Improved Sample Pre-treatment for Determination of Caffeine in Tea Using a Cartridge Filled with Polyvinylpolypyrroridone (PVPP)

Yuji YAMAUCHI, Akiko NAKAMURA, Miki KITAI, Kirara HATANAKA, Iho KOHNO, and Tsuyoshi TANIMOTO*

Faculty of Pharmaceutical Sciences, Doshisha Women's University; Kodo, Kyotanabe, Kyoto 610–0395, Japan.
Received April 2, 2007; accepted July 1, 2007; published online July 3, 2007

We have improved sample pre-treatment for the effective removal of polyphenols and simple analysis of caffeine in tea using a cartridge filled with polyvinylpolypyrroridone (PVPP). Nearly 100% of catechins were removed from the green tea sample and caffeine was completely recovered in the range of 98.2—101.3% by sample pre-treatment with a PVPP cartridge. Reproducibility of preparing PVPP pre-treatment cartridges was sufficient for quantitative analysis, because RSDs of analytical values for caffeine obtained by using three individual pre-treatment cartridges filled with 10—200 mg PVPP were 0.60—2.8%. The PVPP pre-treatment cartridge also removed polyphenols perfectly and recovered caffeine faultlessly from oolong and black tea samples. Comparison with the conventional method without sample pre-treatment indicated that the present pre-treatment method with a PVPP cartridge was useful for the simple and precise analysis of caffeine in green, oolong and black tea samples.

Key words tea; caffeine; polyphenol; polyvinylpolypyrroridone; pre-treatment; HPLC

Caffeine is a methylated xanthine popularly consumed from a wide variety of foods and beverages such as tea and coffee; however, the intake of high amounts of caffeine has been shown to produce negative effects upon premenstrual syndrome[1,2] and pregnancy,[3,4] and to promote infertility[5] and cancer.[6—8] The consumption of even small amounts of caffeine must be often avoided for infants and young children because of its stimulating effect on the central nervous system and cardiac muscle. Tea contains caffeine as one of the major components at the level of about 20—50 mg/g in dry leaves.[9,10] The stimulating effect by the consumption of tea beverages can be attributed to this compound. In recent years, the production of tea beverages and products has been steadily increasing because of the desirable effect of tea polyphenols on human health and the worldwide revaluation of Japanese traditional foods, and the intake of caffeine from tea products is therefore also growing every year. Therefore, it is important to develop a more precise, simpler and faster analytical method to determine caffeine in tea samples for controlling the quality of tea products and human health.

Simultaneous determination of caffeine and polyphenolic compounds in tea has been accomplished by a number of methods,[11] such as high-performance liquid chromatography (HPLC)[12,13] and capillary electrophoresis (CE).[14,15] These methods are very attractive for the precise analysis of tea constituents, but time-consuming and unsuitable for a quick check or the rapid routine assessment of tea quality. On the other hand, only a few methods have been reported for the selective determination of caffeine in tea. The main interferences in achieving this purpose are tea polyphenols. The conventional method to remove these interferences from tea brews is liquid–liquid extraction, which is often injurious to human health and the social environment because of the use of poisonous reagents and solvents such as lead acetate and chloroform.[16] Although solid-phase extraction (SPE) seems a superior method to clean up tea samples, this method is costly, laborious, and time-consuming.[17,18]

Polyvinylpolypyrroridone (PVPP), which is an inexpensive and excellent absorbent of polyphenols,[19] is often used to eliminate tea catechins from tea brews for the determination of caffeine. When a tea sample was mixed with PVPP powder for more than 30 min (PVPP batch treatment), most of the tea polyphenols were removed, while a certain amount remained in the sample solution and interfered with the analysis of caffeine.[20] The batch treatment method with PVPP was also uneconomical, because a lot of PVPP powder was wasted during the treatment procedure. Recently, a pre-column filled with PVPP was reported for the on-line removal of tea polyphenols.[9,10] This method is very economical and fascinating for the simple and rapid HPLC analysis of caffeine in tea samples. Unfortunately, the PVPP pre-column was not applicable to other analytical methodology such as micro-HPLC, CE and spectrophotometric methods, and the reproducibility of preparing the column seemed poor. In this paper, we attempted to improve the sample pre-treatment method using a cartridge filled with PVPP for the effective removal of polyphenols and simple analysis of caffeine in tea. The advantages and limitations of the pre-treatment of a tea sample using the PVPP cartridge as compared with the conventional method for the determination of caffeine in tea are described and discussed.

