HPLC Determination of Taurine in Sports Drinks W

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Background

Taurine (2-aminoethanesulfonic acid) is a conditionally essential amino acid. While it can be synthesized by healthy adults from methionine and cysteine 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 (*1*). One effect of taurine deficiency is retinal dysfunction (*2*).

Because taurine is not incorporated into proteins, it is less familiar to many scientists. A brief survey of general biology textbooks does not find mention of it, and a brief survey of biochemistry textbooks highlights only taurine's ability to conjugate cholic acid to form the bile salt, as glycine does. Still, it is one of the most prevalent free amino acids in the body, concentrated in the heart, central nervous system, retina, skeletal muscle, and liver (where it conjugates bile acids to form bile salts). Concentrations in blood plasma average around 60 μM; the concentration in enhanced areas is in the 2–40-mM range (*2, 3*). While its role in the body is not fully understood, it has been studied extensively and a group of international researchers has held a taurine symposium approximately every two years since 1975. An excellent review of the subject was written by Huxtable (*4*).

Physiological roles of taurine include brain development (*5*) and the development of eyesight (*2*); taurine is also used in the treatment of congestive heart failure (*6*) and epilepsy (*3*). It is a suspected neurotransmitter (*7*). It is present in human milk, but not cow milk (except early in lactation), and has been added to infant formula since the mid-1980s, partly as a result of work by Hayes in which kittens fed a taurine-free diet did not develop sight (*7*).

Taurine was brought to my attention by students interested in its presence in some sports drinks, which were more expensive than others (approximately $2.00 for 8 fl. oz). Labels and Web sites claim that taurine helps "in the production of energy at the cellular level, providing an energy boost and promoting rapid recovery after exertion" (*8*). One site simply claims, "Taurine enhances body functions, maintains general well-being and provides energy boost" (*9*). The products are generally marketed as dietary supplements with the warning that the product is not intended to diagnose, treat, cure, or prevent any disease. This provides an excellent opportunity for students to think about the regulation of some industries relative to others.

One study examining amino acid changes during exercise reports a higher intramuscular taurine level in those who are in athletic training than in those who are not (*10*). While reporting the difference, the authors also point out that no reason for or possible benefit of the increased level is yet known. While the analysis of sports drinks will only determine the amount of taurine present in these drinks, considering the implications of the results provides an excellent opportunity for students to do further background work.

Most published work determines taurine levels in blood, urine, and milk samples, consistent with the research being done on its health effects. The method described here is adapted from these (*11–14*) to take advantage of the simpler composition of a sports drink and to reduce the analysis time.

This *Journal* has published many articles over the years utilizing HPLC analysis of drinks (*15–18*) and more recently of amino acids (*19, 20*). This article adds another to that list, with distinct advantages.

1. The procedure requires a pre-column derivatization, an important and useful technique for students that also helps them understand more about chromatographic techniques, but it does not require the hydrolysis of a peptide.
2. It examines two subjects, amino acids and sports drinks, that many students find interesting.
3. It can easily incorporate other topics on the application of science and the role of nutraceuticals in dietary supplements, a very timely and interesting topic.

Experimental Procedure

Chemicals

Solutions of taurine (available from Aldrich) spanning the range of 10–80 μg/mL were prepared from a stock solution containing 1000 μg/mL. As a preventative measure taurine solutions were wrapped in foil or otherwise protected from light (*21*).

Sports drink samples were diluted before derivatization. Most drinks contained around 1000 mg in 250 mL and a 1:100 dilution was appropriate. If the sample was carbonated, it was degassed.

2,4-Dinitrofluorobenzene (DNFB) is available from Aldrich. The derivatization reaction was performed in a 0.01 M carbonate buffer, pH 9.0. Mobile phase A was 0.01 M pH 6.0 phosphate buffer and mobile phase B was HPLC-grade acetonitrile. The mobile phases were filtered through a 0.45-μm filter and degassed.

Derivatization Reaction

Sanger's reagent, 2,4-dinitrofluorobenzene (DNFB), reacts with terminal amino groups of amino acids and peptides in basic solution to form a dinitrophenyl derivative, which is bright yellow and has an absorption maximum around 360 nm. The pH must be such that the amino group is not protonated, because the nitrogen lone pair is necessary for the reaction mechanism. However, at higher pH levels, the DNFB reacts directly with hydroxide to form 2,4-dinitrophenol, which also absorbs strongly at 360 nm. A pH of 9 is satisfactory for the reaction with taurine. DNFB is only slightly soluble in water and the addition of dimethyl sulfoxide (DMSO) speeds the reaction considerably. To further hasten the reaction, it is performed in a 40 °C water bath.

A 1.0-mL sample, 2.0 mL of 0.01 M pH 9.0 carbonate buffer, 0.5 mL of DMSO, and 0.1 mL of DNFB were combined and vortexed for 30 seconds to mix well. The solution was held at 40 °C for 15 minutes. Next, 6.5 mL of 0.01 M pH 6.0 phosphate buffer (Mobile phase A) was added and the solution was mixed by shaking. The solutions were protected from light after reaction (*12*).

