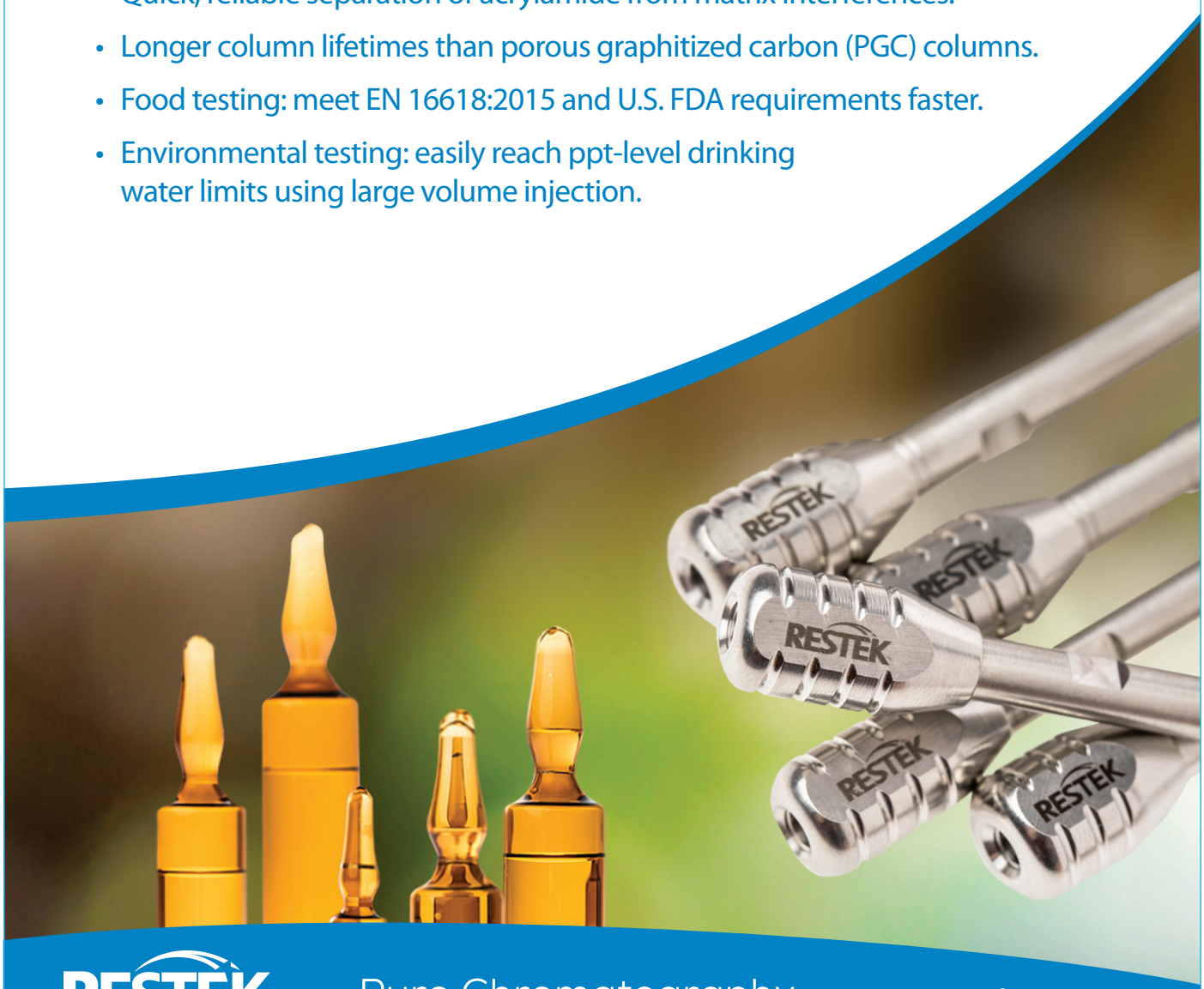
Restek LC

Improve Acrylamide Analysis with Allure Acrylamide LC Columns and Certified Reference Materials

- Quick, reliable separation of acrylamide from matrix interferences.
- Longer column lifetimes than porous graphitized carbon (PGC) columns.
- Food testing: meet EN 16618:2015 and U.S. FDA requirements faster.
- Environmental testing: easily reach ppt-level drinking water limits using large volume injection.



