Using the Biochrom 30 for the Quality Control of Infusion Solutions

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Introduction

Infusion solutions are used in hospitals for patients who are unable to eat properly due to illness or surgery. They are used for fluid replacement, electrolyte balance restoration, and supplemental nutrition.

Many infusion fluids contain a selection of amino acids and are key materials in parenteral nutrition. The infusion fluids are specifically designed to meet the varied protein requirements of critically ill patients.

Intravenous amino acid infusions are also used during general anaesthesia to prevent decreases in core temperature resulting from increased energy expenditure and heat accumulation.

The analysis of the amino acids is of utmost importance to satisfy legislation for the quality control of these solutions.

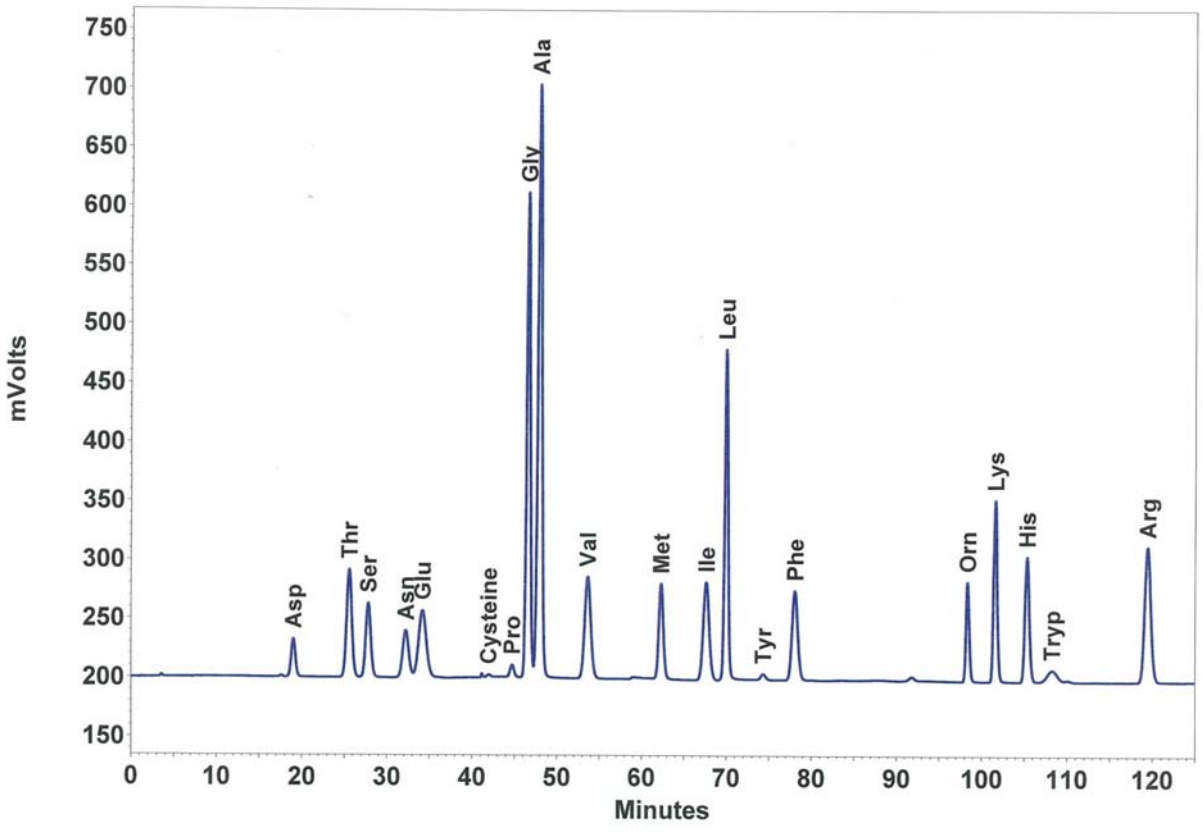


Figure 1: Amino acid infusion (x200 dilution) analysed with the standard lithium high performance program

Experimental data

The Biochrom 30 Amino Acid Analyser is available in various configurations based on either sodium or lithium buffer chemistry depending on the type of product to be analysed, and the amino acids of interest.

