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Trefoil Columns

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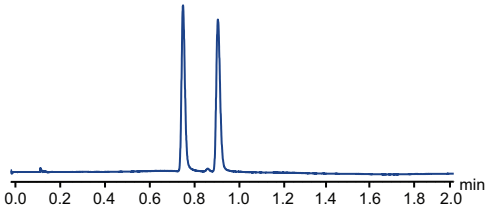
I. INTRODUCTION

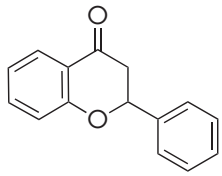
Thank you for choosing a Trefoil® Column. Trefoil Columns are specifically designed to use the complete range of capabilities of the ACQUITY UPC^{2}® System to achieve fast, robust chiral separations to enable both selectivity and speed and to reduce method development time. Trefoil Columns consist of modified polysaccharide coated stationary phases for broad-spectrum chiral selectivity. The higher order structure of the polysaccharides have specific spatial orientations that impart chiral selectivity through well-chosen modifications or selectors.

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 using co-solvents and additive blends that most favorably modulate chiral recognition. These columns are designed to separate enantiomers, stereoisomers, metabolites, degradants, and impurities with greater resolution and speed.

Trefoil Columns for Chiral Separations

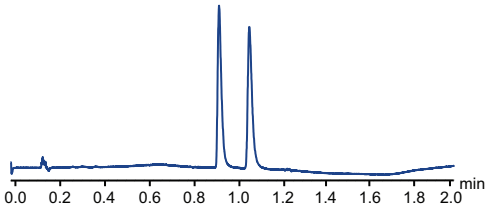
Trefoil AMY1 2.5 µm Columns
Amylose tris-(3,5-dimethylphenylcarbamate)

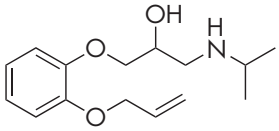




Flavanone

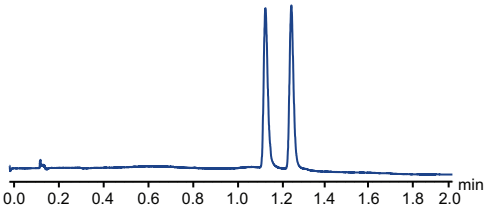
Trefoil CEL1 2.5 µm Columns
Cellulose tris-(3,5-dimethylphenylcarbamate)

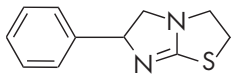




Oxprenolol

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





Tetramisole

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

Trefoil Column: 2.1 x 50 mm
Co-solvent: 1:1 MeOH:IPA w/20 mM NH4OH
Gradient: 3–60% in 1.5 min, 60% for 0.5 min
Flow rate: 1.2 mL/min
Temperature: 40 °C
ABPR: 3,200 psi
UV Detection: 220 nm

The Trefoil packing materials are designed for use with the ACQUITY UPC^2 Systems and are manufactured in an ISO-certified plant using ultra-pure reagents. Each batch of Trefoil material is 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 on the eCord™ Intelligent Chip.

Trefoil Columns are designed and tested specifically for use on ACQUITY UPC^2 Systems. Trefoil 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 Trefoil Columns on any system other than an ACQUITY UPC^2 System.

II. GETTING STARTED

Each Trefoil 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 an 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

An SFC column, while in use, is 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 utilize tubing and connectors which have been designed to meet stringent tolerance levels and to minimize extra-column volume. 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 Trefoil 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 a purity level of 99.97% (food grade) and use high-quality chromatography grade solvents (see section IIIb).
2. Connect both the inlet and outlet of the column to the ACQUITY UPC^2 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. eCord Installation

The eCord button should be attached to the side of the column heater module. The eCord button is magnetized and does not require specific orientation.

d. Column Equilibration

Trefoil 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 missmatch. This can be done by enabling active preheating on an ACQUITY UPC^2 System.

e. Initial Column Efficiency Determination

1. Before use, perform an efficiency test on the column, using suitable analytes dissolved in a weaker injection solvent to avoid peak distortion from strong injection solvent effects (see section IIIb). It is recommended to use Waters Quality Control Reference Material (p/n 186007950) which contains a mixture of standards specifically chosen for the Trefoil Columns. For more information, please review the SFC Columns Brochure (Literature Number 720005706EN).

