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# Factors Affecting Stability of *Z*-Ligustilide in the Volatile Oil of *Radix Angelicae Sinensis* and *Ligusticum Chuanxiong* and Its Stability Prediction

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**ABSTRACT** The purpose of this investigation is to obtain a suitable vehicle for *Z*-ligustilide in the volatile oil of *Radix Angelicae Sinensis* and *Ligusticum Chuanxiong* in which it is stable enough for the application in pharmaceutics, to investigate its degradation laws, and to predict its shelf-life at 25°C. Factors including temperature, light, pH value, co-solvents and antioxidants can all influence the stability of *Z*-ligustilide, thereinto antioxidants could markedly improve its stability in aqueous solution by almost 35%. The suitable vehicle for *Z*-ligustilide contains 1.5% tween-80, 0.3% Vitamin C, and 20% propylene glycol (PG).

Furthermore, the degradation rates of *Z*-ligustilide were found to conform to a rate equation following Weibull probability distribution within a range of degradation ratio, and the equation could be expressed as follow:

$$\ln \ln (1/1-\alpha) = \ln k + m \ln t$$

Where $\alpha$ is degradation ratio; *t* is time; *m* and *k* are constants relating to the degradation rate. The degradation rate will get greater as the increasing of parameter *k*. According to the degradation law obtained from the equation, the drug shelf-life (10% of active ingredient degraded, $T_{90}$) in this vehicle was predicted to be more than 1.77 years at 25°C through Arrehenius equation and accelerating experiments.

The present investigation was undertaken to propose a kinetic treatment that may be applicable to any type of degradation of the active ingredient of pharmaceutical formulation, and also could provide a good foundation for the new drug development of *Z*-ligustilide, especially for injection formulation.

**KEYWORDS** *Z*-ligustilide, *Radix Angelicae Sinensis*, *Ligusticum chuanxiong*, Stability, Weibull probability distribution

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## INTRODUCTION

*Radix Angelicae Sinensis* (Chinese Danggui, CDG) and *Rhizoma Chuanxiong* (Chinese Chuanxiong, CCX), two important Chinese traditional medicines, are the roots of *Angelica sinensis* (Oliv.) Diels and *Ligusticum chuanxiong* Hort., respectively. "Foshousan" is one of the traditional Chinese medical prescriptions composed of them (Wang, 1999; Xu & Chen, 1999; Lv, 2000), and volatile oil is one of the bioactive components of this composite formula (CDG and CCX contain 0.4% and 1.0% volatile oil, respectively). *Z*-ligustilide (Fig. 1, Guang et al., 2001) is the major chemical component in volatile oils both of CDG and CCX, and it accounts for 35% and 58% of the composition, respectively (Li & Ma, 2000). However, *Z*-ligustilide is a very unstable compound. According to the literature (Li & Ma, 2000), 58% of the pure *Z*-ligustilide may degrade when it is conserved under 25°C for 15 days in daylight, and even stored at 4°C in the refrigerator in darkness, it may degrade nearly 15% during 15 days. Recently, it has been reported that *Z*-ligustilide remains relatively stable in certain solvents, for example, its purity decreased from 97.98% to 96.36% when stored in chloroform at 25°C for 25 days, while to 91.24% in cyclohexane under the same reserving condition (Zhou & Li, 2001). Because of the particularly unstable properties of *Z*-ligustilide, furthermore the solvents mentioned are not permitted to be used in pharmaceutics, few dosage forms containing it are used clinically, and there have been no reports concerning *Z*-ligustilide stability in preparations. Up to now, only some per-oral prescriptions, such as softgel capsules and droplets, have been permitted by the State Food and Drug Administration (SFDA) of The People's Republic of China. But even in these prescriptions, *Z*-ligustilide is not regarded as the primary ingredient for content examination because its content is easy to decrease under requirement, and only serves as an assistant ingredient for distinguishability. In addition, because of the absorption problem of oral dosage forms, they are usually used as daily administration for unserious diseases, therefore, for the acute and serious diseases, an injection can bring pharmacodynamic action more quickly than oral dosage forms. The patients tend to accept injection therapy for critical disease in China, so it is necessary to acquire a stable vehicle for *Z*-ligustilide.

