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Analytical methods for quality and quantity control of energy drinks and food supplements, containing amino acids

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Abstract: The need for analytical control of food supplements and "energy" drinks containing amino acids is huge. In the literature there are describes various analytical techniques for their qualitative and quantitative analysis. Most preferred methods is HPLC because of its range, accuracy and speed. Despite the wide variety of methods, there isn't coherent analytical system associated with the standardization of food additives containing amino acids.

Keywords: Amino Acids, Taurine, Control, Food Additives, HPLC

1. Introduction

The sales and consumption of energy drinks and food additives has been on the rise in Worldwide since 2000, and their popularity among people continues to grow. Recently the pharmaceutical and food industries produce a large number of supplements and products which contain amino acids. Called "the building blocks of life", amino acids have an important role in human nutrition and health maintenance. The amino acids have a biological activity and are components in foods and food additives. The food additives contains a different variety of essential and non-essential amino acids that play a critical role in metabolizing nutrients, building muscle tissue, and protecting the body against disease. Taurine is one of the main energy boosting ingredients in many energy drinks. Taurine is an amino acid that can be synthesized or ingested by humans. Nowadays amino acids are used as flavor enhancers, ingredients in cosmetic and pharmaceutical products and as specialty nutrients in the medical field, and the production capacity requirements are constantly increasing. The fact that one food additive or "energy" drink has a plant or animal origin, does not make it safe. This paper reviews analytical methods for characterization, quality and quantity control of "energy" drinks and food additives, containing amino acids.

2. Determination of Sulfur-Containing Amino Acids in Energy Drinks (Taurine)

2.1. Sume Dates of Using of Taurine

Taurine, α-aminoethanesulfonic acid, is a derivative of the sulfur-containing amino acid cysteine [14]. Taurine occurs naturally in food, especially in seafood and meat. The mean daily intake from omnivore diets was determined to be around 58 mg (range from 9 to 372 mg) and to be low or negligible from a strict vegan diet[3]. In another study taurine intake was estimated to be generally less than 200 mg/day, even in individuals eating a high meat diet [4]. According to another study, taurine consumption was estimated to vary between 40 to 400 mg/day [5]. Taurine is present in the diet and is a normal metabolite in humans. It is a metabolic product of sulphur amino acids, mainly biosynthesised from cysteine in the liver. It participates in the formation of bile salts and the detoxification of certain xenobiotics. It is involved in a number of crucial physiological processes including

modulation of calcium flux and neuronal excitability, osmoregulation, and membrane stabilization [6][7]. Human clinical studies show that the oral intake of taurine can influence physiological functions. For example, taurine (3 or 6 g/day) decreased blood pressure in hypertension patients [7][8]. A similar effect was seen in animal models of hypertension but the mechanism of action is unknown. A substantial increase in the plasma concentration of growth hormone was reported in some epileptic patients during taurine tolerance testing (oral dose of 50 mg/kg bw/day), suggesting a potential to stimulate the hypothalamus and to modify neuroendocrine function, similar to that seen with certain other amino acids, such as arginine and histidine [9]. The effect on growth hormone is probably attributable to its known hypoglycaemic action. There is an indication that taurine (2 g/day) has some function in the maintenance and possibly in the induction of the psoriatic state[10]]. Numerous publications describe special effects of taurine in different animal models, e.g. on behavior, blood pressure, serum glucose and serum cholesterol. Taurine was mostly applied in doses of 1000 mg/kg bw/day and above to induce these effects, using the oral route of administration. Intraperitoneal injection of taurine, however, seems to influence behavioral parameters at much lower doses. In one study, even a dose of 1.5 mg/kg bw was reported to decrease psychomotor activity[11]. The intake of taurine from regular consumption of some taurine-containing "energy" drinks is several times higher than that from the rest of the normal human diet. There is only limited information available either from human or conventional animal studies for risk assessment of taurine. There is a lack of scientific evidence to support the safety of taurine present in beverages at concentrations that may result in intakes several-fold higher than that usually obtained from the rest of the diet. Given the available information on involvement of taurine in a number of key physiological processes, together with the very limited data on possible adverse effects of taurine in humans and laboratory animals and the doses at which such effects were reported, the Committee considers it likely that the margin between normal daily intake of taurine from the diet (excluding consumption of "energy" drinks) and an adverse effect level in humans may be relatively small. At present, there is insufficient information on which to set an upper safe level for daily intake of taurine [12]. It may also be necessary to take into consideration, that absorption of taurine from beverages may be more rapid than from a food matrix. As, mentioned earlier, potential interactions between taurine and caffeine, both of which are present in several "energy" drinks, have not been sufficiently investigated. It is unable to conclude that the safety-in-use of taurine in the concentration range reported for taurine in "energy" drinks has been adequately established. Further studies would be required to establish an upper safe level for daily intake of taurine.

