

Date	Analyst	Project No.	Project Name
11.Nov.2005	Alison DeBatt	A169	Ginger

## Evaluation and optimization of methods for the identification of Ginger

### 1. Analytical goal:

Optimize/develop a method for the identification of Ginger (*Zingiber officinale*) using HPTLC fingerprinting and gingerols as reference standards.

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

Literature (see appendix)	Scope	Mobile phase / Stationary phase	Refer to Figure # below
USP 28	Compare samples to ginger reference material. No derivatizing reagent	Ether, hexane (7:3) Silica gel	1
Wagner (1) and Ph.Eur.5	Compare samples to Citral and resorcinol. Derivatization with vanillin reagent	Hexane, ether (40:60) Silica gel F254	2
Wagner (2)	Compare samples to vanillin, capsaicin, borneol, and cineol. Derivatization with Vanillin reagent	Toluene, ethyl acetate (93:7) Silica gel	3

### 3. Experimental evaluation of selected methods

#### 3.1 Materials

##### 3.1.1 Samples

Sample name	Source / Batch	Authentication	Notes
<i>Zingiber officinale</i> (powder extract)	Removed - proprietary information	Unknown	None
<i>Zingiber officinale</i> (tablets)		Unknown	None
<i>Zingiber officinale</i>		Unknown	None
<i>Zingiber officinale</i> (BRM)		Unknown	None
Ginger tincture		no	None
Ginger oil/tincture		no	None
Ginger XRM <i>Zingiber officinale roscoe</i>		yes	None
Ginger BRM <i>Zingiber officinale roscoe</i>		yes	None
Ginger powder (spice)		no	None
Ginger powder Spice		no	None
Fresh ginger root		no	None
Frozen Galangal root		no	None

### 3.1.2 Standards (marker compounds)

Name	Source
6-shogaol	Chromadex 19211-135
6-gingerol	Chromadex 07164-125
8-gingerol	Chromadex 07163-119
10-gingerol	Chromadex 07162-124
Ginger standard mixture	USP FOE129

### 3.2 Results and discussion

**Figure 1:** USP 28 [changes in method: use of HPTLC plates, application volume, and use of gingerol standard USP, reagent: vanillin reagent]

Developing solvent: hexane, ether (3:7)

Derivatizing reagent: Vanillin reagent (sprayed)

Image



Track 1: gingerols (USP standard)

Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume

Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume

Conclusions:

- Separation of substances should be optimized.
- The standards are not seen
- Vanillin reagent does not show fluorescence after derivatization.
- The lower region of the plate is colored after derivatization.

**Figure 2:** Ph.Eur.5 and Wagner 1 [changes in method: use of HPTLC plates, application volume, and use of gingerol standard USP]

Developing solvent: hexane: ether (40: 60)

Derivatizing reagent: Vanillin reagent (sprayed)

Image



Track 1: gingerols (USP standard)

Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume

Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume

Conclusions:

- As Fig. 1

**Figure 3:** Wagner 2 [changes in method: use of HPTLC plates, application volume, and use of gingerol standard USP]

Developing solvent: toluene, ethyl acetate (93:7)

Derivatizing reagents:

**Middle:** Vanillin reagent (Sprayed)

Image



Track 1: gingerols (USP standard)

Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume

Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume

Conclusions:

- Vanillin reagent produces colored zones
- Mobile phase provides good separation but is too weak
- The mobile phase toluene: ethyl acetate (93:7) will be optimized by increasing the ratio of ethyl acetate. (Please see section 4)

**3.3 Conclusions:**

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

✓ Method “Wagner 2” 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”.

#### **4. Method optimization**

##### **4.1 Sample preparation**

USP: Transfer about 0.2 g of pulverized sample to a test tube, add 5 mL of methanol, shake for 30 minutes, and centrifuge

Optimized method: Mix 0.2 g of pulverized sample with 5 mL of methanol, sonicate for 10 minutes, and filter or centrifuge.

Results:



Track 1: gingerols (USP standard)

Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume

Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume

Conclusion:

The optimized method for sample preparation yields similar results as the original method (USP). The application volume of 2 µL is suitable.

