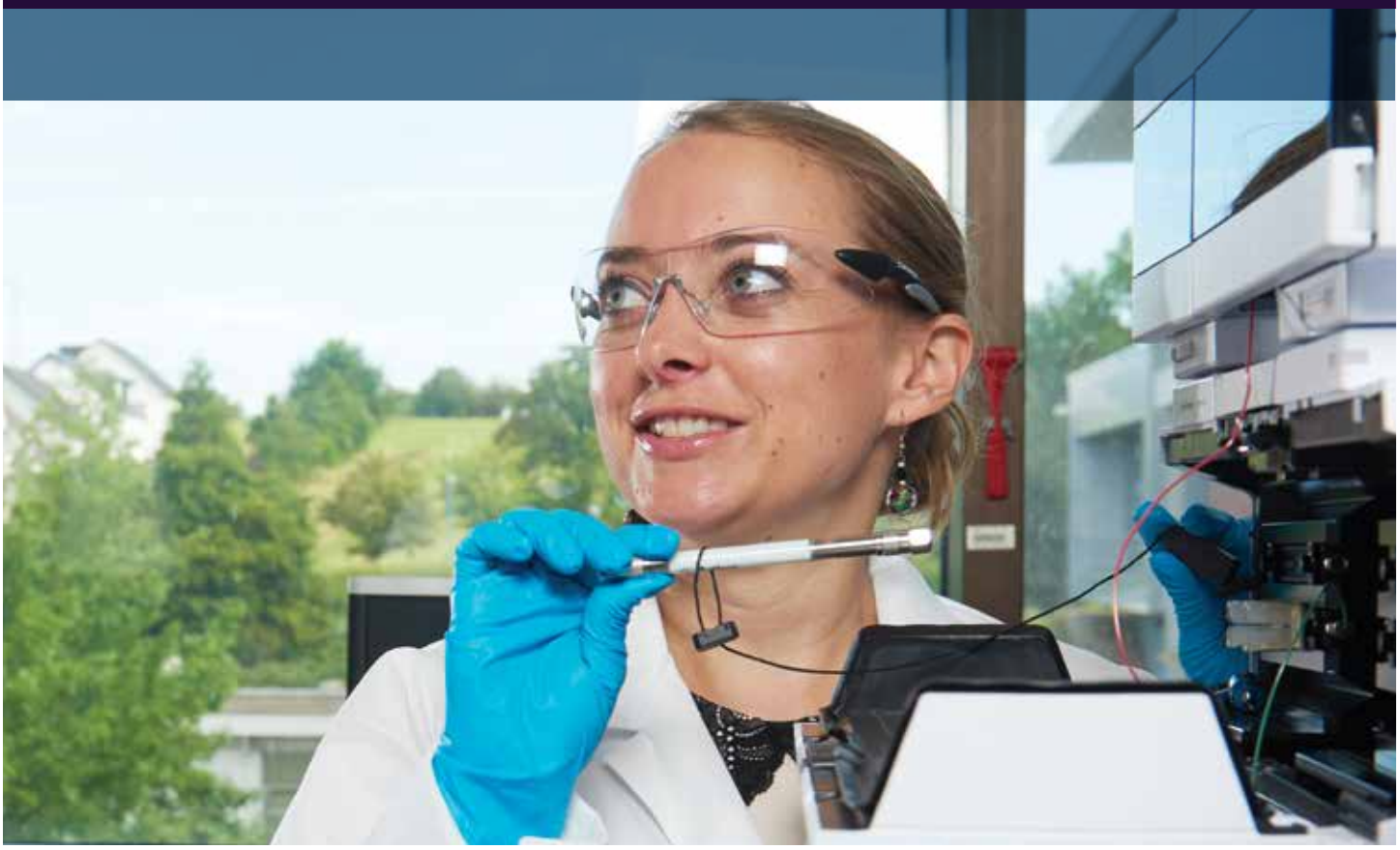
Agilent

Trusted Answers

Agilent Biocolumns

Amino Acid Analysis

"How-To" Guide



Amino Acid Analysis: “How-To” Guide

Accurate results with AdvanceBio end-to-end solution

The Agilent AdvanceBio Amino Acid Analysis (AAA) end-to-end solution optimizes workflow efficiency by combining the advantages of the Agilent InfinityLab LC Series instrumentation and column technology with proven precolumn derivatization chemistry. It is part of the AdvanceBio family that delivers consistent, exceptional performance for the complete characterization of proteins, antibodies, conjugates, new biological entities, and biopharmaceuticals.

This complete, single vendor solution (including chemicals/standards, columns, and application support) provides fast, sensitive, and automated amino acid analysis. It is based on the latest InfinityLab LC Series instrument and column technology. The automated online derivatization in the Agilent 1290/1260 Infinity II vialsampler eliminates tedious manual procedures and delivers reproducible reaction results. AdvanceBio AAA columns provide the speed and resolution of sub-2 µm columns, but with 50 % less backpressure and reduced risk of column clogging.

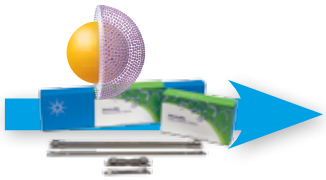
The AdvanceBio AAA solution has evolved from proven Agilent ortho-phthalaldehyde/ 9-fluorenyl-methyl chloroformate (OPA/FMOC) reagents for amino acid derivatization. Together with AdvanceBio AAA columns and standards, these reagents provide an ideal, quantitative and qualitative amino acid analysis that combines speed and sensitivity. When used according to the protocol described in this document, the AdvanceBio AAA solution enables the user to separate the amino acids commonly found in protein/peptide hydrolysates.

.

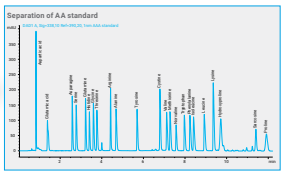
Expert applications and technical support

AdvanceBio AAA reagents and standards
Ready to use

Agilent analytical LC systems
Efficient LC solutions



AdvanceBio AAA columns
Fast, rugged amino acid separation



Fast, reliable data

AdvanceBio AAA columns: Superficially porous particle (SPP) technology

AdvanceBio AAA columns are based on Agilent's innovative 2.7 µm superficially porous particle (SPP) Poroshell technology—particles consist of a 1.7 µm solid core with a 0.5 µm porous shell.

