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

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Abstract

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 (r²) of 0.9998 and 0.9996 for HPLC-PDA 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.

Keywords

Taurine, OPA, Sodium sulfite, HPLC, Spectrophotometer, Energy drinks, Derivatization

Introduction

Energy drinks are carbonated beverages products intended to provide a boost to mental energy. They contain high levels of stimulant ingredients such as caffeine, taurine, guarana, B-complex vitamins and ginseng [1, 2]. Energy drinks is a segment of the market that has experienced high growth, which is forecast to continue. It has been reported that, excessive consumption of energy drinks can lead to potentially harmful effects [3]. Taurine (2-aminoethanesulfonic acid) is one of non-protein and non-essential amino acid which it can synthesize by human body [4, 5]. It is naturally present in the diet and its one of the active ingredient of energy drinks. Taurine (C₂H₇NO₃S) (molecular weight: 125.14 g/mol) is a sulfur-containing β-amino acid which it can dissolve in water. It is an organic weak acid with dissociation constant pKₐ = 4.96 which remains stable in acids and bases [5]. It has physiological effects and pharmacological actions such as a neurotransmitter, antioxidant, modulator of intracellular calcium levels, osmolyte,
profound effects. The authors argue that the use of OPA for derivatization is important due to its simplicity and accuracy in determining taurine amounts in energy drinks.

**Materials and Methods**

**Instrumentation**

**UV-Vis spectrophotometric analysis**

All ultraviolet-visible spectrophotometric measurements were carried out using a double beam UV-Vis spectrophotometer and HPLC with photodiode array detector (PDA). The instrument is equipped with a double beam V-530 spectrophotometer (JASCO, Japan) and a Shimadzu HPLC with PDA detector (Shimadzu Corporation, Kyoto, Japan). The instrument is provided with 1 cm quartz cells. The separation is performed at ambient temperature, and the wavelengths of PDA detector were set in range of 210-400 nm and the quantitative analysis was performed at 298 nm.

**High Performance Liquid Chromatography with Photodiode Array Detector (HPLC-PDA)**

A double beam UV-Vis spectrophotometer and HPLC were used to determine taurine in energy drinks. The chromatographic separation of taurine derivative was carried out on Shimadzu HPLC with PDA detector (Shimadzu Corporation). The instrument equipped with Prominence LC-20AD pump, DGU-20A3R degassing unit, Prominence SIL-20A autosampler, CBM-20A system controller and Prominence SPD-M20A photodiode array detector. An Inertsil ODS-3 (250 mm × 4.6 mm i.d., 5 μm) GL Sciences Inc., Japan) was used as analytical column. The separation was performed at ambient temperature. The mobile phase consisted of acetonitrile and 0.1% trichloroacetic acid (30:70, v/v) with flow rate of 0.8 mL/min. Aliquots of 20 μL of the samples and standards were injected into the chromatographic system using the auto-sampler. The wavelengths of PDA detector were set in range of 210-400 nm and the quantitative analysis was performed at 298 nm.

**Preparation of standard solutions and buffer**

Stock solution of taurine at concentration of 1000 mg L⁻¹ was prepared in distilled water and stored at 4°C. It was appropriately diluted with distilled water to prepare intermediate standard solution (50 mg L⁻¹).

The borate buffer (0.1 M) was prepared by dissolving 0.618 g borax acid and 0.584 g of sodium chloride in 70 mL of distilled water then pH adjusted to 9.5 with 1.0 M potassium hydroxide then the volume was brought up to 100 mL with distilled water.

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**Chemicals and reagents**

Taurine (purity ≥99%) and o-Phthalaldehyde (OPA) (purity 98%) were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium chloride (99.9%) was obtained from CDH (New Delhi, India). Potassium hydroxide pellets (85%) was from Lab Tech Chemical (India). Boric acid (85%) was purchased from VWR International (Leuven, Belgium). Methanol (99.8%) was supplied by Chem-lab NV (Belgium). Water was purified with Daihan Lab Tech (Kyon, Korea). A stock solution of taurine at concentration of 1000 mg L⁻¹ was prepared in distilled water and stored at 4°C. It was appropriately diluted with distilled water to prepare intermediate standard solution (50 mg L⁻¹).

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Preparation of derivatization reagent

Stock solution of OPA was prepared by dissolving 0.156 g of the reagent in 5.0 mL methanol then transferred to a 25 mL volumetric flask and the solution was made up to volume with methanol. This solution was stable for 3 days when kept in refrigerator in the dark.

