DIONEX
Passion. Power. Productivity.

A New Micro LC Front-End to Common MS Platforms

F. Steiner, G. Maio, and F. Arnold, Dionex Softron GmbH, D-82110 Germering, Germany

INTRODUCTION

Powerful LC-MS solutions, mostly involving an electrospray ionization (ESI) interface, have revolutionized separation sciences. Although modern electrospray ionization sources are compatible with elevated LC flow rates of up to 3 mL/min, there is a general benefit in applying flow rates of less than 0.5 mL/min for maximum detection sensitivity, baseline noise reduction and long term robustness of LC-MS methods. This inevitably demands reduction in column internal diameter. For bioanalytical applications—or LC-MS analysis in pharmaceutical, chemical, or environmental research and discovery applications—microbore LC on 1- or 2-mm i.d. columns with flow rates between 50 and 600 µL/min are commonly used.

With LC methods scaled down in column diameter, supplementary benefits adding to the improved ESI-MS compatibility come into play. Micro scale methods exhibit higher detection sensitivity relative to the absolute amount of injected sample and better axial heat dissipation in the column. Both factors increase separation efficiency under certain conditions. Moreover, less mobile phase is consumed, which is also cost effective with respect to solvent disposal.

To achieve optimal results with 1- or 2-mm i.d. columns, a dedicated HPLC instrument is required. In this presentation, we introduce a new micro LC system designed for optimal use of 1–2-mm i.d. columns and coupling to mass spectrometers. The general advantages of micro LC over conventional analytical LC and the performance of the system for fast microbore separation are discussed. The system can be controlled from third party mass spectrometry software via a dedicated high-level driver (DCMS^{Link}).

ULTIMATE™ 3000 MICRO SYSTEM KEY FEATURES

The key features and specifications of this new micro system are:

- Fully integrated micro system with volume optimized fluidics
  - <100 µL gradient delay volume (60 µL in bypass mode)
  - <10 µL extra column volume
- Three pump types available
  - Binary high-pressure micro pump (35 µL gradient delay volume, flow rate range 0.02–10 mL/min)
  - Quaternary low-pressure mixing micro pump (230 µL gradient delay volume, flow rate range 0.01–2.5 mL/min)
  - Dual-ternary low-pressure mixing pump with two full gradient pumps in an enclosure (230 µL gradient delay volume, flow rate range 0.01–2.5 mL/min on each pump channel)
- Dedicated micro autosampler (40 µL gradient delay volume and 2 µL extra column volume contribution)
- 100 Hz 4-channel variable wavelength UV detector with special micro flow cell (1.4 µL detection volume)
- All extra column fluidic parts harmonized to 125-µm i.d. and reduced to optimal length

Figure 1 depicts the micro system mounted in one stack. It highlights one aspect of the modular instrument solution to reduce extra column volume by shortest connection tubing lengths. Figure 2 illustrates which parts of the instrument setup contribute to gradient delay volume or extra column volume, respectively. Reducing these volumes to a minimum is crucial to achieve optimum separation efficiency and separation speed with micro columns, especially in gradient applications.

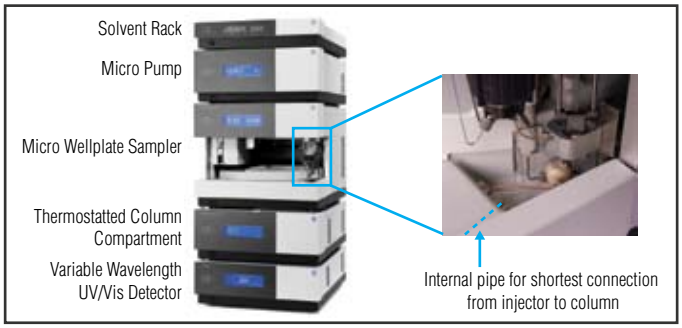

Figure 1. Design of the UltiMate 3000 Micro LC system.