In the case of infusion soluions, lithium systems (also called physiological systems) are often preferred as they enable the separation of amino acids such as cysteine/proline and asparagine/glutamine, which are difficult to separate using the sodium system.

As infusion fluids are used intravenously, manufacturers must abide by very strict legislation in manufacture and quality control. A dedicated amino acid analyser can rapidly deal with complex samples, requiring virtually no sample preparation, making it an essential quality control technique for infusion solutions containing amino acids.

Several infusion samples from various global manufacturers were run on the Biochrom 30 physiological system (lithium system) in the Biochrom Applications Laboratory. The experimental data focuses on two key points for method validation: linearity and repeatability.

Linearity study

An infusion solution was analysed using the standard lithium high performance program.

Cysteine, tyrosine and tryptophan were the amino acids present at the lowest concentrations in the sample. In order to demonstrate the linearity of the Biochrom 30, test solutions containing 80%, 100% and 120% of the specified concentration for the three amino acids of interest were prepared. Each solution was diluted 200 times and injected in 3 replicates.

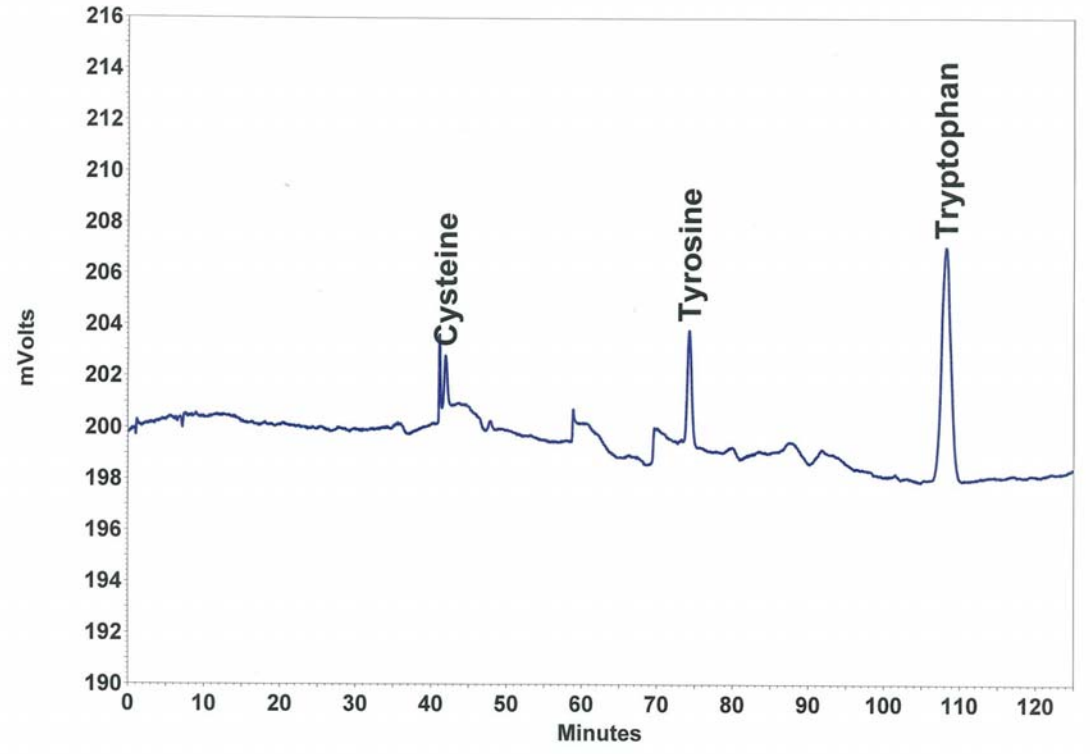
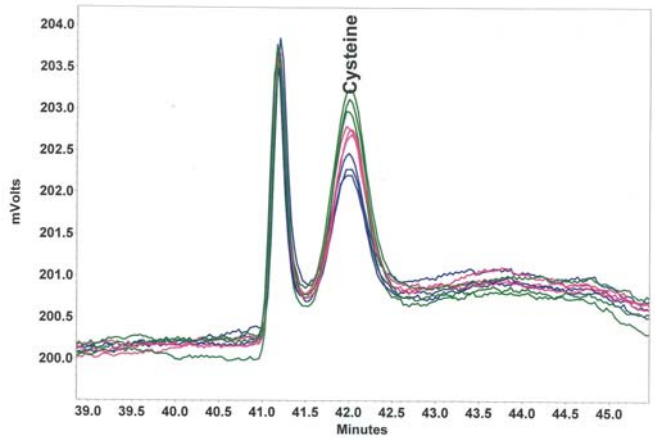
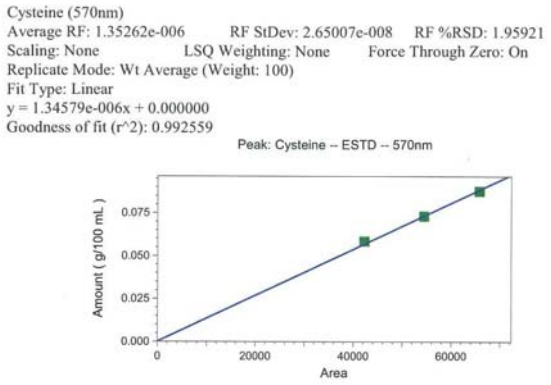
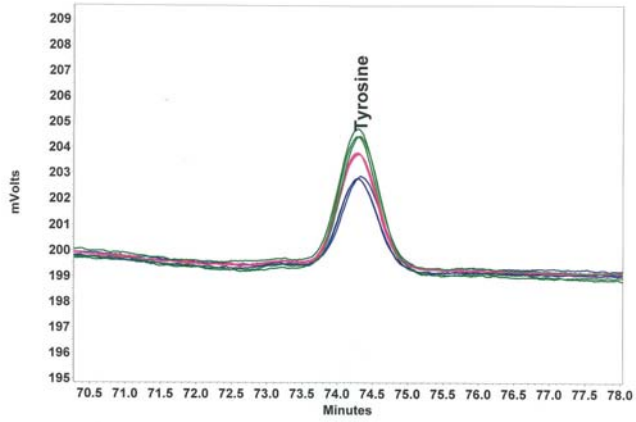
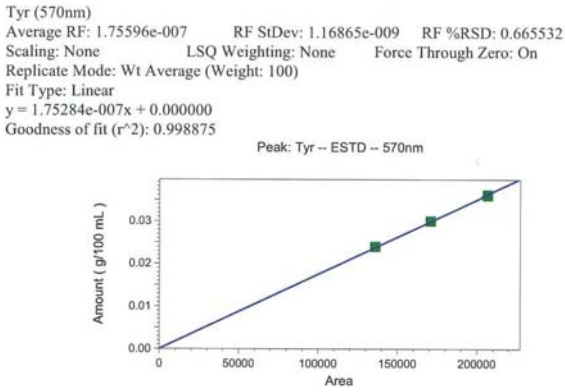


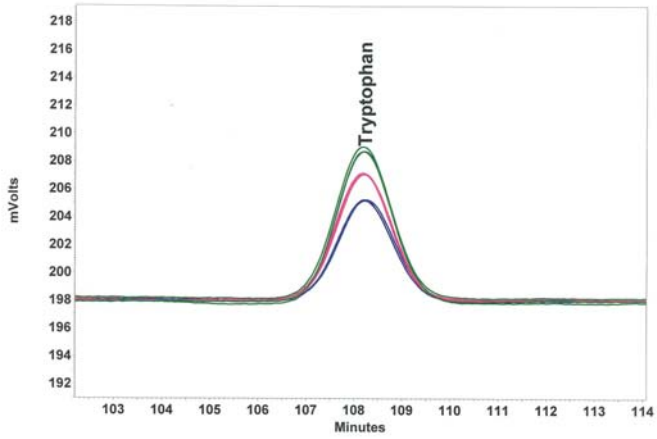
Figure 2: Test solution at 100% of Cysteine (0.73 g/L), Tyrosine (0.30 g/L) and Tryptophan (1.8 g/L) - x200 dilution