The 2.7 µm SPPs provide 40-50 % lower backpressure with 80-90 % of the efficiency of sub-2 µm totally porous particles. The SPPs have a narrower particle size distribution than totally porous particles, which results in a more homogeneous column bed, and reduces dispersion in the column. At the same time, the thin porous shell provides lower resistance to mass transfer. What's more, since the columns incorporate a 2 µm frit, they are as resistant to clogging as 3.5 and 5 µm columns.

Until recently, all silica-based SPP materials possessed limited lifetime in higher pH buffers, including phosphate buffers. To achieve longer lifetimes, it is necessary to protect the base particle by either surface modification or special bonding modification. The surface is chemically modified using a proprietary process to form an organic layer that is resistant to silica dissolution at high pH conditions.

Part of the **InfinityLab** family

AdvanceBio AAA columns: Superficially porous particle (SPP) technology

The AdvanceBio AAA columns ensure excellent selectivity for amino acid analysis.

Fast and rugged amino acid separation

- The speed and resolution of a sub-2 µm column with up to 50 % less backpressure
- More forgiving for dirty samples, due to 2 µm frits
- Unique chemical modification for high pH stability and column lifetime
- Guard column options reduce your operating costs by extending the life of the columns

Everyday efficiency with confidence

- Higher speed and higher resolution thanks to the operating power up to 600 bar and 5 mL/min
- Injector programming for automated online derivatization
- High-sensitivity UV detection based on diode array technology for uncompromised sensitivity for simultaneous multiwavelength detection
- Optional full spectral detection for identification and peak purity analysis
- Wide flexibility for other LC or UHPLC applications with 100 % HPLC compatibility

Agilent detectors – flexibility in detection

Multiple Wavelength Detector:

Uncompromised sensitivity for simultaneous multiwavelength detection.

Diode Array Detector with spectral data:

Identification and peak purity analysis with more selectivity and fewer matrix effects.

Fluorescence Detector:

Superior sensitivity in the multi-signal mode in the femto-mole range.

Advance your confidence: Agilent AdvanceBio Amino Acid Analysis (AAA)

Achieve fast, sensitive, and reproducible separation of amino acids in biological samples

Steps for AAA analysis

1. Prepare HPLC mobile phases
2. Prepare amino acid standards
3. Prepare Internal Standard (ISTD) stock solution
4. Perform online derivatization
5. Set parameters for detection
6. Run high throughput routine analysis
7. Ensure system suitability per European Pharmacacopoeia (Ph. Eur.)
8. Optimize cell culture media and protein hydrolysate standard

Learn more about analyzing amino acids with utmost confidence, visit

www.agilent.com/chem/advancebioaaa

Step 1:

Prepare HPLC mobile phases

Mobile phase A:

10 mM Na_2HPO_4, and 10 mM $Na_2B_4O_7$ pH 8.2

To prepare 1 L, weigh out 1.4 g anhydrous Na_2HPO_4 and 3.8 g $Na_2B_4O_7 \bullet_{10} H_2O$ in 1 L water. Adjust to approximately pH 8.4 with 1.2 mL concentrated HCl, then add small drops of acid and adjust to a final pH of 8.2. Allow stirring time for complete dissolution of borate crystals before adjusting pH. Filter through 0.45 µm regenerated cellulose membranes (p/n3150-0576).

Mobile phase B:

Acetonitrile:methanol:water (45:45:10, v:v:v)

All mobile-phase solvents are HPLC grade.

Mobile phase A is consumed at a faster rate than mobile phase B. Therefore, we recommend preparing 2 L of mobile phase A for every 1 L of mobile phase B.

Injection diluent

The injection diluent is 100 mL of mobile phase A and 0.4 mL concentrated H_3PO_4. This solution is prepared in a 100 mL bottle that should be stored at 4 °C.

0.1 N HCl

Extended amino acid and internal standard stock solutions are prepared in 0.1 N HCl solution. To prepare 0.1 N HCl, add 4.2 mL concentrated HCl (36%) to a 500 mL volumetric flask that is partially filled with water. Mix, and fill to the mark with water. Store at 4 °C.

Derivatization reagents

Derivatization reagents (borate buffers, OPA, and FMOC) are ready-made solutions supplied by Agilent. Simply transfer these reagents from their container into an autosampler vial. Recommended precautions include:

- OPA is shipped in ampoules under inert gas to prevent oxidation. Once opened, the OPA is potent for about 7 to 10 days. We recommend transferring 100 µL aliquots of OPA to microvial inserts and storing in a refrigerator. Replace the OPA autosampler microvial daily.
- FMOC is stable in dry air but deteriorates in moisture. It should also be transferred to microvial inserts in 100 µL aliquots, and stored in a refrigerator. Like the OPA, an open FMOC ampoule transferred to 10 microvial inserts is potent for about 7 to 10 days.
- Borate buffer can be transferred to a 1.5 mL autosampler vial without a vial insert. Replace every three days.

Step 2:

Prepare amino acid standards

Solutions of 17 amino acids (AA) in five concentrations are available from Agilent (10 pmol/µL to 1 nmol/µL) for calibration curves. Store solutions at 4 °C.

To make the extended amino acid (EAA) stock solution, weigh:

- 59.45 mg asparagine
- 59.00 mg hydroxyproline
- 65.77 mg glutamine
- 91.95 mg tryptophan

Add the weighed out amino acids to a 25 mL volumetric flask, fill halfway with 0.1 N HCL and shake or sonicate until dissolved. Then fill to mark with water for a total concentration of 18 nmol/µL of each amino acid.

For the high-sensitivity EAA stock solution, take 5 mL of this standard-sensitivity solution and dilute with 45 mL water (1.8 nmol/µL). Solutions containing extended standards are unstable at room temperature. Keep them frozen and discard at first signs of reduced intensity.

Step 3:

Prepare Internal Standard (ISTD) stock solution

For primary amino acid ISTD stock solutions, weigh 58.58 mg norvaline into a 50 mL volumetric flask. For secondary amino acids, weigh 44.54 mg sarcosine into the same 50 mL flask. Fill halfway with 0.1 N HCl and shake or sonicate until dissolved. Finally fill to mark with water for a final concentration of 10 nmol for each amino acid/µL (standard sensitivity). For high-sensitivity ISTD stock solution, take 5 mL of standard-sensitivity solution and dilute with 45 mL of water. The final concentration of the high-sensitivity ISTD is 1 nmol for each amino acid/µL. Store at 4 °C.

