



A proposal for the quality control of *Tanacetum parthenium* (feverfew) and its hydroalcoholic extract

*Juliana Siqueira Chaves, Fernando Batista Da Costa**

Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Ciências Farmacêuticas, Laboratório de Farmacognosia, 14040-903 Ribeirão Preto-SP, Brazil

Artigo

RESUMO. “Uma proposta para o controle de qualidade de *Tanacetum parthenium* (tanaceto) e seu extrato hidroalcoólico”. Neste trabalho, é apresentada uma proposta para o controle de qualidade físico e químico do pó de *T. parthenium* (tanaceto) e do seu extrato hidroalcoólico obtido por percolação. A lactona sesquiterpênica partenolideo, o principal componente ativo da espécie, foi quantificada por CLAE e seu teor foi de 0,49% no pó e 1,06% no extrato. O teor de flavonóides totais foi determinado por espectroscopia no UV e foi de 0,54% no pó e 1,05% no extrato hidroalcoólico. A santina, principal flavonóide da espécie, foi isolada e posteriormente identificada no extrato por CLAE. Uma vez que o partenolideo também pode ser encontrado em outras espécies de Asteraceae, a análise da santina é importante para certificar a autenticidade do material vegetal. Os resultados obtidos neste trabalho confirmam a autenticidade do material e a eficiência do processo extrativo.

Unitermos: *Tanacetum parthenium*, Asteraceae, controle de qualidade, quantificação por CLAE, extrato hidroalcoólico, flavonóides, partenolideo.

ABSTRACT: In this study, we present a proposal for the physical and chemical quality control of the powder of the species *T. parthenium* (feverfew) and its hydroalcoholic extract obtained by percolation. The sesquiterpene lactone parthenolide, the main active compound of this plant, was quantified by HPLC and its content was found to be 0.49% in the powder and 1.06% in the extract. The total content of flavonoids, determined by UV spectroscopy, was found to be 0.54% in the powder and 1.05% in the hydroalcoholic extract. Santin, the main flavonoid of this species, was isolated and further identified in the extract by HPLC. Since parthenolide can also be found in other Asteraceae species, the analysis of santin is important to certify the authenticity of the plant material. The results confirmed the authenticity of the plant material and the efficiency of the extraction procedure.

Keywords: *Tanacetum parthenium*, Asteraceae, quality control, HPLC quantification, hydroalcoholic extract, flavonoids, parthenolide.

INTRODUCTION

Tanacetum parthenium (L.) Schultz Bip. (Asteraceae) is known in folk medicine as feverfew. This species has been recognized since ancient times as a medicine against a wide range of diseases such as rheumatism, asthma, toothache, and for the alleviation of the “holy fire”, fever and mainly migraine (Knight, 1995; Oliveira et al., 2007; Carvalho et al., 2008). Since the time of Dioscorides (50 B.C.), feverfew has been used in the prevention of migraine, through the Middle Ages up to the present (Palevitch et al., 1997).

Phytochemical studies carried out with this species have shown the presence of many constituents, mainly sesquiterpene lactones, and also some flavonoids. The sesquiterpene lactone parthenolide is the major compound and the chemical marker of *T. parthenium* (Knight, 1995).

The commonly used feverfew extracts trigger the release of serotonin (5-HT) from platelets, thus inducing a wide variety of aggregation agents (Pfaffenrath et al., 2002). The efficacy of the extract in migraine prophylaxis was also assessed in randomized, placebo-controlled, double-blind and cross-over clinical studies (Gruenwald et al., 2000).

Pharmacological investigations of feverfew and parthenolide have demonstrated that both present anti-inflammatory effect through the inhibition of eicosanoids and the transcription factor nuclear factor kappa-B (NF- κ B) (Gruenwald et al., 2000; Garcia-Piñeres et al., 2001; 2004). Further studies revealed that flavonoids such as santin are also important anti-inflammatory constituents of the species (Long et al., 2003; Williams et al., 1999).