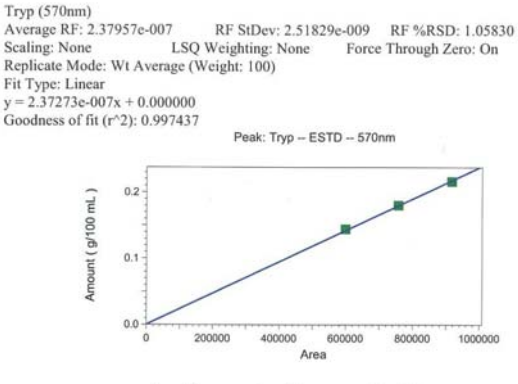


Figure 3: Linearity results for Cysteine, Tyrosine and Tryptophan at 80%, 100%, and 120% of their specified concentrations

The data showed very good repeatability on 3 replicates, and the 3-point linear calibration curve (forced through zero) gave regression coefficients greater than 0.992.

Accelerated program and repeatability studies

Since infusion liquids usually contain a limited number of amino acids the analysis time can be greatly reduced. The short program used below enables the analysis time to be reduced by up to 44% while still retaining an excellent separation.

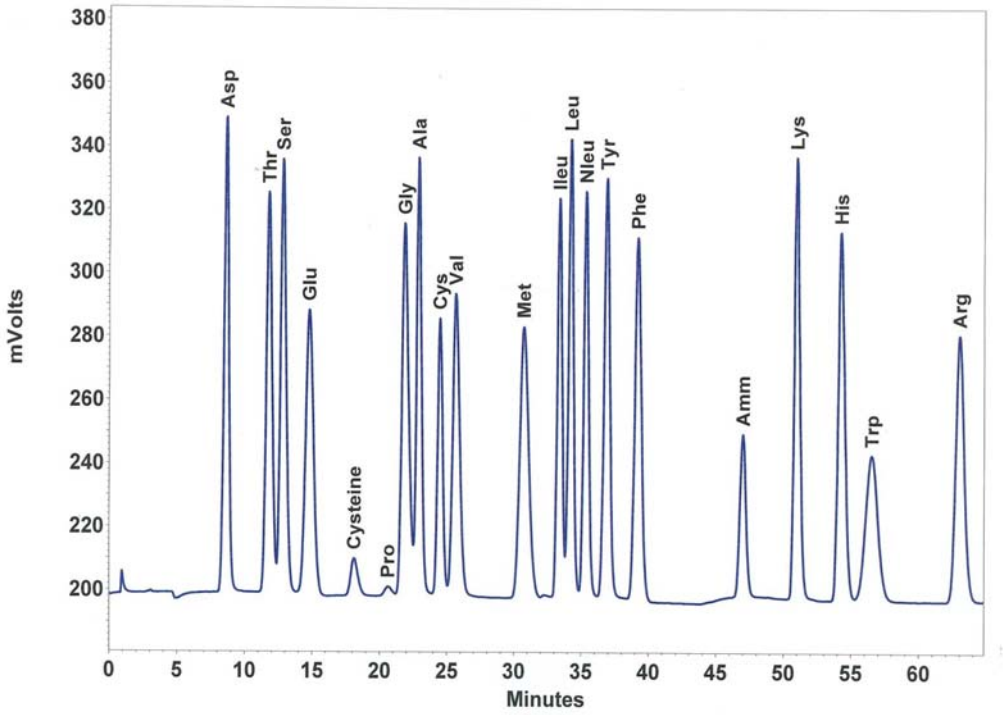


Figure 4: Calibration standard 5 nmol/ 20µL
(prepared using Sigma amino acid solution AA-S-18 and added amino acids)

