Your Lab on Autopilot

HPLC/SFC laboratory automation

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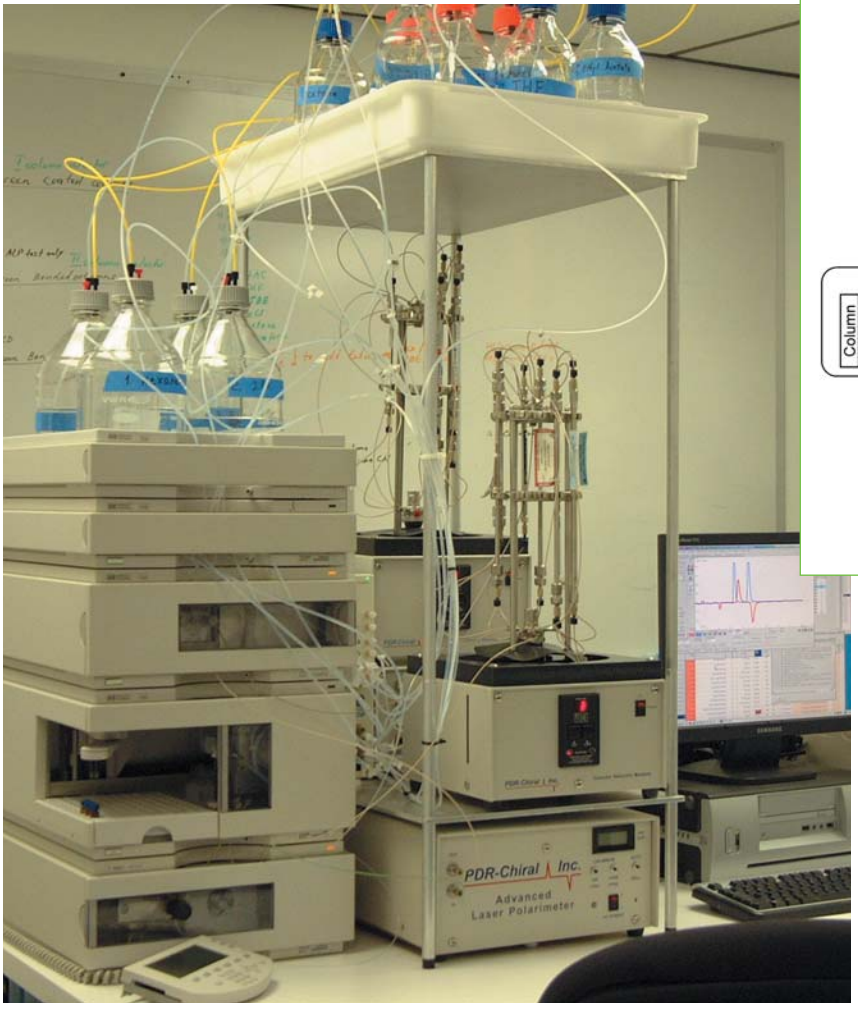
We at PDR-Chiral began automating our contract HPLC/SFC method development and preparative purification laboratory many years ago. This article briefly describes our procedural and developmental efforts in the automation of method development and preparative peak collection.

Operating procedure

We view our contract HPLC/SFC laboratory as a "job assembly line" running coordinated methods and processes. The laboratory is set up as a continuous aisle with systems on either side. Laboratory personnel can walk or roll chairs up and down this aisle to easily monitor and control multiple systems. We can log on to all laboratory computers via the Internet using encrypted software and enjoy remote control of those systems from inside our facility and around the world. Remote control is especially comforting when we are enjoying a three-day weekend but have automated method screening and prep jobs running 24/7. We can not only remotely monitor jobs, but we can stop and start any run and change as many parameters as we wish. By using this arrangement and the appropriate automation, it is possible for a few people to operate many systems on a nearly continuous basis.

