

Date	Analyst	Project No.	Project Name
5.March 2003	ES / BI	P36	Feverfew

## Evaluation and optimization of methods for identification of Feverfew

### 1. Analytical goal:

The fingerprint should allow the identification of Feverfew (*Tanacetum parthenium*) using parthenolide as chemical reference material. The adulterants Mexican feverfew, Chamomile, and Roman chamomile should be discriminated.

### 2. Paper review of methods from literature:

Literature (see appendix)	Scope	Mobile phase / Stationary phase	Refer to Figure # below
USP (1) Ph.Eur.4	Presence of parthenolide	Toluene, acetone (85:15) Derivatization with vanillin	1a
USP (2)	Differentiation of Chamomile and Roman chamomile	Ethyl acetate, water, anhydrous formic acid, glacial acetic acid (10:2.7:1.1:1.1) Derivatization with 2-aminoethyl diphenylborinate in methanol followed by polyethylene glycol	1b
Private communication (Phyto-Technologies)	--	Toluene, ethyl acetate (9:1) Derivatization with sulfuric acid	1c
	--	Chloroform, ethyl acetate, formic acid (2:1:1) Derivatization with sulfuric acid	1d
	--	Cyclohexane, ethyl acetate (1:1) Derivatization with sulfuric acid	1e
	--	Chloroform, acetone (6:1) Derivatization with sulfuric acid	1f

### 3. Experimental evaluation of selected methods

#### 3.1 Materials

##### 3.1.1 Samples

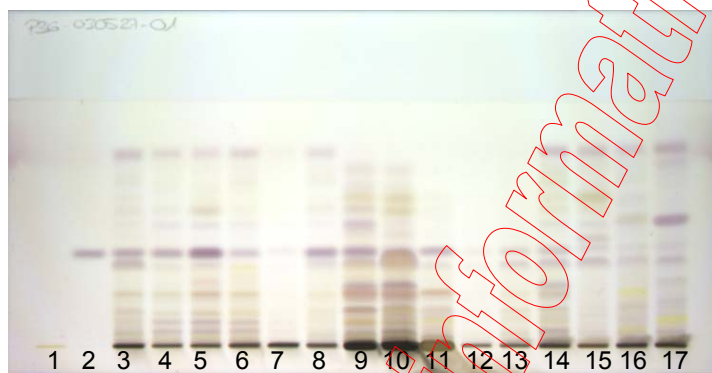
Sample name	Source / Batch	Authentication	Notes
Feverfew whole plant mixed	Removed - proprietary information	Yes	
Feverfew whole plant mixed (BRM)		Yes	
Feverfew flowers		Yes	
Feverfew leaves		Yes	
Feverfew stems		Yes	
Feverfew powdered extract 0.7% parthenolide		-	
Feverfew hydro alcohol extract (1:5), 68-74% alcohol from dried herb		-	
Feverfew hydro alcohol extract (1:5), 55-60% alcohol, yielding 1% parthenolide from dried herb		-	
Feverfew hydro alcohol extract (undefined strength), 40-50% alcohol		-	

from dried herb	Removed - proprietary information		
Feverfew dry extract .5%		-	
Feverfew freeze dried flower/leaf, Capsules 0.1-0.2%parthenolide		-	
<i>Matricariae flos</i>		Yes	
<i>Chamomillae romanae flos</i>		Yes	
Mexican feverfew ( <i>Tanacetum parthenium</i> )		Yes	
<i>Artemisia</i> sp.		No	Old

### 3.1.2 Standards (marker compounds)

Name	Source
Parthenolide	ChromaDex, 00-16071-101
Rutin	Merck, 115F318517

### 3.2 Results and discussion

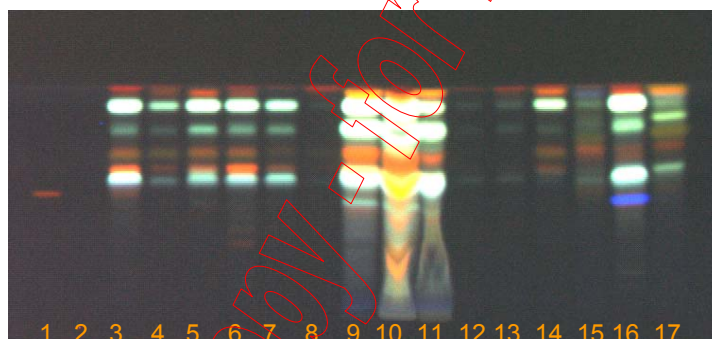


#### Track assignment

- 1: Rutin
- 2: Parthenolide
- 3-7: Feverfew samples
- 9-11: Feverfew liquid extracts
- 8, 12-14: Feverfew dry extracts
- 15: Chamomile (*Matricaria recutita*)
- 16: Artemisia
- 17: Roman Chamomile

