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Research Article

Selected Physical and Chemical Properties of Feverfew (*Tanacetum parthenium*) Extracts Important for Formulated Product Quality and Performance

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Abstract. The objectives of this research are: (1) to assess selected formulation-relevant physical properties of several commercial Feverfew extracts, including flowability, hygroscopicity, compressibility and compactibility (2) to develop and validate a suitable extraction method and HPLC assay, and (3) to determine the parthenolide content of several commercial Feverfew extracts. Carr's index, minimum orifice diameter and particle-particle interaction were used to evaluate powder flowability. Hygroscopicity was evaluated by determining the equilibrium moisture content (EMC) after storage at various % relative humidities. Heckle analysis and compression pressure-radial tensile strength relationship were used to represent compression and compaction properties of feverfew extracts. An adapted analytical method was developed based on literature methods and then validated for the determination of parthenolide in feverfew. The commercial extracts tested exhibited poor to very poor flowability. The comparatively low mean yield pressure suggested that feverfew extracts deformed mainly plastically. Hygroscopicity and compactibility varied greatly with source. No commercial feverfew extracts tested contained the label claimed parthenolide. Even different batches from the same manufacturer showed significantly different parthenolide content. Therefore, extract manufactures should commit to proper quality control procedures that ensure accurate label claims, and supplement manufacturers should take into account possible differences in physico-chemical properties when using extracts from multiple suppliers.

KEY WORDS: botanical; compactibility; compressibility; feverfew; flowability; hygroscopicity; parthenolide.

INTRODUCTION

An evaluation of the physical, chemical and mechanical properties of a drug substance is usually the first step in the development of an oral solid dosage form. An understanding of the raw material provides an essential foundation upon which to predict any problem which may occur in formulation and process development and, ultimately, in manufacture.

Foremost among important physical properties are flowability, hygroscopicity, and compactibility. The flow of powder affects almost every step of manufacture including transfer, storage, blending, feeding and compaction. Poor flowability may result in great difficulty in processing the material, especially in high-speed production. Hygroscopicity and moisture content play important roles in particle–particle interactions and may contribute to poor flowability as well as adversely affect both physical and chemical stability. Compactibility is also an important indicator of the processibility of the material. High-dose drugs often lack critical physical properties that can not be compensated for except by the addition of a large amount of excipients or by adopting a granulation process. The behavior of powder under compression can be used to help select a suitable compression setting and production speed during scale-up. In addition, the relationship between tensile strength of compacts and compression force (or pressure) needs to be determined because the final unit needs to achieve a certain tensile strength and friability to stand up to handling and transportation.

Tanacetum parthenium, commonly known as feverfew, has a long history of usage in Europe to prevent migraine headaches and treat rheumatoid arthritis. In recent years it has become more and more popular in America. The daily dose of feverfew has not been clearly defined yet; however, a Canadian monograph suggests a daily dose of 50–250 mg feverfew dried leaf containing at least 0.2% parthenolide and not exceeding the equivalent of 4 mg parthenolide per day (1). The possibility thus of a high dose of feverfew product formulation makes it necessary to understand the physical

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properties of commercial feverfew extracts. However, a review of the literature reveals no published paper addressing this aspect of feverfew.

Like most botanicals, feverfew is chemically very complex, containing sesquiterpene lactones, flavonoid glycosides, pinenes and other compounds. Parthenolide 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. Feverfew products are required to contain no less than 0.1% parthenolide in France and 0.2% parthenolide in the US, UK and Canada (2).

Many methods have been reported to determine parthenolide content in feverfew using different extraction solvents, procedures, and analytical methods. Extraction solvents used include chloroform (3), petroleum ether (4), acetone (5), acetonitrile (6), 90% acetonitrile (7), methanol (8), 50% methanol (9), alcohol, and others. Soxhlet extraction (4), heating in water bath (8), stirring (5,7), and sonication (6,9) are commonly reported extraction methods. Analytical methods include NMR, GC and HPLC, among which, HPLC is the most common.

Although various extraction solvents and procedures have been reported, little has been reported so far that compares the effectiveness of extraction systems. Zhou et al. (7) compared the extraction efficiency of Soxhlet extraction and the stirring method. The Soxhlet method described includes a 24 h extraction in the Soxhlet apparatus under raised temperature (60°C), evaporating to dryness and then redissolving the residue for HPLC analysis. They found that compared to Soxhlet extraction, bottle-stirring for 30 min at room temperature can reach the same or even higher extraction efficiency if a suitable extraction solvent was selected. In addition, the bottle-stirring method provided more reproducible results because this extraction procedure is much simpler. They also compared five pure organic solvents and their mixtures with different ratios of water for parthenolide extraction. They found 90% acetonitrile to exhibit the highest extraction efficiency. However, they did not investigate the possible role sonication might play in extraction. In addition, methanol, which is the solvent of the official USP extraction method, was not included in the solvent comparison.

