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*Published in:*  
Journal of Separation Science

*DOI:*  
[10.1002/jssc.200700001](https://doi.org/10.1002/jssc.200700001)

Published: 01/08/2007

*Document Version:*  
Peer reviewed version

[Link to publication](#)

*Citation for published version (APA):*  
Yi, T., Zhang, H., Xie, J., & Xue, D. (2007). A new procedure for the preparative separation and isolation of Z-ligustilide from the roots of *Angelica sinensis*. *Journal of Separation Science*, 30(12), 1973-1978.  
<https://doi.org/10.1002/jssc.200700001>

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# A New Procedure for the Preparative Separation and Isolation of *Z*-ligustilide from the Roots of *Angelica sinensis*

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## Abstract

A new procedure for the separation and isolation of *Z*-ligustilide from the roots of *Angelica sinensis* (AS) was developed, and the storage conditions for *Z*-ligustilide were optimized. Using the present procedure, *Z*-ligustilide was enriched by decomposing the *Z*-ligustilide dimers yielding *Z*-ligustilide and dissolving the polar impurities in hot water. Then, the crude *Z*-ligustilide was further purified by a semi-preparative HPLC system. The spiked and non-spiked samples were used for the evaluation of the proposed procedure. Recoveries obtained were varied from 86.2 to 90.7% and RSDs from 4.0 to 6.6%. The yield and purity of the isolated *Z*-ligustilide was found to be 4.57 mg/g and 99.6%, respectively. The results of stability test have shown that the presence of oxidant contributes to *Z*-ligustilide degradation, therefore argon was chosen as a shielding gas for storage. The overall procedure is efficient and convenient which is considered suitable for the preparative separation of *Z*-ligustilide from AS.

**Keyword:** *Z*-ligustilide; *Angelica sinensis*; semipreparative HPLC; shielding gas

## 1 Introduction

The roots of *Angelica sinensis* (AS), one of the well-known Chinese herbal medicines, have been used to invigorate blood circulation and disperse blood stasis [1, 2]. It is recommended as a tonic, hematopoietic and anti-inflammatory for the treatment of menstrual disorders, amenorrhea, dysmenorrhea, anemia, constipation and rheumatic arthralgia in traditional Chinese medicine [3].

Z-ligustilide, a characteristic bioactive compound found in AS, inhibits induced proliferation of aortic smooth muscle cells and decreased blood viscosity. It is usually used for the treatment of arteriosclerosis and platelet aggregation [4, 5]. More and more studies as well as official standards chose Z-ligustilide as a chemical marker for the quality evaluation of AS and other related herbs, eg., the the rhizome of *Ligusticum chuanxiong* [2, 6, 7]. The need of Z-ligustilide as a reference standard is greater. However, the existing literature on the separation of Z-ligustilide from various herbs are mainly focused on the finding of new compounds, and these mentioned methods are relatively complicated and time consuming for the separation of a single marker compound [8-11]. Thereby, a new and efficient procedure for the preparative separation of Z-ligustilide is desirable.

In this study, a modified semi-preparative HPLC method for the preparative separation of Z-ligustilide from AS with satisfactory efficacy was present. By decomposing Z-ligustilide dimers yielding Z-ligustilide and dissolving the polar impurities in hot water, the enrichment of Z-ligustilide in AS was reached, and the capacity of the crude Z-ligustilide loaded onto semi-preparative HPLC was decreased. From the results of stability test, Z-ligustilide should be kept in an argon-filling ampoule against oxidation. The overall procedure is efficient and convenient which is considered suitable for the preparative separation of Z-ligustilide from AS.

