

## B30-21 Rapid Analysis of Homocitrulline and the Branched Chain Amino Acids

Jean-Philippe Veyssier & David Thomas  
Biochrom Ltd, Cambridge, UK

**Using the Biochrom 30+ Amino Acid Analyser, the levels of the branched amino acids leucine, isoleucine, and valine, as well as alloisoleucine and homocitrulline can be determined accurately within a single run.**

A shortened program developed on the Biochrom 30+ Amino Analyser allows the separation and quantification of the branched chain amino acids valine, isoleucine, and leucine using norleucine as the internal standard. In addition, L-alloisoleucine and homocitrulline can be well resolved and quantified.

The separation of the various leucine isomers (isoleucine, leucine and alloisoleucine bearing the same molecular weight) can be an issue on other types of analytical methods such as LC-MS or HPLC. The Biochrom 30+ Amino Acid Analyser Ion Exchange Chromatograph with Post-Column Derivatization using Ninhydrin reagent and lithium citrate buffers allows full resolution of all those isobaric compounds.

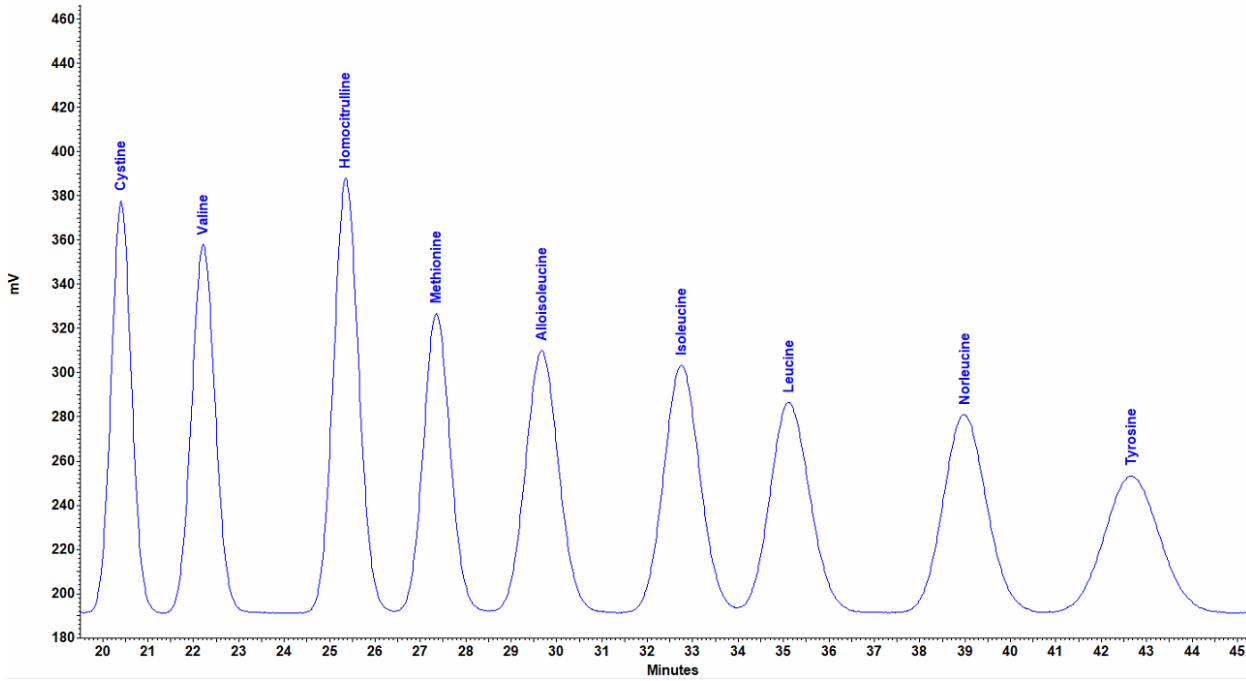
In addition, homocitrulline which is difficult to separate from methionine on a regular full profile amino acid run, is fully separated from it using this short method, thus enabling accurate identification and quantification of both compounds.

If extended, this program also enables the determination of phenylalanine.

The separation is achieved using a regular 20 cm x 4.6 mm Lithium High Performance column (p/n 80-6002-48) using predominantly lithium buffer 3 and some lithium buffer 1 during the column regeneration step. The Biochrom Physiological Standard (p/n 80-6002-80) spiked with homocitrulline was used as reference material.

The program enables between 17 and 22 analyses to be performed a day, depending on the number of amino acids required.

**Biochrom Physiological Standard p/n 80-6002-80 spiked with homocitrulline**



Sample: Physiological Amino Acid Standard Amount Loaded: 10 nmol  
 Column Type: PEEK Column Number: \_\_\_\_\_ Resin Batch: \_\_\_\_\_  
 Bed Length (mm): 200 Diameter (mm): 4.6 Instrument Serial Number: \_\_\_\_\_  
 Test Number: \_\_\_\_\_

Flow Rate (ml/h): Buffer 30 Nin 20  
 Back Pressure (bar): \_\_\_\_\_

	<u>Buffer</u>	<u>Molarity</u>	<u>pH</u>
Buffer 1 -	Lithium Buffer 1	0.2 M	2.80
Buffer 2 -	Lithium Buffer 2	0.3 M	3.00
Buffer 3 -	Lithium Buffer 3	0.5 M	3.15
Buffer 4 -	Lithium Buffer 4	0.9 M	3.50
Buffer 5 -	Lithium Buffer 5	1.65 M	3.55
Buffer 6 -	Lithium Regeneration Buffer 6	0.3 M	~12
Reagent	Ninhydrin		
	Ultrosolve		

---

Nin Flow Rate:

20.0 ml/h

<u>No.</u>	<u>Time</u>	<u>Temp</u>	<u>Buffer</u>	<u>Pump</u>	<u>Nin</u>	<u>Rec</u>	<u>Commands</u>
1	01:00	35°C	3	30.0ml/h	ON	OFF	
2	00:00	35°C	3	30.0ml/h	ON	OFF	Reset
3	01:00	35°C	3	30.0ml/h	ON	OFF	Load
4	02:00	35°C	3	30.0ml/h	ON	ON	
5	00:00	35°C	3	30.0ml/h	ON	ON	Reset
6	15:00	35°C	3	30.0ml/h	ON	ON	
7	30:00	60°C	3	30.0ml/h	ON	ON	
8	05:00	80°C	6	30.0ml/h	ON	ON	
9	02:00	80°C	3	30.0ml/h	ON	ON	
10	15:00	70°C	1	30.0ml/h	ON	ON	
11	10:00	70°C	3	30.0ml/h	OFF	OFF	
12	01:00	65°C	3	30.0ml/h	ON	OFF	
13	01:00	65°C	3	30.0ml/h	ON	OFF	