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# Validation of Taurine Determination Method in Energy Drinks by High Performance Liquid Chromatography

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ABSTRACT: The presence of taurine in energy drinks stimulates the central nervous system and intensifies brain activity, reducing fatigue and creating alertness. Consuming high doses of taurine can cause adverse symptoms such as headache, irritability, and kidney problems. Therefore, it is necessary to monitor the constituents of energy drinks to ensure the level of taurine in the products. The standard concentration defined for energy drinks is 400-1500 mg/liter and acceptable daily intake (ADI) is equal to 21 mg/kg/day. In this research, high performance liquid chromatography (HPLC) was used to measure and validate the analysis method of 10 different brands of energy drinks. After validation of the method, 10 drink samples were collected from Tehran city and tested for taurine content. In order to separate taurine, a gradient system with a mobile phase of phosphate buffer solution/acetonitrile/ methanol and deionized water (45:45:10) was used and measurement was carried out using ultraviolet detector at 338 nm wavelength. The results indicated that the lowest concentration of taurine among all the examined samples is related to the Red Bull brand with an amount of 116.46 mg/L and the highest amount is related to the Happy Life brand with a concentration of 2006.68 mg/L. It was taken the limit of detection (LOD) was calculated as 27.18 mg/L and the limit of quantification (LOQ) as 90.60 mg/L. Comparison of the obtained results with international standards showed that the taurine content of these drinks is lower than the standard limit in most of the examined samples.

**Keywords**: Energy Drinks, High Performance Liquid Chromatography, Taurine.

## Introduction

Energy drinks are a group of products that can increase the physiological and brain function of the body by reducing the effects of fatigue and sleep pollution, and as a result, increase alertness and increase body energy (Ariffin *et al*., 2022; Heckman *et al*., 2010). Energy drinks are divided into low-calorie and high-calorie categories according to the use or non-use of sugar in the formulation (Hafner *et al*., 2021). Two types of elements that can usually be found in the components of these drinks, except glucose and sucrose, are caffeine and taurine in additive form (Muli *et al*., 2021). Although other elements such as caffeine, riboflavin, pyridoxine and various types of plant extracts such as ginseng are also present (Wassef *et al*., 2017). Today, hundreds types of energy drinks are produced and consumed in the world, the difference in which, in addition to color and taste, is the quality of the two main ingredients: caffeine (a stimulant of the central nervous

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system) and taurine (a type of amino acid with positive effects on the heart, muscles and detoxification). These two compounds are distributed and absorbed throughout the body by the blood circulation system until they reach the final destination, which is the brain, and act as a stimulating and energizing compound (Ulenius *et al*., 2019). Taurine is a natural amino acid, with the chemical name (2-aminoethane sulfonic acid, $C_2H_7NO_3S$, MW=125.15), a derivative of the amino acid cysteine (Ariffin *et al*., 2022). This composition is one of the main components of energy drinks. Taurine, as a natural compound in energy drinks, stimulates the nervous system, increases energy, relieves fatigue, invigorates, increases endurance, relieves stress and improves sports performance. Taurine is also present in common food sources such as meat and fish, and the range of daily intake of taurine in the diet is reported to be 40 to 400 mg (Wójcik *et al.,* 2010). In recent years, the trend of adding this amino acid to energy drinks and other products has increased greatly, and its limit in energy drinks is 400-1500 mg (Costa-Valle *et al*., 2018).

Consuming high doses of taurine can cause adverse symptoms such as headache, irritability, and kidney problems, therfore measuring taurine in foods and drinks is considered important to control consumption (Ehlers *et al.,* 2019). Therefore, the purpose of this research is to validate the method and measure the amount of taurine in Iranian energy drinks and compare it with the permissible limit and international standards by high-performance liquid chromatography method.

Among the energy drinks, we can mention the production of Lipovitan D in Japan (1962) and Red Bull in Australia (1987). Amino acids, especially taurine, can be mentioned among the important compounds in energy drinks except caffeine (Ariffin *et al*. 2022).

Taurine is a natural amino acid and a derivative of 2-aminoethanesulfonic acid, which is obtained from the metabolism of methionine and cysteine (Tevatia *et al*., 2015). The chemical structure of taurine and its main biosynthesis pathway are shown in Figure 1. The structure of taurine does not have a carboxyl group, but contains a sulfonate group (Ripps and Shen 2012). The biosynthesis of taurine starts from cysteine. The enzyme cysteine dioxygenase (CDO) catalyzes the conversion of L-cysteine to cysteine sulfinate, then decarboxylation through cysteine sulfinate decarboxylase (CSD) and as the last step requires the oxidation of hypotaurine (2-aminoethane sulfinate) to taurine (Ripps and Shen, 2012).

