MAST - Modular analytical separation technology

Trajan Scientific and Medical

Lewellwyn Coates[1,2], Andrew Gooley[1,2], Paul R. Haddad[1], Brett Paull[1], *Hans-Jürgen Wirth[1,2]
1) ARC Centre for Portable Analytical Technologies (ASTech), University of Tasmania, Private Bag 75, Hobart 7000, Tasmania, Australia
2) Trajan Scientific and Medical, 7 Argent Place, Ringwood, Victoria 3134, Australia

Abstract

The aim of this work was to develop a miniature liquid chromatography system mainly aimed at online reaction monitoring.

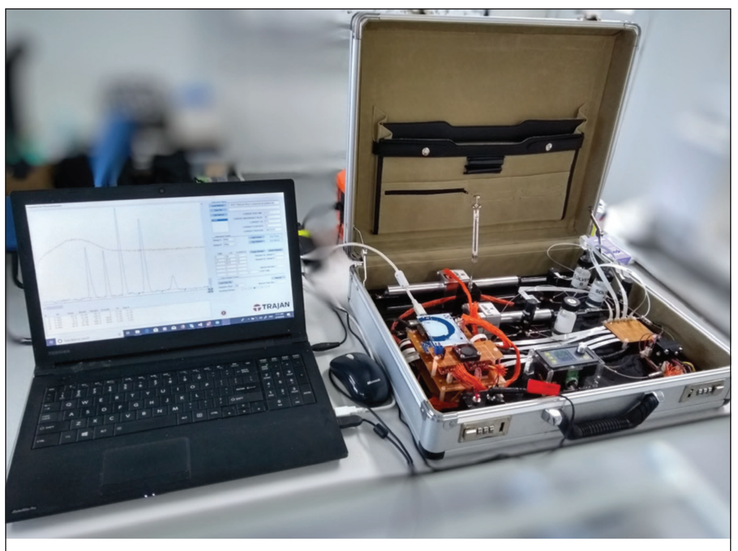
The system has the following design criteria:

- **Cost/performance ratio: The modular design using syringe based solvent pumps and LED based detection allows for an optimized performance for a task while keeping the costs down.**
- **Small: A small footprint would allow the system to be placed directly next to the reactor e.g. inside the fume hood.**
- **Modular: Rather than being an all-round instrument the modular nature of the system allows a custom configuration for the task ahead. Configurations will allow for isocratic and gradient separations, dilution/ derivatization of samples and automated sampling (see Figure 1.).**
- **Performance: While aiming at the use of micro- and capillary columns (0.15-1 mm ID and up to 100 mm long) the system has to be able to pass system suitability tests required by the US Pharmacopeia.**

The current system is powered by stepper motor driven syringe pumps, Labsmith miniature valves, a detector using UV-LEDs and a photodiode and an Arduino based control structure. User control is through a Windows-based software. Potentially, the whole system can be run off batteries and controlled via a smart device for remote applications.

The system

The system is a modular design - for example the four pumps are identical (same stepper motor, same housing, same syringe, same connection).



Components

1	Solvent pump A	7	Purge valve	13	Waste container
2	Solvent pump B	8	Injection valve with loop	14	Sample vials 1-6
3	Sample injection pump	9	8-port selection valve	15	End-of-syringe sensors
4	Sample dilution pump	10	Column	16	Pressure sensor
5	Refill valves	11	UV detector		
6	Microfluidic mixer	12	Solvent reservoirs		

Figure 1. The MAST system in its envisaged most complex configuration: a gradient separation system with automated injection and sample dilution/derivatization.

The pumps

The system uses syringe pumps to transport the liquids. The syringe plunger is moved by a high torque stepper motor which requires 3200 µ-steps to perform a single revolution. The syringe can have 100- 500 µl volume. Depending on the syringe size this equates to 0.7 to 3.3 nL per µ-step. The stepper motor generated 110 Nm torque. Smaller syringe IDs allow to produce higher pressures as the force generated by the motor applies to a smaller area. Measured maximum pressures are slightly lower than the calculated pressures due to internal friction and point of pressure sensing. Measured pressures reach from 100 bar for the 500 µl syringe to 230 bar for the 250 µl syringe and 330 bar for the 100 µl syringe.

The valves

The valves used are LabSmith Microfluidic Products (LabSmith, Livermore, CA). The system uses up to four 3-port valves (AV201) with a swept volume of 160 nL, a 6-port injection valve (AV303) with a 600 nL external loop and an optional 8-port selection valve (AV801). The 8-port selection valve allows the automated injection of the sample, two standards, a resolution solution, a blank and a wash solution, while the other two ports go to the injection valve and to waste. All valves are motorized and are controlled from the user software.

The detector

The detector consists of an Agilent 12 nL z-cell in a 3D-printed housing, a UV-LED (255 nm) and a photodiode sensor. We found that the sensitivity of the detector is a function of current applied to the LED and that a cooled cell allowed us to increase the current/sensitivity. The cooled housing is made of machined aluminum with an integrated fan (Figure 2.).

