National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences[1], Department of Pharmacognosy, School of Pharmacy[2], The University of Mississippi, MS, USA, Facultad de Química, Departamento de Farmacia[3], Universidad Nacional Autónoma de México. Ciudad Universitaria Coyoacan, México

Quantification of parthenolide in *Tanacetum* species by LC-UV/LC-MS and microscopic comparison of Mexican/US feverfew samples

B. Avula[1], A. Navarrete[1, 3], V. C. Joshi[1], I. A. Khan[1, 2]

Received September 5, 2005, accepted October 5, 2005

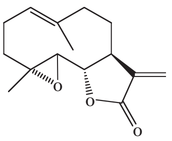
Prof. Dr. Ikhlas A. Khan, National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS 38677, USA
Dr. A. Navarrete, Facultad de Química, Departamento de Farmacia, Universidad Nacional Autónoma de México. Ciudad Universitaria Coyoacan, 04510 Mexico D.F. Mexico
ikhan@olemiss.edu
(Authors share equal contribution)

Pharmazie 61: 590–594 (2006)

LC-UV and LC-MS methods have been developed which permit the analysis of parthenolide in different Mexican/US feverfew samples and commercial products. The study was undertaken to confirm the presence of parthenolide in Mexican plant samples and its comparison with US feverfew samples. The best results were obtained with a Phenomenex Luna C18 (2) column using gradient mobile phase of water and acetonitrile:methanol (9 : 1). Elution was run at a flow rate of 1.0 mL per min and ultraviolet detection at 210 nm. The results obtained using LC-UV were comparable to those obtained using LC-MS. Parthenolide was detected in all the samples analyzed and is the major chemical constituent of feverfew. The samples collected in Oaxaca, Mexico (0.28%) and Puebla, Mexico (0.25%) showed the highest content of parthenolide. All Parthenium samples were also examined under light and fluorescent microscopy.

1. Introduction

Feverfew (*Tanacetum parthenium* (Linn.) Schulz-Bip., Asteraceae) has been shown to be of value in migraine prophylaxis (Berry 1984). The physiological activity, chemical composition and clinical effects of feverfew have been investigated extensively (Abad et al. 1995; Knight 1995; Vogler et al. 1998). The anti-migraine properties and bioactivity of *T. parthenium* are ascribed mainly to the sesquiterpene lactone parthenolide (Heptinstall et al. 1992). It is the major chemical constituent of feverfew. However, parthenolide content in feverfew is known to vary widely (Cutlan et al. 2000). In Mexico, this plant is known as Santamaría (Romo de Vivar and Jiménez 1965) and it has been reported that *T. parthenium* grown in Mexico does not contain parthenolide (Awang et al. 1991; Heptinstall et al. 1992). Literature reports the determination of parthenolide in feverfew plant samples by HPLC (Abourashed and Khan 2000), HPTLC (Abourashed 2004), supercritical fluid chromatography (Guo et al. 2002), GC (Abourashed and Khan 2001) and LC/NMR (Awang et al. 1991). The study was undertaken to confirm the absence or presence of parthenolide in Mexican plant samples. In the present work, a LC-UV method and a more sensitive LC-MS method were developed to analyze samples of *T. parthenium* collected from five different places in Mexico during the same seasonal period. The parthenolide content was compared with samples of *T. parthenium* and *T. vulgare* obtained from USA. The analytical method was used to analyze commercial products containing *T. parthenium*. In addition a comparative microscopic study was performed between the samples from Mexico and the USA.



Parthenolide

2. Investigations, results and discussion

2.1. Chemical analysis

For LC-UV analysis, parthenolide standard solutions were prepared from the stock solutions of 0.9, 5, 10, 25, 50 and 100 μg/mL. Each solution was injected three times beginning with the most dilute concentrations. Linear regression analysis of the calibration plot gave the equation $y = 2.41 \times 10^4 x$, with a correlation coefficient (R^2) of 0.9999.