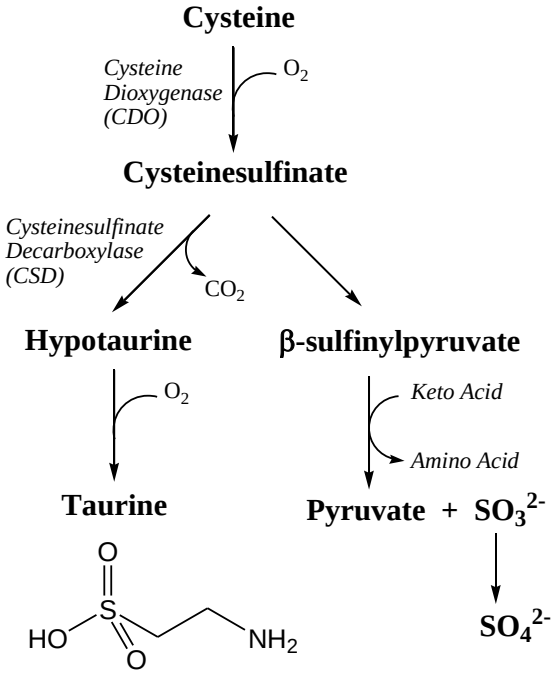


**Fig. 1.** The chemical structure of taurine and its formation steps (Ripps and Shen 2012).

This diagram briefly shows the main stages of conversion of L-Cysteine to Taurine. Cysteine deoxygenase (CDO) enzyme catalyzes the conversion of L-

Cysteine to cysteine sulfinate and the oxidation of hypotaurine leads to the production of taurine.

Taurine is one of the most abundant amino acids in the brain and spinal cord, leukocytes, heart and muscle cells, eye retina and almost all tissues of the body, therfore it is the most abundant amino acid in the body after glutamine (Shao and Hathcock 2008). Taurine has many roles in the human body, for example, one can mention the roles of regulating osmotic pressure and the immune system, regulating water balance, energy levels, heart rate, as well as muscle contraction, insulin secretion, apoptosis and proliferation of beta cells. Also, this amino acid plays an antioxidant role in the body and is effective in protecting the immune system and plays an effective role in the formation of bile salts (Ripps and Shen 2012). The main role of taurine in the body is maintaining hydration and the balance of electrolytes in the body's cells, helping digestion by forming bile salts, creating a balance in the transfer of minerals into cells such as calcium, helping the main activities and central nervous system and the role of antioxidant and its effect in regulating the body's immune system. There is evidence that taurine consumption reduces blood pressure in adults (Schaffer and Kim 2018). Recent studies show that taurine supplements used in mice have been effective in preventing weight gain during a high-calorie diet and controlling blood sugar. Although many amino acids play an important role in muscle building, taurine does not play a direct role in muscle building and does not establish a bond with other amino acids, because taurine is not one of the 20 main amino acids in protein building. The reasons for using taurine include increasing the level of water transfer into muscle cells and retaining more water in them (such as creatine), increasing the level of alertness, increasing the power of blood pumping by the heart, reducing blood pressure and preventing dehydration (Ripps and Shen 2012; Bagci and Okten 2022; Ito *et al*., 2012). Taurine plays an important role in the production of nitric oxide, where lowering taurine levels contributes to the reduction of nitric oxide production. Also, by widening the channels, it is effective in transferring the blood flow, which results in the transfer of a large volume of oxygen and nutrients into the cells (Ripps and Shen 2012; Schaffer and Kim 2018). There is increasing evidence that taurine depletion leads to a wide variety of pathological disorders, including cardiomyopathy, renal dysfunction, pancreatic beta-cell dysfunction and loss of photoreceptors in the retina according to the reports, taurine has very wide anti-inflammatory effects (Tao *et al*., 2022). Taurine supplements have been suggested to deal with and treat epilepsy, heart failure, cystic fibrosis and diabetes (Rafiee *et al*., 2022). Also, taurine has shown resistance to neurotoxins in animal studies (Owoeye *et al*., 2018). In addition, homotaurine, as one of the taurine analogs, has shown anti-amyloid activity, which theoretically can protect against the progression of Alzheimer's disease and facilitate its treatment (Manzano *et al*., 2020). Taurine is present in common food sources such as meat and fish, and the amount required by the body is provided in this way. The average daily intake of taurine in the diet is 40 to 400 mg (Wójcik *et al*., 2010). The trend of adding this amino acid to energy drinks and other products has increased in recent years, which has been accompanied by more studies on this amino acid. However, the conducted studies show a decrease in the percentage of use of this amino acid

