

Determination of parthenolide from dissolution testing of powdered feverfew products

Abstract

Botanical dietary supplements are widely available without requirements for proof of safety or efficacy, but with requirements to be produced according to current Good Manufacturing Practices. Since 2007, cGMP's mandated under federal law include standards for manufacture, storage and distribution and the assurance of quality and integrity of products sold to consumers. While standards require compliance with content claims made in labeling, there remain no assurances of bioavailability. Historically, feverfew is a dietary supplement taken by sufferers of migraine headaches. We examined the dissolution profiles of commercially obtained powdered feverfew products and analyzed them for the presence and quantity of parthenolide, the proposed active constituent and marker compound. Encapsulated feverfew products were purchased at local retail markets throughout metropolitan Chicago, Illinois, USA, and subjected to dissolution testing in the laboratory. Aliquots removed from testing vessels over a 2 hour period were extracted and analyzed by hplc. Analysis of each sample demonstrates whether or not parthenolide is detectable, depending on the conditions dissolution. Parthenolide in encapsulated powdered feverfew products may not withstand the extreme pH of the stomach. Therefore, it may be best to recommend that dietary supplements containing powdered feverfew be taken on an empty stomach.

Keywords: dietary supplements, feverfew, *tanacetum parthenium*, dissolution testing, HPLC

Volume 2 Issue 4 - 2015

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Received: July 1, 2015 | **Published:** July 20, 2015

Abbreviations: Å, angstrom; AUC, area under the curve; cGMP, current good manufacturing practices; °C, degrees celcius; hplc, high performance liquid chromatography; hr, hour; µg, microgram; µL, microliter; µ, micron; mg, milligram; mL, milliliters; mL/min, milliliters per minute; mm, millimeters; nm, nanometer; C18, octadecylsilane; PC, personal computer; RS, reference standard; rpm, revolutions per minute; UV, ultraviolet; USP-NF, united states pharmacopeia-national formulary; w/v: unit weight per unit volume; w/w: unit weight per unit weight

Introduction

Feverfew (*Tanacetum parthenium*) (L.) Schultz-Bip. (Asteraceae), a member of the daisy family, is historically used to treat or prevent migraine headache. Parts of the plant contain several potentially active chemical constituents, including flavanoid glycosides, sesquiterpenes, monoterpenes, polyacetylenes, pyrethrin, melatonin and tannins.^{1,2} Parthenolide, a sesquiterpene lactone, is thought to be one of the most biologically active components and is frequently used as a marker compound when performing chemical analysis of supplements containing the herb. The source of feverfew is very important, as plants from the US, Mexico and Serbia has been found previously to contain little or no parthenolide following chemical analysis. The leaves contain the highest concentration of parthenolide and contain a higher concentration when harvested during flowering, in the spring.^{1,3} The concentration of parthenolide may degrade on storage at room temperature, with concentrations falling between 35-50% in 9months.⁴ These factors make proper formulation of feverfew particularly important relative to clinically relevant expectations. Preparations sold in the United States, United Kingdom and Canada are standardized to contain at least 0.2% parthenolide.

Currently, there are no recognized standard methods for assuring that the constituents of feverfew are available for absorption in the body, and research has focused on determining if marker compounds, such as parthenolide, are present in labeled amounts. Several groups have published feverfew extraction methods, with the USP-NF having published an official monograph for Powdered Feverfew.⁵ In this method, methanol is used to extract parthenolide, and high-performance liquid chromatography (hplc) is used to determine parthenolide content, using acetonitrile:water (9:11) as the mobile phase. This extraction method differs from others published in the literature that use extraction media such as acetone, aqueous acetonitrile, ethanol, chloroform, and petroleum ether.^{4,6-8} Mobile phases used in hplc analysis may also vary, though combinations of acetonitrile and water are the most common.

In their work analyzing the dissolution behavior of commercial feverfew products Jin, Madieh and Augsburg described that products demonstrated substantially different dissolution profiles between one another, and that release performance was not consistent from brand to brand.⁶ This, combined with variation in label claims for content and dose, may account for different product behavior in the consumer.⁹ The focus of this work centered upon the analytical determination of parthenolide from commercially obtained encapsulated powdered feverfew products by simple dissolution testing of products at various conditions of pH, without using biorelevant dissolution media. Extracts of dissolution media were examined according to USP monograph by hplc using USP Parthenolide RS as a reference and USP Rutin RS as internal standard in the analyses.

