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Simultaneous determination of eight flavonoids in propolis using chemometrics-assisted high performance liquid chromatography-diode array detection

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ABSTRACT

A fast analytical strategy of second-order calibration method based on the alternating trilinear decomposition algorithm (ATLD)-assisted high performance liquid chromatography coupled with a diode array detector (HPLC-DAD) was established for the simultaneous determination of eight flavonoids (rutin, quercetin, luteolin, kaempferol, isorhamnetin, apigenin, galangin and chrysin) in propolis capsules samples. The chromatographic separation was implemented on a Wondasil™ C18 column (250 mm × 4.6 mm, 5 μm) within 13 min with a binary mobile phase composed of water with 1% formic acid and methanol at a flow rate of 1.0 mL min^{-1} after flavonoids were only extracted with methanol by ultrasound extraction for 15 min. The baseline problem was overcome by considering background drift as additional compositions or factors as well as the target analytes, and ATLD was employed to handle the overlapping peaks from analytes of interest or from analytes and co-eluting matrix compounds. The linearity was good with the correlation coefficients no less than 0.9947; the limit of detections (LODs) within the range of 3.39–33.05 ng mL^{-1} were low enough; the accuracy was confirmed by the recoveries ranged from 91.9% to 110.2% and the root-mean-square-error of predictions (RMSEPs) less than 1.1 μg/mL. The results indicated that the chromatographic method with the aid of ATLD is efficient, sensitive and cost-effective and can realize the resolution and accurate quantification of flavonoids even in the presence of interferences, thus providing an alternative method for accurate quantification of analytes especially when the complete separation is not easily accomplished. The method was successfully applied to propolis capsules samples and the satisfactory results were obtained.

1. Introduction

Propolis is a naturally occurring resinous hive product gathered by worker honeybees from buds and barks of different plant species [1,2]. Propolis possesses a multitude of biological and physiological properties such as anti-oxidant, anti-inflammatory, antimicrobial, antifungal, anticancer, antiulcer, cardioprotective, neuroprotective and immunomodulatory effects, and among others [2]. It has been used as folk medicine since ancient times, and listed as an official drug in London pharmacopeia of the 17th century, and more and more scientific evidences demonstrate that propolis could also be used in the treatment of rhinitis, asthma, diabetes, and neurological diseases, and so on [3]. Therefore, propolis has recently gained an increasing popularity as a natural product for its plenty of beneficial effects and has been extensively used in health foods, beverages and cosmetics and has a potential for research and development of new pharmaceuticals to maintain health and prevent diseases [2–4].

Although the chemical composition of propolis is highly complex and is significantly variable depending on the phytogeographical areas, collection time and botanical source, the varieties of pharmaceutical activities of propolis are mainly associated with its biologically active polyphenols fraction, including flavonoids and phenolic acids [2]. Flavonoids are ubiquitous secondary plant metabolites, which are not indispensable for plant survival but are bioactive across kingdom, and exhibit diverse therapeutic and health-promoting values for animal systems, e.g., anti-oxidation, anti-proliferative, antitumor, anti-inflammatory and pro-apoptotic activities [5]. Studies show that flavonoids have been used to prevent and treat many diseases, including acne, respiratory infection, gastrointestinal disease and urinary tract infections, and are expected to be developed to be a class of new anti-infection agent [6]. Higher intake of some dietary flavonoids may significantly lower the risk of suffering from Parkinson's disease [7]. Hence, there is a pressing need to develop methods applied to the determination

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of flavonoids in flavonoids-rich foods, in this case, propolis capsules, for clinic research as well as quality control.

Numerous methods have been established to determine flavonoids in propolis and its products, including fluorescent spectrometry [8], capillary electrophoresis (CE) [9], micelle electrokinetic capillary chromatography [10], near infrared spectroscopy (NIRs) [11], gas chromatography–mass spectrometry (GC–MS) [12,13] and HPLC coupled with different detectors, such as HPLC-UV [14], HPLC–MS [15,16] and HPLC-DAD [16–19], and so on. Among them, fluorescent spectrometry is frequently suitable for total flavonolids determination or the quantification of the individual flavonoids; CE is characterized by time-saving, tiny sampling and low-cost, but its poor repeatability largely limits its practical applications; GC usually requires sample extraction and derivatization of analytes; chromatographic methods are indisputably the most widely applied and popular methods due to the excellent separation ability and the capability to analyze multiple compounds simultaneously. However, all these methods often demand a complicated preliminary extraction and purification step and in the case of HPLC–MS, the high cost also restricts its use. Furthermore, high background signals arising from co-eluted interferences and peak broadening make the HPLC-related methods problematic, posing challenges on the simultaneous quantification of flavonoids in propolis. Although there have been some successful cases obtaining the satisfactory resolution by adding an organic modifier to the mobile phase, optimizing the mobile phase conditions, or varying detecting systems, they are almost at the cost of spending time and resources. And there is also no guarantee to ensure the complete separation when ever increasing complex samples are analyzed in which case univariate calibration is unserviceable for it requires full selectivity for target analytes.

