

# 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<sup>-1</sup>, sodium sulfite concentration of 202 mg L<sup>-1</sup> 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<sup>-1</sup> and 0.5-15 mg L<sup>-1</sup> with correlation coefficient (*r*<sup>2</sup>) of 0.9998 and 0.9996 for HPLC-PDA and spectrophotometer respectively. The LODs were 0.109 mg L<sup>-1</sup> and 0.141 mg L<sup>-1</sup> 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 (C<sub>2</sub>H<sub>7</sub>NO<sub>3</sub>S) (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 pK<sub>a</sub> = 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,

protect the liver and benefit the gallbladder [5]. The various physiological functions of taurine are explained with amino terminal group in the structure and sulfonic acid group moiety [6]. 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 [7, 4]. The mean daily intake of taurine from diet was estimated to vary between 40 to 400 mg [8]. Some energy drinks contain high level of synthetic taurine up to 4000 mg L<sup>-1</sup> [3], 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 [9]. 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 [10, 4]. For these reasons it has been banned in some Scandinavian countries [10]. 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), evaporative light scattering detector (ELSD) and mass spectrometer detector (MS). To a lesser extent, microchip capillary electrophoresis (MCE) [11], amino acid analyzer, Fourier Transform infrared (FTIR) [1], spectrofluorimetric method [12] and nuclear magnetic resonance spectroscopy (H1-NMR) [13]. Since taurine has no chromophore, a derivatization step is important to permit its detectability using optical detection [1, 5]. Many reagents have been used for taurine derivatization such as 2,4-dinitrofluorobenzene (DNFB) [2], 2-[2-(dibenzocarbazol)-ethoxy]ethyl chloro-formate (DBCEC) [14], ninhydrine [15], Hantzsch reagent, tetra-cyanoethylene [12], 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride (DMS-Cl) [15], phenol and sodium hypochlorite [16], 4-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) [17], fluorescamine [18], and *O*-phthalaldehyde (OPA) [19-21].

OPA is common cheap reagent that is used for derivatization of free amino acids including taurine followed by HPLC analysis [19, 21]. OPA reacts rapidly with primary amine and amino acids in presence of reducing agent (alkylthiol or sulfite group) to form isoindole ring [21-23]. The compound 1-alkylthio-N-alkylisoindoles which produce from the reaction of amino acids and OPA-alkylthiol reagent is unstable due to further reaction with excess OPA in the derivatization matrix. Sodium sulfite is used in combination with OPA as a derivatization agent in order to improve amino acid derivatives stability [24]. Hence in this study, we used OPA and sodium sulfite for taurine derivatization. HPLC with fluorescence and electrochemical detection (ED) are the most frequent methods for determination of taurine after pre-column derivatization with OPA [19-21, 25]; but these detectors are not available in many laboratories. Although the OPA fluorometric method has low to nanomolar's detection limit [26], however, the concentration of taurine is high in

energy drinks and thus fast analysis and cheap method is critical issue to consider. Due to availability of spectrophotometer and HPLC with photodiode array detector (PDA), this study aimed to optimize and validate spectrophotometric and HPLC-PDA methods for determination of taurine in energy drinks after derivatization with OPA-sodium sulfite reagent.

## Materials and Methods

### Instrumentation

#### UV-Vis spectrophotometric analysis

All ultraviolet-visible spectrophotometric measurements were carried out using a double beam V-530 (JASCO, Japan), the instrument is provided with 1 cm quartz cells.

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

The chromatographic separation of taurine derivative was carried out on Shimadzu HPLC with PDA detector (Shimadzu Corporation, Kyoto, Japan). 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<sup>-1</sup>. 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.

### 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 (Kyonggi, Korea). Acetonitrile HPLC grade (99.9%) was purchased from Duksan (Korea). Trichloroacetic acid (80%) was from Scharlau (European Union). Hydrochloric acid (37%) was purchased from Sham Laboratory (Addra, Syria). Sodium sulfite (99%) was from BDH chemicals (United Kingdom).

### Preparation of standard solutions and buffer

Stock solution of taurine at concentration of 1000 mg L<sup>-1</sup> 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<sup>-1</sup>).

The borate buffer (0.1 M) was prepared by dissolving 0.618 g boric 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.

### 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 ( $\text{Na}_2\text{SO}_3$ ) (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 ( $50 \text{ mg L}^{-1}$ ) 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- $\text{Na}_2\text{SO}_3$  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  $40 \text{ mg L}^{-1}$  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.