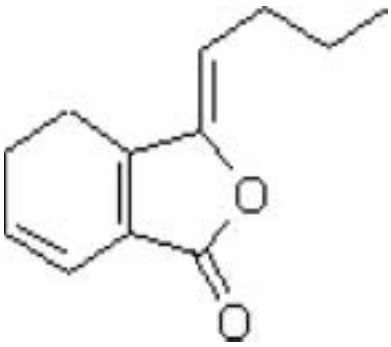

**FIGURE 1** Chemical Structure of *Z*–ligustilide.

In this study, in order to achieve a suitable vehicle to improve *Z*-ligustilide chemical stability, factors that may affect its stability were investigated and finally provide a suitable injectable vehicle for *Z*-ligustilide. The degradation laws of *Z*-ligustilide in different vehicles were investigated in detail, and it was found that it didn't conform to the accustomed zero, first-or second-orders. However, Weibull probability distribution was approved to be applicable in these cases (Naoya, 1975), and the equation could be expressed as follows:

$$\ln\ln\left(\frac{1}{1-\alpha}\right) = \ln k + m\ln t \qquad (1)$$

where $\alpha$ is degradation ratio, $t$ is time; $m$ and $k$ are constants relating to the degradation rate. The degradation curve can be a linear plot using a Weibull probability paper although it is too inconvenient in practice. However, plot $\ln\ln\left(\frac{1}{1-\alpha}\right)$ *vs.* ln t, a linear plot can be obtained through Microsoft excel without a particular Weibull probability paper, and, usually, in the same system, the slopes of the linear plot are basically the same value. In most occasions, parameter $k$ is a constant related to the degradation rate. Therefore, the degradation degree can be compared by studying the parameter $k$. Collectively, the degradation rate gets greater as the increasing of parameter k obtained from the linear plot. In general, such conducting manner is clear and comprehensible. This theory may propose a new kinetic treatment that is applicable to any type of degradation of the active ingredient of pharmaceutical formulation. By using the results of short-term degradation studies at elevated temperatures and Arrehenius

equation, the shelf-life, time at which remaining percents of *Z*-ligustilide is 90% ($T_{90}$) can be calculated. This provides a good basis for the development of injectable formulations containing this volatile oil.

## MATERIAL

CDG and CCX were purchased from Tianjin Chinese herb medicine factory. The volatile oil was prepared by steam distillation for two times in our laboratory. Deionized water was obtained using SZ-93 water distillation equipment (Shanghai Yarong Biochem. Co., China). An HH-S 11.2 isothermal water bath was obtained from Jiangsu Dongtai electrical appliances plant (China). Ethanol, glycerol, propylene glycol (PG), and methanol were purchased from Tianjin Bodi Chem. Co. Ltd. (China). Tween-80, also known as polysorbate–80, and was obtained from Beijing Yili Chem. Co. (China). $Na_2S_2O_5$, $NaHSO_3$, and sodium oleate were obtained from Tianjin Kemiou Chem. Co. Ltd. (China). Vitamin C (VC) was kindly provided by Northeast General Pharmaceutical Factory. Methanol was HPLC grade, and the other chemicals were analytical grade.

## METHODS

### HPLC Instrumentation and Chromatographic Conditions

A Shimadzu LC-10AT *VP* HPLC–DAD system consisting of a vacuum degasser, binary pump, autosampler, thermostated column compartment, and diode array detector (DAD) (SPD -M10A *VP*, Shimadzu, Japan) was used for quantitative analysis. A wavelength of 329 nm was chosen for *Z*-ligustilide detection. The separation of *Z*-ligustilide was achieved by using an ODS ($C_{18}$) column (5 μm, 200 mm × 4.6 mm, Cat.No. 99902, Serial No. 8021348, Dikma Corporation, America). The eluent was a 70:30 (v/v) mixture of methanol: $H_2O$ (Mao et al, 2002). The injection volume was 20 μl, and the flow rate was 1.0 $mL.min^{-1}$. Retention time of *Z*-ligustilide was approximately 12.1 ± 0.2 min at 35°C. The DAD connected to the HPLC provided verification of peak homogeneity.

### Preparation of Standard Solution

Stock solution for standard curves of *Z*-ligustilide in methanol was freshly prepared. The mobile phase was used for all sample dilutions. The standard curves were determined from a series of concentrations of *Z*-ligustilide solutions covering the range 10 to 80 $\mu g.mL^{-1}$. Each standard calibration curve of *Z*-ligustilide was found to be linear over the concentration range mentioned above. The correlation coefficients ($r^2$) were greater than 0.9996. From the results of the *Z*-ligustilide HPLC assay, it was found that both intra-day and inter-day validations were less than 2% with regard to both precision (represented by the coefficient of variation, or CV %) and bias (represented by the standard deviation, or S.D %).

