

Quality control of proteins - basis for special medical food - by post-column derivatization with ninhydrin



Introduction

Formula Food for special medical use, referred to as "special medical food", is to meet the special needs of nutrition or diet for people with eating restriction, dyspepsia, metabolic disorder or specific disease state, a specially prepared formula.

When the target population is unable to use ordinary diet or daily diet to meet their nutritional needs, special medical use formula food can play a nutritional support role as a nutritional supplement. In view of the specific metabolic state of different diseases, the formula food for special medical use has put forward special regulations on the corresponding nutrient content, which can better adapt to the specific disease state or the nutritional requirement of a certain stage of the disease, to provide targeted nutritional support for patients is an effective way for clinical nutritional support.

It is necessary to analyze the content and quality of protein and whether its amino acid composition meets the needs of human body. Amino acid analyzer is a special instrument for analyzing amino acid in national standard method.

In this application note, two dietary supplements are to be tested for their ingredients.

Supplement I is a whitish colored powder that is used as a dietary supplement.

Supplement II is a ready-to-drink beverage.

Both supplements consists of a balanced mixture of essential and non-essential amino acids, carbohydrates, fats, minerals and vitamins and is administered as part of a "PKU diet" for children (< 8 years) and adults.

For Supplement II, data on ingredients are already available from a testing laboratory and will be verified by further analysis.

Sample Preparation & Analysis of Supplement I

5000 mg of the homogenized sample is transferred to a 500 mL volumetric flask and this is filled up to the measuring mark with 3 molar HCl. The sample was completely dissolved.

50 μ L of the sample solution is mixed together with 450 μ L Sample dilution Buffer as well as another 500 μ L Sample dilution Buffer containing norleucine (200 nmol/mL). The sample is then ready for analysis.

Sample Preparation & Analysis of Supplement II

First, the beverage sample is homogenized by vigorous shaking. Then 1000 μ L are transferred to a reaction vessel and centrifugated at 14500 rpm for 7 min. A white precipitate with a yellowish colored supernatant is formed at the bottom of the tube. From the supernatant, 400 μ L is taken and 100 μ L of sample precipitation buffer is added to perform the protein precipitation. The sample was incubated for 1 hour at 4 °C and then filtered through a micro filter. 10 μ L of the particle free solutions were mixed with 790 sample dilution buffer containing norleucine. The sample is then ready for analysis.

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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.



Figure 1: Amino Acid Analyzer ARACUS

Table 1: Determined amino acids concentration of the whitish powder supplement I.

Amino Acid	concentration of amino acid mg / 100 g
Asp	7.7
Thr	4.1
Ser	4.8
Glu	15.1
Gln	Not detected
Gly	3.6
Ala	4.5
Val	5.5
Cys	1.5
Met	1.6
Ile	4.3
Leu	7.0
Tyr	5.9
His	2.4

Trp	1.4
Lys	5.4
NH ₄	0.1
Arg	3.8
Pro	7.2

Table 2: Determined amino acids concentration of the ready-to-drink supplement II. With ARACUS (A) in comparison to the results of a testing laboratory (B).

Amino Acid	(A) concentration of amino acid g/ 100 mL	(B) concentration of amino acid g/ 100 mL
Tau	34.5	35.5
Asp	0.43	0.56
Thr	0.45	0.45
Ser	0.39	0.40
Gly	0.54	0.57
Ala	0.32	0.32
Val	0.62	0.59
Cys	0.03	0.22
Met	Not detected	0.15
Ile	0.57	0.53
Leu	0.94	0.91
Tyr	0.06	0.81
Phe	Not detected	Not added
His	0.35	0.34
Trp	0.19	0.18
Lys	0.69	0.70
Arg	0.64	0.60
Pro	0.78	0.65
Carnitin	Not analysed	4.7

