

Eksigent's Microfluidic Flow Control Applied to Fast LC/MS of Pharmaceutical Small Molecules

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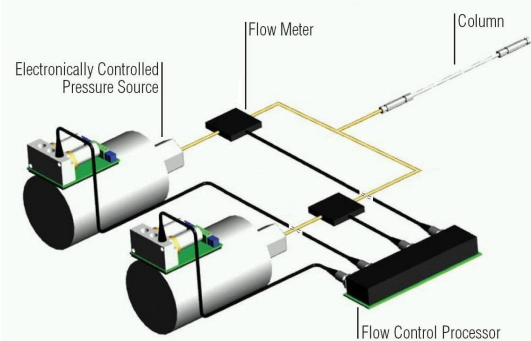


Abstract

Traditional approaches to faster chromatography have involved higher column pressures, shorter columns and faster flow rates. This alternative, micro flow approach uses far less solvent at traditional HPLC pressures, resulting in less waste generation. Here, the micro flow LC system has been combined with mass spectrometry, and the benefits and limitations of capillary LC/MS with fast gradients are explored. Finally, the system is demonstrated in an open-access pharmaceutical research mode using Masslynx® software from Micromass.

Eksigent's Microfluidic Flow Control (MFC), Fig. 1, is part of the ExpressLC-100®, a new capillary HPLC system that provides the benefits of capillary LC while maintaining the gradient reproducibility and ruggedness of conventional HPLC systems. Flow rates from 200nL to 30µL per minute are optimal. MFC provides accurate, extremely fast and reproducible gradient control at these micro flow rates by continuously monitoring and adjusting HPLC flow with an embedded processor.

Fig. 1: A Look Inside Eksigent's MFC Pumping System



Microfluidic Flow Control (MFC) Pumping Technology Advantages:

- Flow meters measure actual flow rate of each mobile phase
- Active Flow Control uses feedback to control variable pressure source of each phase
- Accurate flow rate is maintained regardless of system backpressure or viscosity

LC to MS Interface

In order to avoid post-column band broadening, the ExpressLC-100 was positioned near the mass spectrometer. A length of 30 micron I.D. fused silica tubing was used to connect flow out of the 45 nanoliter UV cell directly into the ESI source of the mass spec. – see Fig. 2. Including the UV cell, post column system volume is kept under 46 nanoliters. Precolumn system volume is 300 nanoliters from mixer to column.

Micro Flow LC to MS Advantages:

- 30 centimeters of 30 micron ID fused silica is fed directly into modified ESI MS source, adding less than 1 nanoliter of system volume,
- No flow splitting necessary, see Fig. 3, negligible backpressure increase
- No measurable band broadening from UV cell to MS

Fig. 2: Connecting the Eksigent Pump and Micromass LCT MS

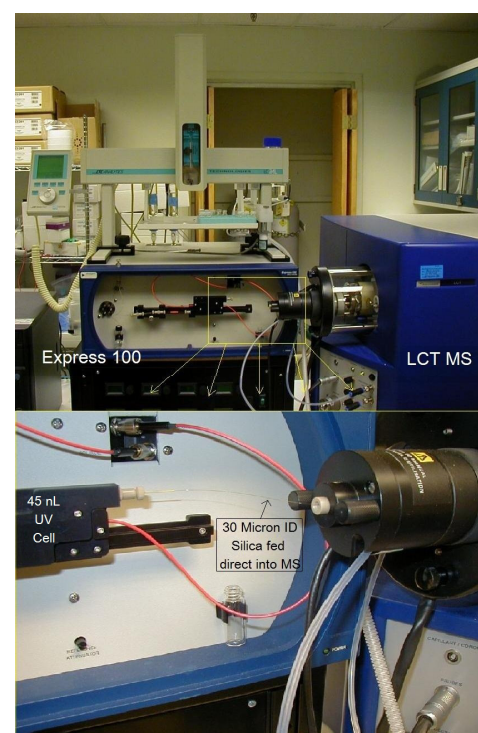
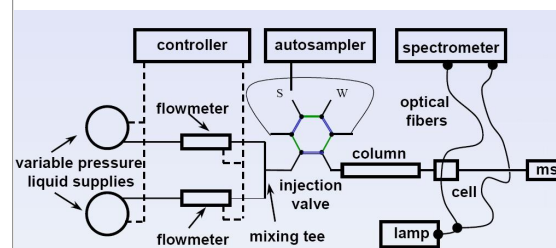


Fig. 3: Overall Liquid Flow Schematic – No Flow Splitting



Experimental

Injector: CTC LC PAL
Pump: Eksigent ExpressLC-100 Single-Channel binary gradient HPLC system
Mass Spectrometer: Micromass LCT® MS
Column: ChromXP C18, 3 micron, 50X0.3mm
Mobile Phase: H₂O + 0.1% Formic, ACN + 10% THF + 0.02% Formic
Flow: 12 microliters to 16 microliters per minute
Column Temperature: Ambient
Detection Wavelength: 220-260 nm
Injection Volume: 20 nanoliters
Gradient: 5% ACN to 100% ACN in 30 seconds, holding 100% ACN for additional 30 seconds

