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On-line monitoring of *in-vitro* oral bioaccessibility tests as front-end to liquid chromatography for determination of chlorogenic acid isomers in dietary supplements

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ABSTRACT

A novel fully automated *in-vitro* oral dissolution test assay as a front-end to liquid chromatography has been developed and validated for on-line chemical profiling and monitoring of temporal release profiles of three caffeoylquinic acid (CQA) isomers, *namely*, 3-CQA,4-CQA and 5-CQA, known as chlorogenic acids, in dietary supplements. Tangential-flow filtration is harnessed as a sample processing approach for on-line handling of CQA containing extracts of hard gelatin capsules and introduction of protein-free samples into the liquid chromatograph. Oral bioaccessibility/dissolution test assays were performed at 37.0 ± 0.5 °C as per US Pharmacopeia recommendations using pepsin with activity of ca. 749,000 USP units/L in 0.1 mol/L HCl as the extraction medium and a paddle apparatus stirred at 50 rpm. CQA release rates and steady-state dissolution conditions were determined accurately by fitting the chromatographic datasets, *namely*, the average cumulative concentrations of bioaccessible pools of every individual isomer monitored during 200 min, with temporal resolutions of ≥ 10 min, to a first-order dissolution kinetic model. Distinct solid-to-liquid phase ratios in the mimicry of physiological extraction conditions were assessed. Relative standard deviations for intra-day repeatability and inter-day intermediate precision of 5-CQA within the 5–40 μg/mL concentration range were < 3.4% and < 5.5%, respectively. Trueness of the automatic flow method for determination of 5-CQA released from dietary supplements in gastric fluid surrogate was demonstrated by spike recoveries, spanning from 91.5–104.0%, upon completion of the dissolution process. The proposed hyphenated setup was resorted for evaluating potential differences in dissolution profiles and content of the three most abundant chlorogenic acid isomers in dietary supplements from varied manufacturers.

1. Introduction

Nowadays, healthy eating and good nutrition are topics of major interest worldwide. Therefore, pharmaceutical companies invest considerable efforts in developing novel food supplements, usually served in capsules or tablets that contain one or several dietary ingredients to enhance nutritional supply. They might include vitamins, minerals, herbs or other botanicals, amino acids, and additional substances, such as enzymes, glandular tissues, metabolites, or probiotics [1].

Unlike tablets, capsules are favoured in pharmaceutical and medical therapies because of their “easy-to-swallow” properties supplemented by the advantage of active substance enclosure. Therefore, consumers do not experience neither odour nor bitterness of drugs [2]. Capsules could be made using hard or soft shell components [3,4]. Hard-shelled capsules are typically made of gelatin, a naturally occurring polymer with notable hygroscopic properties [5], because of its biodegradability [6,7] and biocompatibility [8,9] in physiological environments. Shells of gelatin capsules are prepared from a molten gel mass (gelatin) and a plasticizer dissolved in an aqueous vehicle [2]. The most usual sources of gelatin production are pig skin (46%), bovine hides (29.4%) and pig and cattle bones (23.1%) [10].

Gelatin capsules are readily melted in water at a temperature above 30 °C. Drug release formulations are thus expected to dissolve in the human digestive tract at the physiological temperature under the action of gastric pH and digestive enzymes [11]. In fact, the dissolution behaviour of gelatin capsules is extraction medium and experimental conditions dependent. According to US Pharmacopoeia (USP) [12], the addition of enzymes, such as pepsin or pancreatin, to the dissolution medium is allowed in dissolution testing. The addition of pepsin is recommended when the medium

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is water or, alternatively, a physiologically relevant medium with a pH below than 6.8. The critical factor, however, is the activity of pepsin. The recommended activity in drug dissolution testing is to not exceed 750,000 USP units per liter [3].

Green coffee extract has attracted a great deal of attention over the past few years as a food supplement commonly delivered in gelatin capsules for quick weight loss. Significance of green coffee is attributed to the presence of chlorogenic acids (CGAs), naturally occurring phenolic compounds found largely in the majority of higher plants [13,14]. They are a family of esters formed between quinic and certain *trans*-cinnamic acids, mostly caffeic, ferulic or *p*-coumaric acids. CGAs account for many positive health effects on the human body with recognised antioxidative and anticancer properties, and, most importantly, for promoting weight loss on the basis of their capacity to slow the release of glucose into the bloodstream after a meal [15,16]. Green coffee beans are the highest source of CGA, particularly caffeoylquinic acids (CQAs), ranging from 4 to 14% [17].

There have been a plethora of studies dealing with CGAs content in coffee or other foodstuffs, such as sweet potatoes [17,18], tomatoes [19], apples, and oranges [20], just to name a few. The most abundant and concomitantly most effective CGA related to health promotion as a functional food ingredient is 5-caffeoylquinic acid (5-CQA), often inaccurately called "*chlorogenic acid*". It is however reported that this compound might lead to up to 9 isomers – particularly 3–CQA and 4-CQA in aqueous heated solutions [21].

In this paper, a novel fully automated flow-set up integrating on-line tangential filtration as a front-end to liquid chromatographic separations is proposed for chemical and temporal profiling of dissolution tests of green coffee bearing food supplements and investigation of the release rates of three CQA acids (mostly 5-CQA, but 3-CQA and 4-CQA as well) using biomimetic digestive fluid as a proxy for bioaccessibility in the human gastric fluid. To the best of our knowledge, this is the first article reporting tangential flow filtration as automatic sample processing approach in real-time monitoring of oral bioaccessibility/dissolution test assays of drugs or dietary supplements coupled to HPLC.

In contrast to pharmaceutical dosage forms, food supplements are not subjected at present to stringent regulatory control & quality assurance tests [22]. Our system is presented as a viable approach to speed up assays of green coffee bean-based food supplements to quantify the actual content of 5-CQA and isomers thereof (3-CQA and 4-CQA), detect potential cases of product adulteration and characterize the rates of CQA release under physiologically simulated experimental conditions.

2. Materials and methods

2.1. Samples, chemicals and materials

5-caffeoylquinic acid (5-CQA), hydrochloric acid (37%), glacial acetic acid (100%), ammonia solution (25%), orthophosphoric acid (85%), acetonitrile (HPLC gradient) and Coomassie Blue G250 dye were purchased from Sigma Aldrich (St. Louis, USA). Hard-shelled capsules of green coffee extracts, namely, Vieste-Zelená káva (Volt Retail Ltd., Great Britain) and Café Slank (Espadiet SL, Granollers, Spain), were purchased in local Czech and Spanish pharmacies. These samples are further identified as ZK (Zelená káva) and CS (Café Slank) capsules, respectively, throughout.

Two dialyfiltration modules in series (Vivaflow 50, Sartorius Stedim Biotech, Goettingen, Germany) housing a polyethersulfone (PES) hydrophilic membrane each with a molecular-weight cut-off of 5 kDa and featuring low binding protein characteristics-to prevent membrane fouling in the course of dissolution testing-were selected for on-line tangential filtration/sample clean-up. The active membrane surface area of a single module for transfer of low molecular weight species is about 50 cm^2, with 15 mm wide and 300 μm deep flow channels for the donor compartment integrated in a polycarbonate case. The two modules with a nominal transfer area of 100 cm^2 are interconnected via an 8 cm-long PVC tubing (4.0 mm ID). Syringe filters made of polytetrafluoroethylene-PTFE, Nylon-NYL, polyvinyldiene difluoride – PVDF (Merck Millipore, Madrid, Spain) with pore size of 0.22 or 0.45 μm and cartridge diameters ranging from 17 to 33 mm were also tested in in-line sampling protocols.

The physiologically based extraction medium was prepared by dissolving a metered amount of pepsin (1.34 g) with an activity of 559 USP units/mg (Sigma) in 1 L of 0.1 mol/L HCl so as to cope with USP specifications of biomimetic gastric fluid composition containing $<$750,000 USP units of pepsin per litre [3].

2.2. Flow system and software for automation of unit operations

A diagrammatic description of the flow-through setup accommodating in-line dialyfiltration for automatic monitoring of dissolution testing of dietary supplements as a front end to liquid chromatographic separations is illustrated in Fig. 1. The flow system was assembled as follows: A 1000 mL glass beaker filled with

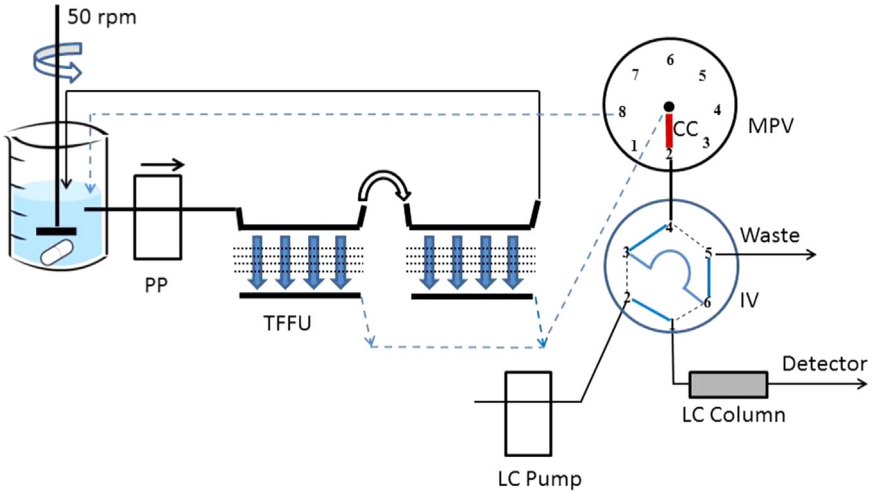

Fig. 1. Diagrammatic description of the flow setup with on-line tangential filtration for automatic kinetic dissolution tests of food supplements as a front end to HPLC. PP – peristaltic pump, TFFU – two tangential flow filtration units, MPV – multi-position valve, IV – injection valve, LC-liquid chromatography, CC-communication channel.

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