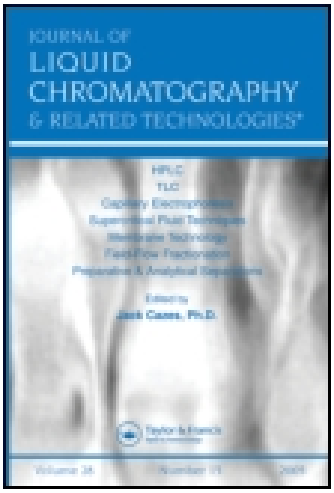

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# INVESTIGATION OF CHEMICAL COMPONENTS VARIATION IN MAXING SHIGAN DECOCTION BY HPLC-DAD

**Min He,[1] Yizeng Liang,[1] Zhimin Zhang,[1] Yaping Li,[2] Zhongda Zeng,[1] Dongsheng Cao,[1] Yonghuan Yun,[1] and Jun Yan[1]**

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□ *Many efficacious herbal prescriptions were used to cure a variety of diseases, each herb contains different components that generate their distinctive efficacy. Maxing Shigan decoction is such an example. Its chemical components were complicated, which couldn't be analyzed by the simple qualitative and quantitative method. So, the similarity and difference of chemical components in Maxing Shigan decoction, single herbs, and disassembled prescriptions were investigated by high-performance liquid chromatography diode array detector (HPLC–DAD) combined with chemometrics methods, which include smoothing and filtering, ordinary manual linear deduction, adaptive iteratively reweighted penalized least squares (airPLS), heuristic evolving latent projections (HELP), and alternative moving window factor analysis (AMWFA). These methods possess practical value in ordinary laboratory when facing complicated components analysis. All resolved pure peaks, including ephedrine, pseudoephedrine, methylephedrine, amygdalin, liquiritin, glycyrrhizic acid, and benzoic acid in the Maxing Shigan decoction, were determined. The dissolved ratios of seven bioactive constituents and other major mutative constituents among Maxing Shigan decoction and other disassembled prescriptions were comprehensively investigated and compared. The comparative results of chemical compositions can help us better understand the chemical composition variation of Maxing Shigan decoction due to the cooking process.*

**Keywords** airPLS, AMWFA, dissolved ratios, HELP, HPLC–DAD, Maxing Shigan decoction

## INTRODUCTION

In China, many efficacious herbal prescriptions, including Maxing Shigan decoction, originated from *Shanghanlun*, a classical ancient book about Traditional Chinese Medicines (TCMs) written by Zhang zhongjing in Han Dynasty, where different patterns were named according to the decoction used for various diseases.[1] Maxing Shigan decoction has been

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widely used for thousands of years due to its reliable therapeutic efficacy. *Ephedra herb* is monarch drug in the Maxing Shigan decoction, which is one of the oldest medical herbs in TCMs. It was considered to be effective for inducing diaphoresis and allaying asthma. The active ingredients of *ephedra herb* are considered as ephedrine alkaloids, an internationally hot research topic.[2] In addition, there are also many active ingredients such as Ephedrannin A and B,[3] polysaccharide,[4] and so on.

In Chinese medicine theory, multiple herbs are usually combined to treat diseases, and chemical component combinations are considered as the material foundation of a compound prescription. Compound prescription is an entire substance from a multi-target to multi-level aspect. However, decocting procedure may induce chemical transformations and change global quality, such as Du-Shen-Tang.[5] Maxing Shigan decoction is composed of *ephedra herb, gypsum fibrosum* (*GF*), *semen armeniacae amarum*, and *prepared glycyrrhizae radix*, which does not exhibit a diaphoretic effect with warm property.[6–8] In this way, a combination of herbs is very important to establish the properties of herbal medicines. When *ephedra herb* is combined with other herbs, the total effectiveness of herbal prescription might be sometimes changed. Therefore, it is very important to quantify the chemical compositions in Maxing Shigan decoction.

Previously,[9] studies on single herbs and compound prescriptions were limited to specific chemical compounds with known molecular structures, the compounds with unknown molecular structures and their interaction among herbs were neglected. While chemometric resolution methods are gradually used for herbal quality control, it helps us to analyze and interpret useful information from raw data. So, the multi-compound approach was proposed which used both chemical compounds with known structures and those with partial chemical information, such as retention time, mass spectra, or ultraviolet (UV) spectra;[10,11] a more comprehensive understanding of Chinese medicine could be obtained by using this approach.

HPLC–DAD data from decoction contain baseline drift, a number of overlapped and even embedded peaks. These problems make it difficult to conduct comparative analysis among different samples. Hence, baseline drift should be corrected and overlapped peaks should be resolved before qualitative and quantitative analysis. airPLS[12] was shown to be a competitive method for baseline correction. Chemometric resolution methods, such as HELP[13,14] and AMWFA,[15–19] have proved to be effective in resolving the two-dimensional data into pure chromatograms and pure spectra of each component, which can be further used for qualitative and quantitative analysis. So, it is possible to conduct research on the concentration changes or chemical variation in the process of decoct among Maxing Shigan decoction, simple herbs, and other disassembled prescriptions.

In this investigation, seven major compounds of known structures and biological activity in the Maxing Shigan decoction, including ephedrine, pseudoephedrine, methylephedrine, amygdalin, liquiritin, glycyrrhizic acid, and benzoic acid, were identified by their retention time and UV spectra compared with reference substances. The multi-compound chemometric approach, which could resolve the overlapping peaks, was used in this investigation. The dissolved ratios of all the peaks, among Maxing Shigan decoction and other disassembled prescriptions, were comprehensively investigated and compared. The comparative results of chemical compositions can help us well understand the chemical composition variation of Maxing Shigan decoction due to the cooking process.



## EXPERIMENTS

### Materials

All the herbs used in this study were purchased from Hunan Academy of Chinese Medicine, then identified by Xiangya Hospital, China. *Ephedra herb* is the dry stems of *ephedra sinica* from Inner Mongolia, and *semen armeniacae amarum* and *prepared glycyrrhizae radix* were prepared according to relevant standard procedure. Ephedrine, pseudoephedrine, methylephedrine, amygdalin, liquiritin, glycyrrhizic acid, and benzoic acid were purchased from the Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). The solvents, such as acetonitrile, methanol, and phosphoric acid, were of HPLC grade. Water is deionized water. Other reagents and chemicals were of analytical grade.

