# Sensitive and Reliable Amino Acid Analysis in Protein Hydrolysates using the Agilent 1100 Series HPLC

Technical Note

Pharmaceutical

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## Abstract

This technical note demonstrates the performance of amino acids analysis on the Agilent 1100 Series modules and systems for LC. Detailed information regarding instrumental and chromatographic conditions and performance are given. The precision for retention times over 10 runs is as low as 0.2 % RSD, and the RSD of areas is between 0.6 and 5 %. The limit of detection for the Agilent 1100 Series diode array detector (DAD) is below 5 pmol with a signal-to-noise ratio of 2. For the Agilent 1100 Series fluorescence detector (FLD) the limit of detection is below 100 fmol except for Cys-SS-Cys. Due to better selectivity we recommend the use of the FLD below 100 pmol. The linearity correlation factor for well resolved amino acids is between 0.99998 and 0.99999 in the range from 10 pmol up to 1000 pmol for DAD.

A special amino acid report is shown and hints for maintenance and troubleshooting are included. Ordering information about columns, standards, chemicals and capillaries, and Agilent Application Services is also included.


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## Introduction

Since the introduction of automated amino acid analysis on the HP 1090 Series HPLC system using a two step precolumn derivatization, this technique has become a well accepted and routine method for the analysis of primary and secondary amino acids in protein hydrolysates. This technical note will demonstrate that amino acid analysis can be done on the Agilent 1100 Series modules and systems for LC using the binary pump to obtain even better performance than was achieved with the HP 1090 Series. The conditions used are the same, that were used on the HP Amino-Quant 2 based on the HP 1090 Series system.

## Experimental

### HPLC Instrumentation:

The following Agilent 1100 series modules were used:

- high pressure gradient pump
  order number: G1312A
- online vacuum degasser
  order number: G1322A
- autosampler
  order number: G1313A ( or
  thermostatted autosampler
  order number: G1327A)
- thermostatted column
  compartment
  order number: G1316A
- diode array detector for
  concentrations above 100 pmol
  order number: G1315A
- fluorescence detector for
  concentrations below 100 pmol
  order number: G1321A
- Agilent ChemStation
  order number: G1319A
- Software for amino acid reports
  part number G1300-10013
  (user contributed software).

One modification was made for the standard high pressure gradient pump. The solvent mixer (part number G1312-87330) was replaced by a capillary (part number 1090-87610) or Upchurch mixer to reduce delay volume.

### Amino acid Standards

Five different concentrations of amino acid standards were used for evaluating the precision of retention times and areas, and the limit of detection and linearity. These were 10, 25, 100, 250 and 1000 pmol/µl. The standards contained the following compounds:

| | |
|---|---|
| Asp | Aspartic acid |
| Glu | Glutamic acid |
| Ser | Serine |
| His | Histidine |
| Gly | Glycine |
| Thr | Threonine |
| Ala | Alanine |
| Arg | Arginine |
| Tyr | Tyrosine |
| Cys-SS-Cys | Cystine |
| Val | Valine |
| Met | Methionine |
| Phe | Phenylalanine |
| Ile | Isoleucine |
| Leu | Leucine |
| Lys | Lysine |
| Pro | Proline |

### Derivatization Reagents

The online derivatization was performed using ortho-phthalaldehyde (OPA) for the primary amino acids and 9-fluorenylmethyl chloroformate (FMOC) for the secondary amino acids. A 0.4 N borate buffer was used with pH 10.4.

## Preparing mobile phases, standards and derivatization reagents

### Mobile phase A:

1. Weigh 1.36 ± 0.025 g of sodium acetate tri-hydrate and transfer it into a 800 ml glass beaker.

2. Add 500 ml of purified water and stir until all crystals are completely dissolved.

3. Add 90 µl of triethylamine (TEA) and mix.

4. Adjust the pH to 7.20 ± 0.05 by adding a few drops of 1–2 % acetic acid.

5. Add 1.5 ml of tetrahydrofuran (THF) and mix.

### Mobile phase B:

1. Weigh 1.36 g ± 0.025 g of sodium acetate trihydrate and transfer it into a 200 ml glass beaker.

2. Add 100 ml of purified water and stir until all crystals are dissolved.

3. Adjust pH to 7.20 ± 0.05 by adding a few drops of 1–2 % acetic acid.

4. Add this solution into a mixture of 200 ml acetonitrile and 200 ml methanol and mix.

### Derivatization reagents:

For convenience use microvial kit from Agilent (part number 9301-1388)

### OPA reagent

1. Place several microvials in the microvial rack.

2. Open one ampule of the OPA reagents. This is the approximate amount needed for ten days. Preparing more could cause problems with oxidation.

