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APPLICATIONS

HPLC-UV Analysis of Gingerol in Ginger Root

Method Status: Scientifically Valid per cGMPs for Dietary Supplements

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Introduction

Identity testing of raw materials for potency and verification of label claim data in nutraceutical formulations are requirements of dietary supplement cGMPs. Manufacturers and contract testing labs alike are looking for accurate and scientifically valid methods that are suitable for use with different formulations. The complex nature of nutraceuticals and botanicals often requires long analysis and difficult sample cleanup steps to resolve matrix interferences.

There were two primary goals of this project: 1) to optimize our pre-exisiting method to reduce total analysis time using newer high efficiency HPLC technologies and 2) evaluate the quantitative results obtained by using newly available gingerol and shogaol reference standards versus a traditional quantitation method that relied on using capsaicin as a reference standard.

Botanical Information

Ginger root

Botanical Name

Zingiber officinale

Common Names

Ginger, Jiang, Ardraka, Shunthi

Plant Description

Ginger originates from India and is now cultivated in many tropical countries. It is a perennial reed-like plant with leafy stems and grows to 3-4 feet tall. Producing clusters of white and pink flower buds that blossom into yellow flowers, it is often used as landscaping in subtropical homes.

Therapeutic Use Overview

The ginger rhizome is used as a spice in many cuisines in food, beverages and confections. It is also an important component of the traditional medicines of India, China, Indonesia and Japan. It has been used for motion sickness, nausea, indigestion and osteoarthritis. The gingerol compounds it contains are well known as antioxidants.

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. The fully porous Luna® 2.5 µm HST C18 100 x 2.0 mm and the Kinetex® Core-Shell Technology 5 µm C18 50 x 4.6 mm were from Phenomenex, Torrance, CA. All chromatographic conditions are specified on their corresponding chromatograms in **Figures 1** through **7**.

The ginger root biomass reference material, ginger extract reference material, 6-Gingerol, 8-Gingerol, 10-Gingerol, 6-Shogaol, 9-Shogaol, 10-Shogaol, Capsaicin and the analytical method on the Luna HST C18 were provided by ChromaDex®. The formulated products were purchased from a local health foods store.

All materials were dissolved in methanol/water (80:20) with 0.1 % TFA added as a stabilizer. Before injection, samples were filtered through a 0.45 µm Phenex™ PTFE syringe filter.

Figure 1.
Analytical Reference Standards Using Luna HST 2.5 µm Column

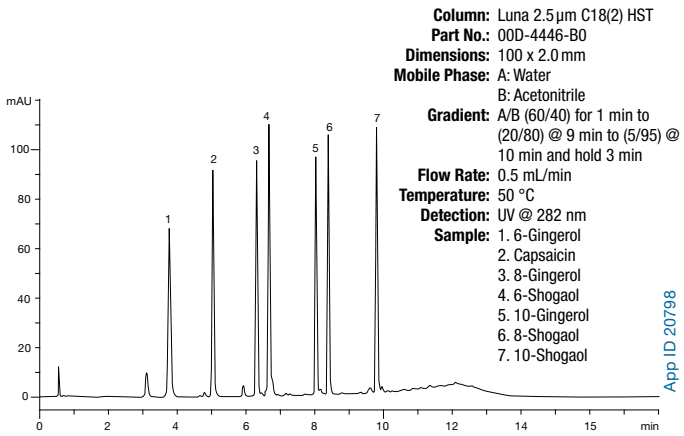

Figure 2.
Analytical Reference Standards using Kinetex 5 µm Core-Shell Technology Column

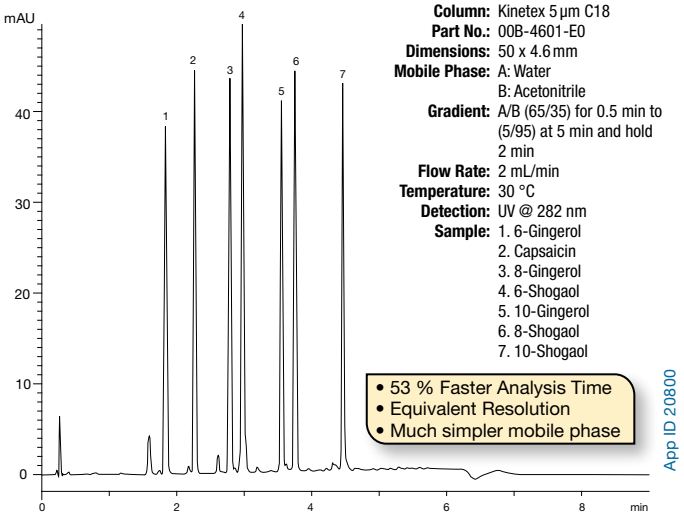

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Figure 3.
Ginger Root Biomass Sample Using Luna® HST 2.5 µm Column

