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February 11, 2020

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5001 Campus Drive
College Park, MD 20740

Attention: Dr. Susan Carlson
Re: GRAS Notification – *Dihydroquercetin*

Dear Dr. Carlson:

GRAS Associates, LLC, acting as the Agent for Blue California, is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notification for *Dihydroquercetin.* Along with Blue California's determination of safety, an Expert Panel of qualified persons was assembled to assess the composite safety information of the subject substance with the intended use as an ingredient in non-alcoholic beverages (up to 0.02 g per L), flavored fermented milk and dairy products (up to 0.02 g per kg), and chocolate products (up to 0.07 g per kg).The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

William J. Rowe, President
Agent for Blue California
GRAS Associates, LLC
11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852
wrowe@nutrasource.ca

Enclosure: GRAS Notification for Blue California – *Dihydroquercetin*

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: 2019/08/22

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☐

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Hadi Omrani	Technical Director - Regulatory Affairs
Organization *(if applicable)*	
Blue California	
Mailing Address *(number and street)*	
30111 Tomas	

City	State or Province	Zip Code/Postal Code	Country
Rancho Santa Margarita	California	92688	United States of America

Telephone Number	Fax Number	E-Mail Address
949-635-1991 X29	949-635-1984	hadi@bluecal-ingredients.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
William J. Rowe	President
Organization *(if applicable)*	
GRAS Associates	
Mailing Address *(number and street)*	
11810 Grand Park Ave, Suite 500	

City	State or Province	Zip Code/Postal Code	Country
North Bethesda	Maryland	20852	United States of America

Telephone Number	Fax Number	E-Mail Address
519-341-3667	888-531-3466	wrowe@nutrasource.ca

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Dihydroquercetin

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

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6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Dihydroquercetin is intended to be used as an ingredient in conventional non-alcoholic beverages (up to 0.02 g per L), flavored fermented milk and dairy products (up to 0.02 g per kg), and chocolate products (up to 0.07 g per kg).

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Blue California
(name of notifier)

has concluded that the intended use*(s)* of Dihydroquercetin
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Blue California agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them;
(name of notifier)

agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

30111 Tomas, Rancho Santa Margarita, CA 92688
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Katrina Emmel on behalf of William J. Rowe, President	2/11/2020

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Multiple Appendices -- Appendices 1 through 6	

GRAS Notification

of

Dihydroquercetin
(DHQ)

Food Usage Conditions for General Recognition of Safety

on behalf of

Blue California
30111 Tomas
Rancho Santa Margarita, CA 92688

2/11/20

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FIGURES

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FOREWORD

Blue California based its Generally Recognized as Safe (GRAS) assessment of dihydroquercetin (DHQ) primarily on the composite safety information, i.e., scientific procedures with corroboration from history of use. The safety/toxicity of DHQ, history of use of DHQ, and compositional details, specifications, and method of preparation of the subject ingredient were reviewed. In addition, a search of the scientific and regulatory literature was conducted through December 2, 2019 with particular attention paid to adverse reports, as well as those that supported conclusions of safety. Those references that were deemed pertinent to this review are listed in Part 7. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

At Blue California's request, GRAS Associates, LLC ("GRAS Associates") convened an Expert Panel to complete an independent safety evaluation of Blue California's DHQ preparation. The purpose of the evaluation is to ascertain whether the intended food uses of DHQ as described in Part 3 are generally recognized as safe, i.e., GRAS, under the intended conditions of use, as concluded by Blue California. In addition, Blue California has asked GRAS Associates to act as Agent for the submission of this GRAS notification.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

A. Basis of Exclusion from the Requirement for Premarket Approval Pursuant to Subpart E of 170[1]

Blue California has concluded that its ≥ 95% DHQ preparation, BC-DHQ™, is GRAS under Section 201(s) of the Federal Food, Drug, and Cosmetic (FD&C) Act.[2] This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based primarily on scientific procedures as described in the following sections. The evaluation accurately reflects the intended conditions of food use for the designated BC-DHQ™ preparation.

Signed:

Agent for Blue California

William J. Rowe
President
GRAS Associates LLC

Date: 2/11/20

[1] See 81 FR 54960, 17 August 2016. Accessible at: https://www.gpo.gov/fdsys/pkg/FR-2016-08-17/pdf/2016-19164.pdf (Accessed 2/2/2020).
[2] Available at: https://legcounsel.house.gov/Comps/Federal%20Food,%20Drug,%20And%20Cosmetic%20Act.pdf (Accessed 2/2/2020).

11810 Grand Park Avenue
Suite 500
North Bethesda, MD 20852

B. Name and Address of Responsible Party

Blue California
30111 Tomas
Rancho Santa Margarita, CA 92688

As the Responsible Party, Blue California accepts responsibility for the GRAS conclusion that has been made for its ≥ 95% DHQ preparation, which is also referred to BC-DHQ™, as described in the subject safety evaluation; consequently, Blue California's BC-DHQ™ preparation, which meets the conditions described herein, is not subject to premarket approval requirements for food ingredients.

C. Common Name and Identity of Notified Substance

The common name of the ingredient to be used on food labels is dihydroquercetin and is abbreviated as DHQ throughout this document.

D. Conditions of Intended Use in Food

Blue California's BC-DHQ™ preparation, which contains ≥ 95% DHQ, is intended to be added as an ingredient in various food categories as described in Part 3. The serving levels reflect good manufacturing practices principles in that the quantities added to foods should not exceed the amounts reasonably required.

E. Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30(a) and (b), Blue California's BC-DHQ™ preparation (≥ 95% DHQ) has been concluded to be GRAS based on scientific procedures as discussed in the detailed description provided below.

BC-DHQ™ is not subject to premarket approval requirements of the FD&C Act based on Blue California's conclusion that the substance is GRAS under the conditions of its intended food use.

Blue California certifies, to the best of our knowledge, that this GRAS notice is a complete, representative, and balanced assessment that includes all relevant information available---both favorable and unfavorable---that is pertinent to the evaluation of the safety and GRAS status of the subject ≥ 95% DHQ preparation. The preparation of this safety evaluation also included a comprehensive literature search through December 2, 2019.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notice will be maintained at the offices of Blue California, Rancho Santa Margarita, CA, and will be made available during customary business hours.

Blue California certifies that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA). No non-public, safety-related data were used by the Expert Panel to reach a GRAS conclusion.

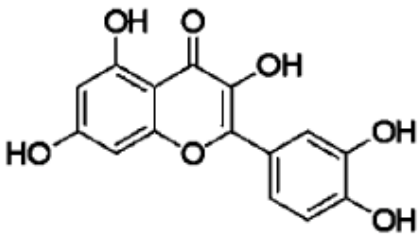
PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Chemical Identity of Ingredient

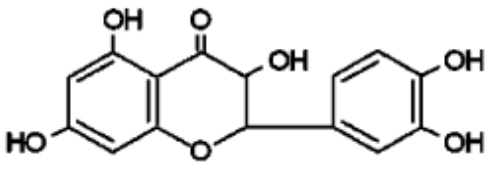
Flavonoids are a diverse chemical class of secondary metabolites universally found in the plant kingdom (Fowler and Koffas, 2009). Vegetables and fruits contain many flavonoids in the form of flavonols, flavones, and flavanones. It is estimated that the dietary intake of flavonoids ranges from 0.05 to 1 gram per person per day (Stevens et al., 1999). The total flavonol and flavone intakes are reported to be between 3 and 65 mg per day, where the lowest reported intake is in Finland and the highest intake is in Japan (Justesen et al., 2000).

Dihydroquercetin, which is commonly referred to in the literature as taxifolin, is a flavanol that is structurally similar to quercetin. The chemical structures of quercetin and DHQ are provided in Figure 1. DHQ was confirmed to be in the (2R,3R)-*trans* configuration by circular dichroism, proton nuclear magnetic resonance (^{1}H-NMR), and ^{1}H-^{1}H correlation spectroscopy 2-dimensional nuclear magnetic resonance (COSY 2D NMR) analyses (Appendix 1).

Figure 1. Chemical Structures of Quercetin and Dihydroquercetin[a]



Quercetin



Dihydroquercetin

[a] Adapted from Vladimirov et al. (2009).

Common or Usual Name:	Dihydroquercetin
Chemical Name:	(2R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one; (2R,3R)-3,3',4',5,7-pentahydroxyflavanone
Synonyms:	Taxifolin; Diquertin; (2R,3R)-*trans*-Dihydroquercetin, (2R,3R)-Dihydroquercetin; Taxifoliol; Dystylin; Catechin hydrate; (+)-Dihydroquercetin; (+)-Taxifolin; DHQ
CAS Number:	480-18-2
Molecular Formula:	$C_{15}H_{12}O_7$
Molecular Mass:	304.25 daltons

Dihydroquercetin has been the subject of numerous studies and US patents since the early 1950s when Giessman and Lischner first determined the chemical structure (Gupta et al., 1971).

In certain foods, such as peanuts, white wine, and onions, DHQ is present as both an aglycone and a glycoside (Itaya and Igarashi, 1992; Oi et al., 2012; Singleton and Trousdale, 1983). Additionally, DHQ has also been identified as a component of bee pollen (Silva et al., 2009). A list of foods in which DHQ has been identified is provided in Table 8.

