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Simultaneous determination of umbelliferone and scopoletin in Tibetan medicine *Saussurea laniceps* and traditional Chinese medicine *Radix angelicae pubescentis* using excitation-emission matrix fluorescence coupled with second-order calibration method

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ABSTRACT

A chemometrics-assisted excitation-emission matrix (EEM) fluorescence method is presented for simultaneous determination of umbelliferone and scopoletin in Tibetan medicine *Saussurea laniceps* (SL) and traditional Chinese medicine *Radix angelicae pubescentis* (RAP). Using the strategy of combining EEM fluorescence data with second-order calibration method based on the alternating trilinear decomposition (ATLD) algorithm, the simultaneous quantification of umbelliferone and scopoletin in the two different complex systems was achieved successfully, even in the presence of potential interferents. The pretreatment is simple due to the "second-order advantage" and the use of "mathematical separation" instead of awkward "physical or chemical separation". Satisfactory results have been achieved with the limits of detection (LODs) of umbelliferone and scopoletin being 0.06 ng mL^{-1} and 0.16 ng mL^{-1}, respectively. The average spike recoveries of umbelliferone and scopoletin are 98.8 ± 4.3% and 102.5 ± 3.3%, respectively. Besides, HPLC-DAD method was used to further validate the presented strategy, and *t*-test indicates that prediction results of the two methods have no significant differences. Satisfactory experimental results imply that our method is fast, low-cost and sensitive when compared with HPLC-DAD method.

1. Introduction

Traditional Chinese medicine (TCM) has a long history in preventing and curing human diseases in oriental countries and is gaining more and more attention all over the world due to its low toxicity and good therapeutic performance. It has been widely used in oriental medical history, especially in China, and plays an important role in treatment of various kinds of disease [1,2]. For example, the traditional Tibetan medicine *Saussurea laniceps* (SL) has a good therapy in some diseases, such as rheumatoid arthritis, stomach ache and dysmenorrhea [3]. It is also known with another common name, "snow lotus". "Snow lotus", derives from several species of the genus Saussurea in the family Compositae, is a kind of well-known herbal medicine in China and widely used in dealing with pain and inflammatory conditions [4]. Although Tibetan medicine *Saussurea involucrate* (SI) and *Saussurea medusa* (SM) also have the common name "snow lotus", SL significantly prevents nociceptive production and inflammatory response to a greater degree than either of the other two among the three herbs in the aspect of physiological effects, exerts more potent effects than other species against experimental edema and pain in animal models [3,4]. Therefore, SL has a higher value in the treatment of painful and inflammatory diseases. *Radix angelicae pubescentis* (RAP), known as "Duhuo" in China, is a traditional Chinese medicine used for the treatment of rheumatic disease (Pharmacopoeia of the People's Republic of China, 2010). Pharmacological studies indicate that RAP possesses anti-inflammatory, analgesic [5,6], antiparasitic [7], anticancer and platelet aggregation inhibitory activities [8]. Coumarins are the main active components of SL and RAP. For example, more than 60 coumarins have been isolated and identified from RAP [8,9].

Coumarins comprise a very large class of phenolic substance found in plants and are made of fused benzene and α-pyrone rings [10]. To date, at least 1300 coumarins have been identified, principally as secondary metabolites in tissues of plants which belong to the families Umbelliferone, Rutaceae, Solanaceae, Compositae and others [10]. Pharmacological studies reveal that coumarins have been used in the treatment of a diverse range of diseases, such as brucellosis, burns, rheumatic disease and even cancers [11]. It is also noticed that some coumarins have been linked to phototoxic, mutagenic, carcinogenic, and hepatotoxic effects [12,13]. Coumarin derivatives including umbelliferone (7-hydroxycoumarin) and scopoletin are accepted as major active components in SL and RAP. Previous studies have reported biological activities of coumarin derivatives [14]. For instance, umbelliferone has been demonstrated to act as an antibacterial, anti-

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inflammatory, antirheumatic, and immunomodulatory agent and have positive effects on cholesterol metabolism. Scopoletin has analgesic, antiparasitic, anti-cholinergic and age-impaired memory ameliorative activities. With these various functions in mind, it is not surprising that coumarin derivatives impact significantly on the clinical efficacy and safety of herbal medicine. Thus, as the major active components of the two common medicines, umbelliferone and scopoletin are thought to be the most important bioactive markers for the basic quality control of SL and RAP and other plant-derived drugs from them. Therefore, it's very necessary to establish a fast, sensitive and low-cost method to determine umbelliferone and scopoletin in SL and RAP simultaneously.

