Rapid Analysis of 5-aminolevulinic acid

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5-aminolevulinic acid can be fully separated from Phenylalanine using a short program on the Biochrom 30 Amino Acid Analyser. This enables quick screening and quantification of this compound found in urines and plasma of subjects suffering from lead poisoning or metabolic disorders like porphyria.

Introduction

5-aminolevulinic acid (ALA) is an early precursor in the synthesis of the heme, which is involved in the composition of haemoglobin, and is synthesised in the mitochondrion.
An enzyme deficiency in the porphyrin pathway (5-aminolevulinate dehydrogenase) leads to insufficient production of heme and accumulation of ALA. This anomaly is encountered in hepatic porphyria and is one of the types of porphyrias, inherited or acquired disorders of certain enzymes in the heme biosynthetic pathway.

The principal problem in these deficiencies is the accumulation of porphyrins and their chemical precursors (such as ALA), which are toxic to tissue in high concentrations.

Levels of ALA in plasma also increase in cases of lead poisoning, due to the disturbance by lead of the essential enzyme 5-aminolevulinate dehydrogenase.

Lead poisoning is sometimes mistaken for porphyria but the distinction is that lead poisoning usually causes anaemia while true porphyria does not.

Studies [1] and [2] showed that a correlation exists between plasma ALA and blood lead concentrations, pointing to a possible use of plasma ALA concentrations as a toxicological indicator of exposure to lead. These studies also shows evidence for the contribution of ALA to the pro-oxidant effects and toxicity of lead in humans and other animals.

In this application note, we separate and quantify ALA in samples in 35 minutes using a Biochrom 30 Amino Acid Analyser.

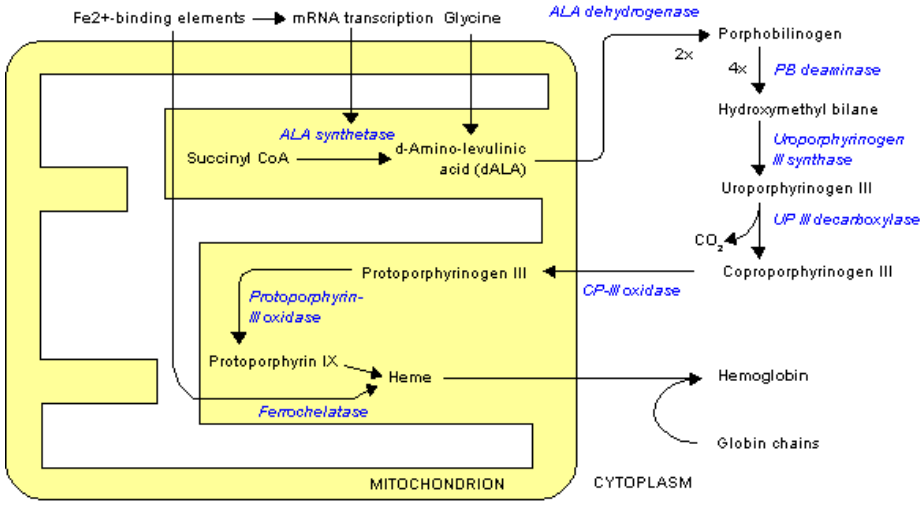

Figure 1. *Metabolic pathway of 5-aminolevulinic acid*

Experimental conditions

The main issue encountered with ALA separation on cation exchange columns is that it co elutes with Phenylalanine when run with a standard program. By modifying this program, it is possible to partially separate these two peaks which is enough for screening but not suitable to perform quantification.

In order to improve the separation, a short program has been developed and allows full separation of ALA from Phenylalanine.

The program uses mainly Lithium Citrate Buffer DII and is performed on a standard 20cmx4.6mm column. The buffer flow rate is set at 25mL/h and the Ninhydrin flow rate at 20mL/h. The total runtime injection to injection is 59 minutes and ALA elutes in 35 minutes. ERNDIM samples from the 2008 cycle have been used to show the capability of the method on real plasma matrices.