Materials and methods

The ten dietary supplement products studied, labeled A-I and K,

were purchased at local health food stores throughout metropolitan Chicago, Illinois, USA. Brand and manufacturer information is available upon request to the authors. All products were gelatin capsules unless otherwise noted. Ethyl acetate, Buffer Solution pH 4.0, hydrochloric acid, and hplc grade acetonitrile were obtained from Fischer Scientific (Fair Lawn, NJ). Monobasic sodium phosphate was obtained from Sigma Chemical (St. Louis, MO). Anhydrous sodium sulfate, parthenolide standard, rutin standard, and hplc grade methanol were obtained from Aldrich (Milwaukee, WI). Phosphoric acid was obtained from Acros Organics (Morris Plains, NJ) and compressed helium, USP was obtained from Airgas (Brookfield, IL). Ultra-pure water used to prepared dissolution media was obtained from a Milli-Q Plus water purification system (Millipore, Molsheim, France).

All dissolution analyses were performed prior to passage of product expiration dates. Two capsules of each dietary supplement product were placed in the basket of a Van Kel VK 7000 dissolution apparatus and 750 D temperature controller (Edison, New Jersey) at 50rpm and 37°C for 2hours. Tests were carried out in aqueous dissolution media of pH 1.0, 3.0 and 7.0; three tests were conducted at each pH. An Accumet pH meter 10 (Fischer, Fair Lawn, New Jersey) was used to adjust the pH of dissolution media. Aliquots of 10mL were removed from each vessel after 1 hour and 2hours. Each sample was exhaustively extracted with 10mL of ethyl acetate in a separatory funnel. Organic layers were dried by filtration through approximately 5 grams of anhydrous sodium sulfate as a drying agent, transferred to round-bottom flasks and evaporated to a small volume under reduced pressure at 40°C using a RE111 Rotavapor with 461 water bath (Büchi, Postfach, Switzerland) and Model B-169 vacuum aspirator (Brinkmann, Westbury, New York). Extracts were transferred to small vials, evaporated to dryness under reduced pressure and stored at 4°C until reconstituted with 1mL of methanol for hplc analysis.

Analysis by hplc was performed using Breeze software for PC (Waters, Cambridge, Massachusetts) with acetonitrile: water (9:11) as an isocratic mobile phase delivered using a model 1525 binary pump (Waters, Cambridge, Massachusetts) at the rate of 1.0mL/min. A Model

2487 variable wavelength UV-Visible detector (Waters, Cambridge, Massachusetts) was set to detect at wavelengths 210 and 254nm. Standard curves were prepared daily using parthenolide standard solutions (0.0002% w/v:0.2% w/v). The weight of parthenolide in standard solutions was plotted against AUC at 210nm, calculated as the average of six analyses performed in triplicate for each of two samples. A Gemini column (5 μ , C18, 100Å, 250X4.6mm) and Guard Cartridge (C18 4 X 3.0mm) provided the matrix for chromatographic separation (Phenomenex, Torrance, California).

Each sample (200 μ L) was transferred to a glass autosampler vial insert and combined with either 50 μ L of solvent, 25 μ L of standard parthenolide solution (0.2% w/v) plus 25 μ L of solvent, or 25 μ L of standard parthenolide solution (0.2% w/v) plus 25 μ L of Rutin RS solution (0.1% w/v) as an internal standard. Injections of 10 μ L volume were analyzed using a Waters 717 plus autosampler (Cambridge, Massachusetts). The parthenolide/rutin spiked samples were used to determine and confirm parthenolide retention volume. The parthenolide concentration of each sample was calculated as the average of six analyses from the AUC at its retention volume performed in triplicate for each of two samples, and compared to the standard curve prepared on the day of analysis.

Results and discussion

Dissolution testing was performed by the method and under conditions previously described by Augsburg and colleagues.⁸ Analysis by hplc was performed according to USP monograph.⁵ Results of hplc analyses are given in Table 1. Wavelengths used during detection were 210nm, the absorbance maximum wavelength for parthenolide, and 254nm to detect overlapping chromophores. Parthenolide content is calculated in milligrams. Parthenolide is detectable at the sub-nanogram level and calculated in micrograms recovered at 1 or 2hours, as shown in Table 2. Even the samples tested to contain the highest concentration of parthenolide still contained less than the amount stated on the product labels by an order of magnitude. Several factors may explain these results.

Table 1 Parthenolide extraction and analysis.

