Determination of taurine in dried sha-chong (*Sipunculus nudus*) by high performance liquid chromatography

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Abstract. The paper studied the determination of taurine in dried sha-chong (*Sipunculus*) by high performance liquid chromatography. The method showed the wide detection range from 0.0 to 500 µg/mL with the linear dependent coefficient of 0.9965 with the limit of detection (LOD) 3.74 ng/mL (S/N=3) and the precision of 4.39 % (relative standard deviation, RSD). The standard addition recovery rate was between 82.5 % ~ 101 %. Finally, the method was successfully applied in detection of the taurine in dried sha-chong and other aquatic products.

1. Introduction

As one of the sulfur-containing amino acids with the simple structure in animals, taurine (C₂H₇NO₃S, 2-aminoethanesulfonic acid) possesses slightly acid and the stability to heat. Taurine combines cholic acid with the form of combination in human and animal bile, while it exists in the form of free in brain, ovary, heart, liver, milk, pineal, pituitary, retina, adrenal and other tissues [1-2]. Taurine is an essential amino acid for human body although it does not participate in the synthesis of protein, but it plays an important role in the development of nervous system of fetus and infant [3-4]. As an active substance regulating the normal physiological activities of the body, taurine acts as the functions of anti-inflammatory, maintaining the osmotic pressure balance of the body, regulating nerve conduction, participating in endocrine activities, increasing the ability of heart contraction, improving the immune ability of the body and enhancing the anti-oxygen ability of cell membrane [5-9]. Therefore, taurine can be widely used in medicine, food additives, biochemical reagents and so on [10-11]. Taurine in the body mainly comes from the diet, especially the seafood.

A variety of detection methods have been extensively developed with titration [12], colorimetric [13-14], thin layer chromatography scanning [15-16], amino acid analysis [17], high performance liquid chromatography (HPLC) and so on [18-19]. Nevertheless, the interferences from other amino acids and the large relative errors limits the application of titration, colorimetric, thin layer chromatography scanning. Amino acid analysis requires the expensive instruments. Owing to the advantages of facile to operation, good stability, high sensitivity and accuracy, HPLC was adopted to detect the taurine in...
Lycium barbarum [20], milk powder [21], beer [22], solen gouldi [23], takifugu obscurus [24], ostrea plicatula [25-26], pinctada martensi [19] and mytilus edulis [27]. However, to our knowledge, HPLC was applied in the determination of taurine in dried sha-chong (sipunculus nudus), which has been less reported.

Herein we establish a suitable HPLC method for the detection of taurine in sipunculus nudus and other aquatic products. The established method showed a good linearity in the taurine range 0.0-500.0 μg/mL (R^2= 0.9958) with the limit of detection (LOD) 3.74 ng/mL (S/N=3) and the precision of 4.39 % (relative standard deviation, RSD). The standard addition recovery rate was between 82.5 % ~ 101 %. Finally, it was successfully applied in the detection of the taurine in the aquatic products.

2. Materials and methods

2.1. Sample collection
The aquatic products were mainly involving of fresh samples and dry products, such as carp (Carassius), grass carp (Ctenopharyngodon idellus), crayfish (Procambarus clarkia), prawn (Penaeus vannamei), clam (Meretrix), and dried sha-chong (Sipunculus nudus). All the samples were transported back to the laboratory in the car refrigerators, thawed, and homogenized, and kept the fresh samples in the -20 °C freezer and the dry samples in the 4 °C freezer for refrigeration for further using.

2.2 Instrument. The instrument of high performance liquid chromatography (1100) with UV detector was purchased from Agilent Technologies Company. High speed centrifuge (CF16RX-II) was purchased from HITACHI company. Turbine mixer was purchased from IKA. Ultrasonic vibrator was from.

2.3 Reagent.
The taurine calibration (≥99 %) was charged from Bei jing Solarbio life sciences company. Glacial acetic acid, hydrochloric acid, anhydrous sodium carbonate, sodium acetate, methylamine hydrochloride, dansyl chloride, zinc acetate, potassium ferrocyanide were all purchased from Sinopharma chemical reagent Co. Ltd., and acetonitrile (chromatographically pure) were from J.T.baker. Ultrapure water was 18.2 MΩ·cm. Other reagents were analytical degrade without special instructions.

2.4 The Sample pretreatment.
Homogenized dried sha-chong (Sipunculus nudus) samples of 2.0 (±0.01 g) were accurately weighed in the centrifuge tubes with the volume of 50 mL, then 10 mL of ultrapure water was added into the tubes, which kept for 2 h to ensure that the dry samples absorb water adequately. Another 30 mL warm ultrapure water (40 °C) were added into the tubes. The samples were shaken vigorously on the turbine mixer and extracted for 10 min on ultrasonic oscillator. 1.00 mL 15 % of potassium ferrocyanide solution and 1.00 mL 30 % of zinc acetate solution were successively added into the reaction systems. After being fixed the volume to 50.00 mL with ultrapure water and stirred adequately, the sample solutions were centrifuged at 10000 r/min for 10min, and the supernatants were taken for reserve. The supernatants was kept at 4 °C in the dark and kept stable for 24 hours. 1.00 mL of the obtained supernatants, 1.00M sodium carbonate buffer and 1.00M dansyl chloride solutions were accurately added into 25 mL colorimetric tubes and mixed thoroughly. The derivative reactions began in dark for 2h at room temperature with shaking for 1 min in the first 1 h, and stop at 0.10 mL methylamine hydrochloride solution adding into the system. Keep the stock solutions in dark until the precipitations were complete. The supernatants were filtered for further application through 0.45 μm filter membrane. The derivatives can be protected in dark for 48 hours at 4 °C.