B. Manufacturing Processes

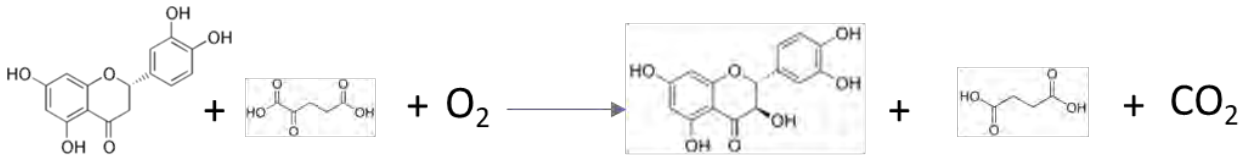
Blue California uses an enzymatic bioconversion reaction to produce BC-DHQ™ high purity DHQ from eriodictyol, a bitter-masking flavanone extracted from plant materials. Eriodictyol is converted to DHQ by flavanone 3β-hydroxylase (F3H), a ubiquitous enzyme found in higher order plants that catalyzes the following reaction:

$$\text{Flavone} + \text{2-oxoglutarate} + O_2 \leftrightarrow \text{dihydroflavonol} + \text{succinate} + CO_2$$

Blue California uses a nonpathogenic and nontoxigenic strain of wild-type *Escherichia coli* K12 W3110 to produce F3H. The microbe is a gram-negative, non-spore forming, facultative anaerobe, with a long history of safe industrial use. *E. coli* K12 is the most commonly used industrial strain and is GRAS under 21 CFR 170.36.

The conversion of eriodictyol to DHQ, by F3H enzyme in the presence of 2-oxoglutarate, is shown in Figure 2.

Figure 2. Bioconversion of Eriodictyol to Dihydroquercetin



eriodictoyl 2-oxoglutarate oxygen dihydroquercetin succinate carbon dioxide

1. Fermentation Process

The glycerol stock of *E. coli* W3110 strain (carrying apple F3H gene) is removed from storage at -70°C, thawed to room temperature, and grown in 50-mL LB culture seed media at 37°C. After 16 hours, the growing Seed Culture 1 is transferred to 2-L LB culture seed media as Seed Culture 2. When the cells read OD_{600} = 5, they are transferred to 500-L fermenters.[3] This Seed Culture 3 is then transferred to a 60-ton production fermenter.

The *E. coli* W3110 strain cells are cultured in the presence of a peptone yeast extract[4] for 24 hours and then harvested by centrifugation. The cells are passed through a homogenizer, and the resulting mixture is separated by another centrifugation step. The supernatant is passed through an ion exchange column which retains the F3H enzyme. F3H is then eluted from the column with sodium chloride solution and mixed with a reaction buffer in a 60-ton reaction tank with slow agitation. The reaction buffer is prepared with ferrous sulfate ($FeSO_4$) and disodium phosphate (Na_2HPO_4), after which the pH is adjusted with phosphoric acid (H_3PO_4).

Eriodictyol, derived from orange peel, is dissolved in methanol and fed into the reaction tank containing the enzyme-buffer mixture. The reaction is allowed to proceed to completion, which is verified by high performance liquid chromatography (HPLC) analysis. The reaction mixture is then heated to 85°C for 20 minutes to denature the enzymes, and the supernatant is removed for down-stream processing.

2. Extraction and Purification

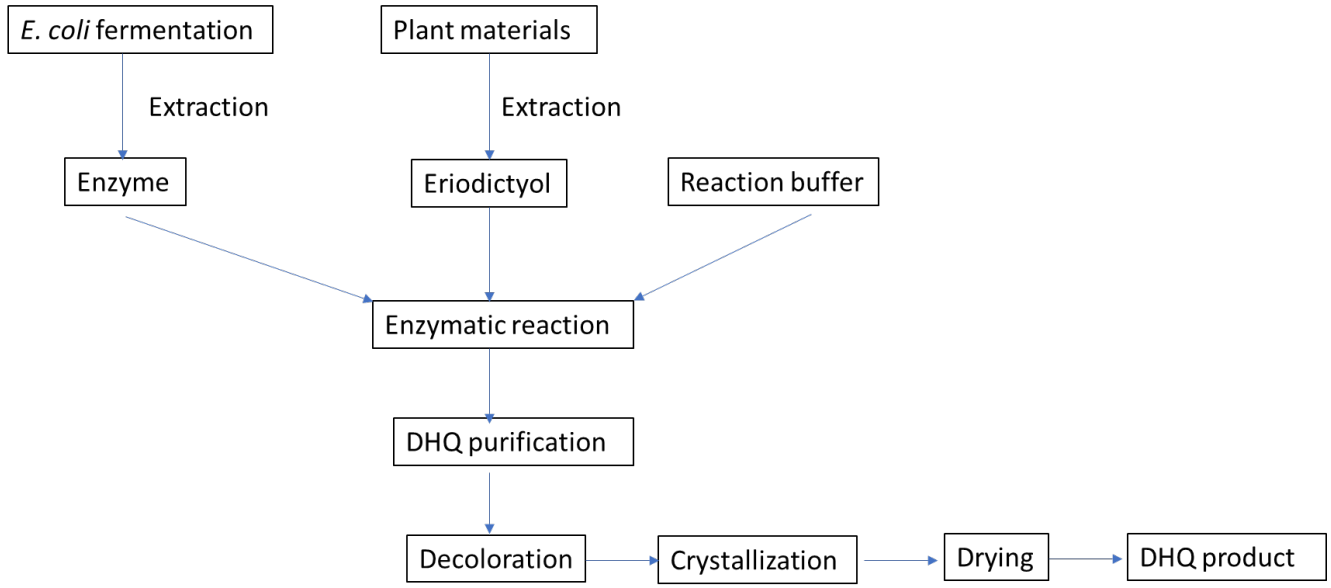
The enzymatic conversion mixture is centrifuged and the supernatant is transferred to an ion-exchange resin column. The column is washed with warm water and DHQ is eluted with food grade ethanol. The eluent is condensed with a wipe-film evaporator, and the condensate is then transferred to a crystallization tank and crystallized by chilling. The crystals are subsequently re-dissolved in water and the solution is passed through activated charcoal to remove any colorant from fermentation. The resulting high purity DHQ preparation is dried in a baking oven and crushed into fine powder.

[3] Blue California uses older, larger cells to perform the measurement.
[4] Peptone yeast extract aids in *E. coli* cell growth, and ultimately increases enzyme production.

A manufacturing process flow chart for the production of BC-DHQ™ high purity DHQ is provided in Figure 3.

Raw materials used in the manufacturing process are suitable food-grade materials and are used in accordance with applicable US Federal Regulations and Current Good Manufacturing Practice (CGMP). All resins and processing aids are food grade materials. Supporting documentation for the raw materials and processing aids is provided in Appendix 2.

Figure 3. Manufacturing Flow Chart for BC-DHQ™



C. Product Specifications

1. Specifications for Dihydroquercetin

There are no known established standardized specifications for DHQ; however, specifications for Ametis JSC's taxifolin-rich *Larix gmelinii* preparation were reviewed and reported on by the European Food Safety Authority (EFSA) (Turck et al., 2017). Blue California has adopted product specifications for our DHQ that are comparable to Ametis JSC's specifications, as reported to EFSA (Turck et al., 2017) for DHQ as a consumable human food substance. Ametis JSC's specifications and specifications for Blue California's DHQ preparation are shown in Table 1.

Table 1. Specifications and Analysis for Blue California's BC-DHQ™

Physical and Chemical Parameters	Ametis JSC's Dihydroquercetin Specifications[a]	Blue California's BC-DHQ™	
		Specification	Method
Appearance Form & Color	White or straw-coloured powder	Off white to white powder	Visual
Moisture	≤ 10%	≤ 5%	USP
Bulk Density	NS	≥ 0.15 g/mL	USP
Tap Density	NS	≥ 0.30 g/mL	USP
Particle Size	NS	> 95% through Mesh #60 sieve	USP
Taxifolin	≥ 90.0% (on dry basis)	≥ 95% (as dihydroquercetin, on dry basis)	HPLC
Ethanol	< 5,000 mg/kg	< 1,000 ppm	USP
Methanol	NS	< 200 ppm	USP
Dichlorodiphenyltrichloroethane (DDT)	≤ 0.05 mg/kg	NS[b]	NA
Heavy Metals	NS	< 10 ppm	USP
Lead	≤ 0.5 mg/kg	< 0.5 ppm	ICP-MS
Arsenic	≤ 0.02 mg/kg	< 0.5 ppm	ICP-MS
Cadmium	≤ 0.5 mg/kg	< 0.5 ppm	ICP-MS
Mercury	≤ 0.1 mg/kg	< 0.5 ppm	ICP-MS
Total Viable Count	≤ 10,000 cfu/g	< 5,000 cfu/g	AOAC
Enterobacteria + Div. Gram-Negative Bacteria	≤ 100 cfu/g	NS	NA
Total Coliform	NS	< 100 cfu/g	AOAC
Total Yeast & Mold	≤ 100 cfu/g	< 100 cfu/g	AOAC
E. coli	Negative in 1 g	Negative	AOAC
Salmonella spp.	Negative in 10 g	Negative	AOAC
Staphylococcus aureus	Negative in 1 g	NS	NA
Pseudomonas spp.	Negative in 1 g	NS	NA

[a] From Turck et al. (2017)

[b] Blue California does not have a specification for DDT since BC-DHQ™ is derived from a fermentation process. However, DDT was an analyte in pesticide screens conducted on five representative lots of BC-DHQ™ (Appendix 5) and no concerns were noted upon review.

NS – Not specified; NA – Not applicable; USP – United States Pharmacopeia; HPLC – High Performance Liquid Chromatography; ICP-MS – Inductively Coupled Plasma-Mass Spectrometry; AOAC – Association of Official Analytical Chemists; ppm – Parts per million; cfu – Colony forming unit

2. Specifications for Blue California's Dihydroquercetin Preparation and Supporting Methods

The compositions of five non-consecutive lots of Blue California's BC-DHQ™ preparation and product specifications, are provided in Table 2.

