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

Special Issue Article

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Abstract 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.

Keywords *taurine – soft drinks – liquid chromatography – food and food supplements quality control – mass spectrometry*

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is a sulfur-containing organic amino acid which can be derived by biosynthesis from cysteine metabolism (Brosnan & Brosnan, 2006). The biosynthesis of taurine from cysteine is shown in Figure 1a.

Taurine is found in high abundance in skeletal muscle of humans (Balshaw et al., 2013; Bongiovanni et al., 2020; Seidel et al., 2019; Waldron et al., 2018; Wen et al., 2019; Wu, 2020). The rich sources of taurine are eggs, milk, meat, and sea food. The daily intake of taurine is between 10 and 400 mg (EFSA, 2009). Laidlaw et al. (1990) reported that in humans, with a decreased daily intake of taurine (e.g., vegans), taurine is stored in the body and its urinary excretion is lower. Taurine plays a crucial role in several metabolic and physiological processes such as glucose and lipid regulation (Carvalho et al., 2020; Murakami, 2015), energy metabolism (Murakami, 2015), anti-inflammatory modulation (Qaradakhi et al., 2020), and antioxidant action (Ibrahim et al., 2020; Surai et al., 2021). In some metabolic and physiological processes, there are also some correlations between taurine and oncological diseases. It is expected that taurine could be used as a novel tumor

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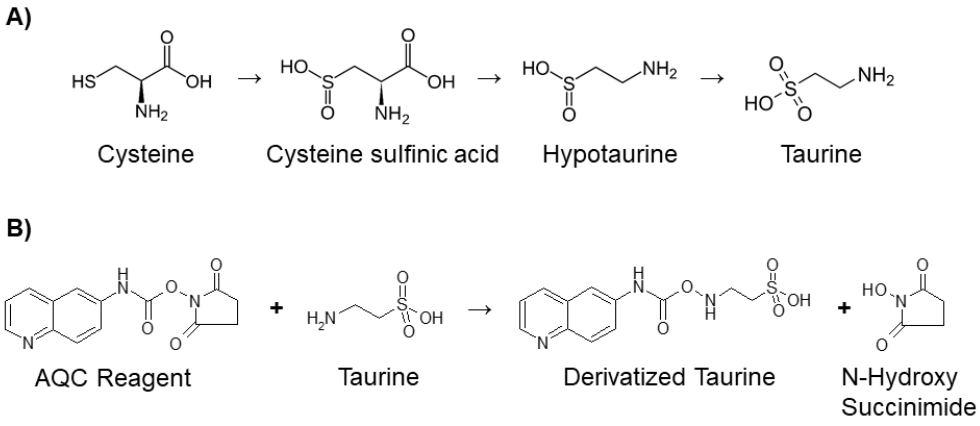

Figure 1. The biosynthesis of taurine from cysteine (a) and illustrative scheme of derivatization process of taurine using AccQ•Tag Ultra reagent (b).

marker for enhanced detection of breast cancer through downregulation of angiogenesis and enhancement of tumor cell apoptosis (El Agouza et al., 2011). An inhibition effect was demonstrated on the lung cancer cell line and on the growth of xenograft tumors, while p53 upregulated modulator of apoptosis (PUMA) is crucial in taurine-induced growth suppression (Tu et al., 2018). The same PUMA modulator plays an important role in taurine-induced apoptosis pathway in human colorectal cancer (Zhang et al., 2014). Highly sensitive techniques can be used to monitor taurine levels as a biomarker of various diseases, including cancer.

Recently, energy drinks and beverages containing taurine are becoming increasingly popular due to their energizing effects. These beverages typically also contain caffeine, vitamins, guarana, or ginseng. Excessive consumption of energy drinks may result in a variety of physiological responses such as arrhythmias, tremor, agitation, gastrointestinal upset, and insomnia (Gunja & Brown, 2012; Lévy et al., 2019). The intake of taurine from energy drinks (1 L of energy drink contains on average 3180 mg of taurine) far exceeds the mean daily intake from omnivore diets (about 60 mg) (Lévy et al., 2019). Therefore, it is essential to control the maximum amount of taurine found in the food and beverages consumed by humans.

