HPLC/SFC/CCC Lab Automation

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We began automating our HPLC/SFC contract purification lab in the late 1990s, adding CCC about 2010. This paper describes the lessons we learned and the unique products we developed to improve both throughput and predictability while significantly reducing labor and overall cost to purify. Products were initially developed for internal use.

We develop flow charts for processing compounds along a deterministic path. Flow charts are reviewed and incrementally optimized as we gain more experience and bring more capability online. We pay particular attention to how quickly jobs move through the lab and use total elapsed time from receipt to shipment as our primary metric, without sacrificing quality.

Studying the flow of jobs through our lab clearly indicates that rotovap time is about equivalent to prep separation/collection time but method development and scale up studies take significantly longer. We focused our product development on automating method development and prep peak collection aimed at 24/7/365 unattended operation.

Various criteria are used to determine if a compound is ready to leave method development. Is separation good enough to support the required analysis or purification? Does the method scale up with enough efficiency to complete the prep job in a reasonable amount of time and at a reasonable cost? Will the compound be stable in the eluent when using a 40˚C rotovap bath?

An HPLC/SFC/CCC lab should be viewed as an assembly line running a continuous stream of related methods and processes. With proper work flow and 24/7 automation of bottle-neck operations, productivity will increase significantly. Under most circumstances we can develop a method and purify 50+ grams in one week using the procedure outlined below.

Mo Put sample in method development station and screen solvents and columns for separation methods.

Tu Select methods indicating reasonable separation and check overnight stability at 50°C in candidate eluents. Can be skipped, if you are sure of stability.

We Measure sample solubility in eluents that passed stability test.
Evaluate loading, impurities, and cycle time at the analytical level and plug values into Prep Predictor to predict prep performance.
Verify loading, impurities, and cycle time at prep scale.
Start prep run, after collecting a few injections verify purity.

Th Evaporate collected peaks.

Fr Analyze dry powder and ship.

We give careful consideration to problem separations -- otherwise they are likely to block progress on normal separations. Often it is best to complete some easy jobs in a busy cue and apply extra time to difficult separations.

Laboratory techniques and procedures must be able to be repeated many times with consistent results. Any technique or procedure that does not meet this requirement is not robust and should not be a part of your operating procedure. Find a better way and/or fix equipment.

Productivity increases are realized by running continuously overnight and on weekends (5x increase) and by using previously optimized methods, sequences and procedures rather than making each step a research project (more than 2x increase). If operated properly a collective 10x increase in throughput is reasonable to assume.

Learn to read system pressure for an early indication of impending system shut down due to clogged frits, filters, and columns. Each system should have a chart of pressure at routine flow rate and eluent through each flow path or column. If you run 24/7/365, anticipating problems will avoid middle-of-the-night shut-downs and delivery delays.

Clean systems at the end of each job. We automatically wash the entire system with MeOH then IPA at the end of each screening sequence in method development. This avoids problems with retained compounds and/or solvents that are not miscible.

Use progressive maintenance and try to stay ahead of problems. Use additional frits and filters to protect columns, valves, etc.

Arrange the lab for easy access to busy locations. Usually it is necessary to schedule down time when making incremental changes. Instill a flexible and continuous improvement strategy.

An accurate listing of the elapsed time for each step in a typical purification job usually shows that there is as much need for low technology improvements as there are for high technology improvements. Optimize processing steps and your lab in view of the processes and be willing to make incremental changes. For example, we usually spend about as much time drying collected peaks as it takes to separate them – so rotovap time is just as important as prep separation time (SFC helps). Method development takes the longest time.

Method development and prep laboratories should be set up as continuous isles with systems on both sides of the isle. Rear access is important for ongoing maintenance and configuration optimization. Lab personnel can walk or roll chairs up and down isles to monitor and orchestrate multiple systems easily. Wide passage ways can be cut between 2 or more rooms to make a continuous isle long enough to include multiple method development, prep, and rotovap systems. Using this arrangement and appropriate automation it is possible for one person to operate many systems on a near continuous basis.