### Investigation of Stabilization of *Z*-ligustilide

The volatile oil of the composite formula was dissolved in different vehicles to obtain stock solutions with the help of tween-80 (1.5%), and the concentration of *Z*-ligustilide in each stock solution was about 1.5 $mg.mL^{-1}$. All stock solutions were sealed in 2 mL glass ampoules and tested under different conditions. Furthermore, all the ampoules were kept in darkness except for the experiments to investigate the effects of light. At predefined intervals, three ampoules were sampled, and the contents were filtered through a 0.45 μm membrane filter after being mixed together. The filtrate was measured immediately by HPLC after diluting to a chosen concentration with mobile phase to determine the residual content of *Z*-ligustilide.

Preliminary experiments were conducted to test the effects of light, temperature, and some co-solvents on the stability of *Z*-ligustilide. Among those, the effects of light and temperature were conducted in aqueous solutions, and the effect of light was reviewed by placing the ampoules under 4500 l× illumination for 10 days, while the effect of temperature was examined under 4, 25, 40, and 60°C, respectively. The co-solvents included 20% ethanol, glycerol, and PG(v/v), and the experiments were conducted by boiling the ampoules at 100°C for 0.5 h first, and then placing them at 25°C for 50 days.

As the basis of the preliminary experiments, factors which might influence the stability, including the effects of co-solvents, pH value, antioxidants, the combined effects of co-solvent and antioxidant, the amount of antioxidant, were investigated in detail by accelerated tests. All the ampoules tested were kept in

**TABLE 1** Details of Each Factor to Be Tested in Accelerated Experiments

| Factors tested | Vehicles adopted | Details of each factor |
|---|---|---|
| Combination of co-solvents | Semiaqueous vehicles containing different amount of co-solvents | 20% glycerol<br>40% glycerol<br>20% propylene glycol<br>15% propylene glycol & 25% glycerol<br>25% propylene glycol & 15% glycerol |
| pH | 40% phosphate buffer solutions at different pH and 60% water | pH 5.0<br>pH 5.8<br>pH 6.5<br>pH 7.4 |
| Antioxidants | Aqueous solution containing 0.2% of different antioxidants | $Na_2S_2O_5$<br>$NaHSO_3$<br>Sodium oleate<br>VC |
| Combination of VC and co-solvents | Semiaqueous vehicles containing 0.2% VC and different amount of co-solvents | Aqueous solution<br>20% glycerol<br>20% propylene glycol<br>15% glycerol & 25% propylene glycol |
| Amount of VC | Semiaqueous vehicles containing 20% propylene glycol and different amounts of VC | 0.1%<br>0.2%<br>0.3%<br>0.4% |

an isothermal water bath at 80°C without light for 24 h, and sampled at 0, 1, 2, 3, 5, 7, 9, 12, and 24 h. The residual content of *Z*-ligustilide at each time point was compared with the content at 0 h to evaluate its stability under different conditions, and the results were analyzed to find a law that the degradation of *Z*-ligustilide may confirm to. The details of each factor tested are given in Table 1.

The $T_{90}$ for *Z*-ligustilide in the semiaqueous solution was predicted by the classical thermal methods (Sumie et al, 1987). The accelerated stability tests were conducted at four temperatures (70, 80, 90, 100°C, respectively) the same as the accelerated experiments mentioned previously, and the results were assayed through Arrhenius equation. The semiaqueous vehicle consisted of 1.5 $mg.mL^{-1}$*Z*-ligustilide (equal to 3.2 $mg.mL^{-1}$ oil), 1.5% tween-80, 0.3% VC, 20% PG, and 80% water.

## RESULTS AND DISCUSSION

As mentioned, the pure *Z*-ligustilide is liable to degrade. In the volatile oil extracted from CDG and CCX, *Z*-ligustilide only accounts for 47% or so, in this case, other components in the oil and the pharmaceutical excipients used in preparations may all influence the *Z*-ligustilide stability when they coexist in the same vehicle. Therefore, it was very difficult to clarify exactly how *Z*-ligustilide was stabilized or degraded. However, one of the degradation pathways can be examined when the other conditions are fixed, then the factors influencing *Z*-ligustilide degradation can be controlled correspondingly. Some possible influences that could lead to *Z*-ligustilide degradation will now be discussed in detail. Moreover, the degradation rate law of *Z*-ligustilide in each vehicle is approached to find a suitable equation.