RESTEK Pure Chromatography www.restek.com

A Better Solution for Acrylamide Analysis

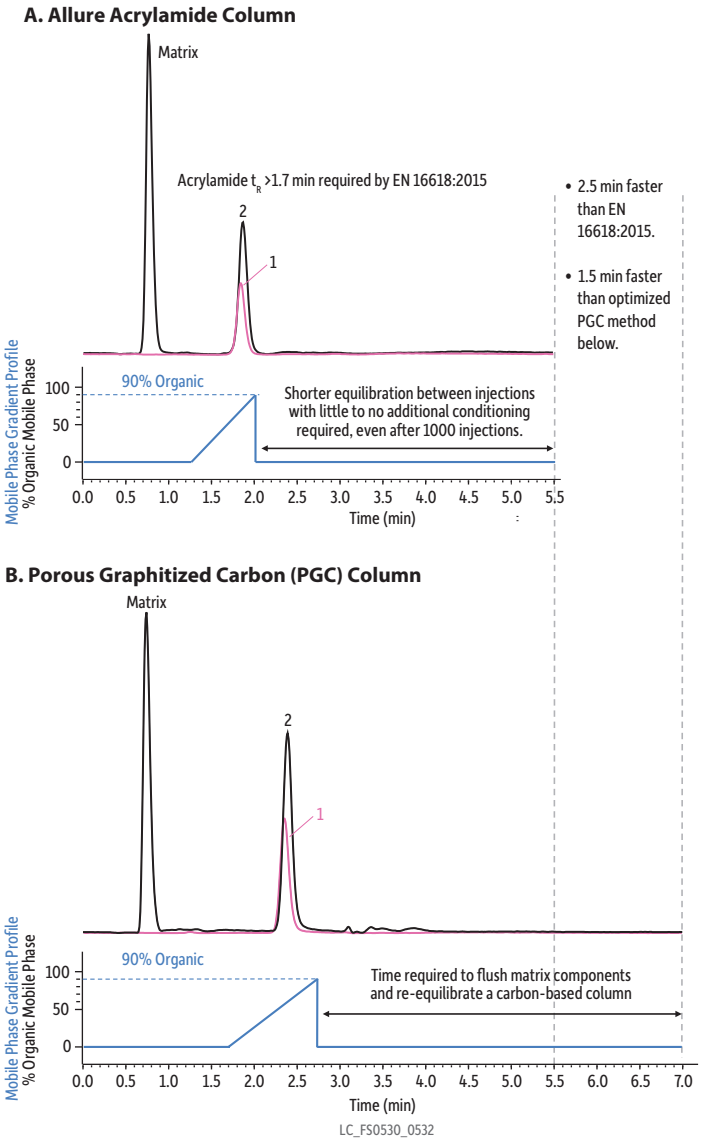
The analysis of acrylamide in food or drinking water is challenging because of sample complexity, matrix interferences, and the need to detect extremely low concentrations. Allure Acrylamide columns were developed specifically for this purpose and provide excellent retention characteristics for separating acrylamide from matrix components. Manufactured with small pore particles and a proprietary bonded stationary phase, they have a unique affinity for small polar molecules such as acrylamide.

In addition to Restek's specialized column chemistry, our application-specific formats, guard columns, and reference standards make it easier to ensure data quality, meet method requirements, and significantly increase sample throughput for acrylamide analysis.

Two Column Formats for Optimal Application-Specific Performance

- **50 x 2.1 mm** for faster analysis times and fewer column changes for food and drinking water with SPE sample prep.
- **150 x 3.0 mm** for using large volume injection to meet ppt-level sensitivity requirements for drinking water.

Figure 1: Labs can increase sample throughput using an Allure Acrylamide column because much less equilibration time is needed compared to a PGC column, even with a complex matrix like potato chips.



