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Study of VD_3-β-Clodextrin Inclusion Complex

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Abstract

Vitamin D is responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc by involving in the metabolism. However, its use in additive field is limited by its low aqueous solubility and chemical stability. So trace amounts of VD_3 was wrapped in β-CD molecule by the method of saturated aqueous vacuum drying, in order to improve its stability, uniformity and solubility in food and feed additive. The inclusion complex was characterized by NMR, IR techniques and compared with original VD_3 in the aspect of stability and bioavailability. Results of orthogonal design experiments show that the optimum technology of inclusion is that the feed ratio of β-CD to VD_3 is 15:1, being stirred for 5 hours at 80˚C. Dispersion of VD_3 in the inclusion complex is more uniform, while stability and absorption rate of inclusion complex are significantly higher than original VD_3.

Keywords

VD_3, β-Clodextrin, Inclusion Complex, NMR, IR, Quality Evaluation

1. Introduction

Vitamin D, also known as cholecalciferol, including vitamin D_2 (ergocalciferol,) and vitamin D_3 (cholecalciferol), whose chemical name is 9,10-open-loop cholesteric 5,7,10(19-) leukotriene-3β-alcohol, and vasoactive substance is 25-hydroxy vitamin D_3-abbreviated [25-(OH)-D_3] (calcifediol, INN). In recent years, the demand for VD_3 is on the rise, which is widely used in areas of food additives, pharmaceutical preparations and feed additives. Vitamin D is responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc by involving in the metabolism. In the liver, vitamin D_3 is converted to calcifediol, while vitamin D_2 is converted to 25-hydroxy vitamin D_2 [25-(OH)-D_2]. These two specific vitamin D metabolites are measured in serum to determine personal vitamin D status. Part of the calcifediol is converted by the kidneys to calcitriol, the

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biologically active form of vitamin D. Calcitriol circulates as a hormone in the blood, regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone. Calcitriol also affects neuromuscular and immune function [1]-[3]. Therefore vitamin D has gradually become an important auxiliary material in food industry. But because of its very small added amount, whether it can be uniformly dispersed in the raw material becomes extremely important. Since the vitamin D molecule has many Olefinic bonds, it is unstable and easy to be oxidized in humid environment [4] [5]. In the aspect of increasing the solubility, reducing volatility, enhancing chemical stability of food additive, extending food shelf life and stabilizing food color, β-CD is widespread used [6]. In this study, VD_3-β-cyclodextrin inclusion complex is prepared by saturated aqueous vacuum drying method, in order to improve its stability and uniformity in food and feed additive [7].

2. Experimental

2.1. Materials and Animals

Original VD_3; β-CD; acetonitrile; 25-hydroxy vitamin D_3 ELISA kit; ether. Wistar rats (male, weighing 200 ± 20 g).

2.2. Preparation of the Standard Solution and Test Solution

VD_3 standard were precisely Weighed 10.05 mg, placed into 50 ml measuring flask, and dissolved by methanol to make the stock solution whose VD_3 concentration was 201.0 μg/ml. Then the stock solution was shaken well and precisely taken 2 ml to a 10 ml volumetric flask, Which Methanol was added to in order to make the standard solution whose VD_3 concentration was 40.20 μg/ml. The determinand was precisely Weighed 80 mg to 10 ml measuring flask, and completely wetted up by adding 5 ml dimethyl sulfoxide. Then the particles were ultrasound dissolved. Methanol was added to dilute the solution to the mark. Then the solution was filtered through 0.45 μm organic microporous membrane. The filtrate was the test solution.

2.3. Inclusion Orthogonal Design

By pre-experiment study, the main factors of influencing the effect of inclusion by saturated aqueous solution vacuum drying method were the original feed ratio of β-CD and VD_3, the stirring temperature and stirring time. So L9 (3^4) orthogonal design was used and the inclusion rate was the evaluation index to choose the optimum process (**Tables 1-3**). Among them, the inclusion rate = amount of drug in inclusion compound/total dosage × 100%.

2.4. Preparation of Samples

2.4.1. Preparation of the VD_3-β-CD Inclusion Complex

B-CD was weighed 15 g, and was suspended in 150 ml distilled water, heated to 60˚C, to make saturated aqueous solution of β-CD; VD_3 was weighed 1.0 g and dissolved in 5 ml distilled water. The original VD_3 aqueous solution was added dropwise to the β-CD aqueous solution, stirred for 5 h (80˚C, 600 r/min), then frozed for 24 h at 4˚C, vacuum filtered. The filter cake was dried in an electrothermal vacuum oven at room temperature. The VD_3-β-cyclodextrin inclusion complex was obtained by smashing the dried filter cake, weighed, and sifted through the 100 mesh sieve to be reserved [8]-[10].