Calibration curves may be made using two to five standards depending on experimental need. Typically 100 pmol/µL, 250 pmol/µL, and 1 nmol/µL are used in a three-point calibration curve for "standard sensitivity" analysis.

The following tables should be followed if an internal standard or other amino acid (for example, the extended amino acids) is added. Table 1 describes "standard sensitivity" concentrations typically used in UV analysis. Table 2 is typically used for "high sensitivity" fluorescence analysis.

Table 1. Standard sensitivity calibration standards

	Concentration of Final AA Solution (pmol/µL)		
	900	**225**	**90**
Take 5 mL of 18 nmol EAA Dilute with water	5 mL -	5 mL 15 mL	5 mL 45 mL
Diluted EAA mix	5 mL	20 mL	50 mL
Take 5 mL of diluted EAA mix	5 mL	5 mL	5 mL
Add 10 nmol ISTD solution	5 mL	5 mL	5 mL
EAA-ISTD mix	10 mL	10 mL	10 mL
Take 100 µL of EAA-ISTD mix	100 µL	100 µL	100 µL
For 1 nmol AA, add	900 µL	-	-
For 250 pmol AA, add		900 µL	-
For 100 pmol AA, add	-	-	900 µL
Final AA solution with EAA and 500 pmol/µL ISTD	1 mL	1 mL	1 mL

Table 2. High sensitivity calibration standards

	Concentration of Final AA Solution (pmol/µL)		
	90	**22.5**	**9**
Take 5 mL of 1.8 nmol EAA Dilute with water	5 mL -	5 mL 15 mL	5 mL 45 mL
Diluted EAA mix	5 mL	20 mL	50 mL
Take 5 mL of diluted EAA mix	5 mL	5 mL	5 mL
Add 1 nmol ISTD solution	5 mL	5 mL	5 mL
High-sensitivity EAA-ISTD mix	10 mL	10 mL	10 mL
Take 100 µL of EAA-ISTD mix	100 µL	100 µL	100 µL
For 100 nmol AA, add	900 µL	-	-
For 25 pmol AA, add		900 µL	-
For 10 pmol AA, add	-	-	900 µL
Final AA solution with EAA and 50 pmol/µL ISTD	1 mL	1 mL	1 mL

Step 4:

Perform online derivatization

Depending on the autosampler model, the automated online derivatization program differs slightly. For the Agilent G7129A vial sampler with 100 µL capillary*, with injection program is as follows:

1. Draw 2.5 µL from borate vial (p/n5061-3339)
2. Draw 1.0 µL from sample vial
3. Mix 3.5 µL in air five times
4. Wait 0.2 minutes
5. Draw 0.5 µL from OPA vial (p/n 5061-3335)
6. Mix 4.0 µL in air 10 times default speed
7. Draw 0.4 µL from FMOC vial (p/n 5061-3337)
8. Mix 4.4 µL in air 10 times default speed
9. Draw 32 µL from injection diluent vial
10. Mix 20 µL in air eight times
11. Inject
12. Wait 0.1 minutes
13. Valve bypass

* Note: other autosampler models may have a different volume capillary installed, which will require adjustment of volumes

The location of the derivatization reagents and samples is up to the analyst and the sampler tray configuration. Using the G7129A with a 2 × 56 well plate tray (p/n G2258-44502), the locations are:

- Vial 1: Borate buffer
- Vial 2: OPA
- Vial 3: FMOC
- Vial 4: Injection diluent
- P1-A-1: Sample

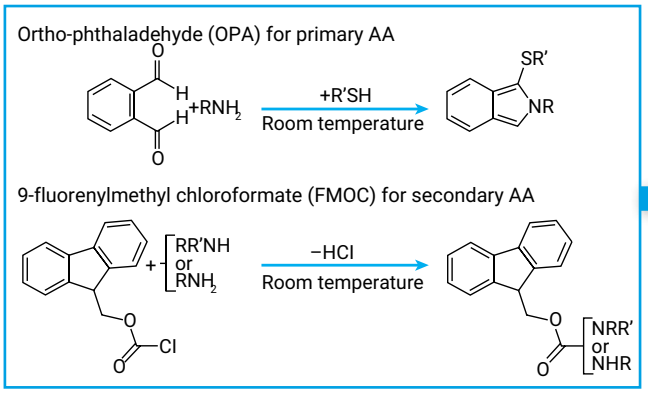
Note: Use the correct vials, closures, and pumps parameters

Conical vial inserts with polymer feet (Figure 1A) are required to hold the OPA and FMOC reagents because of the limited volumes involved. The inserts are compatible with wideopening screw-top (Figures 1B and 1C) or crimp-top vials. For this procedure, an airtight seal is needed for both FMOC, which is highly volatile, and OPA, as it slowly degrades in the presence of oxygen. Snap-cap vials should therefore not be used in this procedure. Be careful not to use vials or caps designed for other instruments, to prevent damage to the auto injector.

Pump parameters for all methods include compressibility (×10-6 bar) A: 40, B: 80, with minimal stroke A, B of 20 µL.

Figure 1. Insert, vial, and cap for amino acid analysis using the Agilent 4226A autosampler: A) Conical insert (Agilent p/n 5181-1270), B) amber wide opening vial (Agilent p/n 5182-0716), and C) screw cap (Agilent p/n 5182-0721).

Increase precision with Autosampler automation



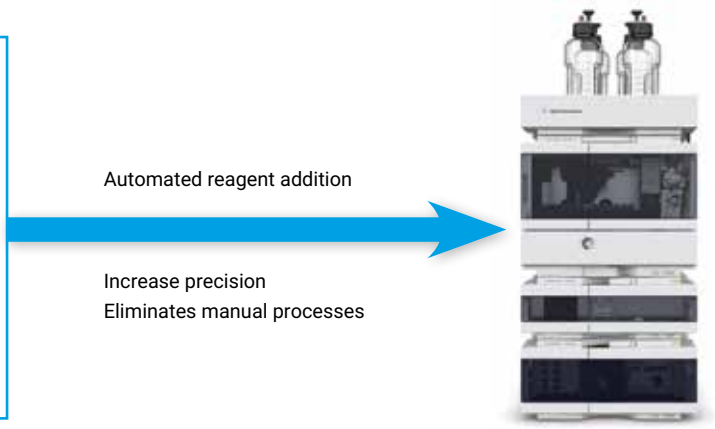


Figure 2. Online derivatization of OPA and FMOC: Separation of polar amino acids on RP-phase and detection by UV and Fluorescence

Step 5:

Set parameters for detection

Thermostatted column compartment (TCC)

Left and right temperatures should be set to 40 °C. Enable analysis when the temperature is within ± 0.8 °C.

