Rapid Extraction and High-Performance Liquid Chromatographic Determination of Parthenolide in Feverfew (*Tanacetum parthenium*)

Joseph ZiQi Zhou,* Xiaolan Kou, and Doug Stevenson

Department of Research and Development, Nature's Sunshine Products, Inc., 1655 North Main Street, Spanish Fork, Utah 84660

A rapid and sensitive method for quantifying parthenolide in feverfew herb (*Tanacetum parthenium*) was developed that is significantly faster than those reported in the literature. The extraction system consisted of acetonitrile/water (90:10, v/v) in a bottle with stirring for 30 min. Both Soxhlet and bottle-stirring extractions were studied. Samples were analyzed using high-performance liquid chromatography with a Cosmosil C18-AR column (150 × 4.6 mm, 5 μm, 120 Å). The mobile phase consisted of acetonitrile/water (55:45, v/v) with a flow rate of 1.5 mL/min and UV detection at 210 nm. Analysis time was 6 min, with a detection limit of 0.10 ng on column. The calibration curve was linear over a range of 0.160–850 μg/mL parthenolide with R^2 = 0.9999. Replicate tests indicated good reproducibility of the method with an RSD% = 0.88 (n = 10). Spike recovery of parthenolide was found to be 99.3% with an RSD% = 1.6 (n = 6).

Keywords: *Parthenolide; feverfew; Tanacetum parthenium; HPLC; extraction*

INTRODUCTION

Feverfew (*Tanacetum parthenium*), native to Europe and the British Isles, is commonly found along fields and roadsides and is also grown in flower gardens throughout Europe and the United States. The short perennial grows 15–60 cm tall and blooms from July to October with yellow flowers (Wren, 1994; Baily et al., 1978). The herb's medicinal effects have been known for centuries, and it traditionally is recommended for fevers and headaches. Feverfew has also been traditionally used for the treatment of anemia, earache, and indigestion; as an abortifacient; and as a remedy to eliminate parasites from the intestines (Johnson et al., 1985; Murphy et al., 1988; Duke, 1988; Murray et al., 1991). Much of the herb's efficacy has been attributed to parthenolide (Figure 1), the predominant sesquiterpene lactone present in feverfew (Groenewegen and Heptinstall, 1990). Studies in Great Britain indicate that the frequency and duration of migraine attacks are reduced if feverfew is taken daily (Johnson et al., 1985; Murphy et al., 1988).

Some work has been done to determine parthenolide in feverfew. Yoshioka used chloroform/petroleum ether to extract sesquiterpene lactones, including parthenolide, and used NMR for identification (Yoshioka et al., 1970). Marchand used chloroform extraction and developed a high-performance liquid chromatography (HPLC) gradient method for analysis (Marchand et al., 1983). In further work Rey reported chloroform Soxhlet extraction and HPLC analysis (Rey et al., 1992). Other extraction methods are also reported (Bloszyk et al., 1978; Smith et al., 1992; Commission Francaise de Pharmacopee, 1987). Heptinstall's group tried a chloroform stirring extraction (Groenewegen et al., 1990) and a
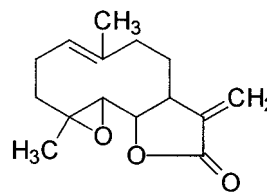
Figure 1. Structure of parthenolide, molecular weight = 248.32.

petroleum ether Soxhlet extraction (Awang et al., 1991) combined with HPLC and ^{1}H NMR analysis. They also tested an acetone extraction and HPLC derivatization method (Heptinstall et al., 1992). Recently, Heptinstall et al. reported the extraction efficiencies of a series of ethanol/phosphate-buffered saline and acetonitrile/phosphate-buffered saline mixtures for the resuspension of acetone extracts (Brown et al., 1996). The most widely used method in the herbal industry among the cited methods is Soxhlet extraction using petroleum ether combined with HPLC analysis (Awang et al., 1991), and this method with some modifications now is under consideration to become one of the United States Pharmacopoeia methods (USP, 1997). The common weakness in the reviewed methods is the effectiveness of the extraction system. Most of the cited research focused on either qualitative identification of the lactone family, the development of HPLC conditions, or bioactivity studies of parthenolide. Little has been done so far to find an efficient solvent system to quantitatively extract parthenolide in feverfew. In addition, the technique of dissolving or resuspending the extract in solvent included in these published methods makes the methods less accurate and cumbersome.

