Notice

DESCRIPTION CN115124593A

A method for synthesizing high-purity GHK-Cu

[0001]

Technical Field

[n0001]

This invention relates to the synthesis of glycylhistyllysine copper, and particularly to a method for synthesizing high-purity glycylhistyllysine copper, especially to a method for synthesizing glycylhistyllysine copper crystals.

[0003]

Background Technology

[n0002]

Glycylhistyllysine (GHK) is an endogenous collagen tripeptide isolated from human plasma. It has a strong affinity for divalent copper ions and can spontaneously form a complex with them, GHK-Cu.

The composition of GHK-Cu is: glycyl-L-histidyl-L-lysine–copper. The copper ion Cu²⁺ is not yellow like copper metal, but appears blue in aqueous solution, so GHK-Cu is also known as blue copper peptide.

According to current research results, its main functions include: effectively promoting collagen production, increasing angiogenesis and antioxidant capacity, stimulating the production of glucosamine, helping the skin restore its self-repair ability; and promoting the growth and differentiation of epithelial cells, thereby accelerating wound healing.

In terms of its role in skin tissue, it has antioxidant properties, promotes collagen production, and assists in wound healing. Scientists have discovered that the wrinkle-reducing effect of copper ions is mainly due to the transport of divalent copper ions with biochemical effects into cells through the amino acid complex (peptide), thereby exerting physiological functions. However, how to synthesize high-purity GHK-Cu more efficiently is a challenge.

[n0003]

The application number is 201810868124.

X's Chinese application discloses a method for directly reacting the product obtained by freeze-drying the purified crude GHK peptide with copper acetate in a 1:1 or 2:1 ratio to generate GHK-Cu. The disadvantages of this method are that the content of acetate ions in the reaction with copper acetate is difficult to control, which affects the yield. In addition, this method is relatively complicated, costly, and not suitable for mass production.

[n0004]

Chinese patent application No. 201711221991.6 discloses a low-cost method for synthesizing GHK acetate, which involves deprotecting Tit-Gly-His(Tit)-Lys(Trt)-OH in acetic acid and triisopropylsilane to generate GHK acetate.

Although this method can reduce the cost of producing GHK acetate, an excess of acetate ions in the synthesis of GHK-Cu can prevent GHK-Cu from crystallizing.

[n0005]

Chinese patent application No. 201310751827.1 discloses a method for synthesizing GHK tripeptide. Specifically, the method involves synthesizing Boc-Gly-His(Boc)-LYS(Ac)-OH, followed by deprotection with trifluoroacetic acid to obtain trifluoroacetate of GHK, and then generating GHK acetate by reversed-phase chromatography and ion-exchange chromatography.

The disadvantage of this method is that the deprotected trifluoroacetate needs to be purified by reversed-phase chromatography to remove trifluoroacetic acid from the product, and then converted into acetate by ion exchange chromatography. This method is costly and not suitable for large-scale production.

[n0006]

Existing GHK-Cu production technologies basically utilize the reaction of GHK acetate with copper acetate at room temperature to obtain the product. This method is prone to affecting the yield of GHK-Cu because the acetate content in the reaction system is relatively high and the acetate ion content is difficult to control.

[n0007]

As can be seen from the above, in the process of synthesizing GHK-Cu from existing GHK products, it is difficult to efficiently synthesize high-purity GHK-Cu, especially GHK-Cu crystallization, due to the interference of anions (such as acetate and trifluoroacetate) brought by the salts carried in the GHK products.

[0010]

Summary of the Invention

[n0008]

The purpose of this invention is to provide a method for synthesizing high-purity glycylhistyllysine copper (GHK-Cu). This method utilizes anion exchange resin to remove anions from GHK products (such as GHK acetate, GHK trifluoroacetate, etc.) brought by salts, and then uses recrystallization to efficiently synthesize GHK-Cu products with high purity and full crystal particles.

[n0009]

Therefore, the present invention provides a method for synthesizing high-purity GHK-Cu, comprising the following steps:

[n0010]

S1. Add copper salt to the solution of GHK product, stir evenly to obtain a mixed solution, wherein the GHK product contains by-product anions;

[n0011]

S2. Add the mixed solution to the anion exchange resin for reaction, and then filter to obtain the mixed solution after reaction;

[n0012]

S3. After concentrating the mixed solution after the reaction, add an aqueous ethanol solution and heat to 40°C～70°C to dissolve by ultrasonication to obtain a redissolved solution;

[n0013]

S4. Cool the redissolved solution to obtain GHK-Cu crystals.

[n0014]

In step S1, preferably, the copper salt is selected from at least one of copper acetate, copper chloride, copper sulfate and copper carbonate, and more preferably, the copper salt is copper acetate and/or copper chloride.

[n0015]

In step S1, preferably, the molar ratio of GHK to copper salt in the solution of the GHK product is 1 to 2:1.

[n0016]

In step S1, the byproduct anion is at least one of trifluoroacetate, acetate, hydrochloride, phosphate and citrate anions, with the most common byproduct anions being at least one of trifluoroacetate, acetate and hydrochloride anions.

[n0017]

In step S1, preferably, the concentration of GHK in the solution of the GHK product is 50 mg /ml-500 mg/ml, and more preferably, 100 ± 20 mg/ml.

[n0018]

In step S2, preferably, the anion exchange resin is a strong base anion exchange resin.

[n0019]

In step S2, preferably, the anion exchange resin has been pretreated, and the pretreatment step includes: washing the anion exchange resin with alkali and then washing it until neutral.

[n0020]

In step S3, preferably, the concentration involves removing 50wt%-100wt% of the solvent in the mixed solution by vacuum evaporation.

[n0021]

In step S3, preferably, the concentration of ethanol in the aqueous ethanol solution is 65% -85% by volume, preferably 78% by volume.

[n0022]

In step S3, preferably, the ethanol-water solution is heated to 55°C.

[n0023]

In step S4, preferably, the redissolved solution is cooled to ≤40°C, and the cooling is performed in stages, specifically including: first cooling to 20-30°C, and then cooling to 2-8°C.

[n0024]

The beneficial effects of this invention are as follows:

[n0025]

(1) The method of the present invention uses anion exchange resin to desalt GHK products by removing anions. After removing anions, it is beneficial for copper salt to complex with GHK to synthesize GHK-Cu crystals. Furthermore, combined with the principle of recrystallization, GHK-Cu crystals are obtained by heating and dissolving in an ethanol aqueous solution and then cooling. Therefore, the GHK-Cu product obtained by the method of the present invention has high purity, high yield and convenient crystallization. At the same time, the present invention is simple to operate, low in cost and high in efficiency.

[n0026]

(2) The method of the present invention broadens the sources of GHK products, which can be GHK acetate or GHK trifluoroacetate, etc. Furthermore, the content of anions in GHK products no longer affects the yield of GHK-Cu, which is conducive to further reducing the cost and industrialization of the method.

[n0027]

(3) The method of the present invention utilizes the addition of an ethanol solution with a concentration of 65%-85% by volume and the principle of recrystallization to precipitate GHK-Cu from the ethanol solution to obtain a high-purity GHK-Cu product.

Using an ethanol solution with a concentration of 65%-85% not only allows the precipitated GHK-Cu crystals to have larger particle size, higher yield, and higher purity, but also helps to reduce costs.

[n0028]

(4) The method of the present invention raises the temperature of a 65-85% volume ethanol solution to 40-70°C, which accelerates the dissolution rate and solubility of GHK-Cu products in ethanol and improves the purity and yield of the products.

If heating is not used, the solubility of GHK-Cu in ethanol will decrease, which will hinder recrystallization, reduce the yield, or make it difficult to obtain GHK-Cu crystals.

[0032]

Detailed Implementation

[n0029]

The following provides a detailed description of the embodiments of the present invention: These embodiments are implemented based on the technical solution of the present invention, and provide detailed implementation methods and processes. However, the scope of protection of the present invention is not limited to the following embodiments. Experimental methods in the following embodiments that do not specify specific conditions are generally performed under conventional conditions.