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**
50 mm	0.2	0.4
150 mm	0.5	1.0

Table 1. Empty column volumes in mL (multiply by 10 for flush solvent volumes).

III. COLUMN USE

To ensure the continued high performance of Trefoil 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 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.

Trefoil Columns are designed for supercritical (compressed CO_2) applications and may be used with compressed CO_2 and the following co-solvents (individually and in miscible combinations): alcohols (typically methanol, ethanol, isopropanol), acetonitrile, and alkanes (typically heptane and hexane). Under SFC conditions, the co-solvent elution strength is in the following order: methanol > ethanol > isopropanol > acetonitrile > alkanes. When dissolving samples for analysis, use an injection solvent that is weaker to avoid peak distortion. Employing blends of methanol, isopropanol, acetonitrile, and/or heptane commonly maintains analyte solubility while avoiding strong injection solvent peak distortion.

Water tends to have an irreversible effect on selectivity. While not damaging the polysaccharide, the selectivity will be permanently effected upon exposure.

Trefoil Columns contain a modified polysaccharide coating which can be damaged when exposed to stronger solvents. Solvents that must be avoided include:

- Ketones such as acetone, methyl ethyl ketone (MEK)
- Halogenated solvents such as chloroform, dichloromethane, methylene chloride
- Ethyl acetate
- Tetrahydrofuran (THF)
- Methyl tert-butyl ether (MTBE)
- Toluene
- Dimethylformamide
- Dimethyl sulfoxide
- N-Methylformamide

c. Additives

Trefoil 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, organic amines (such as diethylamine and triethylamine) and ammoniated methanol. Typical concentrations of 20 mM or 0.2% can be used safely. 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

Trefoil Column hardware and packing materials are packed and tested to pressures of up to 6,000 psi (414 bar or 41 Mpa).

e. Temperature

The recommended maximum operating temperature for Trefoil Columns is 40 °C.

f. Band Spreading Minimization

The ACQUITY UPC² System has been 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 1 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.

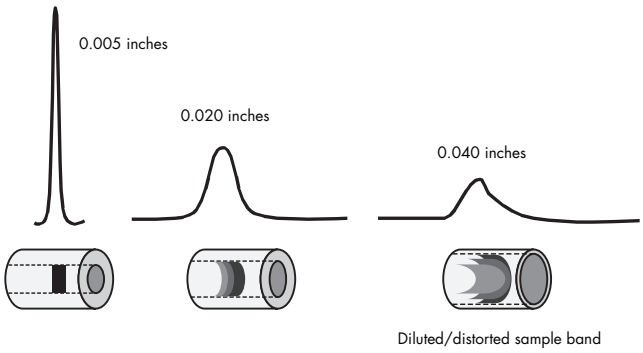


Figure 1: Effect of connecting tubing on system.

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, ADDITIVES, 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 to improve cleaning.Flush columns with 10 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.

Polar samples	Non-polar samples
1. Methanol	1. Isopropanol
2. Isopropanol	2. Heptane
3. Methanol	3. Isopropanol
4. Mobile phase	4. Mobile phase

Table 2. 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 (eg, ammonia, organic amines, formic acid, trifluoroacetic acid) can be used successfully with all Trefoil 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.

c. Storage

If additives were used, flush the column using a minimum of 5 column volumes 50% MeOH/CO_2. Store the columns in 100% CO_2.

VI. INTRODUCING ECORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

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 2. 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.

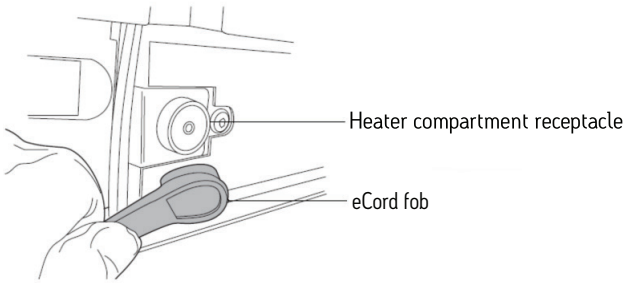


Figure 3. Installing the eCord Intelligent Chip.

c. Column Use Information

The eCord Chip provides the user 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. ACQUITY UPC^2 COLUMN FAMILY

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

Torus™ Columns Care and Use Manual – 720005203EN

Viridis® Columns Care and Use Manual – 720004349EN

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