doi:10.2533/chimia.2007.186 CHIMIA **2007**, *61*, No. 4

Chimia 61 (2007) 186–189

ISSN 0009–4293

New Trends in Fast Liquid Chromatography

Dao T.-T. Nguyen§, Davy Guillarme, Serge Rudaz, and Jean-Luc Veuthey*
§SCS Poster Prize Winner

Abstract: Analytical laboratories are currently interested in enhancing overall productivity by increasing sample throughput and reducing analysis time. Different approaches are proposed in liquid chromatography (LC) to perform fast or ultra-fast separations with cycle times of less than 5 or 1 min, respectively. Among these approaches, the use of monolithic supports, high temperature LC (HTLC), short columns and ultra-performance LC (UPLC) are described and compared in this study. A comparison of the above LC approaches is presented through Knox curves and pressure plots based on experimental data. Fast separations of pharmaceutical compounds are presented in order to illustrate the interest of these techniques and compare them with conventional LC separations.

Keywords: Fast liquid chromatography · HTLC · Monolith · Short columns · UPLC

1. Introduction

The pharmaceutical industry is interested in new technologies for increasing productivity for routine analytical work, method development, process monitoring and quality control. High-throughput methods are also mandatory in various fields such as the separation of drugs and metabolites (*e.g.* therapeutic drug monitoring, pharmacokinetic studies[1]), substances of environmental interest (*e.g.* determination of pollutants[2]), food additives or biological samples (*e.g.* proteomics, metabolomics[3]).

In liquid chromatography (LC), there are different approaches used to reduce analysis time and obtain fast or ultra-fast methods with cycle times less than 5 or 1 min, respectively. Some examples are: the reduction of column length (≤50 mm), the increase of mobile phase flow rate, the reduction of particle size (≤2 μm), the use of ultra-high pressure (>400 bar) and high temperature (≤200 °C), as well as the use of monolithic supports. These approaches are obviously not equivalent since it is not always straightforward to decrease analysis time while maintaining good chromatographic performance.[4]

This study presents advantages and drawbacks of the above-mentioned fast LC approaches with the help of Knox curves and pressure plots based on experimental data. Several separations of pharmaceutical compounds were performed to illustrate the interest of fast LC.

2. Monoliths

Monolithic columns are made of a single rod of porous material, prepared by a polymerization process generating a bimodal structure (macropores and mesopores).[5] The separation performance is based on macropore and mesopore size. With this bimodal structure it is possible to work at high flow rates without excessive backpressure (high permeability) and loss in efficiency. Monolithic columns are prepared from organic polymers (*e.g.* polymethacrylates, polystyrenes) or inorganic polymers (*e.g.* silica, zirconia, titania). The most widely used and commercially available monolithic columns (provided by Merck and Phenomenex, under the trademarks Chromolith® and Onyx®, respectively) are based on silica polymers which feature macropores of 2 μm and mesopores of 13 nm. In spite of undeniable benefits, this technique presents some drawbacks such as the limited number of commercially available column geometries (*i.e.* internal diameter of 4.6 mm, 3.0 mm or 100 μm) and a low resistance to extreme pH (2 < pH < 8) or high pressure (ΔPmax = 200 bar).

Fig. 1 presents the comparison of a conventional stationary phase (column packed with 5 μm silica particles) with several fast-LC approaches. The comparison is based on Knox curves (Fig. 1a) and pressure plots (Fig. 1b) obtained for all strategies for the isocratic separation of parabenes using butylparabene as a reference compound. The Knox curve obtained with the monolithic support demonstrated a minimum plate height H_{min} of 8.8 μm while the column packed with 5 μm particles exhibited a value of 11.8 μm. These results are in agreement with a previous study, demonstrating that the efficiency of the monolithic support was equivalent to a column packed with 3.5 μm particles (Fig. 1a).[6] Furthermore, at a linear velocity higher than the optimal value, efficiency remained satisfactory because this support possesses a low mass transfer resistance.

