Supplementary material for publication via *JCE Online*

to accompany

HPLC Determination of Taurine in Sports Drinks

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PURPOSE/BACKGROUND

The amino acid taurine (2-aminoethanesulfonic acid) is present in many sports drinks claiming to provide you with some special advantage. While taurine is not incorporated into proteins, our bodies contain a good amount (approximately 70 g in a 70 kg person) of it. It has been linked to physiological processes involving brain development and eyesight, and it has been used in the treatment of congestive heart failure and epilepsy. While taurine is suspected to be involved in many physiological roles in our bodies, mechanisms for its action have not yet been fully developed. It is one of the amino acids our bodies can synthesize or it can be provided in our diet. Infants (especially pre-term infants) are not generally able to synthesize taurine and it is provided from mother's milk. Because of its importance in development, it has been included in infant formulas since the mid-eighties.

In this lab, you will be using HPLC to determine the amount of taurine in a sports drink. You may bring your own drink, or one will be provided for you. Because not all sports drinks contain taurine, make sure the label mentions it.

Taurine does not absorb light of any convenient wavelength and must be derivatized in order to be detected by the absorbance detector of the HPLC. We will be utilizing Sanger's reagent (1-fluoro-2,4-dinitrobenzene, or more commonly 2,4-dinitrofluorobenzene (DNFB)) for the derivatization. In basic solution (when the amino group of taurine is not protonated, and the nitrogen lone pair is available) DNFB undergoes nucleophilic aromatic substitution with taurine to form a 2,4-dintitrophenyl taurine derivative which absorbs well at 360 nm. The reaction is not specific to taurine and any amino group or hydroxide can act as the nucleophile. You may want to review the mechanism (which is thought to proceed through a carbanion intermediate) in your organic chemistry textbook. Sanger's reagent has been used for years to label terminal amino groups of proteins.

The derivatization reaction will be done at 40^oC in a water bath for 15 minutes at a pH of 9.0. The mixture is then brought to a pH of 6.0 before injection onto the HPLC column.

SOLUTIONS AND SAMPLE PREPARATION

You will need several solutions for this lab. Instructions are given here for their preparation, though they may be provided for you by the instructor or other groups who have already completed this lab.

0.01 M pH 9.0 carbonate buffer

For 250 mL: Dissolve 0.200 g $NaHCO_3$ and 0.014 g Na_2CO_3 in 250 mL deionized water. Test the pH with a pH meter and adjust with NaOH or HCl if necessary.

Mobile phase A: 0.01 M pH 6.0 phosphate buffer

For 1000 mL: Dissolve 1.298 g $NaH_2PO_4 \cdot H_2O$ and 0.159 g $Na_2HPO_4 \cdot 7H_2O$ in 1000 mL deionized water. Test the pH with a pH meter and adjust with NaOH or HCl if necessary.

Vacuum filter through a 0.45 μm filter and degas by sonicating under vacuum for 10 minutes.

Mobile phase B: HPLC grade acetonitrile

Vacuum filter through a 0.45 μm filter and degas by sonicating under vacuum for 10 minutes.

Taurine standard solutions

Prepare solutions that are 10, 20, and 50 μg/mL by appropriate dilution (1:100, 2:100, and 5:100) of the provided 1000 μg/mL stock solution.

Sports drink sample

If the drink is approximately 250 mL and the label claims it contains 1000 mg, dilute it 1:100. Otherwise, perform an appropriate dilution (based upon the label) which will bring it into the range covered by your standard solutions above. If the drink is carbonated, degas it by sonication under vacuum for 10 minutes before diluting it.

DERIVATIZATION REACTION

The procedure is the same for standard solutions and sample.

Into a test tube, pipet

1.0 mL sample

2.0 mL 0.01 M pH 9.0 carbonate buffer

0.5 mL methyl sulfoxide (DMSO)

0.1 mL DNFB

Vortex for 30 seconds and place in a 40°C water bath for 15 minutes. At the end of 15 minutes, pipet (using a graduated pipet) 6.5 mL of mobile phase A (0.01 M pH 6.0 phosphate buffer) into the reaction mixture.

