Determination of taurine in energy drinks by high-performance liquid chromatography

Brad McConn
Department of Chemistry, Concordia College, 901 8th St S, Moorhead, MN 56562

Abstract

The concentration of taurine in two energy drinks, Monster Lo-Carb and 5-Hour Energy, was found by using high-performance liquid chromatography. The popularity of energy drinks has been on the rise since 2006, and it is important to have an efficient way to determine the concentrations of ingredients in the drinks. The experimental results from this procedure agree well with the amounts of taurine listed in the Nutrition Facts on the bottles of the drinks. This, along with the linear curve produced, supports the conclusion that this procedure provides a successful method of detecting and determining the concentration of taurine in energy drinks.

Introduction

The sales and consumption of energy drinks has been on the rise in America since 2006, and their popularity among college students continues to grow. Their appeal is in the fast-acting energy boost it gives to the consumer from ingredients including caffeine and carbohydrates, as well as other energy boosting supplements like taurine, tyrosine, citicoline and guarana among others. While there is no doubt that these drinks do give an individual a significant burst of energy, the effects these beverages have on the body are not well known.

As mentioned above, taurine is one of the main energy boosting ingredients in many energy drinks. Taurine is an amino acid that can be synthesized or ingested by humans. The average daily intake of taurine varies greatly but is generally between 40 and 400 mg, while the body contains a total of about 70 g in a 70 kg person. It is known to be important in neurological development, and to that effect has been included in baby formulas since the 1980’s. For the average adult, intake of up to 3000 mg/day is considered safe.

Taurine has also been linked to increased athletic performance, although this finding remains controversial. A study done in 2003 concluded that taurine supplementation increased VO(2) max and exercise time to exhaustion, and for this reason it is attractive as an ingredient in energy drinks. However many studies have pointed out the need for more research in this area and question the effects that the small amounts found in energy drinks would have.

The most common methods for the detection of taurine include high performance liquid chromatography (HPLC) and ultraviolet visible absorption spectrometry (UV-Vis). The most common method, and the one that this study utilized, is HPLC, as it is relatively low cost and requires a low sample volume. The two drinks examined were Monster Lo-Carb and 5-hour ENERGY. As taurine does not absorb UV/Vis radiation in significant amounts, a derivatization reaction is needed. In this study Sanger's reagent, 2,4-dinitrofluorobenzene (DNFB), will be utilized. DNFG reacts with the amino groups of
amino acids in basic solution to form a dinitrophenyl derivative, which has a maximum absorption of around 360 nm. For this reaction to occur, the pH must be high enough that the amino group is not protonated, but low enough to ensure that the DNFB does not react directly with hydroxide to form 2,4-dinitrophenol. A pH of 9 is suitable for the reaction with taurine.

**Experimental**

**Preparation of Carbonate Buffer**

A 10 mM pH 9 carbonate buffer solution was made for use in the derivitization reaction by dissolving 0.2081 g of NaHCO₃ (Sigma-Aldrich) and 0.0138 g of Na₂CO₃ (Sigma-Aldrich) in 250 mL of deionized (DI) water and then adding hydrochloric acid drop-wise until a pH of 9 was reached. The remaining solution was then diluted with DI water to a final volume of 250 mL.

**Preparation of Phosphate Buffer for Mobile Phase**

A 10 mM pH 6 phosphate buffer solution was made for the HPLC mobile phase by dissolving 1.298-g of NaH₂PO₄·H₂O (Fisher) and 0.1584-g of Na₂HPO₄·7H₂O (Fisher) in DI water and then adding NaOH until a pH of 6 was reached. The solution was then diluted with DI water to a final volume of 2 L and filtered and degassed in preparation for the HPLC.

**Preparation of Standard Solutions**

Standard taurine solutions were made as close to 10, 20 and 50 ppm as possible. A solid taurine sample (Sigma-Aldrich) of 0.2519 g was dissolved in DI water in a 500-mL volumetric flask and diluted to the mark. Three 50-mL volumetric flasks were obtained and 1.0, 2.0 and 5.0 mL of the previous solution were added to each, respectively. The flasks were then diluted to volume with DI water.

**Sample Preparation**

Approximately 25 mL of the energy drink Monster Lo-carb was poured into a 125-mL Erlenmeyer flask and degassed by sonicking for ten minutes. A dilution of 1:125 was done using DI water. For 5-hour ENERGY, a dilution of 1:200 was performed using DI water.

**Derivitization Reaction**

The derivitization procedure was the same for the standards and the samples. Into a test tube, 1.0 mL of sample, 2.0 mL of the carbonate buffer, 0.5 mL of methyl sulfoxide (DMSO) and 0.1 mL of 2,4-dinitrofluorobenzene (DNFB, Acros) were pipeted. The solution was shaken for 30 s and placed in a 40°C water bath for 15 min. At the end of the 15 min, 6.5 mL of the phosphate buffer was added to the mixture.
Instrument Preparation

For the analysis of the samples, a Varian ProStar HPLC system with Model 410 autosampler and reversed phase column (Phenomenex Kinetex XB-C18 100 Å) with a 2.1-μm particle size and column dimensions of 4.6 mm x 50 mm was used. The instrument was operated at a flow rate of 1.0 mL/min with a sample injection volume of 10 μL. An isocratic elution of 80:20 (v/v) of the phosphate buffer/acetonitrile was used. The detection wavelength was 360 nm, the peak absorbance for the taurine derivative.