### Instrumentation and Conditions

Chromatographic analysis was performed on an DION U-3000 Series (DION, Germany), equipped with P680 HPLC pump, ASI-100 autoplate-sampler, VD170U detector, Chromeleon workstation, and a thermostatically controlled column compartment. Chromatographic separation was carried out at 25°C on a Sepax HP-$C_{18}$ column (4.6 mm × 250 mm, 5 μm). The mobile phase consisted of 0.02 M potassium dihydrogen phosphate, 0.1% triethylamin, and adjusts pH to 3.05 (A) and acetonitrile (B), using elution gradients for analysis of samples. The gradient elution for analysis of samples was as follows: 0–20 min, 5–10% B; 20–40 min, 10–22%; 40–50 min, 22% B; 50–85 min, 22–45% B; 85–90 min, 45% B. The flow rate was kept at 0.8 mL/min, and the injecting volume was set at 20 μl.

## Standard Solution Preparation

Standard stock solutions (1000 μg/mL) of ephedrine, pseudoephedrine, methylephedrine, amygdalin, liquiritin, glycyrrhizic acid, and benzoic acid were prepared in methanol and stored away from light at 4°C, respectively. Working solutions of lower concentration were prepared by making appropriate dilution of the stock solution.

### *Preparation of Maxing Shigan Decoction, Simple Herbs and Other Disassembled Prescriptions Samples*

The experiment was immediately performed after all medicines were purchased. They were dried for 4 hr at 40°C and finely powdered and sealed. An herbal sample of Maxing Shigan decoction comprised the following medicines: 2.25 g of *ephedra herb*, 2.25 g of *semen armeniacae amarum,* 1.5 g of *prepared glycyrrhizae radix*, and 4.5 g of *gypsum fibrosum.* Each medicine was prepared using the classical method. *Ephedra herb* (soaked for 30 min) was boiled in 30 mL of water for 20 min, and then the scum on the liquid surface was skimmed. Other herbs (soaked for 30 min) were then added into the decoction, and further boiled for 30 min. Then, the solution was centrifuged (1,500 × g for 10 min) and the supernatant was collected. The residue was further boiled with the same method for three times, and all the supernatants were combined. The extraction procedure was strictly conducted according to the same experimental conditions. Then, the cooled extraction solution were collected and kept at a cool and lucifuge condition for less than 4 hr. The final volume was adjusted to 100 mL. All solutions were filtered through 0.45 μm filters before analyze. In order to check if this procedure will introduce the chemical transformations, the correlation coefficient between the chromatograms from different storage times, say 1 hr and 4 hr, is more than 0.9995, which means there is no significant difference during the storage period. A single herb control sample solution, including 2.25 g of *ephedra herb*, 2.25 g of *semen armeniacae amarum,* and 1.5 g of *prepared glycyrrhizae radix,* were made according to the method above. The disassembled prescriptions, including the whole prescription without *gypsum fibrosum,* the whole prescription without *semen armeniacae amarum*, and the whole prescription without *prepared glycyrrhizae radix,* were also prepared according to the method described above. All samples were repeated three times.

## Data Resolved by Chemometrics Methods

In this study, the samples of Maxing Shigan decoction were prepared from four herb species (*ephedra herb, gypsum fibrosum, semen armeniacae amarum*, and *prepared glycyrrhizae radix*). Then, HELP was conducted to

resolve the overlapped peaks. AMWFA was employed to perform the comparative analysis of decoction among seven groups and resolve the embedded peaks. The qualitative identification of these chemical components was carried out by UV spectra combined with reference substance. The dissolved ratios of seven mark bioactive constituents and other major mutative constituents among Maxing Shigan decoction and other disassembled prescriptions were investigated.

All data analysis was carried out on a Pentium IV (Intel) personal computer. HELP method was implemented in C++ and MFC with nice graphic user interface by our laboratory. AMWFA and airPLS method programs were coded in MATLAB 2010 (a) for windows.



## RESULTS AND DISCUSSION

### Identification of Major Bioactive Constituents in Maxing Shigan Decoction

Ephedrine, pseudoephedrine, methylephedrine,[2] and amygdalin[20] are considered as active ingredients responsible for the treatment of asthma in oriental medicine. Liquiritin and glycyrrhizic acid[21,22] are the main active ingredients in *Glycyrrhizae Radix*. They were usually integrated as pharmaceutical preparations such as cough–cold syrup. Different components have their own physical and chemical properties, their structures are shown in Figure 1. From the plot, one can see that their structures are diverse. In this respect, a method for simultaneously analyzing them is needed.

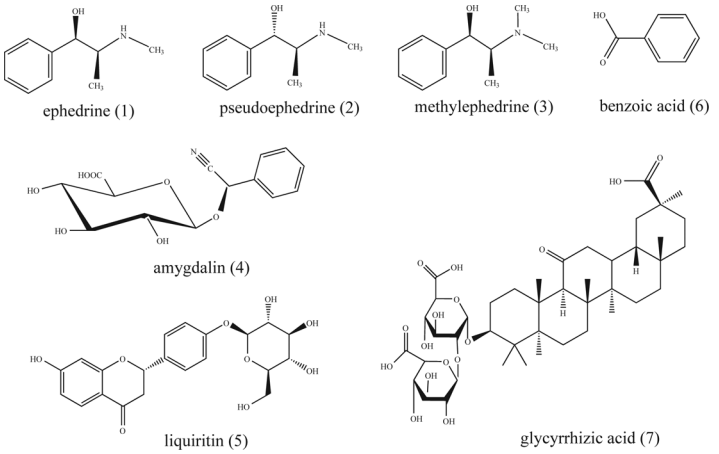


**FIGURE 1** Structures of the major active components in Maxing Shigan decoction.

Seven reference compounds, including ephedrine, pseudoephedrine, methylephedrine, amygdalin, liquiritin, benzoic acid and glycyrrhizic acid, were analyzed by HPLC-DAD from 190–399 nm. Their retention times (RT) are 18.9 min, 19.9 min, 22.2 min, 31.7 min, 46.0 min, 48.6 min, and 82.5 min, respectively. The HPLC chromatograms of Maxing Shigan decoction extract monitored at 210 nm, 250 nm, 276 nm were shown in Figure 2. From this plot, one can see that there were more than 100 components in the chromatogram. Reversed-phase LC-method was employed in the current work for the separation of these seven bioactive constituents or more. To this end, reverse phase C18 columns using acetonitrile and 0.1% phosphoric acid as a mobile phase were tested, which does not show more superiority than the method above, but low pH keeps the method abandoned. Thus, water-tolerant chromatographic columns were selected.

