Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

ACQUITY ULTRAPERFORMANCE LC BY DESIGN

Resolution, Speed, Sensitivity

Waters® ACQUITY UltraPerformance LC® Systems have been holistically designed with innovative technologies to dramatically improve resolution, speed, and sensitivity.

PROVEN INNOVATION, EXTRAORDINARY RESULTS

In 2004, separation science entered a new dimension in performance with the first successful harnessing of the separation power of sub-2 µm particle chemistries. This new realm of chromatography, known as UltraPerformance LC (UPLC®), delivered a new level of efficiency and productivity when compared to traditional HPLC techniques. Chromatographers now have the ability to work at these higher efficiencies with a much wider range of linear velocities, flow rates, and backpressures to obtain superior results.

UPLC technology has been adopted successfully in laboratories around the world for the most demanding separations and meets all the requirements for a highly robust, dependable, and reproducible system. Combining UPLC instrumentation, informatics, and chemistries with Waters MS technologies, you can gain high quality information faster, and make a meaningful impact on your business and scientific bottom line – making science possible.

PROVEN ADVANCEMENTS IN CHROMATOGRAPHIC SCIENCES

- Takes advantage of small, pressure-tolerant particles
- High-pressure fluidic modules (up to 15,000 psi)
- Minimized system volumes and optimized flow paths
- Column Manager for rapid column switching and faster method development
- Sample Manager with reduced cycle times and negligible carryover
- High-speed detectors (optical and mass)
- Integrated system software
  - Novel communication protocols
  - Advanced diagnostics

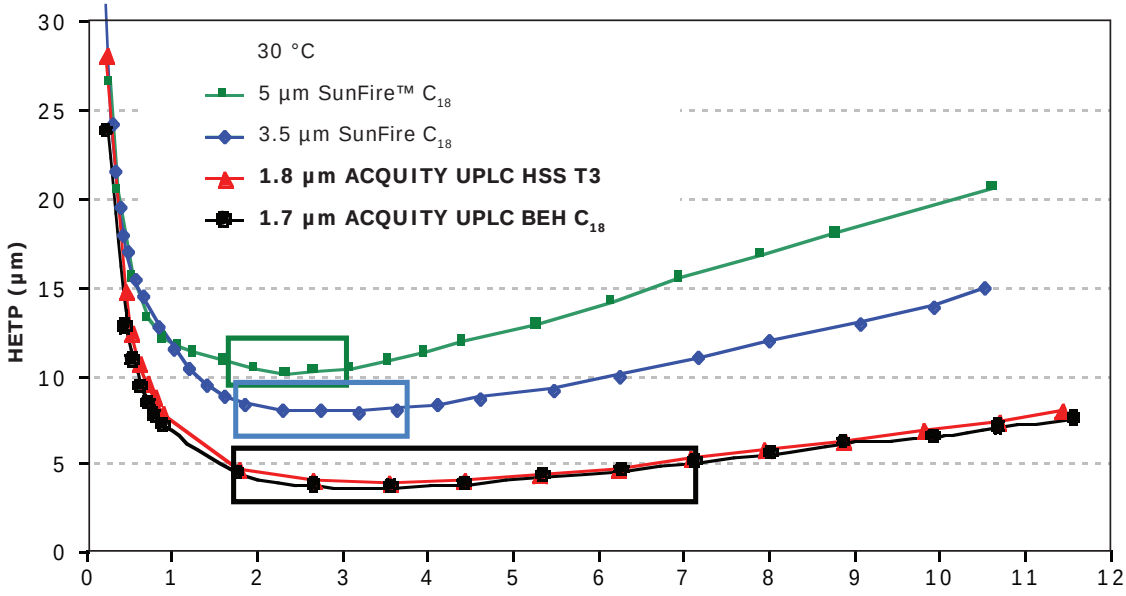
"The results showed that all of the major degradation products and impurities were separated by the UPLC approach but **in half the time required by the HPLC**. The UPLC method was also beneficial as it gave a **flatter baseline, sharper peaks, and very good retention time precision**. This meant that **less time was required to integrate the UPLC data** than the HPLC data as less manual intervention was needed."

WREN SA, TCHELITCHEFF P.
Use of ultra-performance liquid chromatography in pharmaceutical development.
J Chromatogr A.
2006 Jun 30;1119 (1-2):140-6.

THE THEORY AND SCIENCE BEHIND UPLC

Chromatographic theory states that increasing efficiency increases resolution. Reducing the stationary phase particle size has been exploited for more than 40 years as the driver for increasing efficiency. However, HPLC system designs have struggled to take advantage of the smallest particle sizes. Not only do small particles require systems to operate at backpressures above current limitations (>6000 psi/400 bar), but they also require low-dispersion fluidics that do not compromise performance. Waters ACQUITY UltraPerformance LC Systems have been designed to fully utilize all the advantages of small particles and begin where conventional HPLC systems fall short. With a holistic design approach to all the integral component parts – from sample introduction to data capture, with fast optical and MS detectors – Waters UPLC Systems encompass a design philosophy that has redefined separation sciences in today's marketplace.

van Deemter curves are used to determine the mobile phase flow rate where column efficiency will be maximized. As particle size decreases, the corresponding Height Equivalent to Theoretical Plate (HETP) also decreases, resulting in higher chromatographic efficiency. These higher efficiencies are available over a much wider range of flow rates than with larger particle sizes. As a result, the speed of the assay can be optimized without sacrificing resolution.