Diode array detector (DAD)

Signal A: 338 nm	10 nm bandwidth	Reference wavelength 390 nm	20 nm bandwidth
Signal B: 262 nm	16 nm band-width	Reference wavelength 324 nm	8 nm bandwidth
Signal C*: 338 nmix	10 nm band-width	Reference wavelength 390 nm	20 nm bandwidth

*Signal C is not required if the instructions below are followed.

To detect both OPA and FMOC derivitized amino acids in a single chromatogram it is necessary to switch detector wavelength between the last eluting OPA derivitized amino acid, lysine (peak 20 in the standard), and before the first eluting FMOC derivitized amino acid, hydroxyproline (peak 21 in the standard).

Determining the appropriate transition point using the DAD is possible by initially collecting two channels (Signal A 338 nm, to detect OPA derivitized amino acids and Signal B 262 nm, to detect FMOC derivitized amino acids). This will determine the ideal point at which to switch the wavelength during the run. Subsequent runs can be made using a single channel with the detector timetable function used to program a wavelength switch from 338 to 262 nm at the appropriate time. Between the elution of OPA-lysine and FMOC-hydroxyproline, allow time for both OPA and FMOC derivitized amino acids to be detected in a single chromatogram.

Peak width settings of > 0.01 minutes are used for all columns.

Fluorescence detection

FLD should always be the last detector module in the flow stream to avoid damage to the pressure sensitive flow cell (max 20 bar).

Peak width 0.01 min, stop time 18 min (adjust as needed)

Excitation 340 nm; Emission 450 nm; Filter 390 nm (Default filter)

Timetable Signal:

0.00 min Excitation 340 nm, Emission 450 nm; Gain (as needed)

5.53 min Excitation 260 nm, Emission 325 nm;

PMT Gain 10 (as needed; transition between lysine and hydroxyproline)

To determine the transition point needed with fluorescence detection (FLD), it is necessary to perform two separate runs: the first using Excitation 340 nm, Emission 450 nm to detect the OPA derivitized amino acids and the second using Excitation 260 nm, Emission 325 nm to detect the FMOC derivitized amino acids. Both OPA and FMOC derivitized amino acids can be detected in a single chromatogram, using the detector timetable function. This function programs a wavelength switch at the appropriate point after the last eluting OPA derivitized amino acid, lysine (peak 20 in the standard) and before the first eluting FMOC derivitized amino acid, hydroxyproline (peak 21 in the standard).

Note: If the concentration of amino acids is below 100 pmol, we recommend the use of fluorescence detection.

Gradient program

Time (min)	% B
0	2
0.35	2
13.4	57
13.5	100
15.7	100
15.8	2
18	end

Flow rate: 1.5 mL/min for 4.6 mm id columns and 0.62 mL/min for 3 mm id columns.

Inj. volume: 1 µL with needle wash at the port for 7 s.

Typical Separations

A separation of 20 amino acids using an AdvanceBio AAA column is shown in Figure 3.
The following parameters are noted:

- No change in elution profile of amino acids with and without NaN_3 in mobile phase.
- NaN_3 is used only as a preservative to contain bacterial/fungal growth.
- Filtering the mobile phase using 0.45 µm filter is highly recommended. Note: If the concentration of amino acids is below 100 pmol, we recommend the use of fluorescence detection.

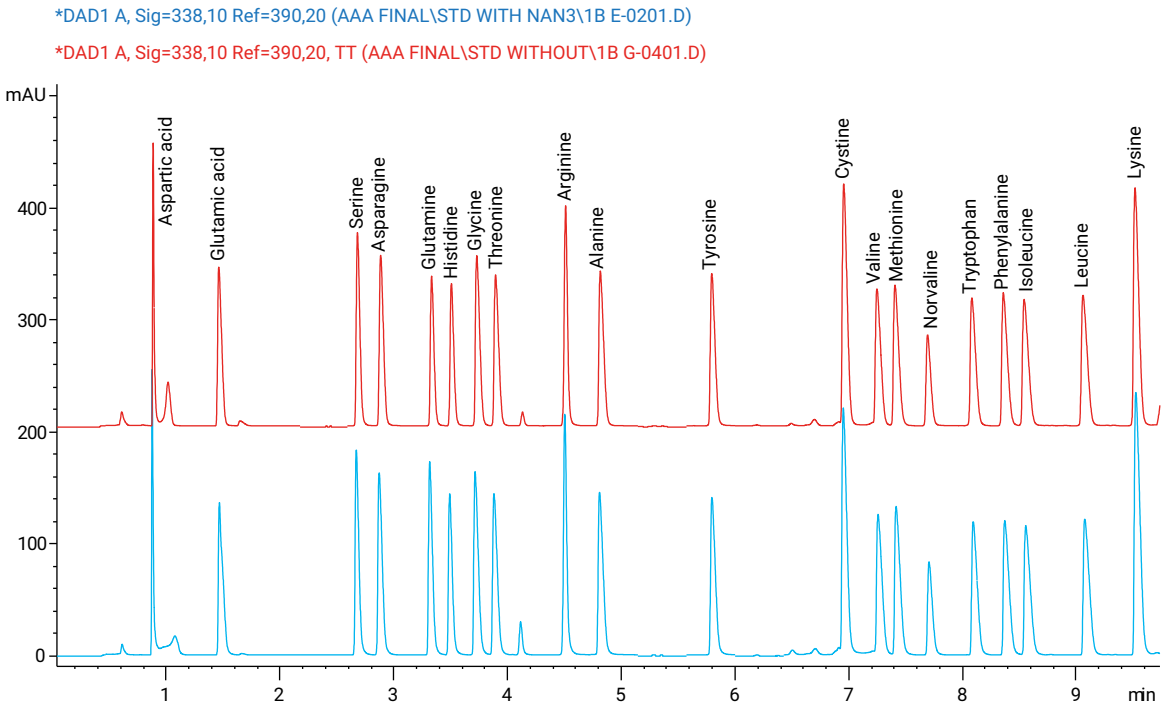


Figure 3. Separation of 20 amino acid standards using an Agilent AdvanceBio AAA 4.6 x 100 mm column with and without 5 mM sodium azide in mobile phase.