This paper describes a rapid and sensitive extraction of parthenolide from feverfew. The best extraction system was determined after numerous solvent systems were examined. and results from bottle-stirring and Soxhlet extraction are compared. In addition, a signifi-

* Author to whom correspondence should be addressed: telephone (801) 798-4178; fax (801) 798-4108; e-mail JosephZ@Natr.com.

Extraction and HPLC Determination of Parthenolide in Feverfew

J. Agric. Food Chem., Vol. 47, No. 3, 1999 **1019**

cant improvement in HPLC conditions as compared to those reported in the literature is given. For more than two years this method has successfully been implemented to routinely analyze parthenolide in feverfew from a variety of sources.

MATERIALS AND METHODS

Materials. Feverfew powder from different lots used in this study was obtained from Galilee Herbal Remedies (Kfar Hanassi, Israel). Parthenolide standard was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). All other reagents used were purchased from Fisher Scientific (Pittsburgh, PA) and were of HPLC grade.

Extraction Solvent Selection and Optimization. Parthenolide content in feverfew powder was first analyzed using the method described by Awang et al. (1991). Using this method, 4 g of feverfew powder was extracted in a Soxhlet apparatus for 24 h using 450 mL of petroleum ether (30−60 °C). The resulting extract was then reduced to dryness using a rotary evaporator with a bath temperature of 40 °C followed by vacuum pumping for ∼2 h. The remaining residue was treated with 100 mL of acetonitrile, filtered with a 0.45 μm PTFE filter, and injected directly onto the column.

To get the best extraction system, other pure solvents, such as acetonitrile, ethanol, acetone, and chloroform, and the solvents combined with different amounts of water were each tested on the same lot of feverfew sample. Both Soxhlet and bottle-stirring extractions were used. In the Soxhlet method, the above extraction procedures were exactly followed. In the bottle-stirring method, 1.5 g of feverfew powder was weighed into a 120 mL amber glass bottle containing a magnetic stir bar, and then 100 mL of extraction solvent was added. The bottle was sealed with a Teflon-lined cap. After 24 h of stirring, the clear extract was filtered through a 0.45 μm PTFE syringe filter and injected onto the column. When using chloroform as the solvent, an extra step was necessary. The chloroform extract was reduced to dryness using nitrogen and then recovered with 100 mL of acetonitrile for HPLC analysis.

Profile of Extraction Efficiency versus Time. The extraction efficiency−time profile was obtained using the bottle-stirring method. In this method, 100 mL of extraction solvent was transferred to a 120 mL glass bottle containing a magnetic stir bar and placed on a magnetic stirring plate with moderate stirring. To the bottle was added 1.5 g of accurately weighed powdered feverfew, and aliquots of 1 mL were drawn from the extract at times of 0.5, 1, 3, 5, 7, 10, 15, 20, 30, and 60 min and 24 h. The extract samples were filtered through a 0.45 μm PTFE syringe filter prior to HPLC analysis. The bottles remained closed with a Teflon-lined cap except when sampling.

Liquid Chromatography. An HP 1090 series II liquid chromatograph (Hewlett-Packard Co.) with a photodiode array detector (DAD) was used. The instrument control and integration were accomplished with Hewlett-Packard 3D Chemstation software. Initial samples were analyzed with HPLC conditions as described by Awang et al. (1991), which include a Brownlee Spheri-10 RP-18 column (250 × 4.6 mm, 10 μm), a mobile phase of acetonitrile/water (45:55, v/v) at a flow rate of 2 mL/min, and DAD detection at 210 nm. The injection size was 20 μL.

