

Short communication

PLE in the analysis of plant compounds

Part I. The application of PLE for HPLC analysis of caffeine in green tea leaves

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Abstract

A broad spectrum of sample preparation methods is currently used for the isolation of pharmacologically active compounds from plant and herbal materials. The paper compares the effectiveness of infusion, microwave assisted solvent extraction (MASE), matrix solid-phase dispersion (MSPD) and pressurised liquid extraction (PLE) as sample preparation methods for the isolation of caffeine from green tea leaves. The effect of PLE variables, such as extraction temperature, pressure and time, on the yield of caffeine from the investigated matrix is discussed.

The obtained results revealed that PLE, in comparison with other sample preparation methods applied, has significantly lower efficacy for caffeine isolation from green tea leaves. The evaluation of PLE conditions leads to the conclusion that elevated pressure applied in the PLE process is the factor hindering the extraction.

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1. Introduction

Due to the character and complexity of plant material the analytical procedure of plant constituents examination involves the application of a sample preparation procedure, which allows to fully isolate the analysed substance from the plant matrix. A broad range of extraction techniques (Soxhlet extraction, percolation, maceration, digestion, extraction under reflux, steam distillation, etc.) are currently used for this purpose [1–4], most frequently exhaustive extraction in the Soxhlet apparatus. Although this is a relatively simple method, it suffers from such disadvantages as long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility [5]. Recently, an innovative sample preparation technique, pressurised liquid extraction (PLE), has been more and more often applied [6–10]. The grow-

ing popularity of PLE results mainly from the possibility of eliminating the drawbacks mentioned above. Moreover, PLE allows for using extrahents at elevated pressure and hence also at temperatures above their normal boiling point.

PLE was originally developed for sample preparation in environmental analysis [6,11]. More recently, PLE application has been extended onto other sample types: biological, pharmaceutical and foodstuffs [12,13]. An important and interesting employment of PLE is the extraction of chemical constituents from plant and herbal materials [8,9,14–16], where PLE appears to be the most effective sample preparation method.

Tea is probably one of the most popular beverages in the world due to its sensory properties, stimulating effects and potential health benefits [17–19]. To understand the mechanisms behind these effects, a great deal of scientific effort has been made to isolate and identify active components in tea [20]. One of them is caffeine, which has attracted much scientific and public attention during the past years. Many

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analytical procedures have been developed to determine caffeine in tea leaves [21–25]. In most of them, the applied sample preparation stage is very simple and relies on the infusion of tea in water at higher temperatures. The infusions applied in the reported research differ mainly in their duration (from 10 min to a few days) and temperature (60–100 °C) [21–24]. According to the opinion presented in [21], triple infusion of the same sample at 70 °C, 40 min each time, leads to full recovery of caffeine from tea. Because in many cases, PLE is recognised as the most effective sample preparation method, the question appears if also works for caffeine analysis in tea. This paper discusses caffeine yields from green tea leaves obtained using the following sample preparation methods: PLE, infusion, ultrasonic assisted infusion, microwave assisted solvent extraction (MASE) and matrix solid-phase dispersion (MSPD).

2. Materials and methods

2.1. Materials and reagents

Yunnan green tea and coffee (Caffe dé Collumbia) were purchased locally. A sufficiently large representative sample of green tea leaves (ca. 500 g) was ground with a Braun cutting mill to obtain particles of 0.2–0.4 mm. Caffe dé Collumbia, which is ground coffee, was extracted without further manipulation. Exactly weighted portions of the samples were used for the tested extraction procedures and MSPD.

Methanol (HPLC grade) and ortophosphoric acid (analytical-reagent grade) were obtained from the Polish Factory of Chemicals POCh (Gliwice, Poland). E. Merck (Darmstadt, Germany) provided potassium dihydrogen phosphate and caffeine standard (1,3,7-trimethylxanthine). Water, purified on a Milli-Q system from Millipore (Millipore, Bedford, MA, USA), was used throughout the experiments. Neutral glass (fraction 0.4–0.6 mm) was applied as a dispersing agent in the PLE extraction cell.

