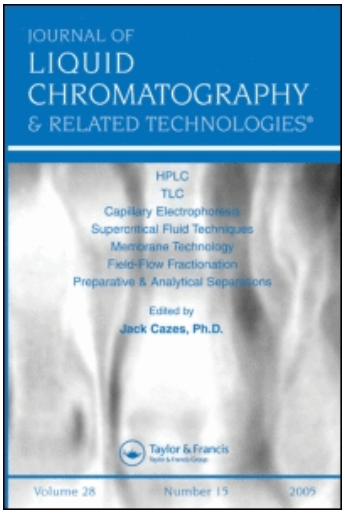

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Microwave-Assisted Extraction and Fingerprint Studies of *Schisandra Chinensis* (*Turcz.*) by High Performance Liquid Chromatography

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Microwave-Assisted Extraction and Fingerprint Studies of *Schisandra Chinensis* (*Turcz.*) by High Performance Liquid Chromatography

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Abstract: In order to choose an appropriate extraction method, samples of *Schisandra Chinensis* (*Turcz.*) *Baill* were extracted with different methods and microwave-assisted extraction was found to be the best one for extraction of *Schisandra Chinensis* (*Turcz.*) *Baill.* The contents of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin in 10 samples collected from different regions in China were determined by HPLC. The chromatograms of ten samples were applied to establish the fingerprint of *Schisandra Chinensis* (*Turcz.*) *Baill* based on high performance liquid chromatography. The fingerprint consisting of 18 common peaks showed good stability and repeatability, with RSD less than 1.5% for retention time in HPLC. The fingerprint is suitable to identify and differentiate samples from different regions in China and can be used for quality control.

Keywords: *Schisandra chinensis* (*Turcz.*) *Baill*, Microwave-assisted extraction (MAE), Fingerprint analysis, High performance liquid chromatography (HPLC)

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INTRODUCTION

Schisandra Chinensis (*Turcz.*) *Baill* has been widely used as a traditional Chinese medicine in China and Japan. The fruits of *Schisandra Chinensis* (*Turcz.*) *Baill* are effect as a tonic, a sedative, an antitussive, and an anti-aging drug.[1] The major active compounds found in the fruits of *Schisandra Chinensis (Turcz.) Baill* are lignans, which have a dibenzocyclooctadiene skeleton, such as schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin. The effects of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin on alanine transaminase, aspartate aminotransferase, albumin and total protein in serum show that they can afford protection against CCl_4 induced hepatic damage.[2] Modern medicine research suggests that these lignans have a protective effect on the liver and an immuno-modulating effect.[3]

In view of the above beneficial effects of *Schisandra Chinensis* (*Turcz.*) *Baill* and, in order to further study it deeply, an efficient and convenient method for extraction of *Schisandra Chinensis* (*Turcz.*) *Baill* from natural plants is warranted, due to the extraction of *Schisandra Chinensis* (*Turcz.*) *Baill* from medicinal plants by classical methods being tedious, time consuming, and requiring a large number of solvents. Recently, there are some papers about using non conventional methods such as supercritical CO_2 extraction technique for extraction of *Schisandra Chinensis* (*Turcz.*) *Baill* have been reported.[4–6] To our knowledge, there have been no reports about using microwave-assisted techniques for extraction of *Schisandra Chinensis* (*Turcz.*) *Baill* published.

Currently, the microwave-assisted technique is widely used in the field of sample preparation for many chemistry laboratory applications.[7–9] The use of microwave-assisted techniques for treatment of solid and liquid samples enables the analyst to adapt the sample characteristics to the requirements of the analytical technique easily and quickly. The microwave-assisted technique has also been employed for the extraction of a variety of analytes from several herbs,[10–12] thus reducing treatment time and solvent consumption than conventional extraction techniques. Nevertheless, the development of microwave-assisted extraction (MAE) methods is still in progress.

The aim of the present paper is to develop a MAE method for the extraction of *Schisandra Chinensis* (*Turcz.*) *Baill* from natural plants, establish the characteristic fingerprint of *Schisandra Chinensis* (*Turcz.*) *Baill* with HPLC techniques. The fingerprint can help to identify and differentiate samples from different geographical origins and can be used for quality control.