In recent years, various analytical methods for the determination of taurine in energy drinks have been developed. Chromatographic methods (especially, high-performance liquid chromatography [HPLC]) coupled with various detection techniques (e.g., ultraviolet spectrophotometry [UV], mass spectrometry [MS], etc.) are the dominant ones (Lajin & Goessler, 2018; Orth, 2001; Rai et al., 2016; Yan et al., 2012). From the analytical point of view, it is necessary to perform a derivatization procedure before performing the own analysis. This is due to the lack of a chromophore group in the taurine molecule (Todorova, 2015) that significantly helps in its detection using convenient detection techniques. There are several types of derivatization reagents for precolumn derivatization or fluorescent labeling, such as 2,4-dinitrofluorobenzene (DNFB) (Yan et al., 2012), 4-fluoro-7-nitrobenzofurazan (NBD-F) (Sawabe et al., 2008), 4-chloro-7-nitro-1,2,3-benzofurazan (NBD-Cl) (Omar et al., 2017; Omer et al., 2018), phenylisothiocyanate (PITC) (Battaglia et al., 1999), 5-dimethylaminonaphtalene-1-sulfonyl chloride (dansyl chloride [DNS-Cl]) (Manzi & Pizzoferrato, 2013), *o*-phthalaldehyde (OPA) (Ziegler et al., 1992), 2-mercaptoethanol (BME) (Smith et al., 2004), and ninhydrin (Draganov et al., 2014). The vast majority of the developed methods deal with the use of reversed phase C18 columns and isocratic elution modes (Ahmad et al., 2022). For example, an HPLC–UV method with precolumn derivatization by DNFB was able to analyze taurine in beverages and milk samples within 40 min with the limit of detection (LOD) at 0.07 µg/mL concentration value (Yan et al., 2012). On the contrary, the determination of taurine content in soft drinks without precolumn derivatization and HPLC–MS analysis was characterized by significantly worse LOD value (30 µg/mL). However, the time of analysis was significantly improved (the analysis took only 2 min) (Catharino et al., 2011). Further, HPLC approaches based on the use of hydrophilic interaction chromatography (HILIC) separation column without precolumn derivatization and MS detection offered LOD values in the range of 0.01–30 ng/mL with the time of analysis between 2.5 and 3.5 min (Marchei et al., 2005; Ricciutelli et al., 2014). As can be seen, the HPLC–MS approaches are the preferred ones in such type of analyses.

The aim of this study was the full validation and application of a simple, accurate, and robust method based on ultrahigh-performance liquid chromatography hyphenated with mass spectrometry (UHPLC-MS) for the determination of taurine content in soft drinks sold in Slovakia. Compared to other methods, our approach can detect taurine in 1.96 min with the LOD of 4.29 µmol/L (i.e., 0.52 µg/mL). Several brands of soft drinks commercially available in Slovakia (Hell, Maxx, Tiger, and Red Bull) were successfully tested for taurine levels. This is the first study in which the method has been applied for the determination of taurine in soft drinks. In the future, it

can be used to control the taurine content in food, beverages, and various food supplements (i.e., quality control).

EXPERIMENTAL

Instrumentation

Liquid chromatography (LC)-MS experiments were performed with the use of Acquity UPLC H-Class (Waters, Prague, Czech Republic). The UHPLC system was fitted with a 2.1 mm × 100 mm, 1.6 µm particle size Cortecs UPLC C18 column. The mobile phase was acetonitrile and water in LC-MS grade, and both contained 0.1% formic acid. The flow rate was 0.5 mL/min, and the column temperature was 55 °C. The injection volume was 1 µL and was constant for all samples and the taurine standard. The separation was performed under gradient elution conditions summarized in Table 1.

The detection was performed with the use of a single quadrupole mass spectrometry detector – QDa detector (Waters) – equipped with an electrospray (ESI) ionization source working in positive (ESI+) mode. The ion source setup is as follows: capillary voltage – 0.8 kV, probe temperature – 600 °C, source temperature – 150 °C, cone voltage – 30 V, and desolvation gas – nitrogen.

Chemicals and samples

Analytical-grade taurine was obtained from Sigma Aldrich (Steinheim, Germany) and a deuterated internal standard (IS) of D5-L-glutamine was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). LC-MS–grade formic acid (HFo) and acetonitrile (ACN) were purchased from Sigma Aldrich, and LC-MS–grade water was purchased from Merck (Darmstadt, Germany). Also, 0.1 mol/L hydrochloric acid (HCl) p.a. quality was obtained from Sigma Aldrich. The derivatization reagent and diluent AccQ•Tag Ultra and borate buffer (pH 8.6) were obtained from Waters.