### Influence of Light, Temperature and Co-solvents

Effects of light and temperature were shown in Table 2. When the samples were exposed to an illumination of 4500 lx for 5 days, about 86.47% of the *Z*-ligustilide was photo-degraded, and 10 days later almost all of it disappeared, and thus it proved that *Z*-ligustilide was very sensitive to illumination. To make sure of this conclusion, one more experiment was conducted, which was to seal the aqueous solution in glass and amber ampoules, respectively, and

**TABLE 2** **Effects of Light and Temperature on Stability of *Z*-ligustilide at Different Times**

| Time (day) | Remaining percentage of *Z*-ligustilide (%) | | | | |
|---|---|---|---|---|---|
| | 4°C | 25°C | 40°C | 60°C | Illumination |
| 0 | 100 | 100 | 100 | 100 | 100 |
| 5 | 100 ± 1.41[a] | 99.45 ± 0.4 | 93.17 ± 0.51 | 71.61 ± 0.72 | 13.53 ± 1.05 |
| 10 | 99.81 ± 0.62 | 98.33 ± 1.35 | 86.48 ± 0.87 | 64.75 ± 0.98 | 2.08 ± 1.48 |

[a]Mean ± S.D. of three measurements.

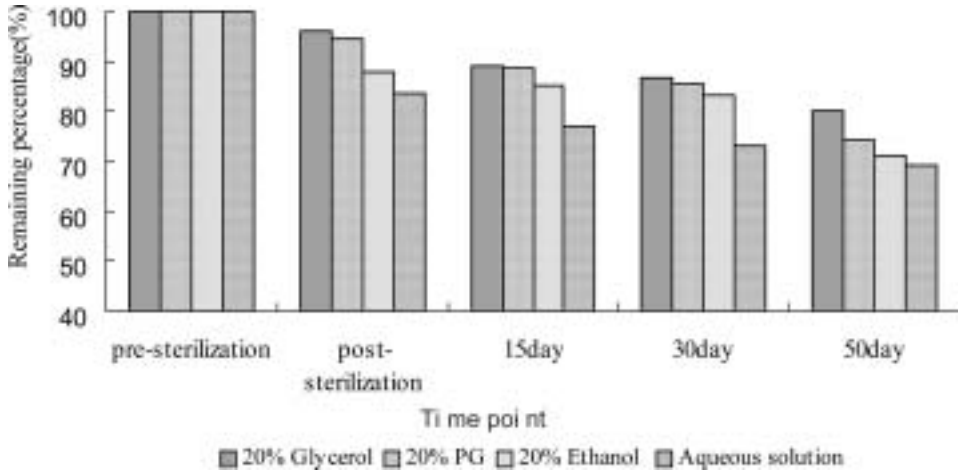


**FIGURE 2** **Effects of Co-solvents on Stability of *Z*-ligustilide.**

then expose them to 4500 lx illumination for 10 days as before. The results showed that the degradation of *Z*-ligustilide in the amber ampoules was about 3.36% after 5 days, 5.25% after 10 days. The results showed that the amber ampoule could by and large prevent the degradation caused by light, but compared to the samples placed in absolute darkness, the amber ampoules can't resist the light utterly, and this indicated that *Z*-ligustilide needs to be saved strictly without light. The conclusion can prove that photo degradation is a major degradation pathway.

The degradation rate of *Z*-ligustilide was increased remarkably as the improvement of temperature storing, however, when stored at 4°C in the dark for 10 days, over 99% of the *Z*-ligustilide remained. This result accords with the conclusion brought by Van't Hoff that when the ambient temperature was increased by 10°C, the reaction rate could improve by 2–4 times. Arrhenius pointed the relation between reaction rate and temperature at 1889 by Arrhenius equation:

$$\ln k = -\frac{E_a}{RT} + \ln A \qquad (2)$$

That is when the ambient temperature increased, all the possible degradation rate of *Z*-ligustilide, including photo-degradation, hydrolysis, oxidation and so on, can be accelerated. Thus *Z*-ligustilide should be conserved at as lower temperature as possible.

Additionally, some co-solvents (Fig. 2) reduced the degradation rate and improved the thermal stability of *Z*-ligustilide, and this content will be discussed in detail subsequently.