2.4.2. Preparation of the Physical Mixture

The calculated and exactly weighed (1:1 molar ratio) amounts of VD_3 and β-CD were pulverized in a ceramic

Table 1. L_9 (3^4) factors-levels of the inclusion process.

Level	A (β-CD/VD_3)	B (stirring temperature)	C (stirring time)
1	5:1	40˚C	1 h
2	10:1	60˚C	3 h
3	15:1	80˚C	5 h

Table 2. L_9 (3^4) orthogonal experimental results.

Test number	A	B	C	D	Inclusion rate
1	1	1	1	1	0.3068
2	1	2	2	2	0.1258
3	1	3	3	3	0.4036
4	2	1	3	3	0.1955
5	2	2	1	1	0.2012
6	2	3	2	2	0.2884
7	3	1	2	2	0.3093
8	3	2	3	3	0.5114
9	3	3	1	1	0.2825
k_1	0.84	0.81	0.79	0.79	
k_2	0.69	0.84	0.72	0.72	
k_3	1.10	0.97	1.11	1.11	
Range	0.14	0.05	0.17	0.13	
SSj	0.03	0.005	0.043	0.029	

Table 3. Variance analysis.

Source of variation	SS	DOF	Variance	F value	Critical value	Significance
1	0.030	2	0.015	23.905	4.459	*
2	0.005	2	0.003	4.069		
3	0.043	2	0.021	34.324		*
4	0.029	2	0.014	22.814		*
Overall error	0.005	8	0.001			

Notes: $^*P < 0.05$.

mortar and carefully mixed.

2.5. Quality Assessment of the VD_3-β-CD Inclusion Complex

VD_3, β-CD, the VD_3-β-CD inclusion complex made by the best technology and the physical mixture were weighed 10 mg each calculated by the inclusion rate. IR spectra were performed under the same conditions to verify whether the inclusion complex was formed by comparing infrared absorption peak.

Stability constants were measured by the method of thermostatic acceleration. VD_3 test sample and inclusion complex were put in thermostatic water bath (25˚C). Samples of 2 ml were taken at 60, 120, 180, 240, 300 minute, and filtered through 0.45 μm microporous membrane. Finally, the filtrate was measured to record peak area.

30 male Wistar rats were randomly divided into two groups, and intragastric administrated pure VD_3 and VD_3 inclusion complex according to 4.5 μg VD_3 per kilogram body weight. Concentration of 25-hydroxy VD_3 in serum was measured by euzymelinked immunosorbent assay (ELISA). The pharmacokinetic parameters of 25-hydroxy VD_3 in Wistar rats' serum were calculated by PKSolver 2.0 pharmacokinetics software.

3. Results and Discussion

3.1. IR Spectrum

The IR spectroscopic analysis confirmed the interaction and the complex formation between VD_3 and β-CD. IR

spectra of the complex were compared with the physical mixture and pure substances. It can be seen that the stretching vibration peak of O-H in *β*-CD inclusion complex moves significantly to the high band compared with that in *β*-CD (change value was 12 cm^{-1}). And the stretching vibration peak of O-H in *β*-CD inclusion complex becomes stronger and narrower, which shows that association effect between O and H in *β*-CD molecule is weakened by VD_3 intervention, confirming the existence of the inclusion complex (see **Figure 1**).

3.2. Comparison of Stability

Concentration of VD_3 at each test time point was calculated by standard curve method. At certain temperature, a graph of logarithm of VD_3 concentration (log C) versus time (t) is plotted and fitted, whose slope is m, and the degradation rate constant (K) is equal to −2.303 m. Graph of logarithm of degradation rate constant (log K, K = −2.303 m) versus reciprocal of corresponding absolute temperature (1/T) is a fitted straight line. Results of degradation rate constant at the room temperature (K_{25}) is showed in **Table 4**, showing the inclusion complex is significantly higher stable than original VD_3.

3.3. Relative Bioavailability

Elimination rate constant (k), the half-life period ($t_{1/2}$), time of maximum concentration (T_{max}) and mean residence time (MRT) of VD_3-*β*-CD inclusion complex are similar to those of original VD_3, but C_{max}, $AUC_{0-\infty}$ are obviously larger. According to the formula: $F = AUC_{0-t}(TF\text{-}NE)/AUC_{0-t}(TF) \times 100\%$, the relative bioavailability of VD_3-*β*-CD inclusion complex to original VD_3 is calculated, and the result is 209%. Through process improvement, dispersion of VD_3-*β*-CD inclusion complex is more uniform, and absorption rate is significantly higher than original VD_3 (see **Table 5**).