Although parthenolide as well as feverfew have been subject to several chemical and pharmacological

* E-mail: febcosta@fcfrp.usp.br; Tel. +55-16-36024312, Fax +55-16-36024879

investigations, a study describing the standardization of its hydroalcoholic extract including flavonoid analysis and parthenolide quantification is still lacking. Therefore we propose herein a methodology for the quality control of the powder and the hydroalcoholic extract of *T. parthenium* taking into account the parthenolide and flavonoid contents.

MATERIAL AND METHODS

Chemicals

Organic solvents for HPLC analyses (Mallinckrodt Baker, Phillipsburg, NJ, USA) were filtered through 0.45 µm cellulose membranes prior to use. Water was processed in a Millipore, Milli-Q® purifier (Millipore, Bedford, MA, USA). Organic solvents for the other analyses were of analytical grade (Merck or Synth, Brazil). The reagents H₂SO₄, HCl, AlCl₃, AcOH and fructose were obtained from Merck (Brazil). Parthenolide was purchased from Sigma-Aldrich (Steinheim, Germany).

General experimental procedures

The identity of santin was established by ¹H NMR (Bruker DRX 400 spectrometer, 400 MHz, Germany). Flavonoids were quantified in a Hewlett Packard UV-Vis Spectrophotometer, (Kayak, XA, USA), and IR spectra obtained in a Nicolet Protégé 460 spectrophotometer (France).

The HPLC equipment used in the parthenolide quantification consisted of a SLC 10Avp liquid chromatograph (Shimadzu Corp., Japan) with a SPD-M10A diode array detector (DAD), three pumps (LC-10AD), an integration software (Class vp, v. 5.02) and an analytical column Nucleosil 100-5 RP-18, 4.6 x 250 mm, 5 µm, 100 Å (Macherey & Nagel, Durin, Germany). The HPLC system employed in the analysis of santin was identical to the above, but equipped with two pumps only (LC-6AD), a column oven (CTO-10ASVP) and an analytical column Nucleosil 300-5 RP 18, 4.6 x 250 mm, 5 µm, 300 Å (Macherey & Nagel, Durin, Germany).

Plant material

The powder from the aerial parts of *T. parthenium* was purchased from Galilee Herbal Remedies (Kibbutz Kfar Hanassi, Israel).

Characterization of the *T. parthenium* powder

Powder fineness, total ash, acid insoluble ash, swelling index and moisture content: these assays were performed according to the standard methods described in the WHO guidance (World Health Organization,

1988).

Extraction, isolation and identification of santin

Extraction of the *T. parthenium* powder (200 g) using Me₂CO was proceeded through sonication for 15 min. This procedure was repeated two more times and the resulting solvent was evaporated at reduced pressure. The crude extract (8 g) obtained was submitted to vacuum liquid chromatography (VLC) with silica gel 60H (100-200 mesh ASTM, Merck, Brazil), using *n*-hexane and increasing amounts of EtOAc and MeOH as eluents, yielding seven fractions. Fraction 3 (0.98 g) was chromatographed again by VLC using the same solvent system described above. Fraction 1 (0.74 g) was purified by "flash" chromatography using silica gel 60 (230-400 mesh ASTM, Merck, Brazil) as adsorbent. Elution (isocratic) was made with *n*-hexane:CH₂Cl₂:2-propanol 8.0:1.5:0.5 and yielded 28 fractions. Fractions 19-28 (88 mg) were combined and purified by crystallization from *n*-hexane:MeOH, resulting in 18 mg of pure santin. The structure of the flavonoid (Figure 1) was established by comparison of its ¹H NMR spectral data with those reported in the literature (Williams et al., 1999).

Quantification of total flavonoids by UV spectroscopy

The determination of total flavonoids was carried out by UV spectroscopy according to the German Pharmacopoeia (Bundesvereinigung, 1986). The method is based on the use of reagents such as hexamethylenetetramine and AlCl₃, which ionize phenolic groups and form acid complexes, thus changing the shapes and shifting the absorption peaks of the flavonoids in the spectrum (Mabry et al., 1970).

