زانکۆی سەلاحەدین – هەولێر

Salahaddin University - Erbil

Determination of Taurine in Energy Drinks by High Performance Liquid Chromatography (HPLC)

Graduation Research Project

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By:

Jehan Hashim Hamad

Supervised by:

Ms. Sara Hadi Assaf

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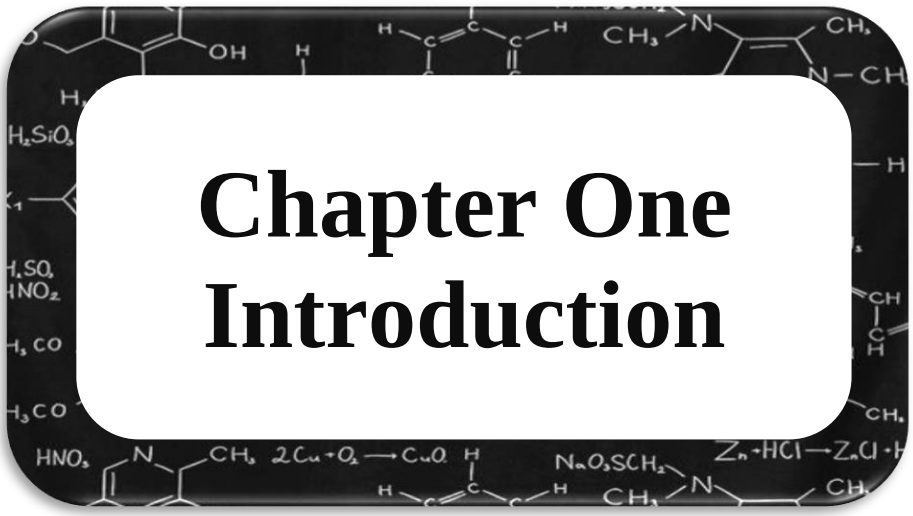
Abstract

Energy drinks have become increasingly popular in recent years, with their consumption being prevalent among individuals seeking enhanced alertness and performance. Taurine, an organic compound found naturally in the human body and commonly added to energy drinks, plays a pivotal role in various physiological processes. However, excessive intake of taurine has been associated with potential health risks, making accurate quantification crucial for regulatory purposes and consumer safety. This research project focuses on the determination of taurine levels in energy drinks using High Performance Liquid Chromatography (HPLC) as the analytical technique. HPLC offers high sensitivity, precision, and efficiency in separating and quantifying individual components within complex matrices like energy drinks.

Keywords: Taurine, Energy Drinks, HPLC.



Chapter One
Introduction

1. Introduction

1.1. Food Analysis

Food analysis is continuously requesting the development of more robust, efficient, sensitive, and cost-effective analytical methodologies to guarantee the safety, quality, and traceability of foods in compliance with legislation and consumers demands. From the so-called wet chemistry used at the beginning of the 20th century (Vilela et al., 2017). The constituents to be detected or determined in food analysis may be elements, radicals, functional groups, compounds, groups of compounds, or phases. Few of the chemical, physical, or physicochemical methods used in food analysis are completely specific or selective. Sometimes, careful adjustment of pH, oxidation or reduction, or complexing of certain groups or elements makes determinations possible. However (except for specific biological methods (e.g., enzymatic, immunological), fractionation procedures generally must be used. Advances in food analysis in the last three decades have resulted from the development of many instrumental methods and from improvements in separation methods (mainly chromatography) that, in turn, utilize more and more instrumental techniques (Pomeranz, 2013). Food analysis, in the 21st century, demands the development of more robust, efficient, sensitive, and cost-effective analytical methods to assure safety, quality, and traceability of food according to legislation and consumers demands. Computer vision is a fast, non-invasive low-cost method for evaluating food quality. Regarding to food composition, analysis by instrumental methods such as chromatographic, spectrophotometric, gravimetric, fluorimetric, rheological, mass spectrometric and more recently NIR and FTIR technologies are available (Tang, F., et al. 2019). In the current context of food trade globalization and due to the recognized impact of the diet on human health, food analysis has become more important than ever. Food analysis has today gone beyond the traditional analysis of the major components of food and is more complex and broader. In addition to the nutritional value of foodstuffs (i.e.,

carbohydrates, proteins, lipids, vitamins, minerals and water), food analysis has been focused on food safety for a long time, mainly in the determination of residues of pesticides and veterinary drugs. Nowadays, food safety analysis encompasses a wide variety of compounds including natural contaminants (e.g., toxins) or anthropogenic contaminants such as persistent organic pollutants (POPs) (Wu et al., 2019).

1.2. Taurine

In 1827, a new compound was isolated from ox bile by German scientists Friedrich Tiedemann and Leopold Gmelin (Surai, P.F., et al. 2021), which is now known as Taurine (2-aminoethanesulfonic acid) is one of non-protein and non-essential amino acid. Taurine is commonly called an amino acid, but it does not contain a carboxyl group and its amino group is located at the β-position. It can be naturally found in mammalian muscles and presents the most abundant free amino acid in the heart, retina, skeletal muscle, leukocytes, brain and animal tissues (Hohmann, M., et al. 2014). While it can be synthesized by healthy adults from decarboxylation methionine or cysteine followed by oxidation of its thiol group and it can be absorbed from foods (generally skeletal muscle) that contain it, preterm infants and children on taurine-free diets have less taurine in their blood plasma because they have not developed the capacity to synthesize it (Orth 2001). Taurine ($C_2H_7NO_3S$) (molecular weight: 125.14 g/ mol) is a sulfur-containing β-amino acid which is soluble in water. It is an organic weak acid with dissociation constant pKa = 4.96 which remains stable in acids and bases (Omer, .M.M.A., et al. 2019).