It is highly recommended to include the ability to monitor lab computers remotely. Our lab computers have internet access behind a firewall but can only access to our protected server via secondary user names and passwords. We control all lab computers remotely via the internet, currently using Team Viewer. Remote monitoring and control is especially comforting when we are enjoying a 3 day weekend but have many automated method development and prep systems running 24/7.

1.1 Method Development

It is not realistic to predict an appropriate separation method just by looking at analyte structure but usually some limiting decisions can be made. Basic automation of method development involves selecting a reasonable set of columns and eluents for the analytes expected. Automation software and hardware then sequentially injects analytes in a set of methods optimized for screening each eluent against all appropriate and available columns. If an HPLC/SFC/CCC system is configured with an Advanced Laser Polarimeter (ALP) it is easy to positively identify enantiomerically pure separations. Gradient methods are best for screening because a single injection covers more eluent variation in less time and a gradient can usually be designed to wash the column near the end of each run.

AutoCCC method development for CounterCurrent Chromatography uses the eluent mixer to create a new "column" for each injection. Long sequences are used for method screening, same as in packed column techniques (HPLC/SFC).

The objective of automated method development should be to develop the ability to run many pre-written and pre-tested methods unattended and without much human effort or attention; and then to write, refine, and maintain an effective set of sequences containing equilibration, separation, and washing methods.

Think of your collection of screening methods as a library with sequences being reading lists – once a method is optimized it can be used forever in different sequences (select or copy/paste). We "select" methods from our library when writing sequences. We seldom write new methods, except when new columns become available.

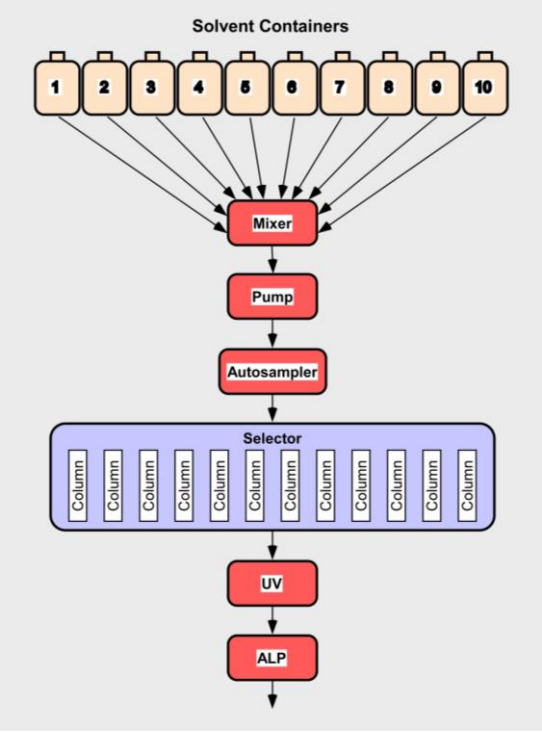
Screen all appropriate columns with a particular eluent, change the eluent, flush/equilibrate, screen all appropriate columns with the new eluent, etc.

It is best to order a sequence of methods to progress from high solvent strength to low solvent strength, always considering miscibility and including equilibration and washing steps to insure that each sample is introduced into consistent and reproducible method conditions. When one of our sequences is complete, not only have we screened a sample on many columns and eluents, but the columns are washed, equilibrated, and ready for the next sample or long term storage. We call this "care and feeding of columns" and we seldom need to replace a column.

When reviewing screening results, be suspicious of runs with no peaks – is the sample stuck on the column?

Be aware of column memory effects with additives --best to have a different set of columns for additives, especially true of chiral columns.

The most important aspect of an automated method development station is the ability to run long sequences with many methods using many columns and gradients from many bottles. We manufacture low pressure gradient eluent mixers that draw from 10 or 20 bottles and 24 position column selectors with heating and cooling. Our software has a common chromatographic user interface that controls method development and prep in HPLC, SFC, and CCC applications. We originally designed this suite of products because we could not buy what we wanted.



1 -- Method Development Plumbing and AutoMDS on Agilent with 20 bottles/24 columns with heating and cooling.

2 – AutoMDS on Agilent with older-style column selectors: 10 bottle/12 column HILIC on left and 10 bottle/24 column Chiral on right with one column selector for neutral and the other for acid additives.

