

# Analytical Methods

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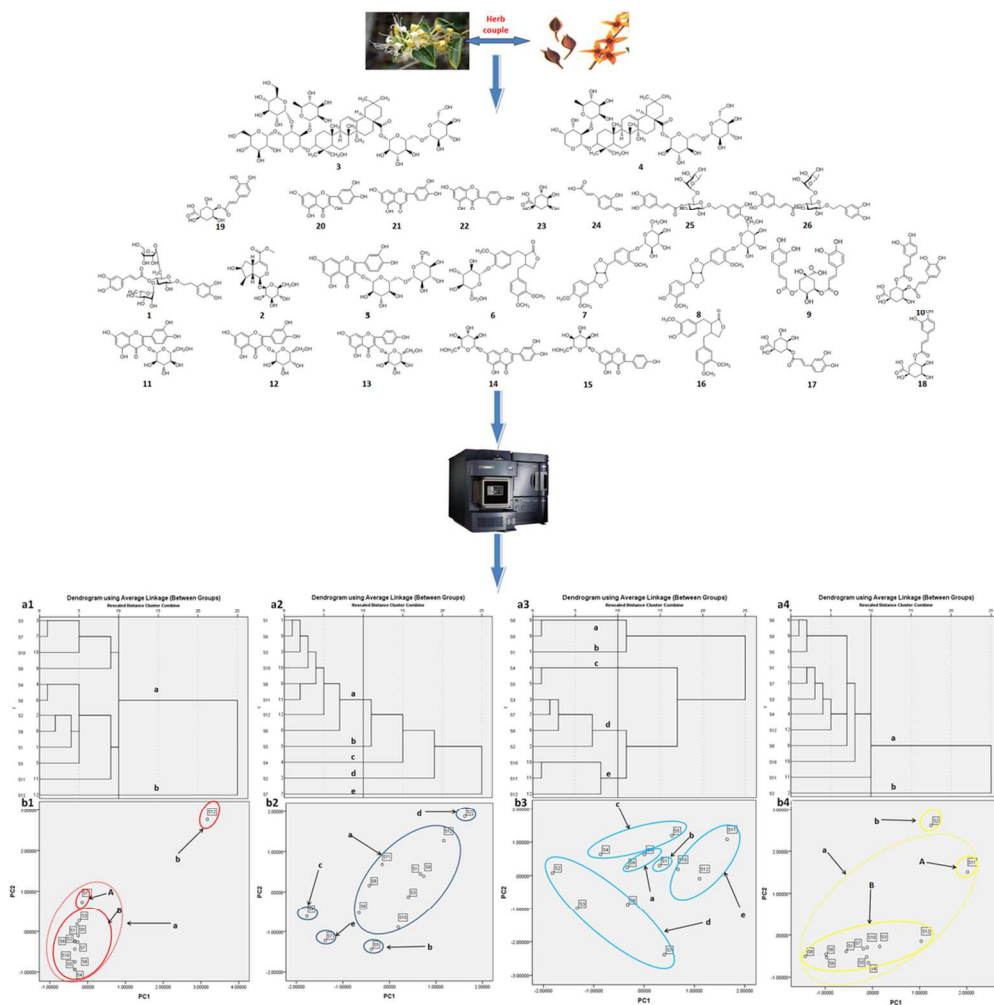
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4 Simultaneous determination of twenty-six components of *Flos Lonicerae*  
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6 *Japonicae* - *Fructus Forsythiae* herb couple using UPLC-ESI-MS/MS,  
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8 Application to its preparations  
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32 **Abstract**

33  
34 In this study, we developed a method using UPLC-ESI-MS/MS for determining the contents  
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36 of forsythoside B, loganin, macranthoidin B, dipsacoside B, rutin, arctiin, phillyrin,  
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38 pinoresinol- $\beta$ -D-glucoside, 3, 5-dicaffeoylquinic acid, 3, 4-dicaffeoylquinic acid, isoquercitrin,  
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40 hyperoside, astragaloside, luteoloside, genistin, arctigenin, neochlorogenic acid, chlorogenic acid,  
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42 cryptochlorogenic acid, quercetin, luteolin, genistein, quinic acid, caffeic acid, isoforsythoside  
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44 and forsythoside A in *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple simultaneously  
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46 with run time of only 8 min. The separation was performed on an Acquity UPLC HSS T3 C<sub>18</sub>  
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48 column (100 mm $\times$ 2.1 mm, 1.8  $\mu$ m) at a flow rate of 0.4 mL $\cdot$ min<sup>-1</sup>, and acetonitrile/methanol (4:1,  
49  
50 v/v)-0.4% formic acid was used as mobile phase. Variations in the intra- and inter-day precision  
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52 of all analytes were below 5.00%, the matrix effect of all the analytes was found to be within  
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54 the acceptable range, and the accuracy was evaluated by a recovery test within the range of  
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56 95.63% - 103.1%. The method successfully quantified the twenty-six compounds in *Flos*  
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3 *Lonicerae Japonicae* - *Fructus Forsythiae* herb couple. Besides, it transpired, through hierarchical  
4 cluster analysis and principal component analysis, that the consistency of *Flos Lonicerae*  
5 *Japonicae* - *Fructus Forsythiae* herb couple as the two important herbs in *Flos Lonicerae*  
6 *Japonicae* - *Fructus Forsythiae* herb couple preparations (Shuang-Huang-Lian oral liquid,  
7 Yin-Qiao-Jie-Du tablet and Fufang Qin-Lan oral liquid except that in Qin-Re-Jie-Du oral liquid)  
8 was relatively good. The results showed that the method was accurate, sensitive and reliable.

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15 **Keywords:** *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couples, UPLC-ESI-MS/MS, Quality  
16 control, PCA, HCA  
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## 1. Introduction

Herbs used together in couples are the basic composition units of Chinese herbal formulas and have special clinical significance in traditional Chinese Medicine (TCM). The herb couples (mixture of two herbs) are much simpler than complicated formulations in composition but retain the basic therapeutic features. *Flos Lonicerae Japonicae* possesses wide pharmacological actions, such as antibacterial, anti-inflammatory, antiviral, antiendotoxin, blood fat reducing, antipyretic, etc [1]. *Fructus Forsythiae* has the effects of antibacterial, antiviral, antioxidant, anti-inflammatory, anti-obesity and antiemetic, etc [2]. The two herbs are the basic components of Chinese herbal preparations such as Shuang-Huang-Lian oral liquid, Qin-Re-Jie-Du oral liquid, Yin-Qiao-Jie-Du tablet and Fufang Qin-Lan oral liquid etc., which are extensively used in clinical practice [3]. We found that the pharmacological effects (anti-bacteria and antiviral) were decreased significantly as the *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple was knocked out from Shuang-Huang-Lian oral liquid, Qin-Re-Jie-Du oral liquid, Yin-Qiao-Jie-Du tablet and Fufang Qin-Lan oral liquid, but increased significantly as the *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple was knocked in, which elucidate the importance of *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in four preparations above [4].

It was reported that *Flos Lonicerae Japonicae* extract in preparations included flavones, isoflavones, organic acids, triterpenoid saponins and iridoids etc., [5-14] and *Fructus Forsythiae* extract in preparations contained phenylethanoid glycosides, lignans, flavones and few saponins etc [15-25]. Some of them displayed many bioactivities *in vivo* or *in vitro* [26, 27]. A reliable UHPLC-LTQ-Orbitrap-MS system has been performed by us to detect qualitatively the compounds in *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple, and successfully found 35 ingredients including 26 obtained reference standards (quinic acid, caffeic acid, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3, 5-dicaffeoylquinic acid, 3, 4-dicaffeoylquinic acid, isoforsythoside, forsythoside A, forsythoside B, rutin, isoquercitrin, hyperoside, astragalgin, luteoloside, genistin, quercetin, luteolin, genistein, phillyrin, Arctiin pinoresinol- $\beta$ -D-glucoside, arctigenin, loganin, Dipsacoside B, Macranthoidin B) and 9 components recognized by literature

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3 data [5-25] (forsythoside D, forsythoside E, monomethylether- $\beta$ -D-glucoside,  
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epipinoresinol- $\beta$ -D-glucoside, epipinoresinol, pinoresinol, phillygenin, sweroside, centauroside). Besides, 35 ingredients, except 9 recognized compounds, can all be considered as common peaks to control quality of *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple, as the ingredients cannot appear in every batch of *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple.

Thus, in order to control the quality of Shuang-Huang-Lian oral liquid, Qin-Re-Jie-Du oral liquid, Yin-Qiao-Jie-Du tablet and Fufang Qin-Lan oral liquid, it is imperative to develop an effective and comprehensive analytical method based on 26 components above to control the quality of *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple.

As we all known, the quality control of TCM usually only focuses on a single component or a limited number of components [28]. Unlike synthetic drugs, it is well known that medicinal herbs and their preparations generally exert their therapeutic effects through the synergic effects of the multiple active ingredients and the multi-targets they are targeting [29]. Chemical fingerprint as a semi-quantification method was applied extensively to the quality control of herbal medicine [30, 31], but its characteristics (long analytical time, subjective selected common peaks, etc.) also limited its application. We propose that the method about rapidly determination 26 components quantitatively and simultaneously combined with chemometric analysis applied to control quality in *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple will be more accurate and convenient than chemical fingerprint.

To date, various analytical methods have been used to analyze *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple preparations, including high performance liquid chromatography (HPLC) [32-36] and capillary electrophoresis (CE) [37-39]. Among them, HPLC-UV was more frequently used [32-34]. One of the wavelengths of UV detection was often set form 190 to 210 nm due to the structure characteristics of saponins and iridoids, which usually caused high baseline noise and poor sensitivity. In such case, the evaporative light scattering detection (ELSD) could serve as an alternative [35, 36]. However, its low sensitivity and uncertainty in peak identification limited its use. In addition, these HPLC methods would take a long analytical time.

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3 Recent success in the use of liquid chromatography coupled with a triple quadrupole mass  
4 spectrometry (LC-MS/MS) for characterizing and quantifying a wide variety of compounds in  
5 complex samples [40-45] suggests that LC-MS/MS might be a technique in the determination of  
6 multiple compounds. For example, Liang et al [41] developed for the simultaneous quantification  
7 of 41 bioactive components in Niu Huang Shangqing pill by HPLC-MS/MS.  
8 Ultra-performance-liquid chromatography coupled with a triple quadrupole electrospray tandem  
9 mass spectrometry (UPLC-ESI-MS/MS) is a powerful tool to solve the problems of above methods  
10 because of its high sensitivity and rapid resolution. Due to the high selectivity of multiple reaction  
11 monitoring (MRM) mode, optimization of chromatographic separation is greatly simplified.  
12 Furthermore, MRM can be used to increase specificity of detection and identification of the  
13 known molecules.  
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16 In this study, a rapid and sensitive UPLC-ESI-MS/MS method was developed for the  
17 simultaneous determination of forsythoside B (1), loganin (2), macranthoidin B (3), dipsacoside B  
18 (4), rutin (5), arctiin (6), phillyrin (7), pinoresinol- $\beta$ -D-glucoside (8), 3, 5-dicaffeoylquinic acid (9), 3,  
19 4-dicaffeoylquinic acid (10), isoquercitrin (11), hyperoside (12), astragalol (13), luteoloside (14),  
20 genistin (15), arctigenin (16), neochlorogenic acid (17), chlorogenic acid (18), cryptochlorogenic  
21 acid (19), quercetin (20), luteolin (21), genistein (22), quinic acid (23), caffeic acid (24),  
22 isoforsythoside (25) and forsythoside A (26) (Fig. S11), containing isomers in the six groups. The  
23 method was fully validated and applied to the determination of the multiple components of *Flos*  
24 *Lonicerae Japonicae* - *Fructus Forsythiae* herb couple. Besides, this method combined with  
25 chemometric analysis could control the quality of *Flos Loniceræ Japonicae* - *Fructus Forsythiae*  
26 herb couple in preparations rapidly.  
27

## 28 **2. Experimental**

### 29 **2.1. Chemicals, reagents and materials**

30 Chlorogenic acid, luteoloside, phillyrin and forsythoside A were purchased from National  
31 Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).  
32 Neochlorogenic acid, cryptochlorogenic acid, 3, 4-dicaffeoylquinic acid, 3, 5-dicaffeoylquinic acid  
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3 and forsythoside B (98% pure) were purchased from Sichuan Weikeqi Bio-tech Co., Ltd. (Sichuan,  
4 China). Isoforsythoside, caffeic acid, quinic acid, genistein, luteolin, quercetin, arctigenin,  
5 genistin, astragalin, hyperoside, pinoresinol- $\beta$ -D-glucoside, arctiin, rutin, isoquercitrin,  
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and forsythoside B (98% pure) were purchased from Sichuan Weikeqi Bio-tech Co., Ltd. (Sichuan, China). Isoforsythoside, caffeic acid, quinic acid, genistein, luteolin, quercetin, arctigenin, genistin, astragalin, hyperoside, pinoresinol- $\beta$ -D-glucoside, arctiin, rutin, isoquercitrin, dipsacoside B, macranthoidin B and loganin (98% pure) were purchased from Chengdu Herbpurify Co., Ltd. (Sichuan, China). Shuang-Huang-Lian oral liquid was manufactured by Harbin third pharmaceutical factory (Harbin, China). Fufang Qin-Lan oral liquid was purchased from Heilongjiang ZBD pharmaceutical Co., Ltd. (Heilongjiang, China). Qin-Re-Jie-Du oral liquid and Yin-Qiao-Jie-Du tablet were purchased from Beijing Tongrentang pharmaceutical Co., Ltd. (Beijing, China). Sodium formate was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

## 2.2. Preparation of standard solutions

A mixed standard stock solution containing forsythoside B (1), loganin (2), macranthoidin B (3), dipsacoside B (4), rutin (5), arctiin (6), phillyrin (7), pinoresinol- $\beta$ -D-glucoside (8), 3, 5-dicaffeoylquinic acid (9), 3, 4-dicaffeoylquinic acid (10), isoquercitrin (11), hyperoside (12), astragalin (13), luteoloside (14), genistin (15), arctigenin (16), neochlorogenic acid (17), chlorogenic acid (18), cryptochlorogenic acid (19), quercetin (20), luteolin (21), genistein (22), quinic acid (23), caffeic acid (24), isoforsythoside (25) and forsythoside A (26) were prepared in methanol containing 0.1% formic acid. The working standard solutions were prepared by diluting the mixed standard solution with 10% acetonitrile/methanol (4:1, v/v) containing 0.4% formic acid and 0.5mM sodium formate to a series of proper concentrations for calibration (dilution factor=1, 2, 4, 8, 16, 32).

## 2.3. Preparation of sample solutions

1 mL of Shuang-Huang-Lian oral liquid, Qin-Re-Jie-Du oral liquid and Fufang Qin-Lan oral liquid were dropped accurately into a 50 mL volumetric flask, respectively. Ultrasonication (35 kHz) was performed at room temperature for 20 min after 20 mL of 50% methanol as extraction solvent were added, then the same solvent was diluted to volume. Besides, 10 Yin-Qiao-Jie-Du tablets (weighting 5.5 g) were ground into powder, from which a sample (0.3 g), with an accurate weight, was taken and transferred into a 50-mL conical flask with stopper, and 50 mL of 50%



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3 methanol as extraction solvent were added. After accurately weighting, Ultrasonication (40 kHz)  
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5 was performed at room temperature for 20 min, and then the same solvent was added to  
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7 compensate for the lost weight during the extraction [46, 47]. After centrifugation ( $9659 \times g$ , 10  
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9 min) above, the supernatant were diluted fifty times for Shuang-Huang-Lian oral liquid,  
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11 Qin-Re-Jie-Du oral liquid and Fufang Qin-Lan oral liquid and ten times for Yin-Qiao-Jie-Du tablet,  
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13 respectively with 10% acetonitrile/methanol (4:1, v/v) containing 0.4% formic acid and 0.5mM  
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15 sodium formate, then filtered through 0.22  $\mu\text{m}$  membrane before injection into the  
16  
17 UPLC-ESI-MS/MS system for analysis.

#### 2.4. UPLC-MS/MS instrumentation and conditions

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22 Chromatographic analysis was performed on a Waters Acquity UPLC system (Waters Co.,  
23  
24 Milford, MA, USA), consisting of a binary pump solvent management system, an online degasser,  
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26 and an autosampler. An Acquity UPLC HSS T3  $\text{C}_{18}$  column (10 mm $\times$ 2.1 mm, 1.8  $\mu\text{m}$ ) was employed  
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28 and the column temperature was maintained at 40  $^{\circ}\text{C}$ . The mobile phase was composed of A (0.4%  
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30 formic acid) and B (acetonitrile/methanol 4:1, v/v) using a gradient elution of 10-11% B at 0-0.5  
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32 min, 11-13% B at 0.5-0.75 min, 13-15% B at 0.75-1.5 min, 15-10% B at 1.5-2 min, 10-30% B at  
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34 2-2.8 min, 30-30% B at 2.8-3.37 min, 30-10% B at 3.37-4 min, 10-10% B at 4-4.3 min, 10-95% B at  
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36 4.3-5 min, 95-95% B at 5-6 min, 95-10% B at 6-7 min and hold for 1 min. The flow rate was set at  
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38 0.4  $\text{mL}\cdot\text{min}^{-1}$ . The auto-sampler was conditioned at 4  $^{\circ}\text{C}$  and the injection volume was 5  $\mu\text{L}$   
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40 (Reported previously).  
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43 Mass spectrometry detection was performed using Xevo Triple Quadrupole MS (Waters Co.,  
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45 Milford, MA, USA) equipped with an electrospray ionization source (ESI). The ESI source was set  
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47 in positive ionization mode for macranthoidin B, dipsacoside B, rutin, arctiin, phillyrin,  
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49 isoquercitrin, hyperoside, astragaln, luteoloside, genistin, arctigenin, quercetin, luteolin,  
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51 genistein, quinic acid, caffeic acid, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid,  
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53 3, 5-dicaffeoylquinic acid and 3, 4-dicaffeoylquinic acid, and in negative ionization mode for  
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55 loganin, pinoresinol- $\beta$ -D-glucoside, isoforsythoside, forsythoside A and forsythoside B,  
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57 respectively. The conditions of MS analysis were designed as follows: Capillary voltage, 3.3 kV;  
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3 Source temperature, 150 °C; Desolvation temperature, 500 °C; Cone gas flow, 50 L·h<sup>-1</sup>; Desolvation  
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5 gas flow, 1000 L·h<sup>-1</sup>. The cone voltage (CV) and collision energy (CE) were set to match the MRM  
6  
7 of each marker. The dwell time was automatically set by the MassLynx software.

## 2.5. Validation of UPLC-MS/MS method

### 2.5.1. Calibration curves, limits of detection (LOD) and quantification (LOQ)

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12 A series of concentrations of standard solution were prepared for the establishment of  
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14 calibration curves. The peak areas were plotted against the corresponding concentrations to  
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16 obtain the calibration curves. LODs and LOQs were determined using diluted standard solution  
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18 when the signal-to-noise ratios (S/N) of analytes were about 3 and 10, respectively. The S/N was  
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20 calculated as the peak height divided by the background noise value.

### 2.5.2. Precision, repeatability, stability and matrix effect

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24 The intra-day and inter-day variations, which were chosen to determine the precision of the  
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26 developed method, were investigated by determining the 26 analytes in six replicates during a  
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28 single day and by duplication the experiments on three consecutive days. Variations of the peak  
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30 area were taken as the measures of precision and expressed as percentage relative standard  
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32 deviations (RSD).

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36 Repeatability was confirmed with six independent analytical sample solutions prepared from  
37  
38 the same batch of sample and variations were expressed by RSD.

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42 Stability was performed by analyzing the same sample solution and mixed standard stock  
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44 solution stored at 25 °C during the overall analytical process at 0, 2, 4, 8 and 12 h respectively. The  
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46 measurement was taken using the RSD of the peak area of each analyte.

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50 The matrix effect was evaluated by comparing the peak areas obtained from samples where  
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52 the matrix was spiked with standard solutions to those obtained from the pure reference  
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54 standard solutions at the same concentration.

### 2.5.3. Recovery

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58 A recovery test was used to evaluate the accuracy of this method. The test was performed  
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60 by adding known amounts of the standards at low (80% of the known amounts), medium (the

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3 same as the known amounts) and high (120% of the known amounts) levels. The spiked samples  
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5 were extracted, processed, and quantified in accordance with the aforementioned methods. The  
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7 average recovery percentage was calculated by the formula: recovery (%)=(observed  
8  
9 amount-original amount)/spiked amount  $\times$  100%.

## 11 **2.6. Identification and quantification**

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13 Identification of target peaks was performed by comparing their UPLC retention times, and  
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15 mass/charge ratios ( $m/z$ ) with those of the standards. In order to further confirm the structures  
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17 of the constituents, standards and samples were analyzed by UPLC-MS/MS in positive and  
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19 negative ion modes. Quantification was performed using linear calibration plots of peak areas  
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21 and concentration.  
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## 23 **2.7. Methods for pattern recognition analysis of samples**

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25 The data for chemical difference in different batches of *Flos Lonicerae Japonicae* - *Fructus*  
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27 *Forsythiae* herb couple in four preparations (Shuang-Huang-Lian oral liquid, Fufang Qin-Lan oral  
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29 liquid, Qing-Re-Jie-Du oral liquid and Yin-Qiao-Jie-Du tablet) was analyzed by HCA and PCA, which  
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31 has been extensively applied to the samples variation. Both HCA and PCA were done by SPSS 20.0  
32  
33 software. Between-group linkage method was applied, and squared euclidean distance was selected  
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35 as measurement.  
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## 37 **3. Results and discussion**

### 38 **3.1. Method development**

#### 39 **3.1.1. Optimization of mass spectrometry**

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41 The stock solutions of the analytes diluted with a mixture of methanol/water (60:40, v/v)  
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43 containing 0.1% formic acid were directly infused along with the mobile phase into the mass  
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45 spectrometer with electrospray ion source. In the MS full-scan model, as our previous report, the  
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47 most abundant ions were  $[M+H]^+$  for rutin, isoquercitrin, hyperoside, astragalin, luteoloside,  
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49 genistin, arctigenin, quercetin, luteolin, genistein, quinic acid, caffeic acid, neochlorogenic acid,  
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51 chlorogenic acid, cryptochlorogenic acid, 3, 5-dicaffeoylquinic acid and 3, 4-dicaffeoylquinic acid,  
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53  $[M+HCOO]^-$  for loganin and  $[M-H]^-$  for pinoresinol- $\beta$ -D-glucoside, forsythoside A, isoforsythoside  
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3 and forsythoside B. However, we found the most abundant ions were  $[M+Na]^+$  for macranthoidin  
4 B, dipsacoside B, arctiin and phillyrin. To date, there have been many literature reports [41, 44, 48]  
5 regarding the quantitative study of analytes using  $[M+H]^+$  in the positive mode and  $[M-H]^-$  in the  
6 negative mode, only a few for  $[M+Na]^+$  in the positive mode [49] owing to their response  
7 instability under the circumstance, but we found that the response showed high sensitivity,  
8 stability and linearity, probably as sodium ion source could be from the sodium formate added to  
9 the solution. The precursor→product ion pairs for MRM detection were generated by the  
10 intellistart procedure, which was embedded in the Masslynx software. For example, the  
11 macranthoidin B, dipsacoside B, arctiin and phillyrin were analyzed by multiple reaction  
12 monitoring (MRM) at m/z transitions of 1421.4→1097.6, 1097.2→347.1, 556.9→395.1 and  
13 556.9→309.1, respectively. The parameter for cone voltage was set as 90 V for macranthoidin B,  
14 90 V for dipsacoside B, 68 V for arctiin and 64 V for phillyrin. The parameter for collision energy  
15 was set as 78 V for macranthoidin B, 76 V for dipsacoside B, 34 V for arctiin and 30 V for phillyrin.  
16 Usually, longer dwell time can improve the sensitivity and accuracy, but the number of MS  
17 transitions channels at the same time increased can directly result in the dwell time decreased.  
18 Therefore, we can manually adjust continuously to MS transitions channels as the function of  
19 DMRM [41, 50-52] to control the dwell time. In short, it is necessary to ensure not only 12-15 points  
20 for each peak but also lots of particles arriving in MS detector in certain period which can be  
21 controlled by dwell time calculated by the MassLynx software. It was shown (Fig.SI2) that the dwell  
22 time for each analyte was more than 0.02 S, and the points for each peak were approximately 15,  
23 which can satisfy the quantification requirement.

### 24 3.1.2. Optimization of chromatography

25 Fig. SI2 showed chromatograms of the 26 reference compounds. No interfering peak was  
26 observed under the assay conditions. All analytes were eluted rapidly within 8 min. Isomers in six  
27 groups, including flavones containing isoquercitrin and hyperoside as isomer and astragaloside and  
28 luteoloside as isomer, phenylethanoid glycosides containing forsythoside A and isoforsythoside as  
29 isomer, and phenolic acids containing neochlorogenic acid, chlorogenic acid and  
30

1  
2  
3 cryptochlorogenic acid as isomer and 3, 5-dicaffeoylquinic acid and 3, 4-dicaffeoylquinic acid as  
4  
5 isomer, except lignans containing arctiin and phillyrin as isomer, had the same precursor and  
6  
7 product ions in mass spectrometry, respectively. Therefore, it was indispensable to separate the  
8  
9 isomers in the five groups with UPLC.

10  
11 It was studied [53] that high strength silica (HSS) T3 C<sub>18</sub> 1.8 μm bonded phase was designed  
12  
13 to retain and separated small water-soluble polar organic compounds, and we also reported  
14  
15 previously that the separations for flavones, isoflavones, phenolic acids and iridoids in *Flos*  
16  
17 *Lonicerae Japonicae* - *Fructus Forsythiae* herb couple *in vivo* were performed by HSS T3 C<sub>18</sub>  
18  
19 column. In the present study, we found (Fig. S12) that an Acquity UPLC HSS T3 C<sub>18</sub> column  
20  
21 (10mm×2.1mm, 1.8μm) elicited a suitable retention and a base-line separation for not only  
22  
23 flavones, isoflavones, phenolic acids and iridoids, but also phenylethanoid glycosides, lignans and  
24  
25 saponins. Besides, since flavones, isoflavones, phenylethanoid glycosides and phenolic acids  
26  
27 showed weak acidic property, formic acid in the UPLC mobile phase at a concentration of 0.4%  
28  
29 was added so as to overcome the peak-tailing effect, and improve the resolution for isomers in  
30  
31 five groups. Interestingly, the LOQ can satisfy the quantification requirements of  
32  
33 pinoresinol-β-D-glucoside, forsythoside A, isoforsythoside and forsythoside B, though the  
34  
35 response was significantly decreased because of ion suppression effect (Table 1).  
36  
37

38  
39 The results (Fig. S12) showed that the gradient elution of the mobile phase was suitable for  
40  
41 the flavones, isoflavones, phenylethanoid glycosides, phenolic acids, lignans, saponins and  
42  
43 iridoids separations, and the method described above could achieve symmetric peak shape, high  
44  
45 resolution ( $R_s > 1.5$ ) among peaks and short run time for the simultaneous analysis of the  
46  
47 twenty-six compounds.

### 48 49 **3.1.3. Optimization of sample preparation**

50  
51 To achieve the optimal extraction conditions, three important factors, namely, extraction  
52  
53 methods, extraction solvents, and extraction time which might influence the extraction efficiency  
54  
55 of the target constituents, were optimized. The different levels of each factor including extraction  
56  
57 solvent (50% methanol, 80% methanol and 100% methanol), extraction method (ultrasonic  
58  
59

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3 extraction versus heat reflux extraction), extraction time (10, 20 and 40 min) were investigated  
4  
5 individually by using univariate approach. The results revealed that for all the components in *Flos*  
6  
7 *Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in preparations, 50% methanol showed  
8  
9 similar extraction efficiencies with 80% methanol and 100% methanol. In addition, ultrasonic  
10  
11 extraction for 20 min was found to be adequate and appropriate for the analysis. The methods of  
12  
13 sample extraction procedure were consistent with the report described previously [46, 47].  
14

15  
16 In order to obtain more stable response of  $[M+Na]^+$  for macranthoidin B, dipsacoside B,  
17  
18 arctiin and phillyrin, each sample containing 0.5mM sodium formate as sodium ion source  
19  
20 enough provided was injected into the UPLC-ESI-MS/MS system for analysis. Actually, the use of  
21  
22 sodiated or other metal adducted ion surrogate has been reported previously [54-57]. The  
23  
24 problem associated with sodiated ion surrogates is it might have variations in matrix factor which  
25  
26 could potentially lead to inaccuracy in quantitation of analytes especially for the quantitative  
27  
28 study of analytes using  $[M+H]^+$  in the positive mode. However, it was shown (Table 1-3) the  
29  
30 method of twenty-six analytes determination exhibited high sensitivity, linearity, precisions,  
31  
32 stability and ignorable matrix effect.  
33

### 34 3.2. Analytical method validation

35  
36 The proposed UPLC-ESI-MS/MS method for quantitative analysis was validated by  
37  
38 determining the linearity, LOD, LOQ, intra-day and inter-day precisions, stability and accuracy. As  
39  
40 shown in Table 1, all calibration curves showed good linearity ( $r^2 > 0.9920$ ) within the test ranges,  
41  
42 and the overall LODs and LOQs were in the range of 0.01391-1.880 ng/mL and 0.04636-6.268  
43  
44 ng/mL, respectively. The RSD values of intra- and inter-day variations, repeatability and stability  
45  
46 of the 26 analytes were all less than 5.00% (Table 2, 3). The matrix effect of all the analytes was  
47  
48 found to be within the acceptable range, and all values were in the range from 95.0% to 105%  
49  
50 (Table 3). The overall recoveries of four preparations laid between 95.63% and 103.1% with RSD  
51  
52 less than 5.00% (Table 3). All the results mentioned above indicated that the established method  
53  
54 was accurate.  
55

### 56 3.3. Sample analysis

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2  
3 This newly developed and validated method was applied to *Flos Lonicerae Japonicae* -  
4  
5 *Fructus Forsythiae* herb couple in preparations (12 batches of Shuang-Huang-Lian oral liquid, 12  
6  
7 batches of Fufang Qin-Lan oral liquid, 12 batches of Qing-Re-Jie-Du oral liquid and 12 batches of  
8  
9 Yin-Qiao-Jie-Du tablet), and the quantification results are summarized in Table 4-7. Not  
10  
11 surprisingly, it was discovered that all of the samples contained a relatively high level of  
12  
13 chlorogenic acid, but different levels of phillyrin, and mostly met the Chinese Pharmacopeia  
14  
15 standards. Phenylethanoid glycosides (isoforsythoside and forsythoside A) and isochlorogenic  
16  
17 acid (3, 5-dicaffeoylquinic acid and 3, 4-dicaffeoylquinic acid) were the second and third highest  
18  
19 contents components among all of the analytes. We found in the previous study that the  
20  
21 pharmacological actions of these components are directly associated with those of the whole  
22  
23 preparation [26, 27], suggesting that if there were additional analytes that should be considered  
24  
25 for inclusion in the pharmacopeia standards, this compounds above would be a preference.  
26  
27

28 To evaluate the variation of *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in all  
29  
30 preparations, hierarchical cluster analysis (HCA) was performed on the basis of the contents of 26  
31  
32 tested compounds from UPLC-MS/MS profiles by SPSS 20.0 for windows (SPSS Inc., Chicago, IL,  
33  
34 USA). The results showed that 12 tested samples of Shuang-Huang-Lian oral liquid (Fig. 1a1),  
35  
36 Fufang Qin-Lan oral liquid (Fig. 1a2), Qing-Re-Jie-Du oral liquid (Fig. 1a3) and Yin-Qiao-Jie-Du  
37  
38 tablet (Fig. 1a4) were divided into two main clusters (a and b), five main clusters (a, b, c, d and e),  
39  
40 five main clusters (a, b, c, d and e) and two main clusters (a and b), respectively, which implied  
41  
42 that *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in Shuang-Huang-Lian oral liquid  
43  
44 and Yin-Qiao-Jie-Du tablet might have consistency better than that in Fufang Qin-Lan oral liquid  
45  
46 and Qing-Re-Jie-Du oral liquid. Moreover, principal components analysis (PCA) was further  
47  
48 performed to assess the variation for samples by SPSS 20.0 for windows. The first two principal  
49  
50 components (PC 1 and PC 2) with >85% of the whole variance, were extracted for analysis. The  
51  
52 scatter plot is shown in Fig. 1b, where each sample was represented as a marker. In the scatter  
53  
54 plot, it was noticeable that Shuang-Huang-Lian oral liquid and Yin-Qiao-Jie-Du tablet (Fig. 1b1,  
55  
56 1b4) were all clearly clustered into two domains, and it was seen (Fig. 1b1) that domain a, which  
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3 was manufactured from 2009 year, was clearly different from the domain b, and subgroups A  
4  
5 divided from domain b, produced from 2010 year, had also difference, compared with subgroups  
6  
7 B, manufactured from 2013 and 2012 years. Besides, we also found (Fig. 1b4) that most of the  
8  
9 samples (Yin-Qiao-Jie-Du tablet) were located in domain a except for S2, which was divided  
10  
11 clearly subgroups again (A and B). The results above indicated that *Flos Lonicerae Japonicae* -  
12  
13 *Fructus Forsythiae* herb couple in the products (Shuang-Huang-Lian oral liquid and  
14  
15 Yin-Qiao-Jie-Du tablet) between 2012 and 2013 year had mostly good consistency. In addition,  
16  
17 the samples (Fufang Qin-Lan oral liquid) (Fig. 1b2) were clearly clustered into five domains, and  
18  
19 S5 was in domain b, S4 was in domain c, S2 was in domain d, S7 was in domain e and mostly were  
20  
21 in domain a from 2012 year, which indicated that *Flos Lonicerae Japonicae* - *Fructus Forsythiae*  
22  
23 herb couple in the products from 2012 year, qualitatively, also had relatively good consistency.  
24  
25 However, there existed significantly difference (Fig. 1b3) of *Flos Lonicerae Japonicae* -*Fructus*  
26  
27 *Forsythiae* herb couple in Qing-Re-Jie-Du oral liquid of different batches, which due to their  
28  
29 instability during the process of production or storage, influenced possibly by the fact that  
30  
31 proportion of *Flos Lonicerae Japonicae* and *Fructus Forsythiae* in Qing-Re-Jie-Du oral liquid as a  
32  
33 complex TCM preparation were relatively low, compared with other herbs, like *Gypsum Fibrosum*  
34  
35 as mineral drug. Generally, all results above (Fig. 1b) from PCA were almost consistent with those  
36  
37 obtained by HCA (Fig. 1a), which indicated that UPLC-MS/MS method combined with HCA and  
38  
39 PCA might be suitable for evaluating quality of TCMs.  
40  
41

#### 42 4. Conclusion

43  
44 In this study, a simple and accurate UPLC-ESI-MS/MS method was developed, for the first  
45  
46 time, to determine the flavones, isoflavones, organic acids, saponins, iridoids, phenylethanoid  
47  
48 glycosides and lignans in *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in the  
49  
50 preparations (Shuang-Huang-Lian oral liquid, Qin-Re-Jie-Du oral liquid, Yin-Qiao-Jie-Du tablet and  
51  
52 Fufang Qin-Lan oral liquid) simultaneously with run time of only 8 min. In addition, this method  
53  
54 could distinguish *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in the preparations  
55  
56 from different batches based on the quantified measurement of 26 analytes and ensuring the  
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3 quality of the main herbs (*Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple) in different  
4  
5 batches' preparations by chemometric analysis.  
6

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9 **Figure legends**

10 **Fig.1** (a) Dendrograms of hierarchical cluster analysis, and (b) the scatter plot obtained by  
11 principal components analysis for *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in  
12 four preparations (**1**: 12 batches of *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple in  
13 Shuang-Huang-Lian oral liquid; **2**: 12 batches of *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb  
14 couple in Fufang Qin-Lan oral liquid; **3**: 12 batches of *Flos Lonicerae Japonicae* - *Fructus*  
15 *Forsythiae* herb couple in Qing-Re-Jie-Du oral liquid; **4**: 12 batches of *Flos Lonicerae Japonicae* -  
16 *Fructus Forsythiae* herb couple of Yin-Qiao-Jie-Du tablet).  
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46 **Table1** Calibration curves, LOD and LOQ data of investigated compounds by UPLC-MS/MS

No.	Analytes	Calibration curves	$r^2$	Linear range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
47 01	Forsythoside B	$y = 3747.3x - 6.2461$	0.9999	8.827-282.5	0.6621	2.207
48 02	Loganin	$y = 1778.3x - 3.8576$	0.9998	7.193-230.2	0.5394	1.798
49 03	Macranthoidin B	$y = 447.92x - 9.4353$	0.9994	9.390-4908	1.409	4.695
50 04	Dipsacoside B	$y = 16164x - 273.34$	0.9999	3.794-3885	0.2844	0.9480
51 05	Rutin	$y = 43637x + 410.05$	0.9971	18.17-581.7	0.04260	0.1420
52 06	Arctiin	$y = 12671x + 73.183$	0.9998	4.301-1101	0.1614	0.5380
53 07	Phillyrin	$y = 759.31x + 113.02$	0.9952	44.17-1413	1.6563	5.521
54 08	Pinoresinol- $\beta$ -D-glucoside	$y = 9133.4x + 232.35$	0.9983	35.16-1125	0.6591	2.197

09	3, 5-dicaffeoylquinic acid	$y = 38033x - 181.41$	0.9992	7.587-1942	0.1422	0.4740
10	3, 4-dicaffeoylquinic acid	$y = 49190x - 5682.4$	0.9987	160.5-5135	1.880	6.268
11	Isoquercitrin	$y = 183684x - 109.97$	0.9994	1.286-82.33	0.09660	0.3220
12	Hyperoside	$y = 15911x + 86.107$	0.9995	55.74-3567	0.2613	0.8710
13	Astragalin	$y = 117820x - 36.196$	0.9994	0.6430-41.17	0.1929	0.6430
14	Luteoloside	$y = 141560x + 3683.9$	0.995	2.592-1327	0.04860	0.1620
15	Genistin	$y = 239372x - 42.399$	0.9998	0.4840-61.90	0.01813	0.06045
16	Arctigenin	$y = 94897x - 76.065$	0.9998	1.092-558.9	0.08190	0.2730
17	Neochlorogenic acid	$y = 92138x + 22702$	0.9928	119.0-7615	0.03480	0.1160
18	Chlorogenic acid	$y = 109779x + 47690$	0.9983	261.41-16730	0.03840	0.1280
19	Cryptochlorogenic acid	$y = 89445x + 8775.4$	0.9988	158.6-5077	0.04650	0.1550
20	Quercetin	$y = 14186x - 4.2639$	0.9979	0.1850-23.74	0.01391	0.04636
21	Luteolin	$y = 52973x - 55.902$	0.9987	0.5490-70.31	0.04110	0.1370
22	Genistein	$y = 54407x - 8.7139$	0.9985	2.573-329.3	0.04830	0.1610
23	Quinic acid	$y = 12182x + 2142.9$	0.9969	57.54-7365	0.1350	0.4500
24	Caffeic acid	$y = 36495x + 669.51$	0.9921	16.68-533.7	0.03900	0.1300
25	Isoforsythoside	$y = 2933.2x - 109.34$	0.9982	60.10-1923	1.127	3.756
26	Forsythoside A	$y = 6245.7x + 380.61$	0.9997	101.9-6519	0.9549	3.183

Table2 Precision levels of the 26 analytes in *Flos Lonicerae Japonicae - Fructus Forsythiae* herb couple

No.	Analytes	Precision (RSD, %)	
		Intra-day (n=6)	Inter-day (n=3)
01	Forsythoside B	4.82	1.09
02	Loganin	4.79	1.93
03	Macranthoidin B	3.67	4.63
04	Dipsacoside B	3.09	3.85
05	Rutin	4.76	3.20
06	Arctiin	2.43	3.10

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07	Phillyrin	3.05	3.66
08	Pinoresinol- $\beta$ -D-glucoside	3.16	2.53
09	3, 5-dicaffeoylquinic acid	4.31	3.90
10	3, 4-dicaffeoylquinic acid	1.89	0.694
11	Isoquercitrin	2.37	2.99
12	Hyperoside	1.71	2.65
13	Astragalin	4.78	0.502
14	Luteoloside	1.28	0.916
15	Genistin	1.72	4.29
16	Arctigenin	2.79	0.440
17	Neochlorogenic acid	2.56	4.75
18	Chlorogenic acid	2.62	4.36
19	Cryptochlorogenic acid	2.54	4.09
20	Quercetin	4.23	1.97
21	Luteolin	4.47	3.13
22	Genistein	1.71	4.55
23	Quinic acid	2.83	1.16
24	Caffeic acid	2.05	1.18
25	Isoforythoside	4.81	2.11
26	Forsythoside A	3.29	2.12

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Table 3 repeatability, stability and recovery levels of the 26 analytes in *Flos Lonicerae Japonicae* - *Fructus Forsythiae* herb couple

No.	Analytes	Repeatability (RSD, %, n=6)				Stability (RSD, %, n=6)				Matrix effect (% n=6)	Recovery (% n=3)				
		SHL	QL	QRJD	YQJD	SHL	QL	QRJD	YQJD	Mixed standard stock solution	SHL	QL	QRJD	YQJD	
01	Forsythoside B	4.41	3.97	1.77	4.04	2.52	4.52	4.93	2.34	1.64	102.60±3.83	99.50±2.15	98.01±2.54	98.77±3.29	100.20±3.30
02	Loganin	2.77	2.32	3.73	1.24	2.54	4.65	3.70	4.14	4.69	97.68±4.18	99.63±2.88	99.12±2.93	97.58±2.18	98.79±2.75
03	Macranthoidin B	4.63	4.35	2.43	4.02	3.93	1.78	4.86	4.22	0.40	101.03±4.83	98.11±1.81	100.50±4.36	97.81±2.57	97.59±0.26
04	Dipsacoside B	2.57	4.28	1.00	4.21	4.24	4.23	4.80	4.19	4.52	96.19±3.71	101.70±2.94	99.44±1.69	97.81±2.57	98.90±4.75
05	Rutin	4.80	3.95	3.75	3.32	4.28	2.42	4.04	3.13	4.58	98.73±4.94	100.90±3.53	101.60±2.43	98.06±1.72	100.80±4.79
06	Arctiin	2.10	4.44	4.25	2.56	3.97	4.19	4.70	4.74	0.43	97.09±2.25	99.33±2.95	101.00±4.98	99.95±4.20	99.61±3.32
07	Phillyrin	3.41	2.12	4.06	1.70	3.63	3.12	3.03	2.93	4.15	98.88±4.42	101.20±3.77	101.40±4.04	97.44±3.17	97.74±1.27
08	Pinoresinol-β-D-glucoside	4.58	2.00	4.05	3.11	4.66	4.59	4.58	4.78	3.59	98.19±3.77	100.80±2.56	102.30±0.99	100.40±4.06	97.20±1.82
09	3, 5-dicaffeoylquinic acid	4.21	4.73	1.73	1.38	4.62	4.48	4.02	4.87	2.66	98.56±3.23	98.80±4.13	102.50±0.35	100.80±1.74	99.70±4.07
10	3, 4-dicaffeoylquinic acid	3.89	4.78	2.98	2.72	4.64	4.09	4.10	4.72	2.22	99.34±1.83	96.46±0.60	99.38±2.33	98.71±2.08	100.90±3.39
11	Isoquercitrin	2.54	4.73	3.34	2.60	4.01	3.50	1.21	4.29	3.63	99.56±1.56	96.39±1.71	99.99±4.52	100.90±3.99	98.31±2.55
12	Hyperoside	2.31	4.02	4.97	3.67	3.83	4.16	4.38	4.85	4.93	99.54±2.54	98.55±2.46	100.30±3.28	99.24±0.86	103.10±0.15
13	Astragalin	3.06	4.26	2.74	2.85	4.22	4.92	4.83	3.27	0.94	98.58±3.89	98.33±1.14	99.45±3.81	97.65±1.34	101.80±1.87
14	Luteoloside	3.67	2.94	2.42	2.41	3.37	3.23	3.98	2.48	4.20	99.87±1.93	100.30±3.89	100.90±0.51	101.30±1.85	99.79±1.88
15	Genistin	2.05	3.60	3.43	4.89	3.40	4.85	3.05	3.55	1.52	99.51±4.43	102.20±2.42	101.10±2.16	102.70±2.58	98.38±0.90
16	Arctigenin	1.92	3.77	3.52	3.44	4.74	4.85	4.89	4.95	1.44	99.36±3.07	99.49±3.22	99.22±3.43	98.59±1.82	99.32±4.43
17	Neochlorogenic acid	3.58	3.52	1.42	2.07	4.98	4.48	4.78	4.91	1.35	97.39±3.84	95.63±0.43	100.60±2.95	100.40±3.28	100.70±4.64
18	Chlorogenic acid	2.88	4.80	3.68	1.30	4.55	4.28	3.90	3.69	1.58	99.39±0.99	98.64±0.55	100.00±1.73	102.00±2.06	97.32±0.61
19	Cryptochlorogenic acid	4.58	3.03	1.11	1.16	4.84	4.72	4.23	4.47	3.74	98.71±4.25	99.21±3.58	99.79±1.85	100.80±2.48	99.32±0.76

20	Quercetin	3.58	4.88	4.45	4.37	2.51	4.66	4.61	2.75	3.62	99.45±2.08	102.10±2.29	98.30±2.83	100.70±2.78	99.05±2.39
21	Luteolin	4.95	2.19	4.95	4.14	2.72	4.67	4.64	1.53	1.58	98.81±3.12	98.35±3.78	97.69±1.55	96.62±2.42	100.50±3.35
22	Genistein	2.30	2.11	4.74	4.78	1.92	2.61	3.91	4.68	3.26	98.67±4.19	98.11±0.81	95.99±0.29	100.00±2.39	98.42±0.56
23	Quinic acid	3.02	2.35	3.00	1.52	2.26	3.93	4.65	4.81	4.32	96.89±2.22	98.80±3.44	96.91±1.92	99.32±2.25	101.50±4.28
24	Caffeic acid	3.47	1.83	4.39	3.02	4.97	3.79	4.92	4.78	4.38	97.09±1.24	99.25±1.94	102.50±0.98	100.90±2.55	101.00±2.37
25	Isoforythoside	3.30	4.20	4.66	3.45	3.82	4.68	4.93	1.96	3.63	102.72±4.93	98.81±3.17	99.29±4.08	100.30±3.82	99.37±3.32
26	Forsythoside A	3.81	2.54	1.07	3.52	4.56	3.23	4.71	4.83	2.84	98.27±3.08	98.81±2.11	101.30±1.89	102.10±1.39	98.44±0.23



Table4 The results of 12 batches of sample analysis of Shuang-Huang-Lian oral liquid (μg/mL)

No	Compoud	S1(13021633)	S2(13012063)	S3(13021622)	S4(13010461)	S5(13010835)	S6(12122851)	S7(12020715)	S8(12012035)	S9(12022231)	S10(12012015)	S11(10080188)	S12(09090221)
01	Forsythoside B	16.09	12.22	16.84	12.89	12.13	12.22	15.64	15.56	20.71	23.29	15.41	19.20
02	Loganin	5.115	10.176	6.708	16.27	19.64	15.99	8.020	12.05	8.020	11.21	35.20	11.77
03	Macranthoidin B	41.04	15.37	49.23	14.25	14.63	17.23	38.44	16.11	32.11	14.25	71.18	1035
04	Dipsacoside B	117.6	90.85	40.05	10.04	133.1	10.14	44.39	95.15	137.2	89.19	89.52	257.2
05	Rutin	178.6	103.5	162.7	134.1	150.6	145.6	167.7	127.9	236.2	179.9	85.56	64.09
06	Arctiin	10.50	8.174	9.213	7.293	6.833	7.595	9.713	6.412	7.398	7.293	8.119	6.460
07	Phillyrin	557.5	572.9	643.6	610.4	510.1	626.0	613.5	589.1	635.4	561.9	626.6	508.2
08	Pinoresinol-β-D-glucoside	91.97	103.2	118.6	70.76	63.63	75.16	103.1	105.3	92.04	105.3	105.7	187.7
09	3, 5-dicaffeoylquinic acid	82.74	81.60	76.98	53.89	118.2	54.41	77.20	82.81	103.8	93.15	55.34	147.7
10	3, 4-dicaffeoylquinic acid	364.0	339.0	298.8	240.1	494.1	246.2	306.2	343.0	433.5	367.6	298.7	640.4
11	Isoquercitrin	4.470	6.073	5.284	3.218	4.313	3.361	5.594	6.948	3.496	8.308	2.153	1.857
12	Hyperoside	253.5	149.1	247.3	183.2	206.6	201.3	234.6	186.5	343.8	255.3	128.9	93.76
13	Astragaln	2.584	4.206	4.072	0.9373	2.741	1.124	4.066	4.635	1.722	5.113	1.074	0.4475
14	Luteoloside	178.3	254.4	797.2	59.01	471.7	60.90	747.7	347.1	202.1	623.1	502.7	280.7
15	Genistin	11.00	7.933	25.14	0.8100	11.86	0.8997	23.35	10.18	12.05	16.08	11.30	6.197
16	Arctigenin	1.548	1.319	1.395	1.400	1.448	1.411	1.335	1.498	1.916	1.657	1.417	1.318
17	Neochlorogenic acid	916.1	869.6	1038.4	892.4	891.6	870.3	1061	956.7	1143	1080	896.3	1112
18	Chlorogenic acid	782.3	733.1	888.5	784.6	805.2	818.7	919.2	818.7	1009	958.8	760.5	993.8
19	Cryptochlorogenic acid	726.9	674.7	810.5	665.0	693.3	678.5	804.2	737.0	890.1	836.6	703.9	896.0
20	Quercetin	1.100	0.4400	1.700	1.090	1.380	0.8000	1.720	0.9400	1.010	1.090	0.3600	0.3900

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21	Luteolin	1.050	4.670	10.86	1.240	7.270	1.160	9.590	4.970	0.8800	8.150	3.020	4.290
22	Genistein	39.93	40.34	41.55	41.62	39.14	38.74	40.34	40.65	40.61	41.36	35.50	36.18
23	Quinic acid	314.7	317.3	241.7	497.3	406.4	482.8	237.3	337.4	434.4	338.3	446.8	585.6
24	Caffeic acid	71.58	67.77	67.34	47.63	51.26	51.46	56.18	68.83	75.02	78.35	143.5	171.7
25	Isoforythoside	333.5	316.1	378.7	268.1	240.1	277.3	329.6	321.0	343.9	276.8	377.2	579.2
26	Forsythoside A	608.0	579.3	684.8	440.1	439.9	478.4	575.9	580.9	666.1	541.5	778.0	1191

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Table5 The results of 12 batches of sample analysis of Fufang Qin-Lan oral liquid ( $\mu\text{g/mL}$ )

No.	Compound	S1(20120506)	S2(20120508)	S3(12021102)	S4(20120803)	S5(B20130106)	S6(20120303)	S7(B20130401)	S8(20120206)	S9(20120403)	S10(20120109)	S11(20120716)	S12(20120910)
01	Forsythoside B	8.762	15.13	7.981	8.722	15.72	4.809	27.14	10.07	10.76	11.08	15.39	15.79
02	Loganin	4.834	6.146	5.865	3.803	7.645	3.990	11.49	4.834	6.239	6.708	7.083	7.177
03	Macranthoidin B	1142	1166	1152	19.38	796.0	940.4	17.43	854.7	1227	1012	899.9	1248
04	Dipsacoside B	374.8	394.7	387.7	11.77	256.3	305.9	10.74	256.0	404.4	338.1	258.3	404.3
05	Rutin	71.90	146.2	53.73	101.9	78.12	49.56	29.06	86.93	64.42	64.54	111.8	96.47
06	Arctiin	17.94	16.95	18.14	11.30	9.940	13.38	10.80	15.02	17.96	13.49	14.85	16.41
07	Phillyrin	795.2	909.4	680.2	784.4	681.6	627.2	677.8	793.8	827.7	766.5	840.7	832.2
08	Pinoresinol- $\beta$ -D-glucoside	293.4	342.7	264.4	193.3	216.4	213.3	265.9	281.0	344.2	285.1	305.9	374.0
09	3, 5-dicaffeoylquinic acid	142.7	193.2	124.3	10.04	121.8	87.37	48.23	84.47	144.6	125.3	91.66	163.0
10	3, 4-dicaffeoylquinic acid	573.5	755.1	521.5	88.38	500.3	366.7	211.9	330.5	529.0	489.1	349.2	590.9
11	Isoquercitrin	2.270	3.956	1.920	1.563	3.239	1.686	1.924	1.858	2.053	2.573	2.547	2.795
12	Hyperoside	100.9	205.7	77.20	154.3	111.5	75.17	42.69	126.9	95.01	104.7	158.2	138.4
13	Astragaln	0.5797	1.148	0.5269	0.3767	0.9939	0.4777	0.5493	0.4809	0.6185	0.7432	0.6091	0.7867
14	Luteoloside	17.37	24.91	17.27	10.28	22.26	12.66	66.02	14.09	17.41	18.53	15.18	19.03
15	Genistin	0.4477	0.6716	0.4765	0.1805	0.4135	0.3094	0.7316	0.3303	0.4350	0.4003	0.3961	0.4916
16	Arctigenin	2.728	3.772	2.3079	1.956	1.362	2.015	1.524	2.516	2.688	1.762	2.897	3.332
17	Neochlorogenic acid	791.4	1014	753.4	234.6	584.4	5112.0	343.9	549.4	816.6	699.1	618.4	917.4
18	Chlorogenic acid	693.1	914.1	633.5	130.0	450.5	388.4	220.8	439.4	709.6	583.3	503.7	810.1
19	Cryptochlorogenic acid	661.4	826.9	612.6	212.7	478.5	417.4	282.5	454.4	662.6	570.7	494.7	734.6
20	Quercetin	0.3494	0.8700	0.4159	0.4271	1.169	0.1853	0.3693	0.4549	0.3450	0.7330	0.6262	0.4169
21	Luteolin	0.8311	1.582	0.6681	0.5676	0.9856	0.7249	0.9604	0.7540	0.6773	0.6866	0.7835	0.8846
22	Genistein	48.14	48.91	43.69	44.34	46.74	44.52	41.95	45.75	46.00	45.47	42.08	42.64

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23	Quinic acid	178.2	226.1	185.6	33.20	73.91	103.1	147.1	88.16	213.2	159.6	125.7	235.7
24	Caffeic acid	61.04	75.24	44.66	26.20	159.3	25.06	186.1	50.16	62.78	112.4	52.00	66.96
25	Isoforsythoside	220.8	316.6	152.8	133.9	354.7	125.5	577.8	218.9	229.7	300.9	265.2	281.7
26	Forsythoside A	404.7	583.4	270.9	228.3	773.0	196.9	1165	370.2	463.6	616.3	522.4	555.2

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Table 6 The results of 12 batches of sample analysis of Qing-Re-Jie-Du oral liquid ( $\mu\text{g/mL}$ )

No.	Compound	S1(13261451)	S2(13261454)	S3(13261452)	S4(13260888)	S5(13260890)	S6(13260510)	S7(13260511)	S8(13260110)	S9(13260311)	S10(13260790)	S11(13260570)	S12(13260410)
01	Forsythoside B	22.40	16.41	14.51	48.75	44.41	47.82	14.76	20.68	20.93	35.58	35.58	15.03
02	Loganin	13.92	15.33	11.77	18.70	15.33	19.92	29.67	33.14	32.20	36.04	31.83	29.67
03	Macranthoidin B	1058	1181	1306	974.7	1040	1273	1290	1122	1234	1176	1201	1371
04	Dipsacoside B	185.2	196.1	246.2	187.2	204.8	247.6	252.3	207.1	227.4	216.6	235.3	263.2
05	Rutin	40.42	29.85	22.74	41.44	37.00	23.22	24.61	38.21	33.99	32.21	32.69	23.33
06	Arctiin	1.067	0.7678	0.7211	0.9319	1.483	0.9227	0.9115	0.4729	0.2883	0.7331	1.142	2.249
07	Phillyrin	17.52	11.21	9.992	40.51	25.03	20.17	0.2261	22.68	22.27	15.71	13.94	21.98
08	Pinoselinol- $\beta$ -D-glucoside	64.62	50.30	41.21	110.8	109.1	44.89	46.33	71.37	62.35	83.60	85.59	46.24
09	3, 5-dicaffeoylquinic acid	235.9	189.2	193.4	194.3	177.7	173.9	330.0	518.7	677.2	587.4	174.8	444.2
10	3, 4-dicaffeoylquinic acid	547.2	453.1	447.1	483.2	453.8	395.9	432.0	464.9	459.9	408.2	423.8	386.9
11	Isoquercitrin	8.940	7.252	6.796	6.591	5.866	6.649	7.282	8.523	7.931	6.637	6.627	6.596
12	Hyperoside	61.42	46.33	38.43	56.75	63.30	38.55	39.19	58.34	47.21	50.14	50.68	36.92
13	Astragaln	3.793	3.401	4.004	2.032	2.154	3.684	4.232	3.907	3.931	2.980	3.048	3.777
14	Luteoloside	113.6	82.39	47.27	59.99	58.61	45.42	49.01	102.1	82.16	55.05	55.37	46.31
15	Genistin	3.852	2.976	2.219	2.602	2.488	2.175	2.431	3.829	3.332	2.565	2.706	2.504
16	Arctigenin	1.790	1.750	1.562	1.851	1.951	1.582	1.311	1.855	1.782	1.770	1.931	1.720
17	Neochlorogenic acid	941.9	804.7	835.2	739.4	744.5	796.8	821.0	862.6	815.7	688.3	733.4	744.3
18	Chlorogenic acid	897.2	758.9	775.4	740.3	706.2	748.5	785.2	815.4	755.7	673.6	672.7	671.4
19	Cryptochlorogenic acid	754.6	655.5	655.8	597.5	616.8	649.9	674.2	694.5	653.7	580.4	601.5	598.6
20	Quercetin	3.070	1.820	2.440	1.655	1.389	1.407	1.743	2.784	3.253	1.826	2.051	2.156
21	Luteolin	4.353	2.789	1.601	1.812	1.676	1.154	1.666	3.347	2.849	1.590	1.658	1.553
22	Genistein	3.805	2.281	1.125	1.331	1.198	0.6898	1.189	2.825	2.340	1.115	1.181	1.079
23	Quinic acid	306.1	280.6	286.4	292.0	309.0	298.5	307.2	302.5	298.4	310.4	322.2	315.6

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24	Caffeic acid	33.87	26.99	25.54	21.36	19.96	24.27	26.63	30.58	28.11	23.10	23.91	24.60
25	Isoforsythoside	163.6	141.1	129.8	128.9	121.5	134.7	136.7	158.9	155.9	127.2	136.4	127.1
26	Forsythoside A	257.2	210.1	196.6	209.3	195.8	213.0	225.1	268.8	268.0	273.3	233.9	199.6

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Table 7 The results of 12 batches of sample analysis of Yin-Qiao-Jie-Du tablet ( $\mu\text{g}/\text{tablet}$ )

No.	Compound	S1(12120753)	S2(13120748)	S3(12120752)	S4(12120749)	S5(13122020)	S6(13122022)	S7(12120751)	S8(13120150)	S9(13122028)	S10(13120314)	S11(13120140)	S12(12120741)
01	Forsythoside B	19.58	24.90	21.58	19.25	20.98	18.37	22.03	21.27	19.20	77.93	18.91	22.86
02	Loganin	33.46	16.21	37.57	31.05	38.18	33.49	41.25	34.58	37.32	33.56	35.17	46.12
03	Macranthoidin B	1485	2002	1487	1566	1509	1568	1404	1405	1336	1448	1866	1498
04	Dipsacoside B	186.0	308.3	204.1	185.0	196.9	197.7	177.5	166.8	173.2	173.9	191.0	192.8
05	Rutin	177.8	399.2	192.9	162.2	171.8	164.3	174.6	158.6	157.9	711.7	336.5	186.6
06	Arctiin	412.0	338.2	449.0	490.5	315.5	301.3	459.1	446.0	295.2	401.4	408.3	498.7
07	Phillyrin	104.5	100.2	110.6	73.60	114.8	137.2	101.8	108.7	147.2	108.1	108.8	112.6
08	Pinoresinol- $\beta$ -D-glucoside	230.4	246.3	241.3	202.5	243.1	218.9	233.2	212.2	216.6	220.0	256.4	227.4
09	3, 5-dicaffeoylquinic acid	61.93	43.08	24.13	26.68	30.25	37.58	51.36	56.05	49.74	53.16	53.93	67.94
10	3, 4-dicaffeoylquinic acid	600.7	690.5	636.2	624.2	622.4	579.4	618.9	557.6	580.2	623.0	729.3	680.1
11	Isoquercitrin	30.14	26.01	33.25	33.20	31.87	31.44	31.64	30.02	33.27	32.05	34.94	35.58
12	Hyperoside	214.1	560.6	227.5	182.8	198.7	206.0	221.2	200.4	197.3	224.8	433.4	241.9
13	Astragaln	11.62	11.73	12.38	12.00	11.97	11.86	11.60	10.98	11.60	11.19	12.37	12.28
14	Luteoloside	236.4	549.3	251.1	260.1	237.7	226.8	249.4	221.8	219.4	266.1	231.9	290.0
15	Genistin	5.816	4.931	6.053	5.636	4.883	4.366	5.326	4.838	4.140	5.582	5.529	5.763
16	Arctigenin	146.2	426.3	158.1	128.3	398.6	356.6	159.9	147.9	350.8	146.3	334.0	155.9
17	Neochlorogenic acid	83.98	133.5	102.9	106.1	80.46	75.89	112.7	101.1	83.30	92.80	128.8	118.8
18	Chlorogenic acid	4606	3513	5033	4773.	4894	4916	5013.	$2142 \times 10^{-1}$	4934	4765	4468	5179
19	Cryptochlorogenic acid	83.98	133.5	102.9	106.1	80.46	75.89	112.7	101.1	83.30	92.80	128.8	118.8
20	Quercetin	7.350	34.33	8.593	5.744	7.766	7.505	5.939	6.986	7.293	7.744	21.32	9.552
21	Luteolin	19.50	12.56	20.52	18.32	18.74	18.40	18.25	17.14	17.77	16.98	15.58	18.09
22	Genistein	18.21	11.47	19.19	17.08	17.47	17.14	17.01	15.91	16.55	15.78	14.38	16.81
23	Quinic acid	1592	1055	1603	1600	1537	1619	1746	1689	1576	1618	1490	1869

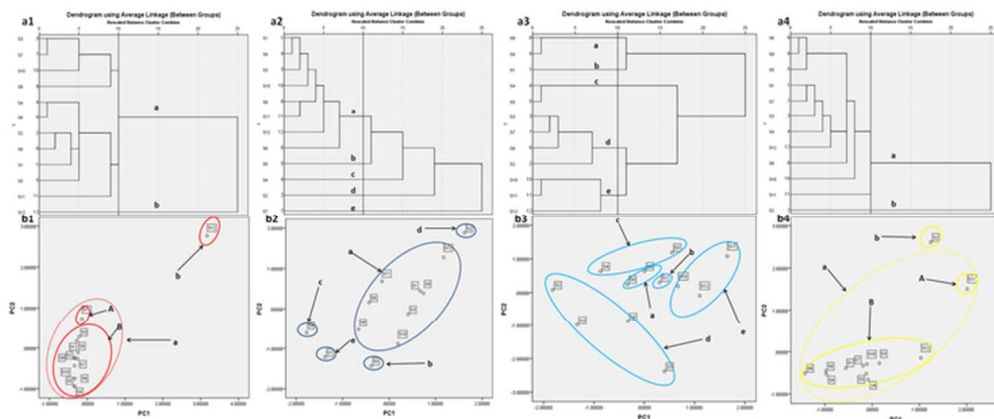
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24	Caffeic acid	75.40	32.85	82.77	66.99	49.46	49.83	63.01	78.48	63.54	70.95	66.74	93.45
25	Isoforythoside	813.8	876.8	818.8	626.8	723.9	666.2	698.8	686.0	612.2	700.2	747.0	699.6
26	Forsythoside A	1670	1738	1752	1462	1610	1499	1659	1544	1456	1591	1839	1683

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