Determination of Contents of Catechins in Oolong Teas by Quantitative Analysis of Multi-components Via a Single Marker (QAMS) Method

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Abstract The catechins in oolong tea are related to its flavor and health benefits. In this paper, a new strategy for a quantitative analysis of multi-components via a single marker (QAMS) was developed and validated to analyze six catechins (epigallocatechin, catechin, epicatechin, epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate) in oolong tea by HPLC. Catechin was chosen as the internal reference substance. The relative correction factors (RCFs) between catechin and the other five catechins were investigated for the QAMS calculation using five different types of chromatographic columns. Meanwhile, the content of each of the six analytes was also determined by a conventional external standard method. Finally, the angle cosine value revealed that there was no significant difference between the QAMS method and the external standard method. The QAMS method established in this study solved the problem of the availability and high cost of some standard substances and would be useful for providing an efficient and feasible quality assessment method for oolong tea and other botanic samples.

Keywords Catechins · Oolong tea · Quantitative analysis of multi-components via a single marker (QAMS) · HPLC

Introduction

In recent years, oolong tea, as a semi-fermented tea beverage, is becoming more popular in the world, especially in southern China and Japan (Hayat et al. 2015). It is well-known for its flavor and significant effects to increase digestion and fat transformation (Zhu et al. 2015). Besides, other studies suggested that oolong tea might play a role in alleviating cardiovascular disease and cancer, reduction of cholesterol, anti-oxidation, anti-bacterial, anti-hyperglycemic, and anti-obesity (Zhu et al. 2002; Zhang et al. 2014; Hosoda et al. 2003).

The catechins in oolong tea are associated with its health benefits. The major catechins in oolong tea are epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), and epicatechin gallate (ECG), the structures of which are shown in Fig. 1 (Chen et al. 2010). The determinations of catechins in oolong tea have been previously reported using an external standard method with HPLC on reversed phase chromatographic columns coupled to a UV-absorbance detector (Rahim et al. 2014; Wang et al. 2012; Zuo et al. 2002; Yi et al. 2015). However, due to the inadequate supply and high price of standard substances, the application of external standard method was limited for multi-component determination in practice. In contrast, a quantitative analysis of multi-components via a single marker (QAMS) method could solve this problem by using one chemical standard to calculate multi-components simultaneously. The QAMS method which has been adopted in Chinese Pharmacopoeia for the quality evaluation of coptidis rhizoma (Chinese Pharmacopoeia Commission 2015), is based on the relative correction factor (RCF)
between each analyte and single standard within certain concentration ranges. This new method was firstly applied for quality evaluation of caulis akebiae (Wang et al. 2006) and is now extensively used in multi-component quantitative analysis for botanic samples (Kuang et al. 2015; Li et al. 2016; Wang et al. 2015).

The aim of the present work was to establish a facile QAMS analytical protocol for simultaneous quantification of six catechins in oolong tea with a single marker. Catechin was selected as an internal marker due to high content in oolong tea and availability at a low price. The RCFs were obtained to calculate the content of the other five components. Furthermore, the results of the QAMS method were compared with those of the external standard method.

**Material and Methods**

**Chemicals and Reagents**

Six catechin standards EGC, C, EC, EGCG, GCG, and ECG (Fig. 1) were purchased from Chengdu Push Bio-technology Co., Ltd. (Chengdu, China). Five oolong tea samples were collected in a local market with different brands. Purified water was purchased from Wahaha Group Co., Ltd. (Hangzhou, China). HPLC grade acetonitrile was purchased from Sigma-Aldrich (MO, USA). Other chemicals and reagents of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

**Instrumentation**

HPLC separation system was consisted of two pumps (LC-20AD, Shimadzu, Tokyo, Japan), an online degasser (DGU-20A3R, Shimadzu), an auto-sampler (SIL-20A, Shimadzu), a diode array detection (MSD-M20A, Shimadzu), and a column heater (HT-230A, Tianjin Heng’ao Technology Development Co., Ltd.). Chromatographic separation was performed on a Shimadzu Wonda Cract ODS-2 analytical column (250 mm × 4.6 mm, 5 μm) using 0.5 % acetic acid solution in water (solvent A) and acetonitrile (solvent B). The column was kept in an insulated compartment at 35 °C. The gradient elution procedure was 10–30 % (B) in 0–40 min. The constant flow rate was 1 mL/min. The sample solution was injected by the auto-sampler with 10 μL.

Four additional analytical columns used were Tianhe Kromasil C18 (250 mm × 4.6 mm, 5 μm), Phenomenex Luna C18 (250 mm × 4.6 mm, 5 μm), Dalian Elite Hypersil BDS C18 (250 mm × 4.6 mm, 5 μm), and Agilent Zorbax SB-aq C18 (250 mm × 4.6 mm, 5 μm).