Table 2. Specifications for Blue California's Dihydroquercetin Preparation

Physical and Chemical Parameters	Blue California BC-DHQ™ Specifications	Results of Batch Numbers				
		7730-160823	7730-161028	7730-170425	7730-170525	7730-170616
Appearance Form & Color	Off white to white powder	Pass	Pass	Pass	Pass	Pass
Bulk Density	≥ 0.15 g/mL	0.16 g/mL	0.15 g/mL	0.16 g/mL	0.17 g/mL	0.16 g/mL
Tap Density	≥ 0.30 g/mL	0.32 g/mL	0.32 g/mL	0.34 g/mL	0.32 g/mL	0.32 g/mL
Particle Size	> 95% through mesh #60 sieve	100%	100%	100%	100%	100%
Dihydroquercetin Assay- HPLC	≥ 95% (on dry basis)	97.8%	97.8%	97.3%	95.2%	97.7%
Loss on Drying	≤ 5%	3.32%	3.71%	3.25%	3.48%	3.82%
Ethanol	< 1,000 ppm	Pass	Pass	Pass	Pass	Pass
Methanol	< 200 ppm	Pass	Pass	Pass	Pass	Pass
Heavy Metals	< 10 ppm	Pass	Pass	Pass	Pass	Pass
Lead	< 0.5 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm
Arsenic	< 0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm
Cadmium	< 0.5 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm
Mercury	< 0.5 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm
Total Plate Count	< 5,000 cfu/g	<1,000 cfu/g	< 1,000 cfu/g	<1,000 cfu/g	< 500 cfu/g	<1,000 cfu/g
Total Coliform	< 100 cfu/g	< 3 cfu/g	< 10 cfu/g	< 3 cfu/g	< 3 cfu/g	< 3 cfu/g
Total Yeast & Mold	< 100 cfu/g	< 10 cfu/g	< 100 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
E. coli	Negative	ND	ND	ND	ND	ND
Salmonella	Negative	ND	ND	ND	ND	ND

cfu – Colony forming unit; g – gram; ND – Not detected; ppm – Parts per million

Blue California analyzes its high purity DHQ preparation by HPLC. A method verification report, which includes representative chromatograms, is provided in Appendix 3. In addition to the presentation of key specifications found in Table 2 for comparison with generally accepted purity standards, certificates of analysis for five representative lots of DHQ are provided in Appendix 4.

Blue California has also analyzed representative lots of material for pesticides (Appendix 5). No concerns were noted upon review.

D. Physical or Technical Effect

Dihydroquercetin is not added to food for a physical or technical effect. It will be added to conventional foods and beverages as an ingredient for those wishing to increase their dietary intake of the ingredient.

E. Stability

1. Published Stability on Dihydroquercetin

Ametis JSC submitted results of a stability study to EFSA on its DHQ material stored in dark glass containers for three months under normal storage conditions (25°C, 65% relative humidity) and for 24 weeks under accelerated conditions (40°C, 75% relative humidity). The taxifolin content of samples stored at 40°C was reported to be 94.5% after one week and 97.5% after 30 weeks. Furthermore, Ametis JSC provided information indicating that soymilk concentrate fortified with taxifolin was observed to have a loss of 6.8% taxifolin at 4°C, 3.2% taxifolin at 10°C, and 10.3% taxifolin at 20°C over a year. The EFSA panel considered the stability data provided by Ametis JSC to be sufficient and did not raise any safety concerns (Turck et al., 2017).

2. Stability Data for Blue California's Dihydroquercetin

Blue California conducted a six-month accelerated stability study on its BC-DHQ™ high purity DHQ at 40 ± 2°C and 75 ± 5% relative humidity. A summary of the accelerated stability results is presented in Table 3.

Table 3. Blue California's BC-DHQ™ Stability Data

Dihydroquercetin Lot# 7730-160823				
Duration	Appearance	Moisture (%)	Dihydroquercetin Assay (%) Dry weight	Total Plate Count
t=0	Beige powder	3.15	97.4	25 cfu/g
1 month	Beige powder	3.15	96.9	30 cfu/g
2 months	Beige powder	3.11	97.6	25 cfu/g
3 months	Beige powder	3.18	97.2	45 cfu/g
6 months	Beige powder	3.21	97.4	30 cfu/g

Dihydroquercetin Lot# 7730-161028				
Duration	Appearance	Moisture (%)	Dihydroquercetin Assay (%) Dry weight	Total Plate Count
t=0	Beige powder	3.56	97.5	20 cfu/g
1 month	Beige powder	3.50	97.6	25 cfu/g
2 months	Beige powder	3.61	97.5	15 cfu/g
3 months	Beige powder	3.60	97.3	30 cfu/g
6 months	Beige powder	3.66	97.4	35 cfu/g
Dihydroquercetin Lot# 7730-170425				
Duration	Appearance	Moisture (%)	Dihydroquercetin Assay (%) Dry weight	Total Plate Count
t=0	Beige powder	3.12	97.2	50 cfu/g
1 month	Beige powder	3.13	96.7	40 cfu/g
2 months	Beige powder	3.20	97.1	35 cfu/g
3 months	Beige powder	3.22	97.2	40 cfu/g
6 months	Beige powder	3.28	97.3	40 cfu/g
Dihydroquercetin Lot# 7730-170525				
Duration	Appearance	Moisture (%)	Dihydroquercetin Assay (%) Dry weight	Total Plate Count
t=0	Beige powder	3.36	95.4	10 cfu/g
1 month	Beige powder	3.42	95.3	15 cfu/g
2 months	Beige powder	3.50	95.5	25 cfu/g
3 months	Beige powder	3.55	95.4	10 cfu/g
6 months	Beige powder	3.62	95.4	25 cfu/g
Dihydroquercetin Lot# 7730-170616				
Duration	Appearance	Moisture (%)	Dihydroquercetin Assay (%) Dry weight	Total Plate Count
t=0	Beige powder	3.76	97.8	30 cfu/g
1 month	Beige powder	3.78	97.6	25 cfu/g
2 months	Beige powder	3.79	97.6	30 cfu/g
3 months	Beige powder	3.82	97.5	15 cfu/g
6 months	Beige powder	3.87	97.8	30 cfu/g

The stability data in the scientific literature for DHQ, along with Blue California's stability testing results for BC-DHQ™, support the position that Blue California's BC-DHQ™ preparation is well-suited for the intended food uses.

In addition, Blue California claims a two-year shelf life for BC-DHQ™.

PART 3. DIETARY EXPOSURE

The subject DHQ preparation is intended to be used as an ingredient in a limited number of human food categories, similar to those categories and use levels evaluated by EFSA for Ametis JSC's 90% DHQ preparation (Turck et al., 2017). The intended food use categories and use levels for Blue California's BC-DHQ™ DHQ preparation are presented in Table 4.

Table 4. Blue California's Intended BC-DHQ™ Food Uses

Food Category	Maximum Level of Use
Non-alcoholic beverages	0.02 g/L
Flavored fermented milk and dairy products	0.02 g/kg
Chocolate products	0.07 g/kg

A. Estimate of Dietary Exposure to BC-DHQ™

In 2016, EFSA reviewed a petition for the use of taxifolin-rich (DHQ-rich) extract from Dahurian Larch (*Larix gmelinii*) as a novel food ingredient at various per serving levels in specific conventional foods: alcohol-free beverages, fermented milk and dairy products, and chocolates, as well as in dietary supplements with a recommended daily dose of 100 mg per day (Turck et al., 2017). The notifier—Ametis JSC—Indicated that their DHQ preparation was intended for use in foods for the general population aged nine years and up.

Estimated intake levels of DHQ were prepared for the European population based on EFSA's Comprehensive Food Composition Database for 'consumers only,' as shown in Table 5. The combined intake from all intended food uses considering the 97.5th percentile intake estimates and 100 mg DHQ per day from supplements resulted in an estimated daily intake of 158 mg DHQ for adults and 146.2 mg DHQ for adolescents. EFSA noted that the estimated dietary intake calculation was conservative.

Table 5. Estimated Daily Intake of DHQ from Addition to Conventional Foods (Europe)[a]

Subpopulation	Food Category	Use Level (g/kg)	Mean (mg/day)	95th Percentile (mg/day)	97.5th Percentile (mg/day)
Adolescents (10 to 17 years of age)	Non-alcoholic beverages	0.0250	10.5	20.4	30.3
	Flavored fermented milk and dairy products	0.019	2.4	5.8	7.5
	Chocolate products	0.070	2.5	7.6	8.4
	Combined consumption for all categories	--	15.4	33.8	46.2

Subpopulation	Food Category	Use Level (g/kg)	Mean (mg/day)	95th Percentile (mg/day)	97.5th Percentile (mg/day)
Adults (Aged 18 years or older)	Non-alcoholic beverages	0.0250	9.4	28.8	36.4
	Flavored fermented milk and dairy products	0.019	4.1	11.2	13.9
	Chocolate products	0.070	2.3	6.0	7.7
	Combined consumption for all categories	--	15.8	46.0	58.0

[a] Adapted from Turck et al. (2017)
Note: The intake estimates were performed at levels slightly different from the proposed intake levels.

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) subsequently conducted a supplementary safety assessment for DHQ by considering also those population groups that were originally excluded---at the request of the applicant (i.e., infants, young children and children up to nine years of age)---for the food categories set out in the application, and by taking into consideration the extension of use of taxifolin from yogurt to a wider range of dairy products, as shown in Table 6. These updated use levels were then used to determine the estimated intake of DHQ for specific population subgroups, as shown in Table 7.