Flow Rate (mL/min)			Linear Velocity (µ, mm/sec)									
1.0 mm I.D.	0.04	0.07	**0.11**	**0.14**	**0.18**	**0.21**	**0.25**	**0.28**	**0.32**	**0.35**	**0.39**	**0.42**
2.1 mm I.D.	0.15	0.30	**0.45**	**0.60**	**0.75**	**0.90**	**1.05**	**1.20**	**1.35**	**1.50**	**1.65**	**1.80**
4.6 mm I.D.	0.70	1.40	2.10	2.80	3.50	4.20	4.90	5.60	6.30	7.00	7.70	8.40

"**Conversion from HPLC to UPLC methodology** alleviated the inherent sample and stability problems owing to the **four-fold reduction in analysis time**; the decreased sample analysis time consequently increases the productivity of the QC laboratory... **(with a) >93% decrease in solvent acquisition and disposal costs.**"

POBLOCKA K, GRANGER T, ROSS R, TAISHIDLER E.
Converting existing HPLC methods to UPLC: Problems, development and optimization.
Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, 58th Annual Meeting, Chicago, Illinois, February 25-March 2, 2007.

ULTRA RESOLUTION

Chromatographers are faced with the challenge of analyzing highly complex mixtures such as peptide digests, impurities and *in vivo* metabolism samples. UPLC technology provides the ideal solution to fully optimize analytic resolution.

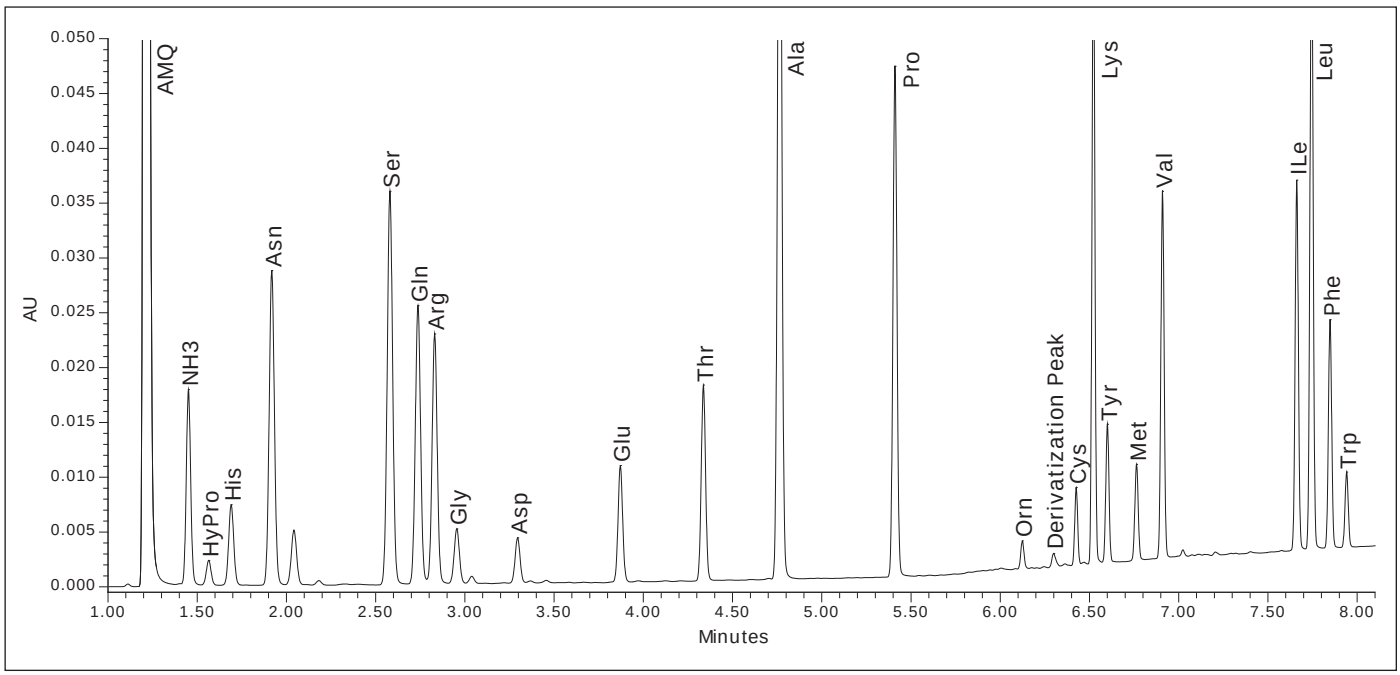
The ACQUITY UPLC® System takes full advantage of the efficiency gains offered by sub-2 µm particles. These particles provide 3X higher efficiency than 5 µm particles and 2X higher efficiency than 3.5 µm particles. And since resolution is proportional to the square root of the inverse of the particle size, sub-2 µm particles provide 70% higher resolution than 5 µm particles and 40% higher resolution than 3.5 µm particles. In gradient separations, the same types of performance gains are also achievable, where peak capacity is used to measure the resolving power.

The increased resolving power of the UPLC particles provides new levels of information about your sample, and also minimizes the amount of time required to develop methods.

According to the resolution equation for isocratic liquid chromatography separations, resolution is proportional to the square root of column efficiency:

$$Rs = \sqrt{\frac{N}{4}} \left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

According to van Deemter chromatographic theory, column efficiency is inversely proportional to particle size: $N \propto \frac{1}{dp}$



Analysis of cell culture media. The analysis of commercial amino acids exemplifies how an application that requires superior resolution can now be combined with speed and sensitivity to deliver a practical solution to lengthy run times and frequent repeat analyses. Even "Fast HPLC" methods cannot guarantee an effective separation such that good business decisions can be made quickly.

"A series of β-blockers was analyzed using both HPLC/MS and UPLC/MS using mobile phases containing acetonitrile and TFA and either H_2O or D_2O. The use of UPLC gave both **faster analysis and higher peak capacity**. The spectra obtained from UPLC/MS were as good as those from HPLC/MS and showed **better signal/noise** performance with the D_2O containing mobile phases."

WREN SA, TCHELITCHEFF P.
UPLC/MS for the identification of beta-blockers.
J Pharm Biomed Anal. 2006 Feb 24;40(3):571-80.