The most widely used method of extraction in the industry is the USP method which is a modification of Soxhlet extraction. The usual 24 h extraction period in the Soxhlet apparatus is replaced by repeated 10 min extractions with methanol in a 60°C water bath. This modification may be intended to minimize exposure to elevated temperature owing to the possible thermal lability of parthenolide. However, the evaporation and resuspension steps still exist, which may reduce the extraction yield and make the assay cumbersome. In the Soxhlet experiment, Zhou et al. (7) found that evaporating the first solvent extract to dryness leaves a green residue in the flask that will not go into solution after the addition of resuspension solvent, even after several hours of mixing. Direct quantitation of the first solvent extract clearly improves the results and makes the method easier and more suitable for the determination of parthenolide in large numbers of samples.

Another problem area in current methods is the adoption of isocratic elution for HPLC analysis. Given the complexity of feverfew, a low ratio of organic solvent may not be sufficient to elute out all the hydrophobic compounds in feverfew which may interfere with the determination of parthenolide. In addition, most of the current methods use water as part of the mobile phase rather than aqueous buffer. Water is simpler than buffer and will not exhibit salt precipitation. However, for a stability-indicating method, it is recommended that separations be developed at a mobile phase pH where the retention of analytes is less affected by changes in pH and which prevents interference from the possible acidic or basic degradation products. Among all published methods, only Abourashed et al. (6) used a buffered mobile phase and gradient elution. However, they used 50 mM NaH_2PO_4 directly and did not adjust pH to its buffering capacity range. In general, most buffers only provide adequate buffering capacity for controlling mobile phase pH to within 1 U of their pKa. Otherwise, the buffer is of little value.

Thus, the objectives of this research are: (1) to assess the flowability, hygroscopicity, and compactibility of several commercial feverfew extracts, (2) to develop an extraction and HPLC analytical procedure which can be used to assay parthenolide content in normal and degraded feverfew extracts and finished products, and then (3) to use the developed extraction and analytical procedure to evaluate the parthenolide content of selected commercial feverfew extracts.

MATERIALS

Feverfew Commercial Extracts

Five feverfew powdered extracts ostensibly standardized to 0.2–1.2% parthenolide were obtained from four nutraceutical companies: A1, A2, B1, C1 and D1 (Table I). A1 and A2 are two different batches from the same company.

Standard

Parthenolide (97% purity) was purchased from EMD Biosciences Inc (San Diego, CA).

Table I. Characterization Based on Claims and Coding of the Commercial Standardized Feverfew Extracts Studied

Source	Plant part used	Claimed carrier(s)	% Parthenolide claimed
A1	Flower	None	0.8
A2	Dried leaves and flower	None	0.8
B1	Leaf	Maltodextrin/cellulose	0.2
C1	Flowering tops	Maltodextrin/silicon dioxide	0.5
D1	Aerial parts	None	1.2

Solvents and reagents

HPLC grade acetonitrile (Burdick & Jackson, Muskegon, MI), potassium phosphate, monobasic (Sigma Chemical Co., St. Louis, MO), reagent grade phosphoric acid 85% (J. T. Baker, Phillipsburg, NJ), HPLC-grade alcohol and methanol (E.M. Science, Gibbstown, NJ) were used. All water was purified using an in-house Milli-Q system (Millipore, Milford, MA).

The excipients were obtained from the following suppliers: microcrystalline cellulose and croscarmellose sodium (Avicel PH102 and Ac-Di-Sol, FMC Biopolymer, Newark, DE), maltodextrin (Maltrin M510, Grain processing Corp., Muscatine, IA), magnesium stearate (Mallinckrodt Baker, Paris, KY) and colloidal silicon dioxide (Cab-O-Sil, Cabot Corp., Billerica, MA). All excipients were USP/NF grade and were used as received.

METHODS

Physical Characterization

Flow Studies and Particle Size Analysis

Carr's compressibility index (C) provides an indirect measurement of flow and is determined from the tapped and bulk densities of the material per Eq. 1 (10).

$$C = \left(\frac{\rho_t - \rho_o}{\rho_t}\right) \times 100 \tag{1}$$

Where ρ_t is tapped density and ρ_0 is loose bulk density. The bulk and tapped densities were measured using a Scott Volumeter and a Stampf jolting volumeter (Shandon Southern Instruments, Inc, Sewickley, PA), respectively, following the USP method (8). Densities were determined in triplicate and the averages were used to calculate the Carr indices.

Minimum orifice diameter studies were performed using a Flodex Powder Flowability Tester (Hanson Research Corporation, Northridge, CA) and the method of Gioia (11). The diameter of the smallest orifice through which the powder exhibits free-flow three times out of three is taken as the flowability index.

The volume mean and median diameters of the commercial extracts were determined using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK). A dry powder feeder was used to deliver the sample. A pump was connected to the feeder to provide different levels of dispersion pressure (55, 80 and 110 psi). Each sample was analyzed in triplicate under each of the three pressures.

Moisture Content and Hygroscopicity

Moisture content was determined using a Computrac MAX 2000XL (Moisture analyzer, Arizona Instrument LLC) based on loss-on-drying. Hygroscopicity was evaluated by determining the equilibrium moisture content (EMC) of each commercial extract after storage at various relative humidities (RH) for 21 days based on method of Callahan (12). Desiccators containing saturated salt solutions and a 25°C precision temperature incubator were used to prepare chambers with relative humidity ranging from 8 to 84%. Calcium sulfate anhydrous desiccant (Drierite, WA Hammond Drierite Company Ltd, Xenia, OH) and purified water were used for the 0% RH and 100% RH chambers, respectively. Duplicate samples of each commercial feverfew extract (~400 mg) placed in two open and numbered weighing boats were stored in each chamber. The equilibrium moisture content [EMC] (%) of samples was determined using equation 2.

$$\mathrm{EMC} = \left(\frac{\mathrm{P}}{\mathrm{P} + 100}\right) \times 100 \tag{2}$$

Where P is % moisture (dry basis)

Heckel Analysis

As required for Heckel analysis, the true density of each material was determined using a model 1305 Multivolume Helium Pycnometer (Micromeritics, Norcross, GA). Compression was performed on an instrumented modified Colton 321 single station tablet press. About 200 mg of powder were accurately weighed and manually filled into the die with 8.7 mm diameter. The die was lubricated prior to each compression with a saturated magnesium stearate solution in acetone. The position of the lower punch was adjusted to provide a peak upper compression pressure of ~100 MPa for each material. The thickness of final tablet is 2.5±0.1 mm. During compression, the upper and lower compression pressure, and upper and lower punch displacement were recorded every 1 ms. Low-pass Fourier transform filtering was performed on the upper and lower force data to maximize the signal to noise ratio using IGOR Pro software (WaveMetrics, Lake Oswego, OR). The data were fit to the Heckel pressure–density relationship as Eq. 3.

$$\ln\left[\frac{1}{1-D}\right] = KP + A \tag{3}$$

Where D is the ratio of the density of the powder mass at the pressure of P to the true density of the powder, K is slope of linear portion of curve and A is Heckel number. The most linear portion of the plot as determined from the second derivative was used to determine K and the mean yield pressure ($1/K$) was calculated. Each sample was analyzed in triplicate.

Compression Pressure–Radial Tensile Strength Relationship

About 450 mg of powder were weighed and compressed to different compression forces on an instrumented rotary press (Stokes B2, Stokes Engineering, Doylestown, PA). Tablets were made one-at-a-time using one set of flat-faced tooling of 11 mm in diameter. Compression pressure was calculated from force per Eq. 4.

$$P = \frac{39.2F}{\pi d^2} \tag{4}$$

Where P is compression pressure in MPa, F is compression force applied in Kg force and d is diameter of tablet in millimeter. The die was lubricated prior to each compression

with a saturated magnesium stearate solution in acetone. Tablets were placed in amber bags and stored in a desiccator containing Calcium sulfate anhydrous desiccant for at least 24 h before measuring their tensile strength.

Tablet breaking force was determined on a motorized tablet hardness tester (Model 2E/106, series 7203, Key industries, Farmingdale, NY) which measures the force required to fracture tablets by diametral compression. The diameter and thickness were measured with a digital caliper (Model 62379-531, Control Company, Friendswood, TX). Six randomly selected tablets were used for each test batch. Radial tensile strength was calculated per Eq. 5.

$$\sigma_x = \frac{2F}{\pi dt} \tag{5}$$

Where σ_x is tensile strength in MPa, F is force required to break tablet in Newton, d and t are diameter and thickness of tablet in millimeter respectively.

Chemical Characterization

One feverfew standardized extract, A1, was used for the entire method development. All experiments in this part were performed in triplicate unless otherwise noted.

Extraction Procedure

Accurately weigh 250 mg of feverfew directly into a 25 ml volumetric flask. Put 20 ml of 50% ethanol into the flask and then the flask is sonicated for 30 min. The final volume of extract is adjusted to 25 ml with 50% ethanol. An adequate volume (~2 ml) is passed through a 0.45 μm nylon syringe filter. The first 1 ml is discarded; the remaining volume is collected in an amber HPLC sample vial for HPLC analysis.

Extraction Solvent Selection

To find the best extraction system, feverfew samples were extracted as described in the extraction procedure except for the extraction solvent. Different pure solvents (acetonitrile, methanol and ethanol) and the solvents combined with different amounts of water (10, 20 and/or 50%) were tested.

Profile of Extraction Efficiency versus Time

Feverfew samples were extracted as described in the extraction procedure except that the sonication time was 2, 5, 10, 20, 30 and 60 min, respectively.

Extraction from Excipient Matrix

To determine if this method can be applied to the extraction of formulations, extraction studies from an example excipient matrix were performed. The excipient matrix consisted of 63% microcrystalline cellulose, 30% maltodextrin, 6% croscarmellose sodium, 0.5% magnesium stearate, 0.5% colloidal silicon dioxide. 250 mg feverfew, the blend of 125 mg excipient matrix/250 mg feverfew extract, or 125 mg excipient matrix were extracted respectively.

Extraction Stability

An extract of feverfew in 50% ethanol was prepared and analyzed. The sample was stored in an amber HPLC sample vial at room temperature for 24 h and reanalyzed to determine 1-day stability of feverfew extract solution.

Stability of Parthenolide Standard

Standard solutions of parthenolide in ethanol (9 μg/ml) were stored at 5°C and protected from light. Stability of standard solution was determined periodically for 2 months.

