Determination of the amino acid profile in blood plasma samples using post-column derivatization and ninhydrin

Introduction

With brand biological functions, amino acids are one of the important products of human being's metabolism. AAs are the basic unit of protein molecules in the bio-organism and the important material basis to maintain homeostasis. In the form of amino acids, daily intake of protein is absorbed by digestive system and transported to the whole body through blood. Defects in related proteins and enzymes involved in amino acid metabolism in the human body or various pathological conditions can lead to abnormal amino acid metabolism and changes in blood amino acid levels. Therefore, it is of great significance to analyze the content of amino acids in blood.

So far, there are more than 20 kinds of amino acids involved in protein synthesis. They exist in two forms, free state in plasma or urine and bound state as peptides and proteins.

The role of amino acids in plasma is particularly important. However, some certain diseases may cause the metabolism of amino acids in body become disorders. Amino acid disorders are divided into two types, one is caused by related gene mutations, due to lacking of some enzymes participating amino acid metabolism and some carrier proteins, genetic diseases is led by absorption obstruct of amino acids in kidney or intestinal tract; the other one is called secondary amino acid disorder, for example burns, severe trauma, illness, which are caused by severe lesions in organs related to amino acid metabolism, like liver and kidney.

Most part of aromatic amino acids and alanine are degraded in liver, while most part of isoleucine, leucine and valine are degraded in muscles, kidneys and brain.

Therefore, it is essential to analyze the content of amino acids in the blood, which can provide necessary scientific basis for clinical diagnosis or observation.

In this application note we would like to show how easy it is to analyze plasma samples with the ARACUS amino acid analyzer. The professional instrument is widely used for its short analysis time and high sensitivity. In this article, plasma samples from human, mouse and pork are first prepared and then analyzed.

Sample Preparation of human blood plasma

400 µl blood plasma and 100 µL precipitation solution were mixed in a 1.5 mL tube and deposit in the refrigerator for 60 min for the protein precipitation. Afterwards the samples were centrifuged. The supernatant was filtered with a membraSpin by centrifugation at 14000 rpm for five minutes. 100 µL of the sample solution is mixed together with 100 µL sample dilution buffer containing norleucine (200 nmol/mL). The particle free solution was used for the injection.

Sample Preparation of mice blood plasma

20 µL blood plasma and 4 µL precipitation solution were mixed in a 1.5 mL tube with 6 µL sample dilution buffer containing norleucine (1 nmol/µL) and 30 µL pure sample dilution buffer. Due to the reduced volume of 60 µL the deposition of the sample in the refrigerator was skipped and the sample was directly centrifuged. The supernatant was filtered with a membraSpin by centrifugation at 14000 rpm for five minutes. The particle free solution was used for the injection.

Sample Preparation of pork blood plasma

400 µl blood plasma and 100 µL precipitation solution were mixed in a 1.5 mL tube and deposit in the refrigerator for 60 min for the protein precipitation. Afterwards the samples were centrifuged. The supernatant was filtered with a membraSpin by centrifugation at 14000 rpm for five minutes. 100 µL of the sample solution is mixed together with 100 µL sample dilution buffer containing norleucine (150 nmol/mL). The particle free solution was used for the injection.

Results

Human blood plasma:

Table 1 compares the results of two independently tested human blood plasma samples. No abnormalities are seen in either sample and the values are within the normal range. The corresponding chromatograms are shown in Figure 2.

Table 1: Determined Amino Acid concentrations of two independently human blood plasma samples.

Amino Acid	(A) concentration of amino acid nmol/ mL	(B) concentration of amino acid nmol/ mL
P-Ser	Not detected	13.6
Tau	47.0	128.7
Urea	> 6800.0	> 6500.0
Asp	3.1	14.1
Thr	148.2	169.7
Ser	105.5	128.3
Asn	69.1	75.1
Glu	31.6	211.6
Gln	627.5	490.9
a-AAA	10.1	16.2
Gly	230.4	382.3
Ala	422.4	560.0
Cit	40.9	30.7
a-ABA	21.8	21.5
Val	260.5	251.8
Cys	56.4	81.5
Met	42.3	17.6
Ile	86.2	59.4
Leu	160.0	168.0
Tyr	82.5	78.8
Phe	57.5	92.4
H - Cysteine	Not detected	Not detected
b-Ala	10.9	21.4
b-AiBA	8.8	Not detected
g-ABA	7.1	15.5
His	89.1	107.4
3Mehis	8.6	13.1