Subsequent samples were analyzed using the following modified conditions: Nacalai Tesque Cosmosil C18-AR column (150 × 4.6 mm, 5 μm, 120 Å); mobile phase of acetonitrile/water (55:45, v/v) (different from 45:55 as shown above) at a flow rate of 1.5 mL/min; and DAD detection at 210 nm. The injection size was 1−20 μL. Spectral information from 190 to 600 nm was acquired with the DAD for the samples including parthenolide calibration standards to confirm the peak identity, purity, and best wavelength setting.

Quantitation. Parthenolide concentrations in feverfew extracts were determined according to an external standard procedure using a multipoint calibration curve. In the Soxhlet method, 80 mg of parthenolide standard was weighed and extracted in the same fashion as the feverfew samples. The resulting extracted solution was reduced to dryness and reconstituted with 100 mL of acetonitrile. Five standards ranging from 25 to 400 μg/mL were then made from the solution. In the bottle-stirring method, six parthenolide standards were independently made and extracted in the same manner as the feverfew samples. The concentrations of the standards ranged from 0.160 to 850 μg/mL. All injections for both standards (STD) and feverfew extract samples (SMP) were run in the following sequence (e.g., six-point calibration and two feverfew extract samples): STD1 (two injections), STD2 (2), ..., STD6 (2), SMP1 (three injections), SMP2 (3), STD1 (2), STD2 (2), ..., STD6 (2). The calibration injections bracketed unknown sample injections. Peak area was used for quantitation.

Method Reproducibility and Spike Recovery Measurement. Reproducibility was determined by performing two sets of five replicate analyses on the same lot of feverfew within a one-week time period. New calibrations were made for each day of analysis. Intralaboratory reproducibility was also examined by having the same lot of feverfew analyzed by different persons in different laboratories.

Spike recovery was measured by analyzing a replicate of six feverfew samples, which was the same lot as above, with a known amount of parthenolide standard added (∼7.5 mg): spike recovery = $[(PN_{total} - PN_{nonspike})/PN_{spiked}] \times 100\%$, where PN_{total} is the total amount of parthenolide found in the feverfew samples including the known amount of parthenolide standard spiked in the sample, $PN_{nonspike}$ is the average amount of parthenolide as found in the above replicate analysis without parthenolide spiked, and PN_{spiked} is the amount of parthenolide standard spiked.

Statistical Analysis. At least 3 replicates were performed in the extraction solvent selection and optimization for each solvent test, 5 replicates in the profile determination of extraction efficiency versus time, 10 replicates in the method reproducibility measurement, and 6 in the spike recovery examination. Three injections were made on each sample for HPLC analysis. Microsoft Excel 97 was used to compute means, standard deviations, and relative standard deviations (RSD) or coefficients of variation and to do linear regression analysis for standard calibrations, including the calculation of both linear equations based on the method of least squares and linear correlation coefficient (R^2). All mean values were subjected to analysis of variance (ANOVA) at $p < 0.05$ to determine statistical significance.

RESULTS AND DISCUSSION

Optimum Extraction System and Profile of Extraction Efficiency versus Time. Figures 2 and 3 show the results of the parthenolide extraction from feverfew using both the bottle-stirring and Soxhlet methods and various kinds of solvent systems. The results are the averages from at least three trials for each solvent. The same lot of feverfew sample was used in the tests.

It is clear that acetonitrile with 10% water (v/v) using the bottle-stirring method extracted the highest percentage of parthenolide from feverfew and is the best extraction system. The results from both ethanol and acetone systems are comparable. In contrast, the extraction with petroleum ether showed the worst efficiency of extracting parthenolide, indicating that petroleum ether, a nonpolar solvent, is not a good choice for parthenolide extraction.

