Anionic Polar Pesticide Column

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I. INTRODUCTION

Thank you for choosing Waters™ Anionic Polar Pesticide Column. This column enables scientists to analyze and separate highly anionic polar pesticides on HPLC, UHPLC, or UPLC™ instrumentation. This column is specifically designed for excellent peak shape, with added selectivity for analysis of various polar pesticides such as glyphosate operating under HILIC/WAX conditions. This column is 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 step of the process controls retention characteristics, while the second provides the unique selectivity of the phase.

Column:	Anionic polar pesticide
Stationary phase:	Diethylamine
Particle shape:	Spherical
Particle size:	5 µm
Pore size:	130 Å
Pore volume:	0.7 cc/g
Surface area:	185 m^2/g
Endcapped:	Proprietary

The packing materials used for the Anionic Polar Pesticide Column are optimized for use with the ACQUITY™ UPLC Systems. The Anionic Polar Pesticide Column is manufactured in an ISO-certified plant using ultra-pure reagents. Each batch of particles are tested, and the results are held to narrow specification ranges to assure excellent, reproducible performance. A Performance Chromatogram and Certificate of Batch Analysis is provided, and the information is included on the eCord Intelligent Chip. With the column part number and lot number, this same column performance data and batch information can be found on the Waters' website at www.waters.com/coa.

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

II. GETTING STARTED

Each Anionic Polar Pesticide 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 UPLC chromatographic batch test. The Performance Test Chromatogram is specific to each batch 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, in accordance with laboratory requirements.

a. 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 UPLC Systems. VanGuard Pre-columns are packed with the same chemistries and frits as the 2.1 mm I.D. Anionic Polar Pesticide columns. VanGuard Pre-columns are designed to be attached directly to the inlet side of Anionic Polar Pesticide 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.*

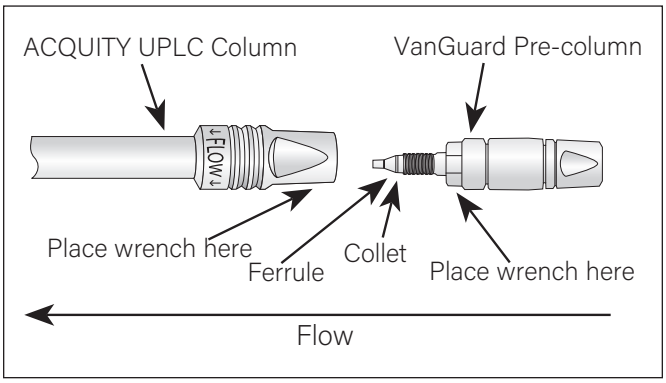


Figure 1. Connecting a VanGuard Pre-column to a column.

Installation Instructions

1. Remove VanGuard Pre-column from the box and shipping tube and then remove plastic plug.
2. Orient the pre-column so that the male end is facing up and carefully remove the rubber O-ring that holds collet and ferrule in place during shipping (collet and ferrule are not yet permanently attached).
3. Orient the Anionic Polar Pesticide Column perpendicular to the work surface so that the column inlet is on the bottom (facing down), with the column outlet on top (facing up).
4. From below, insert VanGuard Pre-column into Anionic Polar Pesticide Column inlet and hand-tighten (collet and ferrule are not yet permanently attached).
5. While pushing the the VanGuard Pre-column into the column inlet, turn the assembled column and pre-column 180˚ so that the pre-column is now on top.
6. Tighten with two 5/16" wrenches placed onto the flats of the Anionic Polar Pesticide Column and VanGuard Pre-column hex nut (male end) as shown above.
7. Tighten 1/4 turn to set collet and ferrule.
8. Check that the ferrule is set by loosening the connection and inspecting the ferrule depth. A properly set ferrule depth will resemble other connections in the ACQUITY UPLC Systems.
9. Reattach the pre-column by connecting the VanGuard Pre-column with the analytical column.

b. Column Installation (with or without a VanGuard Pre-column)

Notes: It is not recommended to run with 100% water for an extended period of time on this column. The Anionic Polar Pesticide Columns are shipped dry.