3 – AutoMDS & AutoPrep on DE CCC.

1.2 Prep Purification

Prep purifications are roughly divided at the one kilogram level. Above one kilogram processes and systems tend to be well developed and optimized for low solvent consumption and low total cost. Simulated moving bed systems (SMB) dominate this region. Below one kilogram processes tend to be optimized for quick turn-around and system flexibility. HPLC was the traditional choice but SFC is becoming more common because there is less liquid to be evaporated after collection and column back pressure is lower allowing higher flow rates and faster processing. So SFC has 2 advantages: faster methods and more concentrated fractions (because the CO2 goes away).

At the under one kilogram level it is usually best to run HPLC or SFC methods with baseline separation between peaks. Injections are overlapped or stacked such that pure peaks are being collected from one injection while the next injection's elution is clean and not contaminating the collections. This results in reduced cycle time by allowing injections much closer together than would be possible if each injection's elution ended before the next injection began. When stacking, be careful to track impurities so they do not end up in the collected fractions.

Baseline separation between peaks, sometimes loading down, is usually more efficient when the entire operation is considered. Overlapping peaks can lead to concern over purity and the need to dry-down overlapped regions and inject again. During this time the HPLC/SFC/CCC is not available for other jobs without proper cleaning and setup.

Because there is a practical limit to the volume that can be injected onto a particular column, injection loading is often limited as much by solubility as by column loading limits. Trying to achieve maximum useful loading often implies nearly saturated sample solutions. This can result in precipitation build up in the system or column, especially if the sample solution is different from the eluent composition. If a method yields too much separation when the practical loading limit is reached, it is usually best to increase the organic modifier percentage in the eluent so as to drive the peaks closer together (but not overlapping) and reduce the cycle time. Processing rate is injected mass divided by cycle time.

A good method is usually the most important aspect of prep purification. Separation is clearly a requirement but solubility, impurities, and stability are also important. We developed a "Prep Predictor" spreadsheet that accepts inputs like injected mass, cycle time, eluent composition, flow rate, and collection valve open/close times. This spreadsheet predicts values like total run time, total solvent consumed, and total solvent collected. This information can be used to compare methods and accurately plan and/or cost a job. Examples follow and the Prep Predictor is available for download at www.pdr-separations.com .

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Prep Predictor LC

Material to be Separated	g	200.000
Injection Concentration	mg/mL	90.0
Injection Volume	mL	1.000
Material per Injection	mg	90.000
Number of Injections		2222.2
Cycle Time	min	10.0
Time Req'd	Hours	370.4
Time Req'd	Days	15.4
Flow Rate	mL/min	20.0
Total Eluent Req'd	L	444.4
Solvent B	%	35.0
Total Solvent A Req'd	L	288.9
Total Solvent B Req'd	L	155.6
Total Eluent Collected	L	311.1
Fraction 1 Open	min	5.00
Fraction 1 Close	min	7.00
Eluent Collected, Fraction 1	L	88.9
Fraction 2 Open	min	8.00
Fraction 2 Close	min	13.00
Eluent Collected, Fraction 2	L	222.2
Column, ID	cm	2.1
Column, Packed Length	cm	25.0
CSP Density	g/cc	0.6
CSP (calculated)	g	52.0

