

Separation of Alloisoleucine and Saccharopine on the Biochrom Amino Acid Analysers



Amino acids: Alloisoleucine, Saccharopine.

Material: Biochrom 20/20+/30/30+ Lithium Buffer Systems equipped with a High performance or high resolution column.

Methods: Lithium Accelerated, High Performance or High Resolution programme.

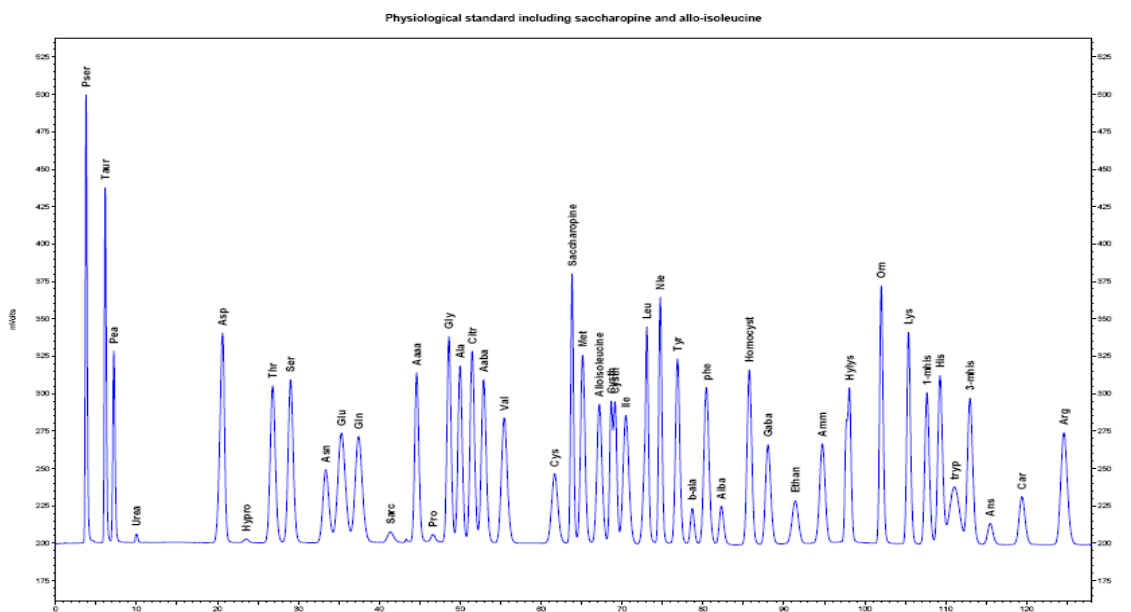
Alloisoleucine is a specific marker produced in the metabolic disorder Maple Syrup Urine Disease and is derived from the amino acid Leucine. This amino acid tends to co-elute with Cystathionine on a standard programme. This optimisation note describes how to modify the programme in order to obtain full separation of Alloisoleucine.

This method also allows the separation of Cystine and Saccharopine.

Methodology:

To improve the separation between Alloisoleucine and Cystathionine, the time of buffer 2 (Step 5, Lithium Citrate B) has to be increased until a satisfactory resolution is achieved between the two compounds. Proceeding by 1 minute increments is preferable. Increasing the time of buffer 2 excessively will cause Cystathionine and Isoleucine to co elute so a compromise has to be found.

Then the temperature at step 6 needs to be increased in consequence to maintain a good resolution between Citrulline and 2-aminobutyric acid.



Optimised Elution Programme

	<u>Buffer</u>	<u>Molarity</u>	<u>pH</u>	<u>Batch No.</u>
Buffer 1 -	Lithium Citrate Buffer (A)	0.20	2.80	15282
Buffer 2 -	Lithium Citrate Buffer (B)	0.30	3.00	15245
Buffer 3 -	Lithium Citrate Buffer (CII)	0.50	3.15	14616
Buffer 4 -	Lithium Citrate Buffer (DII)	0.90	3.50	15259
Buffer 5 -	Lithium Citrate Buffer	1.65	3.55	15371
Buffer 6 -	Lithium Hydroxide Solution	0.30	n/a	15257
Reagent	Ninhydrin			15466
	Ultrsolve			15432

Title: LiHP
Filename: C:\Program Files\BioSys\Programs\2008\103776 LiHP Saccharopine & Alloileu.prg
Comments: Optimised for the separation of Saccharopine and Alloisoleucine

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	31°C	1	25.0ml/h	ON	OFF	
2	00:00	31°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	31°C	1	25.0ml/h	ON	OFF	Load
4	04:00	31°C	1	25.0ml/h	ON	ON	
5	33:30	31°C	2	25.0ml/h	ON	ON	
6	12:00	41°C	3	25.0ml/h	ON	ON	
7	06:00	62°C	3	25.0ml/h	ON	ON	
8	26:00	62°C	4	25.0ml/h	ON	ON	
9	38:00	77°C	5	25.0ml/h	ON	ON	
10	06:00	77°C	6	25.0ml/h	ON	ON	
11	06:00	77°C	1	25.0ml/h	ON	ON	
12	02:00	31°C	0	OFF	OFF	OFF	
13	30:00	31°C	1	31.2ml/h	OFF	OFF	
14	06:00	31°C	1	25.0ml/h	ON	OFF	



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