Smaller particles enable extraordinary increases in analysis speed without sacrificing resolution. As particle size is decreased, column length can also be reduced proportionally to keep efficiency constant:

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

Moreover, van Deemter theory states that optimum flow rate is inversely proportional to particle size:

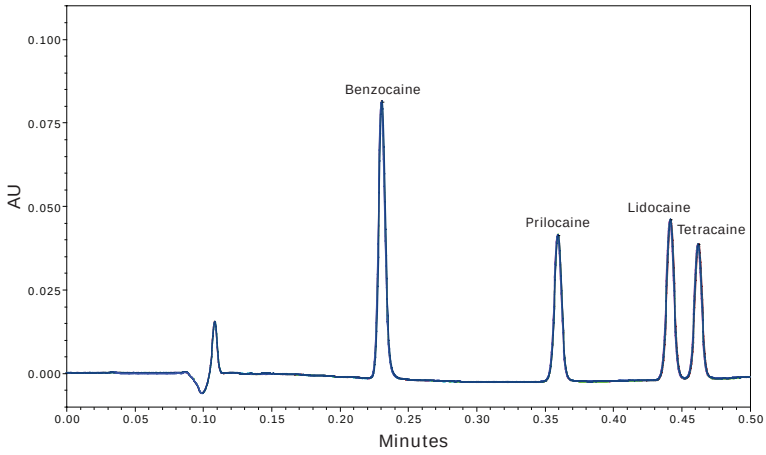
$$Flow\ Rate \propto \frac{1}{dp}$$

ULTRA SPEED

High throughput laboratories have a constant need to generate more information and process more samples per unit time, while maintaining the quality of the data generated.

When transitioning from 5 µm particles to sub-2 µm particles, column length can be reduced by a factor of three (vs. 5 µm particles), while maintaining the same efficiency, and the separation can be run at a 3X higher flow rate. This translates to a 9X increase in throughput with no loss in resolution.

Anesthetics
Av. RT %RSD = 0.103%
Max. Pressure ~ 11,600 psi



Anesthetics method

System:	ACQUITY UPLC System with PDA
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Sample:	Anesthetics (10 µg/mL in 80/20 water/methanol) **6 Replicates**
Injection volume:	2 µL
Temperature:	50 °C
Flow rate:	**1.3 mL/min**
Mobile phase A:	10 mM ammonium bicarbonate pH 10.0
Mobile phase B:	acetonitrile
Gradient:	35 to 70% B in 30 seconds
Detection:	225 nm, 40 Hz, fast
Backpressure:	**11,600 psi**

Injection Set	Caffeine RT %RSD	Acetanilide RT %RSD	2-Acetomidophenol RT %RSD
1 of 6	0.034	0.038	0.028
2 of 6	0.046	0.050	0.035
3 of 6	0.176	0.207	0.155
4 of 6	0.066	0.065	0.048
5 of 6	0.015	0.016	0.020
6 of 6	0.063	0.069	0.045
All Injections	0.087	0.103	0.075

ACQUITY UPLC gradient performance is illustrated by this high throughput analysis. Retention time reproducibility is 0.103% RSD or better for a 1.2-minute total separation time under UPLC operating conditions.

"A **rapid and reliable UPLC method was developed and validated** for the analysis of busulfan in the plasma of patients undergoing bone marrow transplantation... The transition to UPLC from HPLC has **increased throughput five-fold allowing us to treat more patients.**"

XU QA, THAPAR J, THAPAR N, MADDEN TL.
Development and validation of a UPLC method for analysis of busulfan in cancer patient plasma samples.
Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, 58th Annual Meeting, Chicago, Illinois, February 25 - March 2, 2007.

ULTRA SENSITIVITY

The pursuit of increased sensitivity has been mainly focused on the detector, either optical or mass spectrometric. This has been driven by the need to detect compounds at increasingly lower levels (e.g. highly potent pharmaceuticals). Significant gains can be made by employing UPLC technology.

The ACQUITY UPLC System using sub-2 µm particles provides up to 70% higher sensitivity than 5 µm particles and 40% higher sensitivity than 3.5 µm particles. In the case of equal efficiency but faster analysis times, this provides 3X higher sensitivity than 5 µm particles and 2X higher sensitivity than 3.5 µm particles. When productivity is a priority – UPLC delivers.

With the ACQUITY UPLC System, you always achieve more sensitivity. In the case where UPLC is used to generate higher efficiency, peak widths become narrower according to the following equation:

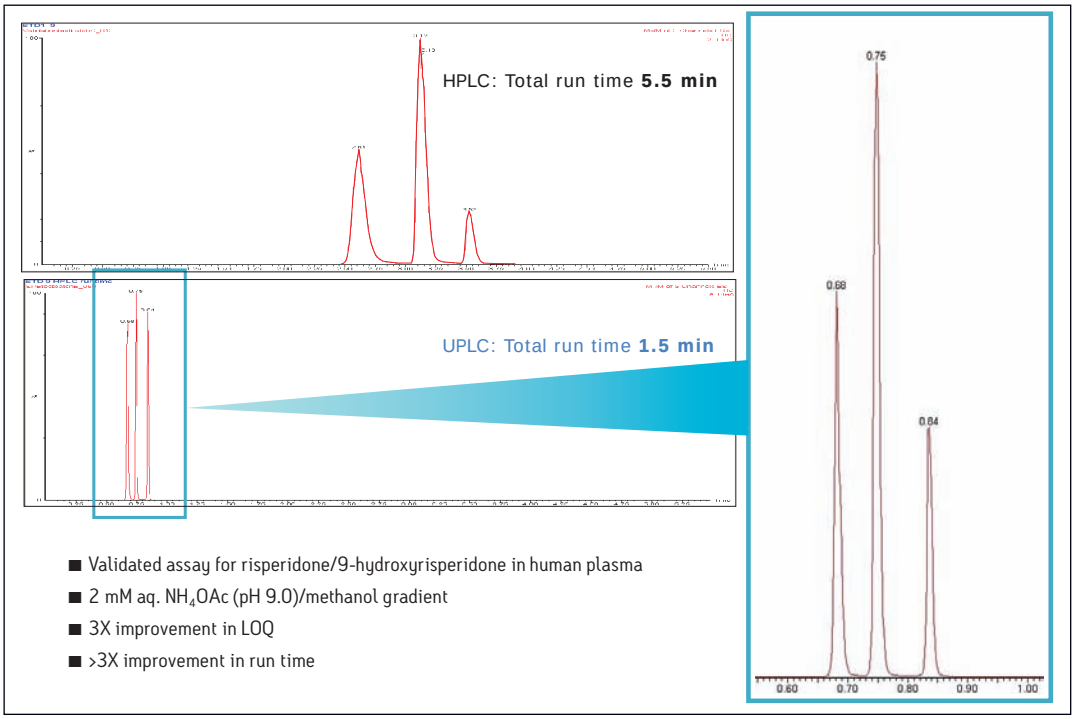
$$N \propto \frac{L}{w^2}$$

And since the peaks become narrower, they also become taller:

$$Peak\ Height \propto \frac{1}{w}$$

Similarly, in the case where UPLC is used to provide the same efficiency with a faster analysis time by using shorter columns, peak height increases according to the following equation:

$$Peak\ Height \propto \frac{1}{L}$$



- Validated assay for risperidone/9-hydroxyrisperidone in human plasma
- 2 mM aq. NH_4OAc (pH 9.0)/methanol gradient
- 3X improvement in LOQ
- >3X improvement in run time

This comparison between an HPLC/MS/MS method and a UPLC/MS/MS method for risperidone/9-hydroxyrisperidone in human plasma demonstrates the advantages of UPLC/MS/MS for bioanalysis. The ACQUITY UPLC System with the Quattro Premier™ XE Mass Spectrometer produced a method with a 1.5 minute run time – more than three times faster than HPLC/MS/MS, with a three-fold improvement in the LOQ.

"For the first time, a semi-automated sample preparation method in 96-well plate format for the determination of EE and NE/LN concentrations in human plasma was **developed and validated over the range of 0.01-2 and 0.1-20 ng/mL**, respectively. UPLC and small particle size columns have **greatly increased sample analysis speed and assay selectivity over conventional HPLC**, proving very useful for the OC methods. The speed, robustness, sensitivity, and selectivity of the method make it suitable for high-throughput quantitative analysis of EE and NE/LN in drug-drug interaction clinical studies."

LICEA-PEREZ H, WANG S, BOWEN CL, YANG E.

A semi-automated 96-well plate method for the simultaneous determination of oral contraceptives concentrations in human plasma using ultra performance liquid chromatography coupled with tandem mass spectrometry.
J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Jun 1; 852 (1-2):69-76.


SAMPLE ORGANIZER
Sample Organizer
Acquity
TUV Detector
Acquity
Waters
Column Heater/Cooler
Acquity
Waters
Sample Manager
Acquity
Waters
Binary Solvent Manager
Acquity
Waters
DETECTOR OPTIONS
ELS, FLR, PDA, TUV, SQD, AND TQD
COLUMN MANAGER OPTIONS
COLUMN HEATER, COLUMN HEATER/COOLER, COLUMN MANAGER
SAMPLE MANAGER
BINARY SOLVENT MANAGER

BINARY SOLVENT MANAGER

Perfecting solvent delivery in UPLC

Achieving high peak capacity separations using small particles requires a greater pressure capability than the typical operating range, 2000-4000 psi (135-270 bar), of HPLC instrumentation. Waters ACQUITY UPLC Systems are designed with an upper pressure limit of 15,000 psi (1034 bar), and routinely operate at 8000-12,000 psi (535-800 bar) to effectively deliver optimal resolution sensitivity and speed.

A solvent delivery system that delivers eluents at these pressures must also manage solvent compressibility, especially with multi-solvent and gradient separation conditions. In the ACQUITY UPLC System, solvent compressibility compensation is both automatic and continuous, ensuring smooth and reproducible flow.

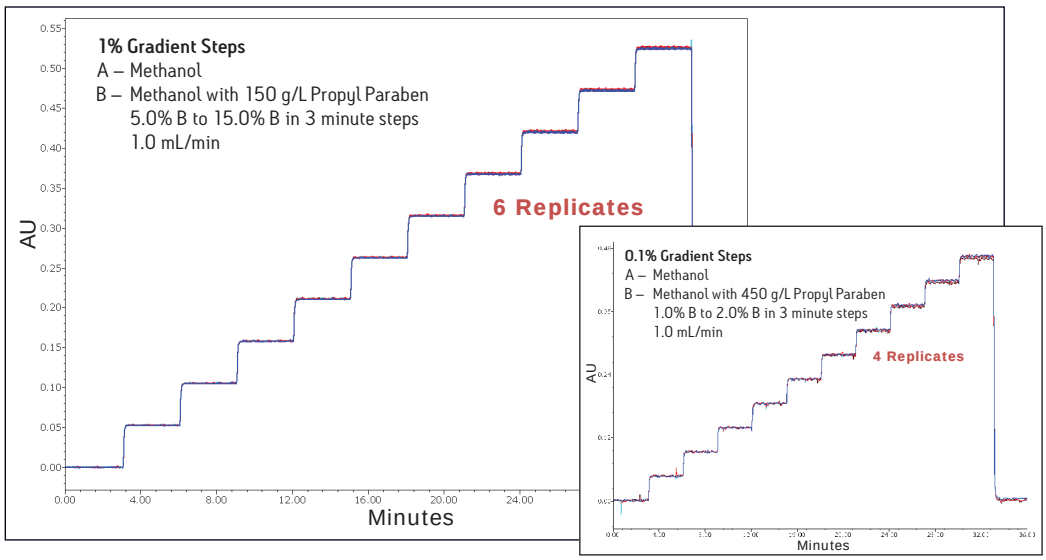
And finally the solvent delivery system must meet the solvent blending needs of UPLC methods, which require minimum delay volumes for optimal utilization of 1 mm-2 mm I.D. ACQUITY UPLC columns, particularly for direct interface with mass spectrometers or for high throughput applications. Efficient solvent mixing is a prerequisite for gradient elution precision, and to facilitate the best performance from LC detectors.

ACQUITY UPLC Binary Solvent Manager addresses the unique challenges of UPLC

- **Advanced Solvent Management**
  Each solvent delivery module incorporates independently-driven pistons, serial flow, and automatic, continuous compressibility compensation.
- **Binary Solvent Capability**
  Two solvent delivery modules operate in parallel to blend two solvents in <120 µL system volume. Each module is equipped with an automated solvent select valve to extend capability to two pairs of solvents.
- **Leak Sensor**
  For reliable unattended operation.
- **In-line Vacuum Degassing**
  PerformancePLUS™ degasser removes dissolved gasses from up to four eluents, plus the two wash solvents used in the ACQUITY UPLC Sample Manager.
- **UPLC Pressure Capabilities**
  Take full advantage of UPLC potential by routinely operating at pressures approaching 15,000 psi (1000 bar).
- **Intelligent Intake Valve (I²Valve)**
  Synchronized valve performance allows for faster system priming and startup times.

Integrating serial and parallel flow

The Binary Solvent Manager utilizes two serial flow solvent delivery modules operating in parallel to enable accurate and precise solvent delivery for pulse-free linear and non-linear gradient profiles. This Solvent Manager also automatically compensates for mixing any viscosity solvent without user intervention.



ACQUITY UPLC Binary Solvent Manager provides unmatched compositional accuracy, even at the low percentage gradient endpoints. The flow delivery performance, gradient profile selection, low internal volume, and advanced software control algorithms enable high performance gradient applications.

SAMPLE MANAGER

Innovative sample management

UPLC sample management is a multifaceted process characterized by fast injection cycles, precise injection volumes, minimal carryover, and temperature control, which together contribute to the speed and sensitivity of this new technology.

Every aspect of the ACQUITY UPLC Sample Manager – sample transport mechanisms, sample temperature control, and sample injection fluidics – is designed to optimize the processing of any two industry-standard microtiter plates or vial holders. Unique pressure-assisted injection technology improves low-volume injection performance with faster injection cycle times, and superior accuracy, while sample carryover is minimized with fully programmable, multi-solvent washing protocols.

ACQUITY UPLC Sample Manager addresses the unique challenges of UPLC with:

- **Flexible Sample Compartment**
  Use two microtiter plates or vial holders in any combination. The ACQUITY UPLC Sample Manager with the addition of the optional Sample Organizer extends sample capacity to: standard microtiter plates, 14 intermediate plates and vial holders, or 8 deep-well plates or vial holders. Any combination can be accessed randomly for transport into the sample injection compartment for processing, or easily accessed by the operator at any time.
- **Controlled Sample Environment**
  Sample temperature is regulated over a range of 4 to 40 °C for sample stability. User-controlled compartment illumination lets you monitor injector needle and sample position.
- **Needle-In-Needle Sample Probe**
  Unique design enables routine injection from mat-covered microtiter plates. Inject 1 µL out of 4 µL for sample-limited applications.
- **Multi-Injection Mode**
  Facilitates fast injection cycles and superior sample injection accuracy for 0.1-50 µL injection volumes.
- **eCord™ Information Management Technology**
  Records lifetime column history and is accessible through the ACQUITY UPLC System instrument console.
- **Leak Sensor**
  For reliable unattended operation.

COLUMN MANAGEMENT

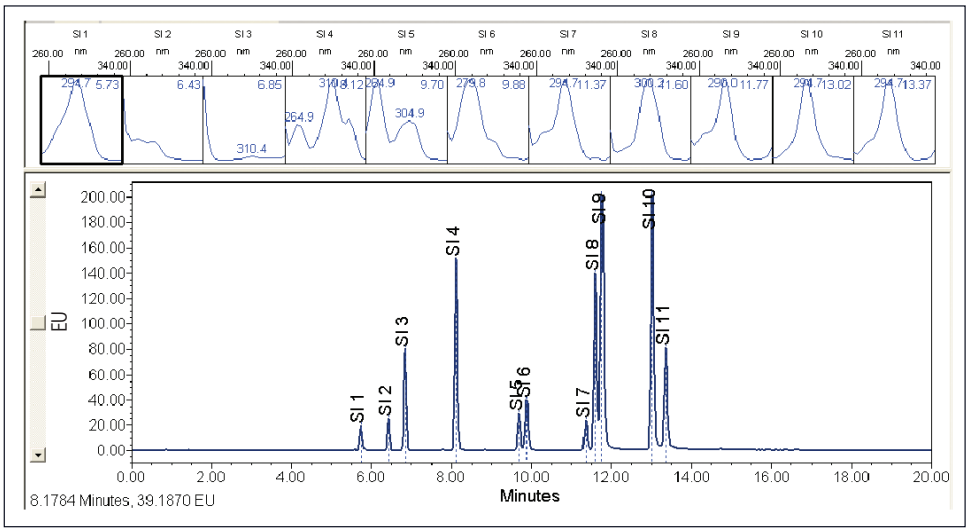
Delivering accurate and efficient column temperature management and control is very important in UPLC applications. The ACQUITY UPLC System offers four types of Column Managers or Column Heater/Cooler combinations depending on the needs of your lab.

Description	Temperature range	Application type	Number of columns	Min I.D.	Max I.D.	Max length
ACQUITY UPLC Column Heater	5 °C above ambient to 90 °C	Routine applications	1	1.0	4.6	150 mm
ACQUITY UPLC Column Heater/Cooler	10 to 90 °C	High efficiency, low dispersion applications	Up to 4 Serial	1.0	4.6	150 mm
ACQUITY UPLC Column Manager	10 to 90 °C	Methods development/ high throughput	Up to 4 Parallel	2.1	4.6	150 mm
ACQUITY UPLC 30 cm Column Heater/Cooler	4 to 65 °C	Support of HPLC columns	Multiple HPLC	2.1	7.8	300 mm

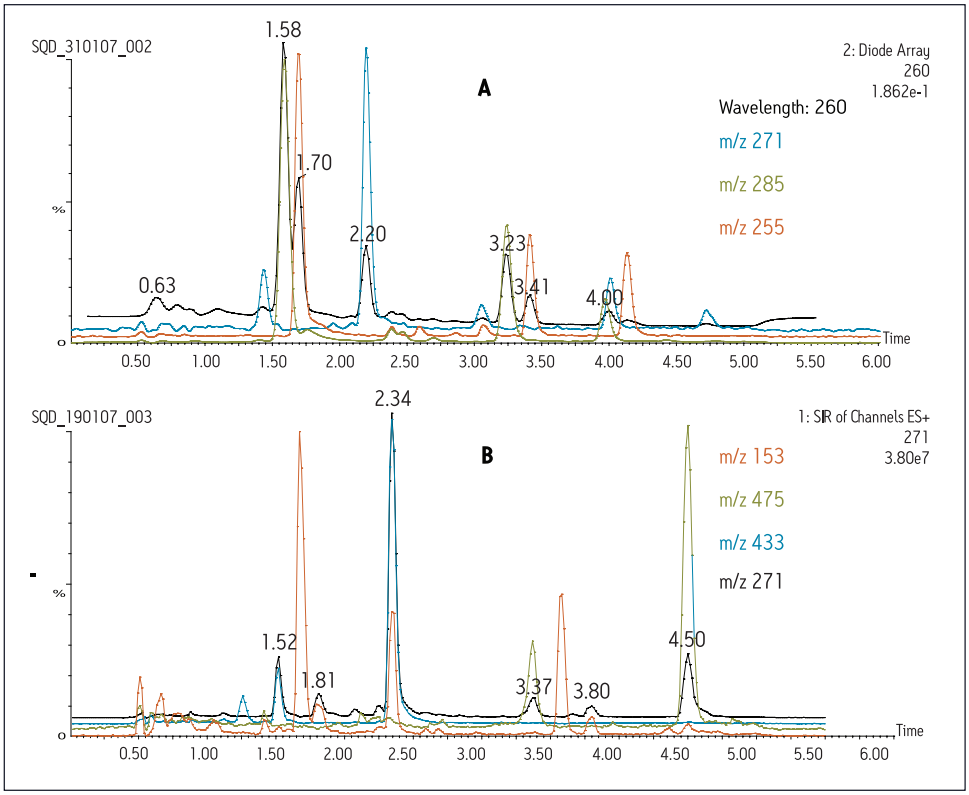
UltraPerformance detection

UPLC systems can generate peak widths as low as 1 second at half-height. This poses challenges for peak detection. A UPLC-compatible detector must have a high sampling rate to allow reproducible integration of the analyte peaks. The detector cell must also have minimal dispersion to preserve the efficiency of the separation. The ACQUITY UPLC detection family includes Fluorescence (FLR), Evaporative Light Scattering (ELS), Photodiode Array (PDA), Tunable UV-Visible (TUV), and single quadrupole (SQ) detectors that have been designed specifically to complement and maintain the significant advantages gained by the use of the ACQUITY UPLC fluidic technology.

The technologies enable high sensitivity detection, while maintaining the ability to acquire high quality spectral data. Novel detector design effectively manages the narrow peaks associated with rapid and/or high peak capacity separations.



For samples with many components, like Polynuclear Aromatic Hydrocarbons (PAHs), ACQUITY UPLC FLR Detector's 3D scan feature easily determines optimal excitation and emission wavelengths.