1Mehis	35.4	Not detected
Trp	51.4	33.7
Orn	105.7	229.6
Lys	201.9	290.0
NH4	48.2	356.9
EOHNH2	5.6	21.9
Arg	52.9	8.4
Hypro	11.6	13.8
Pro	225.9	238.6

Mouse blood plasma:

Table 2 summarizes the results of an examined mouse blood plasma sample. Selected amino acids were analysed. The corresponding chromatogram is shown in Figure 3.

Table 2: Determined Amino Acid concentrations of a mouse blood plasma sample.

Amino Acid	concentration of amino acid µmol/ L
Ser	175.9
Ala	336.1
Lys	335.1
NH4	192.5
Arg	155.2

Pork blood plasma:

Table 3 summarizes the results of a tested pork blood plasma sample. The corresponding chromatogram is shown in Figure 4.

Table 3: Determined Amino Acid concentrations of a pork blood plasma sample.

Amino Acid	concentration of amino acid nmol/ mL
P-Ser	9.2
Tau	358.7
Urea	> 2400.0
Asp	43.1
Thr	410.5
Ser	399.1
Asn	249.0
Glu	235.9
Gln	1033.4
a-AAA	9.3
Gly	1534.0
Ala	1566.6
Cit	122.6
Val	341.6
Cys	45.5
Met	114.2
Ile	186.7
Leu	242.1
Tyr	206.9
Phe	256.8
g-ABA	22.5
His	158.6
3Mehis	< 15.0
Trp	77.0

Car	35.5
Orn	145.6
Lys	255.3
NH4	270.3
Arg	243.6
Hypro	136.0
Pro	983.7

The samples were analyzed by the Amino Acid Analyzer ARACUS, manufactured and distributed by membraPure GmbH worldwide. ARACUS is using the classic routine analysis of amino acids by post-column derivatization with ninhydrin and the detection at 440 nm and 570 nm. For the sample of mouse only at 570 nm.

Figure 1: Amino Acid Analyzer ARACUS

Conclusions

The tests have shown that the ARACUS can also manage with very small amounts of sample, as was the case with the mouse sample, and that sample preparation does not present any problems. Plasma samples of different origin (human, mouse, pork) can also be processed in the same way. Depending on the requirements for the number of amino acids to be analyzed, the method can be varied, resulting in an optimization ratio between the number of amino acids and the run time of the analysis. Fewer amino acids - shorter program.