For LC-MS analysis, parthenolide standard solutions were prepared from the stock solutions of 0.05, 0.1, 0.5, 1.0 and 5 μg/mL. Each solution was injected three times beginning with the most dilute concentrations. Linear regression analysis of the calibration plot gave the equation $y = 7.5 \times 10^6 x$, with a correlation coefficient (R^2) of

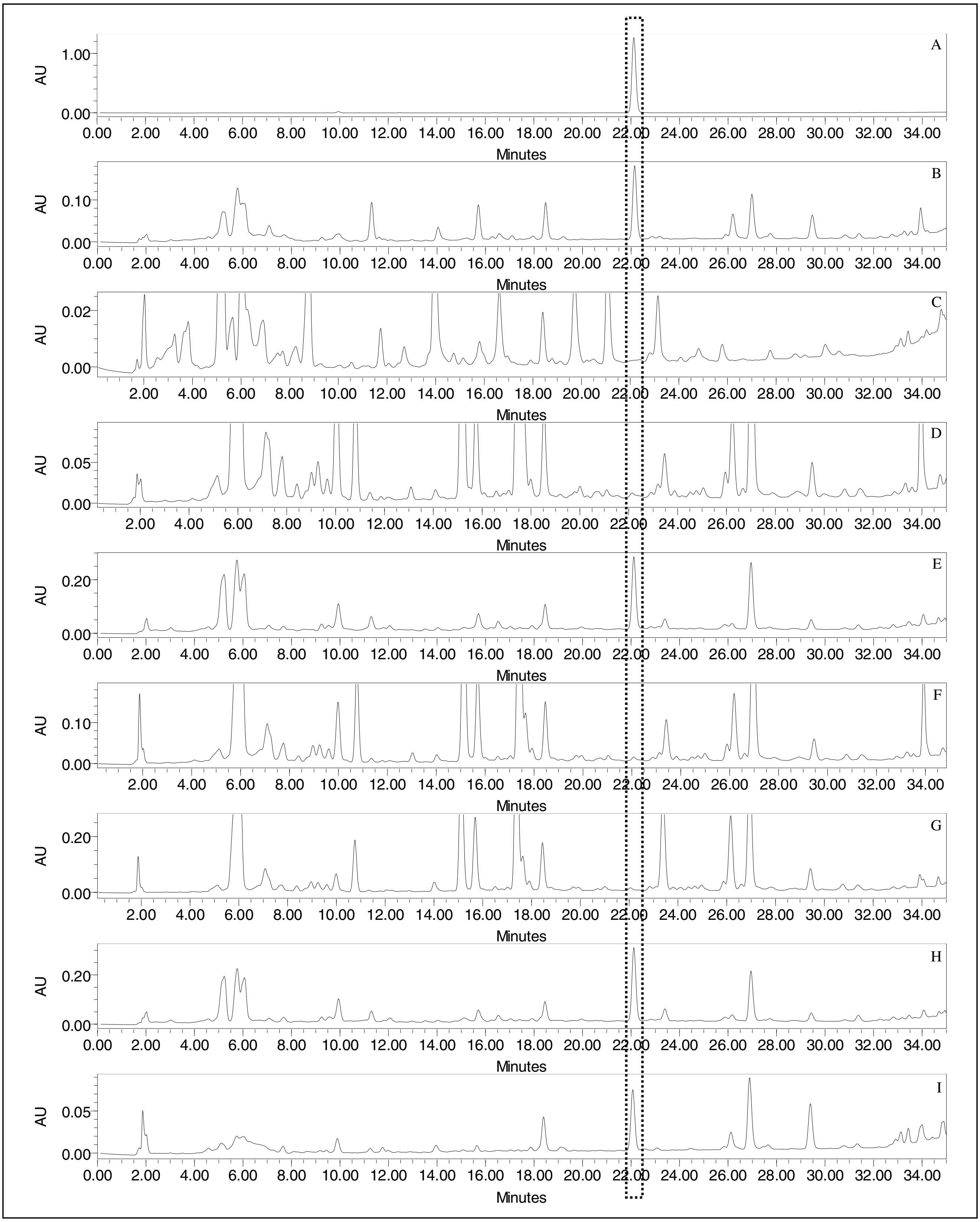


Fig. 1: A typical HPLC chromatogram of parthenolide (A) at 210 nm and HPLC comparison of chromatograms of *T. parthenium* (USA) (B), *T. vulgare* (USA) (C)*, T. parthenium* (Guerrero, Mexico)(D)*, T. parthenium* (Puebla, Mexico) (E), *T. parthenium* (Tulyehualco, Mexico) (F), *T. parthenium* (Edo de, Mexico) (G), *T. parthenium* (Oaxaca, Mexico) (H) and an extract of dietary supplement containing *T. parthenium* (I)

0.9997. Because of the lower limits of detection seen with the LC-MS method, a broader concentration range of calibration standards could be used. No standard solutions at concentrations above 5.0 µg/mL were analyzed.