According to the high permeability of monoliths, large mobile phase flow rates can be used without generating excessive pressure. From a theoretical point of view, pressure generated by this monolith is equivalent to a column packed with 10–11 μm particles.[7]

The separation of a pharmaceutical formulation (Rapidocaine®) containing methylparabene, propylparabene, lidocaine and its by-product 2,6-dimethylaniline was performed under isocratic conditions (Fig. 2). The analysis time was reduced by a factor of six between the monolithic (Fig. 2b) and the conventional (Fig. 2a) support (1.4 min instead of 9.0 min). Some changes in selectivity (peak 4 – lidocaine) were observed, making method transfer more challenging. Moreover, peak fronting observed for lido-

*Correspondence: Prof. J.-L. Veuthey
Laboratory of Analytical Pharmaceutical Chemistry
School of Pharmaceutical Sciences
University of Geneva, University of Lausanne
Boulevard d'Yvoy 20
CH-1211 Geneva 4
Tel.: +41 22 379 63 36
Fax: +41 22 379 68 08
E-Mail: Jean-Luc.Veuthey@pharm.unige.ch

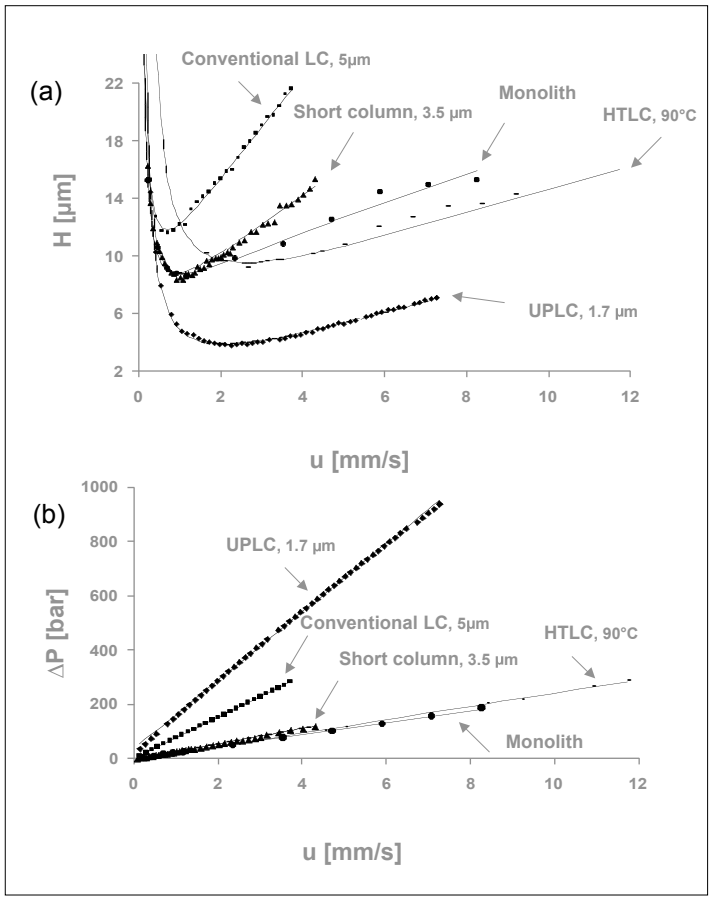

Fig. 1. Comparison of HPLC with several fast-LC approaches. (a) Knox curves, (b) Pressure plots