Table 6. Dihydroquercetin Proposed Uses and Use Levels as Evaluated by EFSA[a]

Food Category	Maximum Level of Use
Unflavored fermented milk products, including natural unflavored buttermilk (excluding sterilized buttermilk) non heat-treated after fermentation	0.020 g/kg
Flavored fermented milk products including heat-treated products	0.020 g/kg
Dehydrated milk	0.052 g/kg
Cream and cream powder	0.070 g/kg
Cheese and cheese products	0.090 g/kg
Unripened cheese	0.090 g/kg
Ripened cheese	0.090 g/kg
Whey cheese	0.090 g/kg
Processed cheese	0.090 g/kg
Fats and oils essentially free from water (including anhydrous milkfat)	0.164 g/kg
Cocoa and chocolate products	0.070 g/kg
Fruit juices	0.020 g/L
Vegetable juices	0.020 g/L
Fruit nectars and vegetable nectars and similar products	0.020 g/L
Flavored drinks with sugar	0.020 g/L
Flavored drinks with sweetener	0.020 g/L

[a] Adapted from EFSA (2017)

Table 7. Dihydroquercetin Intake Estimates for Specific Subpopulations[a]

Population Group (Age Range)	Range of means (mg/kg bw/day)	Range of high intakes (95th percentile)[b] (mg/kg bw/day
Infants (up to 1 year)	0.12-0.34	0.32-0.74
Toddlers (1-3 years)	0.34-0.94	0.74-1.54
Other children (4-9 years)	0.28-0.73	0.66-1.47
Adolescents (10-17 years)	0.19-0.39	0.36-0.76
Adults (18-64 years)	0.09-0.22	0.24-0.52
Elderly (>64 years)	0.05-0.17	0.13-0.32

[a] Adapted from EFSA (2017)
[b] Based on surveys with > 60 consumers

Based on the expanded proposed uses and use levels, the highest estimated 95th percentile intake for a 70 kg adult with a combined DHQ intake from fortified foods (36 mg) and food supplements (100 mg) slightly decreased from 146 mg DHQ per day (Turck et al., 2017) to 136 mg DHQ per day (EFSA, 2017).

For adolescents (aged 14-18 years) with a mean body weight (bw) of 61 kg, the estimated 95th percentile combined DHQ intake from fortified foods (46 mg) and food supplements (100 mg) was estimated by EFSA to be 146 mg per day, slightly higher than the Turck et al. (2017) estimate of 133.8 mg per day. The EFSA Panel noted that this estimate is considered conservative, as the calculations were based on consumption data for a population group of children aged 10-17 years, which included children below 14 years of age with lower body weight and food intake per person.

The EFSA Panel calculated the highest mean and 95th percentile intakes per kg bw amongst all population groups to be for toddlers (1-3 years), as 0.94 and 1.54 mg DHQ per kg bw per day, respectively. Children aged 4-9 years were estimated to have a slightly lower 95th percentile daily intake of 1.47 mg DHQ per kg bw per day.

The EFSA Panel concluded that the taxifolin-rich extract from Dahurian Larch is safe under the proposed conditions of use (EFSA, 2017).

A review of the published literature did not identify any substantiated estimates of daily dietary intakes of DHQ stated to be specific to the US population from the background diet. Based on the anticipated daily consumption of commonly consumed foods, Schauss et al. (2015) estimated individual DHQ exposure in the US to be 426.24 mg per day. The data and methodology used for this calculation were not disclosed.

A non-exhaustive literature search indicates that DHQ is naturally-occurring in many foods common to the human diet, as shown in Table 8. Most of these references do not state the form of DHQ in the foods; however, (2R,3R)-*trans* DHQ is found in apple (Vega-Villa et al., 2009).

Table 8. Dietary Sources of Dihydroquercetin[a]

Dietary Source	Concentration	Reference
Apple flesh	1,300 mg/kg	Vega-Villa et al. (2009)
Apple skin	7,400 mg/kg	Vega-Villa et al. (2009)
Red onions	98 mg/kg	Slimestad et al. (2007)
Tomato	NQ	Turck et al. (2017)
Olive oil	129.4 mg/kg	Carrasco Pancorbo et al. (2004)
Sorghum grain	NQ	Gujer et al. (1986)
White grapes	NQ	Masa et al. (2007)
Strawberries	NQ	Sun et al. (2014)
Mulberries	21 mg/kg (fresh weight)	Zhang et al. (2008)
Açaí	NQ	Gallori et al. (2004)
Peanuts	103.4 mg/kg	Pratt and Miller (1984)
Siberian Pine seed extract	1,720 mg/kg	Lantto et al. (2009)
Thyme essential oil	41.96-93.73 mg/kg	Varga et al. (2015)
Citrus fruits	NQ	Kawaii et al. (1999)
White wine	NQ	Pozo-Bayón et al. (2003)
Beer	1 mg/L	Gerhäuser (2005)
Walnut	NQ	Zhao et al. (2017)
Mexican oregano	1,260 mg/kg[b]	Lin et al. (2007)
Prickly pear	NQ	Dok-Go et al. (2003)
Fenugreek seeds	NQ	Yu et al. (2017)
Almond skin	9.0 mg/kg	Fallico et al. (2011)

[a] Including dihydroquercetin derivatives such as taxifolin deoxyhexose found in açaí
[b] Mean of three samples
NQ – not quantified

In calculating an estimate of DHQ intake in the US from background diet, we considered the commonly consumed foods in the American diet identified as containing the most DHQ: apples and olive oil. According to USDA data, apples are the most consumed fruit in the US diet, with 2017 data showing loss adjusted per capita availability of fresh or processed apple to be 113.8 pounds (51.6 kg) per person (USDA, 2019). Considering the skin of the apple accounts for a tiny

percentage of the weight consumed, the DHQ value for 1,300 mg per kg results in an estimated annual intake of approximately 67,000 mg DHQ or 183.7 mg DHQ per person per day, assuming equal daily intake throughout the year.

Olive oil per capita consumption in 2014 for the United States is reported to be 0.9 kg (International Olive Council, 2016). Using the 129.4 mg per kg value, the annual intake of DHQ per person is estimated at 116.5 or 0.32 mg per person per day, assuming equal daily intake throughout the year.

Assuming that all DHQ from apple and olive oil is in the (2R,3R) *trans* form, the combined amount of (2R,3R) *trans* DHQ from the background diet is estimated to be 184 mg per person per day based on intake from apple and olive oil alone.

Blue California intends to use BC-DHQ™ in conventional foods similar to those identified in the initial EFSA review at various levels as detailed in Table 4. FDA's methodology was applied to estimate mean and high total consumption using USDA survey data on the daily consumption of various food types (FDA, 2006). The corresponding mean total intake value was multiplied by two because the 90th percentile consumption is unlikely to exceed the mean by more than a factor of two (FDA, 2006). FDA methodology is recognized as a method that overestimates consumption. Estimated Daily Intakes (EDIs) for these proposed conventional food categories, concerning the intended use levels, are provided in Table 9.

Table 9. Conventional Foods Dietary Intake Estimations for (2R,3R) *trans* Dihydroquercetin

Food Category	Maximum Use Level of DHQ[a] (g/serving)	USDA Mean Grams of Food Consumed (All Individuals)[b]	RACC serving size (g)[c,d]	Estimated Mean mg DHQ[a] Consumed (All Individuals)	Estimated 90th Percentile mg DHQ[a] Consumed (All Individuals)	Reference Number, Page Number
Non-alcoholic beverages	0.0072	821	360	16.42	32.84	USDA (1997) Table 9.7, page 32
Fermented milk and dairy products[e]	0.0034	8	170	0.16	0.32	USDA (1997) Table 9.4, page 29
Candy containing chocolate	0.0021	4	30	0.28	0.56	Smiciklas-Wright et al. (2002) Appendix B, page 244
Total				16.86	33.72	

[a] (2R,3R) *trans* DHQ
[b] Mean grams food consumed for all individuals taken from Reference 2 or calculated from Reference 1
[c] Reference Amounts Customarily Consumed (RACC) as indicated by FDA, Available at: https://www.fda.gov/downloads/food/guidanceregulation/guidancedocumentsregulatoryinformation/labelingnutrition/ucm513820.pdf (Accessed 7/6/17)
[d] For liquids, assume 1 mL = 1 g
[e] Determined using yogurt United Stated Department of Agriculture (USDA) mean grams of food consumed and RACC serving size

The intended levels of use proposed by Blue California result in estimated daily exposures of 16.86 mg and 33.72 mg (2R,3R) *trans* DHQ for the mean and 90th percentile, respectively. By comparison, the EFSA evaluation estimated adult daily intake from addition to the same food categories to be 15.8 and 46 mg for the mean and 95th percentile, respectively. These similar values indicate that consumption patterns within the US and Europe are similar for these food categories. As such, we consider the similarity in consumption patterns for these food categories would extend to other age groups as well.

It should be noted that the EFSA Panel (Turck et al., 2017) estimated the total daily intake of taxifolin from both conventional foods (46.0 mg for adults and 33.8 mg for adolescents at the 95th percentile) and food supplements (100 mg per day), resulting in an estimated combined 95th percentile (2R,3R) *trans* DHQ intake of 146 mg per day for adults and 133.8 mg per day for adolescents. Interestingly, the subsequent EFSA scientific opinion in which expanded use into a broader range of fermented milk products and added intake assessment of children below nine years of age, resulted in the highest mean and 95th percentile daily estimated intake per kg body weight from fortified foods only, to be in toddlers (0.94 and 1.54 mg, respectively) (Table 7). The 95th percentile (2R,3R) *trans* DHQ intakes for adolescents (14-18 years) were 46 mg (from fortified food) and 100 mg from supplements, totaling 146 mg per day (2.4 mg per kg bw per day based on 61 kg individuals). The 95th percentile intake for adults from fortified food (36 mg) and supplements (100 mg) was 136 mg per day or 1.94 mg per kg bw per day (based on a 70 kg individual). The EFSA intake analyses did not include an estimate of (2R,3R) *trans* DHQ from the background diet. No concerns about the dietary intake levels from novel food use were raised by EFSA, which supports the safety of the proposed use levels of BC-DHQ™ (Table 4).

The estimated intake of (2R,3R) *trans* DHQ from the background diet for the US population based on published literature of DHQ quantified in commonly consumed foods and per capita intake of those foods is 184 mg per day, resulting in an estimated combined mean and 90th percentile intake from background diet and the intended use of 200.86 and 217.72 mg per day, respectively. This equates to 2.87 and 3.11 mg DHQ per kg bw per day for the mean and 90th percentile, respectively.

B. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed in or On Food

No other substances are expected to be formed in or on food under the intended conditions of use for Blue California's BC-DHQ™.

C. Dietary Exposure to Contaminants or Byproducts

There are no known concerns regarding dietary exposure to contaminants or byproducts of DHQ.

PART 4. SELF-LIMITING LEVELS OF USE

There are no known self-limiting levels of use.

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

A. Other Information on Dietary Exposure

1. History of Traditional Medicinal and Human Food Use

There are no known documented medicinal or human food use of DHQ before January 1, 1958.

DHQ occurs in a number of foods that are part of the American diet, including apples, red onions, tomatoes, olive oil, sorghum, white grapes, mulberries, açai, peanuts, thyme, citrus fruits, white wine, and beer. It has also been reported that over 250 taxifolin-containing food supplements, foods, and cosmetic products were registered by the Russian Federation by April 2009, with recommended adult intakes ranging from 5 to 100 mg of taxifolin per day. In addition, taxifolin derived from larch wood is used as an ingredient in dietary supplements in Russia, Switzerland, Canada, and the U.S. (Turck et al., 2017).

2. U.S. Regulatory History

A search of FDA's GRAS Notice (GRN) database[5] using the terms "dihydroquercetin," "DHQ," and "taxifolin" yielded no results.

As noted on their corporate website, Ametis JSC reported "self-affirmed" GRAS status for its (2R,3R) *trans* DHQ product, Lavitol®, in 2009 (Ametis JSC, Date Unknown).

3. Canadian Regulatory History

A search of the Health Canada website, using the terms "taxifolin" and "dihydroquercetin," yielded that taxifolin is considered a natural health product and classified as a Schedule I isolate obtained from plant material. Source materials are cited as *Drimia maritima* (bulb), *Larix dahurica* (wood and root wood), *Larix sibirica* (wood and root wood) *Senegalia catechu* (whole plant) and *Silybum marianum* (seed) (Health Canada, 2019).

4. European Regulatory History

In December 2016, EFSA responded to a novel food application for a taxifolin-rich extract prepared from Dahurian Larch. The EFSA Panel noted that the specifications, representative batch data, and stability data presented by Ametis JSC were sufficient and did not present any safety concerns. Intended uses for the taxifolin-rich extract (~90% DHQ) included non-alcoholic

[5] Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices (Accessed on February 2, 2020)

beverages, fermented milk and dairy products, and chocolate. As a food supplement, a daily dose of 100 mg per day was also proposed. The Panel noted that the provided genotoxicity data "do not raise concern." Using a no observed adverse effect level (NOAEL) of 1,500 mg per kg bw per day derived from a subchronic toxicity study in rats and estimated combined intake levels from conventional foods and dietary supplements, the EFSA Panel determined a margin of safety of 660 for adults, 460 for adolescents, and 960 for children aged 9-14 years. The Panel concluded that taxifolin-rich extract from Dahurian Larch is safe as a novel food under the intended conditions of use proposed by Ametis JSC (Turck et al., 2017).

In November of 2017, following a request from the European Commission, the EFSA NDA Panel conducted a supplementary safety assessment for taxifolin by considering also those population groups which were originally excluded at the request of the applicant (i.e., infants, young children and children up to nine years) for the food categories set out in the application, and by taking into the extension of use of taxifolin from yogurt to a wider range of dairy products. The Panel concluded that the taxifolin-rich extract from Dahurian Larch was safe under the proposed conditions of use (EFSA, 2017).

5. Other Regulatory History

A search of the Food Standards Australia New Zealand (FSANZ) website[6] using the terms "taxifolin" and "dihydroquercetin" resulted in no regulatory results for use as a food additive. A search of the Australian Therapeutic Goods Administration website resulted in a historical document from 2007 entitled "Substances that may be used in Listed medicines in Australia," which cites that taxifolin alone is not permitted for use as a Listed medicine; however, taxifolin as a component of *Pinus pinaster* is eligible for use in Listed medicines (TGA, 2007). Further review of the Australian Register of Therapeutic Goods shows over 30 products currently on the market containing *P. pinaster*.

It has been reported that as of April 2009, over 250 DHQ-containing products were registered with Russian Federation regulatory bodies (Turck et al., 2017). A search of the Ministry of Agriculture of the Russian Federation website using the terms "taxifolin" and "dihydroquercetin" resulted in no results.

PART 6. NARRATIVE

A. Discussion on Safety Data on Dihydroquercetin

From an extensive online database search, current to December 2, 2019, using the terms "dihydroquercetin," "biological activity and dihydroquercetin," and "safety and dihydroquercetin," and search engines (Toxnet, PubMed, and Google Scholar), references were scanned for relevant biological effects and safety data on DHQ. Many of the studies found referred to DHQ by

[6] Available at: https://www.foodstandards.gov.au/Pages/default.aspx (Accessed on February 2, 2020)

one of its synonyms--- taxifolin or diquertin. The novel foods dossier submitted to EFSA by Ametis JSC, a Russian company, was reviewed. The dossier contained unpublished studies and published studies that had no English translations available. In addition, the EFSA scientific opinion on a taxifolin-rich extract from Dahurian Larch, which was adopted on December 13, 2016, was reviewed. In October 2017, EFSA released a statement on the safety of taxifolin-rich extract from Dahurian Larch following the completion of a supplementary safety assessment; this document was also reviewed as it considered those populations which were originally excluded from the December 2016 document at the request of the applicant. The more relevant studies are summarized in the following sections.

1. *in Vitro* Toxicology Studies

The cytotoxicity of a number of flavonoids, including taxifolin, toward cultured human lung embryonic fibroblasts (TIG-1) and human umbilical vein endothelial (HUVE) cells was examined (Matsuo et al., 2005). Taxifolin was found to be slightly toxic to TIG-1 cells and HUVE cells, with LC_{50} concentrations of > 300 µM and > 200 µM (respectively) following incubation at 37°C for 24 hours. As shown in the absorption, distribution, metabolism, and excretion (ADME) section below, the maximum concentration of DHQ that could be present in plasma after consumption of DHQ at the 90th percentile as indicated in this GRAS dossier (approximately 260.84 ng per mL or 0.86 µM) is considerably lower than the concentration that caused toxicity to TIG-1 cells and to HUVE cells.

To evaluate the phototoxic potential of taxifolin, a 3T3 Neutral Red Uptake Phototoxicity Test was conducted according to the Organisation for Economic Co-operation and Development (OECD) TG 432 (Rajnochova Savobodova, 2017). The authors used HaCaT keratinocytes (immortalized human keratinocytes), normal human epidermal keratinocytes, and dermal fibroblasts to better approximate human skin. Taxifolin was found to be nonphototoxic and photostable.

2. Acute and Subacute Toxicity Studies

Schauss et al. (2015) discussed a good laboratory practices (GLP) compliant unpublished acute oral toxicity study in albino outbred rats (gender unspecified) in which no toxicological or gross pathological effects were observed following a single gavage dose of 75, 150, and 1,500 mg per kg bw Lavitol® (91-98% DHQ) as compared to a negative control of potato starch.

In a follow-up GLP-compliant subacute oral toxicity study, male and female albino outbred rats were dosed for seven consecutive days with 10,000 or 15,000 mg per kg bw of Lavitol® (90.94% DHQ) via gavage and the study included concurrent controls (Schauss et al., 2015). Animals were observed for mortality, external appearance, behavior, clinical signs, sensory reactivity to auditory, visual and proprioceptive stimuli and muscle strength. Body weights and food consumption were evaluated as well. Blood was collected on day 0 and day 8 for hematological and biochemical evaluation and urine was collected on days 0 and 8. Animals were euthanized on day eight and underwent a full necropsy with organ weights. There was no difference between

test groups and control animals concerning the quantities of water and food consumed. The administration of Lavitol® did not affect animals' behavior, fur, skin, or mucous membranes. There were no differences in white blood cell count, hemoglobin, hematocrit, basophils, eosinophils, monocytes, or lymphocytes between test and control animals. A decreased red blood cell (RBC) count for females at both doses was evident on day 8, and significant differences in RBC volumes between the control group of female rats and the experimental group of females were noted, but were within normal biological limits. There was no difference in urinalysis between groups and gross pathology analyses revealed no abnormalities. The histopathological and morphological changes observed in all groups and between sexes were considered incidental, physiologically related, and not induced by the test substance.

The effect of DHQ on mean blood pressure and macro- and micro-rheological blood parameters in 17-week-old hypertensive Wistar-Kyoto spontaneously hypertensive (SHR) rats were evaluated following oral dosing at 20 mg per kg bw per day for six weeks (Plotnikov, 2017a). No adverse effects related to DHQ exposure were reported in the SHR rats. In another study, which evaluated the changes in angiotensin-converting enzyme (ACE) activity in the aortas of male normotensive Wistar-Kyoto and SHR rats, the animals were dosed by daily gavage with DHQ at 100 or 300 µg per kg bw for two weeks or vehicle control beginning at postnatal weeks 10-12 or 12-14 (Slashcheva, 2016). SHR rats given 100 µg per kg body weight (bw) per day DHQ from weeks 10-12 exhibited decreased body weight compared to control SHR rats of the same age ($p<0.05$). There was no effect of either dose of DHQ on the body weights of any other group. In normotensive Wistar-Kyoto rats, administration of DHQ at 300 µg per kg bw per day from weeks 12-14 caused a significant reduction in activity of ACE in the aorta ($p<0.05$) but had no effect on blood pressure. The effects observed in this study are not considered to be adverse because the body weight reduction in SHR rats was less than 10% and the reduction in ACE activity in normotensive Wistar-Kyoto rats was not associated with a decrease in blood pressure.