Results and discussion

HPLC Data

Chromatograms for all standard and sample solutions were obtained using the HPLC. Figure 1 shows a chromatogram of a 50 ppm standard. The first two peaks seen in Fig. 1 at 0.62 and 0.74 min are solvent in the solution and the tallest one, at 1.38 min is unreacted DNFB. The next one is the taurine derivative, which has a retention time of ~1.66 min, and the last one, at 7.74 min, is 2,4-dinitrophenol. This is due to a reaction of the DNFB with hydroxide. It does not affect the results and can be ignored.

Figure 1. Chromatogram of the 50 ppm standard at 360 nm.

Figure 2 shows a chromatogram of a Monster-Lo carb sample. In each case, the peak area for the taurine derivative was within the range covered by the standards. The tallest peak, unreacted DNFB, had a retention time of 1.38 min, while the taurine derivative had a retention time of 1.66 min.
Figure 2. A chromatogram of a Monster-Lo carb sample at 360 nm.

*Calibration Curve*

A calibration curve was generated using Excel from four separate runs of the standards. The peak area for each run was plotted against concentration and a linear curve was fit using Excel. This is summarized in Table 1 and Fig. 3.

**Table 1.** A results summary for each of the three standards.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc (ppm)</th>
<th>Run 1 Area (mAU·s)</th>
<th>Run 2 Area (mAU·s)</th>
<th>Run 3 Area (mAU·s)</th>
<th>Run 4 Area (mAU·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1</td>
<td>9.968</td>
<td>54.5</td>
<td>54.3</td>
<td>51.3</td>
<td>52.4</td>
</tr>
<tr>
<td>Standard 2</td>
<td>19.94</td>
<td>113.4</td>
<td>114.1</td>
<td>125.7</td>
<td>125.9</td>
</tr>
<tr>
<td>Standard 3</td>
<td>49.84</td>
<td>307.0</td>
<td>306.9</td>
<td>339.9</td>
<td>339.1</td>
</tr>
</tbody>
</table>

**Figure 3.** The calibration curve generated by the results above.
Sample Data

The results for each sample, Monster Lo-carb and 5-hour ENERGY, are summarized below in Tables 2 and 3, respectively. Two samples of each energy drink were run twice, with the sample being designated by 1 or 2 and the run designated by a or b. For Monster Lo-carb, the retention time for sample 1b deviated greatly from the others. The reason for this is unknown, and the asterisk designates that the sample was thrown out of the final results. The average was found by finding the average of each run and then averaging the two samples. For example, for 5-hour ENERGY, samples 1a and 1b were averaged first, then 2a and 2b. Then those two values were used to find the final result.

Table 2. A summary of the HPLC data for Monster Lo-carb energy.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area (mAU·s)</th>
<th>Retention Time (min)</th>
<th>Conc diluted (ppm)</th>
<th>Conc undiluted (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>218.6</td>
<td>1.66</td>
<td>34.252</td>
<td>4281</td>
</tr>
<tr>
<td>1b*</td>
<td>239.2</td>
<td>2.09</td>
<td>37.47</td>
<td>4684*</td>
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<tr>
<td>2a</td>
<td>206.1</td>
<td>1.65</td>
<td>32.29</td>
<td>4036</td>
</tr>
<tr>
<td>2b</td>
<td>205.2</td>
<td>1.66</td>
<td>32.15</td>
<td>4018</td>
</tr>
</tbody>
</table>

*designates that the point was thrown out

Average 4154
Std Dev 180
% RSD 4.3%

Table 3. A summary of the HPLC data for 5-hour ENERGY.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area (mAU·s)</th>
<th>Retention Time (min)</th>
<th>Conc diluted (ppm)</th>
<th>Conc undiluted (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>270.7</td>
<td>1.64</td>
<td>42.41</td>
<td>8482</td>
</tr>
<tr>
<td>1b</td>
<td>269.4</td>
<td>1.64</td>
<td>42.21</td>
<td>8441</td>
</tr>
<tr>
<td>2a</td>
<td>267</td>
<td>1.67</td>
<td>41.83</td>
<td>8366</td>
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<tr>
<td>2b</td>
<td>267.8</td>
<td>1.66</td>
<td>41.95</td>
<td>8391</td>
</tr>
</tbody>
</table>

Average 8420
Std Dev 59
% RSD 0.70%

HPLC Data Analysis

The literature value for taurine in Monster Lo-carb is 1000mg/serving. The calculated value came out to be 997 mg/serving, giving an error of 0.3%. This was after the point with the larger retention time was thrown out. For 5-hour ENERGY, the literature value is unknown. The drink simply says that it contains 1870 mg of “Energy Blend” per serving, and a review of scientific literature found no reported value. The calculated value came out to be 479.9 mg of taurine per serving. As the calibration curve generated by this method was linear and the data for Monster Lo-carb was very close, the value of 479.9 mg will be assumed to be fairly close to the actual amount of taurine in 5-hour ENERGY.
Conclusions

In this study, the concentration of taurine in Monster Lo-carb and 5-hour ENERGY was determined using high-performance liquid chromatography. An isocratic-elution method was used to create a calibration plot and the peak areas for Monster Lo-carb and 5-hour ENERGY were then used to calculate the concentration of taurine in each. The calculated taurine value was within 0.3% of the literature value, while the literature value of 5-hour ENERGY is unknown. This small error, along with the linearity of the calibration plot and reproducibility of the data suggest that HPLC was a successful method for determining the concentration of taurine in energy drinks.

Acknowledgments

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References

1) Orth, Dale L. Journal of Chemical Education. HPLC Determination of Taurine in Sports Drinks. 2001, 78 (6), 791