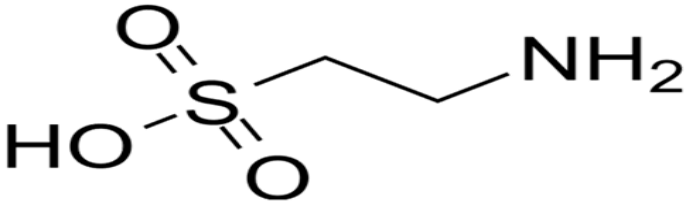


Figure 1. Structural formula of taurine

Taurine plays an important role in many significant physiological processes in living organisms due to its anti-inflammatory and antioxidant effects. Furthermore, it protects against many kidney and cardiovascular diseases, growth retardation, ischemia reperfusion injury, sepsis, diabetes mellitus, epilepsy (including other seizure diseases) and several kinds of cancer. Several psychological effects are engaged to Taurine presence such as diminishing of depression, trauma and schizophrenia (Teresa, P.Y., 1990). It contributes to osmotic regulation and membranes stabilization, detoxication of liver by binding to harmful substances. Taurine aids in cholesterol transformation to bile acids, which causes decreasing of low density lipoproteins in the blood. Further, it indirectly improves strength of bones by increasing production of alkaline phosphatase binding to osteopontin. Utilization of fats and proteins to form and maintain muscles is enhanced with its presence. As pharmaceutical ingredient in ophthalmic drugs, Taurine prevents cataract occurrence, decreases oxidative lens damage and treats retinopathy by influencing retina progression. In contrast this, many times higher concentration of Taurine was found in brain (Ricciutelli, M., et al. 2014). It optimizes central nervous system activity by stimulating thinking processes, works as neurotransmitter and protects brain against cytotoxic effects of glutamate. Recently, it is used for Alzheimer's neurodegenerative disease treatment. On the other hand, several researches showed that a disproportionate intake of Taurine causes side effects such as hypoglycemia, dehydration, decreasing heart frequency while blood pressure is increased, diarrhea, peptic ulcer, dizziness cardiomyopathy and liver toxicity. All these side effects are increased by the combination of Taurine with caffeine, guarana and amino acids in energy drinks (Farag, A.S., et al. 2019). It is added to energy drinks maybe due to its purposed stimulant effects and it may improve athletic performance, improve attention and verbal reasoning skills. The mean daily intake of taurine from diet was estimated to vary between 40 to 400 mg. Some energy drinks contain high level of synthetic taurine up to 4000 mg L-1, hence the daily intake

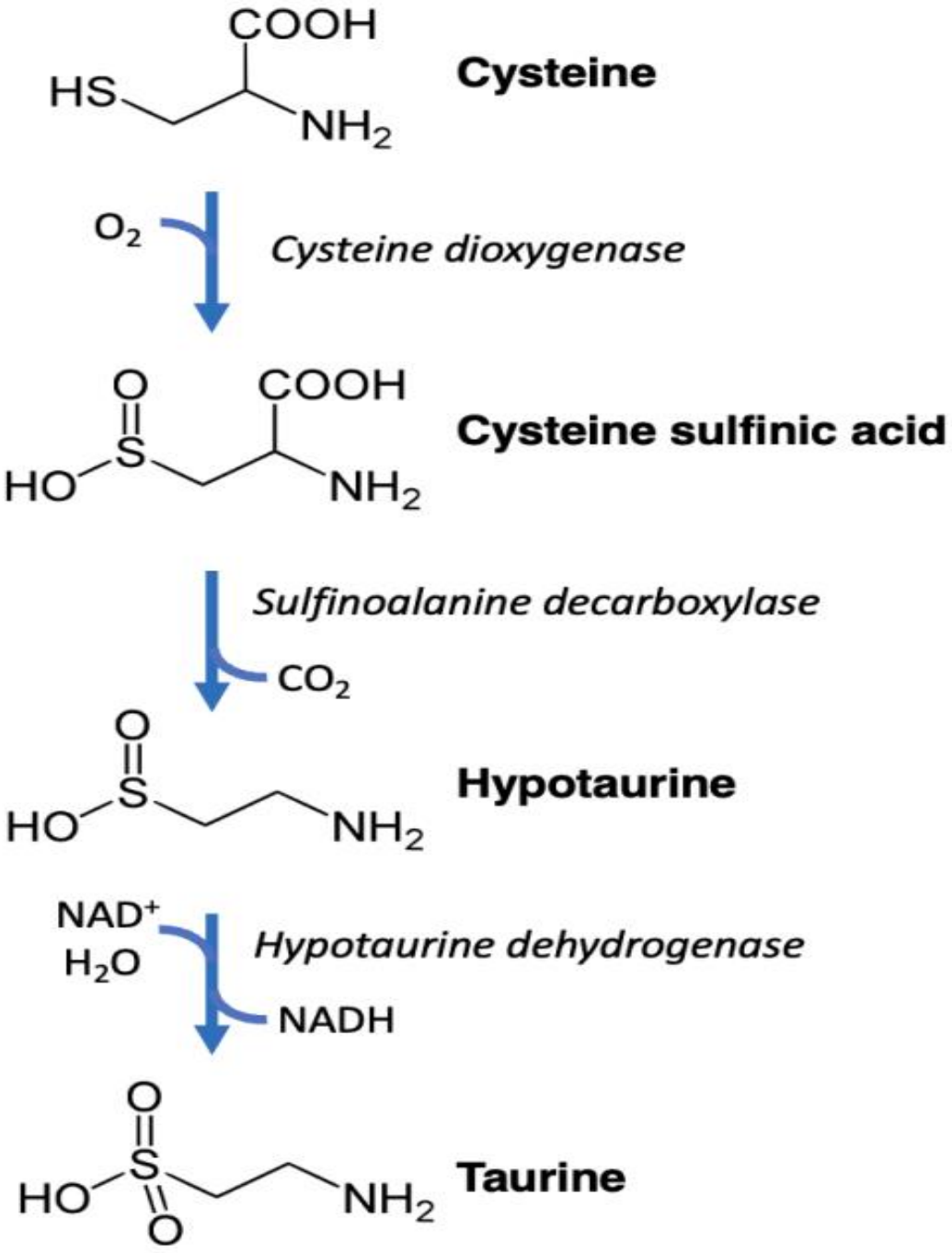
of taurine would be 2000 mg from consumption of 0.5 L of these drinks. This is five times greater than the highest estimated intake of 400 mg/day from naturally occurring taurine in omnivore diets. High doses of taurine greater than 2.0 g per day may cause unintended side effects ranging from high blood pressure to strokes, induction of psoriasis and seizures to heart disease. Therefore, it is important to develop simple and accurate analytical method to measure taurine amount in energy drinks. Several techniques have been developed for the determination of taurine. The most common analytical methods for the measurement of taurine are UV-Vis spectrophotometry and high-performance liquid chromatography (HPLC) coupled to different detectors i.e. UV-Vis, fluorescence detector (FLD), and mass spectrometer detector (MS), amino acid analyzer, spectrofluorimetric (Omer, M.M.A., et al. 2019).