Note: Addition of 5 mM sodium azide (NaN_3) to mobile phase A is optional to prevent microbiological growth and extend shelf life of buffers.

Step 6:

Run high-throughput amino acid analysis

The chromatogram in Figure 4 illustrates typical routine standard sensitivity in high-throughput applications that can be obtained using Agilent AdvanceBio AAA columns. These separations were produced using the Agilent 1260 Infinity II HPLC binary system with AdvanceBio AAA, 100 mm, 2.7 µm columns of different internal diameters, and DAD detection. A single run can be completed in under 20 minutes (including re-equilibration) with adequate resolution. The primary amino acids (1-20, OPA-derivatized), were monitored at 338 nm, while the secondary amino acids (21-23, FMOC-derivatized), were monitored at 262 nm.

The first 20 amino acids in Figure 4, the primary amino acids, are derivatized with OPA. The last three, hydroxyproline, sarcosine, and proline, are derivatized with FMOC. A programmable wavelength switch from 338 to 265 nm takes place after lysine (peak 20) elutes and before hydroxyproline (peak 21) elutes.

- The method can easily be scaled to different column dimensions.
- In this case, the only changes to the method were made by altering the flow rate, changed geometrically with the diameter of the column.
- The low-volume heat exchanger was used with short red tubing to minimize extra column volume.

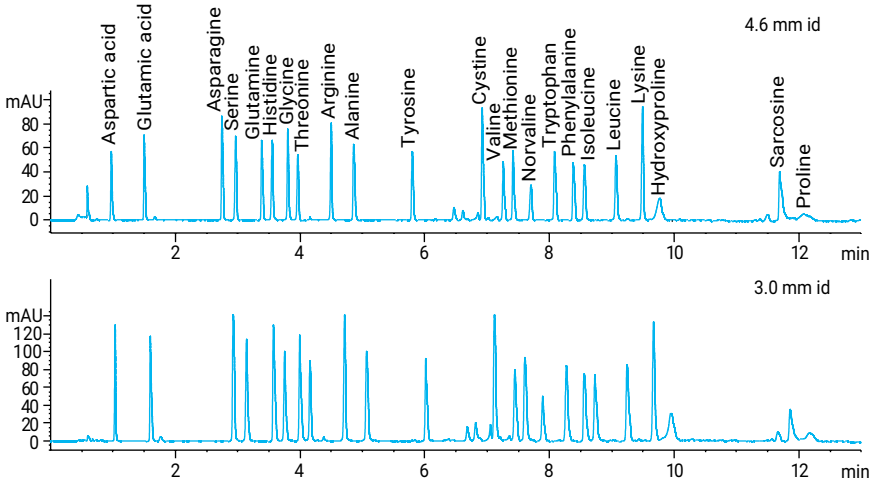


Figure 4. Separation of AA standards using Agilent AdvanceBio AAA columns of different internal diameters using the amino acid method.

Retention time and area precision for 100 pmol and 1000 pmol analysis (n=6)

Table 3. Retention time and area RSD precision for amino acids (100 pmol) separated using an AdvanceBio AAA, 4.6 x 100 mm column (six replicates.)

Amino Acids	Average RT	RT RSD (%)	Area RSD (%)
1. Aspartic acid	0.851	1.270	1.066
2. Glutamic acid	1.428	0.973	1.850
3. Asparagine	2.639	0.605	1.790
4. Serine	2.835	0.629	1.820
5. Glutamine	3.285	0.470	1.560
6. Histidine	3.465	0.430	1.220
7. Glycine	3.681	0.477	1.920
8. Threonine	3.837	0.440	1.950
9. Arginine	4.458	0.251	2.150
10. Alanine	4.764	0.280	3.060
11. Tyrosine	5.762	0.128	1.650
12. Cysteine	6.870	0.067	1.900
13. Valine	7.201	0.084	2.47
14. Methionine	7.363	0.073	1.82
15. Norvaline	7.602	0.073	1.72
16. Tryptophan	8.055	0.054	1.57
17. Phenylalanine	8.341	0.051	1.66
18. Isoleucine	8.503	0.047	1.72
19. Leucine	9.000	0.030	1.70
20. Lysine	9.428	0.028	1.66
21. Hydroxyproline	9.747	0.021	4.13
22. Sarcosine	10.980	0.026	1.15
23. Proline	11.620	0.021	4.36

Table 4. Retention time and area RSD precision for amino acids (1000 pmol) separated using an AdvanceBio AAA, 4.6 x 100 mm column (six replicates).

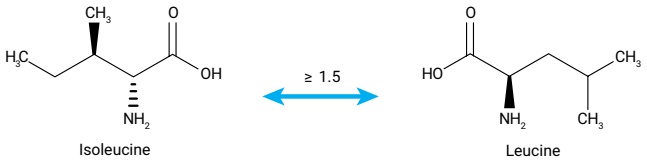
Amino Acids	Average RT	RT RSD (%)	Area RSD (%)
1. Aspartic acid	0.837	0.151	2.60
2. Glutamic acid	1.400	0.512	2.19
3. Asparagine	2.583	0.124	2.13
4. Serine	2.772	0.114	1.74
5. Glutamine	3.220	0.092	1.80
6. Histidine	3.405	0.077	1.39
7. Glycine	3.598	0.068	1.48
8. Threonine	3.766	0.059	2.26
9. Arginine	4.422	0.027	1.66
10. Alanine	4.685	0.031	1.87
11. Tyrosine	5.695	0.034	2.04
12. Cysteine	6.794	0.030	2.22
13. Valine	7.118	0.025	2.40
14. Methionine	7.281	0.025	1.78
15. Norvaline	7.573	0.019	1.77
16. Tryptophan	7.970	0.024	2.03
17. Phenylalanine	8.238	0.027	1.98
18. Isoleucine	8.413	0.025	2.17
19. Leucine	8.925	0.020	1.81
20. Lysine	9.357	0.022	2.00
21. Hydroxyproline	9.718	0.014	3.14
22. Sarcosine	10.961	0.015	5.91
23. Proline	11.911	0.011	2.58

Step 7:

Ensure system suitability as per European Pharmacopoeia

The The European Pharmacacopoeia (Ph. Eur.) defines requirements for the qualitative and quantitative composition of amino acids and mixtures of amino acids. The requirements for allowed impurities are also defined. Manufacturers of amino acids are legally bound to prove that their amino acids meet these specifications before they can distribute their products in Europe.

