

Poster Reprint

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Routine Quantitation of 17 Underivatized Amino Acids by LCMS

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Introduction

HPLC with UV detection has long been the instrument and method of choice for the analysis of amino acids in food and supplements


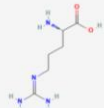
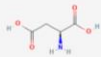



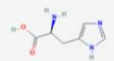










Using UV detection for amino acids usually requires the use of the absorption of the carboxyl group (-COOH). Some amino acids with benzene rings can also be detected, but historically, have been difficult to analyze underivatized with sufficient sensitivity and selectivity. Due to these issues, derivatization methods utilizing a derivatizing reagent that selectively reacts with the amino groups (-NH₂, -NHR) have been used. This derivatization can be accomplished either pre-column or post-column.

When pre-column derivatization is employed, the derivatizing agent is mixed directly with the sample. The yield of the derivatization reaction can be greatly affected by sample matrix, leading to inaccurate detection and quantitation. When post-column derivatization is employed, the derivatization reagent is kept constantly flowing and results in a high rate of consumption of this reagent. Post-column derivatization limits the types of reagents that can be used since the detection of unreacted reagent is not permitted. This method is not suitable for high-sensitivity analysis.

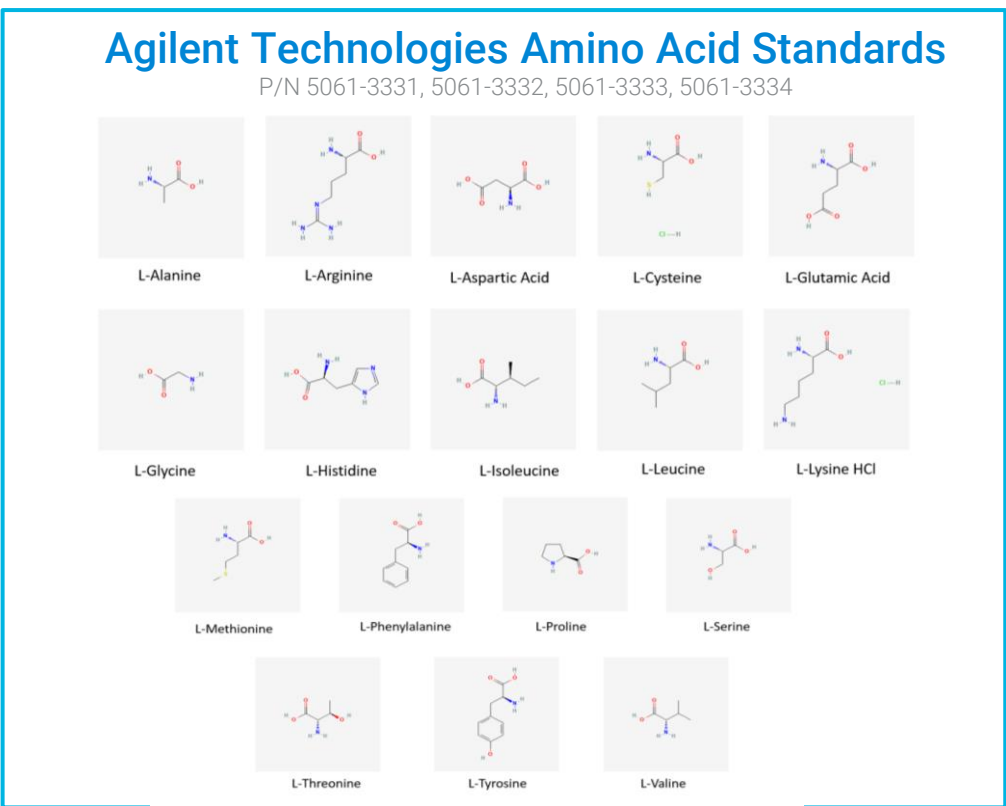
The method presented in this paper does not require derivatization, This method allows for the cost-effective and rapid quantitative analysis of 17 underivatized amino acids in one injection using an Agilent LC/MSD iQ. Two commercially available amino acid supplements were analyzed in this study. Working ranges were selected to be those most useful to industry and all were linear. Good accuracy and precision were observed, both in solvent and in matrix.