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Prep Predictor SFC

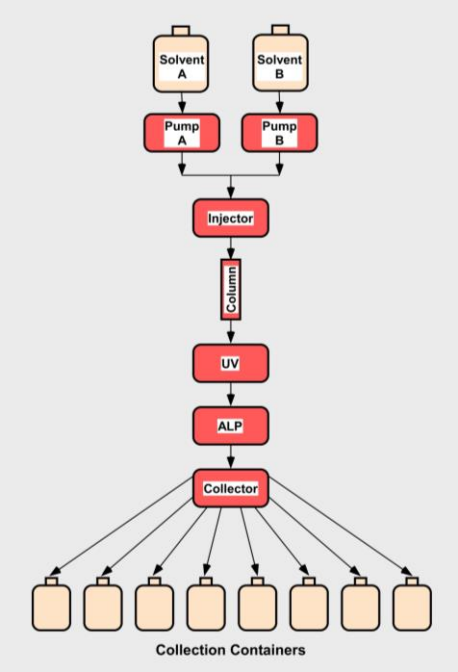
Material to be Separated	g	20.000
Injection Concentration	mg/mL	20.0
Injection Volume	mL	3.000
Material per Injection	mg	60.000
Number of Injections		333.3
Cycle Time	min	5.0
Time Req'd	Hours	27.8
Time Req'd	Days	1.2
Flow Rate	mL/min	50.0
Total Eluent Req'd	L	83.3
Modifier	%	25.0
Total CO2 Req'd	lb	439.2
Total CO2 Req'd	50 lb Tanks	8.783
Total Modifier Req'd	L	20.8
Total Modifier Collected	L	17.9
Fraction 1 Open	min	5.20
Fraction 1 Close	min	6.70
Eluent Collected, Fraction 1	L	25.0
Modifier Collected, Fraction 1	L	6.3
Fraction 2 Open	min	7.00
Fraction 2 Close	min	9.80
Eluent Collected, Fraction 2	L	46.7
Modifier Collected, Fraction 2	L	11.7
Column, ID	cm	2.1
Column, Packed Length	cm	25.0
CSP Density	g/cc	0.6
CSP (calculated)	g	52.0

4 -- Prep Predictor LC and SFC

If all columns are the same length and particle size, scale up is linear with column area ($A = \pi r^2$). In our contract lab we primarily use HPLC/SFC columns in 2 sizes, 4.6 mm and 2.1 cm diameter both with 25 cm length and the same 5 micron particles. We scale our methods between analytical and semi-prep by multiplying analytical parameters by 21 (area of 2.1 cm diameter divided by area of 4.6 mm diameter). In practice we run at 50 mL/min in SFC semi-prep and 50/21=2.38 mL/min in analytical method development. Now our retention times are the same at both scales. Similarly we explore loading at the analytical level and multiply by 21 to predict semi-prep loading. Our semi-prep runs are completely predictable using these techniques.

Key Points for Prep

- Consider the whole job when choosing the best method
  – solubility and impurities can make a big impact
- It is usually inefficient to collect overlap regions, dry, and inject
- It is usually better to make a large number of under loaded injections using an automated 24/7 system to achieve low risk, high purity separations
- Be cautious of precipitation in long runs, especially if sample is not dissolved in eluent
- Select the shortest cycle time that avoids impurities
- Use SFC when appropriate to reduce evaporation volume
- Wash column and system immediately after run
- Be serious about your columns health and condition



5 – Prep Plumbing and AutoPrep on Agilent HPLC

6 – AutoPrep on JCT CCC.