The concentration of parthenolide in the unknown samples was calculated. If this concentration was out of the calibration graph range, the unknown sample was diluted two or more times before it was injected again.

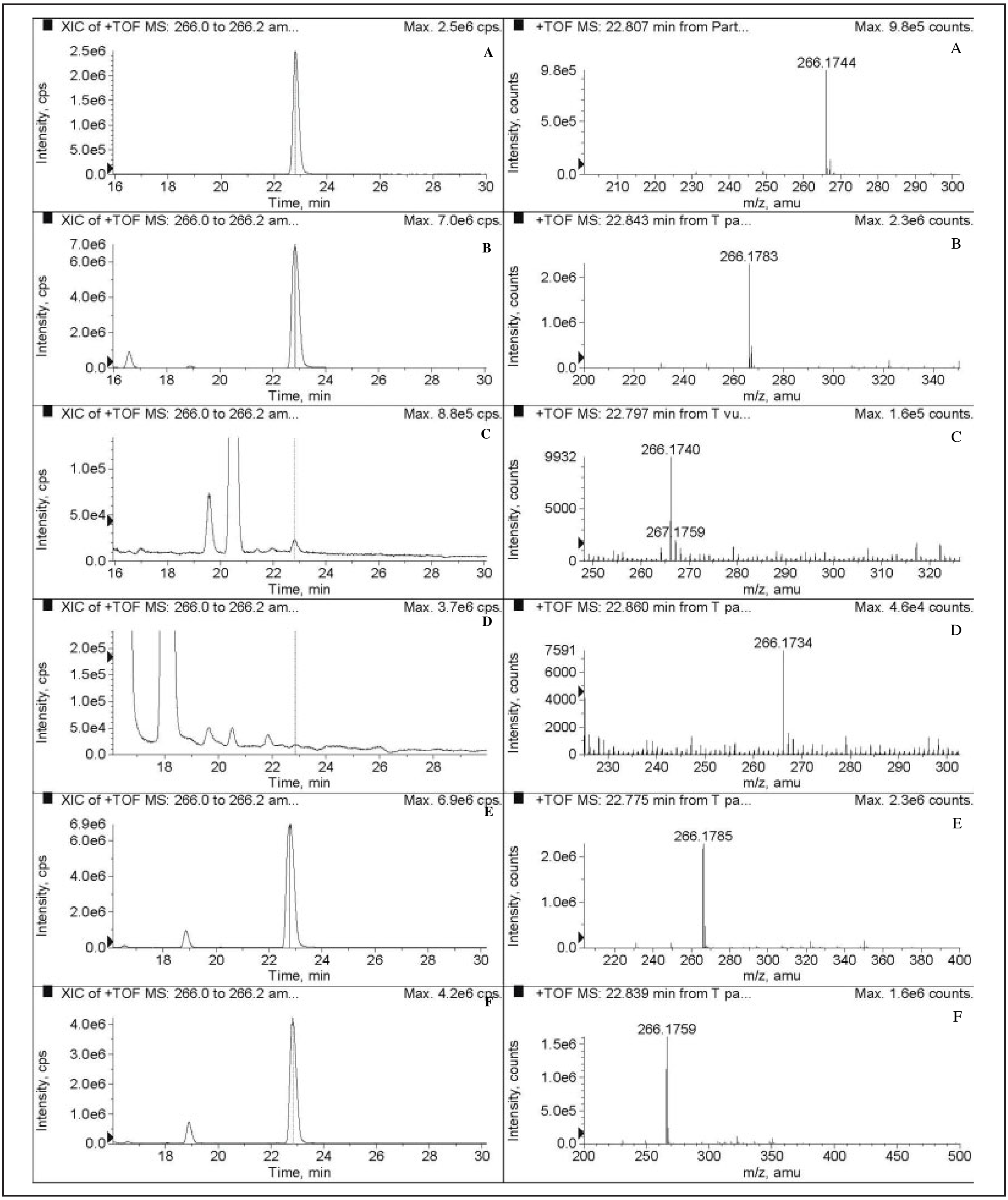


Fig. 2: XIC and mass spectra of the parthenolide (A), *T. parthenium* (USA) (B), *T. vulgare* (USA) (C), *T. parthenium* (Guerrero, Mexico) (D), *T. parthenium* (Puebla, Mexico) (E) and an extract of dietary supplement containing *T. parthenium* (F)

The calibration curve showed a linear correlation between sample concentration and peak area. Intra and inter-day variation were determined with standards. It was performed thrice on three different days and each concentration point was injected in triplicate. Purity of the standards was confirmed by the PDA data of all peaks of interest. In order to determine the accuracy of the method, one sample was spiked with a known amount of the standard compounds and recovery rates were between 98.3% and 103.5%. An indicator for precision is the standard deviation (δ). All samples were injected in triplicate and the standard deviation of standard compounds was below 1.0%. Calibration data indicated the linearity of the detector response for parthenolide from 0.90 to 100.0 μg/mL. The limit of detection and limit of quantification were found to be 0.3 and 0.5 μg/mL respectively for the stan-

Table: Content of parthenolide in plant samples and products containing *Tanacetum parthenium*

	Species	Origin	Parthenolide content (%)	
			LC-UV	LC-MS
Plants samples	*T. parthenium*	Boulder Colorado, USA	0.16	0.17
	T. vulgare	Boulder Colorado, USA	DUL	DUL
	T. parthenium	Guerrero, Mexico	DUL	DUL
	T. parthenium	Puebla, Mexico	0.25	0.29
	T. parthenium	Tulyehualco, Mexico	DUL	DUL
	T. parthenium	Estado de Mexico, Mexico	DUL	DUL
	T. parthenium	Oaxaca, Mexico	0.28	0.30
Capsules	*T. parthenium*	USA	0.06	0.07
Capsules	*T. parthenium*	USA	0.09	0.10
Drops	*T. parthenium*	USA	174[a]	177[a]

[a] in µg/mL, DUL = Detected Under Limit of Quantification

dard compound with PDA detection and with LC-MS (TOF), the limit of detection was 0.01 µg/mL.

