Simple and Rapid UV Spectrophotometry of Caffeine in Tea Coupled with Sample Pre-treatment Using a Cartridge Column Filled with Polyvinylpolypyrrolidone (PVPP)

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We have applied a sample pre-treatment method with a cartridge column filled with polyvinylpolypyrrolidone (PVPP) to the effective removal of polyphenols and simple UV spectrophotometry of caffeine in tea. The absorption maximum length (λ_{max}) for caffeine was close to those for tea catechins in aqueous 1% acetic acid; therefore, the UV spectrum of a non-treated green tea sample had a large absorption wave. In contrast, the absorbance of the green tea sample was gradually reduced by PVPP cartridge treatment using PVPP from 0 to 50 mg, and was nearly constant using a pre-treatment cartridge with more than 100 mg PVPP, because tea catechins were effectively removed and caffeine was mostly recovered from a green tea sample by means of PVPP cartridge treatment. The PVPP pre-treatment cartridge also removed polyphenols successfully from oolong and black tea samples. Comparison with conventional HPLC analysis indicated that the present pre-treatment method with a PVPP cartridge was useful for the simple and selective UV spectrophotometric determination of caffeine in green, oolong and black tea samples.

Key words tea; caffeine; polyphenol; polyvinylpolypyrrolidone; spectrophotometry; UV

Tea is one of the most popular beverages, with coffee, consumed by humans. The three types of tea are green, oolong and black tea. Black tea is produced in many countries such as India, Sri Lanka, Turkey, Kenya and Russia, and is consumed throughout the world including Europe and North America. Oolong tea is made and consumed only in Asian areas around China. Green tea is mainly produced and consumed in Japan and China, however, in recent years, both the consumption and the consumption areas of green tea beverages and articles have been steadily increasing because of recognition of the desirable effect of green tea polyphenols on human health and the worldwide revaluation of Japanese traditional foods.

Tea contains caffeine as one of the major components at the level of about 20—50 mg/g in dry leaves.[1,2] The pharmacological effects of caffeine include stimulation of the central nervous system[3] and the cardiac muscle,[4] and therefore the consumption of caffeine must be avoided by infants and young children because of these undesirable effects. Intake of a high amount of caffeine has also been shown to produce negative effects upon premenstrual syndrome[5,6] and pregnancy[7,8] and to promote infertility[9] and cancer.[10—12] Therefore, it is important to control and monitor the intake of tea containing caffeine by young children and the above patients, because the intake of caffeine from tea products by humans is growing every year. It is essential to develop a more precise, simpler and faster analytical method to determine caffeine in tea to control and maintain the quality of tea and human health.

Simultaneous determination of caffeine and polyphenolic compounds in tea has been accomplished by a number of methods[13] such as high-performance liquid chromatography (HPLC)[14,15] and capillary electrophoresis (CE).[16,17] 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 environment because of the use of poisonous reagents and solvents such as lead acetate and chloroform.[18] Although solid-phase extraction (SPE) seems a superior method to clean up tea samples, this method is costly, laborious, and time-consuming.[19,20] The selective determination of caffeine in tea using UV spectrophotometry[21] and Fourier transform infrared spectroscopy[22] without pre-treatment of a tea sample was also reported, but it seems that these methods are difficult to implement widely, because specific and complex methods are necessary to process the obtained data to determine the objective analytes.

Polyvinylpolypyrrolidone (PVPP), an inexpensive and excellent absorbent of polyphenols,[23] is often used to eliminate polyphenols from extract of plants such as tea. Recently, a pre-column filled with PVPP was reported as a useful tool for the on-line removal of polyphenols from tea samples.[1,2] This method is very economical for simple and rapid HPLC analysis of caffeine in tea samples. Unfortunately, the PVPP pre-column was not applicable to other analytical methodologies such as micro-HPLC, CE and spectrophotometric methods, and the reproducibility of preparing its column seemed poor. In our previous report,[24] we improved the sample pre-treatment method using a cartridge column filled with PVPP for the effective removal of tea polyphenols and the simple HPLC analysis of caffeine in tea. The reproducibility of sample pre-treatment using a PVPP cartridge column was satisfactory for the determination of tea caffeine by the HPLC method. The sample treatment procedure using a PVPP pre-treatment cartridge seemed applicable to a variety of analytical methods. In this paper, we attempted to apply the sample pre-treatment method with a cartridge filled with PVPP to the simple and selective UV spectrophotometric determination of caffeine in tea. The advantages and limitations of UV spectrophotometry of caffeine coupled with the pre-treatment

