Experience and Applications of a New Portable HPLC Machine

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New technologies are continuously being introduced to improve the efficiency of the analytical laboratory. One area where efficiency could be increased is the analysis of samples at the source. This is particularly important in a range of analytical processes and has resulted in a significant rise in the use of hand-held spectroscopic instrumentation, for example. However, it is not just spectroscopic devices that can be employed for at-source sample analysis. Portable LC systems in particular also offer significant advantages for certain applications. This article highlights some of these application areas. It will also look at the recent introduction of a conveniently portable HPLC instrument of conventional scale and performance that can address many of the challenges that analytical scientists face when trying to analyse samples outside of the laboratory. A series of potential and actual applications are discussed along with the tradeoffs involved and the customisation and support required by diverse customer requirements.

Introduction

There is a growing need for scientists to be able to perform analytical measurements at the sample source, instead of collecting samples and returning them to a central laboratory for analysis. This approach can help in terms of reducing the analysis costs but will also have an impact on the timeliness of the data and potentially its integrity as well if the sample degrades. Historically, this has been difficult to achieve due to the size, cost and complexity of the instrumentation that is required to perform the analysis. Recent advances in fluidics and LED technology have facilitated the design of HPLC systems small enough to be fully portable rather than 'luggable'. This allows samples to be analysed in situ, bringing substantial benefits; however, it is important that in doing so there is no compromise in the quality of the data that is being produced. There are a range of such instruments that are currently available [1], including the following:

The Axcend Focus LC® system from Axcend Corp (Provo, UT) was developed in the laboratories of Professor Milton Lee at Brigham Young University. This device is small enough to run on battery power and is designed for use with packed capillaries, meaning that there is less solvent consumption and potentially an increase in the sensitivity of assays compared to those that are run in the laboratory.

SIELC Technologies (Wheeling, IL) have produced the Cromite system, which is quite modular in terms of the column design and also the unique method of introducing a sample into the system. Instrumental control and data collection proceed via a proprietary program in the Cloud.

PolyLC's SmartLife LC™ uses a portable binary high-pressure gradient HPLC. It is designed for use with ordinary columns and using conventional operating conditions, which means that transferring methods to and from a main analytical laboratory becomes easier. This is ideal for labs with HPLC experience. To some extent it's analogous to a modernised version of the MINICHROM machine from 1998 [2], but using nonproprietary control and data software and low-voltage LED-based detection.

The SmartLife LC incorporates two custom-designed reciprocating piston pumps for high-pressure mixing, allowing gradient separations to be performed. The pumps are of the compact scale needed for portability: 12 x 8 x 6 cm each, including the pump head. The pumps are independently controlled and capable of 345 bar (5,000 psi) across the flow range, 0.01 up to 5 ml/min with ± 2% accuracy and low pulsation. The operating parameters compare favourably with modern laboratory-based technology, meaning that it is quite feasible to transfer methodologies from field to lab. The cylindrical detector module is 6 x 4 cm and is powered by the USB hub. LED detectors provide extremely stable output and over 50,000 hours of operation. A Valco manual injection valve is used to introduce the sample. The unit can be powered either with line current or a rechargeable 12V battery. The complete system weighs 10 kg. and fits inside a plastic instrument case.

Solvent bottles are stored within the module and comprise two bottles for the gradient pumps and two bottles for a wash solvent and for waste collection.

Figure 1: The SmartLife LC.

It is rugged enough to ship via common carrier or in the overhead compartment of an airplane.

Figure 2: Stowing a SmartLife LC in the overhead compartment en route to a demonstration in Ghana. When traveling, mobile phases are generally prepared after arrival.

This design aspect potentially avoids the need for service in the field. Malfunctioning units can readily be shipped back to the manufacturer for repairs while a loaner unit goes the other way to minimise downtime. Alternatively, all critical components (pumps; detector; injector valve) are readily accessible and can easily be serviced or replaced.