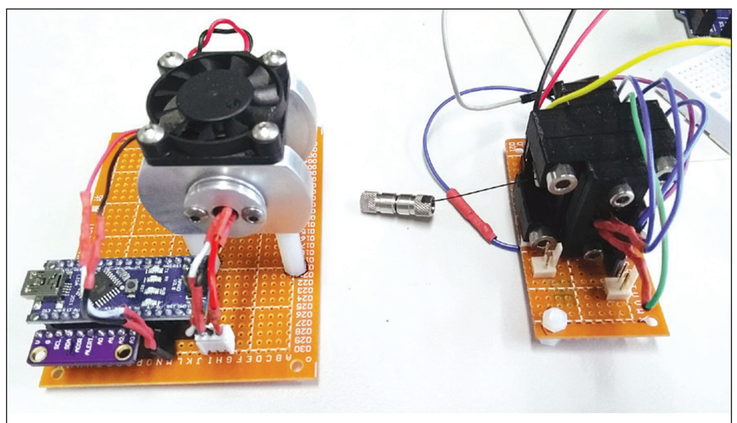


Figure 2. The miniature UV detector unit. Version 1 on the right and the improved, cooled version on the left. LED, cell, photodiode, holder with cooling and controller has a footprint of 70 x 90 mm.

LED design is changing constantly. 235 nm is the lowest wavelength available but other wavelengths are under development. There is also a project to move from single wavelength detection to multi wavelengths detection.

The control architecture

System control is performed on three levels: The user interface is located on a Windows based computer/laptop. It allows the user to set up methods, execute analysis and save the data. Communication between the computer and the system is done via USB or Bluetooth. High level system control is performed by an Arduino Mega board. This board (master) handles communication with the computer and sends control parameters to the individual system components. Finally, the components are controlled by Arduino Nano boards (slaves). These boards carry the majority of calculations.

The control software

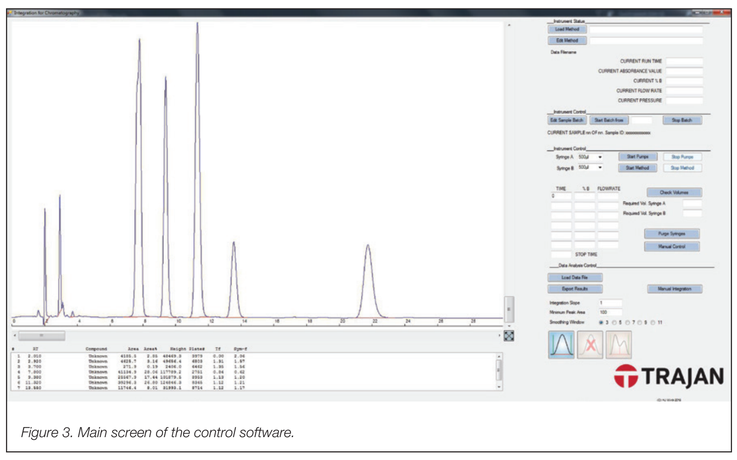


Figure 3. Main screen of the control software.

The interface software (Figure 3.) is written in Visual Basic (Visual Studio 2019) and allows to create, save, load and edit methods, control the instrument, stream data to the hard drive and do basic data analysis. The gradient system can handle up to six gradient steps. In the full version the software will also be able to run sample tables. The master/slave control boards are programmed in the Arduino version of C++ (Arduino 1.8.2).

Pump accuracy and precision

The solvent delivery of the pump was measured gravimetrically. Using a 250 µl syringe flow rates between 5 and 50 µl/min showed a precision of ≤ 1.0% RSD and an accuracy of ≤ 4.0% RSD (Table 1.).

Set flow rate	Measured average	StdDev	% RSD	% Deviation
50	49.35	0.032	0.07	-1.29
25	25.01	0.048	0.19	0.05
20	20.56	0.040	0.19	2.78
15	15.58	0.099	0.63	3.89
10	10.34	0.081	0.78	3.38
5	5.17	0.034	0.66	3.44
3	3.09	0.023	0.74	2.83

Table 1. Accuracy and precision of the syringe pumps.

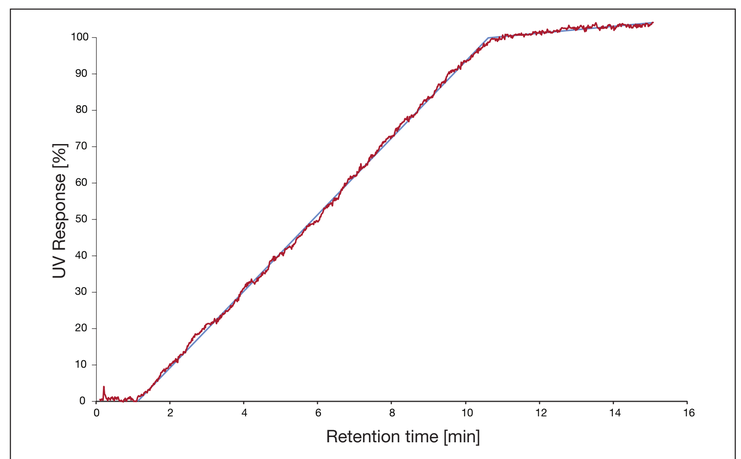


Figure 4. Gradient shape and delay.