Using the best extraction system as determined above, the profile of extraction efficiency versus time on high-potency feverfew was obtained (Figure 4). The results are averages from five trials with a maximum RSD% of 2.3. The most striking result with this system is that in 30 s, >80% of the parthenolide is extracted from the plant material, as compared to the results
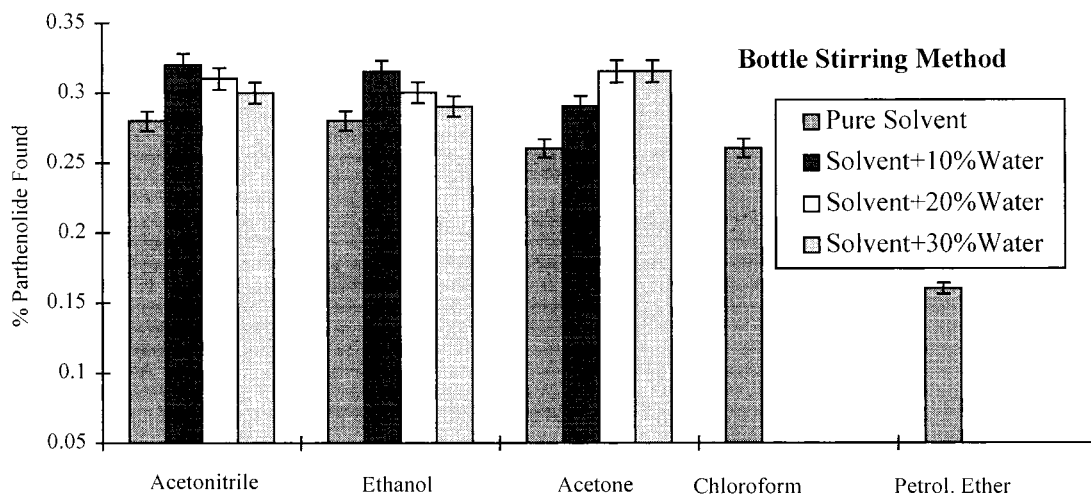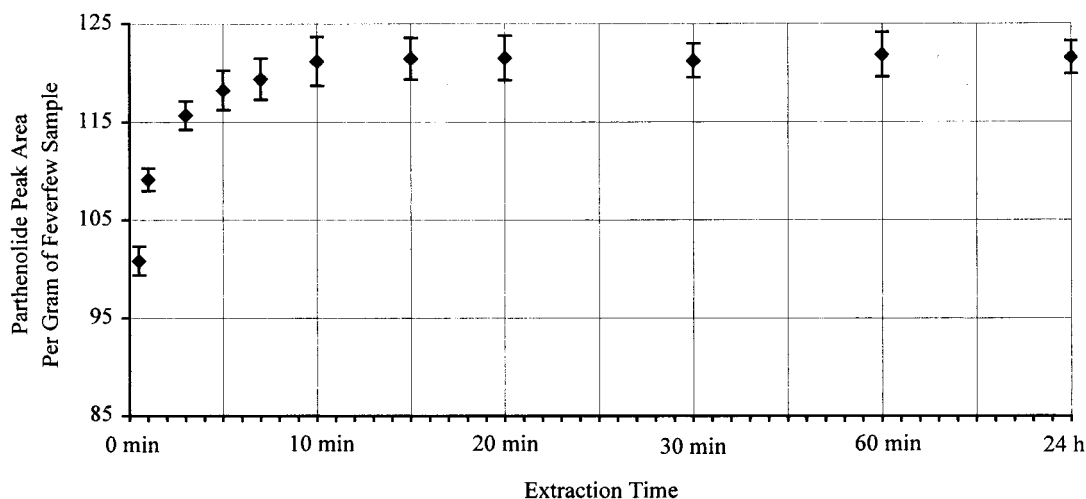
Figure 2. Extraction results in percentage of parthenolide found in the same lot of feverfew sample using the bottle-stirring method and different solvents. HPLC conditions used for quantitation: Nacalai Tesque Cosmosil C18-AR column (150 × 4.6 mm, 5 μm, 120 Å), mobile phase of acetonitrile/water (55:45, v/v) at a flow rate of 1.5 mL/min, DAD detection at 210 nm, and injection size of 10 μL. The results are averages from at least three trials for each solvent. The error bars represent RSD%.
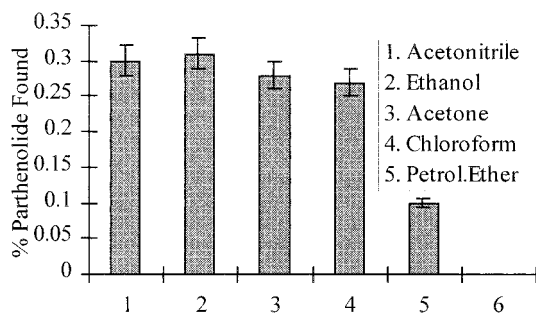
Figure 3. Extraction results in percentage of parthenolide found in the same lot of feverfew sample using the Soxhlet method and different solvents. The HPLC conditions described by Awang et al. (1991) were used for quantitation: Brownlee Spheri-10 RP-18 column (250 × 4.6 mm, 10 μm), mobile phase of acetonitrile/water (45:55, v/v) at a flow rate of 2 mL/min, DAD detection at 210 nm, and injection size of 20 μL. The results are averages from three trials for each solvent. The error bars represent RSD%.