EXPERIMENTAL

Apparatus

The microwave system used to perform the microwave-assisted extraction (MAE) and microwave- and ultrasonic-assisted combination extraction (MAE-USO)

processes was a microwave-ultrasound instrument (CW-2000, Dissolve Sample Testing Technique Limited Company, Shanghai, China). The system can be controlled with an infrared sensor connected to a microcomputer, which ensures the regulation and the collection of different parameters like temperature and power. The vessel was placed into the microwave irradiation cavity and fitted to the condenser. The whole system was open and run at atmospheric pressure.

Ultrasound assisted extraction was performed in an ultrasonic instrument (JL-600DT, Shanghai Jie Li Science and Technology Limited Company, Shanghai, China) and working frequency was 40 KHz and power was 120 W.

The HPLC system was equipped with dionex high performance liquid chromatograph with Chromeleon 4.0 software, P680 pump, ASI-100 automated sample injector, and PDA-100 photodiode array detector. The column was 5 mm Diamonsil™ C18 (200 mm × 4.6 mm I.D.)

Materials and Reagents

Standards of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin were purchased from The National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Samples of *Schisandra Chinensis* (*Turcz.*) *Baill* were collected from different regions of China, which are called *Fructus Schisandrae Sphenantherae* from the south of China and

Table 1. The source of sample

Sample number	Species	Region
1	*Fructus Schisandrae Sphenantherae*	Yunnan
2	*Fructus Schisandrae Sphenantherae*	Zhejiang
3	*Fructus Schisandrae Sphenantherae*	Hubei
4	*Fructus Schisandrae Sphenantherae*	Jiangsu
5	*Fructus Schisandrae Sphenantherae*	Guangdong
6	*Fructus Schisandrae Chinensis*	Liaoning
7	*Fructus Schisandrae Chinensis*	Jilin
8	*Fructus Schisandrae Chinensis*	Tonghua
9	*Fructus Schisandrae Chinensis*	Hebei
10	*Fructus Schisandrae Chinensis*	Heilongjiang

Fructus Schisandrae Chinensis from the north of China, respectively (Table 1). All samples of *Schisandra Chinensis* (*Turcz.*) *Baill* were kept in a desiccator and the dried samples were ground into powder; 2.00 g were accurately weighed for every extraction experiment.

Acetonitrile used in the HPLC analysis was reagent grade. Ethanol and other chemicals used in the experimental work were analytical reagent grade. All aqueous solutions were made up in deionized water. A stock solution of four standards was prepared in acetonitrile and was diluted to the desired concentration.

Stirring Extraction

Stirring extraction was carried out in the conventional equipment with a magnetic stirrer and temperature sensor. The 2.00 g of sample was extracted with 50 mL 75% alcohol for 120 min at 76°C. The solvent extract was centrifuged for 10 min and the upper solution was filtered though a 0.45 mm nylon filter, then directly injected into the HPLC for determination of the contests of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin.

Microwave-Assisted Extraction and Microwave- and Ultrasonic-Assisted Combination Extraction

In the microwave-ultrasound instrument (CW-2000, Shanghai, China), 2.00 g of sample was placed in a 100 mL flask, which was equipped with a cooler at the top, and then extracted with 50 mL 75% alcohol for 45 s at a constant power of 200 W (MAE1), or for 10 min at the constant temperature 76°C (MAE2). In the same instrument, the same sample also was extracted with 50 mL 75% alcohol for 45 s using a microwave- and ultrasonic-assisted combination extraction technique (power, 200 w) (MAE-USO). After extractions, the solvent extracts were treated as in the stirring extraction and the filtrates were directly injected into the HPLC and GC system for determination.

Ultrasonic Extraction

Ultrasonic extraction was carried out on the ultrasonic instrument (JL-600DT, Shanghai, China) and working frequency was 40 KHz. The sample (2.00 g) was extracted with 50 mL 75% alcohol in a 100 mL flask, and kept for sonication for 40 min at room temperature at power 120 W. After extractions, the solvent extracts were treated as in the stirring extraction.

