# Simultaneous quantification of nine major active components in traditional Chinese prescription Mahuang decoction and the influence of herbal compatibility on their contents

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## ABSTRACT

**Background:** Mahuang decoction (MHD), a famous classic traditional Chinese formula, has been extensively applied for treating cold, influenza, asthma, acute bronchitis, and other pulmonary diseases. However, the interaction among four drugs of MHD has not been clearly deciphered from the aspect of molecular composition. **Objective:** To assess the quality of MHD and explore the interplay among different prescription drugs. **Materials and Methods:** A reversed-phase high performance liquid chromatography (RP-HPLC) coupled with diode array detector (DAD) method for the simultaneous separation and determination of nine bioactive components was developed. A somatomedin A (SMA)-phenyl column (4.6 mm × 250 mm, 5 μm) was eluted by a gradient mobile phase contained acetonitrile and 0.05% formic acid-0.05% triethylamine aqueous solution. Four detection wavelengths (210, 252, 278, and 291 nm) were utilized for the quantitative analysis due to the different ultraviolet (UV) spectra of these compounds. **Results:** Satisfactory separation was obtained for all the components, and the assay was fully validated in respects of linearity, precision, stability, and accuracy. It was found that the calibration curves for all analytes showed good linearity ($R^2 \geq 0.9991$) within the test ranges. The relative standard deviations (RSDs) for intra- and interday repeatability were not more than 1.70 and 2.66%, respectively. The spike recoveries of nine components varied from 97.50 ± 1.69 to 99.27 ± 1.37%. **Conclusion:** The established method was successfully applied to analyze nine active compounds in decoction samples of various drug compatibilities of MHD. The variations of contents were obvious for different combinations, which hinted the mutual promotion or inhibition of componential dissolution among four herbs of MHD.

**Key words:** Determination, HPLC-DAD, herbal compatibility, Mahuang decoction, traditional Chinese prescription

## INTRODUCTION

Traditional Chinese prescriptions have been used clinically for thousands of years, and exhibit magical efficacy on prevention and therapy of human diseases. More than 100,000 formulae have been accumulated in long-term practices.[1] However, the compatibility principles and the interactions among different drugs of Chinese compound prescriptions have not been clearly deciphered from the aspect of molecular composition even though it is universally accepted that the joint contribution of multidrugs and multicomponents is responsible for the synergistic and therapeutic effects of Traditional Chinese prescriptions.[2,3] The research has faced many obstacles due to the unimaginable complexity of multi-herb formulae.[4]

Mahuang decoction (Ephedra decoction, MHD), which is a famous classic formula recorded in *Treatise on Febrile Diseases* (Shang Han Lun in Chinese) edited by Zhang Zhongjing in the Han Dynasty, consists of Herba Ephedrae (Ephedra), Ramulus Cinnamomi (Cassia twig), Semen Armeniacae Amarum (Bitter apricot kernel), and

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Radix Glycyrrhizae Praeparatae (Prepared licorice) with a traditional dose ratio of 9:6:6:3. MHD has been extensively applied for the treatment of cold, influenza, acute bronchitis, bronchial asthma, and other pulmonary diseases for its acknowledged activities of inducing diaphoresis and allaying asthma;[5,6] and is considered the typical representation reflecting the essential composition principle of Traditional Chinese prescriptions-'monarch, minister, assistant, and guide'. The four drugs composed MHD have their respective potency when used independently, while after combination in MHD, they not only show the primary or secondary effect, but also supplement and restrict one another, thus form a prescription with great therapeutic function.[7]

Modern pharmacological studies demonstrated that the main bioactive components of MHD include L-ephedrine, D-pseudoephedrine, L-methylephedrine (from Ephedra), cinnamic alcohol, cinnamic aldehyde, cinnamic acid (from Cassia twig), amygdalin (from bitter apricot kernel), liquiritin, and glycyrrhizic acid (from prepared licorice). In the previous reports, the componential interplay between two drugs such as Ephedra and Cassia twig, or Ephedra and bitter apricot kernel has been analyzed and discussed,[8-11] but little information of nine major bioactive components in four drugs was mentioned integrally.

In the present paper, a simple, accurate, and reliable high performance liquid chromatography coupled with diode array detector (HPLC-DAD) method for the simultaneous quantification of the foregoing nine major components contained in MHD was successfully established for comprehensive quality evaluation of this important traditional Chinese formula. Furthermore, the content fluctuations of nine bioactive ingredients were detected in different drug combinations of MHD, with the aim to provide reference for interpreting the compatibility mechanism of MHD as well as other Chinese compound prescriptions.