Software Interface

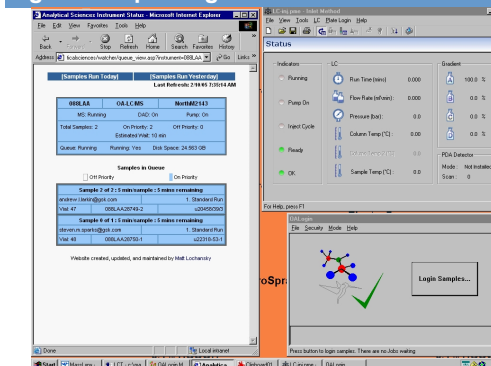
Eksigent's software allows in-depth control of every aspect of the LC/UV system, including injection volume, which can be easily selected from the software interface. UV detection can be selected in the range of 200-380nm. Summed UV absorbance data is generated into an analog signal, which allows connection of this analog data to mass spec.

This analog signal was fed into acquisition by Masslynx Software so that mass spectral confirmation of UV peaks could be reported. Masslynx software initiates sample injection through control of the CTC PAL. Upon injection, the CTC PAL generates a contact closure. This contact closure triggers all data acquisition within Masslynx software and the gradient initiation within Eksigent software. In this way Eksigent and Masslynx software are used together. Additionally, Masslynx software enables open-access use.

Results

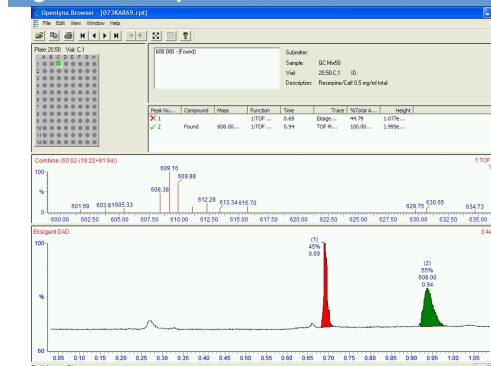
With Masslynx open-access software controlling sample queue, the sample login screen is shown in the right side of Fig. 5. Users press this login button with the mouse pointer, enter their ID and email address to login samples. Additionally, a web based queue watcher was developed to allow users to remotely monitor instrument queue or retrieve data, and this is shown in the left side of Fig. 5.

Fig. 5: Sample Login with Web Queue Watcher



Results are automatically emailed to users, and an example of emailed data results is shown in Fig. 6.

Fig. 6: Data Report with MS Peak Confirmation



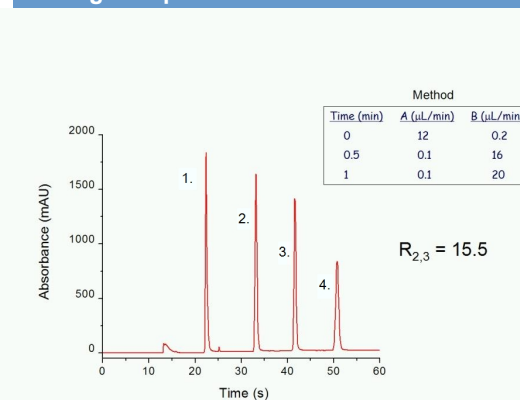
Speedy analysis of 96-well plate formats was made possible with sample to sample cycle times on the order of just over 2 minutes. At 13 microliters per minute, 96 samples could be analyzed using only 2.5 milliliters of total HPLC solvent. This was compared to current sample to sample cycle times of 5 minutes and current solvent consumption of 960 milliliters per 96 samples. Solvent consumption is less than 1% of current techniques and results are generated in less than half the time of current techniques.

Column Resolution

A mixture of four test ingredients, see Fig. 7:

1. 8-Bromoguanosine
2. Diethyl phthalate
3. Di-n-pentyl phthalate
4. Dioctyl phthalate

Fig. 7: Optimized One-Minute Method



Conclusions

- For fast LC/MS, this system is a viable alternative to high pressure or high flow rates without significantly sacrificing resolution or sensitivity
- The Eksigent system is robust, durable and convenient.
- Solvent consumption is on the order of 0.25% of current methods. Therefore hazardous waste generation is 0.25% of current methods and overall liquid costs are 0.25% of current methods.
- Estimated money saved per 10000 samples analyzed is on the order of \$2000 in liquid purchase/disposal costs alone.
- Less sample loading into mass spectrometer.
- Power to experiment with a wide range of columns and methods.
- Easy access to column, UV cell, and injector.
- Cuts current analysis time in half.
- Easy access to mass spec. interface.
- One method applicable to most pharmaceutical small molecules.

Acknowledgements

- Eksigent.com – all the HPLC method development from the people at eksigent