Soxhlet Extraction

For Soxhlet extraction, 2.00 g of sample was put into a 100 mL Soxhlet thimble. The apparatus was fitted with a 250 mL round bottom flask

containing 80 mL of 75% alcohol. The extraction temperature was controlled at 76°C and the flask was heated for 4 h. After cooling, the extract was treated as in the stirring extraction.

HPLC Analysis and Identification

The mobile phase consisted of acetonitrile (A) and water (B). Elution was performed using the following gradient mode: in 0 min, the flow rate to 0.8 mL · min^{-1}, the mobile phase A:B (55:45, v/v); in 10 min, the flow rate to 1.0 mL · min^{-1}, the mobile phase A:B (68:32, v/v); in 30 min, the flow rate to 1.0 mL · min^{-1}, the mobile phase A:B (68:32, v/v); in 35 min, the flow rate to 1.0 mL · min^{-1}, the mobile phase A:B (55:45, v/v) (analysis time: 35 min). The UV spectra from 210 nm to 400 nm were investigated and the detector was set to a wavelength of 254 nm. The temperature was 30°C and the injection volume is 20 mL.

RESULTS AND DISCUSSION

Effect of Extraction Method

The contents of the extraction of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin were obtained by using different extraction techniques, in order to achieve high extraction rates with less consumption of solvent and saving extraction time. Figure 1 depicts the extraction contents of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin from sample 10 by using stirring extraction for 120 min (STR), MAE1 technique for 45 s (power was kept in 200 w), MAE2 technique for 10 min (temperature was kept in 76°C), microwave- and ultrasonic-assisted combination extraction for 45 s (power, 200 w) (MAE-USO), ultrasonic extraction for 40 min (USO) and Soxhlet extraction for 4 h (SOX), respectively. The results show that the MAE1 technique greatly reduced the extraction time and obtained maximum extraction values of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin in a short time compared to the more time consuming Soxhlet, ultrasonic, and stirring extraction techniques.

Fingerprint Development of *Schisandra Chinensis* (*Turcz.*) Baill by HPLC

In order to obtain good resolution and a large number of peaks, optimization of parameters in HPLC was done through investigating the influence of the mobile phase and detection wavelength. In this work, acetonitrile and water were chosen as the optimum mobile phase (see HPLC Analysis and Identification). After the

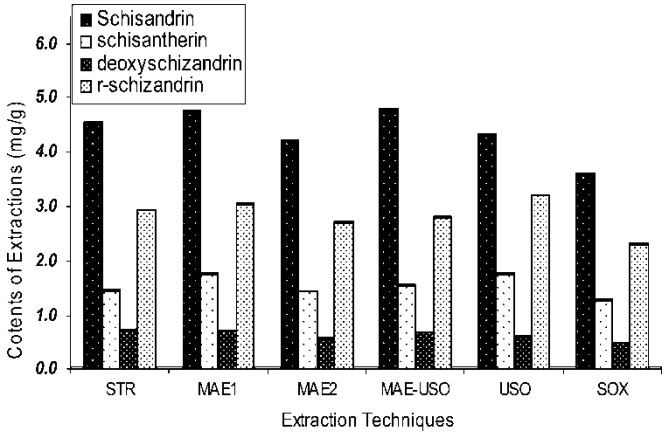

Figure 1. Comparison of the extraction contents of *schisandrin*, *schisantherin*, *deoxyschizandrin*, and *r-schizandrin* from plant material of *Schisandra Chinensis* (*Turcz.*) *Baill* (Sample 10) by different extraction techniques.

preprocessing of baseline correction was performed, most of the major peaks were baseline separated. To obtain a large number of detectable peaks on the HPLC chromatogram, the spectra from 210 nm to 400 nm for all peaks were investigated and 254 nm was selected as the best detection wavelength. At the optimized conditions, a standard three-dimensional chromatogram of