2.2. Determination of Taurine in Energy Drinks

The most common methods for the detection of Taurine include high performance liquid chromatography (HPLC) and ultraviolet visible absorption spectrometry (UV-Vis)[1]. Brad McConn from Concordia College has analysed the concentration of taurine in two energy drinks, Monster Lo-Carb and 5-Hour Energy, by using high-performance liquid chromatography[1]. Preparation of Carbonate Buffer A 10 mM pH 9 carbonate buffer solution was made for use in the derivatization reaction by dissolving 0.2081 g of NaHCO3 (Sigma-Aldrich) and 0.0138 g of Na2CO3 (Sigma-Aldrich) in 250 mL of deionized (DI) water and then adding hydrochloric acid drop-wise until a pH of 9 was reached. The remaining solution was then diluted with DI water to a final volume of 250 mL.

2.2.1. Preparation of Phosphate Buffer for Mobile Phase

A 10 mM pH 6 phosphate buffer solution was made for the HPLC mobile phase by dissolving 1.298-g of NaH2PO4·H2O (Fisher) and 0.1584-g of Na2HPO4·7H2O (Fisher) in DI water and then adding NaOH until a pH of 6 was reached. The solution was the diluted with DI water to a final volume of 2 L and filtered and degassed in preparation for the HPLC.

2.2.2. Preparation of Standard Solutions

Standard taurine solutions were made as close to 10, 20 and 50 ppm as possible. A solid taurine sample (Sigma-Aldrich) of 0.2519 g was dissolved in DI water in a 500-mL volumetric flask and diluted to the mark. Three 50-mL volumetric flasks were obtained and 1.0, 2.0 and 5.0 mL of the previous solution were added to each, respectively. The flasks were then diluted to volume with DI water.

2.2.3. Sample Preparation

Approximately 25 mL of the energy drink Monster Lo-carb was poured into a 125-mL Erlenmeyer flask and degassed by sonicating for ten minutes. A dilution of 1:125 was done using DI water. For 5-hour ENERGY, a dilution of 1:200 was performed using DI water.

