

# Automated Pre-Column Derivatization of Amino Acids by HPLC: Mr. Amino, Meet Mr. Roboto

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## Introduction

Amino acids are the building blocks of life and essential for survival. Unfortunately, they are difficult to measure at low levels using High Performance Liquid Chromatography (HPLC) due to their weak chromophores. Detector sensitivity can be improved by derivatizing the amino acids with OPA (o-phthalaldehyde) and FMOC (Fluorenylmethyloxycarbonyl chloride), but the derivatizations are time-consuming and manually intensive. The solution is to "train" your autosampler to carry out the derivatizations for you! This is done by programming the individual steps into the autosampler's instruction set, drastically improving reproducibility and accuracy. We were successfully able to program both our older and newer generation HPLCs with the instructions to perform this derivatization to save time and money while improving the consistency of the results. We demonstrate the programming, operation, and results obtained while using this method for the determination of amino acids in a variety of samples including recovery sports drinks and fruit juices. In order to take full advantage of this automated sample prep "robot", we developed a high-speed HPLC method for the analysis of the derivatized amino acids.

## Experimental Conditions

**Instrument:** 1200SL & 1100 Agilent HPLCs

**Mobile Phase A:** 10 mM Na<sub>2</sub>HPO<sub>4</sub> + 10 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, pH 8.2

**Mobile Phase B:** Methanol

**Column:** Agilent Eclipse Plus C-18, 4.6mm x 50 mm, 1.8µm particles

**Flow:** 2.0 mL/min

**Gradient Timetable:**

Time	%B (MeOH)
0.00	2.0
0.20	2.0
7.67	57.0
7.77	100.0
12.00	100.0

**Detector:** Diode Array (DAD): Signal 338, BW 10 nm; Ref 390, BW 20 nm

## Materials

Amino Acid Standard: Sigma DL-Amino Acids

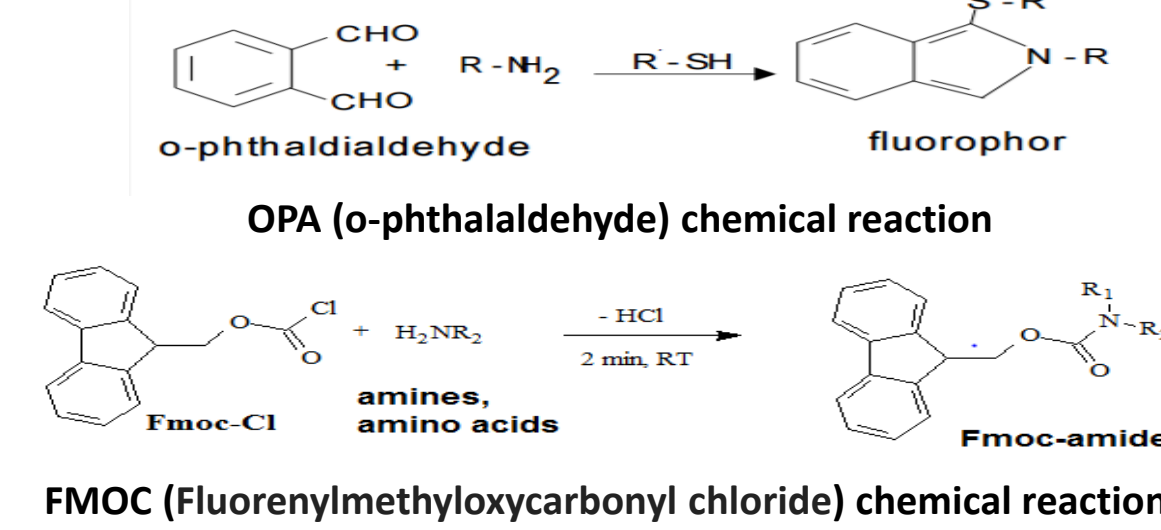
OPA - o-Phthalaldehyde Reagent Sigma Aldrich

FMOC - Fluorenylmethyloxycarbonyl Chloride Sigma Aldrich

Recovery Mix - MP Muscle Pharm - BCAA 3:2:1 amino acid post workout mix

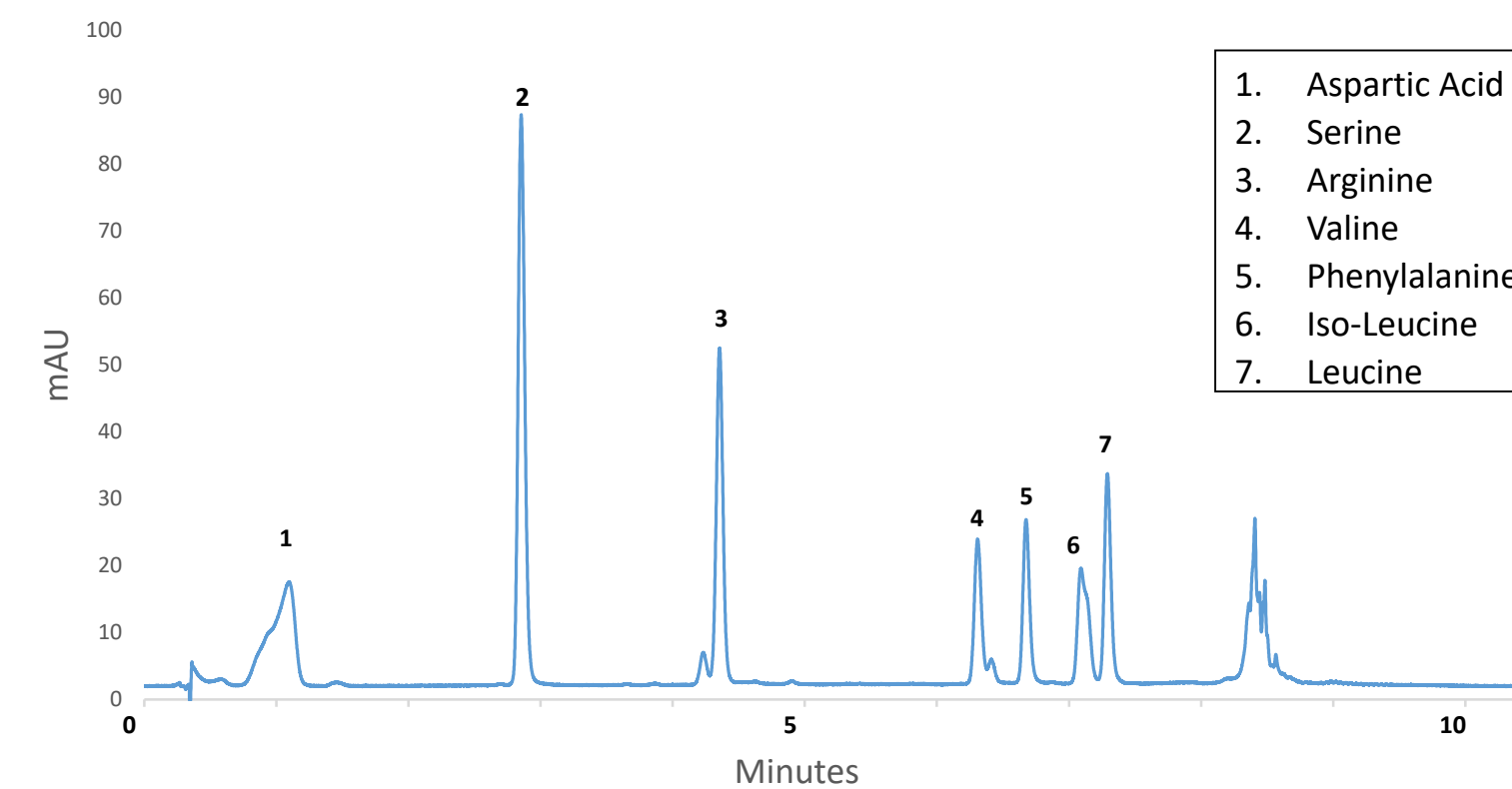
Generic Grape Juice

## Chemical Reaction of Derivatizing Agents



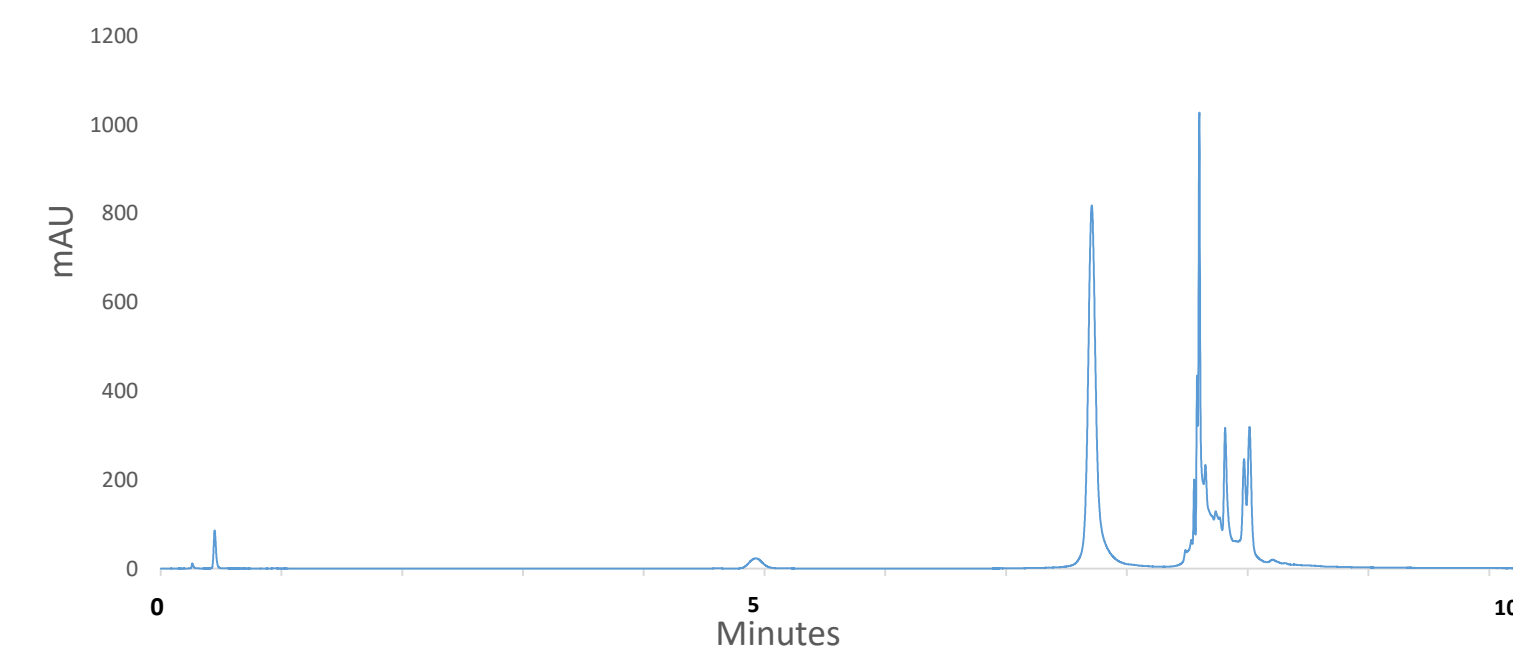
## Derivatized Amino Acids

### Amino Acid Standard with Automated Derivatization

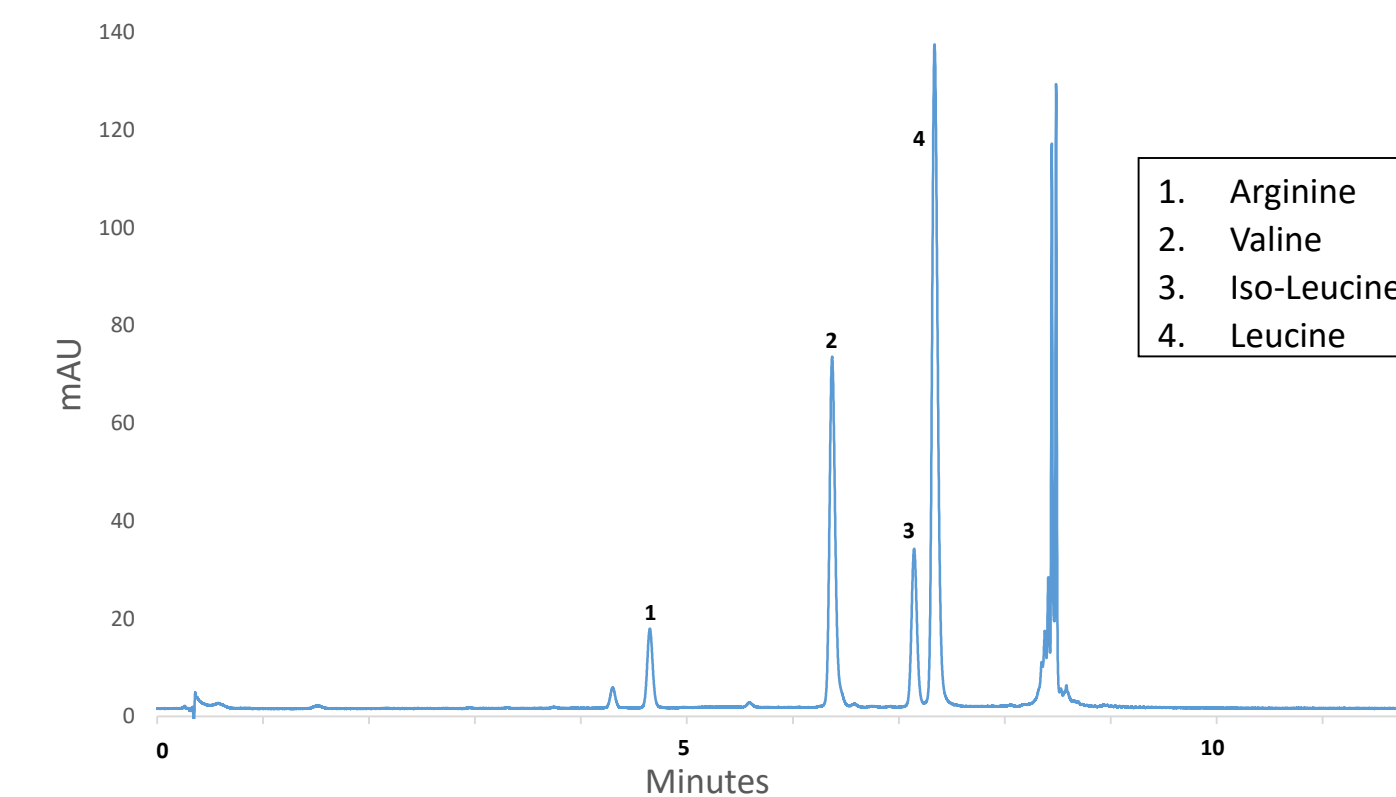


## Non-Derivatized Amino Acids

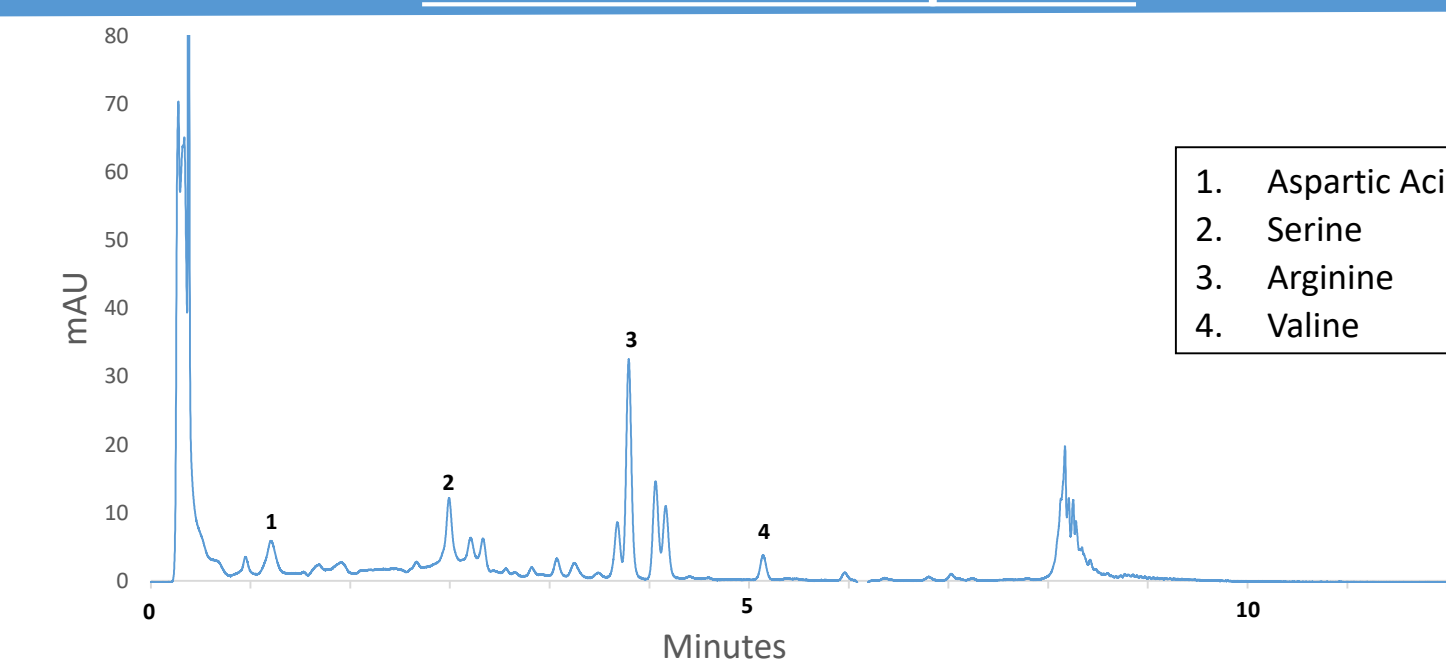