Various criteria are used to determine if a job is ready to advance to the next step. Is separation good enough to support the required analysis or purification? Does the method scale up with enough efficiency to complete a prep job in a reasonable amount of time and cost? Will the compound be stable in the eluent when using a 40°C rotovap bath? Our team has developed a "Prep Predictor" spreadsheet that accepts input parameters such as injected mass, cycle time, eluent composition, flow rate and collection valve open/close times. This spreadsheet then predicts parameters including total run time, total solvent consumed and total solvent collected. We scale our methods between analytical and prep by multiplying parameters by a column scale-up factor. All of this infor-



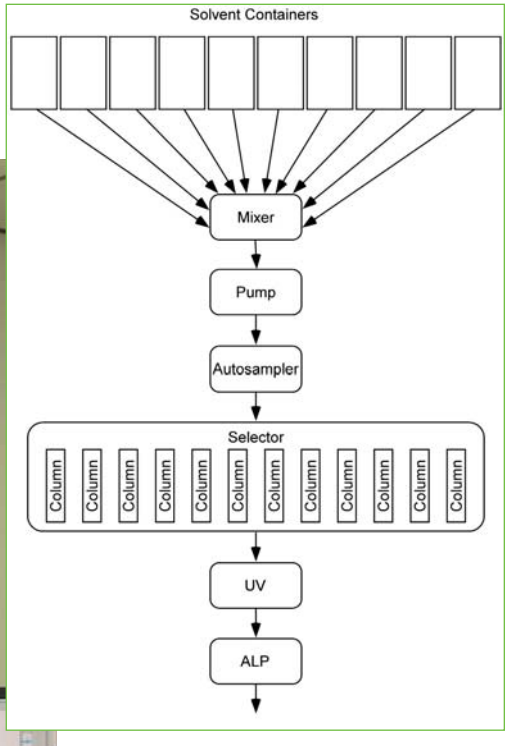


Figure 1: Automated method development system. (a) Generic flow diagram. (b) AutoMDS on Agilent 1100 with two column selectors (22 columns total), mixer (20 solvents) and advanced laser polarimeter.

mation is used to compare competing methods and to accurately plan and cost a job.

Under most circumstances, we can complete method development and purification of 50+ grams of, for example, an unknown racemate, in one week using the procedure outlined here:

Monday: Put sample in automated method screening station and screen solvents and columns for appropriate separation methods.

Tuesday: Select methods indicating separation and check overnight stability at 50°C in candidate eluents.

Wednesday:

- Estimate sample solubility in eluents that pass the overnight 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 level.
- Start prep run.

Thursday: Rotovap collected peaks.

Friday: Analyze dried sample and ship.