1.3. Occurrence of Taurine

1.3.1 Endogenous Sources of Taurine

Taurine is produced endogenously, in the liver mainly via the “cysteine sulfinic pathway” (Figure-1). Cysteine dioxygenase oxidizes cysteine to form cysteine sulfinic acid, which is then decarboxylated by cysteine sulfinic acid decarboxylase to obtain hypotaurine, which is then oxidized by hypotaurine dioxygenase to form taurine. An alternative pathway is trans-sulfuration, in which homocysteine is converted into cystathionine, which is then transformed into hypotaurine by cystathionine gamma-lyase, cysteine dioxygenase, and cysteine sulfinic acid decarboxylase, and finally oxidized to form taurine (Santulli, G., et al. 2023).

Figure 2. Representation of the chemical reactions of the cysteine sulfinic pathway.



1.3.2.Dietary Source of Taurine

Taurine is primarily sourced from various animal-derived foods in the human diet. Meat serves as a prominent source, with beef, lamb, and poultry containing significant amounts of taurine. Seafood, notably fish such as salmon and mackerel, along with shellfish like shrimp, also provide ample taurine. Additionally, dairy products such as milk, cheese, and yogurt contribute to taurine intake. Eggs, particularly the yolks, contain taurine as well (Sampath, W.W.H.A., et al. 2020). While some energy drinks may contain added taurine for its purported benefits, it's important to note that these beverages often provide it in trace amounts compared to natural dietary sources. Overall, a balanced diet rich in animal-based foods typically ensures sufficient intake of taurine, an essential compound for various physiological functions in the human body (Pasantes-Morales, H., et al. 1989). The best natural sources of taurine include:

- **Scallops:** Shellfish have some of the highest taurine content, especially scallops. Whether you cook them or eat them raw, 100 grams of scallops can have up to 827 milligrams of taurine. Other good options include clams at 520 milligrams and mussels at up to 655 milligrams for the same portion.
- **Tuna:** we get it fresh or from a can, tuna is an excellent source of taurine. Though when choosing your fish, darker meat is richer in amino acids than white meat. Some varieties, like yellowfin tuna, contain up to 964 milligrams per 100 grams, while other marine fish have high levels as well. Try cod for its 120 milligrams or salmon with 94 milligrams of taurine per serving.
- **Tilapia:** Freshwater fish are high in taurine as well. Tilapia’s dark muscle has about 972 milligrams for a 150-gram filet, while the white meat has less than 120 milligrams. There’s also the dark meat from carp with 868 milligrams and catfish with almost 700 milligrams for the same serving.
- **Octopus:** Octopus contains about 335 milligrams per 3-ounce portion. Squid has potent levels as well, with 219 milligrams for the same serving.

- **Turkey:** With up to 306 milligrams per 100 grams, turkey has the highest taurine content of any animal meat. But like fish, the meat you choose matters. Only dark turkey meat has these high amounts, while light meat has just 30 milligrams.
- **Chicken:** You can add chicken to almost any recipe and with it, about 170 milligrams of taurine to your meal. However, as with turkey, go for the dark meat for the taurine benefits. Light meat like chicken breast has only 18 milligrams of taurine per 100 grams compared to cuts like chicken thighs.
- **Seaweed:** Because most taurine sources are from animals, seaweed is an excellent option for people on a plant-based diet. Nori, the papery-like seaweed product used in making sushi, has up to 1,300 milligrams of taurine per 100 grams. While we don't eat that much in a single sitting, sprinkling a sheet of nori into a dish or eating it with sushi can add about 40 milligrams of taurine to your meal.
- **Beef:** Beef is rich in nutrients and amino acids, including taurine. While a high intake of red meats is linked to greater rates of chronic diseases, most people can have two to three servings a week without much risk. With these servings, you'll add about 40 milligrams of taurine to your meal (Xu, S.W., et al. 2020).