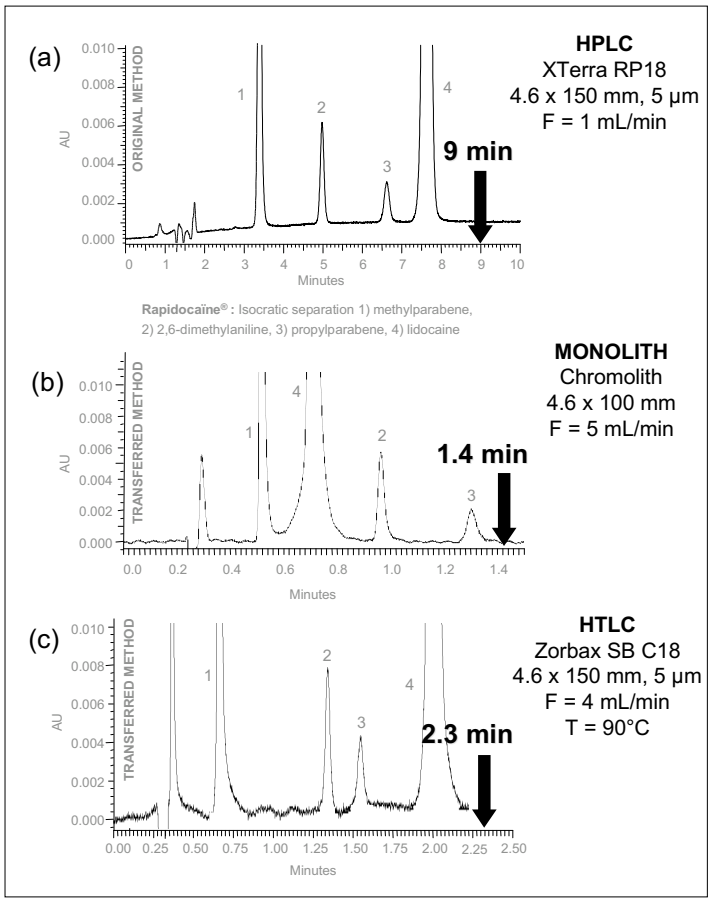

Fig. 2. Isocratic separations of pharmaceutical formulation (Rapidocaine®) obtained using (a) HPLC, (b) monolith, and (c) HTLC at 90 °C

caine could be attributed to overload of the chromatographic support since monoliths present a lower loadability than conventional packed columns.[7]

3. High-temperature Liquid Chromatography (HTLC)

Raising the mobile phase temperature (usually up to 200 °C) allows a reduction of the analysis time by decreasing the mobile phase viscosity and thus increasing the solutes' diffusion coefficients. Additionally, because of a decrease in water polarity at high temperature conditions, the organic content in the mobile phase must be reduced (green chemistry) to maintain equivalent retention factors.[8] However, HTLC suffers from some drawbacks. The first one is the risk of stationary phase degradation, since classical bonded silica phases are unstable at high temperature (>60 °C). The development of a new generation of silica-based columns (hybrid or modified silica) can overcome this problem.[9] Furthermore, the use of high temperature requires dedicated instrumentation to preheat the mobile phase for avoiding peak distortion in the analytical column. Finally, the injected compounds must not be susceptible to thermal degradation under the conditions used.

According to Knox curves (Fig. 1a), HTLC is beneficial in terms of analysis time and efficiency:

i) the minimum of the Knox curve was about 20% better at 90 °C (H_{min} = 9.6 µm) compared to 30 °C;
ii) since mass transfer resistance is better at high temperature, it was possible to maintain good efficiency values, even at high linear velocities;
iii) it is also important to note that the optimum linear velocity (u_{opt}) was shifted to a higher value at 90 °C. Consequently, when temperature is increased, the mobile phase flow rate should be adjusted to higher values. Due to a significant decrease in mobile phase viscosity with temperature (Fig. 1b), high mobile phase flow rates are possible and do not generate excessive backpressure.[10].

An example is reported in Fig. 2c. A reduction in analysis time by a factor of four was obtained (2.3 min instead of 9.0 min) which is directly proportional to mobile phase flow rates (1 and 4 ml/min at 30 °C and 90 °C, respectively). Changes in selectivity[11] and resolution (*e.g.* peaks 2 and 3) were also observed. These changes were attributed to the modification in mobile phase properties with temperature. For this reason, elution order is not always predictable in HTLC, making method transfer difficult.

In general, HTLC is a good solution to reduce analysis time but is limited by the compatibility of silica-based stationary phases at high temperatures.

4. Short Columns

Reducing column length is one of the simplest approaches to decrease analysis time. To limit the loss in efficiency associated with shorter column lengths, a simultaneous reduction in particle size is mandatory (*i.e.* 2.5–3.5 µm *vs.* 5 µm). The use of short columns exhibits several advantages, such as the possibility to quickly transfer an existing method (using simple scaling equations) and to work at higher flow rates (as a result of the low pressure generated). However, due to the limited chromatographic performance in terms of efficiency (*N* being directly proportional to the column length, *L*), this strategy is generally employed for the separation of simple mixtures[12] or in combination with MS detection.[13]

Fig. 3 presents the comparison for the separation of a drug substance and its by-products performed in gradient mode with conventional and short columns. A significant reduction in analysis time was obtained with the short column (*i.e.* 2.0 min *vs.* 22.0 min). It can be noted that several parameters