## 2 Experimental

### 2.1 Instrumentation

#### 2.1.1 Sample extraction

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2 A Branson 5210E–MTH ultrasonic processor (280 W, Branson ultrasonics corporation, CT,  
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4 USA) was used for AS sample extraction. A Labconco Freeze Dry System (Kansas City, MO,  
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6 USA) was used for drying the AS residue after extraction.  
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### 9 10 **2.1.2 HPLC-DAD analysis and semi-preparative HPLC**

11 A PerkinElmer Series 200 HPLC system consisting of a vacuum degasser, binary pump,  
12  
13 autosampler, thermostated column compartment and diode array detector (DAD) (Norwalk, CT,  
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15 USA) was used for quantitative analysis and preparative separation. For chromatographic  
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17 analyses, an alltima C18 column (4.6 mm×250 mm, 5 µm, Deerfield, IL, USA) with a compatible  
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19 guard column was used. The mobile phase consisted of water (A) and acetonitrile (B) using a  
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21 gradient program of 15-20% (B) in 0-10 min, 20-53% in 10-40 min, and 53-100% in 40-60 min.  
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23 The solvent flow rate was 1 mL/min and the column temperature was set at 30 °C. UV 293 nm,  
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25 the maximal absorption wavelength of *Z*-ligustilide, was chosen as the measuring wavelength.  
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30 The crude *Z*-ligustilide was purified with the same HPLC instrument equipped with a 2-mL of  
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32 sample loop and a Supelco C18 semi-preparative column (250 mm × 21.2 mm, 12 µm, Bellefonte,  
33  
34 PA, USA). The mobile phase consisted of water (A) and methanol (B) using an optimal gradient  
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36 elution of 60% B at 0-10 min, 60-100% B at 10-90 min. The flow rate was 10 mL/min and the  
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38 injection volume was 2 mL, respectively.  
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### 42 **2.2 Plant materials and reagents**

43 The roots of *Angelica sinensis* (AS) were collected from Gansu province of China in October,  
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45 2004. Identities of these herbs were confirmed by Prof. Hao Zhang (West China School of  
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47 Pharmacy, Sichuan University, Chengdu, P.R.China.) using geographical origin identification,  
48  
49 macroscopic identification, microscopic identification, and physicochemical identification.  
50  
51 Voucher specimens were deposited in the Herbarium, Sichuan University, China (No. 55:1:7).  
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55 ANC and methanol for mobile phase and extraction solvent were of HPLC grade  
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57 (Lab-scan, Bangkok, Thailand). Deionized water was generated from a milli-Q water system  
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59 (Millipore, Bedford, MA, USA).  
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### 2.3 Enrichment of Z-ligustilide from AS by an ultrasonic method

Dried AS roots were ground to powder (20-40 mesh), a 200 g aliquot of the sample was sonicated in 500 mL of hot water at 80 °C for 30 min (490 W), and cooled. The mixture was filtered through a slow-filter, and the AS residue was sonicated by the above-mentioned method for three times. The AS residue after extraction was lyophilized with a Labconco freeze dry system. The freeze-dried AS residue was extracted in 200 mL of methanol by means of sonication at room temperature for 30 min, and the operation was repeated for three times. The combined extract was evaporated to 10 mL under vacuum ( $T = 35\text{ }^{\circ}\text{C}$ ), and the concentrated extract was injected into the semi-preparative HPLC system for further purification.

### 2.4 Purification of Z-ligustilide from AS using semi-preparative HPLC

The effluent of Z-ligustilide was collected according to the semi-preparative HPLC chromatogram, and then was dried with  $\text{N}_2$  stream ( $T = 35\text{ }^{\circ}\text{C}$ ). The purified Z-ligustilide was identified by  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra with the published data [12], and its purity was determined by HPLC-UV, respectively.

### 2.5 Validation of the procedure

To evaluate the efficacy of the proposed procedure, recoveries were determined with spiked and non-spiked samples. The spiked samples were prepared by adding mixture 2 g of the pure Z-ligustilide to 200 g of AS powder, and then the spiked and non-spiked samples were extracted according to the proposed procedure simultaneously ( $n=3$ ).

### 2.6 Optimization of storage conditions for Z-ligustilide

The isolated Z-ligustilide was sealed in an ampoule (2 mg/ampoule). Influence of heat, light and oxygen on the stability of Z-ligustilide was studied using an orthogonal test  $L_4 (2^3)$  as shown in Table 1.

*Insert Table 1 Here*

## 3 Results and discussion

### 3.1 Choice of plant material for the separation

Our previous research suggested that AS contains more amounts of *Z*-ligustilide and less interfering constituents [6, 13], comparison with the other *Z*-ligustilide-containing herbs, eg., the roots of *Ligusticum chuanxiong*, *Cnidium officinale*, *Angelica sinensis* and *Angelica acutiloba*. Thereby, AS was chosen as plant material for the separation.

