

Rapid and Reproducible Amino Acid Analysis of Physiological Fluids for Clinical Research using LC/MS/MS with the Amino Acid 45/32 kit



API 3200™ LC/MS/MS System

Key Features

- Ability to analyze 45 amino acids in a single analysis
- High throughput with less than 25 minute analysis time per sample
- Single universal protocol for a range of physiological fluids such as plasma, urine, CSF, etc.
- The ability to separate and quantify Alloisoleucine
- Much smaller sample requirement, 40 µl
- Each of 45 amino acids has its own internal standard
- A wide dynamic range with LLOQ from 1-10µM
- A kit that includes the required reagents, standards and controls
- The ability to automate the pipetting steps (optional)

Introduction

Amino acid analysis has applications in many areas of biochemical research, clinical medicine, food and beverage technology. Samples of interest include plasma, urine, CSF, culture media, peptide/protein hydrolysates, animal feed and food products. Current dedicated amino acid analyzer systems have long run times, lack robustness, and are difficult to maintain. Here, we describe a method for the analysis of amino acids in physiological fluids using amine reactive isotope coded tags (iTRAQ® Reagents) in conjunction with LC/MS/MS. The combination of LC/MS/MS with iTRAQ® Reagents provides significantly shorter analysis times, better and robust separation, ability to use labeled internal standards for accurate and robust quantitation, lower detection limits and wide dynamic range.

Analysis

Analysis was carried out by first precipitating the proteins out of the sample with sulfosalicylic acid, and then derivatizing with iTRAQ® Reagents supplied in AAA 45/32 kit. Derivatized samples are then injected onto a reverse phase column and amino acids separated. The amino acids are monitored using a mass spectrometer. Data shown here is using Agilent 1200 HPLC system with Applied Biosystems AAA C18 Column coupled to an Applied Biosystems/ MDS SCIEX API 3200™ Triple Quadrupole Mass Spectrometer.

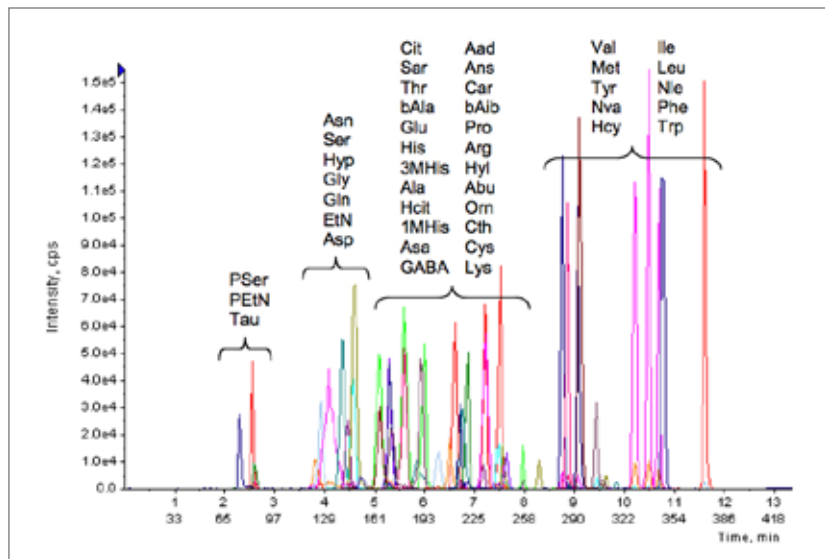


Figure 1 Shows the separation of the 45 Amino Acids in 25 minutes.

Analysis of 45 amino acids in 25 minutes

Figure 1 shows the separation of the labeled amino acids in a physiological standard mix. Fast separations can be achieved since only isobaric (same mass) and isomeric amino acids need to be fully separated for accurate quantitation.

Separation of isomeric and isobaric amino acids

Figure 2 shows the chromatograms of the isobaric and isomeric amino acids sarcosine, alanine, and beta-alanine; 1-methyl histidine; 3-methyl histidine; gama-aminobutyric acid, beta-aminoisobutyric acid, and alpha-aminobutyric acid; valine and norvaline, and isoleucine, leucine, and norleucine.