## MATERIALS AND METHODS

### Reagents and materials

The standard of L-ephedrine (lot number: 171241-201007), D-pseudoephedrine (lot number: 171237-201208), and L-methylephedrine (lot number: 171247-200301) were purchased from the National Institute for Food and Drug Control of China (Beijing, China). The standards of cinnamic alcohol (lot number: 20120421), cinnamic aldehyde (lot number: 20120311), cinnamic acid (lot number: 20120407), amygdalin (lot number: 20110421), liquiritin (lot number: 20120131), and glycyrrhizic acid (lot number: 20101107) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). All these standard substances had over 98% purity. HPLC-grade acetonitrile was supplied by TEDIA (Fairfield, OH, USA), and water was purified by using a Milli-Q ultra-water system (Billerica, MA, USA) and filtered with 0.22 μm membrane. Other reagents including methanol, formic acid, and triethylamine were all of analytical grade.

The four Chinese herbs *Ephedra intermedia* Schrenk et C.A.Mey. (lot number: 20120523), *Cinnamomum cassia* Presl (lot number: 20120409), *Prunus armeniaca* L. var. ansu. Maxim. (lot number: 20120608), and *Glycyrrhiza uralensis* Fischer (lot number: 20120415) were purchased from Hangzhou Tairentang Drug Store (Hangzhou, China), and were identified by Prof. Hong Wang, College of Pharmaceutical Science, Zhejiang Chinese Medical University where the voucher specimens are deposited.

### Apparatus and chromatographic conditions

An Agilent 1260 Infinity Series HPLC system equipped with a G1311C quarternary pump, a G1316A column oven, a G1329A autosampler, and a G1315D photodiode array detector was used for the chromatographic analysis. All separations were performed on a somatomedin A SMA-phenyl column (4.6 mm × 250 mm, 5 μm). The mobile phase was composed of 0.05% formic acid-0.05% triethylamine aqueous solution (A) and acetonitrile (B) which was applied in the gradient elution as follows: 0-15 min, 92-90% A; 15-20 min, 90-90% A; 20-50 min, 90-73% A; 50-55 min, 73-62% A; 55-60 min, 62-62% A; 60-65 min, 62-92% A, and finally, reconditioning the column with 92% A isocratic for 10 min. The flow rate was 1.0 ml/min and the column temperature was set at 30°C. The injection volume was 20 μl with needle wash. The detection wavelengths were set at 210, 252, 278, and 291 nm where the components had their maximum response of ultraviolet (UV) spectrum, respectively.

### Preparation of stock mixed standard solution

Stock mixed standard solution with concentrations of 0.1 mg/ml for L-ephedrine, D-pseudoephedrine, L-methylephedrine, amygdalin, liquiritin, glycyrrhizic acid, and concentrations of 0.01 mg/ml for cinnamic alcohol, cinnamic acid, and cinnamic aldehyde was prepared by 50% methanol.

### Preparation of MHD extract for HPLC quantification

The raw materials were weighed in conformity with MHD formula, that is, Ephedra 9 g, Cassia twig 6 g, bitter apricot kernel 6 g, and prepared licorice 3 g. Ephedra was immersed in 90 ml water for 30 min and first decocted for 30 min. The other three drugs were immersed in 150 ml water for 30 min, then decocted with Ephedra for another 30 min. The aqueous extraction solution was adjusted to 150 ml, filtered through 0.45 μm membrane and injected into HPLC system.

### Preparation of extraction solutions of different herbal combinations

In order to investigate the mutual influence of four drugs in MHD, 14 groups of herbal combinations were designed as follows: (1) Ephedra 9 g; (2) Cassia twig 6 g; (3) bitter apricot kernel 6 g; (4) prepared licorice 3 g; (5) Ephedra 9 g and Cassia twig 6 g; (6) Ephedra 9 g and bitter apricot kernel 6 g; (7) Ephedra 9 g and prepared licorice 3 g; (8) Cassia twig 6 g and bitter apricot kernel 6 g; (9) Cassia twig 6 g and prepared licorice 3 g; (11) bitter apricot kernel 6 g and prepared licorice 3 g; (11) Ephedra 9 g Cassia twig 6 g and bitter apricot kernel 6 g; (12) Ephedra 9 g, cassia twig 6 g, and prepared licorice 3 g; (13) Ephedra 9 g, bitter apricot kernel 6 g, and prepared licorice 3 g; (14) Cassia twig 6 g, bitter apricot kernel 6 g, and prepared licorice 3 g.

Different drug combinations were extracted by the similar way described above. The crude drugs were added 10-fold water, soaked for 30 min then boiled for another 30 min. If there was Ephedra included in the drug combination, it should be preboiled for 30 min in accordance with the traditional theory of Chinese medicine.

### Validation of the method

The calibration was carried out using a series of standard solution prepared by diluting the stock solution to appropriate concentration range. With two replicates per concentration, the calibration curves were plotted with integrated chromatographic peak areas of nine major active components in MHD against the corresponding concentrations.

