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Short communication

# PLE in the analysis of plant compounds Part II: One-cycle PLE in determining total amount of analyte in plant material

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**Abstract**

Pressurised liquid extraction (PLE) is recognised as one of the most effective sample preparation methods. Despite the enhanced extraction power of PLE, the full recovery of an analyte from plant material may require multiple extractions of the same sample. The presented investigations show the possibility of estimating the true concentration value of an analyte in plant material employing one-cycle PLE in which plant samples of different weight are used. The performed experiments show a linear dependence between the reciprocal value of the analyte amount ($E^*$), extracted in single-step PLE from a plant matrix, and the ratio of plant material mass to extrahent volume ($m_p/V_s$). Hence, time-consuming multi-step PLE can be replaced by a few single-step PLEs performed at different ($m_p/V_s$) ratios. The concentrations of rutin in *Sambucus nigra* L. and caffeine in tea and coffee estimated by means of the tested procedure are almost the same as their concentrations estimated by multiple PLE.


*Keywords:* Linear dependence in PLE; One-cycle PLE; Caffeine analysis; Rutin analysis

## 1. Introduction

Among numerous physicochemical separation methods used as sample preparation procedures for chromatographic analysis, extraction methods are the most versatile and frequently employed. A recently developed extraction technique of pressurised liquid extraction (PLE) is recognised as one of the most effective ones. PLE allows us to use extrahents at elevated pressure and, hence, at temperature above their normal boiling point, and in consequence to remove analytes quickly and efficiently from various matrices. However, despite the enhanced extraction power of PLE, the full recovery of an analyte from plant material can require multiple extractions of the same sample. In such cases the time of the entire analytical procedure can be significantly increased and determined by the time of a multiple extraction procedure (depending on the number of extraction steps).

In a simple liquid–liquid extraction process the recovery (fraction) of a substance ($E$) in one-extraction step is given by Eq. (1) [1,2]:

$$E = \frac{K_0 V}{1 + K_0 V} \tag{1}$$

where $K_0$ is a partition coefficient of the substance between two phases, and $V = V_0/V_w$. $V_0$ and $V_w$ are the volumes of the organic and water phases, respectively.

Transforming the above equation into its linear form,

$$\frac{1}{E} = \frac{1}{K_0 V} + C \tag{2}$$

the total amount of substance contained in one of the phases of the liquid–liquid extraction system ($E_{total} = 1/C$) can be easily calculated:

$$\frac{1}{E_{total}} = \lim_{v_w/v_0 \to 0} \left( \frac{V_w}{K_0 V_0} + C \right) \tag{3}$$

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Thus, the finding of the total substance amount existing in one of the phases resolves itself into a few single extraction processes carried out at different ($V_w/V_0$) ratios and into the extrapolation of the ($1/E$) versus ($V_w/V_0$) linear relationship to $V_w/V_0 = 0$.

The question appears at this moment whether one-cycle PLE from plant material can be physicochemically considered as a partition process analogous to those for the liquid–liquid extraction process. It can be expected that, for well-matched extraction conditions, the one-cycle PLE process can also be described by an equation similar to that for the liquid–liquid extraction process (see Eq. (2)), i.e. by the following one:

$$\frac{1}{E^*} = \frac{m_p}{K_0^* V_s} + C^* \quad (4)$$

where $m_p$ and $V_s$ are the weight of plant material and the volume of extractant, respectively.

If it is true, the limit of the $1/E^*$ versus $m_p/V_s$ plot (when $m_p/V_s \to 0$), should be equal to the reciprocal value of the total analyte amount contained in plant material ($E^*_{total}$).

The aim of this paper is to verify experimentally the proposition that one-cycle PLE can be described as a partition process of an analyte between solid matrix and liquid extractant using equations analogous to those for the liquid–liquid extraction process. The research is justified because the estimation of the total analyte amount in plant material using one-cycle PLE can in some cases be less time-consuming than multiple PLE of the same sample.

The problem is discussed with reference to the analysis of the following components:

- rutin in *Sambucus nigra* L. flowers,
- caffeine in green tea,
- caffeine in black tea, and
- caffeine in coffee beans.