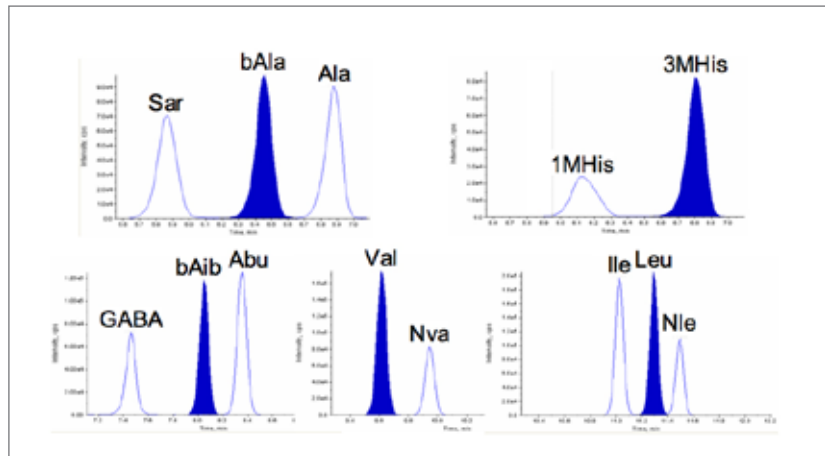


Figure 2 Demonstrates the separation of isomeric and isobaric amino acids.

Alloisoleucine analysis

Figure 3 shows the detection of Alloisoleucine in plasma sample and compares it with a sample without it. Quantitation of Alloisoleucine is done using internal standard Nle. Known amount of Nle is added along with the sulfosalicylic acid solution that is used to precipitate the proteins.

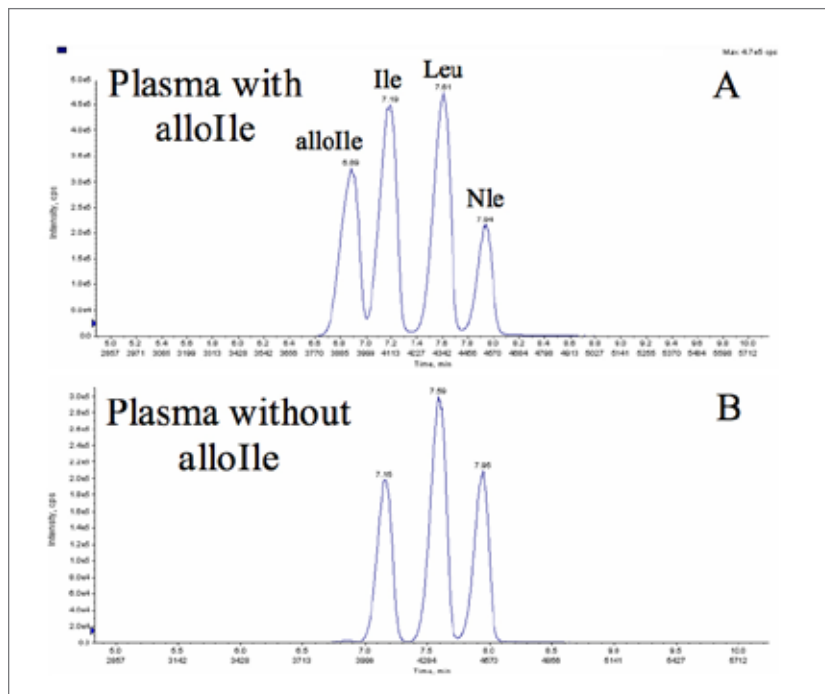


Figure 3 Demonstrates the ability to resolve and quantitate Alloisoleucine in plasma.

Performance

The amino acid quantities obtained using the AAA 45/32 kit correlate well with the results from conventional amino acid analyzers.

The limits of detection from 1 to 10 µM and dynamic range of 3 to 4.5 orders of magnitude also compare favourably (Figure 4).

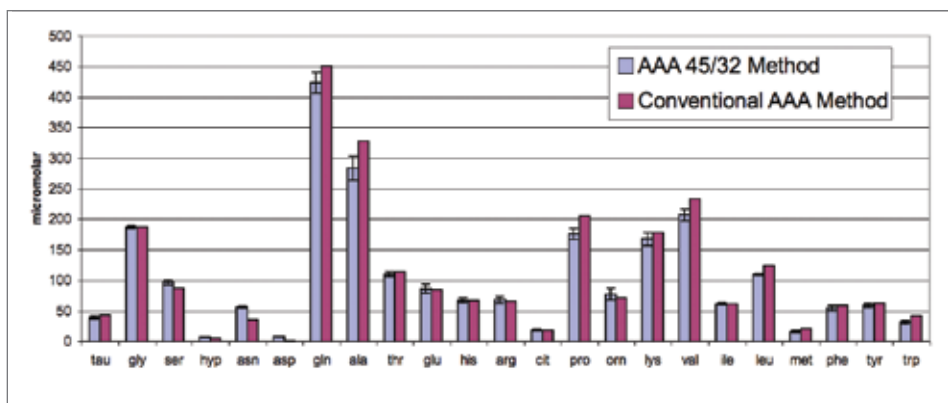


Figure 4 Compares the results of AAA 45/32 method and conventional AAA system (ion-exchange-post-column ninhydrin derivation Beckman Model 7300) for a plasma sample.

