Determination of Taurine in Energy Drinks by HPLC Using a Pre-column Derivative

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A rapid and simple method to determine taurine in energy drinks by pre-column high-performance liquid chromatography was developed using a derivative of 4-fluoro-7-nitrobenzofurazan (NBD-F) without the need for an exclusive instrument. The reaction of taurine with NBD-F finished in 10 min at 60°C. The derivative was measured on a UV-Visible detector (470 nm) by HPLC using a conventional Octadecyl silane (ODS) column. A mixture of disodium hydrogenphosphate-citric acid buffer solution (pH 5.4) containing 10 mmol/l tetrabutylammonium bromide and acetonitrile (7:3) was used as the mobile phase. The recoveries were in the range of 98.2–99.9%, the precision as standard deviation was in the range of 0.3–0.5%, the linearity as a coefficient of correlation value was 0.999 and the specification was confirmed for taurine added to three commercial energy drinks. The content of taurine measured compared to the labeled amount in five commercial energy drinks containing taurine was 92.9–105.1%.

Key words —— taurine, 4-fluoro-7-nitrobenzofurazan, HPLC, pre-column, 2-aminoethanesulfonic acid

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is one of the amino sulfonic acids and is found in the tissues of many animals. Taurine is the main component of many energy drinks as a tonic medicine. Post-column, pre-column and on-column derivatization of taurine by high-performance liquid chromatography has been reported.[1–7] The post-column derivatization method is the most popular approach but it requires ion-exchange chromatography and an exclusive instrument. Pre-column derivatization methods require more than one hour reaction time and is time-consuming. On the other hand, 4-fluoro-7-nitrobenzofurazan (NBD-F) acts as a fluorescent reagent that produces the derivative of the primary and secondary amines and can be purchased easily.[8] These derivatives have a maximum intensity of UV-VIS spectrum near 470 nm and are fluorescent.[9] NBD-F is used for the determination of amino acid.[10,11] In the present study, we developed a rapid and simple determination of taurine in energy drinks by HPLC with pre-column derivatization with NBD-F without an exclusive instrument.

MATERIALS AND METHODS

Materials —— Taurine (2-aminoethanesulfonic acid) was purchased from Wako Pure Chemical Industries (Tokyo, Japan). NBD-F was purchased from Dojindo laboratories (Kumamoto, Japan) and NBD-F solution was prepared by dissolving 1 mmol/l of acetonitrile. All other chemicals used were of HPLC or reagent grade.

Recommended Procedure of Derivatization of Taurine by NBD-F —— 1.0 ml of sample solution, 2.0 ml of 0.1 mol/l phosphate buffer containing 20 mmol/l EDTA (pH 9.0) and 1.0 ml of 1 mmol/l NBD-F were added in a test tube. The tube was tightly capped and mixed, and heated for 10 min at 60°C. Then the tube was cooled in ice water, and 1.0 ml of 0.2 mol/l HCl was added. The solution was measured by HPLC.

Conditions of HPLC —— The column was L-column ODS (150 × 4.6 mm i.d., 5 μm, Chemical Evaluation and Research Institute, Tokyo, Japan). A mixture of disodium hydrogenphosphate-citric acid buffer solution (pH 5.4) containing 10 mmol/l tetrabutylammonium bromide and acetonitrile (7:3) was used as the mobile phase. The flow rate was 1.0 ml/min. The column temperature was 40°C. The injection volume was 20 μl. The HPLC system consisted of a Shimadzu LC10 CLASS-VP system equipped with a Shimadzu SPD-M10AVP diode array detector (wavelength: 470 nm, both Shimadzu, Kyoto, Japan).

Sample and Standard Solutions for Recovery Test of Taurine from Energy Drinks —— Taurine was added to three commercial energy drinks that do not contain taurine, until the concentration of

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taurine was 1500 mg per 100 ml. Furthermore, each sample solution was diluted with water until the concentration of taurine was 0.015 mg/ml. Taurine was dissolved in water to make 3 mg/ml of standard stock solution. Standard stock solution was diluted with water for standard solution. A standard calibration curve was prepared by plotting peak area versus taurine concentration over the range of 0.0015 to 0.15 mg/ml. These solutions were measured by this method and we examined recovery, precision and specification. Linearity was examined by standard solution of taurine.

Determination of Taurine in Energy Drinks —— Five commercial energy drinks containing taurine were determined by this method. Each sample solution was diluted with water until the concentration of taurine was 0.015 mg/ml based on the labeled amount. The content of taurine in drinks were calculated using a standard calibration curve.