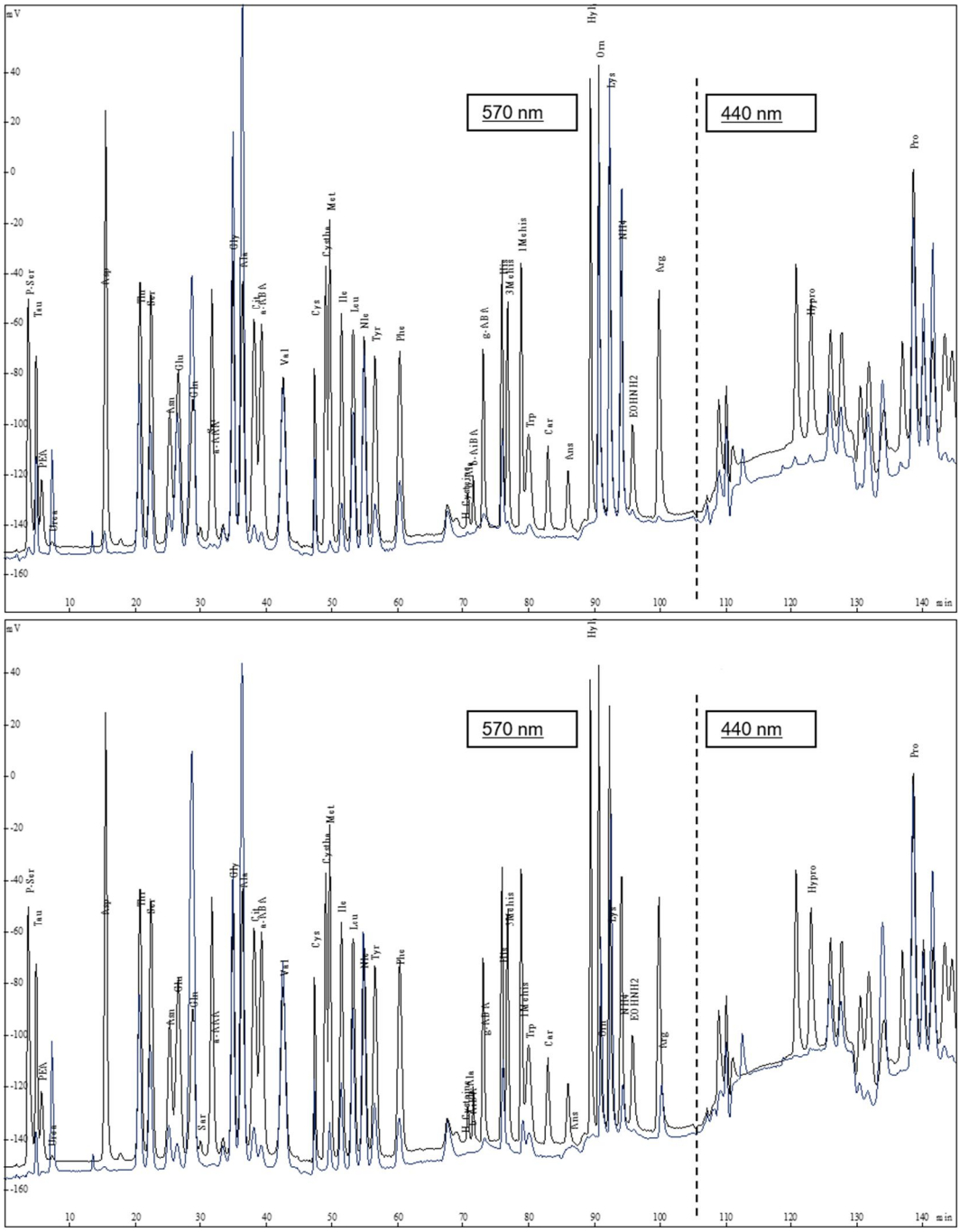

Figure 2: Comparison of a physiological amino acid standard (black) with "human blood plasma A" sample (blue, top image) and " human blood plasma B" sample (blue, bottom image).The detection was performed at 440 nm and 570 nm. The concentrations of the individual amino acids were determined using a known concentration of the standard amino acid mixture.

