ISSN 2278 5221 www.ijpmb
Vol. 3, No. 3, July 20

Review Article

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

K Naresh [1]*, S Bhawani [1] and T Maneesh Kumar [1]

Corresponding Author: K Naresh ð banu.sunkara@gmail.com

Ultra Performance Liquid Chromatography (UPLC) takes the advantage of technological strides made in particle chemistry performance, system optimization, detector design and data processing and control. Using sub 2 mm particles and mobile phases at higher linear velocities and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance. This review introduces the theory of UPLC and summarizes some of the most recent work in the field.

Keywords: UPLC, HPLC, Sensitivity, Resolution

INTRODUCTION

UPLC refers to high Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. UPLC comes from High Performance Liquid Chromatography (HPLC). HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. HPLC is approved technique as it has been used in laboratories worldwide over the past 30-plus years. HPLC technology simply doesn€t have the capability to take full advantages of sub-2 •m,particles (Michael, 2005).

UPLC can be regarded as new invention for,liquid,chromatography. UPLC brings dramatic improvements in sensitivity, resolution and,speed,of analysis can be calculated. It has instrumentation that operates at high,pressure,than that used in HPLC and in this system uses fine,particles (less than,2.5 •m) and mobile phases at high linear velocities decreases the length of column, reduces solvent consumption and,saves time. According to the van Deemter equation, as the particle size decreases to less than 2.5 •m, there is a significant gain in efficiency, while the efficiency

[1] Balaji Institute of Pharmaceutical Sciences, Laknepally, Narsampet, Warangal-506 331,

does not diminish at increased flow rates or linear velocities (Swartz, 2005).

Therefore by using smaller particles, speed,and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed UPLC. The technology takes full advantage of chromatographic principles to run separations Using columns packed with smaller,particles (less than 2.5 •m) and/or higher flow rates for increased,speed, this gives superior resolution and sensitivity,.Now a days in industrial area UPLC refers for some of, the, most recent work field.

CHEMISTRY OF SMALL PARTICLES

Smaller particles provide not only increased efficiency, but also the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Efficiency is the primary separation parameter behind UPLC, since, it relies on the same selectivity and retentivity as HPLC. In the fundamental resolution (Rs) equation: resolution is proportional to the square root of N.

$$Rs = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k+1}$$

System Efficiency — Selectivity — Re sentivity

But since N is inversely proportional to particle size (dp): as the particle size is lowered by a factor of three, from, for example, 5 •m (HPLC scale) to 1.7 •m (UPLC-scale), N is increased by three and resolution by the square root of three or 1.7. N is also inversely proportional to the square of the peak width:

$$N \propto \frac{1}{w^2}$$

This illustrates that the narrower the peaks are, the easier they are to separate from each other. Also, peak height is inversely proportional to the peak width:

$$H \propto \frac{1}{w}$$

So as the particle size decreases to increase N and subsequently Rs, an increase in sensitivity is obtained, since narrower peaks are taller peaks. Narrower peaks also mean more peak capacity per unit time in gradient separations, desirable for many applications, e.g., peptide maps. Still another equation comes into play when migrating toward smaller particles:

$$F_{xxx} \propto \frac{1}{dpc}$$

This relationship also is revealed from the van Deemter plot. As particle size decreases, the optimum flow F opt to reach maximum N increases. But since back pressure is proportional to flow rate, smaller particle sizes require much higher operating pressures, and a system properly designed to capitalize on the efficiency gains; A system that can both reliably deliver the requisite pressures and that can maintain the separation efficiency of the small particles with tightly managed volumes. Efficiency is proportional to column length and inversely proportional to the particle size.

$$N \propto \frac{L}{dp}$$

Therefore, the column can be shortened by the same factor as the particle size without loss

of resolution. Using a flow rate three times higher due to the smaller particles and shortening the column by one third (again due to the smaller particles), the separation is completed in 1/9 the time while maintaining resolution. Although high efficiency, nonporous 1.5-ðm particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. Silica-based particles have good mechanical strength but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitations, but they have their own issues including low efficiencies, limited loading capacities and poor mechanical strength. Packing a 1.7 ðm particle in reproducible and rugged columns was also a challenge that needed to be overcome, however. A smoother interior surface for the column hardware, and re-designing the end frits to retain the small particles and resist clogging were necessary. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations.

PRINCIPLE

The UPLC is based on the principal of use of stationary phase consisting of,particles,less than,2.5 ðm (while HPLC columns are typically filled with,particles,of 3 to 5 ðm). The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency) (Van et al., 1956),

$$H=A+B/v+Cv$$

where;

A,, B€and, C€are constants

v€is the linear velocity, the carrier gas flow rate.