2.5 The calibration curve.
0.0 µg/mL, 5.00 µg/mL, 10.0 µg/mL, 20.0 µg/mL, 50.0 µg/mL, 100.0 µg/mL, 200.0 µg/mL, and 500.0 µg/mL of the calibration taurine were prepared and derived in accordance with the samples. The standard curve was drawn with the concentration of the standard taurine as the abscissa and the response peak area as the ordinate.

2.6 The instrument conditions and determination.
C₁₈ reversed-phase column (250mm×4.6mm, 5 µm) was adopted to detect the taurine. Sodium acetate buffer and acetonitrile were used as mobile phase with the volume ratio of 70 and 30, respectively. The flow rate was set as 1.0 mL/min and the injection volume 20 µL. The taurine was detect using UV detector at 254 nm.

2.7 Calculation formula.
The taurine in the samples was calculated as follows:

\[ X = \frac{C \times V}{m \times 1000} \times 100 \]  

In the formula (1), X, C, V and m denoted the content of taurine in the sample (mg/100 g), the concentration of taurine (µg/mL) calculated from the calibration curve, the constant volume (mL) and the weight of the aquatic product (g), respectively.

3. Results and discussion

3.1. Effects of the derivative reactions times
Figure 1 described the effects of the derivative reactions times. The results show that the peak areas of the derivative reaction time of 3 min (Figure 1 red points) were less than those of 2 h (Figure 1 black points), which indicated the samples were not derivated completely for 3 min. Hence, the time of 2 h was chosen as the derivative reactions in this work, which was consistent with the literature reports.

![Figure 1](image)

Figure 1. The effects of the derivative reactions times. Black points: 2h; red points: 3 min, respectively.

3.2. Influence of water addition.
Dry samples were different from the fresh samples, especially on the content of water, which could course the distinction of the obtained extraction solutions. It was list in table 1 that the results detection of taurine in dried sha-chong without adding water and adding water to restore fresh samples. As obviously shown in table 1, the measured values of taurine without adding water were less than those of adding water, which might be result that the dry samples absorbed some extraction solution. Meanwhile, the relative standard deviation (RSD) of the results adding water was 2.58 %, which was less than 9.55, suggested the more repeatability.
Table 1. The influence of water adding.

| Dried sha-chong | The determination values without adding water / μg/mL | The determination values adding water / μg/mL |
|-----------------|-----------------------------------------------------|---------------------------------------------|
| 1               | 187.2                                               | 360.0                                      |
| 2               | 215.3                                               | 375.0                                      |
| 3               | 226.0                                               | 377.8                                      |
| Average(mg/100g)| 209.5                                               | 370.9                                      |
| RSD (%)         | 9.55                                                | 2.58                                       |

3.3 Linear range, detection limit, recovery and precision of the method.
Each sample was tested third times in parallel to ensure the reliability of the results. The detail experiments were as follows. Low content of grilled fish were chosen as the blank samples, and 20 mg/100 g of taurine were added. 6 samples were measured in parallel with each concentration. Meanwhile, blank experiments were conducted at the same time.

The results were list in Table 2, which indicated the method display well linear with the taurine in the concentration range of 0.0~500.0 μg/mL (R²=0.9958). The recoveries were 82.5%~101%, and the average recover was 87.9% with the RSD of 7.56%. The precision of the method was 4.39%, which was calculated by the eleven times results of the blank samples in parallel. The limit of detection (LOD) is 3.74 ng/mL (S/N=3). The results show that the method presented the good accuracy and the favourable precision, and could be applied to detect the taurine in real samples.

Table 2. The calibration equations, linear range, precision, recovery and LOD of The method.

| Analyses | Linear range / μg/mL | The calibration equations | R² | Spiked /mg·kg⁻¹ | Recovery/ % | Precision /% | LOD /ng·mL⁻¹ |
|----------|-----------------------|----------------------------|----|----------------|-------------|--------------|--------------|
| Taurine  | 0.0 ~ 500.0           | y = 301.6173 + 35.2296x   | 0.9958 | 20.0 | 101,82.5,88.0,85.6,84.7,85.7 | 4.39 | 3.74 |

3.4 Detection of taurine in real samples
Figure 2 present the results of taurine detected by the established method in different samples. According to the data in the figure, it was observed that the fish collected in this paper had low of taurine, which was consistent with the repoted. However, the taurine in dried sha-chong (370.9 mg/100 g) was only lower than that in clam (420.9 mg/100 g), but higher than that in crap (226.8 mg/100 g), grasscarp (75.4 mg/100 g), crayfieh (79.4 mg/100 g) and prawn (65.0 mg/100 g), demonstrated that dried sha-chong was a food with high nutrient and beneficial to physical and mental health.

Figure 2. The taurine detected by the established method in different aquatic products.

4. Conclusion
In summary, we developed the determination method of taurine in dried sha-chong and other aquatic products based on HPLC. The pretreatment conditions of the approach were optimized, including the derivative reactions time and the water adding during the extraction to restore the dry sample to the fresh state. The results show that the derivative reactions time was 2 h and adding water during the extraction avoid the low value and increased the reproducibility. The obtained method revealed a good linearity in the taurine range 0.0–500.0 μg/mL (R^2 = 0.9958) with the limit of detection (LOD) 3.74 ng/mL, the precision of 4.39 % and the recovery rate of 82.5 %–101 %. In conclusion, the method was applied in detection of the taurine in dried sha-chong and other aquatic products involving the fish, shrimp, shellfish. The results demonstrated that dried sha-chong was a food with high nutrient and beneficial to physical and mental health.

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