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# Rapid determination of theaflavins by HPLC with a new monolithic column

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**Abstract**: The quantitative determination of four theaflavin monomers by a rapid reversed-phase high performance liquid chromatographic method was developed. A new RP-18 end-capped column with particle size 2 μm and equilibrated to 35°C in a Shimadzu temperature controller module was used. Four theaflavin monomers were successfully separated in 8 min by the new strategy, comparing to 20–85 min by HPLC in the peer literature reports. Linear gradient elution: from 92% mobile phase A (v) to 76% mobile phase A (v) during early 3 min and then 92% mobile phase A (v) till 8 min at elution flow rate 1.5 ml/min. The limits of detection and quantification were in the range of 0.1–0.3 and 0.4–1.1 mg/l. Satisfactory recoveries of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-gallate were 97.5–102.6, 98.6–102.4, 99.6–105.4, and 95.5–105.4%, respectively. The new method was applied to quantitative analysis theaflavins of tea samples, including 10 black teas, 5 oolong teas, and 5 green teas. This method is suitable for the rapid, accurate and inexpensive quantitative analysis of theaflavins under the basic detection conditions of HPLC.

**Keywords**: chromolith column; determination; high performance liquid chromatography; rapid; tea; theaflavins

Tea (*Camellia sinensis*) has been known as an important beverage plant in the world for thousands of years, and has a great agricultural and commercial application value, especially fermented tea, such as black tea, oolong tea, etc. Theaflavins are important quality chemical constituents in fermented tea which play a decisive role in colour and taste quality. The generalized structure of theaflavins is shown in Figure 1. However, theaflavins are well-known natural powerful antioxidants (Narai *et al*. 2016). Recent studies have shown that they have diverse pharmacological effects including anti-pancreatitis (Collier *et al*. 1997), promoting the blood circulation (Li *et al*. 2004), anti-aging (Betts *et al*. 2011), anti-inflammatory (Jin *et al*. 2013), anti-cancer (Xue *et al*. 2014), anti-bacterial (Liu *et al*. 2016), anti-obesity (Chikako *et al*. 2017), anti-fatty (Hu *et al*. 2017), etc. Therefore, the accurate determination method of theaflavins is important for tea quality chemistry and functional activity research.

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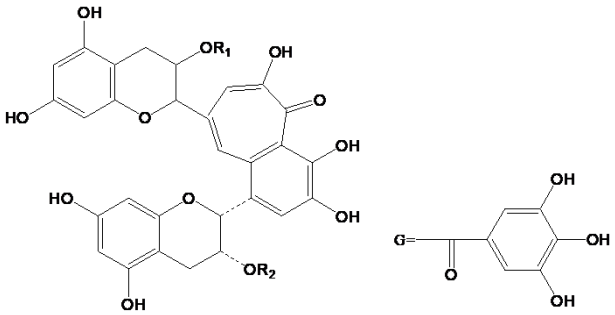


Figure 1. Structure of theaflavins
Theaflavin (TF): $R_1$ – H, $R_2$ – H; theaflavin-3-gallate (TF-3-G): $R_1$ – H, $R_2$ – G; Theaflavin-3'-gallate (TF-3'-G): $R_1$ – G, $R_2$ – H; theaflavin-3,3'-gallate (TF-3,3'-G): $R_1$ – G, $R_2$ – G

Several methods have been reported for the determination of theaflavins in fermented tea, including spectrophotometric one (Lee *et al*. 2000), capillary electrophoresis (Zu *et al*. 2012), electronic tongue (Carloni *et al*. 2013), gas chromatography (Korir *et al*. 2014), ultra-high performance liquid chromatography (Narai *et al*. 2016), thin layer chromatography (Sun *et al*. 2016), molecular imprinted polyacrylamide graphite nanocomposite electrode (Ryan *et al*. 2017), high performance liquid chromatography (Xia *et al*. 1999; Shannon *et al*. 2017), etc. The spectrophotometric method and electronic tongue (ET) are used for the total amount of tea theaflavins, while the theaflavin monomer cannot be analysed. At the same time, this method is very low in repeatability. Thin layer chromatography (TLC) has low cost, convenient operation and simple analysis, but it cannot make the accurate quantitative analysis of theaflavins. Gas chromatography (GC) is advantageous in a certain way due to high sensitivity and accurate quantitative analysis, but the boiling point of theaflavins is too high to gasify, which need to be analysed by derivatization method. Capillary electrophoresis (CE) has an advantage of quick analysis, low amount of sample and organic solvent consumption, but it is less sensitive due to the short sample path length of the CE capillary, which is much more difficult to be equal to the HPLC method in consistency and reproducibility. Ultra-high performance liquid chromatography (UPLC) represents higher separation efficiency in separation science. UPLC methods for the determination of flavonoids and phenolic compounds have been developed. However, a UPLC unit is expensive and requires higher maintenance costs.

High performance liquid chromatography (HPLC) is by far the most popular method for the analysis of tea theaflavins. In these methods, the used stationary phase is based on particulate packing materials of around 5.0 μm in particle size (Xia *et al*. 1999; Shannon *et al*. 2017). Particulate packing materials, however, are beset with problems of backpressures when higher elution flow rates are tested. Separation times of 20 min or more are often required. Monolithic columns represent an innovative type of column for rapid chromatography analysis. In contrast to the conventional HPLC columns, monolithic columns are made from a single piece of porous silica gel, giving them greater porosity and permeability, and also allowing chromatographic analyses to be performed in a fraction of the time previously required.

The present study aimed to develop a new HPLC method using a new monolithic column for the simultaneous determination of four main theaflavin monomers in tea. The method was applied to the determination of theaflavins in black tea, oolong tea, and green tea. This method has shorter analysis time and higher efficiency than the existing HPLC methods reported in the literature, and is less expensive than the ultra-high performance liquid chromatography (UPLC). This method can provide a reference for rapid, accurate and inexpensive quantitative analysis and stability analysis of theaflavins in health food, tea beverage industry and tea food.

## MATERIAL AND METHODS

***Reagents and materials***. Theaflavin (TF; ≥ 99%), Theaflavin-3-gallate (TF-3-G; ≥ 99%), Theaflavin-3'-gallate (TF-3'-G; ≥ 99%) and Theaflavin-3,3'-gallate (TF-3,3'-G; ≥ 99%) standards were purchased from Sigma Aldrich (Germany). Twenty tea samples were randomly collected from China, India, and Sri Lanka.

***Apparatus***. The HPLC system (Shimadzu, Japan), equipped with SPD-20A, UV-visible detector, CTO-20A column oven, LC-20AD quaternary pumping system and DGU-20A3 chromatography interface, and SIL-20A auto-injector, LCsolution software, was used for peak purity determination. The chromatographic separation was performed on a new Chromolith RP-18 column (4.6 mm i.d. × 100 mm, 2.0 μm; Merck KGaA, Germany). The temperature of the column oven was set at 35°C. The UV detector was set at 280 nm. The injection volume was 10 μl. The comparative selection experiments of mobile phase A, mobile phase B, elution flow and elution gradient are as follows.

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### Theaflavin separation effect

***Acetic acid concentrations in mobile phase A***. The mobile phase A employed in the analysis consisted of different acetic acid concentrations (1, 2, 6, 10, 20 and 30%; acetic acid/water, v/v). The mobile phase B employed in the analysis consisted of acetonitrile/ethyl acetate mixture (7:1, v/v). Linear gradient elution: from 92% mobile phase A (v) to 76% mobile phase A (v) during early 3 min and then 92% mobile phase A (v) till 8 min at an elution flow rate of 1 ml/min. The separation effects of theaflavins under different acetic acid concentrations in mobile phase A were compared.

***Ratio of acetonitrile to ethyl acetate in mobile phase B***. The mobile phase A employed in the analysis consisted of 2% (v/v) acetic acid in water. The mobile phase B employed in the analysis consisted of different acetonitrile to ethyl acetate ratios (7:1, 8:1, 9:1, v/v). Linear gradient elution: from 92% mobile phase A (v) to 76% mobile phase A (v) during early 3 min and then 92% mobile phase A (v) till 8 min at an elution flow rate of 1 ml/min. The separation effects of theaflavins under different acetonitrile to ethyl acetate ratios in mobile phase B were compared.

***Elution flow rate***. The mobile phase A employed in the analysis consisted of 2% (v/v) acetic acid in water. The mobile phase B employed in the analysis consisted of acetonitrile/ethyl acetate mixture (7:1, v/v). Linear gradient elution: from 92% mobile phase A (v) to 76% mobile phase A (v) during early 3 min and then 92% mobile phase A (v) till 8 min at an elution flow rate of 1 ml/min (0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0 ml/min). The separation effects of theaflavins under different elution flows were compared.

***Elution gradient***. The mobile phase A employed in the analysis consisted of 2% (v/v) acetic acid in water. The mobile phase B employed in the analysis consisted of acetonitrile/ethyl acetate mixture (7:1, v/v). Linear gradient elution: from 92% mobile phase A (v) to different proportions of mobile phase A (76, 75, 74, 73, 72, 71, 70, 65, and 60%, v) during early 3 min and then 92% mobile phase A (v) till 8 min at an elution flow rate of 1.5 ml/min. The separation effects of theaflavins under different elution gradients were compared.

***Linearity analysis***. The linearity curves were established by analysing standard mixtures of TF, TF-3-G, TF-3'-G and TF-3,3'-DG (0.05–120.00 mg/l). The coefficient of determination ($R^2$) was calculated by linear least-squares regression.

***Limit of detection (LOD) and limit of quantification (LOQ)***. The LOD and LOQ were determined as follows:

$$LOD = 3 \times sd/slope \quad (1)$$

$$LOQ = 10 \times sd/slope \quad (2)$$

where: sd – standard deviation

***Repeatability and reproducibility***. By analysing each of the standard theaflavins (5 mg/l), the repeatability and reproducibility of the TF, TF-3-G, TF-3'-G and TF-3,3'-G peak areas were investigated on the same day (intra-day repeatability) and over six days (inter-day repeatability). The mean, standard deviation and relative standard deviation were calculated by Excel software.

***Recovery***. By analysing each of the standard theaflavins mixtures (5 mg/l), the recovery of a mixture of theaflavin standards to each type of tea samples was studied. The mean, standard deviation and relative standard deviation were calculated by Excel software.

***Analysis of theaflavins in tea samples***. The extraction methods of black tea, oolong tea, green tea samples referred to ISO 14502. Ground black tea, green tea and oolong tea samples (0.2 g) were extracted in 5 ml of 70% methanol at 70°C for 10 minutes. Dispense 5 ml of hot 70% methanol extraction mixture into the extraction tube, stopper and mix on the vortex mixer. Continue heating the extraction tube in the water bath for 10 min, mixing on the vortex mixer after 5 minutes. Remove the extraction tube from the water bath and allow it to cool to room temperature. Remove the stopper and place the tube in the centrifuge at 3500 rpm for 10 minutes. Carefully decant the supernatant into a graduated tube. The extraction of tea residues with 70% methanol was repeated. Combine the two extracts, make up to 10 ml with cold methanol/water extraction mixture and mix the contents. The contents were filtered by 0.45 μm filter. The HPLC injection volume of each sample was 10 μl.

## RESULTS AND DISCUSSION

### Theaflavins separation effect

***Acetic acid concentration in mobile phase A***. The effect of different acetic acid concentrations

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in mobile phase A (1, 2, 6, 10, 20, and 30%; acetic acid/water, v/v) was studied. The increase of the proportion of acetic acid can accelerate the separation process, but there is a problem of overlapping of chromatographic peaks of TF, TF-3-G, TF-3'-G and TF-3,3'-DG. With the increasing proportion of acetic acid, the separation degree of theaflavins decreased. When the acetic acid concentration was 2% (acetic acid/water, v/v), TF, TF-3-G, TF-3'-G and TF-3,3'-G were well separated and the retention time was short around eight minutes. Compared to the mobile phases mentioned by Jin *et al*. (2013) and Liu *et al*. (2016), the present study was more efficient in the analysis of theaflavin monomers in tea samples. However, TF-3-G and TF-3'-G were not well separated (Liu *et al*. 2016; Tao *et al*. 2016) and furthermore they required a longer analysis time of about 30 or 85 min (Jin *et al*. 2013; Liu *et al*. 2016; Chikako *et al*. 2017; Trisita *et al*. 2017).

***Ratio of acetonitrile to ethyl acetate in mobile phase B***. The effect of different ratios of acetonitrile to ethyl acetate (7:1, 8:1, 9:1; v/v) in mobile phase B was studied. When the 7:1 acetonitrile/ethyl acetate ratio of mobile phase B was used, the peaks of TF-3'-G and TF-3,3'-DG were not well resolved. When the 8:1 and 9:1 acetonitrile/ethyl acetate ratios in mobile phase B were used, the peaks of TF-3-G and TF-3'-G were not well resolved.

***Elution flow rate***. The effect of different flow rates (0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0 ml/min) on the separation of the analytes was studied. It was noted that retention time decreased with an increase in flow rate and all the peaks were well resolved. However, when the elution flow rate reached above 1.5 ml/min, the separation degree of theaflavins started to decrease, and TF-3-G, TF-3'-G and TF-3,3'-DG were not well resolved. Therefore, 1.5 ml/min was chosen.

***Elution gradient***. The results showed that the separation degree of theaflavins decreased with an increase in the elution gradient concentration. The 75% mobile phase A (v) at 3 min provided a good separation degree of four theaflavin monomers and a short separation time.

Linear gradient elution: from 92% mobile phase A (v) to 76% mobile phase A (v) during early 3 min and then 92% mobile phase A (v) till 8 min at an elution flow rate of 1.5 ml/min. As comparison, the analytes were also separated by a particulate ODS C-18 column under the same chromatographic separation conditions (flow rate 1.5 ml/min, column temperature at 35°C, the UV detector was set at 280 nm, the injection volume was 10 µl) with the same HPLC conditions (Figure 2B), which contain the same gradient and mixture of theaflavin standards (5 mg/l). The column resulted in a good separation effect, but the separation time was longer (around 32 min).

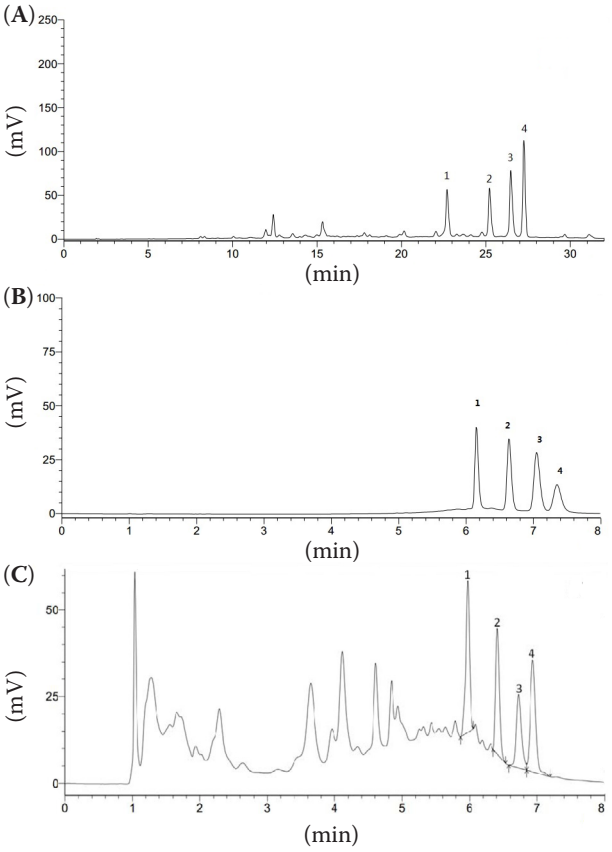


Figure 2. Typical chromatograms for the four types theaflavins by ODS hypersil C18 (**A**), monolithic column (**B**), and typical chromatogram for the black tea by monolithic column (**C**)

1 – theaflavin (TF); 2 – theaflavin-3-gallate (TF-3-G); 3 – theaflavin-3'-gallate (TF-3'-G); 4 – theaflavin-3,3'-gallate (TF-3,3'-G)

Table 1. Analytical characteristics of the deveolped HPLC method

| Analyte | Linear range (mg/l) | $R^2$ | LOD (mg/l) | LOQ (mg/l) | Inter-day (%RSD) | Intra-day (%RSD) |
|---|---|---|---|---|---|---|
| TF | 0.2–95.0 | 0.9999 | 0.25 | 0.83 | 0.66 | 0.02 |
| TF-3-G | 0.2–95.0 | 0.9997 | 0.19 | 0.63 | 0.46 | 0.05 |
| TF-3'-G | 0.2–95.0 | 0.9999 | 0.32 | 1.07 | 0.51 | 0.06 |
| TFDG | 0.2–95.0 | 0.9999 | 0.12 | 0.40 | 0.65 | 0.08 |

$R^2$ – square of regression coefficent; RSD – relative standard deviation ($n$ = 6)

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Similar order of elution to that of the Chromolith column was found (Figure 2A), suggesting a similar separation mechanism between the columns.

***Analytical characteristics***. The analytical characteristics of the new HPLC method were evaluated in terms of linearity, LOD, LOQ, repeatability, reproducibility and recovery. The TF, TF-3-G, TF-3'-G and TF-3,3'-DG standards (2 mg/l) were put into the blank sample (water) for the measurement of LOD and LOQ. The LOD and LOQ for these theaflavins were comparable to those reported by Li *et al*. (2004), Xue *et al*. (2014) and Liu *et al*. (2016). The results showed that the relative standard deviations (RSD) for TF, TF-3-G, TF-3'-G and TF-3,3'-DG ranged between 0.02 and 0.08% (Table 1). The reproducibility over six days was carried out by analysing the same theaflavin standard solution. The results showed that all the samples performed good reproducibility (inter-day and intra-day RSD ≤ 0.7; Table 1). By analysing each of the standard theaflavin mixtures (5 mg/l), the recoveries of a mixture of theaflavin standards to black tea, oolong tea, and green tea samples were studied. The results showed that the samples performed good recoveries (97.5–101.5 ± 1.2%; Table 2). Satisfactory recoveries of TF, TF-3-G, TF-3'-G, and TF-3,3'-DG were obtained (97.5–102.6, 98.6–102.4, 99.6–105.4, and 95.5–105.4%, respectively).

***Comparison with other HPLC methods***. The characteristics of some HPLC methods for the determination of theaflavins and related tea chemical compounds were summarized (Table 3). However, the list is not exhaustive, it rather aims to explain the important characteristics of the reported HPLC methods. The results in Table 3 showed that the shortest time of the theaflavin analysis by HPLC was 20 min, the longest time was 85 minutes. The particle-type stationary phase, Chromolith RP-18 col-

Table 2. Recoveries of theaflavins by the developed HPLC method (%)

| Sample number | Analytes | | | |
|---|---|---|---|---|
| | TF | TF-3-G | TF-3'-G | TFDG |
| 1 | 97.5 ± 0.3 | 98.6 ± 0.4 | 105.4 ± 0.1 | 95.5 ± 0.4 |
| 2 | 102.3 ± 0.3 | 100.5 ± 0.5 | 103.4 ± 0.5 | 96.7 ± 0.1 |
| 3 | 102.6 ± 0.4 | 101.2 ± 0.7 | 103.3 ± 1.3 | 101.1 ± 3.1 |
| 4 | 102.4 ± 0.3 | 101.4 ± 0.2 | 102.4 ± 0.1 | 103.6 ± 0.1 |
| 5 | 101.9 ± 0.2 | 101.9 ± 0.2 | 100.8 ± 0.4 | 105.4 ± 0.1 |
| 6 | 101.5 ± 0.1 | 102.4 ± 0.1 | 99.6 ± 0.2 | 104.3 ± 0.3 |

Values ± sd

Table 3. Some reported HPLC methods for the analysis of tea flavonoids

| No. | Analytes | Detector (280nm) | Column | Dimension | Program | LOD | LOQ | Separation time (min) | References |
|---|---|---|---|---|---|---|---|---|---|
| 1 | theaflavins | PDA | CAPCELL PAK C-18 UG120 | 4.6 mm × 100 mm, 3 µm | gradient | – | – | 40 | Chikako *et al.* (2017) |
| 2 | theaflavins and catechins | UV | Polar-RP 80 A | 4.6 mm × 150 mm, 4 µm | gradient | – | – | 45 | Asako *et al.* (2017) |
| 3 | theaflavins, catechin, and caffeine | UV | VP-DOS C-18 | 4.6 mm × 250 mm, 5 µm | gradient | – | – | 85 | Duiyan *et al.* . (2013) |
| 4 | theaflavins and atechins | PDA | Whatman Partisphere C-18 | 4.6 mm × 125 mm, 5 µm | isocratic | 0.05–500 µg/ml | – | 30 | Honglin *et al.* . (2016) |
| 5 | theaflavins and theasinensins | UV | Cosmosil 5C18-AR-II | 4.6 mm × 250 mm, 5 µm | gradient | 0.25–10 mg/ml | – | 55 | Jinjin *et al.* (2014) |
| 6 | theaflavins | UV-VIS | Gemini C-18 | 4.6 mm × 150 mm, 5 µm | gradient | – | – | 65 | Chen *et al.* (2011) |
| 7 | theaflavins | UV-VIS | LUNA C-18 | 4.6 mm × 250 mm, 5 µm | gradient | – | – | 20 | Sharma *et al.* (2008) |
| 8 | theaflavins | PDA | Hypersil C-18 | 4.6 mm × 150 mm, 5 µm | isocratic | – | – | 30 | Wang *et al.* . (2008) |
| 9 | theaflavins | UV | Nucleosil C18 | 4.6 mm × 100 mm, 3 µm | isocratic | 0.13–0.25 mg/ml | – | 50 | Li *et al.* (2004) |
| 10 | theaflavins | UV | Monolithic C-18 | 4.6 mm × 100 mm, 2 µm | gradient | 0.12–0.32 mg/ml | 0.40–1.07 mg/l | 8 | Our method |

PDA – photo diode array; UV – ultraviolet spectrometry; DAD – diode array detector

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Table 4. Levels of theaflavins in tea samples

| Samples | No. | TF | TF-3-G | TF-3'-G | TF-3,3'-G | TFs |
|---|---|---|---|---|---|---|
| | | (mg/g) | | | | |
| **Black tea** | 1 | 0.779 ± 0.034 | 0.990 ± 0.004 | 0.504 ± 0.021 | 1.223 ± 0.007 | 3.495 ± 0.052 |
| | 2 | 4.656 ± 0.043 | 4.003 ± 0.021 | 4.006 ± 0.039 | 3.169 ± 0.022 | 15.834 ± 0.086 |
| | 3 | 3.935 ± 0.008 | 3.414 ± 0.019 | 3.122 ± 0.007 | 2.665 ± 0.011 | 13.136 ± 0.019 |
| | 4 | 4.021 ± 0.020 | 3.292 ± 0.040 | 2.654 ± 0.021 | 2.118 ± 0.009 | 12.084 ± 0.050 |
| | 5 | 4.211 ± 0.039 | 3.473 ± 0.012 | 3.088 ± 0.045 | 2.718 ± 0.027 | 13.489 ± 0.111 |
| | 6 | 5.044 ± 0.084 | 3.836 ± 0.065 | 3.534 ± 0.106 | 2.736 ± 0.083 | 15.150 ± 0.338 |
| | 7 | 1.860 ± 0.003 | 1.664 ± 0.013 | 1.343 ± 0.026 | 1.808 ± 0.004 | 6.676 ± 0.030 |
| | 8 | 0.802 ± 0.007 | 0.802 ± 0.007 | 0.426 ± 0.023 | 0.586 ± 0.008 | 2.616 ± 0.018 |
| | 9 | 2.156 ± 0.010 | 2.136 ± 0.011 | 1.867 ± 0.014 | 1.533 ± 0.005 | 7.691 ± 0.039 |
| | 10 | 2.680 ± 0.030 | 2.070 ± 0.007 | 1.853 ± 0.007 | 1.372 ± 0.007 | 7.976 ± 0.038 |
| | max | 5.044 | 4.681 | 4.170 | 4.993 | 17.640 |
| | min | 0.779 | 0.598 | 0.185 | 0.372 | 2.020 |
| | avg | 2.387 | 2.392 | 2.383 | 2.422 | 9.581 |
| **Oolong tea** | 11 | 0.400 ± 0.002 | 0.759 ± 0.008 | 0.089 ± 0.003 | 0.405 ± 0.001 | 1.654 ± 0.010 |
| | 12 | 1.057 ± 0.010 | 0.968 ± 0.045 | 0.917 ± 0.026 | 0.895 ± 0.025 | 3.836 ± 0.029 |
| | 13 | 0.810 ± 0.014 | 0.699 ± 0.002 | 0.197 ± 0.013 | 0.345 ± 0.006 | 2.051 ± 0.035 |
| | 14 | 1.357 ± 0.006 | 1.236 ± 0.006 | 1.149 ± 0.061 | 1.036 ± 0.054 | 4.779 ± 0.115 |
| | 15 | 1.092 ± 0.004 | 0.675 ± 0.029 | 0.231 ± 0.005 | 0.379 ± 0.001 | 2.376 ± 0.035 |
| | max | 1.357 | 1.236 | 1.149 | 1.036 | 4.779 |
| | min | 0.400 | 0.675 | 0.089 | 0.345 | 1.654 |
| | avg | 0.943 | 0.867 | 0.517 | 0.612 | 2.939 |
| **Green tea** | 16 | | | lower than LOD | | |
| | 17 | | | lower than LOD | | |
| | 18 | | | lower than LOD | | |
| | 19 | | | lower than LOD | | |
| | 20 | | | lower than LOD | | |
| | max | | | lower than LOD | | |
| | min | | | lower than LOD | | |
| | avg | | | lower than LOD | | |

TFs – TF + TF-3-G + TF-3'-G +TF-3,3-G; LOD – limit of detection

umn, reasonable gradient elution, reasonable elution infusion, reasonable flow rate were employed by our proposed method that provided a short analysis time (8 min) and perfect resolution and separation degree. The effect of our proposed separation method can be comparable with UPLC, but it is less costly and requires lower maintenance costs. It is very suitable for many labs in the world.

***Analysis of tea samples***. The quantification of TF, TF-3-G, TF-3'-G, and TF-3,3'-DG in tea samples was done on the basis of the external standard method using calibration curves (Table 4). The theaflavin content in green tea was not detected. The contents of TF, TF-3-G, TF-3'-G, and TF-3,3'-DG in black tea were higher than in oolong tea and green tea. This may be related to the enzymatic oxidation degree of catechins and other substances in tea leaves. Different equipment has been reported for the quantification of theaflavin content in black tea and oolong tea, such as high performance liquid chromatography (HPLC) (Wang *et al*. 2010, Xu *et al*. 2019), ultra-high performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (HPLC-MS-MS) (Tao *et al*. 2016, Xu *et al*. 2018), high-speed countercurrent chromatography (HSCCC) (Wang *et al*. 2008), electronic tongue (Qin *et al*. 2019), and capillary electro-

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phoresis (Wright *et al*. 2001). The range of theaflavin content in black tea is 9.240–21.318 mg/g (Qin *et al*. 2019). The total concentrations of theaflavins were 3.44, 0.41 and 1.70 g/ml in black, green and oolong teas for low grade, respectively; and for high grade, the total concentrations were 6.87, 0.45 and 0.65 g/ml and the level of total theaflavins in black tea was higher than in other tea samples (Tao *et al*. 2016).

## CONCLUSIONS

A rapid HPLC method using a monolithic column was developed, validated and applied to the simultaneous determination of TF, TF-3-G, TF-3'-G, and TF-3,3'-DG in tea samples. The use of the Chromolith RP-18 column enables to determine theaflavins almost three times faster than by a conventional particulate column (~8 vs. ~20 min). The limits of detection and quantification were in the range of 0.1–0.3 and 0.4–1.1 mg/l, respectively. Satisfactory recoveries of TF, TF-3-G, TF-3'-G, and TF-3,3'-DG were 97.5–102.6, 98.6–102.4, 99.6–105.4 and 95.5–105.4%. Black tea is a kind of fermented tea by withering, rolling, fermentation and drying. Green tea is non-fermented tea. Relatively high levels of theaflavins were found in black tea. The HPLC method using the Chromolith RP-18 column is a new useful analytical technique for the rapid, accurate and inexpensive determination of theaflavins.

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