# A practical application of wavelet moment method on the quantitative analysis of *Shuanghuanglian* oral liquid based on three-dimensional fingerprint spectra

Jing Chen, Bao Qiong Li, Hong Lin Zhai*, Wen Juan Lü, Xiao Yun Zhang

*College of Chemistry & Chemical Engineering, Lanzhou University, South Tianshui Road 222, Lanzhou 730000, Gansu, China*

## ABSTRACT

The overlapping and shifts of peaks and noise signals appear mostly in high performance liquid chromatography (HPLC) experiments. A practical application of wavelet moment method on the quantitative analysis of the main active components in *Shuanghuanglian* oral liquid samples was presented based on the determination of HPLC coupled with photodiode array detector (PAD). The wavelet moments were calculated from the divided regions in the grayscale images of three-dimensional (3D) HPLC-PAD fingerprint spectra according to the target peak(s), and then used to establish linear models, respectively. The correlation coefficients ($R$) were more than 0.9980 within the test ranges. The intra- and inter-day variations were less than 1.13% and 1.10%, respectively. The recovery ranged from 96.2% to 102.7%. The overall LODs and LOQs were less than 0.2 μg/mL and 0.7 μg/mL, respectively. Our study indicated that wavelet moment approach could defuse the overlapping and shifts of peaks and noise signals in the chromatographic determination owing to its multi-resolution and inherently invariance properties. Thus the analytical time was shortened, and the obtained results were reliable and accurate.

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## 1. Introduction

High-performance liquid chromatography (HPLC) is a versatile analytical technique that has been widely used in analytical field owing to its high resolving ability, quick response and simple operation. HPLC coupled with photodiode array detector (PAD) can provide three-dimensional (3D) fingerprint spectra, which consists of the absorptions at each retention time point of every scanning wavelength. HPLC-PAD technologies have been employed for the qualitative and quantitative analyses of the multiple target components such as the simultaneous determination of flavonoid, saponins and polyacetylenes in Folium Ginseng and Radix Ginseng [1], the fast analysis of four phenolic acids in olive [2], the simultaneous qualitative and quantitative analysis of phenolic acids and flavonoids [3], the comprehensive evalution of Danhong injection [4], the determination of melamine in protein supplements [5], the simultaneous determination of pre-emergence herbicides in environmental samples [6], the simultaneous determination of 10 active components in traditional Chinese medicine 'YIGONG' capsule [7]. Although many investigations were carried out under

the selected single wavelength from 3D HPLC-PAD spectra, several second-order calibration methods, which process 3D spectra directly, have been reported such as trilinear parallel factor analysis (PARAFAC) [2], N-way principal component analysis (N-PLS) [8,9] and alternating trilinear decomposition (ATLD) [10–12]. On the other hand, image processing techniques were introduced to the 3D spectra analyses by our research group, in which the grayscale density or Zernike moment methods had been applied to quantitative analysis of three compounds [13,14] and wavelet moment method had been applied for similarity analysis [15] based on 3D HPLC-PAD spectra. The advantages of all the methods mentioned above are that they can reveal the systemic characteristic of complex samples without selecting single-fixed wavelength, and obtain more reasonable results.

Moment invariants, which provide invariance abilities of translation, rotation and scaling in image descriptions, have been widely applied to extract features in image analysis in many fields. Especially, wavelet moment method possesses powerful multi-resolution ability and can present more details, which is particularly useful for handling the overlapping and shifts of peaks as well as noise signals in HPLC experiments. In our previous work, wavelet moment method was tried to apply on the simultaneous quantitative analysis of three components in simple artificial simulation mixtures based on 3D fluorescence spectra [16], and

the obtained results revealed the application potential of our ideal.

Apart from overlapping peaks, the difference of 3D HPLC-PAD spectra from 3D fluorescence spectra is that it has several difficulties such as retention time shifts, baseline drift and noise signals. In order to further verify and perfect the quantitative analysis using wavelet moment method based on 3D HPLC-PAD spectra, we applied the improved method to the quantitative determination of the main active components in real Shuanghuanglian (SHL) oral liquid samples. By means of the analysis of obtained models and the validation of approach, we comprehensively estimated the performance of wavelet moment method on the quantitative analysis of 3D HPLC-PAD spectra.

## 2. Experimental and data analysis

### 2.1. Experimental

#### 2.1.1. Reagents and samples

Shuanghuanglian (SHL) oral liquid consisted of three main active organic compounds (chlorogenic acid, forsythin, and baicalin), and their chemical structures were illustrated in Fig. 1.

Acetonitrile and methanol were of high performance liquid chromatography grade (HPLC-grade) (DIKMA, USA); phosphoric acid was of analytical grade (FUCHEN, China). The standards of chlorogenic acid, forsythin and baicalin were of HPLC-grade (ABCR, Germany). The real samples with seven batches of Shuanghuanglian (SHL) oral liquid were purchased from different markets (Lanzhou, China).