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method using a PVPP cartridge as compared with the conventional HPLC determination of caffeine in tea are described and discussed.

Experimental

Reagents and Chemicals Caffeine was purchased from Nacalai Tesque (Japan). (−)-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.

Apparatus A Shimadzu (Japan) UV-3600 spectrophotometer system equipped with a Shimadzu UVProve Version 2.20 software for acquisition, storage and manipulation of spectrophotometric data was used. Tosoh (Japan) T-9M-UV-10 quartz glass cells (10 mm optical path length, 4 mm optical path width, 1.4 ml capacity) were used.

The HPLC system consisted of a GL-Sciences (Japan) PU-611C pump, a Tosoh AS-8020 auto-injector, a GL-Sciences Model-554 column oven, a Shimadzu 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) 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.[24]

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. 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).[24]

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 ultra-sonic bath for 30 min. 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 fifty times with an aqueous 1% (v/v) acetic acid solution. The prepared solution was used as a non-treated tea sample for UV spectrophotometry and HPLC analysis.

Three 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 2 ml fraction was collected and used as the treated tea sample for UV spectrophotometry and HPLC analysis.

One hundred milligrams of the PVPP powder were added to 50 ml non-treated tea sample. The suspension was allowed to stand for 30 min with frequent mixing and filtered through a No. 5 filter paper. The resultant solution was used as the batch-treated tea sample[1,23] for UV spectrophotometry and HPLC analysis.

Recovery Test A powdered tea sample was extracted according to the above preparation procedure. One milliliter of a filtered extract and the appropriate amount of a known standard solution of caffeine were mixed and filled up to 50 ml with an aqueous 1% (v/v) acetic acid solution. The resultant solution was used as a non-treated tea sample with added standard, and was pre-treated by a PVPP cartridge column according to the above-mentioned procedure.

Results and Discussion

Spectrophotometric Characteristics of Caffeine and Tea Catechins The aqueous 1% (v/v) acetic acid solution containing 20 μM caffeine was examined by UV spectrophotometry. An absorption peak for caffeine at the absorption maximum length (λ_{max}) of 273.0 nm was observed as shown in the literature.[21,25] The maximum molar absorptivity (ε_{max}) for caffeine at 273.0 nm was 9.81×10^3 M^{-1} cm^{-1} under this condition, and a good linear relationship was observed between the absorbance at 273.0 nm and the concentration of caffeine from 1 to 100 μM; the slope and r were 9.70×10^3 M^{-1} and 0.999, respectively. UV absorption spectra of 20 μM (−)-epigallocatechin gallate (EGCg), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg) and (−)-epicatechin (EC) in aqueous 1% acetic acid were also measured, and the λ_{max} and ε_{max} for those tea catechins are shown in Table 1 along with reference values.[26] The absorption spectra of tea catechins were so close to that of caffeine that the presence of tea catechins might interfere with the precise determination of caffeine in tea by UV spectrophotometry. These results indicated that the methodology to effectively remove catechins from tea samples was indispensable for the selective and rapid determination of caffeine in tea with UV spectrophotometry.