Quantification of parthenolide by HPLC

The parthenolide (Figure 2) content was assessed by an adaptation of the method described by Zhou et al. (1999) with the aid of a calibration curve.

Stock solutions: pure parthenolide (5.0 mg) was placed in a volumetric flask and the volume was completed to 5 mL with MeCN. Standard solutions with concentrations of 1, 2, 10, 50, 100, 250, 500, and 750 µg/mL were prepared from the parthenolide stock solution (1 mg/mL) by means of serial dilution with MeCN.

Preparation of sample for analysis: 150 mg of the *T. parthenium* powder was extracted with 10 mL of MeCN-H₂O 9:1, sonicated for 30 min and centrifuged for 12 min. For the hydroalcoholic extract, the solvent was evaporated under vacuum and 150 mg of the dried extract was extracted in the same way as above. The supernatant was passed through a 13 mm, 0.45 µm nylon membrane (Sartorius, Spain). Aliquots (20 µL) of standard solutions of parthenolide or sample

were submitted to HPLC in triplicate. A mobile phase consisting of a mixture of MeCN-H₂O 45:55 was used for the elution, run at a flow rate of 1.0 mL/min and UV detection at 210 nm.

Method validation. (a) *Calibration curve and linearity*: a calibration curve was constructed with standard parthenolide solutions in the concentration range of 1 to 750 µg/mL. Injection volumes were 20 µL. Each sample was analyzed in triplicate and the average detector responses were used to construct the curve (Brasil, 2002). (b) *Precision and accuracy*: three standard solutions in three concentration levels (50, 250 and 750 µg/mL) in 10 replicates were employed to observe the standard deviation of the method in the same day (intra-assay precision). For inter-assay precision, a 250 µg/mL standard solution was analyzed in 10 replicates during three days. The accuracy was obtained for those three nominal concentrations of parthenolide (50, 250 and 750 µg/mL) performed in quintuplicate (Brasil, 2002). (c) *Specificity*: the purity of the parthenolide samples was checked by analyzing the peaks from the chromatogram using DAD (Abourashed and Khan, 2000). (d) *Detection limit (DL) and quantification limit (QL)*: the standard solution (1 µg/mL) was injected three times and the signal height and baseline noises were averaged as follows: DL = 3 x signal height x standard amount/baseline noise, and QL = 10 x signal height x standard amount/baseline noise (Brasil, 2002).

Preparation and characterization of the hydroalcoholic extract

The extract was obtained by percolation of the *T. parthenium* powder in 70% ethanol according to the German Pharmacopoeia specifications, as described by Schmidt and List (1989). Two parts of the extract were obtained from one part of the powder. This process was performed in three steps: 1) pre-swelling, for 2 h with a 1:3 liquid to powder proportion; 2) intermediate maceration (24 h); 3) percolation (4-6 drops/min per 100 g of powder).

Characterization of the hydroalcoholic extract

Loss on drying: performed according to the German Pharmacopoeia (Hartke and Mutschler, 1987). *Density, pH, and alcohol determinations*: performed according to the Brazilian Pharmacopoeia (Farmacopéia Brasileira, 1977). *Total flavonoids*: the quantification of total flavonoids was carried out by UV spectroscopy according to the German Pharmacopoeia (Bundesvereinigung, 1986). *TLC analysis*: samples were eluted with C₆H₆-Me₂CO 9:1 in glass plates coated with 0.25 mm of silica gel 60 GF₂₅₄ (Merck, Brazil); the reagent used for the detection of spots was a 1% MeOH solution of fructose with 2% H₂SO₄ 1:1; the plates were kept during 2-4 min at 110 °C until the appearance of

colored spots (Drozd and Bloszyk, 1978); the retention times of the spots were compared to that of a parthenolide standard. *Qualitative analysis of santin*: the qualitative analysis of santin in the *T. parthenium* extract as well as the sample preparation were carried out according to a method described for HPLC analysis of flavonoids in *Passiflora* species (Pereira et al., 2004). Santin was suspended in 1 mL of MeOH 60% (1 mg/mL), filtered through a 0.45 µm nylon membrane (Sartorius, Spain) and directly injected into the HPLC system. Injection volumes were 50 µL. *Parthenolide content*: see above.