during the years 2004 to 2008 from 27% to 21% in energy drinks (Heckman *et al*., 2010). According to previous studies, among 49 energy drinks that were analyzed, 99% were below the Acceptable Daily Intake (ADI) for taurine (González-Vázquez *et al*., 2020). There is no official international law that sets a Maximum Allowable Level (MAL) for taurine in energy drinks. For this reason, the manufacturer is not obliged to include information about the amount of this amino acid in his product. However, due to the dangers of excessive consumption of energy drinks, it is necessary to know the effect of the ingredients and implement accurate measurements to prevent negative consequences for the consumer. In Iran, there is no standard procedure for measuring the amount of taurine. Increasing consumption of taurine in the diet increases its concentration in plasma and tissues such as skeletal muscles, heart and brain (Wu 2020). Consuming this amount has no side effects and consuming 6 grams of taurine can be used as a supplement before aerobic exercise (Carvalho *et al*., 2020). Taurine is a very essential amino acid and its deficiency in some premature babies disrupts the conversion of Cystathionine into cysteine. Therefore, for this reason, it is added to many children's auxiliary supplements as a precaution (Zlotkin *et al*., 1981). Also, the high amount of taurine in the hypothalamus is related to the improvement of nerve function (Jakaria *et al*., 2019). Also, the effect of taurine association with several types of hormones including norepinephrine, dopamine and growth hormone has been determined, but the direct mechanism of their effect on their secretion is not known (Schaffer and Kim 2018; Terry *et al*., 1982). Studies show that there are several possible biochemical mechanisms that taurine plays a role in muscle contraction. Intracellular taurine modulates the function of Ca-ATPase (SERCA) of the sarcoplasmic reticulum network and ultimately leads to an increase in calcium accumulation through increasing the maximum SR calcium pumping. When the intracellular taurine concentration is higher than the physiological range, calcium accumulation does not change, but the decrease in taurine level leads to a significant decrease in calcium (Merckx and De Paepe 2022). The intense activity of skeletal muscle cells leads to an increase in the production of reactive oxygen species (ROS), which helps the oxidation of contractile proteins, including the inhibition of SERCA (Bouviere *et al*., 2021). Also, taurine prevents the production of ROS in the mitochondrial respiratory complex (Baliou *et al*., 2021). This process is through the change of the mitochondrial tRNA, which affects the synthesis of proteins involved in electron transfer in the complex (Jong *et al*., 2021). In addition to the oxidation of proteins, ROS in hypoxic muscles cause DNA damage, which may be blocked by taurine (Thirupathi *et al*., 2020). Taurine supplementation reduces insulin and endothelial markers in people with T2 diabetes, oxidative stress and inflammation (Moludi *et al*., 2022). The amount of taurine is often not disclosed in the food list. Therefore, the purpose of this research is to validate and investigate the amount of taurine in 10 different brands of energy drinks collected from the city of Tehran using high performance liquid chromatography (HPLC).

## Materials and Methods

In this research, which was conducted with the aim of investigating the toxicity of energy drinks by measuring the amount of taurine in them, various materials, equipment and methods were used as

described below.

All chemicals were obtained from Merck Chemical Company, Germany with CAS number of Methanol (67-56-1), Acetonitril (75-05-8), Boric acid (10043-35-3), Sodium hydroxid (1310-73-2), Ortho-phthalaldehyde (OPA) (643-79-8), 3-mercaptopropionic acid (107-96-0), Taurine (107-35-7), Water for chromatography (7732-18-5). The list of energy drinks, the company and the name of the producing countries are given in Table 1.

**Table 1.** List of energy drinks, company and name of producing country

| Samples | Production company | Production country |
|---|---|---|
| Energy drink | 24 hours | Iran |
| Energy drink | Big Bear | Iran |
| Energy drink | Black Bruin | Turkey |
| Energy drink | Black Royal | Iran |
| Energy drink | Black Wolf | Iran |
| Energy drink | Fire Power | Iran |
| Energy drink | Happy Life | Iran |
| Energy drink | Hype | Iran |
| Energy drink | Jaguar | Spain |
| Energy drink | Red Bull | Germany |

The names of Equipment and the used devices, their model, the production company and the country of manufacture are as follows: Magnetic stirrer (Hanna,Singapore); pH meter pH211 (Hanna, Romania); Spectrophotometer Ultraspec III (Pharmacia LKB, Sweden); Filtration set (MILIPORE, France ); Filtration vacuum pump (WELCH, USA); HPLC 1100 Series (Agilent, Germany); Autosampler syringe (Hamilton, USA); Autosampler vials with caps (Agilent; USA); Phenomenex column C18 (4.6×150) (Phenomenex, USA); Digital balance TE IS3S (Sartorius, Germany); Samplers (10, 100, 500 and 1000 ML) (Eppendrof, Germany); Sonicator TECNO-6AZ S.P.A. (Tecno gaz, Italy).