Agilent Technologies Amino Acid Standards

P/N 5061-3331, 5061-3332, 5061-3333, 5061-3334

			 Cl-H	 Cl-H
L-Alanine	L-Arginine	L-Aspartic Acid	L-Cysteine	L-Glutamic Acid
				 Cl-H
L-Glycine	L-Histidine	L-Isoleucine	L-Leucine	L-Lysine HCl
				
L-Methionine	L-Phenylalanine	L-Proline	L-Serine	
				
L-Threonine	L-Tyrosine	L-Valine		

Agilent Technologies Amino Acid Standards
P/N 5061-3331, 5061-3332, 5061-3333, 5061-3334



Experimental

Instrumentation

All experiments in this study were performed using an Agilent 1260 Prime Infinity II LC consisting of an Agilent 1260 Infinity II Vial Sampler (G7129C), an Agilent 1260 Infinity Flexible pump (G7104C), and an Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) coupled to an Agilent Infinity Lab LC/MSD iQ (G6160A) mass spectrometer. The system was controlled by Agilent Open Lab CDS software, version 2.6. Data processing was performed using the same Agilent Open Lab CDS software.



Parameter	Settings
Analytical Column	Agilent HILIC-Z, 3.0x150mm, 2.7 µm Part Number 693975-301
Column Temperature	25.0° C
Injection Volume	1.0 µL
Run Time	14.00 minutes
Post-run Time	8.0 minutes
Flow Rate	0.6 mL/minute
Mobile Phase A	Aqueous 0.1% Formic acid
Mobile Phase B	Acetonitrile
Mobile Phase C	10mM Aqueous Ammonium Formate
Quaternary Pump Gradient	<u>Time(min)</u> <u>Flow(mL/min)</u> <u>%A</u> <u>%B</u> <u>%C</u>
	0.00 0.60 5.00 90.00 5.00
	5.00 0.60 15.00 70.00 15.00
	6.00 0.60 20.00 60.00 20.00
	7.00 0.60 40.00 30.00 30.00
	9.00 0.60 45.00 10.00 45.00
	10.00 0.60 45.00 10.00 45.00
MS Ion Source	Electrospray
Nitrogen Gas Temperature	325° C
Gas Flow	11.0 L/min
Nebulizer	50 psi
Capillary Voltage	3500 V

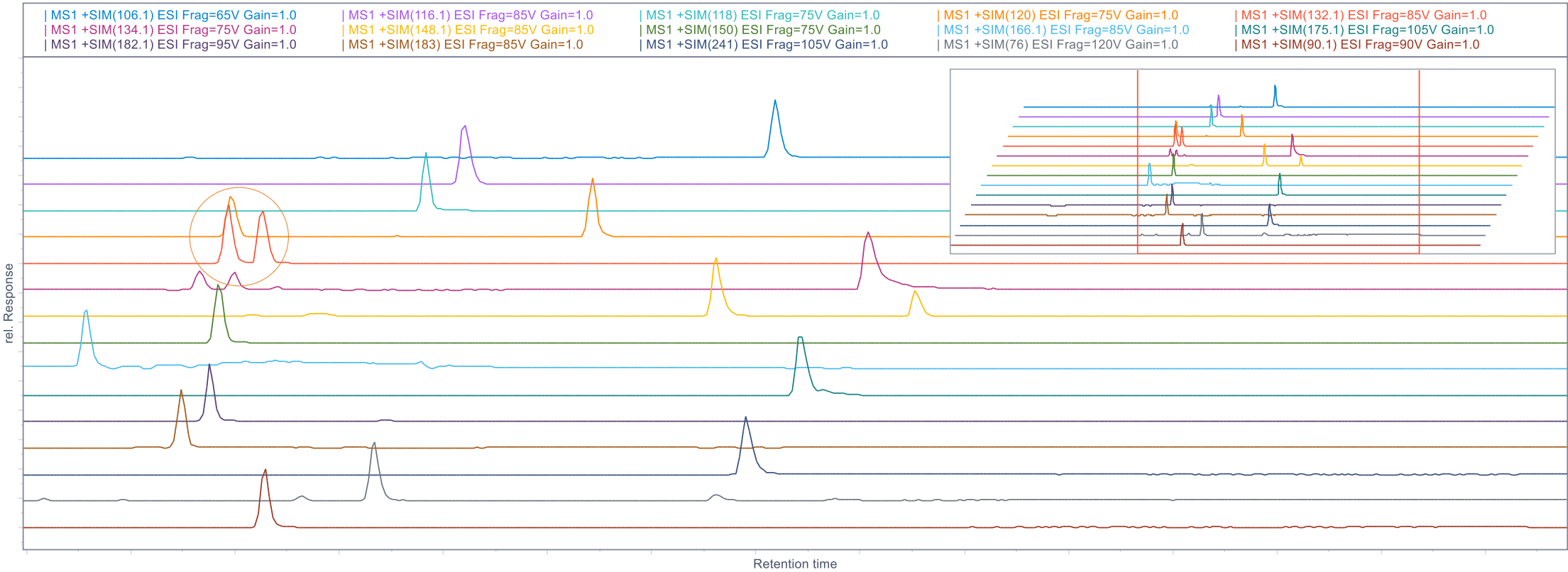
Linearity and Range - Amino Acids in Water