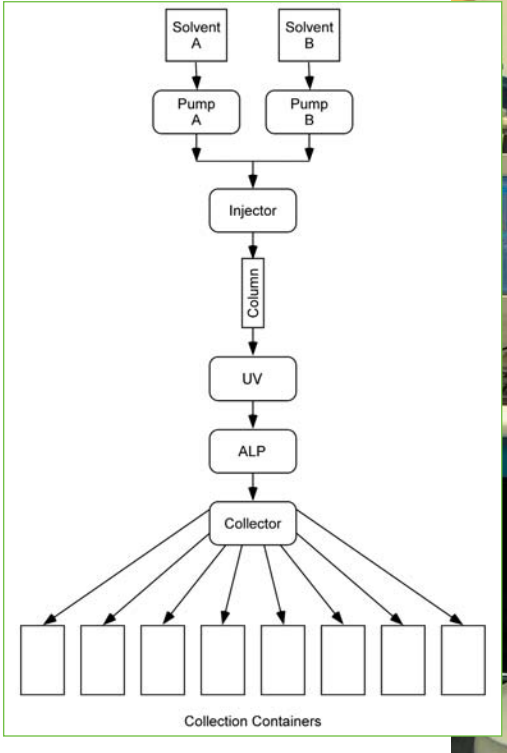
Method development

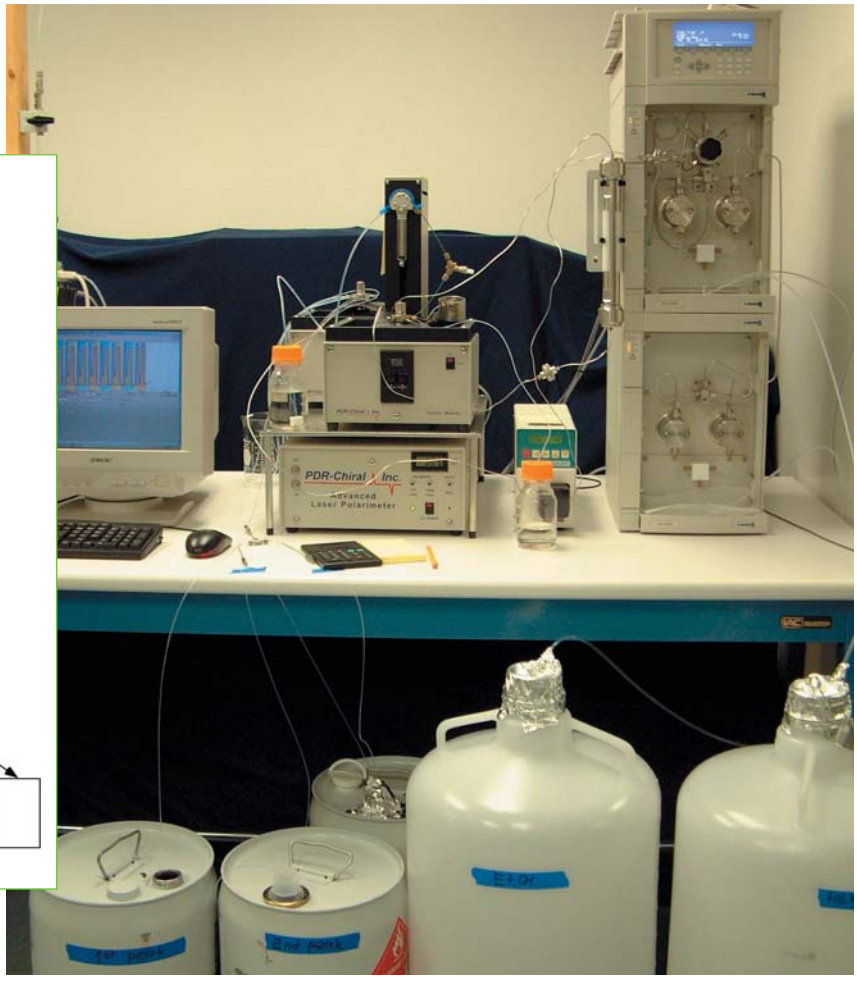
Our automated method screening systems run many prewritten and pretested methods unattended. Once a reasonably complete collection of sequences has been built, running a new sample only involves selecting the proper sequence for each sample vial. Think of your collection of screening methods as a library with sequences being reading lists—once a method is optimized, it can be used forever in different sequences. A well-written sequence includes equilibration and rinsing methods to keep the columns clean and the chromatography stable.

A problem with most HPLC/SFC systems is that they are limited in the number of columns and solvents that can be configured in a sequence. PDR-Chiral developed low-pressure gradient solvent mixers with 10 to 24 ports (bottles) and 12-position column selectors (typically 11 columns and bypass). We have also developed AutoMDS software to directly control the HPLC/SFC, our mixers and up to eight column selectors. This means we can have up to 24 bottles and 88 columns under single keyboard control. Figure 1b shows the method screening system, which has 22 columns in two column selectors, a 20-bottle, low-pressure gradient mixer and can screen chiral and achiral methods. This system can also screen tandem column configurations.

Our low-pressure gradient mixers are an engineering extension of the standard quaternary low-pressure gradient mixers that are part of most HPLC systems. We use the same solenoid actuated diaphragm valves assembled in a compact rosette manifold. This valve assembly is locally controlled by a single-board computer (SBC) mounted inside the mixer chassis. The SBC stores and executes a method continuously until changed by

Figure 2: Automated prep system. (a) Generic flow diagram. (b) AutoPrep with Gilson pumps, Knauer UV, ALP, injector and collector showing two solvent containers on the lower right and two peak collection containers on the lower left.





software on the controlling computer. Since the SBC is dedicated to the mixer, there are no timing irregularities.

In the mixer control window of AutoMDS, specific solvent names can be entered for each mixer port, and specific solvent names and gradient details print in the report along with other method details for full documentation. The mixer control window also includes viscosity compensation for each solvent and cycle time adjustments to be compatible with any pump.