1.4. Physiological Function of Taurine

1.4.1.Antioxidant Properties

Taurine involves in a variety of physiological functions related to homeostasis of skeletal muscle, the retina, the central nervous and cardiovascular systems. Major biological functions of Taurine are summarized in Table-1 (Schaffer and Kim 2018). Taurine possesses several antioxidant properties that contribute to its potential health benefits. Taurine's antioxidant properties make it a potentially valuable compound for protecting cells and tissues from oxidative damage, which is implicated in various age-related diseases, neurodegenerative disorders, cardiovascular conditions, and other health problems. However, further research is needed to fully elucidate the mechanisms underlying taurine's antioxidant effects and its therapeutic potential in oxidative stress-related diseases (Seidel, U., et al. 2019). Here are some key ways in which taurine exhibits antioxidant activity:

- **Scavenging Free Radicals**: Taurine has been shown to act as a scavenger of various reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide radicals, hydroxyl radicals, and peroxynitrite. By neutralizing these harmful molecules, taurine helps prevent oxidative damage to cells and tissues.
- **Enhancing Antioxidant Enzyme Activity**: Taurine can enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes play crucial roles in the cellular defense against oxidative stress by converting harmful ROS into less reactive species.
- **Protecting Lipids**: Taurine has been reported to protect cellular membranes and lipids from oxidation. Lipid peroxidation, a process initiated by ROS, can lead to cell damage and contribute to various diseases. Taurine's ability to inhibit lipid peroxidation helps maintain membrane integrity and function.

- **Modulating Inflammatory Responses**: Taurine has anti-inflammatory properties that can indirectly contribute to its antioxidant effects. By reducing inflammation, taurine helps decrease the production of ROS and RNS generated during inflammatory processes, thereby mitigating oxidative stress.
- **Chelating Metal Ions**: Taurine can chelate metal ions such as copper and iron, which are involved in generating ROS through Fenton reactions. By sequestering these metal ions, taurine helps prevent their participation in oxidative processes, reducing oxidative stress (Belal, S.A., et al. 2018).

Table 1. Regulatory roles of Taurine in biological functions.

Function
Antioxidant
Membrane stabilisation
Mitochondrial integrity maintenance
Vitagene activation
Bile acid conjugation
Ca homeostasis
FA metabolism/oxidation
Energy metabolism
Osmoregulation
Thermoregulation
Detoxification
Neuroprotection
Anti-inflammatory
Immunomodulation

1.4.2.Role in Neurotransmission

Taurine is not classified as a classical neurotransmitter, plays a vital role in neurotransmission through its multifaceted actions within the nervous system. Acting as a neuromodulator, taurine influences neuronal excitability by regulating the activity of ion channels, particularly chloride channels. By activating glycine and GABA receptors, which mediate chloride influx into neurons, taurine helps modulate the overall excitability of neuronal circuits, thereby balancing neurotransmission (El Idrissi and Trenkner 1999). Additionally, taurine contributes to calcium homeostasis within neurons, crucial for neurotransmitter release, synaptic plasticity, and neuronal survival. Its ability to regulate intracellular calcium levels ensures the proper functioning of neurotransmission processes. Moreover, taurine exhibits neuroprotective properties by scavenging free radicals and reducing oxidative stress, thereby preserving neuronal integrity and function, essential for efficient neurotransmission. Furthermore, while taurine itself is not a primary neurotransmitter, it can influence the release of other neurotransmitters like glutamate and GABA, contributing to the regulation of synaptic transmission and neuronal excitability. Lastly, taurine's involvement in neuronal development, particularly during early brain development, underscores its importance in establishing normal neuronal connectivity and neurotransmission pathways. Overall, taurine's multifaceted contributions to neurotransmission highlight its significance in maintaining proper neuronal function and overall brain health (Wu and Prentice 2010).

1.4.3.Impact on Cardiovascular Health

Taurine found abundantly in the heart and vascular tissues, exerts significant positive effects on cardiovascular health. Its impact spans various physiological mechanisms crucial for maintaining a healthy cardiovascular system. Firstly, taurine plays a pivotal role in blood pressure regulation by acting as a vasodilator, promoting the relaxation of blood vessels. This vasodilatory effect helps reduce peripheral resistance, thereby contributing to the management of hypertension (Swiderski, J., et al. 2023). Additionally, taurine influences the renin-angiotensin-aldosterone system, mitigating the production of angiotensin II, a potent vasoconstrictor, thus further aiding in blood pressure control. Furthermore, taurine possesses antiarrhythmic properties, which contribute to stabilizing the heart's rhythm. By modulating ion channels involved in generating action potentials and regulating calcium flux within cardiac cells, taurine helps prevent abnormal heart rhythms, such as atrial fibrillation and ventricular arrhythmias. These actions are crucial for maintaining proper cardiac function and reducing the risk of life-threatening arrhythmias (Higuchi, M., et al. 2012). Taurine's antioxidant and anti-inflammatory properties also play a vital role in cardiovascular health. Its anti-inflammatory effects help alleviate inflammation within arterial walls, further reducing the risk of cardiovascular diseases. Another significant impact of taurine on cardiovascular health is its ability to improve lipid profiles. Studies have shown that taurine supplementation can lead to reductions in total cholesterol, LDL cholesterol, and triglyceride levels, while increasing levels of HDL cholesterol. These favorable changes in lipid metabolism contribute to lowering the risk of atherosclerosis and coronary artery disease. Additionally, taurine enhances myocardial function by improving myocardial contractility—the heart's ability to pump blood effectively. By modulating calcium handling within cardiac cells and protecting against ischemia-reperfusion injury, taurine supports optimal heart function and resilience to cardiac stressors (Sarnobat, D., et al. 2023).