### Amino Acid Standard without Derivatization



## Amino Acids in Recovery Mix



## Amino Acids in Grape Juice



## Automated Injection Technique



1. Draw 12.5 µL of Borate
2. Draw 5.0 µL of sample
3. Mix 17.5 µL in air, max speed, 5 times
4. Wait 0.2 min
5. Draw 2.5 µL of OPA
6. Mix 20.0 µL in air, max speed, 10 times
7. Draw 5.0 µL of FMOC
8. Mix 25 µL in air, max speed, 10 times
9. Draw 5.0 µL of Water
10. Mix max amount in air, max speed, 8 times
11. Inject
12. Wait 0.1 min
13. VALVE Bypass

## Conclusions

Sample preparation can be a very time consuming and error prone procedure in the lab. By employing an HPLC autosampler to do most of the sample prep, the contamination and reproducibility issues are drastically reduced. By automating the derivatization process, a lab can run a more samples with better reproducibility and lower labor requirements. This automated sample prep procedure was implemented on both a 1200SL series HPLC and an older 1100 series HPLC. Both generations of instruments were used to detect a variety of amino acids (Arginine, Valine, Iso-Leucine, Leucine, Aspartic acid, and Serine) in grape juice and a sports recovery drink. Note that this method yields both qualitative and quantitative results.

Automated derivatization is not limited to amino acid determination, but may also be applied to a variety of other useful derivatization procedures, thus expanding the utility of HPLC, improving the reproducibility of laboratory measurements, and reducing the overall costs of analysis.

## Acknowledgements

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**Axion**  **Training Institute, Inc.**  
**Analytical Laboratories, Inc.**