HPLC

You will be instructed in the lab on the operation of the HPLC and integrator. You will be using a 150 x 4.6 mm Alltech Econosphere C18 3µ column. Check that the 5.0 µL sample loop is attached to the injector and be certain that the gradient program is as shown below. Remember that all samples must be filtered through a 0.45 µm filter before injection!

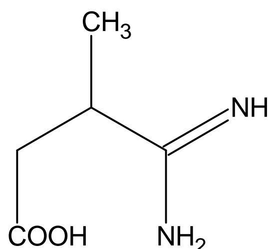
Gradient program

Run Time (minutes)	%A	%B	Flow rate (ml/minute)
Initial	90	10	1.0
10	75	25	1.0
15	50	50	1.0
19	50	50	1.0
22	90	10	1.0

QUESTIONS/RESULTS/CALCULATIONS

Each chromatogram should contain at least three peaks. The first (at approximately 5.4 minutes corresponds to 2,4-dinitrophenol. The second (at approximately 7.6 minutes) corresponds to the dinitrophenyl derivative of taurine. The third (at approximately 17.5 minutes) corresponds to unreacted DNFB.

1. A plot of taurine peak area versus taurine concentration should be linear and can be used to determine the concentration of taurine in your sample. Compare this concentration to the concentration stated on the product label. Is there a <u>significant</u> difference?
2. I stated above that the first peak is due to the presence of 2,4-dinitrophenol. This chemical is neither present in sports drinks, nor is it an impurity in the DNFB. How did it get into your sample?
3. Some drinks also contain creatine whose structure is shown below. These drinks do not show an additional peak in the chromatogram. Why doesn't creatine react with DNFB to form a dinitrophenyl derivative? You might want to make some comparisons to the similar amino acid arginine.



Instructor Notes

We use a Waters HPLC system: two 510 pumps controlled by a 680 controller, with a Rheodyne 7725i manual injector and a 486 tunable absorbance detector. The detector is attached to a Spectra-Physics SP4270 integrator via the 10 mV full scale output. With the detector AUFS set at 1.0 and a 5 μL injection, we use an attenuation of 4, corresponding to 0.4 absorbance units full scale on the paper. Typically, the DNFB peak is tallest (around 0.3 absorbance units) while the other peaks are around 0.1 absorbance unit. An unidentified but small (another order of magnitude smaller than DNP-taurine peak) sometimes occurs around 9.5 minutes. It appears to be a DNFB byproduct and does not interfere with the separation. A sample chromatogram is presented in Figure 1 as a reference.

We use regular volumetric pipets for all of the measurements except the 6.5 mL of mobile phase A (graduated pipet) and the 0.1 mL of DNFB. While DNFB's melting point is variously reported in different ranges spanning 23-30°C, it is consistently a viscous liquid at our room temperatures. We use a micropipetter for the delivery of 0.1 mL of it to the reaction mixture.

Some references indicate that taurine solutions and dinitrophenyl taurine derivatives are light sensitive, hence the caution about protecting taurine solutions from light. In practice, we have observed no degradation, even in samples left unprotected overnight. This could be an interesting area for further study by students.

An alternate gradient scheme utilizes a 50/50 (vol./vol.) mixture of acetonitrile and water for mobile phase B. For this mobile phase, the following gradient program would be used.

Gradient program for 50/50 acetonitrile/water as mobile phase B

Run Time (minutes)	%A	%B	Flow rate (ml/minute)
Initial	80	20	1.0
10	50	50	1.0
15	0	100	1.0
19	0	100	1.0
22	80	20	1.0

It can be seen that the percent acetonitrile is the same in both programs. Some pump systems operate more smoothly with the above program, chiefly because they avoid problems with mixing acetonitrile and water by doing most of it outside the pumps. The advantage to the program recommended in the article and used by our students is that you do not have to make up any mobile phase B. It saves a little time, but probably more importantly results generally in less wasted acetonitrile.

