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The Solution and Solid State Stability and Excipient Compatibility of Parthenolide in Feverfew

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ABSTRACT

The objectives of this research were to evaluate the stability of parthenolide in feverfew solution state and powdered feverfew (solid state), and explore the compatibility between commonly used excipients and parthenolide in feverfew. Feverfew extract solution was diluted with different pH buffers to study the solution stability of parthenolide in feverfew. Powdered feverfew extract was stored under 40°C/0%~75% relative humidities (RH) or 31% RH/5~50°C to study the influence of temperature and relative humidity on the stability of parthenolide in feverfew solid state. Binary mixtures of feverfew powered extract and different excipients were stored at 50°C/ 75% RH for excipient compatibility evaluation. The degradation of parthenolide in feverfew solution appears to fit a typical first-order reaction. Parthenolide is comparatively stable when the environmental pH is in the range of 5 to 7, becoming unstable when pH is less than 3 or more than 7. Parthenolide degradation in feverfew in the solid state does not fit any obvious reaction model. Moisture content and temperature both play important roles affecting the degradation rate. After 6 months of storage, parthenolide in feverfew remains constant at 5°C/31% RH. However, ~40% parthenolide in feverfew can be degraded if stored at 50°C/31% RH. When the moisture changed from 0% to 75% RH, the degradation of parthenolide in feverfew increased from 18% to 32% after 6-month storage under 40°C. Parthenolide in feverfew exhibits good compatibility with commonly used excipients under stressed conditions in a 3-week screening study.

KEYWORDS: Feverfew, Botanical, Parthenolide, Stability, Excipient compatibility.

INTRODUCTION

Tanacetum parthenium, commonly known as feverfew, has a long history of use in Europe to prevent migraine headaches and treat rheumatoid arthritis. Like most botanicals, feverfew is chemically complex, containing sesquiterpene lactones, flavonoid glycosides, pinenes, and other compounds. Parthenolide (Figure 1) has been thought to be the most active chemical component in feverfew and is widely used as an active marker for standardization and quality control.[1] Feverfew products are required to contain no less than 0.1% parthenolide in France and 0.2% parthenolide in the United States, United Kingdom, and Canada.[1]

Wide variations between actual content and label claims of parthenolide in feverfew extracts and formulated products have been frequently reported.[2-5] The poor quality of feverfew products can be attributed to many possible factors that can be traced back to the source of feverfew raw material. Feverfew grown in the United Kingdom and Germany is well known to have high parthenolide content, while plants from the United States, Mexico, and Serbia appear to be nearly devoid of parthenolide.[1] The leaves, flowers, and seeds contain higher parthenolide levels than the stalks and roots. Harvesting the plants in spring yields a much higher concentration of parthenolide than harvesting in the fall.[6] Plant processing, such as drying temperature,[7] extraction solvent, and methods,[8] can also affect the parthenolide content in feverfew.

The influence of source and processing cannot completely explain the low parthenolide content in feverfew, particularly in the case of extracts that have ostensibly been standardized to a fixed level. In this case, the instability of parthenolide may be a cause for poor parthenolide content. Tanko et al[9] concluded that parthenolide in the whole dried feverfew leaves declined as a function of time over 120 days of storage, whether it is stored in freezer, refrigerator, or at room temperature. Temperatures in the range of –15°C to 24°C did not significantly affect the parthenolide decrease. Heptinstall et al[10] showed that parthenolide in powdered feverfew leaves could drop by 50% in 9 months if kept at room temperature and unprotected from light. Smith and Burford[11] studied the stability of a supercritical fluid extract of feverfew at room temperature and found its parthenolide level dropped from 0.38% to 0.18% in 2 years. Although these experiments are not formal stability studies, they indicate that parthenolide content in feverfew, no matter if it is whole or in a powdered crude material or an extract, could change greatly over a period of 1 to 2 years. However, all suppliers of the commercial

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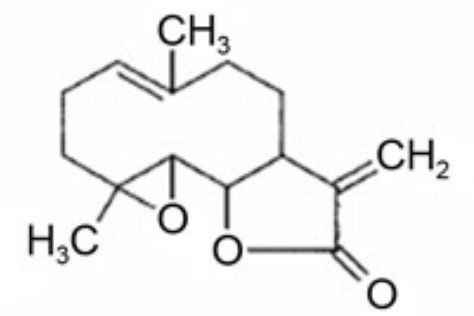


Figure 1. Structure of parthenolide.

feverfew extracts that were studied in this research claimed at least a 2-year shell life or retest period. According to International Conference on Harmonization guidelines, the degradation of active compounds (drugs) cannot exceed 5% of the initial value during shelf life.[12] It is doubtful if commercial feverfew extracts can meet ICH requirements during their claimed shelf life.