Stock solution of sodium sulfite (Na₂SO₃) (0.25 M) was prepared by dissolving 1.575 g in distilled water in a 100 mL volumetric flask and made up to volume with distilled water. Derivatization reagent working solution was prepared by mixing 0.6 mL of OPA stock solution and 0.3 mL of sodium sulfite stock solution then completes the volume to 5.0 mL with borate buffer (0.1 M). The reagent was prepared daily and kept in amber bottle.

Samples

Five different commercial brands of energy drinks containing taurine namely red bull, tornado, krating daeng, bison and tiger were purchased from local market in Khartoum, Sudan. All the samples were kept in a refrigerator until the analysis has been done.

Optimized derivatization method

Aliquots of taurine intermediate standard solution (50 mg L⁻¹) over the volume ranges 0.10-1.6 mL and 0.10-2.0 mL were transferred to a set of 5.0 mL volumetric flasks and the volume were adjusted to 1.6 mL and 2.0 mL with distilled water for UV-Vis spectrophotometer and HPLC-PDA analysis respectively. After addition of 0.4 mL OPA-Na₂SO₃ intermediate solution, the reaction is allowed to proceed for 5.0 min in dark. For HPLC analysis, the pH of the mixtures was adjusted to 3.0 by addition 0.1 mL of HCl (0.5 M) to prevent the analytical column from damage. Afterwards, the solution is made up to volume with distilled water. Finally, the obtained derivative was analyzed by measuring its absorbance at 323 nm either using spectrophotometric determination against reagent blank or by chromatographic separation coupled with PDA detection.

Determination of taurine in energy drinks Samples

Approximately 50 mL of each energy drink sample was poured into a 100 mL beaker and degassed by sonicating for 30 min in a Bandelin Sonorex ultrasonic bath (Berlin, Germany). Then, the pH of each degassed sample was adjusted to 7.0 with potassium hydroxide (1.0 M). The samples were diluted with distilled water until the concentration of taurine was 40 mg L⁻¹ for all energy drink samples based on the labeled amount. Then 0.5 mL of the samples were subjected to derivatization as described in section 2.6.

Results and Discussion

Derivatization reaction and absorption spectra

The reaction between amino acids and OPA exists in presence of thiol compounds. The most common used thiols with OPA for taurine derivatization are 2-mercapto- propionic acid [19] and 2-mercapto-ethanol [20] which have unpleasant stench, toxic effects and produced unstable product [27]. Therefore, in this work odorless sodium sulfite was used instead of alkylthiol to react with OPA and taurine under experimental conditions and produce relatively stable N-alkyl 1-isoidonle sulfonate derivative figure 1. The overlapped absorption spectra of taurine in water, OPA in methanol and derivative taurine-OPA-sulfite was shown in figure 2. The taurine and OPA exhibit maximum absorption peak at 201 nm and 251 nm, respectively, while the derivatization product absorbed at 227 and 323 nm. In spite of the absorption of derivatization product is higher in 227 nm, the wavelength 323 nm was used for all spectrophotometric measurements to enhance the selectivity.

Optimization of derivatization conditions

Experiments were conducted to investigate the optimal reaction conditions using UV-Vis spectrophotometer. The main parameters affecting on the derivatization reaction between taurine and OPA-Na₂SO₃ such as pH, concentration of OPA and Na₂SO₃, dilution solvent, time of the reaction were studied.

Effect of pH

The derivatization reaction took place under basic conditions [22]. Therefore, the effect of pH on the absorbance of derivatization product was investigated in range of 8.0-11 using 0.1 M borate buffer. The results show that the absorbance of taurine derivative increases with increasing the pH of
Effect of OPA and Na$_2$SO$_3$ concentration on derivatization

The effect of OPA concentration was studied over the range (5-150) mg L$^{-1}$ in the final solution. It was found that, increasing the concentration of OPA increase the reaction yield up to an amount of 60 mg L$^{-1}$ and then leveled off. Therefore, a concentration of 60 mg L$^{-1}$ was considered optimum.

Also, the influence of Na$_2$SO$_3$ concentration was investigated over the range (50-353 mg L$^{-1}$). It was observed that, the response of taurine derivative increase with the rise of concentration of Na$_2$SO$_3$ solution and becomes maxima at concentration of 202 mg L$^{-1}$. Therefore, the concentration of 202 mg L$^{-1}$ was chosen to ensure the highest absorbance of product.

Effect of time on derivatization

By following the reaction over various periods of time (0.5–10 min), it was found that the reaction was completed in 5.0 min and then the response is slightly declined with prolonged reaction time. This may be due to instability of OPA derivatives at room temperature over a long time. So, the reaction time was set to 5.0 min for the further experiments.

Effect of diluting solvent

Different solvents, such as acetonitrile, ethanol, methanol, acetone and water were tested as diluting solvents for derivatization product. The results showed that water is the best solvent as the highest absorbance value was obtained.

According to optimization studies, the optimized conditions used for further studies were found as borate buffer pH 9.5, OPA concentration of 60 mg L$^{-1}$, concentration of Na$_2$SO$_3$ of 202 mg L$^{-1}$, reaction time of 5.0 min.

Validation of the method

The current method was validated by evaluating several parameters, such as linear range, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability) (RSD%) and accuracy (recovery).

Linearity and limit of detection and quantification

For the linearity measurement for the taurine-OPA/Na$_2$SO$_3$ derivative, six standard solutions containing taurine were prepared over the range from 0.5 to 16 mg L$^{-1}$ and 0.5 to 20 mg L$^{-1}$ for UV-Vis spectrophotometer and HPLC-PDA analysis, respectively. Then the calibration curves were constructed by plotting absorbance (spectrophotometer) or peak area (HPLC-PDA) against the concentration of taurine. Good linearities were obtained with correlation coefficients ($r^2$) of 0.9996, 0.9998 for UV-Vis spectrophotometer and HPLC-PDA respectively as shown in table 1.

The limit of detection (LOD) and limit of quantification (LOQ) of these methods were calculated from the calibration curve data. They were determined according to the following formula: LOD = 3.3×SDa/b, and LOQ = 10×SDa/b, where: SDa is the standard deviation of intercept, b is the slope [17]. LOD and LOQ were found to be 0.141, 0.109 mg L$^{-1}$ and 0.423, 0.328 mg L$^{-1}$ for UV-Vis spectrophotometer and HPLC-PDA analysis respectively, table 1.

Precision

The precision of these methods was estimated by intra-day repeatability and inter-day reproducibility. The intra-day repeatability was evaluated by analyzing six replicates of taurine standard derivative (8.0 mg L$^{-1}$) over one day. The inter-day reproducibility was determined by analyzing twelve replicates of the taurine standard derivative (0.8 mg L$^{-1}$) over three days. The precision is presented as the percentage relative standard deviation (RSD%).

Accuracy

The accuracy of these methods was determined by recovery test. Aliquots of 1.0 mL of diluted energy drink samples containing taurine at concentration level of 20 mg L$^{-1}$ were spiked with aliquots of 0.2, 0.5 and 1.0 mL taurine intermediate standard solution (50 mg L$^{-1}$). Then the derivatization for spiked samples was the same as described previously in section 2.6. The final concentrations of added amount of taurine standard were 2.0, 5.0 and 10 mg L$^{-1}$. Good percentage recoveries in range (93.3-105%) and (90.2-104%) were obtained for UV-Vis spectrophotometer and HPLC-PDA analysis respectively, table 3.

Applications of the methods

The proposed UV-Vis spectrophotometer and HPLC-
PDA methods were applied to determine taurine content in some energy drink samples available in local markets namely (red bull, tornado, krating daeng, bison and tiger). Figure 3 (A-B) shows the chromatograms of taurine standard derivative (8.0 mg L\(^{-1}\)) and derivatized tiger energy drink sample (4.0 mg L\(^{-1}\)) spiked with taurine at concentration of 2.0 mg L\(^{-1}\) determined by HPLC-PDA. As seen in figure 3, no interference peak from other ingredients was observed, which could be attributed to the selectivity of the method. As indicated in table 4, the obtained taurine concentrations of analyzed energy drinks were very close to the concentration values in the labels. The percentage was (94.75-102%) and (95.15-100.8%) that indicate the high accuracy of the two proposed methods UV-Vis spectrophotometer and HPLC-PDA respectively, for the determination of the studied analyte.