## Effects of the Combination of Glycerol and PG

As indicated in Fig. 2, all the co-solvents examined can reduce the degradation of *Z*-ligistuilde to a different extent, and they in principle are acceptable in pharmaceutics, but for injection, the glycerol and PG are better than ethanol because of less irritation. So glycerol and PG were selected to examine the effect of co-solvent to its stability further through accelerating experiments at 80°C, and the results are shown in Table 3.

The data dealt with adopting traditional zero-order, first-order, and second-order reaction rate equation,

**TABLE 3** **Effects of Glycerol and Propylene Glycol and Their Combinations on Stability of *Z* -ligustilide**

| Time (hours) | Remaining percentage of *Z*-ligistuilde (%) | | | | |
|---|---|---|---|---|---|
| | 15% glycerol &25% PG | 25% glycerol &15% PG | 20% PG | 40% glycerol | 20% glycerol |
| 0 | 100 | 100 | 100 | 100 | 100 |
| 1 | 89.23 ± 0.65[a] | 90.57 ± 0.24 | 85.85 ± 0.56 | 86.95 ± 1.03 | 86.81 ± 0.75 |
| 2 | 86.24 ± 0.88 | 87.17 ± 1.30 | 81.62 ± 0.86 | 83.51 ± 1.07 | 83.54 ± 0.45 |
| 3 | 84.25 ± 0.67 | 85.14 ± 0.58 | 78.93 ± 1.08 | 79.60 ± 0.98 | 79.67 ± 0.56 |
| 5 | 80.91 ± 0.94 | 81.73 ± 1.85 | 74.27 ± 0.96 | 76.34 ± 0.68 | 75.55 ± 0.37 |
| 7 | 77.14 ± 0.20 | 78.64 ± 0.45 | 72.56 ± 1.56 | 73.53 ± 0.32 | 73.27 ± 0.35 |
| 9 | 75.52 ± 0.37 | 76.50 ± 0.68 | 71.49 ± 0.88 | 72.37 ± 0.75 | 71.92 ± 0.56 |
| 12 | 74.47 ± 0.58 | 75.08 ± 0.52 | 70.64 ± 0.67 | 70.81 ± 0.53 | 69.93 ± 0.36 |
| 24 | 72.24 ± 0.34 | 72.90 ± 0.25 | 65.92 ± 0.69 | 67.14 ± 0.86 | 66.14 ± 0.25 |

[a]Mean ± S.D. of three measurements.

and the results indicated that they apparently are not applicable in this case, that is, the concentration of *Z*-ligustilide and time can't be expressed as follow:

$$C_t = C_0 - kt \text{ (zero-order)};$$
$$\ln C_t = \ln C_0 - kt \text{ (first-order)};$$
$$\frac{1}{C_t} = \frac{1}{C_0} + kt \text{ (second-order)}$$

However, the degradation rate of active ingredient of drugs in homogeneous or heterogeneous system was found to conform to a rate equation as follows:

$$\ln\ln\left(\frac{1}{1-\alpha}\right) = \ln k + m \ln t \qquad (1)$$

Where α is degradation ratio; *t* is time; *m* and *k* are constants.

In these cases, plot $\ln\ln\left(\frac{1}{1-\alpha}\right)$ *vs.* ln t, five linear plots of degradation rate of *Z*-ligustide (correlation coefficients between 0.9505 ~ 0.9972) in co-solvents could be obtained (Fig. 3). It could be found that the intercepts of 20% glycerol, 40% glycerol, and 20% PG were almost identical (–1.8049, –1.7773, and –1.7962), while the combination can markedly reduce it (–2.3622 and –2.3359), although the slopes were different from each other. This indicates that *Z*-ligistuilde may degrade through hydrolysis, and when some co-solvents substituted a part of water in the vehicle, the chance of hydrolysis was reduced, and *Z*-ligustilide was stabilized

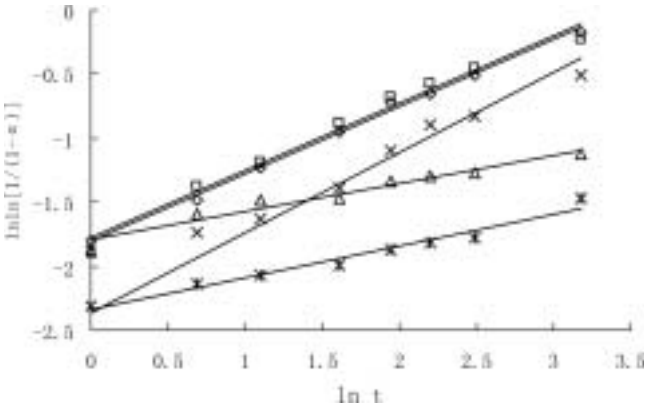


**FIGURE 3** **Regression Lines of Degradation Ratio of *Z*-ligustilide in Different Co-solvent *vs*. ln *t*. including 20%glycerol (◇), 40% glycerol (□), 20% PG (△), 25% glycerol & 15% PG (×) and 15% glycerol & 25% PG (*).**

to a greater degree, but organic solvent content is not in proportion to the extent of degradation, and when the content reaches a certain value, the degradation will not reduce continually. However, it could be found that in comparison with glycerol or PG alone, the combined solutions were preferable to reduce the degradation rate of the drug in the oil in spite of their proportions. The possible reason may be that glycerol and PG have some initial effect, which can prevent the occurrence more, but the detailed effect is to be discussed in future.

## Effects of pH

According to earlier investigations, it is known that *Z*-ligustilide is very sensitive to pH, and the relatively stable pH range is between 5.0–7.4. When pH values were higher than 8.0, it was hydrolyzed rapidly. In

order to minimize the degradation arousing by pH, stability experiments with *Z*-ligustilide were conducted at various pH values among 5.0–7.4. According to Eq. 1, plot $\ln\ln\left(\frac{1}{1-\alpha}\right)$ *vs.* ln t, the five linear plots were (correlation coefficients between 0.9788–0.9979) parallels, and the slopes were all close to 0.3445. Plotting parameter *k vs.* the pH value, the pH profile of *Z*-ligustilide was shown in Fig. 4. It was found that the pH profile had a "V" shape and the minimum value of *k* was at pH value 5.8. This may be interpreted that *Z*-ligustilide has a lactone bond (Fig. 1), so it is liable to hydrolyze catalyzed by a specific acid (hedonism ion) or specific base (hydroxide ion)(Bi, 2000; Cui, 2002), and suitable pH value can reduce such reaction as much as possible, that is, if the pH value was adjusted to 5.8, the hydrolysis degradation was reduced to a considerable extent. This accords with the results of the above experiments.

## Effects of Various Antioxidants

Although *Z*-ligustilide was more stable in semiaqueous solution than in aqueous solution, it still underwent marked degradation, so some antioxidant may be useful to the stability of it. To find an effective antioxidant for *Z*-ligustilide, several antioxidants available were investigated by adding them to the aqueous solutions, and the amount of antioxidant was 0.2% (g.100 $mL^{-1}$). Figure 5 showed the remaining contents of *Z*-ligustilide in the different antioxidant solutions during the test period. The initial concentration of *Z*-ligustilide in all the solutions was 700 $\mu g.mL^{-1}$ or so. However, soon after $Na_2S_2O_5$ and $NaHSO_3$ were added, the measured *Z*-ligustilide contents fell to 98.99 and 208.73 $\mu g.mL^{-1}$, respectively, and a new peak of production degraded from Z-ligustilide appeared on the HPLC chromatogram. This indicated that both $Na_2S_2O_5$ and $NaHSO_3$ (maybe including other antioxidants containing the element sulphur) could react with *Z*-ligustilide to produce new compounds. In addition, the drug degradation curve of the solution containing 0.2% sodium oleate showed no difference from the referent solution containing no antioxidant. However, when 0.2% VC was added, the stability of *Z*-ligustilide was substantially improved, and only 12.69% of the drug was degraded after 24 h at 80°C. This suggests that the degradation of *Z*-ligustilide in solution is largely due to oxidation. In this case, the oxidant might be the oxygen dissolved in the solution and remaining in the ampoules, and this was confirmed by nitrogen flooding. The percentage degradation of *Z*-ligustilide without nitrogen after incubating the ampoules in boiling water for 0.5 h was 17.42 ± 0.62, while it was 13.49 ± 0.36 in the ampoules full of nitrogen according to our experiments. Thus, it could be concluded that the remaining oxygen played an important role in the oxidation reactions of *Z*-ligustilide. However, depending on the method used for removal, some oxygen may remain in solution. Simple flooding was not effective enough, and to remove oxygen completely by boiling was not feasible because *Z*-ligustilide is also unstable to heat. The use of an antioxidant is a great help in minimizing the decomposition of *Z*-ligustilide. In substance, the antioxidants added are all strong reducers (except