Luo et al. [17] carried out the simultaneous determination of ten flavonoids using HPLC-DAD by modifying the mobile phase with THF (tetrahydrofuran), which may do harm to human body, in a little long time (no less than 45 min) and the recoveries for chrysin and galangin was not satisfactory enough which may be explained by the fact that they were not separated perfectly from other substances under that condition.

Nevertheless, the coupling of second-order chemometric tools and HPLC-DAD data provides a useful and economical alternative to solve this type of problems for its intrinsic property "second-order advantage", i.e., analytes of interest can be quantified even in the presence of potential uncalibrated interferences containing in real samples, which is accomplished through increasing selectivity by mathematical separation means [20–24]. It is a promising and interesting strategy which has been successfully used in a number of cases [25–33] and will be increasingly exploited by analysts.

This paper presents a simple and fast chromatographic method assisted with the alternative trilinear decomposition algorithm (ATLD) [34] for the first time to simultaneously quantify eight mostly found flavonoids in propolis capsules. The quantification was done by dividing chromatograms into four sub-sections and separately developing specific model for each set of flavonoids in separate three-way arrays using ATLD. This method can handle the heavy-overlapped peaks and there is no complex pretreatment procedure, except that flavonoids were extracted with methanol by ultrasound extraction.

2. Theory

2.1. Trilinear component model

In the case of HPLC-DAD measurements, a given sample generates a 2D (two-dimensional) chromatographic data with the size of $I \times J$, where I denotes the number of retention time of the first dimension provided by the chromatograph itself and J is the number of spectral wavelength of the second dimension provided by the detector. One way to analyze this type of data is to arrange the data arrays measured for a group of samples (containing not only the calibration samples but also the unknown test samples) into a three-way data arrays $\underline{\mathbf{X}}$ ($I \times J \times K$), where K represents the number of samples which can be considered as the third dimension. Provided that $\underline{\mathbf{X}}$ follows a trilinear component model, it can be mathematically expressed as a function of elution times, spectral wavelength and component concentrations and could be written as follows:

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

where N denotes the total number of detectable factors including calibrated analytes of interest, uncalibrated substances as well as background in the matrices; x_{ijk} represents the elements of the array $\underline{\mathbf{X}}$ in the ith retention time, jth wavelength and kth sample; e_{ijk} means the corresponding elements of the three-way residual array $\underline{\mathbf{E}}$ with the same size as $\underline{\mathbf{X}}$; while a_{in}, b_{jn} and c_{kn} symbolize the elements (i, n), (j, n) and (k, n) of the normalized chromatogram matrix $\mathbf{A}(I \times N)$, the normalized spectrum matrix $\mathbf{B}(J \times N)$ and the relative concentration matrix $\mathbf{C}(K \times N)$, respectively.

2.2. ATLD algorithm

ATLD, an algorithm without any constraints developed by Wu et al. [34], is one of the most commonly used methods for processing second-order data obtained from the hyphenated instruments (in particular HPLC-DAD) due to its convergence and "second-order advantage". Followed by estimating the number of factors and initializing the matrices $\mathbf{A}$ and $\mathbf{B}$ randomly, it updates alternately $\mathbf{A}$, $\mathbf{B}$ and $\mathbf{C}$ from the following Eqs. (2)–(4) based on an alternating least-squares principle and the Moore-Penrose generalized inverse computations with singular value decomposition (SVD) and then minimizes the loss function written as Eq. (5) until a certain stopping criterion is satisfied, usually $\varepsilon = 10^{-6}$, calculated according to Eq. (6).

$$\mathbf{a}_i^T = diag(\mathbf{B}^+ \mathbf{X}_{i..} (\mathbf{C}^T)^+) \tag{2}$$

$$\mathbf{b}_j^T = diag(\mathbf{C}^+ \mathbf{X}_{.j.} (\mathbf{A}^T)^+) \tag{3}$$

$$\mathbf{c}_k^T = diag(\mathbf{A}^+ \mathbf{X}_{..k} (\mathbf{B}^T)^+) \tag{4}$$

$$\sigma = \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} \left(x_{ijk} - \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} \right)^2 \tag{5}$$

$$\left| \frac{\sigma^{(m)} - \sigma^{(m-1)}}{\sigma^{(m-1)}} \right| \leq \varepsilon \tag{6}$$

where $\mathbf{a}_i$, $\mathbf{b}_j$ and $\mathbf{c}_k$ are the ith, jth and kth row vectors of $\mathbf{A}$, $\mathbf{B}$ and $\mathbf{C}$, respectively; $\mathbf{X}_{i..}$, $\mathbf{X}_{.j.}$ and $\mathbf{X}_{..k}$ mean the frontal, horizontal and horizontal slices of $\underline{\mathbf{X}}$; the superscript T denotes the transpose of a matrix while + represents the Mooore-Penrose generalized inverse and *diag* refers to the $N \times N$ diagonal matrix from the corresponding vector argument. After finishing the iterative procedure, the concentrations of target analytes in unknown samples can be estimated by separate pseudounivariate regression of the relative concentration scores against their reference values.

The ATLD based second-order calibration has the advantages of faster convergence, insensitive to excessive factors and more robust as the introduction of Moore-Penrose pseudoinverse computation in the iterative procedure when compared to the traditional PARAFAC. More details about ATLD and its comparison with other algorithms can be found in our previous works [24,34,35].