The limits of detection and quantification under the chromatographic condition were calculated at a signal-to-noise (S/N) ratio of 3 for limit of detection (LOD) and 10 for limit of quantification (LOQ), respectively.[12]

Intra- and interday variations were chosen to determine the precision of the developed method. The relative standard deviation (RSD) was taken as a measure of precision. Intra- and interday repeatability was determined on six replicates within 1 day and 5 consecutive days, respectively.[13]

The stability of analytical solution at ambient temperature was investigated by analyzing sample solution at 0, 2, 4, 8, 12, and 24 h. The RSD values of peak areas were used for evaluation ($n$ = 6).

To evaluate accuracy, MHD samples were spiked with various amounts of standard solution. The spiked solutions of each concentration level were prepared in triplicate and their peak areas were used to calculate the recoveries.

## RESULTS AND DISCUSSION

### Optimization of HPLC conditions

Since nine analytes in MHD belong to alkaline, acid and neutral compounds, respectively, and alkaloids in Ephedra, or phenylpropanoids in Cassia twig have analogical chemical structure, appropriate chromatographic conditions are critically important for good separation. In our experiment, different columns, mobile phases, and elution programs were employed. Flow rate and over temperature were also optimized. Eventually, a SMA-phenyl column was used to improve the resolution of Ephedra alkaloids, and eluted by acetonitrile-0.05% formic acid-0.05% triethylamine aqueous solution with a flow rate of 1.0 ml/min at 30°C which was found suitable for the simultaneous determination.

Considering the individual UV absorption characteristics of different compounds [Figure 1], the photodiode array detector was set at 210 nm for L-ephedrine, D-pseudoephedrine, L-methylephedrine, amygdalin, and

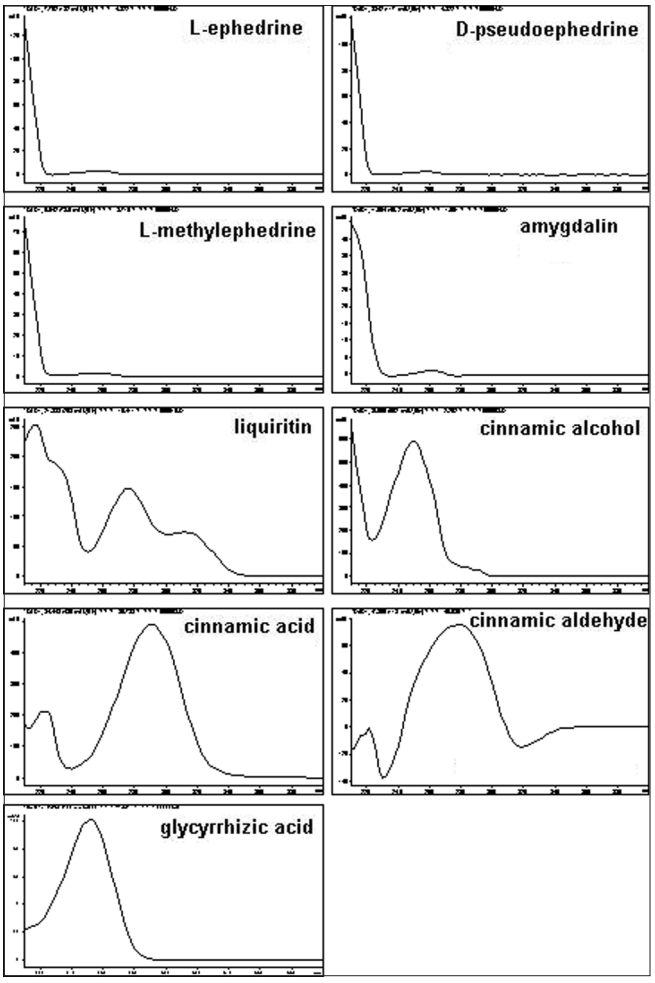


**Figure 1:** Ultraviolet absorption spectra of the nine active components contained in mahuang decoction

liquiritin; 252 nm for cinnamic alcohol and glycyrrhizic acid; 278 nm for cinnamic acid; and 291 nm for cinnamic aldehyde. Under this proposed analytical condition, the nine marker constituents were sufficiently resolved and successfully separated, and excellent agreement between standard and sample spectra was found in all analyzed samples [Figures 2 and 3].

### Calibration curves and the limits of detection

Calibration equations of mixed standard solutions, coefficients of determination ($R^2$), linear range, and the detection limits of all analytes were presented in Table 1. All calibration curves were constructed from peak areas of the reference standards versus their concentrations. The results of LOD and LOQ were also given in Table 1.

### Precision, stability and accuracy

It was found that overall intra- and interday variations of nine components were not more than 1.70 and 2.66%, respectively; suggesting that the developed method was precise. And the sample solution was stable for at least 24 h at room temperature [Table 2].

Accuracy was determined by adding three different quantities (low, medium, and high) of the authentic standards to the known amounts of MHD samples.