Apparatus and Chromatographic Conditions

The HPLC system consisted of a Hitachi L-7100 pump, L-7250 auto-sampler and L-7400 UV-detector. Separation was accomplished on a Gemini C18 column, 150×4.6 mm id, 5 μm particle size with a Security Guard cartridge system (Phenomenex, Torrance, CA). Chromatographic data was processed with D-7000 HPLC system manager software (Merck/Hitachi).

The mobile phase consisted of solvent A (10 mM KH_2PO_4 in H_2O, adjusted pH to 3.0 with phosphoric acid), and solvent B (acetonitrile) and degassed with helium. Elution was run at a flow rate of 1.5 ml/min with a linear gradient (41% B in A for the first 7.5 min, then gradually increased to 70% B in A in 2.5 min, then keep 70%B in A for another 5 min, then gradually decrease to 41% in 2 min, and then equilibrate for another 4 min) and UV detection at 210 nm. The column temperature was maintained at 25°C during all the determinations. All injections were performed in triplicate unless otherwise stated.

Method Validation

Identification and system suitability. To identify peaks in chromatograms, the retention times of chromatographic peaks were compared with the retention time of parthenolide standard. The mobile phase ratio was adjusted sequentially to vary retention times to see if there is the appearance of peak splitting or shoulders. A system suitability table of peaks of interest was generated by D-7000 HSM software.

Linearity. A calibration curve was prepared by serial dilution of the external standard parthenolide (3.6, 9, 18, 45, 90 μg/ml). The slope, intercept and correlation coefficient were calculated by linear regression analysis.

Spike Recovery. A known amount of parthenolide (~2.5 mg) was added to 250 mg feverfew sample. Extraction was carried out as described above and parthenolide content was determined. The spike rate (R) was calculated according to Eq. 6.

$$\mathrm{R} = \frac{2\mathrm{PN}_{\mathrm{total}} - \mathrm{PN}_{\mathrm{nonspike}}}{\mathrm{PN}_{\mathrm{spike}}} \times 100\% \tag{6}$$

Where $\mathrm{PN}_{\mathrm{total}}$ is the total amount of parthenolide found in the sample, $\mathrm{PN}_{\mathrm{nonspike}}$ is the average amount of parthenolide of feverfew without parthenolide spiked, and $\mathrm{PN}_{\mathrm{spiked}}$ is the amount of parthenolide standard spiked.

Method Reproducibility. Reproducibility was determined by performing three sets of three replicate analyses within a 1-month period.

Table II. Flowability of Commercial Feverfew Extracts

Feverfew source	Bulk density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Minimum orifice diameter (mm)	Particle size under 110 psi (D50, mm)
A1	0.557±0.014	0.803±0.001	30.6	22	75.9±1.4
A2	0.525±0.005	0.744±0.005	29.4	20	55.5±0.5
B1	0.513±0.007	0.915±0.004	42.6	18	20.5±0.0
C1	0.487±0.007	0.802±0.006	39.3	16	41.9±0.3
D1	0.404±0.013	0.704±0.029	43.9	22	34.2±1.0

Parthenolide Content in Several Commercial Feverfew Extracts

The method validated above was applied to five commercial feverfew extracts to determine their parthenolide content.

Statistical Analysis

Where appropriate, the Student's *t*-test or analysis of variance (ANOVA) was used to compare the data. The data were considered to be significant when $p<0.05$. Spearman rank correlation coefficient (SRCC) was used to indicate the rank correlation between two variables. The correlation was considered to be significant when SRCC exceeds the range between −0.9 and 0.9 ($N=5$).

RESULTS AND DISCUSSION

Physical Properties

Characterization of Standardized Feverfew Extracts

Table I shows that different companies use different plant parts (flower, leaf or the whole aerial part) to produce their extracts. Even for the same company, the plant parts used may vary with production batch. Different excipients may be added for standardization. These variations may cause significant differences in physical and chemical properties among different manufactures or different batches from the same manufacturer.

Flowability and Particle Size Analysis

Flowability is not an inherent material property, but a result of the combination of many material physical properties that affect flow such as density, particle size and shape, cohesive strength and internal friction, and even the equipment used for handling. So flowability can never be adequately evaluated by a single test or expressed by a single value (13). In the present study, Carr's index, a very commonly applied test of flowability, is combined with the minimum orifice diameter to reflect extract flowability.

Carr's index measures the change in powder density after tapping. A large increase in density generally indicates high inter-particle cohesion and friction and poor powder flow. Generally Carr indices less than 15% are indicative of free-flowing powders; indices greater than 40% usually correspond to very poor flow (10). Based on this standard, all commercial feverfew extracts tested have poor to very poor flowability; A1 and A2 should flow much better than B1, C1 and D1 (Table II).