In order to provide more qualitative information, the major peaks and their corresponding UV spectra from 190–399 nm in acid mobile phase are shown in Figure 3. From these UV spectra, we concluded that peak 28 may be triterpenoid saponin without α, β-unsaturation ketone, and peak 62 and 69 may be flavonoids or their glycoside. Peak 71 may possess the similar structure with liquiritin, peak 73 may possess the similar structure with chlorogenic acid, while peaks 90 and 92 may possess the similar structure with glycyrrhizic or glycyrrhetinic acid. Of course, these are tentatively qualitative analyses and further confirmation is necessary.

## Baseline Correction, Overlapped Peaks Resolved by HELP

The baseline drift of HPLC–DAD chromatogram in this test is very serious, which will blur signals and deteriorates analytical results. Thus, it is

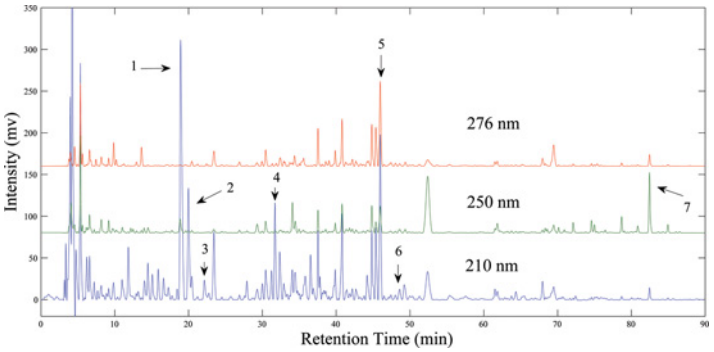


**FIGURE 2** The baseline correction result from the chromatograms data of Maxing Shigan decoction at 210 nm, 250 nm, and 276 nm using the ordinary manual linear deduction combined with the airPLS method. 1, Ephedrine; 2, Pseudoephedrine; 3, Methylephedrine; 4, Amygdalin; 5, Liquiritin; 6, Benzoic acid; 7, Glycyrrhizic acid. (Color figure available online.)

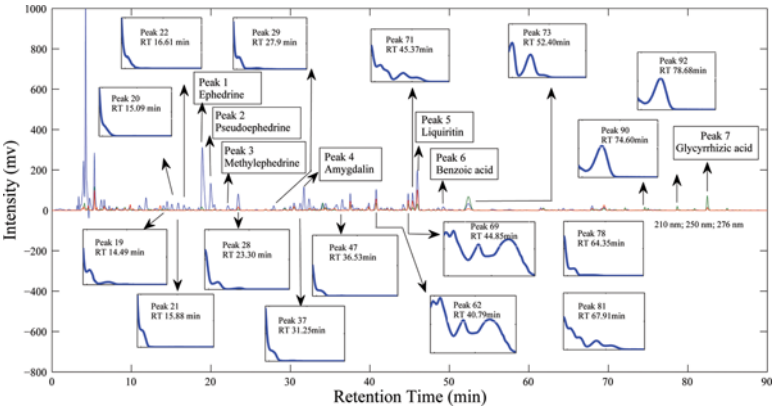


**FIGURE 3** The major peaks and their corresponding UV spectra from 190–399 nm in acid mobile phase. (Color figure available online.)

necessary to correct baseline drift to perform further data analysis. Here the ordinary manual linear deduction combined with the airPLS[12] method can satisfy our requirement from 190–399 nm simultaneously. airPLS changes weights of sum squares errors (SSE) between previously fitted baseline and original signals iteratively. The weights of SSE are obtained adaptively using the difference between previously fitted baseline and original signals. Firstly, the steepest fraction was corrected by the ordinary manual linear deduction, and then the whole chromatogram data from 190–399 nm were corrected automatically by airPLS method. The baseline correction result from the chromatograms data of Maxing Shigan decoction at 210 nm can be seen in Figure 4.

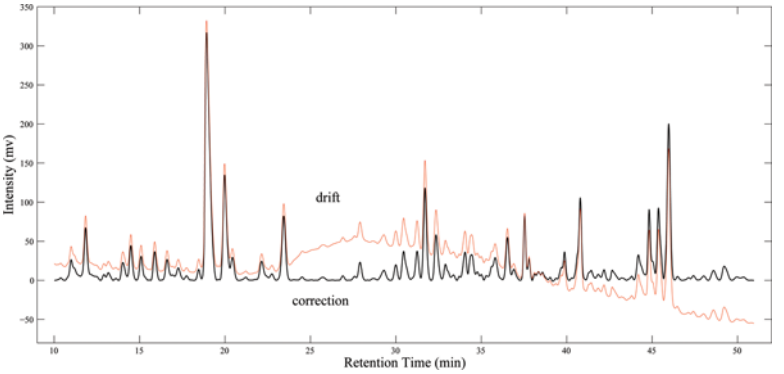


**FIGURE 4** The baseline correction result from the chromatogram data of the sample of Maxing Shigan decoction monitored at 210 nm from 10–51 min. (Color figure available online.)

All the chromatograms from seven groups are very complex analytical systems. There exist some overlapped and embedded peaks. Because of these, the simple qualitative and quantitative method will definitely fail between Maxing Shigan decoction and the other six groups. Moreover, we can't calculate their peak area accurately and further get the information about their chemical component variation. Overlapped peaks shown in Figure 5a are a good example, which is the segment in the range of 37.24–38.07 min taken from the sample of ephedra herb. HELP[13,14] is a classic chemometrics method to resolve the overlapping peaks in two-dimensional chromatographic data matrix, after baseline correction, the number of components, the selective region marked by H1, H1 + H2, H2, and zero-component region of each component were determined using the evolving latent projective graph and rankmap on the basis of the eigenstructure tracking analysis (Figures 5b and 5c). Finally, two pure chromatographic profiles and UV spectra resolved by means of full rank resolution are shown in Figure 5d and e.

