Detection and silica gel column separation of VB12- carbonyl- putrescine drug nanocarrier

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Abstract

One biologically active derivative of vitamin B12 (cyanocobalamin) has synthesized in which putrescine was attached to the ribose-5′-hydroxyl group of vitamin B12 (VB12- carbonyl-putrescine). It potential to act as oral delivery agent for proteins nanospheres used the vitamin B12 uptake system. The separation and purification with preparative silica column chromatography procedure for VB12- carbonyl-putrescine was eatablished in this paper. The separation process was monitored by TLC. ESI-MS was used to detect the molecular weights. The result showed that the preparative silica column chromatography had a better resolving ability for separating the VB12-carbonyl-putrescine. The mobile phases used were water/ ammonia solution containing n-butanol and isopropanol. From the ESI-MS spectrum, the molecular weights of the component was 1469Da. Separated 6mg VB12- carbonyl- putrescine once time, TLC method was very sensitive to the quality analysis of VB12- carbonyl-putrescine. The preparative silica column chromatography is a better separation and purification method for preparing VB12-carbonyl-putrescine.

Keywords

Drug nanocarrier, vitamin B12 derivative, silica column chromatography

1. Introduction

The vitamin B12 uptake system possesses enormous potential as an absorption enhancing mechanism for orally administered proteins and peptides, the vitamin B12 derivatives uptake system was evaluated by determining their affinity for intrinsic factor (IF) [1;2]. The ribose-5′-hydroxyl group of vitamin B12 was activated through the use of 1,1′-carbonyldi-(1,2,4-triazole) (CDT)[3], subsequent addition of an aminoal-kane diacid dihydrazide gave rise to vitamin B12 derivatives suitable for attachment to various proteins, peptides, or nanospheres to enable oral delivery utilizing the vitamin B12 uptake system[4]. The ribose-5′-carbamate derivatives was found to possess similar affinity for intrinsic factor as that of the e-monocarboxylic acid of vitamin B12[5]. Previous studies have synthesized the 5′-hydroxyl group on an acid anhydride, namely VB12-carbonyl-putrescine. The further use of this derivate limited by the yield and purity. In this work, the separation and purification procedure for the VB12-carbonyl-putrescine was established by the use of Silica gel column.

2. Material and methods

2.1 Materials

Vitamin B12, 1,4-butanediamine (putrescine), N,N'-dicarbonyl (1,2,4-triazole) (CDT) were purchased from Sigma-Aldrich. Diisopropylethylamine (DIEA),1,4-butanediamine (putrescine) were purchased from Xiya reagent co.,LTD. Silica purchase from Yantai industrial chemical.

2.2 Synthesis of vitamin B12- carbonyl- putrescine

Solid 1,1′-carbonyldiimidazole (CDT,0.05mg) was added to cyanocobalamin (vitamin B12,0.1mg) previously dissolved in dimethyl sulfoxide (2.5 mL) at 30℃, and the mixture was reacted in sonication bath for 30 min. DIEA(200μL) and putrescine(500μL) were added and sonication bathing continued for a further for 2h at room temperature. The mixture was poured into ethyl acetate (10 mL) and left to stand to stop the reaction. The mixture was centrifuged at 8000 rpm at 4 ℃ for 5 min. The

supernatant was poured off and the residue washed with acetone. The solid product was dried under a stream of nitrogen.[6; 7]

2.3 Separation and purification of vitamin B12-carbonyl-putrescine by Silica gel column chromatography

Silicon powder (5μm-10μm modified, 2.5g, pre-activated at 110 ℃ for 12h) and n-butanol (13ml) were added to the mortar, the mixture was ground for 60sec and then poured into a chromatographic column(dimensions of 10mm×200mm) . Allowed the gel to settle. Do not let the gel to dry. The mobile phases solution were the mixture of n-butyl alcohol, isopropyl alcohol and deionized water with ratio of 9:6:5, 2% ammonia water was added to the solution and let stand for 5min before use, 10mg vitamin B12-carbonyl-putrescine dissolved into 1ml mobile phase solution. Apply the sample to the prepared column until it enter into the column completely and elute immediately with the mobile phases solution. Flow rate of 3ml/min was applied. The eluent with vitamin B12 and its derivatives distinctive red color were collected. The eluted fractions were removed off organic solvents under vacuum rotary evaporation, the concentration were vacuum freeze-dried.[8]

Spotting the fractions eluted from Silica gel column chromatography with capillary tube to the silica TLC aluminum plates (5cm×10cm). The developing solvent system was n-butyl alcohol, isopropanol, deionized water and ammonia acetate-acetic acid (45: 30:25:2)[9].