1. Before connecting the column (or VanGuard and analytical column combination) to the LC system,
   a. Ensure your LC and flow path are clean. For more information, see Section IV of this manual.
   b. Prime the solvent lines, to waste, with mobile phase A (0.9 % formic acid in water) and mobile phase B (0.9 % formic acid in acetonitrile) for at least 3 minutes.

Then connect the inlet end of the column to the injector outlet. Arrows on the column indicate the correct direction of solvent flow.

c. eCord Installation

The Anionic Polar Pesticide Column includes an eCord button which can be attached to the ACQUITY UPLC Systems and should be attached to the side of the instrument's column heater module. The eCord button is magnetized and does not require specific orientation.

d. Column Conditioning and Equilibration

To obtain the retention time and peak shape performance, it is necessary to equilibrate the column with a minimum of 20 column volumes under the initial gradient conditions.

Note: It is not recommended to run with 100% water for an extended period of time on this column.

1. After installing the column in step b, flush the column with 50:50 mobile phase A:B at a flow rate of 0.5 mL/minute for approximately 25 minutes.
2. Equilibrate the column with the method's initial gradient conditions. Allow the column the equilibrate with a minimum of 20 column volumes and the pressure (delta pressure <30 psi) and temperature to stabilize for a minimum of 25 minutes. (Refer to Table 1 for a listing of empty column volumes.)

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

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

e. Useful Functional Tests for Benchmarking a New Column

It may be useful to benchmark the performance of your Anionic Polar Pesticide Column with glyphosate standard as a representative benchmark of the intended application.

It is recommended to run six injections of blank (50:50 water: methanol +0.5% formic acid) solvents before running your samples.

The below method describes LC methodologies for this column. Please ensure your LC is clean. For more information on LC cleaning and maintenance, see section IV.

LC conditions:

LC system	ACQUITY UPLC I-Class PLUS with Sample manager with (FL)
Column	2.1 x 100 mm column
Mobile phase A	0.9% formic acid in water
Mobile phase B	0.9% formic acid in acetonitrile
Strong wash	10:90 Acetonitrile:Water
Weak wash	90:10 Acetonitrile:Water
Seal wash	10% Methanol in water
Column temperature	50 °C
Sample temperature	10 °C
Injection volume	10 µL
Flow rate	0.5 mL/min
MS system	Xevo™ TQ-S micro
Ionization mode	ESI negative
MRM transitions	Glyphosate 168 > 62.9 168 > 150

Gradient method:

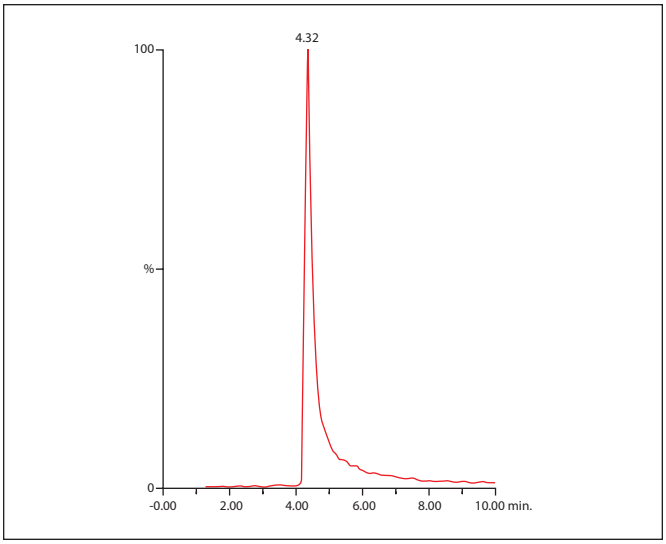
Time (min)	%A	%B	Curve
Initial*	10.0	90.0	–
4.00	85.0	15.0	2
12.0	85.0	15.0	6
18.0	10.0	90.0	1

** The gradient method shown is for the ACQUITY UPLC I-Class PLUS System (BSM), where there is a gradient start delay of 320 µL after injection required (shown in the Figure below).*

For the ACQUITY UPLC H-Class PLUS System (QSM), please use the Column Calculator on your instrument PC to maintain the method's performance.

