ACQUITY UPC² Columns for chiral and achiral separations

Acquity UPC²®

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Acquity UPC²

ACQUITY UPC² System and ACQUITY UPC² Columns.

ACQUITY UPC² Instruments and Columns

The ACQUITY UPC²® System gives scientists the ability to precisely vary mobile phase strength, pressure, and temperature. With this ability to fine-tune the resolving power and selectivity of the system, scientists can exercise better control over the retention of analytes for separating, detecting, and quantifying structural analogs, isomers, and enantiomeric and diasteriomeric mixtures – all compounds that are often a challenge to separate by any other means. A key benefit of the ACQUITY UPC² System is the ability to use inexpensive and non-toxic compressed liquid CO_2 as a primary mobile phase.

THE ABILITY TO HANDLE CHIRAL AND ACHIRAL SEPARATIONS WITH UNEQUALED SPEED AND UNPARALLELED CONFIDENCE

The ACQUITY UPC^2 System is a member of the ACQUITY® platform family which embraces a holistic design concept that emphasizes the functional relationship between the various instrument components, column chemistries, software, and the separation system as a whole. They are designed to achieve separation excellence and provide a complete solution with the ACQUITY brand.

The ACQUITY UPC^2 System brings the technology advances of the ACQUITY portfolio to the world of traditional SFC-based separations by providing liquid CO_2-based separations for the performance and reliability required by ACQUITY users.

The ACQUITY UPC^2 Trefoil™ and Torus™ column chemistries, combined with the ACQUITY UPC^2 platform, will enable separation scientists to better access the power of normal-phase chromatography with the ease and reliability of reversed-phase chromatography.

The ACQUITY UPC^2 Trefoil and Torus column chemistries provide the ability to handle chiral and achiral separations with unequaled speed and unparalleled confidence.

Trefoil™ Technology

ACQUITY UPC^2 Trefoil Columns

These columns are the best choice for fast, robust chiral separations.

- Optimized particle size, column dimension, and flow rates
- Takes full advantage of mass spectrometry detection
- Faster results with method development protocol
- High quality, consistent, and reproducible columns

Torus™ Technology

ACQUITY UPC^2 Torus Columns

A family of achiral chemistries designed to set a new performance standard.

- Excellent peak shapes with or without additives
- Wide range of unique selectivities with unique ligands
- Highest efficiency and QC-ready robustness
- Modified ligand designed for lipids and fat-soluble vitamins

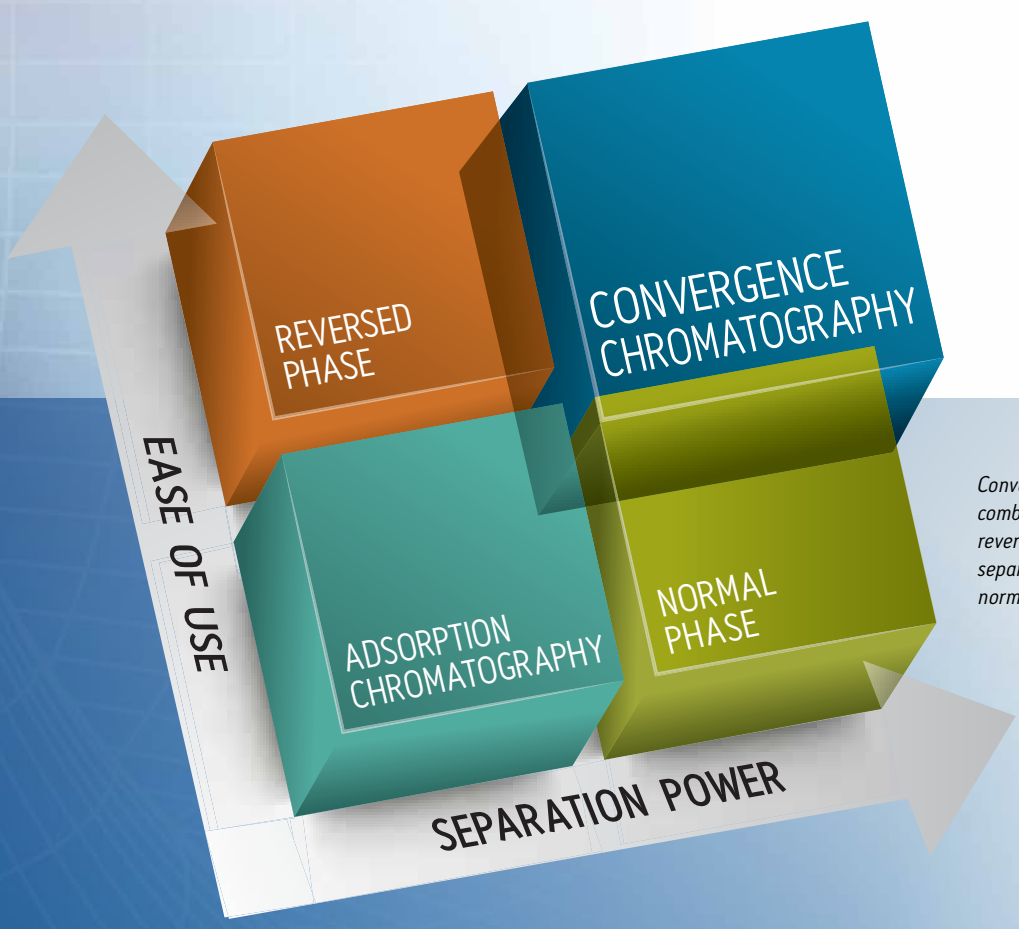
Convergence Chromatography

In 2012, Waters introduced UltraPerformance Convergence Chromatography™ (UPC$^{2®}$), a category of separation science that provides an exceptional increase in selectivity to the chromatography laboratory. UPC^2 is a holistically-designed chromatographic technology that uses compressed CO_2 as the primary mobile phase to leverage the chromatographic principles and selectivity of normal-phase chromatography while providing the ease-of-use and method development simplicity of reversed-phase LC.

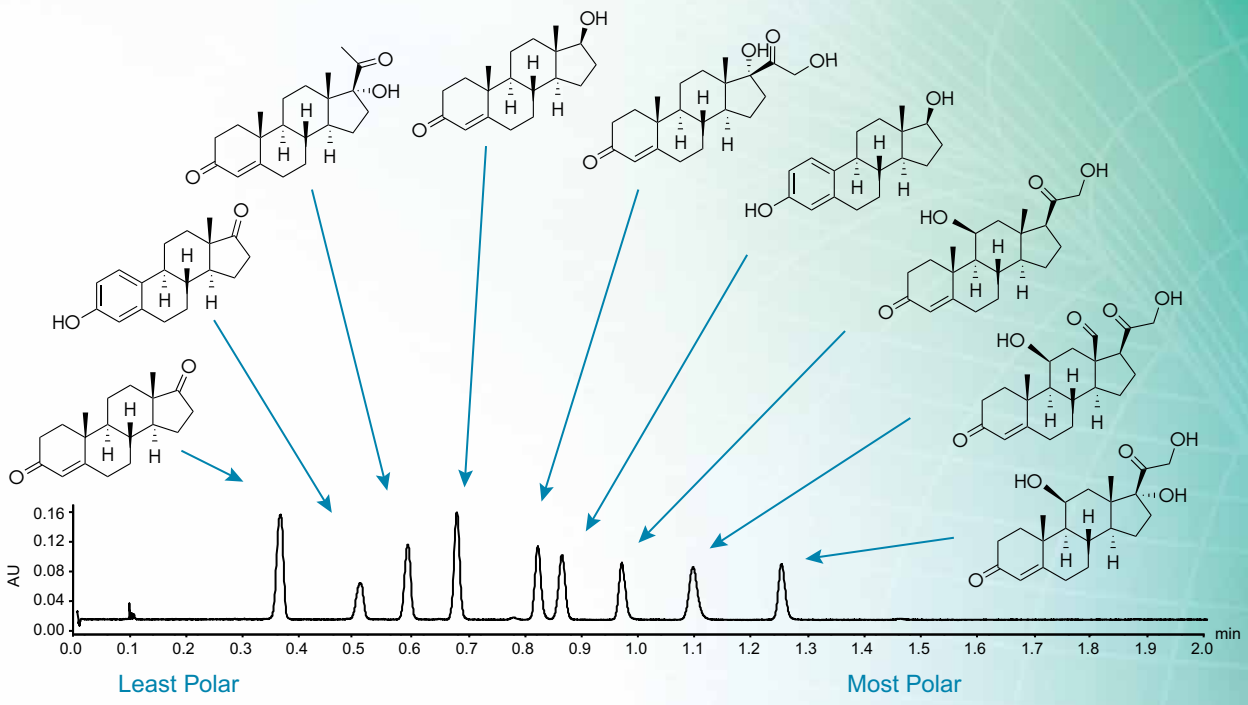
Most often tied with its SFC origins, supercritical fluid chromatography, which exploited density differences of liquefied gases in a supercritical state, the technique has evolved to include the use of co-solvents in a sub-critical state when it was found that chromatography can be performed in either state. Convergence chromatography is the term used since breaking down these barriers.

The miscibility of CO_2 with a wide range of polar and non-polar organic solvents, has made the liquid CO_2-based mobile phase versatile enough to separate a much wider range of compounds than reversed-phase chromatography, especially for mixtures containing polar compounds. Not only can you use CO_2-based solvents with both polar and non-polar stationary phases, you can influence the chromatography by modulating solvent gradients with a much wider choice of columns (including chiral columns) using the same mass spectrometry-compatible co-solvents.

The unique feature of convergence chromatography is not the state or the condition of the solvent, rather the ability to combine or converge the separation of a much wider variety of compounds with one chromatographic system.



Convergence chromatography combines the ease-of-use of reversed-phase LC with the separation power of normal-phase LC.



