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Development and validation of UHPLC–MS/MS method for determination of eight naturally occurring catechin derivatives in various tea samples and the role of matrix effects

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ABSTRACT

A complete analytical procedure combining optimized tea infusion preparation and validated UHPLC–MS/MS method was developed for routine quantification of eight naturally occurring catechin derivatives in various tea samples. The preparation of tea infusions was optimized in terms of temperature, time and water-to-tea ratio in green, white and black teas. The catechins were analyzed using ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry in a run of only 4 min including equilibration of the system. The UHPLC–MS/MS method was fully validated in terms of inter/intra-day precision, accuracy, linearity ($r^2 > 0.9991$), range (50–5000 ng/ml), LOD (1.5–7.5 ng/ml) and LOQ (5–25 ng/ml). Validation of the method included also the determination of the matrix effects that were evaluated in both flavored and unflavored green, white and black teas. Dilution of the resulting tea infusions appeared to be crucial for the matrix effects and also for subsequent catechin quantification in real tea samples in order to fit into the linear range of the UHPLC–MS/MS method. This complete procedure for catechin quantification was finally applied to real sample analysis represented by 70 commercial tea samples.

1. Introduction

Besides water, tea is the most widely consumed beverage in the world, which is due to its health benefits, gustatory properties, stimulant effects and cultural dimension [1]. Post-harvest processing of leaves of *Camellia sinensis* (L.) (Theaceae) is an important factor determining a type of tea and affecting tea polyphenol content and also polyphenol composition. According to the level of fermentation, three basic types of tea are distinguished i.e. non-fermented green tea, partially fermented oolong tea and fully-fermented black tea. Fermentation, an enzymatic oxidation process, in the case of tea, converts monomeric phenolic compounds into dimers, oligomers and polymers [2]. To avoid oxidation in post-harvest processing, tea leaves resulting in green tea are subjected to fixing process that inactivates enzymes such as polyphenol oxidase, polyphenol peroxidase, and ascorbic acid oxidase [1,3]. Therefore, monomeric catechins predominate in green tea whereas dimeric theaflavins and polymeric thearubigins in black tea [4]. Black tea is the most popular type of tea representing approximately 76–78% of the worldwide tea production and consumption, green tea 20–22% and oolong tea less than 2% [5]. Black tea is consumed primarily in North America, Europe and India, whereas green tea in Japan and China [6].

Catechins, flavan-3-ol derivatives, are colorless and water-soluble compounds. The major catechins contained in fresh tea leaves include (−)-epigallocatechin gallate, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epicatechin [7]. During post-harvest processing and storage of tea leaves, catechins are prone to oxidation, epimerization, polymerization and degradation. Temperature, humidity, oxygen, metal ions, pH of the system and tea ingredients are responsible for these chemical changes [8]. Tea catechins show various health beneficial effects and they are best known for their antioxidant activity [9,10]. Theaflavins contained in black tea leaves also act as antioxidants [11]. Similar activity to catechins was reported by Leung et al. [12]. Anti-microbial [13,14], anti-viral [15,16] and anti-fungal [17,18] effects were observed as well. Due to anti-oxidant, anti-inflammatory, anti-proliferative and anti-platelet activity catechins are assumed to decrease cardiovascular risk [19] and they are also hypothesized to reduce the risk of several types of cancer [6].

Analysis of catechins in biological matrices is typically performed using liquid chromatography coupled to mass spectrometry

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(MS), UV or PDA detector. The major drawback of most of the published HPLC methods is a long chromatographic run of about 20–45 min [20–26]. It results in high costs of analysis, high solvent consumption and also potential analyte degradation [27]. Only few faster HPLC methods [28–30] were published in the past years taking still about 10 min. Use of columns packed with sub-2 μm particles in ultra-high performance liquid chromatography (UHPLC) allows to reduce time of chromatographic separation while maintaining the same resolution [31,32]. Surprisingly, UHPLC has been quite scarcely employed in analysis of catechins. Eight naturally occurring catechins were successfully separated using UHPLC–UV in a run of only 1.7 min by Spáčil et al. [33]. In addition, even faster (0.5 min) UHPLC separation of seven catechin derivatives was reported by Guillarme et al. [34]. Nevertheless, none of these methods was fully validated for determination of catechins in real samples. Recently, fast UHPLC–UV method published by Naldi et al. [35] was fully validated for qualitative and quantitative analysis of 6 catechins and caffeine that were separated in a run of 3 min. In spite of higher selectivity and sensitivity of MS detection, it has been quite rarely used for this purpose. The development of UHPLC–MS/MS method that was partially validated was previously published by our group [36]. In this paper the focus was put on the optimization of MS conditions. To our knowledge a fast and selective UHPLC–MS/MS method that is fully validated for determination of 8 naturally occurring catechin derivatives in various tea samples catechins in various tea samples has not been published yet.

Sample preparation step of tea leaves prior to LC–MS analysis of catechins usually involves tea infusion preparation, filtration through a membrane filter and subsequent dilution. Despite all the advantages that LC–MS brings, it suffers from a major drawback called matrix effects. Is the sample treatment involving infusion preparation, filtration and dilution sufficient enough for reliable quantification of catechins in terms of overcoming matrix effects and providing acceptable validation results? Therefore, evaluation of this phenomena was included in the method validation. To our knowledge, no studies have been published evaluating matrix effects of catechins in tea samples. The aim of this work was to develop a complete procedure for routine quantification of catechins contained in various tea samples. Development of this procedure involved optimization of conditions for tea infusion preparation and development and validation of fast UHPLC–MS/MS method for quantification of all eight naturally occurring catechins in tea samples. Conditions for infusion preparation were optimized in terms of temperature, time and water-to-tea ratio. Validation of the method included also evaluation of matrix effects in both unflavored and flavored green, white and black teas. The resulting procedure was applied for quantification of catechins in tea samples commonly available for tea consumers in the Czech Republic to demonstrate its applicability to real sample analysis and to compare the total amount of catechins in flavored and unflavored green, white and black teas.

2. Experimental

2.1. Chemicals and reagents

The following standards of catechins: (−)-catechin gallate (CG), (−)-epicatechin gallate (ECG), (±)-catechin hydrate (C), (+)-epicatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-gallocatechin gallate (GCG) and (−)-epigallocatechin gallate (EGCG) were purchased from Sigma–Aldrich (Steinheim, Germany). Acetonitrile, methanol and mobile phase additives such as formic and acetic acid, all of them LC–MS grade, were obtained from Sigma–Aldrich (Steinheim, Germany). LC–MS grade water was prepared by Milli-Q reverse osmosis system (Millipore, Bedford, MA, USA) immediately prior to use. Green, white and black teas used for validation of UHPLC–MS/MS method and optimization of sample preparation step were obtained from Oxalis (Slušovice, Czech Republic). The other tea samples were purchased from local supermarkets or specialized tea shops in the Czech Republic and France.

2.2. Instrumentation and analytical conditions

ACQUITY Ultra Performance LC™ (UPLC) system (Waters, Milford, MA, USA) consisting of binary solvent manager and sample manager was coupled with Micromass Quattro micro™ API benchtop triple quadrupole mass spectrometer (Waters, Milford, MA, USA). All UHPLC analyses were performed on the analytical column CSH C_{18} (100 mm × 2.1 mm, 1.7 μm) (Waters, Milford, MA, USA) and the column was maintained at 40 °C. Samples were separated using a gradient elution with 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B). The flow rate was set at 0.3 ml/min and the chromatographic run time was 4.0 min including equilibration of the system. The gradient started with 8.5% of solvent B, increased to 40.0% over 2.1 min and in 2.2 min the percentage of solvent B ramped to original conditions (8.5%). The injection volume was 5 μL. All the ion source and ion optic parameters were optimized and they were finally set as follows: capillary voltage 1.0 kV, extractor voltage 2.0 V, hexapole voltage 0.2 V, cone voltage 35 V (GC, EGC, C, EC), 30 V (EGCG, GCG, ECG, CG), cone gas flow rate 70 L h^{-1}, desolvation temperature 450 °C and desolvation gas flow rate 600 L h^{-1}. The resulting most intense SRM transitions with optimized collision energies and dwell times are shown in Table 1. Scheduled SRMs were used in order to maximize the dwell times for individual transitions. The data were acquired and processed using MassLynx™ software version 4.1 (Waters, Milford, MA, USA).

2.3. Standard solutions

Stock standard solutions of GC, EGC, C, EC, GCG, EGCG, CG and ECG were prepared by dissolving each compound in acidified methanol (0.1% formic acid) to give a solution with a concentration of 1.0 mg/mL. Formic acid was added due to the stability reasons. Stock standard solutions were stored at 4 °C until further dilution and they were prepared fresh every two weeks. Mixture consisting of 0.1% formic acid in water and 0.1% formic acid in methanol (50/50, v/v) was used for preparation of stock standard solutions as for dilution of natural samples.

2.4. Sample preparation

Both tea forms i.e. loose leaf tea and tea bag tea were homogenized before quantification of catechins. Loose leaf tea was taken from the bottom, middle and top of the pack and thoroughly mixed. Although smaller size of tea leaf particles would provide higher extraction efficiency, loose leaf tea samples were not crushed or rubbed to keep the conditions of tea infusion preparation close to the real procedure. Three tea bags of one and the same analyzed tea were cut open and their content was mixed. 1 g of resulting tea samples was weighed and subjected to sample preparation. Conditions for tea infusion preparation were optimized for green (Formosa gunpowder), white (Snow buds) and black (Keemun) teas. Both green and white tea infusions were prepared using 100 ml of water at 90 °C maintained for 20 min. During infusion preparation, tea samples were mildly stirred every 5 min. Black tea infusions were prepared according to the same procedure as used for green and white teas except temperature (100 °C). After infusion preparation, the resulting sample was filtered through hydrophilic 0.22 μm pore