A Bio-activity Guided in Vitro Pharmacokinetic Method to Improve the Quality Control of Chinese Medicines

Prof. Joan Z. Zuo, School of Pharmacy
The Chinese University of Hong Kong

Background

• The current method of quality control for Chinese medicines (CM) involve stability testing using any designated marker(s) present in the product.

• The shortcoming of the current approach is that these marker(s) are chosen entirely arbitrarily which may have no relevance to the product activity inherent in the herbs.

• Without good QC, the efficacy and safety of TCM products are difficult to establish and world acceptance of TCM is unlikely.

There is a need for an improved method that can provide quality advancement of CM.
Objectives

• To demonstrate the feasibility of a bio-activity guided in-vitro pharmacokinetic method (BAPK) for quality control of both single herb or complex formulae products
  – Si-Wu-Tang (SWT) product

Part I: Bioavailability

Part II: Bioactivity
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

↓

Dissolution

↓

Gastrointestinal (GI) metabolism

↓

GI absorption

↓

Identification of relevant bioactive marker(s)

→

Stability analysis of the identified markers
Part I:
Study Design and Procedures

I. Literature search (data mining) to identify active components in SWT.

II. HPLC-DAD and HPLC-MS/MS assay method development for simultaneous determination of these markers.

III. Preliminary test of raw herbs in accordance with Chinese Pharmacopeia (CP) 2005.

IV. Manufacture of CU-SWT and CU-Si Wu He Ji in accordance with CP 2005 to serve as a “reference” product.

V. Determination of contents of CU-SWT and marketed products.

VI. Identification and comparison of relevant markers via bio-activity guided pharmacokinetics approach.

VII. Stability evaluation of the identified relevant markers in CU-SWT and CU-Si Wu He Ji.

VIII. Establish the most stable marker(s) for SWT product.
### Part I Results: Data Mining

<table>
<thead>
<tr>
<th>Herb</th>
<th>Major active components identified from data mining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelicae</td>
<td>Ferulic acid</td>
</tr>
<tr>
<td></td>
<td>Ligustilide</td>
</tr>
<tr>
<td>Chuanxiong</td>
<td>Ligustrazine</td>
</tr>
<tr>
<td></td>
<td>Ligustilide</td>
</tr>
<tr>
<td></td>
<td>Ferulic acid</td>
</tr>
<tr>
<td></td>
<td>Butylphthalide</td>
</tr>
<tr>
<td></td>
<td>Senkyunolide A</td>
</tr>
<tr>
<td>Paeoniae</td>
<td>Paeoniflorin</td>
</tr>
<tr>
<td></td>
<td>Paeonol</td>
</tr>
<tr>
<td></td>
<td>Gallic acid</td>
</tr>
<tr>
<td>Rehmanniae</td>
<td>Catalpol</td>
</tr>
</tbody>
</table>
Part I Results
Development of HPLC-DAD Assay and Method Validation

HPLC-DAD method development

HPLC-DAD system: Waters 2695 Separation Module
Waters 996 Photodiode Array Detector and Autosampler

LC column: Thermo ODS Hypersil, 4.6 x 250 mm, 5 um, with Waters Delta-Pak C18 guard column, 4.6 x 0.5 mm, 5 um

Mobile phase: 0.04% v/v phosphoric acid and 0.04% v/v diethylamine in water : ACN, gradient elution

Flow rate: 1 mL/min

Temperature of LC column: ambient
Detection: 210 to 400 nm
Quantification: internal standard method
Part I Results
HPLC-DAD chromatograms of the nine analytes of interests

(a) 280 nm

(b) 230 nm

(c) 320 nm

Remark: Gallic acid (GA), senkyunolide A (SA), paeoniflorin (PF), ferulic acid (FA), Z-ligustilide (Lig), butylphthalide (Bu), ligustrazine (TMP) and paeonol (PO)
Part I Results: CP Tests of Raw Herbs

(a) Radix Paeoniae Alba

1. Test herb (source 1)
2. Test herb (source 2)
3. Reference herb
4. Paeoniflorin standard

(b) Radix Rehmanniae Preparata

1. Test herb
2. Reference herb
3. Catalpol standard
Part I Results: CP Tests of Raw Herbs

(c) Radix Angelicae Sinensis

(d) Rhizoma Chuanxiong

1. Test herb (root)
2. Test herb (body)
3. Test herb (head)
4. Reference herb

1. Reference herb
2. Test herb
Part I Results:  CP Tests of Raw Herbs

Content of raw herbs:

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Batch</th>
<th>FA</th>
<th>Lig</th>
<th>SA</th>
<th>Bu</th>
<th>TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelicae</td>
<td>#1</td>
<td>0.058</td>
<td>1.26</td>
<td>0.031</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>0.069</td>
<td>1.82</td>
<td>0.027</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>0.051</td>
<td>0.16</td>
<td>0.021</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>0.046</td>
<td>0.69</td>
<td>0.035</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>0.036</td>
<td>0.70</td>
<td>0.023</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Chuanxiong</td>
<td>#1</td>
<td>0.130</td>
<td>2.41</td>
<td>0.350</td>
<td>0.063</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>0.025</td>
<td>1.68</td>
<td>0.270</td>
<td>0.049</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>0.081</td>
<td>2.83</td>
<td>0.270</td>
<td>0.028</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>0.110</td>
<td>2.60</td>
<td>0.350</td>
<td>0.048</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>0.110</td>
<td>1.08</td>
<td>0.300</td>
<td>0.033</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Remark:  N.A. = not applicable, N.D. = not detectable

- variation among batches of raw herbs was observed
- batch #4 and #5 of Angelicae do not comply with 0.05% ferulic acid (FA) content as stated in CP (2005)
- Ligustrazine (TMP) was not detectable in Chuanxiong

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## Part I Results: CP Tests of Raw Herbs

### Content of raw herbs: continued

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Batch</th>
<th>GA</th>
<th>PF</th>
<th>Cat</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeoniae</td>
<td>#1</td>
<td>0.120</td>
<td>2.06</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>0.076</td>
<td>2.11</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>0.065</td>
<td>0.53</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>0.088</td>
<td>2.36</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>0.034</td>
<td>1.25</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Rehmanniae</td>
<td>#1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.004</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.005</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.004</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.001</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

Remark: N.A. = not applicable, N.D. = not detectable

- batch #3 and #5 of Paeoniae do not comply with 1.6% paeoniflorin (PF) content as stated in CP (2005)
- very low content of catalpol (Cat) in Rehmanniae; paeonol (PO) cannot be detectable in Paeoniae
Part I Results – Manufacture CU-Si Wu He Ji

The method was based on the protocol described in CP (2005)

A: Radix Angelicae Sinensis 250 g
C: Rhizoma Chuanxiong 250 g
P: Radix Paeoniae Alba 250 g
R: Radix Rehmanniae Praeparata 250 g

A+C
Soaking in water (RT) 0.5 h

A+C
Steam distillation

Water Phase

P + R

Residue

1 h Filtration

Aqueous phase

1.5 h Filtration

Aqueous phase

1.5 h Filtration

Aqueous phase

1 h Filtration

Aqueous phase

Combination

Concentrated to small volume

Add ethanol to 55% (v/v)

Stood for 24 h

Adjust density to 1.26~1.30 (55~60°C)

Filtration

Ethanol phase

Filtration

Ethanol phase

Combination

Dilute with water

Filtration

Product
Part I Results – Manufacture CU-SWT

The method was modified from CP (2005)

A: Radix Angelicae Sinensis 250 g
C: Rhizoma Chuanxiong 250 g
P: Radix Paeoniae Alba 250 g
R: Radix Rehmanniae Praeparata 250 g