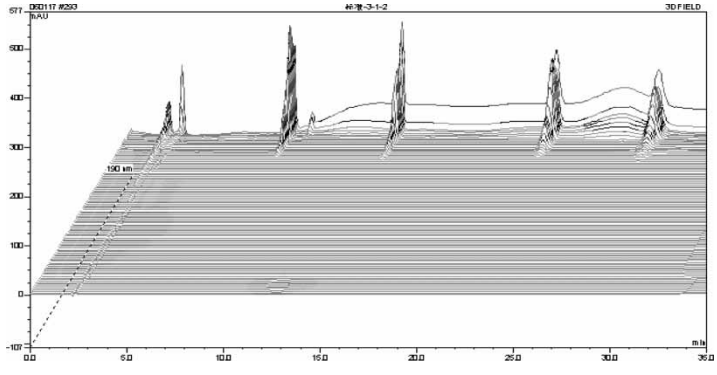

Figure 2. 3D figure of chromatogram of *schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin* (from right to left) HPLC conditions: The Diamonsil^{TM} C18 column (200 mm × 4.6 mm I.D., 5 μm), mobile phase was A (acetonitrile) and solvent B (H_2O) in the gradient mode as follows: t = 0 min, the flow rate to 0.8 mL · min^{-1}, the mobile phase A:B (55:45, v/v), t =10 min, the flow rate to 1.0 mL · min^{-1}, the mobile phase A:B (68:32, v/v); in 30 min, the flow rate to 1.0 mL · min^{-1}, the mobile phase A:B (68:32, v/v), t = 35 min, the flow rate to 1.0 mL · min^{-1}, the mobile phase A:B (55:45, v/v), photodiode array (PAD) detection at 254 nm.

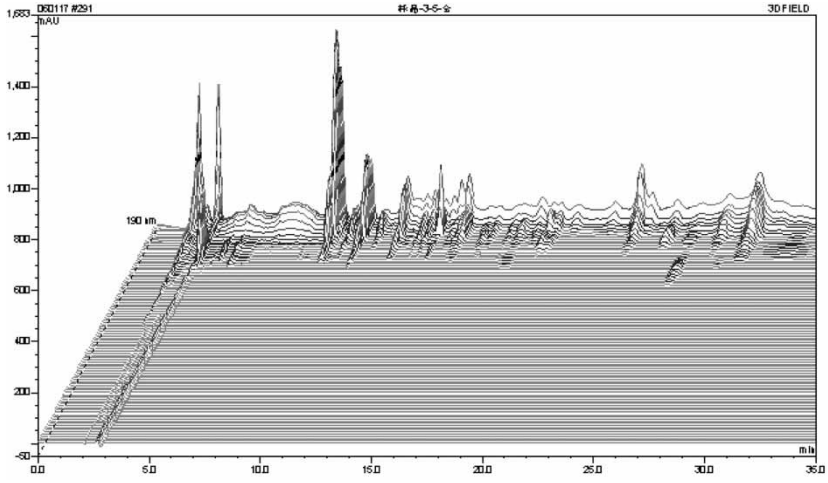

Figure 3. 3D figure of chromatogram of *Schisandra chinensis* (*Turcz.*) *Baill* (Sample 10). HPLC conditions as in Figure 2.

schisandrin, *schisantherin*, *deoxyschizandrin*, and *r-schizandrin* was determined and is shown in Figure 2. Similarly, Figure 3 is the three-dimensional chromatogram of sample 10. In the optimized condition, chromatograms of ten samples were merged into one, which is shown in Figure 4.

It is notable from Figure 4 that chromatograms of ten samples from different regions are quite different, which means that the contents of *schisandrin*, *schisantherin*, *deoxyschizandrin*, and *r-schizandrin* of samples are affected by regions. In order to compare their similarity, 10 samples were divided into two groups: group 1 consisted of samples 1–5 from the south

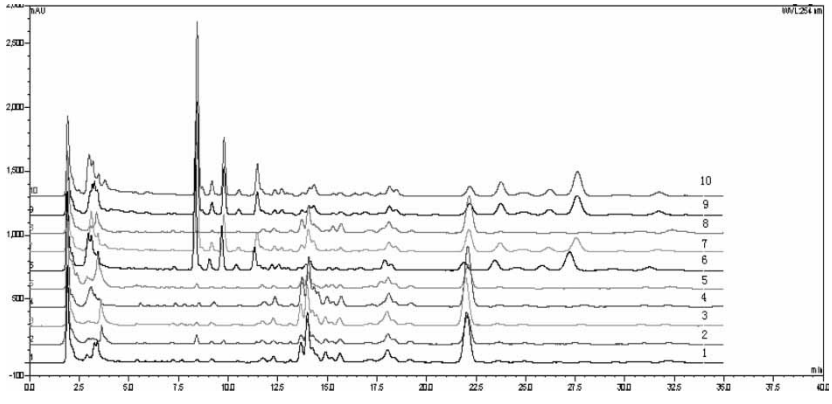

Figure 4. Chromatogram of *Schisandra chinensis* (*Turcz.*) *Baill* from different regions HPLC condition as in Figure 2.