2.2. Sample preparation methods

For statistical purposes each sample preparation procedure was repeated three times in given experimental conditions.

2.3. Infusion

The samples of powdered green tea leaves (0.1 g) were extracted with 10 ml of water at 70 °C for 40 min. Each sample was extracted three times with fresh portions of water, and the combined extract was transferred to a 50 ml volumetric flask and filled up to its volume with water.

During ultrasonic assisted infusion, a sample of green tea (0.1 g) was extracted in the same way as above. In this case, the vial with extract was occasionally placed in ultrasound bath (4 × 2 min) (Sonic 2 type, Polsonic, Warsaw, Poland). The extracts were pooled together into a 50 ml

volumetric flask and filled up to its volume with water. The obtained extracts were subjected to the HPLC analysis.

2.4. Matrix solid-phase dispersion

Tea portions of 0.2 and 0.8 g of either C18 sorbent (Supelclean LC-18, Supelco Park, Bellefonte, PA) or 0.8 g of quartz sand were weighed precisely and mixed in a glass mortar. After the addition of water (1.5 ml), the whole mixture was ground with a glass pestle until a homogeneous pulp was obtained (approximately 10 min). The sides of the mortar and the pestle were scraped occasionally with a spatula to ensure the best possible homogenisation. After homogenisation the blend was transferred with a spatula to a syringe barrel with a filter disc at the bottom. The sample was covered with another filter disc and compressed using a plunger. In the case of C18 sorbent the mortar, the pestle and the spatula were rinsed with methanol–water mixture (80–20%, v/v) whereas in the case of sand with water, the rinsed solution was transferred into the syringe barrel. Portions of the same methanolic mixture or water were then added to the column and the sample was allowed to elute dropwise by applying a slight vacuum. Samples (25 ml) were collected. Separate experiments proved that:

- caffeine concentration in the 20th ml of the eluate is very low and has no influence on the final result;
- caffeine recoveries from C18 phase obtained using 80% aqueous methanol as an eluent solution are greater than 98%;
- caffeine recoveries from quartz sand using water as an eluent are greater than 99%.

The obtained samples were subjected to the HPLC analysis.

2.5. Pressurised liquid extraction

PLE was performed with a Dionex ASE200 instrument (Dionex Corp., Sunnyvale, CA, USA). The green tea samples (0.5 g) were mixed with inert material (neutral glass) and placed into a 22-ml stainless steel extraction cell. The employment of a dispersion agent, such as neutral glass, is recommended in order to reduce the volume of the solvent used for extraction [26]. The cell content was extracted with water in two modes: one-cycle PLE and multiple PLE of the same sample. The conditions of the tea extraction procedure (temperature, time and pressure) are given in Section 3. In the case of ground coffee beans, the PLE conditions recommended as default by Dionex Corp. were applied, i.e. temperature 100 °C, pressure 60 bar and 10 min static extraction time.

The volume of the collected extracts was between 25 and 31 ml, depending on the packing density of the extraction cells. The system was washed with water between the runs. The obtained extracts were transferred to

100 ml volumetric flasks and filled up to its volume with water.

2.6. Microwave assisted solvent extraction

MASE was performed with Plasmotronika UniClever BMZ bath. Portions of ground green tea leaves (1 g) were mixed with water. The obtained suspensions were irradiated with microwaves in open and closed system in 40% generator power during 30 min. The obtained extracts were transferred to 100 ml volumetric flasks subsequently filled up to their volume with water.