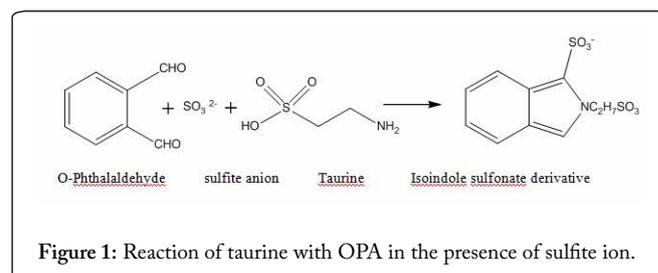


Figure 1: Reaction of taurine with OPA in the presence of sulfite ion.

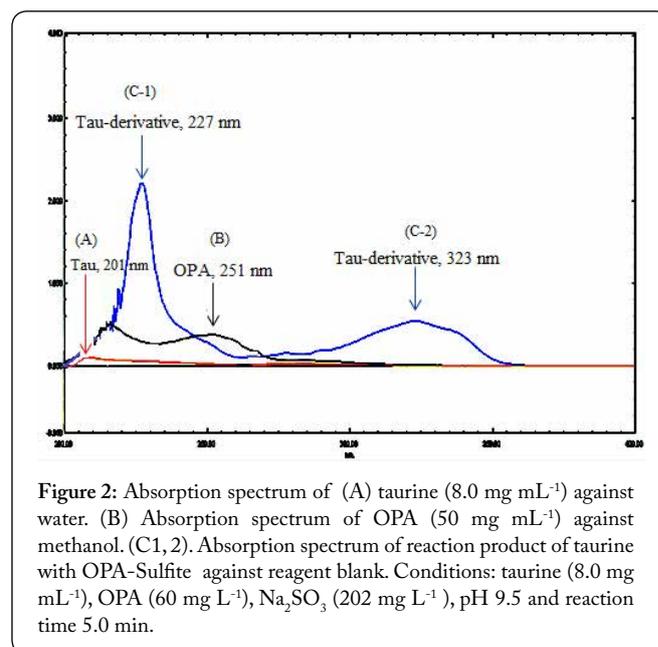


Figure 2: Absorption spectrum of (A) taurine ( $8.0 \text{ mg mL}^{-1}$ ) against water. (B) Absorption spectrum of OPA ( $50 \text{ mg mL}^{-1}$ ) against methanol. (C1, 2). Absorption spectrum of reaction product of taurine with OPA-Sulfite against reagent blank. Conditions: taurine ( $8.0 \text{ mg mL}^{-1}$ ), OPA ( $60 \text{ mg L}^{-1}$ ),  $\text{Na}_2\text{SO}_3$  ( $202 \text{ mg L}^{-1}$ ), pH 9.5 and reaction time 5.0 min.

### 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- $\text{Na}_2\text{SO}_3$  such as pH, concentration of OPA and  $\text{Na}_2\text{SO}_3$ , 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

borate buffer up to pH 9.5 and then decrease. This result is in agreement with that reported by Klongnganchui et. al., for determination of taurine after pre-column derivatization with OPA. Thus, pH of 9.5 was selected for derivatization reaction. However, most of the published works for derivatization of amino acids with OPA has been done with buffer at pH 10 or 10.4 [19, 21, 25].

#### Effect of OPA and Na<sub>2</sub>SO<sub>3</sub> concentration on derivatization

The effect of OPA concentration was studied over the range (5-150) mg L<sup>-1</sup> 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<sup>-1</sup> and then leveled off. Therefore, a concentration of 60 mg L<sup>-1</sup> was considered optimum.

Also, the influence of Na<sub>2</sub>SO<sub>3</sub> concentration was investigated over the range (50-353 mg L<sup>-1</sup>). It was observed that, the response of taurine derivative increase with the rise of concentration of Na<sub>2</sub>SO<sub>3</sub> solution and becomes maxima at concentration of 202 mg L<sup>-1</sup>. Therefore, the concentration of 202 mg L<sup>-1</sup> 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<sup>-1</sup>, concentration of Na<sub>2</sub>SO<sub>3</sub> of 202 mg L<sup>-1</sup>, 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<sub>2</sub>SO<sub>3</sub> derivative, six standard solutions containing taurine were prepared over the range from 0.5 to 16 mg L<sup>-1</sup> and 0.5 to 20 mg L<sup>-1</sup> 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<sup>2</sup>) 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 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<sup>-1</sup> and 0.423, 0.328 mg L<sup>-1</sup> for UV-Vis spectrophotometer and HPLC-PDA analysis respectively, table 1.

**Table 1:** Quantitative parameters for determination taurine- OPA/sulfite derivative using UV-Vis spectrophotometer and HPLC-PDA.

Parameter	Spectrophotometer	HPLC-PDA
Concentration range (mg L <sup>-1</sup> )	0.5-15	0.5-20
Equation	Y= 0.073x - 0.0004	Y= 38967x + 349.3
Regression coefficient (r <sup>2</sup> )	0.9996	0.9998
LOD (mg L <sup>-1</sup> )	0.141	0.109
LOQ (mg L <sup>-1</sup> )	0.423	0.328

#### 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<sup>-1</sup>) over one day. The inter-day reproducibility was determined by analyzing twelve replicates of the taurine standard derivative (0.8 mg L<sup>-1</sup>) over three days. The precision is presented as the percentage relative standard deviation (RSD%). The intra-day precision for UV-Vis spectrophotometer and HPLC-PDA were 1.278% and 1.816%, respectively. The inter-day precision for UV-Vis spectrophotometer and HPLC-PDA were 2.236% and 2.858%, respectively, which indicate that the proposed methods were adequately precise, table 2.

**Table 2:** Intra-day and inter-day precisions for the determination of taurine-OPA-sulfite derivative with UV-Vis spectrophotometer and HPLC-PDA.

Parameter	Spectrophotometer	HPLC-PDA
Intra-day precision (RSD%) (n = 6)	1.278	1.816
Inter-day precision (RSD%) (n = 12)	2.236	2.858

#### 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<sup>-1</sup> were spiked with aliquots of 0.2, 0.5 and 1.0 mL taurine intermediate standard solution (50 mg L<sup>-1</sup>). 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<sup>-1</sup>. 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-

**Table 3:** Percentage recovery (n = 3) for determination of taurine in energy drink samples.

Sample	UV-Vis spectrophotometer				HPLC-PDA			
	Sample content (mg L <sup>-1</sup> )	Added amount (mg L <sup>-1</sup> )	Found (mg L <sup>-1</sup> )	Recovery% ± SD (n = 3)	Sample content (µg L <sup>-1</sup> )	Added amount (µg L <sup>-1</sup> )	Found (µg L <sup>-1</sup> )	Recovery% ± SD (n = 3)
Tornado	4.0	2	5.972	98.6 ± 0.02	4.0	2.0	5.760	96.0 ± 0.05
	4.0	5	9.072	101 ± 0.01	4.0	5.0	8.527	90.5 ± 0.01
	4.0	10	13.94	99.4 ± 0.02	4.0	10.0	13.621	96.2 ± 0.16
Krating-daeng	4.0	2	6.099	105 ± 0.01	4.0	2.0	5.840	92.0 ± 0.03
	4.0	5	9.116	102 ± 0.01	4.0	5.0	8.783	95.6 ± 0.09
	4.0	10	13.99	99.9 ± 0.06	4.0	10.0	13.882	98.2 ± 0.12
Bison	4.0	2	6.130	106 ± 0.05	4.0	2.0	5.804	90.2 ± 0.07
	4.0	5	8.970	99.4 ± 0.12	4.0	5.0	9.197	104 ± 0.10
	4.0	10	13.33	93.3 ± 0.14	4.0	10.0	13.525	96.6 ± 0.18
Tiger	4.0	2	5.994	99.7 ± 0.11	4.0	2.0	5.841	95.2 ± 0.04
	4.0	5	9.143	103 ± 0.08	4.0	5.0	8.866	97.3 ± 0.07
	4.0	10	13.84	98.4 ± 0.11	4.0	10.0	13.932	99.3 ± 0.17
Red Bull	4.0	2	5.950	97.5 ± 0.08	4.0	2.0	6.086	104 ± 0.06
	4.0	5	8.850	97.0 ± 0.16	4.0	5.0	8.976	99.5 ± 0.09
	4.0	10	13.43	94.3 ± 0.12	4.0	10.0	13.415	94.1 ± 0.16

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<sup>-1</sup>) and derivatized tiger energy drink sample (4.0 mg L<sup>-1</sup>) spiked with taurine at concentration of 2.0 mg L<sup>-1</sup> 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<sub>2</sub>SO<sub>3</sub> 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

