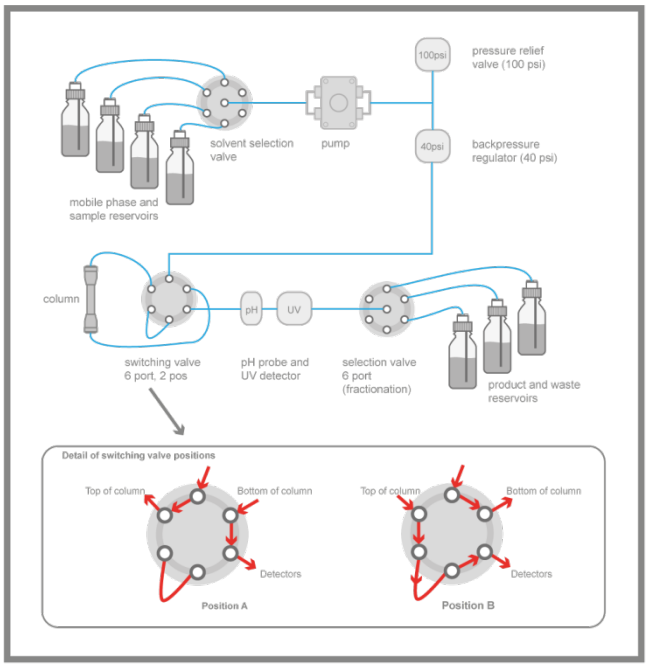

Fig. 1 – Diagram of the immunoaffinity separation system.

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.

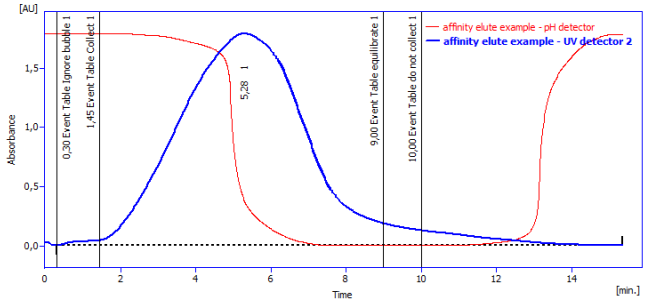

Fig. 2 - Example of the elution cycle in immunoaffinity separation

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- Immunoafinity 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.

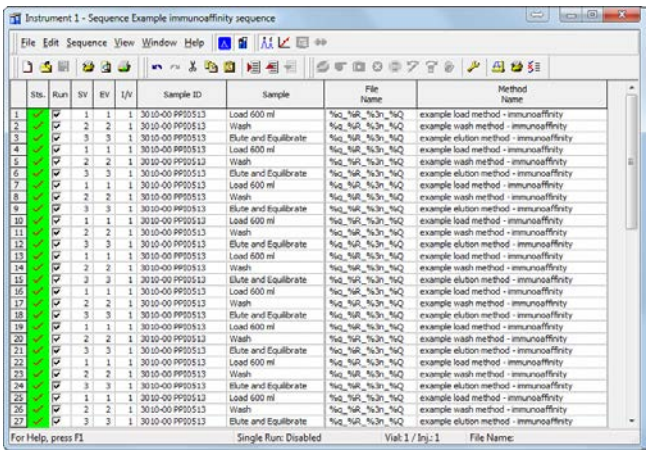

Fig. 3 - Example of sequence table for immunoaffinity separation

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.

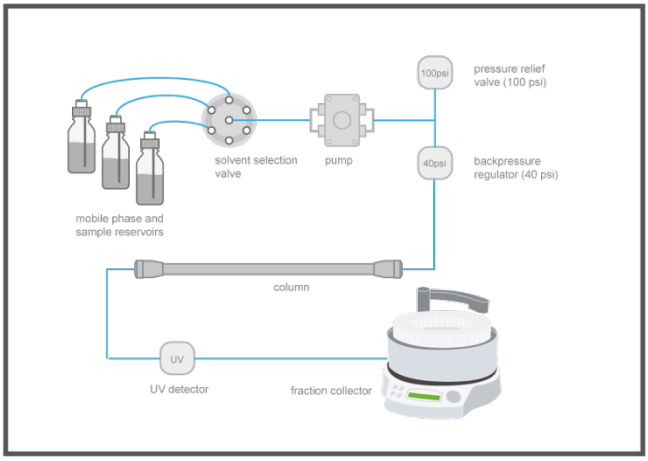

Fig. 4 - Diagram of the Stacked Injection SEC system

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.

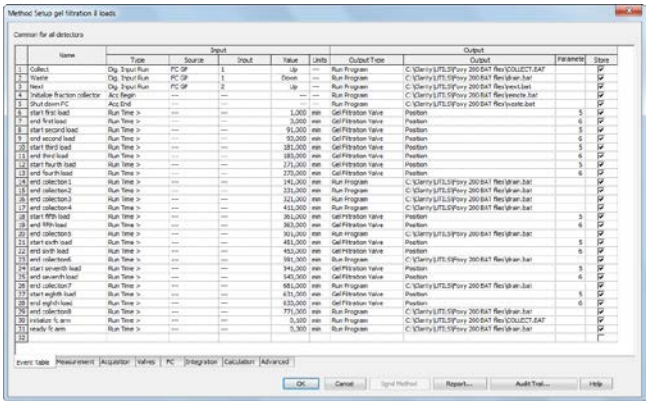

Fig. 5 - Example of Stacked Injection Method Event Table

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.

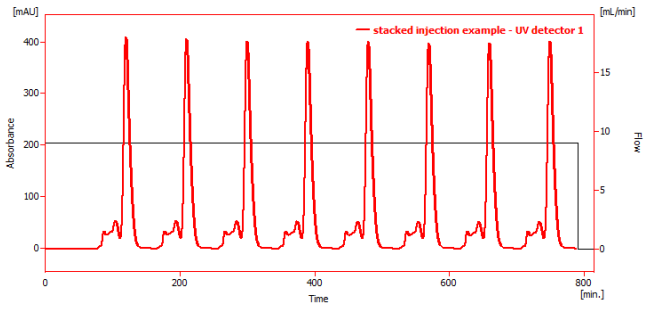

Fig. 6 - Example of stacked injection SEC separation

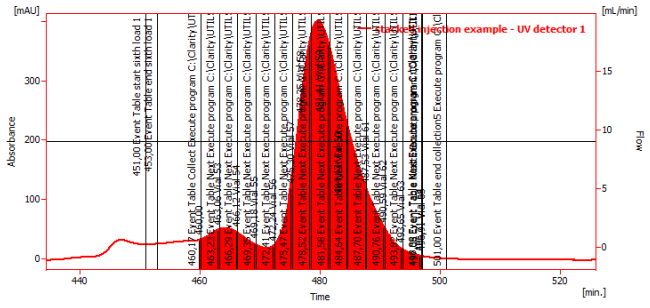

Fig. 7 - Detail of stacked injection SEC separation cycle

Conclusion

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

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- e.g. Colibrick 4 ch	U34
Add on instruments (optional – to control more systems from 1 station)	C55

Table 3 - Clarity Software Components

Authors

- Scott Horn, Southern Biotechnology Associates, Birmingham, USA, www.southernbiotech.com
- Ivan Vinš, DataApex Ltd., Prague, Czech Republic; www.dataapex.com, vins@dataapex.com