2.2.4. Derivatization Reaction

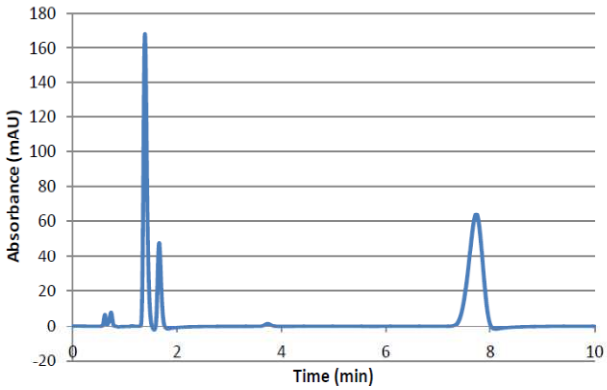

Figure 1. *Chromatogram of the 50 ppm standard at 360 nm. [1].*

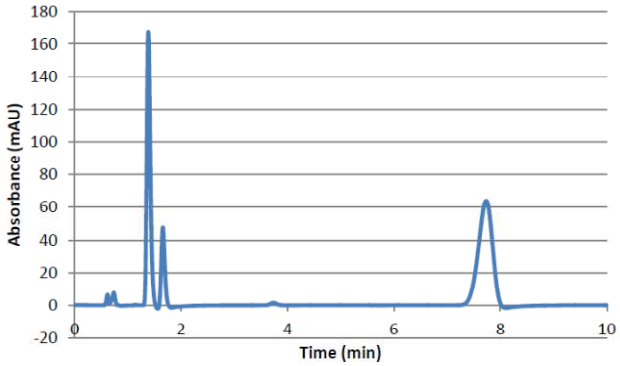

Figure 2. *Cchromatogram of a Monster-Lo carb sample at 360 nm. [1].*

The derivatization procedure was the same for the standards and the samples. Into a test tube, 1.0 mL of sample, 2.0 mL of the carbonate buffer, 0.5 mL of methyl sulfoxide (DMSO) and 0.1 mL of 2,4-dinitrofluorobenzene (DNFB, Acros) were pipeted. The solution was shaken for 30 s and placed in a 40˚C water bath for 15 min. At the end of the 15 min, 6.5 mL of the phosphate buffer was added to the mixture [1].

A calibration curve was generated using Excel from four separate runs of the standards. The peak area for each run was plotted against concentration and a linear curve was fit using Excel. This is summarized in Table 1 and Fig. 3.

Table 1. *A results summary for each of the three standards.*

Sample	Conc (ppm)	Run 1 Area (mAU-s)	Run 2 Area (mAU-s)	Run 3 Area (mAU-s)	Run 4 (mAU-s)
Standart 1	9.968	54.5	54.3	51.3	52.4
Standart 2	19.94	113.4	114.1	125.7	125.9
Standart 3	49.84	307.0	306.9	339.9	339.1

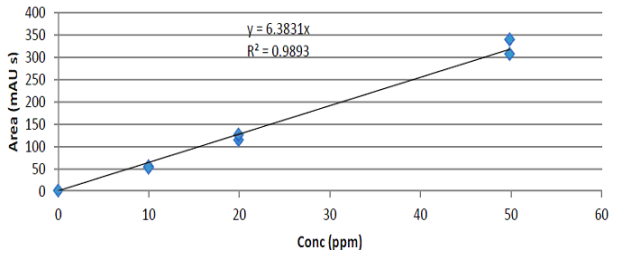

Figure 3. *The calibration curve generated by the results above.*

3. Determination of Amino Acids in Food Additives

The components in biological fluids - amino acids, peptides, and proteins must be isolated and purified to allow their evaluation, and standardizing. The separation is achieved by various methods based on differences in aggregate charge, molecular size, solubility, affinity for specific substances and others properties. In clinical practice, these methods are used for diagnosis of diseases and for the preparative purification and preparation of other organic products.

Electrophoresis is a method for separating charged molecules, or larger particles, based mainly on differences in the cumulative charge. The direction of movement of one of the poles of an external electric field is determined by the sign of the combined charge of the molecules. The speed is determined by the ratio of charge/molecular weight. Furthermore, the separation depends on the intensity of the field and the shape of the molecules in the mixture and the structure of the carrier gel (agar, agarose, cellulose acetate, polyacrylamide gel, paper, or other). Upon selection of the various experimental conditions electrophoresis methods are successfully applied for separation of mixtures of amino acids.

Chromatographic methods are widely applied to identify and quantify amino acids, taking account of their chemical properties and select the appropriate chromatographic conditions. In chromatographic techniques, substances are allocated between static and mobile phase. Separation depends on the relative ability of compounds to bind more strongly with one or the other phase.

Thin layer chromatography (TLC) - except for purposes of a qualitative analysis may be used in semi-quantitative analysis of the amino acids. This analytical approach used to determine the amino acid components with relevant conditions. By means of HPLC assay is carried out on amino acids in the formulation of nutritional supplements. In ion exchange chromatography the amino acids, peptides and proteins are separated on the basis of the difference-coupled. This is one of the most used modern methods of separation, proof and determination of amino acids in protein hydrolysates. In it the hydrolyzate is passed through a column containing ion exchange resin with charged groups that bind oppositely charged amino acids. By creating a gradient of pH and salt concentration of the eluent, the individual amino acids are eluted gradually from the column and separated. Nowadays the ion exchange chromatography is applied in special high pressure apparatus providing an extremely high-resolution, and reproducibility of the results. A high performance liquid chromatography method for the determination of 20 amino acids (AAs), using 1,2,5,6-dibenzocarbazole-9-ethyl chloroformate as a novel fluorescent labeling reagent, has been developed and applied for the analysis of amino acids. The simultaneous separation of 20 AA derivatives was achieved on a Hypersil BDS C(18) column with gradient elution. And the identification of amino acids derivatives was carried out by on-line electrospray ionization mass spectrometry in positive ion mode. The amino acids derivatives were detected with excitation and emission at 300 nm and 395 nm respectively [13].