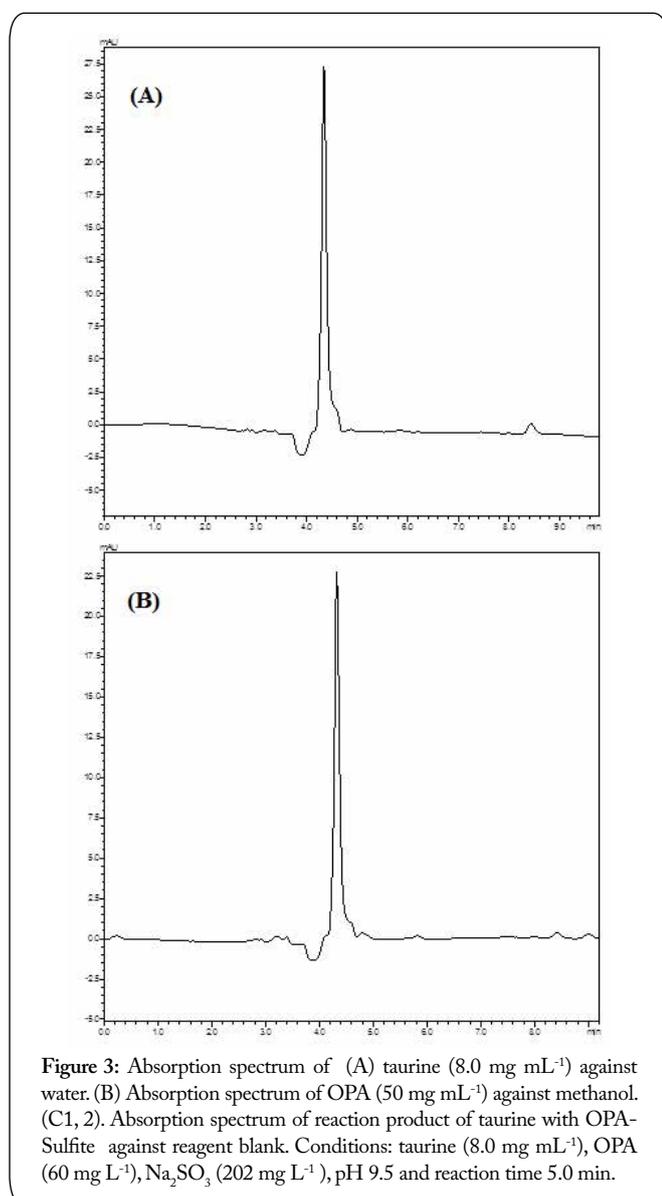
**Table 4:** Comparison between measured taurine contents in this study and labeled amount of taurine in energy drink samples.

Sample	Labeled amount in (mg L <sup>-1</sup> )	Spectrophotometer		HPLC-PDA	
		Conc. mg L <sup>-1</sup> ± SD <sup>a</sup>	Recovery (%)	Conc. mg L <sup>-1</sup> ± SD	Recovery (%)
Tornado	100	102 ± 1.88	102.0	98.36 ± 1.50	98.36
Krating-daeng	4000	4022 ± 12.02	100.5	4033 ± 13.43	100.8
Bison	4000	3790 ± 10.60	94.75	3806 ± 8.48	95.15
Tiger	3200	3181 ± 11.88	99.41	3172 ± 12.44	99.12
Red Bull	4000	3933 ± 12.73	98.33	3937 ± 9.19	98.43

<sup>a</sup> n=3

**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.

Analytical Method	Mobile phase	Column	Derivatization agent	Detection method	LOD & LOQ (mg L <sup>-1</sup> )	Reference
UV-Vis spectrophotometer	-	-	Na <sub>2</sub> SO <sub>3</sub>	UV-Vis	LOD: 0.141 LOQ: 0.423	Present study
HPLC-PDA	Acetonitrile : 0.1% trichloroacetic acid (30:70) %	Inertsil ODS-3 (250 × 4.6 mm i.d., 5 μm)	Na <sub>2</sub> SO <sub>3</sub>	PDA	LOD: 0.109 LOQ: 0.328	Present study
HPLC-FLD	Methanol : Acetonitrile : Phosphate buffer 0.02M, pH 7.5) (8.0:17.5:74.5) %	Agilent C18 (150 × 4.6 mm, 5 μm)	3-mercaptopropionic acid	Fluorescence	LOD: 0.03	[21]
HPLC-FLD	Phosphate buffer ( 0.02 M, pH 4.8): Acetonitrile (65:35) %	Fortis (250 × 4.6 mm, 5 μm)	2-mercaptoethanol	Fluorescence	LOD: 0.001 LOQ: 0.005	[20]
HPLC-FLD	Disodium hydrogen phosphate (0.0125 M, pH = 7.2) : Acetonitrile (94:6) %	Genesis C18 (150 mm, 4 μ)	3-mercaptopropionic acid	Fluorescence	LOD: 0.4 pg mL <sup>-1</sup> LOQ: 1.2 pg mL <sup>-1</sup>	[19]
HPLC-UV/Vis	Phosphate buffer (0.05 M, pH: 5.3) : Methanol (60:40) %	C18 (S10 ODS <sub>2</sub> )	2-mercaptoethanol	UV-Vis	LOD: 0.3	[28]



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.

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