Based on our previous research [13], the major constituents in AS have been identified as ferulic acid (1), senkyunolide I (2), senkyunolide H (3), senkyunolide F (4), coniferyl ferulate (5), senkyunolide A (6), butylphthalide (7), *E*-ligustilide (8), *E*-butylidenephthalide (9), *Z*-ligustilide (10), *Z*-butylidenephthalide (11), angelicide (12), riligustilide (13), tokinolide B (14), levistolide A (15), senkyunolide P (16), *Z*-ligustilide dimer E-232 (17), and *Z*, *Z'*-3,3',8,8'-diligustilide (18) by using the combined HPLC-DAD and mass spectrometric techniques (MS) [6, 14, 15]. This result retrieved that the major types of constituents in AS were phthalide constituents, and the structures of the identified compounds are shown in Figure 1.

*Insert Figure 1 Here*

### 3.1 Enrichment of *Z*-ligustilide and the proposed transformation of instable constituents in AS

In this study, AS powder was sonicated in hot water. *Z*-ligustilide, the predominant compound in AS, is water-insoluble and heat-stable. Compound 1-3 is water-soluble for their multi-hydroxy or carboxylic structures, while compound 4, 6, 7, 9 and 11 are relatively minor constituents. Compound 5 is hydrolyzed in hot water easily [16], and the hydrolysates both are water-soluble. Compound 8 is a trans-isomer of *Z*-ligustilide and compounds 12-18 are dimmers of *Z*-ligustilide, therefore all of them they might be decomposed into *Z*-ligustilide in hot water. The proposed pathway for the transformation of these instable constituents is shown in Figure 2.

*Insert Figure 2 Here*

Extraction procedure, including solvent consumption, extraction times, extraction periods, and extraction temperature was further optimized by a single factor method of optimization.

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Sonication of 30 min for 3 times at 80 °C is effective to enrich Z-ligustilide and remove the impurity as far as possible.

The AS powder and the freeze-dried AS residue were extracted in methanol [13], respectively. The methanolic extracts were injected into HPLC for qualitative comparison (Figure 3, Figure 4). The results demonstrated that the sonication in hot water was effective to enrich Z-ligustilide and remove impurity.

*Insert Figure 3 and Figure 4 Here*

### 3.3 Purification of Z-ligustilide using semi-preparative HPLC

Currently, two strategies for column separation of Z-ligustilide are generally employed. The first strategy is normal phase separation based on normal-phase supports, eg., silica gel [8, 9, 17, 18]. However, due to Z-ligustilide that might involve in the formation of hydrogen bond between phthalide lactones and silica gel, the trailing effect might interfere with the separation. Another strategy is based on reversed-phase supports, such as octadecylsilane bonded silica (ODS) [11, 19-21]. Comparing with the first strategy, ODS can provide superior separation effect with less trailing effect. Nevertheless, the application of this strategy has been limited because of the high cost of reversed-phase supports. As a common drawback, the two strategies are both time consuming and pollutional.

To solve this problem, semi-preparative HPLC technique was used. Semi-preparative HPLC can provide an on-line chromatogram for each peak in the herb extract [22, 23]. In most cases, the purity of the isolated compound and the conveniences of the procedure are satisfactory [24, 25].

In the present purification procedure, the 25- $\mu$ L sample loop and the analytical HPLC column were replaced with a 2-mL sample loop and a semi-prparative HPLC column, respectively. An optimized mobile phase system consisting of methanol and pure water was used for the further purification of Z-ligustilide, and baseline separation of the major constituents was obtained. The retention time of Z-ligustilide was about 21-25 minutes and the non-polar impurities were eluted



