

## ORIGINAL RESEARCH ARTICLE

# Mensuration of natural vitamin A in fish liver using liquid chromatography

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## ABSTRACT

To mensurate the content of vitamin A (vitamin A<sub>1</sub>, vitamin A<sub>2</sub>) in fish liver accurately, methods of sample pretreatment (including water bath saponification, room temperature saponification, and direct extraction) and detection methods (including normal phase chromatography and reversed phase chromatography) were screened and applied to the mensuration of vitamin A in the liver of 9 economic fish species. The results revealed that by reversed phase chromatography vitamin A<sub>1</sub> and vitamin A<sub>2</sub> were effectively divided, and showed good linear relationship within their respective linear ranges ( $R^2 > 0.99$ ). The content of vitamin A extracted by water bath saponification was distinctly higher than the content of Vitamin A by room temperature saponification and direct extraction ( $p < 0.05$ ). Its average recovery rates of vitamin A<sub>1</sub> and vitamin A<sub>2</sub> were respectively 104.52% and 90.94%. The total content of vitamin A in the livers of other freshwater fishes and marine fishes except for the freshwater snakehead and big mouth bass was more than 200  $\mu\text{g}/100\text{ g}$ , and the total content of vitamin A in the liver of marine giant grouper is 14,413.78  $\mu\text{g}/100\text{ g}$ , among the result was the highest. The water bath saponification method with reversed phase chromatography has good precision and is suitable for the mensuration of vitamin A in fish liver.

**Keywords:** fish; liver; vitamin A; extraction method; detection method

Vitamin A (V<sub>A</sub>) is an essential fat soluble vitamin for human body, which can maintain vision. Epithelial tissue. Immunity and other normal physiological functions will lead to night blindness when the human body lacks V<sub>A</sub>. Xerophthalmia, Growth retardation, Immune decline, etc.<sup>[1,2]</sup>. V<sub>A</sub> deficiency is a global health

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problem. About 250 million preschool children lack  $V_A$ <sup>[3]</sup>.  $V_A$  is divided into natural  $V_A$  and synthetic  $V_A$ , and natural  $V_A$  mainly exists in the liver, Dairy, In egg yolk and red yellow plants, synthetic  $V_A$  is mostly used to apply ointment.  $V_{AD}$  oil and other products, but synthetic  $V_A$  has some potential safety hazards, which is easy to lead to hyperlipidemia, Pseudotumor, Eyelid conjunctivitis, etc.<sup>[4,5]</sup>. Natural  $V_A$  includes retinol ( $V_{A1}$ ) and dehydrogenated retinol ( $V_{A2}$ ). Their structures are similar, and the only difference is that  $V_{A2}$  has a conjugated double bond on the 3, 4 carbon bond.  $V_{A1}$  is commonly found in mammals and marine fish, while  $V_{A2}$  is mostly found in freshwater fish<sup>[6]</sup>. The  $V_A$  content in fish liver is rich and economically available, which is an excellent raw material for obtaining natural  $V_A$ <sup>[7]</sup>.

$V_A$  itself is a trace element and its nature is unstable, so it is easy to be exposed to light. Hot. Oxygen. Acid. Alkali damage<sup>[8,9]</sup>, so it is particularly important to choose an extraction method that can accurately reflect the real content of the sample. At present,  $V_A$  extraction methods mainly include saponification method and direct extraction method. Saponification method can be divided into room temperature saponification method and water bath saponification method. Water bath saponification method has a wide range of applications, but the operation is cumbersome and the temperature is high, which may cause certain losses to  $V_A$ . Room temperature saponification method has a mild effect, but it takes a long time; the direct extraction method has shorter extraction time and simpler operation.

The detection method of  $V_A$  includes ultraviolet method. Fluorescence method and liquid chromatography, in which UV method and fluorescence method have low sensitivity and cumbersome operation, and liquid chromatography is the main method to detect  $V_A$  at present. Liquid chromatography is also divided into normal phase chromatography and reverse phase chromatography. Normal phase chromatography allows more fat in the sample, and its mobile phase can also have a large span of polarity range<sup>[10]</sup>. It is stipulated in the Pharmacopoeia 2010 that normal phase chromatography is used to determine CIS and trans  $V_A$  in drugs<sup>[11]</sup>, and the research results of Rebeca and Suana<sup>[12]</sup> show that normal phase chromatography can well separate  $V_A$  isomers with good resolution. Reversed phase chromatography has higher stability<sup>[13]</sup>, which is often used for the analysis and determination of  $V_{A1}$  content. At present, there are few studies on  $V_{A2}$ <sup>[14-16]</sup>. Due to the unstable nature of  $V_{A2}$ , now most research methods only take  $V_{A1}$  content as the total  $V_A$  content, which may cause the test results to be inconsistent with the actual results and cannot truly reflect the  $V_A$  content of the sample. In order to correctly evaluate the  $V_A$  content in fish liver, this study compared normal phase chromatography with reverse phase chromatography, and water bath saponification method with grouper liver as raw material. Room temperature saponification method. Three sample pretreatment methods of direct extraction were compared, and the best sample pretreatment was selected. Detection methods, and compared the types and total content of  $V_A$  in the liver of 9 economic fish, in order to provide preliminary basic data for the development and utilization of new natural  $V_A$  resources.