The minimum orifice diameter measurement may be more of a practical flowability test. The smaller the circular orifice through which the powder can pass, the better its flowability. In contrast to Carr indices, this test showed that C1 flowed the best, followed by B1 and then A2, A1 and D1. The greater flowability of B1 and C1 indicated by the

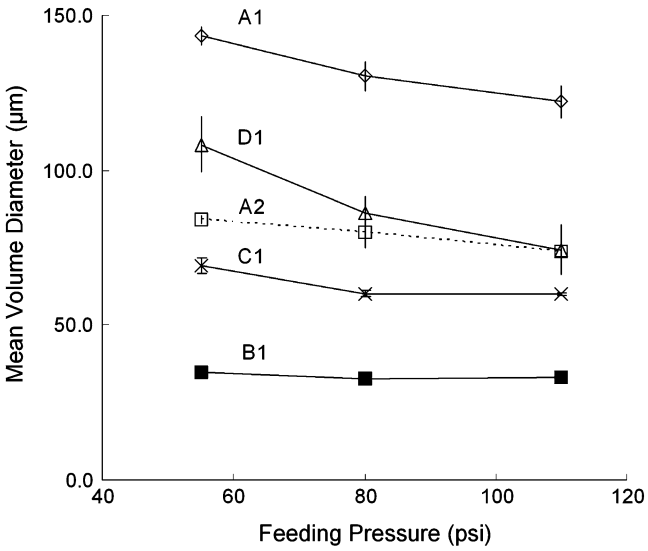

Fig. 1. The mean volume diameter of feverfew extract under different feeding pressure ($n=3$)

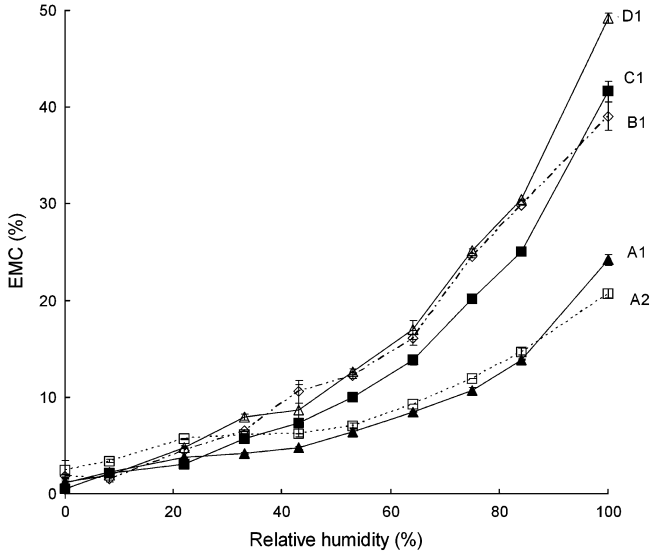

Fig. 2. EMC of feverfew extracts under different relative humidities ($n=3$)

minimum orifice diameter test may be reflective of the excipients suppliers added to B1 and C1. Most particularly, a glidant, silicon dioxide, was added to C1 which may explain why it appeared to be the most flowable among the extracts tested. The lack of agreement of these results with Carr indices (SRCC=0.075) may in part indicate that the latter lacks sufficient sensitivity to predict the changes caused by excipients in these complex compositions. Podczeck and Newton (14) also found that Carr indices could not predict well the effect of small quantities of magnesium stearate on the flow properties of powdered cellulose. It is interesting to compare these results with the observations below made when conducting particle size analysis,

Flowability is largely dependent on interparticulate interactions. A bed of larger particles in which there are fewer contacts between particles generally flows better than a