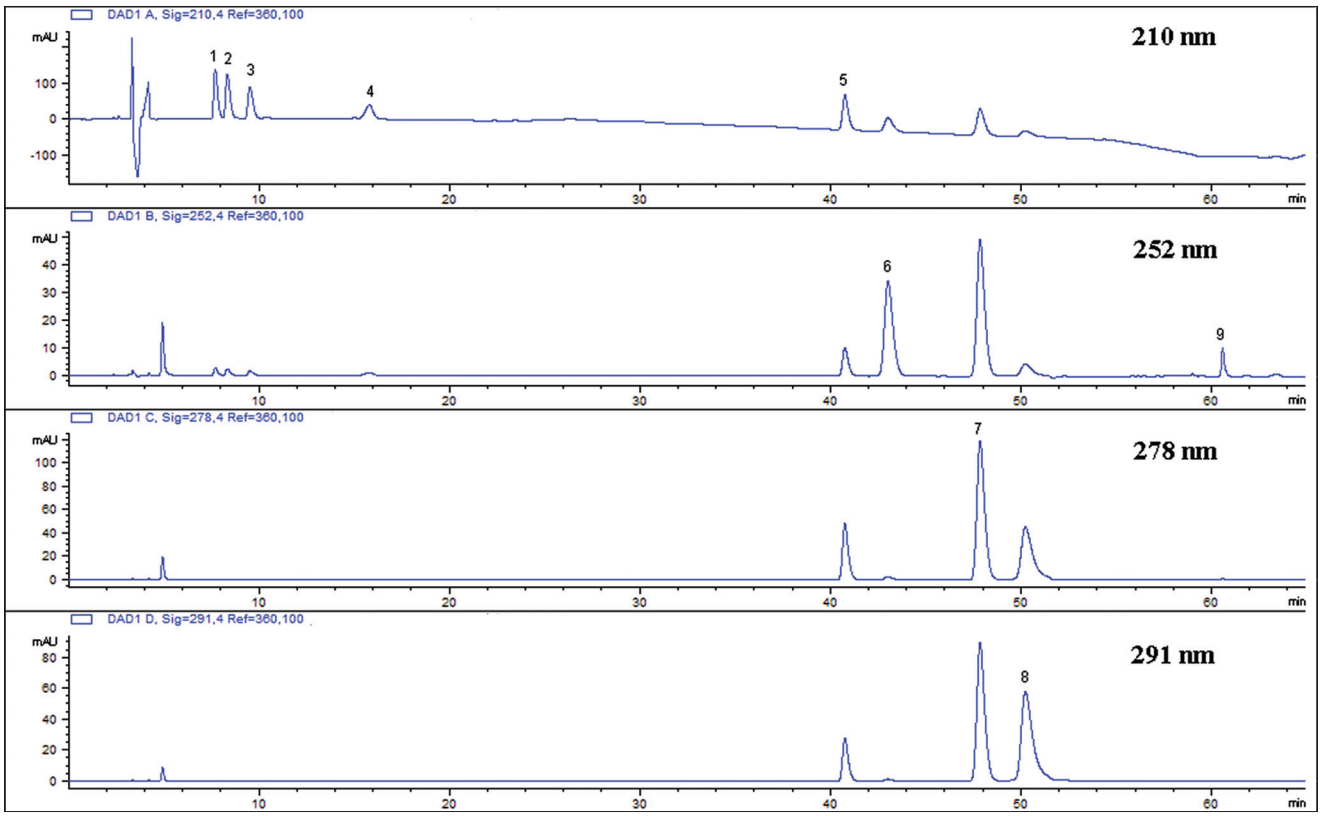


**Figure 2:** High-performance liquid chromatograph chromatogram of a mixed standard solution. 1. L-ephedrine, 2. D-pseudoephedrine, 3. L-methylephedrine, 4. amygdalin, 5. liquiritin, 6. cinnamic alcohol, 7. cinnamic acid, 8. cinnamic aldehyde, 9. glycyrrhizic acid

**Table 1: Calibration equations, coefficients of determination ($R^2$), linear ranges, LODs and LOQs of all analytes**

| Analyte | Calibration equation | $R^2$ | linear range (μg/ml) | LOD (μg/ml) | LOQ (μg/ml) |
|---|---|---|---|---|---|
| L-ephedrine | y=38.23x−78.77 | 0.9991 | 10.0-40.0 | 0.60 | 2.0 |
| D-pseudoephedrine | y=48.04x−120.12 | 0.9992 | 5.00-80.0 | 0.65 | 2.2 |
| L-methylephedrine | y=48.04x+95.38 | 0.9993 | 5.00-60.0 | 0.90 | 3.0 |
| Amygdalin | y=15.56x−32.17 | 0.9995 | 50.0-400 | 1.2 | 4.0 |
| Liquiritin | y=53.96x+31.01 | 0.9992 | 10.0-85.0 | 0.25 | 0.85 |
| Cinnamic alcohol | y=230.34x+25.25 | 0.9995 | 5.00-17.5 | 0.10 | 0.35 |
| Cinnamic acid | y=275.47x−194.29 | 0.9991 | 5.00-30.0 | 0.010 | 0.040 |
| Cinnamic aldehyde | y=105.56x+155.13 | 0.9993 | 5.00-80.0 | 0.0050 | 0.015 |
| Glycyrrhizic acid | y=18.59x−107.40 | 0.9992 | 2.00-200 | 0.040 | 0.15 |