Various methods for determination of umbelliferone and scopoletin in plant extracts have been previously reported in literature. These methods are commonly based on chromatography coupled with different detections, such as HPLC-DAD [15–17], HPLC-UV [18,19], LC-MS/MS [8], micellar electrokinetic capillary chromatography (MECC) [20], capillary electrophoresis (CE) [21] or gas chromatography combined with mass spectrometry (GC–MS) [22]. HPLC coupled with different detection systems has been used as a primary analytical method. The aforementioned methods usually have certain disadvantages since tedious pretreatment procedures are required such as extraction, pre-concentration and purification, which are complex, time-consuming, and high-cost. Therefore, an economical and simple method for simultaneous determination of umbelliferone and scopoletin is of great importance. Fluorescence measurements can be carried out quickly and at low-cost. Usually, the conventional fluorescence data analysis is based on a single wavelength. However, the signals of the analytes of interest may overlap with each other due to the wide spectroscopy range of excitation and emission wavelengths. This makes the determination difficult to a great extent because of the non- or low-selectivity of the single wavelength fluorescence method. Compared with single wavelength fluorescence spectra, excitation-emission matrix (EEM) fluorescence spectra can provide more detailed spectral information and increase the selectivity of spectrofluorimetry method. However, for complex systems, the EEM data are also nonselective for the recorded signals consist of the signals from all of the contributing fluorophores. Thus, the spectra of analytes will overlap with potential interferents heavily. For various chemometric techniques [23–27], multiway calibration methods are an attractive part. The determination of analytes of interest in complex matrices can be achieved successfully by combining second- or high-order instruments with multiway calibration methods [28–30]. Therefore, an alternative is the use of EEM fluorescence coupled with second-order calibration methods. Resolution of excitation and emission profiles together with concentrations for the analytes of interest can be predicted reasonably and accurately by second-order calibration methods, even in the presence of potential interferents attributed to the "second-order advantage" [31–34]. And this property has been exploited in many fields such as pharmaceuticals, food, chemistry and environmental science [35–39].

Traditional Chinese medicine is a complex system, serious spectra overlap will occur between analytes of interest and potential interferents. Besides, the spectra of analytes may also overlap with each other. These spectra overlap will lead to heavy collinearity of the measurement data. Thus, simultaneous determination of umbelliferone and scopoletin in SL and RAP is difficult based on conventional fluorescence methods. As far as we know, the reports about simultaneously quantitative analysis of umbelliferone and scopoletin in the traditional Tibetan medicine SL and Chinese medicine RAP by EEM coupled with second-order calibration methods has not been found in literature. Therefore, in order to simultaneously exploit the sensitivity of fluorescence analysis and resolution abilities of multiway calibration methods, a strategy that combines EEM fluorescence data with second-order calibration method based on the alternating trilinear decomposition (ATLD) algorithm [40] is presented in this work to determine umbelliferone and scopoletin in SL and RAP simultaneously. The sample pretreatment is simple due to the "second-order advantage". Neither complex preprocessing nor auxiliary reagents are involved in the experiments, and the required equipment can be found in laboratories of low complexity. In order to further assess the accuracy of the proposed method, it is validated and compared with the HPLC-DAD method. Satisfactory experimental results imply the proposed method is fast, low-cost and high sensitive, indicating that the use of EEM fluorescence coupled with second-order calibration method has broad prospects for simultaneous determination of multi-components in complex systems regardless of potential interferents.

2. Theory

2.1. Trilinear component model for second-order calibration

Trilinear component model (PARAFAC/CANDECOMP model) [41–43], which was proposed by Harshman [43], Carroll and Chang [42] independently, is regarded as one of the most commonly used models in second-order calibration field. Suppose a given sample produces an EEM at I excitation wavelengths and J emission wavelengths, then a three-way data array $\underline{\mathbf{X}}$ $(I \times J \times K)$ is obtained by stacking EEMs of K samples. The trilinear component model can be expressed as follows:

$$x_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk}, \quad \text{for } i = 1, 2, \ldots, I; j = 1, 2, \ldots, J; k = 1, 2, \ldots, K \tag{1}$$

Here x_{ijk} is the element of $\underline{\mathbf{X}}$, corresponding to the response intensity of sample k at excitation wavelength i and emission wavelength j; a_{in}, b_{jn} and c_{kn} are the elements of matrices $\mathbf{A}$ $(I \times N)$ with normalized excitation profiles, $\mathbf{B}$ $(J \times N)$ with normalized emission profiles and $\mathbf{C}$ $(K \times N)$ with relative concentrations, respectively. The term e_{ijk} represents the element of the three-way residual data array $\underline{\mathbf{E}}$. N stands for the number of factors, which should correspond to the total number of fluorescing species, including the components of interest and the potential interferents as well as background. The scheme of trilinear decomposition was pictorially illustrated in Fig.1.

2.2. Alternating trilinear decomposition method

ATLD method, firstly developed by Wu et al. in 1996, is one of the most famous iterative algorithms. It has been commonly used due to the advantages of being insensitive to excessive component numbers, fast convergence and fully exploiting the "second-order advantage". It is based on the alternating least-squares principle without any constraints, which is able to correlate the signals of spectra with the concentrations of the analytes. More detail description of the ATLD method was reported in the original literature [40].

3. Experimental

3.1. Chemicals and reagents

Umbelliferone and scopoletin were purchased from Aladdin (Shanghai, China) and their structures were shown in Fig.2. Methanol was purchased from Sigma-Aldrich. Acetic acid was purchased from Adamas Reagent Company. All used chemicals were of analytical or higher grades. Ultrapure water was produced by the Milli-Q Gradient A10 system (Millipore, MA, USA). Britton-Robinson buffer solution (B-R buffer) (pH = 10.38) was used.

Stock solutions of umbelliferone (56.4 μg mL^{-1}) and scopoletin (45.2 μg mL^{-1}) were prepared in 25.00 mL brown volumetric flask by dissolving their standards in methanol. They were stored in a freezer at 4 °C. Working solutions were prepared daily by appropriate dilution of the stock solutions with ultrapure water.