Experimental

Reagents and Materials Caffeine was purchased from Nacalai Tesque (Japan). Theobromine, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin and (−)-epigallocatechin gallate were purchased from Wako Pure Chemicals (Japan). PVPP was purchased from Sigma. All other chemicals were of analytical grade and were used without further purification. Deionized and distilled water, and methanol (MeOH) and acetonitrile (MeCN) of HPLC grade were used throughout this study.

Ten kinds of green tea, 4 kinds of oolong tea and 5 kinds of black tea were purchased from markets in Kyoto and Nara Pref., and were stored at −20 °C. Prior to studies, all tea samples were milled using a Sun (Japan) FM-60K mill-mixer.

HPLC Conditions The HPLC system consisted of a GL-Sciences (Japan) PU-611C pump, a Tosoh (Japan) AS-8020 auto-injector, a GL-Sciences Model-554 column oven, a Shimadzu (Japan) SPD-10Avp UV–Vis spectrophotometer, and a Hitachi (Japan) D-2500 calculator. The guard and analytical columns were GL-Sciences Inertsil ODS-3 (3 μm, 1.5×10 mm)

* To whom correspondence should be addressed. e-mail: ttanimot@dwc.doshisha.ac.jp

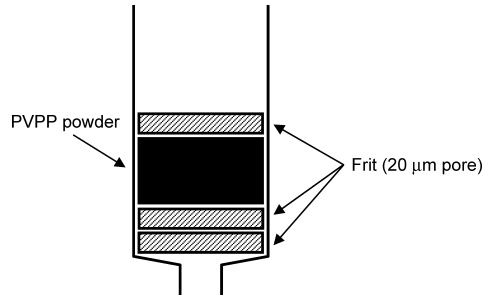

Fig. 1. Schematic Illustration of a PVPP Pre-treatment Cartridge

and Inertsil ODS-3 (3 μm, 2.1×150 mm), respectively. The temperature of the column oven was set at 30 °C. The mobile phase was prepared using MeOH/H_2O/acetic acid (15 : 84 : 1, v/v) and pumped at a flow rate of 0.2 ml/min. Detection wavelength and sample volume were set at 272 nm and 5 μl, respectively.

Preparation of PVPP Pre-treatment Cartridge The PVPP pre-treatment cartridge was prepared by filling a Varian empty reservoir cartridge (9×60 mm, 3 ml capacity) equipped with two frits (20 μm pore) with PVPP powder, and setting a frit on the PVPP powder (Fig. 1). The prepared PVPP pre-treatment cartridge was conditioned with 2 ml MeOH/H_2O/acetic acid (20 : 79 : 1, v/v) followed by 2 ml aqueous 1% (v/v) acetic acid solution using a GL-Sciences GL-SPE vacuum manifold (12 ports).

Sample Preparation and Pre-treatment In a 100 ml volumetric flask, the powdery tea sample (500 mg) was extracted with 80 ml MeCN/H_2O/acetic acid (50 : 49 : 1, v/v) in a Branson (U.S.A.) Bransonic 2510J-MT ultrasonic bath for 30 min.[9] A flask with the mixture was then filled up to 100 ml with the same solution. The resultant solution was filtered through a No. 5 filter paper and diluted ten times with an aqueous 1% (v/v) acetic acid solution. The prepared solution was used as a non-treated tea sample for HPLC analysis.

Two milliliters of a non-treated tea sample was passed through a PVPP pre-treatment cartridge conditioned by the above procedures using a GL-SPE vacuum manifold. The first 1 ml fraction was discarded, and the second 1 ml fraction was collected and used as the treated tea sample for HPLC analysis.

PVPP powder was added to a 50 ml non-treated tea sample. The suspension was allowed to stand for 30 min with frequent mixing and filtered through No. 5 filter paper. The resultant solution was used as a batch-treated tea sample for HPLC analysis.[9,20]

Results and Discussion

Performance of PVPP Pre-treatment Cartridge The efficiency of PVPP cartridge treatment for removing tea polyphenols was compared with that of PVPP batch treatment. As shown in Fig. 2a, the four major catechins and caffeine in the non-treated green tea sample could be well separated under the isocratic HPLC condition adopted in this study, and more than 60 min was required for one analysis of the non-treated green tea sample because of the long retention time of (−)-epicatechin gallate. In the case of PVPP batch treatment,[9,20] the peaks of green tea catechins decreased considerably, but still remained at the level of 0—43.9% on the basis of peak areas as against that in the non-treated green tea sample, as shown in Fig. 2b. Surprisingly, in the case of PVPP cartridge treatment, four green tea catechins and other tea components, which were maybe polyphenols, were removed completely, while almost all caffeine was recovered (Fig. 2c). In the case of the previously reported PVPP pre-column method, it was known that the peak height of caffeine was lowered because of peak broadening, although the tea polyphenols were removed completely.[9] It also seems that the PVPP pre-column is difficult to be applied to other analytical methods such as micro-HPLC, CE and spectrophotometry. Fortunately, the PVPP cartridge-treatment method is applicable to many other analytical methodologies. Although SPE seems a very useful method to clean up tea sample, several kinds of solvents are used to remove needless tea components and recover necessary one. In the case of the PVPP cartridge-treatment, tea brews are only passed through pre-treatment cartridges, therefore showing that PVPP cartridge treatment is a superior method to remove catechins from green tea samples than PVPP batch treatment, the PVPP pre-column method, and SPE method.