**Comparison of present work with other reported studies**

The sensitivity and chromatographic separation conditions for the current modified methods were compared with the previous published studies for determination of taurine after derivatization with OPA reagent. As indicated in table 5 most of used techniques for taurine determination are based on HPLC with fluorescence detection [19-21] which is not available in many laboratories. In present work we used universal instruments i.e. spectrophotometer and HPLC-PDA for detection taurine-OPA-sulfite derivative. In this study, simple mobile phase i.e. (0.1% trichloroacetic acid and acetonitrile) has been used for separation of taurine-OPA-Na\(_2\) SO\(_3\) derivative on HPLC system. Most of the other HPLC methods for taurine determination after derivatization used buffers as a constituent of mobile phase [19-21, 28]. The LODs and LOQs obtained by these current methods are comparable by that one obtained by Ferreira et al. and higher than that achieved with other analytical methods [19-21, 28]. The relatively higher LODs obtained by these methods is due to the use of UV-Vis and PDA as a detection technique while the other methods used the fluorescence detector. Because of the high concentrations of taurine in energy drink samples, the LODs of the introduced methods were satisfactory for the energy drink samples.

**Conclusion**

Rapid, simple and inexpensive UV-Vis spectrophotometric and HPLC-PDA methods has been optimized and validated for determination of taurine in energy drink after derivatization with OPA and sodium sulfite. In this study we found that using OPA and sodium sulfite as derivatization agents is
compatible with taurine analysis in energy drink samples. The proposed methods show good analytical figures of merits and have been applied successively for determination of taurine in some energy drinks available in local markets.

Acknowledgments

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References


### Table 5: Comparison of conditions, LODs and LOQs of present work with other analytical methods for taurine analysis after derivatization with OPA reagent published in literature.

<table>
<thead>
<tr>
<th>Analytical Method</th>
<th>Mobile phase</th>
<th>Column</th>
<th>Derivatization agent</th>
<th>Detection method</th>
<th>LOD &amp; LOQ (mg L⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-Vis spectrophotometer</td>
<td>-</td>
<td>-</td>
<td>Na₂SO₃</td>
<td>UV-Vis</td>
<td>LOD: 0.141, LOQ: 0.423</td>
<td>Present study</td>
</tr>
<tr>
<td>HPLC-PDA</td>
<td>Acetonitrile : 0.1% trichloroacetic acid (30:70) %</td>
<td>Inertial ODS-3 (250 × 4.6 mm i.d., 5 μm)</td>
<td>Na₂SO₃</td>
<td>PDA</td>
<td>LOD: 0.109, LOQ: 0.328</td>
<td>Present study</td>
</tr>
<tr>
<td>HPLC-FLD</td>
<td>Methanol : Acetonitrile : Phosphate buffer 0.02M, pH 7.5 (8:0.17:5.74:5) %</td>
<td>Agilent C18 (150 × 4.6 mm, 5 μm)</td>
<td>3-mercaptopropionic acid</td>
<td>Fluorescence</td>
<td>LOD: 0.03</td>
<td>[21]</td>
</tr>
<tr>
<td>HPLC-FLD</td>
<td>Phosphate buffer (0.02 M, pH 4.8): Acetonitrile (65:35) %</td>
<td>Fortis (250 × 4.6 mm, 5 μm)</td>
<td>2-mercaptopropanethanol</td>
<td>Fluorescence</td>
<td>LOD: 0.001, LOQ: 0.005</td>
<td>[20]</td>
</tr>
<tr>
<td>HPLC-FLD</td>
<td>Disodium hydrogen phosphate (0.0125 M, pH = 7.2): Acetonitrile (94:6) %</td>
<td>Genesis C18 (150 mm, 4 μ)</td>
<td>3-mercaptopropionic acid</td>
<td>Fluorescence</td>
<td>LOD: 0.4 pg mL⁻¹, LOQ: 12 pg mL⁻¹</td>
<td>[19]</td>
</tr>
<tr>
<td>HPLC-UV/Vis</td>
<td>Phosphate buffer (0.05 M, pH: 5.3): Methanol (60:40) %</td>
<td>C18 (S10 ODS)</td>
<td>2-mercaptopropanethanol</td>
<td>UV-Vis</td>
<td>LOD: 0.3</td>
<td>[28]</td>
</tr>
</tbody>
</table>

Figure 3: Absorption spectrum of (A) taurine (8.0 mg mL⁻¹) against water. (B) Absorption spectrum of OPA (50 mg mL⁻¹) against methanol. (C1, 2). Absorption spectrum of reaction product of taurine with OPA-Sulfite against reagent blank. Conditions: taurine (8.0 mg mL⁻¹), OPA (60 mg L⁻¹), Na₂SO₃ (202 mg L⁻¹), pH 9.5 and reaction time 5.0 min.