3. Pipette between 50 and 100 µl into each microvial.

4. Cap all vials. Store vials which are not directly used in the refrigerator.

### FMOC reagent

1. Place several microvials in the microvial rack.

2. Open one ampule of the FMOC reagents. This is the approximate amount needed for ten days.

3. Pipette between 50 and 100 µl into each microvial.

4. Cap all vials. Store vials which are not directly used in the refrigerator.

### Borate buffer

Place 1 ml of borate buffer into a 2 ml vial.

### Water

Place 1 ml purified water into a 2 ml vial.

### Preparing internal standards of 5 nmol and 500 pmol

The secondary amino acids were quantified using sarcosine as an internal standard. The primary amino acids can be quantitated by either using external standard procedures or by using norvaline as an internal standard. If you are working with DTDPA to convert cystine and cysteine to the stable Cys-MPA during hydrolysis, then make up your ISTD stock solutions in borate buffer instead of HCl to avoid solubility problems.

1. 5 nmol ISTD standard:
   Weigh 22.3 mg sarcosine (optional 29.3 mg norvaline) and dissolve in 50 ml 0.1 N HCl.

2. 500 pmol ISTD standard:
   Take 5 ml of 5 nmol ISTD and dilute with 50 ml 0.1N HCl.

### Preparing the calibration standards

For standard sensitivity eight different concentration were used:

1. 900 pmol/µl amino acid standards with 500 pmol/µl internal standards.
   Place 900 µl from standard with 1000 pmol amino acids and 100 µl from ISTD (5 nmol) in a 2 ml vial. Mix and place 100 µl each in microvials

2. 225 pmol/µl amino acid standards with 500 pmol/µl internal standards. Use 250 pmol amino acid standard and follow the same procedure as under 1.

3. 90 pmol/µl amino acid standards with 500 pmol/µl internal standards.
   Use 100 pmol amino acid standard and follow the same procedure as under 1 of this section.

## For high sensitivity three different concentrations were used:

1. 90 pmol/µl amino acid standards with 50 pmol/µl internal standards.
   Place 900 µl from standard with 100 pmol amino acids and 100 µl from ISTD (500 pmol) in 2 ml vial. Mix and place 100 µl each in microvials.

2. 22.5 pmol/µl amino acid standards with 50 pmol/µl internal standards.
   Use 25 pmol amino acid standard and follow the same procedure as under 1.

3. 9 pmol/µl amino acid standards with 50pmol/µl internal standards.
   Use 10 pmol amino acid standard and follow the same procedure as under 1.

| |
|---|
| **Standard sensitivity** |
| 9 nmol/µl extended amino acid with 5 nmol/µl internal standards |
| 2.25 nmol/µl extended amino acid with 5 nmol/µl internal standards |
| 900 pmol/µl extended amino acid with 5nmol/µl internal standards |
| **High sensitivity** |
| 900 pmol/µl extended amino acid with 500 pmol/µl internal standards |
| 225 pmol/µl extended amino acid with 500 pmol/µl internal standards |
| 90 pmol/µl extended amino acid with 500 pmol/µl internal standards |

**Table 1**
**Stock solutions**

## Extended Amino Acids (EAA)

In addition to the previously analyzed 17 amino acids which can be found in hydrolysates (see figure 1), the amino acid supplement kit contains the following amino acids as solids, which are of interest for amino acid analysis in food:

norvaline
sarcosine
asparagine
glutamine
tryptophan
and hydroxyproline

Table 1 shows the stock solutions used for different purposes.

Use these stock solutions in place of the 5 nmol ISTD or 500 pmol ISTD stock solutions previously described, to prepare your calibration standards.

## Preparing internal standards of 10 nmol and 1 nmol

The secondary amino acids were quantitated using sarcosine as an internal standard. The primary amino acids can be quantitated by either using external standard procedures or by using norvaline as an internal standard. If you are working with DTDPA to convert cystine and cysteine to the stable Cys-MPA during hydrolysis, then make up your ISTD stock solutions in a borate buffer instead of HCl to avoid solubility problems.

1. 10 nmol ISTD standard:
   Weigh 44.6 mg sarcosine (optional 58.6 mg norvaline) and dissolve in 50 ml 0.1 N HCl.

2. 1 nmol ISTD standard:
   Take 5 ml of 10 nmol ISTD and dilute with 50 ml 0.1 N HCl.

## Preparing the stock solution of the extended amino acid standards

For standard sensitivity an 18 nmol/µl concentration was used:

Weigh
59.5 mg asparagine
65.7 mg glutamine
91.8 mg tryptophan
59.0 mg 4-hydroxyproline
into a 25 ml graduated flask.

For high sensitivity a 1.8 nmol concentration was used:
5 ml of the 18 nmol EAA was placed into a 50 ml flask and made up to 50 ml with 0.1N HCl

Table 2 details the preparation of the calibration standards for standard sensitivity, with table 3 showing the calibration standards for high sensitivity.

| Concentration | 90 pmol of final solution | 22.5 pmol | 9 pmol |
|---|---|---|---|
| Amount of 18 nmol EAA | 5 ml | 5 ml | 5 ml |
| Dilute with 0,1N HCl | — | 15 ml | 45 ml |
| Amount of diluted solution from above | 5 ml | 5 ml | 5 ml |
| Amount of 10 nmol ISTD | 5 ml | 5 ml | 5 ml |
| Amount of mixed solution from above | 100 µl | 100 µl | 100 µl |
| Amount of 1000 pmol AA standard | 900 µl | — | — |
| Amount of 250 pmol AA standard | — | 900 µl | — |
| Amount of 100 pmol AA standard | — | — | 900 µl |

**Table 2**
**Preparing the calibration standards for standard sensitivity**

| Concentration | 90 pmol of final solution | 22.5 pmol | 9 pmol |
|---|---|---|---|
| Amount of 18 nmol EAA | 5 ml | 5 ml | 5 ml |
| Dilute with 0,1N HCl | — | 15 ml | 45 ml |
| Amount of diluted solution from above | 5 ml | 5 ml | 5 ml |
| Amount of 1 nmol ISTD | 5 ml | 5 ml | 5 ml |
| Amount of mixed solution from above | 100 µl | 100 µl | 100 µl |
| Amount of 100 pmol AA standard | 900 µl | — | — |
| Amount of 25 pmol AA standard | — | 900 µl | — |
| Amount of 10 pmol AA standard | — | — | 900 µl |

**Table 3**
**Preparing the calibration standards for high sensitivity**

## Results

The analysis of amino acids using precolumn online derivatization with OPA and FMOC can be accomplished using UV (figure 1) or fluorescence detection (figure 2). We recommend the use of fluorescence detection if the concentration of amino acids is below 100 pmol.

**Chromatographic conditions**

| | |
|---|---|
| Column: | 200 × 2.1 mm AA column and guard column |
| Mobile phase: | A = 20 mMol NaAc + 0.018 % TEA adjusted to pH 7.2 with 1-2 % acetic acid, B = 20 % of 100 mMol NaAc adjusted to pH 7.2 with 1-2 % acetic acid + 40 % ACN and 40 % MeOH |
| Flow rate | 0.45 ml/min |
| Compressibility A | 50 x $10^{-6}$bar |
| Compressibility B | 115 x $10^{-6}$ bar |
| Stroke: | A and B Auto |
| Gradient: | start with 100%A, at 17 min 60%B, at 18 min 100%B, at 18.1 min flow 0.45, at 18.5 min flow 0.8, at 23.9 min flow 0.8, at 24 min 100%B and flow 0.45, at 25 min 0%B |
| DAD UV Detector | Signal A= 338/10 nm, Ref= 390/20 nm; Signal B= 262/16 nm, Ref= 324/8 nmat; 15 min signal A =262/16 nm, Ref =324/8 nm |
| Oven temp: | 40 °C |
| Post time: | 5 min |

**Injector program**

| | |
|---|---|
| 1 | Draw 5.0 µl from vial 10—borate buffer |
| 2 | Draw 1.0 µl from vial 11—OPA reagent |
| 3 | Draw 0.0 µl from vial 12—water |
| 4 | Draw 1.0 µl from sample |
| 5 | Draw 0.0 µl from vial 12—water |
| 6 | Mix 8 µl in air, max speed, six times |
| 7 | Draw 1.0 µl from vial 14—FMOC |
| 8 | Draw 0.0 µl from vial 12—water |
| 9 | Mix 9 µl in air, max speed, 3 times |
| 10 | Inject |

**FLD Setting Agilent 1100 Series**

| | |
|---|---|
| Excitation | 340 nm |
| Emission | 450 nm |
| PTM gain | 12 |
| at 14.5 min | |
| | Excitation = 266 nm |
| | Emission = 305 nm |
| | PTM Gain 11 |

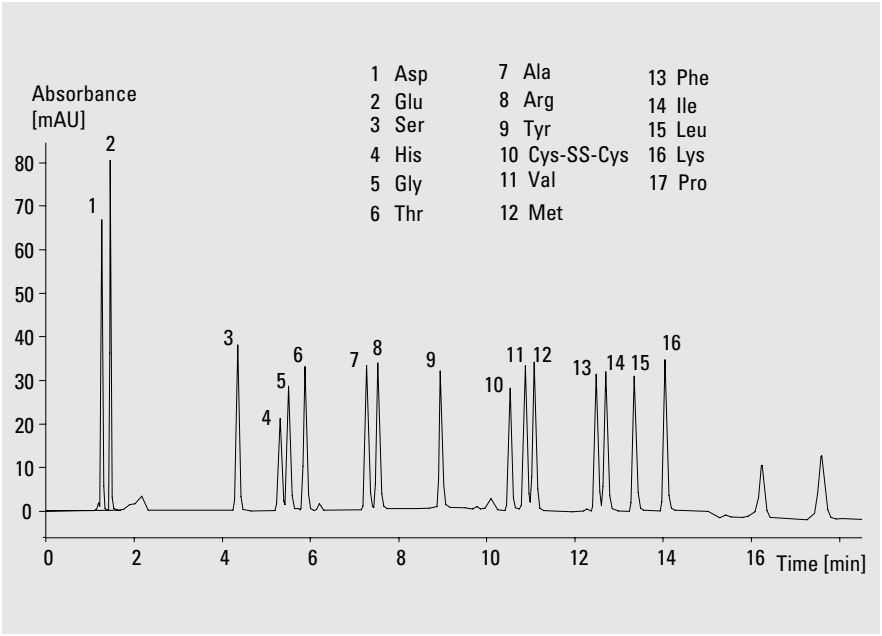



**Figure 1**
**Analysis of 250 pmol/µl amino acid standard with precolumn online derivatization and using DAD-UV detection**

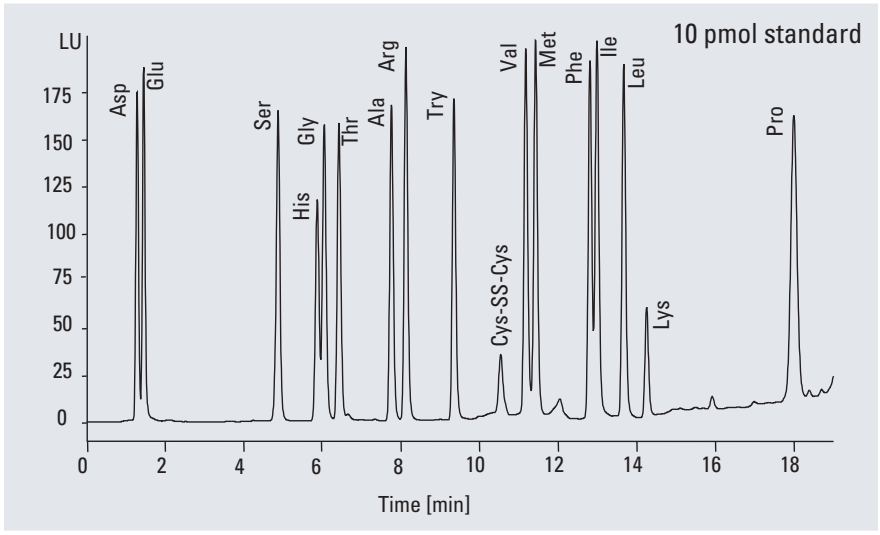



**Figure 2**
**Analysis of 10 pmol/µl amino acids with fluorescence detection**

The limits of detection (LOD) for both detectors is given in table 4.

Linearity for the Agilent Series 1100 DAD is excellent for well resolved peaks in the range from 10 to 1000 pmol/µl using standards (figure 3). In figure 3 the correlation coefficient for four amino acids is between 0.99998 and 0.99999.

| Compound (pmol) | LOD for DAD (pmol) | LOD for FLD (fmol) |
|---|---|---|
| Asp | 0.6 | 19 |
| Glu | 0.5 | 18 |
| Ser | 0.9 | 21 |
| His | 2.1 | 29 |
| Gly | 1.6 | 21 |
| Thr | 1.3 | 21 |
| Ala | 1.3 | 20 |
| Arg | 1.2 | 17 |
| Tyr | 1.2 | 19 |
| Cys-SS-Cys | 1.7 | not measured |
| Val | 1.3 | 17 |
| Met | 1.2 | 16 |
| Phe | 1.4 | 17 |
| Ile | 1.4 | 16 |
| Leu | 1.4 | 18 |
| Lys | 1.2 | 57 |
| Pro | 2.4 | 22 |

**Table 4**
**LOD's for fluorescence and UV detection**

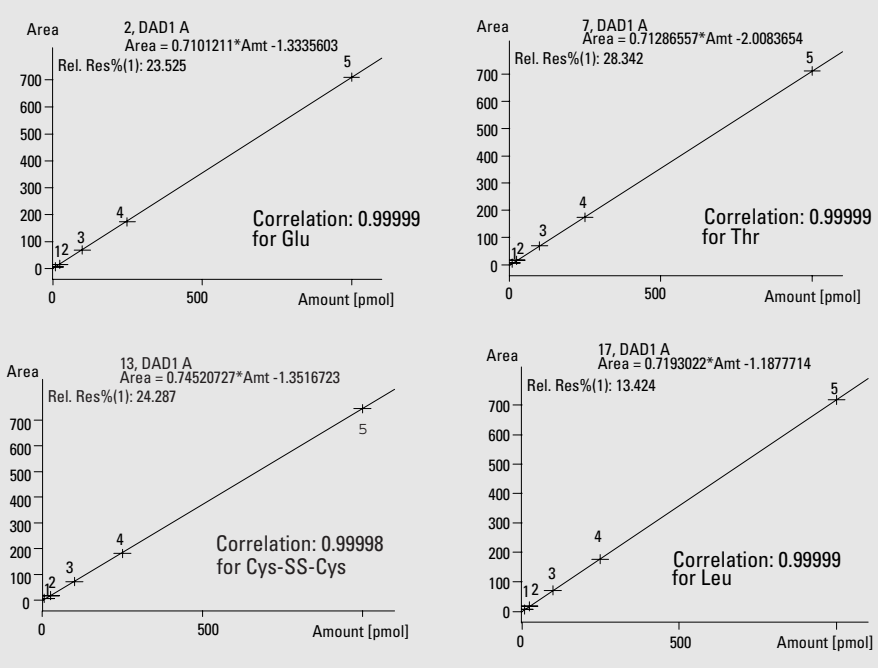



**Figure 3**
**Linearity over the concentration range from 10 pmol/µl to 1000 pmol/µl**

Retention time and area precision for both detectors are shown in table 5. Retention time precision over 6 runs is for both detectors below 0.2 %. Area precision for the DAD over 6 runs is below 5 % for a 100 pmol/µl amino acid standard. For the fluorescence detector the precision of areas is close to 5 %.

## Software

The standard software of the Agilent Chemstation can be used, and no additional macros are needed. A template for the special amino acids report is available in the Amino Acids Reports software with all necessary information and documentation.

| Compound | RSD RT | | RSD Area | |
|---|---|---|---|---|
| | DAD | FLD | DAD | FLD |
| Asp | 0.05 | 0.139 | 0.58 | 0.924 |
| Glu | 0.14 | 0.155 | 0.62 | 0.576 |
| Ser | 0.17 | 0.156 | 0.84 | 1.015 |
| His | 0.12 | 0.155 | 1.67 | 1.778 |
| Gly | 0.12 | 0.118 | 1.05 | 1.124 |
| Thr | 0.11 | 0.113 | 0.80 | 0.739 |
| Ala | 0.12 | 0.120 | 0.82 | 0.767 |
| Arg | 0.08 | 0.094 | 0.67 | 0.905 |
| Tyr | 0.06 | 0.062 | 3.18 | 1.614 |
| Cys-SS-Cys | 0.05 | not measured | 1.99 | not measured |
| Val | 0.05 | 0.058 | 0.84 | 0.919 |
| Met | 0.05 | 0.045 | 0.91 | 1.236 |
| Phe | 0.04 | 0.048 | 0.90 | 1.079 |
| Ile | 0.04 | 0.050 | 1.18 | 0.759 |
| Leu | 0.04 | 0.040 | 1.05 | 0.952 |
| Lys | 0.05 | 0.060 | 4.93 | 5.107 |
| Pro | 0.04 | 0.044 | 4.14 | 4.379 |