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2 after 30 minutes as shown in figure 5. The effective purification was reached and about 40 mL (10  
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4 mL/min, 4 min) of fraction was collected for each injection.  
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7 *Insert Figure 5 Here*  
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9 As a result, the yield of Z-ligustilide was 4.57 mg/g on the air-dry basis and the purity  
10 determined by HPLC was 99.6%. Recoveries obtained were varied from 86.2 to 90.7% and RSDs  
11 from 4.0 to 6.6% for the evaluation. The overall procedure is efficient and convenient which is  
12 considered suitable for the preparative separation of Z-ligustilide from AS.  
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### 18 **3.4 Study of storage conditions for Z-ligustilide**

19 Suitable storage conditions are desirable to ensure the stability of Z-ligustilide in shelf-life. Z-  
20 ligustilide is an oleaginous fluid at room temperature; thus, an ampoule was chosen as the container.  
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28 Effects of heat, light and oxidant usually contribute to the instability of drugs [26], therefore an  
29 orthogonal test was designed to evaluate the effect of these three factors on the stability of Z-  
30 ligustilide in 3 months of storage (Table 1). The results have shown that oxidation and light are  
31 the main factors for the degradation of Z-ligustilide, and it is necessary to chose argon as a shielding  
32 gas because of its inertia and high density [27]. On the other hand, the results confirmed that heat  
33 has little effect on the stability of Z-ligustilide, therefore Z-ligustilide is relatively stable during  
34 sonication in hot water. For a longer storage, the purified Z-ligustilide should be kept in an argon-  
35 filling brown ampoule at about -10 °C against oxidation.  
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## 50 **4 Concluding remarks**

51 The present procedure facilitates ultrasonic vibration in hot water for enriching Z-ligustilide in  
52 AS powder and decreasing the capacity loaded onto semi-preparative HPLC. It will probably be an  
53 effective method for the preparative separation of Z-ligustilide from AS. Also, the results of  
54 stability test have shown that the presence of oxidant contributes to Z-ligustilide degradation,  
55 therefore argon was chosen as a shielding gas for storage.  
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5 **Acknowledgements**  
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7 The author would like to thank Mr. Zhonghui Li (Chemical department, Hong Kong Baptist  
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9 University) for the recording of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra.  
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## 5 References

- [1] The State Pharmacopoeia Commission of P.R.China, *The Pharmacopoeia of the People's Republic of China, Vol. 1*, Chemical Industry Publishing Press, Beijing 2000, pp. 89
- [2] Department of Health, the Government of the Hong Kong Special Administrative Region, *Hong Kong Chinese Materia Medica Standards, Vol.1*, Popular Publishing Press, Hong Kong 2005, pp. 39-48
- [3] Zschocke, S., Classen-Houben, D., Bauer, R., (Wagner H, Bauer R, Xiao PG, Chen JM, Eds.) *Radix Angelicae sinensis* (Danggui), Chinese Drug Monographs and Analysis; Vol. 3(14), 2001
- [4] Ozaki, Y., Sekita, S., Harada, M., *Yakugaku Zasshi* 1989, 109, 402-406.
- [5] Foye, W. O., Wang, X. P., Wang, H. F., *Med. Chem. Res.* 1997, 7, 180-191.
- [6] Yi, T., Leung, K. S. Y, Lu, G. H., Chan, K., Zhang, H., *Chem. Pharm. Bull.* 2005, 54, 255-259.
- [7] Department of Health, the Government of the Hong Kong Special Administrative Region, *Hong Kong Chinese Materia Medica Standards, Vol.2*, Popular Publishing Press, Hong Kong 2007, pp. 55-64
- [8] Su, D. M., Yu, S. S., Qin, H. L., *Acta Pharm. Sin.* 2005, 40, 141-144.
- [9] Huang, W. H., Song C. Q., *Acta Pharm. Sin.* 2003, 38, 680-683.
- [10] Zhang, D. L., Teng, H. L., Li, G. S., Liu, K., Su, Z. G., *Sep. Sci. Technol.* 2006, 41, 3397-3408.
- [11] Lu, X. H., Liang, H., Zhao, Y. Y., *China J. of Chin. Mater. Med.* 2003, 28, 423-425.
- [12] Fischer, F. C., Gijbels, M. J., *Planta Med.* 1987, 53, 77-80.
- [13] Yi, T., Leung K. S. Y, Lu, G. H., Zhang, H., *Planta Med.* 2007, 73, (in press)
- [14] Lin, L. Z., He, X G., Lian, L. Z., King, W., Elliott, J., *J. Chromatogr. A* 1998, 810, 71-79.
- [15] Yi, T., Leung, K. S. Y, Lu, G. H., Zhang, H., Chan, K., *Chem. Pharm. Bull.* 2005, 53, 1480-1483.
- [16] Lu, G.H., Chan, K., Leung, K., Chan, C. L., Zhao, Z. Z., Jiang, Z. H., *J. Chromatogr. A* 2005, 1068, 209-219.