## 1. Materials and methods

### 1.1. Test materials

#### 1.1.1. Raw materials and reagents

A basket of fish was purchased from the Zhanjiang Xiashan wholesale market, including Grass Carp, Snakehead, Gold Pomfret, Variegated Carp, Pearl Gentian Grouper, Tilapia Mossambica, Largemouth Bass, and Longpont Grouper liver. After the fish was killed, the liver was transported back to the laboratory with ice cubes to remove the surface fat and connective tissue. After being washed with precooled normal saline, the surface water was sucked dry with kitchen paper towels. After homogenization, it was immediately frozen in an ice box of -8 °C.

Ascorbic acid, anhydrous sodium sulfate, potassium hydroxide, petroleum ether, and 2,6-Ditert-butyl-p-cresol (BHT) are all analytically pure and obtained from Shantou Xilong Company. Anhydrous ethanol (analytical purity) is from Tianjin Kemio Company, and methanol is chromatographically pure.  $V_{A1}$  standard (purity  $\geq 95\%$ ), sigma company of the United States;  $V_{A2}$  standard (purity  $\geq 93\%$ ), TRC Canada; isopropyl alcohol (chromatographic purity), Shanghai Aladdin company; N-hexane (chromatographic purity), thermofisher company, USA.

### 1.1.2. Instruments and equipment

E<sub>2</sub>695 high performance liquid chromatograph (equipped with 2489 UV detector), waters, USA; fa<sub>2</sub>004 analytical balance, Shanghai Sunny company; N<sup>-1</sup>300 rotary evaporators, Shanghai Ailang company; SHZ-B constant temperature water bath oscillator, Shanghai Xunbo company; MGS<sup>-2</sup>200h nitrogen blower, Shanghai Elang company; ultrasonic cleaning machine, Kunshan ultrasonic company; Z<sup>-1</sup>6kl centrifuge, sigma company of the United States; D<sup>-2</sup>4uv clear ultra pure water all-in-one machine, German Merck company; zls<sup>-3</sup> vacuum centrifugal concentrator, Hunan Hexi company.

## 1.2. Test method

### 1.2.1. Preparation of VA standard solution

25 mg  $V_{A1}$  standard and 2.5 mg  $V_{A2}$  standard were dissolved in 25 mL and 5 mL absolute ethanol respectively to make standard stock solution. Before use, the concentration of  $V_{A1}$  standard stock solution was corrected according to Appendix B of gb<sub>5</sub>009.82-2016. After correction, the mass concentration of  $V_{A1}$  standard stock solution was 1.29 mg/mL.  $V_{A2}$  did not correct its concentration because there was no reference method. Draw 0.25 mL  $V_{A1}$  standard stock solution and 0.4 mL  $V_{A2}$  standard stock solution respectively into the same 10 mL volumetric flask, fix the volume with methanol, and prepare a mixed standard intermediate solution. Accurately absorb 0.1 respectively. 0.25. 0.5. 1.0. 2.0. Put 3.0 mL mixed standard intermediate solution into 5 mL Brown volumetric flask, fix the volume with methanol to the scale, and prepare  $V_{A1}$  and  $V_{A2}$  series mixed standard working solutions.

### 1.2.2. Sample pretreatment (VA extraction)

Because  $V_A$  is unstable, use brown bottles and glass bottles wrapped with tin foil during operation, and try to avoid light exposure.