Total and Free Amino acids can be determinate in liquid food additives and energy drinks using gas chromatograph.

3.1. Chromatogtaphic Conditions

Thermo Scientific GC-FID device, supplied with:

Fast analytical column (l = 10 m, d = 0.25 mm, 1.0 μm 5% diphenylpolysiloxane, 95% dimethylpolysiloxane film);

Injector temperature (split-splittless in split regime)-250 ^{o}C, temperature of flame-ionization detector-320 ^{o}C;

Carrier gas flow rate-2.0 ml/min.

Initial oven temperature (110 ^{o}C) held for 0.3 min., subsequently ramped at a rate of 27 ^{o}C/min until 320 ^{o}C is reached and is held for 5 min. at 320 ^{o}C.

3.2. Test Preparation

An accurately weighed quantity of solution must be extracted with 1000.0 ml 0.1 M HCl. An aliquot part of 1.0 ml of the extract is neutralized by adding of sodium carbonate to pH: 2.5 ÷ 5.0 to obtain 100.0 ml. To 100 µl of the neutralized sample are added 100 µl of solution of internal standard Norvaline. The obtained solutions have to purify by cation-exchange solid-phase extraction. Amino acids in purified samples have to be derivatized with ethylchloroformate. The derivatizing reagent is removed by scavenge with nitrogen. The derivatives of Amino acids is dissolved in aliquot part of isooctane and analyzed by gas chromatography with flame-ionization detector [15].

The identity is proved by the fact, that all data for the retention time (tR) of aminoacids in food additive correspond to the values of tR of the respective aminoacid in reference solution. 13 aminoacids are separated in range of time: 2.1 min.-6.55 min. In the examined food additive form free aminoacids L-Aspartate is with the highest concentration (29.81 mg/dl). The total content of L-Cystine is with the highest concentration (4.41 g/dl) and L-Triptophan is with the lowest concentration (10.01 mg/dl) [15].

4. Conclusion

Amino acids are biologically important organic compounds that contain amine and carboxylic acid functional groups, along with a side-chain specific to each amino acid. Amino acids can be divided to two types, essential and non-essential.

Eight amino acids have been found necessary and essential. The amino acids have a biological activity and are components in energy drinks and food additives. The foods and food additives contain a different variety of essential and non-essential amino acids that play a critical role in metabolizing nutrients, building muscle tissue, and protecting the body against disease. Low consumer`s awareness of food additives containing amino acids in many cases makes this type of product risk [13]. The popularity of energy drinks is rising, and it is important to have an efficient way to determine the concentrations of ingredients in the drinks. The described method for control is successful one for determing the concentration of taurine in energy drinks.