Table 2. The similarity analysis with the relative areas of the common peaks in HPLC fingerprint of *Fructus Schisandrae Sphenantherae*

	Sample number				
The Similarity	1	2	3	4	5
HPLC					
The included angle cosine	0.9857	0.982	0.9916	0.9763	0.8863
HPLC					
Correlation coefficient	0.9856	0.982	0.9916	0.9763	0.885

of China (*Fructus Schisandrae Sphenantherae*), and group 2 consisted of samples 6–10 from the north of China (*Fructus Schisandrae Chinensis*). With two different mathematic methods including the included angle cosine and correlation coefficient calculated with the software of Excel 2002,[13] the data from 10 samples were processed for similarity analysis. Using the relative peak areas of common peaks, similarity analysis was conducted by HPLC (detection conditions as in Figure 2) and the results are shown in Tables 2 and 3, respectively. As shown in Table 2, the values of included angle cosine and correlation coefficient of most of samples are more than 0.97 (excepting a somewhat low of sample 5), which means that most of these samples are alike. From Table 3, the values of included angle cosine and correlation coefficient of all samples are higher than 0.9, except for sample 8, which also shows that these samples from the north of China are similar except for sample 8.

There are about 39 peaks within 35 min in the chromatogram. Among these, 18 common peaks comprise the fingerprint of *Schisandra Chinensis* (*Turcz.*) *Baill* obtained by HPLC (Figure 5). RSD values of relative retention time by HPLC are shown in Table 4. As shown in Table 4, RSD values of the 18 common peaks in the fingerprint are less than 1.5% (maximum 1.3 and average 0.778%), indicating that the HPLC fingerprint has good stability and repeatability. In addition to the common peaks, there

Table 3. The similarity analysis with the relative areas of the common peaks in HPLC fingerprint of *Fructus Schisandrae Chinensis*

	Sample number				
The similarity	6	7	8	9	10
HPLC					
The included angle cosine	0.9555	0.9293	0.6392	0.9105	0.9311
HPLC					
Correlation coefficient	0.9558	0.9289	0.636	0.9098	0.9313

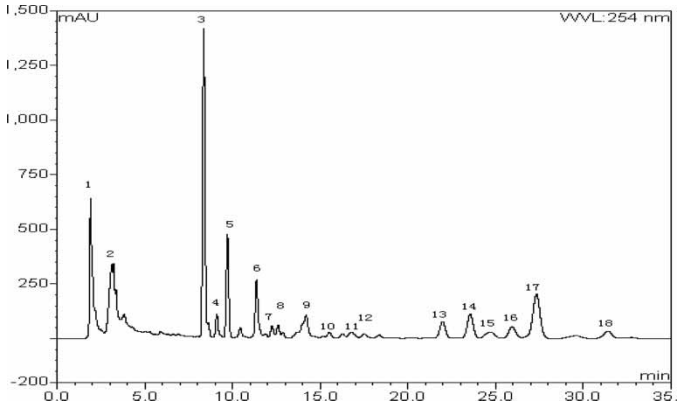

Figure 5. Chromatogram of *Schisandra chinensis* (*Turcz.*) *Baill* (Sample 10). HPLC condition as in Figure 2.

are about 21 uncommon peaks in the chromatogram. Total peak area of the uncommon peaks is less than 10%, which meets the standards.