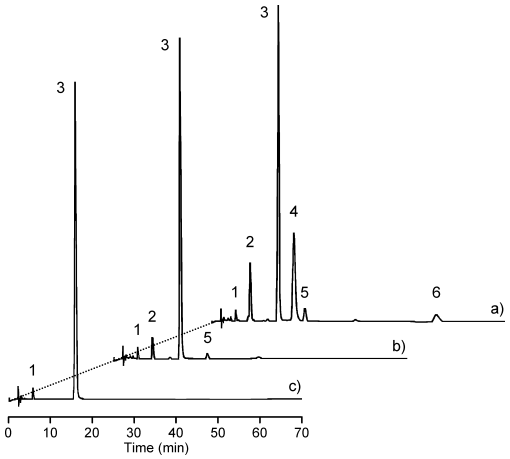

Fig. 2. Typical Chromatograms of (a) Non-treated, (b) PVPP (100 mg) Batch-Treated and (c) PVPP (100 mg) Cartridge-Treated Samples of Green Tea

Peaks: 1, theobromine; 2, (−)-epigallocatechin; 3, caffeine; 4, (−)-epigallocatechin gallate; 5, (−)-epicatechin; 6, (−)-epicatechin gallate.

Table 1. Effect of Amounts of PVPP Powder in Pre-treatment Cartridges on the Recovery of Caffeine and Tea Catechins[a)]

Amount of PVPP (mg)	Recovery (%)[b,c)]				
	Caffeine	EGC	EGCg	EC	ECg
PVPP cartridge treatment					
10	100.3 (0.7)	71.4 (9.0)	37.0 (6.5)	67.3 (4.7)	33.5 (5.5)
20	100.4 (0.6)	21.3 (3.1)	2.9 (1.2)	22.9 (3.0)	ND
30	101.3 (1.2)	ND	ND	ND	ND
50	99.3 (1.2)	ND	ND	ND	ND
100	98.2 (1.9)	ND	ND	ND	ND
150	98.5 (1.1)	ND	ND	ND	ND
200	101.2 (2.8)	ND	ND	ND	ND
PVPP batch treatment					
100	98.6 (0.1)	36.8 (0.6)	ND	43.9 (0.9)	ND
200	99.1 (0.8)	19.2 (0.2)	ND	25.2 (0.4)	ND

a) Tea catechins: EGC, (−)-epigallocatechin; EGCg, (−)-epigallocatechin gallate; EC, (−)-epicatechin; ECg, (−)-epicatechin gallate. *b*) Numbers in parentheses are S.D.s (n=3). *c*) ND: not detected at the sensitivity of S/N=5.

Optimum Condition of PVPP Pre-treatment Cartridge In order to optimize the capability of the pre-treatment cartridge filled with PVPP as an improved tool for effective removal of tea polyphenols and simple analysis of caffeine in tea, the effect of the amount of PVPP powder in the pre-treatment cartridges upon the recovery efficiency for caffeine and removal efficiency for four green tea catechins was examined. Table 1 summarizes the recovery yields of caffeine and tea catechins estimated as the ratios of peak areas on HPLC obtained for respective components in green tea samples

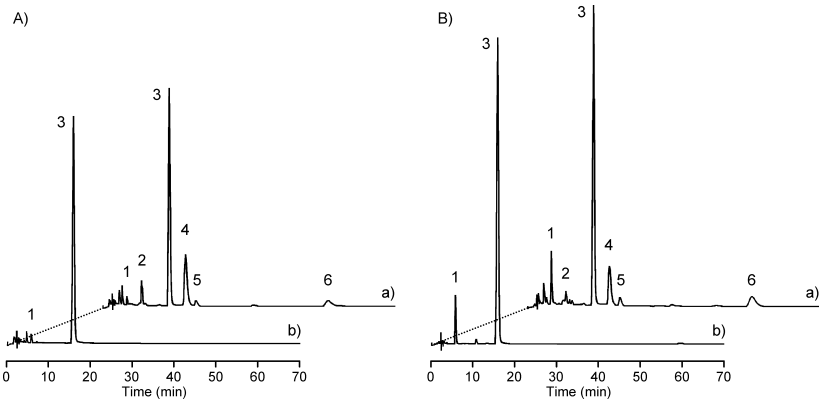

Fig. 3. Typical Chromatograms of (a) Non-treated and (b) PVPP (50 mg) Cartridge-Treated Samples of (A) Oolong and (B) Black Tea
Peaks are the same as in Fig. 2.

treated with PVPP against those obtained from the non-treated samples. In this study, a kind of green tea was used, which contained 27.6 mg/g caffeine, 26.9 mg/g (−)-epigallocatechin, 113.8 mg/g (−)-epigallocatechin gallate, 9.0 mg/g (−)-epicatechin, and 39.6 mg/g (−)-epicatechin gallate.

Green tea catechins were mostly removed by even the cartridge with 20 mg PVPP and totally by that with more than 30 mg PVPP. On the other hand, caffeine in green tea was completely recovered in the range 98.2—101.3% by using all cartridges filled with 10—200 mg PVPP. Compared with PVPP batch treatment, which reduced the contents of tea catechins and recovered caffeine at the level of 98.6—99.1%, PVPP cartridge treatment could have superior capability of removing green tea polyphenols without reducing the caffeine content. Additionally, the reproducibility of preparing the PVPP pre-treatment cartridges seemed satisfactory for the precise analysis of caffeine in green tea, because the RSDs of analytical values for caffeine obtained by using three individual pre-treatment cartridges filled with 10—200 mg PVPP were 0.60—2.8%. While the efficiency of recovering caffeine and removing green tea polyphenols was essentially unchanged using cartridges with 30—200 mg PVPP, 50 mg PVPP was employed as the filling material for the pre-treatment cartridge for further examination from an economical and practical point of view.

In order to investigate the capacity of a pre-treatment cartridge, non-treated tea samples made from 0.1, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, and 3.0 g green tea were treated with cartridges filled with 50 mg PVPP, and the recovery yields of caffeine and four green tea catechins were examined. Non-treated tea sample was not obtained from >3.0 g green tea because of difficulty of experimental operation. Surprisingly, caffeine in 0.1—3.0 g green tea was completely recovered in the range 99.1—102.3%. On the other hand, four catechins were not detected or recovered at the level of $<0.1\%$ in every case. Therefore, the cartridge filled with 50 mg PVPP can be used for sample pre-treatment of tea extracts from 0.1—3.0 g tea leaf.

PVPP Cartridge-Treatment of Oolong and Black Tea Samples Oolong and black tea are produced from the same tea leaves as green tea (*Camellia sinensis* L.); however, both teas contain different kinds and contents of components from green tea, since their production processes differ considerably from that of green tea.[16] In the case of HPLC analysis of non-treated oolong and black tea samples, although polyphenols and caffeine seemed to be well separated within 65 min, the drift of the baseline during successive analysis under the isocratic condition was caused by polyphenolic compounds such as theaflavines in black tea, which had much longer retention times.[16] It was observed that these polyphenols were completely removed by PVPP cartridge treatment (data not shown), and caffeine in PVPP cartridge-treated samples of oolong and black tea could be recovered faultlessly and well-separated chromatographically (Fig. 3). These results clearly indicate that PVPP cartridge treatment is a useful and simple method of removing polyphenols and recovering caffeine not only in green tea but also in oolong and black tea.

As shown in Figs. 2 and 3, caffeine could be analyzed in less than 20 min using PVPP cartridge treatment of tea samples without drift of the baseline during successive analysis under the isocratic HPLC condition. Moreover, PVPP cartridge treatment was simpler and more practical to carry out than the SPE method with C_{18} material,[17,18] and was not time-consuming. Therefore, the analytical time could be effectively shortened and the precision in quantitative analysis could be raised using PVPP cartridge treatment.

Analysis of Caffeine in Green, Oolong and Black Tea Ten kinds of green tea, 4 kinds of oolong tea and 5 kinds of black tea were subjected to the quantitative analysis of caffeine according to both methods with PVPP cartridge treatment and non-treatment. As shown in Fig. 4, a good linear relationship was observed between the results obtained in these methods; the slope, intercept and r were 1.001, -0.197 mg/g and 0.998, respectively. This result indicated that sample pre-treatment with a PVPP cartridge was practical and precise in determining the caffeine in tea samples, and that all three types of tea samples could be treated properly for the simple and selective analysis of caffeine.