4. Conclusions

Because of its very small amount in food additive, the inclusion complex is easy to be mixed unevenly, which

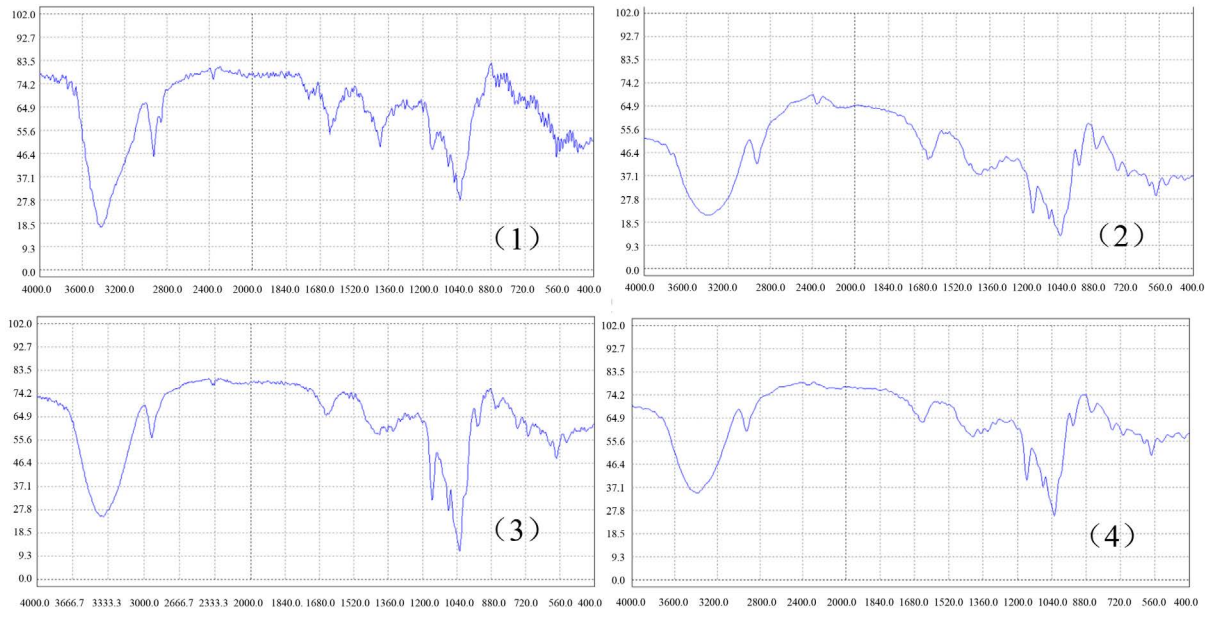

Figure 1. IR spectra: (1) VD_3; (2) *β*-CD; (3) VD_3-*β*-CD inclusion complex; (4) equimolecular physical mixture of VD_3 and *β*-CD.

Table 4. Degradation rate constant and half-life period of original VD_3 and the inclusion.

	m	K	logK	1/T	$t_{1/2}$ (h)	$t_{0.9}$ (h)
original VD_3	-1.06×10^{-2}	2.44×10^{-2}	−1.613	3.4×10^{-3}	28.40	4.30
Inclusion complex	-2.0×10^{-4}	4.61×10^{-4}	−3.336	3.4×10^{-3}	1503.25	227.77

Table 5. The pharmacokinetic parameters of 25-hydroxy VD_3 in Wistar rats' serum.

pharmacokinetic parameters	Unit	VD_3-β-CD inclusion complex	VD_3
K	min^{-1}	7.04×10^{-4}	8.96×10^{-4}
$t_{1/2}$	min	985	774
T_{max}	min	2	5
C_{max}	μg/L	24.09	13.84
$AUC_{0-\infty}$	μg/L·min	1.81×10^4	7.38×10^3
AUC_{0-t}	μg/L·min	7.88×10^3	3.77×10^3
MRT	min	1.72×10^3	1.62×10^3

can even lead poisoning [11]. In this study, trace amounts of VD_3 were wrapped in β-CD molecule by the method of saturated aqueous vacuum drying, in order to improve its stability, uniformity and solubility in food and feed additive. While rats' blood pharmacokinetic study indicates that the bioavailability of VD_3-β-CD inclusion complex is also better. The experiments show that the optimum technology of inclusion is that the feed ratio of β-CD to VD_3 is 15:1, being stirred for 5 hours at 80˚C, which can be widely used in additive to improve safety, effectiveness, and economy.

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