Fig. 1 shows the variations of parthenolide in different samples of *T. parthenium* from Mexico and USA. The extracts were analyzed using LC-UV and LC-MS and concentration of parthenolide was calculated (Table) using this data. Parthenolide was detected in all the samples analyzed. The sample collected in Oaxaca (0.28%) and Puebla (0.25%) showed the highest content of this lactone, followed by the sample collected in Boulder, Colorado, USA (0.16%). The other samples collected in Mexico showed trace amounts. Parthenolide was detected in traceable amounts also in *T. vulgare.* Three commercially available dietary supplements were tested. Two were ground feverfew extracts sold as capsules and one was sold as drops. The content of parthenolide ranged between 0.07–0.1% in capsules and in the drops the concentration was 175 µg/mL. These results were verified by LC/MS (Fig. 2), which is a more sensitive analytical method. The LC-MS experiments for peak confirmation were performed on LC-MS-TOF. The method was also applied for quantification of various plant samples and commercial products. Best results could be obtained in the positive ESI mode. To improve sensitivity the LC flow rate was reduced to 0.6 mL/min, the solvent gradient had to be modified to obtain comparable retention times. Otherwise, the same optimized conditions were used. LC-MS analysis revealed that parthenolide had a molecular peak at 266.18 $[M + NH_4]^+$. No interfering peaks were found at the retention time of interest.

2.2. *Macroscopic observation*

All the *Tanacetum parthenium* (L.) Schultz Bip. samples colleted from Mexico were compared with an authentic specimen deposited at the National Center for Natural Products Research. The anatomical characters were matching the feverfew description in British Herbal Pharmacopoeia (BHP, 1996). In addition to the character mentioned in BHP (1996) we also observed ovate glands/structures on the leaf surface in both *T. parthenium* as well as *T. vulgare* when examined under fluorescent microscope. These glands/structures were observed between the anomocytic stomata and the trichomes. The ovate glands/structures are best visible under UV EX 450−490. At this wavelength the glands are bright green (Fig. 3A). Observation under UV EX 330−380 shows that theses ovate glands are different from the isodimeteric basal cell of the trichome (Fig. 3B). Observation of these glands under UV light could be a useful key identification character to authenticate feverfew in powder form.