Conclusion

The pre-treatment method of tea samples was improved using a cartridge filled with PVPP. When the green tea sample was pre-treated using the PVPP cartridge, tea catechins were totally removed and caffeine was recovered completely from the sample. The reproducibility of preparing PVPP pre-treatment cartridges was satisfactory for the precise analysis of caffeine in green tea on the basis of the RSDs of analytical

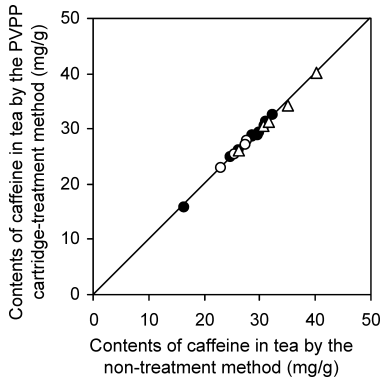

Fig. 4. Correlation of the Quantitative Values of Caffeine in 10 Green (Closed Circle), 4 Oolong (Open Circle) and 5 Black Tea Samples (Open Triangle) Determined by the PVPP (50 mg) Cartridge Treatment Method and the Non-treatment Method

values for caffeine. Sample treatment with a PVPP cartridge was applicable to the pre-treatment of oolong and black tea samples. The faultless recovery of caffeine and effective removal of polyphenols in oolong and black tea were also accomplished by this method. Between the quantitative values obtained from three types of tea samples treated with a PVPP cartridge and those from non-treated samples, a good linear relationship was observed. This result indicated that PVPP cartridge treatment was useful and precise in analyzing all types of tea samples. Moreover, this improved pre-treatment with a PVPP cartridge was not only simple and practical to carry out, but also economical and not time-consuming. To render the present analytical method a more practical tool from the point of view of the routine analysis of many samples, the analytical time for one sample must be significantly shortened. In addition, this method may be applicable to many other analytical methods for the determination of caffeine in various food samples. Further studies along these lines are currently underway in our laboratory.

Acknowledgement This work was supported in part by a Grant-in-Aid from Doshisha Women's University (No. 2007-11).

References

1) Mackay Rossignol A., Bonnlander H., *Am. J. Public Health*, **80**, 1106—1110 (1990).
2) Mackay Rossignol A., Zhang J., Chen Y., Xiang Z., *Am. J. Public Health*, **79**, 67—69 (1989).
3) Fenster L., Eskenazi B., Windham G. C., Swan S. H., *Am. J. Public Health*, **81**, 458—461 (1991).
4) Caan B. J., Golhaber M. K., *Am. J. Public Health*, **79**, 1299—1300 (1989).
5) Wilcox A., Weinberg C., Baird D., *Lancet*, **24**, 1453—1456 (1988).
6) Welsch C. W., DeHoog J. V., O'Connor D. H., *Cancer Res.*, **48**, 2068—2073 (1988).
7) Welsch C. W., DeHoog J. V., O'Connor D. H., *Cancer Res.*, **48**, 2078—2082 (1988).
8) Phelps H. M., Phelps C. E., *Cancer*, **61**, 1051—1054 (1988).
9) Nakakuki H., Horie H., Yamauchi Y., Kohata K., *J. Chromatogr. A*, **848**, 523—527 (1999).
10) Horie H., Nesumi A., Ujihara T., Kohata K., *J. Chromatogr. A*, **942**, 271—273 (2002).
11) Horie H., Kohata K., *J. Chromatogr. A*, **881**, 425—438 (2000).
12) Yang X. R., Ye C. X., Xu J. K., Jiang Y. M., *Food Chem.*, **100**, 1132—1136 (2007).
13) Goto T., Yoshida Y., Masaaki K., Nagashima H., *J. Chromatogr. A*, **749**, 295—299 (1996).
14) Stach D., Schmitz O. J., *J. Chromatogr. A*, **924**, 519—522 (2001).
15) Worth C. C. T., Wiesler M., Schmitz O. J., *Electrophoresis*, **21**, 3634—3638 (2000).
16) Finger A., Kuhr S., Engelhardt U. H., *J. Chromatogr.*, **624**, 293—315 (1992).
17) Naik J. P., Nagalakshmi S., *J. Agric. Food Chem.*, **45**, 3973—3975 (1997).
18) Terada H., Sakabe Y., *J. Chromatogr.*, **291**, 453—459 (1984).
19) Doner L. W., Becard G., Irwin P. L., *J. Agric. Food Chem.*, **41**, 753—757 (1993).
20) Ikegaya K., Takayanagi H., Anan T., *Tea Res. J.*, **71**, 43—47 (1990).