RESULTS AND DISCUSSION

Validation of the HPLC method

The adaptation of the HPLC method for parthenolide quantification was made in order to improve the specificity of the parthenolide peak. The method used is fast (run time = 15 min, Fig. 3) and simple (isocratic elution). The time of analysis is an important factor in analytical work since it optimizes equipment use and reduces solvent consumption.

A previous study (Zhou et al., 1999) showed that 30 min stirring in MeCN-H₂O 9:1 is the best system for extracting parthenolide. The calibration curve showed good linearity (R² = 0.9994) in the concentration range tested. The regression equation was $y = 2.10 \cdot 10^{-5}x - 5.8839$. The percentages of relative standard deviation (RSD) were 0.23 to 3.7 %.

Analyses of UV spectra collected at the beginning, in the middle and at the end of the parthenolide peak showed that the method presents specificity.

The limit of quantification was 0.07 µg and the limit of detection was 0.02 µg. The method resulted in acceptable intra and inter-assay precision (Tables 1 and 2) since the % RSD ranged from 0.34 to 4.1 % for the intra-assay and 0.34 to 2.19 % for the inter-assay tests. The accuracy ranged from 94.09 to 99.99% and is shown in Table 3. These results are in agreement with literature data (Brasil, 2002; Causon, 1997) and show that the method and sample extraction are reproducible.

Characterization of the *T. parthenium* powder and hydroalcoholic extract

Several techniques used in this study have already been described in the guidelines of World Health Organization (WHO, 1998) and pharmacopoeias (Farmacopéia Brasileira, 1977) and are essential to ensure authenticity, safety and stability of the plant material and its preparations. Nevertheless, a publication containing a compilation of all these specifications for *T. parthenium* (Table 4) was not yet available.

The low water content in the *T. parthenium* powder (Table 4) indicates that the plant material is stable. Total ash and acid insoluble ash (Table 4)

Table 1. Intra-assay precision of standard parthenolide for three nominal concentrations (50.0, 250.0 and 750.0 µg/mL, n=10).

Replicates	Standard Solution (µg/mL)		
	50.0	250.0	750.0
1	48.19	238.92	735.51
2	47.01	238.94	729.95
3	47.52	239.61	723.39
4	46.38	240.72	738.09
5	47.05	240.43	742.94
6	42.29	240.75	711.73
7	44.52	239.57	696.51
8	48.86	239.54	705.71
9	46.68	240.19	727.22
10	47.23	241.41	733.98
mean	46.57	240.01	724.50
SD	1.890	0.825	15.18
% RSD	4.06	0.34	2.10

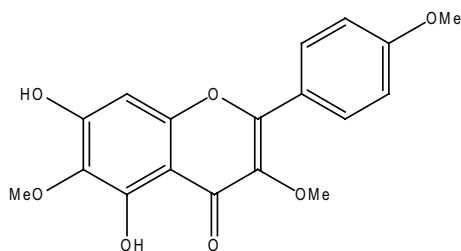
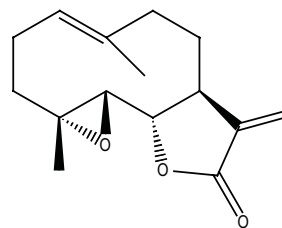
SD = standard deviation; % RSD = relative standard deviation.

Table 2. Inter-assay precision of standard parthenolide for one nominal concentration (250.0 µg/mL, n=10) during three days.

