Determination of Taurine in Quadriceps Femoris by High Performance Liquid Chromatography with Precolumn Derivatization

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Abstract. A method for determination of taurine in mouse quadriceps femoris by high performance liquid chromatography with precolumn derivatization ophthalaldehyde (OPA) was established. The sample was extracted and treated with canon exchange resin. Results showed that taurine in quadriceps femoris was separated and quantified on C18 reversed phase column by high performance liquid chromatographic (HPLC) after derivatization with OPA in 3min, using mixture of methanol and phosphate （V/V = 1:1，pH = 4.9） as mobile phase, rate of flow is 0.6mL/min, detecting at 340nm by UV-detector, L-Glutamine as internal standard. The result showed that the linear ranger of taurine was 6.25-187.7ng/mL, correlation coefficient R2=0.9994, the recoveries were 91.8%-101.8%, RSD=3.2% (n=6). The retention time of taurine is 7.32 min. The concentration of taurine in mouse quadriceps femoris is 3.18mg/g. The protein and amino acid were separated by sample pre-treatment. The method is of good separation effect, simple, reliable, and can be used to analyze the taurine concentration in mammal tissue.

1. Introduction

Taurine (abbreviation Tau) is a sulfur-containing β-amino acid, named after it was first isolated from bovine bile in 1827, also known as taurocholic acid and bovine choline. The content of taurine in animal tissues is higher than that of other organisms. The highest content of taurine is in marine animals, and the content in plants is less. For mammals, poultry and other animals, taurine is a conditionally essential amino acid. They can only synthesize 30-40% of their physiological requirements, and the rest needs to be obtained from food. For rodents, the taurine synthase activity in their bodies is high, and the taurine synthesized by themselves can meet their physiological needs[1]. It has been proved to be effective in mammalian central nervous system[2,3], spinal cord[4], heart[5], retina[6], liver[7], kidney[8], pancreas[9], reproductive system and breast[10]. Has taurine distribution. The research results show that taurine has a wide range of biological effects, can regulate a variety of physiological activities of the body, and has now begun to be widely used in production and human life.

Currently reported detection methods for taurine mainly include neutralization titration [11], thin-layer scanning method[12], high performance liquid chromatography[13], automatic amino acid analysis method[14], spectrophotometric method[15], Capillary electrophoresis, etc.[16], and in these methods, there are shortcomings such as cumbersome operation, long analysis time, large errors, expensive equipment, and interference substances affecting the determination. In this study, high performance liquid chromatography OPA pre-column derivatization method was established for the determination of taurine content in mouse quadriceps femoris muscle. The separation effect was good and the accuracy was high, which can be used as a reference for the rapid and efficient determination of taurine content in mammal tissues.

2. Materials and methods

2.1 Materials

10mM taurine standard stock solution: 0.125 g dissolved in 100 mL ultrapure water. OPA derivatizing agent: accurately weigh 20 mg of OPA and place it in a brown bottle, dissolve it with 2 mL of methanol, then add 80 μL of 2-mercaptoethanol and 18 mL of sodium borate buffer, mix well and place in ice solution for later use. The concentration of L-Glutamine (Glu) standard stock solution (internal standard) is 10 mM: 0.18713 g is dissolved in 100 mL ultrapure water. Taurine, L-Glutamine and OPA are all from sigma of the United States; methanol is chromatographically pure; anhydrous sodium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, sulfosalicylic acid, sodium borate, 732 type strong acid benzene. The ethylene-based cation exchange resin is of domestic analytical grade. The

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mobile phase methanol/phosphate was passed through a 0.22 μm filter membrane and degassed ultrasonically for 30 minutes before use.

High performance liquid chromatograph (Waters, USA), MV 1201 UV-visible detector (Dalian Yilite), KQ 5200 ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.).

2.2 Methods

2.2.1 Chromatographic conditions

C_{18} chromatographic column (Waters, 4.6mm×150mm, 5μm). Mobile phase: methanol/phosphate (V/V=1:1, pH=4.9). Liquid A is methanol and liquid B is the mobile phase. First equilibrate the column with liquid A for 2 hours at a flow rate of 0.2 mL/min, and gradually increase the flow rate to 0.6 mL/min, change the ratio of A and B, so that liquid A will eventually be replaced with liquid B to keep the column pressure stable. The setting parameters of the liquid chromatograph are: maximum pressure 200 kg/cm^2, minimum pressure 0 kg/cm^2, flow rate 0.6 mL/min, UV detector wavelength 340 nm, room temperature 25 ℃.

2.2.2 Preparation of Taurine Standard

Preparation of taurine standard with concentrations of 5 μm, 20 μm, 50 μm, 100 μm, 150 μm: Add 0.05, 0.10, 0.50, 1.00, 1.5mL of 10mM taurine reserve solution to 4 100mL volumetric flasks, and then add 1.00mL of 10.00mM glutamine reserve solution to each flask, at constant volume.