Preparation of standard solutions, IS solutions, and samples

The stock solution of taurine reference substance was prepared by dissolving its powder in 0.1 mol/L HCl to the desired concentration of 1 mol/L. Working solutions of taurine were obtained by proper dilution of the stock solution with 0.1 mol/L HCl. The taurine concentration levels were in the range of 10–500 µmol/L (10, 20, 50, 100, 250, and 500 µmol/L). A volume of 5 µL of each calibration level was taken into the derivatization process.

The stock solution of the IS (D5-L-glutamine) was prepared in 0.1 mol/L HCl to the desired concentration of 1 mol/L. Working solutions of D5-L-glutamine were made by proper dilution of the stock solution with 0.1 mol/L HCl to the desired concentration of 250 µmol/L.

Table 1. UHPLC gradient program for the separation of taurine.

Time (min)	Flow (mL/min)	Mobile phase A (%)	Mobile phase B (%)
Initial	0.5	99	1
0.7	0.5	99	1
1.3	0.5	87	13
3.7	0.5	85	15
7	0.5	60	40
8	0.5	5	95
9	0.5	5	95
9.8	0.5	99	1
10.5	0.5	99	1

Four commercial energy drinks (i.e., Red Bull, Hell, Tiger, Maxx) were obtained from a local store. In all beverages, the content of taurine was declared by the manufacturer to be 4 mg/mL. Before the analysis, 10 mL of each beverage was transferred into 10 mL volumetric flask and then the samples were sonicated in three intervals for 15 min in an ultrasonic bath. After the sonication procedure, the samples were 1000 times diluted with the LC-MS water and then 5 µL of each sample was taken into the derivatization process.

Derivatization procedure

The derivatization procedure of the calibration standards and samples of taurine was carried out according to the following protocol. At first, the derivatization solution was prepared by mixing the AccQ•Tag Ultra (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate [AQC] reagent with 1 mL of the AccQ•Tag Ultra diluent and heating at 55 °C for 15 min. Then, 70 µL of borate buffer (pH 8.6), 5 µL of calibration standard or sample, and 5 µL of IS were transferred into a 1.5 mL Eppendorf vial. The sample was vortexed and then 20 µL of prepared derivatization solution was added. Further, the sample was heated at 55 °C for 10 min. The mechanism of the derivatization procedure of taurine is illustrated in Figure 1b. When the derivatization process was finished, the samples were centrifuged at 30,000 ×*g* for 10 min and then directly transferred to injection vials.

RESULTS AND DISCUSSION

UHPLC-MS method

The UHPLC-MS method, including sample preparation (derivatization) and analysis conditions, was applied based on our previously developed method for analysis of 20 proteinogenic amino acids (Piestansky et al., 2019). It was found that the selected method was fully implementable for

the analysis of nontypical amino acid taurine in soft drinks. The quantitative analytical approach was based on the IS method. The ideal IS in LC-MS approaches is an isotopically labeled version of the analyte (Hansen, 2015). The addition of an IS minimizes errors during the analysis, mainly the loss of analyte during sample preparation/treatment, and prevents the influence of matrix effects, which, in most cases, negatively affect the analysis. Due to the lack of isotopically labeled taurine in our laboratory, we decided to use isotopically labeled D5-L-glutamine as a suitable IS. This selection was made due to the very similar elution properties of both substances. Moreover, previous papers also indicated the use of isotopically labeled L-glutamine as an IS for taurine determination (Gamagedara et al., 2012).

During MS step optimization, it was necessary to identify appropriate *m/z* of taurine and IS derivative (D5-L-glutamine) ions for their unequivocal quantification in selected ion monitoring (SIM) regime of the single quadrupole mass spectrometer. The following *m/z* values of $[M+H]^+$ ions were identified and used in further experiments: 296.1 (taurine), 322.2 (D5-L-glutamine).

Validation

The adapted UHPLC-MS method for taurine determination was validated according to the International Conference on Harmonisation (ICH) Q2(R1) guideline (ICH 2005). Validation parameters such as specificity, linearity, accuracy, precision, detection limit (LOD), quantitation limit (LOQ), and robustness were investigated. All resulting validation and performance parameters are summarized in Tables 2–4. The calibration curves in the water matrix were calculated using TargetLynx XS software (Waters). As a result of the evaluation, a calibration curve was constructed based on the ratio of analyzed taurine to IS D5-L-glutamine. The coefficient of determination (r^2) of taurine was higher than 0.99. It reflects appropriate linearity in the selected concentration range (10–500 µmol/L). The predicted LOD and LOQ values calculated from the calibration lines were 4.29 and 10 µmol/L, respectively.

The precision and accuracy of the method were determined using a pooled sample prepared by mixing 1 mL of each energy drink. The pooled sample was diluted and spiked with an appropriate amount of taurine standard solution to obtain final spiked concentrations of 10, 100, and 500 µmol/L. The precision of the developed method was determined as intra- and inter-day precision (Table 3). The relative standard deviation (RSD) values were in the range of 0.1%–2.62% for the intra-day assay and 0.57%–3.95% for the inter-day assay. Accuracy of the method was measured as the percent of analyte recovered by the assay (expressed as mean bias from nominal concentration, %Nom, for the measurements of three spiked levels of taurine). The values determined during the accuracy test were in the range of 91.5%–108% (Table 4).

Table 2. Overview of several selected operation and validation parameters of the UHPLC-MS method.

	Taurine
t_R (min)	1.96
a (counts)	0.00642644
RSD_a (%), $n = 6$	9.7
b (counts × µmol^{-1} × L)	0.00801683
RSD_b (%), $n = 6$	4.8
r^2	0.9982
Linear range (µmol/L)	10–500
LOD (µmol/L)	4.29
LOQ (µmol/L)	10
N	93,340

Separation efficiency (N) was calculated according to the equation $N = 5.545*(t_R/w_{1/2})^2$, where t_R is the retention time and $w_{1/2}$ is the full width at half maximum of the peak. The calibration curve is expressed by the equation $y = b.x + a$. All calculations and results were obtained from the measurements of samples at LOQ concentration level (i.e., 10 µmol/L).

Table 3. Evaluation of accuracy parameters of taurine tested at three concentration levels (10, 100, and 500 µmol/L).

	Nominal (µmol/L)	**Found (µmol/L)**	**RSD (%)**	**RE (%)**
Intra-day, $n = 5$	10	10.92	2.62	9.2
	100	90.99	0.10	-9.0
	500	533.94	0.72	6.8
Inter-day, $n = 10$	10	10.95	3.95	9.5
	100	92.31	0.57	-7.7
	500	535.30	1.41	7.1

RE – relative error, RSD – relative standard deviation

Table 4. Recovery of taurine tested at three concentration levels (10, 100, and 500 µmol/L).

	Taurine		
Added (µmol/L)	10	100	500
Found (µmol/L)	10.80	91.50	532.70
RSD (%), $n = 5$	0.8	2.7	0.6
Recovery (%)	108.0	91.5	106.5

All observed results were within the acceptance criteria of the ICH guideline.

The small, but deliberate variation of the operational parameters (changes in the separation column temperature ±1°C and mobile phase A composition ±0.01%) in the robustness test resulted in standardized peak area changes not higher than 1.5% when compared to the results obtained