There are no published studies of the stability of feverfew under strictly controlled conditions, nor of the factors that may affect feverfew stability. Actually, few such studies of this kind have ever been reported in the field of dietary supplements owing at least in part to the chemical complexity of botanicals. For chemically defined single compounds, not only the degradation of active compounds, but also the appearance of degradation products can be monitored, and the possible degradation pathways and their affecting factors may be extrapolated. However, such an analysis is almost impossible for botanicals because there are so many compounds in botanicals and biological activity usually cannot be attributed to a single active component. Moreover, the degradation products are generally difficult to separate and the degradation pathway of botanicals may be much more complicated because of the influence of surrounding components acting as reactants or catalyzers.

Any stability evaluation must begin with an examination of chemical structure, which provides some indication of chemical reactivity. Parthenolide includes an ester group and an ether group, which suggest hydrolysis and oxidation may be the most likely pathways. Some papers discussed the possibility of intramolecular cyclization[13] and photo-isomerization,[14] which can occur under chemically synthetic conditions.

Even for a drug intended to be formulated into a solid dosage form, a limited solution phase stability study is necessary to make sure that the drug does not degrade intolerably when exposed to gastrointestinal fluids. This information is also useful in the selection of a granulation fluid and drying conditions.

The stability of drugs in solid state is affected not only by their chemistry, but also by their environment, such as ambient temperature, moisture content, light, and so forth. Excipient compatibility testing is also important in developing a stable dosage form, especially when the active ingredient is likely to be unstable. However, there is still very limited information regarding compatibility testing of botanical extracts. Kopelman et al[15] studied the compatibility of St. John's Wort with commonly used excipients. They found different excipients may protect or accelerate the degradation of different individual phytochemicals in St. John's Wort, which may be attributed to differences in their chemical reactivity, including pH effects, or differences in the "moisture-scavenging" ability of excipients.

Thus, the specific objectives of this study were (1) to evaluate the effect of pH on the stability of parthenolide in feverfew solution state, (2) to evaluate the effect of humidity and temperature on the stability of parthenolide in powdered feverfew extract, and (3) to study the compatibility between commonly used excipients and parthenolide in feverfew.

Materials and Methods

Excipients and Extract

One feverfew extract (A1, Eugene, OR) was used for the stability and compatibility study, in which parthenolide content is ostensibly standardized to 0.8%, but actually is around 0.2%.

Excipients were chosen based on their functionality, widespread commercial use, and physicochemical properties. Fillers (microcrystalline cellulose, starch, dibasic calcium phosphate, lactose, and maltodextrin), lubricants (magnesium stearate, stearic acid, silicon dioxide), and superdisintegrants (croscarmellose sodium, crospovidone, and sodium starch glycolate) were examined. All excipients were US Pharmacopeia/National Formulary grade.

The excipients were obtained from the following suppliers: microcrystalline cellulose and croscarmellose sodium (Avicel PH102 and Ac-Di-Sol, FMC Biopolymer, Newark, DE), starch 1500 (Colorcon, West Point, PA); Fast-Flo Lactose (Foremost, Baraboo, WI), dibasic calcium phosphate (Emcompress, JRS Pharma, Patterson, NY), maltodextrin (Maltrin M510, Grain Processing Corp., Muscatine, IA), crospovidone (ISP, Wayne, NJ), sodium starch glycolate (Primojel, DMV International, Veghel, The Netherlands), magnesium stearate (Mallinckrodt Baker, Paris, KY), stearic acid (Crompton Corp., Memphis, TN), and silicon dioxide (Cab-O-Sil, Cabot Corp, Tuscola, IL).