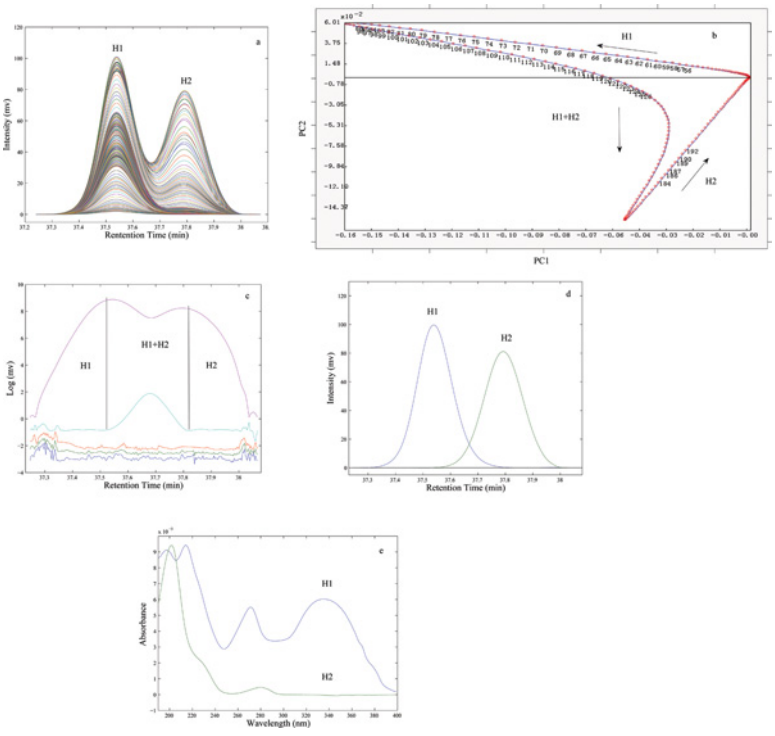


**FIGURE 5** LC-DAD chromatogram data from *ephedra herb* monitored at 190–399 nm after baseline correction (a); the evolving latent projective graph (b); rankmap (c); the pure chromatogram resolved by HELP from *ephedra herb* (d); pure spectra of the components (e). H1: peak 1 resolved by HELP in this Fig, H2: peak 2 resolved by HELP in this figure. (Color figure available online.)

A large number of analytical methods have been developed for the separation and determination of ephedrine-type alkaloids in herbal materials or health products, such as comprehensive two-dimensional gas chromatography,[23] HPLC, capillary electrophoresis, and so on. Most of these methods are not well suited for the quantitative determination of ephedrine-type alkaloids, mainly due to requiring harsh separation condition, lack of separation resolution, and interferences from sample components. The peaks of pseudoephedrine and methylephedrine in Maxing Shigan decoction are such examples. The optimized separation condition keeps them separated from the ephedrine well, but their measurements will be affected by some interfering peaks in Maxing Shigan decoction. Thus, the results of their peak areas will not be accurate and precise enough. With the assistance of the HELP method, their inflow and outflow time were determined, and then the errors from chromatographic profiles could be overcome. HPLC-DAD is a common instrument and widely used in the determination of common compounds. However, it is difficult to determine the complicated chemical components of herbal medicine because different herbs have their own physical and chemical property components, and many chemical reactions occurring among different components during the preparation. It usually requires harsh a separation condition or more such as a comprehensive two-dimensional HPLC system without the help of chemometric methods.[24] HPLC-DAD combined with chemometric methods can satisfy most requirements.

## Embedded Peaks Resolved by AMWFA and Comparison of Components of Different Groups

Although HELP has shown its power, there are still some peaks whose UV spectrum are similar or their inflow and outflow time isn't "first-in-first-out."[25] For the latter problem, there is little selective region for any component, such as the fragment from 29.67–30.23 min in Maxing Shigan decoction, seen in Figure 6a1, for which the HELP method becomes useless. We therefore must resort to other methods, e.g., AMWFA, selective ion analysis (SIA),[26] and so on. AMWFA is more applicable for the HPLC-DAD data. By comparison among three matrices originated from Maxing Shigan decoction (Figure 6a1), *prepared glycyrrhizae radix* (Figure 6a2), and *ephedra herb* (Figure 6a3), one can see that the overlap between the two peaks (Figure 6a1) are very serious. However, the pure chromatogram and pure spectra of the components in Maxing Shigan decoction can be resolved by AMWFA (Figures 6b, and 6c). The specific steps will be explained from the next case analysis below.

The LC-DAD results (**X** matrix) of the fragment from 39.09–40.23 min from Maxing Shigan decoction is shown in Figure 7a. It seems that two

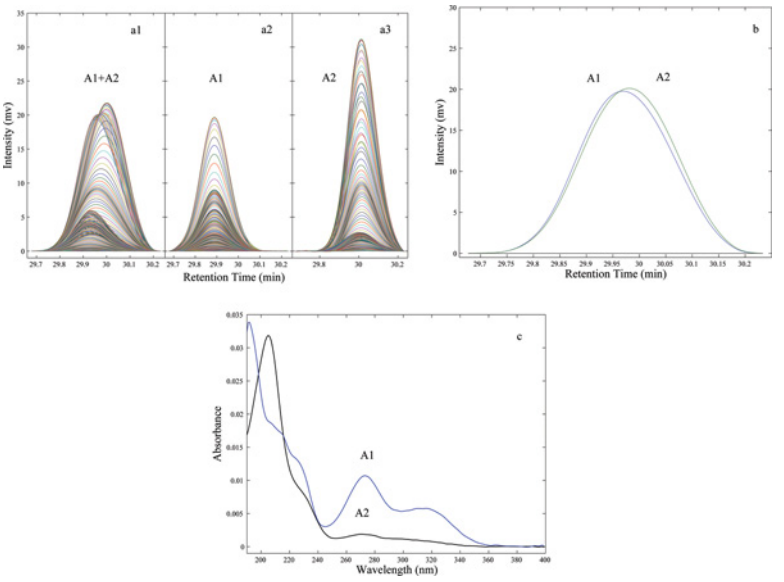


**FIGURE 6** (a) LC-DAD chromatogram data of the fragment from 29.67–30.23 min from Maxing Shigan decoction (a1); *prepared glycyrrhizae radix* (a2); *ephedra herb* (a3) after baseline correction. (b) The pure chromatogram in Maxing Shigan decoction resolved by AMWFA, A1 originated from *prepared glycyrrhizae radix*, A2 originated from *ephedra herb*. (g) Pure spectra of the components. (Color figure available online.)

components are not baseline separated when it was seen from single wavelength chromatograms. In fact, six components at least are overlapped seriously or embedded; the corresponding fragment from *ephedra herb* (**Y** matrix) is also shown in Figure 7a. From this figure, one can see that it is really a difficult problem to be solved. However, we can also resolve this kind of overlapping with the help of the AMWFA method.[13]