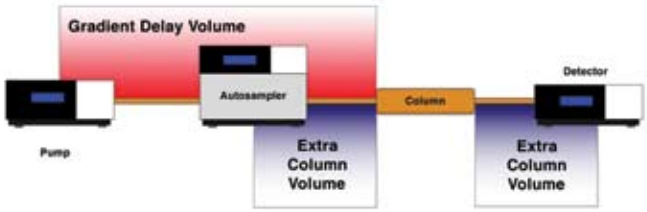

Figure 2. Contribution of system parts to characteristic system volumes.

THE PERFORMANCE OF THE SYSTEM WITH RESPECT TO EFFICIENCY AND SEPARATION SPEED ON MICRO COLUMNS

With separations occurring under isocratic conditions, extra column band broadening mainly affects the efficiency of the early eluting peaks. Peaks with higher retention experience more dilution during elution from the column. Hence, extra column band broadening is of minor importance when the retention factor is high. The extra column volume is only a small percentage of the peak volume. Figure 3 shows the effect of extra column band broadening (isocratic separation of alkylphenones). The mixture was injected on a 4.6-mm i.d. and a 2.1-mm i.d. column in a conventional analytical LC instrument. The dramatic peak broadening with the micro column, especially for early eluting peaks, is obvious.

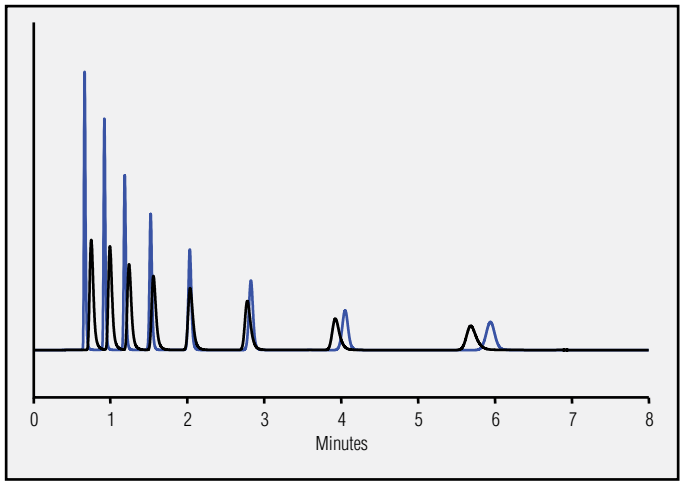

Figure 3. Separation of 8 alkylphenones on a 4.6 x 100-mm Acclaim® 120, 3 µm, C18 column (blue chromatogram) and a 2.1 × 100-mm Acclaim 120, 3 µm, C18 column (black chromatogram) with flow rates and injection volumes adapted (2 mL/min, 10 µL and 0.42 mL/min, 2.1 µL, respectively). Both separations were performed on a conventional analytical instrument.

To characterize the suitability of a system for micro column applications, the achieved plate height is plotted versus the retention factor. The range of 80% of the column plate number must be achieved at a retention factor k between 2 and 3. As shown in Figure 4, this is easily achieved with 2.1-mm i.d. columns on the micro system.

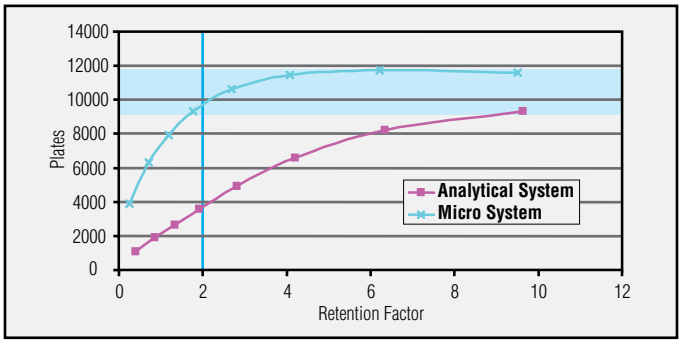

Figure 4. Plate number plotted versus retention factor for the isocratic separation of 8 alkylphenones on a 2.1 × 100-mm Acclaim 120, 3 µm, C18 column operated in a conventional analytical system and in the micro system.