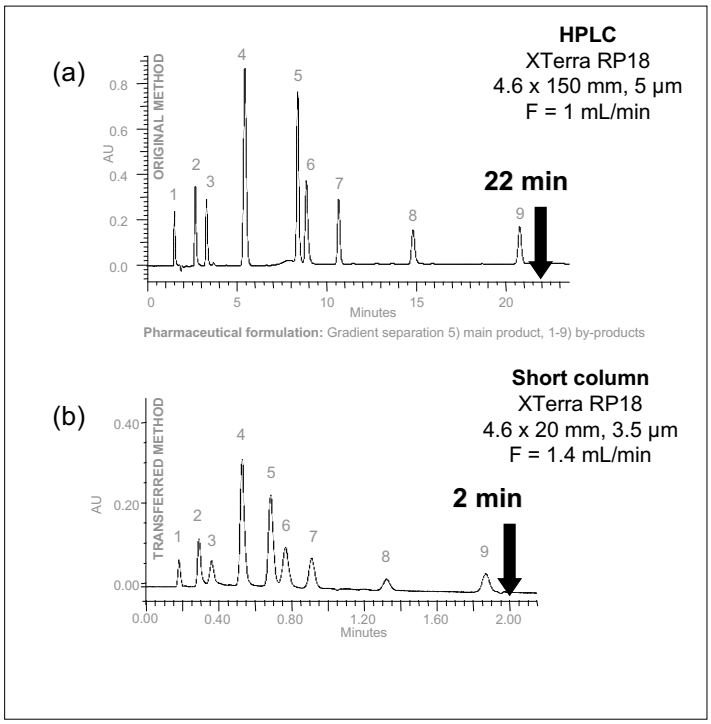

Fig. 3. Gradient separations of a pharmaceutical mixture (main compound and its by-products) obtained using (a) HPLC (L = 150 mm, d_p = 5 µm) and (b) short column (L = 20 mm, d_p = 3.5 µm).

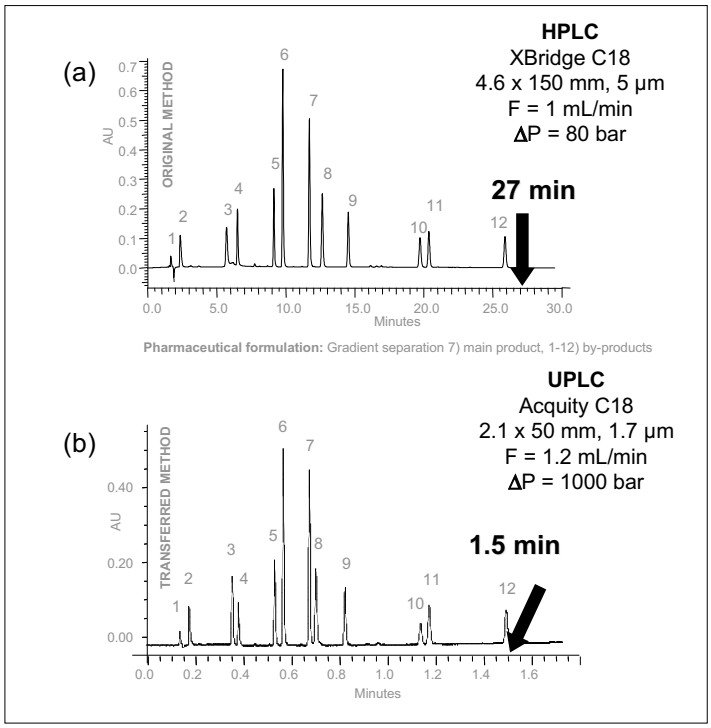

Fig. 4. Gradient separations of a complex pharmaceutical mixture (main compound and its by-products) obtained using (a) HPLC (d_p = 5 µm, ΔPmax = 400bar) and (b) UPLC (d_p = 1.7 µm, ΔPmax = 1000bar)

such as the mobile phase flow rate, injected volume and gradient profile were calculated to avoid any change in selectivity during the method transfer thanks to a Excel® program called 'HPLC calculator' available free on our website.[14] Under these conditions, changes in chromatographic profiles were only attributed to the loss in chromatographic performance (*i.e.* about 60% lower peak capacities) generated by the reduction in column length. The loss in resolution could also be due to the contribution of extra-column band broadening and system dwell volume. Therefore, an optimized LC system is mandatory to reduce peak dispersion.