–***Software used for statistical calculations***

Excel software was used to draw graphs. Also, Agilent software called Value Solution ChemStation software was used to record chromatograms and measure the area under the peaks and integrate.

–***Derivatization of taurine amino acid***

–***Preparation of samples and standards***

In order to prepare the taurine standard solution with a final concentration of 1.7 mg/ml, we weigh 17 mg of the standard was weighed and brought to 10 ml in a volumetric flask with 0.1 N HCL.

In order to prepare soft drinks for derivatization and measurement of taurine content, we take 10ml from each sample was taken and brought to 20ml with 0.1N HCL. In order to remove the gas in soft drinks that will cause damage to the column and cause errors in the result; each sample is placed in an ultrasonic bath for 20 minutes and then filtered with a 0.45 micron syringe filter.

–***Reagent solutions for standard taurine derivatization and soft drinks***

1. First reagent: Borate Buffer 4.0 normal

In order to prepare normal 0.4 borate buffer reagent, first weigh 1.23 grams of boric acid was weighed and added to 50 ml of deionized water. The pH was then set to 10.2 using NaOH. This solution can be stored at 4°C.

2. The second reagent: o-phthalaldehyde (OPA)

In order to prepare this reagent, 100.5 mg of OPA was weighed and 0.34 ml of 3-mercaptopropionic acid was added. Finally, The volume was brought to 10 ml with borate buffer solution prepared in the previous step.

–***Derivatization by HPLC***

1. 2.5 µl is drawn from vial one containing borate buffer with Hamilton syringe in the autosampler part of the device.
2. From the existing sample, that contain energy drink or taurine standard, and its preparation has been carried out in the previous steps, the amount of 0.5 µl is added to the syringe.
3. The obtained mix (3 µl) is set in air mode with the maximum speed of 2X and we wait for 30 seconds.
4. Deionized water is drawn from vial 2 without a cover to wash the needle.
5. Vial No. 3 contains the main derivatization reagent, OPA, at this stage, 0.5 µl is taken from this vial to be added to the sample and borate buffer in the syringe.
6. The resulting mix (3.5 µl) in the air mode of the device with the maximum speed of 6X.
7. Repeat the fourth step
8. At the end, 32 µl of vial 5 containing deionized water is added to the syringe, and from the final mix, 18 µl is injected in air with the maximum speed of 2X.

–***Analysis of derivatized taurine by HPLC***

Analysis of derivatized taurine available in different energy drink companies and standard samples were carried out using high performance liquid chromatography (HPLC). The chromatography column used was a C18 column with a length of 15 cm, an internal diameter of 4.6 mm and a particle diameter of 5 µm, and the temperature of the column was set at 40°C. The detector in the device was UV/Visible and the wavelength ($\lambda_{max}$) was set to 338 nm in the applied settings. The injection was carried out in a volume of 18 µl, with a flow rate of 1 mL/min and a gradient washing type. The total time required for this analysis was 26 minutes.

–***Mobile phase composition***

All organic and aqueous solvents of the mobile phase are HPLC grade and before use in the device, they were re-purified using a filtration set, cellulose acetate filter and vacuum created by a vacuum pump, and for degassing, they were sonicated in an ultrasonic bath for 20 minutes.

A. Phosphate buffer: To prepare this buffer, we weigh 6.21 g of sodium dihydrogen phosphate dihydrate and add it to 1000 ml with deionized water. The pH was adjusted to 7.83 by 10N NaOH.
B. This solvent is a mixture of acetonitrile, methanol and deionized water (45:45:10).

–***Mobile phase gradient program***

The gradient washing program is determined in such a way that at first injection for 1.9 minutes from path A containing phosphate buffer, 100% of the volume is applied as input flow, then until 18.1 minutes from path A, the input flow is reduced to 43% volume and enter the rest of the flow (57%) from path B containing the solvents acetonitrile, methanol and deionized water with the proportions mentioned earlier. Next, from path B, 100% of the input volume is taken until minute 22.3, and finally, until minute 26, the input flow is changed to path A (Table 2).