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- [17] Wu, D. R., Lohse, K., Greenblatt, H. C., *J. Chromatogr. A* 1995, 702, 233-241.
- [18] Schiel, J. E., Mallik, R., Soman, S., Joseph, K. S., Hage, D. S., *J. Sep. Sci.* 2006, 29, 719-737.
- [19] Rodriques, C. M. P., Setchell, K. D. R., *Biomed. Chromatogr.* 1996, 10, 1-5.
- [20] Wahlen, E., Tamasawa, N., Ichimiya, H., Egestad, B., Sjoevall, J., *J. Lipid Res.* 1994, 35, 1902-1906.
- [21] Tomandl, J., Tallova, J., Tomandlova, M., Palyza, V., *J. Sep. Sci.* 2003, 26, 674-678.
- [22] Mazzei, J. L., d'Avila, L. A., *J. Liq. Chromatogr. Relat. Technol.* 2003, 26, 177-193.
- [23] Miliauskas, G., van Beek, T. A., de Waard, P., Venskutonis, R. P., Sudhoelter, E. J. R., *J. Chromatogr. A* 2006, 1112, 276-284.
- [24] Carbognani, L., *J. Sep. Sci.* 2003, 26, 1575-1581.
- [25] Gaul, S., Lehnert, K., Conrad, J., Vetter, W., *J. Sep. Sci.* 2005, 28, 2268-2274.
- [26] Mollica, J. A., Ahuja, S., Cohen, J., *J. Pharm. Sci.* 1978, 67, 443-465.
- [27] Waterman, K. C., Adami, R. C., Alsante, K. M., Hong, J., Landis, M. S., Lombardo, F., Roberts, C. J., *Pharm. Dev. Technol.* 2002, 7, 1-32.