Replicates	Standard Solution (µg/mL)		
	Day 1	Day 2	Day 3
1	238.92	249.39	256.55
2	238.94	252.73	252.89
3	239.61	250.81	252.02
4	240.72	250.05	251.46
5	240.43	250.95	251.05
6	240.75	256.38	239.72
7	239.57	247.75	240.29
8	239.54	249.83	249.98
9	240.19	249.60	251.63
10	241.41	250.77	252.79
Mean	240.01	250.62	249.84
SD	0.825	2.336	5.463
% RSD	0.34	0.96	2.19

values are in accordance with literature specifications and indicate low content of inorganic and silicon contaminants (WHO, 1998). The presence of santin (Fig. 1), parthenolide (Table 4, Fig. 2) and total flavonoids (Table 4) confirm the authenticity of the plant material. All results are acceptable since RSD values were lower than 10%.

The efficiency of the extraction process is influenced by the powder fineness (Table 4), and the swelling index assay (Table 4) shows the extra-amount of solvent that should be added in the extraction (Schmidt

**Figure 1.** Chemical structure of the flavonoid santin.**Figure 2.** Chemical structure of the sesquiterpene lactone parthenolide.

and List, 1989).

The hydroalcoholic extract was obtained by percolation since it is a dynamic and continuous method and is more efficient than maceration (Prista and Alves, 1967). Losses on drying, density, IR and TLC analyses (Table 4) were used to control the extraction procedures.

The UV-visible absorption spectroscopy technique, which was based on the German Pharmacopoeia, allowed the quantification of total flavonoids in the powder and also in the hydroalcoholic extract (Tables 4 and 5).

The result of IR spectroscopic analysis (Table 5) also indicates that the extraction procedure was efficient for parthenolide and flavonoids, which are the most important active compounds of feverfew (Gruenwald et al., 2000).

Several studies suggest that parthenolide, which is thought to be the most active component of *T. parthenium*, should be used as a chemical marker to characterize *T. parthenium*. However, parthenolide has also been reported in other Asteraceae species such as the German chamomile [*Matricaria recutita* (L.) Rausch.] and tansy (*Tanacetum vulgare* L.). Both drugs are sometimes incorrectly supplied as feverfew (Smith and Burford, 1993). Therefore, the identification of santin by HPLC as well as quantification of parthenolide as proposed herein are very useful parameters to characterize *T. parthenium* unequivocally.

The results also showed that the use of hydroalcoholic extracts is more advantageous than the use of the crude powder in order to obtain plant-derived products or phytomedicines containing higher amounts of active compounds such as parthenolide and flavonoids. As demonstrated in the present study, these compounds can be easily analyzed by different qualitative and/or quantitative methods. Although the use of hydroalcoholic extracts is strongly recommended, most of the *T. parthenium*-derived products sold by several pharmaceutical companies and pharmacies are in form of capsules that simply store the powder of the aerial parts of this plant.

In conclusion, a reliable methodology for the quality control of the powder and the hydroalcoholic extract of *T. parthenium* is now reported. It takes into