Table 1. Absorption Maximum Length (λ_{max}) and Maximum Molar Absorptivity (ε_{max}) of Caffeine and Green Tea Catechins[a] in Aqueous 1% (v/v) Acetic Acid

Tea component	Our values		Reference values[b]	
	λ_{max} (nm)	10^{-3} ε_{max} (M^{-1} cm^{-1})	λ_{max} (nm)	10^{-3} ε_{max} (M^{-1} cm^{-1})
Caffeine	273.0	9.81	272.7	9.85
EGCg	273.5	11.2	275	11.5
EGC	269.9	1.51	271	1.45
ECg	276.3	13.9	279	14.0
EC	278.4	4.04	280	3.58

a) Tea catechins: EGCg, (−)-epigallocatechin gallate; EGC, (−)-epigallocatechin; ECg, (−)-epicatechin gallate; EC, (−)-epicatechin. *b*) Caffeine: in water in ref. 25; catechins: in ethanol in ref. 26.

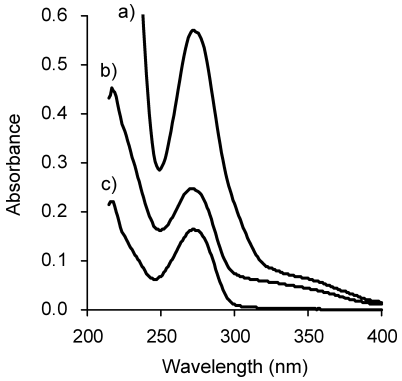

Fig. 1. Typical UV Spectra of (a) Non-treated, (b) PVPP (100 mg) Batch-Treated and (c) PVPP (100 mg) Cartridge-Treated Samples of Green Tea

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. 1a, the UV spectrum of a non-treated green tea sample had a large absorption wave with main and shoulder peaks at absorption lengths of 273 and *ca.* 350 nm, respectively, and the maximum peak absorbance of the main peak was 0.571. The main peak was mainly caused by caffeine and catechins which coexisted in a non-treated green tea sample. Although the origin of the shoulder peak is not yet identified, we guess it to be caused by flavonoids and their glycosides such as kaempferol and quercetin, which have λ_{max} at 263.8 and 363.6 nm, and 253.8 and 367.0 nm in the aqueous 1% (v/v) acetic acid solution, respectively. The

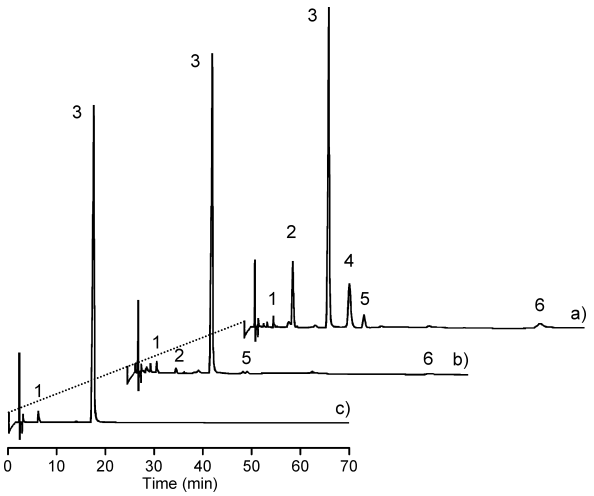

Fig. 2. Typical HPLC Chromatograms of (a) Non-treated, (b) PVPP (100 mg) Batch-Treated and (c) PVPP (100 mg) Cartridge-Treated Samples of Green Tea

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

glycosides of kaempferol and quercetin were known to exist in brew of green tea. On the other hand, the peak absorbance of the UV spectrum of the green tea sample treated with a pre-treatment cartridge with 100 mg PVPP had dramatically decreased to 0.164 at 273 nm. Moreover, the shoulder peak at *ca.* 350 nm had almost completely disappeared from the UV spectrum of a green tea sample (Fig. 1c). In the case of PVPP batch-treatment, the peak absorbance of 0.246 at 273 nm was a little larger than that for the PVPP cartridge-treated sample, and the shoulder peak remained (Fig. 1b).