1.5. Taurine in Energy Drinks

1.5.1. Common Use as an Ingredient

Energy drinks have become ubiquitous in today's fast-paced lifestyle, offering consumers a quick and convenient way to boost alertness and vitality. Taurine, a naturally occurring amino acid, is a common ingredient in many energy drinks. Its inclusion is often linked to purported benefits such as enhanced mental focus, increased energy levels, and improved exercise performance.

- **Cognitive Enhancement:** Taurine is believed to have cognitive-enhancing properties, making it a sought-after ingredient in energy drinks designed to improve mental alertness and focus. Studies suggest that taurine may modulate neurotransmitter activity, contributing to better cognitive performance without the jitteriness associated with high caffeine intake (Chen, C. et al. 2019).
- **Synergistic Effect with Caffeine:** Taurine is frequently combined with caffeine in energy drinks to create a synergistic effect. While caffeine acts as a stimulant, taurine may help mitigate potential side effects such as restlessness and anxiety. The combination is thought to offer a smoother and more sustained energy boost compared to caffeine alone (Santhakumar, A., et al. 2013).
- **Energy Metabolism:** Taurine plays a role in energy metabolism, and its presence in energy drinks is often linked to claims of increased physical stamina and endurance. Some studies suggest that taurine may enhance the muscles' ability to contract efficiently, leading to improved exercise performance (Wen, C., et al. 2019).
- **Antioxidant Properties:** Taurine is known for its antioxidant properties, which may contribute to the overall health benefits associated with energy drinks. Antioxidants help neutralize free radicals in the body, potentially reducing oxidative stress and supporting the body's natural defense mechanisms (Oliveira, M.W., et al. 2010).

- **Electrolyte Balance:** In addition to its role in energy metabolism, taurine is an osmolyte, helping regulate water balance and electrolyte concentrations in cells. This property is particularly relevant in energy drinks marketed as performance enhancers, as it may aid in preventing dehydration during physical activities (Ciechanowska, 1997).
- **Formulation and Regulatory Considerations:** The amount of taurine in energy drinks can vary, and regulatory bodies in different countries may have specific guidelines regarding its permissible levels. Manufacturers often carefully formulate the taurine-caffeine ratio to achieve the desired physiological effects while staying within regulatory limits (Chesney, R.W., et al. 1998).

1.5.2. Purpose and Perceived Benefits

Taurine, an amino acid often included in energy drinks, serves various purposes and is believed to offer several perceived benefits. Primarily, taurine is thought to contribute to an energy boost by influencing mitochondrial function, aiding in the production of cellular energy. This is particularly relevant for those seeking a quick and sustained increase in vitality. Additionally, taurine is associated with mental alertness as it may play a role in neurotransmitter regulation, potentially enhancing cognitive function and focus. The inclusion of taurine in energy drinks is also tied to cardiovascular health benefits, such as improved blood flow and pressure regulation, contributing to an overall sense of well-being. Moreover, taurine's role in osmoregulation makes it valuable for maintaining hydration and electrolyte balance, particularly important for individuals consuming energy drinks during physical activities (Alsunni and Badar 2011). As an antioxidant, taurine may help neutralize free radicals, reducing oxidative stress and supporting overall health. Athletes, in particular, may find taurine beneficial due to its potential impact on muscle contractions, potentially enhancing exercise performance. However, it's crucial to acknowledge that individual responses can vary, and more research is needed to fully understand the mechanisms and long-term effects of taurine in energy drinks. As with any dietary supplement, moderation and consideration of overall formulation, including other ingredients, are key factors in assessing the benefits and potential risks associated with taurine consumption. Individuals with pre-existing health conditions should consult healthcare professionals before incorporating taurine-containing products into their diet (Gwacham and Wagner 2012).

1.5.3. Recommended Daily Intake

Determining the recommended daily intake of taurine in energy drinks is a complex task due to variations in individual tolerance, health conditions, and regulatory standards. Unlike essential nutrients with established dietary reference values, taurine does not have a universally agreed-upon Recommended Dietary Allowance (RDA). The absence of a set standard makes it challenging to establish a one-size-fits-all guideline for taurine intake in energy drinks. In the absence of specific RDAs, regulatory bodies often set upper limits to guide safe consumption. However, these limits may differ across countries, adding to the challenge of providing a globally applicable recommendation. It is crucial for consumers to be aware of and adhere to the taurine limits established by regulatory authorities in their respective regions. Individual tolerance plays a significant role in determining the appropriate intake of taurine. Some people may be more sensitive to taurine's effects, especially when combined with other stimulants commonly found in energy drinks, such as caffeine (Duchan, E., et al. 2010). Health conditions, medications, and other factors can influence how individuals respond to taurine, underscoring the importance of consulting with healthcare professionals, particularly for those with pre-existing medical conditions. While taurine is naturally present in the diet, including in meat and seafood, the concentrations found in energy drinks can significantly exceed typical dietary levels. Responsible consumption involves considering not only the taurine content in energy drinks but also the cumulative intake from other dietary sources. A balanced and varied diet can provide taurine without the need for additional supplementation through energy drinks. Given the lack of consensus on specific daily intake recommendations for taurine, it is prudent for consumers to exercise moderation and adhere to product labeling. Manufacturers often provide information on taurine content per serving, allowing consumers to make informed choices based on their individual preferences and health considerations. It is advisable to read product labels, stay within recommended serving sizes, and

monitor overall stimulant intake, including taurine, to maintain a balanced and healthy lifestyle. Ultimately, individualized decisions regarding taurine intake should consider factors such as age, health status, and sensitivity to stimulants, promoting responsible consumption of energy drinks (Mihaiescu, T., et al. 2024).