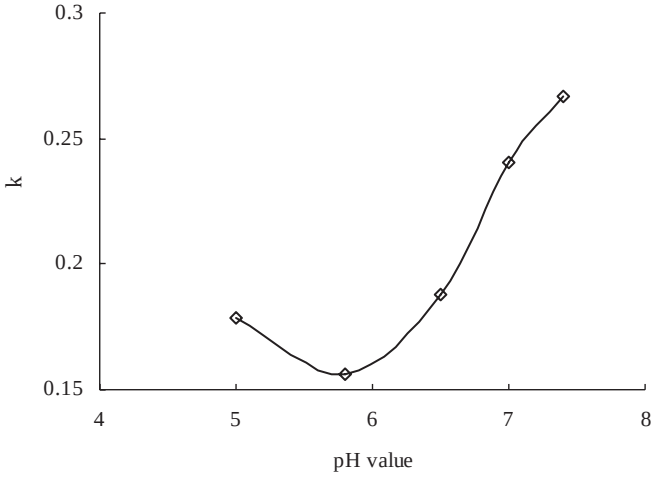


**FIGURE 4** pH Profile for *Z*-ligustilide at 80°C.

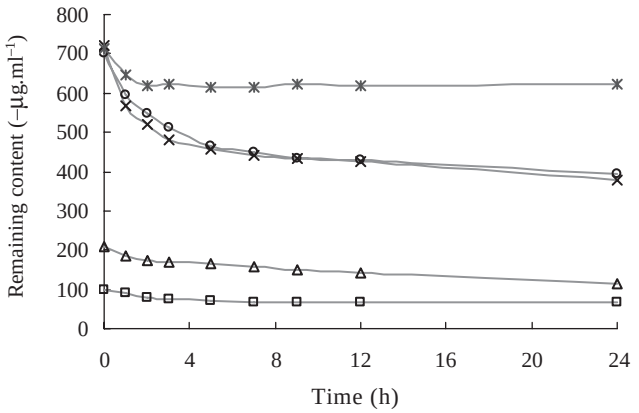


**FIGURE 5** Effects of Various Antioxidants on Stability of *Z*-ligustilide. The Antioxidants Added Were 0.2% Sodium Oleate (○), 0.2%$Na_2S_2O_5$(△), 0.2%$NaHSO_3$ (□), 0.2%VC(*), and No Antioxidant(×).

sodium oleate, which can also be used as stabilizer in many cases), and they work by consuming oxygen at a faster rate than the rate at which *Z*-ligustilide reacts with the oxygen and, in such cases, they protect *Z*-ligustilide until they were completely used up. This means that the use of an antioxidant imposed a lag-time upon the decomposition profile of *Z*-ligustilide. When more antioxidant was added, less drug may be oxidized until the antioxidant is fully saturated. Furthermore, antioxidants can also act by interrupting the oxidative pathways. In such a case they would themselves be regenerated and would function in a manner independent of the elapsed time (Cartensen & Rhodes, 2000).

## Combined Effects of Vitamin C and Co-solvents

The combined effects of co-solvents and antioxidant VC on *Z*-ligustilide stability were investigated by using an aqueous solution (containing 0.2% VC) as reference. Compared with the reference, when 0.2% VC was added, remaining percentage of *Z*-ligustilide in semiaqueous systems was all more than 4.33% greater. It was found that although the slopes of degradation linear plots obtained from Eq. 1 were six to one (0.3333, 0.4418, 0.8645), the parameters *k* of different combinations were close to each others (0.011, 0.009, 0.010). This suggested that, in order to stabilize *Z*-ligustilide enough, both VC and some co-solvents are indispensable. Because there were no marked difference among the solutions of different co-solvents and their combinations, and considering the safety of co-solvents to be injected, 20% PG was chosen to stabilize *Z*-ligustilide along with the antioxidant VC.

