# A practical and novel 'standard addition' strategy to screen pharmacodynamic components in traditional Chinese medicine using Heishunpian as an example

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The things included in this 6-page supplementary information are as follows:

1. Methodology validation of the analysis of UPLC-Q-TOF-MS fingerprints

2. Quantitative analysis of target components in different extracts

3. Stability of different batches of HSP

1. Methodology validation of the analysis of UPLC-Q-TOF-MS fingerprints

The six extract solutions, prepared as in '2.4.1' were used as sample solutions to evaluate method precision by the successive analysis of six injections. Repeatability was calculated using six replicates of each sample. Meanwhile, the analysis of different periods of time in a day (0, 2, 4, 8, 16, and 24 h) was used to evaluate stability.

2 Quantitative analysis of target components in different extracts

2.1 Calibration curves

Linear correlation analysis for each target component was determined in triplicate using six different concentrations of the standard stock solutions. Calibration curves were plotted based on the peak area versus concentration for each analyte.

2.2. Limit of quantitation

The limit of quantitation for each analyte was calculated using the standard stock solution on the basis of a signal-to-noise ratio of 10

2.3. Precision, repeatability, stability, and accuracy

Intraday precision and interday reproducibility were tested. In intraday precision, each stock solution was analyzed six times within one day (n = 6). Interday reproducibility was determined within three consecutive days (n = 6).

To determine repeatability, six samples of each extract from the same source were prepared and analyzed using the method described previously. Variations were expressed as relative standard deviations (RSDs).

Stability was tested for two consecutive days (0, 2, 4, 6, 8, 12, 18, and 24 h) using the

sample solutions. Variations were expressed as RSDs.

The recovery test was used to evaluate the accuracy of the developed method. The contents of each target analyte were calculated based on their respective calibration curves. The same weight of each analyte was spiked into an accurately weighed portion of the sample for six times, and then extracted and analyzed using the previously described method. The recoveries were calculated using the following formulas:

$$Recovery\,(\%) = (amount\ \ found - original\ amount) / amount\ spiked \times 100\%$$

and

$$RSD\,(\%) = (SD/mean) \times 100\%.$$

3. Stability of different batches of HSP

Taking the quality differences among batches of HSP into consideration, we purchased 10 batches at preparatory stage, and authenticated by Dr. Lu Zhang at Tianjin University of Traditional Chinese Medicine (Tianjin, China). After identification, 10 batches of HSP were analyzed by UPLC-Q-TOF-MS, the analytical methods are same as the way in "section 2.3" of revised manuscript. The base peak intensity (BPI) chromatogram of HSP is shown in Fig.1. It can be found the peak area of common peaks in different batches of HSP were different. That indicate there are many diversities in different batches of HSP, and the quality of different batches of HSP is also diverse. In view of the quality of HSP can not be controlled easily and the repeatability of experiment should be guaranteed, S-10 with medium peak area was

used in next experiment. It was processed by specialized company of Chinese herbal medicine and can stand for the majority of market HSP. It was bought from Hua Miao Engineering Technology Development Center of Traditional Chinese Medicine (Beijing, China), (No.305231).

In our study, we screened the target components (TCs, possible pharmacodynamic components) by spectrum-effect relationship analysis. In order to select a suitable batch of HSP, we measured the content of TCs in different batches of HSP, which followed the method in "section 2.5" of manuscript. The results are shown in Table 1. The content ranges of TCs in these 10 batches of HSP are chasmanine: 0.97-30.48 μg/g, mesaconitine: 8.91-32.02 μg/g, hypaconitine: 93.00-315.52 μg/g, deoxyaconitine: 7.94-19.85 μg/g, respectively. The content ranges are wide, which indicates the quality stability of market HSP is poor. Selecting one beach can decrease the error caused by unfixed origin of HSP. The sample we selected (S-10) was processed by specialized company of Chinese herbal medicine. The contents of TCs in S-10 are chasmanine: 14.31 μg/g, mesaconitine: 15.37 μg/g, hypaconitine: 205.96 μg/g, deoxyaconitine: 15.40 μg/g, respectively. They are in medium place of the content range. And compare with the mean, there are little errors, it can stand for the majority of market HSP.

In conclusion, quality difference among batches of HSP is great and difficult to control. So after analyzing the chemical components of HSP, we selected one batch of HSP which can stand for the majority of market HSP to use in next experiment. It also was processed by specialized company of Chinese herbal medicine and its quality is

medium in market. The effect of uncontrollable factor can be decreased and the experiment can be repeated easily.

# TABLES

Table S1 The regression data, LODs and LOQs for target compounds analyzed by UPLC-Q-TOF-MS.

| Compound | Regression equation (y = ax + b) | $R^2$ | Linear range ($\mu g \cdot mL^{-1}$) | LOD ($\mu g \cdot mL^{-1}$) | LOQ ($\mu g \cdot mL^{-1}$) |
|---|---|---|---|---|---|
| Chasmanine | y=34878x-72.188 | 0.9984 | 0.01-0.16 | 0.006 | 0.020 |
| Mesaconitine | y=93869x-217.3 | 0.9987 | 0.01-0.16 | 0.008 | 0.025 |
| Hypaconitine | $y=1.4\times10^5x-488.63$ | 0.9997 | 0.1-1.6 | 0.050 | 0.160 |
| Deoxyaconitine | $y=1.0\times10^5x-139.64$ | 0.9985 | 0.01-0.16 | 0.009 | 0.030 |

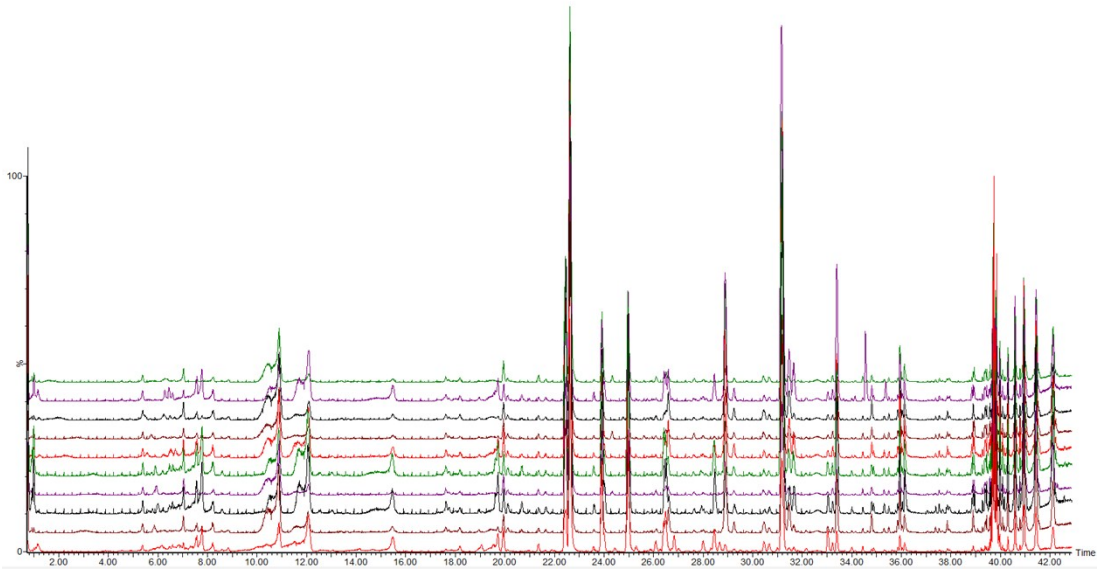


Fig.1. The base peak intensity (BPI) chromatogram of different batches of HSP