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# A validated UHPLC method for the determination of caffeoylquinic and di-caffeoylquinic acids in green coffee extracts using an RP-Amide fused-core column

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ABSTRACT

The presented work describes the development and validation of a rapid UHPLC-UV method using a fused core particle column with an RP-Amide stationary phase for the separation and quantitative analysis of caffeoylquinic and di-caffeoylquinic acids in green coffee extracts. Three caffeoylquinic acids (3-caffeoylquinic acid, 4-caffeoylquinic acid, and 5-caffeoylquinic acid) and two di-caffeoylquinic acids (1,3-di-caffeoylquinic acid, and 3,5-di-caffeoylquinic acid) were separated and analyzed in 8 min. That was possible due to the unique selectivity of the RP-Amide stationary phase for the analyzed acids. The retention behavior of all analytes under different compositions of the mobile phase on different columns was evaluated in this study. The optimal chromatographic separation was performed using an Ascentis Express RP-Amide (100 × 2.1 mm) fused-core column with a particle size of 2.7 μm at a temperature of 30 °C. For validation of the newly developed method, acetonitrile was used as mobile phase B and 5% formic acid, filtrated through a 0.22 μm filter, was used as mobile phase A. They were delivered at a flow rate of 0.9 mL $min^{-1}$ according to the elution gradient program. The detection wavelength was set at 325 nm. A solid-liquid extraction with a solution of methanol and a 5% water solution of formic acid (25 + 75 v/v) using an ultrasonic bath was chosen for the preparation of the available commercial samples of food supplements containing a green coffee extract. Recoveries for all analyzed acids were 98.2–101.0% and the relative standard deviation ranged from 0.3% to 1.4% for intra-day and from 0.3% to 3.0% for inter-day repeatability. The limits of detection were in the range of 0.30–0.53 μg $mL^{-1}$.



## 1. Introduction

Isomers of chlorogenic acid (CGA) are phenolic compounds, cinnamic acid derivatives, with biological effects mostly related to their antioxidant and anti-inflammatory activities. Caffeoylquinic acids (CQA) and di-caffeoylquinic acids (di-CQA) are the main derivatives of chlorogenic acid, which is found in plant extracts [1]. Today, green coffee extract is being used to produce food supplements, because it is a major source of chlorogenic acid [2,3]. These food supplements have attracted a lot of attention for their promise of quick weight loss. Among the health benefits attributed to CGA derivatives are a reduced relative risk of cardiovascular disease and type 2 diabetes mellitus [4,5], antibacterial activity [6], anti-inflammatory effects [7], and the ability to slow the release of glucose into the bloodstream after consuming a meal [8,9]. Nevertheless, the content of caffeoylquinic acids in green coffee extracts might differ depending on the extraction processes used, the loss of compounds during the manufacturing process and the source of the green coffee bean raw materials.

In this presented study, a new UHPLC method for the separation and determination of three caffeoylquinic acids (3-CQA, 4-CQA, and 5-CQA) and two di-caffeoylquinic acids (1,3-di-CQA, and 3,5-di-CQA), which were found in samples of food supplements containing green coffee extract, was developed. Three caffeoylquinic acids are also known as chlorogenic acid (3-CQA), cryptochlorogenic acid (4-CQA) and neochlorogenic acid (5-CQA). Isochlorogenic acid A is another name for 3,5-di-caffeoylquinic acid. Despite the great popularity of green coffee beans and food supplements containing green coffee extract, there are many general problems associated with food supplements (e.g. a legal classification, a lack of guarantees of efficiency and safety, the purity and stability of used substances, undeclared substances, etc.), as well as with the

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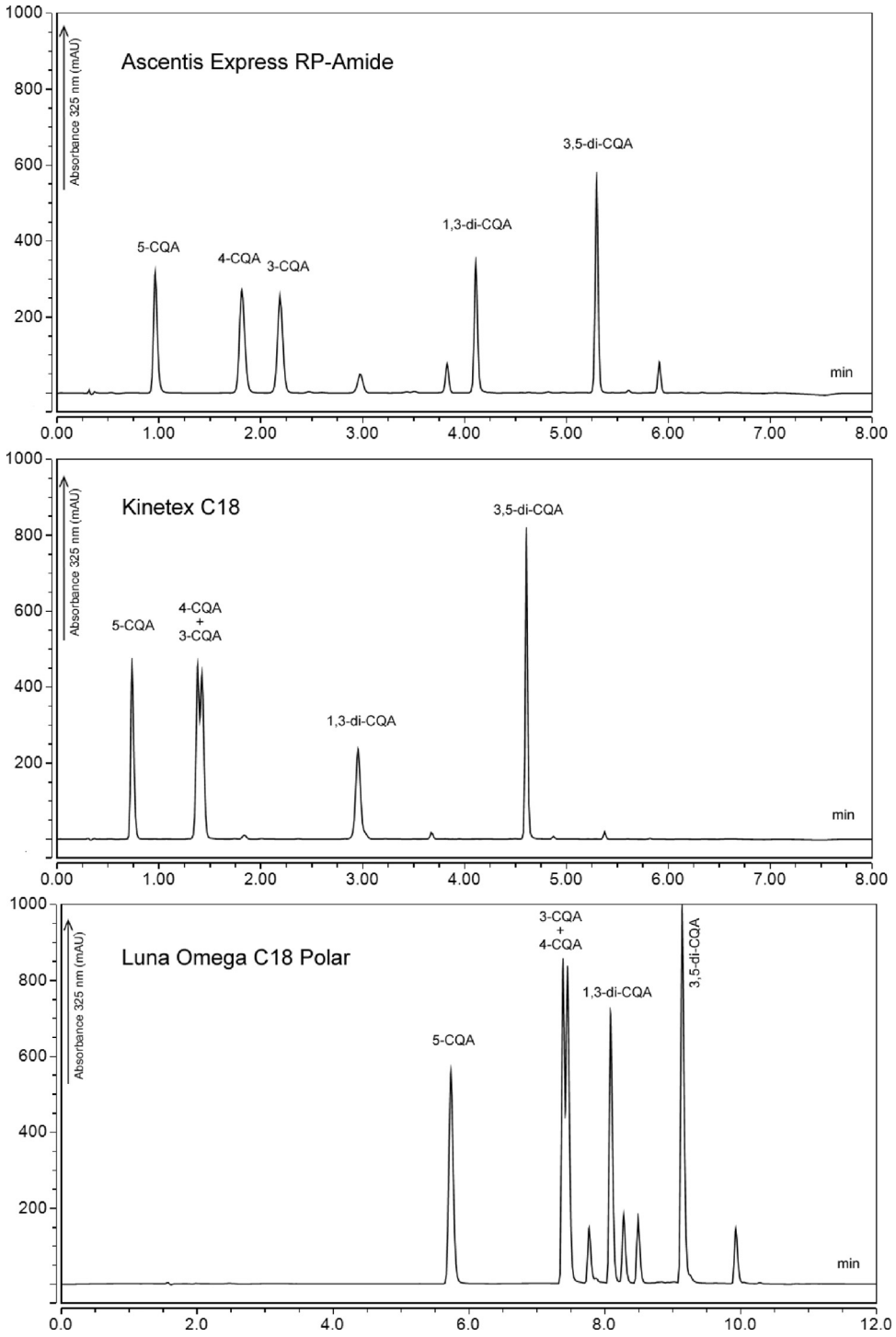


**Fig. 1.** Comparison of caffeoylquinic acids separation on three different stationary phases: Ascentis Express RP-Amide (100 × 2.1 mm, 2.7 μm), Kinetex C18 (100 × 2.1 mm, 2.6 μm), and Luna Omega C18 Polar (150 × 2.1 mm, 1.6 μm) using the optimal chromatographic conditions and 2% formic acid as mobile phase A for all columns.

plant extracts themselves. Producers declare the content of a plant extract at a certain amount in their preparation, but this information tells us absolutely nothing about the content of biologically active substances (chlorogenic acid and its derivatives in this case). As can be seen in previous studies, the potencies of plant extracts vary in different food supplement preparations [10,11]. There is a huge contradiction between the amount of used extract and the content of active substances in one dose, which can have a substantial effect on dosing and lead to an overdose by consumers or to an inefficacy of preparation. To maintain the consumers' trust in the safety and efficacy of products containing plant extracts, new modern analytical methods and studies for the quality assurance of food supplement preparations must be developed.

Several analytical methods for the determination of caffeoylquinic acids in plant materials [12–15], roasted coffee [16,17], brewed coffee, human plasma, urine extracts [1,18], and dietary supplements [19,20] have been published. Liquid chromatography coupled with PDA and MS detection is the most commonly used method for the determination of caffeoylquinic acids [12–20]. Even though mass spectrometry detection provides for the struc-