**Table 5**
**Precision of retention times and areas of DAD and FLD over 6 runs**

## Amino acid reports

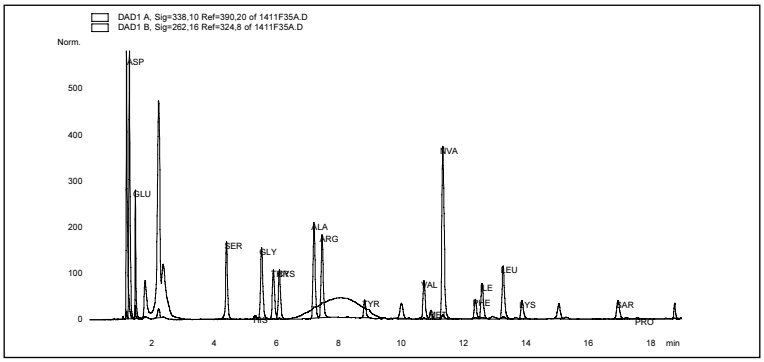
The following report can be obtained using the special method and report template of the Amino acid software kit (G1300-10013). The following information is included on the supplied disk:

- Installation procedure for the method
- Checkout example for the report template
- Instrument optimization procedure for amino acid analysis
- Customizing supplied operation
- Calculation specifications and template and method details
- Common errors

A detailed description on how to obtain and interpret this report is included on the disk as a Word for Windows document which can be printed out. An example of the printout is shown in figure 4.

Amino Acid Analysis Report

```
Acquisition method name:        DATA:AAS.M            Seq. Line:    6
Aquisition operator name:       RG                    Vial#:        35
Sequence name:                  TOXIC.S               Injection#:   1
Sample name:                    LYSOZYME
Data file name:                 C:\HPCHEM\1\DATA\1411F35A.D
Data analysis method name:      C:\HPCHEM\1\METHODS\DEFAA.M
Injected on:                    15 Nov 91    9:28 am
```




| # | Compound | Ret time [min] | Area [mAU*s] | Height [mAU] | Width [min] | Symmetry |
|---|---|---|---|---|---|---|
| 1 | ASP | 1.285 | 226.5 | 80.78 | 0.043 | 0.666 |
| 2 | GLU | 1.477 | 65.8 | 38.79 | 0.025 | 0.468 |
| 3 | SER | 4.401 | 102.1 | 23.48 | 0.065 | 0.859 |
| 4 | HIS | 5.328 | 5.8 | 1.17 | 0.074 | 0.947 |
| 5 | GLY | 5.524 | 113.5 | 21.59 | 0.081 | 0.760 |
| 6 | THR | 5.902 | 74.5 | 14.91 | 0.077 | 0.811 |
| 7 | CYS | 6.094 | 73.4 | 14.86 | 0.075 | 0.847 |
| 8 | ALA | 7.199 | 139.0 | 28.94 | 0.074 | 0.739 |
| 9 | ARG | 7.459 | 122.1 | 25.03 | 0.075 | 0.786 |
| 10 | TYR | 8.833 | 27.9 | 5.51 | 0.077 | 0.705 |
| 11 | VAL | 10.730 | 59.1 | 11.67 | 0.077 | 0.770 |
| 12 | MET | 10.955 | 14.4 | 2.70 | 0.081 | 0.811 |
| 13 | NVA | 11.333 | 281.2 | 52.08 | 0.083 | 0.650 |
| 14 | PHE | 12.380 | 30.9 | 6.02 | 0.079 | 0.803 |
| 15 | ILE | 12.595 | 60.2 | 10.69 | 0.086 | 0.734 |
| 16 | LEU | 13.270 | 88.6 | 15.94 | 0.085 | 0.716 |
| 17 | LYS | 13.875 | 31.1 | 5.51 | 0.086 | 0.700 |
| 18 | SAR | 16.957 | 256.7 | 39.76 | 0.100 | 0.849 |
| 19 | PRO | 17.579 | 21.8 | 1.80 | 0.171 | 1.073 |