**Table 2.** Applied mobile phase gradient washing

| Time (min) | Flow rate (ml/min) | Mobile Phase A% | Mobile Phase B% |
|---|---|---|---|
| 0 | 1 | 100 | 0 |
| 1.9 | 1 | 100 | 0 |
| 18.1 | 1 | 43 | 57 |
| 18.6 | 1 | 0 | 100 |
| 22.3 | 1 | 0 | 100 |
| 23.2 | 1 | 100 | 0 |
| 26 | 1 | 100 | 0 |

– ***Validation***

The method used for the analysis of taurine in energy drinks has been carried out in compliance with ICH rules and by fully studying the scientific and practical criteria of a valid method. In this regard, validation parameters such as checking the linearity range, accuracy and precision studies, repeatability test and Relative Standard Deviation (RSD), Limit of Detection (LOD) and Limit of Quantification (LOQ) have been investigated.

– ***Draw the calibration curve***

In order to draw the calibration curve, it is necessary to determine the linear range. For this purpose, concentrations of 425, 850, 1700, 3400 and 6800 mg/liter of taurine standard were made and after derivatization, they were injected three times into the HPLC device and their sub-peak levels were recorded. Then, by obtaining the average level below the peak of each concentration, the graph of the changes of the levels below the peak according to the concentration was drawn by Excel software, and the calibration curve and its line equation were obtained.

– ***Studies of accuracy and precision of the method***

The accuracy of the method indicates the degree of correspondence and closeness between the results of the analysis and the actual results, which were compared with the standard results in order to check this parameter. Also, the accuracy of the method indicates the closeness of the data obtained from the measurement of the analyte in several independent occasions, which was evaluated.

– ***Determining the detection limit and determination limit***

The detection limit is the lowest value or the lowest concentration of the desired component that can be detected by the analytical device. The analyte should produce a signal that is statistically three times the control signal. Equation (1) was used to determine it.

Equation (1):

$$\mathrm{LOD} = \frac{3S_b}{m}$$

Where; LOD is the Limit of Detection, m is the slope of the calibration curve and $S_b$ is the standard deviation of the control.

The determination limit of a method is the lowest amount or the lowest concentration of the target component that can be quantitatively measured with an analytical device under experimental conditions with acceptable accuracy and precision. The target signal for the detection limit is considered to be ten times the control signal. Equation (2) is used to calculate it.

Equation (2):

$$\mathrm{LOQ} = \frac{10s_b}{m}$$

LOQ is the Limit of Quantification, m is the slope of the calibration curve and $S_b$ is the standard deviation of the control.

– ***Determination of repeatability and Relative Standard Deviation (RSD)***

The repeatability of the method is a measure of the accuracy of the method, it is expressed by a parameter called the percentage of relative standard deviation (%RSD). In the present research, repeatability was performed in one working day, with three repetitions at two concentration levels of 1000 and 2000 mg/liter. The repeatability of the method is calculated according to equation (3) and equation (4), where S is the standard

deviation, X is the area under the peak, $\bar{x}$ is the average of the data, and N is the number of experiments.

Equation (3):

$$S = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2}$$

Equation (4):

$$RSD\% = \frac{S}{X} \times 100$$

### –*Estimation of daily intake of taurine and comparison with ADI values*

According to the average taurine measured in these drinks, the Estimated Daily Intake (EDI) of taurine in an adult is calculated according to the following formula and compared with the ADI amount.

Equation (5):

$$Average\ contamination = \frac{½LOQ \times (NU)_{+}(TCC)}{TA}$$

The average contamination of the method is calculated according to equation (5), where NU is the number of uncontaminated samples, TCC is the total contamination of contaminated samples and TA is the total number of analyzed samples.

Equation (6):

$$EDI = \frac{\text{Per capita consumption} \times \text{Average contamination}}{\text{The average weight of an adult}}$$

## Results and Discussion

### –*Analytical data evaluation*

In order to validate the proposed method, merit values including linear range, repeatability, detection limit and determination limit were evaluated. In general, an analytical process should have a large linear range, low detection limit, suitable repeatability and applicability to analyze real samples.

### –*Determining the linear range and drawing the calibration graph*

The calibration chart was drawn using taurine standard solutions at 5 concentration levels as the area under the peak based on the concentration. The linear equation resulting from the calibration curve was obtained as $y = 0.337x - 8.25$ and correlation coefficient ($r^2$) equal to 0.995. The value of the correlation coefficient equal to 0.995 indicates a good relationship between the value and the answer. Figure 2 shows the drawn calibration graph and Figure 3 shows the chromatograms of different concentrations of taurine. The area under the standard curve of taurine in specific concentrations is given in Table 3.