Leucine (Leu) is a branched-chain α-amino acid, produced by the fermentation process. During this process, isoleucine can be produced as a by-product. The European Pharmacopoeia states that leucine and isoleucine should have a resolution of not less than 1.5 [1]



Isoleucine Leucine

Table 5. System suitability testing using Agilent AdvanceBio AAA columns and AA standards

System Suitability	AdvanceBio AAA, C18, 4.6 × 100 mm, 2.7 µm	AdvanceBio AAA, C18, 3.0 × 100 mm, 2.7 µm
Resolution between Leucine and Isoleucine (≥1.5)	4.5	4.6

Reference:

1. European pharmacopoeia 9.0 (2.2.56)
Amino Acid Analysis

Step 8:

Optimize cell culture media and protein hydrolysate standard

Cell cultures are widely used to produce biopharmaceuticals and other biologically active compounds. The composition of the cell culture media affects the yield and structure of the desired products and must be carefully optimized. Cell culture media is typically composed of mixtures of amino acids, vitamins, carbohydrates, inorganic salts, as well as different peptides, proteins, and other compounds. As the cells grow, they consume nutrients and release target biopharmaceuticals as well as waste products. Amino acids serve as the building blocks of proteins, as well as intermediates in many metabolic pathways. Therefore, amino acids are typically added to cell culture media to provide nutritional requirements for the cells.

Determination of amino acid flux in cultured cells is an important indicator of the metabolic rate and health of those cells. It can also be used as an indicator of the remaining carbon and nitrogenous fuel available. This is especially true in hepatocyte and hepatoma cell lines, where extensive gluconeogenesis, urea production, and protein synthesis may consume larger quantities of amino acids than other cell types.

HPLC with precolumn derivatization is a standard technique in the analysis of amino acids. Precolumn derivatization of free amino acids in solution for HPLC separations with UV or fluorescence detection is at times done manually offline. Some immediate drawbacks to offline derivatization are sources of error due to operator skill, competence, and laboratory technique; extra sample manipulation; extra time required; and increased risk of contamination. Automated online derivatization minimizes these error sources, immediately improves precision, and saves time. A rugged high-resolution HPLC method including online derivatization, therefore, can increase productivity compared to offline methods.

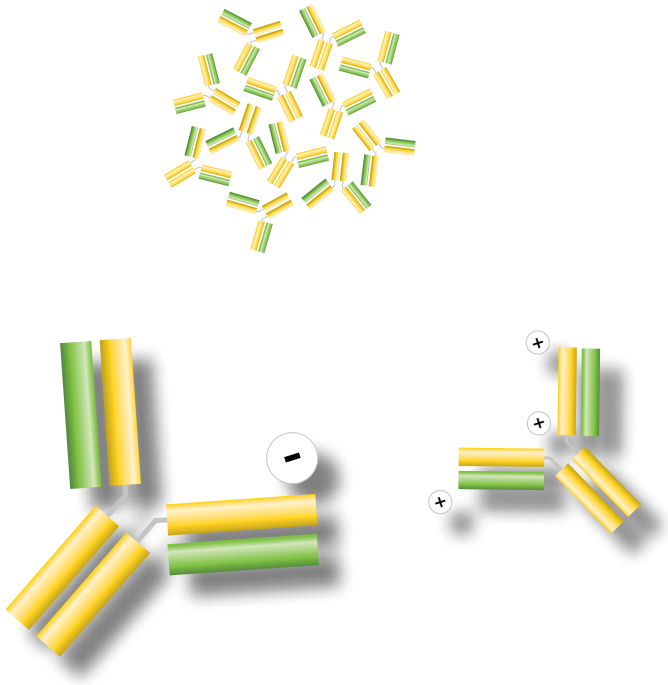
Amino acid compositional analyses of commonly used cell culture media and protein hydrolysate are shown in Figures 5-8. This analysis confirms that the amino acid composition of cell culture media accurately matches with their theoretical composition. Such applications are useful for monitoring and adjusting amino acid composition. This analysis is an essential part of optimizing the manufacturing process to ensure high quality and optimum yield of the final biopharmaceutical product.

The following cell culture media are used for compositional analysis using amino acid method with an AdvanceBio AAA 4.6 x 100 mm column (Figures 5-8).

1. Minimum Essential Medium Eagle (MEM) M4655: L-arginine, L-Cystine, L-Glutamine, L-Histidine, L-Isoleucine, L- Leucine, L-Lysine, L-Methionine, L- Phenylalanine, L-Threonine, L-Tryptophan, L- Tyrosine, and L-Valine.

2. Non-Essential Amino Acid (NEAA) Cell Culture Supplement M7145: L-Alanine, L-Asparagine, L-Aspartic acid, L-Glutamic acid, Glycine, L-Proline, and L-Serine.

3. RPMI 1640 R0083: L-arginine, L-Asparagine, L-Cystine, Glycine, L-Histidine, Hydroxy-L-Proline, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tryptophan, L-Tyrosine, and L-Valine.



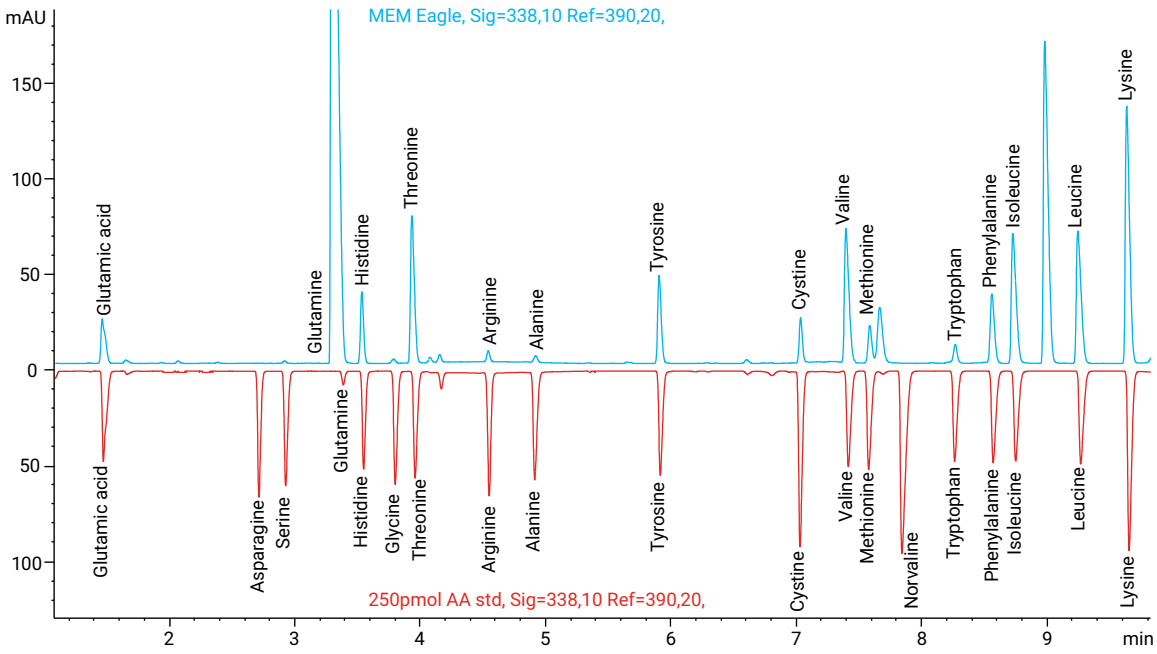


Figure 5. Amino acid analysis of Eagles MEM media (blue trace) and comparison with amino acid standards using the Agilent AdvanceBio AAA solution.

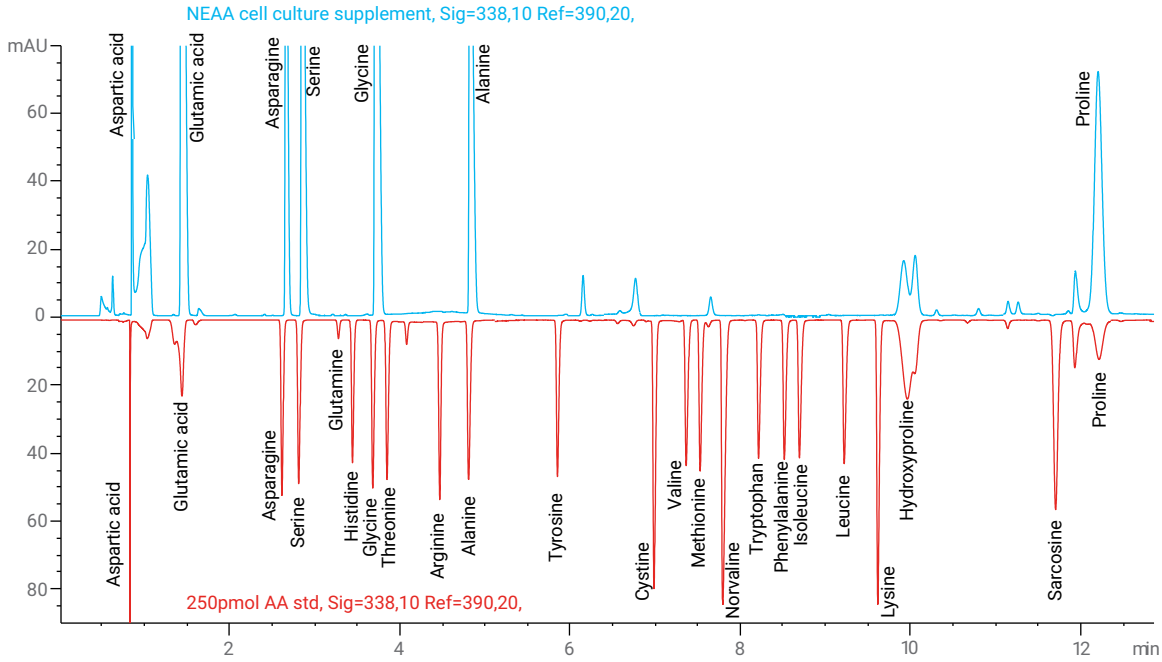


Figure 6. Amino acid analysis of Non-Essential Amino Acid (NEAA) media (blue trace) and comparison with amino acid standard using the Agilent AdvanceBio AAA solution.

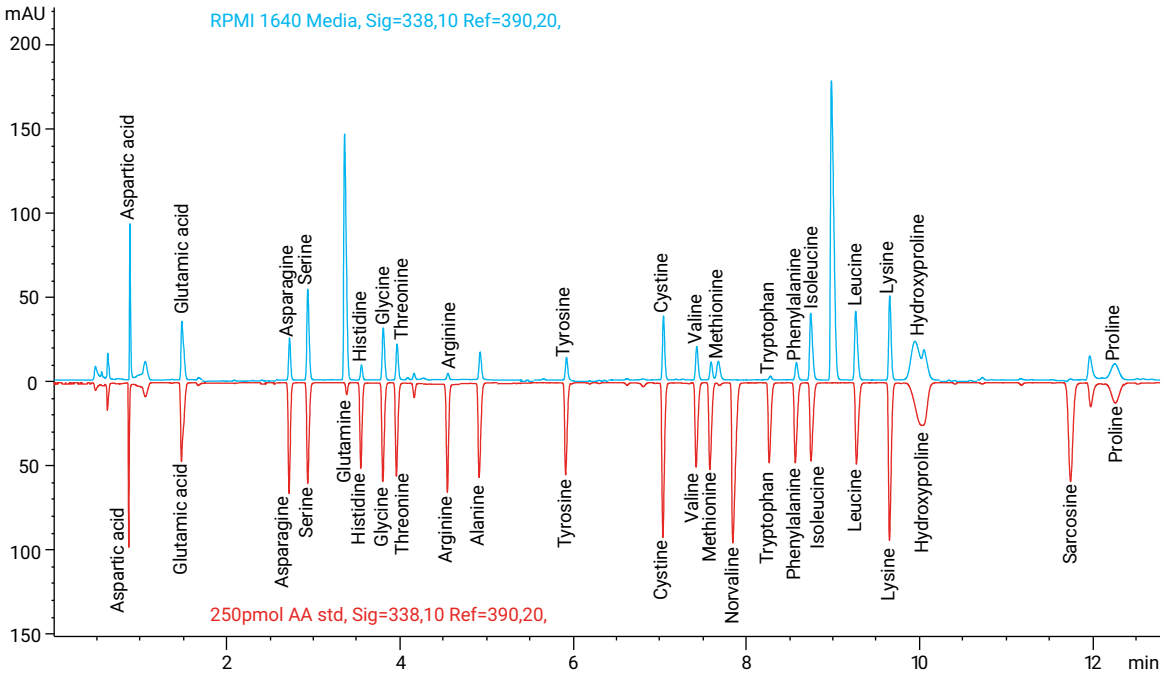


Figure 7. Amino acid analysis of RPMI 1650 media (blue trace) and comparison with amino acid standard using the Agilent AdvanceBio AAA solution.