#### 2.1.2. Preparation of standards and samples solutions

The standard stock solutions were made in methanol with chlorogenic acid of 1650 μg/mL, forsythin of 1580 μg/mL and baicalin of 750 μg/mL. Then, a series of mixed working solutions ($n = 16$) with appropriate concentrations were further mixed and diluted with distilled water, which were used for calibration curves. An aliquot of 0.2 mL of sample was accurately diluted to a 10 mL volumetric flask with water.

#### 2.1.3. Instrument and chromatographic conditions

The system consisted of a Waters (USA) HPLC instrument coupled with a photodiode array detector (Waters 2998), a binary HPLC pump (Waters 1525), manual injector and the reversed phased column (Waters Symmetry C-18 column, $4.6 \times 250$ mm, 5 μm) was used.

The standards and samples were analyzed using a gradient elution program including 0.2% phosphoric acid aqueous solution (A) and acetonitrile (B). The gradient elution program was following: 0–3.5 min, 27% B; 3.5–6.0 min, 27–60% B; 6.0–7.0 min, 60–40% B; 7.0–10.0 min, 40–27% B; 10.0–30.0 min, 27% B. The column temperature was kept in the range from 30 to 34 °C, the photodiode array scanning wavelengths were 220–400 nm, flow rate was set at 1.0 mL/min and the injection volume was 5 μL. Each solution was filtered through a 0.22 μm membrane (Shanghai, China) before injecting into the HPLC-PAD system.

### 2.2. Analytical method

#### 2.2.1. Data

The chromatographic data in ASCII format that obtained on the basis of the experimental conditions mentioned above was exported and a two-dimensional (2D) matrix was created for each sample. The rows and columns of the 2D matrix corresponded the retention time ranged from 2.0 to 8.0 min (722 points) and the wavelength ranged from 220 to 400 nm (153 points), respectively. The data in this matrix was the HPLC intensity at each retention time point of every scanning wavelength, which was regarded as the density matrix of grayscale image in this work.

In order to reduce the impact of irrelevant information and increase the effectiveness of target information extracted, the
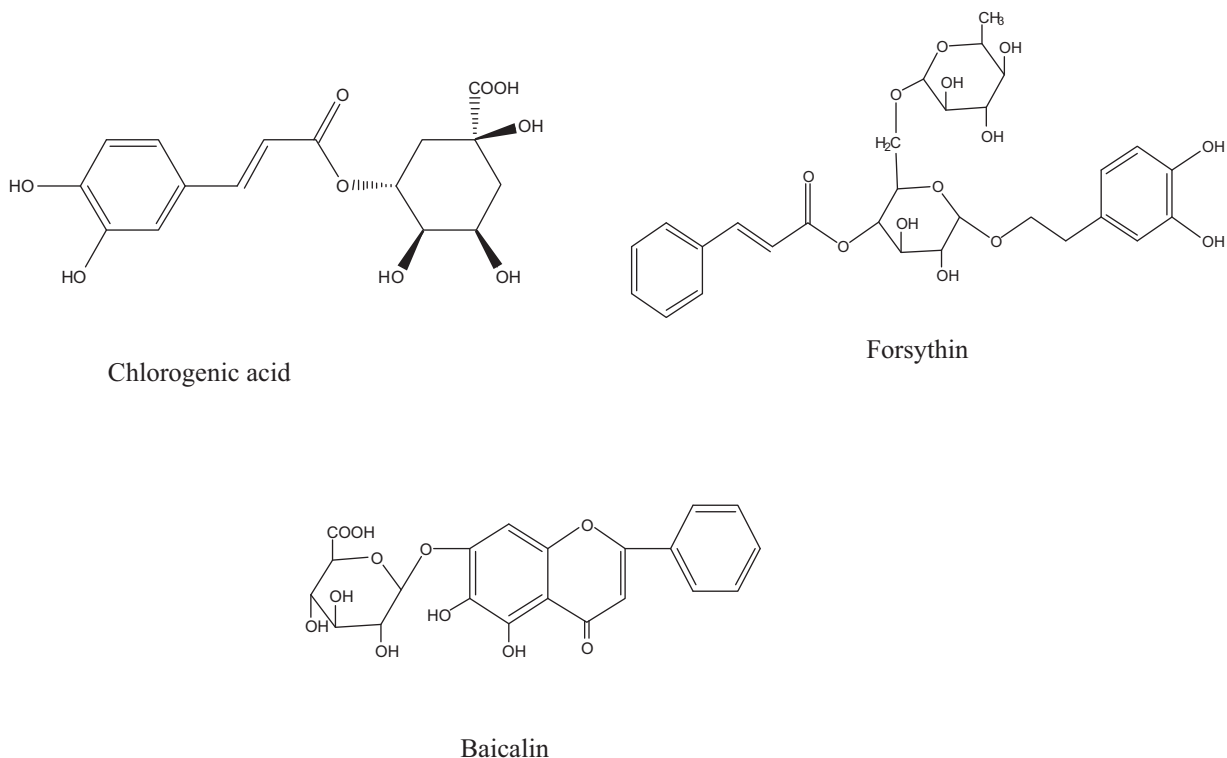


Chlorogenic acid

Forsythin

Baicalin

Fig. 1. The chemical structures of three active components.