The work procedure of the AMWFA method can be described as follows. **X** and **Y** can be firstly decomposed by singular value decomposition (SVD) analysis, the results of multi-component spectral correlative chromatography (MSCC), and inverse projection multi-component spectral correlative chromatography (IP-MSCC) between matrices **X** and **Y,** shown in Figure 7b, which indicates that the UV spectra features of the compounds in peak cluster **X** are highly correlated with that in peak cluster **Y**. Secondly, the moving window searching was conducted between the whole **X** matrix and the whole **Y** matrix with a fixed window size, the two orthogonal bases of loadings, say **E** and **F** $\mathbf{E}=[\mathbf{e}_1, \mathbf{e}_2, \ldots, \mathbf{e}_m]$ of **X,** $\mathbf{F}=[\mathbf{f}_1, \mathbf{f}_2, \ldots, \mathbf{f}_n]$ of **Y**, $m$ and $n$ are the number of chemical components in matrices **X** and **Y**, the region of the number of common components, and the spectral auto-correlative curve was also acquired by drawing similarity vs. retention time, as shown in Figure 7d. When one of the common components in both **X** and **Y** exists,

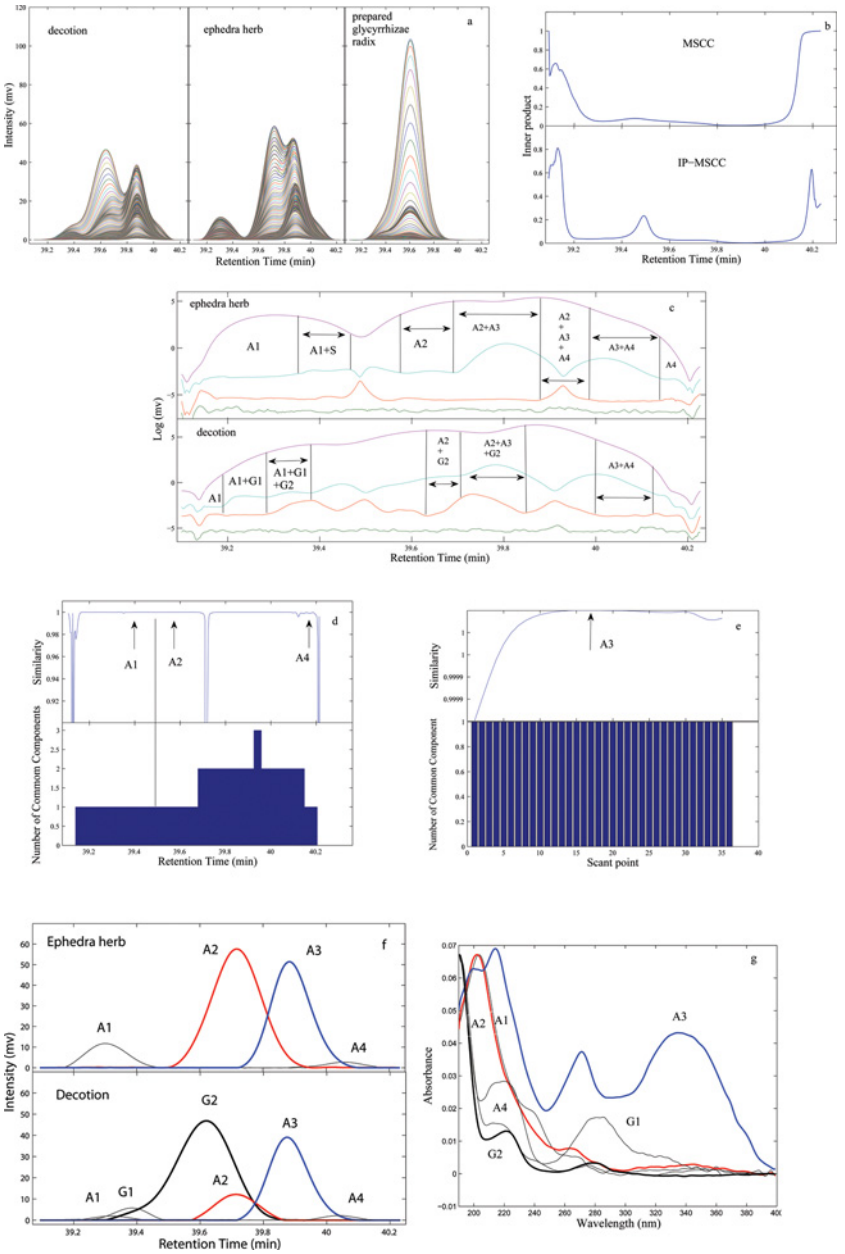


**FIGURE 7** (a) LC-DAD chromatogram data of the fragment from 39.09–40.23 min from Maxing Shigan decoction, *ephedra herb, prepared glycyrrhizae radix* after baseline correction. (b) Results of MSCC and IP-MSCC analysis between Maxing Shigan decoction and *ephedra herb.* (c) Rank map curve results of Maxing Shigan decoction and *ephedra herb.* (d) Results of the number of common component and similarity between Maxing Shigan decoction and *ephedra herb.* (e) Results of the number of common component and similarity between the section of A2 + A3 of *ephedra herb* and A3 + A4 of Maxing Shigan decoction. (f) Resolved pure chromatogram of the main peaks in Maxing Shigan decoction and *ephedra herb.* (g) Pure spectra of the main components. A1, A2, A3, A4 originated from *ephedra herb,* G1,G2 originated from *prepared glycyrrhizae radix.* S is the interference peak. (Color figure available online.)

its spectrum, say $\mathbf{s}_k$, can be written by linear combination of **E** or **F** as equation $\mathbf{s}_k = \sum a_{ik}\mathbf{e}_i = \sum b_{jk}\mathbf{f}_j = \mathbf{E}\mathbf{a}_k = \mathbf{F}\mathbf{b}_k$ $(k = 1, 2, \ldots, c)$, where $\mathbf{a}_k$ and $\mathbf{b}_k$ are linear combination coefficients of bases **E** and **F**. Because of the presence of noises, an objective function[13] is constructed and one obtains $\mathbf{a}_k = \mathbf{E}^T\mathbf{s}_k = \mathbf{E}^T\mathbf{F}\mathbf{b}_k = \mathbf{E}^T\mathbf{F}\mathbf{F}^T\mathbf{s}_k = \mathbf{E}^T\mathbf{F}\mathbf{F}^T\mathbf{E}\mathbf{a}_k$ and $\mathbf{b}_k = \mathbf{F}^T\mathbf{s}_k = \mathbf{F}^T\mathbf{E}\mathbf{a}_k = \mathbf{F}^T\mathbf{E}\mathbf{E}^T\mathbf{s}_k = \mathbf{F}^T\mathbf{E}\mathbf{E}^T\mathbf{F}\mathbf{b}_k$. Because $\mathbf{s}_k$ is a common component in matrices **X** and **Y**, $\mathbf{a}_k$ and $\mathbf{b}_k$ must be the eigenvectors of matrices $\mathbf{E}^T\mathbf{F}\mathbf{F}^T\mathbf{E}$ and $\mathbf{F}^T\mathbf{E}\mathbf{E}^T\mathbf{F}$ with only one value. If there is no pure common component between **X** and **Y**, the value of $d_k$ will be significantly less than 1 and the value of $f(\mathbf{a}_k, \mathbf{b}_k)$ will be closed to 2 or more. So, there are pure common components from 39.14–39.68 min and from 40.15–40.20 min, while there are no pure common components from 39.68–40.15 min. Finally, the corresponding pure UV spectrum of common components marked with A1, A2, A4 could be obtained from the eigenvector on the three flat regions by moving window-evolving factor analysis and spectral auto-correlative curve obtained by formula $\mathbf{s} = \sum a_i\mathbf{e}_i = \sum b_j\mathbf{f}_j = \mathbf{E}\mathbf{a} = \mathbf{F}\mathbf{b}$ during the scanning process.

