Accelerated Analysis of Amino Acids in Physiological Samples

Jean-Philippe Veyssier
Biochrom Ltd, Cambridge, UK

Quality of resolution, accurate identification and quantification are critical in amino acid analysis to help the diagnosis of inborn errors of metabolism. Clinical laboratories require higher throughput of data because of an increased workload. A faster analysis programme has been developed by Biochrom to speed up the analysis of physiological samples whilst maintaining excellent resolution, precision and accuracy on the results.

Introduction

Amino acid analysis using a cation exchange column followed by detection using ninhydrin derivatization gives an unequivocal verdict for the diagnosis of metabolic disorders. It is the method of choice for many clinical labs who need an accurate result on both plasma and urine samples.

The need for faster analysis is required but gain of speed should not mean loss of quality chromatography.

It is critical that key amino acids such as Alloisoleucine, Argininosuccinic acid (ASA), Sulfocysteine or Saccharopine have to be separated from the other free amino acids found in physiological fluids as they play a crucial role in diagnosis. Furthermore, urine analysis should not be neglected as some disorders can only been seen in this type of sample.(1)

Biochrom has developed a fast method for physiological samples that allows the separation of 46 amino acids and derivatives, including Alloisoleucine, Argininosuccinic Acid and Saccharopine.

Method:

The study was performed on the Biochrom Amino Acid Analyzer using 5 Lithium Citrate buffers and a 200mm x 4.6mm analytical column run at increased flow rate (35ml/h) and pressure. Calibration was performed using Amino Acid Standards Acidic/Neutral and Basics (Sigma Aldrich) spiked with Saccharopine, Alloisoleucine and ASA (Sigma Aldrich) at a concentration of 250µM. 20µL of this calibration solution were injected. The separation of all amino acids was achieved by modifying the buffer gradient. The stepwise buffer gradient system allows fine tuning of the separation in the area of interest without disrupting the rest of the chromatography. Aminoethyl Cysteine was used as the internal standard.

The new method was evaluated using not only amino acid standards but also plasma samples from the ERNDIM 2009 and 2010 QC scheme. These samples were prepared from fresh human plasma and spiked with known amounts of test amino acids.(2)

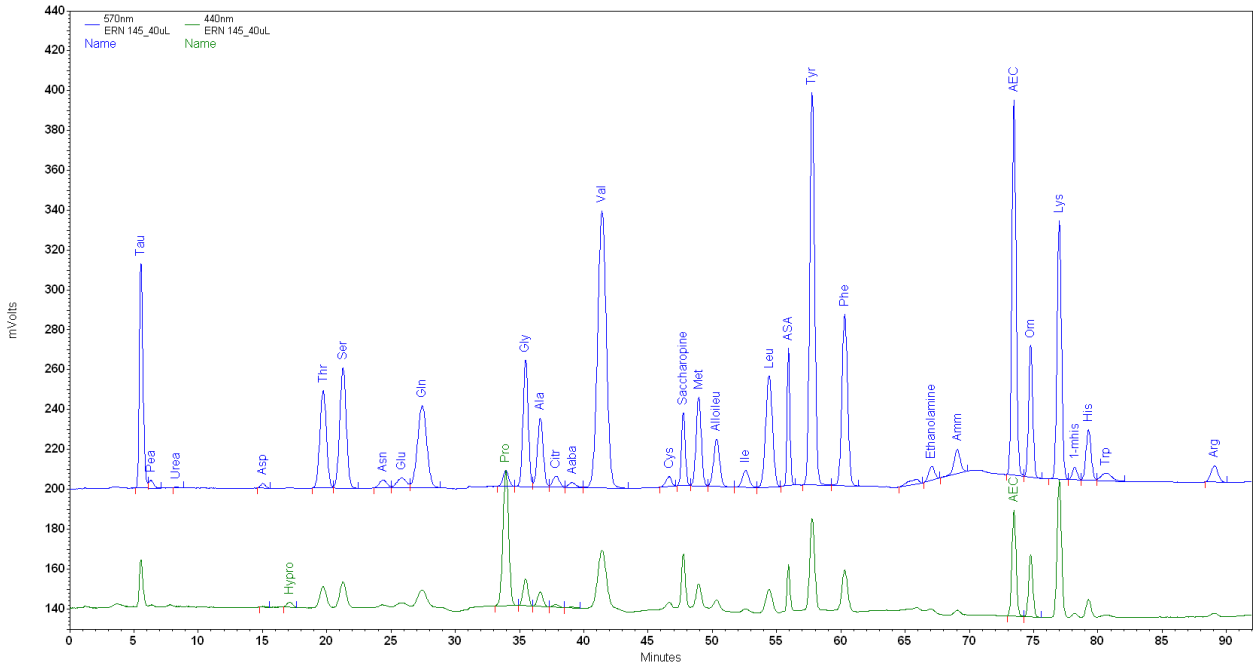

Figure 1. Plasma sample containing unusual amino acids. 570nm and 440nm channel.

Results and discussion

The full amino acid profile was obtained in 90 minutes (120 minutes cycle time). When compared to the standard physiological method, this is 30% faster therefore allowing 4 extra patient samples to be analyzed per 24 hours while lowering the cost per sample due to decreased chemical consumption. The higher flow rates and pressure have a low impact on the life of the column as the pressure generated does not exceed 120 bar (1740 PSI). The quality of the separation is excellent with resolutions better than 1.5 USP (United States Pharmacopeia Standard) on selected amino acids (Table 1). There are no ambiguities on peak identification, even on unusual amino acids.