The instrument control and data collection is controlled locally using Clarity® from DataApex Ltd, which is installed on a tablet that fits inside the lid of the case and operates with Windows 10. Auxiliary devices from other manufacturers can be added to the system as long as the devices are Clarity-compliant (i.e., have the necessary drivers). The tablet is WiFi-capable, facilitating remote troubleshooting, and data can be transmitted anywhere. The USB hub has two readily accessible ports. These can be used for data output or for connecting any USB-connected device. Devices that are not Clarity-compliant can still be controlled within the chromatography program with the use of an appropriate converter box such as the Colibrick from DataApex.

A full-featured HPLC system can be both portable and inexpensive only with some compromises. The main compromise with the SmartLC is the detector. The default detector module measures absorbance using an LED with a single wavelength. Any single wavelength can be selected within the range of available LED's, currently 235 nm and higher. Installation of an alternative LED module takes only several minutes. A dual-wavelength LED module is optional. Other types of detectors can be used, but this could compromise the portability of the device to the point that it may offer few advantages over a conventional HPLC system. Another compromise is the manual injector valve. An autosampler can be used with the instrument, again at the cost of mobility. A column heater is not included in the base design but could be added.

Some Applications where the technology has already been utilised

The instrumentation has been used in a variety of different applications to great effect. The following applications highlight the portability and applicability of this type of instrument.

A. Haemoglobins: Haemoglobin is probably the most widely analysed protein in the world. About 7% of the human race are carriers of a gene for a significant haemoglobinopathy such as sickle cell disease. In parts of sub-Saharan Africa the incidence of carriers is as high as 35%. The version of the SmartLC used for haemoglobin analysis is called SmartLife LC™, and uses a detector wavelength of 415 nm. Six units are now in use in sub-Saharan Africa. An obvious use of a portable HPLC is its use in the field for analysis of haemoglobin variants in remote regions. This can involve either a single drop of blood (4 µl of whole blood suffices for 50 replicate analyses) or archived samples in the form of dried blood spots. The out-of-pocket expense for the reagents and HPLC

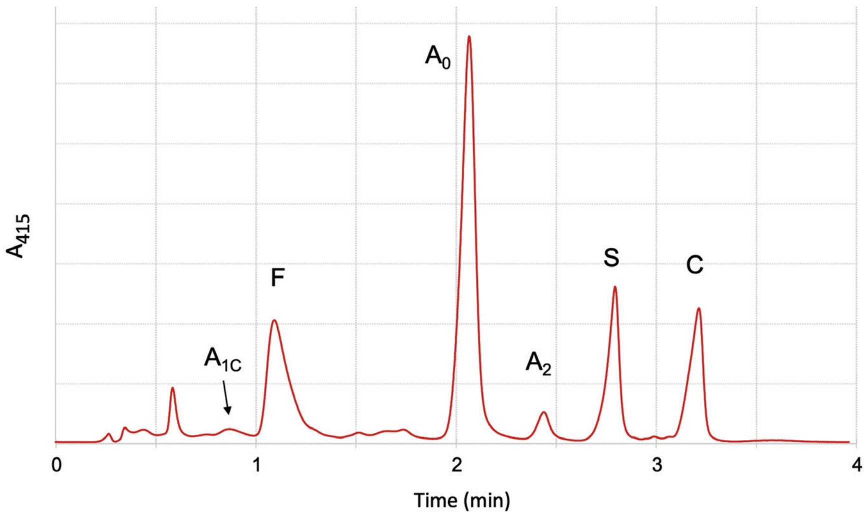


Figure 3: Quick analysis of a composite sample of the major haemoglobin variants. Column: PolyCAT A®, 35x4.6-mm, 3-µm, 1500-Å. Flow rate: 1.5 ml/min. Detection: 415 nm. Backpressure: 125 bar. Mobile phase A: 20 mM bis-tris + 2 mM KCN, pH 6.90. Mobile phase B: 20 mM bis-tris + 2 mM KCN + 200 mM NaCl, pH 6.55. Gradient: 0-0.10', 83-85% A; 0.10-1.65', 85-62% A; 1.65-2.15', 62-52% A; 2.15-2.40', 52-15% A; 2.40-2.90', 15% A; 2.90-3.10', 15-83% A; 3.10-4.00', 83% A.

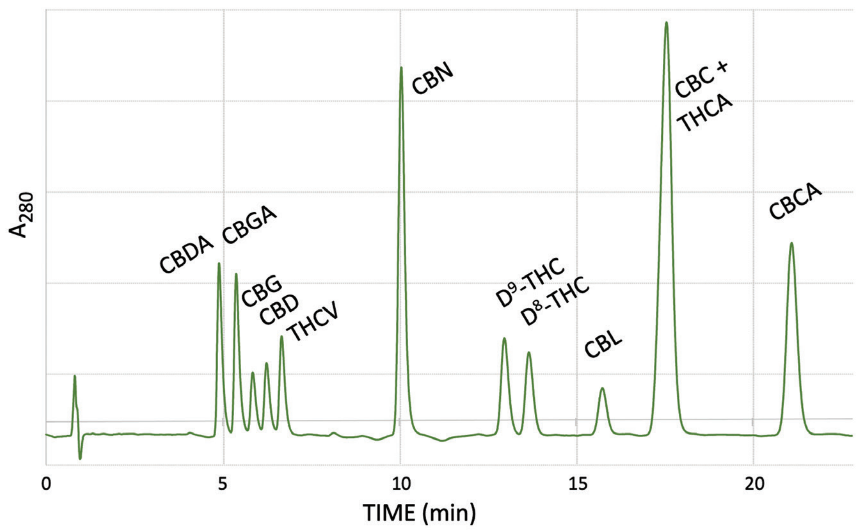
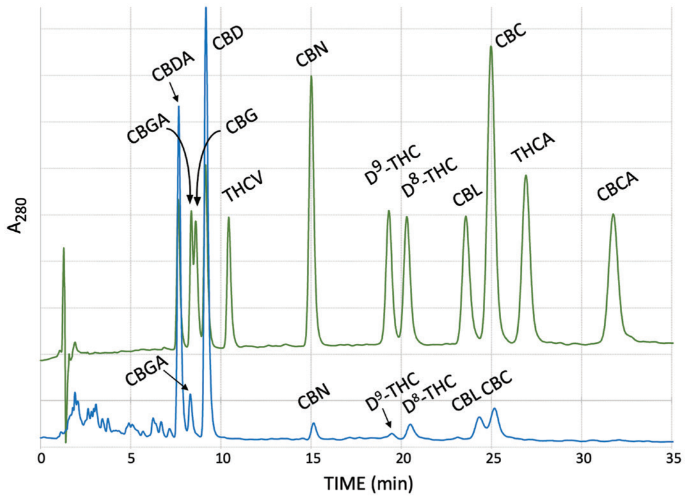


Figure 4: Gradient separation of cannabinoids. Column: Halo® C18, 100x4.6-mm, 2.7 µm, 90 Å. Flow rate: 1 ml/min. Detection: 280 nm. Gradient: 0.1% formic acid with 65-70% ACN in 21' with a 4-minute delay.



Analysis

Cannabinoid	Area %
CBDA	28.1
CBGA/CBG	3.2
CBD	43.6
CBN	2.2
D9-THC	1.2
D8-THC	3.2
CBL	2.6
CBC	4.1

Figure 5: Isocratic analysis of a CBD oil tincture ('For the People' 600 Orange). Column: Orosil C18-HC, 150x4.6-mm, 3 μm, 100 Å. Flow rate: 1 ml/min. Detection: 280 nm. Mobile phase: 25% 0.2% H_3PO_4 with 75% ACN.

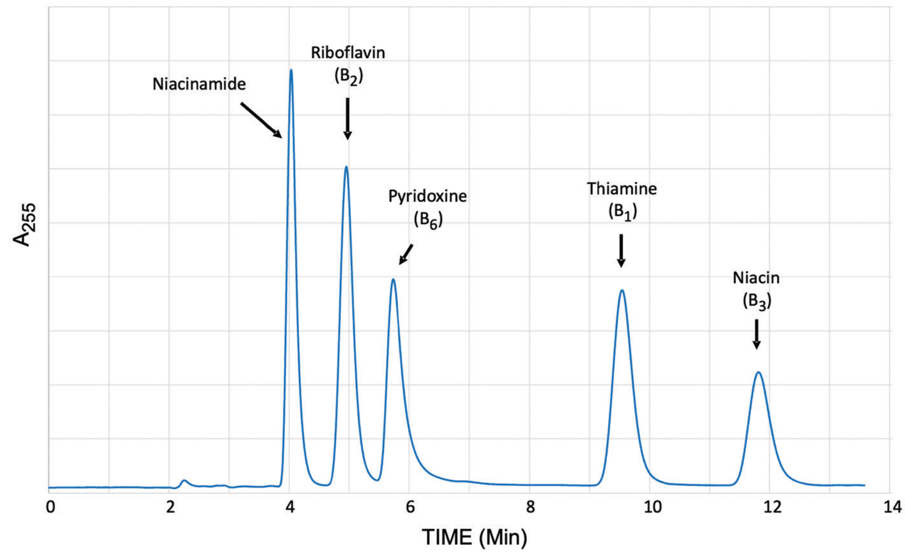


Figure 6: Isocratic separation of B-vitamins via HILIC. Column: PolyHYDROXYETHYL A, 200x4.6-mm, 5 μm, 60 Å. Flow rate: 1 ml/min. Detection: 255 nm. Mobile phase: 40 mM ammonium acetate, pH 5.0, with 75% ACN.

column usage is about US$ 1 per analysis. Figure 3 is a chromatogram of a composite sample of major haemoglobin variants.

B. Cannabinoids: This is one application where analysis in the field could be helpful. An example would be the analysis of extracts of cannabis from different locations at a farm to get more detail about differences in cannabinoid composition in the different places. This version of the machine is called SmartCanna LC™, with a detector wavelength of 280 nm. Figure 4 shows the gradient separation of a composite sample of cannabinoid standards.

Figure 5 shows an isocratic analysis of a CBD oil tincture with an overlay of a composite mixture of cannabinoid standards.

Figure 6 shows the isocratic separation of B-vitamins in the hydrophilic interaction chromatography (HILIC) mode.

Custom Modifications

In addition to the wavelength of the detector module, the most common modification to date has involved the controller. It is somewhat cumbersome to enter data directly on the tablet that comes with the unit; a USB or Bluetooth keyboard and mouse make the task easy. A PC can be substituted for the tablet as the controller, in which case Clarity is then loaded onto the computer and the license transferred to it. The default mobile phase bottles have a 500-ml volume and fit inside the case. There is no reason why they have to be inside the case during operation, and some customers have ordered 2-L. bottles for the purpose instead. They must be carried separately when the unit is moved, a minor inconvenience. Add-ons such as a sample carousel and automatic sample injector would certainly compromise the portability of the device, as mentioned earlier, and would require operation using line current rather than the battery. However, if the machine is to remain in one location for an extended period, then such modifications would increase its capabilities to the point that they approach those of a conventional HPLC.

Future Applications

Portable HPLC machines have not been available long enough to have entered markets which would truly exploit their portability. There are numerous application areas that would benefit from the technology, and move chromatography away from a laboratory based activity to one which is more inclusive of the population. One such niche would be analysis of water samples or fruit in the field in real time, rather than having to transport them back to a central lab. The results could inform the locations being sampled. As the technology in this area develops through the use of lighter components and higher-functioning separation and detection technology, the possibility of moving chromatographic analysis into the world at large becomes a potential reality. This would start to move the technology towards a future where separation science is seen as a means of ensuring a better lifestyle.

References

1. The Analytical Scientist 65 (June 2018) 26-29: Dream Small.

2. V.M. Tulchinsky, D.E. St. Angelo. A Practical Portable HPLC System – MINICHROM, a New Generation for Field HPLC. Field Analyt. Chem. Technol. 2 (1998) 281-285.