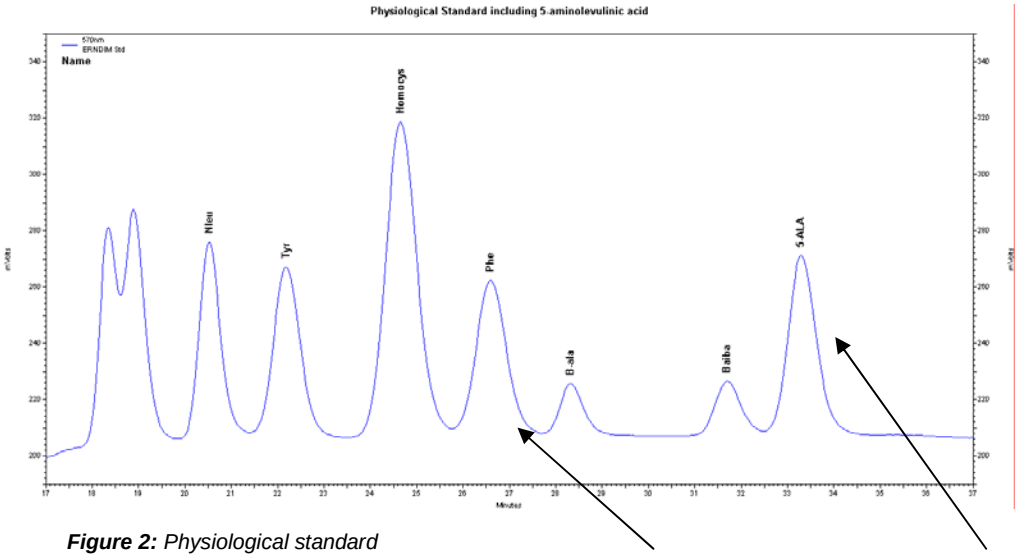

Figure 2: *Physiological standard including 5-aminolevulinic acid*

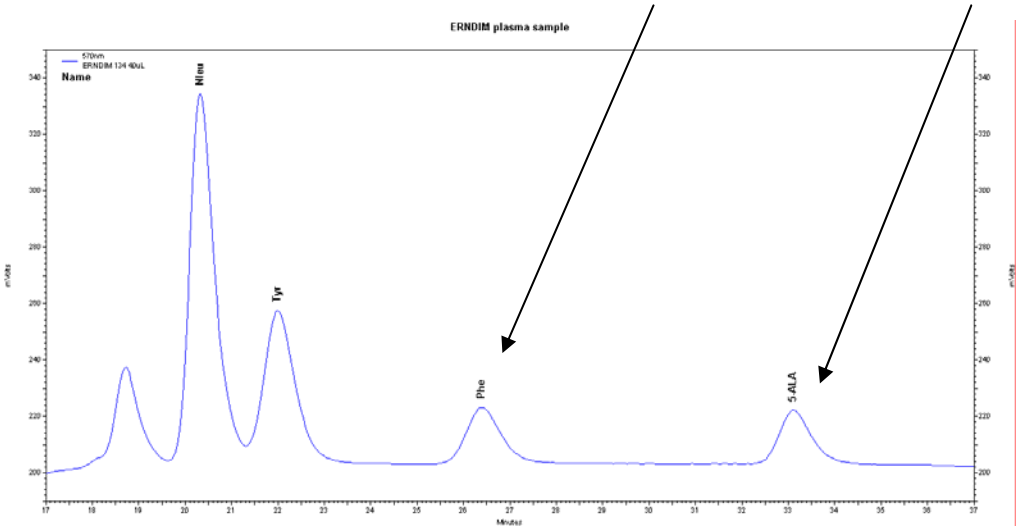

Figure 3: *Plasma sample (ERNDIM)*

Linearity

In order to demonstrate the ability to quantify low levels of ALA, 5 standards with concentrations of 2.5, 5, 10, 20, 30, 40 and 50µmol/L were injected.

The results obtained followed a linear regression curve, with a goodness of fit of **r^2=0.998.**

5-ALA (570nm)
Average RF: 0.000143452 RF StDev: 2.06474e-005 RF %RSD: 14.3932
Scaling: None LSQ Weighting: None Force Through Zero: Off
Replicate Mode: Replace
Fit Type: Linear
y = 0.000130763x + 0.855052
Goodness of fit (r^2): 0.997614

	Level 1	Level 2	Level 3	Level 4
Amount	2.5	5	10	20
Area	13267	39083	71919	137836
RF	0.0001884374764	0.0001279328608	0.0001390453148	0.0001450999738
	45315	34634	68116	82005
Last Area				
Residual	-0.0898837	-0.96566	-0.259392	1.12111
Rep StDev				
Rep %RSD				
Rep 1 Area	13267	39083	71919	137836

	Level 5	Level 6	Level 7
Amount	30	40	50
Area	218524	292994	385074
RF	0.0001372846918	0.0001365215669	0.0001298451726
	41628	94546	16172
Last Area			
Residual	0.570105	0.832188	-1.20846
Rep StDev			
Rep %RSD			
Rep 1 Area	218524	292994	385074