RESULTS AND DISCUSSION

Conditions of Derivatization of Taurine by NBD-F

For the derivatization of taurine by NBD-F, the optimum conditions were measured. The effect of pH of phosphate buffer containing 20 mmol/L EDTA is shown in Fig. 1. The effects of temperature and reaction time on the reaction are shown in Fig. 2. The best conditions for the reaction were pH 9.0, temperature 60°C, and reaction time 10 min on the basis of the peak area intensity of labeled taurine (NBD-Taurine). The UV-VIS spectrum of NBD-Taurine is shown in Fig. 3. The maximum intensity of spectrum was near 470 nm of wavelength. The reproducibility of peak area intensity of NBD-Taurine was 0.62% at 470 nm (Relative Standard Deviation (RSD), $n = 6$). The stability of peak area intensity of NBD-Taurine peak was maintained over 12 hr. Taurine and NBD-F react at equal molarity and NBD-F may be used by components except taurine in drinks. Thus the sample was diluted with water, so the concentration of NBD-F was 8 times greater than taurine in reaction solution.

Recovery Test of Taurine from Energy Drinks

An example of HPLC chromatogram of sample with added taurine is shown in Fig. 4. NBD-Taurine was eluted around 5 min and a decomposition from NBD-F was eluted around 8 min. No other peaks that could interfere with the NBD-Taurine peak by 470 nm were present on the chromatogram in any samples, so high specification was confirmed. The recovery of taurine from samples is shown in Table 1. The range of recovery was 98.2–99.9% with a standard deviation of 0.3–0.5%. The recovery of taurine from sample was satisfactory. The standard calibration curve of taurine is shown in Fig. 5. The linearity was good in the 0.0015 to 0.15 mg/ml range and the coefficient of correlation value was 0.999. The quantitation limit was 0.0005 mg/ml (RSD = 4.2%, $n = 6$).

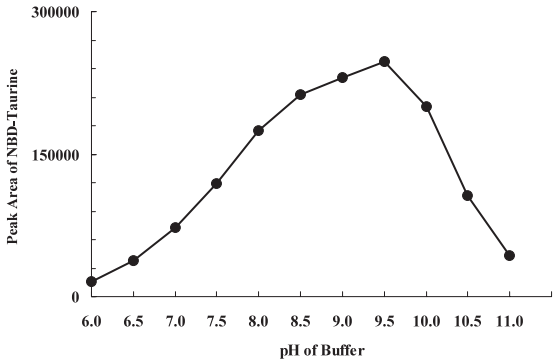

Fig. 1. Effect of pH of the Buffer on the Reaction of Taurine and NBD-F

Mixtures of 1.0 ml of 0.015 mg/ml taurine and 1.0 ml of 1 mmol/l NBD-F were heated for 10 min at 60°C with 2.0 ml of 0.1 mol/l phosphate buffer containing 20 mmol/l EDTA at different pH value. Each point was the average of three experiments.

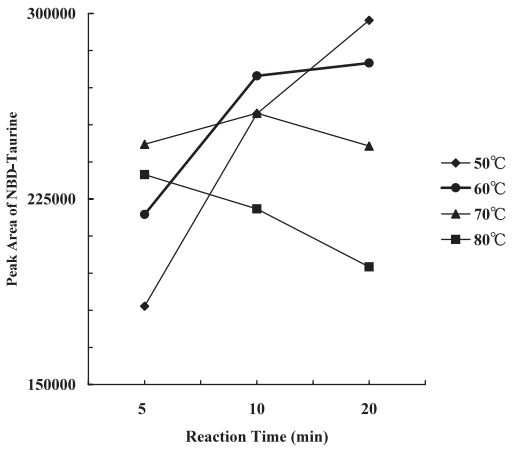

Fig. 2. Effects of Temperature and Time on the Reaction of Taurine and NBD-F

Mixtures of 1.0 ml of 0.015 mg/ml taurine and 1.0 ml of 1 mmol/l NBD-F were heated with 2.0 ml of 0.1 mol/l phosphate buffer containing 20 mmol/l EDTA (pH 9.0) at different temperature and time. Each point was the average of three experiments.

