Using the Biochrom 30 for the ERNDIM quantitative amino acid scheme

Since January 2005, Biochrom has taken part in the ERNDIM[1] quantitative amino acid scheme. In addition to confirming the suitability of the Biochrom 30 for this purpose, the main goals are to gain a better understanding of the difficulties faced by clinical laboratories regarding quantitative amino acid analysis and to help improve this analysis for all Biochrom amino acid analyser users.

The ERNDIM scheme consists of analysing 8 lyophilised samples per year, all prepared from the same basic human serum and spiked with various amounts of analytes. As part of the scheme, 29 amino acids are reported, 25 of them have been common to both the 2005 and 2006 ERNDIM cycles.

The Biochrom 30 enables labs to perform full routine analyses of physiological fluids such as plasma, urine, serum, and CSF. The separation and quantification of amino acids is achieved by ion exchange chromatography followed by post column ninhydrin derivatisation and photometric detection.

Experimental conditions

The ERNDIM scheme analysis was performed on a Biochrom 30 using a lithium high performance program.

The sample was prepared by adding 1 mL of distilled water to the contents of the vial. The sample was then deproteinised using a 10% SSA solution containing Norleucine as the internal standard.

The calibration standard was prepared using the Sigma amino acid standard solution Acidics and Neutrals (A6407) and Sigma amino acid standard solution Basics (A1585) to which were added the required additional amino acids. The calibration solution was also treated with SSA.

[1]ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism)
http://www.erndimqa.nl/
http://www.erndim.unibas.ch/

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2005 CYCLE

A summary of the 2005 results is displayed in the table opposite. The results obtained on the Biochrom 30 for the 2005 cycle were in good correlation with the results obtained for all labs in terms of accuracy, precision and linearity.

•The mean values (concentrations in µmol/L) for most amino acids were within 10% of the values for all labs.

• The overall percentage recovery of added analyte was 102%.

• The precision (CV%) obtained on duplicates was better than 5% for most amino acids .

• The linearity (coefficient of regression (**r**)) for all the amino acids was on average 0.9965.

