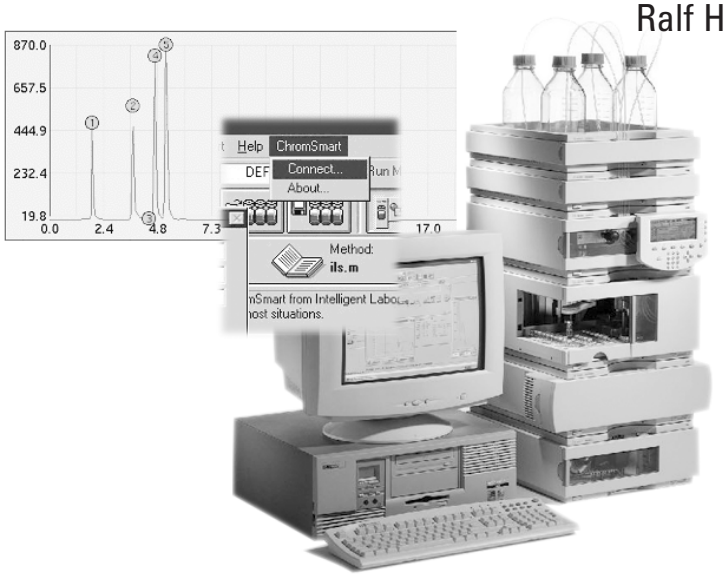
Automated method development using Agilent 1100 Series HPLC systems, Agilent ChemStation and ILS ChromSmart MD software

Application

Jeffrey D. DeCicco
Douglas R. Myers
Ralf Honsberg



Abstract

The development of an HPLC method can be a time-consuming and knowledge-intensive process. Experienced chromatographers often spend a good portion of their effort on routine tasks rather than performing value-added research and development. The ChromSmart MD software from Intelligent Laboratory Solution (ILS) automates these routine tasks and the experimental method development process. ChromSmart uses real-time, expert-system technology to plan, execute, and evaluate methods. This Application Note demonstrates the use of ChromSmart with Agilent 1100 Series HPLC systems and Agilent ChemStation software for automating the experimental method development process.

Agilent Technologies

ChromSmart MD software

ChromSmart integrates HPLC instruments, sample descriptors, experiment constraints, and method objectives to develop separation methods. ChromSmart MD emulates the actions and decisions of analysts by automating the time-consuming, repetitive tasks of method planning, development, execution, and interpretation. ChromSmart MD does these tasks unattended and online.
The analysts begins the method development process by specifying analysis conditions for the initial experiment as well as criteria that the final method must fulfill in terms of chromatographic performance such as retention time and resolution. ChromSmart MD analyses the results of the initial experiment, determines the next logical step required to drive the method towards fulfillment of the criteria, and then executes this step while monitoring the results. ChromSmart MD bases the decisions about which experimental parameters to vary on expert interpretation of the results. With intelligent analysis performed by ChromSmart, there are no lengthy systematic searches covering redundant or irrelevant parameter spaces. Instead, navigating the search space for method planning is done in a more directed manner, substantially increasing the efficiency of the method development process. ChromSmart provides the following capabilities for increasing laboratory productivity and throughput.

- Automated development of HPLC separation methods using the analysts' expert knowledge
- Automated identification of separation conditions, including gradient, isocratic, reversed-phase, normal phase
- Handling of a wide range of samples, including ionic/non-ionic and chiral/non-chiral
- Post-run analyses, method results interpretation, and recommendations for subsequent runs
- Automated search through the analysts' selectivity variable preferences, including control of column switching systems
- Automated equilibration with detection and removal of retained materials
- Total area tracking to stop the method early, if the sample has eluted through the column
- Automated column washing (also known as a zero-injection gradient or ZIG) for new columns or columns with possible retained material