Product	Powdered feverfew/capsule (mg)	Stated parthenolide (%w/w)	Analyzed parthenolide (%w/w)	Parthenolide/ capsule (mg)
A	400	0.4	0.44	1.75
B	350	0.7	0.26	0.90
C	450	0.7	0.34	1.51
D	350	0.7	0.21	0.72
E	455	NA*	0.29	1.34
F	350	0.1 – 0.2	0.16	0.56
G	100	0.6	0.22	0.22
H	380	NA	0.26	1.00
I	380	0.16	0.24	0.93
K	455	NA	0.31	1.39

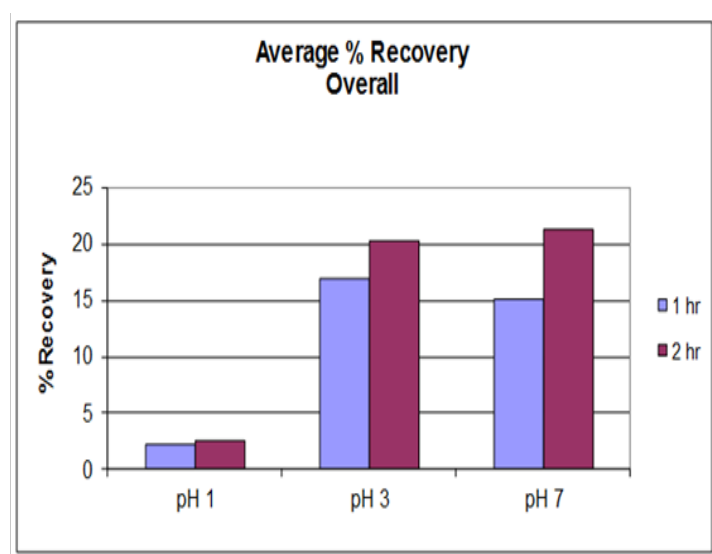
*Not available

Table 2 Average amount of parthenolide recovered, pH3 and pH7

Product	Average amount of parthenolide recovered at pH 3 (µg)		Average amount of parthenolide recovered at pH 7 (µg)	
	1 Hour	2 Hours	1 Hour	2 Hours
A	59	88	127	124
B	199	307	38	98
C	114	73	110	99
D	26	29	58	42
E	132	270	209	482
F	67	55	104	73
G	104	73	70	114
H	268	344	218	304
I	90	153	89	101
K	386	559	368	571

On average, less than 5% of parthenolide, calculated as stated on the label, was recovered from dissolution testing at pH 1, after either 1 or 2 hours. After 1 hour, approximately 15% of parthenolide, calculated as stated on the label, was recovered from dissolution testing at both pH 3 and pH 7. After 2 hours, approximately 20% of parthenolide, calculated as stated on the label, was recovered from dissolution testing at both pH 3 and pH 7, Figure 1.

- It is known that parthenolide is poorly water-soluble. This can impact the amount able to dissolve at any pH. The use of biorelevant dissolution media would present opportunities to improve extraction efficiency from products during dissolution testing.
- A fraction of parthenolide could be lost during manipulation in the step of ethyl acetate extraction prior to hplc analysis.
- In addition to the low levels of parthenolide observed in all samples, the recoverable concentration varied by product, duration of time in the dissolution apparatus and the pH of dissolution media, as shown in Figure 2. This can be explained by several factors:
 - Parthenolide originally contained in products at the time of manufacture may decompose over time and under various environmental conditions during storage, distribution and display on the shelf at commercial retail outlets.
 - Spurious compounds from the complex mixture of plant constituents in the extract may affect retention volume and the ability to integrate AUC accurately.
 - Environmental conditions in the laboratory fluctuate during the course of the day, possibly affecting retention volume and absorbance measurements.
 - Experimental error in the preparation of the daily standard curve may account for unexpectedly low concentrations of parthenolide in some samples.