## Legends for Figure and Tables

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11 **Figure 1.** Chemical structures of the constituents identified in the roots of *Angelica*  
12 *sinensis*  
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18 **Figure 2.** Proposed pathway for the transformation of instable constituents in hot  
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24 **Figure 3.** Typical HPLC chromatogram of the roots of *Angelica sinensis*  
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28 **Figure 4.** Typical HPLC chromatogram of the freeze-dried residue of *Angelica*  
29 *sinensis*  
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34 **Figure 5.** Typical semi-preparative HPLC chromatogram of raw *Z*-ligustilide  
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40 **Table 1.** Study on the effect of heat, light and oxygen on the stability of  
41 *Z*-ligustilide using orthogonal test  $L_4 (2^3)$   
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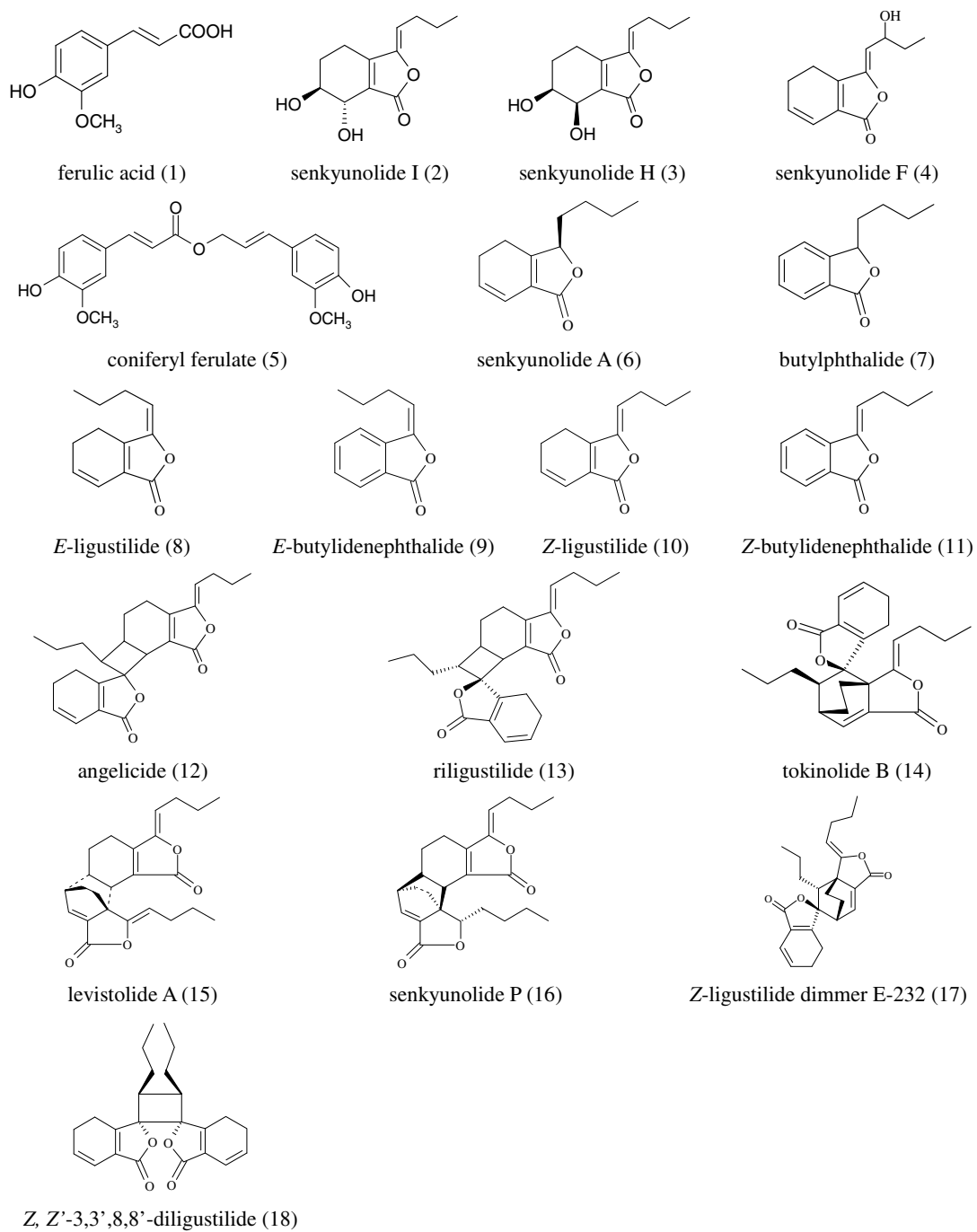