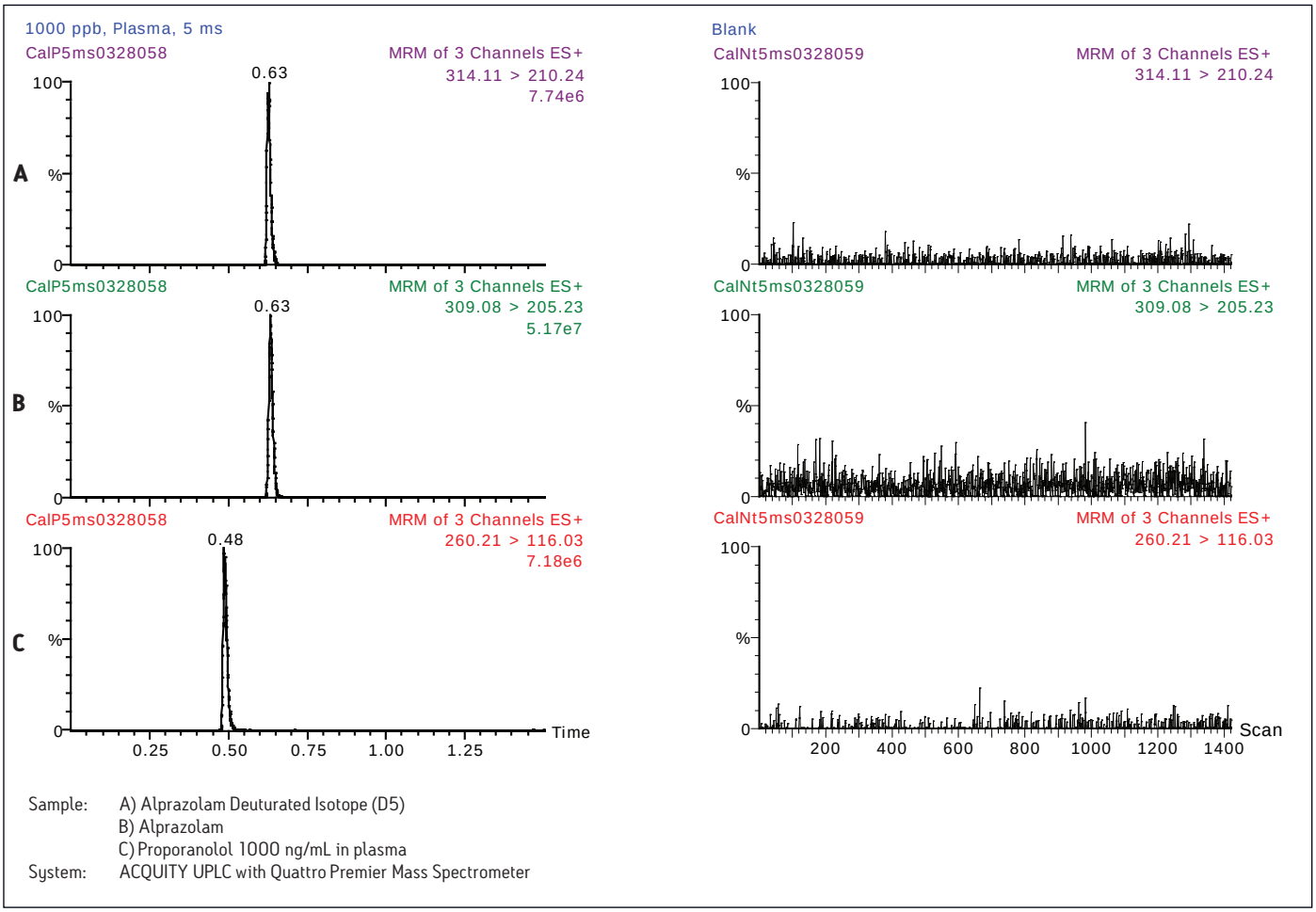


A.) Selected wavelength of 260 nm and full scan UPLC/PDA/MS data with m/z ions for daidzein, genistein, and glycitein, extracted from the TIC and B.) SIR method and the repeions for genistein in the soy supplement.

MINIMAL UPLC CARRYOVER – MS COMPATIBILITY

The ACQUITY UPLC System's sample-handling architecture is designed to ensure exceptionally low carryover and reduced cycle times. And when interfaced with the temperature-controlled Sample Organizer, it increases unattended sample capacity by up to 10 times.

These low carryover characteristics combined with the optimal low flow attributes of UPLC separations take full advantage of both optical detection and electrospray ionization interfaces for UPLC and UPLC/MS applications.



Sample: A) Alprazolam Deuturated Isotope (D5)
B) Alprazolam
C) Proporanolol 1000 ng/mL in plasma

System: ACQUITY UPLC with Quattro Premier Mass Spectrometer

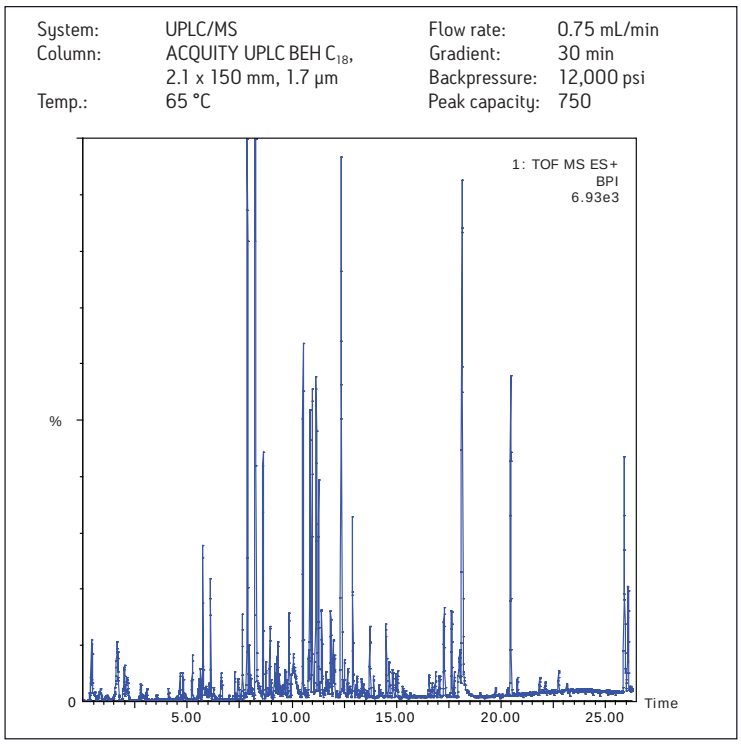
The carryover performance of the ACQUITY UPLC Sample Manager is demonstrated by analysis of plasma standards (left), followed by an injection blank analysis (right). The analytes are monitored by MRM analysis with an assay blank of 0.1 ng/mL.

Extend MS performance

Mass spectrometric detection significantly benefits from the performance characteristics of UPLC. Increased peak concentrations with reduced chromatographic dispersion at lower flow rates promote increased source efficiencies, resulting in at least 3X improvements in sensitivity and improved spectral quality.

ACQUITY UPLC's narrow peaks necessitated an array of complementary, easy-to-use MS detection innovations, from single quad mass detectors to advanced tandem and time-of-flight mass spectrometers (TOF MS), that take full advantage of UPLC performance and throughput facilitating:

- Rapid collection of data points for accurate peak integration
- Polarity switching for acid/base detection
- Multi-mode ionization for detection of a wide range of compounds
- Simplified structural characterization with exact mass

