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Torus Columns

CONTENTS

I. INTRODUCTION

Thank you for choosing a Torus™ Column. Torus Columns are offered with 1.7 µm and 5 µm particles, allowing scientists to transfer SFC achiral separations from analytical to preparative scale. Torus 1.7 µm Columns are specifically designed to use the complete range of capabilities offered by the ACQUITY UPC^2 System to achieve fast, robust achiral separations. Torus Columns simplify the method development process with four completely new and innovative 1.7 µm chemistries for UltraPerformance Convergence Chromatography™. These columns are designed for excellent peak shape, coupled with added selectivity for a wide range of compounds, and improved robustness. Torus 5 µm Columns are offered in both analytical and preparative column formats.

The Torus OBD™ Preparative Columns combine the patented* optimized packing process with a unique set of column designs ensuring increased lifetime and greater reproducibility.

These columns are based on new bonding technology used to create an adaptive selectivity platform. Starting with the ethylene bridged hybrid (BEH) particle, the patent pending bonding consists of a two stage functionalization of chromatographic particles. The first stage controls retention characteristics and is applied to each sorbent. The individual second stage selectors impart the observed selectivity and control peak shape.

(* US Patent # 7,399,410, UK Patent # GB2408469).

Achiral chemistry	Stationary phase	Particle shape	Particle size (µm)	Pore size (Å)	Pore volume (cc/g)	Surface area (m^2/g)	Endcapped
Torus 2-PIC	2-Picolylamine	Spherical	1.7, 5	130	0.7	185	Proprietary
Torus DEA	Diethylamine	Spherical	1.7, 5	130	0.7	185	Proprietary
Torus DIOL	High-density Diol	Spherical	1.7, 5	130	0.7	185	Proprietary
Torus 1-AA	1-Aminoanthracene	Spherical	1.7, 5	130	0.7	185	Proprietary

Table 1. Physical Characteristics.

The Torus packing materials are designed for use with the ACQUITY UPC^2 Systems and for the Waters SFC Purification Systems. Torus Columns are manufactured in an ISO-certified plant using ultra-pure reagents. Each batch of Torus particles are tested and the results are held to narrow specification ranges to assure excellent, reproducible performance. Every column is individually tested and a Performance Chromatogram and Certificate of Batch Analysis is provided . For Torus 1.7 µm Columns, this information is included on the eCord™ Intelligent Chip. For Torus 5 µm analytical and OBD Preparative Columns, Performance Chromatograms and Certificates of Batch Analysis are included with each column. With the column part number and lot number, this same column performance data and batch information can be found on the Waters web site at www.waters.com/coa.

Torus 1.7 µm Columns are designed and tested specifically for use on ACQUITY UPC^2 Systems. Torus Columns will exhibit maximum chromatographic performance and benefit ONLY when used on holistically-designed ACQUITY UPC^2 Systems since these systems and columns were created and designed to operate together. For these reasons, Waters cannot support the use of Torus 1.7 µm Columns on any system other than an ACQUITY UPC^2 System.

II. GETTING STARTED

Each Torus Column comes with a Certificate of Analysis and a Performance Test Chromatogram. The Certificate of Analysis is specific to each batch of packing material and includes the gel batch number, physical characterization, analysis of unbonded particle, analysis of bonded particles, and a SFC chromatographic batch test. The Performance Test Chromatogram is specific to each individual column and contains the following information: gel batch number, column serial number, USP plate count, USP tailing factor, capacity factor, and chromatographic conditions under normal-phase LC conditions. These data should be stored for future reference.

a. Safety Considerations

SFC columns, while in use, are under pressure with CO_2 and possible modifiers as a supercritical fluid. A major safety concern is frostbite caused by adiabatic cooling when CO_2 decompresses from a fluid to a gas at atmospheric pressure. Pay attention to any frosting on the column or system connections. This indicates a leak, usually with temperatures far below 0 °C.

Any small leak could produce a situation where the LEL (lower exposure limit) is reached. Laboratories should be equipped with CO_2 and/or O_2 sensors when carbon dioxide (CO_2) is in use.

b. Column Connectors and Installation

ACQUITY UPC^2 Systems and Waters SFC Systems utilize tubing and connectors which have been designed to meet stringent tolerance levels and to minimize extra-column volumes. For information on system tubing and connectors, please refer to the ACQUITY UPC^2 System Operator's Guide (Literature Number 720004226EN).

Note: *Scale the flow rate up or down accordingly based upon the column I.D., length, particle size, and backpressure of the Torus Column being installed.*

1. Make sure your co-solvent pump is primed and has an adequate solvent/modifier supply before performing injections. It is recommended to use CO_2 with purity level of 99.97% (food grade) and use high-quality chromatography grade solvents (typically methanol) as organic modifiers.
2. Connect both the inlet and outlet of the column to the SFC system.
3. If the column is still filled with a solvent, use a low flow rate and backpressure setting (100 bar) to start pumping CO_2 and modifier through the column.
4. If you see frosting on the column at the inlet or outlet, tighten the finger-tight fitting or compression screw on that side. If you continue to see frosting, turn off the CO_2 and vent the system. Allow the column to depressurize fully before disconnecting the inlet or outlet to troubleshoot the leaking issue.

c. Column Equilibration

Torus Columns are shipped dry. Equilibrate the column with a minimum of 10-column volumes of the mobile phase prior to use. (Refer to Table 2 for a listing of empty column volumes.) Thermal and chemical equilibration are equally important for chromatography. Ensure that the mobile phase is preheated before entering the column to prevent a thermal mismatch. This can be done by enabling active preheating on an ACQUITY UPC^2 instrument.

d. eCord Installation

Torus 1.7 µm Columns include an eCord button which can be attached to the ACQUITY UPC^2 System. The eCord button should be attached to the side of the column heater module; it is magnetized and does not require specific orientation.

e. Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it. Waters recommends using a suitable solute mixture to analyze the column upon receipt. We recommend that weaker solvents are selected as injection solvents. Peak distortion can occur due to strong solvent effects, resulting in lower efficiency values. (Methanol can be considered a strong solvent under SFC conditions.) It is recommended to use Waters Quality Control Reference Material (p/n 186007950) which contains a mixture of standards specifically chosen for the Torus Columns.

ACQUITY UPC^2 Quality Control Reference Materials	
Intended use	Contents
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

2. Determine the number of theoretical plates (N) and use this value as a benchmark for periodic comparisons.

3. Repeat the test at predetermined intervals to track column performance over time. (Slight variations may occur if performance tests are performed on two different ACQUITY UPC^2 Systems due to the quality of the connections, operating environment, system electronics, reagent quality, and column condition.)

Empty column volumes (mL)	Column internal diameter (mm)						
Column length	2.1 mm	3.0 mm	4.6 mm	10 mm	19 mm	30 mm	50 mm
50 mm	0.2	0.4	0.8	4	14	35	98
100 mm	0.3	0.6	1.7	8	28	70	196
150 mm	0.4	0.8	2.5	12	42	106	294
250 mm	0.5	1.0	4	20	70	176	490

Table 2. Empty column volumes in mL (multiply by 10 for flush solvent volumes).

f. VanGuard Pre-columns

VanGuard™ Pre-columns are 2.1 mm I.D. x 5 mm length guard column devices designed specifically for use on the ACQUITY UPC^2 Systems. VanGuard Pre-columns are packed with the same chemistries and frits as our 2.1 mm I.D. Torus Columns. VanGuard Pre-columns are designed to be attached directly to the inlet side of a Torus Column.

Note: *In order to ensure void-free and leak-free connections, the VanGuard Pre-column is shipped with the collet and ferrule NOT permanently attached. Care must be taken when removing the O-ring that holds these two pieces on the pre-column tubing.*

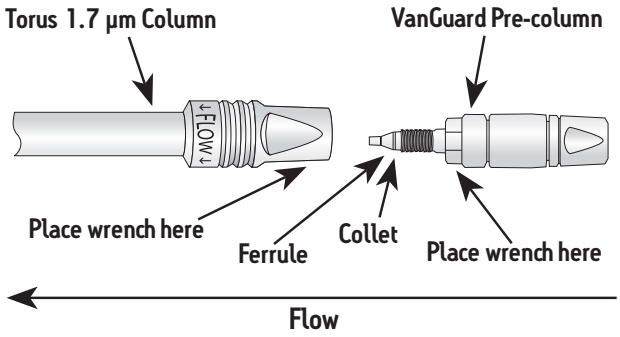

Figure 1. Connecting a VanGuard Pre-column to a Torus 1.7 µm Column.

Installation Instructions

1. Remove VanGuard Pre-column from box and shipping tube and remove plastic plug.

2. Orient pre-column so that male end is facing up and carefully remove rubber O-ring that holds collet and ferrule in place during shipping (collet and ferrule are not yet permanently attached).

3. Orient the Torus Column perpendicular to work surface so that column inlet is on the bottom (facing down), with column outlet on top (facing up).

4. From below, insert VanGuard Pre-column into Torus 1.7 µm Column inlet and hand-tighten (collet and ferrule are not yet permanently attached).
5. While pushing the VanGuard Pre-column into the column inlet, turn assembled column and pre-column 180° so that pre-column is now on top.
6. Tighten with two 5/16" wrenches placed onto Torus Column flats and VanGuard Pre-column hex nut (male end) as shown above.
7. Tighten 1/4 turn to set collet and ferrule.
8. Check that ferrule is set by loosening connection and inspecting the ferrule depth. A properly set ferrule depth will resemble other connections in the ACQUITY UPC^2 System.
9. Reattach pre-column, connect VanGuard Pre-column and analytical column combination to the ACQUITY UPC^2 System, apply mobile-phase flow and inspect for leaks.

III. COLUMN USE

To ensure the continued high performance of Torus Columns, follow these guidelines:

a. Sample Preparation

1. Sample impurities often contribute to column contamination. Waters offers both solid-phase extraction (SPE) and supercritical fluid extraction (SFE) options. For SPE, use Oasis® Solid-Phase Extraction Cartridges/Columns or Sep-Pak® Cartridges of the appropriate chemistry to cleanup the sample before analysis. For more information, visit to www.waters.com/sampleprep. Alternatively, Waters offers the MV-10 ASFE Supercritical Fluid Extraction System for high throughput extractions from a wide variety of sample matrices.
2. Consider preparing the sample in a weak solvent (such as heptane) for the best peak shape and sensitivity. Using weak sample diluents may avoid peak distortion due to "strong solvent effects". In particular, stronger solvents can impact peak shapes of low retaining analytes.
3. If the sample is not dissolved in the mobile-phase modifier, ensure that the sample, solvent and mobile phases are miscible in order to avoid sample precipitation. Filter sample with 0.2 µm membranes to remove particulates. If the sample is dissolved in a solvent, ensure that the membrane material does not dissolve in the solvent. Contact the membrane manufacturer with solvent compatibility questions. Acrodisc® filters are recommended (refer to the Waters Quality Parts,® Chromatography Columns and Supplies Catalog for additional information). Please consider that some analytes can be retained on certain membrane materials and result in lower recovery (or lower detector signal) than expected. Alternatively, centrifugation for 20 minutes at 8000 rpm, followed by the transfer of the supernatant liquid to an appropriate vial could be considered.

b. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet distribution frit of the column. This will result in higher operating pressure, reduced column lifetime, and compromised performance.

c. Additives

Torus Columns can safely be used with commonly used acidic and basic additives used in supercritical fluid chromatography (SFC) such as trifluoroacetic acid (TFA), formic acid, ammonium acetate, ammonium formate, ammonium hydroxide, and organic amines (such as diethylamine and triethylamine) and ammoniated methanol. Typical concentrations of 20 mM or 0.2% can be used safely.

Recommended additives for Torus Column chemistries	20 mM NH_4OH (Ammonium Hydroxide)	20 mM Am. Ac. (Ammonium Acetate)	0.2% TFA (Trifluoroacetic acid)
Torus 2-PIC (2-Picolylamine)	✔	—	✔
Torus DIOL (High density Diol)	—	✔	✔
Torus DEA (Diethylamine)	✔	—	—
Torus 1-AA (1-Aminoanthracene)	✔	✔	✔

Consider the volatility, solubility, and detector compatibility when choosing an appropriate additive. Additives tend to improve peak shape and control the retention characteristics of analytes, but can also impart different selectivities. It is recommended to flush and remove additives and salts from the column before placing the column into storage.

d. Pressure

Torus Column hardware and packing materials are packed and tested to pressures of up to 6000 psi (414 bar or 41 Mpa).

Column Dimensions (mm)	100% CO_2		60/40 CO_2/MeOH	
	Predicted max flow rate (mL/min)	Predicted max pressure (psi)	Predicted max flow rate (mL/min)	Predicted max pressure (psi)
2.1 x 50 mm	3.20	5943	1.55	5928
2.1 x 75 mm	2.25	5925	1.05	5915
2.1 x 100 mm	1.75	5995	0.80	5949
2.1 x 150 mm	1.20	6030	0.55	6053
3.0 x 50 mm	3.75	4295	2.95	5970
3.0 x 75 mm	3.50	5157	2.10	6026
3.0 x 100 mm	3.25	5938	1.60	5991
3.0 x 150 mm	2.30	5947	1.10	6048

Table 3. Useable flow rate ranges and expected ACQUITY UPC² System pressures with Torus 1.7 µm Columns.

e. Temperature

The general guideline for maximum temperature for Torus Columns is 60° C with most common SFC mobile phases and additives. However, some data shows good stability beyond 60° C. This would need to be verified experimentally.

f. Useable Flow-Rate Ranges and Expected ACQUITY UPC² System Pressures

Table 3 estimates maximum flow rate and system pressure which can be achieved without over-pressuring the ACQUITY UPC² System when using Torus 1.7 µm Columns.. The ACQUITY UPC² System has a maximum flow rate of 4 mL/minute and maximum pressure of 6000 psi (~400 bar).

The predicted flow rates and expected backpressure information is based on the ACQUITY UPC² System BPR (Backpressure Regulator) set at 1800 psi (~120 bar).

g. Band Spreading Minimization

The ACQUITY UPC² and SFC systems are designed to minimize band spreading. Deviation from Waters specified tubing could result in deterioration of chromatographic performance due to band spreading induced by inappropriate tubing I.D. Figure 2 shows the influence of tubing internal diameter on system band spreading and peak shape. As can be seen, the larger tubing diameter causes excessive peak broadening and lower sensitivity.

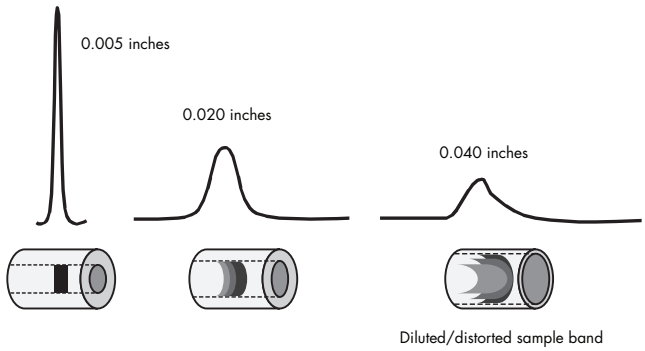

Figure 2: Effect of connecting tubing on system.

IV. TROUBLESHOOTING

1. One of the most common problems with regards to columns is incorrect or insufficient priming of the co-solvent/modifier pump. If no peaks are observed after an injection or unusually long retention times are observed, check the priming of the co-solvent/modifier pump first.

2. If you see frosting on the column at the inlet or outlet, tighten the compression screw on that side. If tightening doesn't work, depressurize the system and the column, and then replace the fitting that is not sealing correctly.

3. If you continue to see frosting on the column, turn off the CO_2 and vent the system. Allow the column to depressurize fully before disconnecting the inlet or outlet. Please contact your Waters representative for additional support.

V. COLUMN CLEANING, EFFECTS OF ADDITIVES, AGING, AND STORAGE

Changes in peak shape, peak splitting, shoulders on the peak, shifts in retention, change in resolution, or increasing backpressure may indicate contamination of the column. Flushing with high concentrations of organic solvent, taking care not to precipitate buffers, is usually sufficient to remove the contaminant. If the flushing procedure does not solve the problem, purge the column using the following cleaning and regeneration procedures.

a. Cleaning

Use the cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column (see Table 4). Placing the column on an ACQUITY UPLC® System (or HPLC system) will enable the use of a wide range of solvents and water to improve cleaning. Flush columns with 20 column volumes of solvent. Increasing column temperature increases cleaning efficiency. If the column performance is poor after cleaning and regenerating, call your local Waters office for additional support.

Note: *Using an ACQUITY UPC^2 System is not recommended for the cleaning and regeneration procedures because recommended solvents and high concentrations of water are immiscible with supercritical CO_2. Rather, the use of an ACQUITY UPLC System is recommended for cleaning and regeneration. (When using the ACQUITY UPLC System for cleaning SFC columns: Unless a Hexane Tetrahydrofuran Compatibility Kit has been installed, running solvents such as THF or Hexane should only be considered when the column cannot be cleaned by running neat, reversed-phase organic solvents such as acetonitrile. Reduce flow rate, lower operationg temperatures, and limit system exposure to THF and/or hexane.)*

Polar samples	Non-polar samples
1. Water (could also be a mixture of acetonitrile and water)	1. Isopropanol (or an appropriate water/ isopropanol mixture)
2. Methanol	2. Tetrahydrofuran
3. Tetrahydrofuran	3. Dichloromethane
4. Methanol	4. Hexane
5. Water	5. Isopropanol (followed by an appropriate water/ isopropanol mixture)
6. Mobile Phase	6. Mobile phase

Table 4: Column cleaning sequence.