*The,A€term is independent of velocity and represents *f* eddy„ mixing. It is smallest when the packed column particles,are small and uniform.

The,B€term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by,v.

*The,C€term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus term is proportional to,v. Therefore it is possible to increase throughput, and thus the,speed,of analysis without affecting the chromatographic performance. The advent of UPLC has demanded the development of a new instrumental system for,liquid,chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size (Lars and Honore, 2003). As shown in Figure 1, smaller,particles,provide increased efficiency as well as the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and,speed. Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as HPLC.

UPLC Stability Indicating Assay

UPLC Conditions

1. Column: 2.1 x 30 mm 1.7 ðm ACQUITY BEH C18

2. Temp: 30…C., A 45 s, 5-85% B linear gradient,
3. Flow rate: 0.8 mL/min was used.
4. Mobile phase: A was 10 mm ammonium formate, pH 4.0, B was acetonitrile. UV detection at 273 nm and 40 pts/s
5. Peaks,in order:,5-nitroso-2, 4, 6-triaminopyrimidine, 4-amino-6-chloro-1, 3-benzenesulfanamide, hydrochlorthiazide, triamterine, and, methylbenzenesulfanamide; 5 •L injection, 0.1 mg/mL each.

Capitalizing on Smaller Particles

Only small particles are not responsible for fast, resolution and sensitivity, some special instrumentation system should be design. So some special kind of system capable of delivering the pressures required, to realize the potential of UPLC have been reported in the literature and elsewhere (Swartz, 2005). In early 2004, the first commercially available UPLC system that embodied these requirements was described for the separation of various pharmaceutical related small organic molecules, proteins, and peptides; it is called the ACQUITY UPLCTM System.,The ACQUITY UPLC System consists of a binary solvent manager, sample manager (including the column heater), detector, and optional sample organizer. The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient mixed under high pressure.

Instrumentation

The UPLC System has been holistically designed to match the performance needs of innovative column chemistries with robust hardware, easy-to-use software and specialized support services. It consists of:

† Small, pressure-tolerant particles
† High-pressure fluidic modules
† Minimized system volume
† Negligible carryover
† Reduced cycle times
† Last response detectors
† Integrated system software and diagnostics

The ACQUITY UPLC System€s high-pressure fluidics optimizes flow rates to make the most of small particle technology. The ACQUITY UPLC System€s sample-handling design is designed to ensure exceptionally low carryover and reduced cycle time. And when interfaced with the Sample Organizer, it increases unattended sample capacity by ten times. High-speed detectors, both optical and mass, contribute to increased sensitivity and help manage the heightened speed and resolution requirements of UPLC.

† ACQUITY UPLC Systems are easily controlled, diagnosed, and monitored via a graphical system console interface. The console offers:
† Quick and easy access to critical instrument parameters.
† Simple system start-up, elegant system status monitoring and predictive performance indicators to ensure maximum productivity.
† Data management capabilities that are supported by both MassLynx‡ and Empower‡ software.
† The ACQUITY UPLC System is also supported by Intelligent Device Management technology with our Connections INSIGHT‡ service, providing instrument diagnostics.

1. Sample injection
2. UPLC columns
3. Column manager and heater or cooler
4. Detectors
5. Softwares
6. Accessories
7. Connection insight service (if provided by mfg.company.water provided it)

Sample Inject

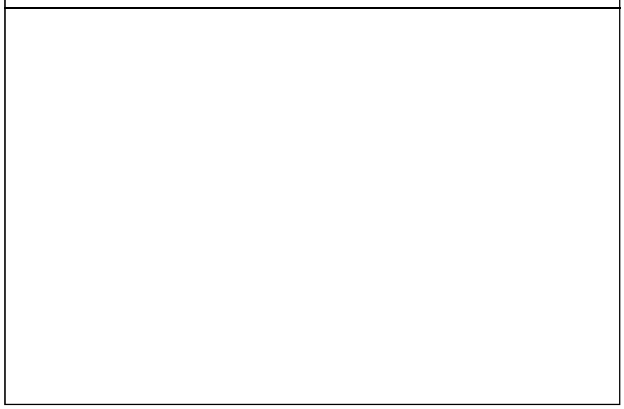
Lee et al. described the design of injection valves and separation reproducibility (Wu et al., 2001) and the use of a carbon dioxide enhanced slurry packing method on the capillary scale for the separation of some benzodiazepines, herbicides, and various pharmaceutical compounds (Wu et al., 1997). Jorgenson et al. modified a commercially available HPLC system to operate at 17,500 psi and used 22 cm long capillaries packed with 1.5 mm C18-modified particles for the analysis of proteins (Tolley et al., 2001). The calculated pressure drop at the optimum flow rate for maximum efficiency across a 15-cm long column packed with 1.7 •m particles is approximately 15,000 psi. Therefore, a pump capable of delivering solvent smoothly and reproducibly at these pressures and that can compensate for solvent compressibility, while operating in both the gradient and isocratic separation modes is required,. With 1.7 •m particles, half-height peak In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalise on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase sensitivity. There are also direct injection approaches for biological samples.