Interfacing with Agilent 1100 Series HPLC and Agilent ChemStation

ChromSmart integrates and controls Agilent 1100 Series 1100 HPLC whereby method control and data acquisition are provided through the Agilent ChemStation software. The communication between ChromSmart and ChemStation occurs through a real-time software bridge. This bridge resides on the same computer as the ChemStation online software. ChromSmart can reside on the same computer or on any computer on the local area network (LAN). Figure 1 shows a typical configuration of equipment. Figure 2 shows how to connect to ChromSmart from the ChemStation's Method and Run Control menu. ChromSmart does not interface directly with the Agilent 1100 Series modules. Instead, ChromSmart collects data in real-time from the ChemStation. ChromSmart evaluates this data in real-time and instructs ChemStation to perform tasks automatically. For example, ChromSmart instructs ChemStation to perform a column equilibration with a given column and mobile phase. During equilibration the UV signal is monitored in real-time. When a stable baseline is detected, ChromSmart instructs ChemStation to stop equilibration and initiate a sample injection. ChromSmart collects the real-time UV spectrum, temperature and flow measurements. In addition, the integrated peak and spectra are collected and analyzed. ChromSmart takes advantage of the advanced control and data analysis capabilities of ChemStation. This includes peak integration and spectral analysis capabilities. For example, ChromSmart can request automatically that ChemStation perform spectral peak purity analysis or spectral comparisons for a set of peaks. ChromSmart also provides advanced equipment monitoring. The objective is to ensure equipment wellness and avoid operating conditions that lead to a shutdown.

For example, back-pressure is monitored in real-time. If the rate of change causes the high-pressure limit to be exceeded, ChromSmart decreases flow or changes the mobile phase concentration. In addition, ChromSmart ensures that columns are protected from mobile compositions that may damage the packing. The Agilent 1100 Series HPLC systems and ChemStation software are very flexible and can incorporate equipment such as automatic column-switching valves. ChromSmart takes advantage of these switching valves and can perform automatically "smart" column screening, using multiple columns during the method development process.

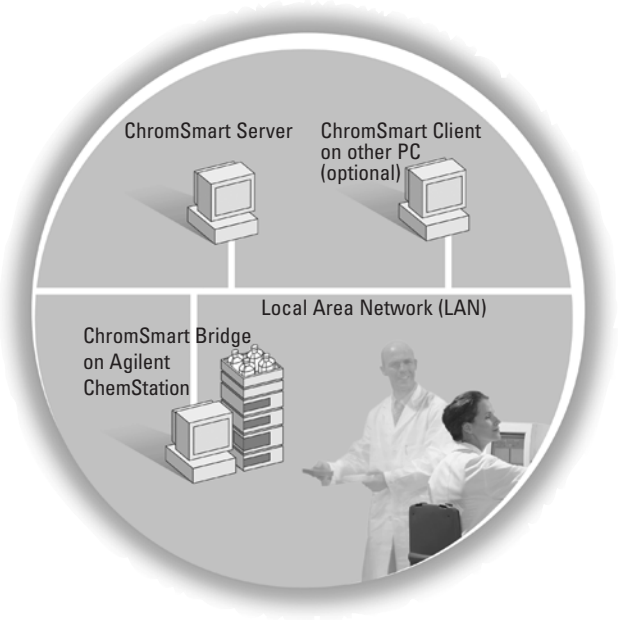


Figure 1
Typical ChromSmart MD configuration

Experimental case study

The following example illustrates how ChromSmart optimized the mobile phase screening process for a reversed phase separation. Minimal configuration was required to start the optimization process. The desired mobile phases and columns to optimize were specified as well as operating preferences, method criteria, and optimization preferences.

Compounds	Concentration
1. Uracil	0.015 mg/mL
2. Phenol	0.7 mg/mL
3. Benzaldehyde	0.05 mg/mL
4. N,N-Diethyl-M-Toluamide	1.0 mg/mL
5. Toluene	4.0 mg/mL
6. Ethyl Benzene	4.0 mg/mL

