Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Purification Systems

Compound purification and isolation can be more efficient – and more productive

With Waters systems, purify using multiple detection choices:

- Ultraviolet/Visible (UV/Vis) Absorbance
- Evaporative Light Scattering (ELS)
- Photodiode Array (PDA)
- Mass Spectrometry (MS)
- Analog

Manage sample injection and collection:

- Manual Injection
- Automated Injection
- Automated Collection
- Automated Injection and Collection

Choose the pump that suits your need:

- Low Pressure Mixing
- High Pressure Mixing

CUSTOMIZED SYSTEMS TO MEET YOUR LAB'S NEEDS

Waters, the leader in preparative chromatography, offers scalable systems to meet your isolation and purification requirements. With a combination of integrated high-performance instrumentation, robust chemistry, easy-to-use software, and world-renowned support services, Waters® Purification Systems reliably isolate and purify microgram to multigram quantities of your compounds of interest. Whether you run a few samples a day or several hundred, your lab will operate with a level of confidence and productivity that multi-vendor systems cannot match.

With Waters purification systems your lab is ready for tomorrow's analyses.

- Robust and reliable systems improve your ability to manage ever-increasing workload demands
- Flexible configurations enable easy scale-up from analytical to preparative chromatography
- Dependable instruments allow for confident, unattended operation
- Easy-to-upgrade systems bring the latest technologies to your labs

Waters AutoPurification™ System with the 2489 UV/Vis Detector.

VERSATILE PURIFICATION AND ISOLATION SOLUTIONS

Purification is used to isolate compounds for drug discovery experiments, to isolate unknown impurities or metabolites, and to purify standards, natural products, and biomolecules. Criteria for performing purification, such as experimental conditions and column and instrument requirements, can vary greatly due to both the number of samples and the amount required. Waters takes complexity out of purification with a versatile solution that is capable of purifying milligrams to multiple grams, in a single system that can be configured to automatically process hundreds of samples. You benefit from high purity and recovery of desired compounds – with minimal system intervention.

Easy-to-use systems

Whether your company manages preparative chromatography in a centralized lab or provides purification capabilities in an Open Access environment, Waters' easy-to-use systems and software allow samples to be run with unattended system operation. This improves productivity by enabling chemists to focus on their results rather than on the instrument.

Waters systems and software make purification easier to manage:

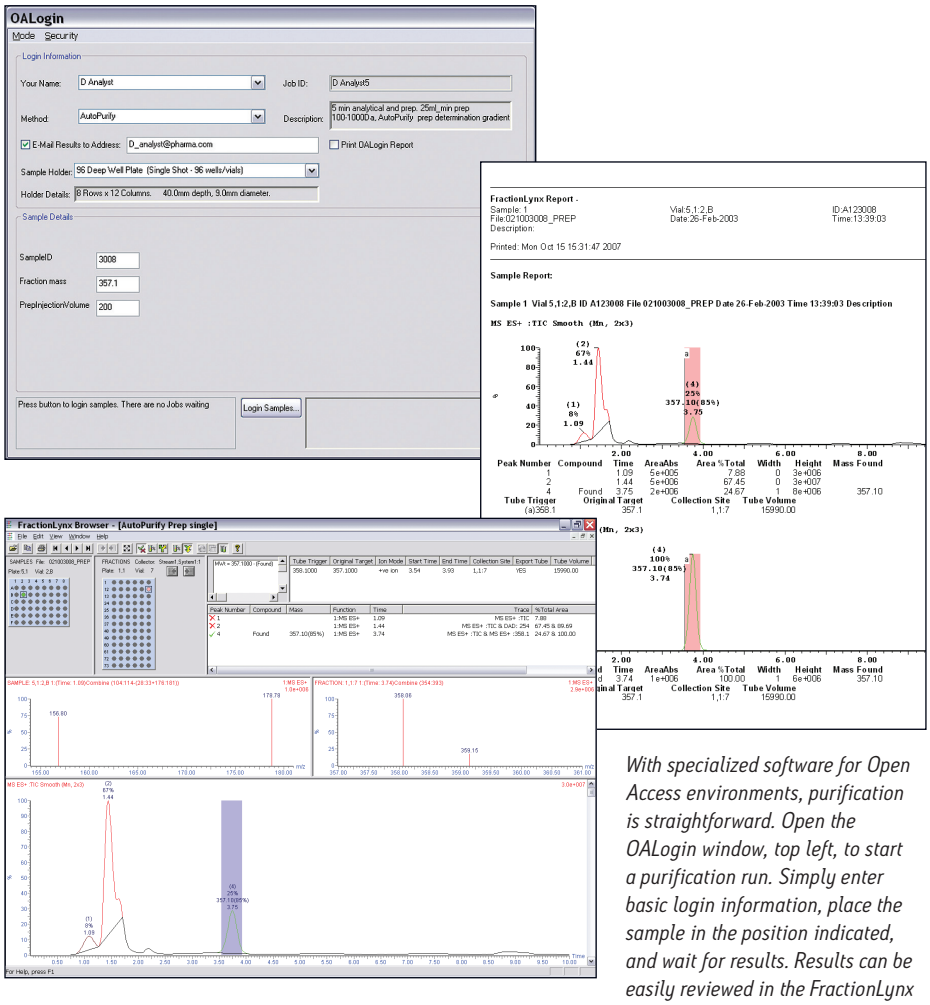
- With Open Access software, chemists can walk up to a system, enter their sample, and have results delivered to their desktop, while an administrator monitors the system remotely
- Fraction collection software provides full-cycle automation, from evaluation and setting collection thresholds, to purification, to fraction analysis
- Solvent monitoring system features tiered responses to instrument status, such as email notification, with intelligent shut-down available
- Remote system monitor tracks the sample queue, instrument, and solvent and waste container status online
- Unique method filtering software enables the user to quickly choose a method from the existing library of methods

Walk-up, walk-away purification runs

Waters Open Access software enables novice to experienced analysts to utilize the full potential of the purification system – with minimal training. Chemists can literally walk up to the instrument, submit samples through an easy-to-follow wizard or a single page login, walk away, and retrieve results online at their convenience.

Results can be automatically emailed or printed in a customized format, or saved as a PDF, and data can be transferred to remote computers.

System management is just as simple. Online, the central administrator can remotely define system users and their privileges for operating instruments across the network.



With specialized software for Open Access environments, purification is straightforward. Open the OALogin window, top left, to start a purification run. Simply enter basic login information, place the sample in the position indicated, and wait for results. Results can be easily reviewed in the FractionLynx browser, bottom, and then printed in a simple report format, top right.

Automated fraction collection

FractionLynx™ Application Manager automates the collection of detected fractions, tracks samples and fractions, and then presents the data in an easy-to-view format. The software can trigger collection using a variety of detector signals including UV/Visible, evaporative light scattering (ELS), MS, and analog.

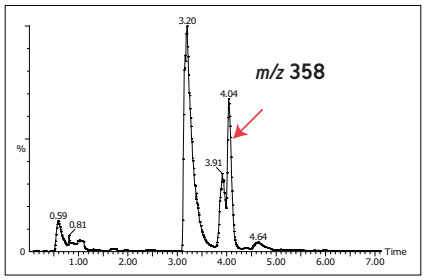
AutoPurify,™ a feature of the FractionLynx Application Manager, further automates this process, from initial analytical screening, through purification, to the analysis of the collected fractions. With AutoPurify, the software automatically selects a focused preparative gradient based on an analytical screening run, which provides better quality purification and eliminates the need for expert manual invention. Automatic determination of the collection threshold on a per-sample basis removes the interference from drifting baselines or high background signals, and increases ease-of-use. Fractions can be re-injected automatically if required, and information exported.