Figure 1

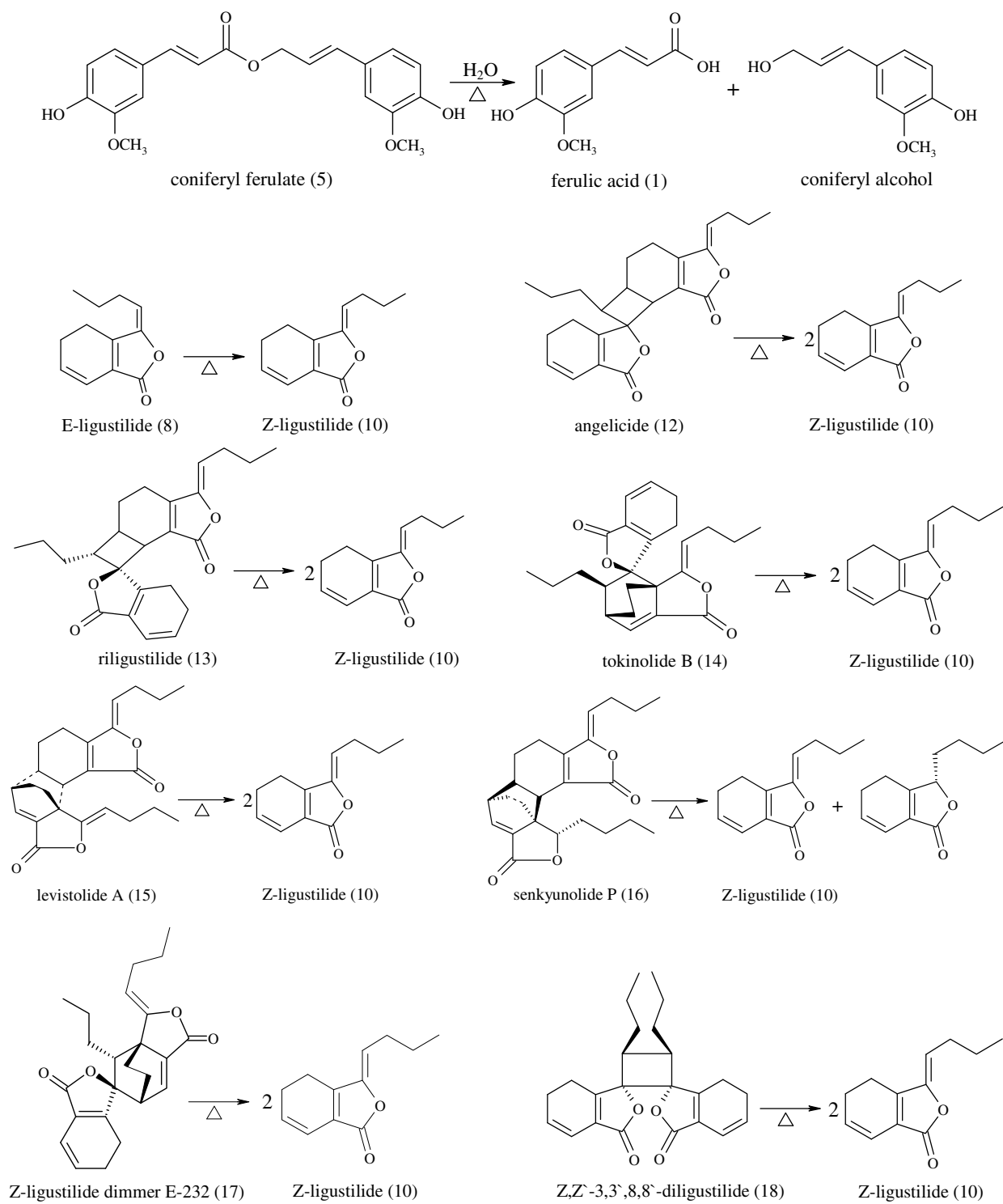


Figure 2

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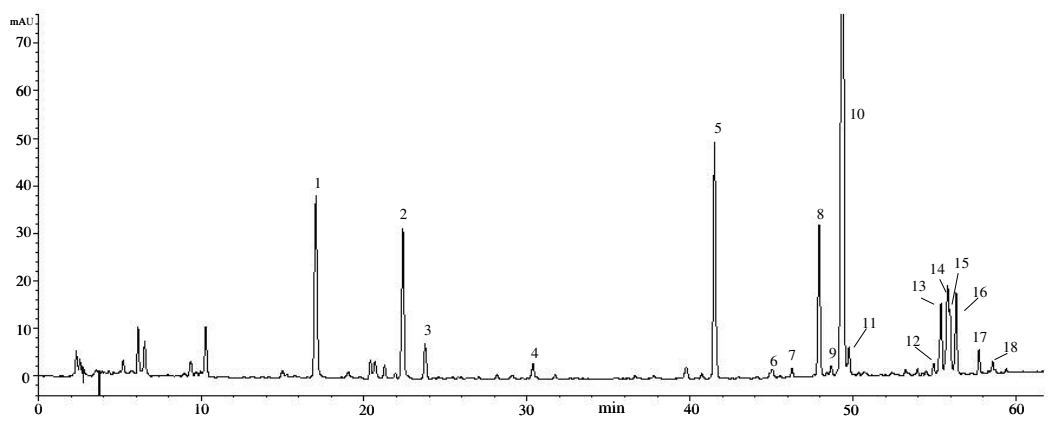
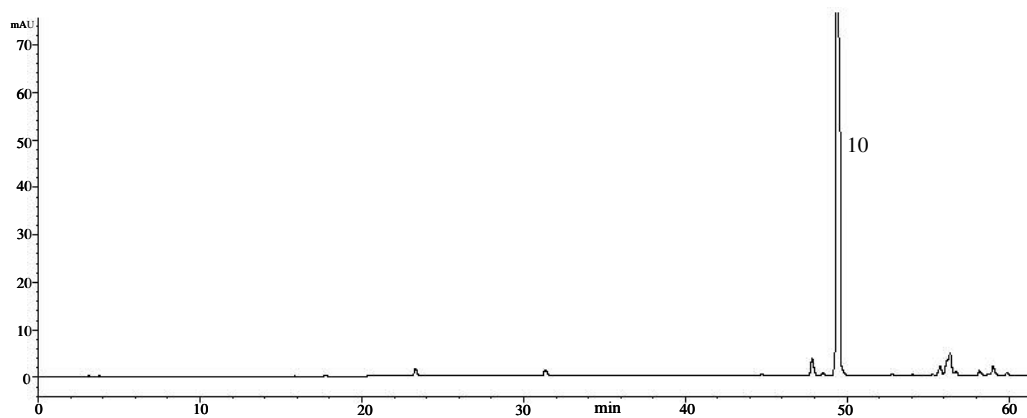