Figure 5 shows the same separation carried out on a 1-mm i.d. column. Even under these demanding conditions, 80% of the column plate number is achieved close to k=3.

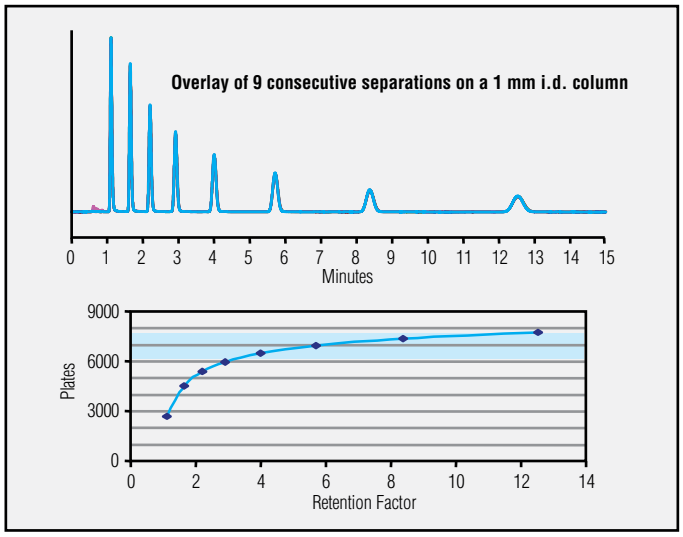

Figure 5. Separation of 8 alkylphenones on a PepMap™ C18 3 µm, 1.0 × 150 mm column operated in the micro system

THE GENERAL BENEFITS WITH THE APPLICATION OF MICRO COLUMNS

The main advantages of HPLC methods involving micro columns are:

- Flow rates are adopted to common ESI-MS requirements (100-500 µL/min)
- Sample volume is saved and improved detection sensitivity for small sample sizes achieved
- Thermostatting of columns for separation at elevated temperature is facilitated (no need for additional eluent pre-heaters that contribute to extra column band broadening)
- Better efficiencies at high pressures are achieved due to better friction heat dissipation
- The cost of solvent and solvent disposal can be tremendously reduced since solvent consumption of a 1-mm i.d. column is 5% that of a 4.6-mm i.d. column

Figure 6 shows the benefit of micro columns with respect to sensitivity. On 3 columns differing in diameter, 0.5 µL of an alkyl phenone mixture at a given concentration was injected. In theory the peak height resulting from the 1-mm i.d. column should be 21-fold higher than that resulting from the 4.6-mm i.d. column. The sensitivity increases with the power of 2 when column i.d. decreases, since the dilution of the sample on the column is directly related to its volume. We observed a 17-fold increase in sensitivity for the 1-mm column compared to the 4.6-mm column.

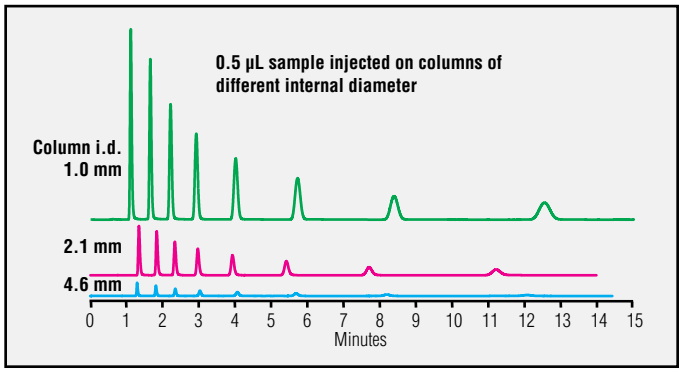

Figure 6. Injection of a 0.5-µL alkylphenone sample on columns of different internal diameters.