The gradient profile was measured by adding a 0.1 vol% of acetone into buffer B and running a blank gradient from 0-100% over 10 minutes. Solvent composition and flow rates are calculated and updated once per second. The gradient profile is linear (Figure 4.) and has a gradient delay of just over 1 minute.

Reproducibility

The following is a repeat injection of six runs of the test mix (Figure 5.)

Sample	Sulfamethazine, Carbamazepine, Ketoprofen, Flavone and Amcinonide at 0.2 mg/ml each
Column	ProteCol C18 125G, 100 x 0.53 mm
Mobile phase A	0.1% FA in water
Mobile phase B	0.1% FA in 80% acetonitrile
Gradient	0-1.5 min 50-94.5%B 1.5-5 min 94.5%B
Flow rate	15 µl/min

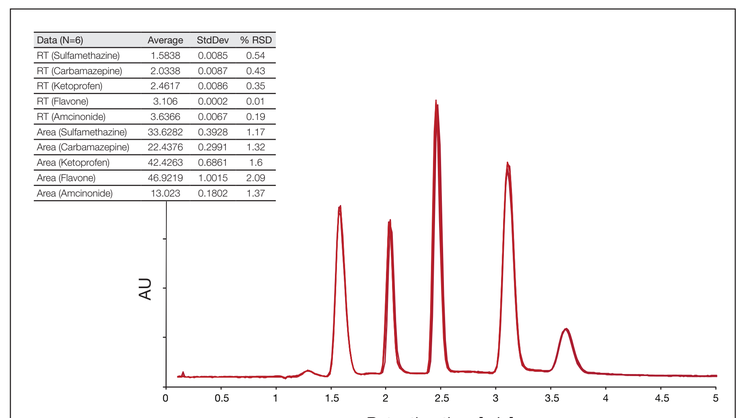


Figure 5. Overlay of six chromatograms of the test mix at 0.2 mg/ml.

Linearity, LOD and LOQ

Linearity of the test mix was tested with concentration reaching from 0.4 to 200 µg/ml.

Sample	Sulfamethazine, Carbamazepine, Ketoprofen, Flavone and Amcinonide
Column	ProteCol C18 125G, 100 x 0.53 mm
Mobile phase A	0.1% FA in water
Mobile phase B	0.1% FA in 80% acetonitrile
Gradient	0-1.5 min 50-87.5%B 1.5-5 min 87.5%B
Flow rate	12 µl/min

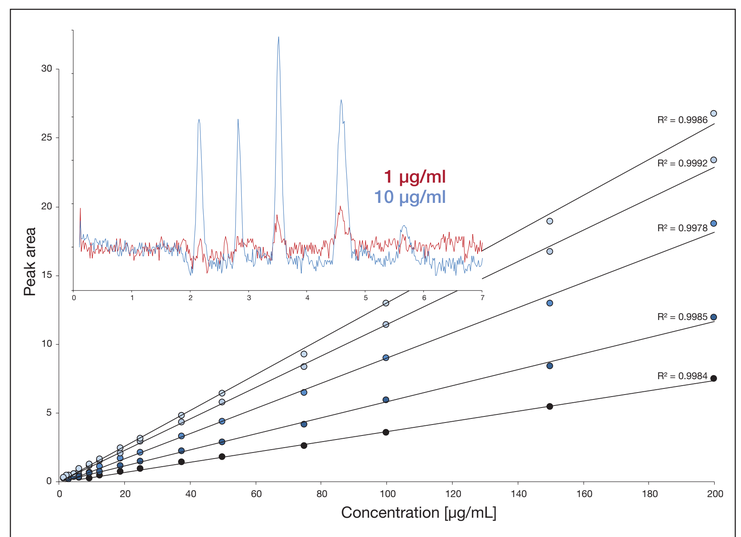


Figure 6. Linearity plot and chromatograms as LOD and LOQ levels.

LOD (S/N >3) started for some compounds at about 1 µg/ml while LOQ (S/N >10) was reached at 10 µg/ml. Linearity was achieved over the concentration range tested (Figure 6.).

Conclusions

From the outset the system was designed to be a cost-effective way to monitor a specific reaction. An important design criteria was the small foot print of the instrument making it possible to place it next to the reactor. While trying to emulate many of functionalities of a conventional HPLC system the targeted application of reaction monitoring does not require the same level of performance as for example trace analysis. Reaction monitoring typically involves the analysis of a few compounds only, the starting materials, the product and a few side products.

Acknowledgements
The project was funded by the Australian Research Council's Industrial Transformation Training Centre (ITTC, Grant No. IC140100022).

Contact info@trajanscimed.com for further information