2.2.3 Preparation of sample pretreatment column

Prepare the sample pretreatment ion exchange column: soak the strong acid styrene cation exchange resin in distilled water for 24 hours, then wash it with 2N NaOH, then wash it with 2N HCl, finally remove the acid solution, and wash with deionized water until it is neutral. Plug the lower mouth of the dropper with glass wool, slowly fill the resin with a height of 5 cm, without air bubbles in the middle of the filler, and soak it vertically in 1M hydrochloric acid for later use.

2.2.4 Sample collection and processing

The experiment used Kunming male rats of the same age, weighing 39±2 g. Manual temperature control 22-26 ℃, light control 14 L : 10 D, free drinking and eating. The mice were sacrificed with a short neck, and the quadriceps femoris of the thighs of 6 mice were collected, and they were labeled 1-6.

Weigh 0.20 g (accurate to 1 mg) quadriceps, cut into small pieces of 1mm^3, homogenize with a glass homogenizer, and extract with 3mL sulfosalicylic acid solution (0.2 mol/L) after homogenization, high speed Centrifuge at a speed of 12000 rpm for 15 minutes. Pipette 400 μL of supernatant into the pretreatment column and rinse with 4 mL ultrapure water. Add 0.5 mL L-Glutamine solution (1 mM) to the solution after passing the column, and dilute to 5 mL with ultrapure water for testing.

2.2.5 Sample derivatization

Accurately draw 20 μL of the solution to be tested, add 20 μL of OPA derivatizing agent, vortex the shaker to mix, and react for 3 min in the dark, and take 20 μL of sample for detection.

2.2.6 Data analysis

The ratio of the peak area of each standard solution sample to the peak area of the internal standard (A_s/A_{ins}) was used as the independent variable, and the ratio of the sample concentration to the internal standard concentration (C_s/C_{ins}) was used as the dependent variable to obtain a linear regr- ession equation and calculate the correlation coefficient.The calculation formula of taurine content is as follows.

$$X = \frac{(a + b \times A_s/A_{ins}) \times C_{ins} \times V_3 \times V_1}{V_2 \times m \times 1000} \times 125.14$$

In the formula m-sampling weight (g); V_1-volume of extract (mL); V_2-volume of sample solution passing through the pretreatment column (μL); V_3-volume of constant volume after passing the column and adding internal standard (mL); 125.14: molar mass of taurine; X-taurine content in the sample (mg/g). All data are expressed as mean ± standard deviation.

2.2.7 Recycling rate determination

Add a certain amount of taurine standard to the sample, use the method 2.2.4 for pretreatment, and measure the sample after the pre-column derivatization reaction according to 2.2.5 to check the recycling rates.

2.2.8 Precision measurement

Precisely draw 6 parts of 20 μL taurine (50 μM) standard solution, derivatize according to 1.2.4 pre-column derivatization method and then inject the samples, continuously measure 6 parts to check the precision.

3. Results and nanalysis

3.1 Derivative reaction

The chemical name of taurine is 2-aminoethanesulfonic acid, the molec- ular formula is $NH_2CH_2CH_2SO_3H$ and the molecular weight is 125.14 Daltons. Taurine has a sulfonic acid group, and most of it is in the form of zwitterions at physiological pH **错误!未找到引用源。**. This characteristic makes taurine more water-soluble and less fat-soluble. Taurine has no UV absorption, so it needs to react with the derivatizing agent, and the derivatized product has UV absorption. Commonly used pre-column derivatization reagents mainly include o-phthalaldehyde

and dinitrofluorobenzene method (DNBF) **错误!未找到引用源。**. This study uses OPA as the derivatizing agent, and the derivatization reaction formula is as follows.

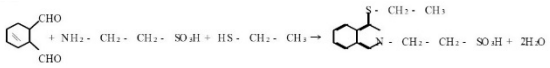

Figure 1 Derivative reaction formula

3.2 3Derivative conditions

This experiment uses the high-performance liquid chromatography OPA pre-column derivatization method. In the experiment, it is found that the derivatization time and sample injection volume will affect the experimental results. Therefore, this experiment uses L-Glutamine as the internal standard, which overcomes the error caused by the difference in the injection volume. However, the derivation time is different, and the ratio of sample peak to internal standard peak area (A_s/A_{ins}) is also different. After experiments, it is found that the longer the reaction time, the smaller the A_s/A_{ins} ratio, but within 2.0-3.0 min, the A_s/A_{ins} changes little (1.05-1.04), indicating that the reaction time of 2.0-3.0min can reduce the error. The derivatization reaction time in this experiment was 3 minutes.

3.3 Chromatographic measurement condition

After repeated tests, room temperature 25 ℃, reaction with OPA for 3 min, mobile phase pH= 4.9, flow rate 0.6 mL/min, UV detector wavelength is 340 nm , the chromatographic separation effect is better, the retention of L-glutamine and taurine The time is 4.09 min and 7.32 min respectively. As shown in Figure 2, there are few impurity peaks and small area, and the internal standard peak and taurine peak can beseparated completely.