obtained in 24 h. Within 10 min, the amount of extracted parthenolide reached the maximum and was constant to 24 h extraction.

The rapid extraction of parthenolide in the manner described above indicates an interesting fact concerning the parthenolide distribution in feverfew. Blakeman and Atkinson studied parthenolide present in the feverfew plant matrix and proposed that parthenolide may be microencapsulated physically within the plant by means of the glandular trichomes. To support this hypothesis, they used scanning electron microscopy to view the results of extraction on the trichomes in the feverfew leaves (Blakeman and Atkinson, 1979). Smith and Burford investigated the extraction of parthenolide from feverfew using a supercritical carbon dioxide extraction technique. It took them only 2 min to extract all of the "free" parthenolide on the surface. However, the extraction was not considered complete. They concluded that the parthenolide was present at different sites in the plant matrix, some from which it can be readily extracted but others where it is more tightly bound and would require the addition of a polar modifier to carbon dioxide to be released (Smith and Burford, 1992). The extraction profile seen in Figure 4 may be explained as follows: the trichomes present on large surface area of feverfew powder account for 80% of the parthenolide,

Figure 4. Profile of extraction efficiency versus time using the bottle-stirring method. Parthenolide amounts extracted are represented by peak area in a relative comparison to each other. The results are averages from five trials with three injections per sample. The error bars shown in the graph represent RSD%. A sample of 1.5 g of high-potency feverfew powder was used in each trial, and the extraction solvent was 100 mL of acetonitrile with 10% water (v/v).

Extraction and HPLC Determination of Parthenolide in Feverfew

J. Agric. Food Chem., Vol. 47, No. 3, 1999 **1021**

Table 1. Volume Effect of Extraction Solvent on Parthenolide Extraction Yield

vol of extraction solvent (mL)	amount of feverfew sample (mg)	% parthen- olide found	av and RSD% on parthenolide	total av and RSD% on parthenolide
60	1537.0	0.932		
60	1539.6	0.934	av = 0.932	
60	1557.5	0.930	RSD% = 0.22	av = 0.928
100	1534.3	0.928		
100	1519.8	0.930	av = 0.928	
100	1521.1	0.926	RSD% = 0.17	RSD = 0.40
400	1627.7	0.927		
400	1568.0	0.922	av = 0.924	
400	1524.2	0.923	RSD% = 0.27%	

which is extracted in the first 30 s. With the combined extraction solvent of acetonitrile and water, the other 20% of the parthenolide is extracted from inside particles or "bound" sites. Equilibrium is reached in 10 min with no change for 24 h. To prove experimentally the completeness of the extraction, an examination focusing on feverfew postextraction residues is needed.