Contents of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin in ten samples from different regions of China were determined by HPLC

Table 4. RSD of the relative retention times in HPLC for common perks of samples 6–10 of *Schisandra Chinensis* (*Turcz.*) Baill

Peak number	RSD (%) $n = 5$
1	0.6
2	1.2
3	0.5
4	0.6
5	0.6
6	1.0
7	1.1
8	1.3
9	0.6
10	0.6
11	0.6
12	0.6
13	0.5
14	0.7
15	1.1
16	0.9
17	0.6
18	0.9

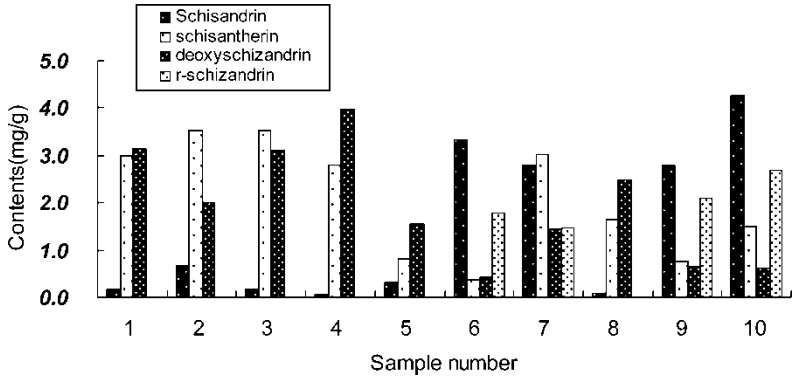

Figure 6. Comparison of the contents of schisandrin, schisantherin, deoxyschizandrin, and r-schizandrin of *Schisandra Chinensis (Turcz.) Baill.* HPLC condition as in Figure 2.

(shown in Figure 6). This reveals that the contents are affected by regions due to the variation in agroclimatic and geographical conditions: the content of deoxyschizandrin is richer in *Fructus Schisandrae Sphenantherae* (samples 1–5), but schisandrin is less in these samples and r-schizandrin is not found in them; schisandrin is richer in *Fructus Schisandrae Chinensis* (samples 6–10), and the content in sample 10 reaches the maximum value of 4.2633 mg/g. The content of r-schizandrin in sample 8 is 0.

CONCLUSIONS

Our study demonstrates that microwave-assisted extraction is the best method for extraction of the samples of *Schisandra chinensis* (*Turcz.*) *Baill*. The fingerprint of *Schisandra chinensis* (*Turcz.*) *Baill* has been established by HPLC, which is suitable to identify and differentiate samples from different regions of China and can be used for quality control.

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REFERENCES

1. Huang, T.; Shen, P.N.; Shen, Y.N. J. Chromatogr. A **2005**, *1066*, 239.

2. Tian, K.; Qi, S.D.; Cheng, Y.Q.; Chen, X.G.; Hu, Z.D. J. Chromatogr. A **2005**, *1078*, 181.
3. Wang, Z.Z.; Zhen, S. J. TCM Res. **1995**, *7*, 119.
4. Yang, S.B.; Chin. J. Chin. Mater. Med. **2001**, *26* (11), 755.
5. Liu, B.; Dean, J.R.; Chin. J. Pharm. **2000**, *31* (3), 101.
6. Xiang, Z.M.; Li, H.L.; Zhang, L.; Chin. J. Chromatogr. **2003**, *21* (6), 568.
7. Kingston, H.M.; Haswell, S.J. *Microwave-Enhanced Chemistry*; Kingston, H.M., Haswell, S.J., Eds.; American Chemical Society: Washington, DC, 1997.
8. Zlotorzynski, A. Crit. Rev. Anal. Chem. **1995**, *25*, 43.
9. Jin, Q.; Liang, F.; Zhang, H.; Zhao, L.; Huan, Y.; Song, D. Trends Anal. Chem. **1999**, *18*, 479.
10. Labbozzetta, S.; Valvo, L.; Bertocchi, P.; Manna, L. J. Pharm. Biomed. Anal. **2005**, *39*, 463.
11. Padron-Sanz, C.; Halko, R.; Sosa-Ferrera, Z.; Santana-Rodriguez, J.J. J. Chromatogr. A **2005**, *1078*, 13.
12. Chemat, S.; Ait-Amar, H.; Lagha, A.; Esvejd, D.C. J. Chromatogr. Chem. Engn. Proc. **2005**, *44*, 1320.
13. Miao, A.D.; Son, D.J. Prog. Pharmaceut. Sci. **2002**, *27*, 51.

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