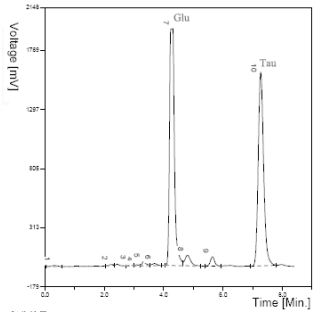

Figure 2 Chromatograms of 100 μM taurine and 100 μM-glutamine (internal standard) standards

3.4 Sample processing

Compared with other samples, animal tissue has its particularity, and the pre-processing method of the sample has a direct impact on the separation effect and the authenticity of the quantification. The main interferences in mouse quadriceps are protein and other amino acids. After the sample is homogenized, 0.2 mol/L sulfosalicylic acid solution is added to remove the protein by denaturation and centrifugation. The supernatant is then passed through 732 strong acid cation exchange resin chromatography. Most of the amino branches are adsorbed on the column, and the taurine Acid can pass through the column, and interference can be reduced after treatment.

3.5 Standard curve and minimum detection limit

Five concentrations of taurine standard solutions were measured. The ratio of peak area to internal standard peak area (As/Ains) of each standard solution was taken as the independent variable, and the ratio of sample concentration to internal standard concentration (Cs/Cins) was taken As the dependent variable to calculate and make the standard curve, the standard curve equation can be obtained: Y=0.8065X+0.0044, as shown in the figure, the linear correlation coefficient is 0.9994. Taking 5 μM as the lowest quantitative detection concentration, and the injection volume of 20 μL, the minimum detection limit is 6.25 ng.

3.6 Recycling rate

Take a sample and add the taurine standard product and then perform the same treatment. The results are obtained after determination. The recycling rates of taurine is calculated by the subtraction method (see Table 1). The average recycling rates of the sample is calculated to be 95.93%. Relative standard deviation RSD=3.7% (n= 6).

Table 1 Recycling rates test results（n= 6）

Number	Addition amount	Measured value	recycling rates
	μg/mL	μg/mL	%
1	3.13	2.87	91.8
2	3.13	2.99	95.6
3	3.13	3.18	101.7
4	3.13	2.92	93.2
5	3.13	2.97	95.0
6	3.13	3.08	98.3

3.7 Precision

The peak area values obtained from the 6 measurements of taurine 50 μM standard product are shown in Table 2. It can be seen that the method is used to determine the content of taurine in muscle tissue with good reproducibility. The average peak area is 7951.78, and the relative standard deviation RSD is 3.2%.

Table 2 Precision measurement（n=6）

number	Peak area	number	Peak area
1	7997.89	4	7954.25
2	7958.88	5	7940.00
3	7934.35	6	7925.34

3.8 Taurine content in quadriceps femoris of mice

The chromatogram of the quadriceps sample is shown in Figure 3. The sample has been pretreated and derivatized for 3 minutes at room temperture 25 ℃, mobile phase pH= 4.9, flow rate 0.6mL/min, 340 nm UV de- tection wav- elength, impurity peak area Small, taurine peaks can be completely separated. The content of taurine in the quadriceps femoris samples of mice 1-6 is shown in Table 3. The average content is 3.18 mg/g, and the coefficient of variation CV is less than 5%.

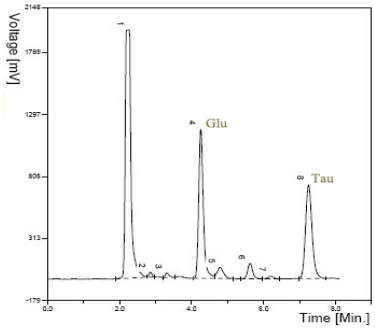

Figure 3 Sample chromatogram

Table 3 Taurine content of quadriceps femoris in mice

Sample	Taurine content CV 100% (mg/g)	Sample	Taurine content CV100% (mg/g)
1	3.09±0.027 0.9	4	3.40±0.13 3.9
2	3.25±0.06 2.0	5	3.04±0.03 1.0
3	3.20±0.05 1.6	6	3.11±0.03 1.0

4. Conclusion and discussion

Taurine is one of the most abundant free amino acids in animals, especially in nerves, muscles, glands and other excitable tissues [1]. Studies have shown that taurine plays an important role in the exercise system and can combat exercise fatigue by increasing the activity of Na^+-K^+-ATPase and Ca^{2+}-ATPase [19,20] in muscle cells. Therefore, it is particularly important to establish a simple and accurate method for the determination of taurine content in mammalian muscle.

In this study, HPLC was used to determine the taurine content in mouse quadriceps femoris muscle samples by OPA pre-column derivatization method, and the optimal experimental conditions for sample pretreatment, ion exchange column, derivatization rea-ction and chromatographic detection were determined. The method has simple pretreatment, low cost, high accuracy and good sensitivity, which can provide a reference for determining the content of taurine in mammalian tissues.

Acknowledgments

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