Amino Acid	RT (min)	Fragmentor Voltage	m/z	r	R ²	Range (pmol/μl)	Range (ppm)
L-Alanine	6.117	90	90	0.99949	0.99899	10 –1000	0.891- 89.1
L-Arginine	7.988	105	175	0.99745	0.99490	10 –1000	1.742 -174
L-Aspartic Acid	7.845	75	134	0.99904	0.99807	10 –1000	1.33 -133.1
L-Cysteine	8.179	105	241	0.99997	0.99994	10 –1000	1.2 -121
L-Glutamic Acid	7.214	85	148	0.99932	0.99864	10 –1000	1.47 -147.1
L-Glycine	6.515	120	76	0.99979	0.99958	10 –1000	0.75 -75
L-Histidine HCL·H ₂ O	4.377	95	210	0.99614	0.99229	10 –1000	2.10 -210
L-Isoleucine	4.652	85	132.1	0.99912	0.99824	10 –1000	1.31 -131
L-Leucine	4.489	85	132.1	0.99888	0.99775	10 –1000	1.31 -131
L-Lysine HCl	4.365	85	183	0.99691	0.99224	10 –1000	1.83 -183
L-Methionine	4.868	75	150	0.99970	0.99840	10 –1000	1.49 -149.2
L-Phenylalanine	4.371	85	166	0.99990	0.99980	10 –1000	1.65 -165.2
L-Proline	5.349	85	116.1	0.99922	0.99844	10 –1000	1.15 -115
L-Serine	6.618	65	106	0.99972	0.99945	10 –1000	1.05 -105
L-Threonine	4.371	75	120	0.99986	0.99982	10 –1000	1.19 -119
L-Tyrosine	5.267	95	182	0.99553	0.99109	10 –1000	1.81 -181.2
L-Valine	5.280	75	118	0.99688	0.99378	10 –1000	1.17 -117.2

Precision and Accuracy - Amino Acids in Water

Amino Acid	Average RT (min)	RT RSD %	Concentration RSD%	Average Accuracy
L-Alanine	6.279	0.293	2.029	101.49
L-Arginine	8.130	0.102	1.5636	101.76
L-Aspartic Acid	8.090	0.089	1.299	102.32
L-Cysteine	8.261	0.083	1.863	101.07
L-Glutamic Acid	7.590	0.147	1.998	100.42
L-Glycine	6.657	0.260	1.593	101.36
L-Histidine HCL·H ₂ O	4.537	0.018	1.172	100.89
L-Isoleucine	4.844	0.378	1.428	100.93
L-Leucine	4.672	0.398	1.4378	101.49
L-Lysine HCl	5.389	0.326	1.218	101.93
L-Methionine	5.045	0.348	1.3132	101.98
L-Phenylalanine	4.539	0.404	1.6932	102.03
L-Proline	5.440	0.335	1.573	102.14
L-Serine	6.754	0.243	1.522	102.17
L-Threonine	6.313	0.280	1.704	102.47
L-Tyrosine	5.387	0.330	1.1617	100.69
L-Valine	5.367	0.346	2.988	100.31