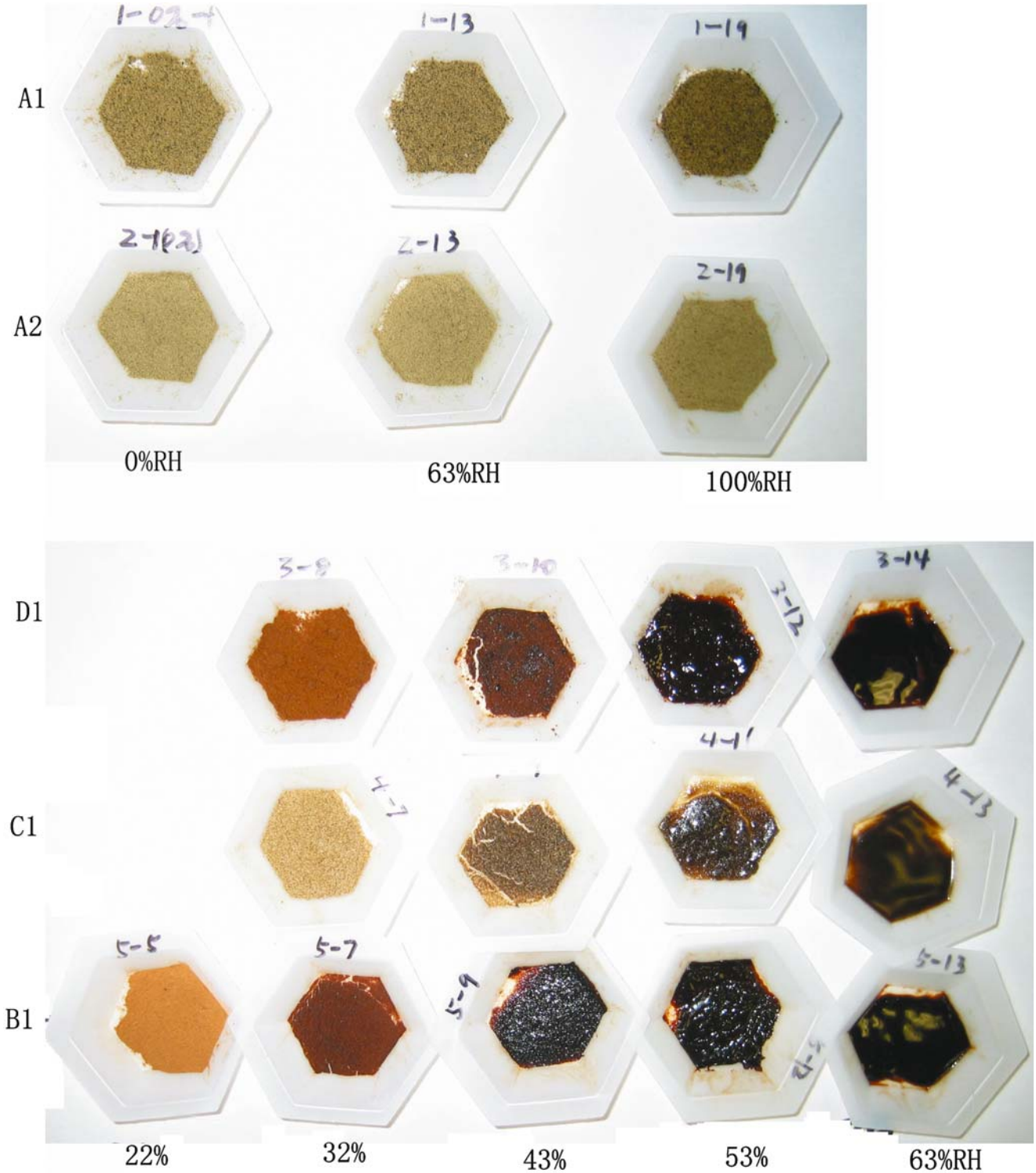

Fig. 3. Changes in physical appearance of feverfew extracts exposed to different relative humidities

Table III. Mean Yield Pressure and Moisture Content of Commercial Feverfew Extracts (n=3)

Feverfew's source	True density (g/ml)	Mean yield pressure (MPa)	Moisture content (%)
A1	1.347	78.1±9.7	4.2±0.1
A2	1.314	49.8±1.8	6.3±0.3
B1	1.417	105.9±10.8	3.6±0.2
C1	1.482	113.7±7.5	3.7±0.2
D1	1.211	37.2±2.3	2.6±0.2

bed of smaller particles. Thus, it was of interest to measure the particle size of the feverfew powdered extracts. Interestingly, the particle size data support the Carr indices better than minimum orifice diameter-based flowability data (SRCC is −0.8 for particle size/Carr indices versus 0.375 for particle size/minimum orifice diameter). For example, B1 and C1 have smaller particle size and exhibited the smallest minimum orifice diameters, but nearly the highest Carr Indices (Table II). Perhaps a better indicator of interparticulate interaction can be derived from the feeding pressure needed for the size analysis. Because the extract powder is sticky, a feeding pressure is needed to separate the aggregate into primary particles during particle size analysis. When the feeding pressure is large enough to achieve apparently complete separation, the measured particle size will be constant and independent of feeding pressure within a certain range. Thus, feeding pressure can be an indirect indicator of the magnitude of particle–particle interactions. Fig. 1 shows that the order of feeding pressure needed is D1 > A1 > A2 > C1 > B1. This order correlates well with the minimum orifice diameter results (SRCC=0.875).

Hygroscopicity

The EMC data (Fig. 2) show that the hygroscopic nature of feverfew extracts varied greatly with source. According to the hygroscopicity classification described by Callahan et al. (12), the A1 and A2 extracts can be ranked as class III materials or moderately hygroscopic, where moisture content increased less than 5% after storage at below 60% RH and less than 50% increases in moisture content occur above 80% RH. Class III materials may or may not require special packaging depending on whether physical stability and appearance of the material are affected. Based on our observation (Fig. 3), there were no apparent physical changes to either A1 or A2 even after exposure to 100% RH.

However, the extracts from the other three providers, B1, C1 and D1, were all very hygroscopic (Class IV materials). This means that moisture increase occurs at relative humidity as low as 40 to 50% and more than 30% moisture content increases are exhibited after storage at above 90% RH. In addition, they began to deliquesce under relative humidity as low as 43% (Fig. 3). Based on ICH guidelines on stability testing (15), a drug substance must not show any physical change under normal and stressed storage conditions. This means that these three extracts can not pass ICH stability testing. They should be modified to render them less hygroscopic.

In the cases of B1 and C1, this high hygroscopicity could be attributed in part to the addition of maltodextrin, which is known to be hygroscopic depending on the degree of starch hydrolysis. In general, an increase in hydrolysis (i.e., dextrose equivalent (DE)) increases sweetness, solubility and hygroscopicity. The hygroscopicity differences among A and D indicate that the material source and extraction procedure can also cause a significant difference in hygroscopicity (e.g. different water–organic solvent ratios (16) and drying methods).

Compression and Compaction Properties

The Heckel model has become universally accepted to characterize a powder's compression properties. The model treats the volume reduction of a powder bed as a first order process, where the pores are the "reactant." This is not expected to be the case when the materials are subjected to very low pressure where particle rearrangement is expected to be the predominant means of volume reduction. At higher compression pressures, a linear relationship often exists between relative density and pressure (17). The slope of this

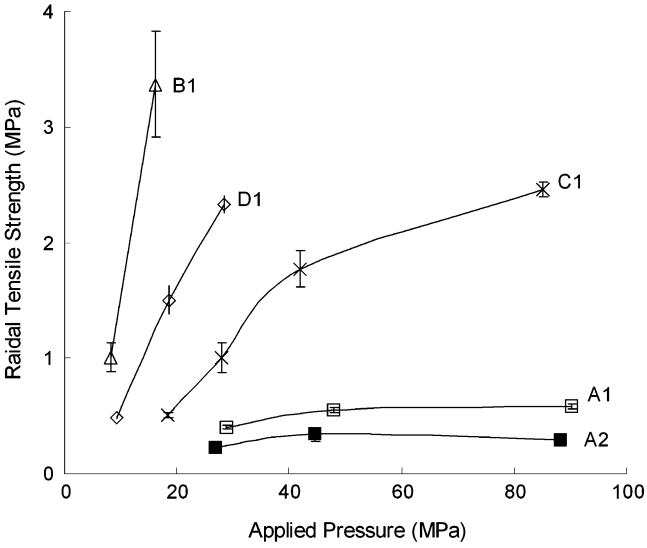

Fig. 4. Radial tensile strength-compression pressure profile of feverfew extracts

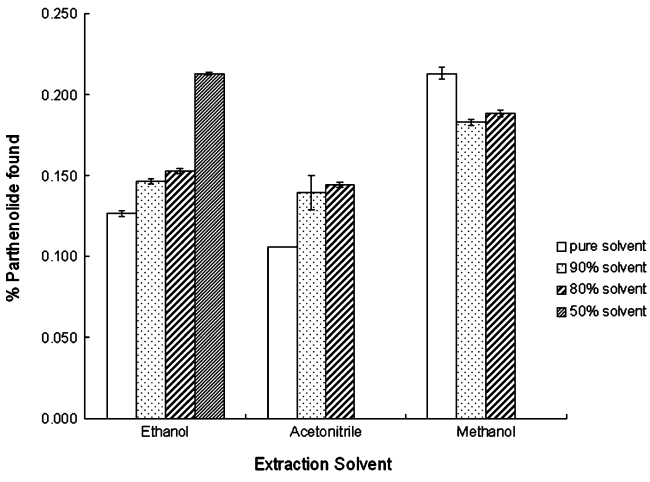

Fig. 5. Extraction results using different extraction solvents (n=3)

linear region (K) is generally accepted to be indicative of the deformation behavior of the material. The reciprocal of K is called the mean yield pressure. Lower mean yield pressures ($1/K$) are generally associated with plastically deforming materials.

All the feverfew extracts tested exhibited good linear densification at compression pressures less than 80 MPa. Table III reveals that the mean yield pressures of these extracts vary in the range of 35–115 MPa. The mean yield pressure of Avicel PH101 was determined as a comparator or reference. Since Avicel is known to deform mainly plastically and its mean yield pressure is around 70 MPa, it can be concluded that these extracts may exhibit plastic deformation, with their apparent plasticity based on mean yield pressure decreasing in the following order: D1 > A2 > A1 > B1, C1.

While Heckel analysis provides information on extract compressibility (i.e. volume reduction under pressure), extract compactibility (i.e., the ability to be compressed into a compact of defined strength) may be of immediate interest. In the present study, compactibility is inferred from the relationship between tablet tensile strength and compression pressure (Fig. 4). It may be observed that the order of mean yield pressures did not correlate well with compactibility (SRCC=0.325). As can be seen from Fig. 4, both A1 and A2 exhibited poor compactibility and their low values of radial tensile strength can not be improved by increasing the compression pressure. The other three extracts revealed good compactibility, especially B1, although it has a much higher mean yield pressure than A1 or A2. The surface properties of a material may be a key determinant for interparticulate bonding. Considering the chemical complexity of botanicals, the different modes of extraction and the different excipients that may be used to prepare the dry extracts, it is perhaps not unexpected that commercial feverfew extracts would exhibit such different compactibilities.

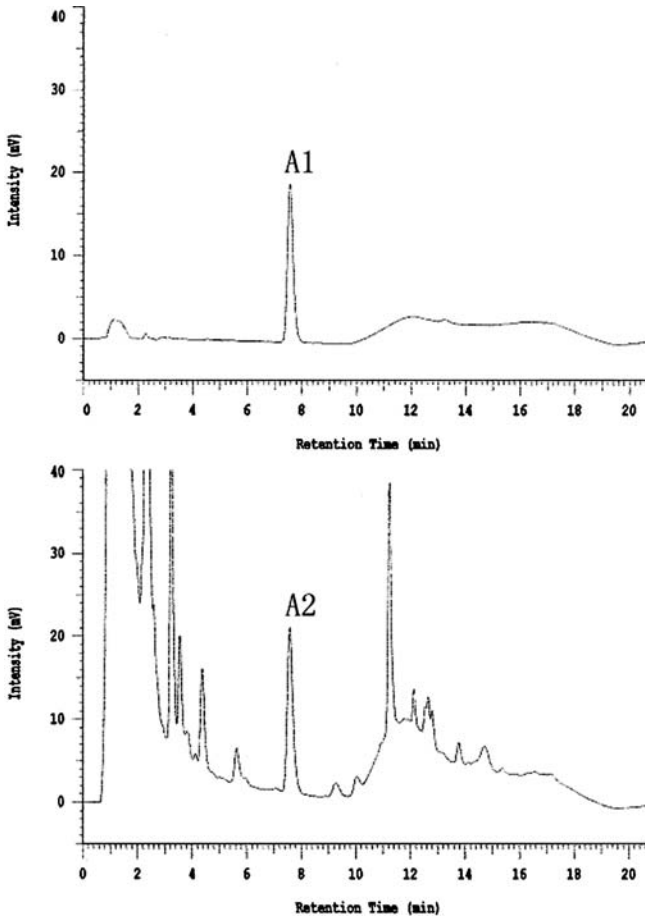

Fig. 6. Parthenolide peak *A1*: standard sample (18 μg/ml) and *A2*: feverfew sample

Chemical Properties

Optimum Extraction System and Procedure

The extraction procedure is optimized based on the comparison of literature methods (Result not showed here). Fig. 5 showed the results of the parthenolide extraction from feverfew using various solvent systems. It's clear that 50% ethanol extracted the highest percentage of parthenolide from feverfew and is the best extraction solvent for feverfew sample tested. This finding is understandable because most extracts are produced by hydro-alcoholic extraction. This extraction solvent may be close to the solvent system used for production, thus maximizing the solubility of whole sample and promoting the availability of parthenolide. However, 50% ethanol is not necessarily the best extraction solvent for all feverfew products due to the difference in chemical composition from different feverfew sources. It was observed that methanol and 50% ethanol are the two best candidates for feverfew extraction. 50% ethanol is a little better for feverfew extract, while methanol works better for feverfew crude material.

The profile of extraction efficiency versus time showed that in the first 5 min, more than 90% of the parthenolide was extracted. Within 20 min, the amount of extracted parthenolide reached the maximum and was constant to 1 h extraction. Therefore, 30 min is a suitable end point for sample extraction. The study on the impact of excipients on the extraction efficiency indicated that this extraction procedure can be used on feverfew finished products.

Validation of HPLC Analytical Method

A modified HPLC analytical condition was developed, with gradient elution and buffered mobile phase. Fig. 6 shows a sample chromatogram of parthenolide standard and feverfew extract. Parthenolide has the same retention time (7.59+ 0.05 min) in these two chromatograms. The pH value of aqueous buffer was set to 3 to get good buffer capacity. Stability testing showed that parthenolide in feverfew extract was stable at pH=3 during run time (18). The varying of

Table IV. Parthenolide Content of Commercial Feverfew Extracts from Different Sources (n=3)

Source	% Parthenolide claimed	% Parthenolide determined
A1	0.8	0.213 ± 0.002
A2	0.8	0.089 ± 0.001
B1	0.2	0.091 ± 0.000
C1	0.5	0.309 ± 0.003
D1	1.2	0

buffer pH from 3 to 7 did not significantly change the retention time and peak area of parthenolide, which indicated the neutral nature of parthenolide. Adjusting the mobile phase ratio sequentially to vary peak retention times didn't cause peak splitting or the appearance of a shoulder on the parthenolide peak. This further confirmed that the peak is pure parthenolide without coeluting peaks. The system suitability of HPLC meets USP requirements. This method proved to be workable for the determination of feverfew in our samples under stressed condition for at least 6 months.

A five-point calibration curve of parthenolide from 3.6 to 90 μg/ml was linear, with correlation coefficients of at least 0.9999. In the spike recovery tests, an average of 99.6% of 2.6± 0.3 mg of parthenolide standard spiked into 250 mg feverfew samples was recovered with an RSD% of 2.1%. The whole method had good repeatability, with RSD=1.2% for different days. Feverfew extract is stable at room temperature for at least 1 day. The ethanol solution of parthenolide stored at 5°C remained stable for at least 2 months.

Parthenolide Content in Commercial Feverfew Extracts

Table IV shows that none of the commercial feverfew extracts analyzed meet their label claims. Even different batches from the same manufacture showed significantly different parthenolide content. One extract that claimed 1.2% parthenolide content didn't contain any detectable parthenolide. To confirm this result, a parthenolide standard solution was added to the extract and then reanalyzed by HPLC. The parthenolide peak appeared in the chromatogram with the same retention time. Therefore, extract manufactures should commit to proper production methods and quality control procedures to ensure that their label claims are accurate.

CONCLUSION

The physical and chemical characteristics of the powdered commercial extract can be significantly affected by the crude material, method of extraction as well as any further processing, and the nature of any excipients added. Feverfew extracts from different manufacturer may exhibit significantly different physico-chemical properties. The large difference between label claims and actual content of parthenolide is a big problem for commercial feverfew extracts. Supplement manufacturers who develop products using these extracts should not rely on certificates of analysis for parthenolide content of extracts and take into account possible differences in physico-chemical properties when using extracts from multiple suppliers.

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