Peaks	Conc. (ng/mL)	Precursor	Product
1. Acrylamide-d3 (IS)	200	75.1	58.1
2. Acrylamide	Endogenous	72.1	55.1

Column	See notes
Temp.:	22 °C
Sample	Acrylamide-d3
Diluent:	Water
Inj. Vol.:	10 µL
Mobile Phase	A. 0.001% Formic acid in water, B. 0.001% formic acid in acetonitrile
Detector	MS/MS
Ion Mode:	ESI+
Mode:	MRM
Instrument	HPLC
Notes	Extracted per EN 16618:2015

Weighed 2.0 g of homogenized potato chips into a 50 mL centrifuge tube. Added 40 mL water followed by the addition of internal standard. Shook by hand for 30 sec, by vortexer for 15 sec, and then on a mechanical shaker for 60 min set to maximum sample extraction agitation. Centrifuged in a refrigerated centrifuge at 10 °C, 3600 x g for 20 min. Removed the aqueous layer after centrifugation, taking care to avoid the top, fatty layer, or the solids at the bottom of the tube. Placed the aqueous extract in an appropriate container.

For cleanup, the first SPE cartridge (multimode SPE column with nonpolar, SAX, and SCX properties, 1000 mg/6 mL) was conditioned with 3 mL methanol and x2, 6 mL aliquots of water. Passed 10 mL of the aqueous extract through the column and collected eluate. For the next cleanup step, the second SPE cartridge (crosslinked polystyrene/poly-DVB SPE column, 500 mg/6 mL) was conditioned with 5 mL methanol and 5 mL water. Passed the eluate from the previous step entirely through the column. Rinsed the loaded cartridge once with 4 mL water and discarded the rinsing solvent. Eluted the acrylamide with 2 mL of 60% methanol in water. Collected the sample and transferred into an evaporation tube. Placed the tube in an evaporator at a temperature no higher than 40 °C to remove the methanol. Evaporated until the final volume was 0.5–0.8 mL using a gentle flow of nitrogen. Transferred the final sample into an autosampler vial and analyzed by LC-MS/MS.

Column Details
A. *Allure Acrylamide column:* 5 µm, 50 mm x 2.1 mm ID analytical column (cat.# 9167552) with 5 µm, 10 mm x 2.1 mm ID guard cartridge (cat.# 916750212).
B. *Porous graphitized carbon column:* 5 µm, 50 mm x 2.1 mm ID analytical column with 5 µm, 10 mm x 2.1 mm ID guard cartridge.

Mobile Phase Gradients (%B)
A. *Allure Acrylamide column:* 0.00 min (0%), 1.00 min (0%), 2.00 min (90%), 2.01 min (0%), 5.50 min (0%), flow = 0.4 mL/min.
B. *Porous graphitized carbon column:* 0.00 min (0%), 1.70 min (0%), 2.70 min (90%), 2.71 min (0%), 7.00 min (0%), flow = 0.4 mL/min.

Get Faster Analyses and Longer Column Lifetimes for Food Applications (50 x 2.1 mm Format)

The porous graphitized carbon (PGC) columns typically used for the analysis of acrylamide in food (e.g., EN 16618:2015) provide adequate retention of acrylamide and enough resolution from matrix components, but they are limited by long run times and short column lifetimes. An Allure Acrylamide column is a better alternative because it can elute matrix compounds quickly and be ready for the next injection faster than a PGC column. As shown in Figure 1, a 50 x 2.1 mm Allure Acrylamide analytical column *with* a guard column meets the EN method 1.7-minute retention time requirement with good separation from the matrix components and in much less time. The use of the phase-matched guard column is beneficial as it provides additional retention for later elution and it also protects the analytical column from matrix components. A per analysis savings of 2.5 minutes (compared to EN 16618:2015) or 1.5 minutes (compared to the optimized PGC column conditions shown in Figure 1) allows more samples to be analyzed per shift, significantly increasing lab output.

As labs using PGC columns approach the 1.7-minute acrylamide retention time system suitability threshold, they face the choice of either replacing the column or conducting a lengthy regeneration procedure in the hope of restoring performance. Both are time-consuming and costly propositions that halt sample throughput. Superior retention time stability can be achieved by switching to an Allure Acrylamide column, as illustrated in Figure 2. The PGC column loses retention almost immediately and ultimately fails the 1.7-minute requirement after 475 injections. In contrast, the Allure Acrylamide column performance remains steady even after 1000 injections. Peak shape also remains unchanged, as shown in Figure 3.

Figure 2: The Allure Acrylamide column still meets EN 16618:2015 system suitability requirements even after 1000 injections—over twice as many passing injections than a typical PGC column.

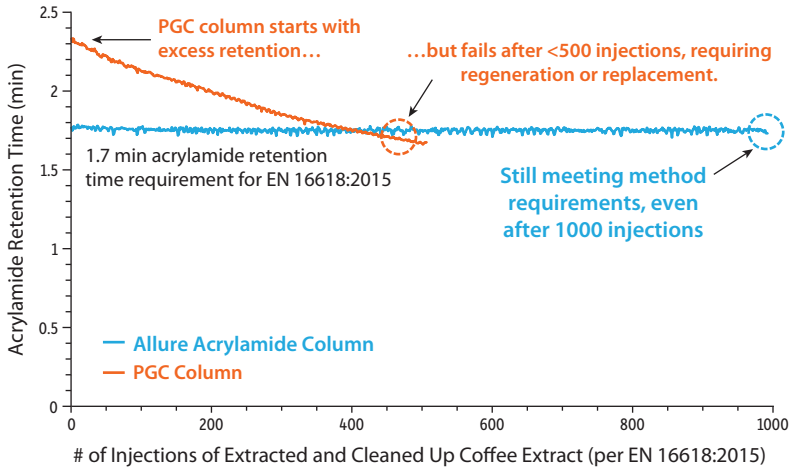
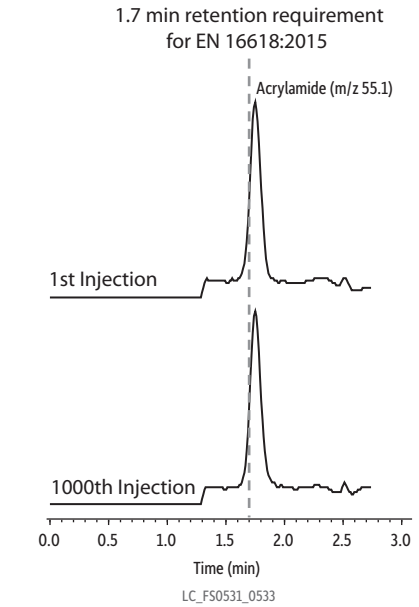


Figure 3: Even after 1000 injections of coffee extract—without any system maintenance or guard column replacement—the Allure Acrylamide column performance remains unchanged.



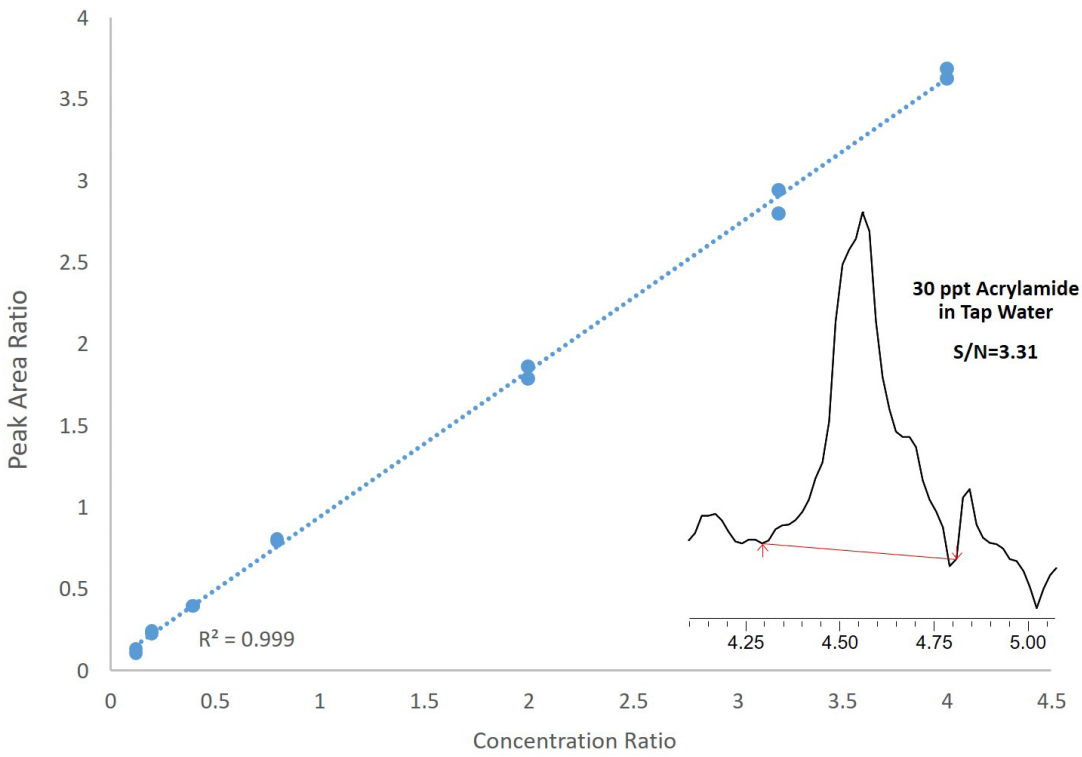
For conditions, visit **www.restek.com** and enter LC_FS0531_0533 in the search.

Easily Reach ppt-Level Drinking Water Limits with Large Volume Injection (150 x 3.0 mm Format)

Low ppt detection and quantitation limits for acrylamide in drinking water are required by various agencies and protocols, and Restek's large volume injection method is an effective approach for meeting 100 ppt LOQ and 30 ppt LOD levels. Drinking water can also be analyzed on a 50 x 2.1 mm column when solid phase extraction (SPE) is used, but for labs wanting to reduce sample preparation time and expense, large volume injection can be an excellent alternative.

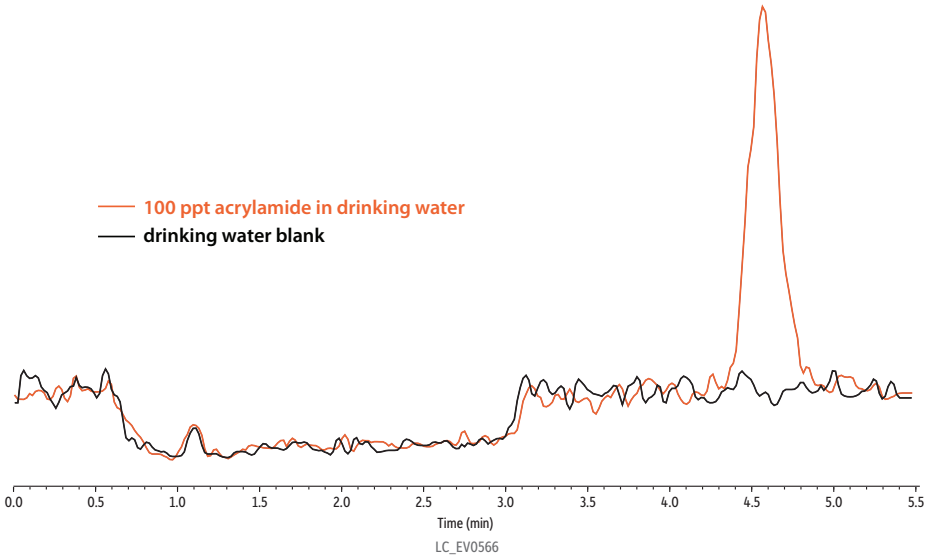
While it is a quick and effective way to gain sensitivity, large volume injection requires a column format that balances adequate capacity with MS-friendly flow rates. The 150 x 3.0 mm Allure Acrylamide column, with its optimized stationary phase selectivity, is sized to both accommodate large volume injections and maintain efficient flow rates into an MS. The result is an analytical column that reliably produces accurate trace-level results for acrylamide in drinking water. As demonstrated in Figure 4, linear responses were obtained across a 30-1000 ppt calibration range. Figure 5 shows a typical response for a 100 ppt QC fortification in comparison to a blank drinking water sample.