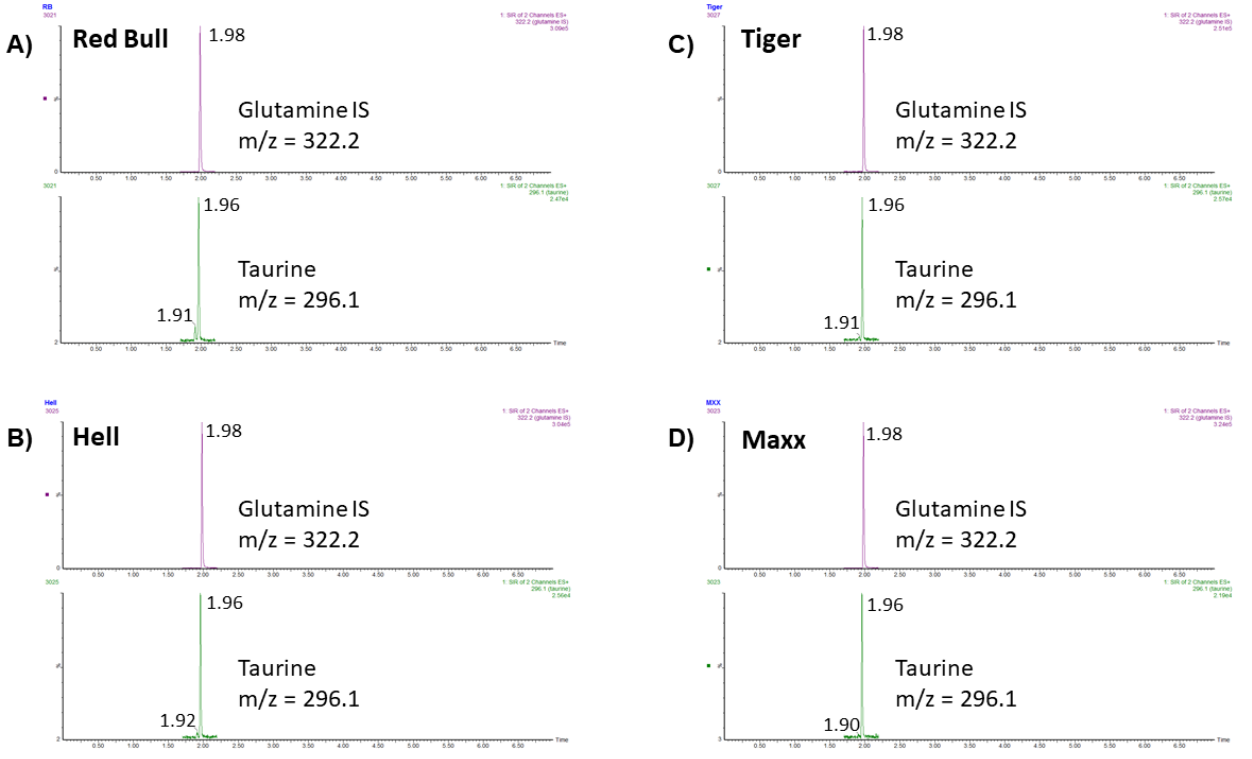

Figure 2. Illustrative chromatograms of taurine and the IS obtained from the analysis of four energy drinks: a) Red Bull, b) Hell, c) Tiger, d) Maxx. For separation and detection conditions, see Section 2.1.

under standard conditions. The presented results clearly show the applicability of the developed method in the field of quality control of commercial products with taurine content.

Application of the UHPLC-MS method for the analysis of taurine in energy drinks

Finally, the validated UHPLC-MS approach was applied to determine the concentration of taurine in four commercially available energy drinks – Red Bull, Hell, Tiger, and Maxx. The manufacturers declared a taurine concentration of 4 mg/mL in each investigated energy drink. . The overview of determined taurine concentrations in such beverages is presented in Table 5. In three samples, the content of taurine was in relatively good agreement with the declared content, but in one case, the content of taurine was only 70% of the declared value. One of the reasons why so low content of taurine was observed in the tested beverage could be degradation of taurine and formation of some degradation products. This hypothesis is supported by Figure 5a, where the presence of another peak with retention time at 1.91 min with the same *m/z* ratio was observed. However, a peak at the same retention position (1.91 min) was observed in all remaining samples (Figure 2b–d). The intensities of these peaks were significantly lower in comparison to those observed in case of Red Bull sample. Therefore, it can be stated that such a low content of taurine could be a result of unsuitable storage of the tested beverage.

Energy drinks can currently be sold in all EU Member States, although some national legislators have decided to take a more specific regulatory approach. In Canada, on energy drinks, the labels contain the maximum daily consumption and include warnings about mixing energy drinks with alcohol (BFR, 2008). The manufacturers of products in Australia and New Zealand have bypassed regulations by classifying them as "dietary supplements" to avoid the 80 mg/250 mL limit for caffeine (Oddy & O'Sullivan, 2009). In 2012, Hungary adopted a "public health tax" that applies to caffeinated energy drinks and other products. Tax for the drinks containing >100 mg of taurine per 100 mL is calculated at the rate of approximately 0.81 €/L (Zámbó et al., 2020). Due to the long-term and widespread consumption of beverages such as coffee and tea that contain caffeine and taurine naturally, energy drinks in developed countries remain largely unregulated (Buxton & Hagan, 2012; Reissig et al., 2009). May be the Hungarian tax or no regulations is the main reason why the manufacturers are not required to meet taurine-declared quantities in soft drinks. An example of the unregulated and uncontrolled amount of taurine in soft drinks can be found in a product sold on the Czech market, which contained 96.1% of the declared amount (Vochyánová et al., 2014), while the Slovak one contained only 70% (see Table 5).

Table 5. Determined concentration of taurine in four tested commercial available energy drinks.

Beverage	Parameters			
	Found ± SD (mg/mL)	RSD (%), $n = 3$	Declared (mg/mL)	% of declared content
Red Bull	2.80 ± 0.07	2.6	4	70.0
Hell	3.77 ± 0.05	1.4	4	94.5
Tiger	3.78 ± 0.01	0.1	4	91.8
Maxx	3.67 ± 0.03	0.9	4	94.3

CONCLUSIONS

In this work, we performed a simple and fast UHPLC-MS analysis of taurine in four commercial energy drinks. Data from the determination of taurine showed that the concentration levels for Red Bull brand were significantly lower (only 70%) than that declared by the brand manufacturer. For other brands, the taurine concentrations were in the range of 91.75%–94.50% that still did not meet the amount declared by the manufacturer.

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CONFLICT OF INTEREST STATEMENT

The authors do not have any conflict of interest concerning the present work.

References

[1] Ahmad W, Yusuf M, Ahmad A, Hassan YA, Amir M, Wahab S. Development and validation of ultra performance liquid chromatography (UPLC) method for the quantitative estimation of caffeine in non-alcoholic soft and energy drinks. J AOAC Int. 2022;105:1146–1152. https://doi.org/10.1093/jaoacint/qsac016

[2] Balshaw TG, Bampouras TM, Barry TJ, Sparks SA. (2013). The effect of acute taurine ingestion on 3-km running performance in trained middle-distance runners. Amino Acids. 2013;44:555–561. https://doi.org/10.1007/s00726-012-1372-1

[3] Battaglia A, Bertoluzza A, Calbucci F, Eusebi V, Giorgianni P, Ricci R, Tosi R, Tugnoli V. (1999). High-performance liquid chromatographic analysis of physiological amino acids in human brain tumors by pre-column derivatization with phenylisothiocyanate. J Chromatogr B. 1999;730:81–93. https://doi.org/10.1016/S0378-4347(99)00188-7

[4] Bundesinstitut für Risikobewertung: New Human Data on the Assessment of Energy Drinks. Federal Institute for Risk Assessment. (https://www.bfr.bund.de/cm/349/new_human_data_on_the_assessment_of_energy_drinks.pdf). 2008. Accessed May 10, 2023.

[5] Bongiovanni T, Genovesi F, Nemmer M, Carling C, Alberti G, Howatson G. Nutritional interventions for reducing the signs and symptoms of exercise-induced muscle damage and accelerate recovery in athletes: Current knowledge, practical application and future perspectives. Eur J Appl Physiol. 2020;120:1965–1996. https://doi.org/10.1007/s00421-020-04432-3

[6] Brosnan JT, Brosnan ME. The sulfur-containing amino acids: An overview. J Nutr 2006;136:1636S-1640S. https://doi.org/10.1093/jn/136.6.1636S

[7] Buxton C, Hagan JE. A survey of energy drinks consumption practices among student-athletes in Ghana: Lessons for developing health education intervention programmes. J Int Soc Sports Nutr. 2012;9:9. https://doi.org/10.1186/1550-2783-9-9

[8] Carvalho MBD, Brandao CFC, Fassini PG, Bianco TM, Batitucci G, Galan BSM, De Carvalho FG, Vieira TS, Ferriolli E, Marchini JS, Silva ASRD, De Freitas EC. Taurine supplementation increases post-exercise lipid oxidation at moderate intensity in fasted healthy males. Nutrients. 2020;12:1540. https://doi.org/10.3390/nu12051540

[9] Catharino RR, Haddad R, Godoy HT, Eberlin MN, Santos LS. (2011). Fast analysis of taurine in energetic drinks by electrospray ionization mass spectrometry. J Braz Chem Soc. 2011;22:801–806. https://doi.org/10.1590/S0103-50532011000400026

[10] Draganov BG, Pencheva IP, Todorova KA. (2014). UV-spectrophotometry determination of taurine in energy drink mixtures. International Journal of Nutrition and Food Sciences. 2014;3:123-126. https://doi.org/10.11648/j.ijnfs.20140302.26

[11] The European Food Safety Authority (EFSA). The use of taurine and D-glucurono-gamma-lactone as constituents of the so-called "energy" drinks. EFSA Journal. 2009;935:1-31. (https://doi.org/10.2903/j.efsa.2009.935). Accessed April 20, 2023

[12] El Agouza IM, Eissa SS, El Houseini MM, El-Nashar DE, Abd El Hameed OM. (2011). Taurine: A novel tumor marker for enhanced detection of breast cancer among female patients. Angiogenesis. 2011;14:321–330. https://doi.org/10.1007/s10456-011-9215-3

[13] Gamagedara S, Shi H, Ma Y. Quantitative determination of taurine and related biomarkers in urine by liquid chromatography–tandem mass spectrometry. Anal Bioanal Chem. 2012;402:763–770. https://doi.org/10.1007/s00216-011-5491-4

[14] Gunja N, Brown JA. (2012). Energy drinks: Health risks and toxicity. Med J Aust. 2012;196:46–49. https://doi.org/10.5694/mja11.10838

[15] Hansen SH. Quantitative and Qualitative Chromatographic Analysis. In: Hansen SH, Pedersen-Bjergaard S. (Eds.), Bioanalysis of Pharmaceuticals. Chichester: John Wiley & Sons; 2015 https://doi.org/10.1002/9781118716830.ch5

[16] Ibrahim MA, Eraqi MM, Alfaiz FA. Therapeutic role of taurine as antioxidant in reducing hypertension risks in rats. Heliyon. 2020;6:e03209. https://doi.org/10.1016/j.heliyon.2020.e03209

[17] International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Topic Q2 (R1): Validation of Analytical Procedures: Text and Methodology. (www.ich.org). 2005. Accessed April 28, 2023.

[18] Laidlaw S, Grosvenor M, Kopple J. (1990). The taurine content of common foodstuffs. J Parenter Enteral Nutr. 1990;14:183–188. https://doi.org/10.1177/0148607190014002183

[19] Lajin B, Goessler W. Sulfur speciation by HPLC-ICPQQQMS in complex human biological samples: Taurine and sulfate in human serum and urine. Anal Bioanal Chem. 2018;410:6787–6793. https://doi.org/10.1007/s00216-018-1251-z

[20] Lévy S, Santini L, Capucci A, Oto A, Santomauro M, Riganti C, Raviele A, Cappato R. European Cardiac Arrhythmia Society Statement on the cardiovascular events associated with the use or abuse of energy drinks. J Interv Card Electrophysiol. 2019;56:99–115. https://doi.org/10.1007/s10840-019-00610-2

[21] Manzi P, Pizzoferrato L. Taurine in milk and yoghurt marketed in Italy. Int J Food Sci Nutr. 2013;64:112–116. https://doi.org/10.3109/09637486.2012.704906

[22] Marchei E, Pellegrini M, Pacifici R, Palmi I, Pichini S. Development and validation of a high-performance liquid chromatography–mass spectrometry assay for methylxanthines and taurine in dietary supplements. J Pharm Biomed Anal. 2005;37:499–507. https://doi.org/10.1016/j.jpba.2004.11.013

[23] Murakami S. Role of taurine in the pathogenesis of obesity. Mol Nutr Food Res. 2015;59:1353–1363. https://doi.org/10.1002/mnfr.201500067

[24] Oddy WH, O'Sullivan TA. Energy drinks for children and adolescents. BMJ. 2009;339: b5268–b5268. https://doi.org/10.1136/bmj.b5268

[25] Omar MMA, Elbashir AA, Schmitz OJ. Capillary electrophoresis method with UV-detection for analysis of free amino acids concentrations in food. Food Chem. 2017;214:300–307. https://doi.org/10.1016/j.foodchem.2016.07.060

[26] Omer M, Omar M, Thiel A, Elbashir A. High performance liquid chromatographic methods for analysis of taurine in energy drinks after pre-column derivatization. Eurasian J Anal Chem. 2018;13:em40. https://doi.org/10.29333/ejac/93422

[27] Orth DL. HPLC determination of taurine in sports drinks. J Chem Educ. 2001;78:791. https://doi.org/10.1021/ed078p791

[28] Piestansky J, Galba J, Olesova D, Kovacech B, Kovac A. Determination of immunogenic proteins in biopharmaceuticals by UHPLC–MS amino acid analysis. BMC Chemistry. 2019;13:64. https://doi.org/10.1186/s13065-019-0581-z

[29] Qaradakhi T, Gadanec LK, McSweeney KR, Abraham JR, Apostolopoulos V, Zulli A. The Anti-inflammatory effect of taurine on cardiovascular disease. Nutrients. 2020;12:2847. https://doi.org/10.3390/nu12092847

[30] Rai KP, Rai HB, Dahal S, Chaudhary S, Shrestha S. Determination of Caffeine and Taurine Contents in Energy Drinks by HPLC-UV. JFSTN. 2016;9:66–73. https://doi.org/10.3126/jfstn.v9i0.16199

[31] Reissig CJ, Strain EC, Griffiths RR. Caffeinated energy drinks—A growing problem. Drug Alcohol Depend. 2009;99:1–10. https://doi.org/10.1016/j.drugalcdep.2008.08.001