The troublesome question is how to resolve the peak in this region of the number of common components equals to two. In Figure 7c, we can see clearly that many regions could be divided in the rank map curve results of *ephedra herb,* the regions marked with A1, A2, and A4 representing the corresponding pure component's regions and the regions marked with A2 + A3, A3 + A4, etc. representing the corresponding mixed component's regions. Similarly, the regions marked with A2 + A3 + G2, A3 + A4 also could be found in the rank map curve of Maxing Shigan decoction. In principle, the pure spectrum could be searched only if the region common components equal one. So, the moving window searching was done between the matrix in the regions marked with A2 + A3 and the matrix in the regions marked with A3 + A4 in the *ephedra herb* and Maxing Shigan decoction, respectively. A pure spectrum could be acquired from the results shown in Figure 7e. The searching also can be used in the regions marked with A2 + A3 + G4 and A3 + A4. Peaks G1, G2 in the studied peak cluster also can be found in the same way as described above. Resolution results of the peaks in Maxing Shigan decoction are shown in Figure 7f, in which A1, A2, A3, A4 originated from *ephedra herb* and G1, G2 originated from *prepared glycyrrhizae radix*. Pure spectra of the components are shown in Figure 7g. Other peaks in the studied samples are determined qualitatively in the same way as described above.

Not all the peaks can be resolved by existing chemometric methods. The UV spectrums of two peaks from 36.19–37.26 min in Maxing Shigan decoction are very similar, so the calculation of peak area can only resort to other methods, such as vertical incision method. All in all, in traditional Chinese medicine liquid chromatograms, complex chemical compositions

and baseline noise make it more difficult to resolve all peaks. In these cases, our methods can help us to resolve the main peaks and eliminate interference.

In Maxing Shigan decoction, 94 pure peaks were determined, 50 peaks originated from *ephedra herb*, 23 peaks originated from *semen armeniacae amarum*, 29 peaks originated from *prepared glycyrrhizae radix*, and 2 new peaks were detected. The components results from Maxing Shigan decoction include retention time, UV spectrum, and source herb are all summarized in Table 1.



## The Analysis of Dissolved Ratios of Components Among Single Herbs, Maxing Shigan Decoction, and Other Disassembled Prescriptions

The material foundation of compound herbal prescription, such as decoction, is chemical component combination, which exerts effect from a multi-target to multi-level aspect.[27] The variations of any ingredient in decoction are likely to change the strength of drug efficacy. The dissolved ratios of active components in herbs are higher or lower than that in the mixed preparation, which may be due to the action between decoction components, such as changing solubility, sediment, or inter-reaction. Therefore, the mixed preparation of herbal decoction is a complicated physiochemical reaction. Only after components' change, including the dissolved ratios changes during the decoction, are clarified, the pharmacological difference between Maxing Shigan decoction and other ephedra prescriptions further can be understood clearly.

According to the resolved chromatogram and UV spectra, the quantitative analysis of most components can be directly calculated, including the dissolved ratios between single herbs and decoction. In Equation (2), $\mathrm{Area}_{\mathrm{decoction}}$, $\mathrm{Area}_{\mathrm{single}}$, $\mathrm{W}_{\mathrm{decoction}}$, and $\mathrm{W}_{\mathrm{single}}$ refer to the peak area of a pure component in decoction, the peak area of a pure component in single crude herb sample, the weight of source herb in the decoction, and the weight of single crude herb:

$$ratios = \frac{Cdecoction}{C\sin gle} = \frac{Areadecoction \times W\sin gle}{Area\sin gle \times Wdecoction} \quad (1)$$

The results of dissolved ratios of seven mark bioactive constituents and other major mutative constituents in Maxing Shigan decoction are shown in Table 2. Alkaloids that came from *ephedra herb* decreased slightly, and amygdalin originating from *semen armeniacae amarum* increased slightly. Through the calculation of dissolved ratios among every decoction, the interacting materials, including decreasing factors or increasing factors,

**TABLE 1** The UV Feature of Major Peaks in Maxing Shigan Decoction and Their Source Herb. 1, Ephedrine; 2, Pseudoephedrine; 3, Methylephedrine; 4, Amygdalin; 5, Liquiritin; 6, Benzoic acid; 7, Glycyrrhizic acid