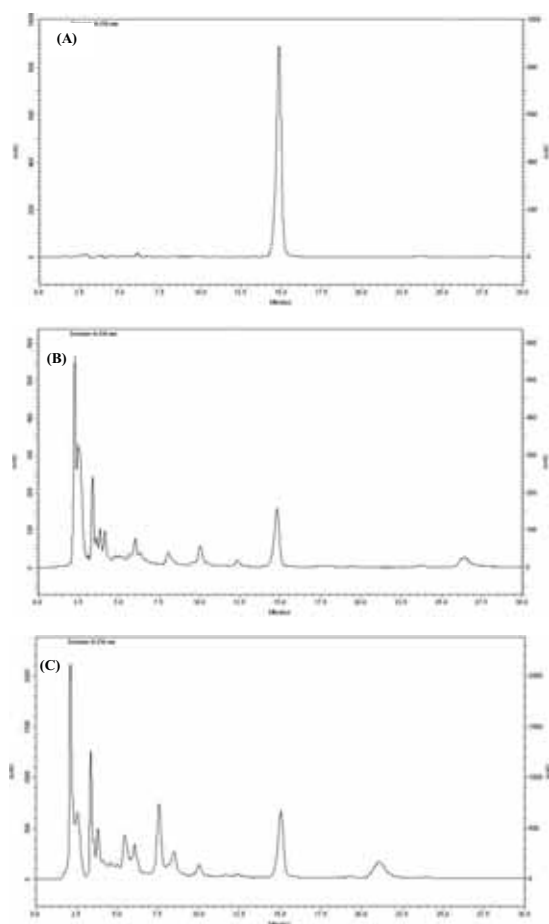


Figure 3. HPLC/DAD traces of parthenolide, mAU x time (min): (A) parthenolide; (B) *T. parthenium* powder; (C) hydroalcoholic extract of *T. parthenium*. Mobile phase: MeCN-H₂O 45: 55; flow-rate 1 mL/min; UV detection at 210 nm.

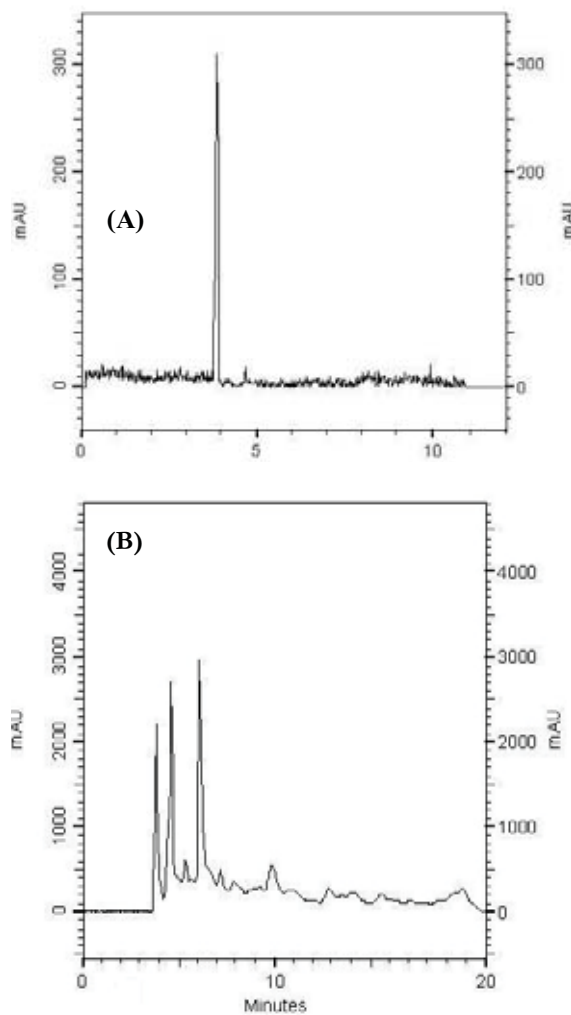


Figure 4. HPLC/DAD traces of santin, mAU x time (min): (A) santin; (B) hydroalcoholic extract of *T. parthenium*. Mobile phase: solvent A (2% formic acid in H₂O), solvent B (MeCN); gradient elution 0-10 min 15% B in A, 10-40 min 15-30% B in A, and 40-50 min 30-15% B in A; flow-rate 0.8 mL/min; UV detection at 241 nm.

Table 3. Determination of accuracy for three nominal concentrations (50.0, 250.0 and 750.0 µg/mL, n=5) of standard parthenolide.

Nominal Values (µg/mL)	True values (µg/mL)	Accuracy	Statistic Analysis for the HPLC Accuracy		
			Mean	SD	% RSD
50.0	49.61	99.21	97.71	1.399	1.43
	47.93	95.87			
	49.27	98.54			
	49.15	98.31			
	48.32	96.64			
250.0	249.83	99.93	99.99	0.529	0.53
	247.75	99.10			
	250.62	100.25			
	250.77	100.31			
	250.95	100.38			
750.0	696.18	92.82	94.09	1.034	1.10
	705.70	94.09			
	715.63	95.42			
	700.52	93.40			
	710.52	94.74			

Table 4. Physical and chemical properties of the *T. parthenium* powder.