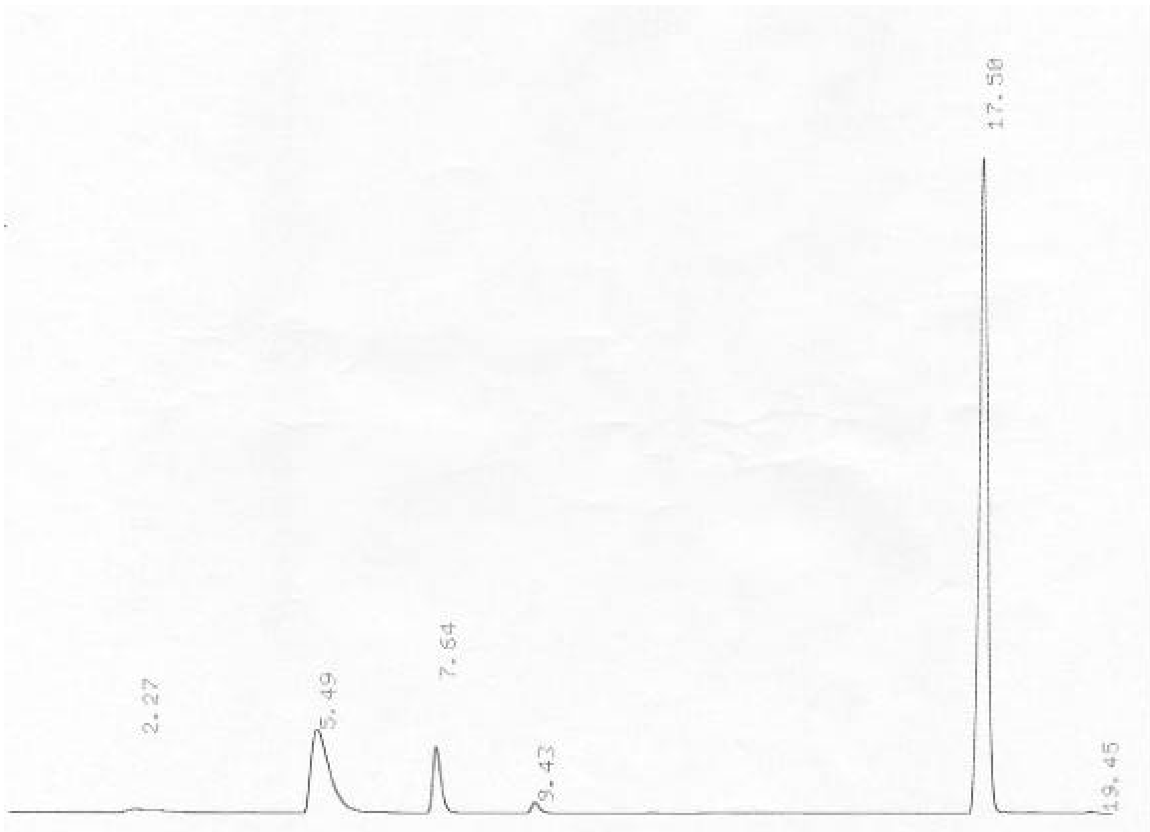


Figure 1: Sample chromatogram of 20 μg/mL sample following experimental procedure described.

CAS Registry Numbers

Acetonitrile, HPLC grade, CH_3CN	*[75-05-8]*
Sodium carbonate, Na_2CO_3	*[497-19-8]*
Sodium bicarbonate, $NaHCO_3$	*[144-55-8]*
Sodium phosphate, monobasic, monohydrate, $NaH_2PO_4 \cdot H_2O$	*[10049-21-5]*
Sodium phosphate, dibasic, heptahydrate, $Na_2HPO_4 \cdot 7H_2O$	*[7782-85-6]*
Taurine, $NH_2CH_2CH_2SO_3H$	*[107-35-7]*
Dimethyl sulfoxide, CH_3SOCH_3	*[67-68-5]*
2,4-dinitrofluorobenzene	*[70-34-8]*