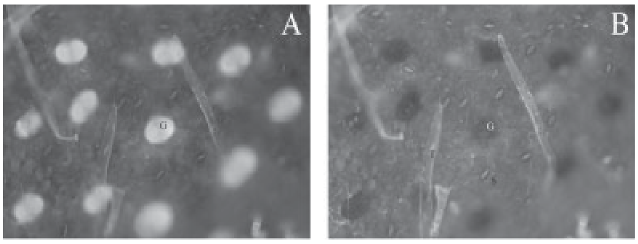


Fig. 3: *Tanacetum parthenium* as examined under fluroscent microscope A: UV EX 450−490 (G) Gland appear fluorescent green B: UV Ex 330−380 (T) Trichomes (blue); (S) Stomata (yellowish brown)

In conclusion, the LC-UV and LC-MS methods developed for the analysis of parthenolide, showed the presence of this lactone in variable amounts in feverfew samples obtained from different locations in Mexico. Analysis using the LC-MS was more sensitive than LC-UV to analyze parthenolide in various plant samples and commercial products. The morphological studies were conducted for Mexican *T. parthenium* and did not show any microscopic differences within the *T. parthenium* samples analyzed from Mexico and USA.

3. Experimental

3.1. *Chemicals*

The standard compound parthenolide (97% purity) was purchased from Sigma (St. Louis, MO, USA), HPLC-grade acetonitrile, methanol and acetic acid were of HPLC grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water for the HPLC mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA, USA). Products of *T. parthenium* were obtained through online.

3.2. *Plant material*

Different populations of *T. parthenium* were obtained from different locations in Mexico (Guerrero, Puebla, Tulyehualco, Estado de Mexico, Oaxaca) in February 2005. Two samples of *T. parthenium* and *T. vulgare* were procured from Boulder, Colorado, USA were also studied. Voucher specimens of all *Tanacetum* samples are deposited at the NCNPR repository.

3.3. *Sample preparation*

Finely powdered dried plant material (0.2 g) or the content of one capsule was sonicated in 2.5 mL of acetonitrile for 15 min followed by centrifugation for 10 min at 3300 rpm. The supernatant was transferred to a 10.0 mL volumetric flask. The procedure was repeated thrice and the respective supernatants were combined. The final volume was adjusted to 10 mL with acetonitrile. The aliquot of the liquid formulations (5.0 mL) were diluted with the equal volume (5.0 mL) of acetonitrile and mixed thoroughly. Prior to use all samples were filtered through a 0.45 µm nylon membrane filter.

3.4. *LC-UV analysis*

The HPLC system consisted of Waters (Waters Corp., Milford, MA, USA) model 6000A pumps, a Waters model U6K injector, a Waters model 680 automated gradient controller, a Waters model 996 photodiode array detector, and a computerized data station equipped with Waters Millennium software. Separation was achieved on a Luna C18 (2) column (Phenomenex, 150 × 4.6 mm I.D.; 5 µm particle size; Phenomenex Inc., Torrance, CA, USA) and operated at 30 °C. The column was equipped with a 2 cm LC-18 guard column (Supelco, Bellefonte, PA, USA). The mobile phase consists of water (A) and acetonitrile : methanol (9 : 1) (B) which were applied in the following gradient elution: 0 min, 80% A: 20% B in next 20 min to 10% A: 90% B. Each run was followed by a 5 min wash with 100% acetonitrile and an equilibration period of 15 min. The flow rate was adjusted to 1.0 mL/min. Parthenolide was detected at 210 nm.