1.6. HPLC Method

HPLC stands for High Performance Liquid Chromatography. It is a powerful analytical technique used to separate, identify, and quantify components in a mixture. In HPLC, a liquid solvent (mobile phase) is pumped under high pressure through a column packed with a stationary phase material. The sample to be analyzed is injected into the solvent stream and carried through the column. As the sample components interact with the stationary phase to varying degrees, they are separated based on their different affinities for the stationary phase and the mobile phase (Moldoveanu, S.C., et al. 2016). This separation process allows for the isolation and identification of individual components within a mixture. HPLC is widely used in various fields such as pharmaceuticals, food and beverage analysis, environmental analysis, forensic science, and many others due to its high sensitivity, accuracy, and ability to handle a wide range of sample types (Singh, R., 2013). Figure.3 shows a basic overview of the HPLC process. The solvent used to separate components in a liquid sample for HPLC analysis is called the mobile phase. The mobile phase is delivered to a separation column, otherwise known as the stationary phase, and then to the detector at a stable flow rate controlled by the solvent delivery pump. A certain amount of sample is injected into the column and the compounds contained in the sample are separated. The compounds separated in the column are detected by a detector downstream of the column and each compound is identified and quantified (Tiwari, A., et al. 2018).

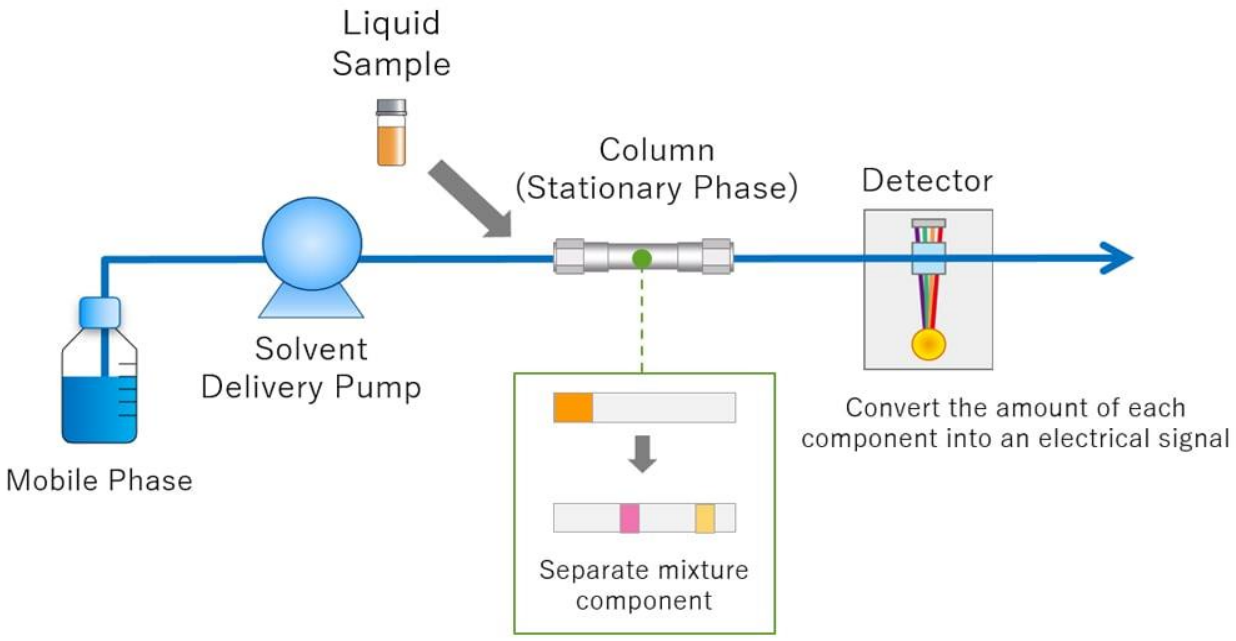


Figure 3. Basic overview of the HPLC process.

There are several types of HPLC techniques, each optimized for specific applications or separation requirements. Some common types of HPLC include:

- **Normal Phase Chromatography (NPC)**: In this technique, the stationary phase is polar (such as silica) while the mobile phase is non-polar. It is typically used for separating compounds based on their polarity.
- **Reverse Phase Chromatography (RPC)**: Here, the stationary phase is non-polar (such as C18) and the mobile phase is polar. Reverse Phase Chromatography is widely used in pharmaceutical and biochemical analysis (Fiorelia, N.E., et al. 2022).
- **Ion-Exchange Chromatography**: In this method, the stationary phase contains charged functional groups, and separation is based on ionic interactions between the analytes and the stationary phase. It is commonly used for separating ions or charged molecules.
- **Size-Exclusion Chromatography (SEC)**: Also known as Gel Permeation Chromatography (GPC), this technique separates molecules based on their size and molecular weight. Larger molecules elute first, while smaller molecules are retained longer due to their penetration into the porous stationary phase.

- **Affinity Chromatography**: This technique utilizes highly selective interactions between a specific ligand attached to the stationary phase and the target analyte. It is commonly used for purifying proteins and other biomolecules.
- **Hydrophilic Interaction Chromatography (HILIC)**: In HILIC, the stationary phase is polar and the mobile phase is non-polar or slightly polar. It is useful for separating polar compounds that may not retain well in traditional reverse phase chromatography.
- **Chiral Chromatography**: This technique separates enantiomers (mirror-image isomers) of chiral compounds. Chiral stationary phases with specific chiral selectors are used to achieve this separation (Ali, A.H., 2022).