[32] Ricciutelli M, Caprioli G, Cortese M, Lombardozzi A, Strano M, Vittori S, Sagratini G. Simultaneous determination of taurine, glucuronolactone and glucuronic acid in energy drinks by ultra high performance liquid chromatography–tandem mass spectrometry (triple quadrupole). J Chromatogr A. 2014;1364:303–307. https://doi.org/10.1016/j.chroma.2014.08.083

[33] Sawabe Y, Tagami T, Yamasaki, K. Determination of taurine in energy drinks by HPLC using a pre-column derivative. J Health Sci. 2008;54:661–664. https://doi.org/10.1248/jhs.54.661

[34] Seidel U, Huebbe P, Rimbach G. Taurine: A regulator of cellular redox homeostasis and skeletal muscle function. Mol Nutr Food Res. 2019;63:1800569. https://doi.org/10.1002/mnfr.201800569

[35] Smith A, Watson CJ, Frantz KJ, Eppler B, Kennedy RT, Peris J. Differential increase in taurine levels by low-dose ethanol in the dorsal and ventral striatum revealed by microdialysis with on-line capillary electrophoresis. Alcohol Clin Exp Res. 2004;28:1028–1038. https://doi.org/10.1097/01.ALC.0000131979.78003.34

[36] Surai PF, Earle-Payne K, Kidd MT. (2021). Taurine as a natural antioxidant: From direct antioxidant effects to protective action in various toxicological models. Antioxidants 2021;10:1876. https://doi.org/10.3390/antiox10121876

[37] Todorova KA. Analytical approaches and methods in quality control procedures of energy food drinks containing caffeine and taurine. International Journal of Nutrition and Food Sciences. 2015;4:1. https://doi.org/10.11648/j.ijnfs.s.2015040101.11

[38] Tu S, Zhang X, Wan H, Xia Y, Liu Z, Yang X, Wan, F. Effect of taurine on cell proliferation and apoptosis human lung cancer A549 cells. Oncol Lett. 2018;15:5473-5480. https://doi.org/10.3892/ol.2018.8036

[39] Vochyánová B, Opekar F, Tůma P. Simultaneous and rapid determination of caffeine and taurine in energy drinks by MEKC in a short capillary with dual contactless conductivity/photometry detection: CE and CEC. Electrophoresis. 2014;35:1660–1665. https://doi.org/10.1002/elps.201300480

[40] Waldron M, Patterson SD, Tallent J, Jeffries O. The effects of an oral taurine dose and supplementation period on endurance exercise performance in humans: A meta-analysis. Sports Med. 2018;48:1247–1253. https://doi.org/10.1007/s40279-018-0896-2

[41] Wen C, Li F, Zhang L, Duan Y, Guo Q, Wang W, He S, Li J, Yin Y. (2019). Taurine is involved in energy metabolism in muscles, adipose tissue, and the liver. Mol Nutr Food Res. 2019;63:1800536. https://doi.org/10.1002/mnfr.201800536

[42] Wu G. Important roles of dietary taurine, creatine, carnosine, anserine and 4-hydroxyproline in human nutrition and health.

Amino Acids. 2020;52:329–360. https://doi.org/10.1007/s00726-020-02823-6

[43] Yan H, Qiao F, Tian M, Row, KH. Application of 2,4-dinitrofluorobenzene pre-column derivatization to quantitative determination of taurine and its intermediate in beverages and milk samples. J Liq Chromatogr Relat Technol. 2012;36:35–43. https://doi.org/10.1080/10826076.2011.644050

[44] Zámbó L, Bakacs M, Illés É, Varga A, Sarkadi Nagy E, Zentai A, Feigl E, Biró K. (2020). Impact assessment of the public health product tax in Hungary. Eur J Public Health, 2020;30(Supplement_5):ckaa166.1231. https://doi.org/10.1093/eurpub/ckaa166.1231

[45] Zhang X, Tu S, Wang Y, Xu B, Wan F. Mechanism of taurine-induced apoptosis in human colon cancer cells. Acta Biochim Biophys Sin. 2014;46:261–272. https://doi.org/10.1093/abbs/gmu004

[46] Ziegler F, Le Boucher J, Coudray-Lucas C, Cynober L. Plasma amino-acid determinations by reversed-phase HPLC: Improvement of the orthophthalaldehyde method and comparison with ion exchange chromatography. J Anal Methods Chem. 1992;14:145–149. https://doi.org/10.1155/S1463924692000270