Assay (n=3)	Result (mean values)	SD	Reference Value	Literature
Powder fineness	Moderately coarse	-	-	WHO, 1988
Total ash	10.53 % w/w	0.47	Max. 15 % w/w	British Herbal Pharmacopoeia, 1990
Acid insoluble ash	5.0 % w/w	0.03	Max. 5 % w/w	British Herbal Pharmacopoeia, 1990
Moisture content	0.54 % w/w	0.03	-	WHO, 1988
Swelling index	0.67 mL	0.06	-	WHO, 1988
Total flavonoids	0.54% w/w	0.04	-	Bundesvereinigung, 1986
Parthenolide content	0.49% w/w	0.01	Min. 0.1 % or 0.2 % w/w	Palevitch et al., 1997

Table 5. Characterization of the hydroalcoholic extract of *T. parthenium*.

Assay (n=3)	Result (mean values)	SD	Reference Value	Literature
pH	5.91	0.02	-	Farmacopéia Brasileira, 1977
Density	0.9331 g/mL	0.002	-	Farmacopéia Brasileira, 1977
Alcohol content	51 % v/v	0.04	-	Farmacopéia Brasileira, 1977
Loss on drying	10.82%.w/w	0.15	-	Hartke and Mutschler, 1987
Total flavonoids	1.05 %. w/w	0.10	-	Bundesvereinigung, 1986
IR spectroscopy	-OH stretch (3378 cm ⁻¹), -CH (2929 - 2862 cm ⁻¹), C=O of γ -lactone ring (1762 cm ⁻¹), C=C (1654 - 1606 cm ⁻¹) and C=C of aromatic rings (1509 - 1447 cm ⁻¹)	-	-	Silverstein et al., 2005
TLC analysis for parthenolide	R _f ^a = 0.49 cm	-	R _f = 0.49 cm	Drozd and Bloszyk, 1978
Parthenolide content	1.06 % w/w	0.006	minimum 0.1 % or 0.2 % w/w	Palevitch et al., 1997

^aR_f: resolution factor = height of mobile phase/height of sample.

account published data, international regulatory demands and governmental guidelines. This methodology may be used as a model by academic researchers as well as pharmaceutical companies for quality control purposes. Once it is properly adapted, it may be applied to other medicinal plants and their extracts.

ACKNOWLEDGMENTS

This work was supported by FAPESP. The authors are grateful to Prof. I. Merfort (Institut für Pharmazeutische Wissenschaften, Lehrstuhl für Pharmazeutische Biologie und Biotechnologie, Albert-Ludwigs-Universität Freiburg, Freiburg i.Br., Germany) for kindly providing a sample of parthenolide. We also thank Mrs. Paula C. P. Bueno (Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto) for discussions and valuable suggestions.

REFERENCES

Abourashed EA, Khan IA 2000. Determination of

parthenolide in selected feverfew products by liquid chromatography. *JAOAC* 4: 789-792.

Brasil 2002. Agência Nacional de Vigilância Sanitária. Guia para validação de métodos analíticos. *DOU* 20/03/2002: Resolução - RE nº 475, 19 de março de 2002: Brasília.

British Herbal Pharmacopoeia 1990. 1st ed. Vol 1. British Herbal Medicine Association. Bournemouth: Dorset.

Bundesvereinigung Deutscher Apothekerverbände (Hgrs) 1986. *Deutscher Arzneimittel-Codex* 2. Frankfurt, Govi, Stuttgart, p.1-3.

Carvalho ACB, Balbino EE, Maciel A, Perfeito JPS 2008. Situação do registro de medicamentos fitoterápicos no Brasil. *Rev Bras Farmacogn* 18: 314-319.

Causon R 1997. Validation of chromatographic methods in biomedical analysis: viewpoint and discussion. *J Chrom B: Biom Applic* 689: 175-180.