Chiral screening examples

Chiral HPLC/SFC has become a mainstream tool in pharmaceutical development. The need to rapidly develop chiral separation methods for analytical and preparative purification applications has increased dramatically in the last 10 years. Chiral HPLC/SFC method development is complicated by the fact that many different eluents and columns are required for a complete screen. The following examples illustrated some common chiral screens.

If screening samples on Daicel IA, IB and IC, newer bonded polysaccharide chiral selectors, it is reasonable to use a 10-port mixer configured with, for example, Hexane, IPA, EtOH, MeOH, ACN, MtBE, Ethyl Acetate, Acetone, THF and DCM.

If screening samples on Daicel OD, AD, AS and OJ, traditional coated polysaccharide chiral selectors, it is reasonable to use a 10-port mixer configured with, for example, Hexane, IPA, EtOH, MeOH, ACN, Hexane + TFA, IPA + TFA, EtOH + TFA, MeOH + TFA, and ACN + TFA. In this arrangement, we would suggest using two sets of columns (eight total), one set being used with the solvents containing TFA and one set is used for solvents without TFA. This avoids the necessity to spend time washing TFA off before running a no-TFA method, as these columns have an additive memory.

If screening samples on ASTEC Chirobiotic T, V or R bonded antibiotic chiral selectors, it is reasonable to use a 10-port mixer configured with three sets of MeOH, ACN and water: one set is pure, one set with acid additive and one set with base additive. These nine bottles can now be creatively combined to create any reasonable combination of MeOH, ACN, water, acid and base, either isocratically or as a gradient.

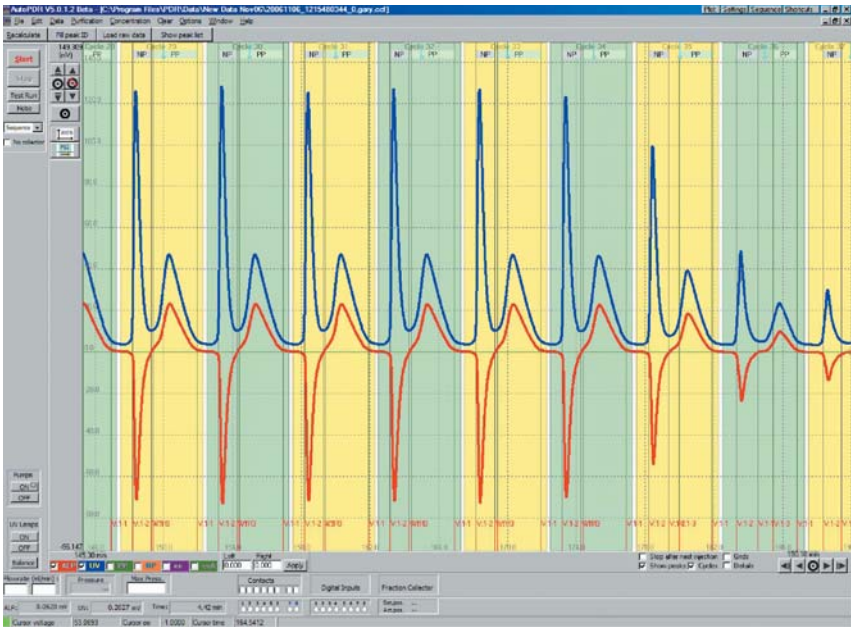


Figure 3: AutoPrep collecting in ALP Only mode

Using an appropriate arrangement of column selectors and mixers, we can configure any high-value combination of columns and solvents into a gradient or isocratic method and sequence using only the mouse and keyboard. Our HPLC/SFC method development station have walk-away automation, broad analyte utility and the flexibility to adapt to a dynamically changing work flow.

AutoMDS and AutoPrep (mentioned below) allow us to use orthogonal detectors, such as UV and an advanced laser polarimeter (ALP), in our method development and prep stations. This significantly extends our capabilities and shortens the development time. UV is usually best for displaying total injected material, including impurities. ALP is much more selective and does not respond to chromophores, but is usually best for displaying and collecting enantiomers.