Table 1
Sample compounds

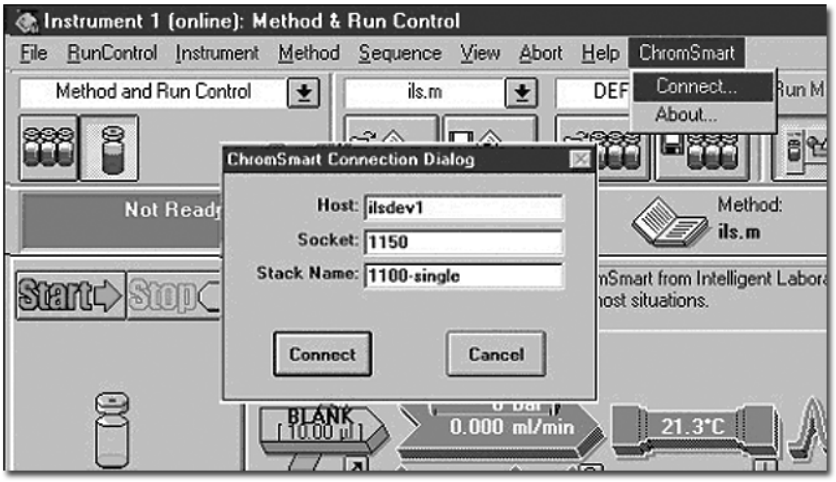


Figure 2
Connecting to ChromSmart from ChemStation

The sample presented to the system contained the six compounds shown in table 1. For this sample, a method of (42 % water / 58 % acetonitrile) was known to provide adequate separation results on a C18 column at a flow rate 1.0 mL/min with a detection wavelength of 245 nm. To run ChromSmart the analyst only has to know the separation type, that is, reversed-phase, and the desired selectivity variables (columns and solvents). In this example, the analyst entered the following information into ChromSmart.

- Reversed-phase chromatography as appropriate separation approach
- Mobile phase selectivity variables in following order:
  1 Ethanol (EtOH)
  2 Methanol (MeOH)
  3 Acetonitrile (ACN)
- C8 column as stationary phase selectivity variable (more columns could be chosen if an automatic column switcher is available on the HPLC system)

The following method and optimization preferences where chosen.

- Perform automatic column washes before sample is injected for the first time or if retained material is suspected on the column.
- Perform automatic column equilibration with minimum equilibration time of 5 minutes and maximum equilibration of 60 minutes.
- Optimize to find best isocratic method with a minimum resolution of 1.0, and retention range between 1.0 and 10.0 k.
- Perform initial scouting gradients between 5% and 80% (strong solvent) in 20 minutes.

Once these preferences were entered in the appropriate dialogs, ChromSmart automatically generates an initial plan of experiments and starts the method development process. This initial plan is based on the optimization and selectivity preferences. The preferences chosen were isocratic optimization with a single selectivity variable type: organic phase. The initial plan consisted of three scouting gradients, one for each solvent. If the scouting gradient suggests an isocratic separation is possible, then the plan is changed dynamically during the method development process to try running an isocratic method. ChromSmart was presented with the sample and allowed to operate in unattended mode. At the conclusion of the method development session, ChromSmart has performed 25 methods in about 6 hours. This included the following:

- 3 scouting gradients
- 9 isocratic experiments
- 3 zero-injection-gradients (ZIG) to wash the column
- 6 column equilibrations

The final result was an isocratic method that met the desired user requirements of resolution and retention. The final method had a mobile phase composition of (81 % water / 19 % acetonitrile) on a C8 column. Figure 3 shows the chromatogram obtained using the final method. Notice that the method was stopped automatically because ChromSmart detected that sample had completely passed through the column.

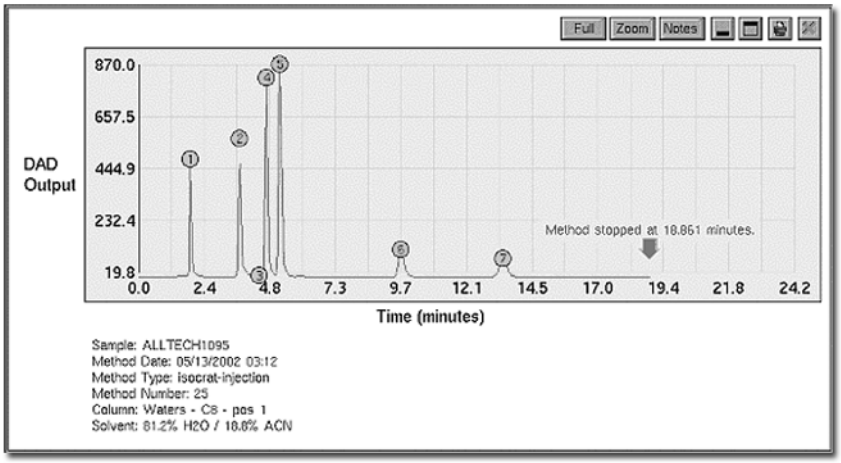
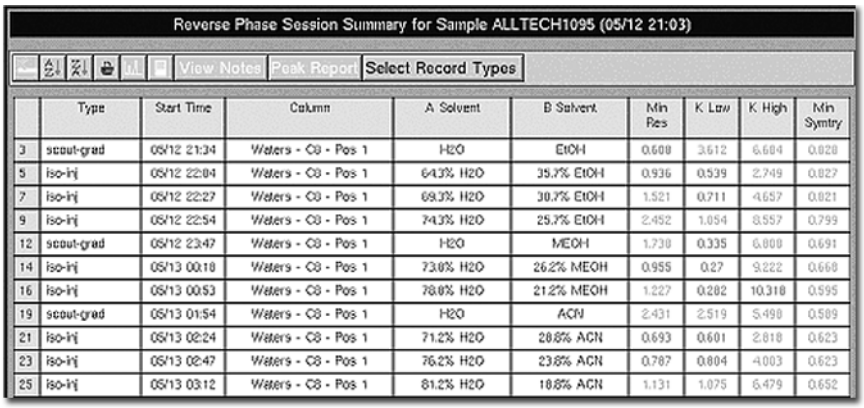


Figure 3
Final method found by ChromSmart for the reversed-phase sample

Browsing method development results in ChromSmart

Several aspects of a method development or sample analysis session can be browsed. This includes a summary of all injections, a slide show of chromatograms, session configuration, the execution plan, formal reports, and an audit trail of the decisions made by ChromSmart. There are two views to browse quickly the results of a session. The first is a spreadsheet summary and the second is a slide show of chromatograms. The results can be queried further to show only methods that involved sample injections, that is, remove ZIGs and equilibrations. Figure 4 shows this view.

From the spreadsheet summary the analyst can review the chromatogram, the decision-making audit trail, a peak report, and overall rating of the method. The values in green represent "good" method conditions that met the users requirements. A more visual approach to review the results is with the slide show option. This option allows the analyst to review the results and notes in a step-by-step fashion.



	Type	Start Time	Column	A Solvent	B Solvent	Min Res	K Low	K High	Min Symtry
3	scout-grad	05/12 21:34	Waters - C8 - Pos 1	H2O	EtOH	0.609	3.612	6.684	0.828
5	iso-inj	05/12 22:04	Waters - C8 - Pos 1	64.3% H2O	35.7% EtOH	0.936	0.539	2.749	0.827
7	iso-inj	05/12 22:27	Waters - C8 - Pos 1	69.3% H2O	30.7% EtOH	1.521	0.711	4.657	0.821
9	iso-inj	05/12 22:54	Waters - C8 - Pos 1	74.3% H2O	25.7% EtOH	2.452	1.054	8.557	0.799
12	scout-grad	05/12 23:47	Waters - C8 - Pos 1	H2O	MEOH	1.730	0.335	6.809	0.691
14	iso-inj	05/13 00:18	Waters - C8 - Pos 1	73.8% H2O	26.2% MEOH	0.955	0.27	9.222	0.668
16	iso-inj	05/13 00:53	Waters - C8 - Pos 1	78.8% H2O	21.2% MEOH	1.227	0.282	10.318	0.595
19	scout-grad	05/13 01:54	Waters - C8 - Pos 1	H2O	ACN	2.431	2.519	5.498	0.589
21	iso-inj	05/13 02:24	Waters - C8 - Pos 1	71.2% H2O	28.8% ACN	0.693	0.601	2.818	0.623
23	iso-inj	05/13 02:47	Waters - C8 - Pos 1	76.2% H2O	23.8% ACN	0.787	0.804	4.003	0.623
25	iso-inj	05/13 03:12	Waters - C8 - Pos 1	81.2% H2O	18.8% ACN	1.131	1.075	6.479	0.652