Figure 4: *Calibration data of ALA*

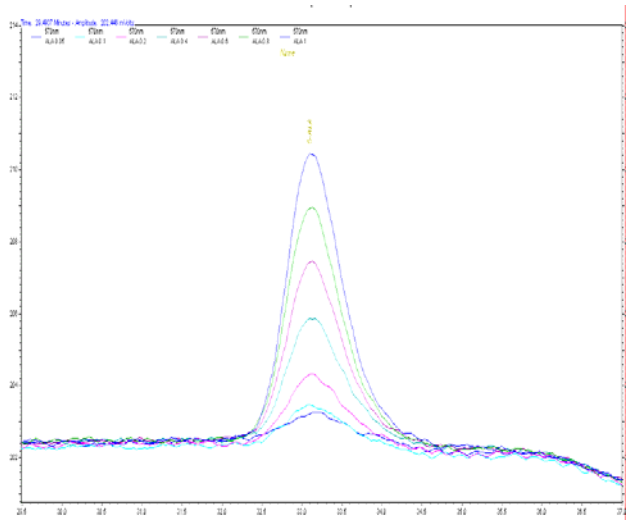

Figure 5: *Overlays of ALA peak at different concentrations*

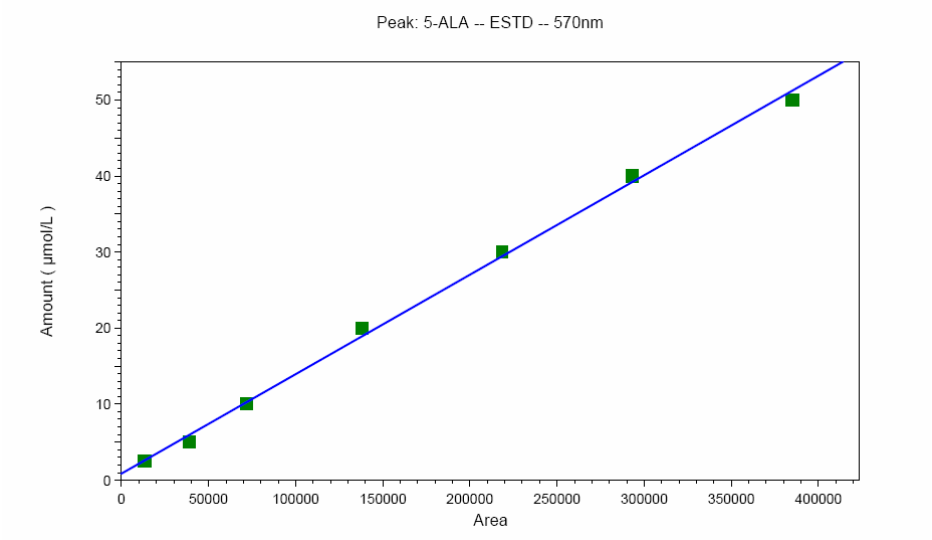

Figure 6*: The calibration curve follows a linear regression relationship*

Repeatability

5 consecutive injections of a Physiological Standard containing ALA were performed. The retention times and areas were compared. The results are shown below:

	ALA Retention time	ALA Area
Run 1	32.231	3102910
Run 2	32.231	3106098
Run 3	32.198	3105840
Run 4	32.231	3114436
Run 5	32.198	3089217
STDEV	0.018	9165
Mean	32.218	3103700
%RSD	**0.06**	**0.30**

***Figure 7**: Repeatability test on ALA peak. Quantity injected is 5nmol.*

The Biochrom 30 shows good repeatability both on retention time and area for aminolevulinic acid, with a respective RSD of 0.06% and 0.3%.

Conclusion

A quick and accurate analysis of 5-aminolevulic acid is now possible using the Biochrom 30 Amino Acid Analyser, avoiding the interference with Phenylalanine. Analysis is performed in less than 1 hour, which allows routine analysis of up to 24 samples a day. No extra chemicals or special columns are required which allows flexibility in the usage of the Biochrom 30.

If you require the ALA short program, please send us an e-mail at support@biochrom.co.uk

References:

[1] Costa et al. Corelation between plasma 5-aminolevulinic acid concentrations and indicators of oxidative stress in lead exposed workers. Clinical chemistry 43, No.7, 1997
[2] Sithisarankul et al. Plasma 5-aminolevulinic acid concentration and lead exposure in children. Environmental Research, Vol.80, No.1, January 1999, pp.41-49(9)