Water contributed to the extraction of parthenolide from feverfew as shown in Figure 2. This may be because many of the components in the plants are hydrophilic. Water molecules attack the water soluble components in feverfew, promoting the organic solvent to catch parthenolide molecules.

Extractions using the bottle-stirring method were also performed by applying different amounts of extraction solvent to the same amount of feverfew sample. This was done to test the volume effect on the extraction yield, that is, to test whether under the testing conditions a change in the extraction solvent volume changes the amount of parthenolide extracted. In the experiment, 60, 100, and 400 mL of acetonitrile/water (90:10, v/v) solvent, respectively, were used. Table 1 shows the average results and RSD% on high-potency feverfew. It indicates that within the testing volume range the amount of parthenolide extracted is independent of the volume of extraction solvent used.

The bottle-stirring method has advantages over the Soxhlet method for parthenolide extraction. These include a much simpler extraction procedure with the benefit of much shorter extraction times. Also, the extraction can be done at room temperature rather than at raised temperatures. This is crucial due to the thermal lability of parthenolide. The bottle-stirring method is also more reproducible. In addition, a mixed solvent system, such as acetonitrile with 10% water, does not work well with the Soxhlet method because the acetonitrile is preferentially distilled over the water and essentially only pure acetonitrile is used for the extraction.

The resuspension step, as described in many published methods, reduces the extraction yield and makes the assay more cumbersome. In the Soxhlet experiment, reducing the first solvent extract to dryness leaves a green residue in the flask that will not go into solution after addition of the resuspension solvent, acetonitrile, even after several hours of mixing. Using the HPLC to directly quantitate the first solvent extract clearly improves the results. For example, analyzing the same petroleum ether extract from the Soxhlet method yielded 0.13% parthenolide without resuspension instead of 0.10% with resuspension.

Chromatographic Conditions Improvement. Figure 5 shows a sample chromatogram of feverfew extract
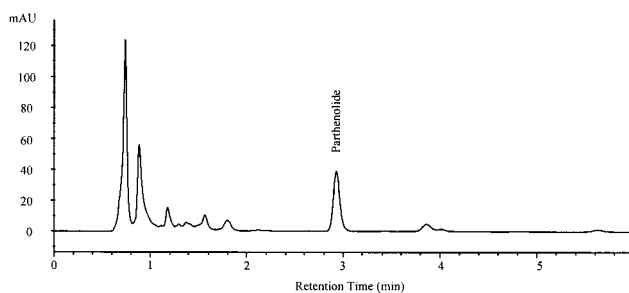
Figure 5. Sample chromatogram of feverfew extract. Conditions: column, Nacalai Tesque Cosmosil C18-4R (150 × 4.6 mm, 5 μm, 120 Å); mobile phasee, water/acetonitrile (45:55, v/v); flow rate, 1.5 mL/min; detection, 210 nm; injection size, 1.0 μL; extraction solvent, 100 mL of acetonitrile with 10% water (v/v); extraction time, 30 min.

under the modified HPLC conditions. Both the retention time and the spectral information acquired with the photodiode array detector from 190 to 600 nm of the sample were compared with the retention time and reference spectrum of a parthenolide standard and are used to identify the parthenolide peak. Comparison of the peak up slope and down slope spectra with the apex spectrum did not show interference or coelution. Adjusting the mobile phase ratio sequentially to vary peak retention times did not cause peak splitting or the appearance of shoulder(s) on the parthenolide peak. This provides further confirmation that the peak is pure parthenolide without coeluting peaks.

The modified HPLC method has higher sensitivity, better separation, shorter retention time, and lower mobile phase flow rate than the Awang method. The detection limit was 0.10 ng on column, and the linearity of a six-point calibration curve from 0.160 to 850 μg/mL had an $R^2 = 0.9999$. Column selection and mobile phase determination are the two of determining factors in HPLC method development. Selecting a newer contemporary column with appropriate packing material, better dimension, and smaller particle size can improve chromatographic performance. Increasing the organic solvent content of the mobile phase for reverse-phase HPLC can decrease the retention time of an analyte. Together these modifications can improve separation, speed of analysis, resolving power, column backpressure, detectability, and solvent consumption per analysis. All of these benefits are clearly demonstrated in the modified HPLC method presented in this study. The column used contains a polymeric end-capped C18 material with low metal content silica and a reduced particle size of 5 μm. In addition, the reduced column dimensions of 4.6 × 150 mm with a mobile phase that has a higher acetonitrile content contribute to shorter run times. These factors are beneficial when in the application of this method in a routine analytical laboratory.

Although the modified HPLC conditions show some advantanges over Awang's, it is also important to note that the results obtained under both chromatographic conditions on the same extract sample (feverfew after extraction) agree with each other within the experimental error, 3%. This agreement indicates again that it is the difference in the extraction procedures rather than the HPLC conditions that causes the difference in the percentage of parthenolide found in feverfew by the two methods. The agreement also supports the conclusion that the peak used for quantitation in the modified HPLC method is true and pure parthenolide.

1022 *J. Agric. Food Chem.,* Vol. 47, No. 3, 1999

Zhou et al.

Table 2. Method Reproducibility Data

test	amount of feverfew sample (g)	% parthenolide found	av and RSD% on parthenolide
1	1.5084	0.959	
2	1.5097	0.965	
3	1.5105	0.974	av = 0.960
4	1.5132	0.951	
5	1.5154	0.950	
6	1.5033	0.962	
7	1.5020	0.962	
8	1.4974	0.956	RSD = 0.88%
9	1.5010	0.948	
10	1.4980	0.969	

The final improved method, consisting of both extraction and HPLC method improvements, is as follows: Accurately weigh 1.5 g of feverfew powder sample into a 120 mL amber glass bottle containing 100 mL of acetonitrile/water (90:10, v/v) solvent, stir the bottle for 30 min using a magnetic stir bar, and filter the extract through a 0.45 μm PTFE syringe filter for HPLC analysis. HPLC conditions were as follows: Nacalai Tesque Cosmosil C18-AR column (150 × 4.6 mm, 5 μm, 120 Å), mobile phase of acetonitrile/water (55:45, v/v) at a flow rate of 1.5 mL/min, and DAD detection at 210 nm. Injection amounts during method development varied from 1 to 20 μL.

It may be mentioned that this method has not yet been tested for its application on other compounds similar to parthenolide.

Method Reproducibility, Spike Recovery. Table 2 shows the method reproducibility data obtained from 10 replicate tests performed on different days in a week on another sample of feverfew. An average of 0.960% parthenolide found in the feverfew with an RSD% of 0.88 indicates good reproducibility of the method. Because parthenolide in feverfew degrades quickly, it is hard to keep an identical sample for an extended reproducibility test. Assays performed by different persons in different laboratories using the same lot of feverfew also showed excellent reproducibility of the method.

In the six spike recovery tests, an average of 99.3% of 7.50 ± 0.30 mg of parthenolide standard spiked into 0.7500 ± 0.0200 g of high-potency feverfew samples in 100 mL of extraction solvent was recovered with an RSD% of 1.6.

LITERATURE CITED

Awang, D. V. C.; Dawson, B. A.; Kindack, D. G.; Crompton, C. W.; Heptinstall, S. Parthenolide content of feverfew (*Tanacetum parthenium*) assessed by HPLC and ¹H-NMR spectroscopy. *J. Nat. Prod.* **1991**, *54*, 1516–1521.