Figure 7 shows the influence of thermal mismatch in a 4.6 × 150-mm LC column at 50 °C column compartment temperature without eluent preheater. The selected application example depicts the analysis of hydrocortisone next to 2 parabens in an ointment. The lower temperature in the column center leads to lower linear flow velocity due to an increased eluent viscosity and higher analyte retention. Both effects produce serious peak distortion. The temperature balance and expected separation performance can be achieved by the use of an eluent preheater. Figure 8 shows a speed up of this application by transfer to a 2.1 × 50 mm column. It can be observed that the thermal mismatch is negligible and the extra column band broadening effect of the eluent preheater is even unfavourable with the micro column. It can be concluded that an eluent preheater is not required here and should not be used for the majority of micro column applications. However, an optimized 2-µL eluent preheater (125-µm i.d.) is available for the system.

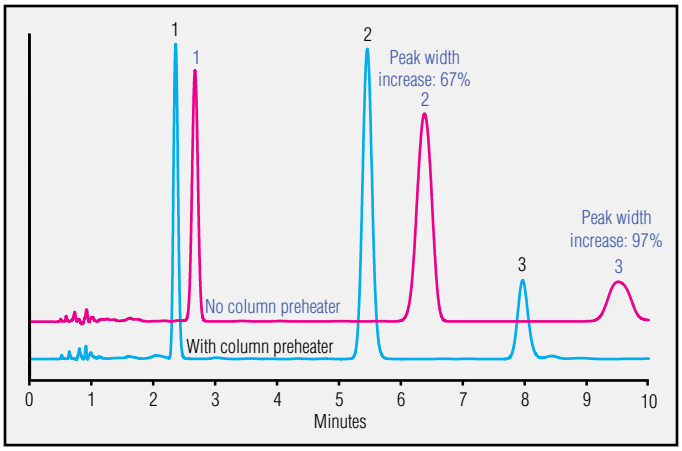

Figure 7. Ointment ingredient separation on a 4.6 × 150-mm Acclaim 120 column (C18, 3 µm) at 50 °C. Obvious thermal mismatch is encountered without eluent preheater. (Peak identification: (1—methylparaben, 2—hydrocortisone, 3—propylparaben).

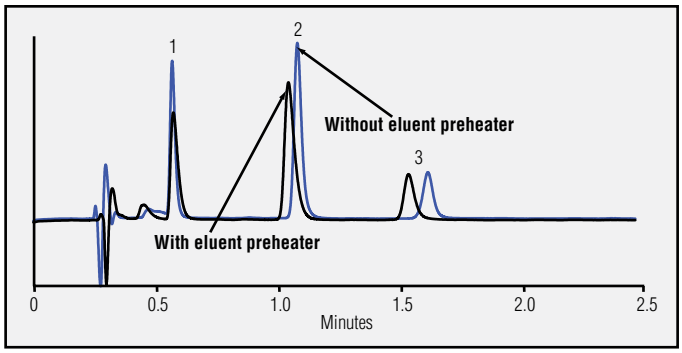

Figure 8. Fast ointment ingredient separation on a 2.1 × 50 mm Hypersil® Gold column (C18, 1.9 µm) at 50 °C. No thermal mismatch is observed while the eluent preheater generates extra column band broadening (for peak identification, see Figure 7 caption).

THE PERFORMANCE OF THE ULTIMATE 3000 MICRO SYSTEM FOR FAST AND ULTRAFAST SEPARATIONS

The beneficial effect of low gradient delay and extra column volume makes the micro system ideal for high speed separations using short micro columns packed with small stationary phase particles (<2 µm). Such columns put high demands on the instrumental performance but enable extreme separation speed. Figure 9A shows the isocratic separation of 5 aromatic compounds on a column of 50-mm length in 85 s. More than 8000 theoretical plates can be achieved on this short column with an optimized instrument. Figure 9B shows a gradient separation of 9 alkylphenones within 20 s on a 30-mm long column at a temperature of 82 °C (a 2 µL eluent preheater was used).