| # | Compound | Ret time [min] | Amount [pmol] | Amount [ng] | Residues Relative to mole % | / mole | ALA |
|---|---|---|---|---|---|---|---|
| 1 | ASP | 1.285 | 511.34 | 58.8 | 18.4 | 24.49 | 21.72 |
| 2 | GLU | 1.477 | 141.22 | 18.2 | 5.1 | 6.76 | 6.00 |
| 3 | SER | 4.401 | 243.88 | 21.2 | 8.8 | 11.68 | 10.36 |
| 4 | HIS | 5.328 | 15.56 | 2.1 | 0.6 | 0.75 | 0.66 |
| 5 | GLY | 5.524 | 261.22 | 14.9 | 9.4 | 12.51 | 11.10 |
| 6 | THR | 5.902 | 169.82 | 17.2 | 6.1 | 8.13 | 7.21 |
| 7 | CYS | 6.094 | 231.97 | 28.1 | 8.3 | 11.11 | 9.85 |
| 8 | ALA | 7.199 | 282.51 | 20.1 | 10.2 | 13.53 | 12.00 |
| 9 | ARG | 7.459 | 247.77 | 38.7 | 8.9 | 11.87 | 10.52 |
| 10 | TYR | 8.833 | 65.62 | 10.7 | 2.4 | 3.14 | 2.79 |
| 11 | VAL | 10.730 | 116.89 | 11.6 | 4.2 | 5.60 | 4.97 |
| 12 | MET | 10.955 | 25.44 | 3.3 | 0.9 | 1.22 | 1.08 |
| 13 | NVA | 11.333 | 500.00 | - | - | - | - |
| 14 | PHE | 12.380 | 62.91 | 9.2 | 2.3 | 3.01 | 2.67 |
| 15 | ILE | 12.595 | 119.58 | 13.5 | 4.3 | 5.73 | 5.08 |
| 16 | LEU | 13.270 | 188.84 | 21.3 | 6.8 | 9.05 | 8.02 |
| 17 | LYS | 13.875 | 74.50 | 9.5 | 2.7 | 3.57 | 3.16 |
| 18 | SAR | 16.957 | 500.00 | - | - | - | - |
| 19 | PRO | 17.579 | 22.71 | 2.2 | 0.8 | 1.09 | 0.96 |

```
Expected molecular weight (AA_ProteinMW) :        14400
Calculated minimum molecular weight :             12770
Expected MW / Calculated MW :                     1.1277
```

**Figure 4**
**An example of the report printout**

## Maintenance

In order to keep the system running smoothly we recommend to do the following:

### Daily

1. Replace derivatization reagents, borate buffer, amino acid standards and wash water, which are placed in the autosampler tray.

2. Recalibration of retention times and response factors daily.

3. Check column and guard column performance using system suitability report.

### Every 2 days

1. Replace mobile phases A and B with freshly-made solvents.

### High column pressure occurs

1. Exchange guard column.

2. If this does not help, exchange analytical column.

## Troubleshooting

### Changes in retention times—amino acids elute earlier

- Poor resolution of His and Gly is caused by absence of triethylamine.

- AA's between Ser and Tyr elute earlier, concentration of THF too high. 0.4 % is already influencing the RT's.

- Low concentration of sodium acetate is influencing the later peaks more than the earlier ones.

- pH too high e.g. 7.6, specially the peaks between 10 and 14 min are eluting too early.

- Oven temperature too high. Nearly all peaks are eluting earlier.

- High concentration of sodium acetate decreases the resolution between Ala/Arg and Val/Met.

- High concentration of acetonitrile lets peaks above 10 min elute earlier.

- High concentration of methanol decreases resolution between Val/Met.

- If concentration of triethylamine is too low resolution between Cys-Cys/Val is getting poor.

### Amino acids elute later

- THF absence is strongly marked throughout the chromatogram. Note especially the splitting of the Glu peak . As the concentration of THF is increased, the peak splitting decreases and the retention time improve.

- If the pH is too low, e.g. 6.8, loss in resolution for Val/Met and Cys-Cys/Val/Met ( for DAD) occurs.

- If the oven temperature is too low e.g. 38ºC all peaks are eluting later.

### Poor chromatographic resolution

- Exhausted guard column.

- Damaged analytical column.

- Post column band broadening due to too long connections.

### Low Intensity chromatogram.

- OPA reagent has deteriorated.

- FMOC reagent has deteriorated.

- Glycine contamination.

## Ordering

Table 6 contains details for ordering instrumentation and supplies.

