A Modified QuEChERS Method Coupled with High Resolution LC-Q-TOF-Mass Spectrometry for Extraction, Identification and Quantification of Isoflavones in the Soybeans

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SUPPORTING INFORMATION

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Fig. S2 IDA-MS spectrum of six authentic isoflavones
Fig. S3 The trend chart of range value for daidzin concentration
Fig. S4 The trend chart of range value for daidzein concentration
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Equations. The equations of RSM design
**Fig. S1** Extracted ion chromatograms of six authentic isoflavone compounds

![Extracted ion chromatograms of six authentic isoflavone compounds](image_url)

**Fig. S2** IDA-MS spectrum of six authentic isoflavones

- **Daidzin**
- **Glycitin**

![IDA-MS spectrum of six authentic isoflavones](image_url)
Genistin

- Spectrum from 05.wiff (sample 1) - d...TOF MS^2 (50 - 1000) from 6.134 min
- Precursor: 433.1 Da, CE: 35.0 CE=35
- Library Spectrum: ranliaomugan, CE=35±15

Daidzein

- Spectrum from 05.wiff (sample 1) - d...TOF MS^2 (50 - 1000) from 8.097 min
- Precursor: 285.1 Da, CE: 35.0 CE=35
- Library Spectrum: huangdouhuangsu-2, CE=35±15

Glycitin

- Spectrum from 05.wiff (sample 1) - d...TOF MS^2 (50 - 1000) from 8.999 min
- Precursor: 271.1 Da, CE: 35.0 CE=35
- Library Spectrum: ranliaomusu, CE=35±15

Genistein
Fig. S3  The trend chart of range value for daidzin concentration

Fig. S4  The trend chart of range value for daidzein concentration
<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compounds</th>
<th>Formula</th>
<th>Ions</th>
<th>Theoretical (m/z)</th>
<th>Experimental (m/z)</th>
<th>Error (ppm)</th>
<th>retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Daidzin</td>
<td>C_{21}H_{20}O_{9}</td>
<td>[M+H]^+</td>
<td>417.11801</td>
<td>417.11830</td>
<td>0.7</td>
<td>6.20</td>
</tr>
<tr>
<td>2</td>
<td>Glycitin</td>
<td>C_{22}H_{22}O_{10}</td>
<td>[M+H]^+</td>
<td>447.12857</td>
<td>447.12892</td>
<td>0.8</td>
<td>6.35</td>
</tr>
<tr>
<td>3</td>
<td>Genistin</td>
<td>C_{21}H_{20}O_{10}</td>
<td>[M+H]^+</td>
<td>433.11292</td>
<td>433.11296</td>
<td>0.1</td>
<td>7.32</td>
</tr>
<tr>
<td>4</td>
<td>Daidzein</td>
<td>C_{15}H_{10}O_{4}</td>
<td>[M+H]^+</td>
<td>255.06519</td>
<td>255.06540</td>
<td>0.9</td>
<td>8.68</td>
</tr>
<tr>
<td>5</td>
<td>Glycitein</td>
<td>C_{16}H_{12}O_{5}</td>
<td>[M+H]^+</td>
<td>285.07575</td>
<td>285.07601</td>
<td>0.9</td>
<td>8.82</td>
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<tr>
<td>6</td>
<td>Genistein</td>
<td>C_{15}H_{10}O_{5}</td>
<td>[M+H]^+</td>
<td>271.06010</td>
<td>271.06018</td>
<td>0.3</td>
<td>9.73</td>
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</table>

**Table S2** The results of analysis of range for daidzin concentration

<table>
<thead>
<tr>
<th>T value: 1.0e+003*</th>
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<tbody>
<tr>
<td>1.5588 1.2544 1.0280 1.0870 1.1460</td>
</tr>
<tr>
<td>1.0557 1.0818 1.3237 1.2582 1.1887</td>
</tr>
<tr>
<td>0.9843 1.0780 1.4054 1.0823 1.2027</td>
</tr>
<tr>
<td>1.0090 1.1934 0.8507 1.1803 1.0703</td>
</tr>
<tr>
<td>R value: 574.48 176.41 554.67 175.96 132.37</td>
</tr>
<tr>
<td>optimal level: 1 1 3 2 3</td>
</tr>
<tr>
<td>order: A; C; B; D</td>
</tr>
</tbody>
</table>
### Table S3 The Results of Analysis of Variance for Daidzin

<table>
<thead>
<tr>
<th>sources of variance</th>
<th>sum of squares</th>
<th>freedom</th>
<th>Standard deviation</th>
<th>F value</th>
<th>$F^*$</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>factor 1</td>
<td>5.5830e+004</td>
<td>3</td>
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<td>14.0456</td>
<td>4.7571;9.7795</td>
<td>high significance</td>
</tr>
<tr>
<td>factor 2</td>
<td>5.6590e+003</td>
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<td>1.8836e+003</td>
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<tr>
<td>factor 3</td>
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<td>4.7571;9.7795</td>
<td>high significance</td>
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<tr>
<td>factor 4</td>
<td>5.2940e+003</td>
<td>3</td>
<td>1.7647e+003</td>
<td>1.3318</td>
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<td>blank list*</td>
<td>2.6559e+003</td>
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<td>6</td>
<td>1.3250e+003</td>
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<tr>
<td>sum</td>
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### Table S4 The results of analysis of range for daidzein concentration

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<tr>
<th>T value:</th>
<th>506.75</th>
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<th>483.54</th>
<th>367.88</th>
<th>405.04</th>
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<tr>
<td></td>
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<td>435.73</td>
<td>498.13</td>
<td>492.80</td>
<td>453.74</td>
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<td>435.32</td>
<td>419.87</td>
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<td>352.17</td>
<td>399.91</td>
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<tr>
<th>R value:</th>
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<th>39.79</th>
<th>210.61</th>
<th>124.92</th>
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<tbody>
<tr>
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<td>48.70</td>
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<table>
<thead>
<tr>
<th>optimal level:</th>
<th>1 1 2 2 2</th>
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</thead>
<tbody>
<tr>
<td>order:</td>
<td>C; A; D; B</td>
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### Table S5 The Results of Analysis of Variance for Daidzein

<table>
<thead>
<tr>
<th>sources of variance</th>
<th>sum of squares</th>
<th>freedom</th>
<th>Standard deviation</th>
<th>F value</th>
<th>$F^*$</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>factor 1</td>
<td>3.1088e+004</td>
<td>3</td>
<td>1.0363e+003</td>
<td>11.2942</td>
<td>4.7571;9.7795</td>
<td>high significance</td>
</tr>
<tr>
<td>factor 2</td>
<td>243.2587</td>
<td>3</td>
<td>81.0862</td>
<td>0.8838</td>
<td>4.7571;9.7795</td>
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</tr>
<tr>
<td>factor 3</td>
<td>6.9443e+003</td>
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<td>2.3148e+003</td>
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<td>4.7571;9.7795</td>
<td>high significance</td>
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<td>1.9705e+003</td>
<td>3</td>
<td>653.8207</td>
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**Table S6**  Analysis of Variance for the Fitted Quadratic Polynomial Model on Daidzin

<table>
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<tr>
<th>sources</th>
<th>sum of squares</th>
<th>df</th>
<th>mean square</th>
<th>F value</th>
<th>p-value</th>
<th>prob&gt;F</th>
<th>significant</th>
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<tbody>
<tr>
<td>Model</td>
<td>28320.60</td>
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<td>3146.73</td>
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<tr>
<td>X1</td>
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<td>0.7579</td>
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</tr>
<tr>
<td>X3</td>
<td>2709.00</td>
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<tr>
<td>X1X2</td>
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<tr>
<td>X1X3</td>
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<tr>
<td>Residual</td>
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<tr>
<td>Lack of fit</td>
<td>11838.31</td>
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<td>3946.10</td>
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<tr>
<td>Pure error</td>
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<td>1647.87</td>
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<tr>
<td>Cor Total</td>
<td>46750.37</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
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</tr>
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</table>

**Table S7**  Analysis of Variance for the Fitted Quadratic Polynomial Model on Daidzein

<table>
<thead>
<tr>
<th>sources</th>
<th>sum of squares</th>
<th>df</th>
<th>mean square</th>
<th>F value</th>
<th>p-value</th>
<th>prob&gt;F</th>
<th>significant</th>
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</thead>
<tbody>
<tr>
<td>Model</td>
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<td>134.79</td>
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<td>0.3414</td>
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<tr>
<td>X1</td>
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<td>97.34</td>
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<tr>
<td>Lack of fit</td>
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<td>149.80</td>
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<td>0.1908</td>
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<td>Pure error</td>
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<td>58.00</td>
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</tr>
</tbody>
</table>
Equations. The equations of RSM design

Equation 1:

\[ x_i = (X_i - X_0) = \Delta X \]  \hspace{1cm} (1)

Where \( x_i \) was the value of the independent variable \( X_i \), \( X_0 \) was the value of \( X_i \) at the center point, and \( \Delta X \) was the step change value.

Equation 2:

\[ Y = b_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} A_{ij} X_i X_j \]  \hspace{1cm} (2)

Where \( Y \) was the predicted variable function and \( A_i, A_{ii}, \) and \( A_{ij} \) were the regression coefficient of linear, quadratic, and interaction terms of independent variables \( X_i \) and \( X_j \).

Equation 3:

\[ Y \text{dai} \text{d} \text{z} \text{i} \text{n} = 207.56 + 19.72X_1 + 5.82X_2 + 18.40X_3 - 44.00X_1X_2 - 31.64X_1X_3 + 39.91X_2X_3 + 2.88X_1^2 + 24.93X_2^2 + 16.75X_3^2 \]  \hspace{1cm} (3)

Equation 4:

\[ Y \text{dai} \text{d} \text{z} \text{i} \text{e} \text{i} \text{n} = 89.35 + 3.09X_1 + 5.26X_2 + 0.92X_3 - 6.37X_1X_2 - 12.46X_1X_3 + 1.90X_2X_3 + 4.82X_1^2 + 1.3 \]