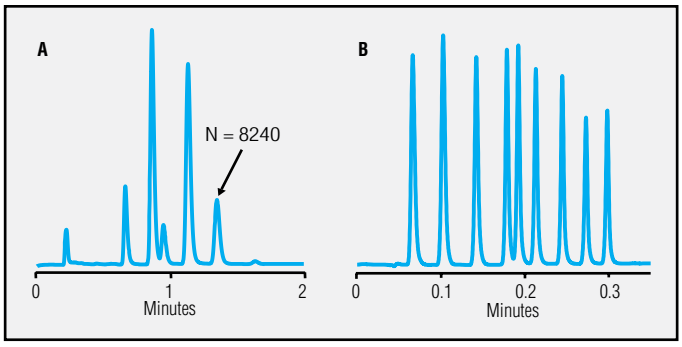

Figure 9. (A) Fast isocratic separation of 5 aromatic compounds (and uracil as inert marker) on a 2.1 × 50-mm Hypersil Gold column (C18, 1.9 µm) at 40 °C and 0.6 mL/min flow rate (column head pressure was 270 bar). (B) Ultrafast gradient separation of 9 alkylphenones on a 2.1 × 30-mm Hypersil Gold column (C18, 1.9 µm) at 82 °C and 2.0 mL/min flow rate (column head pressure was 380 bar).

CONTROL OF THE ULTIMATE 3000 FROM THIRD PARTY MS SOFTWARE

The use of the system as a front end for mass spectrometers is one of the most important areas of application. Especially with high-end MS-MS or MS^n instruments the software integration for single point control of MS and the LC instruments is difficult. With the Dionex Chromatography Mass Spectrometry Link ($DCMS^{Link}$) a high-level driver is available for a seamless integration of the Micro LC system with most common mass spectrometry platforms. The solution is offered for Bruker MS under HyStar™, Thermo MS under Xcalibur™, and ABI/Sciex MS under Analyst® (Figure 10).

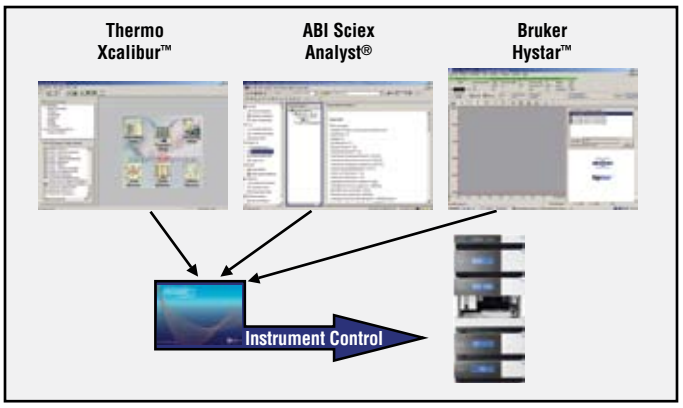

Figure 10. Direct control of the UltiMate 3000 Micro LC system from most common MS software packages.

CONCLUSIONS

- UltiMate 3000 Micro is a dedicated micro LC system with optimized gradient delay and extra column volume
- The system provides excellent separation efficiency with challenging columns of 1-mm internal diameter
- Micro LC provides the advantages of high sensitivity for analytes at trace levels combined with less solvent consumption
- Ultrafast gradient separation within 20 s can be achieved on this instrument
- Micro LC instruments are the ideal front-end for ESI-MS as the column flow rates of 0.2–1.0 mL/min are ideal for robust and sensitive LC-MS
- Control of UltiMate 3000 from third party MS software is possible by the use of a high-level LC instrument driver ($DCMS^{Link}$) embedded in Xcalibur, Analyst and Hystar

Passion. Power. Productivity.

DIONEX

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
Sunnyvale, CA (408) 737-8522 Westmont, IL (630) 789-3660
Houston, TX (281) 847-5652 Atlanta, GA (770) 432-8100
Marlton, NJ (856) 596-0600 Canada (905) 844-9650

www.dionex.com

Europe
Austria (43) 1 616 51 25 Belgium (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10
Germany (49) 6126 991 210 Italy (39) 06 66 51 5052
The Netherlands (31) 161 43 43 03
Switzerland (41) 62 205 99 66
United Kingdom (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282
India (91) 22 28475235 Japan (81) 6 6885 1213
Korea (82) 2 2653 2580

ISO 9001 REGISTERED

LPN 1826-03 10/06