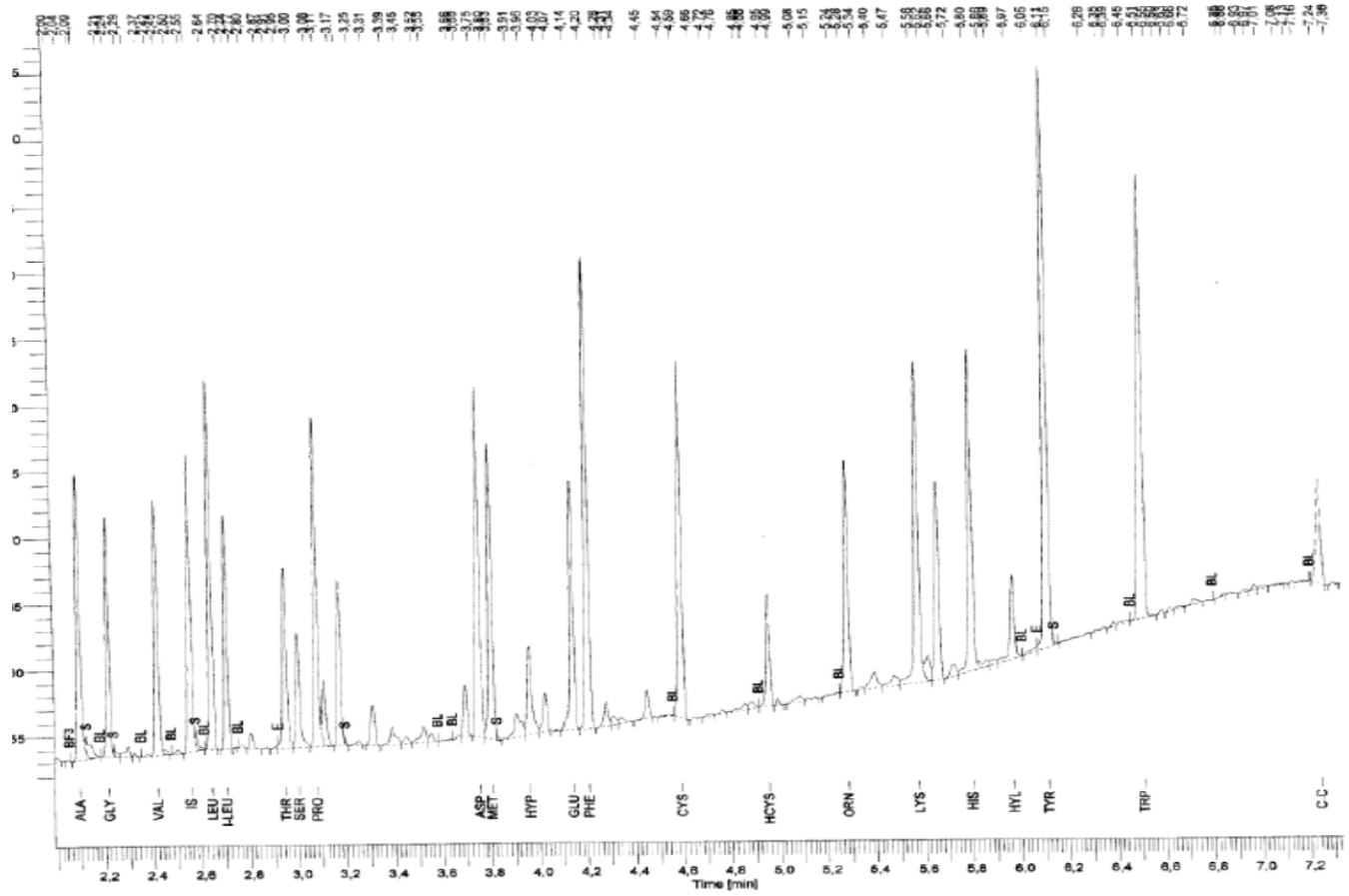

Fig. 4. *Chromatogram of reference standard solution of aminoacids, used for the calibration before the analysis of free aminoacids in sample of Aminogame 1500 solution.[15].*

Table 2. *Retetion time (tR) and peak area (A) for free aminoacids in Aminogame 1500 solution.*

№	Aminoacid	Reference standart for free aminoacids		Free aminoacids in Aminogame 1500 solution	
		t_R	Peak area [Uv sec.]	t_R	Peak area [Uv sec.]
Internal standart	Norvaline	2.554	22011.10	2.551	9933.95
1.	L-Alanine	2.092	23179.74	2.092	182.32
2.	L-Glycine	2.214	17918.37	2.209	338.68
3.	L-Valine	2.417	18844.45	2.417	122.98
4.	L-Leucine	2.641	26847.77	2.636	178.26
5.	L-Isoleucine	2.704	16308.07	2.730	68.74
6.	L-Proline	3.077	26557.31	3.078	545.49
7.	L-Aspartate	3.753	28781.16	3.695	1452.03
8.	L-Methionine	3.804	32676.62	3.797	332.60
9.	L-Glutamate	4.141	21030.56	4.133	199.19
10.	L-Phenylalanine	4.205	40474.83	4.280	759.22
11.	L-Cystine	4.592	29937.50	4.617	98.70
12.	L-Lysine	5.576	31180.66	5.611	202.48
13.	L-Triptophan	6.508	47321.47	6.546	145.27

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