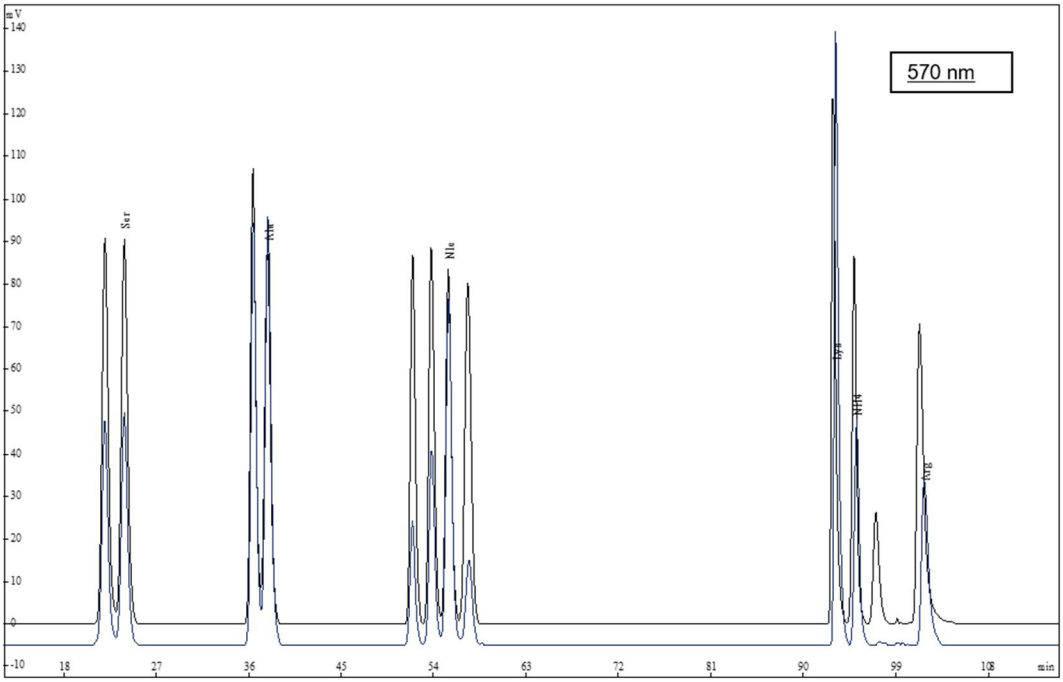

Figure 3: Comparison of a physiological amino acid standard (black) with "mouse blood plasma" sample (blue, top image). Only the amino acids of interest are marked and used for evaluation. The detection was performed at 570 nm. The concentrations of the individual amino acids were determined using a known concentration of the standard amino acid mixture.

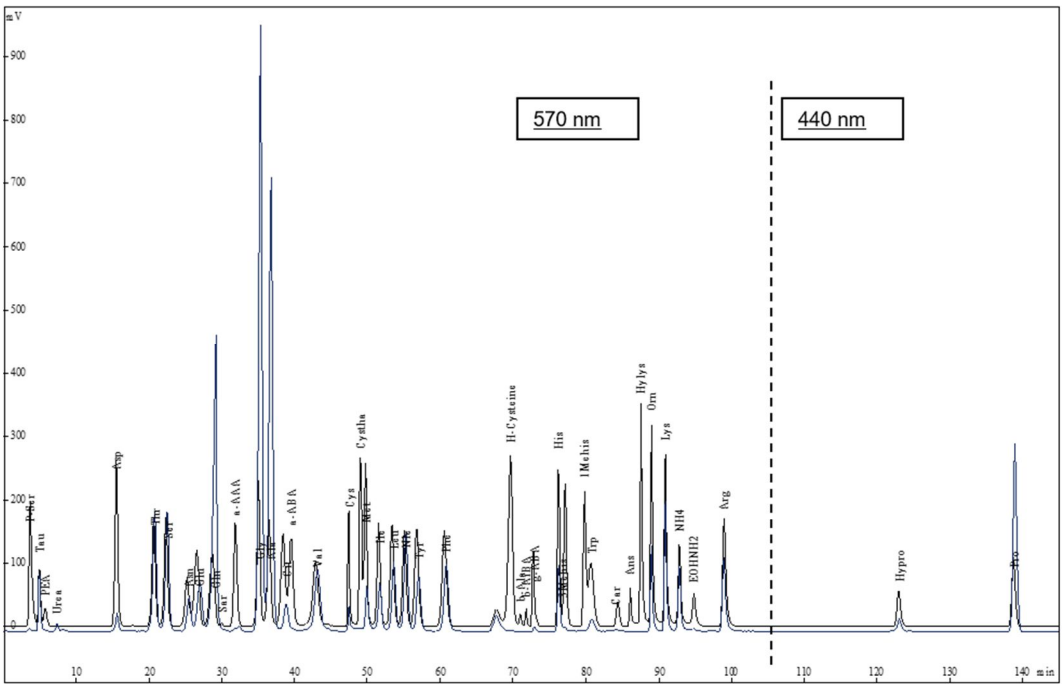

Figure 4: Comparison of a physiological amino acid standard (black) with "pork blood plasma" sample (blue, top image). detection was performed at 440 nm and 570 nm. The concentrations of the individual amino acids were determined using a known concentration of the standard amino acid mixture.

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