The ability of taxifolin to act as an antioxidant *in vivo* was examined by Igarashi et al. (1996). For this study, five-week-old male weanling Wistar rats were divided into three groups of five or six rats each and then exposed to either a control, astilbin (0.074%) added, or taxifolin (0.05%) added diet for ten days (Igarashi et al., 1996). Based on an average initial body weight of 29 g and average food consumption of 11.1 g per day, the average daily amount of taxifolin ingested by the animals was 191.4 mg per kg bw per day. At the end of the dosing period, the animals were anesthetized, blood collected from the heart, and the liver was immediately removed and frozen. Food consumption, body weight gain, total serum cholesterol, high density lipoprotein (HDL)-cholesterol, triacylglycerol and phospholipid, liver lipids and endpoints designed to assess efficacy as antioxidant were measured. There were no adverse effects of taxifolin on any endpoint measured.

The effect of taxifolin on cisplatin-induced oxidative pulmonary damage was investigated in male albino Wistar rats (Unver et al., 2019). There were four groups, with six animals in each group: 50 mg per kg of taxifolin by gavage plus 2.5 mg per kg of cisplatin intraperitoneally group (TC);

2.5 mg per kg of cisplatin intraperitoneally group (CIS); 50 mg per kg of taxifolin by gavage group (TG); and a healthy control group (distilled water by gavage). Taxifolin, cisplatin, and the distilled water were administered at the indicated dose, using the same method daily for 14 days. After 14 days of treatment, animals were euthanized, and blood and lung tissue samples were taken for an assessment of oxidative damage (malondialdehyde, myeloperoxidase, total glutathione and 8-hydroxy-2 deoxyguanosine analyses and histopathological examinations). Results for the TC or CIS groups are not discussed here as they don't directly contribute to the determination of safety of DHQ. Results for a group exposed only to TG were available and could be compared to a control group. Based on the results of the study, the authors concluded that biochemical and histopathological manifestations of oxidative damage were not observed in the blood and lung tissues of the TG group as compared with the control group.

3. Subchronic Toxicity Studies

A GLP compliant subchronic 90-day study in 96 albino outbred rats (48 males and 48 females) to determine the safety of Lavitol® was performed by Schauss et al. (2015). Three experimental groups (12 male; 12 female) received 50, 150, or 1,500 mg per kg bw of Lavitol® each day by gavage, while a fourth group (12 males; 12 female) received 1% potato starch as a vehicle control group. The phytochemical composition of Lavitol® used in this study was 92.20% DHQ, 2.35% aromadendrin, 0.53% eriodictyol, 0.26% quercetin, 0.17% naringenin, and 0.11% pinocembrin. All of the animals in the study exhibited comparable weight gain throughout the dosing period. The quantity of food and water consumed by the animals in all other groups was not significantly different compared with the control animals. There were no abnormal changes in skin and fur appearance, except that the animals administered 50 and 150 mg per kg bw had significantly thicker and fluffier hair compared with the 1,500 mg per kg bw dose group and the control dose group. No abnormal movement was present in any of the groups. Males in the 150 mg per kg bw group were more active during the first month of the study compared with the other groups and controls; however, during the third month of study, males in the 50 and 150 mg per kg bw groups were significantly more active than the males in the 1,500 mg per kg bw group and controls. Stool disturbances were observed in all groups throughout the study, but they were significantly lower in male and female 150 mg per kg bw groups compared with controls. No edema, hyperemia, or pathological excretions were observed in any of the treatment groups. There were no changes in the corneal reflex of any animals tested or any differences in pupil size or width of the palpebral fissure. All indices of hematological analysis were within normal values among groups, and the urine analyses of control and treated animals were within normal ranges. In addition, there were no clinically relevant histopathological differences between experimental and control animals. The authors concluded that there were no adverse effects when Lavitol® was administered orally for 90 days in male and female rats up to a dose of 1,500 mg per kg bw.

In the 2017 EFSA document, a GLP-compliant toxicity study was reported with taxifolin (90.5% DHQ). Wistar albino rats (n= 10 per sex per group) were administered 0, 50, 150, or 1,500 mg per kg bw per day of taxifolin by gavage for 90 days. A high dose recovery group and a control

recovery group were observed for 28 days following the end of the treatment period (n = five rats per sex per group) (EFSA, 2017). The control group received the vehicle, which was a 1% starch solution. No mortality was noted. Absolute body weights in high dose males corresponded with the trend for lower food consumption in this same group. No differences were noted in any other group in either body weights or food consumption. No differences were noted in the ophthalmological examinations, electrocardiograms, and behavioral activity. Minor changes in hematology parameters and clinical biochemistry were noted, but there was no dose response. Urinalysis and relative organ weights were not significantly different between groups. Aggression was observed in two females and three males form the high dose group, one female and two males of the mid dose groups, and one control female; however, there were no differences in behavioral activity between groups (tests performed for this assessment were not stated). Abrasions were observed in high dose females, which was judged to be caused by fighting between the animals. The authors mentioned that the presence of five animals per cage could be a contributing factor to these observations. Gross and microscopic pathological changes were noted in the stomach of one high dose male and female and one high dose recovery group male. EFSA stated that these effects could have arisen from an irritating effect of the test material on the stomach mucosa due to gavage administration. Hypertrophy of the adrenal glands was seen in two males and two females in the high dose group and one male and one female in the high dose recovery group. These effects on the adrenals were thought to be related to stress from the aggressive behavior seen during the study. The EFSA panel concluded that the NOAEL was 1,500 mg per kg bw per day, the highest dose tested.

In a study to evaluate the process of peroxidation in male outbred albino rats following the administration of DHQ for three months, the diets of rats were supplemented daily with a DHQ dose of either 86, 860, or 3,000 mg per kg bw (Chernyak and Shchukina, 2009). A control group receiving only the standard diet was included in the study as well as a reference control group receiving rutin at 86 mg per kg bw. Because this study was designed as an efficacy study, safety endpoints were not included; however, no mortalities or adverse effects were specifically reported.

A study was conducted where the effects of DHQ on microvascularization and microcirculation in the cerebral cortex of SHR rats during the development of arterial hypertension were evaluated (Plotnikov, 2017b). Animals were dosed with 50 mg per kg bw DHQ in 1% starch gel via gavage for six weeks. Concurrent controls, both SHR and normotensive Wistar Kyoto rats received 1% starch gel only. No adverse effects related to exposure to DHQ were reported.

The effect of DHQ on the performance of broiler chickens was assessed by Pirgozliev et al. (2019). For this study, a total of 80 male Ross 308 broilers were allocated to 16 pens of five birds each, and the pens were randomly allocated to eight pens per treatment [a control diet or a diet containing 0.5 g per kg extract of Siberian Larch (85% DHQ)]. The diets were fed over two feeding phases, a grower phase from 7 to 28 days of age and a finisher phase from 28 to 35 days of age. Based on average feed intake of 96.1 g per day (2,788 g per 29 days), an initial

body weight of 123 g, and a DHQ content if 85% in the feed, birds received approximately 64 mg per kg bw per day DHQ. Parameters measured include growth performance variables, color score of left breast meat, weight of proventriculus, gizzard, duodenum, pancreas, jejunum, ileum, caeca, liver, heart, spleen, and the bursa of Fabricius, gastrointestinal (GI) tract and immune organ development, glutathione peroxidase and hemoglobin in blood, hepatic vitamin E content, dietary N-corrected metabolizable energy and nutrient retention coefficients. All birds were healthy throughout the study period and there was no mortality or clinical sign of toxicity. There was no effect of treatment on any parameter measured, except for an increase in the redness index of the breast fillets, which is not considered to be adverse.

4. Chronic Toxicity Studies

Booth and Deeds (1958) investigated the chronic oral toxicity of DHQ in albino rats. Ten weanling rats of each sex were administered dietary levels of 0.125, 0.25, 0.5, and 1.0% DHQ with a control group of 20 per sex receiving the basal diet only. Estimated doses are 125, 250, 500, and 1,000 mg per kg based on estimated food consumption of 10 g per day in a rat of 100 g (IPCS, 2009). At the end of 226 days, 50 percent of the animals receiving 0.5% and 1.0% of the test material were euthanized along with an equal number of animals that were fed the basal diet. The remaining animals in these dose groups were euthanized after 450 days. At the end of 249 days, 50 percent of the animals that were dosed with 0.125% and 0.25% of the test material were euthanized along with an equal number of controls. The remaining animals in these dose groups were euthanized after 650 days. Rats underwent a weekly clinical exam and were weighed weekly as well. During the study, no differences in appearance, behavior, food intake, or growth were noted between experimental animals and controls. No treatment-related deaths occurred. There were no adverse gross nor microscopic changes that were attributable to DHQ, except for vacuolization in the livers of female rats fed 1% DHQ. The authors stated that this was most likely due to fat deposition. The authors concluded that no significant toxicological effects were observed from the long-term administration of DHQ to albino rats at a dietary level of up to 1% (Booth and Deeds, 1958).

In the Schauss et al. (2015) report, a six-month chronic toxicity study in male rats and dogs performed by Shkarenkov et al. (1998; paper and abstract not found) was described. Rats received either control solution, 150 mg DHQ preparation (purity not indicated) per kg bw per day, or 15,000 mg DHQ preparation per kg bw per day via intragastric administration. Dogs received 190 mg DHQ preparation per kg bw per day in their food. The authors mentioned that “except for slight variations in some functional tests, the safety of the preparation was supported by evaluation and analyses of the animal’s organs and tissues.” Further details about the conduct results of these studies were not provided in the Schauss et al. (2015) study. The authors concluded that “in both the rat and dog studies, no evidence of diquertin toxicity was shown.” Although documentation from this study is weak, there is no mention of any of the effects that were observed in a few rats gavaged with 1,500 mg DHQ per kg bw per day in the 90-day study reported by EFSA (2017), even at a 10-fold higher dose.