Limits of detection, dynamic range and reproducibility in control plasma and standards

All 45 Amino Acids were monitored using AAA 45/32 kit but only amino acids for which amounts are available from conventional amino acid analyser for the control plasma are reported.

Comparison With Conventional Amino Acid Analyzer

Amino Acid	Control Plasma Conventional AAA (ninhydrin method) (µM)		Control Plasma 45/32 Kit (µM)	
	Mean	Range	Mean	%CV
Ala	409	327 - 491	423	15%
Abu	14	10.8 - 16.2	17	15%
Arg	21	16.4 - 24.6	30	12%
Asn	22	17.3 - 25.9	39	9%
Asp	21	16.9 - 25.3	21	7%
Cit	24	19.3 - 28.9	26	15%
Glu	295	236 - 354	272	18%
Gln	249	199 - 299	288	9%
Gly	185	148 - 222	198	5%
His	76	60.6 - 91.0	84	9%
Ile	71	56.7 - 85.1	79	7%
Leu	196	157 - 235	218	10%
Lys	160	128 - 192	169	17%
Met	38	30.3 - 45.5	27	14%
Orn	140	112 - 168	154	19%
Phe	82	65.8 - 98.8	92	6%
Pro	199	159 - 239	210	15%
Ser	100	79.9 - 120	109	7%
Tau	43	34.7 - 52.1	45	7%
Thr	119	95.2 - 143	130	18%
Trp	47	37.8 - 56.6	49	7%
Tyr	60	47.6 - 71.4	67	8%
Val	222	178 - 266	226	11%

Detection limits and dynamic range on standards using AAA 45/32 kit

Amino Acid	LLOQ (μM)	ULOQ (μM)	Dynamic Range Orders of Magnitude
Pser	1.0	1000	3.0
PEtN	0.5	10000	4.3
Tau	1.0	5000	3.7
Asn	5.0	10000	3.3
Ser	0.5	5000	4.0
Hyp	0.5	10000	4.3
Gly	0.5	5000	4.0
Gln	0.5	5000	4.0
Asp	0.5	10000	4.3
EtN	0.5	10000	4.3
Cit	0.5	10000	4.3
Sar	0.5	10000	4.3
Thr	1.0	10000	4.0
bAla	1.0	10000	4.0
Glu	0.5	5000	4.0
His	0.5	5000	4.0
3MHis	0.5	10000	4.3
Ala	1.0	5000	3.7
Hcit	5.0	1000	2.3
1MHis	1.0	5000	3.7
Asa	5.0	5000	3.0
GABA	1.0	10000	4.0
Aad	0.5	5000	4.0
Ans	1.0	10000	4.0
Car	1.0	1000	3.0
bAib	0.5	10000	4.3
Pro	5.0	10000	3.3
Arg	5.0	5000	3.0
Hyl	1.0	5000	3.7
Abu	0.5	10000	4.3
Orn	5.0	10000	3.3
Cth	10	10000	3.0
Cys	10	5000	2.7
Lys	0.5	1000	3.3
Val	1.0	5000	3.7
Met	0.5	10000	4.3

Summary

As shown, AAA 45/32 kit based on iTRAQ® Reagents in combination with LC/MS/MS enables a fast (less than 25 min) and accurate, quantitative analysis of forty-five plus amino acids in physiological fluids. Furthermore, this combination also provides low detection limits, a wide linear dynamic range and the ability to robustly resolve key amino acids.

The kits are available for purchase stand-alone, as part of a starter kit or as part of a complete solution that also includes an HPLC, Mass Spectrometer, installation and training. To install the assay, two products are needed, The Starter Kit, Applied Biosystems p/n 4374891 that includes the iTRAQ® Reagents, internal standards, control plasma, user's guide, start-up guide, and a HPLC column, Applied Biosystems p/n 4374841. This starter kit supports the analysis of up to 50 samples.



iTRAQ® Reagents can be purchased stand-alone, as part of a Starter Kit with Standards and Reagents for Amino Acid Analysis or as part of a total solution that includes an HPLC, Mass Spectrometer, Starter Kit, Installation, Familiarization and Warranty.

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