Preparative purification

Techniques for preparative purifications are roughly divided at the 1-kg level. Above 1 kg, processes and systems tend to be well researched and optimized for low solvent consumption and purification cost. Simulated moving bed (SMB)-type systems dominate these applications. Below-1-kg processes tend to be optimized for quick turnaround and system flexibility. HPLC is the traditional choice, but SFC often shows improvements over HPLC due to lower eluent viscosity and less liquid to be evaporated after collection. The following discussion does not necessarily relate to purifications above 1 kg.

At the under-1-kg level, it is usually best to use HPLC or SFC methods exhibiting near-baseline separation between collected peaks. Injections are generally overlapped and stacked closely but carefully together such that peaks being collected are not contaminated by elution from other injections. Collecting the pure regions of the nose and tail from overlapping peaks usually requires that the center be collected, concentrated and injected again in order to recover material that is otherwise lost. We find it less labor intensive and often quicker to lower sample loading and make more injections using automated 24/7 systems (Figures 2 and 4).

For automated prep peak collection, we developed AutoPrep software that includes a new adaptive mode for collecting enantiomeric peaks called ALP Only. The ALP Only collection mode adapts in real time to variations in retention time and loading and always puts the (+) peak in the (+) port, and the (-) peak in the (-) port, regardless of elution order. It is generally possible to

Figure 4: Automated SFC prep system. AutoPrep on Berger Multigram with Knauer UV and ALP.

set optimized default parameters such that only injection volume, cycle time and the number of stacked injections need be entered for each new job (in addition to basic method set up for solvents, pumps and column).

AutoPrep calculates the first derivative of the ALP signal in real time. The sign (+/-) of the ALP first derivative differentiates the two enantiomer peaks so that each can be collected in specific collection ports, i.e., (+) = port 2 and (-) = port 4. A different ALP first derivative threshold value can be set for the start and end of each enantiomeric peak.

In Figure 3, the blue trace is UV and red is ALP. The aqua colored arrows near the top of the plot document injector valve movement; down means "moved to fill loop position" and up means "moved to flush loop onto column" position. Collector valve movement is similarly documented near the bottom in red. V1-1 means Valve 1 moved to Position 1 and V1-2 means Valve 1 moved to Position 2. Across the top of plot peak collection, intervals are marked as NP for negative peak and PP for positive peak. Cycle numbers appear at the top of the plot and cycles have alternating odd/even color backgrounds.

Also notice in Figure 3 that near the end of this run, the peaks are getting smaller. This is because we added pure solvent to the sample solution bottle when it was emptied by the injector to rinse the last of the racemate from the tubing. So the concentration of each injection is falling as pure solvent rinses the tubing. AutoPrep continues to make correct collection decisions as loading is reduced. Similarly, we could change pump speed (flow rate) to change retention times and observe that collection times are still correct because all collection decisions are made based on real-time data.

This ALP Only collection technique has advantages as compared to UV Only collection. The ALP differentiates between enantiomers and ignores achiral impurities. Also, there is no wavelength to adjust and linear dynamic range is very large. This collection technique is very accurate and never puts the wrong peak in the wrong bottle.

Refer to Figure 4. We have separated up to 1 kg of racemate per week on this SFC semi-prep system using AutoPrep in ALP Only collection mode.

Conclusion

These procedures and systems have been in use for a few years and results have been excellent. We are able to run method screening and prep purification largely unattended 24/7 and now view them as background tasks. Overall productivity improvement is more than we expected because procedures are streamlined and compatible with technology improvements. ■

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LC Product News

Application Notes for UFLC System

Application notes are now available for the Prominence ultra-fast liquid chromatograph (UFLC), including notes on xanthine derivatives and triarylmethane ink dyes, soft drinks and urine analysis, available for download at www.ssi.shimadzu.com/fast. One example includes "High-Speed Analysis of Xanthine Derivatives and Triarylmethane Ink Dyes with Ultra Fast HPLC," which details the analysis, retention time and peak area reproducibility using the UFLC system. Research found Shim-pack XR-ODS high-speed columns result in shorter analytical cycle times with systems comprised of conventional hardware, while maintaining high separation efficiency and reproducibility performance. The company also researched the fast and efficient separation of drugs in urine with UFLC, in combination with the LCMS-2010EV mass spectrometer. "Fast Analysis of Drugs in Urine" resulted in an analytical cycle time of 5.5 minutes, including column equilibration