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Products Manufactured

CU-SWT (batch 1 to 3)

CU-Si Wu He Ji (batch 1)

CU-Chuanxiong (batch 1 to 3)

CU-Angelicae (batch 1 to 3)

CU-Rehmanniae (batch 1 to 3)
### Part I Results – Contents of SWT

**(a) SWT product (solid dosage form)**

<table>
<thead>
<tr>
<th>Product</th>
<th>GA</th>
<th>SA</th>
<th>PF</th>
<th>FA</th>
<th>Lig</th>
</tr>
</thead>
<tbody>
<tr>
<td>CU-SWT batch 1</td>
<td>0.987 ± 0.089</td>
<td>0.075 ± 0.002</td>
<td>8.651 ± 0.240</td>
<td>0.525 ± 0.015</td>
<td>1.127 ± 0.224</td>
</tr>
<tr>
<td>CU-SWT batch 2</td>
<td>0.774 ± 0.030</td>
<td>0.049 ± 0.001</td>
<td>6.353 ± 0.057</td>
<td>0.440 ± 0.044</td>
<td>0.473 ± 0.012</td>
</tr>
<tr>
<td>CU-SWT batch 3</td>
<td>0.781 ± 0.101</td>
<td>0.053 ± 0.001</td>
<td>6.362 ± 0.198</td>
<td>0.367 ± 0.015</td>
<td>0.342 ± 0.024</td>
</tr>
<tr>
<td>market-1</td>
<td>0.131 ± 0.009</td>
<td>0.044 ± 0.002</td>
<td>0.054 ± 0.004</td>
<td>0.020 ± 0.002</td>
<td>0.037 ± 0.002</td>
</tr>
<tr>
<td>market-2</td>
<td>0.506 ± 0.070</td>
<td>0.059 ± 0.001</td>
<td>1.930 ± 0.143</td>
<td>0.154 ± 0.041</td>
<td>0.034 ± 0.003</td>
</tr>
</tbody>
</table>

**Remark:**
1. Gallic acid (GA), senkyunolide A (SA), paeoniflorin (PF), ferulic acid (FA) and Z-ligustilide (Lig).
2. Butylphthalide (Bu), ligustrazine (TMP), paenol (PO) and catalpol (Cat) are not detectable.
## Part I Results – Contents of SWT

### (b) Si Wu He Ji (liquid dosage form)

<table>
<thead>
<tr>
<th>Product</th>
<th>Product Code</th>
<th>GA (µg/mL) ± SD (n = 3)</th>
<th>SA (µg/mL) ± SD (n = 3)</th>
<th>PF (µg/mL) ± SD (n = 3)</th>
<th>FA (µg/mL) ± SD (n = 3)</th>
<th>Lig (µg/mL) ± SD (n = 3)</th>
<th>Bu (µg/mL) ± SD (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CU-SWHJ</td>
<td></td>
<td>307.9 ± 13.9</td>
<td>26.5 ± 0.9</td>
<td>2794 ± 225</td>
<td>173.3 ± 10.5</td>
<td>33.2 ± 1.0</td>
<td>N.D.</td>
</tr>
<tr>
<td>market-3</td>
<td></td>
<td>32.7 ± 1.5</td>
<td>2.07 ± 0.04</td>
<td>5795 ± 366</td>
<td>1.62 ± 0.47</td>
<td>1.02 ± 0.04</td>
<td>0.42 ± 0.03</td>
</tr>
</tbody>
</table>

Remark: 1. Gallic acid (GA), senkyunolide A (SA), paeoniflorin (PF), ferulic acid (FA) and Z-ligustilide (Lig) and butylphthalide (Bu).
2. Ligustrazine (TMP), paeonol (PO) and catalpol (Cat) are not detectable (N.D.).
CU SWT products vs. Commercial SWT

Among 9 compounds investigated, ferulic acid, gallic acid, paeoniflorin, senkyunolide A and Z-ligustilide are present in all marketed products and CU-SWT & CU-Si Wu He Ji.

The contents of these components are generally higher in CU SWT products than commercial products (in particular the volatile component Z-ligustilide).

Large variations in contents of each component in marketed products.
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

Dissolution

Gastrointestinal (GI) metabolism

GI absorption

Identification of relevant bioactive marker(s) → Stability analysis of the identified markers
Part I Results – Dissolution Study

Good dissolution of ferulic acid, paeoniflorin, senkyunolide A, gallic acid and Z-ligustilide in SWT products in the dissolution medium (pH 2) within 30 min.
**Part I:** Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product) → Dissolution → Gastrointestinal (GI) metabolism → GI absorption → Identification of relevant bioactive marker(s) → Stability analysis of the identified markers
Part I Results – Metabolism Study

GI metabolism models:
- Rat intestine homogenate
- Human intestine homogenate
- Caco-2 cell lysate

Incubated with markers and SWT products
Detect metabolites by LC/MS/MS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Possible metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeoniflorin</td>
<td>Hydrolysis</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Sulfation, Glucuronation, Methylation</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>Sulfation, Glucuronation, Methylation</td>
</tr>
</tbody>
</table>
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

↓

Dissolution

↓

Gastrointestinal (GI) metabolism

↓

GI absorption

↓

Identification of relevant bioactive marker(s)

→

Stability analysis of the identified markers
Part I Results – Permeability (GI Absorption)

- **Good permeability for ferulic acid, Z-ligustilide, senkyunolide A**
- **Poor permeability for paeoniflorin and gallic acid**

**Ferulic acid**
- Loading Conc: 19.71 µg/mL
- Papp: $1.22 \times 10^{-5}$ cm/s

**Z-Ligustilide**
- Loading Conc: 62.38 µg/mL
- Papp: $1.54 \times 10^{-5}$ cm/s

**Senkyunolide A**
- Loading Conc: 16.11 µg/mL
- Papp: $2.81 \times 10^{-5}$ cm/s
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

→ Dissolution

→ Gastrointestinal (GI) metabolism

→ GI absorption

→ Identification of relevant bioactive marker(s)

→ Stability analysis of the identified markers
Part I Results: – Stability Study

Stability of CU-SWT in various storage conditions

• Temperature at 4, 25 and 40 °C
• Storage period from 0, 1, 2 and 3 months
• Product tested: CU-SWT powder and CU-Si Wu He Ji, both were manufactured by us according to CP 2005
Part I Results: – Stability Study

Stability results of CU-SWT (solid dosage form)

Senkyunolide A, ferulic acid and Z-ligustilide in CU-SWT (solid dosage form) are relatively stable over 3-month period at 4, 25 and 40 °C storage temperatures.
Stability results of CU-Si Wu He Ji (liquid dosage form)

Degradation of senkyunolide A, ferulic acid and Z-ligustilide was observed in CU-SWHJ stored at high temperature.

Part I Results: – Stability Study
Part I: Overall Summary in Selection of Relevant Bioactive Markers

• Three compounds, i.e. **ferulic acid**, **senkyunolide A** and **Z-ligustilide**, could serve as a more relevant bioactive markers for QC of SWT due to their good dissolution, permeability and stability characteristics.

• **Paeoniflorin**, a marker compound designated by the Chinese Pharmacopeia, is poorly permeable and thus not suitable to be considered as a “relevant bioactive” marker.
Are we done yet?

• **Limitation with these markers**: lack of ability to represent the overall activity of a given SWT product.

• Recently, a landmark study was published in Science showing that differentially expressed genes (DEGs) identified via microarray processing and analysis can serve as signature for wide various of chemical components [1].

• We hypothesize that such DEGs can serve as unique composite fingerprint for a given CM product which could be useful for quality control and identity of such product.
Part II: Objective

• To investigate the applicability of the different expression genes (DEGs) derived from special microarray processing and analysis in serving as unique fingerprint for a given SWT product and its four single herbs.
**Part II: Project scheme**

- SWT product/herbs samples
  - LC/MS/MS & MTT
- Donor chamber
- Receiver chamber
- Caco-2
- Absorbed fraction
- Desalting and concentrate

**MCF7**

**Extract RNA**

**Microarray processing**

**DEGs**

**Multi-gene RT-PCR panel**

**Real-time PCR**

**Verification**

- Specificity
- Consistency

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Experimental design of sample treatment for microarray processing / real-time PCR

- Methanol (Control)
  - 1%
  - 1
  - 2
  - 3

- CU-SWT permeates S
  - 1:2
  - 1:8
  - 1:32
  - 1
  - 2
  - 3

- J-SWT permeates J
  - 1:2
  - 1:8
  - 1:32
  - 1
  - 2
  - 3

- Standard mixture (FA, Lig and SA) permeates M
  - 1:2
  - 1:8
  - 1
  - 2
  - 3

- CU-Angelicae permeates A
  - 1:2
  - 1:8
  - 1
  - 2
  - 3

- CU-ChuanXiong permeates C
  - 1:2
  - 1:8
  - 1
  - 2
  - 3

- CU-Paeoniae permeates P
  - 1:2
  - 1:8
  - 1
  - 2
  - 3

- CU-Rehmanniae permeates R
  - 1:2
  - 1:8
  - 1
  - 2
  - 3

6hrs
Total RNA isolation

Microarray/Real time PCR
The Principal Components Analysis (PCA) and Venn Diagram of the differentially expressed genes for microarray data of CU-SWT permeates treatment.
Identification of differentially expressed genes (DEGs), construction of multi-gene RT-PCR panel and method validation.

Step I: DEGs selection criteria:

1. Fold change (FC) greater than a pre-defined threshold (e.g. FC > 1.5 or FC < -1.5);
2. The p < 0.01 based on unpaired sample t-test;
3. The expression fold change in a dose-dependent manner;
4. The gene selected was significantly consistent with the drug’s therapeutic use;
5. The expression of genes selected was consistent in three different batches of microarray work.

Step II: Constructed the multi-gene RT-PCR panel for each treatment group using the DEGs identified.

Step III: Verified the expression fold changes of DEGs using real-time RT-PCR.
# Part II Results

The differentially expressed genes (DEGs) for treatment groups

<table>
<thead>
<tr>
<th>CU-SWT</th>
<th>J-SWT</th>
<th>Standard mixture</th>
<th>CU-Chuanxion</th>
<th>CU-Angelica</th>
<th>CU-Paeonia</th>
<th>CU-Rehmannia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>TRIM65</td>
<td>SLC7A11</td>
</tr>
<tr>
<td>PDK4</td>
<td>CYP1A1</td>
<td>CYP1A1</td>
<td>ALDH1A3</td>
<td>CYP1A1</td>
<td>CYP2B6</td>
<td>CYP1A1</td>
</tr>
<tr>
<td>ST3GAL1</td>
<td>AKR1C1/AKR1C2</td>
<td>HMOX1</td>
<td>INPP4B</td>
<td>ALDH1A3</td>
<td>OR2H1</td>
<td>HMOX1</td>
</tr>
<tr>
<td>TNFRSF21</td>
<td>SLC7A5</td>
<td>PDK4</td>
<td>MSMB</td>
<td>INPP4B</td>
<td>NAP1L5</td>
<td>CXCR7</td>
</tr>
<tr>
<td>THBS1</td>
<td>GCLM</td>
<td>ALDH1A3</td>
<td>NEDD9</td>
<td>LRP8</td>
<td>TMCO6</td>
<td>ALDH1A3</td>
</tr>
<tr>
<td>PIGW</td>
<td>TXNRD1</td>
<td>PHLDB2</td>
<td>CCNK</td>
<td>TNFSF10</td>
<td>LCE1E</td>
<td>GCLM</td>
</tr>
<tr>
<td>GPER</td>
<td>CDH18</td>
<td>PDE4DIP</td>
<td>HECTD1</td>
<td>SAMD9</td>
<td>OSBPL8</td>
<td>S100A7</td>
</tr>
<tr>
<td>PCDH10</td>
<td>CCL28</td>
<td>MAP3K1</td>
<td>OSBPL8</td>
<td>MBNL2</td>
<td>RBAK</td>
<td>ATRX</td>
</tr>
<tr>
<td>TNFSF10</td>
<td>METTL7A</td>
<td>ZC3H11A</td>
<td>LARS</td>
<td>SAMHD1</td>
<td>ESCO1</td>
<td>CP</td>
</tr>
</tbody>
</table>