Y: Peak area of the analyte; x: Concentration in μg/ml; LOD: Limit of detection; LOQ: Limit of quantification

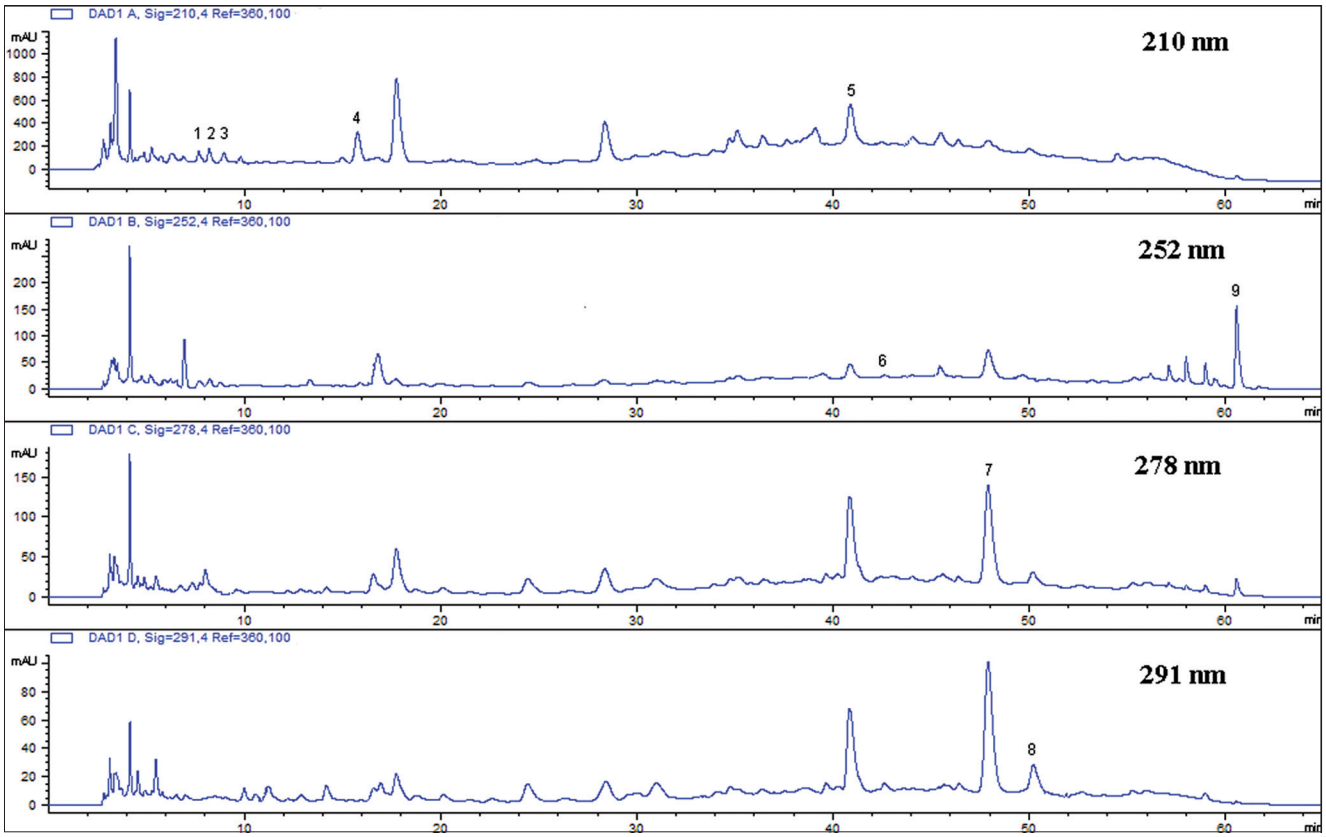


**Figure 3:** HPLC chromatogram of MHD water extract. 1. L-ephedrine, 2. D-pseudoephedrine, 3. L-methylephedrine, 4. amygdalin, 5. liquiritin, 6. cinnamic alcohol, 7. cinnamic acid, 8. cinnamic aldehyde, 9. glycyrrhizic acid