The reproducibility of the calculated concentrations (Table 2) on the ERNDIM sample duplicates presented (142-145) demonstrates the reliability of the method. A higher difference was observed for ASA (12.9% RSD), which is due to the unstable nature of this compound: it converts easily to a pair of lactones. These compounds, referred to as Anhydrides I and II are also detectable chromatographically.

Inter-laboratory results provided on the ERNDIMQA Website [2] showed that the fast method performed well with 90% of the results in the 10%-90% percentile range (cumulative score).

The linearity of the method was also checked. All amino acids gave a linear response between 2.5-1500µmol/L. Amongst them, 23 were linear between 1-1500µmol/L (Table 3) and 13 were linear between 0.5 and 1500µmol/L (Pser, Asp, Thr, Ser, Gly, Ala, Citr, Aaba, Leu, Nleu, Tyr, Phe and Hcy).

Amino Acids	USP Resolution on selected amino acids
Methionine/Alloisoleucine	1.7
Leucine/Argininosuccinic acid	2.2
Alloisoleucine/Isoleucine	2.5
Isoleucine/Leucine	1.9
Cystine/Saccharopine	1.5

Table 1: USP resolutions in plasma sample

Amino Acids	ERN 142 (µmol/L)	ERN 145 (µmol/L)	% RSD
Alloisoleucine	94.9	95.5	0.4%
Argininosuccinic acid	288.0	239.8	12.9%
Isoleucine	36.6	35.9	1.4%
Leucine	250.0	241.8	2.3%
Saccharopine	87.2	82.2	4.2%

Table 2: Reproducibility in plasma sample

Conclusion

The overall analysis time is 30% faster compared to the standard physiological method while the cost of chemicals can be reduced by up to 25% when running the ninhydrin at 20ml/h.

This new method combines the separation quality of the Biochrom dedicated Amino Acid Analyzer with a faster analysis run time. The results demonstrate that the method is both accurate and reproducible. This method enables metabolic laboratories to save time without compromising analytical quality and provides a beneficial and economical alternative to other methods.

This method is included as standard on all new Biochrom 30+ instruments (80-6000-50) and is compatible with the Biochrom 30.

For more information, please visit our website:
www.biochrom.co.uk

Or contact us at: enquiries@biochrom.co.uk.

References:

1. W. Blom & J.G.M Huijmans, Differential diagnosis of inherited amino acid metabolism or transport disorder, Amino Acids (1992) 2; 25-67.

2. ERNDIM QA website, http://www.erndimqa.nl/

23 selected amino acids	Correlation coefficient (Linearity calculated between 1-1500µmol/L)
Phosphoserine	0.999
Taurine	0.999
Phosphoethanolamine	0.999
Aspartic acid	0.999
Threonine	0.999
Serine	0.999
A-amino adipic acid	0.999
Glycine	0.998
Alanine	0.998
Citrulline	0.999
α-aminobutyric acid	0.999
Cystine	0.999
Methionine	0.999
Leucine	0.998
Norleucine	0.998
Tyrosine	0.998
B-alanine	0.998
Phenylalanine	0.999
Homocystine	0.999
Lysine	0.998
1-methyl histidine	0.998
Histidine	0.999
3-methyl histidine	0.998

Table 3: Correlation coefficients on 23 selected amino acids.

BioSys Program

Sample:	Sigma Physiological Fluid Standard A/N and Basics
Amount Loaded:	10 nanomoles
Injection volume:	20µL
Column Type:	PEEK column packed with Ultropack 8 Cation Exchange Resin
Bed Length (mm):	200
Diameter (mm):	4.6

	Buffer	**Nin**
Flow Rate (ml/h):	35	25

Buffers
Buffer 1 - Lithium Citrate Buffer A
Buffer 2 - Lithium Citrate Buffer B
Buffer 3 - Lithium Citrate Buffer CII
Buffer 4 - Lithium Citrate Buffer DII
Buffer 5 - Lithium citrate Buffer 3.55
Buffer 6 - Lithium hydroxide Solution
Reagent - Ultra Ninhydrin solution/Ultrosolve Plus

Title: **Lithium Physiological Accelerated Program**
Reagent Flow Rate: 25.0 ml/h

No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
1	01:00	32°C	1	35.0ml/h	ON	OFF	
2	00:00	32°C	1	35.0ml/h	ON	OFF	Reset
3	01:00	32°C	1	35.0ml/h	ON	OFF	Load
4	02:45	32°C	1	35.0ml/h	ON	ON	
5	26:30	32°C	2	35.0ml/h	ON	ON	
6	13:30	40°C	3	35.0ml/h	ON	ON	
7	01:00	50°C	3	35.0ml/h	ON	ON	
8	19:30	63°C	4	35.0ml/h	ON	ON	
9	25:45	77°C	5	35.0ml/h	ON	ON	
10	04:00	77°C	6	35.0ml/h	ON	ON	
11	04:00	77°C	1	35.0ml/h	ON	ON	
12	02:00	50°C	0	OFF	OFF	OFF	
13	20:00	50°C	1	35.0ml/h	OFF	OFF	
14	04:00	32°C	1	35.0ml/h	ON	OFF	

Biochrom Ltd 22 Cambridge Science Park,
Milton Rd, Cambridge CB4 0FJ UK
Tel: +44 (0)1223 423723
Fax: +44 (0)1223 420164
Email: enquiries@biochrom.co.uk
www.biochrom.co.uk

Biochrom US 84 October Hill Road,
Holliston, MA 01746-1388 USA
Tel: (Toll free): 877-BIO-CHROM (877-246-2476)
Fax: 508-429-5732
Email: sales@biochrom-us.com
www.biochrom-us.com

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