**Figure 1** Average percent parthenolide recovery, overall.

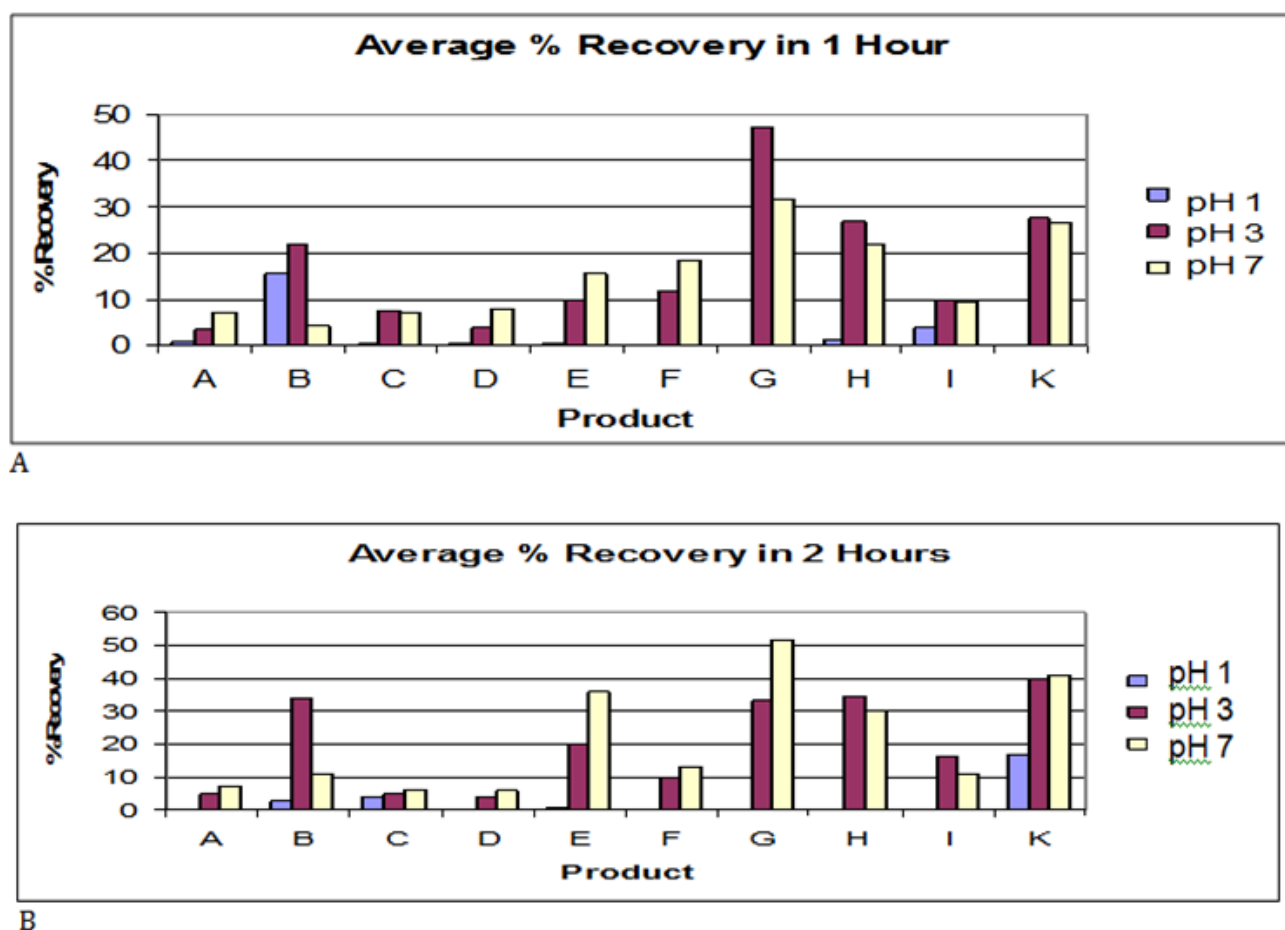


Figure 2 Average percent parthenolide recovery. A) 1 hour; B) 2 hours

Conclusion

Parthenolide in commercial feverfew capsules may be poorly bioavailable due to low aqueous solubility and the inability to withstand the extreme pH of the stomach. Although products may contain higher concentrations of parthenolide than detected in this dissolution study, according to label claim, consumers are advised to purchase only those products which bear the symbol of a reliable independent testing laboratory. To avoid lengthy residence time in the harsh environment of the stomach, it may also be advisable to take encapsulated powdered feverfew products on an empty stomach with plenty of water. As a final, noteworthy point of discussion, all of the products tested in this study were analyzed prior to their date of expiration, and were manufactured prior to the release of cGMP guidelines under US federal law in July, 2007.

Acknowledgements

The authors acknowledge funding from the Department of Pharmaceutical Sciences, Midwestern University Chicago College of Pharmacy; Dr. Elizabeth Langan, for her assistance in calculations and students, Richard Grace, David Hilgoth, Randall Knoebel, Jennifer Lukowski, Michael Messuri and Virginia Yu for their assistance with extraction and analysis.

Conflict of interest

The author declares no conflict of interest.

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