Separation

The experiment requires an HPLC system capable of gradient elution and absorbance detection at 360 nm. We use a 150 × 4.6-mm Alltech Econosphere C18 3µm column, but other C18 columns should work; a guard column is advised. The flow rate is 1.0 mL per minute for an injection volume of 5.0 µL. The gradient program begins at 10% B, ramps to 25% B at 10 minutes, then ramps more quickly to 50% B by 15 minutes and is held at 50% B until the run time is at 19 minutes. All ramping is linear and the system is returned to the initial conditions after 19 minutes.

Hazards

DNFB is a skin-sensitizing agent to some people and gloves should be used when handling it (*22*). Appropriate caution should be exercised in handling the organic solvents.

Results and Discussion

Chromatograms of the standards and sports drinks consistently yield three peaks at 5.4, 7.6, and 17.5 minutes, corresponding to 2,4-dinitrophenol, the DNP–taurine derivative, and unreacted 1-fluoro-2,4-dinitrobenzene. The DNP–taurine derivative peak area is linear from 10 to 80 µg/mL. Most sports drinks containing taurine claim to contain 1000 mg per 250 mL, which corresponds to 40 µg/mL after the 1:100 dilution. Reproducibility is good; relative standard deviations are typically around 1%. Of three brands tested, two were consistent with the label but the third was very low at 830 mg. Another brand (untested) of sports drink (*23*) claims 5000 mg/serving! For comparison, a study using taurine in the treatment of congestive heart failure used a dose of 6000 mg/day (*6*).

[W]Supplemental Material

Detailed instructions and questions for students and notes for the instructor are available in this issue of *JCE Online*.

Literature Cited

1. Gaull, G. E. *J. Am. Coll. Nutr.* **1986,** *5,* 121–125.
2. Geggel, H. S; Ament, M. E.; Heckenlively, J. R.; Martin, D. A.; Kopple, J. D. *N. Engl. J. Med.* **1985,** *312,* 142–146.
3. Oja, S. S.; Kontro P. In *Handbook of Neurochemistry*, Vol. 3, 2nd ed.; Lajtha, A., Ed.; Plenum: New York, 1983; Chapter 18, pp 501–533.
4. Huxtable, R. J. *Physiol. Rev.* **1992,** *72,* 101–163.
5. Gaull, G. E. *J. Pediatr. Gastroenterol. Nutr.* **1983,** *2* (Suppl. 1), S266–S271.
6. Azuma, J.; Sawamura, A.; Awata, N.; Hasegawa, H.; Ogura, K.; Harada, H.; Ohta, H.; Yamauchi, K.; Kishimoto, S. *Curr. Ther. Res.* **1983,** *34,* 543–557.
7. Hayes, K. C.; Carey, R. E.; Schmidt, S. Y. *Science* **1975,** *188,* 949–951.
8. Hansens' Natural; *http://www.hansens.com/pages/product_frm.htm* (accessed Mar 2001); choose Energy under Functional Beverages to access taurine information.
9. INDRINKS Beverages and Food for Fun and Function. *http://www.indrinks.com/Products/BlueOx/index.htm* (accessed Mar 2001).
10. Graham, T. E.; Turcotte, L. P.; Kiens, B.; Richter, E. A. *J. Appl. Physiol.* **1995,** *78,* 725–735.
11. Chen, Z.; Xu, G.; Specht, K.; Yang, R.; Shiwang, S. *Anal. Chim. Acta* **1994,** *296,* 249–253.
12. Morton, R. C.; Gerber, G. E. *Anal. Biochem.* **1987,** *170,* 220–227.
13. Stocchi, V.; Palma, F.; Piccoli, G.; Biagiarelli, B.; Cucchiarini, L.; Magnani, M. *J. Liq. Chromatogr.* **1994,** *17,* 347–357.
14. Ferreira, I. M. P. L. V. O.; Nunes, M. V.; Mendes, E.; Remião, F.; Ferreira, M.A.; *J. Liq. Chromatogr. Rel. Technol.* **1997,** *20,* 1269–1278.
15. DiNunzio, J. E. *J. Chem. Educ.* **1985,** *62,* 446–447.
16. Strohl, A. N. *J. Chem. Educ.* **1985,** *62,* 447–448.
17. Delaney, M. F.; Pasko, K. M.; Mauro, D. M.; Gsell, D. S.; Korologos, P. C.; Morawski, J.; Krolikowski, L. J.; Warren, F. V. Jr. *J. Chem. Educ.* **1985,** *62,* 618–619.
18. Bildingmeyer, B. A.; Schmitz, S. *J. Chem. Educ.* **1991,** *68,* A195–A200.
19. Clapp, C. H.; Swan, J. S.; Poechmann, J. L. *J. Chem. Educ.* **1992,** *69,* A122–A126.
20. Barton, J. S.; Tang, C.-F.; Reed, S. S. *J. Chem. Educ.* **2000,** *77,* 268–269.
21. Lau, D.-W.; Luk, S.-F.; Chiu, T. P. Y. *Analyst* **1990,** *115,* 653–655.
22. Thompson, J. S.; Edmonds, O. P. *Ann. Occup. Hyg.* **1980,** *23,* 27–33.
23. RipFastOnLine; *http://www.ripfast.net/male/shocktub.html* (accessed Mar 2001).