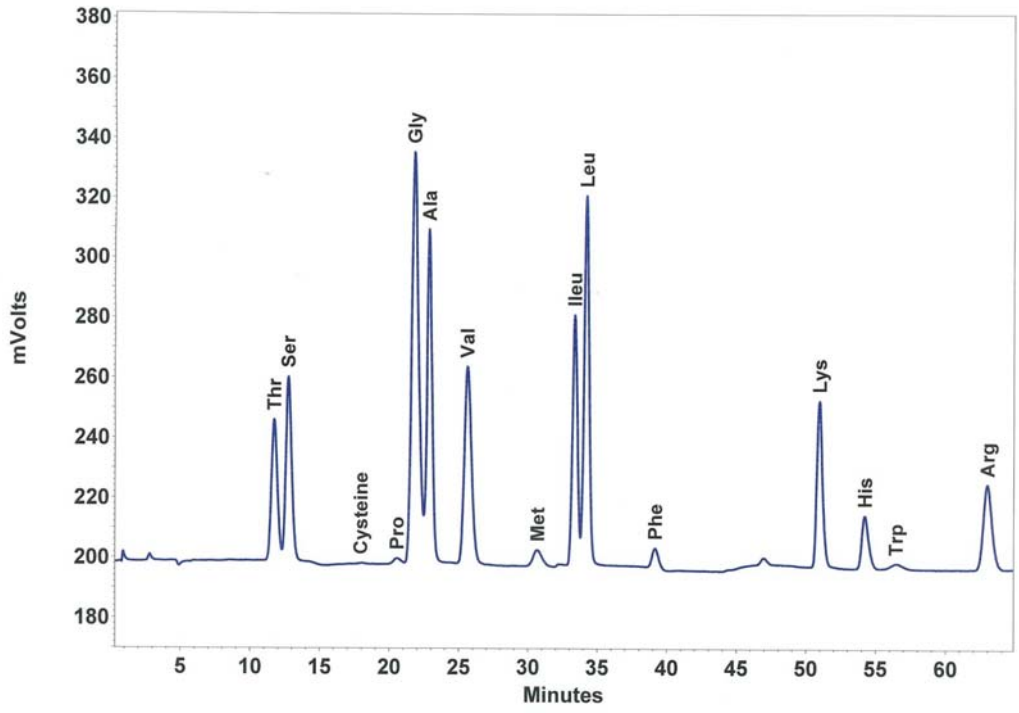


Figure 5: Infusion fluid (x 200 dilution)

Repeatability

The sample was analysed 6 times consecutively using the accelerated program. An overlay of the chromatograms is displayed below (cf. figure 6).

