Identification of the authentic quality of Longdanxiegan pill by systematic quantified fingerprint method based on three wavelength fusion chromatogram

SUN Guoxiang,* ZHANG Jingxian

[College of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China]

Abstract The three wavelength fusion high performance liquid chromatographic fingerprints (TWFP) of Longdanxiegan pill (LHXG) was established to identify the quality of LHXG by the systematic quantified fingerprint method. The chromatographic fingerprints (CFPs) of the 12 batches of LHXG were determined by reversed-phase high performance liquid chromatography. The technique of multi-wavelength fusion fingerprint was applied during processing the fingerprints. The TWFPs containing 63 co-possessing peaks were obtained when choosing baicalin peak as the referential peak. The 12 batches of LHXG were identified with hierarchical clustering analysis by using macro qualitative similarity $S_m$ as the variable. According to the results of classification the referential fingerprint (RFP) was synthesized from 10 batches of LHXG. Taking the RFP for the qualified model all the 12 batches of LHXG were evaluated by the systematic quantified fingerprint method. Among the 12 batches of LHXG 9 batches were completely qualified the contents of 1 batch were obviously higher while the chemical constituents quantity and distributed proportion in 2 batches were not qualified. The systematic quantified fingerprint method based on the technique of multi-wavelength fusion fingerprint can effectively identify the authentic quality of traditional Chinese medicine.

Key words high performance liquid chromatography HPLC systematic quantified fingerprint method three wavelength fusion fingerprint macro qualitative similarity macro quantitative similarity Longdanxiegan pill LHXG
Table 1 TCM quality orders divided by the systematic quantified fingerprint method

<table>
<thead>
<tr>
<th>Grade</th>
<th>$S_m$</th>
<th>$P_m$/%</th>
<th>$\alpha$</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$\geq 0.95$</td>
<td>$95 - 105$</td>
<td>$\leq 0.05$</td>
<td>best</td>
</tr>
<tr>
<td>II</td>
<td>$\geq 0.90$</td>
<td>$90 - 110$</td>
<td>$\leq 0.10$</td>
<td>better</td>
</tr>
<tr>
<td>III</td>
<td>$\geq 0.85$</td>
<td>$80 - 120$</td>
<td>$\leq 0.15$</td>
<td>good</td>
</tr>
<tr>
<td>IV</td>
<td>$\geq 0.80$</td>
<td>$75 - 125$</td>
<td>$\leq 0.20$</td>
<td>fine</td>
</tr>
<tr>
<td>V</td>
<td>$\geq 0.70$</td>
<td>$70 - 130$</td>
<td>$\leq 0.30$</td>
<td>moderate</td>
</tr>
<tr>
<td>VI</td>
<td>$\geq 0.60$</td>
<td>$60 - 140$</td>
<td>$\leq 0.40$</td>
<td>common</td>
</tr>
<tr>
<td>VII</td>
<td>$\geq 0.50$</td>
<td>$50 - 150$</td>
<td>$\leq 0.50$</td>
<td>defective</td>
</tr>
<tr>
<td>VIII</td>
<td>$&lt; 0.50$</td>
<td>$0 - \infty$</td>
<td>$&gt; 0.50$</td>
<td>inferior</td>
</tr>
</tbody>
</table>

$$
S_n = \frac{1}{2} S_f + S_f' = \frac{1}{2} \left( \frac{\sum_{i=1}^{n} x_i y_i}{\sqrt{\sum_{i=1}^{n} x_i^2 \sum_{i=1}^{n} y_i^2}} + \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} y_i} \right)
$$

$$
P_n = \frac{1}{2} C + P = \frac{1}{2} \left( \frac{\sum_{i=1}^{n} x_i y_i}{\sum_{i=1}^{n} y_i} + \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} y_i} \right) \times 100\%
$$
\[ \alpha = \left| 1 - \frac{\gamma_s}{\gamma_t} \right| = \left| 1 - \frac{P}{C} \right| \]

2

2.1

Agilent 1100 DAD Chem Station Agilent 1260 RE52 Sartorius2B110S KQ-50B KDM DAD

2.2

DAD 200

2.3

HPLC

Century SIL C18 BDS 200 mm \times 4.6 mm

<table>
<thead>
<tr>
<th>α</th>
<th>1 - γs/γt</th>
<th>1 - P/C</th>
</tr>
</thead>
</table>
孙国祥,等:基于三波长融合谱的系统指纹定量法鉴定龙胆泻肝丸的真实质量

按表时间段融合指纹谱,以黄芩苷的保留时间和峰面积为参照,计算各指纹峰相对保留时间的相对标准偏差(\(\%RSD\))均小于1.0%,相对峰面积的\(\%RSD\)均小于0.8%,表明检测系统的进样精密度良好。

图绿原酸(\(\%RSD\))、龙胆苦苷(\(\%RSD\))、黄芩苷(\(\%RSD\))、甘草酸(\(\%RSD\))的谱图和样品的融合谱图。

稳定性!精密吸取1号供试液,分别在制备样品后24,72,120,180和240 h进样测定,记录1号,2号和3号峰波长下的色谱图。按表时间段融合指纹谱,以黄芩苷为参照物峰,按峰出现率\(\%\)计,确定12个指纹峰。

以平均值法计算生成准对照指纹图谱(\(\bar{F}\))并计算5批样品的双定性相似度。由于宏定性相似度代表不同样品化学指纹数量和分布比例与对照指纹图谱的相似性程度,因此以\(\bar{F}\)为参量进行系统聚类分析,结果(见图).

将3个厂家的\(\bar{F}\)供试液分别进样检测,记录1号,2号和3号波长下的色谱图。按表时间段融合指纹谱,以黄芩苷峰(1号峰)为参照物,按峰出现率\(\%\)计,确定5个指纹峰。

以平均值法计算生成准对照指纹图谱(\(\bar{F}\))并计算5批样品的\(\bar{F}\)值并按表的限度划分\(\bar{F}\)的质量级别(见表).

仅2号和3号质量较差,其他批样品的质量均良好以上。

Table 3 三波长融合指纹图谱的建立和系统指纹定量法鉴定评价

<table>
<thead>
<tr>
<th>Sample</th>
<th>(S_m)</th>
<th>(P_m/%)</th>
<th>(\alpha)</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2</td>
<td>0.87</td>
<td>77.6</td>
<td>0.08</td>
<td>IV</td>
</tr>
<tr>
<td>S3</td>
<td>0.88</td>
<td>83.0</td>
<td>0.04</td>
<td>III</td>
</tr>
<tr>
<td>S4</td>
<td>0.90</td>
<td>77.0</td>
<td>0.01</td>
<td>III</td>
</tr>
<tr>
<td>S5</td>
<td>0.89</td>
<td>88.3</td>
<td>0.05</td>
<td>III</td>
</tr>
<tr>
<td>S6</td>
<td>0.93</td>
<td>97.0</td>
<td>0.05</td>
<td>III</td>
</tr>
<tr>
<td>S7</td>
<td>0.94</td>
<td>128.6</td>
<td>0.13</td>
<td>V</td>
</tr>
<tr>
<td>S8</td>
<td>0.93</td>
<td>111.4</td>
<td>0.01</td>
<td>III</td>
</tr>
<tr>
<td>S9</td>
<td>0.94</td>
<td>111.8</td>
<td>0.09</td>
<td>III</td>
</tr>
<tr>
<td>S10</td>
<td>0.96</td>
<td>82.4</td>
<td>0.01</td>
<td>III</td>
</tr>
<tr>
<td>S11</td>
<td>0.91</td>
<td>118.0</td>
<td>0.13</td>
<td>III</td>
</tr>
<tr>
<td>S1</td>
<td>0.78</td>
<td>50.7</td>
<td>0.03</td>
<td>VII</td>
</tr>
<tr>
<td>S12</td>
<td>0.75</td>
<td>62.6</td>
<td>0.12</td>
<td>VI</td>
</tr>
</tbody>
</table>
3.6 LDXGP

\[ S_m \geq 0.85 \]
\[ 51 \leq S_{1} \leq 57 \]
\[ 75\% \leq \alpha \leq 125\% \]
\[ S_{11} \leq 87 \]
\[ \alpha = 0.13 \]
\[ t = 0 \]
\[ 9 \leq t \leq 1 \]
\[ t = 2 \]
\[ 12 \leq t \leq 3 \]

4

\[ 2.2^3 \]
\[ 25\% \]
\[ 50\% \]
\[ 75\% \]
\[ 95\% \]
\[ 80\% \]
\[ 24\ h \]
\[ 30 \min \]
\[ I \]
\[ I \]
\[ 75\% \]
\[ 25\% \]
\[ HPLC-M$\]

5

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