Drozd B, Bloszyk E 1978. Selective detection of sesquiterpene lactones by TLC. *Planta Med* 33: 379-384.

Farmacopéia Brasileira 1977. 3rd ed. Andrei: São Paulo.

Garcia-Piñeres AJ, Castro V, Mora G, Schmidt, TJ, Strunck E, Pahl HL, Merfort I 2001. Cysteine 38 in p65/NF- κ B plays a crucial role in DNA binding inhibition by sesquiterpene lactones. *J Biol Chem* 276: 39713-

- 39720.
- Garcia-Piñeres AJ, Lindenmeyer, MT, Merfort, I 2004. Role of cysteine residues of p65/ NF- κ B on the inhibition by the sesquiterpene lactone parthenolide and N-ethyl maleimide, and on its transactivating potential. *Life Sci* 75: 841-856.
- Gruenwald J, Brendler T, Jaenicke C 2000. *PDR for Herbal Medicines*, 2nd ed. Medical Economics Company: Montvale, New Jersey; 306-309.
- Hartke K, Mutschler E 1987. Deutsches Arzneibuch - 9. Ausgabe 1986, Kommentar, 305-306: Stuttgart: Wissenschaftliche.
- Knight DW 1995. Feverfew: chemistry and biological activity. *Nat Prod Rep* 12: 271-276.
- Long C, Sauleau P, David B, Lavaud C, Cassabois V, Ausseil F, Massiot G 2003. Bioactive flavonoids of *Tanacetum parthenium* revisited. *Phytochemistry* 64: 567-569.
- Mabry TJ, Markham KR, Thomas MB 1970. *The Systematics Identification of Flavonoids*, 1st ed. Springer-Verlag: New York.
- Oliveira FQ, Gobira B, Guimarães C, Batista J, Barreto M, Souza M 2007. Espécies vegetais indicadas na odontologia. *Rev Bras Farmacogn* 17: 466-476.
- Palevitch D, Earon G, Carasso R 1997. Feverfew (*Tanacetum parthenium*) as a prophylactic treatment for migraine: a double-blind placebo-controlled study. *Phytother Res* 11: 508-511.
- Pereira CAM, Yariwake JH, Lanças FM, Wauters J, Tits M, Angenot LA 2004. HPTLC densitometric determination of flavonoids from *Passiflora alata*, *P. edulis*, *P. incarnata* and *P. caerulea* and comparison with HPLC method. *Phytochem Anal* 15: 241-248.
- Pfaffenrath V, Diener HC, Fischer M, Friede M, Zepelin HHH 2002. The efficacy and safety of *Tanacetum parthenium* (feverfew) in migraine prophylaxis - a double-blind, multicentre, randomized placebo-controlled dose-response study. *Cephalalgia* 22: 523-532.
- Prista LN, Alves AC 1967. *Técnica Farmacêutica e Farmácia Galênica*, 1st ed. Fundação Calouste Gulbenkian: Lisboa.
- Silverstein RM, Webster FX, Kiemle D 2005. *Spectrometric Identification of Organic Compounds*, 7th ed. John Wiley & Sons Inc: New York.
- Schmidt PC, List PH 1989. *Phytopharmaceutical Technology*, 1st ed. Boca Raton: CRC Press, Boston.
- Smith R, Burford MD 1993. Comparison of flavonoids in feverfew varieties and related species by principal components analysis. *Chemometr Intell Lab Syst* 18: 285 - 291.
- Williams CA, Harbone JB, Geiger H, Hoult JRS 1999. The flavonoids of *Tanacetum parthenium* and *T. vulgare* and their anti-inflammatory properties. *Phytochemistry* 51: 417-423.
- World Health Organization 1998. *Quality Control for Medicinal Plants Material*. Geneva: 53-61.
- Zhou JZ, Kou X, Stevenson D 1999. Rapid extractions and high-performance liquid chromatographic determination of parthenolide in feverfew (*Tanacetum parthenium*). *J Agric Food Chem* 47: 1018-1022.