UPLC Columns

The design and development of sub-2 •m particles is a significant challenge, and researchers have been active in this area for some time, trying to capitalize on their advantages 2-4. Although high efficiency, nonporous 1.5 •m particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. Silica-based particles have good mechanical strength but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitations, but they have their own issues including low efficiencies, limited loading capacities, and poor mechanical strength. In 2000, Waters introduced XTerraˆ, a first generation hybrid chemistry that took advantage of the best of both the silica and Polymeric column worlds. XTerra columns are mechanically strong, with high efficiency and operate over an extended pH range. They are produced using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups. But in order to provide the necessary mechanical stability for UPLC, a second generation Bridged Ethyl Hybrid (BEH) technology was developed called ACQUITY BEH, these 1.7 •m particles derive their enhanced mechanical stability by bridging the methyl groups in the silica matrix. Packing 1.7 •m particles into reproducible and

rugged columns was also a challenge that needed to be overcome. Requirements include a smoother interior surface of the column hardware and re-designing the end frits to retain the small particles and resist clogging. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations. All ACQUITY UPLC BEH columns also include eCord‡ microchip technology that captures the manufacturing information for each column including the quality control tests and certificates

Figure 1:€ACQUITY UPLC BEH Column Chemistries



of analysis.

Column Manager and Heater Cooler

The ACQUITY UPLC Column Manager, with automatic column switching, is for high productivity UPLC sample processing, and its Column Heater/Cooler enables labs to use temperature as a method parameter. The ACQUITY UPLC Column Manager allows users to take full advantage of the performance, range of stationary phases, and mechanical strength offered by ACQUITY UPLC BEH Columns. The Column Manager provides temperature regulation from 10…C to 90…C, automated switching for up to four,columns with,dimensions to 2.1 mm in internal diameter (I.D.) and 150 mm in length, as well as a bypass channel for flow injections. The ACQUITY UPLC System consists of a binary solvent manager, sample manager including the column heater, detector, and optional sample organizer. The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. There are built-in solvent select valves to choose from up to four solvents. There is a 15,000-psi,pressure,limit (about 1000 bar) to take full advantage of the sub-2 ðm particles. The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection process, and,a series,of pressures transducers facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness and a needle calibration sensor increases accuracy. Injection cycle time is 25 s without a wash and 60 s with a dual wash used to further decrease carry over. A variety of microtiter plate formats (deep well, mid height, or vials) can also be accommodated in a thermostatically controlled environment. Using the optional sample organizer, the sample manager can inject from up to 22 microtiter plates. The sample manager also controls the column heater. Column temperatures upto 65^{o} can be obtained.

Detectors

Half-height peak widths of,less than,one second are obtained with 1.7 ðm particles, which gives significant challenges for the,detector. In order to integrate an analyte peak accurately and reproducibly, the,detector sampling rate must be high enough to capture enough data points across the peak. The,detector,cell must have minimal dispersion (volume) to preserve separation

efficiency. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations, depending on the detection technique. MS detection is significantly enhanced by UPLC; an increased peak concentration with reduced chromatographic dispersion at lower flow rates promotes increased source ionization efficiencies. Waters detectors enhance ability to analyze a variety of compounds. Its ACQUITY UPLC detectors.

† The Photodiode Array (PDA),

† Tunable UV (TUV),

Evaporative Light Scattering (ELS) are optimized for UPLC system technology by virtue of their low dispersion characteristics, high data acquisition rates, and reliable performance with cost-effective maintenance support and parts.

Tunable UV (TUV)

The ACQUITY Tunable UV/Visible,detector,cell consists of a light guided flow cell equivalent to an optical fibre. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10 mm flow cell path length with a volume of only 500 mL. Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems.[26].

UPLC is an ideal inlet for the sensitivity and specificity offered by mass spectrometry. The low dispersion, high-speed detection performance of Waters MS Technologies, in combination with the performance characteristics of UPLC, can dramatically extend, detection capabilities.

Softwares

ACQUITY UPLC Systems can be easily controlled, diagnosed and monitored via a graphical system console interface with Empower‡ and MassLynx‡ software. Both Empower and MassLynx provide the dynamic data processing,and information management tools to convert the,results,generated by the ACQUITY UPLC System into valuable knowledge.

Accesories

Waters is continually extending the functionality of the ACQUITY UPLC System: eCord‡ technology, available on all ACQUITY UPLC columns, records column history. Sample Organizer that increases system capacity by more than 10 times. FlexCart system platform improves usability, accessibility and convenience

Connection€INSIGHT ™€Service

Connections INSIGHT‡ uses Intelligent Device Management technology to provide diagnostic information for the ACQUITY UPLC Systems. Connections INSIGHT creates a virtual technical support presence in your lab, enabling Waters to provide you with proactive and timely service, with the highest level of support and satisfaction.

METHOD OPTIMIZATION GUIDELINES AND OBSERVATIONS

During the course of optimizing the UPLC method, considerations to expedite future method transfers were developed, and the following recommendations were made: ‰ Increase elution solvent strength to reduce run times taking advantage of the high resolution potential of UPLC

columns. Increase mobile phase flow rate secondarily to solvent strength in order to promote longer column lifetimes. While high mobile phase linear velocities with good resolution are possible, as with any column, routine operation at 80% maximum rated pressure led to shortened lifetimes. UPLC operation around 8000 psi or less provided comparable or lower column cost per assay than HPLC. Maintaining low flows as much as possible also reduces solvent and waste disposal costs, although these are already an order of magnitude less than HPLC. Reduce column re-equilibration times by taking advantage of the low system dwell volume. Programmed changes in the mobile phase take time to reach the column. The small UPLC dwell volume (measured as 110 ðL, 15% of that of the HPLC) allowed in part the abbreviation of the original assay. Column re-equilibration accomplished during next sample loading in the UPLC, further increasing throughput. Reduce injection volumes appropriately for the column diameter to achieve good peak shapes. Peak splitting occur when too large of a strong sample solvent bolus overwhelms the packing at the column head. While this assay method tolerated 5 ðLinjections, volumes of 1Š3 ðLare more typical starting points. Smaller injection volumes may be compensated by enhanced peak height from use of the high resolution columns and by the low carryover from the UPLC injector (measured as 10% of the HPLC carryover for this analyte) to achieve an equivalent or even lower LOQ). An alternative to smaller injection volumes might be to lower sample solvent strength to accomplish sample focusing on the head of the column. Utilize partial loop-fill injections in preference to full loop-fill. Partial loop-fill precision was good even at volumes up to 80% of the loop total volume . Typical laboratory practice is to limit sample volume injections to roughly 50% of the total loop volume. The UPLC injection system, which utilizes air-gap sandwiching of the sample, allows better utilization of the sample loop and higher injection precision, reducing the need for use of the full loop-fill mode. From a practical point of view, full loop fill requires substantially greater sample movement considering overfill functions. This likely increases subsequent needle washing, this may impact sample throughput and increase wear of the washing hardware. Larger sample volume transfers also increases exposure to sample particulates, lowering long-term instrument reliability. If full loop-fill mode is utilized, perhaps for very high precision requirements ensure adequate loop overfilling. A significant laminar flow velocity differential in the loading sample between its wall interface and center is created in the very narrow bore tubing of the UPLC injector. Overfilling the sample loop by at least four loop volumes was found necessary to fully displace wash solvent from the 5 ðLinjector loop. For this instrument, the manufacturer has determined and set as the default the optimum overfill volume with typical sample solvents for each sample loop size. Operators can specify other overfill volumes for unusual sample compositions. Choose the proper composition and volume of weak sample wash to obtain good peak shape. A portion of the weak sample wash solvent will be co-injected with partial-loop filled samples. The weak solvent wash should therefore mimic the initial conditions mobile phase in solvent strength. Utilizing the weak wash

Figure 2: Chromatograms (from top to bottom): Original HPLC, Initial Scaling to UPLC Showing Peak Shape Improvement and Possibility for Further Method Optimization, and Final UPLC Method. Order of Peak Elution: Internal Standard (IS) Then Cpd A

solvent as sample diluents in the sample loop may enhance sample focusing onto the column. The volume of the weak wash must be sufficient to purge the former strong wash solvent from the loop.

ADVANTAGES

1. Decreases run time and,increases,sensitivity
2. Provides the selectivity, sensitivity, and dynamic range of LC analysis
3. Maintaining resolution,performance.
4. Expands scope of Multiresidue Methods
5. UPLC€s fast resolving power quickly quantifies related and unrelated compounds
6. Faster analysis through the use of a novel separation material of very fine particle size
7. Operation cost is reduced
8. Less solvent consumption
9. Reduces process cycle times, so that more product can be produced with existing
10. Resources
11. Increases,sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need to re-work material.
12. Delivers real-time analysis in step with manufacturing processes
13. Assures end-product quality, including final release testing

DISADVANTAGES

1. Due to increased pressure requires more maintenance and reduces the life of the columns of this type. So far performance similar or even higher has been demonstrated

by using stationary phases of size around 2 ðm without the adverse effects of high pressure.

2. In addition, the phases of less than 2 ðm are generally non-regenerable and thus have limited use.

APPLICATIONS

i. With UPLC increased resolution in shorter run times can generate more information faster without sacrifices. Higher sample throughput with more information per sample may decrease the time to market, an important driving force in today€s pharmaceutical industry. The corresponding HPLC separation takes in excess of 12 min; UPLC accomplishes the same separation in under 30 s.

ii. UPLC can also be used to significantly improve the success of the drug discovery process. Drug discovery is heavily dependant upon the early prediction of metabolic fate and interactions of drug candidate molecules.

iii. Sensitivity, selectivity, and analysis time (sample throughput) are also some of the challenges analysts face when analyzing environmental samples such as soil and water. Explosives residues in soil or environmental waters are of both forensic and environmental interest. These types of assays prove challenging because of the selectivity needed to resolve positional isomers.

iv. In addition, for complex samples like natural product extracts, added resolution can provide more information in the form of additional

Comparision Between UPLC and HPLC

Characteristics	HPLC Assay	UPLC Assay
Column	150×3.2mm	150×2.1mm
Particle size	3 to 5µm	Less than 2 µm
Flow rate	3.0 ml/min	0.6 ml/min
Needle wash	methanol	methanol
Injection volume	5µL	2 µL
Column temperature	30°C	65°C
Maximum back pressure	35-40 MPa	103.5 MPa
Total run time	10 min	1.5 min
Plate count	2000	7500
USP resolution	3.2	3.4
Delay volume	750 µL	110 µL

Note: Total solvent consumption (including 0.5 min of delay time in between injections). Ac
Acetonitrile : 0.53 ml, Water: 0.66 ml.

peaks. HPLC versus UPLC separation comparison of a ginger root extract sample where both speed and resolution are improved.

v. UPLC coupled with MS technology provided parent and fragment mass information of lipids in one chromatographic run, thus, providing an attractive alternative to current LC methods for targeted lipid analysis as well as lipidomic studies.

vi. Applications areas of UPLC specified in Waters literature include high throughput library screening, metabolite identification and bioanalysis, peptide mapping, stability indicating analyses, and quantitative analysis.

POTENTIAL AREAS OF USE

i. Analysis of complex mixtures (e.g., impurity profiles, formulation inerts).

ii. At-line analysis in manufacturing (analysis at the vessel) .

iii. Analysis of large amounts of samples ðFor LC/MS to get better spectra (improved signal to noise).

CONCLUSION

UPLC gives increased resolution, speed and sensitivity for liquid,chromatography,therefore, due to UPLC new chemistry and instrumentation technology can provide more information per unit of work. UPLC has main advantage over others is reduction of analysis time which also helps to reduce solvent consumption. A negative aspect of UPLC could be the higher back pressure than in conventional HPLC. This back pressure can be reduced by increasing the column temperature. But it seems that UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional HPLC.

REFERENCES

1. Michael E Swartz (2005), ƒUPLCTM: An,Introduction,and Review Waters Corporation, Milford, Massachusetts, USA„, Journal of Liquid€Chromatography€& Related Technologies, Vol. 28, pp. 1253Š 1263.

2. Swartz M E (2005), ƒUltra Performance Liquid Chromatography (UPLC): An,Introduction„, Separation Science Re-Defined, LCGC Supplement, May, pp. 8-13.

3. Van Deemter J J, Zuiderweg F J and Klinkenberg A (1956), Chem. Eng. Sci., Vol. 5, p. 271.

4. Lars Y and Honore H S (2003), J. Chromatogr., A, Vol. 1020, pp. 59Š67.

5. Swartz M E (2005), ƒUltra Performance Liquid Chromatography (UPLC): An,Introduction,, and review„, Journal of Liquid Chromatography€& Related Technologies, Vol. 28, pp. 1253Š1263.

6. Wu N, Lippert J A, Lee M L (2001), J. Chromatogr. A , Vol. 911, p. 1.

7. Lippert J A, Xin B, Wu N and Lee M L (1997), J. Microcol. Sepn., Vol. 1, p. 631.

8. Tolley L, Jorgenson J W and Mosely M A (2001), Anal. Chem., Vol. 73, pp. 2985.