The samples will be prepared as follows:

1 g of pulverized sample is mixed with 10 mL of methanol, sonicated for 10 minutes, and filtered or centrifuged.

##### **4.2 HPTLC methodology**

All previous experiments were already performed with HPTLC methodology.

##### **4.3 Derivatization**

Vanillin reagent is compared to anisaldehyde reagent and sulfuric acid reagent.

Results:

Old



New



**White light**



Vanillin reagent

Track 1: gingerols (USP standard)

Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume

Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume



Anisaldehyde reagent



UV 366 nm  
Sulfuric acid reagent

**Conclusion:**

All reagents provide similar results. Vanillin reagent is difficult in handling and doesn't show colored zones under UV 366 nm. Anisaldehyde reagent will be chosen because it yields the more colorful chromatogram prior and after derivatization.

**4.4 Mobile phase**

**Plate One:**

Developing solvent: toluene, ethyl acetate (90:10)

Reagent name: Anisaldehyde reagent

**Results:**

Old



New



Track 1: gingerols (USP standard)

Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume

Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume

**Conclusion:**

Solvent strength is too low; the ratio of ethyl acetate will be increased further.

**Plate Two:**

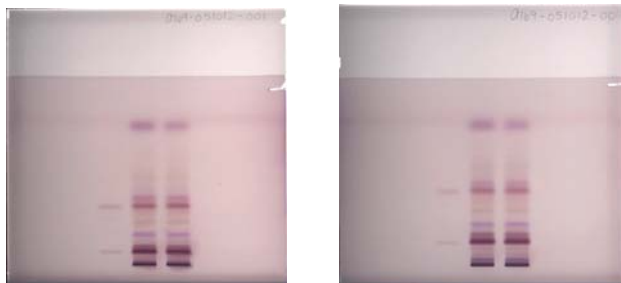
Developing solvent: toluene, ethyl acetate (85:15)

Derivatizing reagent: Anisaldehyde reagent

**Results:**

Old

New



Track 1: gingerols (USP standard)  
Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume  
Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume

**Conclusions:**

Separation has improved; the ratio of ethyl acetate will be increased further.

**Plate Three:**

Developing solvent: toluene, ethyl acetate (80:20)

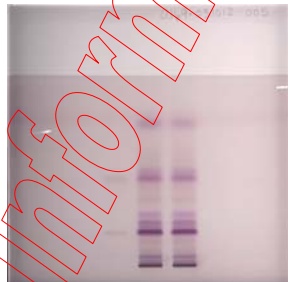
Derivatizing reagent: Anisaldehyde reagent

**Results:**

Old



New



Track 1: gingerols (USP standard)  
Track 2: 0.2 g BRM in 2 mL of methanol (sonicated), 2 µL application volume  
Track 3: 0.2 g BRM in 5 mL of methanol (shaken), 5 µL application volume

**Conclusion:**

Separation has improved; the mobile phase will be used to evaluate a broader range of samples and standards.

**Plate Four:** Evaluate the mobile phase toluene, ethyl acetate (80:20) with a broader range of samples and all available standards.

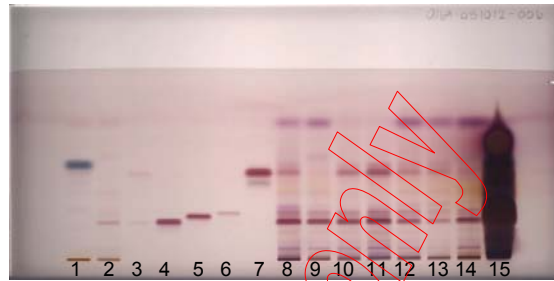
Developing solvent: toluene, ethyl acetate (80:20)

Derivatizing reagent: Anisaldehyde reagent

**Results:**

Old

New



Track 1: USP gingerol mixture  
Track 2: 0.2 g BRM in 2 mL  
of methanol (sonicated),  
2  $\mu$ L application volume  
Track 3: 0.2 g BRM in 5 mL  
of methanol (shaken),  
5  $\mu$ L application volume

Track Assignment (New Plate)

