The Use of Portable Capillary Liquid Chromatography for Common Educational Demonstrations Involving Separations

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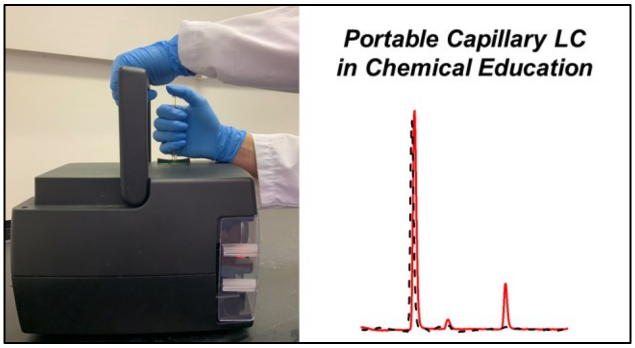
ABSTRACT

High-performance liquid chromatography is one of the primary techniques covered in the undergraduate analytical chemistry curriculum. This Technology Report describes the use of a portable capillary-scale instrument that can provide similar performance to a benchtop instrument but generate less solvent waste and enable operation in non-laboratory settings. Comparisons between the two instrument types were made for single-standard calibration analysis of caffeine in diet soda and aspirin content in over-the-counter tablets, with relative percent differences between the standards and samples under 5% for both instrument types and both samples. The capability to use the instrument in lecture and outreach demonstration activities was also explored. Portable instruments can serve similar pedagogical purposes to traditional instruments as well as provide a platform to introduce discussions on green analytical chemistry based on differences in solvent waste generation and power consumption.

GRAPHICAL ABSTRACT



KEYWORDS
Second-Year Undergraduate, Analytical Chemistry, Chromatography, Instrumental Methods, Laboratory Equipment

INTRODUCTION

High-performance liquid chromatography (HPLC) has been identified as an important part of the undergraduate curriculum[1,2] and HPLC systems are designated as instruments that are essential to a “vigorous undergraduate program” in the ACS Guidelines for Bachelor’s Degree Programs.[3] However, the size of standard HPLC instruments typically precludes their use outside of the traditional laboratory setting, limiting the opportunities for lecture demonstrations and outreach activities. To overcome these limitations, compact and/or portable HPLC instrumentation can be employed.[4–6] The typical design of such instruments focuses on the combination of the various instrument components (pump, injector, column, and detector) into a single module with a smaller size and lower power requirements than a standard benchtop instrument. One design approach is the use of analytical-scale pumps and columns to employ methods that typically use 0.2 – 2.0 mL/min.[7–11] While easiest for method translation from existing methods on standard instrumentation, the volumes of solvent and solvent waste are not always compatible with instrument operation in non-laboratory settings. To overcome these challenges, portable capillary-scale LC instruments designed for columns with smaller inner diameters operating at significantly (*i.e.*, 2 – 3 orders of magnitude) lower flow rates can be used, thus requiring less mobile phase and generating less solvent waste.[12–15]

In this Technology Report, a portable capillary LC instrument with typical operating flow rates in the 0.5 – 3 μL/min range was investigated for use as a platform for common demonstrations of HPLC analysis. These separations have typically been performed as part of undergraduate laboratory experiments with standard instrumentation and are expanded here to demonstrations in classroom and/or outreach settings based on the reduction in instrument size and weight. This specific instrument, the Focus LC (Axcend, Provo, UT), provides simplified column installation through the use of cartridges that combine capillary LC columns (150 μm inner diameter) and on-capillary absorbance detection, while minimizing footprint (740 cm^2) and weight (~8 kg).[12] This instrument has previously been used for the analysis of over-the-counter pharmaceutical compounds[12], drugs of abuse[12,16], and methylxanthines in river water downstream from a water treatment plant.[17] Here, potential ways that this (and similar) portable capillary HPLC instruments can be used in a variety of educational settings are described.

EDUCATIONAL APPLICATIONS OF PORTABLE CAPILLARY LC

Real-World Sample Analysis

Two common undergraduate laboratory experiments involving HPLC are the analysis of caffeine in various beverage samples[18–22] and the characterization of aspirin tablets.[23–27] Here, example separations of these two common applications were obtained using the portable capillary LC instrument and compared to a standard benchtop HPLC instrument (Figure 1). To maximize the number of students that can gain hands-on experience with an instrument during a single laboratory period (165 minutes), Rowan University has typically utilized a single-point calibration approach for the quantitation of caffeine in diet soda as part of its second-year undergraduate analytical chemistry laboratory course. This same approach was compared between the portable (**Figure 1A**) and standard (**Figure 1B**) instruments using a caffeine standard of 0.129 mg/mL, which is the same amount listed by the manufacturer for the diet soda sample used here. The percent relative difference in peak area was 4.56% with the portable capillary HPLC and 3.13% with the standard instrument, demonstrating the similar performance of both systems. Because many institutions use the analysis of aspirin as an introduction to HPLC, the separation of a prepared aspirin standard and a diluted aspirin solution obtained through tablet dissolution (both expected at 3.25 mg/mL) were also performed on both instruments (**Figures 1C** and **1D**). In this example, the percent relative difference in area was 2.51% and 0.22% for the portable and standard HPLCs, respectively. Although some baseline drift can occur during runs (see **Supporting Information**), overall quantitation is not affected. For the compact HPLC, retention time repeatability is generally under 1%RSD, and peak area repeatability is generally under 4%RSD (six replicates both). Occasional issues with analyte carryover in the injector that can reduce peak area repeatability are typically resolved by flushing the loop with acetonitrile; this is less of an issue with needle wash procedures in the autosampler of the benchtop HPLC. For these two samples that are commonly investigated in undergraduate laboratories, the portable capillary LC system provided sufficient performance to match the expected results obtained on a standard benchtop HPLC in a typical undergraduate laboratory setting.