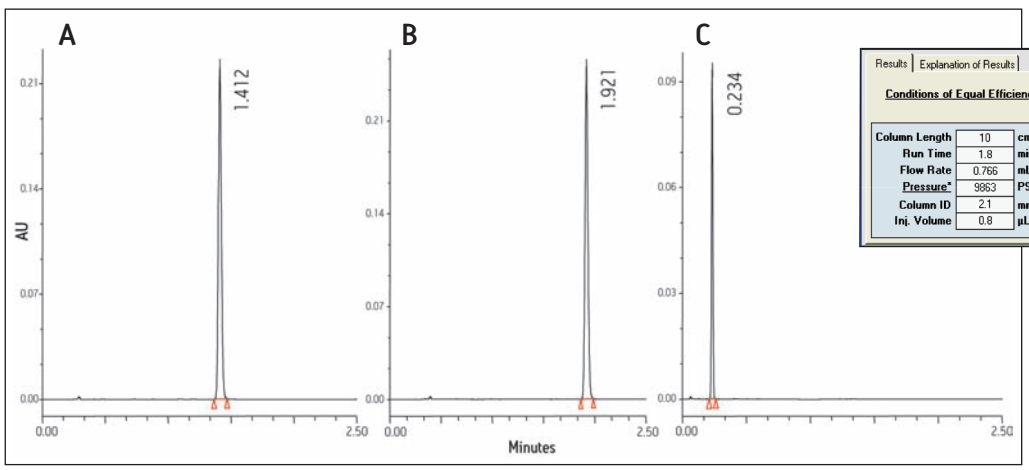


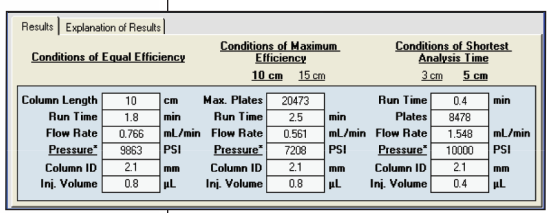
This example shows the natural products analysis of ginseng extract using UPLC/oa-TOF MS with the ACQUITY UPLC System and the LCT Premier™ XE Mass Spectrometer for the ultimate in high resolution exact mass performance.

Effortlessly move your methods to UltraPerformance LC technology and help increase your lab's productivity

Taking advantage of the latest analytical technologies such as UPLC can be a critical element in increasing laboratory productivity and giving your business a competitive advantage. Waters provides you with the tools to move seamlessly and rapidly from HPLC to UPLC, so you can quickly capture its benefits – from both a process and financial point of view.

The ACQUITY UPLC console calculator facilitates the rapid transfer of an HPLC method to UPLC condition. The calculator allows users to input the current HPLC conditions, including column length, mobile phase, and flow rate; and then to optimize the UPLC separation that best suits the analytical column needed.



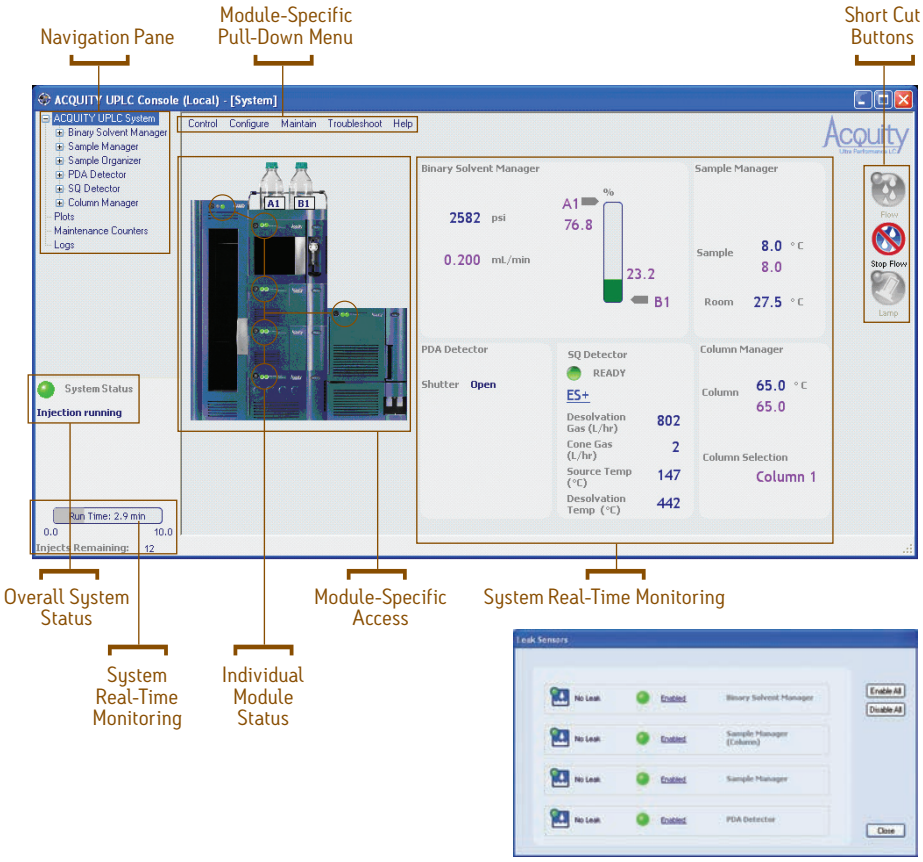


ACQUITY UPLC console calculator.

The original HPLC separation of simvastatin gave a retention time of 9.28 minutes. When the HPLC method was scaled to UPLC by using the ACQUITY UPLC console calculator three results were generated: A.) the Equal Efficiency conditions, B.) the Maximum Efficiency conditions and C.) the Shortest Analysis Time conditions.

Instrument console: control panel

ACQUITY UPLC Systems are equipped with a customizable instrument console that enables operators to stay in control. All instrument functionality – instrument control, interactive system monitoring, status monitoring, and user diagnostic capabilities, including features for unattended operation such as leak sensors – are accessible through this common interface software. The instrument console has the same functionality and appearance when used with either Empower™, MassLynx™, or third-party data systems.



connections Insight®

Connections INSIGHT® remote, intelligent services provide secure, web-based system monitoring, instant alert notification and a direct link to Waters technical experts. These services help you maximize ACQUITY UPLC System uptime, increase laboratory productivity and allows Waters to continually monitor your system's performance to anticipate potential performance issues.

In the event system issues occur, the service automatically alerts Waters and provides information for analysis. This can be done by granting Waters temporary system access for real-time problem resolution.

Connections INSIGHT remote, intelligent services also provide monthly performance status reports that summarize overall system operation and use of consumables, enabling you to better manage your laboratory resources.

For more information on the ACQUITY UPLC System, call your local Waters Sales Representative or visit **www.waters.com/uplc**.

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August 2007 720000880EN LB-AC

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