Track 1: Frozen Galangal  
Track 2: Fresh Ginger  
Track 3: USP standard mixture  
Track 4: 6 - gingerol  
Track 5: 8 - gingerol  
Track 6: 10 - gingerol  
Track 7: 6 - shogaol  
Track 8: *Zingiber officinale* (BRM)  
Track 9: *Zingiber officinale*  
Track 10: *Zingiber officinale* (tablets)  
Track 11: *Zingiber officinale* (powder extract)  
Track 12: Ginger powder  
Track 13: Ground ginger spice  
Track 14: Ginger tincture  
Track 15: Ginger oil/tincture (too concentrated)

**Conclusion:**

Separation is acceptable for the samples and standards that are available; the mobile phase will be optimized by increasing the ratio of ethyl acetate.

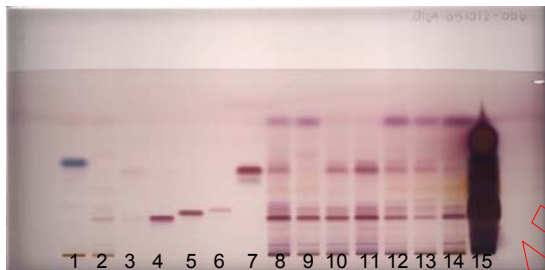
**Plate Five:**

Developing solvent: toluene, ethyl acetate (75:25)

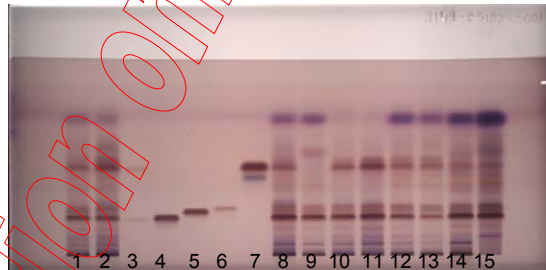
Derivatizing reagent: Anisaldehyde reagent

**Results:**

Old



New



Track Assignment (Old Plate)

- Track 1: Frozen Galangal
- Track 2: Fresh Ginger
- Track 3: USP gingerol mixture
- Track 4: 6 - gingerol
- Track 5: 8 - gingerol
- Track 6: 10 - gingerol
- Track 7: 6 - shogaol
- Track 8: *Zingiber officinale* (BRM)
- Track 9: *Zingiber officinale*
- Track 10: *Zingiber officinale* (tablets)
- Track 11: *Zingiber officinale* (powder extract)
- Track 12: Ginger powder
- Track 13: Ground ginger spice
- Track 14: Ginger tincture
- Track 15: Ginger oil/tincture (too concentrated)

Track Assignment (New Plate)

- Track 1: *Zingiber officinale*
- Track 2: Ginger XRM *Zingiber officinale roscoe*
- Track 3: USP gingerol mixture
- Track 4: 6 - gingerol
- Track 5: 8 - gingerol
- Track 6: 10 - gingerol
- Track 7: 6 - shogaol
- Track 8: *Zingiber officinale* (BRM)
- Track 9: *Zingiber officinale*
- Track 10: *Zingiber officinale* (tablets)
- Track 11: *Zingiber officinale* (powder extract)
- Track 12: Ginger powder
- Track 13: Ground ginger spice
- Track 14: Ginger tincture
- Track 15: Ginger oil/tincture

**Conclusion:**

Separation is still improved; the mobile phase toluene, ethyl acetate (75:25) will be used for the identification of *Zingiber officinale* and products derived thereof.



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

Results:

Old



New



##### Track Assignment (Old Plate)

Track 1: USP gingerol mixture

Track 2: 0.2 g BRM in 2 mL  
of methanol (sonicated),  
2 µL application volume

Track 3: 0.2 g BRM in 5 mL  
of methanol (shaken),  
5 µL application volume

##### Track Assignment (New Plate)

Track 1: *Zingiber officinale*

Track 2: Ginger XRM *Zingiber officinale roscoe*

Track 3: USP gingerol mixture

Track 4: 6 - gingerol

Track 5: 8 - gingerol

Track 6: 10 - gingerol

Track 7: 6 - shogaol

Track 8: *Zingiber officinale* (BRM)

Track 9: *Zingiber officinale*

Track 10: *Zingiber officinale* (tablets)

Track 11: *Zingiber officinale* (powder extract)

Track 12: Ginger powder

Track 13: Ground ginger spice

Track 14: Ginger tincture

Track 15: Ginger oil/tincture

Conclusion:

The mobile phase toluene, ethyl acetate (75:25) is suitable for the separation of various Ginger components. Anisaldehyde as derivatizing reagent provides colored zones, which are well detected under UV 366 nm and in white light.