**Table 2: The intra- and interday precision and stability of all analytes (*n*=6)**

| Analyte | Intraday precision | | Interday precision | | Stability | |
|---|---|---|---|---|---|---|
| | Mean±SD (μg/ml) | RSD % | Mean±SD (μg/ml) | RSD % | Mean±SD (μg/ml) | RSD % |
| L-ephedrine | 38.98±0.47 | 1.20 | 37.63±0.93 | 2.47 | 37.72±0.81 | 2.15 |
| D-pseudoephedrine | 50.59±0.80 | 1.58 | 49.96±1.33 | 2.66 | 48.52±1.22 | 2.51 |
| L-methylephedrine | 48.10±0.82 | 1.70 | 47.87±1.22 | 2.55 | 33.46±0.64 | 1.91 |
| Amygdalin | 49.21±0.70 | 1.42 | 48.59±1.26 | 2.59 | 294.05±4.29 | 1.46 |
| Liquiritin | 50.16±0.70 | 1.40 | 48.99±1.21 | 2.47 | 67.91±1.68 | 2.47 |
| Cinnamic alcohol | 4.99±0.07 | 1.40 | 4.88±0.10 | 2.05 | 9.66±0.25 | 2.59 |
| Cinnamic acid | 5.14±0.01 | 0.19 | 5.08±0.13 | 2.56 | 20.95±0.49 | 2.34 |
| Cinnamic aldehyde | 5.17±0.08 | 1.55 | 5.04±0.12 | 2.38 | 20.34±0.50 | 2.46 |
| Glycyrrhizic acid | 49.56±0.67 | 1.35 | 49.12±1.19 | 2.42 | 96.38±0.74 | 0.77 |

RSD: Relative standard deviation; SD: Standard deviation

The recoveries of L-ephedrine, D-pseudoephedrine, L-methylephedrine, amygdalin, liquiritin, cinnamic alcohol, cinnamic acid, cinnamic aldehyde, and glycyrrhizic acid were 98.30 ± 1.07% (RSD = 1.09%), 98.81 ± 1.40% (RSD = 1.42%), 99.27 ± 1.37% (RSD = 1.38%), 98.30 ± 1.56% (RSD = 1.59%), 97.50 ± 1.69% (RSD = 1.73%), 97.86 ± 1.19% (RSD = 1.22%), 98.71 ± 1.15% (RSD = 1.17%), 99.23 ± 1.52% (RSD = 1.53%), 98.50 ± 1.43% (RSD = 1.45%), respectively [Table 3]. The results demonstrated that the corresponding assay method was reliable and reproducible.

### Quantitative determination of nine active components in MHD and different herbal compatibilities samples

The newly developed analytical method was subsequently applied to determine the nine compounds in MHD and different drug combinations. All samples were extracted and analyzed in triplicate and the contents were shown in Table 4. The content variations of the representative compounds in four herbal drugs were diagramed in Figure 4.

Compared with those in the single Ephedra extract, the contents of three Ephedra alkaloids were decreased when