The instrumental portion of this method was evaluated using standard solutions of seventeen amino acids in acidified water. The calibration curve was generated utilizing the load-on-column method to minimize propagation of any pipetting errors. The calibration range was chosen to be 10 –1000 pmol/μl. The calibration curves of all seventeen amino acids were linear. Note the baseline resolution of leucine and Isoleucine in circle below.



Amino Acids in Supplement #1

Supplement #1 was analyzed in this study and consisted of a capsule containing a powdered mix of L-Leucine, L-Isoleucine and L-Valine only. Four capsules (one serving) were dissolved in one liter of water and the resulting solution was analyzed. It is noted that exact concentrations were not known. Recoveries indicate no matrix effects.

Amino Acid Precision	Retention Time (min)	Retention Time (RSD%)	Concentration (RSD %)	Amino Acid Recovery (pmol/mcL)	Calculated Amount*1	Quantitated Unspiked Conc	Calculated Spiked from Unspiked Quant*2	Quanted Spike (spiked with 100 pmol)	Recovery %
L-Leucine	4.495	0.444	3.097	L-Leucine	590	631	667	685	103
L-Isoleucine	4.670	0.438	3.165	L-Isoleucine	387	340	406	406	100
L-Valine	5.203	0.345	5.200	L-Valine	331	394	455	455	100

*1Amino acid amounts are given as a range by the manufacturer. Actual exact amounts are not known.

*2 900 µL unspiked aa solution + 100 µL of 1000 pmole spike
(0.9(unspiked quant) + 100 pmol = calculated spike)

Amino Acids in Supplement #2

Supplement #2 was analyzed in this study and consisted of capsules containing a powdered mix of L-Leucine, L-Lysine, L-Threonine, L-Isoleucine, L-Valine, L-Phenylalanine, L-Histidine, L-Tryptophan and L-Methionine. Three capsules (one serving) were dissolved in one liter of water and the resulting solution was analyzed for L-Phenylalanine, L-Leucine, L-Isoleucine, L-Methionine and L-Valine. No matrix effects were observed.

Amino Acid Precision	Retention Time (min)	Retention Time (RSD %)	Concentration (RSD %)	Amino Acid Recovery (pmol/µL)	Quantitated Unspiked Conc	Calculated Spiked Conc	Quantitated Spiked Conc	Recovery %
L-Phenylalanine	4.244	0.058	3.037	L-Phenylalanine	284.3	355.9	399.9	112
L-Leucine	4.394	0.071	2.911	L-Leucine	914.3	922.9	960.4	104
L-Isoleucine	4.572	0.067	2.967	L-Isoleucine	509.9	558.9	575.6	103
L-Methionine	4.761	0.058	2.357	L-Methionine	123.2	210.9	226.1	107
L-Valine	5.109	0.073	1.783	L-Valine	690.6	721.5	728.6	100

Conclusions

This method, in concert with the Agilent Technologies Agilent 1260 Prime Infinity II LC coupled with an Agilent iQ Mass Spectrometer, has been shown to be effective, sensitive, accurate, and robust for the screening and analysis of amino acids in nutritional supplements. The ability to analyze seventeen underivatized amino acids in one injection makes this method a time and cost-effective tool for commercial and industrial label verification

References

1 Quantitation of Amino Acids in Soy Flour, Dried Cow's Milk Powder, and Corn Silage by Triple Quadrupole LC/MS/MS
Hui Zhao and Doug Postl Agilent Technologies, Inc. Mike Van Amburgh, Debbie Ross, Andres Felipe Ortega, and Andrew LaPierre Department of Animal Science, Cornell University 5994-3044EN

2. <https://www.Shimadzu.com> "Analytical Methods for Amino Acids."

<https://www.agilent.com/en/promotions/asms>

This information is subject to change without notice.

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