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Technical note

Rapid, direct determination of polyphenols in tea by reversed-phase column liquid chromatography

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Abstract

Column liquid chromatography on a C_{18}-bonded silica column with water–methanol–acetic acid as eluent was used to determine polyphenols and caffeine in tea. Without any pretreatment, catechin, epicatechin gallate, epigallocatechin gallate, epigallocatechin, epicatechin and caffeine were separated successfully within 15 min. The detection limits ($S/N=3$) of polyphenols studied were 1.8–24 mg/l at a detection wavelength 270 nm. The linear range of the peak area calibration curves for the analytes were over two orders of magnitude with a correlation coefficient of 0.996–0.999. Using this method, some Chinese tea samples were analyzed with a good reproducibility (RSD are below 5%).

Keywords: Tea; Polyphenols

1. Introduction

Polyphenols are substantial ingredients of tea. A number of studies [1–3] within the last several years show that polyphenols play an important role in the prevention of certain forms of human cancer. In the food industry, antioxidation of green tea and its catechins have also been studied [4,5].

There are many methods to determine polyphenols in tea, such as near-infrared reflectance spectroscopy [6], micellar electrokinetic chromatography [7], spectrophotometry and column liquid chromatography (LC) [8–13]. LC is the most suitable method for the analysis of polyphenols in tea. By current LC methods, good results have been obtained. These methods can be divided into two main categories.

The first category of methods can obtain a good separation efficiency without pretreatment but need a long analytical time of more than 35 min [8–10], the other category need some pretreatment procedure in spite of a shorter analytical time of less than 20 min [11–13]. So, it is imperative to develop a new method which can have both of these two advantages: short analytical time and no pretreatment procedure.

In this study, a rapid reversed-phase LC method was developed for the determination of the main polyphenols and caffeine in tea. Catechin (D-C), epicatechin gallate (L-ECG), epigallocatechin gallate (L-EGCG), epigallocatechin (L-EGC), epicatechin

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(L-EC) and caffeine were separated successfully within 15 min.

2. Experimental

2.1. Apparatus

A Varian 5000 LC (USA) was used in this study. It consists of a single-piston reciprocating pump, microprocessor-controlled proportioning valves, a manual loop sample injector, a model 100 UV–VIS detector and a model 401 data handling system.

2.2. Chemicals and samples

D-C, L-EGCG, L-EC, L-ECG and L-EGC were purchased from Sigma Chemical Co. (USA) with purities greater than 98%. Caffeine was obtained from the Chinese Academy of Military Medical Science (China), Methanol (HPLC grade) from Tianjin Shield Co., other chemicals used were of analytical grade.

Stock solutions of each of polyphenol and caffeine were prepared as aqueous solutions with concentrations of 1000–2000 mg/l. The standard mixture was prepared using these stock solutions.

Three kinds of Chinese green tea: Yunnan tea, Guangdong tea and Jinghua tea, were used as samples. One gramme of dry tea was put in about 180 ml water and boiled for about 1 h. Then the tea samples were filtered through a 0.45 μm membrane filter and transferred into a 200 ml flask. The tea solutions were injected to HPLC using a 20-μl loop injector.

2.3. LC conditions

A Zorbax SB-C_{18} column (250 mm×4.6 mm I.D., 5 μm) was used. The aqueous solution of 30% methanol containing 0.1% acetic acid was used as eluent with a flow-rate of 1.0 ml/min. The detection wavelength was 270 nm.

3. Results and discussion

3.1. Separation of the main polyphenols and caffeine

Several methanol aqueous-based mobile phases were studied, such as water–methanol in combination with acetonitrile, dimethylformamide (DMF), phosphoric acid and acetic acid. When some alkaline solvents such as DMF were added to the mobile phase, the signal for polyphenols was inhibited greatly, and more noise was observed compared with some acidic compounds such as acetic acid in the mobile phase. It was found that a water–methanol–acetic acid eluent is the best for the separation of the polyphenols and caffeine. A series of experiments were done to compare the effect of the concentration of acetic acid. It was proved that there was little effect on the separation when the concentration of acetic acid in the mobile phase was between 0.05 and 0.5%. The retention time decreased a little when the concentration of acetic acid was increased. The suitable proportion of water/methanol/acetic acid is 70:30:0.1 (v/v). The chromatograms of a standard mixture and a tea sample at optimum conditions are shown in Fig. 1.

3.2. Parameters for quantitative analysis

The detection wavelength was selected as 270 nm in this study. Though higher detection sensitivity can be obtained for all analytes at 210 nm, interference from other coexisting organic compounds was so great that the separation and quantitative analysis of the interested polyphenols became difficult, and the linearity of the calibration curves was not comparable to that obtained at 270 nm.

The detection limits ($S/N=3$) and linear range for the five polyphenols and caffeine were investigated. The linear range of the calibration curves of peak areas for the analytes were over two orders of magnitude with a correlation coefficient of 0.996–0.999. The linear range of the peak height calibration curves was narrower than that of peak areas. The detection limits of all analytes were 1.8–24 mg/l which is suitable for the direct analysis of the main polyphenols and caffeine in tea.

