

An Advanced, Interactive, High-Performance Liquid Chromatography Simulator and Instructor Resources

Paul G. Boswell,^{*,†} Dwight R. Stoll,[‡] Peter W. Carr,[§] Megan L. Nagel,^{||} Mark F. Vitha,[⊥] and Gary A. Mabbott[#]

[†]Department of Horticultural Science, University of Minnesota, St. Paul, Minnesota 55108, United States

[‡]Department of Chemistry, Gustavus Adolphus College, Saint Peter, Minnesota 56082, United States

[§]Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, United States

^{||}Department of Chemistry, Penn State Greater Allegheny, McKeesport, Pennsylvania 15132, United States

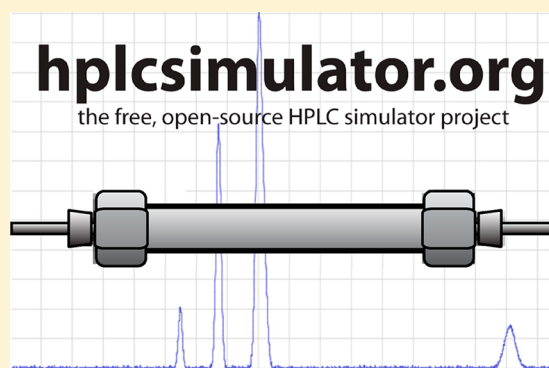
[⊥]Department of Chemistry, Drake University, Des Moines, Iowa 50311, United States

[#]Department of Chemistry, University of St. Thomas, St. Paul, Minnesota 55105, United States

S Supporting Information

ABSTRACT: High-performance liquid chromatography (HPLC) simulation software has long been recognized as an effective educational tool, yet many of the existing HPLC simulators are either too expensive, outdated, or lack many important features necessary to make them widely useful for educational purposes. Here, a free, open-source HPLC simulator is described that we developed. The Web-based simulator is uniquely sophisticated, yet accessible to a diverse user group with varied expertise in HPLC. It features intuitive controls and indicators for a wide range of experimental conditions, and it displays a graphical chromatogram to provide immediate feedback when conditions are changed. The simulator can be found at hplcsimulator.org. At that Web site, a number of example problem sets are also provided that educators can easily use to incorporate the simulator into their curriculum. Comments from students who used the simulator in an undergraduate analytical chemistry class were positive.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Computer-Based Learning, Internet/Web-Based Learning, Chromatography, HPLC, Separation Science, Student-Centered Learning



High-performance liquid chromatography (HPLC) is an important part of the undergraduate chemistry curriculum, but it can be a difficult technique to master. This difficulty can be attributed to the large number of often-interrelated experimental variables that influence the outcome of each chromatographic analysis. For a student to gain an intuitive feel for how the important experimental parameters and their interplay affect HPLC separations, they would need to perform a large number of experiments. Given the time it takes to run individual separations and the constraints of a typical analytical chemistry laboratory session, it is unreasonable, if not impossible, for students to gain this type of experience.

Instead, computer-based HPLC simulators have long been used as a lab exercise that students can use to learn about and experience the fundamental principles of chromatography.^{1–5} HPLC simulators offer the advantages that they are easy-to-use, relatively inexpensive, there is no risk of damage to the instrumentation, no consumption of supplies, less supervision is required, and because simulators provide instant feedback, it can be more engaging than running real HPLC instruments. Of course, simulators should not be a replacement for using real HPLC instrumentation in the lab; rather, they should be used

as a supplementary tool to enhance the value of time spent on a real instrument. HPLC simulators allow students to explore the fundamental principles of HPLC more efficiently than they could in the lab.

For these reasons, we set out to incorporate an HPLC simulator into our undergraduate and graduate course curricula. Several commercial HPLC simulators were available including Drylab (Molnar-Institute),⁶ LC & GC Simulator (Advanced Chemistry Development),² and ChromSword (Iris Tech).⁷ Although they are high functioning, they were outside our budget. Even if we could have purchased them, we would have preferred to introduce students to a resource that they could continue to use after they graduate and no longer have ready access to our computer facilities.

Therefore, we set out to find a suitable HPLC simulator that was either free or low-cost. After an exhaustive Internet and literature search, we found six HPLC simulators (Table 1). Two of those simulators stood out. HPLCSIM⁴ is an unusually sophisticated simulator. It handles both isocratic and gradient

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Table 1. Free and Low-Cost HPLC Simulators

Name	Web Address	Format
HPLCSIM	http://edu.chem.tue.nl/ce/simulations.htm	DOS application
HPLC for Windows	http://pubs.acs.org/doi/pdf/10.1021/ed072p1086.2	Windows application
HPLC Simulation	http://www.colby.edu/chemistry/NMR/hplc.html	Web page (HTML)
HPLC Simulation	http://www.kabyn.com/hplc/index.php	Flash application
Chromulator	http://www.ohio.edu/people/gu/CHROM/	Windows application
Chromatographic Separations	http://www.chem.uoa.gr/applets/AppletChrom/Applet_Chrom2.html	Java Applet

elution and gives control over a wide range of experimental parameters. HPLC for Windows^{5,8} is another notable HPLC simulator. It also handles a range of experimental conditions, but it puts a stronger emphasis on the practical considerations of running HPLC equipment (e.g., making sure to flip the power switch on first). Though potentially useful, both of these pieces of software run only on 32-bit Windows. As 64-bit computers continue to become the norm, the software is destined to become obsolete.

Evidently there was a need in the educational community for a free HPLC simulator that is both sophisticated and able to run on modern operating systems, so we developed one. In this article, we describe a new, free, open-source HPLC simulator we created and made available at hplcsimulator.org,⁹ where we also provide several example problem sets to help instructors easily incorporate the simulator into their own curriculum. We

discuss the key features of the HPLC simulator, other resources available on the Web site, and an example of its use in an analytical chemistry class. The simulator's inner workings are described in the Supporting Information

SIMULATOR DEVELOPMENT

Calculations

The calculations performed by the HPLC simulator are given in the Supporting Information for interested readers and for reference, but it is unnecessary to understand them to be able to use the HPLC simulator; even someone new to HPLC should find it easy-to-use and intuitive. Briefly, the HPLC simulator calculates gradient or isocratic elution chromatograms based on experimental measurements of the retention behavior of 22 compounds on an Agilent Zorbax SB-C18 column.¹⁰ Each time any user-controlled experimental parameter is changed (e.g., temperature, flow rate, etc.), the HPLC simulator recalculates each of the simulated parameters (e.g., retention time, backpressure, etc.) and then redraws the chromatogram.

Experimental Measurements

A Hewlett-Packard (Palo Alto, CA) 1090 HPLC system and an Agilent (Santa Clara, CA) Zorbax SB-C18 column were used for all experiments. Mixtures of 3 or 4 solutes were generally injected at once. Solvents were mixed online. In a typical experiment, k was measured for each compound at four eluent compositions (e.g., 20, 30, 40, and 50% organic modifier in water). From a linear fit to these measurements, the log k_w and S (see eq 8 in the Supporting Information) were determined at

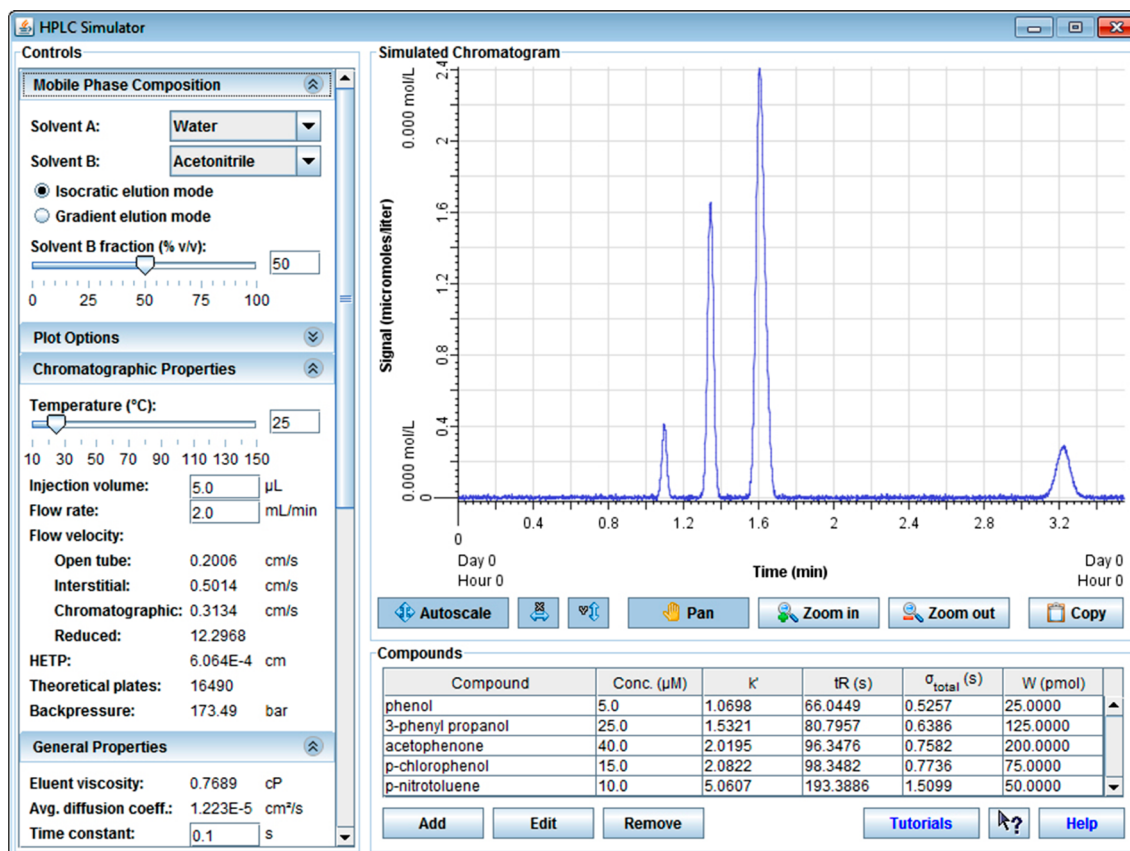


Figure 1. The HPLC simulator interface.

one temperature. Then, the temperature was raised and the retention of each compound was measured at another four eluent compositions; the eluent compositions were slightly weaker to maintain useful k values (>1). A second set of $\log k_w$ and S was calculated at the new temperature. Last, values of A' , B' , a' , and b' were calculated for each compound from the two values of $\log k_w$ and S (see eqs 9 and 10 in the Supporting Information). These values are used by the HPLC simulator to calculate retention times under a range of temperatures and solvent compositions.

Software

The HPLC simulator was written in Java (Oracle Corporation, Redwood Shores, CA) for compatibility with the version 1.6.x compiler. Additional details on included libraries, how to compile the software, and a link to the source code can be found at hplcsimulator.org in the development section.

Features

The new HPLC simulator (version 1.1.2 at this time) is located at hplcsimulator.org (Figure 1).⁹ It can be launched directly from an Internet browser or offline, and runs on both Windows and Mac operating systems. The only notable system requirement is that the Java Runtime Environment (version 1.6.0 or above) must be installed. The vast majority of computers already have it installed (though it may need to be updated), but in the case that it is not installed or out of date, the Web site automatically directs the user to the Web site where the latest version can be downloaded.

The simulator features controls and indicators for a wide variety of experimental parameters and displays a graphical, simulated chromatogram. It provides immediate feedback any time experimental parameters are changed by updating all of the indicators and redrawing the chromatogram and in the case of the slider controls, the chromatogram and indicators update *while* the slider bar is being dragged. The controls and indicators are listed in Table 2. In gradient elution mode, some parameters change during the run. Thus, the simulator can also draw a second plot that shows any one of the following

parameters as a function of time: % organic modifier at the column inlet, backpressure, mobile phase viscosity, retention factor of a selected compound, and position of a selected compound along the column (e.g., see Figure 2). The simulator also features online help to explain the function of the controls and how values are calculated.

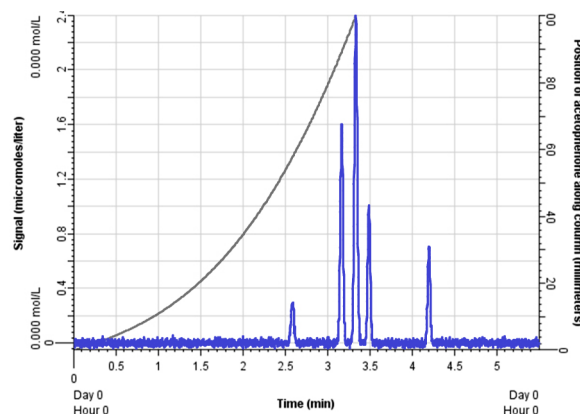


Figure 2. A screenshot of the simulated chromatogram (blue line; the left y axis shows intensity) showing a plot of the position of a compound (the middle peak) along the column as a function of time (gray line; the right y axis shows position) during a 5 min gradient from $\phi = 0.05$ to 0.95 .

The goal in developing the HPLC simulator was to design a tool that could be used to teach the *fundamental principles* of HPLC, not to teach the more practical aspects of operating a real HPLC instrument. For example, on-off switches, over-pressure warnings, time delays (chromatograms are drawn instantly), and so forth were omitted. No attempt was made to simulate any specific type of real-life detector. Instead, an ideal, concentration-sensitive detector was simulated that gives a response equal to the total concentration of compounds eluting from the column.

In addition to the simulator, the Web site also offers a number of other useful resources. Under the "Educational Resources" tab, four problem set are available that were designed to be used in undergraduate analytical chemistry courses. The problem sets cover a range of concepts and instructors are welcome to use them as they are or modify them. Under the "Forum" tab is a discussion forum where users can publicly ask questions, share ideas, or request additional features. Finally, the "Development" tab contains all of the files and information one would need to view or make modifications to the source code.

■ CLASSROOM EXERCISE INVOLVING THE HPLC SIMULATOR

One effective way to use the simulator is to introduce it in class before discussing solvent strength in reverse-phase separations. The following exercise was used in both a quantitative analysis course composed of upper-level chemistry and biochemistry majors and in a four-year-level instrumental analysis course. The students were first given a worksheet (which can be downloaded under the Resources tab) that asks them to go to the HPLC simulator Web site and adjust the fraction of organic solvent, ϕ , to see what effect it has on a chromatogram containing five compounds (a–e). Students easily found a value of ϕ that gives good resolution and they saw that small changes

Table 2. Controls and Indicators for the HPLC Simulator

Controls	Indicators
Organic modifier selection (currently acetonitrile or methanol)	Flow velocity (open tube, interstitial, chromatographic, and reduced)
Temperature	Total porosity
Injection volume	HETP
Flow rate	Reduced plate height
Detector time constant	Number of theoretical plates
Noise level	Backpressure
Column length and diameter	Eluent viscosity
Particle size	Average analyte diffusion coefficient
Interparticle and intraparticle porosity	Void volume
Reduced van Deemter terms	Void time
Sample composition (22 compounds to choose from)	Retention factor of each solute
% organic modifier in eluent (in isocratic mode)	Retention time of each solute
Gradient program (may enter complex gradient programs with an unlimited number of steps)	Peak width of each solute
Nonmixing dwell volume (in gradient mode)	
Mixing dwell volume (in gradient mode)	

Table 3. Calculation of Minimum Selectivity at Various Solvent Compositions for Window Analysis

φ^a	Compound a	Compound b	Compound c	Compound d	Compound e	Minimum selectivity (a)	Closest pair
	$\ln(k_a)$	$\ln(k_b)$	$\ln(k_c)$	$\ln(k_d)$	$\ln(k_e)$	$\ln(k_2) - \ln(k_1)$	
0.100	3.662	4.707	4.920	5.403	4.644	0.063	b and e
0.120	3.534	4.556	4.772	5.243	4.512	0.044	b and e
0.140	3.407	4.405	4.624	5.083	4.380	0.025	b and e
0.160	3.280	4.254	4.476	4.923	4.249	0.006	b and e
0.180	3.153	4.103	4.329	4.764	4.117	0.014	b and e
0.200	3.025	3.952	4.181	4.604	3.985	0.033	b and e
0.220	2.898	3.801	4.033	4.444	3.853	0.052	b and e
0.240	2.771	3.651	3.885	4.284	3.722	0.071	b and e
0.260	2.643	3.500	3.737	4.124	3.590	0.090	b and e
0.280	2.516	3.349	3.590	3.965	3.458	0.109	b and e
0.300	2.389	3.198	3.442	3.805	3.326	0.116	c and e
0.320	2.262	3.047	3.294	3.645	3.195	0.099	c and e
0.340	2.134	2.896	3.146	3.485	3.063	0.083	c and e
...
0.800	-0.793	-0.576	-0.253	-0.190	0.032	0.063	c and d

^aThe optimal condition is bolded.

in φ have a significant effect on retention and selectivity. They also changed particle size and saw the effect on peak width and pressure. These are excellent lessons in themselves, but the simulator also provided an opportunity to generate data quickly, permitting the student to build a predictive model. Such an exercise can be valuable practice in learning the process of doing science.

The students then found a mathematical relationship between the retention of a compound and the organic solvent fraction, φ : Was it linear, was it quadratic, or what was it? Knowledge about how components in a mixture behave could save time finding the optimum separation conditions in the lab. The students gathered data for one compound and saw what type of equation fits. Six or seven data points between $\varphi = 0.20$ and 0.80 were enough to get a good idea. The students easily evaluated the retention factor, k , entered it into a spreadsheet with the corresponding value of φ , and fitted the data with an equation. This was a good time to discuss different models to fit data using the regression feature in the spreadsheet; that is, what constitutes a good fit? Some students stopped with a linear model, but a brief discussion about residuals helped them see that the residuals follow a pattern with linear choice. The residuals were not random; the model was not very good. Soon someone discovered that $k = a e^{-b\varphi}$ worked best as it produced small random residuals.

The discussion was taken further. Assuming that the retention of all compounds followed an exponential function of φ (of course, each has its own set of constants for a and b), how can the equations for the other four compounds in the mixture be found? It helped to point out that if $k = a e^{-b\varphi}$, then $\ln(k) = \ln(a) - b\varphi$. At minimum, data was needed from two experiments to evaluate the parameters for each compound (of course, collecting more data points would improve the precision of the measured parameters). As a class, the slope and intercept were found for each compound linking $\ln(k)$ and φ . Now students could make predictions!

Laub and Purnell^{11,12} demonstrated a strategy called "window analysis" that was used here. It found favorable conditions using selectivity factors for the pair of compounds in a mixture that were the most difficult to separate. With the simulator parameters, $\ln(k)$ could be calculated for each compound. The students generated a table of $\ln(k)$ at small

increments in φ . (A partial table is shown in Table 3.) At each value of φ , the most similar values of $\ln(k)$ were found by inspection, calculate the difference and then its absolute value. The result is $\ln(k_2) - \ln(k_1) = \ln(\alpha)$ where α is the selectivity factor. In other words, the smallest value for the selectivity factor among these compounds at the specified value of φ was found. A plot of the minimum $\ln(\alpha)$ versus φ produced triangular "windows". The selectivity was the most favorable for separation under the conditions associated with the peak of the tallest window (in Table 3, the optimal condition is bolded). The students found that these conditions match those found by trial and error. It took only 10–15 min to become comfortable with the simulator and nearly an hour to bring the class to the point of introducing window analysis. The actual construction of the window plot was assigned as homework following the session. Of course, this approach neglects any consideration of band spreading. However, that point makes an excellent place to launch into the investigation of parameters that influence bandwidth, something that can also be explored with the simulator (even before looking at the van Deemter equation).

■ PEDAGOGICAL EFFECTIVENESS OF THE HPLC SIMULATOR

A small-scale experiment with one of our undergraduate analytical chemistry classes was performed to test the pedagogical effectiveness of using the simulator to teach some of the fundamental concepts of HPLC. All 28 students in the class were given a short homework assignment, but only half of them were given access to the HPLC simulator. Those students who were told about the simulator were selected randomly and given no training or special instructions in the operation of the software. The next day, they were all given a follow up quiz to assess their understanding of the material. The mean score of those students who used the simulator was 12.5/15, whereas those students who did not use the simulator had a mean score of 11.7/15 (significant at the 67% confidence level). The students were also asked to rate their confidence in their answers to the quiz questions on a scale of 1 to 10 (10 being the most confident). The mean confidence of those students who used the simulator was 7.9/10, whereas for those students who did not use it was 6.5/10 (significant at the 93% confidence level). These results were encouraging, but larger,

more thorough studies would help to better understand more effective ways to incorporate the simulator into the classroom.

Comments written in by students who used the simulator were overwhelmingly positive. Not one student expressed difficulty in using the controls, making changes to the conditions, or understanding the information output by the simulator. The comments mainly focused on the HPLC simulator being easy-to-use and intuitive, and that they appreciated being able to see the effect of a change in conditions on the chromatogram. We continue to look for new and more effective ways to use the HPLC simulator in course curriculum and welcome any suggestions.

■ CONCLUSIONS

The described HPLC simulator is a highly functional, easy-to-use tool that can be used for teaching a great number of important fundamental concepts in liquid chromatography. Other free and low-cost HPLC simulators exist, but they have significant shortcomings that limit their utility. We believe that the free, Web-based software discussed in this manuscript overcomes those limitations and could find broad use in the classroom. Moreover, the downloadable problem sets lower the barrier for instructors to incorporate the simulator into their curriculum.

Students who used the HPLC simulator in a course had overwhelmingly positive comments and we expect the HPLC simulator will offer many benefits to both students and instructors. It provides a medium for instructors to easily demonstrate theoretical concepts, it quickly gives students an intuitive feel for HPLC before entering the lab, and it acquaints students with an online resource that could be a useful aid in their future work.

We will continue to improve the simulator and update it regularly. High priorities include adding more stationary phases (including normal phases), adding more compounds, and simulating peak tailing. We welcome any comments, suggestions, and feature requests on the hplcsimulator.org discussion forum. We have also made the source code publicly available on the Web site so that users may customize the software.

■ ASSOCIATED CONTENT

⑤ Supporting Information

Explanation of the chromatographic parameters in the HPLC simulator and their calculation. This material is available via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: bosw0011@umn.edu.

Notes

The authors declare no competing financial interest.

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