ChromaDex

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APPLICATIONS

Chlorogenic Acids from Green Coffee by HPLC

Method Status: Scientifically Valid per cGMPs for Dietary Supplements

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Introduction

The antioxidant content of a green coffee extract is typically reported as the total concentration of chlorogenic acids, which comprise eight primary compounds. Chlorogenic acid itself is the primary peak detected in an HPLC/UV trace and can be as much as 50 % of the total peak area. When assaying the raw material product, it is essential to quantitate all chlorogenic acid like compounds to ensure accurate results.

However, the verification of label claim data in a finished product can be quite challenging due to the complex nature of the formulation, especially if eight compounds must be identified. Many botanicals are often co-formulated to contain multiple active compounds, which can make it difficult to interpret HPLC chromatograms. In this work, we also propose a method for finished products by monitoring only the content of chlorogenic acid. While this method is not meant to give a true determination of the total antioxidant content, it does provide information about the consistency of the finished product.

Botanical Information

Green coffee

Botanical Name

Coffea canephora and *Coffea arabica* are the two most commercially cultivated species

Common Names

Coffee, Arabica and Robusta

Plant Description

An evergreen shrub or small tree that grows to 15 feet in height when un-pruned, the coffee plant has glossy dark green leaves approximately 4-6 inches long and 2.5 inches wide. Clusters of white fragrant flowers bloom simultaneously generating green oval berries which turn yellow then red as they ripen. The term green coffee refers to coffee beans that have not yet been roasted.

Therapeutic Use Overview

Chlorogenic acid is a polyphenolic compound that is known to be a natural antioxidant which neutralizes free radicals in the body. It can also play a significant role within the body's natural biological processes due to its anti-inflammatory and antioxidant activities.

Experimental

HPLC analysis was performed using an Agilent® 1100 LC system (Agilent Technologies Inc., Palo Alto, CA, USA). The system was optimized in order to reduce dead volume and improve performance including increasing the UV scan rate, changing the injector needle seat, re-plumbing the system with red PEEKsil™ Tubing (SGE), and using a semi-micro flow cell. Two analytical methods were compared using chlorogenic acid standards (ChromaDex) from green coffee extract. The first method utilized a Luna® 5 µm C18(2) 250 x 4.6 mm fully porous HPLC column. The second method utilized a Kinetex® 5 µm C18 100 x 4.6 mm core-shell HPLC column. After comparing methods, the preferred method was used to analyze chlorogenic acids in commercially available products which were purchased from a local health store.

Figure 1.
Green Coffee Extract Sample Using a Luna 5 µm HPLC Column

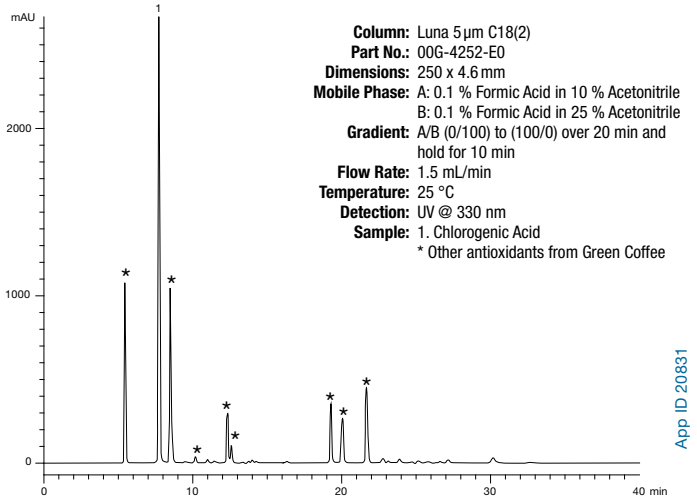

Figure 2.
Green Coffee Extract Sample Using a Kinetex 5 µm Core-shell HPLC Column

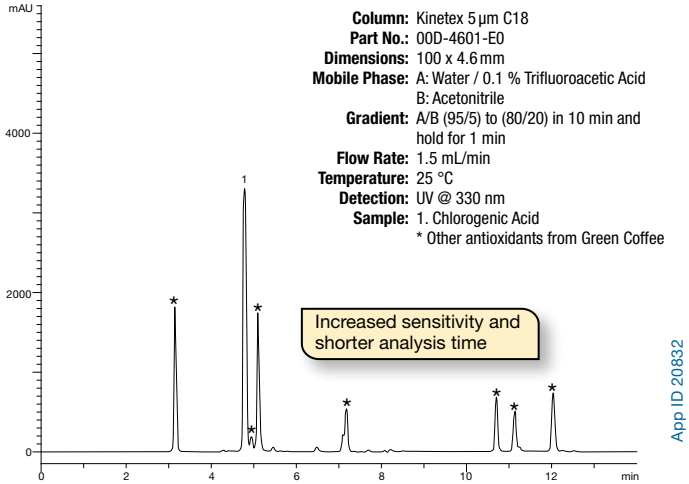

Figure 3.
Calibration Curve for Chlorogenic Acid from 1.25 to 500 µg/mL

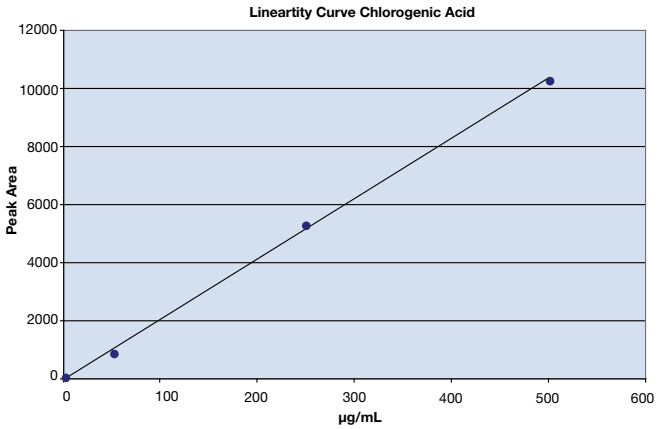

Table 1.
Calibration Curve from Kinetex® 5 µm Core-Shell HPLC Column Based on ChromaDex Reference Standards

	Linearity Equation	R^2	LOQ
Chlorogenic Acid	y = 20.754x – 24.563	0.9997	1.25 µg/mL

Table 2.
Determination of Method Accuracy

	250 µg/mL	500 µg/mL
	Area	Area
Sample 1	254.05	497.83
Sample 2	256.27	495.80
Sample 3	255.87	499.55
Average	255.40	497.73
STDEV	1.18	1.88
% CV	0.46 %	0.38 %

Figure 4.
Analysis of Brewed Coffee Sample Using Kinetex 5 µm Core-Shell HPLC Column

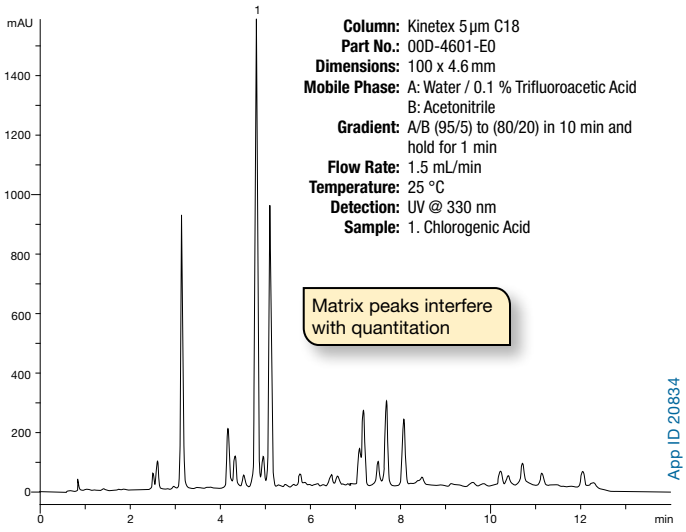

Figure 5.
Analysis of Commercially Available Green Coffee Energy Drink Using Kinetex 5 µm Core-Shell HPLC Column

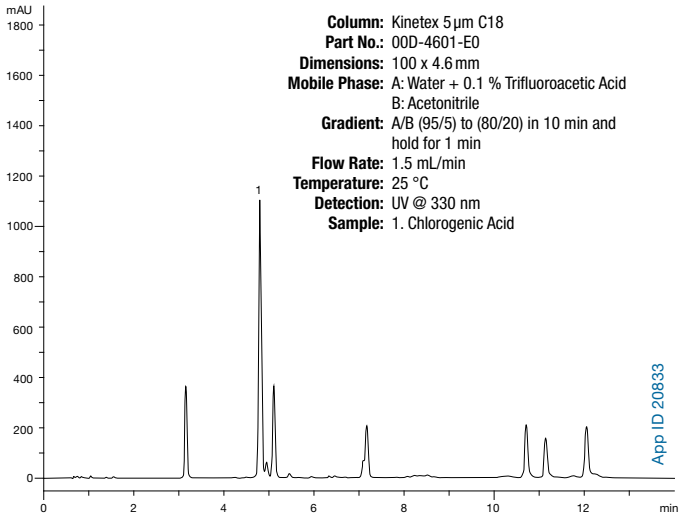

Table 3.
Reference Values for Chlorogenic Acid from Different Products Using a Kinetex 5 µm Core-Shell HPLC Column

Material	Total Antioxidant Content	Concentration of Chlorogenic Acid
Green Coffee Extract	450 mg/g	188 mg/g
Green Coffee Energy Drink (355 mL)	Unable to Determine	0.21 mg/mL
Ground Black Coffee	Unable to Determine	6.5 mg/g

Results and Discussion

The original method supplied by ChromaDex Inc, was meant to provide total antioxidant content in a raw material extract. The method required a total of 40 minutes to elute all chlorogenic acids and re-equilibrate the column (**Figure 1**). The major component in the raw material extract is chlorogenic acid and accounts for almost 50 % of the total content.

Kinetex Core-Shell Technology allows scientists to achieve substantially higher chromatographic efficiencies at much lower pressures than the equivalent fully porous material. Kinetex core-shell columns enhance the performance of any existing HPLC platform, including UHPLC systems.

For those labs that have older HPLC systems with pressure limitations, Kinetex 5 µm core-shell columns allow for substantial improvement in chromatography.

The increased efficiency of the Kinetex core-shell 5 µm column allowed us to reduce the column length to 100 mm and achieve similar if not better resolution. When the gradient was scaled to the new column dimensions, chlorogenic acid eluted in only 4.9 minutes and the total analysis was completed in only 15 min, a 64 % reduction in total analysis time. The corresponding decrease in peak width due to the increase in column efficiency caused the chlorogenic acid peak to go beyond the linear range of the UV detector (**Figure 2**). Samples had to be diluted in order to bring the signal intensities within the linear range. When analyzing botanicals and nutraceuticals, the determination of content in a finished product formulation can be very challenging. The potential for coelutions with other matrix components in a pill, tincture, or other formulation becomes greater the more peaks one has to

monitor. Even something like brewed coffee can produce a large number of additional matrix peaks that interfere with quantitation (**Figure 4**). It would be quite difficult to develop a method that would completely resolve all eight peaks in a reasonable amount of time.

Since chlorogenic acid is the main component in a green coffee extract, using it as a reference compound for assay of finished products greatly simplified the analysis. The increased efficiency of Kinetex® Core-Shell Technology allowed us to completely resolve chlorogenic acid from other matrix components in the brewed coffee and to achieve a more accurate quantitation.

Having demonstrated that the new method was suitable for testing the primary analyte in a finished product, we performed experiments to determine linearity, accuracy, range, and limit of quantitation (LOQ) of Kinetex Core-Shell Technology. Methods were shown to be linear in a range of 0.244 to 500 µg/mL (**Figure 3**). Chlorogenic acid had a very strong absorbance at 330 nm, limiting the range at which the method was linear.

The LOQ was determined to be 1.25 µg/mL for chlorogenic acid (**Table 1**). Accuracy and precision were determined at two different levels, 250 and 500 µg/mL and were found to be less than 1 % indicating that the method was quite reproducible (**Table 2**).

The final experiment tested the method for quantitation of chlorogenic acid in a commercially available formulation (**Figure 5**). The drink itself did not specify the content of chlorogenic acids, but we were able to detect 0.21 mg/mL or 74.55 mg total per serving.

If the same lot of green coffee raw material had been used for the finished product, we could have determined that about 1.12 g/L of the raw material had been added to the formulation.

Conclusion

The benefits of green coffee chlorogenic acid are currently being discussed extensively, and may lead to many nutraceutical companies adding green coffee chlorogenic acid to their formulation. We have proposed two methodologies for the determination of green coffee extracts.

The first method provides a total antioxidant content in a raw material sample. The second method allows for accurate quality control testing in formulated products. Analysis times for both methods were reduced by over 60 % by using Kinetex Core-Shell Technology columns.

Analysis of the green coffee extract raw material using the new Kinetex Core-Shell Technology HPLC method resulted in values that were consistent with the supplied certificate of analysis. We were able to easily resolve chlorogenic acid in both brewed coffee and a commercially available formulation containing green coffee extract.

ChromaDex Ordering Information

Phytochemical Reference Standards

Description	Quantity	Part No.
Chlorogenic Acid (P)	5 mg	ASB-00003450-005
Chlorogenic Acid (P)	10 mg	ASB-00003450-010
Chlorogenic Acid (P)	25 mg	ASB-00003450-025
Chlorogenic Acid (P)	50 mg	ASB-00003450-050
Chlorogenic Acid (P)	250 mg	ASB-00003450-250

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Phenomenex Ordering Information

Kinetex® Core-Shell HPLC Columns

5 µm Columns (mm)	50 x 2.1	SecurityGuard™ ULTRA Cartridges* 3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard ULTRA Cartridges* 3/pk
XB-C18	00B-4605-AN	AJ0-8782	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-AN	AJ0-8782	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
PFP	00B-4602-AN	AJ0-8787	00B-4602-E0	00D-4602-E0	00F-4602-E0	00G-4602-E0	AJ0-8773
Phenyl-Hexyl	00B-4603-AN	AJ0-8788	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
		for 2.1 mm ID					for 4.6 mm ID

* SecurityGuard ULTRA cartridges require holder, Part No. AJ0-9000.

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Scientifically Valid

Section 21CFR111.320 of cGMPs for Dietary Supplements requires you to "identify and use an appropriate scientifically valid method for each established specification for which testing or examination is required to determine whether the specification is met". The FDA does not elaborate on what is considered a scientifically valid method in the cGMPs. ChromaDex has defined scientifically valid as a method that meets minimum linearity, precision, sensitivity and range requirements. These requirements are outlined in an FDA laboratory document, ORA LABORATORY PROCEDURE Food and Drug Administration, ORA-LAB.5.4.5. This laboratory guidance document defines minimal performance attributes for selected methods of analysis and has been applied by ChromaDex to the selection of methods that are fit for purpose in the dietary supplements industry. According to the above definition, the method detailed in this document is considered scientifically valid as applicable to the cGMP requirements. Product specific, full method validations according to AOAC guidelines can be applied to customer samples upon request, to further document method performance in specific samples and matrices.

guarantee

If Phenomenex products in the technical note do not provide at least an equivalent separation as compared to other products of the same phase and comparable dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

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