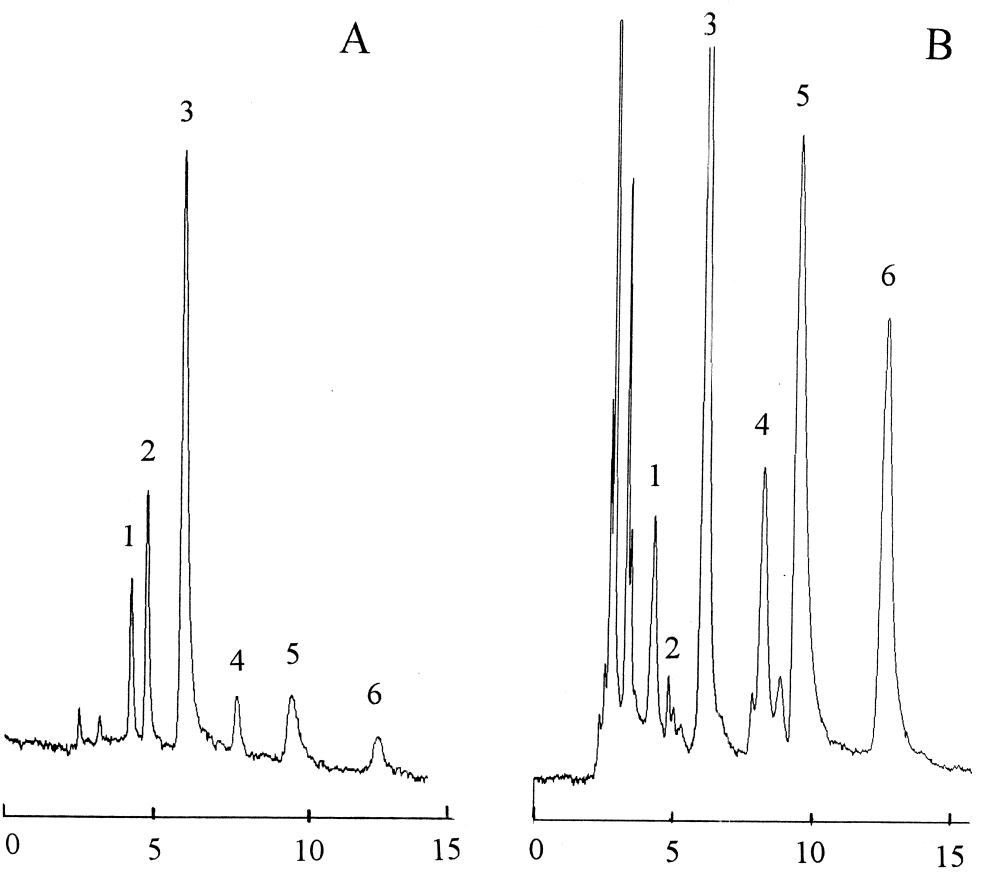

Fig. 1. LC-UV (270 nm) of a standard mixture (A) and Yunnan tea (B) using a 30% methanol containing 0. 1% acetic acid with flow-rate of 1. 0 ml/min. Peak identification: 1. L-EGC; 2. D-C; 3. L-EGCG; 4. L-EC; 5. caffeine; 6. L-ECG. The standard mixture contained 100 mg/l of L-EGC, 160 mg/l of D-C, 54 mg/l of L-EGCG, 120 mg/l of L-EC, 20 mg/l of caffeine and 100 mg/l of L-ECG.

3.3. Analysis of tea samples

Three kinds of Chinese green tea, Yunnan tea, Guangdong tea and Jinghua tea, were analyzed for the contents of five polyphenols and caffeine. The three kinds of tea are the popular Chinese tea, and Yunnan tea is produced in the south-west of China. Although no pre-dialysis was done, the five polyphenols and caffeine which were the main ingredients of the tea were separated successfully from each other and from other nonidentified coexisting compounds in tea within a shorter time (about 15 min) (see Fig. 1).

The results are shown in Table 1. Because of

Table 1
Determination results of polyphenols and caffeine in tea (%, $n=5$)[a]

Tea	D-C	L-EGCG	L-EC	L-ECG	L-EGC	Caffeine
Yunnan	0.56	10.4	13.3	4.54	6.22	3.05
Guangdong	0.57	6.71	7.22	2.68	7.92	2.68
Jinghua	0.35	5.55	7.50	1.42	3.94	2.77

[a] RSDs were below 5%.

weather, soil and species, obvious differences in the contents of polyphenols can be seen. L-EGCG, L-EC and L-EGC are the main polyphenols in all three Chinese tea, but the content of L-EC is highest in Yunnan tea and Jinghua tea while L-EGC is highest in Guangdong tea. The caffeine is the most purine base, there are no obvious differences in the caffeine contents of the three kinds of tea. The total content of polyphenols in the three teas was also determined by spectrophotometry using ferrous tartrate as a colour reagent. The total content of polyphenols in Yunnan tea is 35–40%, while in the other teas it is 20–30% according to our research and the literature. So Yunnan tea is a very suitable raw material for extracting polyphenols. For Yunnan tea and Guangdong tea, the sum of the content of the five polyphenols by LC are approximate to the total content of all polyphenols by spectrophotometry. However, for Jinghua tea, the total content of polyphenols by the spectrophotometry is 24.1% while the sum of the content of the five polyphenols by HPLC is only 18.76%. This means some other polyphenols, which are 20% of the total polyphenols in Jinghua tea, have not been identified yet.

In conclusion, the HPLC method developed in this work is useful for the analysis of the main polyphenols and caffeine in tea. The method is rapid and simple, five main polyphenols and caffeine can be separated and determined within 15 min with a direct injection of tea extract without any pretreatment.

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