3.5. *LC-MS-TOF analysis*

The liquid chromatograph used was an Agilent Series 1100 comprising the modular components: quaternary pump, a vacuum solvent microdegasser, an autosampler with 100-well tray and an online DAD. Separation was achieved on a Luna C18 (2) column (Phenomenex, 150 × 4.6 mm I.D.; 5 μm particle size). The column was equipped with a 2 cm LC-18 guard column (Supelco, Bellefonte, PA, USA). The mobile phase consists of water (0.1% acetic acid) (A) and acetonitrile : methanol (9 : 1) (B) which were applied in the following gradient elution: 0 min, 75% A: 25% B in next 30 min to 25% A: 75% B and in next 5 min to 100% B. The flow rate was adjusted to 0.6 mL/min. The DAD was programmed to acquire the UV-spectra from 190–400 nm (step size 4 nm). The MS analysis was performed on an Agilent Series 1100 SL equipped with an ESI source. All acquisitions were performed under positive ionization mode with a capillary voltage of +4000 V. Nitrogen was used as nebulizer gas (45 psig) as well as drying gas at 7.0 L/min and drying gas temperature at 300 °C. Data acquisition and processing was done with the software Analyst QS.

3.6. *Microscopic analysis*

3.6.1. *Light microscopy*

For microscopic comparison within the samples, 2 g of each sample was ground and digested in 10% nitric acid with 2–3 crystals of sodium hydroxide and heated at 40–60 °C for 15–20 min. The macerated samples were then centrifuged and transferred to water. From water the samples were passed through grades of alcohol 20–98% and mounted on glass slide using “Cytoseal” mounting media. The slides were observed under a Nikon Eclipse E600 microscope.

3.6.2. *Fluorescence microscopy*

All samples were examined under a Nikon ECLIPSE 600 microscope with fluorescence attachment. Digital photomicrographs were taken using a Kodak camera attached to the microscope; images were processed using Adobe Photoshop Software. Samples were observed under UV EX 330–380 and at UVEX 450–490.

Acknowledgement: This research is funded in part by “Botanical Dietary Supplements: Science-Base for Authentication” funded by Food and Drug Administration grant number FD-U-002071-01. A. Navarrete acknowledges CONACYT project C01–018 for partial support.

References

Abad MJ, Bermejo P, Villar A (1995) An approach to the genus *Tanacetum* L. (Compositae): Phytochemical and Pharmacological Review. Phytother Res 9: 79–92.

Abourashed EA (2004) Validation and application of an HPTLC method for the determination of parthenolide in feverfew herb products. J. Planar Chromatogr.-Mod. TLC 17: 375–378.

Abourashed EA, Khan IA (2000) Determination of parthenolide in selected feverfew products by liquid chromatography. J AOAC Int 83: 789–792.

Abourashed EA, Khan IA (2001) GC determination of parthenolide in feverfew products. Pharmazie 56: 971–972.

Awang DVC, Dawson BA, Kindack DG, Crompton CW, Heptinstall S (1991) Parthenolide content of feverfew (*Tanacetum parthenium*) assessed by HPLC and ^{1}H-NMR spectroscopy. J Nat Prod 54: 1516–1521.

Berry M (1984) Feverfew faces the future. Pharm J 232: 611–614.

British Herbal Pharmacopoeia (1996) Exeter, U.K.: British Herbal Medicine Association.

Cutlan AR, Bonilla LE, Simon JE, Erwin JE (2000) Intra-specific variability of feverfew: Correlation between parthenolide, morphological traits and seed origin. Planta Med 66: 612–617.

Guo Y, Wu S, Li F (2002) Determination of parthenolide in feverfew using supercritical fluid chromatography. Tianran Chanwu Yaniu Yu Kaifa 14: 47–48.

Knight DW (1995) Feverfew: chemistry and biological activity. Nat Prod Rep 12: 271–276.

Heptinstall S, Awang DVC, Dawson BA, Kindack D, Knight DW, May J (1992) Parthenolide content and bioactivity of feverfew (*Tanacetum parthenium* (L.) Schultz-Bip.) Estimation of commercial and authenticated feverfew products. J Pharm Pharmacol 44: 391–395.

Romo de Vivar A, Jiménez H (1965) Structure of santamarine, a new sesquiterpene lactone. Tetrahedron 21: 1741–1745.

Vogler BK, Pittler MH, Ernst E (1998) Feverfew as a preventive treatment for migraine: as systematic review. Cephalgia 18: 704–708.