Figure 4
Summary of method that involved sample injections only

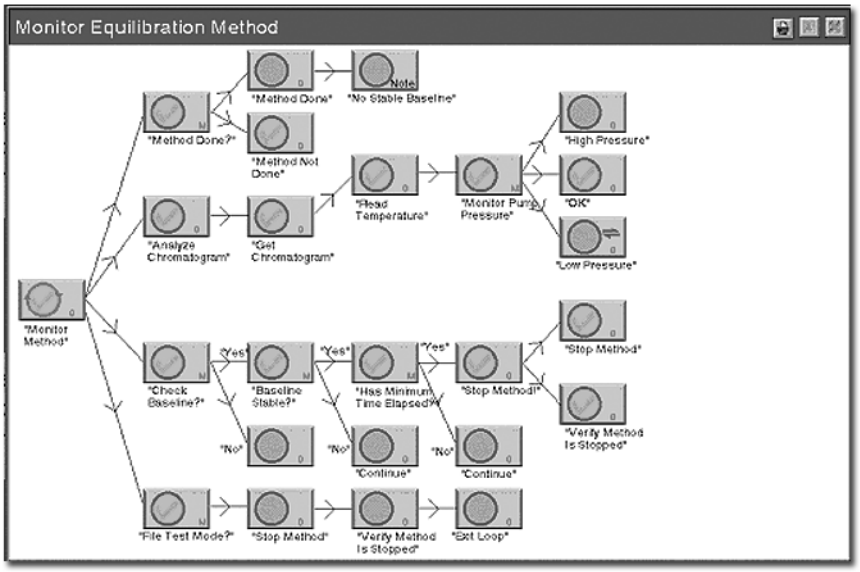


Figure 5
Equilibration decision tree

Using decision trees in ChromSmart

ChromSmart uses automated rules of thumb to analyze chromatographic data, make recommendations, and execute those recommendations. Rules of thumb can range from heuristics to the analysis of model predictions. A good example of a rule of thumb for reversed-phase chromatography is the "Rule of 3": There is approximately a three-fold increase in k for a 10 % decrease in B (strong solvent). This rule would follow an analysis that analyzes the current experimental results and determines that a decrease in % B is required, that is, the peaks are bunched left or an increase in selectivity is required.
These rules of thumb are abundant in the field of chromatographic analysis. Often analysts are implicitly making decisions based upon these rules of thumb. To simplify

matters, rules of thumb are separated into two categories: in-method and post-method. In-method refers to decisions made and actions taken during an actual experiment. Such rules of thumb are concerned with equipment and method monitoring. Post-method refers to decisions made after an experiment is run and may look back at a history of results. Many analysts may perform this analysis implicitly by simply observing the chromatogram. It is possible to summarize such rules by simply taking a step-by-step approach to the decision making process. The first step is to summarize the information that is relevant. In this case, the UV signal level and the method run-time are important. The second step is to summarize the relevant rules.
A flow sheet is a useful tool to explore, depict, and test the information and rules. Once the information and rules are summarized, it is possible to capture and execute them as ChromSmart decision trees. Within ChromSmart, decision trees are essentially automated flow sheets. Figure 5 shows a portion of the in-method automatic equilibration decision tree.

References

1.
The "Rule of 3" can be found in the reference: L.R. Snyder, J. J. Kirkland, J.L. Glajch, Practical HPLC Method Development, *Second Edition*, *Wiley*, *New York*, **(1997).**

Links

Further information about ChromSmart MD software can be found on the Web at www.chromsmart.com.

Jeffrey D. DeCicco, Ph.D., is Marketing Manager and Douglas R. Myers, Ph.D., is President at Intelligent Laboratory Solutions Inc., Naperville, IL, USA.
Ralf Honsberg is Product Manager for Data Systems at Agilent Technologies GmbH, Waldbronn, Germany.

www.agilent.com/chem/nds

Published March 1, 2003
Publication Number 5988-8963EN

Agilent Technologies