Figure 4: Excellent linearity was achieved across a calibration range of 30-1000 ppt using 100 µL injections. (Inset: 30 ppt LOD sample, S:N = 3.31.)



All data for the calibration curve and the 30 ppt LOD were collected using the same instrument conditions shown in Figure 5.

Figure 5: Large volume injection using a 150 x 3.0 mm Allure Acrylamide column is an effective way to meet sensitivity requirements for acrylamide in drinking water (100 ppt LOQ sample vs. matrix blank).



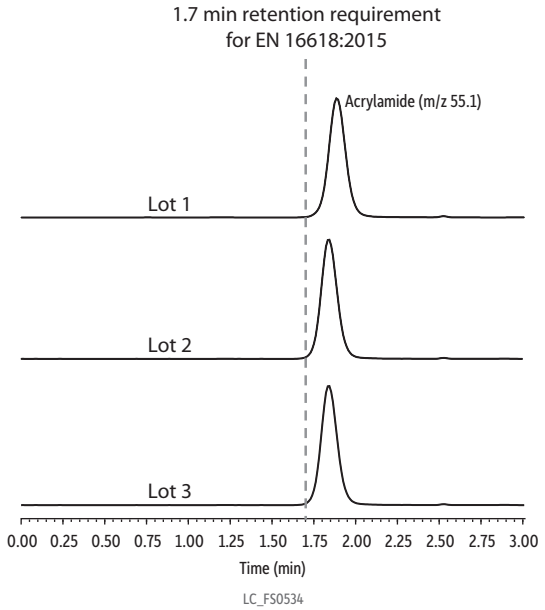
Peaks	t_R (min)	Conc. (ng/L)	Precursor	Product
1. Acrylamide	4.586	100	72.1	55.1

Column Allure Acrylamide (cat.# 916756E)
Dimensions: 150 mm x 3 mm ID
Particle Size: 5 µm
Pore Size: 60 Å
Guard Column: Allure Acrylamide guard cartridge 10 mm, 2.1 mm ID, 5 µm (cat.# 916750212)
Temp.: 22 °C
Sample
Diluent: Tap water
Conc.: 100 ng/L
Inj. Vol.: 100 µL
Mobile Phase
A: 0.001% Formic acid in water

Time (min)	Flow (mL/min)	%A
0.00	0.8	100
5.5	0.8	100

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument HPLC
Notes Tap water samples 1) fortified with 100 ppt acrylamide and 2) neat. Direct injection with 100 µL injection volume.

Figure 6: Rugged, reversed-phase Allure Acrylamide LC columns deliver reproducible results column to column and lot to lot.



For conditions, visit **www.restek.com** and enter LC_FS0534 in the search.

Consistent Performance Injection to Injection and Column to Column

Developed to withstand 100% aqueous mobile phases and elute coextracted matrix components rather than strongly retaining them, Restek Allure Acrylamide guard and analytical columns deliver the same robust performance injection to injection and column to column (Figures 3 & 6). Our stringent manufacturing procedures and rigorous quality testing ensure that when the time comes to replace your Allure Acrylamide column, you will get the same great chromatographic performance with the new column that you relied on with the old one.

Improve Acrylamide Analysis with a Long-Lasting LC Column and a Cost-Effective Internal Standard

Allure Acrylamide LC Column

The Allure Acrylamide LC column provides targeted acrylamide retention as well as improved isolation from matrix interferences to aid in low-level detection and quantitation in food or drinking water matrices. The Allure Acrylamide column is stable enough to last for hundreds of injections of difficult food matrices, such as potato chips/crisps or coffee, without significant loss of acrylamide peak shape or retention. The 150 x 3.0 mm column format accommodates large volume injections, which are used to reach the extremely low limits required for acrylamide in drinking water. The proprietary ligand and bonding process result in much greater stability and longer lifetime compared to carbon-based stationary phases, and an embedded polar group ensures compatibility with 100% aqueous mobile phases. The Allure Acrylamide column is ideal for analyzing acrylamide in drinking water, especially if large volume injections are used to increase sensitivity, and it also provides dependable performance for EN 16618:2015 and U.S. FDA draft procedures for food applications.