Knox curves and pressure plots are shown in Fig. 1. As expected, lower theoretical plate heights were obtained with the column packed with 3.5 µm particles (H_{min} = 8.8 µm) compared to 5 µm. The optimal linear velocity was also shifted to a higher value when particle size decreased ($u \cdot d_p$ = constant). Regarding the pressure plots and considering Darcy's law, the pressure generated by the short column (20 mm) packed with 3.5 µm particles should be approximately 3–4 times lower than a conventional HPLC column (150 mm, 5 µm); considering the concomitant effect of decreasing column length and particle size on backpressure.

5. Ultra-Performance Liquid Chromatography (UPLC)

The use of small particles and very high pressure for performing ultra-fast and/or highly efficient analysis has been described by Jorgenson *et al.*[15] Recently, Waters commercialized, under the trade name UPLC, a system compatible with ultra-high pressure (up to 1000 bar) and columns packed with sub-2 µm particles. Today, several manufacturers offer columns packed with sub-2 µm particles and systems compatible with high pressures (*e.g.* 1200 RR LC® by Agilent, Rheos Allegro Ultra HPLC® by Flux, X-LC® by Jasco, Accela® by Thermo, Acquity UPLC® by Waters). According to the fundamental equations of chromatography, small particles can generate high efficiencies (N being directly proportional to $1/d_p$) and short analysis time ($u_{opt} \cdot d_p$ = constant). However, a significant increase in backpressure is generated according to Darcy's law.[16]

Fig. 4 presents the comparison of columns packed with conventional silica-based (5 µm) and hybrid silica ethylsiloxane (1.7 µm) particles for the separation of a pharmaceutical mixture (main compound and its by-products, at similar concentration levels). Knox curves and pressure plots obtained under LC and UPLC conditions are reported in Fig. 1. As expected from theory, a higher efficiency was obtained with small particles (H_{min} = 3.9 µm for 1.7 µm *vs.* 11.8 µm for 5 µm) and the optimal linear velocity was increased (u_{opt} = 2.11 mm/s for 1.7 µm *vs.* 0.72 mm/s for 5 µm). Finally, columns packed with 1.7 µm particles exhibited a lower mass transfer resistance permitting a separation at high flow rate without losing resolution.

The chromatographic separation was performed in gradient mode with two columns of different geometries but packed with the same stationary phase. By adapting the mobile phase flow rate, injection volume and gradient profile in UPLC, the analysis time was reduced by a factor of 18 (only 1.5 min *vs.* 27 min) without loss of chromatographic performance.

Therefore, UPLC probably represents the best approach to reduce the analysis time while maintaining chromatographic performance. This approach is also well adapted to obtain high efficiency values, in the range of 10'000 ≤ N ≤ 100'000 plates, with suitable longer columns.

6. Conclusion

Depending on the application and according to the available LC system (conventional or dedicated), numerous solutions exist to increase productivity in LC. Fast LC methods presented in this work allow a significant decrease in analysis time compared to conventional LC.

The approach to perform fast LC must be selected in agreement with analytical constraints (*e.g.* LC instrumentation, column geometry, stationary phase, complexity of the analyzed mixture, required resolution, use of MS detector, column stability in high temperature or high pressure conditions, potential degradation of compounds with temperature).

Fig. 5 summarizes each approach, with their advantages and drawbacks. Monoliths and short columns are compatible with a