5. Reproductive/Developmental Toxicity Studies

Schauss et al. (2015) conducted GLP compliant prenatal and postnatal developmental toxicity studies of Lavitol® in 80 pregnant female rats. Following a 14-day acclimation and observation period, rats were mated over 13 days by placing one male with two females during two estrus cycles. Mating was confirmed by the presence of sperm in a vaginal smear, and this was considered gestational day 1. Female rats were divided into four groups as follows: group 1 (n= 20), the control group, received 3 mL of a 1% starch solution per day; group 2 (n = 20) was administered 75 mg per kg bw of Lavitol® during the period of organogenesis—from gestational day 6 to 16; group 3 (n =20) was given 1,500 mg per kg bw per day of Lavitol® during the same period; and group 4 (n = 20) received 75 mg per kg bw per day of Lavitol® during the period of implantation, organogenesis, and fetogenesis—from the 1st to the 19th days of gestation. Animals were dosed via daily gavage based on the most recent body weight taken on days 1, 8, 14, 17, 18, 19, 20, and 21 of gestation. The phytochemical composition of Lavitol® used in this study was 92.19% DHQ, 3.57% aromadendrin, 0.58% eriodictyol, 0.33% quercetin, 0.17% naringenin, and 0.17% pinocembrin. During the dosing period, animals were observed three times daily for signs of pharmacological and/or toxicological effects and included general appearance, behavioral changes, and locomotor activity. Mean group body weight, percentage of body weight gain, feed and water consumption were determined during the study. Blood and urine were collected from females before mating, on day 20 of gestation prior to delivery in that group, and on day 18 or 20 for those euthanized on those days. In each group, five pregnant females were euthanized on day 18 and another five were euthanized on day 20 of gestation. The remaining pregnant females in each group were allowed to give birth and raise the offspring until weaning on day 25 – 30 of lactation. There were no signs of toxicity during the dosing period. All females gained weight during the gestation period, and no statistically or biologically relevant differences were noted in hematological or clinical chemistry parameters between groups and controls. No spontaneous abortions were recorded in any of the Lavitol® groups and no significant differences were found in the number of corpora lutea/dam, implantation sites, resorptions, late fetal deaths, non-live implants or the percent pre- and post-implantation loss, gender ratio differences, or combined fetal weights.

The fetuses collected on day 18 or 20 were examined for the shape of the body, head size, limb extension, sex, digits, skin, umbilical region, anus and genitalia, nares, pinna, eyes, and oral cavity. Two-thirds of the fetuses from each litter underwent a skeletal examination and one-third were fixed in Bouin’s fluid and underwent a visceral examination. The litters allowed to continue to weaning were evaluated for the number and sex of the pups, the numbers of stillbirths and live births, and the presence of gross abnormalities. The date of detection of primary fur, ear unfolding, incisor eruption, eye opening, testes descent and vaginal patency were recorded. Pups were weighed every three days from day 3 to 42 and the crania-caudal size of each pup was measured up to day 42 to determine if somatic neural growth was affected. Multiple sensory-motor reflex changes were evaluated up to day 42. Blood was collected from 15 pups of each sex from each group following fasting and evaluated for hematological and clinical chemistry

parameters. Pups were euthanized on day 43 and underwent a complete gross pathological examination. Microscopic evaluations were conducted on the heart, liver, kidneys, spleen, adrenal gland, and testicles. Lavitol® did not affect litter size, physical development, survival, reflex measurements, behavioral variables, or gross examination, and histopathology revealed no abnormalities related to exposure. The authors concluded that Lavitol® exposure did not result in embryotoxic or teratogenic effects on the development of offspring.

In the EFSA report from 2017, a developmental toxicity study is reported in which pregnant female rats (n=20) were dosed with taxifolin rich extract (at least 90% taxifolin) at 0, 75, or 1,500 mg per kg bw from gestation day 6 to 16 by gavage. This study was conducted in compliance with GLP as per the Ministry of Health of the Russian Federation. The endpoints for the study included clinical signs, mortality, body weight, functional observations, clinical chemistry, fetus survival and gross, skeletal and visceral examination of the fetuses and for the offspring, body weight, sensory motor evaluation, clinical chemistry, necropsy, and histopathological examinations were done. All dams survived and no clinical signs of toxicity were noted during the prenatal dosing. No adverse effects were noted in the fetuses with respect to litter size, weight, the formation of organs and general development. The conclusion was that no embryotoxic or teratogenic effects were seen at dose levels up to 1,500 mg per kg bw.

The effect of DHQ and other phenolics on the morphology, functions, and redox processes in the reproductive cells of four-month old male Wistar rats has been studied in a model of experimental pathospermia (Borovskaya et al., 2018). Experimental pathospermia was stimulated by intravenous (i.v.) injection of the antitumor drug etoposide that significantly suppresses spermatogenesis. Animals in the experimental groups (n=10 per each) received 10.4 mg per kg DHQ or the other phenolics five days before and five days after etoposide administration. A group of intact animals (baseline) consisted of 10 rats. Total sperm count, percentage of degenerative forms of mature spermatozoa, percentage of mobile spermatozoa and prooxidant/antioxidant activity in sperm were determined. There were no adverse effects of treatment with DHQ on any parameter measured in the study.

Blue California concludes that the totality of evidence from GLP-compliant toxicity studies supports the safety of DHQ at up to 1,500 mg per kg bw in rats. Blue California also notes the similarity between the developmental toxicity study summarized in the ESFA report from 2017 and the Schauss et al. (2015) study, though it is unclear if the studies are independent or duplicates.

6. Genotoxicity/Mutagenicity Studies

a. Comet Assay

Schauss et al. (2015) demonstrated that Lavitol® does not induce DNA damage in a GLP-compliant single-cell gel electrophoresis Comet assay. The Lavitol® used in this study had a phytochemical composition of 97.51% DHQ, 1.55% aromadendrin, 0.1% eriodictyol, and 0.15%

quercetin. Single oral doses of 15 or 2,000 mg Lavitol® per kg bw, methyl methanesulfonate as a positive control, or 1% ethanol as a negative control, were administered to 8- to 10-week-old male CBAxC57B1/6 mice (n=20; five per group). These doses correspond to the daily therapeutic dose of 15 mg per kg bw and a subchronic dose that exceeded the therapeutic dose by >100 times that dose. Gel electrophoresis results showed that these doses of Lavitol® did not induce DNA damage in cytogenic preparations of femoral bone marrow, blood samples, and liver samples from the experimental animals. DHQ also tested negative for DNA damage in an *in vitro* Comet assay in human peripheral blood cells at 100 µg per mL, 250 µg per mL, and 500 µg per mL (Živković et al., 2019).

Zhanataev et al. (2008) studied the genotoxic properties of a DHQ preparation derived from larch (FlavitPure, 90% DHQ) in male and female C57B1/6 mice (animal numbers not specified) using a DNA-Comet assay. To test for the induction of DNA damage, a DHQ preparation was administered either repeatedly or as a single dose. In the repeated-dose study, the DHQ preparation was administered as daily doses of 0.15 and 1.5 mg per kg bw for five days, and then the animals were euthanized three hours after the last dose. In the single-dose study, the respective DHQ preparations were administered once as doses of 15, 150, and 2,000 mg per kg, and the animals were euthanized three hours later. Concurrent vehicle (1% ethanol) and positive (cyclophosphamide) controls were included in the study. Gel electrophoresis results showed that there were no significant differences between test animals and controls for either sex or at any dose level. The authors concluded that DHQ does not exhibit any genotoxic effects.

b. Micronucleus Test in Human Lymphocytes

A GLP-compliant micronucleus test conducted according to OECD Guideline 487 showed that Lavitol® did not increase the induction of micronuclei in cultured human lymphocytes *in vitro* in the presence and absence of S9 activation mix (rat liver tissue homogenate used in biological assays) (Schauss et al., 2015). The phytochemical composition of Lavitol® used in this study was 97.5%, 1.55% aromadendrin, 0.10% eriodictyol, and 0.15% quercetin. The maximum final concentration to which the cells were exposed was 3,043 mg per mL, dosed at 1% volume per volume (v/v), to enable testing up to 10 mmol per L.

c. Chromosomal Aberration Test

Lavitol® (93.7% DHQ) did not increase bone marrow metaphases in mice treated with a single dose (15 or 2,000 mg per kg bw) compared to a single dose-treated cyclophosphamide control and negative control groups (Schauss et al., 2015*).* The GLP compliant study was conducted using CBAxC57B1/6 mice of both sexes. Following an acclimation period, a single dose of 15 or 2,000 mg per kg bw was administered orally in one experiment and another experiment, mice were dosed orally with 15 mg per kg bw for five consecutive days. Before euthanasia, animals were dosed with colchicine and then euthanized 2.5 hours later. Femoral bone marrow smears were prepared and then analyzed. There were no significant differences between sexes. It was

concluded that Lavitol® was not genotoxic in mice, either following a single oral dose up to 2,000 mg per kg bw or following repeated doses at 15 mg per kg bw per day for five days.

Zhanataev et al. (2008) performed a chromosome aberration test to determine the genotoxicity of a DHQ preparation (Flavit Company, 90% DHQ) in male and female C57B1/6 mice (number not specified) that included concurrent vehicle (1% ethanol) and positive (cyclophosphamide) controls. The respective DHQ preparations were administered to mice in doses of 1.5 mg per kg bw and 150 mg per kg bw once per day for five days. The level of chromosome aberrations in both sexes and both dose levels did not significantly differ from controls. The authors concluded that DHQ does not exhibit any DNA-damaging activities in mammals.

d. Mutagenicity

A study investigated the mutagenic effects of quercetin and taxifolin on tester strains of *Salmonella typhimurium* TA102 and *Escherichia coli* WP-2 uvrA (Makena et al., 2009). Taxifolin was determined to be not mutagenic in the presence or absence of S9 mix in both TA102 and WP-2 uvrA 2, regardless of the presence of iron or nicotinamide adenine dinucleotide phosphate (NADPH) generating system (NGS). Quercetin, however, was shown to induce mutations in the presence or absence of S9 mix, iron, or NGS. The authors concluded that a minor structural variation between the two plant polyphenols could elicit a marked difference in their genotoxicities.

