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Short communication

Rapid determination of caffeine in tea leaves

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Abstract

For the purpose of efficient screening of low-caffeine tea shoots, a method for the rapid determination of caffeine was developed using high-performance liquid chromatography. Polyvinylpolypyrrolidone was packed in a pre-column and used to remove polyphenols from tea extracts on-line. The concentrations of caffeine extracted from powdered tea leaves at 50°C during 1 day could be analyzed in 2–5 min intervals. The pre-column and the analytical column could be used for the analysis of more than 2000 samples. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Tea leaves; Caffeine; Polyphenols

1. Introduction

As tea contains a lot of polyphenols, which are known to have high antioxidant activity, it is believed that drinking tea is advantageous to human health. However tea also contains caffeine, which stimulates the central nervous system and causes irritation of the gastrointestinal tract. It is desirable to remove caffeine from tea leaves. Until now no practical manufacturing methods to produce high-quality teas with low caffeine content have been developed.

Our purpose is to breed tea cultivars that contain no or very little caffeine in the leaves. For the screening of shoots with low caffeine content, we must determine caffeine in several thousand shoot samples. Several methods for the determination of caffeine in tea have already been reported [1], but these methods are too much time- and labor-consuming for our purpose.

Ikegaya et al. [2] developed a relatively rapid HPLC (high-performance liquid chromatography) method to determine caffeine; with this method, caffeine in tea extracts can be determined in 10 min. However, a laborious pretreatment to remove polyphenols from sample solutions is required before the HPLC analysis, because tea leaves contain polyphenols in much higher concentrations than caffeine. They used PVPP (polyvinylpolypyrrolidone) to remove polyphenols manually, as PVPP is water-insoluble and binds phenolic compounds effectively.

We have developed a HPLC method using a PVPP pre-column to remove polyphenols from tea extracts on-line [3]. As the PVPP pre-column perfectly removed polyphenols in tea extracts, pretreatment to remove polyphenols would not be needed when determining caffeine in tea extracts using this pre-column. In this report we improve the PVPP pre-

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column method to determine caffeine in tea extracts more rapidly.

2. Experimental

2.1. HPLC conditions

PVPP (Sigma) previously suspended in methanol was packed into a short column (10 mm×4.6 mm I.D., Nomura Chemical, Seto, Japan) and the column was placed before the analytical column (75 mm×3 mm I.D., 3 μm, ODS-UG-3, Nomura Chemical). An LC-10 system (Shimadzu, Kyoto, Japan) consisting of a pump, a column oven (40°C), a 20 μl injection loop, an autoinjector and a diode-array detector was used for this research. The flow-rate of the mobile phase (methanol–water–acetic acid, 40:59:1, v/v) was 0.6 ml/min. The wavelength of the detection was 272 nm and the peak heights of the chromatograms were used to calculate the concentrations of caffeine.

2.2. Sample preparations

Tea shoots were collected in the field and dried at 70°C and crushed in a porcelain mortar to make the samples uniform powder. The powder (100 mg) was weighed and extracted with 100 ml of water for 1 day at 50°C. The extract was passed through a membrane filter (cellulose acetate, DISMIC-13CP, Advantec, Tokyo, Japan) and the filtrate was used for the HPLC analysis.

3. Results and discussion

For the purpose of analyzing caffeine in tea leaves, samples are usually extracted with hot or boiling water [2]. We have previously shown that caffeine can be extracted from tea leaves even at room temperature, although that takes quite a long time [4]. In this experiment tea samples were extracted with water at 50°C during 1 day. Under these conditions caffeine could be quantitatively extracted from tea leaves and we could safely handle hundreds of samples at the same time using a conventional incubator.

The tea extracts contain a lot of polyphenols, which made the rapid determination of caffeine difficult. We tried the PVPP pre-column method for a more rapid determination of caffeine.

Downstream of the PVPP pre-column, a C_{18} analytical column was connected directly. When the length of the analytical column becomes longer, the pressure becomes higher. PVPP is a soft polymer and becomes tightly packed under high pressure (more than 10 MPa). When PVPP was packed too tightly, it became impossible to flow the mobile phase. We chose a shorter column (75 mm) in this report; the corresponding lower pressure (less than 60 MPa) prevents too tight packing of PVPP.

While the interference of polyphenols could be overcome by using the PVPP pre-column, tea leaves still contain other components (e.g., ascorbic acid) that may possibly interfere with the determination of caffeine. When we used the mobile phase methanol–water–acetic acid (74:25:1, v/v) developed by Ikegaya et al. [2], the peaks of the interfering substances overlapped with that of caffeine. The content of methanol had to be reduced, so the mobile phase used in this experiment was methanol–water–acetic acid (40:59:1, v/v).

The chromatograms of a tea sample are shown in Fig. 1. Without the pre-column, several peaks appeared and interfered with the determination of caffeine in the real sample (Fig. 1a). With the pre-column other peaks almost disappeared, and it became easy to determine caffeine (Fig. 1b). In this method the calibration curve showed a high correlation coefficient (r^2=0.999) between 0.1 and 100 mg/l when injecting 20-μl caffeine solutions. The linearity is satisfactory for our purposes.

The content of caffeine in tea leaves determined by this method were compared with the results by two other HPLC methods; the method of Ikegaya et al. [2] (method 1) and the isocratic separation of caffeine and other compounds using a C_{18} column (method 2) [5] (Table 1). In our method, the time for the analysis (5 min) was shorter than that of the other methods, and the reliability was not inferior to that of the other methods. Our method is much easier to use than method 1, and much more rapid than method 2.

More than 2000 tea samples could be analyzed in 5 min intervals without changing any of the columns,

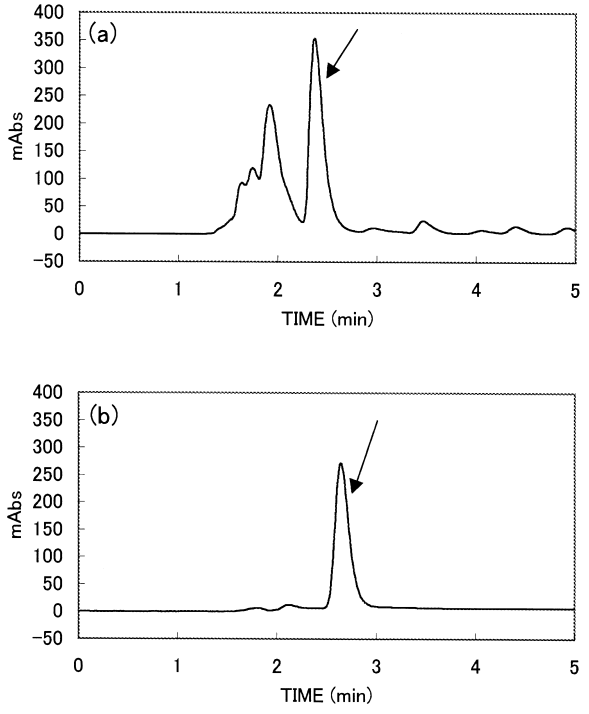

Fig. 1. HPLC–UV chromatograms of tea extracts with and without PVPP pre-column: (a) without pre-column, (b) with pre-column. ↓: peak of caffeine.

Table 1
Comparison of the caffeine content among the three analytical methods

Tea	Caffeine content (%)		
	Method 1 [1]	Method 2 [5]	New method
Sample 1	2.58 (0.6)[a]	2.63 (0.5)	2.63 (0.6)
Sample 2	2.23 (0.6)	2.29 (0.7)	2.32 (0.3)
Time (min)[b]	10	50	5

[a] Relative standard deviation ($n=3$) in parentheses.
[b] Time required for the HPLC analysis of one sample.

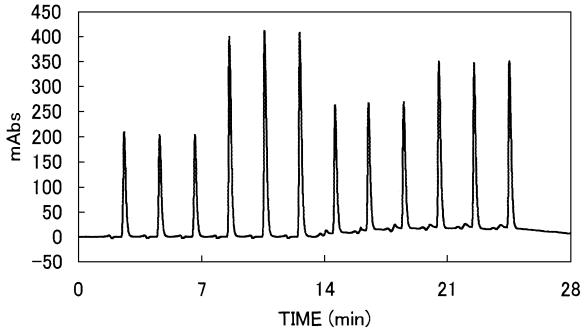

Fig. 2. Continuous determination of caffeine in tea extract using the same HPLC system of Fig. 1. Each sample was injected three times in the order; 20 mg/l of caffeine, 40 mg/l of caffeine, tea extract A and tea extract B.

when injecting 5 μl of sample from the autoinjector. The pre-column could be stored at room temperature for more than 6 months. The stability of the pre-column was sufficient for our purposes. We are now applying this method to the tea cultivars with less caffeine content.

Since no other peak appeared after that of caffeine in our system (Fig. 1b), it seemed possible to inject plural samples successively, like in flow injection analysis. Standards and tea samples were injected in 2-min intervals from the manual injector (20 μl loop) and the absorbance was recorded continuously for 30 min (Fig. 2). In this method the caffeine content in tea could be measured in 2-min intervals. We believe that this is the fastest method for determination of caffeine in real samples using conventional HPLC.

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