Bailey, L. H.; Bailey, E. Z. *A Concise Dictionary of Plants Cultivated in the United States and Canada*; MacMillan Publishing: New York, 1978.

Blakeman, J. P.; Atkinson, P. Antimicrobial properties and possible role in host-pathogen interactions of parthenolide, a sesquiterpene lactone, isolated from glands of *Chrysanthemum parthenium. Physiol. Plant Pathol.* **1979**, *15*, 183–192.

Bloszyk, E.; Geppert, B.; Drozdz, B. Quantitative determination of sesquiterpene lactones in plant material by infrared spectroscopy. Sesquiterpene of the compositae, part xx. *Planta Med.* **1978**, *34*, 79–86.

Brown, A. M. G.; Lowe, K. C.; Davey, M. R.; Power, J. B.; Knight, D. W.; Heptinstall, S. Comparison of extraction procedures for parthenolide in *Tanacetum parthenium* "feverfew". *Phytochem. Anal.* **1996**, *7*, 86–91.

Commission Francaise de Pharmacopee. *French Pharmacopoeia*; Grand Camomille, Maisonneuve: Paris, 1987.

Duke, J. A. *CRC Handbook of Medicinal Herbs*; CRC Press: Boca Raton, FL, 1988.

Groenewegen, W. A.; Heptinstall, S. A comparison of the effects of an extract of feverfew and parthenolide, a component of feverfew, on human platelet activity in-vitro. *J. Pharm. Pharmacol.* **1990**, *42*, 553–557.

Heptinstall, S.; Awang, D. V. C.; Dawson, B. A.; Kindack, D.; Knight, D. W.; May, J. Parthenolide content and bioactivity of feverfew (*Tanacetum parthenium* (L.) schultz-bip.). Estimation of commercial and authenticated feverfew products. *J. Pharm. Pharmacol.* **1992**, *44*, 391–395.

Johnson. E. S.; Kadam, N. P.; Hylands, P. J. Efficacy of feverfew as prophylactic treatment of migraine. *Br. Med. J.* **1985**, *291*, 569–573.

Marchand, B.; Behl, H. M.; Rodriguez, E. Application of high-performance liquid chromatography for analysis and isolation of sesquiterpene lactones. *J. Chromatogr.* **1983**, *265*, 97–104.

Murphy, J. J.; Heptinstall, S.; Mitchell, J. R. A. Randomized double-blind placebo-controlled trial of feverfew in migraine prevention. *Lancet* **1988**, *ii*, 189–192.

Murray, M. T. *The Healing Power of Herbs, the Enlightened Person's Guide to the Wonders of Medicinal Plants*; Prima Publishing: Rocklin, CA, 1995.

Rey, J. P.; Levesque, J.; Pousset, J. L. Extraction and high-performance liquid chromatographic methods for the gamma lactones parthenolide (*Chrysanthemum parthenium Bernh.*), marrubiin (*Marrubium vulgare L.*) and artemisinin (*Artemisia annua L.*). *J. Chromatogr.* **1992**, *605*, 124–127.

Smith, R. M.; Burford, M. Supercritical fluid extraction and gas chromatographic determination of the sesquiterpene lacton parthenolide in the medicinal herb feverfew (*Tanacetum parthenium*). *J. Chromatogr.* **1992**, *627*, 255–261.

USP, Pharmacopeial Previews. In *Pharmacopeial Forum*; USP: Rockville, MD, 1997; Vol. 23, No. 3, pp 3993–3996.

Wren, R. C. *Potters New Cyclopaedia of Botanical Drugs and Formulations*; Health Science Press: Nr. Holsworthy, N. Devon, U.K., 1994.

Yoshioka, H.; Renold, W.; Fischer, N. H.; Higo, A.; Mabry, T. J. Sesquiterpene lactones from *Ambrosia confertiflora* (compositae). *Phytochemistry* **1970**, *9*, 823–832.