Retest the column after using the cleaning procedure to determine if the specific problem has been fixed. If so, continue using the column, avoiding samples and solvents that may clog the column inlet.

b. Effects of Additives

Use of co-solvent additives, such as ammonia or organic amines, can be used successfully with all Torus Columns. Consider the volatility and detector compatibility when choosing an appropriate additive. Additives tend to improve peak shape and control the retention characteristics of analytes, but can also impart different selectivities. An ammonia additive works particularly well on Torus DIOL and Torus 1-AA Columns to improve peak shapes and reduce retention variability. Ion pairing additives should be used with caution on Torus phases, particularly those with exposed amines (e.g. Torus 2-PIC and Torus DEA). Counter ions, such as trifluoroacetate, can bind very strongly or irreversibly with the stationary phase. It is recommended that dedicated columns be used with particular ion pairing additives.

c. Aging

Research at Waters Corporation confirms that there may be changes in retention with SFC columns due to packing material surface changes. This is commonly referred to by SFC industry experts as "aging or retention drift." Mobile-phase additives and methanol can cause changes to the silica or hybrid surface resulting in retention shifts. When working with a new column, the column can be flushed with CO_2/methanol to eliminate or reduce further shifts in retention. Storage in 100% CO_2 is recommended to ensure the column retention and selectivity will be the same from the end of one test to the beginning of another. The 100% CO_2 will halt the aging process and results in no additional retention shifts.

d. Storage

Purging out any additives with a CO_2/methanol mobile phase is recommended and storing columns in pure CO_2.

VI. INTRODUCING ECORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

Supplied with the Torus 1.7 µm Columns, the eCord Intelligent Chip will provide the history of a column's performance throughout its lifetime. The eCord is permanently attached to the column to assure that the column's performance history is maintained in the event that the column is moved from one instrument to another.

Figure 3. eCord Intelligent Chip.

At the time of manufacture, tracking and quality control information will be downloaded to the eCord. Storing this information on the chip will eliminate the need for a paper Certificate of Analysis.

Once the user installs the column, the software will automatically download key parameters into a column history file stored on the chip. In this manual, we explain how the eCord will provide a solution for easily tracking the history of the columns, reduce the frustration of paperwork trails, and give users the reassurance that a well-performing column is installed onto their instruments.

b. Installation

Install the column into the ACQUITY® Column Manager. Plug the eCord into the side of the column heater. Once the eCord is inserted into the column heater the identification and overall column usage information will be available allowing the user to access column information on their desktop.

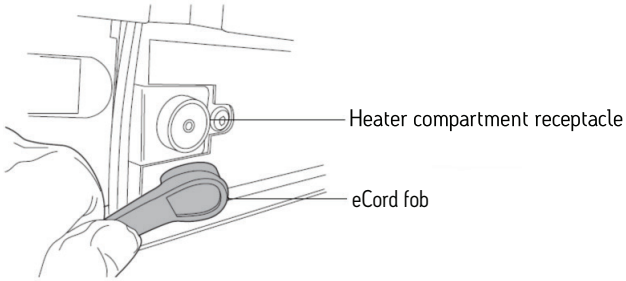

Figure 4. Installing the eCord Intelligent Chip.

c. Column Use Information

The eCord Chip provides the customer with column use data, column dimensions, and serial number. The overall column usage information includes the total number of samples, total number of injections, total sample sets, date of first injection, date of last injection, maximum pressure, and temperature. The information also details the column history by sample set including date started, sample set name, user name, system name, number of injections in the sample set, number of samples in the sample set, maximum pressure and temperature in the sample set, and if the column met basic system suitability requirements. Up to 50 sample sets can be stored on the eCord Chip. In addition, the eCord provides two-way communications between the eCord Chip and Empower® Software.

VII. WATERS SFC COLUMN FAMILY

The Torus Columns Care & Use Manual is one of three manuals for the Waters SFC Column family.

Trefoil™ Columns Care & Use Manual - 720004828EN

Viridis® Columns Care & Use Manual - 720004349EN

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