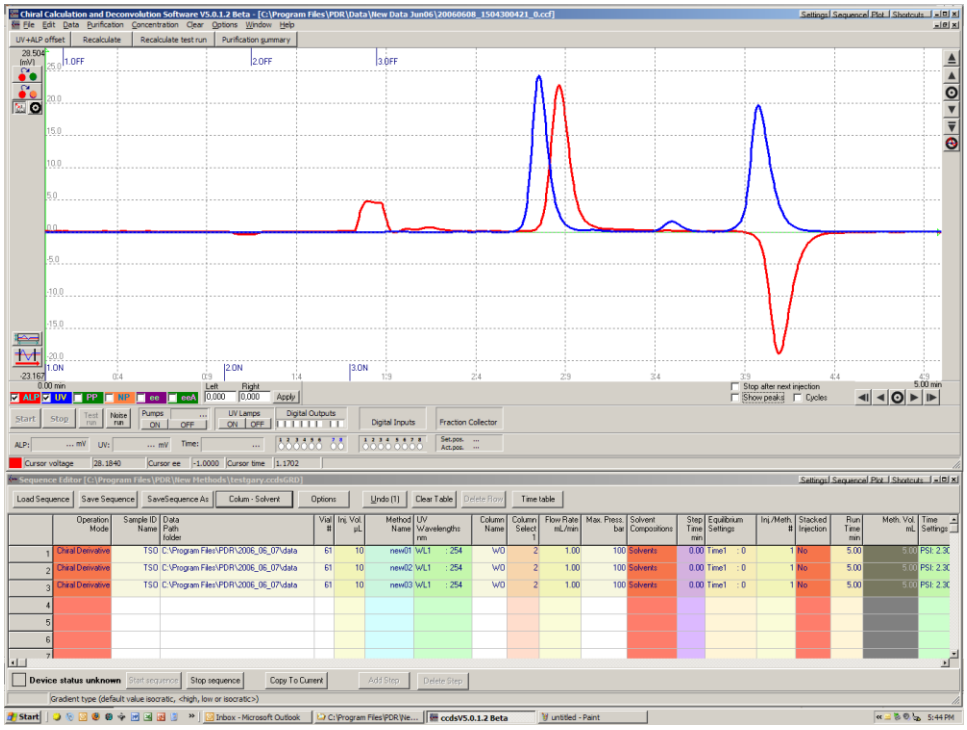
1.3 Software

Our software was originally developed in-house in the late 1990s because we could not find an acceptable product. All development has been in-house and focused on rapid method development and purification of compounds with minimum labor and maximum flexibility. A single user interface monitors and controls any operation. All methods and sequences are hardware independent and use chromatographic parameters that do not depend on the specific hardware being controlled. If we ask for 1 mL/min and 269 nm, who cares about the pump and detector manufacturers? We have drivers that translate chromatographic parameters into hardware commands for specific units, so we can control mixed-vendor hardware.

AutoMDS and AutoPrep software/hardware systems are designed to automate chromatographic method development and purification of achiral and chiral compounds. See key features below.

- Hardware independent methods and sequences displayed in an enhanced method table.
- Queue window for running any combinations of vials, methods, and sequences in a continuous fashion.
- Data Processing Tool that process large data sets (many injections) and filters them for methods giving suitable results.
- Robust control of collection and recycle valves using detector signal amplitudes, polarities, derivatives, calculated plots, time, and logical combinations.
- Unique Racemate collection mode that always puts the positive enantiomer in the positive collection port and the negative enantiomer in the negative collection port regardless of elution order or time.
- Reintegrate and Recalculate functions are used to optimizing peak detection parameters for proper peak area integration in analytical or for proper peak collection in prep applications.
- Chiral calculation of specific rotation and ee. The ee can be plotted and used for peak collection valve control in prep applications.
- Detector data files created by other programs can be imported via ASCII.txt format for post run calculations unique to our software.

Methods and sequences are at the heart of HPLC/SFC/CCC applications. AutoMDS and AutoPrep present methods and sequences in an enhanced method table where each row is a method and each column is a method parameter or method parameter group. To view a method parameter group, double click inside a parameter group cell and a window will pop up. When you click outside the pop up window, it will close. This pop up window feature allows complete method parameter sets to be presented in a single compact row of our enhanced method table. Users can select, edit, write, and run methods individually via the Method window, in a sequence of methods in the Sequence window, or in any creative combination of sample vials, methods, and sequences in the Queue window. The Queue table includes overwrite options for key method parameters (sample name, file prefix, data path, vial number, injection volume, etc.) and can be used for continuous operations. Since our methods and sequences are hardware independent they can be transferred to comparable systems configured with hardware from multiple suppliers.



7 – AutoPDR

1.3.1 AutoMDS

Features:

- Easily Write, Edit, and Run Methods and Large Sequences
- Single Keyboard Control of up to 88 Columns - including tandem configurations - and Random Gradient Mixing from up to 24 Solvent Bottles
- Supports 24/7 Continuous Unattended Operation
- Calculates Specific Rotation, Enantiomeric Excess, and a full-set of Achiral Chromatographic Parameters
- Sequences and Methods are Included

1.3.2 AutoPrep

Features:

- Most Advanced Control of Injection, Detection, and Collection Available
- Collect using UV and/or ALP with +/- Derivative, +/- Voltage, +/- ee, Time, and Logical Combinations
- Combine Data from UV and ALP Detectors to calculate Real Time ee
- Includes “Prep Predictor” -- a Spreadsheet that calculates All Relevant Job Parameters like Run Time, Solvent Consumed/Collected, K/K/D, etc.
- Collection by Polarimeter +/- Derivative requires very little sample specific set up and is Extremely Robust