The Effect of pH Value on the Stability of Parthenolide in Feverfew Solution State

A high concentration feverfew extract was prepared by extracting 2.5 g feverfew with 25 mL 50% ethanol and then centrifuging at 3000 rpm for 5 minutes. Then 1 mL of the

Table 1. Observed First-order Rate Constant and $t_{1/2}$ for Parthenolide Degradation in Buffered Aqueous Feverfew Solutions

pH	Buffer	K (1/Day)	$t_{1/2}$ (Day)	R^2
1	HCl	9.504	0.073	0.9996
3	Glycine	0.074	9.365	0.9974
5	Acetate	0.007	99.000	0.9888
7	Phosphate	0.010	69.300	0.9959
9	Glycine	25.488	0.027	0.9970

supernatant was added to each of five 10-mL of volumetric flasks and brought to 10 mL with the appropriate aqueous 0.1 M buffer (pH values of 1, 3, 5, 7, and 9). The buffers used appear in Table 1. The filtrate was kept in amber vials at room temperature. Parthenolide level was analyzed at certain time intervals to evaluate the impact of pH on parthenolide stability. The rate constant for each pH was calculated from the plot of log (% remaining drug) versus time.

Every experiment in this study was run in triplicate unless specifically mentioned otherwise.

The Effect of Relative Humidity on the Degradation of Parthenolide in Feverfew Solid State

Approximately 250 mg of feverfew extract was placed in each of several open glass vials. The exact weight of extract and vial were recorded. The filled vials were kept at the same temperature (40°C) and different relative humidities (RH) (0%, 31%, 53%, and 75%). Saturated salt solutions were used to provide certain RH. Parthenolide content was determined at 1, 2, 3, and 6 months. The parthenolide content before storage was also determined as a control and considered 100%.

The extraction was performed as follows: 5 mL of 50% ethanol was added to each glass vial containing feverfew. After 5-minute sonication, the extracts were transferred to 25-mL volumetric flasks. The glass vials were rinsed with 5 mL of 50% ethanol 3 times and the rinse solutions were returned to the respective volumetric flasks. The volumetric flasks were sonicated for another 25 minutes and then filtered and assayed using a high-performance liquid chromatography (HPLC) analysis procedure, which had previously been developed in our laboratory.[5]

The Effect of Temperature on the Degradation of Parthenolide in Feverfew Solid State

Approximately 250 mg of feverfew extract was placed in each of several open glass vials and stored at 31% relative humidity and various temperatures (5°C, 25°C, 40°C, and 50°C). Parthenolide content was determined at 1, 2, 3, and 6 months and expressed as percentage of parthenolide content before storage.

Feverfew-Excipient Compatibility

Binary blends of feverfew (75%, 250 mg) and fillers (19%, 50 mg), disintegrants (6%, 17 mg), and lubricants (1%, 2.5 mg) were prepared by weighing feverfew and the appropriate amount of excipient into inert glass vials and blended on a vortex for 10 seconds. The open glass vials were kept at 50°C/75% RH for 3 weeks and then parthenolide content was determined. Samples of the binary blends and feverfew stored in closed glass vials at 5°C were used as control.

Statistical Analysis

All groups in the excipient compatibility study were subjected to analysis of variance (ANOVA) at P less than .05 to determine statistical significance using SPSS software (SPSS, Inc., Chicago, IL).

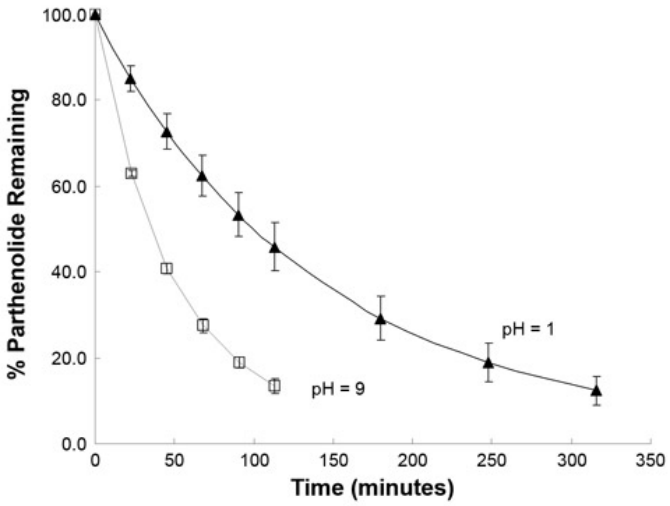
Results and Discussion

The Effect of pH on the Degradation Kinetics of Parthenolide in Feverfew Solutions