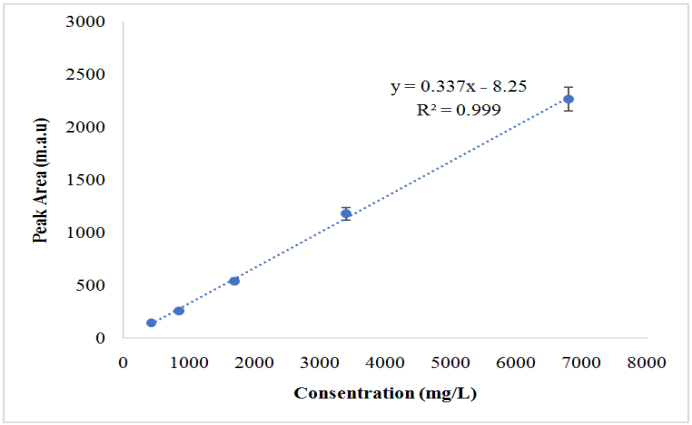


**Fig. 2.** Calibration diagram of taurine standard in different concentrations.

**Table 3.** The area under the standard curve of taurine in specific concentrations

| Area (m.A.U) under the peak | Taurine (mg/L) concentration |
|---|---|
| 145 | 425 |
| 259 | 850 |
| 545 | 1700 |
| 1182 | 3400 |
| 2268 | 6800 |

–***Detection limit and Quantification limit***

The limit of detection (LOD) was equal to 27.18 mg/L and the limit of Quantification (LOQ) was equal to 90.60 mg/L, were obtained using equations 1 and 2. The obtained results indicate that the introduced method has an optimal detection limit and determination limit.

–***Repeatability***

The repeatability of the method, which is a measure of the accuracy of the method, was calculated according to equations 3 and 4. The calculation results are given in Table 4. According to the obtained results, good repeatability was obtained.

**Table 4.** Results of repeatability of taurine measurement by derivatization

| Analyte | Concenteration (mg/L) | RSD%, (n=3) |
|---|---|---|
| Taurine | 1000 | 3.65 |
| | 2000 | 2.49 |

–***Taurine concentration in energy drinks***

In order to investigate the effectiveness of the proposed method to measure taurine in energy drinks, 10 samples of energy drinks with different brands were examined. The resulting chromatograms are shown in Figure 4. According to the chromatograms, there is a significant amount of taurine in every 10 samples. The amount of taurine in each sample was calculated through the calibration curve and according to the line equation, which are given in Table 5.