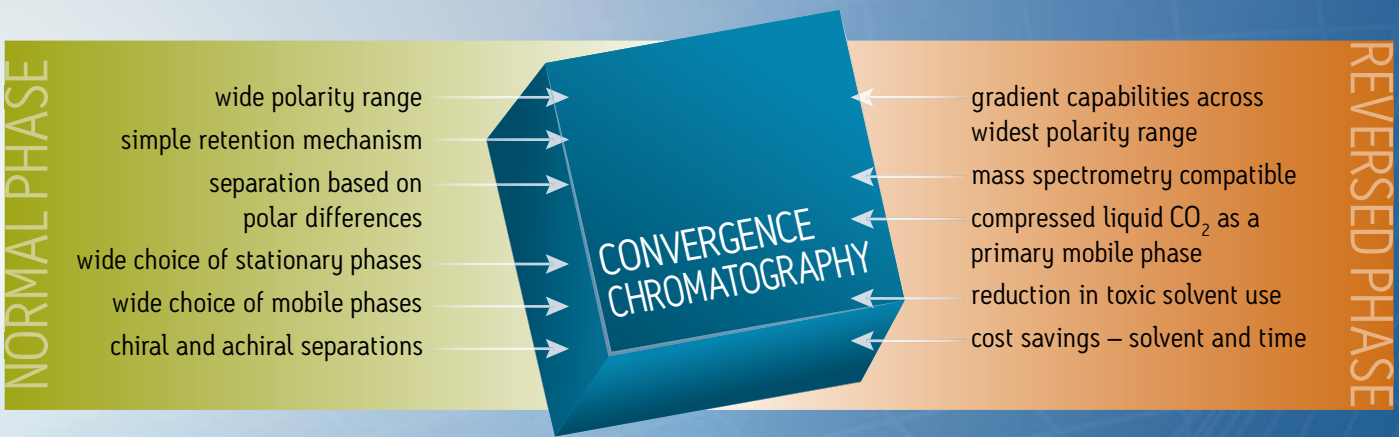
*Convergence chromatography can provide separations according to the overall polarity of the molecules as shown with a family of closely-related steroids.**

RETENTION MECHANISMS IN CONVERGENCE CHROMATOGRAPHY

Convergence chromatography has similar retention mechanisms to normal-phase chromatography in that it generally elutes compounds from column stationary phases according to their polarity in the mobile phase. Whereas in reversed-phase chromatography polar compounds are eluted first, often causing separation challenges, in convergence chromatography, polar compounds are retained and elute last. In the example chromatogram above we see that the general elution profile for a range of neutral steroids is from least polar to most polar.

The powerful orthogonal capability of normal-phase separations is elevated to a mainstream technique with the use of compressed liquid CO_2 as the primary mobile phase in convergence chromatography. This allows the use of gradients across the widest polarity range and brings full mass spectrometry detection capabilities into everyday laboratory use. The separation of most compounds and mixtures that are soluble in organic solvents is made possible. More than that, compounds that are often a challenge to separate by any other means such as structural analogs, isomers, and enantiomeric and diastereomeric mixtures are now simpler and easier to separate using convergence chromatography.

CONVERGENCE CHROMATOGRAPHY: THE POWER OF NORMAL PHASE **WITH** THE EASE OF REVERSED PHASE



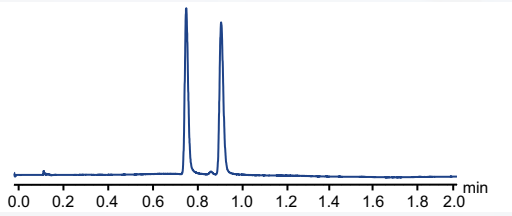
* C. Hudalla *et al.*, Method Development for the Analysis of Endogenous Steroids Using Convergence Chromatography with Mass Spectrometric Detection. Waters Application Note 720004692EN, 2013.

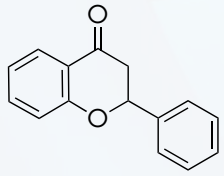
Trefoil™ Technology

ACQUITY UPC² Trefoil Columns

ACQUITY UPC² Trefoil Columns are uniquely designed for the ACQUITY UPC² System to enable both selectivity and speed in chiral separations and to reduce method development time. Trefoil Columns are based on modified polysaccharide-based stationary phases for broad-spectrum chiral selectivity.

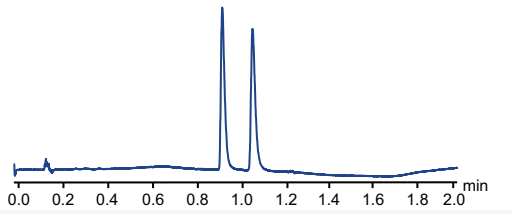
ACQUITY UPC² Trefoil AMY1 2.5 µm Columns
Amylose tris-(3,5-dimethylphenylcarbamate)

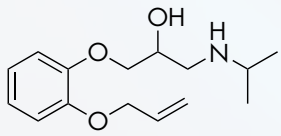




Flavanone

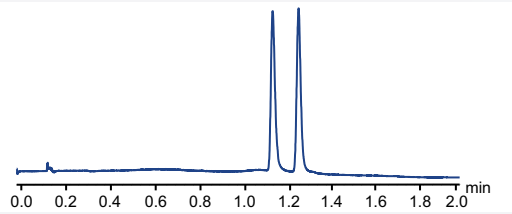
ACQUITY UPC² Trefoil CEL1 2.5 µm Columns
Cellulose tris-(3,5-dimethylphenylcarbamate)

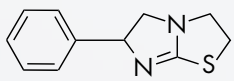




Oxprenolol

ACQUITY UPC² Trefoil CEL2 2.5 µm Columns
Cellulose tris-(3-chloro-4-methylphenylcarbamate)





Tetramisole

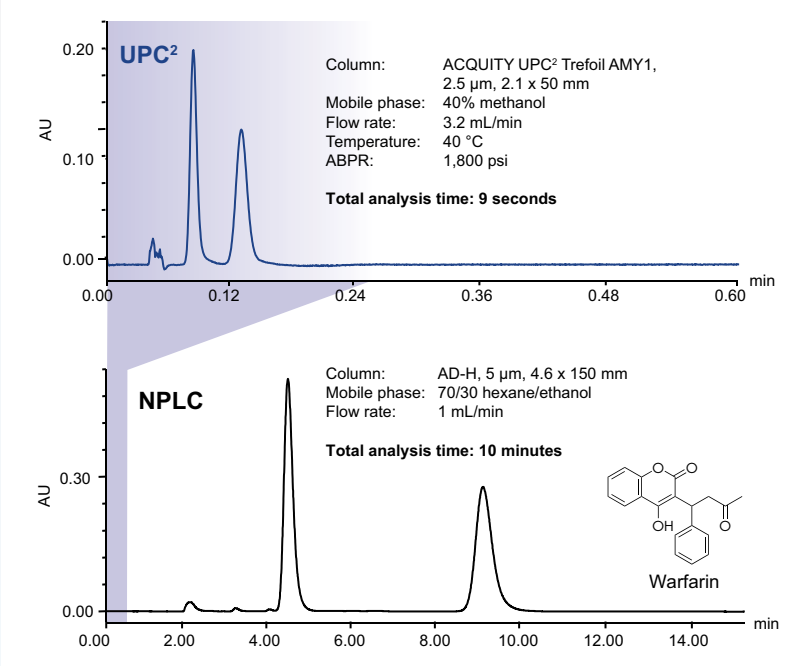
Chiral separations were all run using the 2-minute screening method.

BROAD SELECTIVITIES

Trefoil modified polysaccharide-based stationary phase provides broad-spectrum chiral selectivity. ACQUITY UPC² Trefoil AMY1, CEL1, and CEL2 column chemistries are complementary to each other and independently offer different retention characteristics for separating chiral compounds. Selectivity can be further enhanced by blends of modifiers and additives that most favorably modulate chiral recognition. These columns are designed to separate enantiomers, stereoisomers, metabolites, degradants, and impurities with greater resolution and speed.

TRANSFER OF NORMAL-PHASE CHIRAL TO CONVERGENCE CHIRAL METHODS

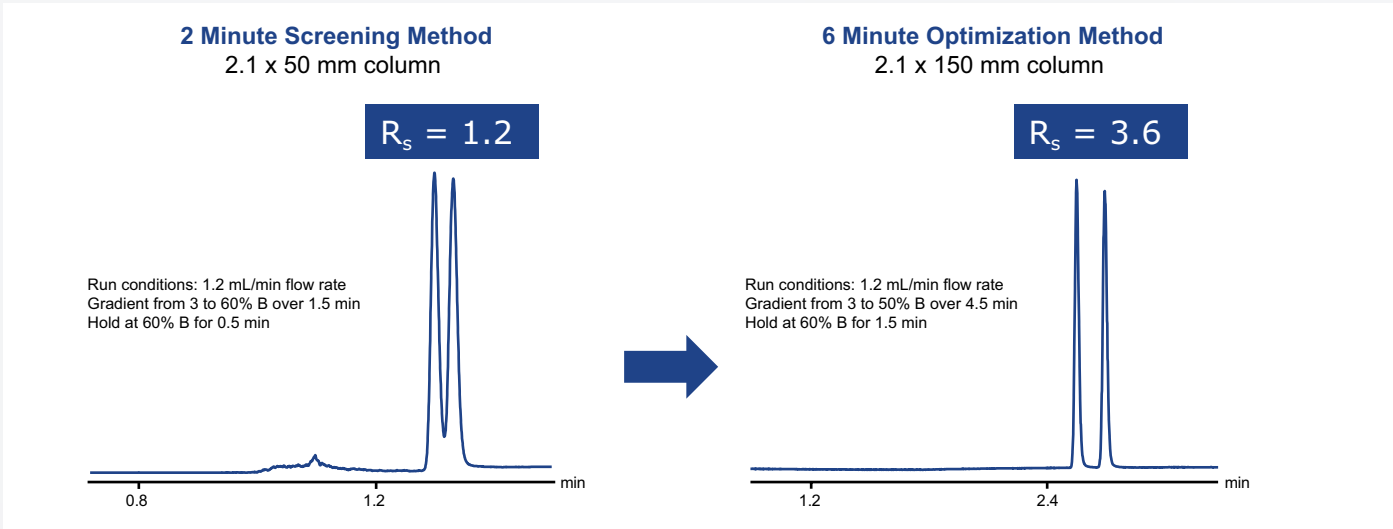
Legacy normal-phase (NPLC) chiral methods can be easily transferred to the ACQUITY UPC^2 System using ACQUITY UPC^2 Trefoil Columns. Many of these old methods have undesirable characteristics such as long run times and often use chlorinated solvents in combination with THF or hexane which are costly to purchase and dispose. With simple redevelopment, new cost effective methods can be obtained using inexpensive and non-toxic compressed liquid CO_2 as the primary mobile phase and can be coupled to mass spectrometers for greater information.



UPC^2 can be greater than 30 times faster, 75 times less solvent per run, 100 times lower cost per analysis.