| | $t_R$ (min) | Wmax (nm) | Source Herb | No. | $t_R$ (min) | Wmax (nm) | Source Herb |
|---|---|---|---|---|---|---|---|
| 1 | 18.92 | 190, 206 | *EH* | 45 | 35.28 | 190, 216, 272, 315 | *PGR* |
| 2 | 19.98 | 190, 206 | *EH* | 46 | 35.85 | 190, 206 | *SAA* |
| 3 | 22.15 | 190, 205 | *EH* | 47 | 36.53 | 190, 206 | *SAA* |
| 4 | 31.71 | 190, 206 | SAA | 48 | 36.90 | 190, 206 | *SAA* |
| 5 | 45.97 | 190, 216, 278, 312 | *PGR* | 49 | 37.53 | 199, 215, 272, 335 | *EH* |
| 6 | 48.59 | 194, 230 | *EH* | 50 | 37.80 | 203 | *EH* |
| | | | SAA | 51 | 38.15 | 190, 206 | *SAA* |
| 7 | 82.46 | 190, 253 | *PGR* | 52 | 38.33 | 190, 225, 260 | *EH* |
| 8 | 9.18 | 190, 254 | *EH* | 53 | 38.58 | 193, 220, 272, 335 | *EH* |
| 9 | 9.85 | 190, 298 | SAA | 54 | 38.76 | 192, 224 | *EH* |
| | | | *PGR* | 55 | 39.30 | 205, 238 | *EH* |
| 10 | 10.98 | 190, 206 | *EH* | 56 | 39.37 | 190, 218, 282 | *PGR* |
| | | | SAA | 57 | 39.61 | 190, 223, 278 | *PGR* |
| | | | *PGR* | 58 | 39.72 | 204, 270 | *EH* |
| 11 | 11.83 | 208, 235 | *EH* | 59 | 39.87 | 215, 272, 338 | *EH* |
| 12 | 12.92 | 205, 268 | *EH* | 60 | 40.50 | 199, 215, 272, 318 | *PGR* |
| 13 | 13.18 | 205, 220, 318 | *EH* | 61 | 40.61 | 205, 270, 338 | *EH* |
| 14 | 13.33 | 190, 220 | *EH* | 62 | 40.79 | 199, 215, 272, 338 | *EH* |
| 15 | 13.62 | 200, 230, 285 | SAA | | | | *PGR* |
| | | | *PGR* | 63 | 41.30 | 190, 225, 276 | *PGR* |
| 16 | 14.02 | 190, 205 | *SAA* | 64 | 41.82 | 201, 272 | *EH* |
| 17 | 14.10 | 200, 250 | *EH* | 65 | 42.95 | 190, 206 | *SAA* |
| 18 | 14.49 | 190, 205 | *EH* | 66 | 43.66 | 203, 276 | *EH* |
| 19 | 14.49 | 190, 210, 255, 300 | *SAA* | 67 | 44.19 | 192, 220, 280 | *EH* |
| 20 | 15.09 | 190, 200 | *SAA* | 68 | 44.36 | 190, 220 | *SAA* |
| 21 | 15.88 | 190, 205 | *EH* | 69 | 44.85 | 213, 272, 338 | *EH* |
| 22 | 16.61 | 190, 205 | *EH* | 70 | 45.09 | 190, 268, 338 | *EH* |
| | | | SAA | 71 | 45.37 | 190, 217, 277 | *PGR* |
| 23 | 17.25 | 190, 205 | *SAA* | 72 | 49.21 | 206, 275 | *EH* |
| 24 | 18.46 | 190, 205, 220 | *SAA* | 73 | 52.40 | 200, 250 | *SAA* |
| 25 | 20.43 | 196, 219, 278 | *SAA* | 74 | 55.36 | 190, 290 | *EH* |
| 26 | 22.35 | 190, 215, 275 | *PGR* | | | | *PGR* |
| 27 | 22.75 | 210, 265 | *EH* | 75 | 57.56 | 197, 225, 283, 348 | *EH* |
| 28 | 23.43 | 192, 223, 278 | *PGR* | 76 | 61.52 | 196, 355 | *EH* |
| 29 | 27.90 | 190, 206 | *SAA* | 77 | 61.85 | 193, 255 | *PGR* |
| 30 | 29.31 | 195, 205, 255 | *EH* | 78 | 64.35 | 190, 221, 275 | *EH* |
| 31 | 29.95 | 192, 213, 273 | *PGR* | 79 | 65.18 | peak cluster | New |
| 32 | 30.01 | 206, 230, 278 | *EH* | 80 | 65.50 | 190 | *EH* |
| 33 | 30.40 | 195, 215, 290 | *SAA* | 81 | 67.91 | 190, 210, 232, 276 | New |
| 34 | 30.45 | 220, 268 | *EH* | 82 | 69.15 | 190, 225, 308 | *EH* |
| 35 | 30.50 | 192, 213, 273 | *PGR* | 83 | 69.46 | 205, 278 | *EH* |
| 36 | 30.60 | 190, 205 | *SAA* | 84 | 70.10 | 190, 253 | *PGR* |
| | | | *EH* | 85 | 70.71 | 190, 210 | *EH* |
| 37 | 31.25 | 190, 206 | *SAA* | 86 | 70.88 | 190, 252 | *PGR* |
| 38 | 32.45 | 192, 215, 280,318 | *EH* | 87 | 71.73 | 190, 225 | *EH* |
| | | | *PGR* | 88 | 72.11 | 190, 253 | *PGR* |
| 39 | 33.71 | 193, 215, 280, 315 | *PGR* | 89 | 72.43 | 190, 235, | *EH* |

(*Continued*)

**TABLE 1** Continued

| | $t_R$ (min) | Wmax (nm) | Source Herb | No. | $t_R$ (min) | Wmax (nm) | Source Herb |
|---|---|---|---|---|---|---|---|
| 40 | 33.98 | 190, 220 | *EH* | 90 | 74.60 | 190, 253 | *PGR* |
| 41 | 34.33 | 190, 216, 272, 315 | *PGR* | 91 | 74.99 | 190, 252 | *PGR* |
| 42 | 34.49 | 204, 252, | *EH* | 92 | 78.68 | 190, 253 | *PGR* |
| 43 | 34.75 | 206 | *EH* | 93 | 80.87 | 190, 253 | *PGR* |
| 44 | 35.00 | 207, 260, 350 | *EH* | 94 | 84.94 | 190, 253 | *PGR* |

*ephedra herb* (*EH*); *semen armeniacae amarum* (*SAA*); *prepared glycyrrhizae radix* (*PGR*); *gypsum fibrosum* (*GF*).



can also be simply deduced. The dissolved ratios of peak 9 in the Maxing Shigan decoction is 0.36, but the dissolved ratios of peak 9 in the whole prescription without *gypsum fibrosum* decoction is 1.01. So, *gypsum fibrosum* was considered a decreasing factor. The major mutative components in Maxing Shigan decoction and their interacting herb are shown in Table 2.

In TCM theory, *prepared glycyrrhizae radix* reconciles all the drugs. From Table 2, it was also participated in the most substance variation. But, the most interesting question is the role of *gypsum fibrosum* in the dissolved

**TABLE 2** The Major Mutative Components in Maxing Shigan Decoction and its Interacting Herb. 1, Ephedrine; 2, Pseudoephedrine; 3, Methylephedrine; 4, Amygdalin; 5, Liquiritin; 6, Benzoic acid; 7, Glycyrrhizic acid

| | | | Interacting Materials | | | | | Interacting Materials | |
|---|---|---|---|---|---|---|---|---|---|
| No. | $t_R$ (min) | Dissolved Ratios | Decreasing Factors | Increasing Factors | No. | $t_R$ (min) | Dissolved Ratios | Decreasing Factors | Increasing Factors |
| 1 | 18.92 | 0.96 | – | – | 46 | 35.85 | 0.71 ↓ | *GF* | – |
| 2 | 19.98 | 0.95 | – | – | 47 | 36.53 | 4.39 ↑ | – | *EH* |
| 3 | 22.15 | 0.98 | – | – | 48 | 36.90 | 1.07 ↑ | – | *EH* |
| 4 | 31.71 | 1.07 | – | *PGR* | 50 | 37.80 | 0.50 ↓ | *PGR* | *GF* |
| 5 | 45.97 | 0.58 ↓ | *SAA* | – | 66 | 43.66 | 0 | *SAA* | – |
| 6 | 48.59 | 0.81 ↓ | *GF* | *PGR* | 67 | 44.19 | 0.82 ↓ | *PGR* | – |
| 7 | 82.46 | 0.40 ↓ | *SAA, GF* | – | 73 | 52.40 | 1.36 ↑ | *GF* | *PGR* |
| 9 | 9.85 | 0.36 ↓ | *GF* | – | 76 | 61.52 | 1.90 ↑ | – | *PGR* |
| 11 | 11.83 | 0.76 ↓ | *SAA, PGR* | – | 78 | 64.35 | 0.81 ↓ | *PGR* | – |
| 15 | 13.62 | 0.14 ↓ | *EH, GF* | – | 79 | 65.18 | new | – | *PGR* |
| 22 | 16.61 | 0.61 ↓ | *GF, PGR* | – | 81 | 67.91 | new | – | *PGR* |
| 24 | 18.46 | 0.62 ↓ | *GF, PGR* | – | 83 | 69.46 | 0.81 ↓ | *PGR* | – |
| 28 | 23.43 | 0.85 ↓ | *GF* | – | 88 | 72.11 | 0.58 ↓ | *SAA, EH, GF* | – |
| 29 | 27.90 | 0.83 ↓ | *GF, PGR* | – | 90 | 74.60 | 0.50 ↓ | *SAA, EH, GF* | – |
| 37 | /31.25 | 1.22 ↑ | *EH* | *GF* | 91 | 74.99 | 0.50 ↓ | *SAA, EH, GF* | – |
| 43 | 34.75 | 0.21 ↓ | *PGR, SAA* | – | 92 | 78.68 | 0.49 ↓ | *SAA, EH, GF* | – |
| 44 | 35.00 | 0.29 ↓ | *PGR, SAA* | – | 93 | 80.87 | 0.47 ↓ | *SAA, EH, GF* | – |
| 45 | 35.28 | 0.47 ↓ | *SAA* | – | 94 | 84.94 | 0.39 ↓ | *SAA, EH, GF* | – |

*ephedra herb* (*EH*); *semen armeniacae amarum* (*SAA*); *prepared glycyrrhizae radix* (*PGR*); *gypsum fibrosum* (*GF*).

ratios of decoctions. Mahuang decoction and Maxing Shigan decoction, initially recorded in Shanghanlun by Zhang Zhong-jing in Han Dynasty, are two of the most important exterior-releasing prescriptions in TCMs. The Mahuang decoction contains *ephedra herb, cinnamon bark, semen armeniacae amarum*, and *prepared glycyrrhizae radix* and has a stronger diaphoretic effect and warming property than *ephedra herb* alone; while Maxing Shigan decoction is composed of *ephedra herb, gypsum fibrosum, semen armeniacae amarum*, and *prepared glycyrrhizae radix*, it does not exhibit a diaphoretic effect with warm property. In this way, a combination of herbs is very important to establish the properties of herbal medicines. *Gypsum fibrosum* is the monarch drug in Maxing Shigan decoction, and it not exist in Mahuang decoction. *Gypsum fibrosum* may be the substance that cause the pharmacological difference of two decoction.

Because *gypsum fibrosum* belong to sulfates mineral, and the characteristics of zero UV absorption make the comparison between Maxing Shigan decoction and the whole prescription removing *gypsum fibrosum* easier and more clear, two kinds of chromatograms monitoring from 190–399 nm were used. In order to observe the dissolved ratios variation, three kinds of single wavelength chromatograms were used, the correlations coefficients of two kinds of chromatograms at 210 nm, 250 nm, and 276 nm were 0.992, 0.985, and 0.915, respectively. Besides peak 9, peak 15, peak 22, peak 24, peak 6, peak 73, peak 81, and other peak clusters, there were other dissolved ratios variation slightly.

Two possibilities about the role of *gypsum fibrosum* are assumed. Firstly, *ephedra herb* and *gypsum fibrosum* have their own pharmacological activity when they were used in different dosages. The synergistic or restrain action is achieved by enhancing or weakening the pharmacological activity after the oral mixed preparation was used. Secondly, due to the cooking process, the concentrations of some chemical components with special biological activity in mixed preparation are changed. Therefore, the dissolved ratios variation of the chemical components after adding *gypsum fibrosum* shouldn't be neglected in analyzing the reason of the unique therapeutic effects.

## CONCLUSION

This study introduces an analytical procedure for the simultaneous determination of ephedrine, pseudoephedrine, methylephedrine, amygdalin, liquiritin, glycyrrhizic acid, and benzoic acid or more with excellent precision and accuracy. In this article, by using the chemometrics methods, HPLC-DAD and gradient elution, the chemical compositions of Maxing Shigan decoction and other disassembled prescriptions were analyzed and then compared regarding their qualitative and relatively quantitative

characteristics. The comparative results of chemical compositions were significant to help us to understand the chemical composition variation of Maxing Shigan decoction due to the cooking process. Furthermore, the chemometric methods could greatly enhance the accuracy of quantitative and qualitative results together with gradient elution. The comparison also showed that AMWFA could be a convenient and fast tool for doing comparative analysis in complicated systems. Ordinary manual linear deduction combined with airPLS method is a good method to correct baseline drift.

The study of Maxing Shigan decoction in our work shows that the mixed preparation of herbal decoction is not as simple as "$1+1=2$" in math. There is no doubt that many active components in herbs such as *ephedra herb, semen armeniacae amarum, prepared glycyrrhizae radix*, and *gypsum fibrosum*, etc., interact in the cocktail form. Hence, the unique bioactivity and therapeutic effects are shown in the mixed preparation of Maxing Shigan decoction.



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