#### Water bath saponification method

Referring to gb<sub>5</sub>009.82-2016, accurately weigh 1.6 g of grouper liver in a conical flask, add 20 mL of water, mix well, add 1.0 g of ascorbic acid and 0.1 g BHT as antioxidants, add 30 mL of absolute ethanol, and then add a certain amount of potassium hydroxide solution with a mass fraction of 50% as saponification solution, mix well, put a cork on the back cover, oscillate in a constant temperature water bath at 80 °C, saponify for 30 min, and immediately cool with ice water after saponification. Transfer the saponified matter into a 250 mL Brown separating funnel with 30 mL water, add petroleum ether to vibrate and extract for 5 min, move the lower aqueous phase to another separating funnel for the second extraction, combine the ether phase and wash it to neutral, then filter it into a 1 L rotary evaporation bottle through 3G anhydrous sodium sulfate, concentrate it on the rotary evaporator until it is nearly dry, dissolve the residue in fractions with methanol, dilute the  $V_A$  content extracted by different pretreatment methods and the liver of different fish to 10 mL, and pass 0.22  $\mu$ m filter membrane to be tested.

#### Room temperature saponification method

Refer to the method of Liu et al.<sup>[17]</sup> and make some changes. Accurately weigh 1.6 g of grouper liver of dragon pontoon into a conical flask, add 20 mL of water, 1.0 g of ascorbic acid and 0.1 g BHT, add 30 mL of

absolute ethanol, and then add a certain amount of potassium hydroxide solution with a mass fraction of 50% as saponification solution. After mixing, purge it under nitrogen flow for 5 min, plug the bottle mouth with a rubber soft plug, and oscillate overnight. After being extracted with petroleum ether, wash it with water to neutral, concentrate it with rotary evaporator, and then fix the volume with methanol to 10 mL, and pass 0.22  $\mu\text{m}$  filter membrane to be tested.

#### Direct extraction method

Refer to the method of Nimalaratne et al.<sup>[18]</sup> with slight changes. Accurately weigh 1.6 g of grouper liver of dragon pontoon into a flat bottom flask, add 20 mL of n-hexane, then add 1.0 g of ascorbic acid and 0.1 g BHT, mix well, and then conduct ultrasonic treatment (ultrasonic power is 240 W, ultrasonic frequency is 40 KHz) for a certain time. Then centrifuge at 5000 r/min for 10 min, take the supernatant, spin it on the vacuum centrifugal concentrator at 25 °C to dry, fix the volume with methanol to 10 mL, and pass 0.22  $\mu\text{m}$  filter membrane to be tested.

### 1.2.3. Detection conditions of VA by liquid chromatography

$V_A$  was detected by reversed phase chromatography and normal phase chromatography respectively. Reversed phase chromatography condition: watersbehc<sub>18</sub> column (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ); the mobile phase is methanol water (volume ratio 96:4); flow rate 0.8 mL/min; injection volume 10  $\mu\text{L}$ . Normal phase chromatography conditions: phenomenxlunasilica chromatographic column (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ); the mobile phase is n-hexane isopropanol (volume ratio 99.7:0.3); flow rate 1 mL/min; injection volume 10  $\mu\text{L}$ .

### 1.2.4. Standard curve drawing and sample detection

Put the mixed standard working solution of  $V_{A1}$  and  $V_{A2}$  series into liquid chromatography for detection, and draw the standard curve with the mass concentration ( $x$ ) of  $V_{A1}$  and  $V_{A2}$  as the abscissa and the corresponding peak area ( $y$ ) as the ordinate. Inject the sample solution into the liquid chromatography for detection, get the peak area, and then get the mass concentration of  $V_{A1}$  and  $V_{A2}$  according to the standard curve, and calculate the  $V_A$  content according to the following formula.

$$X_1 = \rho \times V \times f \times 100/m \quad (1)$$

$$X_2 = (\rho \times V \times f \times 100/m) \times 0.4 \quad (2)$$

$$X_3 = X_1 + X_2 \quad (3)$$

where:  $X_1$ ,  $X_2$ ,  $X_3$  is  $V_{A1}$  respectively.  $V_{A2}$  content and total  $V_A$  content,  $\mu\text{g}/100 \text{ g}$ ;  $\rho$  is the mass concentration of  $V_A$  in the sample calculated according to the standard curve,  $\mu\text{g}/\text{mL}$ ;  $V$  is the constant volume, mL;  $f$  is the conversion factor (the conversion factor of  $V_A$  is 1);  $m$  is the mass of the sample, g; 0.4 is the  $V_{A2}$  conversion coefficient, and the  $V_{A2}$  activity is 40% of  $V_{A1}$ , which is converted during calculation<sup>[19]</sup>.