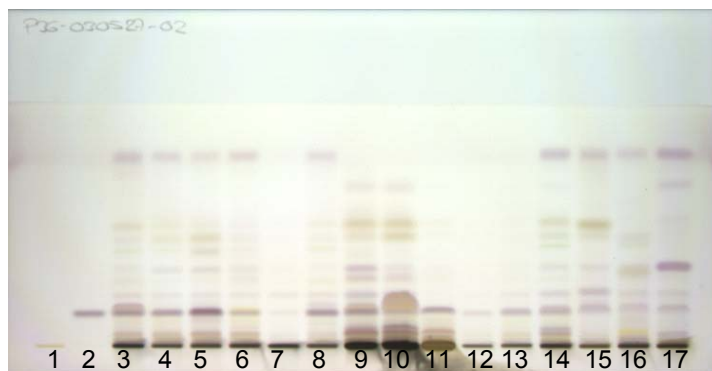
1a Method USP(1) and PhEur4

[changes from original method: use of HPTLC plate and derivatization with sulfuric acid]



1b Method USP (2)

[changes from original method: use of HPTLC plate]



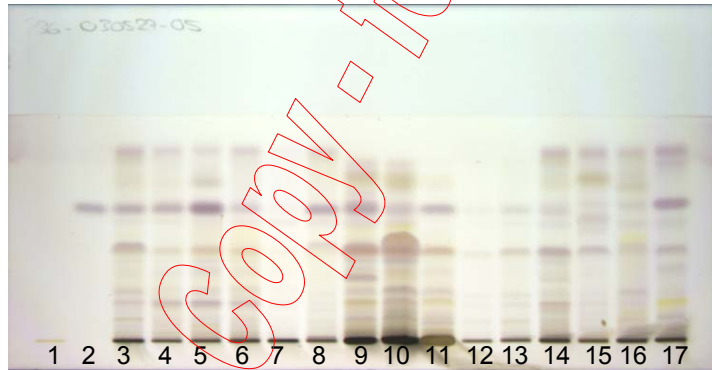
1c Private communication



1d Private communication



1e Private communication



1f Private communication

Track assignment

- 1: Rutin
- 2: Parthenolide
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- 16: Artemisia
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The only mobile phase showing no zones at the position of parthenolide in the adulterants is: cyclohexane, ethyl acetate (1:1) in Figure 1e. In all other mobile phases (Figures 1a, 1c, 1d, 1f), the adulterants show a zone at the position of parthenolide, which interferes with the identification of Feverfew.

The mobile phase shown in Figure 1b (USP 2) doesn't give useful additional information.

### **3.3 Conclusions:**

Check one

... Method from literature is suitable → continue with section 5

X Method (Fig. 1e) needs optimization → continue with section 4

... No suitable method is found → refer to SOP 70.002.01 "Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof".

*The mobile phase: cyclohexane, ethyl acetate (1:1) will be used.*

## **4. Method optimization**

### **4.1 Sample preparation**

Different sample preparations were tested and results compared side by side after chromatography on HPTLC plate.

1) **USP 1:** Reduce about 10 g of Feverfew to a fine powder, and transfer about 1.0 g of the powder, accurately weighed, to a suitable flask. Add 20 mL of methanol, heat the flask on a water bath at 60°C for 15 minutes, cool, and filter. Evaporate the filtrate under reduced pressure to dryness, and dissolve the residue in 2.0 mL of methanol. **Time required: 30-40 min**

2) **Alternative:** 1 g powdered raw material is mixed with 10 mL of methanol, then sonicated for 10 min and filtered through filter paper. **Time required: 15 min**

Results:



Track assignment

1: 2 µL extract 1

2: 5 µL extract 1

3: 2 µL extract 2

4: 5 µL extract 2

Mobile phase: cyclohexane, ethyl acetate (1:1), derivatization with vanillin reagent.

Sample: Feverfew whole plant mixed, AHP#198

Conclusion

Both extraction methods yield the same profile of extracted compounds, only the intensity of zones differs slightly. The simplified extraction method can be used.

*Extraction will be performed as follows: 1 g of milled sample is extracted by sonication with 10 mL of methanol for 10 min. The solution is centrifuged or filtered and the supernatant used as test solution. Application volume: 5µL.*

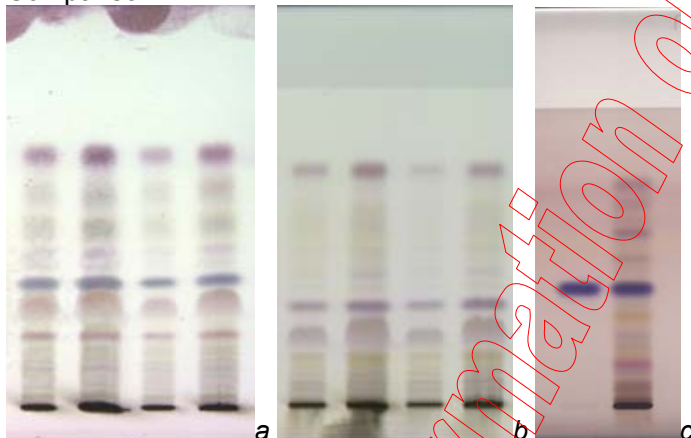
#### **4.2 HPTLC methodology**

No comparison between TLC and HPTLC was performed. All methods were tested using HPTLC methodology directly.

#### **4.3 Derivatization**

Results:

Comparison:



[mobile phase is not the same for all plates] Sample in a and b: as in 4.2 (Feverfew whole plant mixed, AHP#198). Sample in c: Parthenolide and Feverfew whole plant mixed (BRM), AHP#195.

Derivatization by a) spraying the plate with vanillin reagent, b) dipping the plate in sulfuric acid reagent, and c) dipping the plate in anisaldehyde reagent.

#### **Conclusion**

Vanillin reagent can only be used for spraying, the resulting chromatograms appear “wet” (sulfuric acid is very hygroscopic). The best colors are obtained after derivatization with anisaldehyde reagent: parthenolide appears as a very characteristic blue zone in the middle of the chromatogram.

*Derivatization will be performed by dipping the plate in anisaldehyde reagent followed by heating at 100°C for 3 min. Detection will be performed under white light.*

#### **4.4 Mobile phase**

No further optimization.

#### **4.5 Method including all optimized parameters**

Results:



Conclusion  
Result ok.

#### **4.6 Conclusions**

Check one

X Analytical goals achieved → continue with section 5

... Analytical goals not achieved → refer to SOP 70.002.01 "Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof".

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Date	Analyst	Project No.	Project Name
03.Oct.2005	Alison DeBatt	A175	Feverfew

For all following experiments the BRM Feverfew whole plant mixed AHP#195 was used.

## **5. Evaluation of stability (pre-validation)**

### **5.1 Stability of analyte during chromatography**

Result:

No zones are located outside of the diagonal; therefore the sample is stable during chromatography.

Image: A175-051003-2D



#### **Acceptance criteria:**

The sample is stable during chromatography if all zones are located on the diagonal connecting the application position with the intersection of the two solvent fronts.

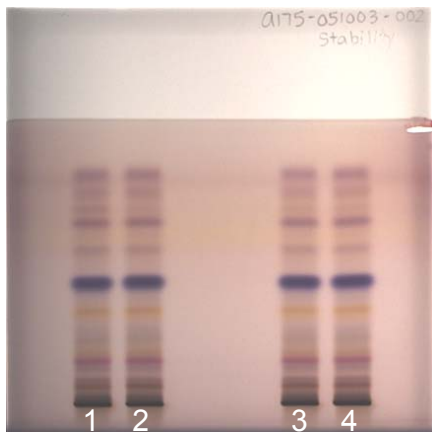
Pass: Yes

### **5.2 Stability of analyte in solution and on the plate**

Result:

No differences are seen between the tracks; therefore the sample is considered stable for at least 3 hours in solution and on the plate.

Image: A175-051003-SolutionStability



1. Sample on the plate for 3 hours prior to chromatography (A)
2. Fresh sample applied immediately prior to chromatography (B)
3. Sample prepared 3 hours prior to chromatography (in solution) (C)
4. Fresh sample applied immediately prior to chromatography (identical with 2) (B)

**Acceptance criteria:**

The sample is stable for at least 3 hours in solution and 3 hours on the plate prior to chromatography if no differences are seen between the four tracks.

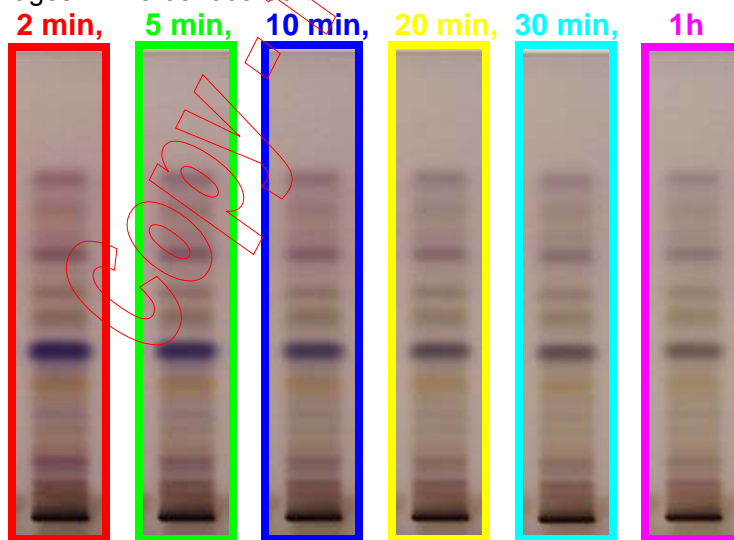
Pass: Yes

**5.3 Stability of result (for documentation)**

Result:

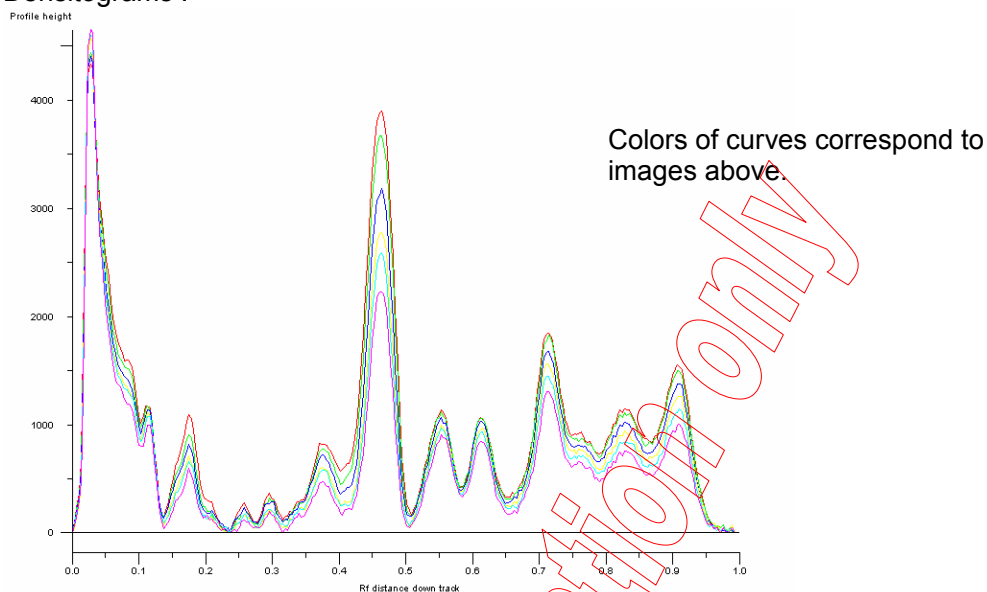
The intensity of the zones decreases over time, but no fractions appear or disappear. Images should be taken within the first 10 minutes after derivatization. After that time, parthenolide begins to turn to gray.

Images: A175-051003-001





**Densitograms :**



**Acceptance criteria:**

The derivatization/visualization yields a stable result, if there is no significant change in the image within 30 min.

Pass: Yes, with the following limitation: the plate should be documented within 10 min after derivatization.

**5.4 Conclusion**

X Stability tests passed → Use FO 70.002.02 “Method to be validated” for method write up, then validate method according to SOP 70.002.01 “Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof”. If the method is not intended to be validated, use FO 70.002.06 “Application Note” for method write up.

... Stability tests failed → restart with section 4 or refer to SOP 70.002.01 “Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof”.

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Date:

Signed:

Date of review:

Name:

Title:

Signature: