ORIGINAL ARTICLE

Characterization and mapping of the multi-component release kinetics of a Traditional Chinese Medicine dosage form using a modified LC/MS/MS method and chemomic release kinetic theory

Hai-yan Li, Xiang-yong Cu, Feng Gao, Peter York, Qun Shao, Xian-zhen Yin, Tao Guo, Zhen Guo, Jing-kai Gu, Ji-wen Zhang

Center for Drug Delivery System, Shanghai Institute of Materia Medica, State Key Laboratory of Drug Research, Chinese Academy of Sciences, Shanghai 201203, China

Research Center for Drug Metabolism, Jilin University, Changchun 130021, China

Institute of Pharmaceutical Innovation, University of Bradford, Bradford, West Yorkshire BD7 1DP, United Kingdom

College of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, China

Received 3 May 2011; revised 27 May 2011; accepted 2 June 2011

KEY WORDS
Chemomic release kinetics; Liuweidihuang pills; Principal component analysis; Hierarchical clustering analysis; Multi-component

Abstract  It is essential to develop effective methods for the quality control of the traditional medicine with multiple components. However, few researches on the quality control have been conducted to interpret the holistic characteristics of the traditional medicine in terms of dissolution/release. In this study, the multi-component release kinetics of Traditional Chinese Medicine (TCM) dosage forms was characterized and mapped by multivariate analysis techniques in the field of ‘omics’. The Liuweidihuang pill was used as a model formulation. The multi-component release kinetics of the concentrated and water-honeyed Liuweidihuang pills at rotation speeds of 50 and 100 rpm were analyzed by chemomic release kinetic theory and modified LC/MS/MS method. Mass features of 103 (concentrated pills) and 101 (water-honeyed pills) were selected with a linear correlation coefficient ≥0.99 between mass responses and concentrations. To compose the chemomic standard spectrum, the relative abundance of both mass features was no less than 1%

*Corresponding authors. Tel.: +86 21 50805901, +86 0431 85619955.
E-mail addresses: gujk@mail.jlu.edu.cn (Jing-kai Gu), jwzhang@mail.shcnc.ac.cn (Ji-wen Zhang).

2211-3835 © 2011 Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association. Production and hosting by Elsevier B.V. All rights reserved.

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association. doi:10.1016/j.apsb.2011.06.007

Production and hosting by Elsevier
1. Introduction

Traditional medicine has played an important role in maintaining human health and improving the quality of human life for thousands of years in China. Nowadays, about 80% of the Asian and African population relies on traditional medicine for at least part of their primary health care. According to the World Health Organization (WHO), traditional medicines include herbal, Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Islamic medicine, traditional Chinese medicine (TCM), acupuncture, Muti, and Ifé. As one of the most important contributors to traditional medicine, TCM philosophy not only considers single factors responsible for a certain disease but also addresses the balance of the whole organism ("holism") and its interaction with the environment.

However, the characteristics of multiple components challenge the quality control for TCM-based pharmaceutical products. Up to now, the application and modernization of TCMs is still limited because of the lack of effective quality control methods for products with multiple components. Furthermore, there is neither in vitro dissolution/release control nor in vivo pharmacokinetic data supporting for compounded TCM agent yet. The dissolution/release tests are one of the most important elements for the characterization of solid oral dosage forms and drug delivery systems (DDS). These tests are mainly used to confirm product quality and batch-to-batch consistency in order to optimize formulations and process conditions for the dosage forms of chemical pharmaceuticals. More importantly, the in vitro dissolution/release tests also provide a basis for the correlation of in vitro dissolution data with in vivo bioavailability, which would benefit the product development to a great extent.

Therefore, it is necessary to develop a new method for the recognition of the multi-component and "holistic" nature of TCM, the key to the effective evaluation of the dissolution/release performance of traditional dosage forms and DDS of TCM. In the previous reports, we have proposed a new theory and method for the release/dissolution kinetics of TCM based on the overall characteristics of the chemome of the TCM. In this study, multivariate analysis methods in bioinformatics and modern sciences named as "-omics", such as principal component analysis (PCA) and hierarchical clustering analysis (HCA), were applied to characterize and visualize the release kinetics of TCMs. The Liuweidihuang pill, one of the most known ancient Chinese herbal formulas, was used as a model formulation.

The Liuweidihuang pill is widely used for the treatment of various disorders, such as backache, alopecia, menoxenia, and sore waist and knees. Recently, Liuweidihuang pills have been reported to increase the number of T lymphocytes and regulate the production of cytokines. As a classic Chinese herbal formula, Liuweidihuang pills are composed of Rehmanniae Radix, Corni Fructus, Dioscoreae Rhizoma, Alismatis Rhizoma, Poria and Paeoniae Suffruticoso Cortex. Only two bioactive components in the pills, loganin and paenol, are measured through current official quality control practices. A number of methods have been developed for the quantitative determination of bioactive components in Liuweidihuang pills, such as HPLC–UV, GC, LC/MS/MS, MEKC, and HPLC–DAD–ELSD. These researches were all based on the determination of single component(s), such as gallic acid, paenol, ursoic acid, loganin, and oleanic acid, or a small number of components. There is still no available compendial standard of quality control on the release kinetics for all TCM solid dosage forms (tablets, capsules, etc.) based on the TCM holistic principle.

In this study, the multi-component release performance of Liuweidihuang pills was quantified through LC/MS/MS based on chemonomic release kinetics theory initially. The chemonomic release reproducibility of the components in Liuweidihuang pills was characterized by score plots from PCA. The chemonomic release of Liuweidihuang pills was visualized through heat maps from HCA.

2. Theory development

The basic theory relating to the chemome and the standard spectrum of the chemonomic concentrations has been described in detail in our previous articles. Mass features with an acceptable linearity ($r > 0.99$) were chosen to frame the chemome. The term "chemome" was defined as the assembly of chemical compounds contained in the TCM preparation.
dissolved in a medium, and defined as a cluster composed by moieties of the components:

$$M = [M_1, M_2, M_3, \cdots, M_{m-1}, M_m]$$  \hspace{1cm} (1)

where $M_i$ denotes the amount of the $i$th compound whilst $m$ denotes the number of components. The standard spectrum of the chemomic concentrations is referred to the assembly of the chemical compound concentration in the solution of the TCM preparation. In this paper, the standard spectrum of the chemomic concentrations consisted of the ratio of the response of each mass feature to the response of the internal standard on LC/MS/MS spectrum. The chemomic concentration can be represented by the term $G$, and the standard spectrum of chemomic concentration ($G_S$) is given by

$$G_S = \frac{M_i}{R} = [R_{S,1}, R_{S,2}, R_{S,3}, \cdots, R_{S,m-1}, R_{S,m}]$$  \hspace{1cm} (2)

where $R$ is the ratio of the response of each mass feature to the response of the internal standard in the standard chemome solution. The chemomic concentration $G_T$ is a characteristic parameter obtained by comparing the chemomic spectrum of the test sample to the chemomic standard spectrum:

$$G_T = [R_{T,1}, R_{T,2}, R_{T,3}, \cdots, R_{T,m-1}, R_{T,m}]$$  \hspace{1cm} (3)

where $R$ denotes the determined response value of each mass feature.

3. Materials and methods

3.1. Materials

The LC–MS/MS system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) and Applied Biosystems SCIEX API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) using electrospray ionization (ESI). Concentrated Liuweidihuang pills (lot number: 8072560) and water-honeyed Liuweidihuang pills (lot number: 9030542) were obtained from Beijing Tong Ren Tang Biosystems/MDS Sciex, Concord, ON, Canada) using electrospray ionization (ESI). Concentrated Liuweidihuang pills (lot number: 8072560) and water-honeyed Liuweidihuang pills (lot number: 9030542) were obtained from Beijing Tong Ren Tang Technologies Co., Ltd. (Beijing, China).

3.2. Analytical methods

3.2.1. Stock solution

Sixteen concentrated Liuweidihuang pills (sixty for water-honeyed pills) were precisely weighed and then thoroughly comminuted to ensure complete mixing. Samples of the fine powders, equal to the average pill weight (eight for concentrated pills and thirty for water-honeyed pills), were weighed and transferred to a dissolution beaker containing 900 mL degassed distilled water. The paddle dissolution method was then employed for 10 h. A sample of solution from the dissolution vessel was filtered using a 0.45 μm membrane, and the standard chemicomic stock solution was obtained by diluting the filtered solution with water at a ratio of 1:10. The solution of standard reference substance diazepam with the concentration of 100 ng/mL was prepared as the internal standard solution.

3.2.2. LC–MS/MS conditions

The chromatography elution was performed using 10 mmol/L ammonium acetate in purified water as solvent A and methanol as solvent B, with a ratio of 10:90 v/v. The flow rate was set at 0.2 mL/min. The injection volume was 20 μL. ESI was performed in positive ion mode with nebulizer. The ion spray needle voltage and heater gas temperature were set at 3000 V and 400 °C, respectively. The pressure of GS1 and GS2 was 40 psi in each case. The pressure of curtain gas was 15 psi. The MS acquisition was performed in Q1 full scan mode (100–1000 amu).

3.2.3. Data collection

Data collection was performed under “LC–MS/MS conditions”. The full scan chromatography was recorded for 2.5 min. After subtracting the background response within 1.6–2.2 min, the data collected within 0.5–1.0 min was used for further analysis.

3.2.4. Standard series solution

Stock solutions with volumes of 1, 1.25 (solution A), 1.67, 2.5 (solution B), 5 and 10 mL (solution C) were transferred to 10 mL volumetric flasks, and diluted to 10 mL with distilled water as the standard series solutions.

3.2.5. Chemomic standard spectrum

100 μL of the stock solution and 100 μL of the internal standard solution were precisely transferred and mixed. Samples with 20 μL of the mixed solutions were then analyzed according to the operation procedure of “Section 3.2.3, Data collection”. The ratios of the response of all the mass features to the response of the internal standard were recorded and exported.

3.2.6. Mass features selection

100 μL of the standard series solution and 100 μL of the internal standard solution were precisely transferred and mixed for the analysis of linearity. Samples with 20 μL of the mixed solutions were then analyzed according to the data collection operation. The ratios of the response of all the mass features to that of internal standard were exported. The chemomic concentrations of individual standard series solutions were obtained by comparison with the chemomic standard spectrum. In order to meet the requirement of LC/MS/MS quantitative analysis, mass features with a linearity coefficient value ($r$) ≥ 0.99 were selected to construct the chemomic standard spectrum.

3.2.7. Precision

Standard solutions of A, B and C were sampled and assayed six times in parallel to evaluate the precision of the method by correlation matrix analysis.

3.3. Determination of the chemomic release kinetics

The release kinetics of the components from the Liuweidihuang pills was determined using the second dissolution method detailed in Chinese pharmacopoeia 2010. Distilled water was used as the dissolution medium and the temperature was set at 37 °C. Aliquots of 5 mL solution samples were collected at 20, 30, 60, 90, 120 and 180 min and 5 mL distilled water was added immediately after collection of each sample solution. The sample solution was filtered through a 0.45 μm membrane, then
examined by LC/MS/MS and the resulting data were processed accordingly to calculate the chemomic release kinetics. The chemomic release kinetics at two different rotation speeds of 50 and 100 rpm were determined.

4. Results and discussion

4.1. Mass features selection

To meet the requirement of subsequent quantitative analysis, the MS responses of the chosen mass features in the standard series solutions should be linear. Also, the correlation coefficient (r) of linear regression should be close to unity. Therefore, mass features with $r \geq 0.99$ were selected to construct the chemome. Mass features of 103 ($m/z$ from 104.35 to 717.65) and 101 ($m/z$ from 104.35 to 869.95) were selected for concentrated Liuweidihuang pills and water-honeyed pills, respectively. The linearity of the chemomic concentration in Liuweidihuang pills was appropriate for the release test.

4.2. Chemomic standard spectrum

In the chemomic standard spectrum, $X$-axis was $m/z$ value of individual mass feature and $Y$-axis was the ratio of the response of each mass feature to that of internal standard where the internal standard bar was set to be 100 (Fig. 1).

4.3. Precision

Owing to the multi-component characteristics of Liuweidihuang pills, the precision in our study was evaluated by correlation matrix analysis, which is usually used to assess the similarity of observations with multiple variables in statistics. As shown in Table 1, the correlation coefficients between six samples of various solutions indicated that the reproducibility of the analytical method met the requirement of our study well ($r \geq 0.985$ for concentrated pills and $r \geq 0.994$ for water-honeyed pills).

4.4. Chemomic release kinetics

For dosage forms composed of single component, the release kinetics was readily described by a dissolution curve, with the time as $X$-axis and $R$ (%, cumulative fraction of drug released) as $Y$-axis. For the TCM dosage forms composed of multi-components, three-dimensional graphs were employed to represent the chemomic release kinetics. As shown in Fig. 2, representative three-dimensional graphs were produced by Sigmaplot software (Version 10.0, Systat Software, Inc.), with the time as $X$-axis, mass feature ($m/z$) as $Y$-axis and the relative cumulative release fraction as $Z$-axis. The contour plots were provided at the top surfaces of the grids simultaneously. Similar to our previous study, Kalman filtering was also employed to process the multiple component release data whilst the chemomic concentration of the sample was obtained. The chemomic release profiles were then plotted as shown in Fig. 3A.

Based on the characteristics of multiple components for TCM, the cumulative release percent of each mass feature at any sampling time was a point in multidimensional space. The distance between each point and the origin could be calculated by the Euclidean distances equation. With an increase in sampling time, the cumulative release percent of each mass feature changed. The spatial position of each point also shifted correspondingly. The shift tendency was similar to the cumulative and increasing trend of multi-component release. Thus, the distance between each point at any sampling time and the origin exhibited the cumulative and increasing trend of multi-component release (Fig. 3B). For the calculation of the Euclidean distances, samples at different times were considered as the observations, and the cumulative release percentage of each mass feature was considered as the characteristic variable of the observations.

As shown in Figs. 2 and 3, the cumulative quantity of chemome released and the distance values at 50 rpm for water-honeyed pills were the smallest. For concentrated pills, three-dimensional and distance curves at 50 and 100 rpm exhibited no distinct differences. Thus, there was no obvious effect of rotation speed on chemomic release kinetics of concentrated Liuweidihuang pills. For water-honeyed pills, the cumulative quantity of chemome released and distance value at 50 rpm was smaller than that at 100 rpm, and thus the effect of rotation speed on the chemomic release kinetics in this case was considerable. Considering the reproducibility of the chemomic release at different time points, or the release variation of each mass feature during certain time intervals, little information was gained from Figs. 2 and 3. Therefore, the three-dimensional Kalman filtered release profiles and

Figure 1  Chemomic standard spectrum of concentrated Liuweidihuang pills (A) and water-honeyed pills (B).
Table 1  Correlation matrix to evaluate the method precision of LC–MS/MS analysis.

<table>
<thead>
<tr>
<th></th>
<th>Concentrated pills</th>
<th></th>
<th>Water-honeyed pills</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>0.989</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>0.991</td>
<td>0.995</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>L4</td>
<td>0.990</td>
<td>0.996</td>
<td>0.993</td>
<td>1</td>
</tr>
<tr>
<td>L5</td>
<td>0.985</td>
<td>0.997</td>
<td>0.992</td>
<td>0.995</td>
</tr>
<tr>
<td>L6</td>
<td>0.986</td>
<td>0.997</td>
<td>0.992</td>
<td>0.996</td>
</tr>
<tr>
<td>M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>0.999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>0.998</td>
<td>0.998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>0.997</td>
<td>0.997</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>0.998</td>
<td>0.998</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>M6</td>
<td>0.998</td>
<td>0.998</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>H1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>0.997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>0.993</td>
<td>0.995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>0.996</td>
<td>0.995</td>
<td>0.998</td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>0.995</td>
<td>0.995</td>
<td>0.997</td>
<td>0.998</td>
</tr>
<tr>
<td>H6</td>
<td>0.993</td>
<td>0.992</td>
<td>0.996</td>
<td>0.998</td>
</tr>
</tbody>
</table>

L1–L6, M1–M6 and H1–H6 represent 6 duplicates of solutions a, b and c, respectively.
distance graphs merely described the release kinetics conventionally, and failed to demonstrate the chemomic release performance of all the individual components.

4.5. Reproducibility of the chemomic release of Liuweidihuang pills

Principal component analysis (PCA) is a useful technique for the classification of data and has been well reported for its principles previously\(^1\). PCA reduces the dimensionality of a data set (sample) by finding a new set of variables, which is smaller than the original set of variables but retains most of the information. The new variables, called principal components (PCs), are uncorrelated and ordered by the fraction of total information each retains. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. In PCA analysis, the score plot provides the distribution and clustering of the samples whilst the loading plot provides the distribution and clustering of the variables. Thus, the PCA score plot was used in this study to illustrate the reproducibility of the multiple component release of Liuweidihuang pills.

Two data sets with 36 observations (six parallel chemomic release samples of 0–20, 20–30, 30–60, 60–90, 90–120 and 120–180 min) were used for PCA analysis. The data set for concentrated pills contains 103 independent variables and that for water-honeyed pills contains 101 independent variables. The PCA analysis was conducted using SIMCA-P software (Umetrics AB, Umea, Sweden, demo version) on the above data sets for dissolution of the concentrated pills at 50 rpm, concentrated pills at 100 rpm, water-honeyed pills at 50 rpm and water-honeyed pills at 100 rpm. Outliers were determined with a Hotelling’s test\(^2\). The time point zero was not included in the computations since it contained no information (i.e. all percentages dissolved are equal to zero), while it influenced the number of degrees of freedom for building the limits. PCA score plots are shown in Fig. 4A–D.

Each point on the score plot represented an individual chemome released within certain time intervals and each point on the loading plot represented a mass feature (data not shown). For example, in Fig. 4A, points 1–7 represented the chemome released within 0–20 min of six parallel operations. If the chemome released within 0–20 min exhibited perfect reproducibility, these seven points should overlap with each other. In fact, ideal reproducibility did not exist and the points were simply distributed closely. Therefore, the PCA score plot was applied to evaluate the reproducibility of the chemome released between different time intervals, rotation speeds and preparations.

In Fig. 4, the seven orange points were located closely in all the four figures (A–D). It indicated that the chemome released within 0–20 min exhibited a relatively good reproducibility regardless of different rotation speeds and preparations. The chemome release performance within 0–20 min was stable and influenced by neither rotation speed nor preparation type, while the distributions of the other five groups (20–30, 30–60, 60–90, 90–120 and 120–180 min) were dramatically changed in all four figures. This observation demonstrated that the chemome released within these five time intervals exhibited relatively poor reproducibility and thus the chemome release...
performance within these time intervals was unstable and influenced by the rotation speed or preparation type.

The effect of the preparation type on the chemomic release was also clearly demonstrated from Fig. 4. The score plots of concentrated Liuweidihuang pills (Fig. 4A and B) showed more extensive separation of the six time interval groups than water-honeyed pills (Fig. 4C and D). Therefore, concentrated Liuweidihuang pills presented better chemomic release reproducibility than water-honeyed pills. This was probably associated with the difference in preparation process between concentrated pills and water-honeyed pills. The former was prepared from the extracted solutions of the herbals while the latter was prepared using the original powders made directly from the herbals. On the other hand, the effect of rotation speed during the dissolution test (50 and 100 rpm) on the chemomic release was less obvious than that of the preparation type. For concentrated pills, the chemomic release reproducibility of 90–120 and 120–180 min at 100 rpm was better than that at 50 rpm, whilst the reproducibility for water-honeyed pills was similar at the two different rotation speeds tested.

The multi-component chemomic release kinetics was a dynamically changing process. The PCA score plot also depicted a time-related trajectory of samples. In Fig. 4, the chemome released in different time intervals was diverse. From the left hand side to the right hand side (along $t\[1\]$), the level of the chemome released increased with the corresponding time interval. The group of 0–20 min was located at the right side along $t\[1\]$, far away from the other points (Fig. 4A–D). This indicated that the chemome released within 0–20 min was the largest of all the time intervals, which was consistent with the results of Figs. 2 and 3. The location tendency of the other five groups was uncertain in the above four figures, illustrating the asynchronicity of the chemomic release. In Fig. 4A (concentrated pills at 50 rpm), the rank of the chemome released was 0–20 $>$ 30–60 $>$ 60–90 $=$ 120–180 $>$ 20–30 $>$ 90–120 min. In Fig. 4B (concentrated pills at 100 rpm), the order was 0–20 $>$ 30–60 $>$ 20–30 $>$ 60–90 $=$ 120–180 $>$ 90–120 min. The location of the 20–30 min group changed dramatically in the plot.

4.6. Visualization of the chemomic release characteristics of Liuweidihuang pills

In order to improve visualization and interpretation of the chemomic release performance and generate a panorama concerning the release variation of the mass features during certain time intervals, a two-way unsupervised hierarchical clustering analysis (HCA) was applied. In HCA, rows and columns were clustered simultaneously to obtain groups as similar as possible to produce results that are easily visualized and interpreted$^{21}$. HCA has been extensively used in the field of genomics, proteomics and metabonomics. Here, considering the common features between the multi-components for TCMs and the omic sciences, it was applied to map the chemomic release characteristics of TCM.

In this study, a two-way HCA was carried out using PermutMatrix (version 1.9.3)$^{22}$. The entire data set (36 x 103 or 36 x 101) was submitted for the two-way HCA, with the rows of mass features and the columns of different time intervals. Z-score transformation of rows normalization was employed to ensure that the chemome released at each time interval had a median value of 0 and a standard deviation of 1. The analysis was carried out using the Euclidean distance as the dissimilarity criterion for clustering and seriation, and Ward’s method as the criterion for aggregation$^{23}$. Then, a heat map of the chemome released in different time intervals was
obtained to visualize the release performance of the multi-component chemome. In the heat map generated by HCA, each colored cell represented the chemome released at each time interval according to the color scale at the top of the figure. Fig. 5A–D depicted the hierarchically clustered heat maps of the chemome released in six different time intervals for concentrated Liuweidihuang pills and water-honeyed Liuweidihuang pills at rotation speeds of 50 and 100 rpm.

The heat maps (Fig. 5) provided clusters of the columns horizontally and the rows vertically. The heat maps also readily showed changes in the chemomic release increments along different clusters of columns (time intervals) and different clusters of rows (mass features). Among the maps of Fig. 5A–D, the columns of “0–20 min” were the best clustered, agreed with the previous results from Fig. 4A. In Fig. 5A, six time intervals were binned into approximately five clusters. Except for the columns of “60–90 min”, other five time intervals were all well clustered. Likewise, in Fig. 5B, six time intervals could be binned into approximately five clusters. Apart from the columns of “120–180 min”, the other five time intervals were all well clustered. In Fig. 5C and D, the distances between clusters were so short that it was very difficult to bin the time intervals into five clusters. Thus it could be concluded that the columns of the six time intervals were mixed with each other as shown in Fig. 5C and D, which are consistent with the results and observations shown in Fig. 4A.

Three clusters of the rows were marked out in the heat maps. As determined previously, the mass features of concentrated pills were different from those of water-honeyed pills. Although the mass features were not identified qualitatively in this study, some useful information about the clustering behavior of the mass features was obtained. In the metabonomic research, the HCA was employed to cluster mass features to discriminate the “metabolites signature”. In the heat maps of metabonomic research, biomarkers could be identified. The altered concentration of the biomarkers in different observations (columns) was noted and used to discriminate the observations. Unlike the metabonomic research, the heat maps in this study were used to show the integrity and the synchronicity of the mass features and then the holism of TCM. As expected, there were no major changes in individual clusters of mass features along different time intervals (Fig. 5A–D). This finding was a persuasive rationale for the visualization of multi-component release by two-way HCA. This method directly proved the integrity of the release kinetics of multi-component TCMs.

5. Conclusions

Based on the PCA techniques used in the field of bioinformatics and multivariate statistics, and HCA in the field of omics-named sciences, combined with LC/MS/MS and chemomic release kinetic theory, the in vitro release characteristics of the TCM dosage form, pills of Liuweidihuang prepared by two different processes, were represented, assessed and visualized directly. Novel techniques were employed in this research to meet the requirement of multiple components consistent with the holism of TCM. The correlation matrix was used to evaluate the precision of the analysis; three-dimensional graphs, the Kalman filtering method and the Euclidean distance were used to frame the tendency of the multiple component release kinetic profiles in a simple and easy-to-read approach; the PCA score plots were used to evaluate the release reproducibility and the HCA heat map to visualize the entire release profiles. It was indicated, in this research, that these techniques provided new choices and new vision to the delivery system design and quality control of dissolution/release of TCM and other similar ethnic medicines with multiple components.
Acknowledgment

This work was supported by the Shanghai Science and Technology Development Funds (No. 09dZ1973300) and Key Projects in the National Science & Technology Pillar Program (No. 2009ZX09304-003).

References