### 1.2.5. Data processing

$V_A$  content is expressed by “mean  $\pm$  standard deviation” ( $n = 3$ ). The  $V_A$  content extracted by different pretreatment methods and the  $V_A$  content in the liver of different fish were analyzed by one-way ANOVA. If the difference was significant, Duncan method was used for multiple comparisons, and  $P < 0.05$  was used as the judgment standard for the significance of the difference.

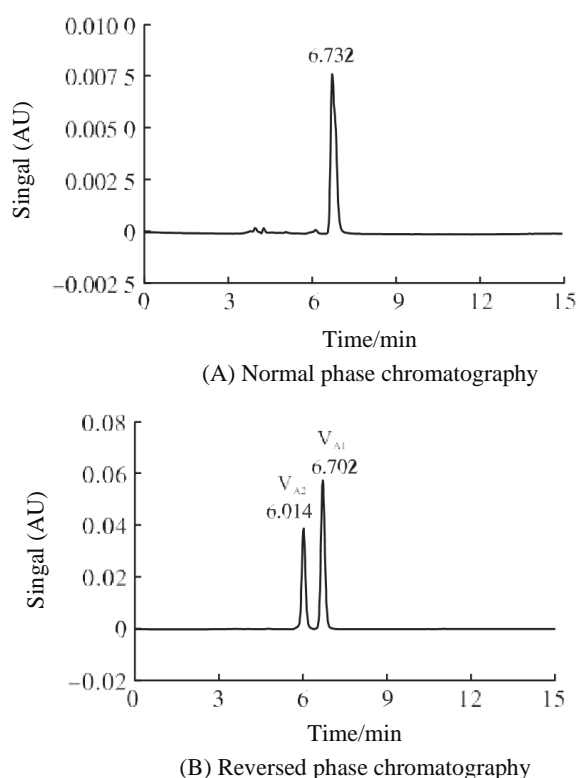
## 2. Results and analysis

### 2.1. Establishment of VA detection method

$V_{A2}$  plays the same important role in visual regulation as  $V_{A1}$ . The mutual transformation of  $V_{A1}$  and  $V_{A2}$  is an adaptive response of animals to light changes.  $V_{A1}$  has the maximum absorption peak at 325 nm,  $V_{A2}$  has the maximum absorption peak at 350 nm, and  $V_{A2}$  is unstable. The change is fast, so 350 nm is taken as the

common measurement wavelength of both<sup>[14]</sup>. In order to more accurately refer to the method of Nimalaratne et al.<sup>[18]</sup>, with slight changes. Accurately weigh 1.6 g of grouper liver of dragon pontoon into a flat bottom flask, add 20 mL of n-hexane, then add 1.0 g of ascorbic acid and 0.1 g BHT, mix well, and then conduct ultrasonic treatment (ultrasonic power is 240 W, ultrasonic frequency is 40 KHz) for a certain time. Then the  $V_A$  content in the sample was determined by centrifugation at 5000 r/min. In this study, normal phase chromatography and reverse phase chromatography were compared.

Mix  $V_{A1}$  and  $V_{A2}$  with standard working solution ( $V_{A1}$  mass concentration is 12.9  $\mu\text{g/mL}$ ,  $V_{A2}$  mass concentration is 8  $\mu\text{g/mL}$ ) were detected by normal phase chromatography and reverse phase chromatography, and the obtained liquid chromatogram is shown in **Figure 1**.



**Figure 1.** Liquid chromatogram of mixed standard solution in normal phase and reverse phase chromatography.

It can be seen from **Figure 1** that only one single peak can be detected by normal phase chromatography, and  $V_{A1}$  cannot be separated. Separate  $V_{A2}$  and replace the mobile phase. The mobile phase ratio and flow rate cannot be separated; and reverse phase chromatography,  $V_{A1}$ ,  $V_{A2}$  separation is in good condition.

Add 19.35  $\mu\text{g/mL}$   $V_{A1}$  and 12  $\mu\text{g/mL}$   $V_{A2}$  mixed standard solution was continuously injected for 6 needles, and the stability of the test method was expressed by relative standard deviation (RSD). The results showed that the average RSD of reverse phase chromatography was 0.38%, which was less than 4.48% of normal phase chromatography, indicating that the stability of reverse phase chromatography was higher. Therefore, reversed phase chromatography was selected as the method to detect  $V_{A1}$  and  $V_{A2}$  in this study. Using reversed-phase chromatography, draw the standard curve according to 1.2.4, and the results are shown in **Figure 2**. It can be seen from **Figure 2** that the linear equations of  $V_{A1}$  and  $V_{A2}$  are  $y = 29,100x - 5515$  and  $y = 29,100x - 5515$  and  $y = 87,906x - 7591.5$  respectively, and the correlation coefficients ( $R^2$ ) are 0.9998 and 0.9999 respectively, which both have a good linear relationship in the range of 0.645 ~ 19.35  $\mu\text{g/mL}$  and 0.4 ~ 12  $\mu\text{g/mL}$ , respectively.