**Fig. 3.** Chromatograms of different concentrations of taurine standard

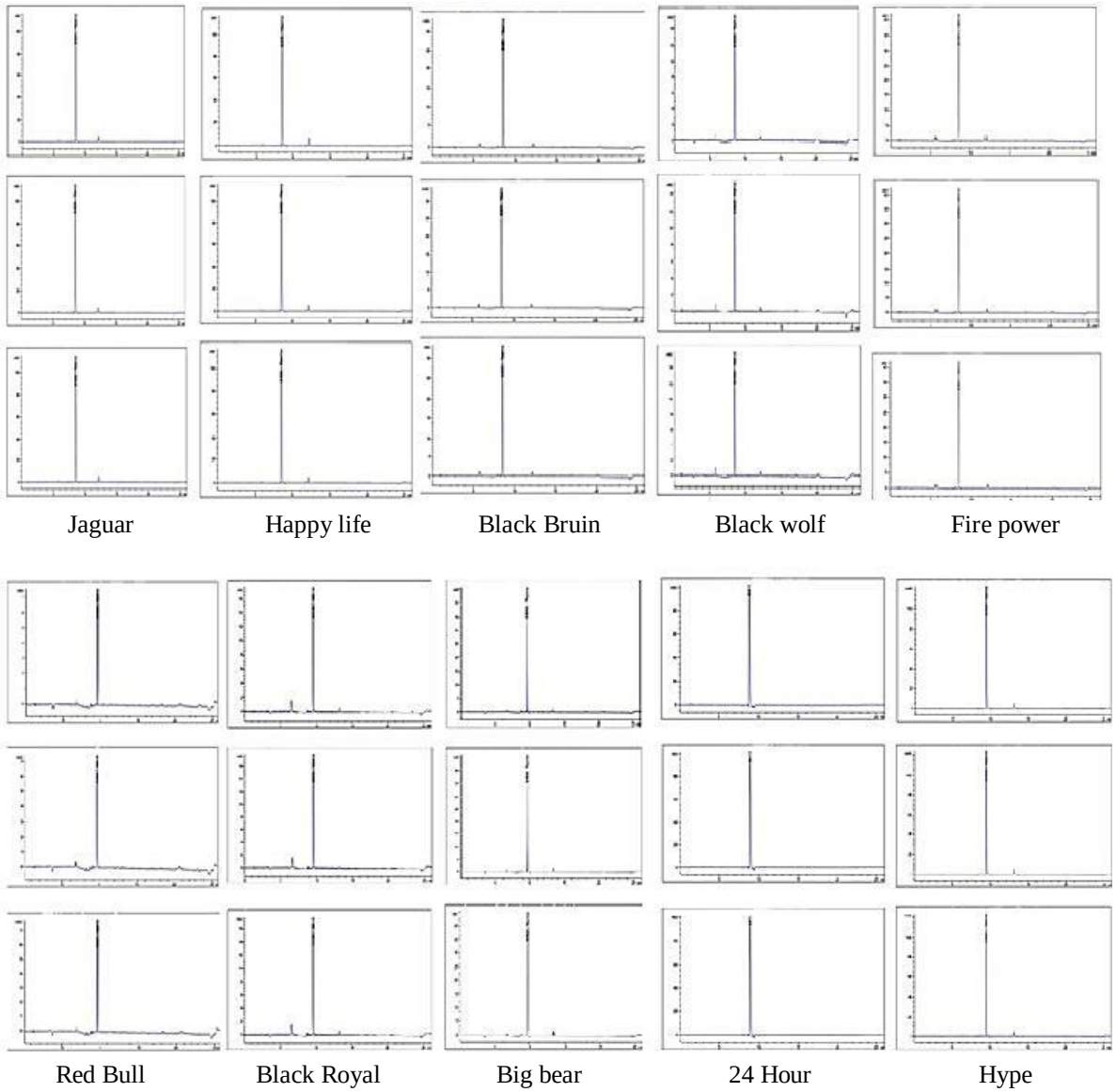


**Fig. 4.** Chromatogram of energy drinks related to different producing companies

**Table 5.** The results of taurine analysis in different energy drink companies (repeated three times)

| No | brand name | Average retention time (min) | Average area under the peak (m.A.U.) | Calculated concentration of taurine (mg/L) |
|---|---|---|---|---|
| 1 | Jaguar | 8.52 | 665 | 1998 |
| 2 | Happy life | 8.50 | 668 | 2006.68 |
| 3 | Black Bruin | 8.54 | 142 | 445.84 |
| 4 | Black wolf | 8.53 | 63 | 211.42 |
| 5 | Fire power | 8.50 | 185 | 573.44 |
| 6 | Red Bull | 9.57 | 31 | 116.46 |
| 7 | Black Royal | 9.57 | 70 | 232.19 |
| 8 | Big bear | 9.54 | 189 | 585.31 |
| 9 | 24 Hour | 8.90 | 583 | 1754.45 |
| 10 | Hype | 9.50 | 613 | 1843.47 |

The lowest level of taurine concentration is related to the Red Bull brand with 116.46 milligrams per liter of taurine and the highest level is related to the Happy Life brand with 2006.68 mg/L of taurine.

–***Estimated daily intake***

According to the per capita consumption of 30 liters of energy drinks per year in a 60 kg adult, the average daily intake of taurine from energy drinks was calculated according to equation 5 and 6 and the values were compared with the acceptable daily intake. Based on the results, the acceptable daily intake (ADI) is equal to 21 mg/kg/day and the estimated daily intake (EDI) of taurine in the sample of energy drinks in adults is equal to 22.65 mg/kg/day were obtained. The hazard index (HI) was obtained 1.078%.