FractionLynx also provides a variety of fraction collection options:

- One-to-one mapping to retain plate format
- Secondary collection of peaks that are not of primary concern, but that are not classified as waste
- Waste collection

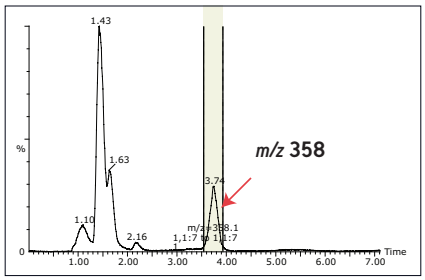
Identification

TIC chromatogram of the analytical-scale analysis of the crude compound indicates partial separation between the compounds.



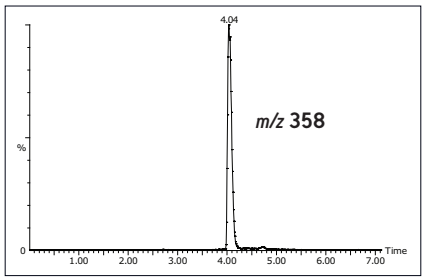
Isolation, with fraction collection highlighted

TIC chromatogram after purification. Successful separation was achieved using a focused gradient and fraction collection indicated by the shaded area.



Confirmation

TIC chromatogram of the analysis of the collection fraction indicates a successful purification.



AutoPurify enables you to analytically evaluate your sample. The software detects the presence of the target compound and measures its purity to determine the purification process.

Build a preparative chromatography system to match your needs, workload, and budget

Choose from a variety of high performance components to configure a preparative chromatography system that's right for your lab. Whether your application requires low or high pressure mixing, low or high flow rates, manual or automated injection and collection, you choose the exact level of functionality and capacity that your application requires. And since Waters Purification Systems are upgradable, you don't have to worry about your investment as your workload increases.

Sample Load	Column ID	Fluid Handling Unit	Max Flow Rate
mg -10s mg	3.9 - 19 mm	1525 Binary HPLC Pump* and EF Kit	22.5 mL/min
mg - g	4.6 - 30 mm	2535 Quaternary Gradient Module	50 mL/min
mg - g	4.6 - 50 mm	2545 Binary Gradient Module*	150 mL/min
mg - g	4.6 - 50 mm	2545 Quaternary Gradient Module	150 mL/min
mg - 10s g	7.8 - 75 mm	2555 Quaternary Gradient Module	300 mL/min

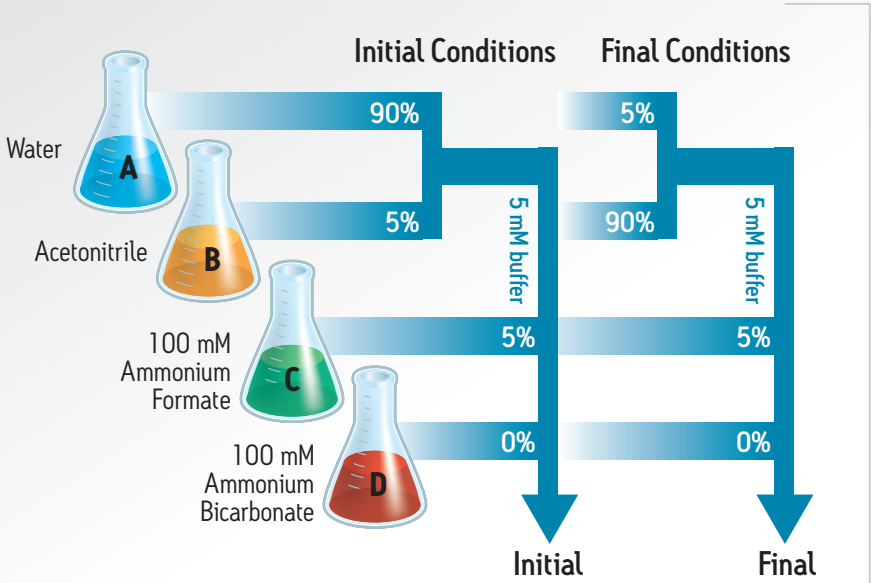
*High pressure mixing

Choose a solvent delivery system based on the amount of material you will be loading on the column and the size of the column.

Low-pressure mixing systems such as the Waters 2545 Quaternary Gradient Module, shown here, with the 2489 UV/Vis Detector and the Fraction Collector, allow you to purify a few samples a day.

Automated solvent management

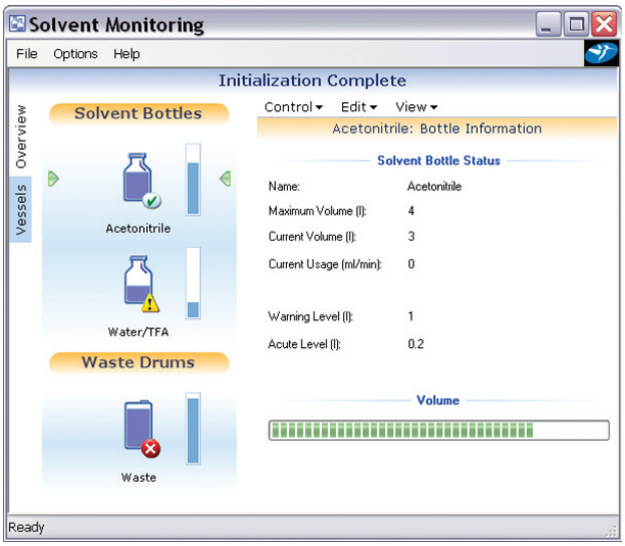
Auto•Blend™ Technology enables you to generate gradients automatically without having to manually alter the buffer concentration. Auto•Blend programming technology improves uptime since no pre-mixing of mobile phases is required. You can build both column cleanup and system flushing into your methods. In addition to method development, Auto•Blend simplifies maintenance by offering straightforward eluent management, increasing the flexibility and efficiency of your laboratory.



Auto•Blend Technology improves uptime by mixing solvents according to your method requirements.

Solvent monitoring

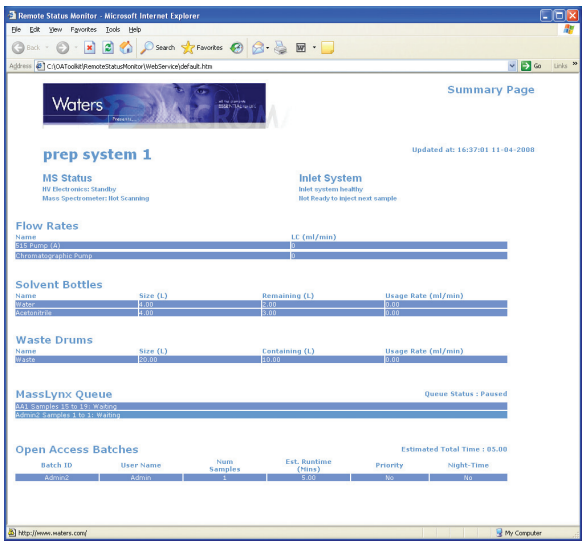
Waters Purification Systems use multiple solvent reservoirs. Each solvent reservoir and waste container is uniquely identified in the solvent monitor console. Email notification when a warning level has been reached is available.



Each solvent reservoir and waste container has its own status level shown in symbol format: OK= check mark, Warning level= exclamation point, and Acute level = cross mark. Each solvent reservoir and waste container is labelled as well as the initial and acute warning level parameters are entered by the user in the vessel status page. The current volume of solvent in the Acetonitrile reservoir is 3 L, with an initial warning level of 1 L and an acute level of 0.25 L.

Remote status

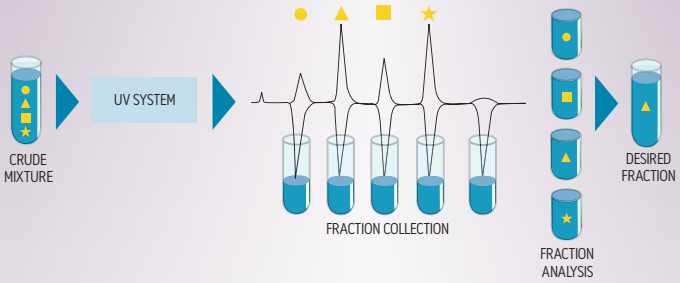
The remote status monitor tracks the sample queue, instrument, and solvent status from any PC on the network. This allows for greater efficiency as the system user does not have to return to the instrument to check on its status.



The web-based remote status monitor provides access to the instrument status information remotely.

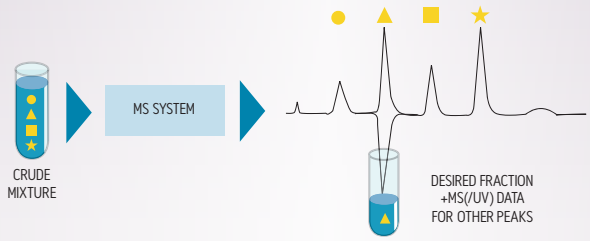
UV SYSTEMS

When fraction collection is based on UV absorbance, multiple fractions may result, requiring further analysis to determine the fraction of interest.



MS SYSTEMS

When fraction collection is triggered by mass detection, fewer fractions result since you collect only the peak containing the mass of interest.



Run samples around the clock with unprecedented versatility and consistency. Add the System Fluidics Organizer (SFO) to easily manage column selection and column regeneration.

- 2767 Sample Manager
- 2545 Binary Gradient Module
- System Fluidics Organizer
- 2489 UV/Vis Detector
- MassLynx™ Software

The Waters AutoPurification System, built around the SQ Detector 2, a compact, single quadrupole, atmospheric pressure ionization mass detector, is designed for everyday mass-directed purification applications. Switch up to five columns (three analytical- and two preparative-scale) with a single command. The system's fluidics layout, enhanced software features, and hardware capabilities combine with Waters' proprietary Optimum Bed Density (OBD™) prep column technology* for simplified operation.

- 2767 Sample Manager
- 2545 Binary Gradient Module
- System Fluidics Organizer
- 2998 Photodiode Array Detector
- 2424 Evaporative Light Scattering Detector
- SQ Detector 2
- MassLynx Software

"Implementing MS-directed purification has enabled our chemists to double throughput – over and above what they had been able to produce with traditional purification techniques."

Lab manager, global pharmaceutical company

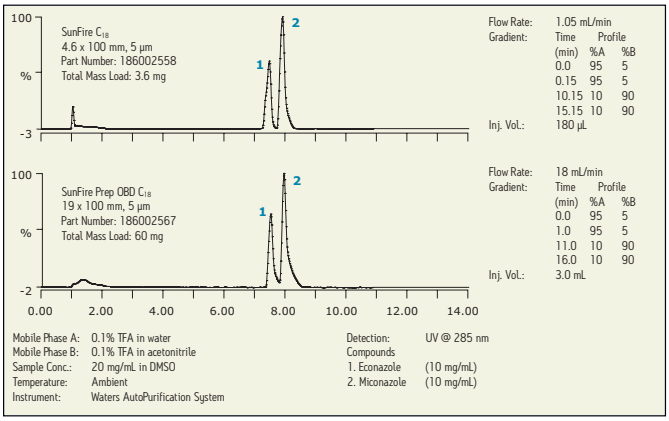
**UK patent # GB2408469*

EFFICIENCY AND SCALABILITY

The isolation and purification of critical compound pairs is a major challenge to purification scientists. Often a successful analytical separation will not scale up directly due to the reduced performance of the preparative column.

The OBD Column design, in combination with the efficient SunFire™ Prep OBD Column particles, ensures equivalent chromatographic performance from analytical to preparative dimensions, eliminating the need for any subsequent time-consuming method redevelopment.

Scale-up of the separation of two antifungal critical pairs on SunFire Columns



Purification chemists require the flexibility to achieve the best combination of particle size and column dimension to easily purify even the most complex of samples. XBridge™ Prep OBD Columns provide the flexibility required for fast purification method development. Wide pH operating range and high loadability have ensured that XBridge Columns provide reliable solutions to the most challenging purification problems.

The combination of the rugged XBridge packings and the OBD design takes preparative column performance to a new level, ensuring direct scalability, maximum efficiencies, and the longest column lifetimes.

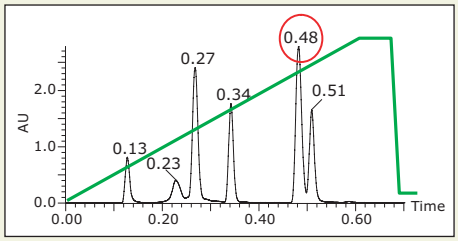
SCALING FROM ANALYTICAL UPLC TO PURIFICATION

Waters UltraPerformance LC® (UPLC®) Technology has been widely accepted by chromatographers because of its improvements over HPLC in sensitivity, resolution, and speed of separations. Now scientists can use this technology in the sample screening process as a tool to evaluate samples prior to purification as well as a tool for rapid analysis of fractions.

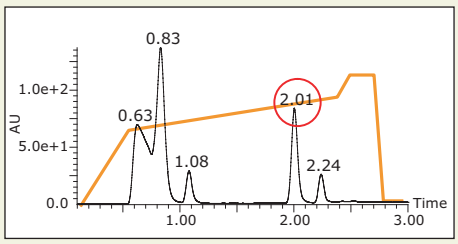
Scaling up from analytical UPLC to preparative HPLC is possible with the use of focused gradients. The efficiency of UPLC can be carried through to purification, offering a substantial increase in throughput and productivity.

In a preparative environment, where the compound of interest is being isolated from the other components in the sample, retaining analytical resolution is not as important as isolating and collecting the single compound of interest.

ACQUITY UPLC analytical separation



Separation of the compound of interest using a 3-minute focused gradient



[Top] The UPLC separation of a sample shows the compound of interest eluting at 0.48 min, and is partially resolved from the peak at 0.51 min.

[Bottom] Using the focused gradient in a preparative run, the separation and isolation of the compound of interest was achieved in less time, in this case in just 3 minutes.

Columns designed for purification

"Being able to load more compound per injection is valuable for saving time without sacrificing purity. Mass recoveries using the Prep OBD Columns are excellent and are higher than other columns I have used. Even after 1,000+ injections, the columns are still performing as they did fresh out of the box."

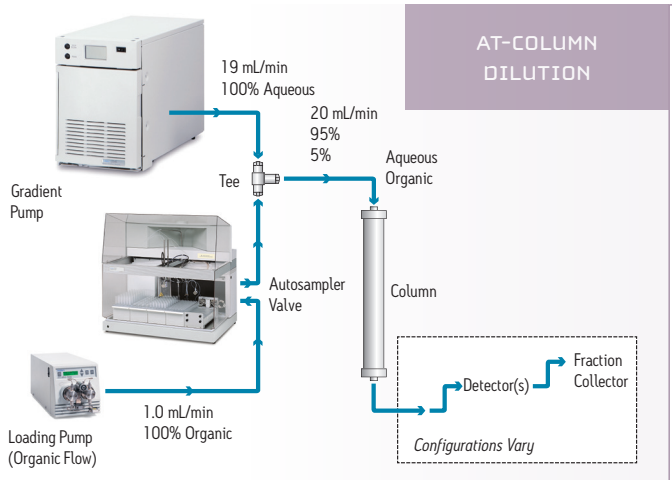
Michael J. Mayer, Ph.D., Senior Research Scientist II, Discovery Sciences – Medicinal Chemistry, AMRI, Albany, NY, U.S. (Contract Laboratory)

XBridge OBD Columns.

WATERS AT-COLUMN DILUTION TECHNOLOGY

This patented* technique allows scientists to increase mass load and injection volumes and enhance the overall performance of their preparative columns. At-Column Dilution was developed specifically for injecting large volumes of relatively strong sample diluents. Such injections may distort the chromatography in a conventional system. If injection artifacts limit mass capacity or chromatographic resolution, the effects can be ameliorated by applying At-Column Dilution. Additionally, At-Column Dilution often increases system ruggedness and column lifetime by preventing bulk precipitation in the sample loop or in the column itself.

At-Column Dilution and conventional separation methods example



Mobile Phase A: Water with 0.1% FA
Mobile Phase B: Acetonitrile with 0.1% FA
Flow Rate: 22.7 mL/min
Loading Pump Flow Rate: 1.2 mL/min

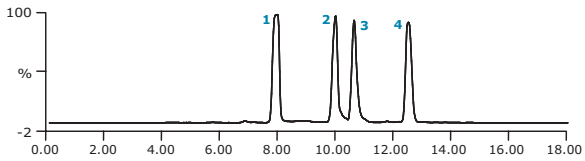
Compounds:
1. Sulfamethazine (25 mg/mL)
2. Althiazide (25 mg/mL)
3. Bendroflumethiazide (25 mg/mL)
4. Amcinonide (25 mg/mL)

Extra holding time is used to ensure sample is fully loaded before gradient starts.
Same gradient slope is used as to the separation without ACD.

Gradient

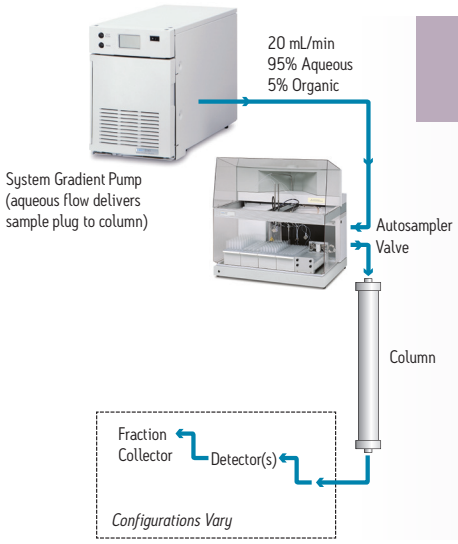
Time	Flow Rate	%A	%B
Initial	22.7	100	0
4.00	22.7	100	0
14.00	22.7	5	
95			
17.34	22.7	5	95

SunFire Prep OBD C_{18}, 5 µm, 19 x 100 mm
Part Number: 186002567
Injection Volume: 1 mL DMSO
Total Mass Load: **100 mg**



At-Column Dilution method

The At-Column Dilution procedure eliminates the sample solvent effect enabling significantly higher sample loading.



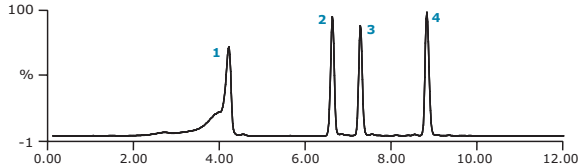
Mobile Phase A: Water with 0.1% FA
Mobile Phase B: Acetonitrile with 0.1% FA
Flow Rate: 23.9 mL/min

Compounds:
1. Sulfamethazine (10 mg/mL)
2. Althiazide (10 mg/mL)
3. Bendroflumethiazide (10 mg/mL)
4. Amcinonide (10 mg/mL)

Gradient

Time	Flow Rate	%A	%B
Initial	23.9	85	15
2.00	23.9	85	15
10.00	23.9	5	95
11.00	23.9	5	95

SunFire Prep OBD C_{18}, 5 µm, 19 x 100 mm
Part Number: 186002567
Injection Volume: 1 mL DMSO
Total Mass Load: **40 mg**



Conventional preparative separation method

Distortion of peak 1 is due to the large volume of DMSO injection. This effect limits the loading capacity for this component.

*U.S. patent # 6,790,361 B2

Partner with Waters to address purification and isolation challenges

We will work with you to develop a complete system solution – instrumentation, chemistry, software, and support – designed for your unique application.

- Our fully integrated solutions provide the flexibility, reliability, and ease-of-use you want for confident and productive sample purification.
- With a Waters Purification System, we link everything together into a single-vendor solution, increasing your system uptime by simplifying maintenance and support.
- Systems are easy to upgrade to expand instrument control and data management capabilities.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

July 2012 720000791EN LB-IGS

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com