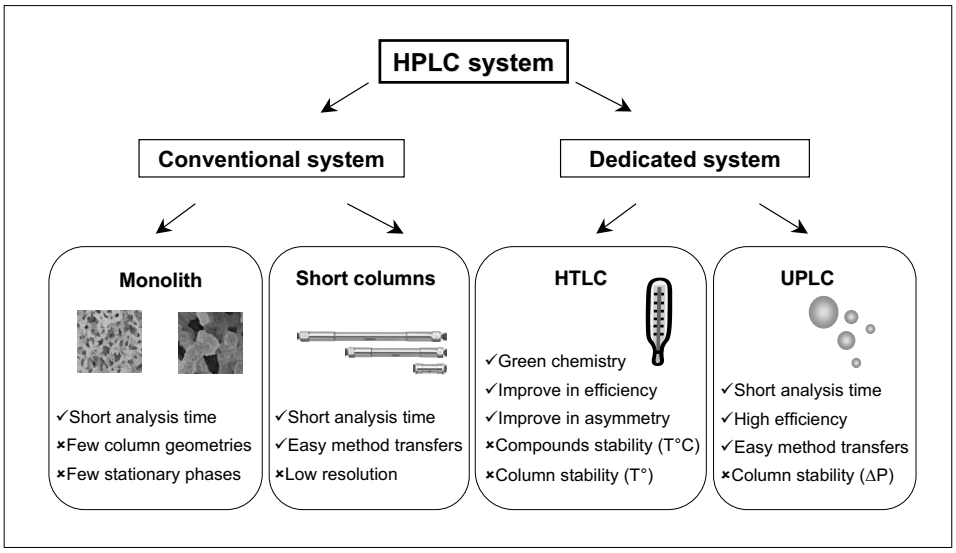

Fig. 5. How to select the best fast LC approach

conventional LC system, but only few column geometries and chemistries are available for monoliths and, short columns are only adapted to simple mixtures or with MS detection. On the other hand, HTLC and UPLC necessitate a dedicated LC system. In order to preheat correctly the mobile phase in HTLC, an efficient oven is mandatory and columns should be compatible with high temperature. For UPLC, an ultra-high pressure-compatible system and corresponding sub-2 µm columns are required.

Combination of fast LC strategies could be used to reduce more drastically the analysis time. This approach is currently under investigation in our laboratory.

Acknowledgements

The authors would like to thank Agilent for the loan of the column oven Metalox 200C and UV detector G1315C, and gratefully acknowledge Waters for the loan of the UPLC system and the generous gift of Acquity and XTerra columns.

Received: December 18, 2006

[1] K. Heinig, F. Bucheli, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2003**, *795*, 337.
[2] T. Koal, A. Asperger, J. Efer, W. Engewald, *Chromatographia* **2003**, *57* (Suppl.), 93.
[3] R. S. Plumb, J. H. Granger, C. L. Stumpf, K. A. Johnson, B. W. Smith, S. Gaulitz, I. D. Wilson, J. Castro-Perez, *Analyst* (Cambridge, U. K.) **2005**, *130*, 844.
[4] D. T. T. Nguyen, D. Guillarme, S. Rudaz, J. L. Veuthey, *J. Sep. Sci.* **2006**, *29*, 1836.
[5] K. Miyabe, G. Guiochon, *J. Sep. Sci.* **2004**, *27*, 853.
[6] F. C. Leinweber, U. Tallarek, *J. Chromatogr., A* **2003**, *1006*, 207.
[7] K. Cabrera, *J. Sep. Sci.* **2004**, *27*, 843.
[8] D. Guillarme, S. Heinisch, J. L. Rocca, *J. Chromatogr., A* **2004**, *1052*, 39.
[9] Y. Yang, *LC-GC Eur.* **2003**, *16*, 37.
[10] B. Yan, J. Zhao, J. S. Brown, J. Blackwell, P. W. Carr, *Anal. Chem.* **2000**, *72*, 1253.
[11] J. Li, P. W. Carr, *Anal. Chem.* **1997**, *69*, 2202.
[12] A. Nguyen Minh Nguyet, L. Tallieu, J. Plaizier-Vercammen, D. L. Massart, Y. Vander Heyden, *J. Pharm. Biom. Anal.* **2003**, *32*, 1.
[13] T. Goto, Y. Ito, H. Oka, I. Saito, H. Matsumoto, H. Sugiyama, C. Ohkubo, H. Nakazawa, H. Nagase, *Anal. Chim. Acta* **2005**, *531*, 79.
[14] *http://www.unige.ch/sciences/pharm/fanal*, November, **2006**.
[15] A. D. Jerkovich, J. S. Mellors, J. W. Jorgenson, *LC-GC Eur.* **2003**, *16*, 20.
[16] D. T. T. Nguyen, D. Guillarme, S. Rudaz, J. L. Veuthey, *J. Chromatogr., A* **2006**, *1128*, 105.