2.4 Detection the molecular weight of vitamin B12- carbonyl- putrescine with ESI-MS

The molecular weight of vitamin B12- carbonyl- putrescine analyzed on a 4000 Q TRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA).[10]

3. Results and Discussion

3.1 Separation and detection of vitamin B12-carbonyl-putrescine synthetic products with Silica gel column chromatography

Two fractions of red colored products were get from the silica gel column chromatography. TLC detection results showed (Fig 1, Tab 1) the Rf value for vitamin B12-carbonyl-putrescine and Vitamin respectively are 0.15 and 0.40.

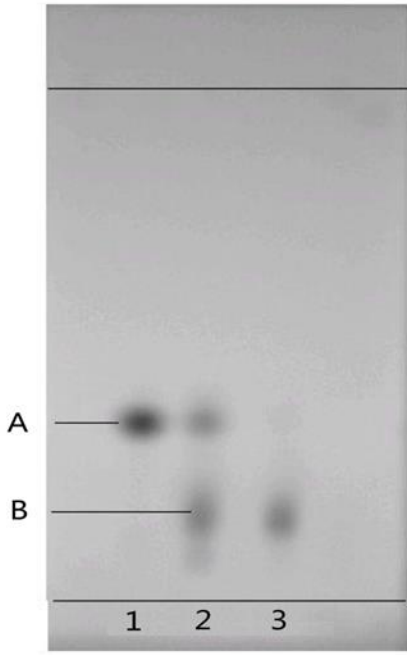

Fig.1 TLC for synthetic product, lane 1 is vitamin B12, lane 2 is the synthetic product before purification, and lane 3 is the fraction after purification.

Tab. 1 Rf values of synthetic product

	Rf
A	0.40
B	0.15

3.2 ESI-mass analysis Molecular Weight of B12-carbonyl-putrescine

The result showed (Fig 2) the molecular weight of the purified product is 1469Da, which is accordance with the theoretical value.

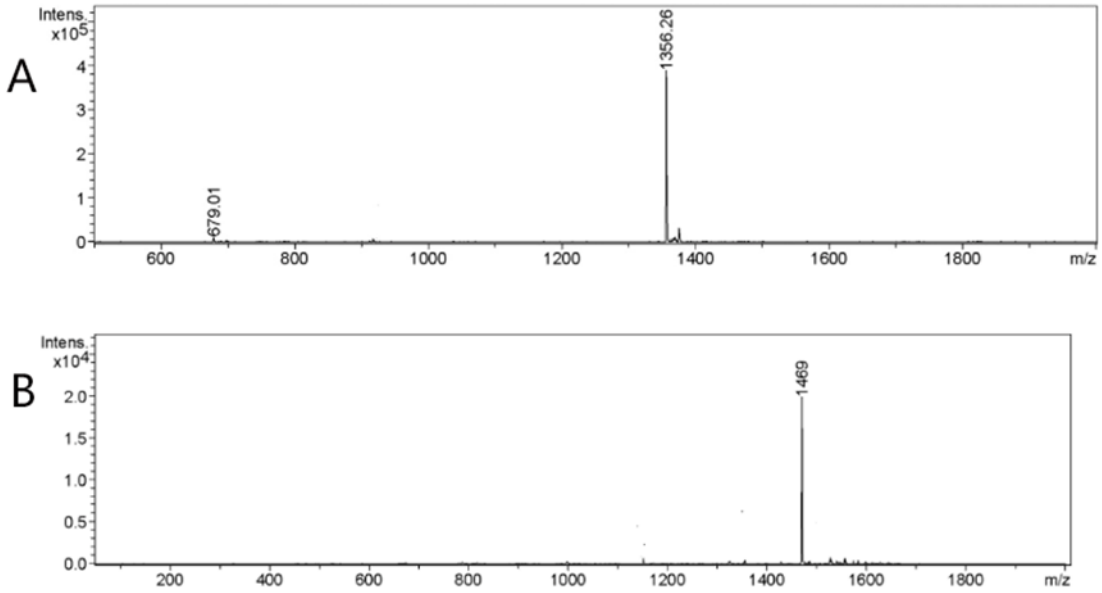

Fig.2 ESI—MS for vitamin B12 and vitamin B12-carbonyl-putrescine.
A is vitamin B12，B is vitamin B12-carbonyl-putrescine.

4. Conclusion

The detection and purification procedure for vitamin B12-carbonyl-putrescine was established in this paper. With the silica gel column chromatography, vitamin B12-carbonyl-putrescine can be better separated and purified. Silica TLC used as the detection method for the product, and ESI-MS was used for analyzing the molecular weight of the product.

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