The degradation of parthenolide in feverfew solution appears to fit a typical first-order reaction (Figure 2). The observed rate constants (K) and $t_{1/2}$ are summarized in Table 1. The pH of feverfew solution has a dramatic effect on parthenolide stability. When logK is plotted against pH value, a V-shape profile is generated, which is a typical pH-profile for a nonionizable compound (Figure 3). Parthenolide is comparatively stable when the environmental pH is in the range of 5 to 7, becoming unstable when pH is less than 3 or more than 7. These results, as well as the chemical structure of parthenolide, suggest that hydrolysis may be the predominant degradation pathway of parthenolide in feverfew solution that is subject to specific-acid, water, and specific-base catalyzers.

The Effect of Moisture Content and Temperature on Parthenolide Degradation in Feverfew Solid State

Figure 4 shows 6-month stability data of parthenolide in feverfew extract at 31% RH and different temperatures. Parthenolide content remains constant after 6-month storage in a refrigerator. At 25°C, parthenolide in feverfew begins to degrade slowly after 3 months. Parthenolide in feverfew shows significant decomposition in the first or second month if stored at 40°C or higher. The parthenolide degradation can reach 40% after 6-month storage at 50°C. Figure 5 shows the influence of different environmental RH on parthenolide stability in feverfew. Apparently, RH can significantly accelerate the degradation of parthenolide in feverfew in the solid



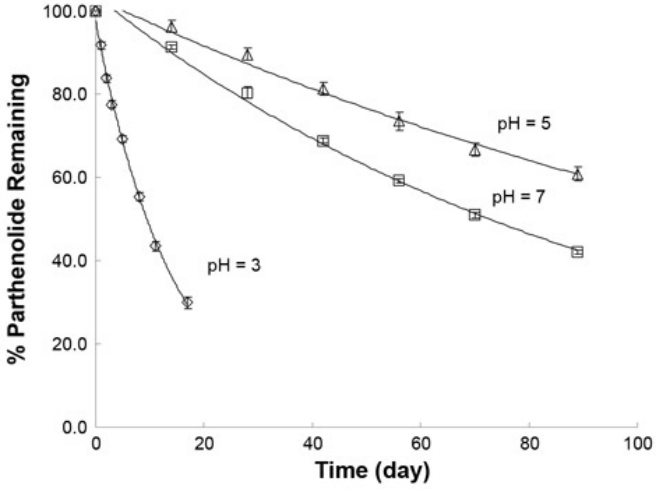


Figure 2. Parthenolide degradation in buffered aqueous feverfew solution over the pH range 1 to 9.

state. When the relative humidity changed from 18% to 32%, the parthenolide degradation increased from 18% to 32% in 6 months.

Different from kinetics in solution, parthenolide degradation in feverfew in the solid state does not fit any obvious reaction model. This may be attributed to the multiple reaction pathways expected in complex botanicals. In the solution state, the reactant molecules are much more likely to interact with each other and the reaction will follow one or more pathways that are favored energetically. In the case of parthenolide, hydrolysis may be the predominant pathway, especially when acid or base catalysts are present. However, in the solid state, because parthenolide is present in a very low concentration, the decomposition pathway of one molecule may be more related to the chemistry of the surrounding molecules, which makes multiple pathways highly possible.