Figure 2 shows HPLC chromatograms[24] of non-treated, PVPP batch-treated and PVPP cartridge-treated green tea samples, which were the same samples used for UV spectrophotometry. In the case of non-treatment, caffeine and four major catechins in green tea were detected, the peaks of which were sharp and well separated. The peaks of four catechins in the PVPP batch-treated green tea sample decreased considerably, but they remained to some extent. With PVPP cartridge treatment, green tea catechins were removed completely, while almost all of the caffeine was recovered clearly; the recovery yield was 99.8%. These results indicated that the large absorbance of the UV spectrum of the non-treated green tea sample was due to the presence of tea polyphenols, and the dramatic decrease of the absorbance at 273 nm of the green tea sample pre-treated with a PVPP cartridge was owing to the effective removal of tea polyphenols from an extract of green tea. However, tea components with λ_{max} at *ca.* 350 nm, which were perhaps thought to be flavonoid glycosides, could not be yet identified and determined by our HPLC analysis, so the recovery or removal efficiency of them could not be examined using the HPLC method.

Optimum Condition of PVPP Pre-treatment Cartridge In our previous report, the optimum conditions for pre-treatment of tea sample for the HPLC analysis were determined, but they were not suitable for the UV spectrophotometry. In the case of the UV measurement, unknown tea components with λ_{max} at *ca.* 350 nm interfered with analysis of tea caffeine. In order to optimize the capability of a pre-treatment cartridge filled with PVPP as a tool for effective removal of tea polyphenols and simple UV spectrophotometry of caffeine in tea, the effect of the amount of PVPP powder in pre-treatment cartridges on the UV absorbance of a tea sample was examined (Fig. 3). The absorbance of a green tea sample at 273 nm was gradually reduced by PVPP cartridge treatment at amounts of PVPP from 0 to 50 mg. When pre-treatment cartridges with more than 100 mg PVPP powder were used, the absorbance at 273 nm was nearly constant. In the case of oolong and black tea samples, the same phenomena were observed. On the basis of HPLC analysis, recovery yields of caffeine in oolong and black tea samples treated with a pre-treatment cartridge with 100 mg PVPP were 100.3% and 100.2%, respectively. These results showed that a pre-treatment cartridge with more than 100 mg PVPP was efficient for removing tea components such as catechins, except caffeine, from not only green tea but also oolong and black tea samples. Consequently, PVPP cartridge treatment seemed a simple and precise analytical tool for the spectrophotometric determination of caffeine in tea samples. For further examination, 100 mg PVPP was employed as the filling material for a pre-treatment cartridge.

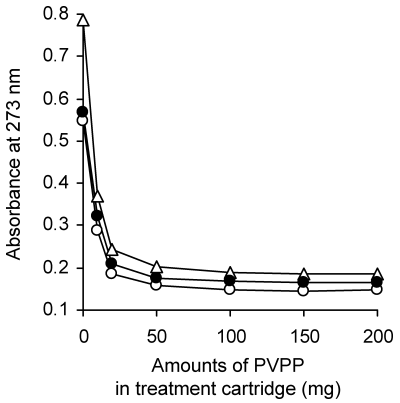

Fig. 3. Effect of Amounts of PVPP Powder in Pre-treatment Cartridges on the UV Absorbance of PVPP Cartridge-Treated Samples of Green Tea (Closed Circle), Oolong Tea (Open Circle) and Black Tea (Open Triangle) at 273 nm

Analysis of Caffeine in Green, Oolong and Black Tea Ten kinds of green tea, four kinds of oolong tea and five kinds of black tea were subjected to quantitative analysis of caffeine according to the spectrophotometric method both with and without PVPP cartridge treatment, and quantitative values were compared with those determined by the conventional HPLC method. As shown in Fig. 4, with the UV method without PVPP cartridge treatment, analytical values of caffeine were about four times as large as those by the HPLC method and the correlation between them was poor; the slope and *r* of the correlative line were 4.068 and 0.763, respectively. On the other hand, analytical values obtained by the UV method with PVPP cartridge treatment well corresponded to those by the HPLC method; the slope and *r* were 1.134 and 0.935, respectively. These results indicated that sample pre-treatment with a PVPP cartridge was helpful in determining caffeine in tea samples by UV spectrophotometry, and that all three types of tea samples could be treated properly for simple and selective UV spectrophotometric analysis of caffeine. Additionally, the reproducibility (within-run precision) of UV analysis of tea caffeine using a PVPP