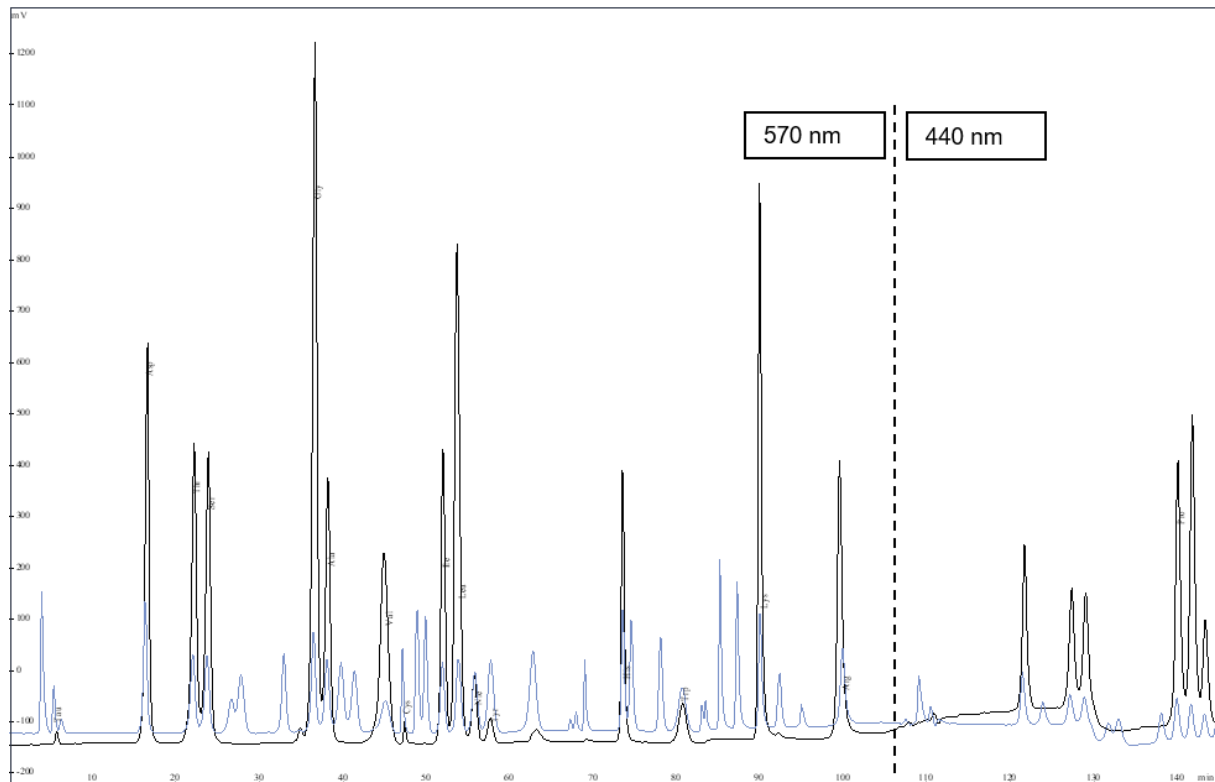
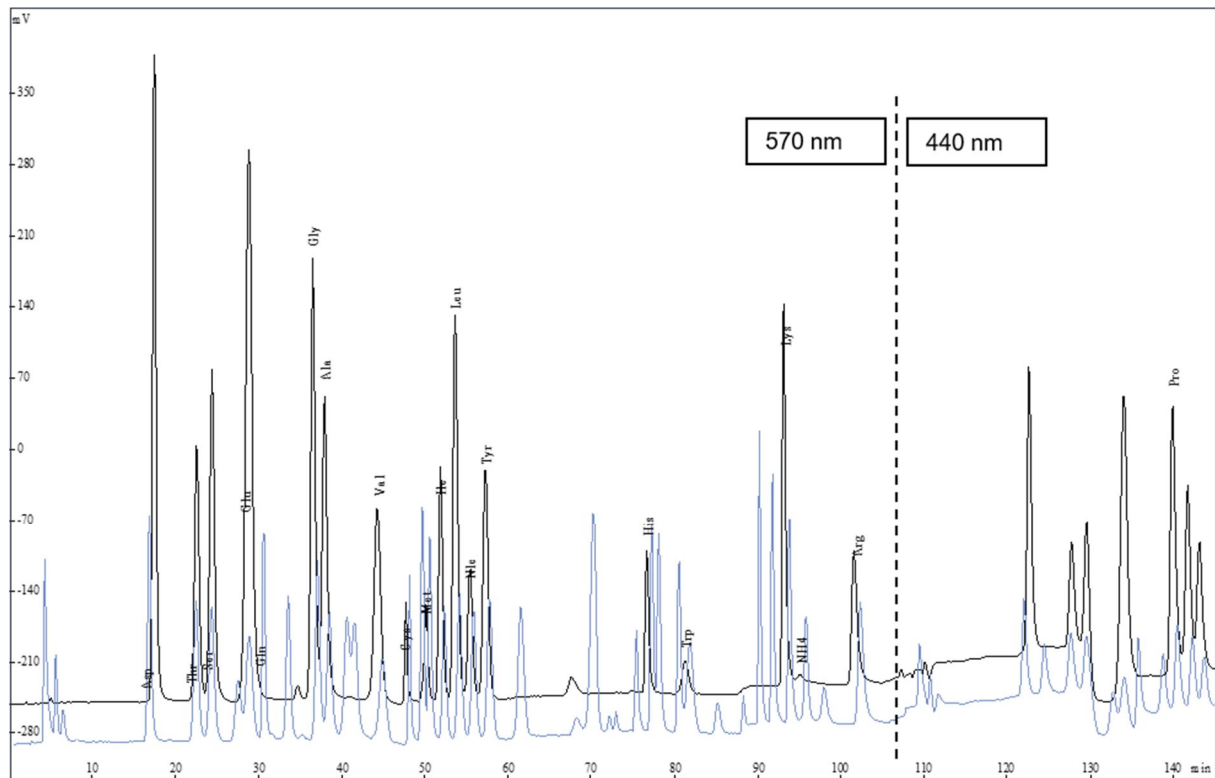


Figure 2: Comparison of a physiological amino acid standard (blue) with "supplement I" sample (black, top image) and "supplement II" sample (black, bottom image). The detection was performed at 440 nm and 570 nm. The concentrations of the individual amino acids were determined using a known concentration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

Conclusion

These results show that ARACUS can be used to determine amino acids in "special medical foods" that must meet the special nutritional needs of people with dietary restrictions, in this case due to a metabolic disorder called "phenylketonuria, PKU", the absence of phenylalanine.

Due to the simple processing of the samples, it does not matter whether the sample is present as a powder or already in a liquid state. It could be shown that both types of food supplements could be prepared without much time consumption. As predicted, the absence of phenylalanine was confirmed in both samples. By the already available results of the other laboratory the results of Supplement II could be verified and thus the feasibility of the sample preparation could be proven.

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