Figure 3



**Figure 4**

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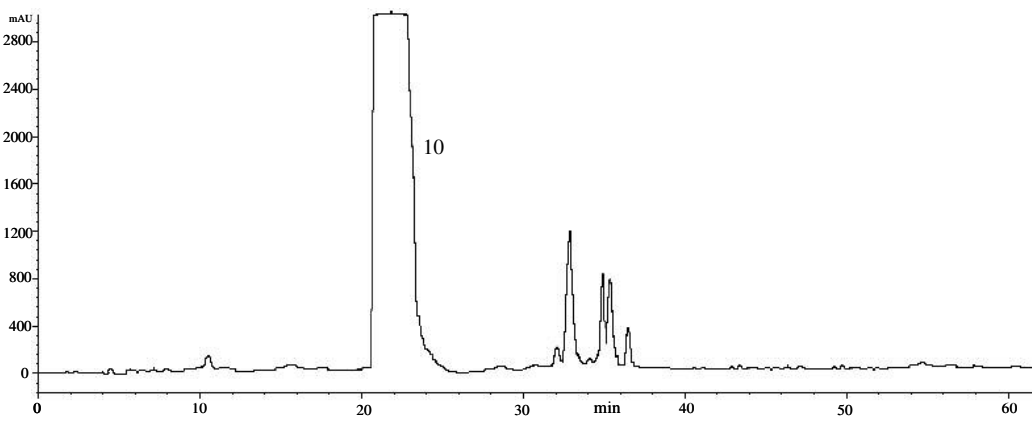


Figure 5

**Table 1**

Trial No.	Three factors on the stability of Z-ligustilide			Content (%, n = 3)
	Temperature	Light	Air	
1	+	+	+	$y_1 = 76.3$
2	+	-	-	$y_2 = 87.2$
3	-	+	-	$y_3 = 85.6$
4	-	-	+	$y_4 = 82.2$
$I_j$	$I_1 = y_1 + y_2$ $= 163.5$	$I_2 = y_1 + y_3$ $= 161.9$	$I_3 = y_1 + y_4$ $= 158.5$	
$II_j$	$II_1 = y_3 + y_4$ $= 167.8$	$II_2 = y_2 + y_4$ $= 169.4$	$II_3 = y_2 + y_3$ $= 172.8$	
$k_j$	$k_1 = 2$	$k_2 = 2$	$k_3 = 2$	
$I_j/k_j$	81.75	80.95	79.25	
$II_j/k_j$	83.9	84.7	86.4	
Extreme deviation ( $D_j$ )	2.15	3.75	7.15	

+, room temperature, sunlight, and unsealed ampoule for 3 months, respectively;  
 -, -10 °C, brown ampoule, and be sealed under argon for 3 months, respectively.