NEW CHIRAL METHODS USING CONVERGENCE CHROMATOGRAPHY ON ACQUITY UPC^2 PLATFORM

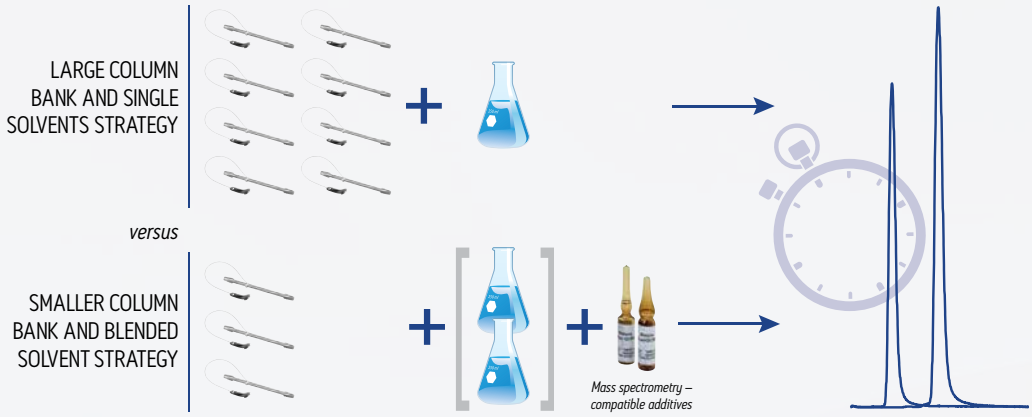
Faster method development is possible when combining the dependable, high performance, low dispersion analytical UPC^2 instrument with the Trefoil Technology stationary phases. Using short, narrow bore columns with a small number of well selected co-solvents and mass spectrometry-compatible additives enables this holistic combination to achieve routine gradient screening runs in 2 minutes. High efficiency, narrow bore columns use 46% less solvent than traditional 4.6 mm columns. Increased sensitivity, reduced solvent use, and faster methods while using mass spectrometry detection offers peace of mind confirmation.



An example of the increased resolution expected when moving from the 2 minute screening method to the 6 minute optimization method.

Chiral Method Development

Method development scientists are seeking faster approaches to achieve their desired chiral separations in the shortest amount of time and with the fewest number of method screening injections. Waters is investigating an approach to achieve the maximum chiral separation power for each of the ACQUITY UPC^2 Trefoil Columns and to increase the probability for achieving desired separations. An experimental study using 55 racemic compounds was performed on the ACQUITY UPC^2 System with the ACQUITY UPC^2 Trefoil Columns using 44 different blends of co-solvents and mass spectrometry-compatible additives. The blends that most favorably modulated chiral recognition were selected. This allows Waters to recommend method development steps that achieve the highest enantiomer separation success rate with the least number of steps on the ACQUITY UPC^2 Trefoil Columns.



The study data analysis for the three chiral stationary phases (CSPs) – ACQUITY UPC^2 Trefoil AMY1, CEL1, and CEL2 – showed that 44 out of 55 compounds (80%) were resolvable. Of those resolvable compounds, 96% of them could be separated using just four runs via optimal pairing of the blends and the ACQUITY UPC^2 Trefoil Columns. Using single solvents instead of optimal blends separated only 82% of these compounds. This demonstrates the advantage of using this optimal path screen with the blends of solvents and additives given in the table below.

Routine method development for chiral compounds is therefore possible within 10 minutes using the three CSP's and four blended gradient runs. The method development strategy and the study that led to it is made possible using ballistic 2-minute gradient runs with short, small I.D. columns containing the efficient 2.5-µm particle size Trefoil Technology chemistries, which are optimized for convergence chromatography.

STEP	COLUMNS AND BLENDS	CUMULATIVE % SUCCESS
1	**AMY 1** Ethanol/Isopropanol/Acetonitrile Ammonium Acetate	46%
2	**CEL 1** Methanol/Isopropanol Trifluoroacetic Acid	73%
3	**CEL2** Ethanol/Acetonitrile Trifluoroacetic Acid	86%
4	**AMY 1** Ethanol/Isopropanol Trifluoroacetic Acid	96%

2-Minute Screening Method
ACQUITY UPC^2 Trefoil 2.1 x 50 mm Columns
1.2 mL/min flow rate

2.5 min cycle time per step

Chiral Separations with Mass Spectrometry

High resolution chiral separation methods developed on ACQUITY UPC^2 Trefoil Columns and the ACQUITY UPC^2 System can easily be coupled with mass spectrometry providing unprecedented levels of information about stereoselective samples. This was not previously possible due to the limitations with interfacing normal-phase LC and MS.

SINGLE QUADRUPOLES

Waters single quadrupole mass spectrometers are a novel approach that can be integrated into existing UPC^2 configurations in order to increase sensitivity and complement the results obtained when using only UV detectors. When single quadrupole MS data is combined with the UV response, it allows the analyst to determine a wider range of analytes in one analytical run with an increased level of confidence.

ACQUITY UPC² System with ACQUITY QDa® Detector.

TANDEM QUADRUPOLES

Analysis within the realm of bioanalysis/DMPK requires the highest levels of resolution, sensitivity, and efficiency, especially for chiral molecules. Most reversed-phase and normal-phase LC systems would fail with this chiral analysis. The use of the ACQUITY UPC^2 System and ACQUITY UPC^2 Trefoil Columns provides a greater level of impact in the form of simplified workflow for DMPK, the ability to separate compounds having structural similarity, and true orthogonality.

ACQUITY UPC² System with the Xevo® TQ-S.

TIME-OF-FLIGHT

In drug metabolism, methods are generally developed in the absence of metabolite standards. Typically, most biologically mediated drug metabolism processes make compounds more polar to facilitate their excretion from the body. If the parent drug is itself relatively polar, reversed-phase retention of polar metabolites is even more challenging. The use of ACQUITY UPC^2 System, ACQUITY UPC^2 Trefoil Columns, and Xevo G2-XS QTof mass spectrometry provides scientists with the tools necessary to identify, quantify, and confirm using accurate mass determination with high resolution.

ACQUITY UPC² System with the Xevo G2-XS QTof.

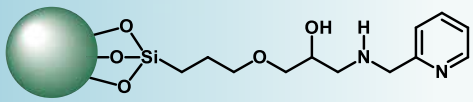
Torus™ Technology

ACQUITY UPC² Torus Columns

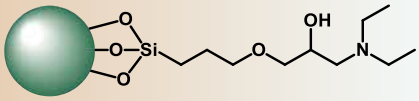
ACQUITY UPC² Torus Columns are specifically designed to use the complete range of capabilities of the ACQUITY UPC² System to achieve fast, robust achiral separations. ACQUITY UPC² Torus Columns simplify the method development process with four completely new and innovative 1.7 µm chemistries for convergence chromatography. These columns are designed for excellent peak shape that eliminate or reduce the need for additives, and offer added selectivity for a wide range of compounds and improved robustness.

The Torus phases are based on a new patent-pending two-stage functionalization of ethylene bridged hybrid (BEH) particles. Modification of the stationary-phase surface during traditional SFC separations has been identified as a primary source of chromatographic variation. The ACQUITY UPC² Torus family of columns addresses this issue through a two stage bonding process which protects the stationary-phase surface from these undesired reactions, resulting in chromatographically robust columns. The initial bonding provides a hydrophilic surface that controls the retention characteristics of the sorbent, and is responsible for minimizing unwanted surface interactions, which lead to retention and selectivity changes over time. The second step of the functionalization is responsible for the individual selectivity and peak shape characteristics of each of the Torus chemistries. The results of these steps are a series of stationary phases with broad ranging selectivities, which maintain robust chromatographic performance over the lifetime of the column.

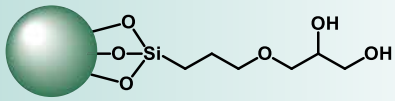
ACQUITY UPC² Torus 2-PIC, 1.7 µm Columns
2-Picolylamine



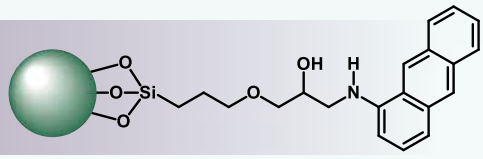
ACQUITY UPC² Torus DEA, 1.7 µm Columns
Diethylamine



ACQUITY UPC² Torus DIOL, 1.7 µm Columns
High Density DIOL



ACQUITY UPC² Torus 1-AA, 1.7 µm Columns
1-Aminoanthracene



1.7 µm PARTICLES

Chromatographers seek solutions that deliver more resolution and robust separations. The ACQUITY UPC² System, with the low system volume, can fully realize the speed and greater efficiencies with columns packed with 1.7 µm particles.

Achiral Torus UPC^2 Method Development

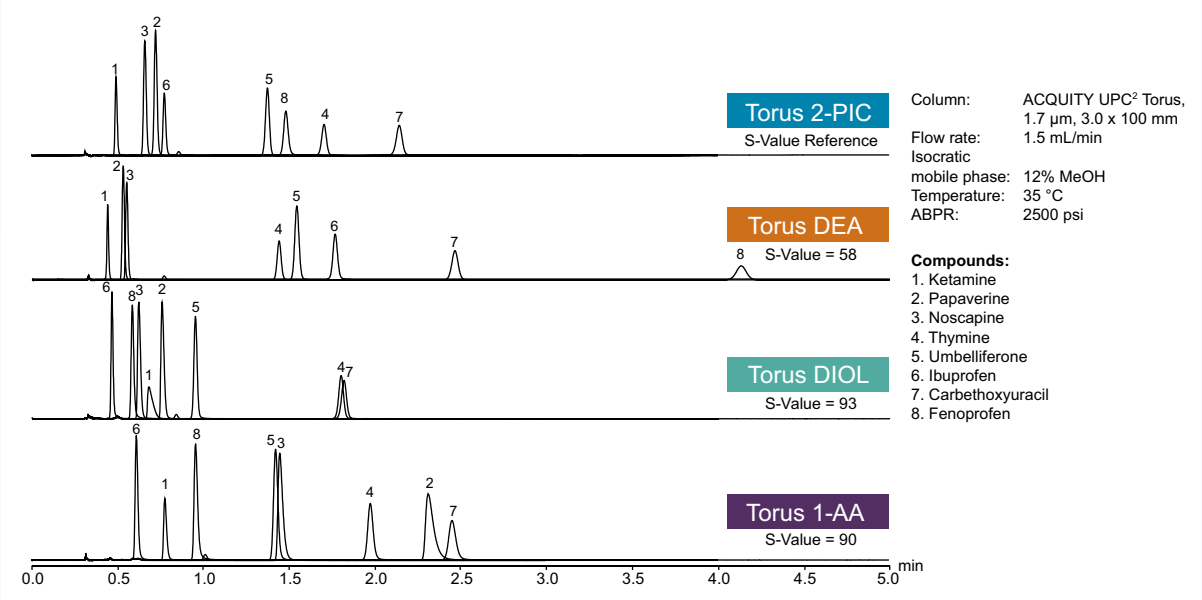
For method development, it is crucial to have a series of columns that have significantly differing selectivities and good retentivity. The Torus chemistries were specifically chosen to provide a breadth of selectivities for acids, bases, and neutral analytes. The synthesis process has been optimized to yield stationary phases with excellent peak and tailing characteristics both with and without additives. The development of robust methods requires columns that do not exhibit changes in performance over time (retention or selectivity).

Recommended Starting Conditions for ACQUITY UPC^2 Torus Columns

BASIC COMPOUNDS	ACIDIC COMPOUNDS	NEUTRAL COMPOUNDS	MIXTURES or UNKNOWNS
TORUS 2-PIC	TORUS 2-PIC	TORUS 2-PIC	TORUS 2-PIC
TORUS DEA	TORUS DIOL	TORUS 1-AA + NH_3	TORUS DIOL
	TORUS DIOL + NH_3	TORUS 1-AA + TFA	TORUS DEA
			TORUS 1-AA + NH_3
			TORUS 1-AA + TFA

Recommended ACQUITY UPC^2 Torus Column and separation condition steps can help speed up the method development process.

Selecting the most suitable column and separation conditions can be challenging for the method development scientist. The ACQUITY UPC^2 Torus Column family is designed to maximize the selectivity choices while providing the optimum peak shape and efficiencies. The four Torus chemistries provide a wide range of selectivities (S-values*) that simplify the method development challenge.



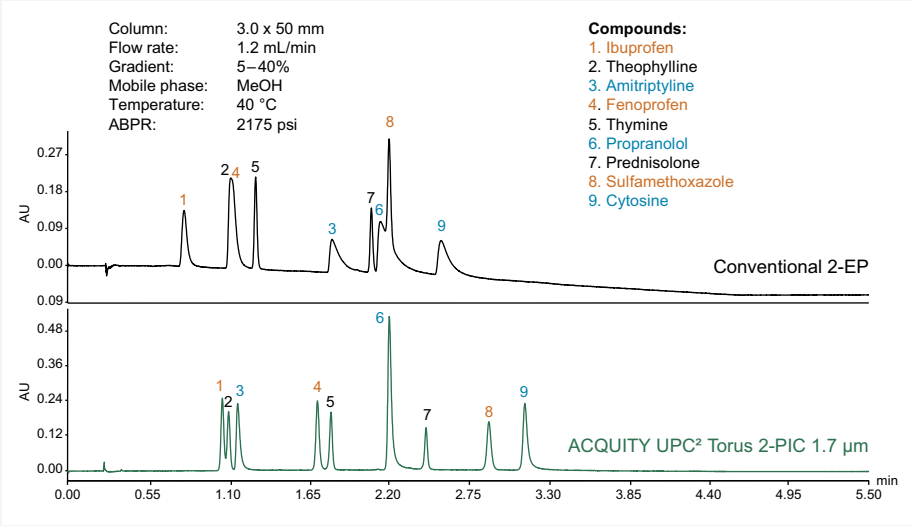
ACQUITY UPC^2 Torus family of columns exhibit different selectivities for acid, bases, and neutral compounds.

* (1) U.D. Neue, E.S. Grumbach, J.R. Mazzeo, K. Tran, and D.M. Wagrowski-Diehl "Method development in reversed-phase chromatography" Chap. 6 in: I.D. Wilson, ed. Bioanalytical Separations, Handbook of Analytical Separations, Vol. 4 Elsevier, Amsterdam (2003); (2) U.D. Neue, J.E. O'Gara, and A. Méndez "Selectivity in reversed-phase separations: influence of the stationary phase" *J. Chromatogr. A* 1127(1–2): 161–174 (2006); (3) U.D. Neue and A. Méndez "Selectivity in reversed-phase separations: general influence of solvent type and mobile phase pH" J. Sep. Sci. 30(7): 949–963 (2007).

ACQUITY UPC² TORUS 2-PIC COLUMNS
2-Picolylamine

ACQUITY UPC² Torus 2-PIC Columns are designed for general use and are the first choice for a wide range of applications with acidic and basic compounds. The Torus 2-PIC phase demonstrates enhanced performance compared to conventional 2-ethylpyridine (2-EP), displaying improved peak shape, added retention, and novel selectivity.

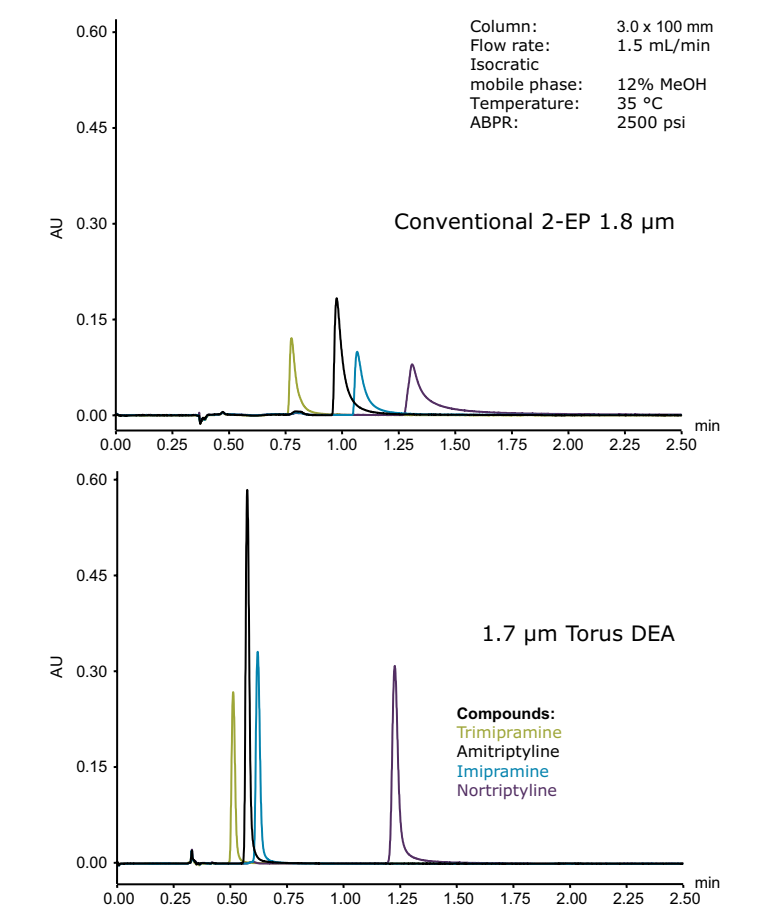
In addition, these columns provide extra flexibility to method development as they can be used with or without additives, while still maintaining exceptional peak shape for a wide range of compounds.



Torus 2-PIC has excellent peak shape characteristics with or without the use of additives for wide ranges of acidic and basic compounds.

ACQUITY UPC² TORUS DEA COLUMNS
Diethylamine

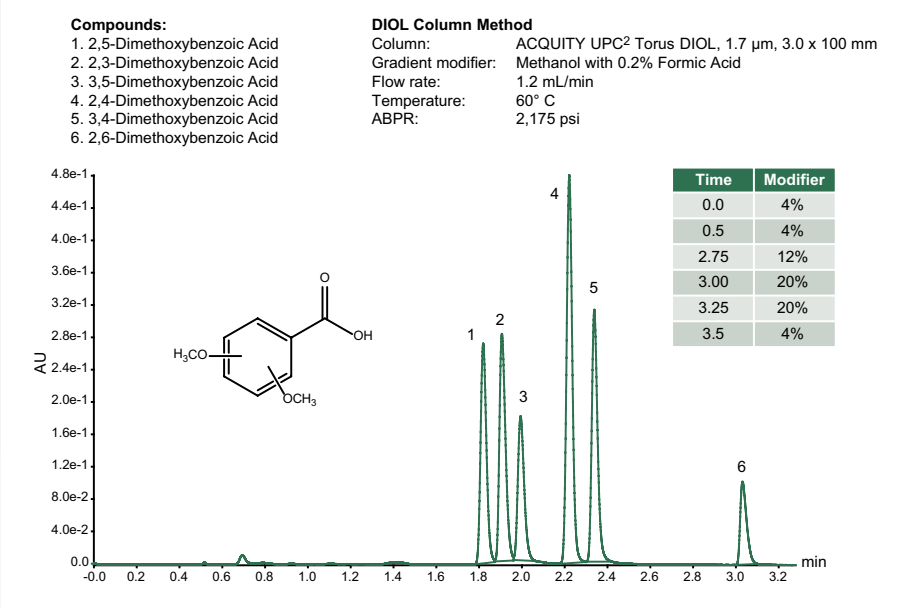
ACQUITY UPC² Torus DEA Columns provide a complementary selectivity to the 2-PIC phase and are designed to provide superior peak shape for very strong bases, with or without additives.



Torus DEA exhibits excellent peak shape for strong basic compounds without the use of additives when compared to a silica 2-EP column.

ACQUITY UPC^2 TORUS DIOL COLUMNS
High Density DIOL

ACQUITY UPC^2 Torus DIOL Columns were developed to provide additional selectivity choices within the Torus family. The high density DIOL surface bonding offers chromatography similar to that from the traditional unbonded silica phases, but adds overall method robustness when utilized with acidic or basic additives.

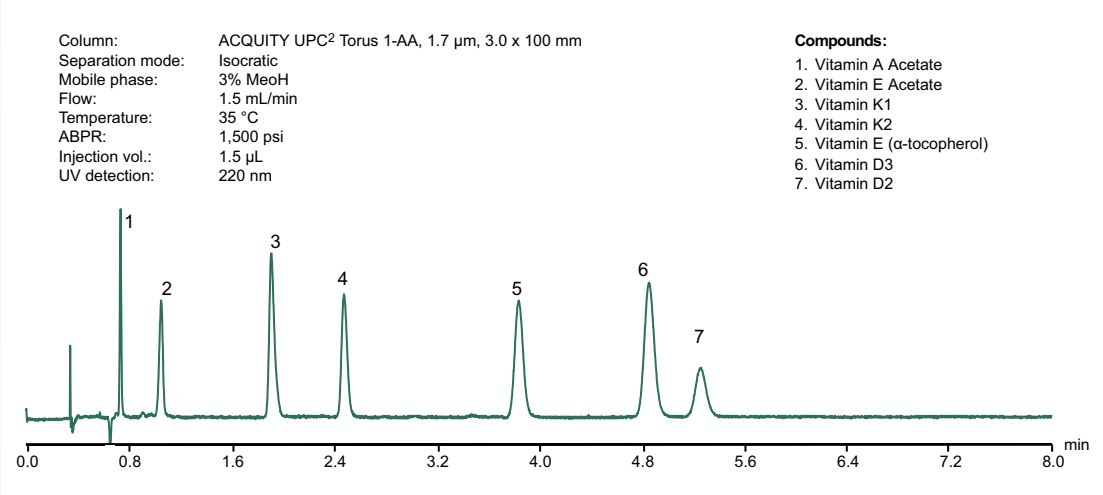


Torus DIOL Columns show good peak shapes for acidic compounds demonstrated by the separation of 6 isomeric forms of dimethoxybenzoic acid.

ACQUITY UPC^2 TORUS 1-AA COLUMNS
1-Aminoanthracene

ACQUITY UPC^2 Torus 1-AA Columns are designed to be the superior choice for separating neutral compounds such as polar and non-polar steroids, and hydrophobic compounds such as lipids and fat-soluble vitamins. This chemistry also provides an orthogonal selectivity (S > 90) to the 2-PIC phase, making it the perfect choice in method development of:

- Hydrophobic (lipophilic) compounds
- Free fatty acids
- Fat-soluble vitamins
- Lipids
- Natural products
- Steroids

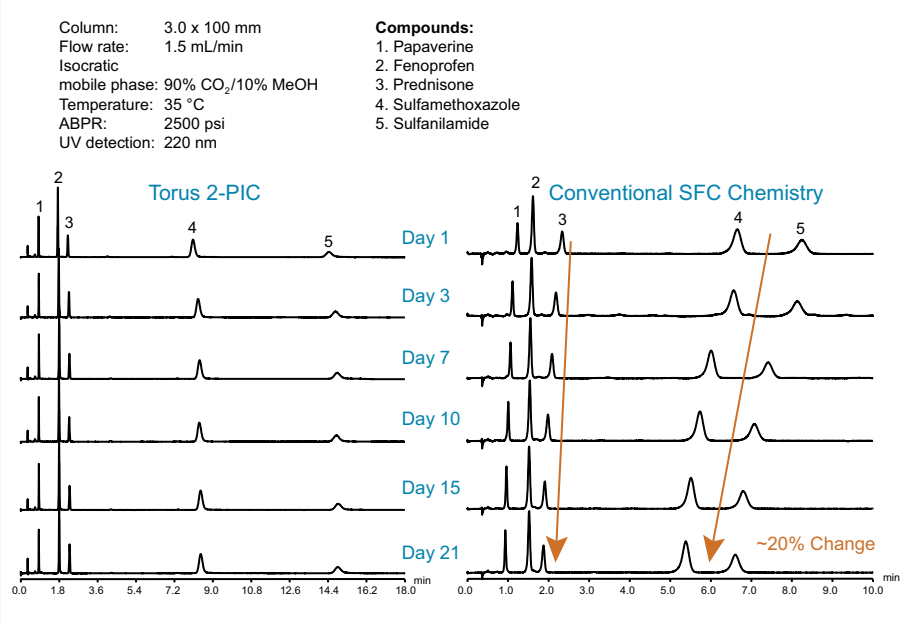


Torus 1-AA Column shows good peak shape and resolution of fat-soluble vitamins.

ACHIRAL SEPARATIONS

INCREASED ROBUSTNESS WITH ELIMINATION OF RETENTION DRIFT

One common concern with silica-based SFC columns is changes in retention while in use. Studies have shown that both selectivities and retention times may shift over time.* In some cases, the column may need to be regenerated in order to establish original performance. ACQUITY UPC^2 Torus Columns are designed to eliminate changes in selectivity and retention leading to greater method robustness and extended column lifetimes.

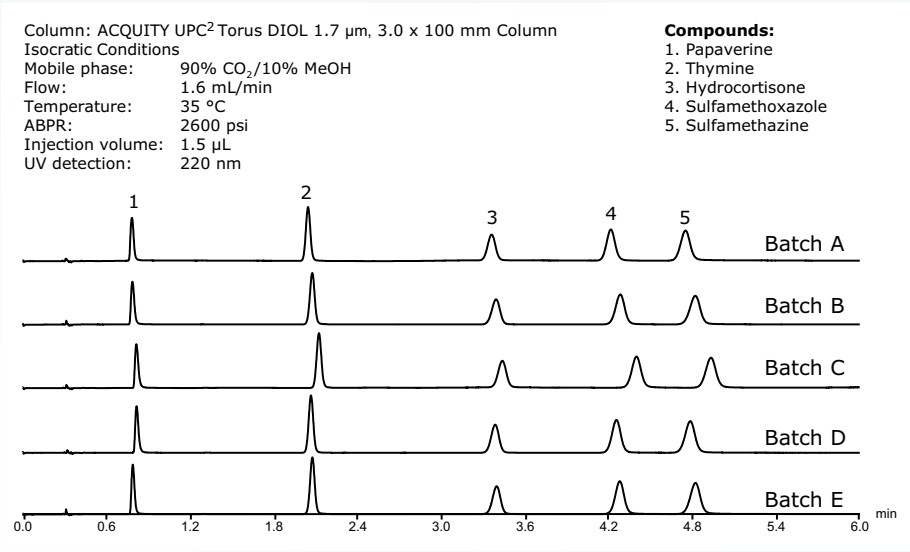


ACQUITY UPC^2 Torus Columns can benefit both method development and QC scientists with the added performance of maintaining retention and selectivity over time.

BATCH-TO-BATCH CONSISTENCY – OPTIMIZED FOR QUALITY CONTROL LABORATORIES

ACQUITY UPC^2 Torus Columns benefit separation scientists with their changing needs throughout the product development process – from early discovery to QC.

In the early development stages, short, sensitive, high resolution methods are the primary goal, while in QC laboratories, the chromatographic focus is on minimizing method variability.



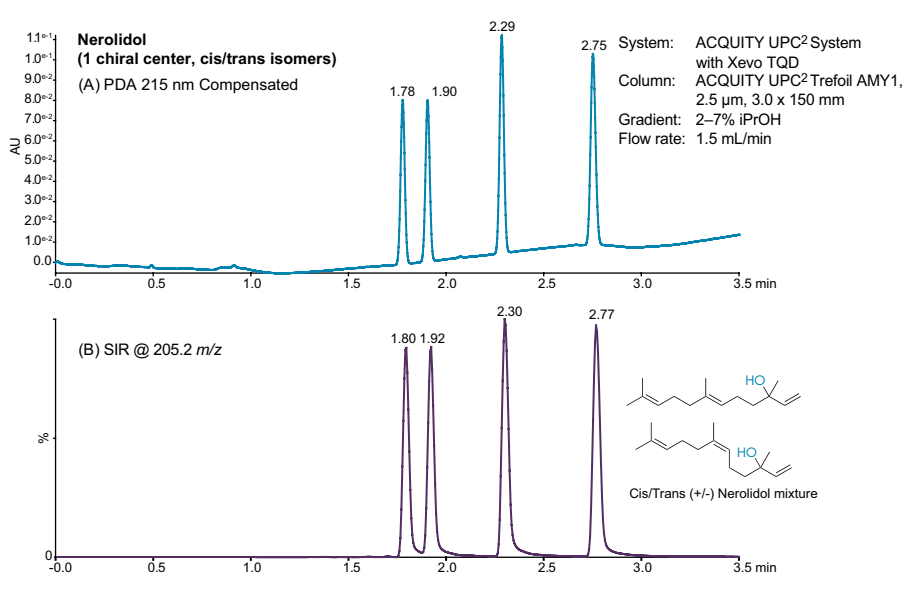
ACQUITY UPC^2 Torus Columns maintain reproducible performance and batch-to-batch consistency, as shown in this overlay of UPC^2 separations on columns from multiple batches.

* K. Ebinger, H.N. Weller, *J. Chromatogr. A*, Comparative assessment of achiral stationary phases for high throughput analysis in supercritical fluid chromatography. 2014 http://dx.doi.org/10.1016/j.chroma.2014.01.060

APPLICATIONS

ISOMER AND STEREOISOMER SEPARATIONS WITH MASS SPECTROMETRY

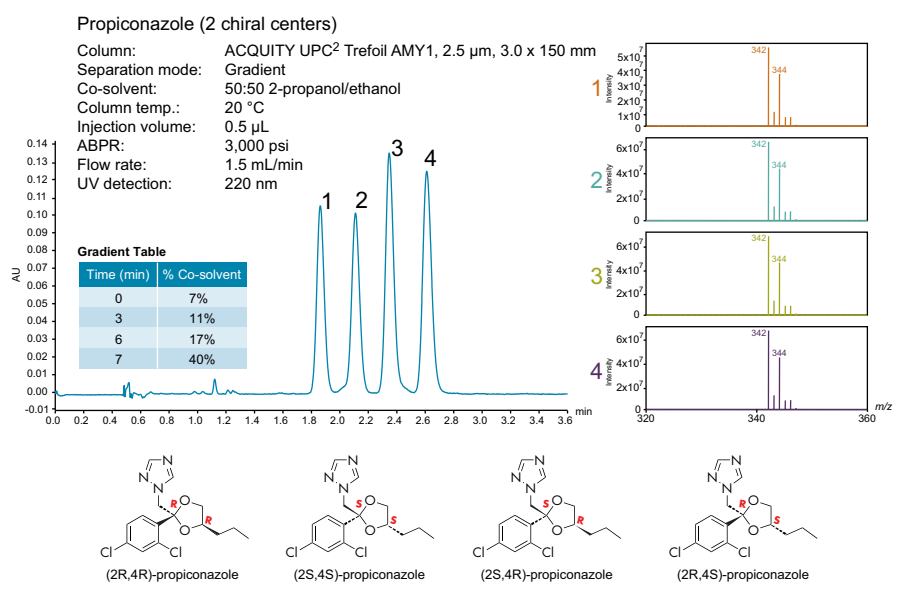
Historically, chiral separations of fragrance compounds have primarily been carried out using chiral stationary phases (CSPs) in capillary gas chromatography (GC) where the analysis time can range from 15 to 50 minutes. In contrast a cis/trans (+/-) nerolidol mixture can be separated on ACQUITY UPC^2 Trefoil Columns in 3 minutes. Unlike traditional normal-phase separations, which require tedious sample preparation, the UPC^2 System allows for diluted samples to be directly injected. In addition, due to the compatibility of the ACQUITY UPC^2 System with MS, the masses of these peaks can be quickly determined, thus confirming the presence of multiple stereoisomers in the sample.



Four stereo isomers of nerolidol (found in essential oils) can be quickly separated on ACQUITY UPC^2 Trefoil Columns in 3 minutes. This shows excellent isomer and chiral resolution in the same run.

MULTIPLE CHIRAL CENTERS WITH MASS SPECTROMETRY

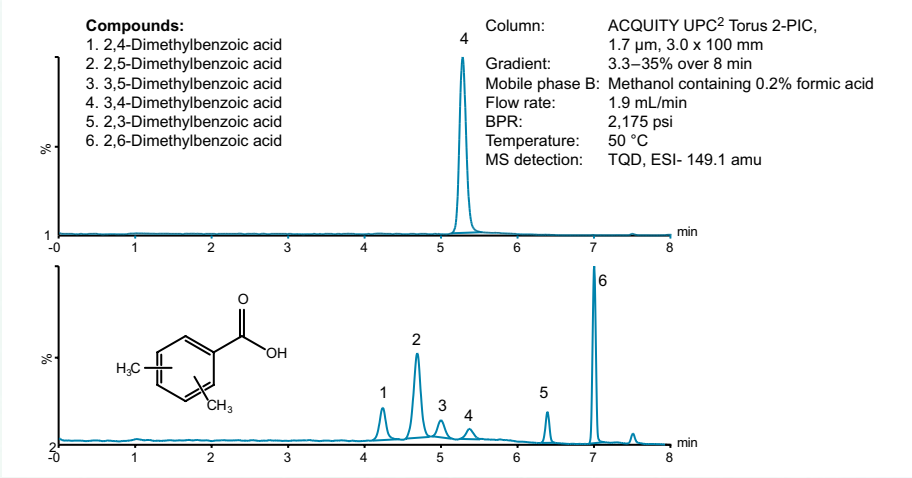
The separation of stereoisomers that can now be achieved using the ACQUITY UPC^2 Trefoil Columns allows for the resolution of more complicated mixtures, such as those resulting from compounds which contain multiple chiral centers. In the example shown here, the enantiopurity of propiconazole (a triazole fungicide) can be rapidly (<3 mins) measured in a pesticide formulation sample. The identity of the stereoisomers and other formulation components can be confirmed using simultaneous mass spectrometry detection.



Analysis of the four stereoisomers of propiconazole with an ACQUITY UPC^2 Trefoil AMY1 Column.

POSITIONAL ISOMERS CAN BE RAPIDLY AND EFFICIENTLY SEPARATED USING ACQUITY UPC² TORUS COLUMNS ON THE UPC² SYSTEM

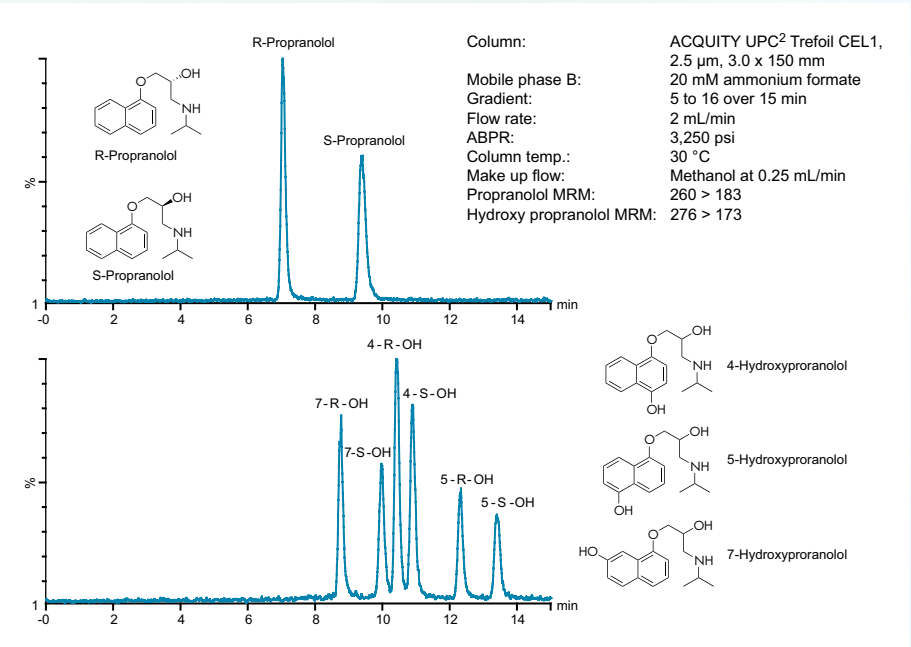
Positional isomers are compounds that only differ in the location of the substituent groups. Each one of these isomers differs only slightly in physical properties from the others. For this reason, positional isomer mixtures can often be a serious challenge to separate. Convergence Chromatography (UPC²) is known to be a highly effective technique for separating structurally similar compounds. Using an ACQUITY UPC² Torus 2-PIC Column, it is possible to separate the positional isomers of dimethylbenzoic acid (DMBA) including the 3,4-DMBA which is a known metabolic marker in urine for trimethylbenzoic acid (TMB) exposure.



1,2,4-Trimethylbenzene (TMB or pseudocumene) occurs naturally in coal tar and petroleum crude oil. It is a major component (typically 40%) of a petroleum refinery distillation fraction known as the C9 aromatic fraction. The largest users of isolated TMB are chemical companies that make trimellitic anhydride. Companies also use it to make dyes and drugs.

ENANTIOMERIC SEPARATION OF PROPRANOLOL AND THEIR ASSOCIATED HYDROXYLATED METABOLITES USING UPC²-MS/MS

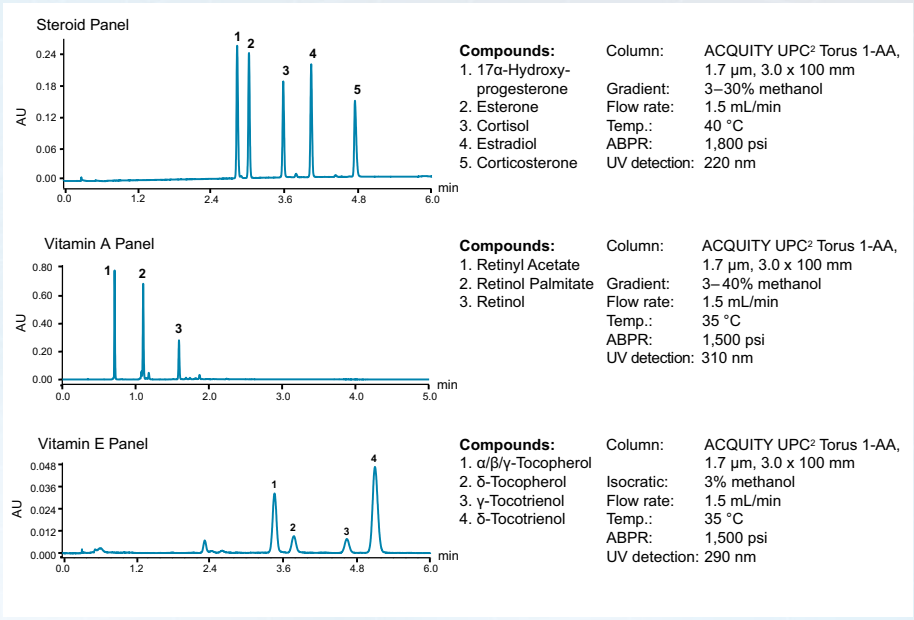
Many drug candidates, as well as their metabolites, contain one or more chiral centers. The ability to identify and monitor the various chiral forms of a compound and its metabolites is an essential step during the drug development process, and is easily achieved using convergence chromatography. The combination of ACQUITY UPC² Trefoil CEL1 Column with mass spectrometry detection facilitated the easy enantiomeric separation of parent compound propanolol and its three hydroxy metabolites 4-hydroxypropranolol, 5- hydroxypropranolol, and 7-hydroxypropranolol in under 15 minutes using an ACQUITY UPC² Trefoil Column.



Separation of propranolol and hydroxy metabolites. Note that each hydroxy metabolite has an R and S form.

ENHANCED RESOLUTION OF STEROIDS, VITAMIN A, AND VITAMIN E

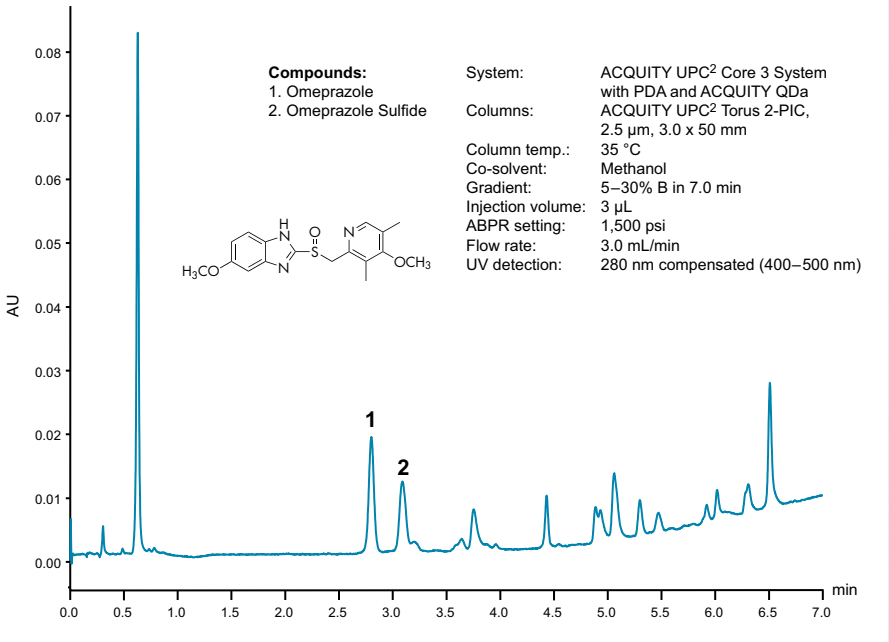
The ACQUITY UPC^2 System harnesses the power of supercritical fluid chromatography to assist in the separation of complex hydrophobic samples such as steroid and fat-soluble vitamin samples. Using the Torus 1-AA Columns with the UPC^2 platform, an analyst can rapidly perform the analysis of these types of samples.



Steroid and vitamin panels using ACQUITY UPC² Torus 1-AA Column.

HIGH RESOLUTION AND SPEED FOR IMPURITY PROFILING

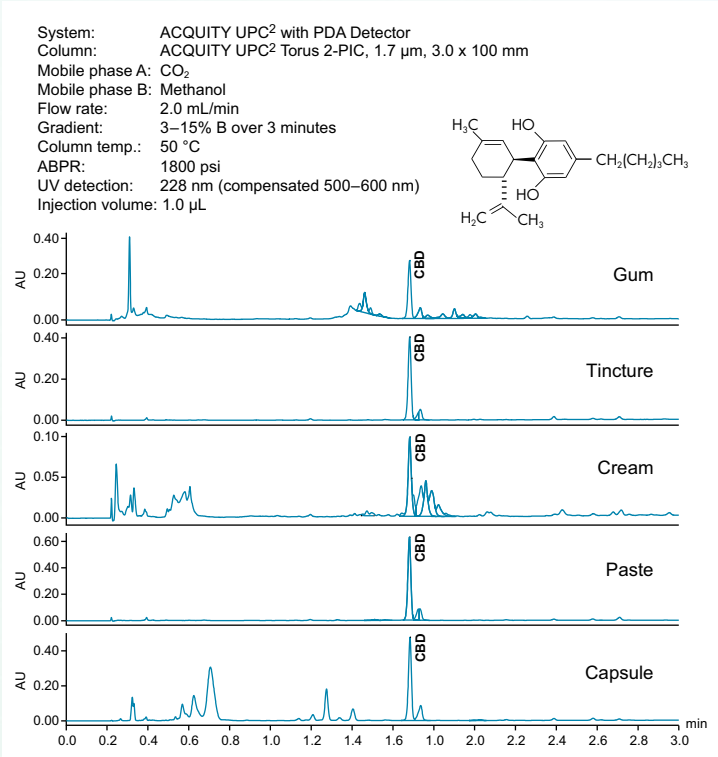
During the manufacture of pharmaceutical compounds, it is important to fully understand any possible degradation products. This is especially true in the case of pharmaceutical compounds administered orally. The ACQUITY UPC^2 Torus 2-PIC Column was used to analyze the acid degradation products of formulated omeprazole tablets. Both the active pharmaceutical ingredient and its acid degradation peaks are well separated and could be identified by either standard injections or by using a mass spectrometry detector.



Analysis of the acid degradation products of an Omeprazole tablet.

RAPID QUANTITATIVE ANALYSIS OF CANNABIDIOL (CBD) FROM 5 CONSUMER PRODUCT FORMULATIONS

Cannabidiolic acid (CBDA) is produced in large abundance in some therapeutic hemp cultivars. Cannabidiol (CBD), is the heat induced decarboxylation product of CBDA and is non-psychoactive and thought to have a wide scope of potential medicinal benefits. CBD has traditionally been administered by smoking or vaporizing (thereby converting CBDA to CBD), however alternative formulations (*e.g.* topical creams) are now widely available. The therapeutic hemp is processed to ensure that any CBDA is present in these products as CBD. Separation of CBD from excipient materials was achieved in 3 minutes per sample and proved suitable for quantitation. This methodology is suitable for laboratories performing quality control or product quality monitoring of CBD content with a wide range of product formulations.



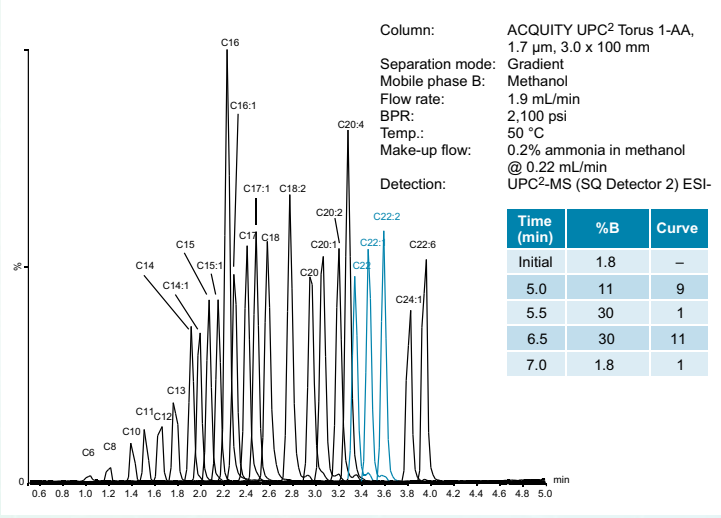
Cannabidiol (CBD) analysis from five different formulations.

FREE FATTY ACID LIPID SEPARATIONS WITHOUT DERIVATIZATION

The elution order of free fatty acid (FFA) species depends on the length and the number of double bonds on the fatty acid chain. In a typical RPLC separation, the longer and the more saturated the acyl chain, the longer the retention time.

In a separation utilizing an ACQUITY UPC^2 Torus 1-AA Column, however, both increasing chain length and increasing degrees of unsaturation increase the retention time of the FFA. This reduces co-eluting lipid species in complex biological samples containing saturated and unsaturated FFA species with different carbon chain lengths, resulting in a simplified analysis.

In addition, the organic layer extract containing the lipids can be injected directly into the system, omitting the need for solvent exchange for compatibility with reversed-phase LC methods.



The UPC^2-MS FFA analysis provides a simple and fast method with a significant reduction in analysis time compared to alternative techniques such as GC-MS, which requires FAME derivatization.

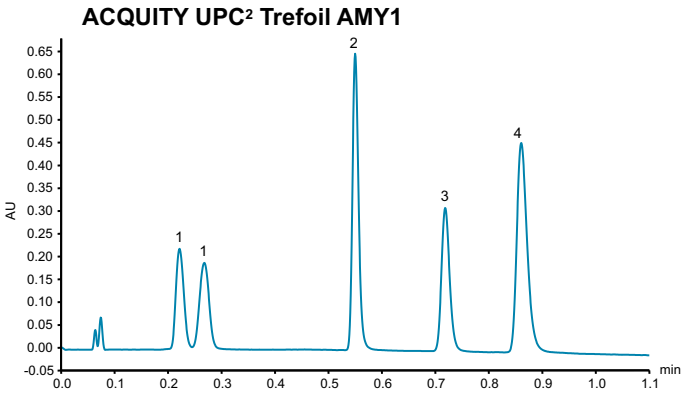
UPC² Quality Control Reference Material

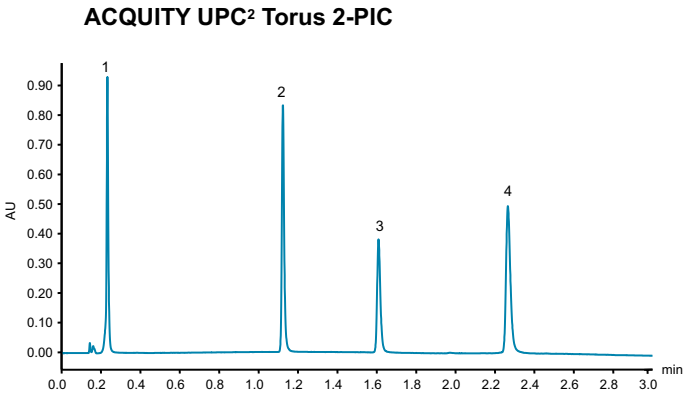
Quality Control (QC) Reference Materials contain mixtures of standards specifically chosen to provide an easy and reliable way to monitor the performance of any chromatographic system. Using a QC Reference Material, you can be assured that your column and system are ready to analyze your samples. Regular use of QC Reference Materials also provides an opportunity to benchmark your chromatographic systems and trend performance over time, making it easier to proactively identify problems and resolve them faster.

ACQUITY UPC² Quality Control Reference Materials

Intended Use	Contents	Part Number
Provides convergence chromatographic performance information for both chiral and achiral modes.	1. 0.50 mg/mL (+/-) *trans*-Stilbene oxide 2. 0.50 mg/mL Thymine 3. 0.50 mg/mL Sulfamethoxazole 4. 0.50 mg/mL Sulfamethizole In a 1 mL solution of 75:25 ACN:MeOH	186007950

Single QC Reference Material for ACQUITY UPC² Trefoil and Torus Columns on a ACQUITY UPC² System





Compounds:
1. *trans*-Stilbene oxide
2. Thymine
3. Sulfamethoxazole
4. Sulfamethizole

System:	ACQUITY UPC²
Columns:	AMY1, 2.5 µm, 2.1 x 50 mm 2-PIC, 1.7 µm, 3.0 x 50 mm
Co-solvent:	Methanol
Flow rate:	2.0 mL/min (3.0 x 50 mm) 2.7 mL/min (2.1 x 50 mm)
Gradient:	See tables
Column temp.:	50 °C
Injection volume:	2.0 µL
Detection (UV):	240 nm
ABPR:	2,000 psi

2.1 x 50 mm Column Gradient

Time	%A	%B
0.00	97	3
1.11	40	60
1.39	40	60
1.42	97	3
2.00	97	3

3.0 x 50 mm Column Gradient

Time	%A	%B
0.0	97	3
3.0	40	60
3.5	40	60
3.6	97	3
5.0	97	3

Chromatograms of UPC² QC Reference Material run on Trefoil and Torus Columns.

The UPC² QC Reference Material is designed for use with both ACQUITY UPC² Trefoil and Torus Columns. This four compound mixture was optimized with the following key chromatographic performance factors in mind:

- Compounds are well separated and cover a wide chromatographic elution range
- Contains a chiral compound to test chiral separation power
- Contains an ionizable compound, to test mass spectrometer performance
- All four compounds are compatible with UV detection

To locate additional information for standards specific to calibration, qualification, and tuning of instruments and detectors, as well as a more comprehensive listing of available standards and reagents, please visit asr.waters.com.

ACQUITY UPC^2 Trefoil Columns Ordering Information

ACQUITY UPC^2 Trefoil Columns				
Dimensions	Particle Size	AMY1	CEL1	CEL2
2.1 x 50 mm	2.5 µm	186007457	186007461	186007654
2.1 x 150 mm	2.5 µm	186007458	186007462	186007655
3.0 x 50 mm	2.5 µm	186007459	186007463	186007656
3.0 x 150 mm	2.5 µm	186007460	186007464	186007657

ACQUITY UPC^2 Trefoil Column Method Development Kits	
Description	Part No.
ACQUITY UPC^2 Trefoil Column Screening Kit, 2.1 x 50 mm columns (AMY1, CEL1, CEL2), 3/pk	176003577
ACQUITY UPC^2 Trefoil Column Optimization Kit, 3.0 x 150 mm columns (AMY1, CEL1, CEL2), 3/pk	176003578

ACQUITY UPC^2 Torus Columns Ordering Information

ACQUITY UPC^2 Torus Columns					
Dimensions	Particle Size	2-PIC	DEA	DIOL	1-AA
VanGuard™ Pre-Column, 2.1 x 5 mm, 3/pk	1.7 µm	186007604	186007622	186007613	186007631
2.1 x 50 mm	1.7 µm	186007596	186007614	186007605	186007623
2.1 x 75 mm	1.7 µm	186007597	186007615	186007606	186007624
2.1 x 100 mm	1.7 µm	186007598	186007616	186007607	186007625
2.1 x 150 mm	1.7 µm	186007599	186007617	186007608	186007626
3.0 x 50 mm	1.7 µm	186007600	186007618	186007609	186007627
3.0 x 75 mm	1.7 µm	186007601	186007619	186007610	186007628
3.0 x 100 mm	1.7 µm	186007602	186007620	186007611	186007629
3.0 x 150 mm	1.7 µm	186007603	186007621	186007612	186007630

ACQUITY UPC^2 Torus Column Method Development Kits	
Description	Part No.
ACQUITY UPC^2 Torus Column Screening Kit, 2.1 x 50 mm columns (2-PIC, DEA, DIOL, 1-AA), 4/pk	176003579
ACQUITY UPC^2 Torus Column Method Development Kit, 3.0 x 100 mm columns (2-PIC, DEA, DIOL, 1-AA), 4/pk	176003580

Torus™ Technology

Additional Ordering Information

ACQUITY UPC^2 BEH, CSH, and HSS Columns

Dimensions	Particle Size	BEH 2-EP	BEH	CSH Fluoro-Phenyl	HSS C_{18} SB, 1.8 µm
VanGuard Pre-Column, 2.1 x 5 mm, 3/pk	1.7 µm	186006575	186006557	186006566	186006616
2.1 x 50 mm	1.7 µm	186006576	186006558	186006567	186006617
2.1 x 75 mm	1.7 µm	186006577	186006559	186006568	186006618
2.1 x 100 mm	1.7 µm	186006578	186006560	186006569	186006619
2.1 x 150 mm	1.7 µm	186006579	186006561	186006570	186006620
3.0 x 50 mm	1.7 µm	186006580	186006562	186006571	186006621
3.0 x 75 mm	1.7 µm	186006581	186006563	186006572	186006622
3.0 x 100 mm	1.7 µm	186006582	186006564	186006573	186006623
3.0 x 150 mm	1.7 µm	186006688	186006686	186006687	186006685

Dimensions	Particle Size	BEH 2-EP	BEH	CSH Fluoro-Phenyl	HSS C_{18} SB
VanGuard Pre-Column, 2.1 x 5 mm, 3/pk	3.5 µm	186006651	186006633	186006642	186006624
2.1 x 50 mm	3.5 µm	186006652	186006634	186006643	186006625
2.1 x 75 mm	3.5 µm	186006653	186006635	186006644	186006626
2.1 x 100 mm	3.5 µm	186006654	186006636	186006645	186006627
2.1 x 150 mm	3.5 µm	186006655	186006637	186006646	186006628
3.0 x 50 mm	3.5 µm	186006656	186006638	186006647	186006629
3.0 x 75 mm	3.5 µm	186006657	186006639	186006648	186006630
3.0 x 100 mm	3.5 µm	186006658	186006640	186006649	186006631
3.0 x 150 mm	3.5 µm	186006659	186006641	186006650	186006632

ACQUITY UPC^2 Method Development Kit

Description	Part No.
ACQUITY UPC^2 Method Development Kit, 3.0 x 100 mm (BEH 2-EP, BEH, CSH Fluoro-Phenyl, HSS C_{18} SB), 4/pk	176003050
ACQUITY UPC^2 Column Screening Kit, 2.1 x 50 mm (BEH 2-EP, BEH, CSH Fluoro-Phenyl, HSS C_{18} SB), 4/pk	176003091

Viridis® Analytical SFC Columns

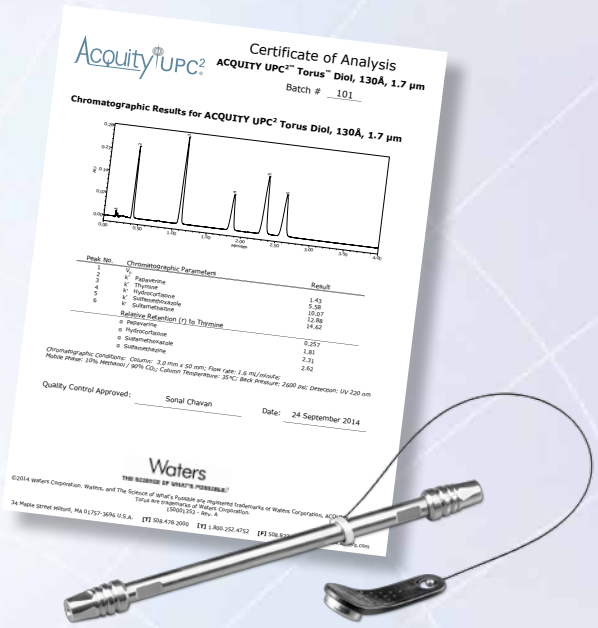
Dimension	Particle Size	BEH 2-EP	BEH	CSH Fluoro-Phenyl	Silica 2-EP	Silica
2.1 x 150 mm	5 µm	186006545	186006544	186006543	186006542	186006541
3.0 x 50 mm	5 µm	186005750	186005719	186005688	186005800	186005804
3.0 x 100 mm	5 µm	186005751	186005720	186005689	186005801	186005805
3.0 x 150 mm	5 µm	186005752	186005721	186005690	186005802	186005806
3.0 x 250 mm	5 µm	186005753	186005722	186005691	186005803	186005807
4.6 x 50 mm	5 µm	186005754	186005723	186005692	186004935	186004908
4.6 x 100 mm	5 µm	186005755	186005724	186005693	186004936	186004909
4.6 x 150 mm	5 µm	186005756	186005725	186005694	186004937	186004910
4.6 x 250 mm	5 µm	186005757	186005726	186005695	186004938	186004911

Viridis Preparative SFC Columns

Dimension	Particle Size	BEH 2-EP	BEH	CSH Fluoro-Phenyl	Silica 2-EP	Silica
OBD™ 10 x 50 mm	5 µm	186008256	186008252	186008248	186008232	186008228
OBD 10 x 100 mm	5 µm	186008257	186008253	186008249	186008233	186008229
OBD 10 x 150 mm	5 µm	186008258	186008254	186008250	186008234	186008230
OBD 10 x 250 mm	5 µm	186008259	186008255	186008251	186008235	186008231
OBD 19 x 50 mm	5 µm	186005762	186005731	186005700	186004943	186004916
OBD 19 x 100 mm	5 µm	186005763	186005732	186005701	186004944	186004917
OBD 19 x 150 mm	5 µm	186005764	186005733	186005702	186004945	186004918
OBD 19 x 250 mm	5 µm	186005765	186005734	186005703	186004946	186004919
OBD 30 x 50 mm	5 µm	186005766	186005735	186005704	186004947	186004920
OBD 30 x 75 mm	5 µm	186005767	186005736	186005705	186004948	186004921
OBD 30 x 100 mm	5 µm	186005768	186005737	186005706	186004949	186004922
OBD 30 x 150 mm	5 µm	186005769	186005738	186005707	186004950	186004923
OBD 30 x 250 mm	5 µm	186005770	186005739	186005708	186004951	186004924
OBD 50 x 50 mm	5 µm	186005771	186005740	186005709	186004952	186004925
OBD 50 x 100 mm	5 µm	186005772	186005741	186005710	186004953	186004926
OBD 50 x 150 mm	5 µm	186005773	186005742	186005711	186004954	186004927
OBD 50 x 250 mm	5 µm	186005774	186005743	186005712	186004955	186004928

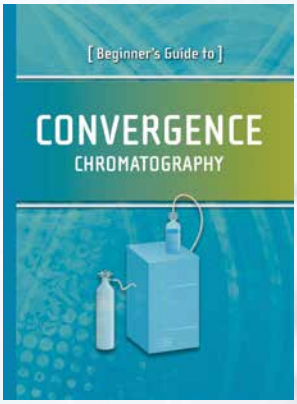
Your Complete UPC² System

Attached to each ACQUITY UPC² Analytical Column is an eCord™ that stores information which is accessed via the ACQUITY UPC² workstation, including all column manufacturing QC data and its Certificate of Analysis.



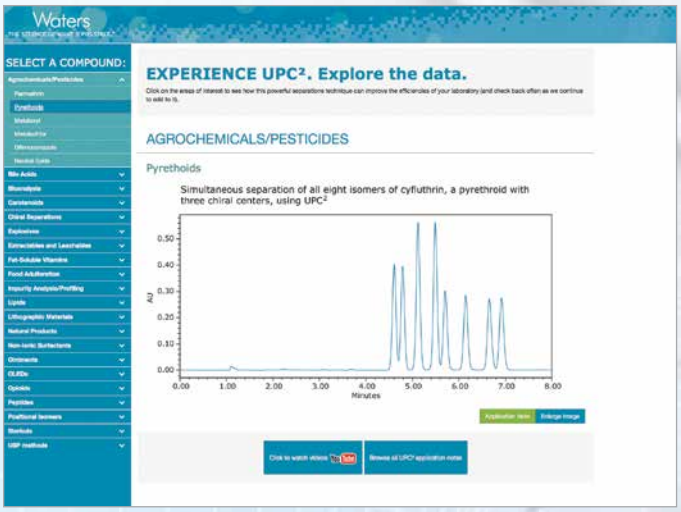
Literature

Description	Literature Code
Viridis SFC Columns Brochure	720003326EN
ACQUITY UPC² System Brochure	720004225EN



Beginner's Guide to Convergence Chromatography

A 64-page book, describes this broad-based, complementary analytical platform that is taking its place as an essential separation technique for analytical laboratories. This primer contains dozens of illustrations, diagrams, and chromatograms describing the fundamentals of convergence chromatography and some of the many applications of the technique. It also outlines the technologies impact on helping scientists overcome analytical challenges and simplify laboratory workflow. For more information, visit: **www.waters.com/CCBook**



UPC² Data Microsite

A large collection of UPC² data developed by Waters scientists, categorized by compound. To browse the selection, visit: **upc2.waters.com**

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Austria 43 1 877 18 07

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Germany 49 6196 400 600

Hong Kong 852 2964 1800

Hungary 36 1 350 5086

India 91 080 49292200 03

Ireland 353 1 448 1500

Israel 9723 3731391

Italy 39 02 265 0983

Japan 81 3 3471 7191

Korea 82 2 6300 9200

Malaysia 603 7841 1688

Mexico 52 55 52 00 1860

The Netherlands 31 76 508 7200

Norway 47 6 384 6050

Poland 48 22 101 5900

Portugal 351 21 893 61 77

Puerto Rico 1 787 747 8445

Singapore 65 6593 7100

Spain 34 93 600 9300

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