Most of the suppliers of the commercial feverfew extracts claim at least a 2-year shell life or retest period under room

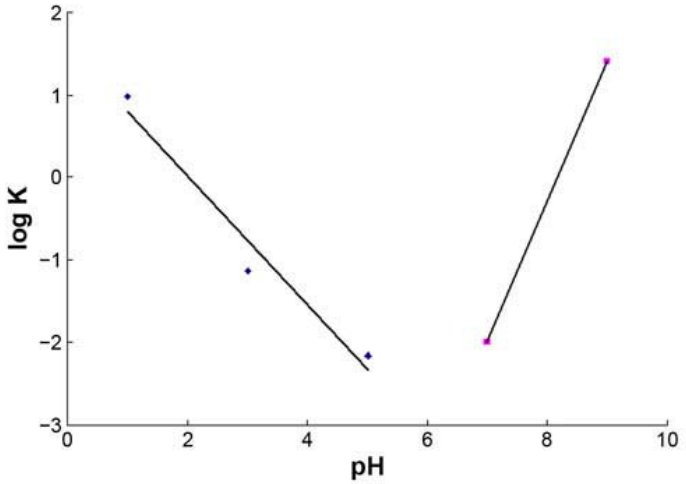


Figure 3. pH-rate profile of parthenolide in feverfew solution.

temperature storage. However, this research showed that parthenolide had significantly degraded after 6-month storage under 25°C/31% RH. This observation raises concern about attaining stability adequate to ensure potency over a reasonable product shelf-life. Because this experiment showed parthenolide can remain stable for at least 6 months under 5°C/31% RH, a substantial improvement in parthenolide stability may be expected if feverfew products are stored under refrigeration. In addition, the multiple degradation pathway and unpredictability of degradation behavior of parthenolide in feverfew solid state indicated that the shelf life of feverfew should be proposed on the basis of a stability study performed over the entire proposed shelf life. This rule may be applicable for all botanicals considering their typical chemical complexity.

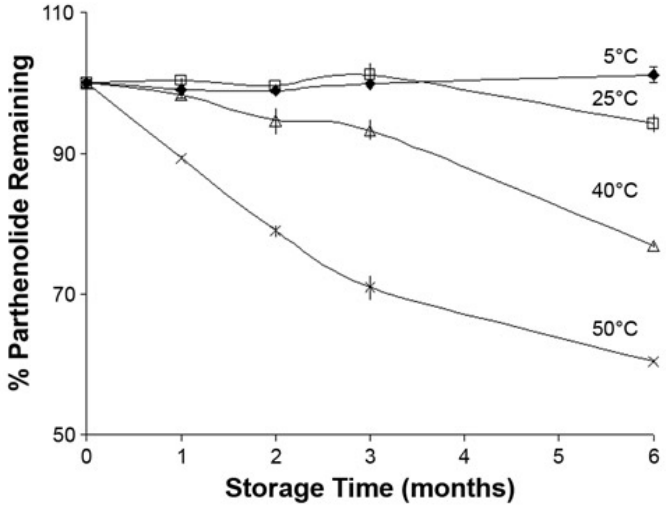


Figure 4. Stability of parthenolide in powdered feverfew extract under 31% RH at different temperatures (n = 3).

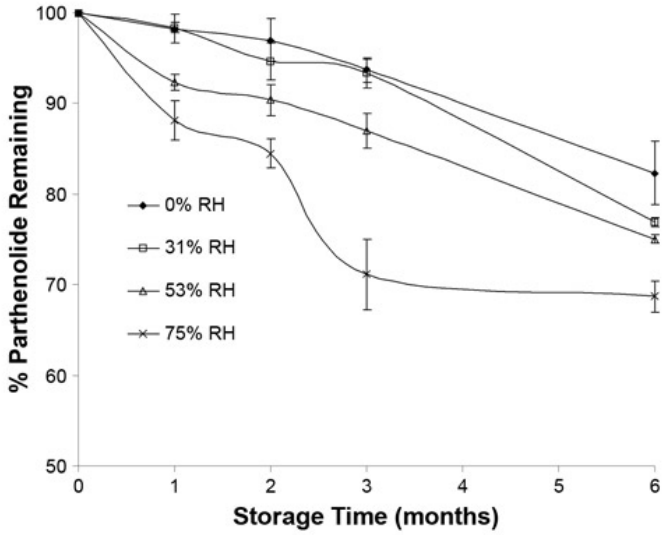


Figure 5. The Stability of parthenolide in powdered feverfew extract at 40°C and different relative humidities (n = 3).