**Table 3: The spike recoveries of all analytes (*n*=6)**

| Analyte | Sample content (mg) | Added amount (mg) | Measured amount (mg) | Recovery (%) | Average (%) | RSD (%) |
|---|---|---|---|---|---|---|
| L-ephedrine | 2.83 | 2.85 | 5.65 | 98.95 | 98.30 | 1.09 |
| | 2.83 | 2.85 | 5.58 | 96.49 | | |
| | 2.83 | 2.85 | 5.65 | 98.95 | | |
| | 2.83 | 2.85 | 5.64 | 98.60 | | |
| | 2.83 | 2.85 | 5.61 | 97.54 | | |
| | 2.83 | 2.85 | 5.66 | 99.30 | | |
| D-pseudoephedrine | 3.64 | 3.65 | 7.22 | 98.08 | 98.81 | 1.42 |
| | 3.64 | 3.65 | 7.34 | 101.37 | | |
| | 3.64 | 3.65 | 7.23 | 98.36 | | |
| | 3.64 | 3.65 | 7.19 | 97.26 | | |
| | 3.64 | 3.65 | 7.26 | 99.18 | | |
| | 3.64 | 3.65 | 7.24 | 98.63 | | |
| L-methylephedrine | 2.51 | 2.50 | 4.99 | 99.20 | 99.27 | 1.38 |
| | 2.51 | 2.50 | 4.98 | 98.80 | | |
| | 2.51 | 2.50 | 5.03 | 100.80 | | |
| | 2.51 | 2.50 | 5.03 | 100.80 | | |
| | 2.51 | 2.50 | 4.94 | 97.20 | | |
| | 2.51 | 2.50 | 4.98 | 98.80 | | |
| Amygdalin | 21.30 | 21.35 | 42.17 | 97.75 | 98.30 | 1.59 |
| | 21.30 | 21.35 | 42.82 | 100.80 | | |
| | 21.30 | 21.35 | 42.27 | 98.22 | | |
| | 21.30 | 21.35 | 41.93 | 96.63 | | |
| | 21.30 | 21.35 | 42.01 | 97.00 | | |
| | 21.30 | 21.35 | 42.52 | 99.39 | | |
| Liquiritin | 5.20 | 5.00 | 9.98 | 95.60 | 97.50 | 1.73 |
| | 5.20 | 5.00 | 10.18 | 99.60 | | |
| | 5.20 | 5.00 | 10.15 | 99.00 | | |
| | 5.20 | 5.00 | 10.06 | 97.20 | | |
| | 5.20 | 5.00 | 10.10 | 98.00 | | |
| | 5.20 | 5.00 | 9.98 | 95.60 | | |
| Cinnamic alcohol | 0.72 | 0.70 | 1.41 | 98.57 | 97.86 | 1.22 |
| | 0.72 | 0.70 | 1.41 | 98.57 | | |
| | 0.72 | 0.70 | 1.39 | 95.71 | | |
| | 0.72 | 0.70 | 1.41 | 98.57 | | |
| | 0.72 | 0.70 | 1.40 | 97.14 | | |
| | 0.72 | 0.70 | 1.41 | 98.57 | | |
| Cinnamic acid | 1.56 | 1.55 | 3.08 | 98.06 | 98.71 | 1.17 |
| | 1.56 | 1.55 | 3.09 | 98.71 | | |
| | 1.56 | 1.55 | 3.10 | 99.35 | | |
| | 1.56 | 1.55 | 3.08 | 98.06 | | |
| | 1.56 | 1.55 | 3.07 | 97.42 | | |
| | 1.56 | 1.55 | 3.12 | 100.65 | | |
| Cinnamic aldehyde | 1.53 | 1.52 | 3.06 | 100.66 | 99.23 | 1.53 |
| | 1.53 | 1.52 | 3.01 | 97.37 | | |
| | 1.53 | 1.52 | 3.01 | 97.37 | | |
| | 1.53 | 1.52 | 3.06 | 100.66 | | |
| | 1.53 | 1.52 | 3.05 | 100.00 | | |
| | 1.53 | 1.52 | 3.04 | 99.34 | | |
| Glycyrrhizic acid | 6.78 | 6.80 | 13.65 | 101.03 | 98.50 | 1.45 |
| | 6.78 | 6.80 | 13.51 | 98.97 | | |
| | 6.78 | 6.80 | 13.38 | 97.06 | | |
| | 6.78 | 6.80 | 13.40 | 97.35 | | |
| | 6.78 | 6.80 | 13.45 | 98.09 | | |
| | 6.78 | 6.80 | 13.48 | 98.53 | | |

RSD: Relative standard deviation

**Table 4: Contents of nine components in MHD and different herbal compatibilities samples (μg/ml, *n*=3)**

| Group | L-ephedrine | D-pseudoephedrine | L-methylephedrine | Amygdalin | Liquiritin | Cinnamic alcohol | Cinnamic acid | Cinnamic aldehyde | Glycyrrhizic acid |
|---|---|---|---|---|---|---|---|---|---|
| Ephedra | 49.47±1.20 | 14.79±0.12 | 26.69±0.72 | | | | | | |
| Cassia twig | | | | | | 6.18±0.15 | 22.03±0.35 | 46.50±0.41 | |
| Bitter apricot kernel | | | | 277.40±2.39 | | | | | |
| Prepared licorice | | | | | 64.95±1.37 | | | | 146.29±2.15 |
| E+C | 46.44±1.16 | 13.55±0.15 | 25.86±0.94 | | | 8.91±0.10 | 26.14±0.30 | 27.76±0.25 | |
| E+B | 52.15±1.23 | 19.61±0.09 | 47.98±1.16 | 309.20±2.29 | | | | | |
| E+P | 33.65±0.79 | 14.12±0.15 | 25.15±0.86 | | 63.62±0.98 | | | | 121.80±1.82 |
| C+B | | | | 344.60±3.37 | | 3.78±0.05 | 20.29±0.31 | 27.63±0.64 | |
| C+P | | | | | 57.44±1.33 | 5.06±0.04 | 20.19±0.26 | 30.40±0.26 | 126.62±1.96 |
| B+P | | | | 319.40±4.56 | 64.27±0.77 | | | | 140.52±1.72 |
| E+C+B | 37.03±0.67 | 35.17±0.11 | 34.84±1.07 | 323.70±5.15 | | 9.23±0.13 | 25.83±0.21 | 29.14±0.28 | |
| E+C+P | 33.59±1.02 | 6.85±0.10 | 26.11±0.84 | | 69.77±0.69 | 8.41±0.06 | 23.35±0.14 | 22.57±0.48 | 115.99±2.12 |
| E+B+P | 36.59±0.95 | 53.23±1.52 | 31.46±0.75 | 318.40±4.78 | 71.62±1.13 | | | | 118.26±1.85 |
| C+B+P | | | | 298.00±5.63 | 56.05±1.41 | 3.20±0.05 | 20.55±0.30 | 23.13±0.37 | 108.15±1.34 |
| E+C+B+P | 37.02±0.88 | 48.50±1.23 | 35.86±0.90 | 303.60±6.04 | 63.17±1.69 | 10.59±0.21 | 20.86±0.54 | 20.49±0.56 | 98.85±2.06 |