ID	Length	qty.	cat.#
5 µm Particles			
2.1 mm	50 mm	ea.	9167552
3.0 mm	150 mm	ea.	916756E

Allure Guard Cartridges

Description	Particle Size	Size	qty.	cat.#
Allure Acrylamide Guard Cartridge	5 µm	10 x 2.1 mm	3-pk.	916750212

25023

Replacement Cap Frit Filters for Trident Guard Cartridges

Replacement guard cartridges can cost as much as an analytical column, so why not protect them, too? The removable cap frit filter in a Trident direct helps prevent clogged cartridges to extend the life of your column, your cartridge, and your budget.

- Use 2 mm cap frit filters with 1.0–2.1 mm ID analytical columns.
- Use 4 mm cap frit filters with 3.0–4.6 mm ID analytical columns.

Description	ID	Porosity	qty.	cat.#
Replacement Cap Frit Filters	4 mm	2.0 µm	5-pk.	25022
	4 mm	0.5 µm	5-pk.	25023
	2 mm	2.0 µm	5-pk.	25057
	2 mm	0.5 µm	5-pk.	25990

Replacement Trident PEEK Ferrules

Description	qty.	cat.#
Replacement Trident PEEK Ferrules	10-pk.	27476

Trident LC Column Protection System

Redesigned to be more rugged and easier to use!

- Match your needs with three levels of protection: filter only, cartridge only, or filter and cartridge.
- Durable metal tip with replaceable PEEK ferrule means easy installation onto column without tools.
- Improved thread design and materials create an optimal seal that releases and reseals easily, allowing multiple installations without galling and binding.
- Easy-to-remove cap frit simplifies filter replacement.
- Direct connection eliminates tubing and connectors that increase system volume and leak potential.
- Low-dead-volume design has negligible effect on chromatography.

27470

Description	Type	Includes	qty.	cat.#
Trident LC Column Protection System	Level 1: Filter Holder Only	filter holder; cap frit filter (4 mm, 2.0 µm); and PEEK ferrule	ea.	27470
	Level 1: Filter Holder Only	filter holder; cap frit filter (4 mm, 2.0 µm); and PEEK ferrule	4-pk.	27471
	Level 2: Cartridge Holder Only	cartridge holder and PEEK ferrule	ea.	27472
	Level 2: Cartridge Holder Only	cartridge holder and PEEK ferrule	4-pk.	27473
	Level 3: Filter Holder and Cartridge Holder Power Pack	filter holder; cap frit filter (4 mm, 2.0 µm); cartridge holder; and PEEK ferrule	ea.	27474
	Level 3: Filter Holder and Cartridge Holder Power Pack	filter holder; cap frit filter (4 mm, 2.0 µm); cartridge holder; and PEEK ferrule	4-pk.	27475

* Fittings on all LC columns have 10-32 threads; however, seat depth varies. An improper seat will yield a poor connection and may affect chromatography. While all Restek LC columns will provide a zero-dead-volume connection when used with a properly installed Trident LC column protection system, analysts should consult the manufacturer for non-Restek column connections. A detailed discussion about port configurations can be found at https://www.restek.com/Pages/faq_lc

Acrylamide

Description	CAS #	Conc. in Solvent	CRM?	Max Shelf Life on Ship Date	Min Shelf Life on Ship Date	cat.#
Acrylamide	79-06-1	1000 µg/mL in methanol, 1 mL/ampul	Yes	24 months	6 months	30494 (ea.)

Acrylamide-d3

Deuterium-labeled acrylamide is an excellent and cost-effective isotopically labeled internal standard choice for the analysis of acrylamide in food or environmental samples.

Description	CAS #	Conc. in Solvent	CRM?	Max Shelf Life on Ship Date	Min Shelf Life on Ship Date	cat.#
Acrylamide-d3	122775-19-3	500 µg/mL in acetonitrile, 5 mL/ampul	Yes	24 months	6 months	30153 (ea.)

RESTEK

Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

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