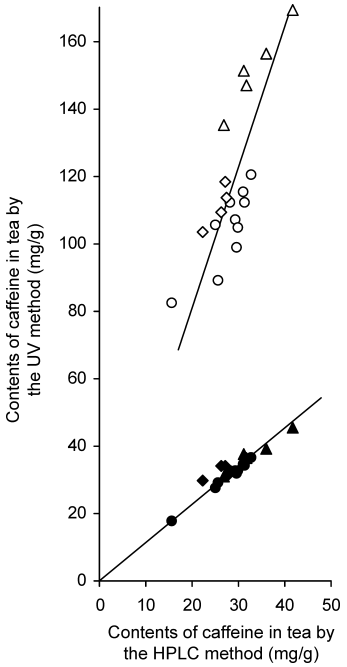

Fig. 4. Correlation of Quantitative Values of Caffeine in 10 Green (Circle), 4 Oolong (Diamond) and 5 Black (Triangle) Tea Samples Determined by the Conventional HPLC Method and Those by the UV Method with (Closed Symbol) and without (Open Symbol) PVPP Cartridge Treatment

pre-treatment cartridge seemed satisfactory, because the RSD of analytical values for caffeine in green tea obtained by using seven individual pre-treatment cartridges filled with 100 mg PVPP was 0.96%. Between-run precision was also evaluated from consecutive measurement for 6 d. The analytical values of tea caffeine obtained on each day deviated by 1.8% ($n=6$). The recovery of caffeine from green tea, including the 31.1 mg/g caffeine, spiked with 10 and 20 mg/g standard caffeine was 101.0 and 97.5%, respectively.

Conclusions

A simple and rapid method of sample pre-treatment for UV spectrophotometric determination of caffeine in tea was developed using a cartridge filled with PVPP. When a non-treated green tea sample was subjected to UV spectrophotometric analysis, a large absorption wave was observed because λ_{max} for caffeine was close to those for tea catechins. In contrast, the spectrophotometric absorbance of a PVPP cartridge-treated green tea sample was adequately reduced and was nearly constant using a pre-treatment cartridge with more than 100 mg PVPP because of the effective removal of tea polyphenols and the complete recovery of tea caffeine from the sample. Sample treatment with a PVPP cartridge for the UV spectrophotometric determination of caffeine was also applicable to the pre-treatment of oolong and black tea samples. Quantitative values of caffeine in three types of tea samples obtained by the UV spectrophotometric method were compared with those by the conventional HPLC method. In the case of UV spectrophotometry without PVPP cartridge treatment, analytical values of caffeine in tea did not correspond to those obtained by the HPLC method. On the other hand, the employment of PVPP cartridge treatment for tea samples substantially improved the correlation between quantitative values of tea caffeine by UV spectrophotometry and those by HPLC. These results indicated that sample pre-treatment with a PVPP cartridge was useful for the simple and selective determination of caffeine in all types of tea by UV spectrophotometry. We have previously reported that the pre-treatment of tea brew using a PVPP cartridge column made the HPLC analysis of tea caffeine very simple and precise.[24] However, the HPLC method does not spread in field analysis by tea cultivators and the medium and small manufacturers of tea leaf products, because it is expensive and needs skilled hands. On the other hand, UV spectrophotometry is a cheaper method than HPLC, and does not need special skill. Therefore, the development of simple and rapid UV spectrophotometry coupled with the sample pre-treatment using a PVPP cartridge column in this study offers the technique for analysis of tea caffeine to field workers and small manufacturers. Further detailed studies for the determintation of caffeine in commercially available tea beverages bottled in cans and PET-bottles using this UV method are currently underway in our laboratory.

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