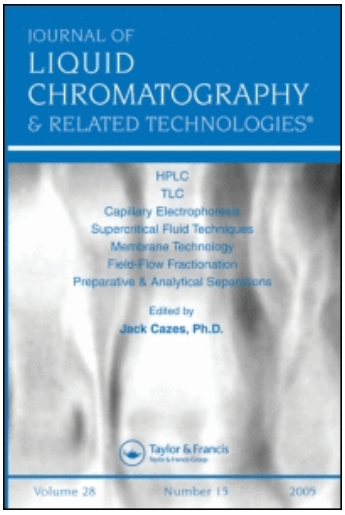

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Qiang He[a]; Yuanping Lv[b]; Ling Zhou[b]; Bi Shi[a]
[a] National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu, P. R. China [b] The Department of Food Science and Engineering, Sichuan University, Chengdu, P. R. China

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SIMULTANEOUS DETERMINATION OF CAFFEINE AND CATECHINS IN TEA EXTRACTS BY HPLC

Qiang He,[1] Yuanping Lv,[2] Ling Zhou,[2] and Bi Shi[1]

[1]*National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu, P. R. China*
[2]*The Department of Food Science and Engineering, Sichuan University, Chengdu, P. R. China*

□ *A simple, rapid, and precise HPLC method was developed for the separation and quantification of catechins and caffeine in green tea extracts. Employing a Hypersil ODS C_{18} column (150 × 4.0 mm) and a diode UV detector (280 nm), it was found that a satisfactory separation can be achieved by a proper gradient elution using methanol and 0.2% acetonitrile solution. Five catechins (epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin (EC), gallocatechin gallate (GCG) and epicatechin gallate (ECG)) and caffeine in tea extracts were well separated on the chromatogram within 15 min. The method exhibited satisfactory repeatability (RSD ≤ 1.53%, n = 3) and accuracy (98.7%–102.4%, RSD ≤ 3.50%, n = 3), and reached a simultaneous determination of catechins and caffeine when used for a commercial sample of Chinese green tea extracts. This HPLC method might be suitable for the quality assurance of tea products and catechin-containing preparations.*

Keywords caffeine, catechins, determination, HPLC, polyphenol, tea

INTRODUCTION

Tea is perhaps one of the most popular beverages all over the world. Recently, tea and tea extracts have attracted much attention since many studies have showed that tea consumption is closely associated with health benefits.[1,2] For example, it has been found that regular drinking of tea can reduce the incidence of cancers and heart diseases, together with other effects such as anti-inflammation and anti-obesity.[3–5] These beneficial effects are mainly attributed to catechins naturally occurring in tea and tea extracts. Catechins are polyphenolic compounds with flavon-3-ol

Correspondence: Bi Shi, National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu, 610065, P. R. China. E-mail: sibitannin@vip.163.com

structure, as shown in Figure 1, and have been proved to be strong antioxidants[6,7] and free radicals scavenging agents.[8] Many investigations demonstrated that the biological activities of catechins are different, depending on their structures. The anti-oxidative activities of main catechinic compounds, assessed using a linoleic acid emulsion, were found in the order of EGCG > ECG = EGC > EC.[9–11]

It was also proved that the galloylated catechins exhibit relative higher free radical scavenging capacity than non-galloylated ones.[10,12] Thus, much attention has been paid to component and content of catechins in tea and tea extracts in researches and applications since they are varying with origins, seasons and the method of processing. On the other hand, caffeine, the coexisting component with catechins in tea is also concerned by researches because it has been proved that caffeine has some negative physiological effects.[13]

In addition to direct consumption of tea drinks, tea extracts have been widely used in pharmaceutical and food areas. For example, tea polyphenols, whose major compositions are catechins, have been employed as natural antioxidants in food industry in many countries. At the same time, some catechin-containing pharmaceutical products have been commercialized. Obviously, no matter how one uses tea and tea extracts, exact and convenient determination of catechins and caffeine is an extremely important work because it is a quality and health related event.

Many methods for determining catechins and caffeine in tea and the derived products have been developed.[14–16] Among these methods, liquid chromatography is the most applied technique. But, usually a low resolution is obtained even though a sophisticated gradient elution and long eluting time are used. Then, a simple, fast and precise HPLC method

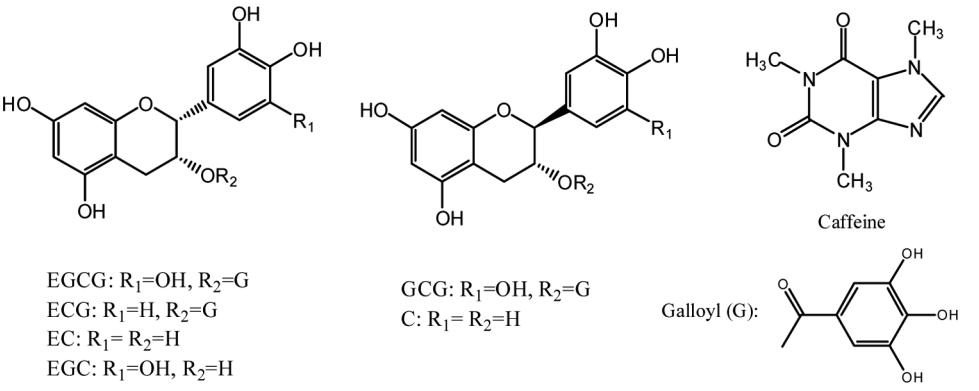

FIGURE 1 Chemical structures of typical catechins and caffeine.

was developed in this study for the separation and determination of catechins and caffeine in green tea extracts.

EXPERIMENTAL

Materials and Sample Preparation

Fifty grams fresh commercial Chinese green tea was extracted by soaking it in 500 mL hot water (80°C) with stirring for 1 h. After filtration, the filtrate was concentrated to 100 mL in a rotary evaporator and extracted by using chloroform (50 mL × 2) to remove caffeine, lipids, and chlorophyll. The aqueous phase was extracted by using ethyl acetate (100 mL × 2), and then a sample of tea extracts, mainly containing catechins, was prepared when the ethyl acetate phase was evaporated.

Reagents and Standards

The CH_3CN and CH_3OH used in mobile phases were all HPLC grade solvents. The standard samples, catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin gallate (GCG), epigallocatechin gallate (EGCG) and caffeine, were purchased from Sigma Chemical Co. All other reagents were of analytical grade.

Preparation of Sample Solutions

Tea extracts (10 mg) and catechinic samples (7.5 mg) were dissolved in 10 mL ethanol/water (1:1, v/v) solution respectively with the aid of ultrasonic, and then scaled to 25 mL. 7.5 mg caffeine was dissolved in 10 mL acetone/water (7:3, v/v) solution and then scaled to 25 mL. Sample solutions used for HPLC analysis were prepared by diluting the stock solutions above. The sample solutions were filtered through a 0.45 μm membrane (Millipore) before HPLC analysis.

HPLC Determination

An Agilent 1100 liquid chromatograph system equipped with a Hypersil ODS C_{18} column (150 × 4.0 mm) was employed for analyses. All the determinations were undertaken at 25°C. Mobile phases included aqueous solution of 0.2% acetonitrile (eluent M1) and methanol (eluent M2). The mobile phases were filtered (0.45 μm, Millipore) and then ultrasonically degassed prior to use. The gradient elution was used in the way that the eluent M2 was linearly increased from 0 to 50% during the period of

0–12 min, and then increased from 50% to 100% during 13–20 min. The flow rate and sample volume injected were 1.2 mL/min and 20 μL, respectively. Eluate was detected with a diode array detector at 280 nm. The chromatographic peaks of the analytes were identified by comparing their retention times with those of standards. Quantification was carried out by integration of peak by using external standard method.

RESULTS AND DISCUSSION

Separation of Catechins and Caffeine by HPLC

Catechinic compounds have similar physical and chemical properties due to their analogous chemical structures, as shown in Figure 1. So, the choice of mobile phases and the use of proper gradient elution are crucial to ensure all components to be efficiently separated. The HPLC conditions for separation of catechins and caffeine were optimized by our preliminary assays, where different combination of acetonitrile, methanol and water were tested. Consequently, it was found that the gradient elution system of methanol-0.2% acetonitrile, as described above, showed the best performance.

Figure 2 shows the HPLC chromatograms obtained from a mixture of standards (A) and a sample of tea extracts (B), and the retention times of catechins and caffeine are presented in Table 1. It can be seen that a satisfactory separation of caffeine and individual catechins, except for catechin (C) and epigallocatechin (EGC), can be achieved within 15 min under the HPLC conditions described above. The column can be recycled by further elution with methanol. The effect of galloyl group on retention time can be found according to the data in Table 1. The ratio of retention time of epicatechin gallate (ECG) to that of epicatechin (EC) is nearly equal to that of epigallocatechin gallate (EGCG) to EGC for both standards and tea extracts determinations, suggesting that the presence of gallate residue in catechin molecule might have a consistent effect on the retention time. This observation is in agreement with the report of Wang et al.[17]

The total content of catechins is commonly used to characterize catechin-containing products, and a higher content means a better quality of product. Meanwhile, the content of esterified catechins such as ECG, GCG, and EGCG in catechin-containing products is of greater concern, because they are recognized to possess higher biological activities than non-esterified ones.[18,19] The peak of C is overlapped with that of EGC, as shown in Figure 2. But this does not affect the determination of esterified catechins as well as caffeine. On the other hand, it can be suggested that this overlap would result in only a small error for quantifying total

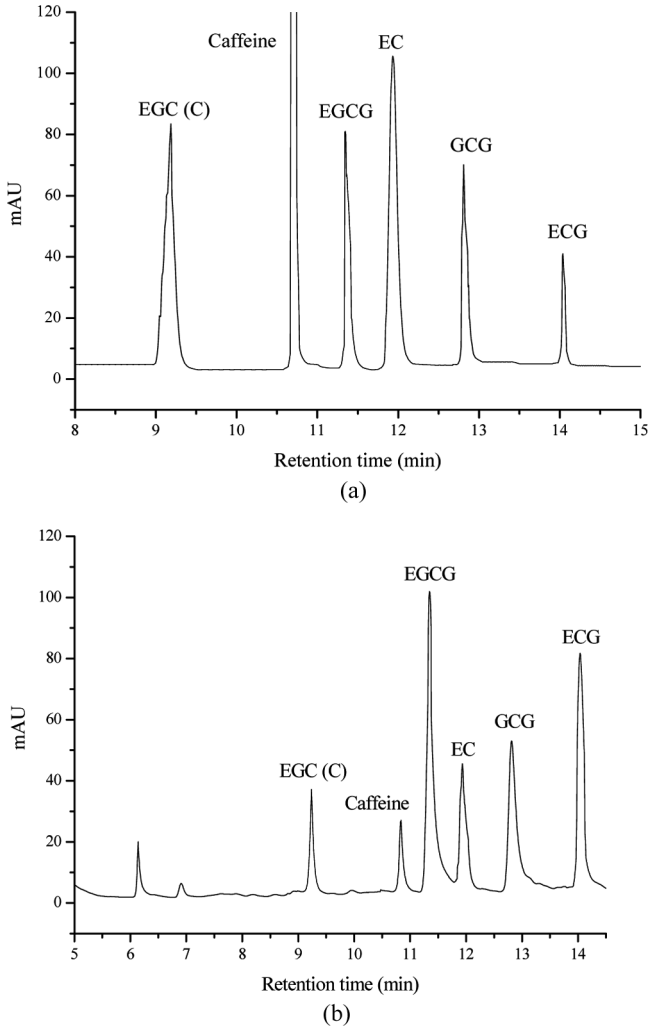

FIGURE 2 HPLC chromatograms of standards (A) and tea extracts (B) (mobile phase: M1 = aqueous solution of 0.2% CH_3CN, M2 = CH_3OH; Column: Hypersil ODS C_{18}, 150 × 4.0 mm, 5 μm; Flow rate: 1.2 mL/min; Detector: DAD, 280 nm; Injection volume: 20 μl; Gradient: 0–12 min with 0–50% M2, 13–20 min with 50–100% M2).

TABLE 1 Retention Times (min) for Catechins and Caffeine ($n = 3$)

Component	EGC (C)	EGCG	EC	GCG	ECG	Caffeine
Standards	9.186 (0.38[a])	11.346 (0.50)	11.932 (0.19)	12.812 (0.66)	14.032 (0.45)	10.679 (0.71)
Extracts	9.219 (1.41)	11.352 (1.27)	11.949 (1.53)	12.851 (0.48)	14.109 (1.13)	10.709 (1.10)

[a]RSD values (%) are given in parentheses.

catechins since the amount of C in tea extracts is usually much lower than those of other individual catechins.[14,20] Therefore, it can be concluded that the main catechins and caffeine in tea extracts could be simultaneously determined by the HPLC method presented in this study.

Validation of the Method

The repeatability, linearity, and accuracy of the proposed method were investigated. To test the repeatability, a mixture solution of standards and a solution of tea extracts were analyzed in triplicate, respectively. The retention times for catechins and caffeine under the HPLC conditions were determined to study the accumulation of errors resulting from different injections. A coincidence of retention times was observed, as presented in Table 1. The relative standard deviations (RSD) for all the analytes in standards solution are within 0.71%, while the RSD values for tea extracts solution are within 1.53%, demonstrating the high repeatability of the method.

The linearity of the assay was evaluated by using a series of standard solutions containing single component, in which the concentration of catechinic component ranged from 3 mg/L to 300 mg/L and that of caffeine was 3–150 mg/L. The calibration curves were obtained by plotting peak areas *vs.* concentrations and the results of regression analysis of the curves are shown in Table 2. The correlation coefficients of the determinations for all the standards are greater than 0.9899, showing a good linearity of determinations for catechins and caffeine.

Recovery tests were conducted in order to evaluate the accuracy of the HPLC method. Analyses were carried out in triplicate before and after addition of 1 mg standard sample into 25 mL stock solution of tea extracts. The results are presented in Table 3 and the recoveries of catechins and caffeine are within the range of 98.7–102.4% with

TABLE 2 Calibration Curves of Catechins and Caffeine

Component	Calibration Curve[a]	Correlation Coefficient (R)
EGC	$Y = 8.1066X + 23.866$	0.9998
EGCG	$Y = 11.603X - 330.99$	0.9996
EC	$Y = 8.6739X - 13.361$	0.9997
GCG	$Y = 14.402X + 9.1619$	0.9915
ECG	$Y = 15.083X + 14.155$	0.9899
Caffeine	$Y = 24.315X - 16.1$	1.0000

[a]Y is the peak area (mAU × sec) and X is the concentration of catechin or caffeine (mg/l); concentration range: 3–300 mg/L for catechins, 3–150 mg/L for caffeine.

TABLE 3 Recoveries of Catechins and Caffeine in Tea Extracts ($n = 3$)

Component	EGC	EGCG	EC	GCG	ECG	Caffeine
Amount added (mg/l)	40	40	40	40	40	40
Recovery (%)	102.4	100.2	101.6	99.5	100.6	98.7
RSD (%)	3.50	2.11	2.05	1.45	1.24	1.18

TABLE 4 Content of Catechins and Caffeine in Tea Extracts ($n = 3$)

Component	EGC (C)	EGCG	EC	GCG	ECG	Caffeine
Content (w/w, %)	5.1	40.9	6.3	10.9	30.4	2.4
RSD (%)	1.55	1.72	1.68	2.14	1.28	1.41

RSD values ranging from 1.18% to 3.50%, verifying the high accuracy of the method.

A blank injection was performed after the analyses of standards and tea extracts and no memory effect was observed. All these results confirm the validity of this analytical method for determination of catechins and caffeine in tea extracts.

Quantitative Measurement of Tea Extracts

The solution of tea extracts was analyzed three times under the optimal HPLC conditions mentioned above. The chromatogram of the tea extracts is illustrated in Figure 2b and the calculated contents of catechins and caffeine are given in Table 4. There is residue of caffeine (2.4%, w/w) in the tea extracts, although chloroform was employed to remove caffeine during the preparation of the tea extracts. The esterified catechins, including EGCG, GCG and ECG, account for more than 82% (w/w) of the tea extracts, and the total content of catechins in the extracts amounts to 93.6% (w/w). Considering the RSD values listed in Table 4, it could be believed that the HPLC method developed in this study can be used for quantitative measurement of tea extracts with satisfactory precision.

CONCLUSION

It has been demonstrated that the HPLC assay proposed in this research is a simple, rapid and precise method for analysis and quantification of catechins and caffeine in tea extracts. This method could also be suitable for quality assurance of tea products and for determination of catechins and caffeine in catechin-containing preparations.

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