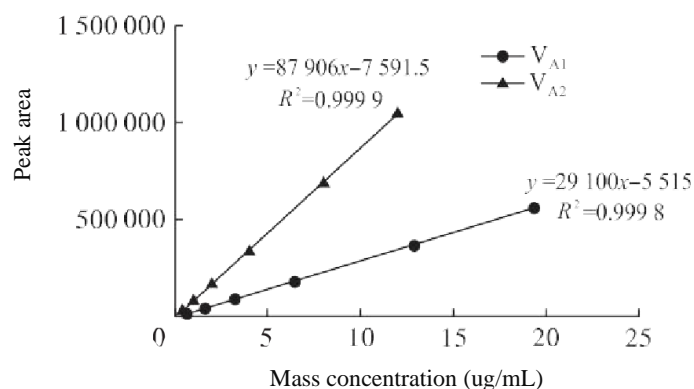


Figure 2. Standard curve of  $V_{A1}$ ,  $V_{A2}$ .

## 2.2. Determination of sample pretreatment method

### 2.2.1. Determination of extraction conditions for three sample pretreatment methods

#### Water bath saponification method

Saponification method is to remove the coexisting lipid compounds in the sample by saponification reaction between alkali and oil, convert  $V_A$  esters into free  $V_A$ , and separate the free  $V_A$  wrapped by oil for determination. Potassium hydroxide solution with a mass fraction of 50% is suitable for the saponification needs of most samples<sup>[20]</sup>. The increase of the amount of alkali is conducive to complete saponification. If the amount of alkali added is insufficient, the saponification reaction is not complete, which will cause the determination result to be low. However, if too much alkali is added, a large amount of water is required to wash it to neutral in the water washing process, resulting in waste. Moreover,  $V_A$  is a sensitive component, and too much alkali will cause certain oxidative damage to  $V_A$ . The  $V_A$  in the liver of grouper was extracted by water bath saponification with different dosage of potassium hydroxide (mass fraction 50%), and the total  $V_A$  content was detected by reversed-phase chromatography. The results are shown in **Figure 3**.

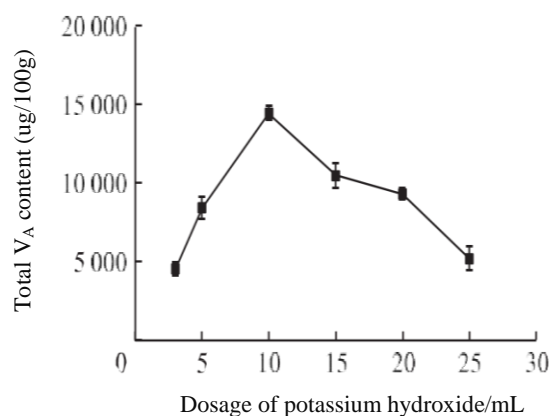


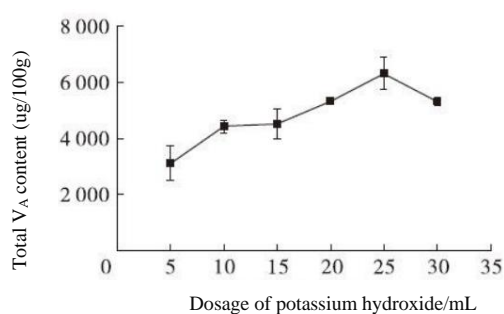
Figure 3. Effect of potassium hydroxide dosage on water bath saponification method.

It can be seen from **Figure 3** that with the increase of the dosage of 50% potassium hydroxide, the total  $V_A$  content first increased and then decreased. When the dosage of 50% potassium hydroxide was 10 mL, the total  $V_A$  content reached the maximum. Then, with the increase of the dosage of 50% potassium hydroxide, the total  $V_A$  content began to decrease, which may be because too much alkali was added, which accelerated the oxidative damage of free  $V_A$ . Therefore, in the water bath saponification method, the dosage of 50% potassium hydroxide is 10 mL.

### Room temperature saponification method

Using room temperature saponification method,  $V_A$  in the liver of grouper was extracted with different amounts of potassium hydroxide (mass fraction 50%), and the total content of  $V_A$  was detected by reverse phase chromatography. The results are shown in **Figure 4**.

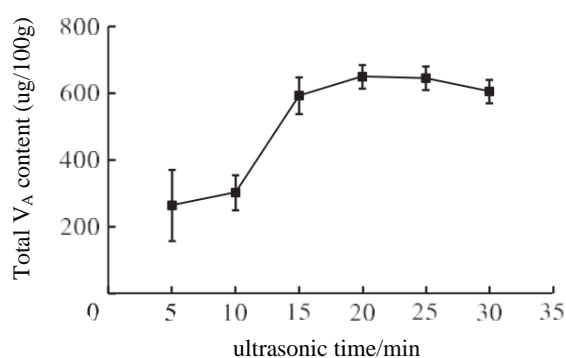
It can be seen from **Figure 4** that with the increase of the dosage of 50% potassium hydroxide, the total  $V_A$  content showed a trend of gently increasing at first and then decreasing. When the dosage of 50% potassium hydroxide was 25 mL, the total  $V_A$  content reached the maximum value, and then increased the dosage of potassium hydroxide, and the total  $V_A$  content decreased. Therefore, in the room temperature saponification method, the dosage of 50% potassium hydroxide is 25 mL.



**Figure 4.** Effect of potassium hydroxide dosage on room temperature saponification method.

### Direct extraction method

The direct extraction method uses the cavitation effect of ultrasound to assist the extraction of organic solvents, so as to accelerate the release of substances in cells<sup>[21]</sup>. If the ultrasonic time is too short, the  $V_A$  extraction effect is not good, and if the ultrasonic time is too long, it will cause a waste of energy. Using direct extraction method,  $V_A$  in the liver of grouper was extracted under different ultrasonic time, and the total content of  $V_A$  was detected by reversed-phase chromatography. The results are shown in **Figure 5**.



**Figure 5.** Effect of ultrasonic time on direct extraction method.

It can be seen from **Figure 5** that with the extension of ultrasonic time, the total  $V_A$  content method showed a trend of rising first and then stable. The total  $V_A$  content was the highest at 20 min, but there was no significant difference with the total  $V_A$  content at 15 min. Therefore, the ultrasonic time was selected as 15 min.

### 2.2.2. Comparison of precision of different extraction methods

According to the conditions determined in 2.2.1, three sample pretreatment methods were used to extract  $V_A$  from the liver of grouper, and the total content of  $V_A$  was detected by reversed-phase chromatography. The

samples were injected four times in a day, and the precision was measured for three days. The relative standard deviation (RSD) was used to express the precision (the greater the RSD, the smaller the precision). The intra day RSD and intra day RSD of different extraction methods were calculated, and the results are shown in **Table 1**.

**Table 1.** RSD comparison of three sample pretreatment methods (%).

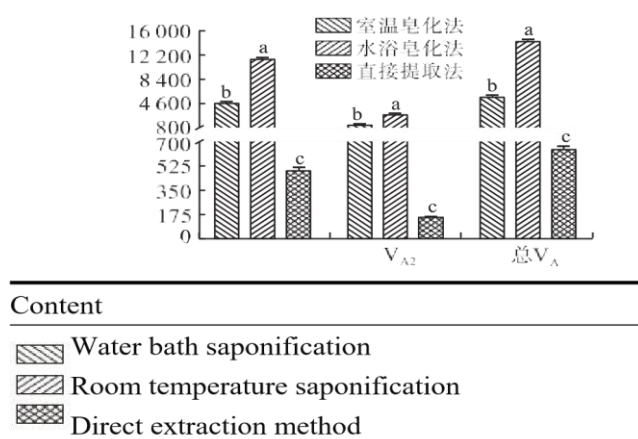
Pretreatment method	Average intraday RSD	Average daytime RSD
Water bath saponification room	1.07	10.64
Temperature saponification direct	1.39	2.77
Extraction method	6.91	10.44

It can be seen from **Table 1** that the daily RSD of water bath saponification method and room temperature saponification method are 1.07% and 1.39% respectively, and the difference between these two extraction methods is small, while the difference with the daily RSD of direct extraction method (6.91%) is large; the daytime RSD of room temperature saponification method is the smallest, which is 2.77%, while the daytime RSD of water bath saponification method and direct extraction method is larger. This may be because the reaction temperature of room temperature saponification method is relatively mild, while the reaction temperature of water bath saponification method is higher,  $V_A$  is unstable and sensitive to temperature, and the longer the storage time is, the more  $V_A$  loss is caused, while the direct extraction method is prone to emulsification in the ultrasonic process, resulting in the instability of the whole system, which has a certain impact on the detection, thus making its RSD larger, which is consistent with the research results of Jiang et al.<sup>[22]</sup>.

The intraday RSD of the three sample pretreatment methods is less than 10%, and the intraday RSD is less than 15%, which meets the requirements of the analytical method<sup>[23]</sup>.

### 2.2.3. Comparison of $V_A$ content with different extraction methods

According to the conditions determined in 2.2.1, three sample pretreatment methods were used to extract  $V_A$  from the liver of grouper, and the content of  $V_A$  was detected by reversed-phase chromatography. The results are shown in **Figure 6**.



**Figure 6.** Comparison of  $V_A$  content in the liver of longpont with three pretreatment methods.

Note: different letters indicate that there are significant differences between different methods in the same category,  $P < 0.05$ .

It can be seen from **Figure 6** that among the three sample pretreatment methods, water bath saponification method  $V_{A1}$ . The content of  $V_{A2}$  and total  $V_A$  were significantly higher than that of saponification and direct



extraction at room temperature ( $p < 0.05$ ), while that of saponification at room temperature  $V_{A1}$ . The content of  $V_{A2}$  and total  $V_A$  were significantly higher than that of direct extraction ( $p < 0.05$ ).

The results of this study showed that the direct extraction method had the lowest  $V_A$  content. Different extraction methods are applicable to different test objects, although the direct extraction method is effective on mink kidney<sup>[12]</sup>. The extraction effect of  $V_A$  from milk powder<sup>[18]</sup> and vegetable oil<sup>[22]</sup> is good, but according to the results of this study, this method is not suitable for the extraction of  $V_A$  from the liver of grouper. The room temperature saponification method has no heating treatment, the effect is more mild, and the reaction rate is also low. The fat content of this research object is high, so the saponification reaction time is longer at room temperature. However, long-term exposure to alkaline environment has caused damage to  $V_A$ , which may be the reason why the  $V_A$  content of room temperature saponification method is lower than that of water bath saponification method. After comparative analysis, combined with the test results in **Table 1**, the water bath saponification method is selected to pretreat the samples.

#### 2.2.4. Recovery rate of water bath saponification method

To homogenize the liver of grouper longpont, press low.  $V_{A1}$  is added at medium and high levels.  $V_{A2}$  standard is pretreated by water bath saponification method, and then the  $V_A$  content is detected by reversed-phase chromatography to calculate the spiked recovery. The results are shown in **Table 2**.

**Table 2.** Standard addition recovery rate.

VA	Content in sample/ $\mu\text{g}$	Scalar addition/ $\mu$	Measured value/ $\mu\text{g}$	Recovery rate/%	Average recovery/%
$V_{A1}$	11.73	4.69	16.62	14.26	14.52
	11.45	6.87	18.21	98.4	
	11.59	9.27	21.87	11.9	
$V_{A2}$	2.82	1.16	3.79	83.62	90.94
	2.90	1.64	4.37	89.63	
	2.74	2.26	4.99	99.56	

It can be seen from **Table 2** that the recovery rate of  $V_{A1}$  standard addition is between 98.40% and 110.90%, and that of  $V_{A2}$  standard addition is between 83.62% and 99.56%. The recovery rate is good. Based on the above results, this study chose the water bath saponification method to extract  $V_A$ .

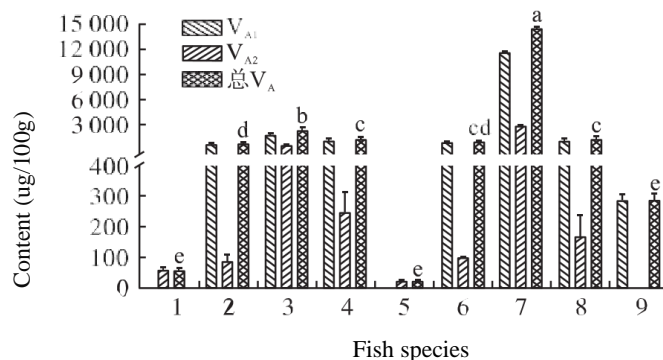
Because the RSD of the water bath saponification method is relatively large during the day, it needs to be determined as soon as possible on the day of extraction.