**Sample Preparation**

An accurately weighed 60 mg grinded powder and 25 mL of 50 % methanol (v/v) were added to a flask in an ultrasonic bath for 15 min. The extraction process was repeated two times. The solution was combined and diluted with 50 % methanol (v/v) to a final volume of 50 mL. Then, the solution was filtered through a 0.45-μm syringe filter with a polytetrafluoroethylene membrane into an HPLC vial. Finally, an aliquot of 10 μL of the solution was injected for HPLC analysis.

**Preparation of Standard Solution**

Six stock solutions were prepared by dissolving each reference standard respectively into a 10-mL volumetric flask with 50 % methanol (v/v) to volume. The concentrations were as follows: EGC 6.2 mg/mL, C 5.1 mg/mL, EC 9.5 mg/mL, EGCG 4.9 mg/mL, GCG 4.9 mg/mL, and ECG 4.7 mg/mL, respectively.

Work solution I was obtained by a mixture of stock solutions with the volume ratio of 2.5:15:0:6.3:0.8:1.5. Work solutions II–V were then diluted step by step with 50 % methanol (v/v) to the additional four concentration levels. All the standard solutions were kept in a refrigerator at 4 °C before use.
Principle of QAMS Method

Within a certain range (i.e., linear range), the quantity of each component (mass or concentration) is proportional to detector response, as shown in Eq. (1) with $i$ representing the number of components contained in the sample and $A_i$ standing for the area under the curve of a certain component. In multicomponent quality assessment, a characteristic component of the sample which is readily available, e.g., $k$ in Eq. (1), is selected as an internal standard. The relative correction factors (RCFs) between the internal standard and each of the other components to be analyzed can be determined via Eq. (2), using the component $m$ as an example. Based on Eq. (2), the concentration of component $m$ ($C_m$) can be calculated using Eq. (3).

$$C_i = f_i \times A_i \quad (i = 1, 2, \ldots, k, \ldots, m) \quad (1)$$

$$f_{km} = \frac{f_k}{f_m} = \frac{C_k \times A_m}{C_m \times A_k} \quad (2)$$

$$C_m = \frac{C_k \times A_m}{f_{km} \times A_k} \quad (3)$$

Establishment of QAMS Method for Catechin Determination

In order to calculate $f_{km}$ for each analyte, it is important to select a suitable internal standard (reference analyte) which should be stable, readily available, at low cost and high abundance in each sample. In the present study, catechin met all the requirements mentioned above and thus was selected as an internal standard. $f_{km}$ of catechin to each analyte could be calculated using Eq. (2). With the results of $f_{km}$, the content of each analyte ($C_m$) in the samples could be calculated using Eq. (3).

Assessment of the QAMS Method and External Standard Method

To assess the similarity of QAMS method and external standard method, cosine ratio value ($C_{ir}$) was calculated using Microsoft Excel 2010 software. $C_{ir}$ is a vector that calculates the angle between two groups of variables in Euclidian geometry. $C_{ir}$ is defined as in Eq. (4).

$$C_{ir} = \frac{\sum_{k=1}^{n} X_{ik} \cdot X_{rk}}{\sqrt{\left(\sum_{k=1}^{n} X_{ik}^2\right) \left(\sum_{k=1}^{n} X_{rk}^2\right)}} \quad (4)$$

where $X_{ik}$ is the value of variable $k$ in sample $i$ and $X_{rk}$ is the value of variable $k$ in common mode.

Results and Discussion

Optimization of Chromatographic Conditions

A DAD detector was set to scan from 200 to 400 nm. According to the 3D plot of retention time-absorption intensity-wavelength of the oolong tea sample (Fig. 2), UV detector was set at 280 nm for quantitative analysis of six catechins based on the baseline stability, non-peak interference, and maximum absorption.

The mobile phase was optimized through comparisons of different solvents, solvent ratio, and gradient profile. Generally, acetonitrile has several advantages: best separation, shortest analyzing time, and lowest column pressure. An acidified mobile phase could minimize peak tailing and improve theoretical plate number (Li et al. 2012; Zuo 2014). Thus, 0.5 % acetic acid–water (A) and acetonitrile (B) were chosen.
as mobile phases with a gradient elution. Under the above chromatographic conditions, the representative chromatograms of the oolong tea extract and standard mixture are shown in Fig. 3.

Table 1 The RCFs with different chromatographic columns of the QAMS method

| Chromatographic column         | EGC  | C    | EC   | EGCG | GCG  | ECG  |
|--------------------------------|------|------|------|------|------|------|
| Shimadzu Wonda Cract ODS-2     | 1.48 | 1.00 | 1.15 | 2.74 | 2.47 | 1.67 |
| Tianhe Kromasil C18            | 1.51 | 1.00 | 1.16 | 2.76 | 2.48 | 1.69 |
| Phenomenex Luna C18            | 1.49 | 1.00 | 1.14 | 2.76 | 2.48 | 1.68 |
| Dalian Elite Hypersil BDS C18  | 1.42 | 1.00 | 1.09 | 2.69 | 2.41 | 1.61 |
| Agilent Zorbax SB-aq C18       | 1.53 | 1.00 | 1.20 | 2.81 | 2.47 | 1.72 |
| Mean                           | 1.49 | 1.15 | 2.75 | 2.46 | 1.67 |      |
| SD                             | 0.042| 0.040| 0.043| 0.030| 0.040|
| RSD (%)                        | 2.82 | 3.45 | 1.58 | 1.21 | 2.41 |
decisive influence on the separation effect of the resolution, symmetry factor, retention time, and theoretical plate number. Thus, the RCF between each analyte and reference substance should be fully assessed in order to better control the related parameters. In the present study, catechin was chosen as the internal reference substance with characteristics of being stable, easily obtainable, low cost, and high abundance in each sample. The RCFs were investigated by five different types of chromatographic columns. As shown in Table 1, the results were obtained according to Eq. (2) with good reproducibility, which were acceptable for quantitative analysis.

### Determination Result of Catechins in Oolong Tea by QAMS Method

Using catechin (C) as the internal reference, the contents of the five other catechins, epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), gallo catechin gallate (GCG), and epicatechin gallate (ECG) in five batches of oolong teas were obtained by a QAMS method and are listed in Table 2, according to Eq. (3). It was observed that among the six components, the mean content of EC (10.5 ± 6.4 mg/g) accounted for the lowest content in oolong tea. Conversely, the mean content of C (249.9 ± 108.3 mg/g) was in the highest content.

### Determination by External Standard Method

Firstly, the validation was implemented under the present chromatographic conditions using a Shimadzu Wonda Cract ODS-2 analytical column. The linearity was plotted based on linear regression analysis of the integrated peak areas (y) versus injection concentration of each standard (x, μg/mL) at five different concentrations. The limit of detection (LOD) and limit of quantification (LOQ) were determined at the signal-to-noise ratio of 3:1 and 10:1, respectively. The inter-day and intra-day precisions were evaluated by relative standard deviation (RSD) under six repeated injections in 1 and 3 days, respectively. The accuracy of the analytical procedure was evaluated using the repeatability and recovery test. The repeatability was assessed by analyzing prepared oolong tea samples (six independent portions). Recovery tests were carried by spiking standards at the middle concentration level to samples (six independent portions) with known content from the same batch. The ratio of the detected and added amounts was used to calculate the recovery.

All the methodology validation data were summarized in Table 3, which indicated that the established external standard method was satisfactory for catechin determination in oolong tea. The results were calculated as shown in Table 1.

### Similarity Evaluation of the QAMS Method and External Standard Method

To assess the consistency of the results, vectorial angle was used to evaluate the similarity between the QAMS method and external standard method. Angle cosine value, ranged from 0 to 1, is a commonly used parameter in the similarity evaluation of traditional Chinese medicine fingerprint (Qing et al. 2011; Wang et al. 2002). The larger the values, the higher the similarity.
the similarity of the target sample will be. When they are equal to 1, the targets are identical. In this work, the data, as shown in Table 1, were above 0.999371, which indicates no significant differences between the QAMS method and external standard method.

**Conclusions**

In the present study, a quantitative analysis of multi-component via a single marker (QAMS) method was developed for the simultaneous quantitative analysis of six catechins in five batches of oolong teas by HPLC. Catechin was chosen as the internal reference substance. The relative correction factors (RCFs) between catechin and five other catechins were investigated for QAMS calculation by five different types of chromatographic columns. For comparison, the content of six analytes was also determined by a conventional external standard method. Finally, the angle cosine value was calculated and showed that there was no significant difference between the newly established QAMS method and traditional external standard method. The QAMS method established in this study will be useful for providing an efficient and feasible quality assessment of oolong tea as well as otherbotanic samples, which solves the problem of absence or high cost of some standard substances.

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**Compliance with Ethical Standards**

**Conflict of Interest** All the authors declare that they have no competing interests.

**Ethical Approval** This article does not contain any studies with human or animal subjects performed by any of the authors.

**Informed Consent** Informed consent is not applicable for the nature of this study.

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