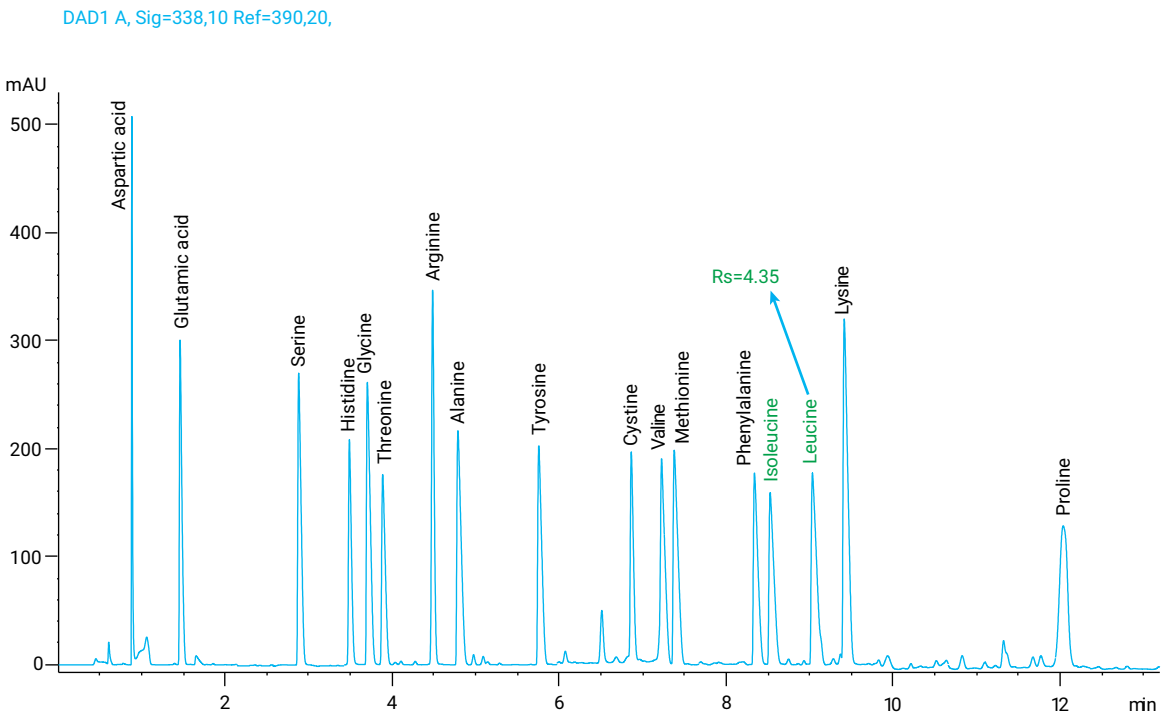


Figure 8. Amino acid analysis of protein hydrolysate. The resolution between leucine and Isoleucine with the AdvanceBio AAA, 4.6 x 100 mm, 2.7 µm column is much higher than the reported value for system suitability requirement.

Maintenance and troubleshooting

The Agilent AdvanceBio AAA solution includes technical and application support. The following maintenance and troubleshooting tips are recommended to keep your InfinityLab LC Series instrument systems running smoothly.

Daily Maintenance:

- Replace derivatization reagent, borate buffer, amino acid standards, and wash water, which are placed in autosampler tray.
- Recalibration of retention times and response factors.
- Check column and guard column performance using system suitability report.
- Every two days replace mobile phase A and B with freshly made solvents

Troubleshooting:

Poor chromatographic resolution

- Exhausted guard column
- Damaged analytical column
- Post column band broadening due to too long connections.
- Always use short red tubing with the low-volume heat exchanger be to minimize extracolumn volume

Low Intensity Chromatogram

- OPA reagent has deteriorated
- FMOC reagent has deteriorated
- Glycine contamination

Ordering Information

Columns, supplies and chemicals	Size	Part No.
AdvanceBio AAA LC column	4.6 x 100 mm, 2.7 µm	655950-802
AdvanceBio AAA guard columns	4.6 x 5 mm, 2.7 µm, 3/pk	820750-931
AdvanceBio AAA LC column	3.0 x 100 mm, 2.7 µm	695975-322
AdvanceBio AAA guard columns	3.0 x 5 mm, 2.7 µm, 3/pk	823750-946
Borate Buffer	0.4 M in water, pH 10.2, 100 mL	5061-3339
FMOC Reagent	2.5 mg/mL in ACN, 10 x 1 mL ampoules	5061-3337
OPA Reagent	10 mg/mL in 0.4 M borate buffer and 3-mercaptoproprionic acid, 6 x 1 mL ampoules	5061-3335
Dithiodipropionic Acid (DTDPA) reagent	5 g	5062-2479
Inserts, with polymer feet	250 µL, 100/pk	5181-1270
Vial, screw top, amber with write-on spot	2 mL, certified, 100/pk	5182-0716
Cap, screw, green, PTFE/white silicone septum	100/pk	5182-0721
Vial, screw top, clear, flat bottom	for LC, 6 mL, certified, 100/pk	9301-1377
Cap, screw	for 6 mL vials, 100/pk	9301-1379
Septum	for 6 mL vials, 100/pk	9301-1378
AA standard	1 nmol/µL, 10 x 1 mL	5061-3330
AA standard	250 pmol, 10/pk	5061-3331
AA standard	100 pmol/µL, 10 x 1 mL	5061-3332
AA standard	25 pmol/µL, 10 x 1 mL	5061-3333
AA standard	10 pmol/µL, 10 x 1 mL	5061-3334
Amino acids supplement kit		5062-2478

Learn more about the Agilent AdvanceBio family of innovations, designed specifically for biomolecule characterization, visit
www.agilent.com/chem/advancebio

Learn more:
www.agilent.com/chem/advancebio

Buy online:
www.agilent.com/chem/store

U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

Published in the USA, March 01, 2020
5991-7694EN
DE.3926157407

Agilent
Trusted Answers