Note: Red color: up-regulated genes; Black color: down-regulated genes
Part II Results

Specificity test

Test Products (total 21 different products):

- **Two batches of CU-SWTs** (CU-SWT batch1 and CU-SWT batch2)
- **Three marketed SWTs** (J-SWT, SWT_NBF, SWTHJ)
- **Four single herbal extracts** (CU-Ang, CU-ChuanX, CU-Pae, CU-Reh)
- **Single chemical standards** (ferulic acid, ligustilide, senkyunolide A) and their mixture
- **Eight independent products** either marketed products (Named M1~M8).

Treat MCF-7 cells and extract RNA

Two sets of multi-gene RT-PCR panels for CU-SWT and J-SWT were tested against 21 different products by real-time PCR

Consistency evaluation

- Test Products: Eight replicate extracts of CU-SWT on 3 separate days
- Treat MCF-7 cells and extract RNA
- The expression fold changes of the CU-SWT DEGs in these products were tested using real-time PCR.
Part II Results:

Data analysis of **intra-day and inter-day consistency** by CU-SWT DEGs panel for CU-SWT

<table>
<thead>
<tr>
<th>CU-SWT DEGs</th>
<th>Coefficient of Variations (CV)</th>
<th>Intra-day (8 batches)</th>
<th>Inter-day (3 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC7A11</td>
<td></td>
<td>3.3~5.6%</td>
<td>6.9%</td>
</tr>
<tr>
<td>PDK4</td>
<td></td>
<td>18.6~26.7%</td>
<td>27.0%</td>
</tr>
<tr>
<td>ST3GAL1</td>
<td></td>
<td>25.0~29.6%</td>
<td>13.0%</td>
</tr>
<tr>
<td>TNFRSF21</td>
<td></td>
<td>16.3~31.7%</td>
<td>14.3%</td>
</tr>
<tr>
<td>THBS1</td>
<td></td>
<td>14.9~17.8%</td>
<td>7.9%</td>
</tr>
<tr>
<td>PIGW</td>
<td></td>
<td>19.8~30.6%</td>
<td>21.1%</td>
</tr>
<tr>
<td>GPER</td>
<td></td>
<td>14.1~27.3%</td>
<td>20.5%</td>
</tr>
<tr>
<td>PCDH10</td>
<td></td>
<td>9.6~22.5%</td>
<td>19.5%</td>
</tr>
<tr>
<td>TNFSF10</td>
<td></td>
<td>12.6~25.9%</td>
<td>23.0%</td>
</tr>
</tbody>
</table>
Identify DEGs of two SWTs (CU-SWT, J-SWT), its four single herbal extracts (CU-Angelica, CU-Chuanxiong, CU-Paeoniae and CU-Rehmanniae) and standard mixture (ferulic acid, ligustilide and senkyunolide A) using microarray processing and analysis.

A panel of specific genes can be constructed using real-time RT-PCR to represent unique “fingerprint” of SWT and single herb tested.

Convenient and cost-effective manner for “identity test” of the product during its manufacturing and testing of counterfeit products.
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