#### **4.6 Conclusions**

✓ Analytical goals achieved → continue with section 5

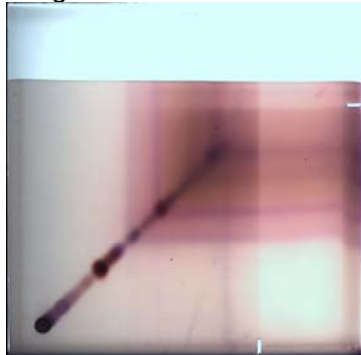
... Analytical goals not achieved → refer to SOP 70.002.01 "Evaluation, development, optimization, and validation of methods for identification of medicinal plants and products thereof".

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

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

Result: No zones are located off of the diagonal, therefore, the sample is considered stable during chromatography

Image: A169-051110-2D-Anis



#### **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.

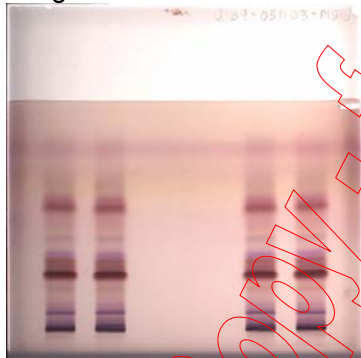
Pass: YES

Fail: -

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

Result: No differences are seen in zone intensity between the tracks and there are no zones that appear or disappear, therefore, the sample is considered stable for at least 3 hours in solution and on the plate.

Image: A169-051003-Plate Stability



#### **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.

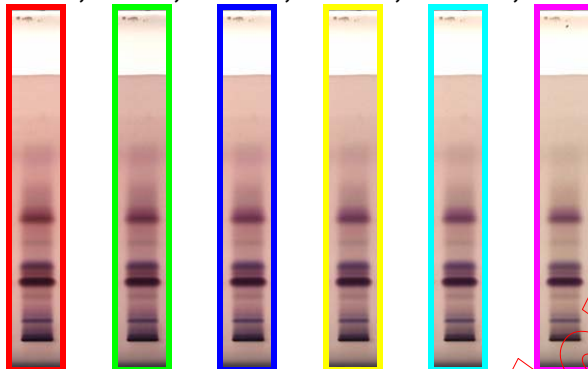
Pass: YES

Fail: -

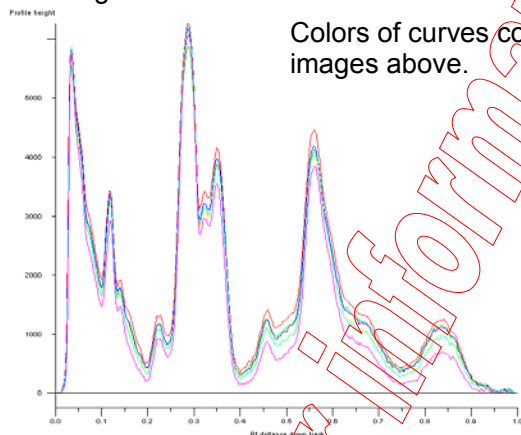
### **5.3 Stability of result (for documentation)**

Result: Zone intensity decreases slightly over time, as does the background color, but there are no zones that appear or disappear; therefore the derivatized plate is considered stable for at least 1 hour.

Images: A169-051110-ImageStability-Anis  
2 min, 5 min, 10 min, 20 min, 30 min, 1h



Densitograms :



**Acceptance criteria:**

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

Pass: YES

Fail: -

**5.4 Conclusion**

✓ 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”.

Printed

Date:

Signed:

Date of review:

Name:

Title:

Signature: