

CASE STUDY:

University of Tasmania

University of Tasmania is a pioneer in the research on separation science methods such as liquid chromatography and other analytical chemistry techniques.

CHALLENGE

High pressure liquid chromatography (HPLC) has many uses including protein purification, routine process monitoring in pharmaceutical and beverage manufacturing, quality control, and biotech research. In HPLC, different compounds of the mixture pass through a column at varying rates due to differences in their partitioning behavior between the mobile phase and the stationary phase. Quantification of these components is done by UV-Vis spectroscopy, mass spectrometry or refractive index measurements. UV-Vis spectroscopy is the commonly preferred method of detection since many analytes absorb in the UV spectrum while solvents remain transparent at these wavelengths. Nearly 80 percent of all known chemicals and materials can be identified with HPLC.

Conventional bench top HPLC systems are bulky with a relatively large footprint. Miniaturization and the use of smaller columns increase sensitivity and reduce solvent consumption and, thus, cost and the amount of waste. In addition, the portability of smaller systems allows them to be used both in the lab and in the field. Miniature, portable liquid chromatography (LC) systems enable more widespread use in environmental analysis and point-of-care diagnostics since sample decomposition during transport can be avoided while reducing analysis time and cost.

Current HPLC UV detectors typically use deuterium lamps as their primary light source because of the high stability of light output through the duration of a measurement as well as the relatively high light output of the lamps at their HPLC-relevant UV wavelengths. However, deuterium lamps require a very stable power supply to maintain their performance and a warm-up period of up to 30 min to allow the lamp to reach thermal equilibrium. For this reason, most lamps are left on while not in use so that the instrument is ready as needed—wasting much of the lamp's useful life. Deuterium lamps are also bulky, require expensive, high power (20 W – 30 W) supplies, and lamp housing, adding significantly to the total system cost.

MINIATURE, PORTABLE
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“INCREASING LIFETIME OF UV LED DETECTORS ENABLES REDUCTION IN THE COST OF OWNERSHIP. LOW COST, ROBUST SYSTEMS WITH HIGH SENSITIVITY ARE KEY TO THE ADOPTION OF MINIATURE LC SYSTEMS IN NEW FIELD APPLICATIONS.”

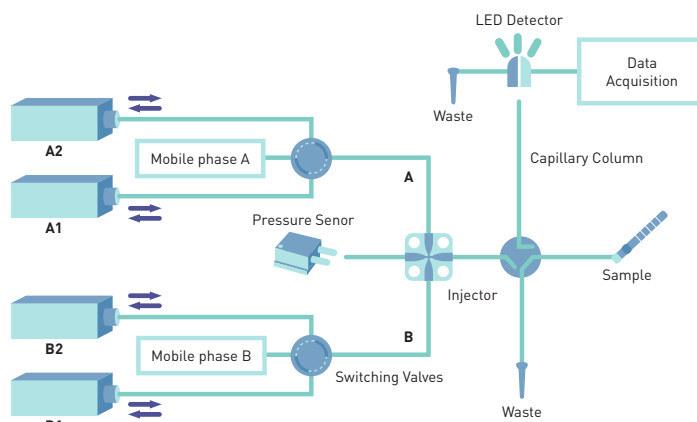
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LEDs are ideal light sources for miniature LC instruments because of their small footprint and inexpensive power supply. In addition, the emission from LEDs can be easily fiber coupled, which is an advantage in applications where the flow cell needs to be isolated. However, the adoption of UVC LEDs (250 nm – 280 nm) has been slow to date, due largely to the poor lifetime of the earliest commercialized devices which lead to frequent, costly replacements.

SOLUTION

Many of the previous portable LC designs were primarily in-house fabricated, and not widely available to users. University of Tasmania explored a modular design using off-the-shelf components, while keeping the system portable and the cost relatively low. The availability of long lifetime, small footprint UVC LEDs from Crystal IS has enabled designers to replace deuterium lamps with UVC LEDs for detection in LC systems.

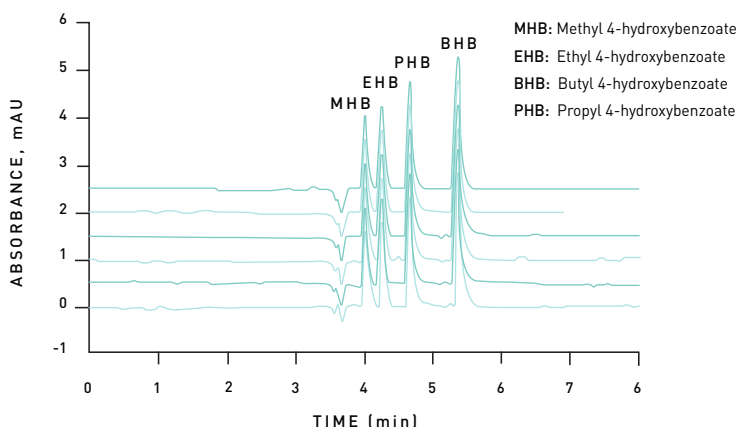
FIGURE 1



Schematic of the portable medium pressure liquid chromatography (MPLC) system.

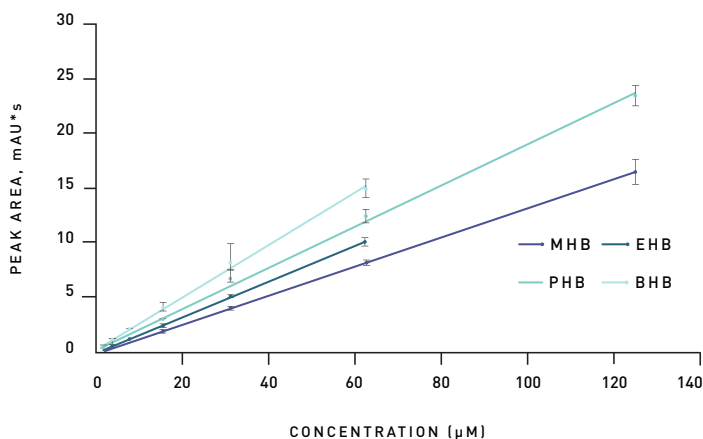
The separation performance of University of Tasmania’s UV detector was tested using parabens mixtures. Good separation of parabens was achieved as shown in the chromatogram (Figure 2). The linearity plot (Figure 3) also shows satisfactory performance over a wide concentration range.

FIGURE 2: SEPARATION OF MODEL MIXTURES OF PARABENS USING A 255 NM LED*



CONDITIONS: Concentration of each analyte was 60 μM methyl 4-hydroxybenzoate (MHB), 0.16 mM ethyl 4-hydroxybenzoate (EHB), 0.16 mM propyl 4-hydroxybenzoate (PHB), and 0.17 mM butyl 4-hydroxybenzoate (BHB); isocratic separation eluent: 50 mM ammonium acetate - acetonitrile 50/50 (v/v); FLOW RATE: 0.5 $\mu\text{L min}^{-1}$; COLUMN: 30 cm \times 100 μm i.d.; injection volume: 4 nL; detection: on-capillary photometric with a 255 nm LED (Optan LED from Crystal IS).

FIGURE 3: CALIBRATION PLOT FOR MHB, EHB, BHB AND PHB*



CONDITIONS: Eluent: 50 mM ammonium acetate - acetonitrile 80/50/20 (v/v); FLOW RATE: 1 0.5 $\mu\text{L min}^{-1}$; COLUMN: 14 30 cm \times 100 μm i.d.; injection volume: 4 nL; detection: on-capillary photometric with a 255 nm LED (Optan LED from Crystal IS).

*REFERENCE: Y. Li et al., "Miniaturised medium pressure capillary liquid chromatography system with flexible open platform design using off-the-shelf microfluidic components," *Analytica Chimica Acta* 896 (2015), 166-176

Crystal IS ADVANTAGE

LEDs offer instantaneous response, low power consumption and design freedom over traditional light sources. In addition, Crystal IS deep UV LEDs provide:

- >Excellent stability of light for lower detection limits
- >Superior spectral quality for measurement linearity over a wide concentration range
- >Little or no radiated heat, which is ideal for heat sensitive samples

Crystal IS[™]
High Performance UVC LEDs

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