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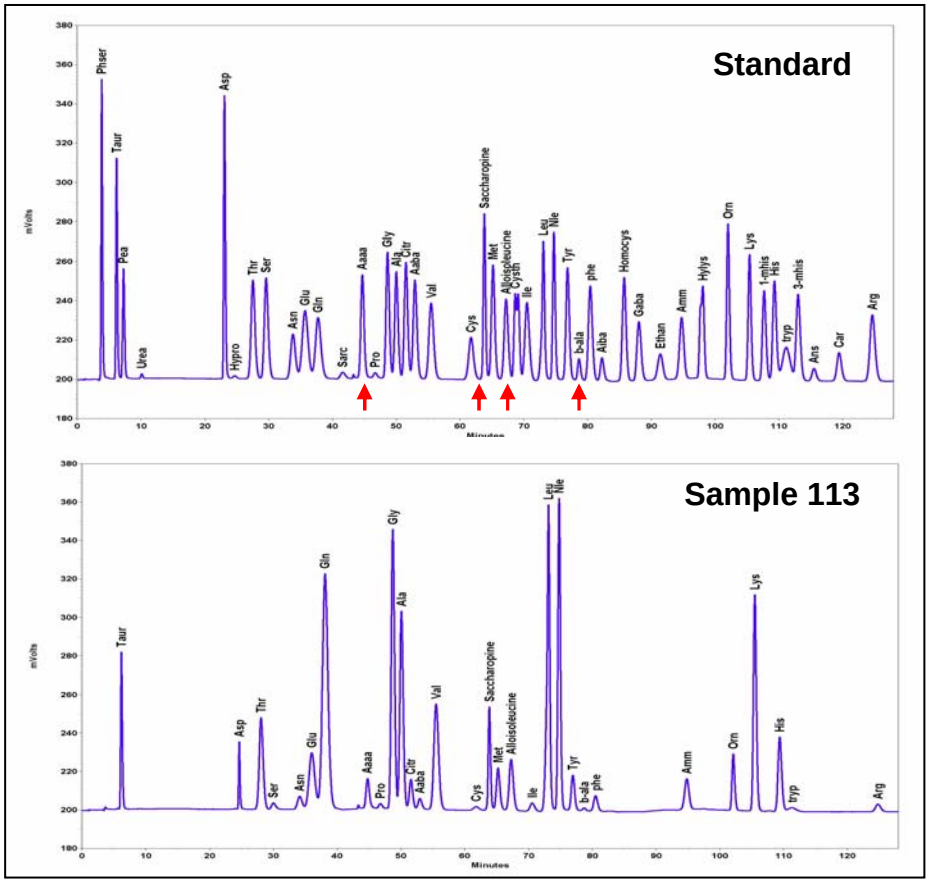
Analyte	Accuracy (Mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (% added analyte)		Data all labs	
	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	Nr. of Labs	Inter Lab CV
Alanine	412	403	2.20%	4.80%	0.9994	0.9962	103%	98%	182	7.50%
alpha-Amino adipic acid	129	122	2.60%	6.70%	0.9996	0.9976	118%	109%	163	15.80%
alpha-Amino butyric acid	14.1	13.3	2.20%	9.80%	0.9889	0.9457	103%	103%	167	20.20%
Arginine	127	124	5.00%	5.10%	0.9992	0.9979	98%	97%	181	10.30%
Asparagine	34.4	27.7	7.00%	10.50%	0.9963	0.9856	126%	94%	159	25.00%
Aspartic Acid	24.7	27.3	4.50%	7.80%	0.998	0.9916	81%	96%	178	18.90%
beta-Alanine	53.9	50.4	14.30%	11.70%	0.9947	0.9918	98%	94%	157	108.90%
Citrulline	81.1	80.3	1.30%	6.30%	0.9947	0.9959	105%	98%	178	21.40%
Cystine	27.9	31.2	5.30%	10.90%	0.9826	0.9847	81%	76%	166	75.30%
Glutamic acid	161	160	3.90%	7.40%	0.9971	0.9899	112%	110%	180	11.60%
Glutamine	746	627	3.90%	6.80%	0.9981	0.9959	103%	93%	179	10.20%
Glycine	305	301	2.20%	5.00%	0.9993	0.9967	100%	99%	182	8.00%
Histidine	153	153	2.30%	4.90%	0.9986	0.9964	96%	94%	181	9.40%
Histidine 1-Methyl	19.5	18.6	3.80%	9.20%	0.9994	0.9956	103%	103%	158	128.00%
Hydroxyproline	26.9	22	49.10%	18.00%	0.9945	0.9805	126%	98%	146	253.10%
Isoleucine	191	185	2.90%	4.60%	0.9987	0.9986	97%	96%	182	7.90%
L-allo Isoleucine	108	107	3.50%	5.70%	0.9981	0.9966	100%	98%	142	14.10%
Leucine	513	502	2.00%	4.50%	0.9995	0.9963	101%	97%	182	7.40%
Lysine	238	231	1.30%	4.50%	0.9993	0.997	99%	94%	182	7.30%
Methionine	116	109	3.50%	4.90%	0.9974	0.9972	103%	94%	181	14.80%
Ornithine	113	108	1.30%	4.80%	0.9988	0.9975	105%	99%	182	9.70%
Phenylalanine	367	347	0.90%	4.00%	0.9993	0.999	95%	92%	182	7.20%
Proline	225	218	3.80%	6.10%	0.9986	0.9965	93%	89%	164	10.10%
Saccharopine	182	104		7.00%	1	0.9951	98%	96%	105	19.30%
Serine	70.4	67	4.40%	8.50%	0.9738	0.9805	118%	110%	180	32.70%
Taurine	138	136	2.30%	5.10%	0.9992	0.997	99%	98%	179	10.40%
Threonine	131	127	2.40%	4.90%	0.9989	0.9963	100%	99%	179	8.00%
Tyrosine	226	214	1.80%	4.10%	0.998	0.9974	98%	93%	182	7.20%
Valine	311	300	3.00%	4.20%	0.9987	0.9974	103%	101%	182	6.40%
Overall	181	169	5.10%	6.80%	0.9965	0.9926	102%	97%	171	30.60%



The additional compounds are marked by a red arrow on the chromatogram opposite. α-Amino Adipic Acid and ß-Alanine which are present in the Sigma standard are routinely separated.

Following suitable optimisation allo-Isoleucine can be separated from Methionine and Cystathionine, and Saccharopine from Cystine.