The Compatibility of Feverfew with Excipients

Differential scanning calorimetry (DSC) and quantitative assay after isothermal stress testing are two methods commonly used for drug-excipient interaction studies. Because of the chemical complexity of botanicals, isothermal stress testing was selected for this study. Conventional isothermal stress testing typically involves challenging drug-excipient mixtures with moisture, as the majority of drug degradation reactions involve moisture. Sample exposure to moisture may be accomplished by increasing the RH of the environment, or by adding a fixed amount of water. Serajuddin et al[16] discussed that the addition of a predetermined amount of water is more suitable for compatibility studies because it can remove the unpredictability from excipient hygroscopicity. The amount of water added varies from 5% to 20% according to the literature.[16-18] However, in this compatibility study, 50°C/75% RH was selected as a stress condition because it was found to be difficult to distribute water into feverfew extract homogeneously and that the practice led to increased variability. Under 50°C/75% RH, parthenolide could degrade ~20% in 3 weeks.

Table 2 shows feverfew (parthenolide) exhibited good compatibility with commonly used excipients under the conditions tested. Under 5°C storage conditions, the addition of excipients almost did not affect the stability of parthenolide except for Emcompress (dicalcium phosphate dihydrate). Under stressed conditions, compared with feverfew-only samples, most binary blends of excipient and feverfew did not exhibit significant differences in parthenolide degradation. Significant differences from the feverfew-only samples were found only with the Fast Flo (lactose) and Emcompress blends, but even these differences were small. Indeed, in separate comparison, there were no significant differences between these 2 and the other 3 commonly used fillers. The slight incompatibility between Emcompress and feverfew may be attributable to the alkaline nature of dibasic calcium phosphate.

Although only 2 fillers exhibited significant effects on parthenolide stability, there appeared to be an overall tendency toward lower parthenolide content in all feverfew-filler mixtures. In this study, feverfew was considered as if it were a high-dose drug for formulation purposes, so only ~20% filler was added. A higher percentage of filler in the formulation may cause a more severe effect.

In the present study, a single extract component thought to be the main source of activity of the botanical was studied. When activity cannot be assigned to a single defined compound, several components of interest may need to be considered. In this latter case, the level of complexity increases greatly. For example, compatibility with individual excipients

Table 2. Effect of Commonly Used Excipients on the Stability of Parthenolide in Feverfew

		% Parthenolide Found (0 Day)	% Parthenolide Found (After 3 Wks)	
			5°C	50°C, 75% RH
Control	Feverfew Only		0.200 ± 0.003	0.157 ± 0.001
Feverfew + Filler	MCC		0.198 ± 0.003	0.154 ± 0.003
	Starch 1500		0.197 ± 0.002	0.154 ± 0.002
	Fast Flo Lactose	0.202 ± 0.004	0.199 ± 0.001	0.152 ± 0.003*
	Emcompress		0.194 ± 0.001*	0.152 ± 0.002*
	Maltodextrin		0.201 ± 0.000	0.154 ± 0.002
Feverfew + Disintegrant	Crospovidone		0.199 ± 0.001	0.158 ± 0.001
	Ac-Di-Sol		0.200 ± 0.002	0.156 ± 0.003
	Promojel		0.202 ± 0.001	0.156 ± 0.001
Feverfew + Lubricant	Magnesium Stearate	0.198 ± 0.004	0.157 ± 0.001	
	Stearic Acid	0.199 ± 0.001	0.156 ± 0.001	
	Cab-O-Sil	0.198 ± 0.003	0.156 ± 0.002	

*Represents significant difference compared with control ($P < .05$).

may vary from one extract component to the next. In such cases, it may be useful to define a phytochemical profile consisting of several components of interest with the goal of achieving stability based on the best possible survival of the profile as a whole. One way to do this is to use similarity metrics to assess changes to the phytochemical profile that might occur under various storage conditions, as previously demonstrated for St. John's Wort.[16]

CONCLUSION

The results from this study indicate that hydrolysis may be the predominant degradation pathway of parthenolide in feverfew solution, whereas parthenolide degradation in feverfew solid state may follow multiple pathways. Parthenolide in feverfew exhibits good compatibility with several commonly used excipients under stressed conditions in a 3-week screening study. The results of this study suggest that the shelf life of botanicals may best be proposed on the basis of a stability study performed over the entire proposed shelf life.

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