Determination of Taurine in Commercial Energy Drinks

The content of taurine in five commercial en-

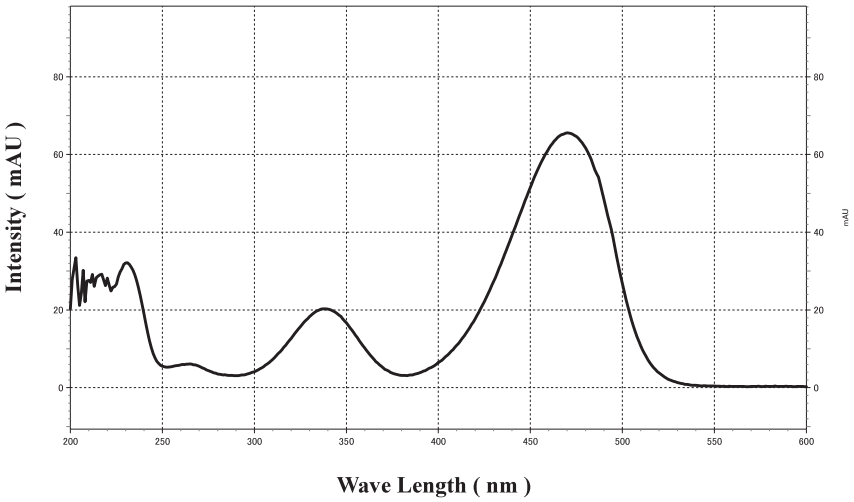

Fig. 3. UV-VIS Spectrum of NBD-Taurine

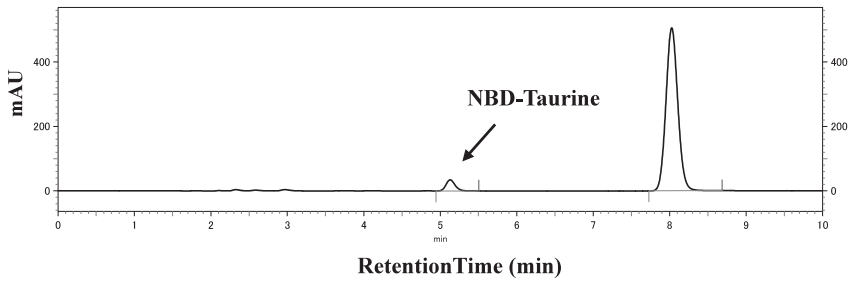

Fig. 4. HPLC Chromatogram of Energy Drink with Added Taurine
The peak of NBD-Taurine was eluted around 5 min and a decomposition from NBD-F was eluted around 8 min.

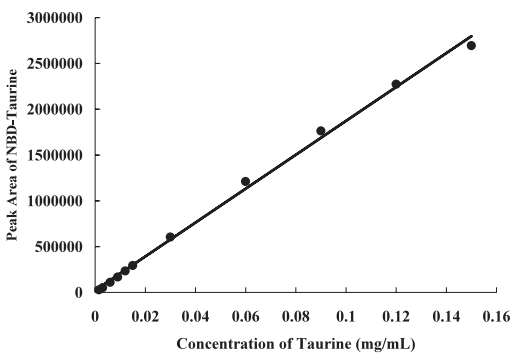

Fig. 5. Standard Calibration Curve of NBD-Taurine
Eleven different concentrations of taurine (0.0015, 0.003, 0.006, 0.009, 0.012, 0.015, 0.03, 0.06, 0.09, 0.12 and 0.15 mg/ml) were measured. The coefficient of correlation value was 0.999. The regression equations were $Y = 184942X + 22752$. Each point was the average of six experiments.

Table 1. Recoveries of Taurine from Energy Drinks

Sample	Recoveries (%)[a)]
Drink-1	99.4 ± 0.3
Drink-2	98.2 ± 0.5
Drink-3	99.9 ± 0.3

a) Mean ± S.D. (n = 6).

Table 2. Content of Taurine in Commercial Energy Drinks

Sample	Labeled amount (mg/100 ml)	Content (mg/100 ml)[a)]	(%)[b)]
Drink-1	1000	932 ± 5.2	93.2
Drink-2	1500	1394 ± 5.3	92.9
Drink-3	1500	1506 ± 8.6	100.4
Drink-4	2000	1926 ± 4.2	96.3
Drink-5	3000	3153 ± 17.7	105.1

a) Mean ± S.D. (n = 6), *b*) measured data against labeled amount.

ergy drinks containing taurine is shown in Table 2. The content of taurine compared to the labeled amount was 92.9–105.1%.

In this study, we developed a rapid and simple method to determine taurine in energy drinks by HPLC without needing an exclusive instrument. Taurine was derivatized by NBD-F at 60°C in 10 min. The derivative was determined at 470 nm using a UV-VIS detector. Because the derivative was retained on a conventional ODS column using tetrabutylammonium bromide as the counter ion, an

expensive ion-exchange column was not necessary. Therefore, this taurine determination method will be a suitable daily quality test for smaller laboratories.

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