#### 2.3. Comparison of $V_A$ content in liver of 9 economic fish species

A basket of nine common economic fish was studied, including Grass Carp, Snakehead, Gold Pomfret, Variegated Carp, Pearl Gentian Grouper, Longpont Grouper, Tilapia Mossambica, and Largemouth Bass. Among these, Grass Carp, Snakehead, Variegated Carp, Tilapia Mossambica, and Largemouth Bass are freshwater fish, while the others are saltwater fish. The liver of these fish was pretreated by water bath saponification method, and then the  $V_A$  content was detected by reversed-phase chromatography. The results are shown in **Figure 7**.

It can be seen from **Figure 7** that among the nine kinds of fish, the total content of  $V_A$  in the liver of grouper is 14,413.78  $\mu\text{g}/100\text{ g}$ , significantly higher than other fish ( $p < 0.05$ ). The total content of  $V_A$  in silver carp liver was 2337.36  $\mu\text{g}/100\text{ g}$ , lower than grouper, but significantly higher than other fish ( $p < 0.05$ ). The total content of  $V_A$  in the liver of Pearl gentian grouper and tilapia was 1265.13 respectively. One thousand two hundred and eighty-seven point eight five  $\mu\text{g}/100\text{ g}$ , significantly higher than that of snakehead. Grass

Carp. The total content of  $V_A$  in the liver of golden pomfret was 1019.71  $\mu\text{g}/100\text{ g}$ , the total  $V_A$  content in Grass carp liver is 765.03  $\mu\text{g}/100\text{ g}$ , both of which are significantly higher than that of snakehead. Big mouth bass and point basket fish ( $P < 0.05$ ), snakehead. Compared with other fish, the total content of  $V_A$  in the liver of largemouth bass and point basket fish was 57.01, respectively, 23.02. Two hundred and eighty-seven point five six  $\mu\text{g}/100\text{ g}$ . The liver of snakehead and Largemouth Bass only contains  $V_{A2}$ , that of basket fish only contains  $V_{A1}$ , and that of the other six species of fish all contain  $V_{A1}$ ,  $V_{A2}$  two types of  $V_A$ .



**Figure 7.** Comparison of  $V_A$  content in liver of 9 economic fishes.

Note: 1) Snakehead; 2) Grass Carp; 3) Variegated carp; 4) Tilapia mossambica; 5) Largemouth Bass; 6) Gold Pomfret; 7) Grouper with dragon pontoon; 8) Pearl gentian grouper; 9) Order a basket of fish. The total  $V_A$  content was analyzed, and different letters showed significant differences,  $P < 0.05$ .

In this study, the liver of freshwater fish contains  $V_{A2}$ . Generally speaking,  $V_{A2}$  mostly exists in freshwater fish, but with the deepening of research, it is found that  $V_{A2}$  also exists in many seawater fish. In this study, four species of marine fish were determined, including Golden Pomfret, Grouper with dragon pontoon,  $V_{A2}$  exists in the liver of three kinds of marine fish, pearl gentian grouper. Kondrashev and Lamash<sup>[24]</sup> also found  $V_{A2}$  with a high proportion in the bodies of two kinds of marine fish, hexagramma bifida and sardines. Studies have shown that fat soluble vitamins cannot be excreted from the body through body fluids and are easy to accumulate in the body. The content of  $V_A$  in the liver increases with the increase of  $V_A$  level in the diet, and the two are significantly linearly and positively correlated<sup>[25]</sup>. The nine kinds of fish selected in this study are all artificially raised. Among these kinds of fish, the grouper is the largest individual, the longest breeding time, and the  $V_A$  content accumulated in the body is also the highest. The types and contents of  $V_A$  in the liver of different fish are different from those of fish. Individual size. Their own diet and feeding management level are related to many factors<sup>[26]</sup>.

### 3. Conclusion

In this paper, the content of  $V_A$  in fish liver was determined by screening the sample pretreatment methods and detection methods of  $V_A$  extraction in fish liver. It was determined that water bath saponification method could extract  $V_A$  from fish liver at most, and reverse phase chromatography was used to detect and separate  $V_{A1}$ ,  $V_{A2}$  is in good condition. With high stability, water bath saponification combined with reversed-phase chromatography is suitable for the analysis and determination of  $V_A$  in fish liver.  $V_A$  is abundant in the liver of fish. Among the nine fish species tested, the total content of  $V_A$  in the liver of other fish species, except snakehead and largemouth bass, reached 200  $\mu\text{g}/100\text{ g}$ , of which the total content of  $V_A$  in the liver of sea water fish Dragon Grouper is the highest, reaching 14,413.78  $\mu\text{g}/100\text{ g}$ . The research results provide preliminary basic data for the development of new natural  $V_A$  resources.

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## Conflict of interest

The authors declare no conflict of interest.

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