## Amini acid standards

Each amino acid standard contains the following amino acids:

| | |
|---|---|
| Asp | Aspartic acid |
| Glu | Glutamic acid |
| Ser | Serine |
| His | Histidine |
| Gly | Glycine |
| Thr | Threonine |
| Ala | Alanine |
| Arg | Arginine |
| Tyr | Tyrosine |
| Cys-SS-Cys | Cystine |
| Val | Valine |
| Met | Methionine |
| Phe | Phenylalanine |
| Ile | Isoleucine |
| Leu | Leucine |
| Lys | Lysine |
| Pro | Proline |

| Order Number | | Description |
|---|---|---|
| **Instrumentation** | | |
| G1312A | | **Agilent 1100 Series binary pump** |
| G1312-67301 | | 0.12 mm id capillary |
| or | | |
| A-330 Upchurch semi prep filter | | 5022-2165 |
| G1313A | | **Agilent 1100 Series autosampler** |
| #011 | | Vial kit |
| or | | |
| G1327A | | **Agilent 1100 Series thermostatted autosampler** |
| #011 | | Vial kit |
| *Optimum performance of the thermostatted autosampler requires the following capillary connections:* | | |
| G1312-67305 | (Pump to autosampler) | Outlet capillary |
| 5021-1823 | (Autosampler to column compartment) | Flexible tubing 400 mm, 0.12 mm id (no fittings) |
| G1316-87303 | (Column compartment to inlet column) | Capillary, 70 mm, 0.12 mm id |
| 5021-1823 | (Outlet column to DAD) | Flexible tubing 400 mm, 0.12 mm id (no fittings) |
| G1322A | | **Agilent 1100 Series vacuum degasser** |
| G1316A | | **Agilent 1100 Series column compartment** |
| G1315A | | **Agilent 1100 Series diode-array detector** |
| #018 | | Standard flow cell (10 mm, 13 µl) |
| G1321A | | **Agilent 1100 Series fluorescence detector** |
| G1319A | | **Agilent ChemStation** |
| **Supplies** | | |
| **5063-6588** | **Amino Acid Separation Kit contains:** | |
| 79916AA-572 | | Column for amino acid analysis, 200 × 2.1 mm |
| 79916KT-110 | | Guard columns, Hypersil ODS, 20 × 2.1 (3 per pack) |
| 79900CH-010 | | Guard cartridge holder |
| 79841-87609 | | Column connector, 35 mm, 0.12 mm id. |
| 9301-1388 | | Crimpcap microvials (100 per pack) |
| 5181-1210 | | Crimpcaps, butyl rubber (100 per pack) |
| **Amino Acid Standards** | | |
| 5061-3330 | | 1 nmol/µl AA standard 10 × 1 ml ampules |
| 5061-3331 | | 250 pmol/µl AA standard 10 × 1 ml ampules |
| 5061-3332 | | 100 pmol/µl AA standard 10 × 1 ml ampules |
| 5061-3333 | | 25 pmol/µl AA standard 10 × 1 ml ampules |
| 5061-3334 | | 10 pmol/µl AA standard 10 × 1 ml ampules |
| 5062-2478 | | Supplemental Amino Acid Kit includes 1 g each norva line, sarcosine, asparagine, glutamine, tryptophan and 4-hydroxyproline |
| **Reagents for amino acid analysis** | | |
| 5061-3335 | | OPA reagent, 10 mg/ml each in 0.4 M borate buffer o-phthalaldehyde (OPA) and 3-mercaptoproprionic acid, 6 ampules |
| 5061-3337 | | FMOC reagent, 2.5 mg/ml in acetonitrile, 9-fluorenyl methylchloroformate, 1 ml |
| 5061-3339 | | Borate buffer, 100 ml |
| 5062-2479 | | DTDPA (dithiodipropionic) reagent for analysis of cysteine, 5 g |
| **Software** | | |
| G1300-10013 | | Software for special method set up and reporting—software description included as document file (User contributed software) |
| 5968-5796E | | Sensitive and reliable amino acid analysis in protein hydrolysates using the Agilent 1100 Series HPLC |
| **Support *(not included in kit)*** | | |
| H1170A | | Application Optimization Service—onsite, including travel from 1–10 continuous days |

**Table 6**
**Ordering information**

## Agilent chemical analysis professional services for chromatography

If you do not have the technical recources available to set up new applications and methods, you may choose to use Agilent's Application Optimization Service (AOS).

Our application experts are specialists in implementing and optimizing applications, integrating new analytical technologies into the laboratory, and complying with global quality and regulatory standards.

Each application expert has a minimum of five years practical laboratory experience and is factory trained—at an R&D level. Most have advanced degrees in science or technology.

With the Agilent Technologies AOS application experts at your side, you can:

- accelerate integration of new analytical technologies into your laboratory operations to remain competitive
- decrease research and development cycle time to reduce the time it takes to get your products to market
- establish a benchmark for instrument performance to ensure confidence in analytical results.

Additionally, all of our application experts adhere to ISO-registered quality processes.

For the latest information and services visit our world wide web site:
http://www.agilent.com/chem

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