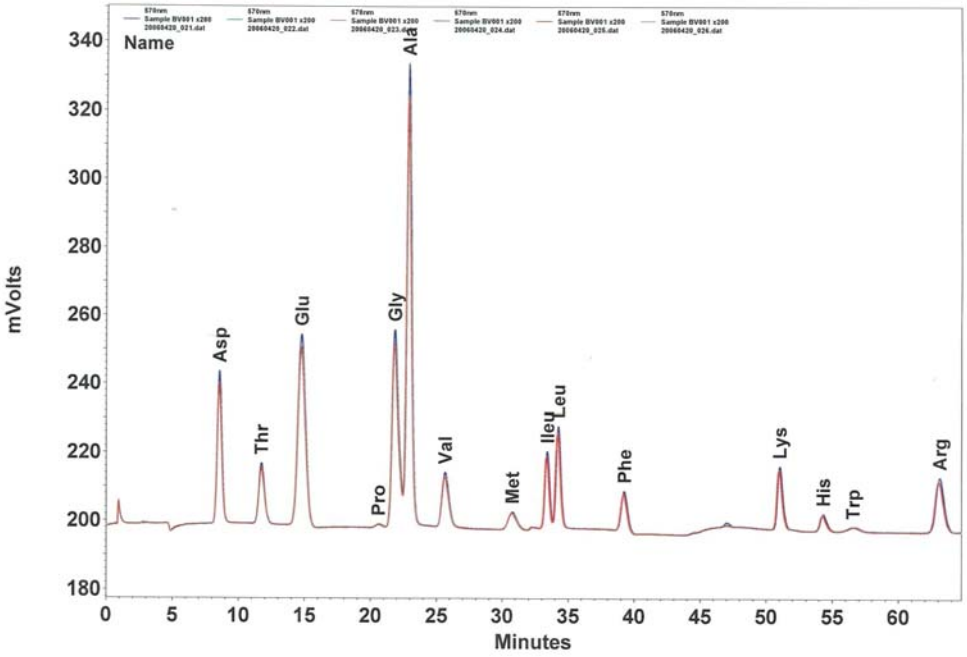


Figure 6: Overlay of 6 replicates

Compound	Concentration (g/L)	RSD (%)
L-Alanine	8.4	0.03
L-Arginine base	3.1	0.02
L-Aspartic acid	3.7	0.02
L-Glutamic acid	8.6	0.02
L-Glycine	3.4	0.04
L-Histidine base	0.6	0.02
L-Isoleucine	2.2	0.03
L-Leucine	2.6	0.02
L-Lysine base	1.9	0.02
L-Methionine	0.8	0.02
L-Phenylalanine	1.6	0.02
L-Proline	3.7	0.02
L-Threonine	1.6	0.02
L-Tryptophane	0.6	0.02
L-Valine	2.0	0.03

Table 1: Results obtained on 6 replicates

The Biochrom 30 gave an excellent repeatability of areas and retention times over 6 runs. The precision on concentrations was typically better than 0.05%.

	Buffer	Molarity	pH	Batch No.
Buffer 1 -	Lithium Citrate Buffer	0.20	2.80	13783
Buffer 2 -	Lithium Citrate Buffer	0.30	3.00	13536
Buffer 3 -	Lithium Citrate Buffer CII	0.50	3.15	13562
Buffer 4 -	Lithium Citrate Buffer DII	0.90	3.50	13518
Buffer 5 -	Lithium Citrate Buffer	1.65	3.55	13519
Buffer 6 -	Lithium Hydroxide Solution	0.30		13222
Reagent	Ninhydrin			13378
	Ultrosolve			13545

Title: **Physiological High Performance**
Filename: C:\Program Files\BioSys\Programs\Fast Protein hydrolysate Li.prg

Nin Flow Rate: 25.0 ml/h

No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
1	01:00	30°C	2	35.0ml/h	ON	OFF	
2	00:00	30°C	2	35.0ml/h	ON	OFF	Reset
3	01:00	30°C	2	35.0ml/h	ON	OFF	Load
4	04:00	30°C	2	35.0ml/h	ON	ON	
5	09:00	30°C	3	35.0ml/h	ON	ON	
6	06:00	55°C	3	35.0ml/h	ON	ON	
7	12:00	55°C	4	35.0ml/h	ON	ON	
8	20:00	85°C	5	35.0ml/h	ON	ON	
9	07:00	90°C	5	35.0ml/h	ON	ON	
10	06:00	90°C	6	35.0ml/h	ON	ON	
11	05:00	90°C	2	35.0ml/h	ON	ON	
12	02:00	30°C	0	OFF	OFF	OFF	
13	15:00	30°C	2	35.0ml/h	OFF	OFF	
14	05:00	30°C	2	35.0ml/h	ON	OFF	

Figure 7: Biosys program for accelerated analysis of infusion solutions

Conclusion

A dedicated ion exchange liquid chromatography system provides rapid, specific amino acid analysis for quality control laboratories in the pharmaceutical industry.

Compared to other techniques, the Biochrom 30 offers several advantages for the analysis of infusion solutions

- Because ninhydrin reacts specifically with amino acids there is no interference with other compounds typically found in infusions such as vitamins and electrolytes.
- Long life columns can tolerate various matrices and high salt content of infusion solutions.
- No sample preparation is required other than dilution.
- Short programs are available for the analysis of specific products.

The Biochrom 30 offers full automation for the fast and reliable analysis of infusion fluids to a high standard that satisfies the strict pharmaceutical legislation.