Chapter Two

Literature Review on The Determination of Taurine in Energy Drinks by High Performance Liquid Chromatography (HPLC)

2. Literature Review on The Determination of Taurine in Energy Drinks by High Performance Liquid Chromatography (HPLC)

2.1. Determination of Taurine in Energy Drinks by HPLC Using a Pre-column Derivative

A rapid and simple method to determine taurine in energy drinks by pre-column high-performance liquid chromatography was developed using a derivative of 4 fluoro-7-nitrobenzofurazan (NBD-F) without the need for an exclusive instrument. The reaction of taurine with NBD-F finished in 10 min at 60°C. The derivative was measured on a UV-Visible detector (470nm) by HPLC using a conventional Octadecyl silane (ODS) column. A mixture of disodium hydrogenphosphate-citric acid buffer solution (pH 5.4) containing 10 mmol/l tetrabutylammonium bromide and acetonitrile (7:3) was used as the mobile phase. The recoveries were in the range of 98.2-99.9%, the precision as standard deviation was in the range of 0.3-0.5%, the linearity as a coefficient of correlation value was 0.999 and the specification was confirmed for taurine added to three commercial energy drinks. The content of taurine measured compared to the labeled amount in five commercial energy drinks containing taurine was 92.9-105.1% (Sawabe, Y., et al. 2008).

2.2. High Performance Liquid Chromatographic Methods for Analysis of Taurine in Energy Drinks after Pre-column Derivatization

Simple and efficient high performance liquid chromatography (HPLC) with photodiode array (PDA) and fluorescence (FLD) detection methods have been validated for determination of taurine in energy drinks. These methods are based on pre-column derivatization of taurine with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) at alkaline medium to form colored fluorescent product. In the both validated HPLC methods, the derivatization product is separated on Intersil ODS-3 analytical column with acetonitrile and 0.1% trichloroacetic acid (30:70, v:v) as mobile phase. The eluted derivative is detected at 472 nm for HPLC-PDA and 472 nm/530 nm (Ex/Em) for HPLC-FLD. The methods were validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy (recovery). Good linearities were achieved for taurine (r2> 0.9998 and 0.9993) in the concentration range of 5-50 mg L^{-1} and 5-50 µg L^{-1} for HPLC-PDA and HPLC-FLD respectively. The LODs were 0.296 and 0.616 × 10^{-3} mg L^{-1} for HPLC-PDA and HPLC-FLD respectively. The precision for peak area were 0.78 and 0.61% for HPLC-PDA and HPLC-FLD respectively. Recoveries of taurine ranging from (92-103.3%), (n = 3) were obtained. The validated method was successfully applied for the determination of taurine in some selected energy drinks available in local markets (Omer, M., et al. 2018).

2.3. Simultaneous determination of taurine, glucuronolactone and glucuronic acid in energy drinks by ultra high performance liquid chromatography–tandem mass spectrometry (triple quadrupole)

In this work, we present for the first time a rapid and robust UHPLC–MS/MS method for analyzing taurine, GlcLA and GlcA in energy drinks simultaneously and without derivatization. The separation of three analytes was achieved using a Kinetex Hilic analytical column (100 mm × 4.6 mm i.d.) and a mobile phase formed by water (A) and acetonitrile (B) both with formic acid 0.1% at a flow rate of 0.8 ml min^{-1} with isocratic elution in 3.5 min. Calibration curves were calculated using the method of standard addition in a concentration range from 2 to 6 mg/100 ml for taurine (R^2 > 0.987), from 0.4 to 1.2 mg/100 ml for GlcLa (R^2 > 0.997), and from 0.2 to 0.6 mg/100 ml for GlcA acid (R^2 > 0.998). The validated method was applied to the analysis of nine commercial energy drinks. The level of taurine found ranged from 0.01 to 0.45 g/100 ml, and it matched with that reported in the labels of the analyzed energy drink samples (Ricciutelli, M., et al. 2014).

2.4. Optimization of HPLC Analysis Method for Taurine And Caffeine in Energy drinks

This paper presents the optimization of a rapid, inexpensive, reliable and selective isocratic (HPLC) method for the simultaneous determination of caffeine and taurine in energy drinks with two common detectors in series: evaporating light scattering detector (ELSD) and (UV) detector. Satisfactory analysis results were obtained on an Astec apHera NH_2 column using methanol/water (30:70 v/v) as mobile phase. The optimized method was used for the analysis of commercial energy drinks containing large amounts of carbohydrates (100 $g \cdot L^{-1}$) and considerably lower amounts of taurine and caffeine (4 and 0.6 $g \cdot L^{-1}$, respectively). The advantages of this method consist of its lack of preliminary samples treatment and also the fact that basic LC instrumentation was employed (Finaru and Elfakir 2018).

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

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 (Zabihi Negin, et al. 2023).

2.6. Determination of Caffeine and Taurine Contents in Energy Drinks by HPLC-UV

Energy drinks are non-alcoholic beverage intended to enhance the psycho-physiological responses in human, which is especially popular among young generation in Nepal. It is normally high caffeinated drink added with other ingredients such as carbohydrates, amino acids, B-group of vitamins etc. In this study, 10 brands of energy drink available in Nepalese markets were taken then analyzed for quantitative determination of Caffeine and Taurine by HPLC-UV method. From the result obtained, pH and TSS values of energy drinks were found in the range of 2.96-3.81 and 6.64-18.21 respectively. Likewise, the Caffeine and Taurine content in same samples were found in the range of not detected (ND) to 35.78 mg/100 ml and ND to 387.5 mg/100 ml respectively. Only the 6 samples out of 10 were confirmed caffeine content as per claimed in label, while only 3 samples were confirmed for Taurine content as per label claimed. Based on this pilot study, the majority of samples did not meet the label claims in term of Caffeine and Taurine, which apparently indicated the misbranding of such drinks. Since, there is no any regulation for such energy drinks in Nepal, it seems to be a great challenge for regulation of their safety and misbranding (Rai, K.P., et al. 2016)

2.7. Determination of taurine in soft drinks by an ultrahigh-performance liquid chromatography-mass spectrometry method

Taurine (2-aminoethanesulfonic acid) is a free sulfur-containing β-amino acid widely distributed in many mammalians. Owing to the energizing effects, it is mostly used in soft drinks and supplements for athletes. Regular intake of soft drinks may lead to an overdose of caffeine, taurine, and guarana and loss of bone mass, overweight, hypertension, and in older age, osteoporosis and cardiovascular diseases. Therefore, it is essential to control the maximum amount of taurine consumed by humans in the food and beverages. Here, a fast, simple, accurate,

and robust method based on ultrahigh-performance liquid chromatography hyphenated with mass spectrometry (UHPLC-MS) was successfully applied for the determination of taurine in selected soft drinks sold in Slovakia. The method was characterized by coefficient of determination higher than 0.99, and the predicted value of the limit of detection was 4.29 μmol/L. The analyzed levels of taurine in selected commercial drinks ranged from 2.8 to 3.78 mg/mL. The concentration in one brand of the investigated drinks was found to be extremely low (about 70%) compared to the declared content by the manufacturer (Chalova, P., et al. 2023).

2.8. Liquid Chromatographic and Spectrophotometric Determination of Taurine in Energy Drinks Based on O-Phthalaldehyde-Sulfite Derivatization

Rapid and efficient high-performance liquid chromatographic and UV-Vis spectrophotometric methods have been optimized and validated for taurine determination in energy drinks. Taurine was derivatized with o-phthalaldehyde and sodium sulfite in alkaline media prior to analysis. The optimum derivatization parameters were found to be 0.1 M borate buffer at pH 9.5, reaction time 5.0 min, o-phthalaldehyde concentration of 60 mg L^{-1}, sodium sulfite concentration of 202 mg L^{-1} and water as diluting solvent. The analytical parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy were investigated. The methods were linear in range 0.5-20 mg L^{-1} and 0.5-15 mg L^{-1} with correlation coefficient (r^2) of 0.9998 and 0.9996 for HPLCPDA and spectrophotometer respectively. The LODs were 0.109 mg L^{-1} and 0.141 mg L^{-1} for HPLC-PDA and spectrophotometer respectively. The precision (RSD%) of intra-day and inter-day of the methods were 1.816-1.278% and 2.858-2.236% respectively, for HPLC-PDA and spectrophotometer respectively. Recoveries of taurine ranging from 90% to 105%, (n = 3) were obtained. The methods were successfully applied for determination of taurine in energy drink samples (Omer, M.M.A., et al. 2019).

2.9. Determination of taurine in energy drinks and oral liquids by pre-column derivatization

Taurine in energy drinks and oral liquid was determined by pre-column derivatization with dansyl chloride. The derivatives were detected by high performance liquid chromatography (HPLC). HPLC with variable wavelength detector was selected, and the wavelength was set at 254 nm.C18 column was used to separate the derivatives, and the sodium acetate solution and acetonitrile (30:70, volume ratio) was used as mobile phase for isometric elution. The column temperature was set at 25 °Cand the sample injection volume was 5 μL. When the concentration of taurine was 5 μg/mL-50 μg/L, the peak area of taurine derivatives had a good linear relationship with the concentration and the correlation coefficient was 0.999. The combination of single factor and orthogonal experiment was used to optimize the derivatization experiment conditions. The results of the test had shown that the peak area of the derivative reached the maximum when derivatization temperature was 35 °C, derivatization time was 35 min, the volume of derivatization reagent was 300 μL and the pH value of the derivative solution was 9.5. Under the experimental conditions, the relative standard deviation (RSD)of the peak area of the six groups was 1.4% and the repeatability was good. The content of taurine in energy drinks and oral liquid was detected, and the recovery rate was 90.0%-108.0%. The deviation between the measured value and the identified value was -10.0%-+10.0%, the deviation of the parallel test is less than 3.0%. Therefore, this method had the advantages of easy preparation and mild reaction conditions (Bin, X., et al. 2019).

Conclusion

In conclusion, the utilization of High-Performance Liquid Chromatography (HPLC) for the determination of taurine in energy drinks offers a robust and reliable analytical method. Through the optimization of parameters such as mobile phase composition, column selection, and detection wavelength, accurate quantification of taurine can be achieved with high sensitivity and precision. The analysis of taurine content in energy drinks is crucial due to its physiological effects and regulatory requirements. Monitoring taurine levels ensures consumer safety and compliance with industry standards. HPLC provides an efficient means to quantify taurine accurately, facilitating quality control and assurance processes in the production of energy drinks. Overall, the application of HPLC in the determination of taurine in energy drinks represents a valuable tool for both analytical chemists and regulatory authorities in ensuring product quality, safety, and compliance with established standards. Continued research and refinement of HPLC methodologies will further advance our understanding and monitoring of taurine levels in energy drinks, contributing to the enhancement of consumer health and well-being.

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زانکۆی سەلاحەدین- هەولێر

Salahaddin University-Erbil

دیاریکردنی تاورین لەناو خواردنەوە وزە بەخشەکان بەڕێگای HPLC

پڕۆژەی دەرچوونە پێشکەشە بە بەشی کیمیایی کۆلێژی زانست زانکۆی سەڵاحەدین-هەولێر وەک بەشێک لە پێداویستییەکانی بەدەستهێنانی بڕوانامەی بەکالۆریۆس لە زانستی کیمیادا

ئامادەکردنی:

جیهان هاشم حمد

بەسەرپەرشتی:

م.سارا هادی

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