E: Ephedra, C: Cassia twig, B: Bitter apricot kernel, P: Prepared licorice, MHD: Mahuang decoction

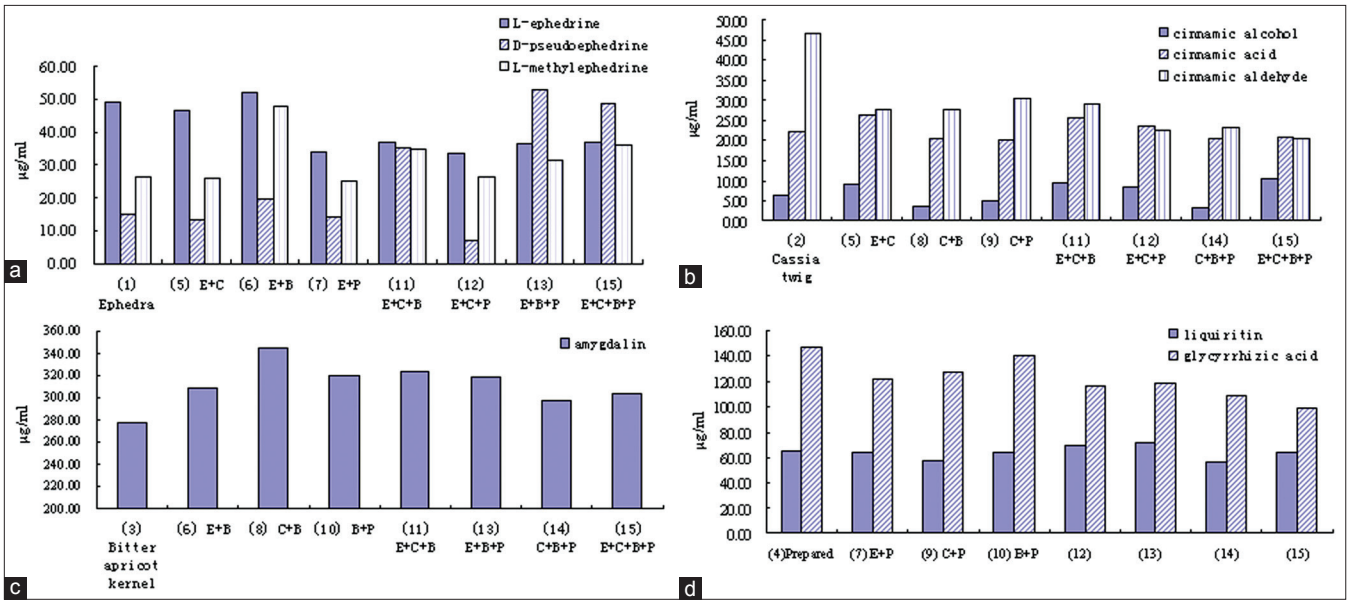


**Figure 4:** Content variations of nine bioactive components in different herb compatibilities of MHD. (a) Content variations of L-ephedrine, D-pseudoephedrine, and L-methylephedrine in Ephedra. (b) Content variations of cinnamic alcohol, cinnamic acid and cinnamic aldehyde in Cassia twig. (c) Content variation of amygdalin in bitter apricot kernel. (d) Content variations of liquiritin and glycyrrhizic acid in prepared licorice

Ephedra was combined with Cassia twig or prepared licorice, while markedly increased after Ephedra in combination with bitter apricot kernel. In the whole formula of MHD, the significant decrease in the content of L-ephedrine contrasted with the increase of those of D-pseudoephedrine and L-methylephedrine; as for three phenylpropanoids in Cassia twig. Ephedra elevated the levels of cinnamic alcohol and cinnamic acid, but degraded the level of cinnamic aldehyde. Besides, their content was all decreased due to the present of bitter apricot kernel or prepared licorice. Amygdalin, as the unique representative ingredient of bitter apricot kernel in our study, was found content increment no matter which drug combined with it. Compared to single decoction, liquiritin in prepared licorice had no significant content change after combination with other three drugs, but the content of glycyrrhizic acid declined obviously because of multi-herb decoction.

## CONCLUSION

A reliable HPLC-DAD method was developed and applied to the simultaneous determination of nine bioactive compounds in MHD. With the fine validation results, the proposed method could be used to scientifically assess the quality of this traditional Chinese formula. Moreover, a comparative study of the contents of these nine ingredients in different herb-herb compatibilities was achieved based on this method, which was important for further elucidation of the composition mechanism of MHD.

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