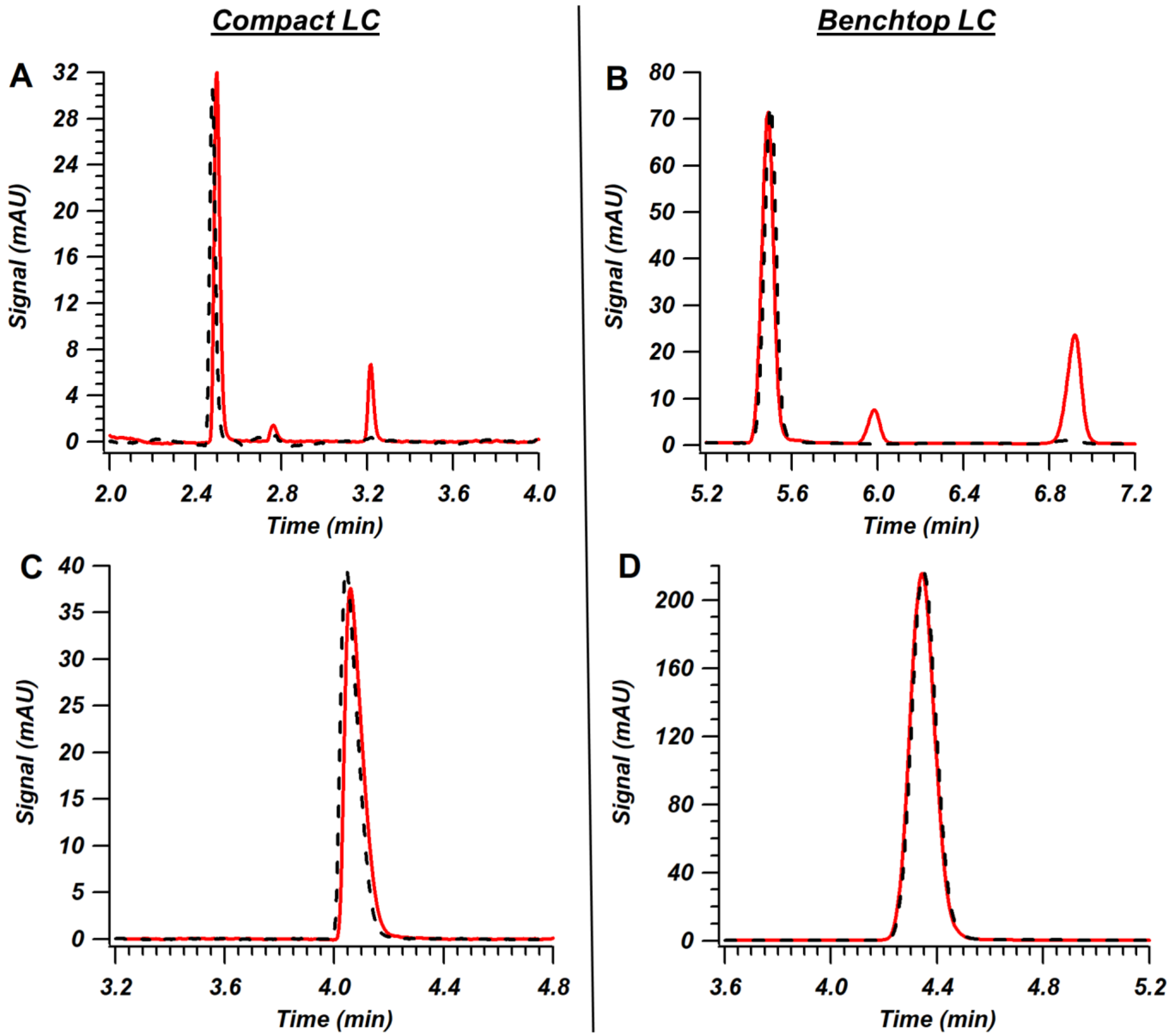


Figure 1. Chromatograms demonstrating HPLC separations that are often performed in undergraduate laboratories. The separation of a diet soda sample is shown in panels A and B, with the red solid trace showing the diet soda sample and the black dashed trace showing a caffeine standard. Panels C and D depict the analysis of aspirin, with the red solid trace obtained from a tablet dissolution sample and the black dashed trace from an aspirin standard. Panels A and C were obtained using a

portable capillary HPLC instrument and panels B and D were obtained using a standard benchtop HPLC instrument. Full experimental details are available in the Supporting Information.

In terms of pedagogical development around the laboratory experiments, portable instrumentation can serve a very similar role to benchtop instruments in terms of general HPLC analysis, as shown above. For courses in which a calibration curve is generated for these types of experiments to improve quantitation (*e.g.*, 0.1 – 0.5 mg/mL for caffeine or 1 – 5 mg/mL for aspirin), it can also be used in place of larger sized instruments. Additionally, the use of portable capillary LC instrumentation opens up various approaches to system comparison (advantages and disadvantages) that can also be included in discussions around the experiment(s) (see activity included in **Supporting Information**). In terms of advantages, the primary focus is likely to be in the area of green analytical chemistry.[28–30] The most obvious comparison is in terms of solvent consumption. For student use, the *Capillary Flow* function within the freely available Molecular Weight Calculator program[31] (Pacific Northwest National Laboratory, Richland, WA) can help simplify calculations regarding the effects of column dimensions on system pressures and flow rates. For more in-depth discussions, power consumption between portable and benchtop instruments can be compared, as can energy consumption related to travel requirements between a central laboratory and on-site analysis. In terms of limitations, the use of an on-capillary absorbance detector inherently limits the pathlength relative to a traditional flow cell used in standard instrumentation, which connects directly to course material related to Beer’s Law. This can also be related to differences between analytical- and capillary-scale columns in terms of injected sample volume and observed peak volume, and how those factors impact the concentration of sample at the detector.[32,33] The use of a single wavelength LED source in the portable instrument can also be compared to a typical photodiode array detector that is used in benchtop instruments. Here, the standard HPLC detector wavelength was set at 254 nm (a common value for such experiments), which was very close to the LED wavelength of 255 nm. Another standard HPLC experiment is the analysis of capsaicin in peppers and hot sauces[34–36], and this analysis is typically performed at 280 nm. On-capillary absorbance detectors with 275 nm LEDs are also available, so differences between the 255 nm and 275 nm LEDs could be observed by swapping column cartridges or with a dual LED design (**Figure S2**).[37] A C18 stationary phase was used for this work, although column cartridges with other stationary phase materials can also be used if needed for specific applications. A key analytical chemistry topic in undergraduate education is the comparison of various techniques for the analysis of a sample, and investigations such as these provide discussion topics related to the differences between variations of a single technique.

Lecture & Outreach Demonstrations

In addition to laboratory experiments, portable instrumentation is more readily adaptable for classroom lecture and outreach demonstrations when compared to standard sized instruments.[38–41] Because of the common theme of acid-base equilibrium in second-year undergraduate analytical chemistry courses, some instructors relate this topic to chromatographic analysis in terms of shifting retention related to changes in pH.[42,43] Here, a qualitative demonstration of the change in retention time for benzoic acid at pH values of 2.1 (below the pK_a), 4.2 (at the pK_a), and 6.1 (above the pK_a) was achieved using both portable and benchtop HPLC instruments (**Figure 2**). As expected, based on previously designed undergraduate activities focused on this topic, the fully protonated form at low pH has a much higher retention time than the deprotonated charged species at higher pH. The retention time observed when the mobile phase pH is at the pK_a fell between the two. Analysis can be in real-time during a lecture on this topic, as the instrument control window can be checked intermittently over time to check on the observed retention shifts. This type of demonstration can also be integrated into other parts of the chemistry curriculum, including overlap with second-year organic chemistry courses discussing the effects of pH on liquid-liquid extractions[44] or comparisons to column chromatography.[45]

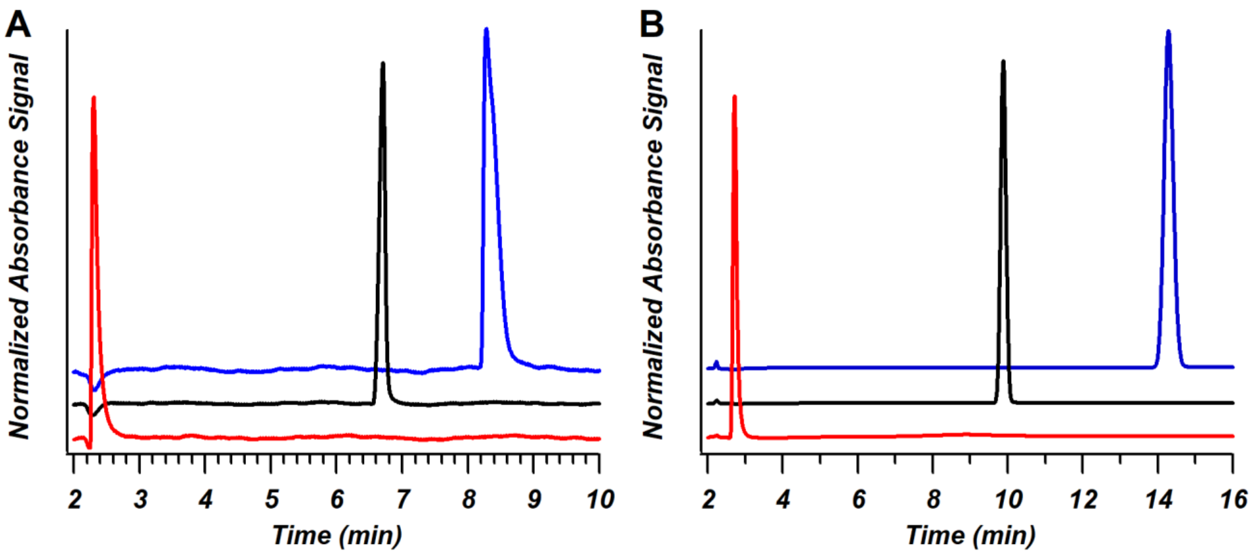
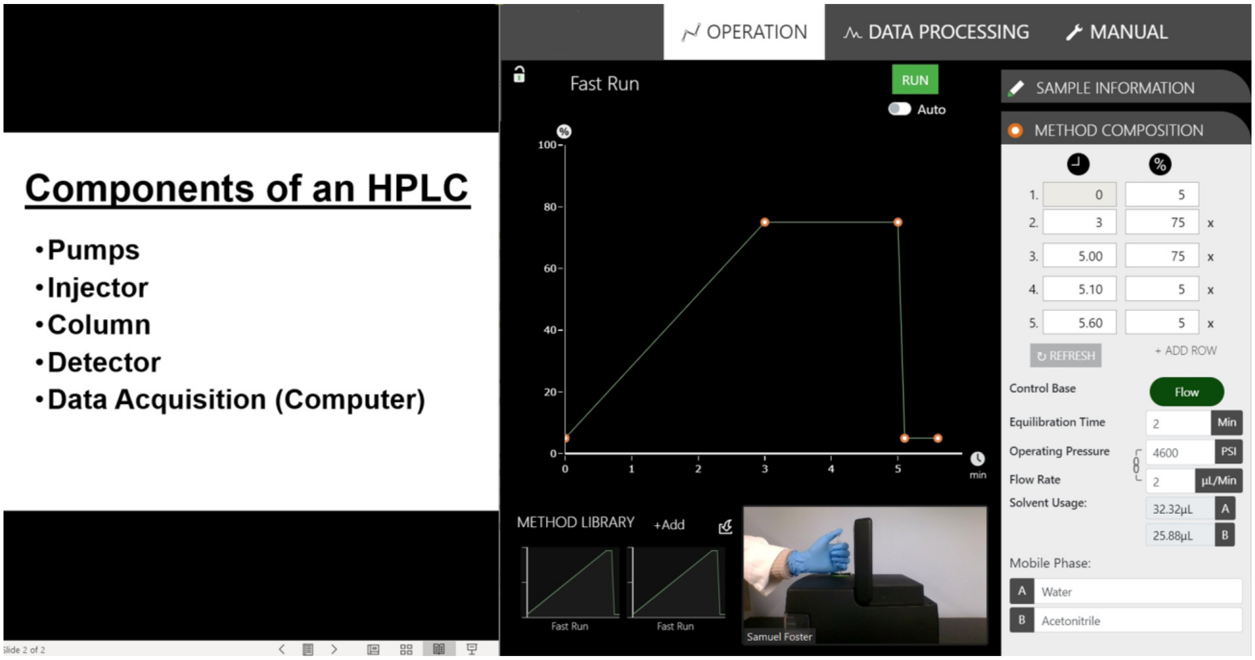


Figure 2. Retention time shift of benzoic acid with changing mobile phase pH. Blue trace is pH = 2.1, black trace is pH = 4.2, and red trace is pH = 6.1. Chromatograms (offset by 10% and normalized to peak signal for clarity) were obtained with the portable capillary HPLC in panel A and using a standard benchtop HPLC in panel B. Full experimental details are available in the Supporting Information.



Components of an HPLC

- Pumps
- Injector
- Column
- Detector
- Data Acquisition (Computer)

Fast Run

METHOD COMPOSITION

1.	0	5
2.	3	75
3.	5.00	75
4.	5.10	5
5.	5.60	5

Control Base: Flow

Equilibration Time: 2 Min

Operating Pressure: 4600 PSI

Flow Rate: 2 µL/Min

Solvent Usage: 32.32µL A; 25.88µL B

Mobile Phase:
A Water
B Acetonitrile

Figure 3. Representative screenshot of video conferencing panel combining slide-based educational material, live instrument feed (through USB camera pointed at instrument on presenter computer), and instrument control panel (which can be controlled through TeamViewer) as part of remote outreach activity.

Because in-person outreach opportunities are currently limited due to pandemic-related restrictions, strategies that have been developed for remote learning related to chemical instrumentation were adapted for outreach to a high school chemistry course. Here, general introductory material related to chromatography was presented to students, while a simultaneous live feed of the instrument in operation and the instrument control panel were also shown to students. The TeamViewer approach to instrument control[46] was used to enable students in the remote setting to set up the gradient method as well as start each chromatographic run (**Figure 3**). In this demonstration, a qualitative demonstration showing an injection of a caffeine standard followed by subsequent injections of standard and caffeine-free diet soda samples provided a direct illustration of chemical analysis using HPLC. Spectral matching approaches have been previously reported for outreach activities related to pigment analysis in art[47,48], and can similarly be applied here through the use of qualitative identification by retention time. Because the amount of solvent waste generated for such demonstrations is well below 1 mL, it can easily be contained within the solvent vial compartment and transported back to laboratory settings for proper disposal upon completion of the activity.

CONCLUSIONS

The various separations demonstrated here provide examples of how portable capillary LC instrumentation can be used as a replacement for (or in addition to) standard HPLC instrumentation in the undergraduate chemistry curriculum. The portability and significant reduction in chemical waste generation also make it a platform that is well suited for chemistry outreach activities involving chromatographic separations. In the future, further reductions in waste generation will be explored through the use of food-grade ethanol-based solvents as organic mobile phase components[49,50], which expands the discussion of green analytical chemistry into not just volumes of solvent used, but also the type of solvent being used. Furthermore, portable ion chromatography instrumentation[51,52] is another emerging topic in this area, which will expand the types of laboratory experiments that can be performed with these platforms. As with portable spectroscopic measurements, new opportunities for curriculum development now exist with the modern capabilities of portable and compact HPLC instrumentation. With the rapid expansion of lower cost approaches to chemical separations in recent years[53,54], opportunities to perform on-site, instrument-based activities utilizing chemical separations will only continue to grow.

ASSOCIATED CONTENT

Supporting Information

Experimental details on the various separations described in this study and an activity related to this topic can be found in the Supporting Information. The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.1c00342

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CONFLICT OF INTEREST STATEMENT

MP, SWF, SK, and JPG are involved in collaborations on system design and operation with the manufacturers of instrumentation described in this work. This was an independent study and no discounted equipment or supplies were provided for the purposes of this report.

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