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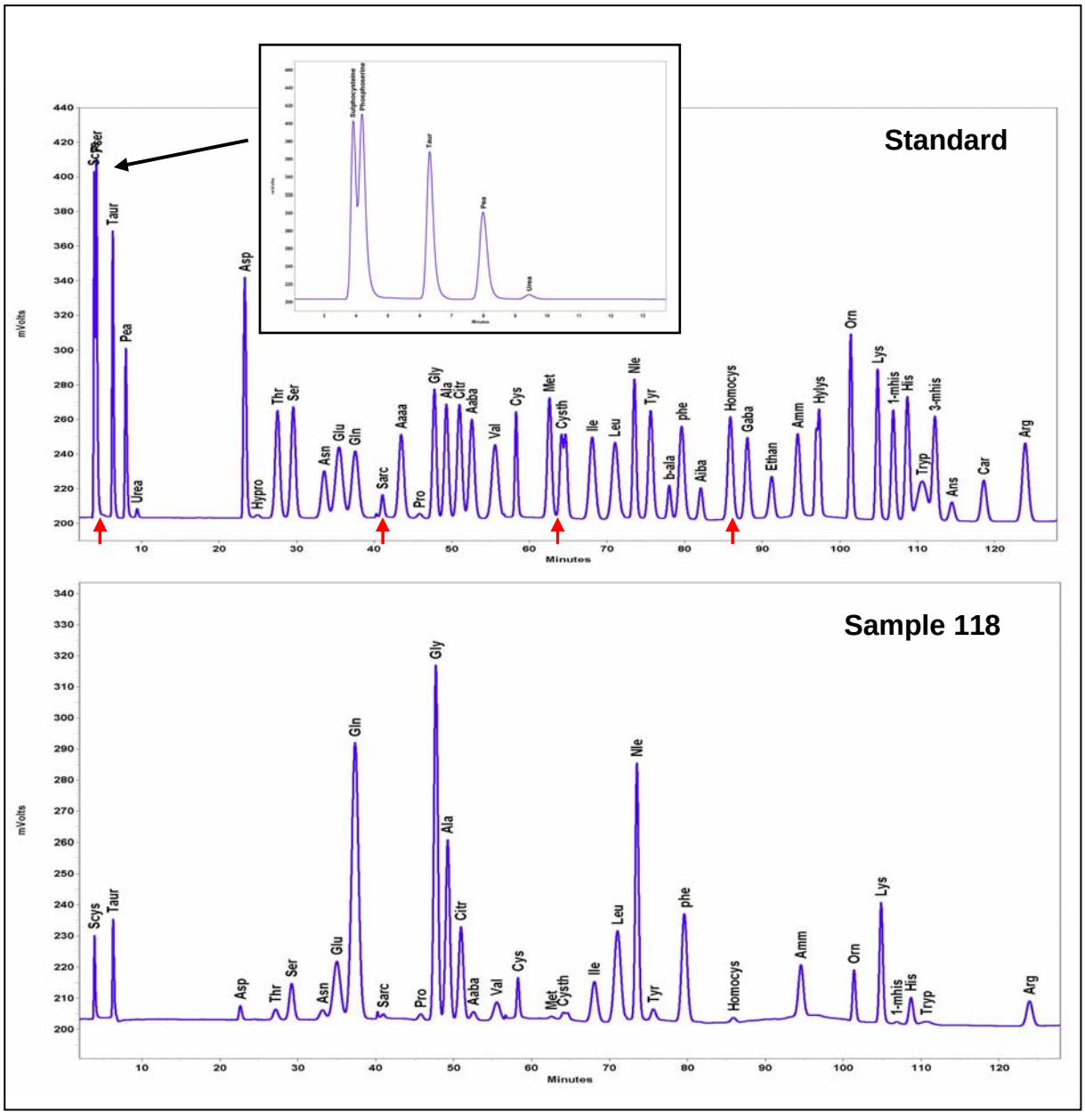
2006 CYCLE

The additional amino acids required for the 2006 cycle are Cystathionine, Homocystine, Sarcosine and Sulphocysteine. The first three, being part of the Sigma standard are usually well resolved using the standard lithium high performance program.

Sulphocysteine however is more difficult to quantify as it often coelutes with Phosphoserine. Various options are available for accurate quantification of Sulphocysteine.

The standard lithium high performance program can be optimised in order to improve the separation between the two peaks as shown in the chromatogram below. Alternatively a short program such as the one described in Applications note B30-4 can be used. Calculating the ratio between the 570 and the 440 channels for Sulphocysteine and Phosphoserine can also be useful if there is any doubt in the peak identification as they are significantly different.



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