## 2. Material and methods

### 2.1. Materials and reagents

Dried flowers of *S. nigra* L. (black elder) were purchased from a local pharmacy (Herbapol-Lublin, Lublin, Poland). Green and black tea (Yunnan) and black coffee (Caffe dé Collumbia) were obtained from a local grocer's. A sufficiently large representative samples of black elder flowers and green or black tea leaves (ca. 500 g) was ground with a Braun cutting mill to obtain particle size of 0.2–0.4 mm. Caffe dé Collumbia, which is ground coffee, was extracted without additional grounding.

Methanol (HPLC grade and analytical-reagent grade), acetonitrile (HPLC grade), glacial acetic acid (analytical-reagent grade) and orthophosphoric acid (analytical-reagent grade) were obtained from the Polish Factory of Chemicals POCh (Gliwice, Poland). E. Merck (Darmstadt, Germany) provided potassium dihydrogen phosphate and standards of rutin (quercetin-3-rutinoside) and caffeine (1,3,7-trimethylxanthine). Water purified in 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. Pressurised liquid extraction

PLE was performed with a Dionex ASE-200 (Dionex Corp., Sunnyvale, CA, USA). To reduce the volume of solvent used for the extraction, the exactly weighted portions of the examined materials were mixed with neutral glass and placed into a 22 ml stainless steel extraction cell [3]. The extraction conditions were as follows:

1. for *S. nigra* L. flowers:
   - extrahent: methanol/water mixture (80/20% v/v),
   - extraction temperature: 100 °C,
   - pressure: 100 bar, and
   - static extraction time: 10 min(the conditions were established in a separate investigation as optimal for the PLE of rutin from *S. nigra* L. [4]);
2. for tea and coffee:
   - extrahent: water,
   - temperature: 100 °C,
   - pressure: 60 bar, and
   - static extraction time: 10 min.These conditions are recommended as default by Dionex Corp.

The collected extracts (25–31 ml) were transferred into 50 ml laboratory flasks and filled up to their volume with pure extrahent. $V_s = 50$ ml was assumed as extrahent volume in all calculations. Between extraction runs, the system was washed with the extraction solvent.

Two types of extraction procedure were performed:

(a) one-cycle extraction of different weight portions (in the range of 0.1–2.0 g) of the investigated materials;
(b) multiple extraction of 0.5 g portion of the materials of the same sample. (Multiple PLE was curried out until the analyte amount appearing in the subsequent extraction step was lower than 0.1 % of that obtained in the first portion.)

All extraction procedures were repeated five times.The resulting solutions were subjected to the HPLC analysis.

### 2.3. HPLC analysis

Analytes concentrations in the obtained extracts were determined by means of HPLC. Measurements were carried out using a Dionex liquid chromatograph DX600 (Dionex Corp., Sunnyvale, CA, USA) consisting of a chromatography enclosure LC20 with a PEEK automated Rheodyne injection valve having 25 μl sample loop; a gradient pump GP50; a UV–vis detector AD25, and a photodiode array detector PDA100. The

chromatographic data were stored and the whole system was under the control of the PeakNet6 data acquisition system.

Chromatographic separations were performed applying a Prodigy ODS-2 column (5 μm, 250 mm × 4.6 mm ID, Phenomenex, Torrance, CA, USA) with a guard column of the same firm. The column and the guard column were placed in the oven and the analyses were performed at 25 °C in the case of rutin, and at 30 °C for caffeine (Column Thermostat, JetStream II Plus, Knauer, Warsaw, Poland). The mobile phase composition was optimised to receive the peaks separated from other components of the examined extracts. In rutin analysis a mixture composed of $CH_3CN$ and water containing 5% glacial acetic acid (25:75% v/v) was employed as mobile phase (flow rate 1 ml/min) in the chromatographic separations. The detection wavelength in the applied AD-25 was set at 350 nm. Simultaneously, during the course of each run, the absorbance spectra from PDA100 (in the range 190–750 nm) were collected. In caffeine analysis 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 was set at 272 nm.

The identification of the peaks were carried out by comparing the retention time of the peaks and their UV–vis spectra with those of the reference standards.

The concentrations of the analytes in the resulting extracts were calculated from the calibration curve.

### 2.4. Statistical analysis

Statistical analysis was performed by means of Student's *t*-test for nondependent samples.

## 3. Results and discussion

The comparison of analyte concentration estimated in the examined material by one-cycle PLE with the independently established true concentration value of the analyte is the simplest way to verify the applicability of the method of quantifying the total analyte amount in plant matrix and the accuracy of measurement. The true total rutin amount in the flowers of *S. nigra* L. and caffeine amount in green and black tea and coffee were determined by performing multiple (so-called exhaustive) extractions of the same sample under the PLE conditions described in experimental. As it was mentioned, each process of multiple extraction was carried out until the analyte amount appearing in the subsequent extracting portion was lower than 0.1% of that obtained in the first portion. The results of these investigations are listed in the second column of Table 1 and are in good agreement with the literature data [5–8]. The values in brackets, marked with one asterisk, reflect the number of extraction steps performed and can be treated as a measure of extraction kinetics. The next column of Table 1 contains the analyte amount extracted from the plant material in the first extraction step of the mentioned above multiple extraction process. The data in brackets, labelled with two asterisks, represent the analyte recovery.

As appears from Table 1, the extraction kinetics of rutin from black elder flower and caffeine from both types of tea are similar in the applied PLE conditions. The isolation of caffeine from coffee is significantly easier. Almost total amount of this analyte gets extracted in the first step of the PLE procedure. Caffeine amounts extracted from tea and coffee in the consecutive steps of the PLE process were discussed in first part of the paper [9].

The dependences between the reciprocal of the analyte yield obtained in one-cycle PLE and the ratio of the material mass to the extrahent volume applied in this process are shown in Fig. 1. ($V_s$ = 50 ml was used in all calculations). According to the obtained data the dependences are linear ($R^2$ is located in the range 0.954–0.996). From the equations describing the adequate relationship (see Fig. 1A–D), the lines are known to have positive slope, except the one for coffee. The dependence corresponding to caffeine in coffee is almost parallel to the $m_p/V_s$ axis. Its run is not strange if one takes into account the easiness of caffeine extraction from coffee. Hence, the very slight negative slope of the plot results probably from experimental errors.

The ($E^*_{total} = 1/C^*$) values calculated from the presented relationships (see Section 1) are listed in the fourth column of Table 1. They are very close to the analyte amounts es-

Table 1
Rutin and caffeine amount estimated in the used materials by multi-step exhaustive PLE in default conditions and by linear dependence $1/E^* = f(m_p/V_s)$

| Analyte | Analyte amount (mg/g) estimated employing | | | Analyte range calculated from Eq. (4) taking two critical ($m_p/V_s$) pairs of points |
|---|---|---|---|---|
| | Multiple PLE | | $1/E^{a} = f(m_p/V_s)$ dependence | |
| | Total amount | Amount obtained in first extraction step | | |
| Rutin in black elder flowers | 29.03 ± 1.16 (7)[a] | 25.86 ± 1.04 (89.1)[b] | 29.50 | 29.05–30.66 |
| Caffeine in green tea | 35.62 ± 2.51 (9)[a] | 28.10 ± 0.93 (78.9)[b] | 35.71 | 28.36–39.90 |
| Caffeine in black tea | 36.31 ± 1.87 (8)[a] | 27.76 ± 1.02 (76.4)[b] | 36.50 | 34.02–37.17 |
| Caffeine in coffee | 22.40 ± 0.63 (2)[a] | 22.40 ± 0.69 (99.9)[b] | 22.62 | 22.54–22.62 |

[a] Number of extraction steps performed.
[b] Recovery in %.

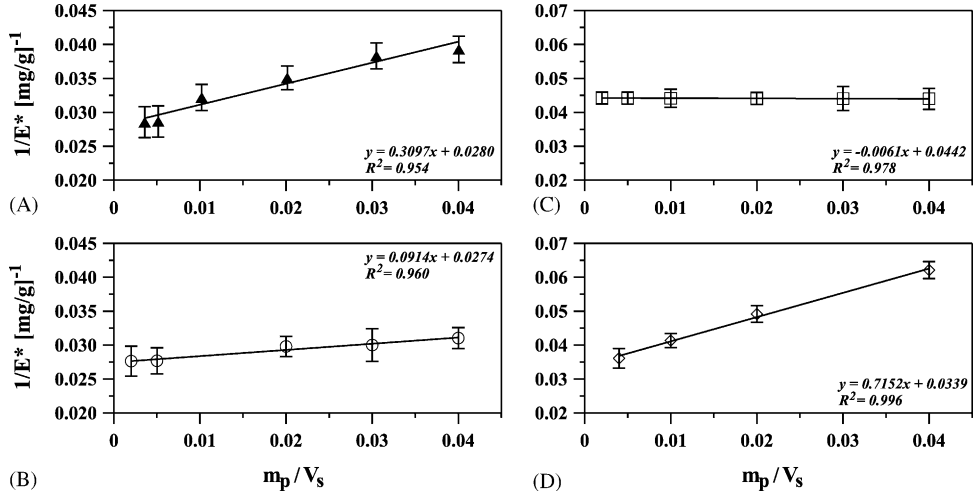


Fig. 1. The dependence between the reciprocal of the analyte yield ($E^*$) and the ratio of the plant matrix mass to extrahent volume ($m_p/V_s$) for: (A) caffeine in green tea; (B) caffeine in black tea; (C) caffeine in coffee; (D) rutin in *S. nigra* L. flowers ($n = 5$).

timated by multiple (exhaustive) PLE (see relevant data in columns 2 and 4 of Table 1). The comparison of the results shows that in the extraction of caffeine and rutin from the investigated matrices one-cycle PLE can be physicochemically considered as a partition process analogous to those for the liquid–liquid extraction process. The ($1/E^*$ versus $m_p/V_s$) function is linear and its limit (when $m_p/V_s \rightarrow 0$) equals to the reciprocal value of the total analyte amount contained in plant material ($E^*_{total}$).

The similarity of the concentration values confirms the accuracy and reliability of the proposed way, which is a shorter procedure for analytes requiring several extraction steps in multiple PLE. The presented experiments were repeated a few times at 4, 5 or 6 ($m_p/V_s$) ratios. It was done only to verify experimentally if the discussed dependences are linear and if their intersection with 0Y axis equals to the reciprocal value of the total analyte amount in plant ($E^*_{total}$). However, such number of extractions is not significantly smaller than in multi-step PLE. The most convenient would be the result obtained using only two ($m_p/V_s$) ratios.

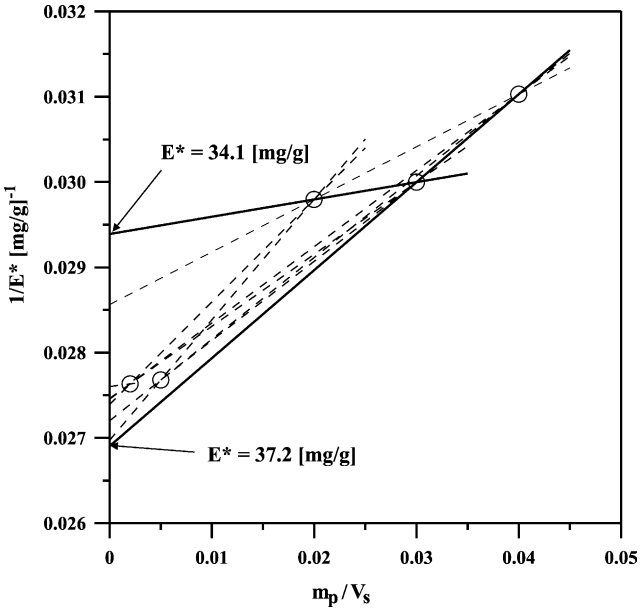


Fig. 2. Dependences between the reciprocal of caffeine yield ($E^*$) in black tea and the ratio of plant matrix mass to extrahent volume ($m_p/V_s$) plotted for each pair of experimental points. Solid lines show the relation for the critical points pair.

The last column of Table 1 lists the concentration range of the analytes calculated for two ($m_p/V_s$) ratios and gives the lowest and the highest concentration (for two so-called critical points). To obtain this range, straight lines were drawn through each pair of points. Fig. 2 is a graphic representation of the calculation performed for black tea. As appears from these data, in the case of caffeine in coffee and black tea, and in the case of rutin in black elder the maximum and the minimum analyte concentration differs less than 6% from the value established by multiple extraction. A bigger difference is observed for green tea.

The presented investigations show that the linear dependence exists between reciprocal value of the analyte amount ($E^*$) extracted in single-step PLE from a plant matrix, and the ratio of plant material mass to the extrahent volume ($m_p/V_s$). This dependence permits to estimate the total amount of the analyte (its true concentration value) in plant matrices using only a few experimental points (at least two). Hence, multi-step PLE can be successfully replaced by single-step PLE performed at different ratios of plant mass to solvent volume to save time and extrahent used. The described way of determining analyte concentration in plant material looks promising and requires further examination involving a larger number of plants and substances, especially those which are difficult to recover in the PLE process.

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