[n0030]

In the embodiments of the present invention, the acetate sample of GHK has an acetate content of 10-40% and a GHK content of 60%-75%.

[n0031]

Example 1: Crystallization of GHK trifluoroacetate

[n0032]

Weigh 4.14 g of GHK trifluoroacetate (GHK content 70% by volume, trifluoroacetate content 25% by volume) sample, add 20 ml of pure water to dissolve, then add 1.7 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use.

[n0033]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature (25°C) to crystallize. After the sample returns to room temperature, place it at 2-8°C for 12 hours (the purpose of the segmented cooling in this example is to slowly cool down and increase the particle size of the crystals). Pour off the blue solution. The resulting crystals are GHK-Cu(1:1). Dry the crystals to obtain 3.80g of GHK-Cu(1:1), with a yield of 91.8%.

[n0034]

Example 2: Crystallization of GHK acetate

[n0035]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume) sample, add 40 ml of pure water to dissolve, then add 1.7 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0036]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(1:1). Dry the crystals to obtain 3.8g of GHK-Cu(1:1), with a yield of 96.9%.

[n0037]

Example 3: Crystallization of GHK hydrochloride

[n0038]

Weigh 3.92 g of GHK hydrochloride sample (GHK content 72% by volume, hydrochloride content 25% by volume), add 40 ml of pure water to dissolve, then add 1.7 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0039]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(1:1). Dry the crystals to obtain 3.61g of GHK-Cu(1:1), with a yield of 92.1%.

[n0040]

Example 4:

[n0041]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0042]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% ethanol aqueous solution to the rotary evaporator flask and heat it to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature

(25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.81g of GHK-Cu(2:1), with a yield of 97.1%.

[n0043]

Example 5

[n0044]

Weigh 2.01 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume) sample, add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0045]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat it to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(1:1). Dry the crystals to obtain 3.65g of GHK-Cu(1:1), with a yield of 93.1%.

[n0046]

Example 6

[n0047]

Weigh 4.00 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.10 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0048]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat it to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.81g of GHK-Cu(2:1), with a yield of 97.1%.

[n0049]

Example 7

[n0050]

Weigh 4.80 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume) sample, add 40 ml of pure water to dissolve, then add 2.10 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0051]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes.

Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat it to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.80g of GHK-Cu(2:1), with a yield of 96.9%.

[n0052]

Example 8

[n0053]

Weigh 3.92 g of GHK acetate (70% GHK, 25% acetate) sample, dissolve in 40 ml of pure water, then add 2.10 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside. Weigh 20 g of strong-base anion exchange resin into a sintered glass funnel, rinse three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin, react for 5-10 minutes, then filter under vacuum. Collect the filtrate and filter again under vacuum. The filtration was repeated three times, and the resin was washed three times with pure water. The filtrate was collected and evaporated under reduced pressure using a rotary evaporator. Then, 20 ml of the prepared 78% vol% ethanol aqueous solution was added to the rotary evaporator flask, and the solution was heated to 55°C and sonicated to dissolve it. The dissolved sample was first placed at room temperature to crystallize. After the sample returned to room temperature (25°C), it was placed at 2-8°C for 12 hours. The blue solution was then discarded, and the resulting crystals were GHK-Cu(2:1). After drying, 3.7 g of GHK-Cu(2:1) was obtained, with a yield of 96.1%.

[n0054]

Example 9

[n0055]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume) sample, add 8 ml of pure water to dissolve, then add 8.98 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0056]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat it to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.75g of GHK-Cu(2:1), with a yield of 95.8%.

[n0057]

Example 10

[n0058]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0059]

Weigh 20g of weakly basic anion exchange resin into a sintered sand funnel. Rinse it three times with sodium carbonate solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.42g of GHK-Cu(2:1), with a yield of 87.2%.

[n0060]

Comparative Example 1

[n0061]

Excess anions are not removed without using anion exchange resins.

[n0062]

Weigh 3.92 g of GHK acetate (70% GHK and 25% acetic acid) sample, dissolve it in 40 ml of pure water, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, add 20 ml of the prepared 78% ethanol aqueous solution, heat to 55°C, and then place it at room temperature. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution, and no GHK-Cu crystals are obtained.

[n0063]

As can be seen from Comparative Example 1, it is difficult to synthesize GHK-Cu crystals without using anion exchange resin to remove excess anions.

[n0064]

Example 11

[n0065]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0066]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium carbonate solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 65% ethanol aqueous solution to the rotary evaporator flask and heat to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.42g of GHK-Cu(2:1), with a yield of 87.2%.

[n0067]

Example 12

[n0068]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0069]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium carbonate solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 70% ethanol aqueous solution to the rotary evaporator flask and heat to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the sample to obtain 3.58g of GHK-Cu(2:1), with a yield of 91.3%.

[n0070]

Example 13

[n0071]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0072]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium carbonate solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 85% ethanol aqueous solution to the rotary evaporator flask and heat to 55°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the sample to obtain 3.78g of GHK-Cu(2:1), with a yield of 96.4%.

[n0073]

Comparative Example 2

[n0074]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0075]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium carbonate solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of ultrapure water to the rotary evaporator flask and heat it to 55°C. Place the heated sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Discard the blue solution. No GHK-Cu product was obtained.

[n0076]

As can be seen from the comparison between Examples 11-13 and Comparative Example 2, the mixed solution for removing byproduct anions needs to be dissolved again with an aqueous ethanol solution; otherwise, it is difficult to synthesize GHK-Cu crystals.

[n0077]

Example 14

[n0078]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0079]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the strong anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 40°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.58g of GHK-Cu(2:1), with a yield of 91.3%.

[n0080]

Example 15

[n0081]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0082]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 45°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.77g of GHK-Cu(2:1), with a yield of 96.1%.

[n0083]

Example 16

[n0084]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0085]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 50°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature

(25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the sample to obtain 3.79g of GHK-Cu(2:1), with a yield of 96.6%.

[n0086]

Implementation of Column 17

[n0087]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0088]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% ethanol aqueous solution to the rotary evaporator flask and heat it to 65°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.77g of GHK-Cu(2:1), with a yield of 96.1%.

[n0089]

Implementation of Column 18

[n0090]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0091]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% vol% ethanol aqueous solution to the rotary evaporator flask and heat to 70°C for ultrasonic dissolution. Place the dissolved sample at room temperature to crystallize. After the sample returns to room temperature (25°C), place it at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystals are GHK-Cu(2:1). Dry the crystals to obtain 3.77g of GHK-Cu(2:1), with a yield of 96.1%.

[n0092]

Comparative Example 3

[n0093]

Weigh 3.92 g of GHK acetate (GHK content 70% by volume, acetate content 25% by volume), add 40 ml of pure water to dissolve, then add 2.1 g of copper chloride dihydrate, stir well to obtain a mixed solution, and set aside for later use;

[n0094]

Weigh 20g of strong base anion exchange resin into a sintered sand funnel. Rinse it three times with sodium hydroxide solution, then rinse it with pure water until the pH of the filtrate is 7. Slowly add the mixed solution to the strong base anion exchange resin and react for 5-10 minutes. Filter the solution and collect the filtrate. Repeat the filtration process three times. Rinse the resin three times with pure water and collect the filtrate. Dry the filtrate under reduced pressure using a rotary evaporator. Add 20ml of the prepared 78% ethanol aqueous solution to the rotary evaporator flask. Without heating, place the flask directly at 2-8°C for 12 hours. Pour off the blue solution. The resulting crystal is GHK-Cu(2:1). Dry the crystals to obtain 2.57g of GHK-Cu(2:1), with a yield of 65.6%.

[n0095]

As can be seen from the comparison between Examples 14-18 and Comparative Example 3, when the mixed solution for removing byproduct anions is redissolved using an ethanol aqueous solution, it needs to be heated to 40-65°C, otherwise it is difficult to synthesize GHK-Cu crystals.

[n0096]

Of course, the present invention may have other various embodiments. Without departing from the spirit and essence of the present invention, those skilled in the art can make various corresponding changes and modifications according to the present invention, but these corresponding changes and modifications should all fall within the protection scope of the present invention.