## Effects of Vitamin C Content

According to Eq. 1, the degradation linear plots (correlation coefficients between 0.9576–0.9922) of *Z*-ligustilide in the solutions containing 20% PG and various amounts of VC (0.1%, 0.2%, 0.3%, and 0.4%) could be acquired. These four linear plots were apparently parallels (*m* near to 0.4415 the same as that of 20% PG). Plot parameter *k vs.* the amount of VC (Fig. 6), it could be found that the more VC was added, the less *Z*-ligustilide degraded. When the amount of VC added exceeded 0.3%, the remaining

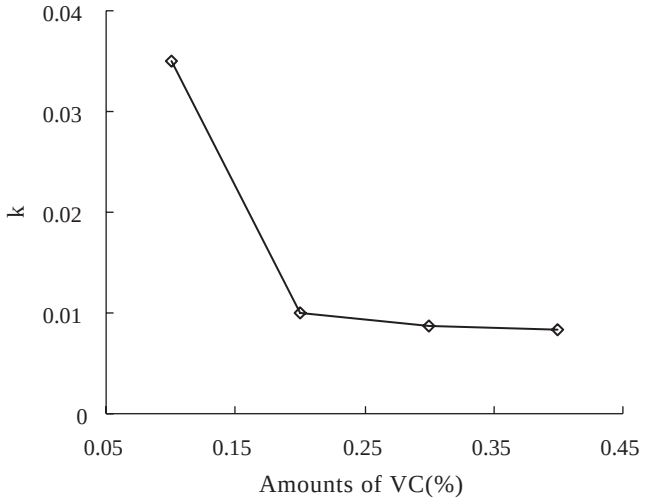


**FIGURE 6** **Regression Lines of Parameter k *vs.* Amount of VC.**

content of *Z*-ligustilide remained over 97%, and there was no clear difference between its degradation in solutions of 0.3% and 0.4% VC, maybe because the amount of oxidant (oxygen, etc.) is definite, and 0.3% VC is adequate to consume it. Thus 0.3% VC is able to stabilize *Z*-ligustilide in the semiaqueous solution.

## Arrhenius Plot and $T_{90}$ Prediction in the Semiaqueous Vehicle

Based on the research, a relatively suitable vehicle was obtained for the stability of *Z*-ligustilide, which contained 20% PG and 0.3% VC, and the pH value of the vehicle was 5.8.

As concluded above, degradation rate law of *Z*-ligustilide at these four elevated temperatures conformed to Weibull distribution, too. Parameters m of these four degradation linear plots (correlation coefficients between 0.9639 ~ 0.9969) were about 0.442, and parameters lnk were –4.3452, –4.0294, –3.6464, and –3.2728, respectively.

According to the Arrehenius equation

$$\ln k = -\frac{Ea}{RT} + \ln A \qquad (2)$$

where T is the temperature in degrees Kelvin. Plot parameters –lnk *vs.* 1000/T, another linear plot representing the relation between lnk and temperature (T) (correlation coefficient $r = 0.9971$) could be obtained, and the equation can be expressed as follow:

$$-\ln k = \frac{4845.5}{T} - 9.7365 \quad (3)$$

Therefore, -lnk at room temperature (25°C, 298.15 K) was calculated to be 6.5154. Thus, the degradation equation at room temperature can be expressed as follow:

$$\ln\ln\left(\frac{1}{1-\alpha}\right) = 0.442 \ln t - 6.5154 \quad (4)$$

Following prolonged testing of *Z*-ligustilide stability, α of *Z*-ligustilide after 180 days at room temperature was 5.9%; this could prove the accuracy of Eq. 5. According to this equation, the shelf-life ($T_{90}$) could be calculated to be almost 1.77 years. From the results, it can be concluded that *Z*-ligustilide degrades more quickly at the first period of storage, and when the factors that can induce the degradation are used out; the degradation will be slowed down markedly.

## CONCLUSION

Oxidation, hydrolysis, and photodegradation were shown to be the major degradation routes of *Z*-ligustilide, and these can be restricted by adding a suitable amount of VC, using co-solvents, adjusting the pH to a suitable range, and storing the drug in the dark. A semiaqueous vehicle, which contains 1.5% tween–80, 0.3% VC, 20% PG, and 80% water, was developed and proved to be more suitable for stabilization of *Z*-ligustilide. The Weibull probability distribution was applied to the degradation rate of *Z*-ligustilide in the vehicles, and it exhibited good approximation within the vehicles adopted; however, the parameters *m* and *k* of different vehicles differed from each other. According to this law, the degradation rate of *Z*-ligustilide can be studied by comparing the parameter *k*, and it degrades more quickly as increasing of *k*. The Arrhenius equation can be applied to these parameters, too. As predicted by the Arrhenius plot of the degradation rate, the shelf-life ($T_{90}$, 25°C) of *Z*-ligustilide in the oil was 1.77 years. This provides a good foundation for improving the *Z*-ligustilide stability and designing new preparations, and the theory of degradation rate can be applied in other cases.

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