***Note:** the parameters included are intended to support the startup of this column for the analysis of glyphosate. Additional method information is available in the Quanpedia™ Databases (http://marketplace.waters.com) and application notes (www.waters.com/polarpesticides). For optimum MS performance, compound optimization is recommended (including MS source parameters). For method extension and optimization on your Waters' Systems, please contact your local Waters office to discuss specialist, application support.*

3. Example chromatography of glyphosate solvent standard achieved on the Anionic Polar Pesticide Column



III. COLUMN USE

To ensure the continued high performance of the Anionic Polar Pesticide Columns, follow these guidelines:

a. Sample Preparation

It is highly recommended to use the methods provided with this column (www.waters.com/polarpesticides) however if you are developing a method of your own, it is important to remember the following for proper performance and column longevity.

1. Consider preparing the sample in organic solvents (Methanol or Acetonitrile) for the best peak shape and sensitivity. It is not recommended to use 100% water as a diluent since the separation is operated under HILC condition. Using organic sample diluents may avoid peak distortion due to "strong solvent effects". In particular, stronger solvents can impact peak shapes of low retaining analytes.
2. 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. All samples must be filtered before analysis to ensure removal of particulates.
3. Please use polypropylene vials (p/n: 186005222) for this application as glyphosate fixates to glass.

b. Solvents

To maintain maximum column performance, use high quality LC-MS 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

The Anionic Polar Pesticide Column can safely be used with commonly used high purity acidic and basic additives used in LC-MS such as formic acid, ammonium formate and ammonium hydroxide. 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

The Anionic Polar Pesticide Columns is packed to pressures designed for use on UPLC/UHPLC/HPLC instrumentation.

Column dimensions	*Maximum operating column pressure	Maximum operating column pressure
2.1 x 50 mm	1,800 psi	124 bar
2.1 x 100 mm	3,600 psi	248 bar
2.1 x 150 mm	4,800 psi	331 bar

** Pressure drop across the column, does not include added system pressure*

e. Temperature

The general guideline for maximum temperature for the Anionic Polar Pesticide Column is 60° C with most common LC mobile phases and additives. However, some data shows good stability beyond 60° C. This would need to be verified experimentally.

f. pH

The Anionic Polar Pesticide Columns can be used routinely under HILIC conditions between pH 2 to 7. As with any LC column, operating at the extremes of pH, pressures and temperatures will result in decreased column lifetime.

g. Flow rate

The Anionic Polar Pesticide Columns can be used with flow rates between 0.1 and 0.7 mL/min. Any flow rates should be acceptable as long as they fall within the pressure limits.

IV. TROUBLESHOOTING

To achieve reliable performance of the method, ensuring sensitivity and peak shapes are acceptable, contamination of the LC system should be eliminated. For cleaning of the Waters LC system and other troubleshooting please refer to the startup guide.

Please contact your local Waters representative if you need more guidance and advice on troubleshooting or cleaning your ACQUITY liquid chromatography system.

For non-Waters LC systems, please contact your supplier for specific information on liquid chromatography system cleaning.

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 the column while taking care not to precipitate buffers, is usually sufficient to remove the contaminant. Using a two step flushing procedure, divert the flow to waste and flush the column with an appropriate volume of 20:80 acetonitrile:water, and gradually return to 100% acetronitrile. Be sure to use a flow rate that does not over pressurize the system or column. 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.

Flush the Anionic Polar Pesticide Columns with 50:50 acetonitrile:water to remove polar contaminants. If this flushing procedure does not solve the problem, purge the column with 5:95 acetonitrile:water. To clean polar contaminants from the Anionic Polar Pesticide Column, run a 10-minute gradient from 0–100% water and return to the initial gradient method condition. Please note that as aqueous concentration increases, backpressure will rapidly increase as well. Reduce flow rate when operating at greater than 60% aqueous. Repeat if necessary.

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. If the column performance is poor after cleaning and regenerating, call your local Waters office for additional support.

b. Storage

Purging out any additives with water/acetonitrile (50:50) and storing in 100% acetonitrile is recommended. Alternate storage solvents can be used but may impact initial column equilibration.

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