According to the obtained results from the present research, out of ten energy drinks under investigation, 4 samples named Happy life, Jaguar, Hype and 24 Hour respectively have taurine content more than 1500 mg/L determined by European Food Safety Authority (EFSA). The average concentration of taurine in 10 energy drink samples was equal to 977 mg/L, therefor each person will get an average of 977 mg/L of taurine by drinking one bottle of energy drinks. Also according to the results of this research, Happy life brand has the highest amount of taurine and Red Bull brand has the lowest amount of taurine. In this study, the comparison of the acceptable daily intake and the estimated daily intake of taurine in the sample of energy drinks did not show much difference, therefor the hazard index for taurine consumption in energy drinks is low and this shows that there is no danger for the consumers of these products. The results of a previous study showed that the caffeine content in energy drinks can be between 75 to240 mg, while the average amount of taurine is 342.28 mg/100 ml (Erdmann *et al*., 2021). The safe margin for regular users of energy drinks is on average 125 ml to 350 ml per person per day (EC Scientific Committee on Food, 2003). The combination of caffeine and taurine together has side effects on the developing adolescent brain, and reports have shown that age is an important factor in the toxicity of caffeine and taurine (Curran and Marczinski, 2017). In an experimental study on rabbit heart, treatment with caffeine and taurine caused ventricular arrhythmia (Ellermann *et al*., 2022).

In this project, an accurate and sensitive analysis method was used to measure taurine in 10 samples of drinks available in the market for validation. The obtained results indicated that this method was completely scientific and practical. Therefor the calibration curve for the taurine standard was linear in the range of 425-6800 ppm and its correlation coefficient was 0.99.

In a previous study, the amount of taurine and caffeine was measured by FT-MIR coupled to chemometrics method simultaneously in 50 energy drinks. The content of taurine and caffeine in 50 samples was between 0 and 69.51 mg/100 ml and 14.92 and 1126.17 mg/100 ml, respectively (González-Vázquez *et al*., 2020). In a previous study, taurine concentration in 20 different energy drinks was investigated using $^{1}$H-NMR and LC-UV/vis. Due to the high agreement with LC-UV/vis data and adequate recovery rates (between 97.1% and 108.2%), $^{1}$H-NMR measurements provide a suitable method for the quantification of taurine in energy drinks (Hohmann *et al*., 2014). In another study in 2019, an electroanalytical method based on oxidation voltammetry was developed for the quantitative

determination of taurine in energy drinks and this method was validated using a standard high-performance liquid chromatography (HPLC) method (Farag *et al.,* 2019). Due to the importance of taurine, many studies have been conducted to measure it, for example, a study in 2008 in Japan on 5 types of taurine-containing energy drinks is taken to compare the concentration listed on the can with the amount measured in the laboratory by HPLC. In this research, the reported numbers (932-3153 mg/100ml) were close to the reported numbers with an acceptable standard deviation (RSD = 4.2%). The measured taurine content compared to the labeled amount in five commercial energy drinks containing taurine was 92.9-105.1% (Sawabe *et al.*, 2008). Also, in 2019, a similar study was conducted in Sudan by Musa Ali Omar. Among the 5 types of energy drinks containing taurine, the amount of taurine added to sample number 1 with a concentration of 98.36 mg/L is the lowest and the rest of the samples are 3172-4033 mg/L. Also, LOD was equal to 0.109 mg/L and LOQ was equal to 0.328 mg/L (Omer *et al.*, 2019). In another study in 2012, the amount of taurine in two brands of Monster Lo-Carb and 5-Hour was determined by HPLC. In Monster Lo-Carb drink, the amount listed on the can was 1000 mg/serving, and the measured amount was obtained 997 mg/serving, while in 5-Hour drink, nothing was written on the can, but the amount obtained by the measurement was 479.9 mg/serving (Mc Conn, 2012). According to another study in 2016 in Nepal, 10 samples of energy drinks produced from different countries (Thailand-Turkey-UK- Korea-Germany) which are available in Nepal were analyzed for their caffeine and taurine content and compared to label claims. Taurine assay was performed by HPLC with UV-visible detector using pre-column derivatization with 2,4-dinitrofluorobenzene. By comparing the obtained results and the claimed values, it was found that the values did not match in 6 samples (sample number 7 from Turkey claimed that it did not have taurine, while its value was found to be 3332.1 mg/L) *(Rai et al., 2016).*

## Conclusion

In this study, a sensitive and accurate analytical method by HPLC was used to measure taurine in energy drinks for validation. The most commonly reported doses for taurine in energy drinks are 400-1500 mg per day. However, the maximum amounts to cause toxicity are much higher and even doses higher than 1500 mg seem to be well tolerated. Comparing the results with international standards shows that the taurine content of these drinks is lower than the standard in most of the examined samples. In general, consumption of energy drinks should be limited to one or two cans of energy drinks per day. Of course, the amount of taurine received from other sources should also be considered. Overall, according to the best available evidence, taurine in recommended amounts has no negative side effects. While there has been no direct problems with taurine supplements, deaths in young athletes in Europe have been linked to consumption of energy drinks containing taurine and caffeine.

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