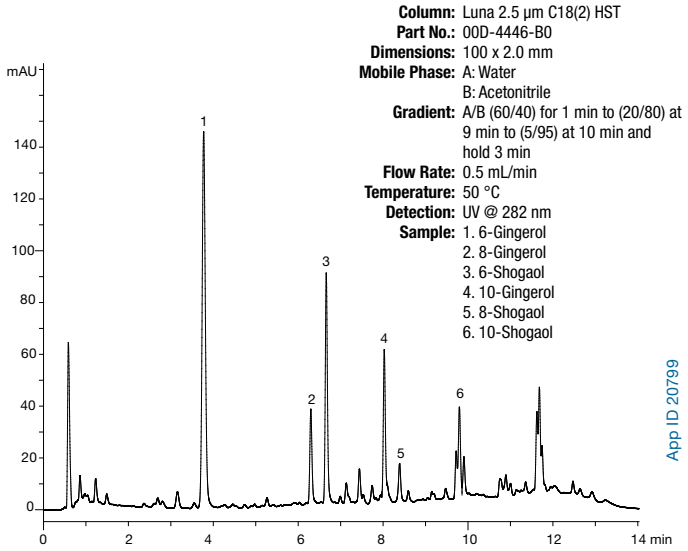


Figure 4.
Ginger Root Biomass Sample Using Kinetex® 5 µm Core-Shell Technology Column

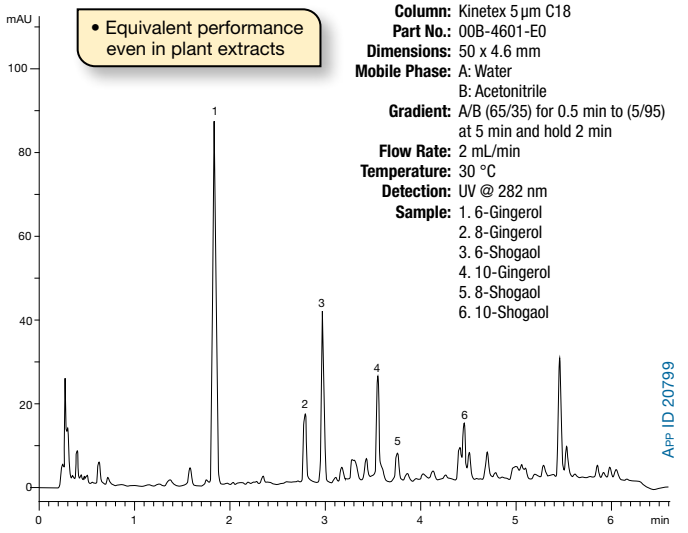


Figure 5.
Representative Calibration Curve for Gingerols from 1.66 µg/g to 500 µg/mL

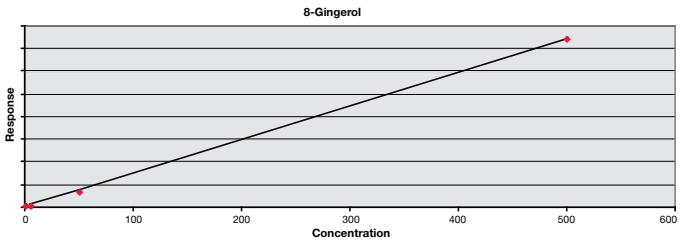


Table 1.
Calibration Curve from Kinetex 5 µm Core-Shell Technology Column Based on ChromaDex Reference Materials

Compound	Linearity Equation	R^2	LOQ
6-Gingerol	Y = 0.7255x – 0.8877	0.9999	1.66 µg/mL
8-Gingerol	Y = 0.7403x – 0.5055	0.9999	1.66 µg/mL
6-Shogaol	Y = 0.8887x – 0.8620	0.9999	1.66 µg/mL
10-Gingerol	Y = 0. 5122x – 0.3766	0.9999	1.66 µg/mL
8-Shogaol	Y = 0.7898x – 0.3010	0.9999	1.66 µg/mL
10-Shogaol	Y = 0.7822x – 1.4783	0.9999	1.66 µg/mL

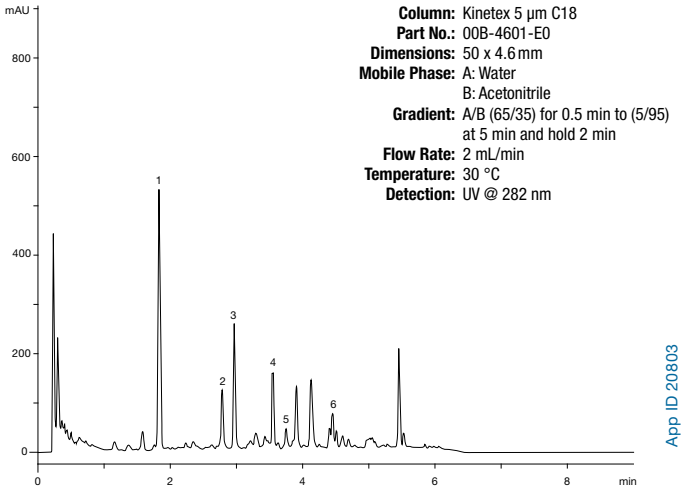
Table 2.
Comparison of Method Accuracy at 500 µg/mL Using Individual Standards vs. Capsaicin Reference

Compound	ChromaDex Standards	Capsaicin
6-Gingerol	100.0 %	83.8 %
8-Gingerol	100.0 %	82.0 %
6-Shogaol	100.0 %	68.4 %
10-Gingerol	100.0 %	118.6 %
8-Shogaol	100.0 %	76.9 %
10-Shogaol	100.0 %	77.8 %
Accuracy based on three replicated injections of the of 500 µ/mL standard		

Table 3.
Comparison of Quantitative Values from a Ginger Root Biomass Sample

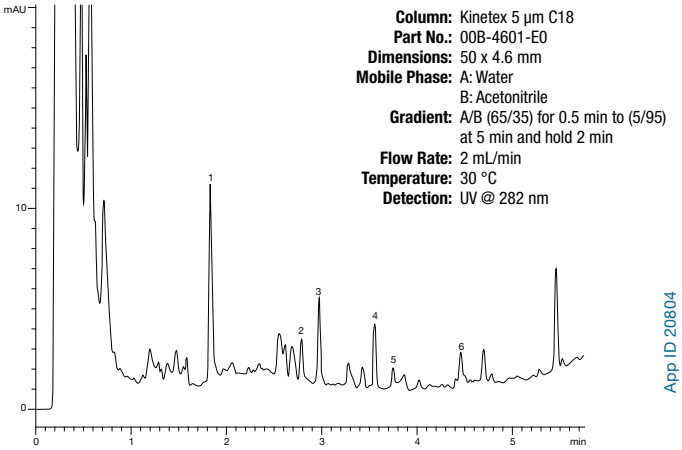
	Gingerol Extract (µg/g)		Gingerol Biomass (µg/g)	
Compound	Reference Standards	Capsaicin	Reference Standards	Capsaicin
6-Gingerol	232	280	866	801
8-Gingerol	70	46	255	136
6-Shogaol	125	118	629	495
10-Gingerol	74	68	251	192
8-Shogaol	16	16	91	78
10-Shogaol	43	45	177	161
Total	**583**	**573**	**2,270**	**1,863**
ChromaDex reference standards allow for more accurate determination of gingerol content, especially in difficult formulations				

Figure 6.
Analysis of Liquid Filled Gel Cap Formulation Using Kinetex 5 µm Core-Shell Technology Column



Peak	Compound	Conc./Capsule (mg)
1	6-Gingerol	15.08
2	8-Gingerol	3.13
3	6-Shogaol	5.84
4	10-Gingerol	5.16
5	8-Shogaol	0.8
6	10-Shogaol	2.03
	Total	32.04
	Label Claim	30

Figure 7.
Analysis of Powder Filled Gel Cap Formulations Using Kinetex® 5 µm Core-Shell Technology Column



App ID 20804

Peaks	Compound	Conc./Capsule (mg)
1	6-Gingerol	0.144
2	8-Gingerol	0.026
3	6-Shogaol	0.05
4	10-Gingerol	0.055
5	8-Shogaol	0.012
6	10-Shogaol	0.025
	Total	0.399
	Label Claim	???

Results and Discussion

The original method supplied by ChromaDex was already optimized on a high efficiency Luna® HST 2.5 µm fully porous column and is much faster than similar published separations. The methodology has been in routine use by ChromaDex for contract analytical testing for the past 5 years and has been shown to provide good separation and accurate results for gingerol components in a variety of botanical products.

The Kinetex Core-Shell Technology allows scientists to achieve substantially higher chromatographic efficiencies at much lower pressures than the equivalent fully porous material. The Kinetex materials enhance the performance of any existing HPLC platform, including UHPLC systems. For those labs that have older HPLC systems without high pressure capabilities, the Kinetex 2.6 µm and 5 µm columns can substantially improve the useable lifetime of these systems.

The increased separation ability of the Kinetex 5 µm column allowed the column length to be shortened by 67 % to a 50 mm length. This reduction in length, combined with gradient conditions that were scaled to match the new column dimensions reduced the analysis time by over 50 %. Resolution and sensitivity were similar to the existing method (**Figures 1** and **2**).

When analyzing botanicals and nutraceuticals, the separation of standards can often be misleading since the plant extract can contain many other endogenous components that could lead to poor results. To demonstrate specificity of the new method, ginger root biomass reference materials were compared using the fully porous Luna HST method versus the Kinetex Core-Shell Technology method. Resolution from matrix components was similar on both columns (**Figures 3** and **4**).

Having demonstrated that the new method provided equivalent results, we performed experiments to determine linearity, accuracy, range, and limit of quantitation (LOQ) of the new Kinetex method. Methods were shown to be linear over a range of 5 to 2,000 µg/mL. The LOQ was determined to be 1.66 µg/mL for all of the compounds. This methodology produced a Signal to Noise ratio of > 10 for the 10-Gingerol peak, which was the smallest peak of interest from the isolated standard (**Table 1**).

There are several different ways to quantitate the components of interest with this methodology. One way is to produce a linearity curve for each component based on the ChromaDex reference standard and use the corresponding linear equation from this data for each component. Another is to spike each sample with a known amount of capsaicin and quantitate against this internal standard, which has been done historically because there were no suitable reference materials available.

The quantitation based on individual reference standards provided higher accuracy than the capsaicin method (**Table 2**). When this quantitation was applied to real samples, the calculated concentration in the ginger extract gave higher calculated values for total gingerol (**Table 3**). When calibrating for compounds in a difficult matrix, it is always preferred to use a reference standard of the actual compound of interest. When using alternative compounds, there is always the potential for unknown co-elutions that could bias the data high or low.

The final experiment was to analyze commercially available formulations and determine if the results were similar to the label claim. To ensure that we properly tested our new assay, we attempted to choose difficult formulations such as those with multiple active components and those with gel cap pills. Analysis of two formulated products showed that the values obtained are consistent with label claims.

Conclusion

HPLC analysis times were reduced by over 50 % by adapting the current method from the Luna HST to the Kinetex Core-Shell Technology method. We performed experiments to verify that the methodology was suitable for nutraceutical products. Final results indicate that this method is robust and ready for routine sample analysis.

Quantitative determination using individual reference standards was the best way to achieve accurate quantitation, especially in formulations containing other botanical products. The label claims of some products are also quite difficult to interpret, making it difficult for a consumer to understand the value of the botanical they are taking. Providing standardized reference methods for analysis is the first step to ensuring quality in nutraceutical products.

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 application 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.

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ChromaDex Ordering Information

Phytochemical Reference Standards

Description	Quantity	Part No.
6-Gingerol (P)	5 mg	ASB-00007164-005
6-Gingerol (P)	10 mg	ASB-00007164-010
6-Gingerol (P)	25 mg	ASB-00007164-025
8-Gingerol (P)	5 mg	ASB-00007163-005
8-Gingerol (P)	10 mg	ASB-00007163-010
8-Gingerol (P)	25 mg	ASB-00007163-025
10-Gingerol (P)	5 mg	ASB-00007162-005
10-Gingerol (P)	10 mg	ASB-00007162-010
10-Gingerol (P)	25 mg	ASB-00007162-025
6-Shogaol (P)	5 mg	ASB-00019211-005
6-Shogaol (P)	10 mg	ASB-00019211-010
6-Shogaol (P)	25 mg	ASB-00019211-025
8-Shogaol (P)	5 mg	ASB-00019212-005
8-Shogaol (P)	10 mg	ASB-00019212-010
8-Shogaol (P)	25 mg	ASB-00019212-025
10-Shogaol (P)	5 mg	ASB-00019214-005
10-Shogaol (P)	10 mg	ASB-00019214-010
10-Shogaol (P)	25 mg	ASB-00019214-025
Capsaicin(methyl-n-vanillyl-6-Noneneamide)(P)	5 mg	ASB-00003135-005
Capsaicin(methyl-n-vanillyl-6-Noneneamide)(P)	10 mg	ASB-00003135-010
Capsaicin(methyl-n-vanillyl-6-Noneneamide)(P)	25 mg	ASB-00003135-025
Ginger Standards Kit (P)	7x 5 mg	KIT-00007615-005
Ginger Standards Kit (P)	7x 10 mg	KIT-00007615-010

Botanical Reference Materials

Description	Quantity	Part No.
Ginger (Zingiber officinale) Root (BRM)	5 g	ASB-00030290-005
Ginger (Zingiber officinale) Root (RGBRM)	5 g	ASB-00030863-005
Ginger (Zingiber officinale) Root peeled (VBRM)	5 g	ASB-00030956-005

Phenomenex Ordering Information

Kinetex® Core-Shell HPLC Columns

5 µm Columns (mm)		SecurityGuard™ ULTRA Cartridges*					SecurityGuard ULTRA Cartridges*
	50 x 2.1	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	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.

guarantee

If Phenomenex products in this 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.

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