2.7. HPLC analysis

HPLC measurements were performed on a Dionex liquid chromatograph (Dionex Corp., Sunnyvale, CA, USA) consisting of a chromatography enclosure (LC20) with a PEEK automated injection valve equipped with a 25 μ l sample loop; a gradient pump (GP50); an absorbance detector (AD25) and a photodiode array detector (PDA100). The whole chromatographic system was under the control of the PeakNet6 data acquisition system. Chromatographic separations were carried out using a Prodigy ODS-2 column (5 μ m, 250 mm \times 4.6 mm i.d.) (Phenomenex, Torrance, CA, USA) and a security guard column of the same material placed in the oven at 30 °C (Column Thermostat, JetStream II Plus, Knauer, Warsaw, Poland). The mixture of potassium dihydrogen phosphate–orthophosphoric acid buffer (pH 3.0) with methanol (70:30%, v/v) was used as mobile phase (flow rate 1 ml/min). The detection wavelength in the applied AD-25 was set at 272 nm. During the course of each run, the absorbance spectra from PDA100 (in the range 190–750 nm) were collected continuously.

The identification of the caffeine peak was carried out by comparing the retention time of the peak ($t_R = 7.2$ min) and its UV–vis spectra with that of the reference standard. The concentrations of caffeine in the resulting extracts were calculated from the calibration curve. Each extract was HPLC-analysed three times.

2.8. Statistical analysis

The data are presented as mean value \pm S.D. Statistical analysis was performed by means of Student's *t*-test for non-dependent samples.

3. Results and discussion

The caffeine amounts estimated in powdered green tea leaves using different sample preparation methods are collected in Table 1. The data can be divided into two groups: those higher than 34.5 mg/g of dry weight and those lower than 28 mg/g of dry weight.

Table 1
Caffeine amount estimated in green tea using different sample preparation method (mean value \pm S.D.)

| Sample preparation method ^a | Caffeine amount in green tea (mg/g) |
|--|-------------------------------------|
| Classical infusion | 34.5093 \pm 1.98 |
| Ultrasonic assisted infusion | 36.0796 \pm 1.94 |
| MSPD with C18 | 36.4512 \pm 1.56 |
| MSPD with sand | 35.8698 \pm 2.01 |
| Opened MASE | 27.6572 \pm 1.82 |
| Closed MASE | 18.9945 \pm 1.03 |
| PLE at 70 °C | 16.2400 \pm 0.84 |

^a Condition, see experiment.

As mentioned in Section 1, in the case of caffeine analysis in tea, infusion is the most frequently applied and recommended as a sample preparation method. The triple infusion of the same tea sample at 70 °C described in [21] is a classical example of the method. The amount of caffeine in green tea samples estimated in this way belongs to the first group of data, but ultrasonic assisted infusion reveals a little bit more caffeine. Still more caffeine is found in tea using MSPD. Moreover, there is no significant difference in the amount of the analyte when C18 sorbent or quartz sand is employed in the MSPD process. It should be noticed, however, that the differences in the first group of data can be treated as measurement errors, the more so that the distribution of values in this group is relatively narrow.

The discussed results prove that the isolation of caffeine from powdered green tea leaves is very easy. Hence, the lowest caffeine amount estimated using PLE, assumed to be one of the most effective sample preparation methods, is a very striking finding (see Table 1). It needs to be stressed here that the PLE value was obtained using three-cyclic PLE at 70 °C and 40 bars (each cycle lasted 10 min). With respect to the presented results, we can speculate that after the PLE procedure some amount of caffeine still remains in the tea matrix. Thus, to explore the most effective PLE conditions, three independent series of multiple-PLE of tea (so-called exhaustive PLE) differing in extraction temperature (other PLE conditions the same as before) were performed. Their results are presented in Table 2, which contains also the total

Table 2
Caffeine amount extracted at different temperatures in subsequent PLE steps from green tea (values recalculated per 1 g samples)

| Cycle number | Caffeine amount (mg) extracted at | | |
|-----------------------|-----------------------------------|------------------|------------------|
| | 70 °C | 100 °C | 150 °C |
| 1 | 9.84 \pm 0.41 | 28.10 \pm 0.93 | 31.06 \pm 1.21 |
| 2 | 3.79 \pm 0.11 | 3.20 \pm 0.16 | 3.03 \pm 0.15 |
| 3 | 2.61 \pm 0.13 | 1.28 \pm 0.07 | 0.93 \pm 0.05 |
| 4 | 1.63 \pm 0.09 | 1.01 \pm 0.07 | 0.29 \pm 0.03 |
| 5 | 1.24 \pm 0.08 | 0.74 \pm 0.06 | 0.13 \pm 0.01 |
| 6 | 0.87 \pm 0.07 | 0.57 \pm 0.05 | – |
| 7 | 0.61 \pm 0.05 | 0.39 \pm 0.04 | – |
| 8 | 0.48 \pm 0.04 | 0.24 \pm 0.02 | – |
| 9 | – | 0.09 \pm 0.01 | – |
| Total caffeine amount | 21.07 \pm 0.88 | 35.62 \pm 1.35 | 35.44 \pm 1.34 |

amounts of caffeine obtained at all extraction steps carried out within each series. It appears from the data that at least five PLE extraction steps at 150 °C should be performed in order to obtain the tea extract containing the caffeine amount that does not significantly influence (less than 0.1 mg) the total analyte content. More than nine steps are required when PLE is performed at 100 °C. In both cases, the total amount of isolated caffeine (five-cycle PLE at 150 °C and nine-cycle PLE at 100 °C) is similar to that obtained by infusion (see Table 1). A nine-step PLE is vastly insufficient to reach a similar value when extraction temperature is 70 °C.

The results at 70 °C evidently prove a significantly lower efficiency of PLE in caffeine isolation from tea. This conclusion is especially confusing when one takes into account the easiness of caffeine extraction from tea, the general opinion about the excellence of PLE, and the complicated and costly PLE equipment. It should be noticed, however, that our results were obtained performing PLE at 40 bars for 10 min. Due to the easiness of caffeine isolation from tea, these conditions were assumed sufficient for the full isolation of the compound. Yet a simple matrix such as tea may require very special PLE conditions for full caffeine recovery. In order to test this hypothesis, more experiments were performed investigating the influence of extraction time and extraction pressure on the yield of caffeine from tea. As appears from Fig. 1, illustrating the influence of extraction time at 100 °C and 100 bars, there are no substantial changes in caffeine yield when the PLE process is longer than 10 min. Experiments with extraction pressure (see Fig. 2) show that the yield of caffeine from green tea at lower temperatures (70 and 100 °C) are pressure-dependent. At these temperatures, the extracted caffeine amount clearly diminishes above 100 bars. On the curve corresponding to 70 °C, a distinct maximum at 60 bar exists. There is no essential pressure influence on the caffeine yield from tea when PLE is carried out at 150 °C. Moreover, caffeine yields at this temperature are the highest. The PLE experiments were not performed above 150 °C as

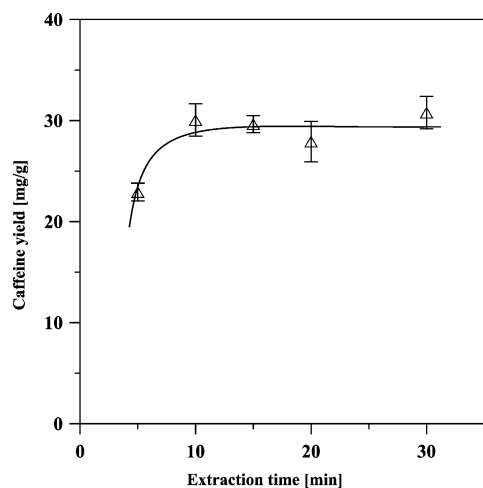


Fig. 1. Dependence between caffeine yield obtained from the green tea samples in PLE process performed at 100 °C and 100 bar and extraction time.

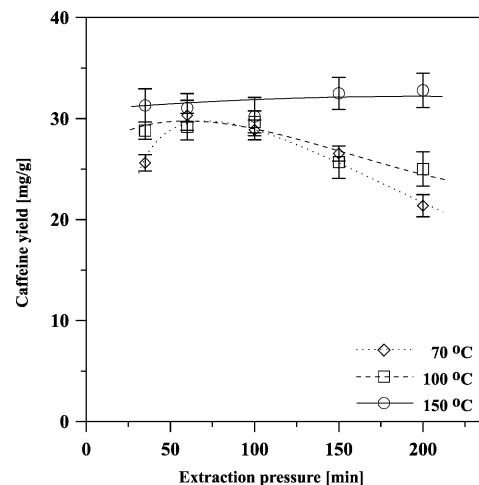


Fig. 2. Effect of extraction pressure on the caffeine yield obtained from the green tea samples in PLE process carried out at 70, 100 and 150 °C. Extraction time 10 min. The results for 100 and 150 °C were obtained in one-cycle PLE process, whereas the results for 70 °C in ten-cyclic process.

the obtained extracts were very turbid, dense and difficult to process further.

It should be remarked that the data in the last column of Table 2 were obtained at the extraction conditions of 150 °C, 40 bars, 10 min, which found to be optimal PLE conditions for caffeine extraction from tea. Thus, at least a few steps are required at the optimal PLE conditions for the recovery of the amount of caffeine obtained during simple classical infusion.

The presented data leads to the conclusion that the elevated pressure in PLE is the factor hindering the extraction of caffeine from tea. One can ask why? A probable answer is suggested by the values in Table 3 showing caffeine yields from coffee using different sample preparation methods.

As results from this data, PLE is considerably more effective than classical and ultrasonic assisted infusion when caffeine is extracted from coffee. Moreover, in the case of coffee most of caffeine (more than 99.9%) is extracted in the first cycle of the PLE process performed in conditions recommended as default by Dionex Corp. (i.e. 100 °C, 60 bars, 10 min). Hence, it can be concluded that different kinetics

Table 3

Caffeine amount estimated in coffee beans using different sample preparation method

| Sample preparation method ^a | Caffeine amount (mg/g) in coffee beans |
|--|--|
| Classical infusion | 15.3723 ± 0.86 |
| Ultrasonic assisted infusion | 16.5718 ± 0.76 |
| MSPD with C18 | 22.4110 ± 1.03 |
| MSPD with sand | 21.9982 ± 1.15 |
| Multiple PLE | |
| First cycle | 22.3996 ± 0.69 |
| Second cycle | 0.0017 ± 0.0003 |
| Third cycle | 0.0006 ± 0.0002 |
| Total | 22.4019 ± 0.70 |

^a Condition, see experiment.

of caffeine isolation in the PLE process of tea and coffee is connected with the properties (rigidity) of tea and coffee matrices. Probably the elevated pressure used in PLE squeezes the soft tea matrix making the diffusion of caffeine from the inside to the outside of the matrix difficult or hindering the penetration of the inner matrix by the extrahent. This explanation is additionally supported by the results obtained using MASE as a sample preparation process. The amount of caffeine extracted from tea in the closed MASE mode (in the closed extraction vessel overpressure is created, 25–27 bar) is significantly lower than in the case of the open MASE mode (performed at atmospheric pressure), see Table 1.

The obtained results show that while PLE is very effective for analyzing caffeine in coffee, it seems to be less effective for tea. In the case of tea:

- pressure is the hindering factor of caffeine extraction;
- the increase of caffeine amount with temperature in the examined temperature range is observed;
- above 10 min extraction time, the effect of time is insignificant.

One-cycle PLE (even conducted in the best PLE conditions) yield only 88% of the total caffeine amount.

These results prove that the expensive PLE (in respect of equipment price) is not always as effective as commonly believed. Sometimes it is better to apply simpler and cheaper sample preparation methods.

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