Blue California concludes that the results of these *in vitro* and *in vivo* genotoxicity and *in vivo* mutagenicity studies do not raise any concerns about the safety of DHQ.

7. Clinical Studies

The clinical studies summarized in Part 6.A.7 have been previously reviewed by EFSA as part of the novel food application by Ametis. While several of the publications cited in the Ametis application are obscure and unavailable for review, Blue California notes that the EFSA Panel did not raise any concerns regarding the safety of DHQ.

Multiple clinical studies investigating various efficacy endpoints were identified. These studies were reviewed for information on adverse events and tolerability of the ingredient. A summary of the findings is provided below.

a. Clinical Studies on Dihydroquercetin

Several clinical studies have been performed on DHQ. Although the studies were designed as efficacy studies, none stated that treatment-related adverse effects were observed (Table 10).

Table 10. Clinical Studies of Dihydroquercetin

Subjects	Treatment	DHQ Dose	Duration	Endpoints Measured	Results	Reference
112 male patients with acute pneumonia Age: 19-40 yrs.	Standard therapy (ST) (n=50); ST + oral α-tocopherol acetate + i.v. sodium thiosulfate (n=32); ST+ taxifolin (n=30)	40-60 mg/day	Two weeks	TBARs in plasma, lung X-ray, pulmonary fibrosis, symptoms of pneumonitis	No adverse effects of taxifolin on endpoints measured. No side effects of treatment.	Kolhir et al. (1998) as cited in Ametis, 2010
29 patients with discirculatory encephalopathy Age: 56-78 yrs	ST + Capilar	80 mg/day	18-21 days	Psychoemotional conditions	No adverse effects of Capilar on endpoints measured. No side effects of treatment.	Zavolokov and Ilyuhina (2001) as cited in Ametis, 2010
100 hypertensive patients with atherosclerosis Age: 50-70 yrs	ST + Capilar (n=68) ST + placebo (n=32)	80 mg/day	12 weeks	Hemodynamic and biochemical parameters, endothelial function, and neurological status (including headache frequency and strength)	No adverse effects of Capilar on endpoints measured. No side effects of treatment.	Britov and Aparina (2006) as cited in Ametis, 2010
42 patients with chronic microcirculatory disturbances Age: 50-76 yrs.	ST + Capilar (n=28) ST + placebo (n=14)	75 mg/day	Three months	Blood circulation parameters (rheology, blood circulation, the strength of capillary walls)	No adverse effects of Capilar on endpoints measured. No side effects of treatment.	Kozlov et al. (2006) as cited in Ametis, 2010
60 patients with atherosclerosis of lower extremities Age: 39-75 yrs	ST + Capilar (n=20) ST + oral Capilar + Capilar-cream (n=20) ST + placebo (n=20)	60 mg/day	Two months	Pain free walking distance, ischemic pain, microcirculation	No adverse effects of Capilar on endpoints measured. No side effects of treatment	Koshkin and Nastavsheva (2008) as cited in Ametis, 2010
30 patients with IHD after aorta-coronary shunting surgery Age: 32-68 yrs	Basic rehabilitation + Capilar (n=20) Basic rehabilitation (n=10)	60 mg/day	12-17 days	Circulation parameters and psychoemotions	No adverse effects of Capilar on endpoints measured. No side effects of treatment.	Shakula et al. (2007) as cited in Ametis, 2010
40 male patients with chronic pulmonary obstructive disease Age: 30-65 yrs	ST + Capilar (n=20) ST (n=20)	80 mg/day	18-21 days	Circulation, respiratory and cardiovascular parameters	No adverse effects of Capilar on endpoints measured. No side effects of treatment.	Shakula et al. (2008) as cited in Ametis, 2010
40 patients with diabetes mellitus, Age: 56.2±8.5 yrs 20 heathy control subjects	ST+ Diquertin (n= 40 patients) ST + placebo (n = 20 controls)	120 mg/day	12 weeks	Sensitivity to insulin; HbA1c levels	No adverse effects of Diquertin on endpoints measured. No side effects of treatment.	Nedosugova (2006) as cited in Ametis, 2010
37 patients with diabetes- related onychomycosis of feet and hands Age: 30-68 yrs	ST + Diquertin (n=20) ST (n=17)	120 mg/day	12 weeks	MDA levels, intoxication parameters	No adverse effects of Diquertin on endpoints measured. No side effects of treatment.	Davudova and Zoloeva (2009) as cited in Ametis, 2010

Capilar – taxifolin; HBA1c – glycated hemoglobin; IHD – ischemic heart disease; i.v.= intravenous; MDA – malondialdehyde; Menting et al. (1994)– standard therapy; TBARs – thiobarbituric acid reactive substances; yrs – years

b. Clinical Studies on Ascovertin and Laviocard

Ascovertin is a complex of 20 mg DHQ and 50 mg ascorbic acid per tablet, and it is used as a drug in Russia for health conditions with an underlying mechanism of oxidative stress (Neveu, 2006). This combination is currently available in the US as a dietary supplement, albeit at a different proportion.[7] Laviocard is a very similar preparation containing both taxifolin (30 mg) and ascorbic acid (70 mg). Several clinical studies have been performed on Ascovertin and Laviocard, none of which stated that treatment-related adverse effects were observed (Table 11).

Table 11. Clinical Studies of Ascovertin and Laviocard

Subjects	Treatment	DHQ Dose	Duration	Endpoints Measured	Results	Reference
40 patients with IHD	ST+ Ascovertin (One tab x three/day) (n = 20) ST+ placebo (n = 20)	60 mg/day	Three months	Hemorheological status; number of anginal episodes/week	No adverse effects of Ascovertin on endpoints measured. Side effects were not discussed.	Tyukavkina et al. (2001) as cited in Ametis, 2010
41 patients w/cerebral atherosclerosis Age = 60.4±4.8 yrs (median) Ten healthy controls	ST + Ascovertin (n= 21 patients) 1st week: Two tab x three/day 2nd-3rd weeks: One tab x three/day ST (n=10 patients) Healthy controls (n=10)	1st week: 120 mg/day 2nd-3rd weeks: 60 mg/day	Three weeks	Hemorheological parameters and subjective symptomology	No adverse effects of Ascovertin on endpoints measured. No side effects of treatment.	Plotnikov et al. (2005) as cited in Ametis, 2010
48 patients w/arterial hypertension of the II & III degree Age = 60±5 yrs (median) Ten healthy controls	ST + Ascovertin (n=38 patients) ST (n=10 patients) Healthy controls (n=10)	1st week: 120 mg/day 2nd-3rd weeks: 60 mg/day	Three weeks	Hemorheological parameters	No adverse effects of Ascovertin on endpoints measured. No side effects of treatment.	Plotnikov et al. (2005) as cited in Ametis, 2010
29 patients with NIDDM Age = 56±4 yrs (median) diagnosed w/NIDDM for 8.5±3.6 years (median), w/fasting blood glucose level of 7.8±1.8 mmol/L Ten healthy controls	ST + Ascovertin (n=19 patients) ST (n=10 patients) Healthy controls (n=10)	1st week: 120 mg/day 2nd-3rd weeks: 60 mg/day	Three weeks	Hemorheological parameters	No adverse effects of Ascovertin on endpoints measured. No side effects of treatment.	Plotnikov et al. (2005) as cited in Ametis, 2010
51 patients w/IHD Age: ≤ 65 yrs 10 healthy controls	ST + Ascovertin (n = 17 patients w/IHD without	1st month: 80 mg/day 2nd-3rd	Three months	Number of episodes of stenocardia; number of nitroglycerin	No adverse effects of Ascovertin on	Plotnikov et al. (2005) as cited in

[7] For example, Life Extension Vitamin C with Dihydroquercetin 1,000 mg, available for direct purchase from Life Extension, as well as Swanson Health Products and Amazon.com. This product contains 1,000 mg Vitamin C and 10 mg dihydroquercetin -3-rhamnoside.

Subjects	Treatment	DHQ Dose	Duration	Endpoints Measured	Results	Reference
	prior MI + 14 pts w/IHD w/prior MI) 1st month: One tab x four/day after meal 2nd-3rd month: One tab x four/day ST + placebo (n= 10 patients w/IHD without prior MI + 10 patients w/IHD w/prior MI) Healthy controls (n=10)	month: 60 mg/day		taken; tolerability to physical exercise; hemorheological parameters; antioxidant parameters	endpoints measured. No side effects of treatment	Ametis, 2010
48 women undergoing operations on the ovaries Age: 20-34 yrs	ST+ Taxifolin:Ascorbic acid (1:2.5) (n=20) Basic therapy: 23 patients Four days before operation to 10 days after the operation	120 mg/day	14 days	Endogenous antioxidants, commissures, lipid peroxidation products; pregnancy	No adverse effects of treatment on endpoints measured. No side effects of treatment	Plotnikov et al. (2005) as cited in Ametis, 2010
35 patients with chronic venous insufficiency (CVI); 25 patients with atherosclerosis	ST + Laviocard (Lavitol®) one tab/day (n=30, 15/condition) ST (n = 30, 20 CVI + 10 atherosclerotic)	30 mg/day	30 days	Blood and circulation parameters	No adverse effects of treatment on endpoints measured. No side effects of treatment	Plotnikov et al. (2005) as cited in Ametis, 2010

CVI – chronic venous insufficiency; IHD – ischemic heart disease; NIDDM – non insulin dependent diabetes mellitus; ST – standard therapy; tab – tablet; yrs – years

c. Conclusion from Clinical Studies with DHQ, Ascovertin and Laviocard

Blue California has reviewed clinical studies that have been performed with DHQ, Ascovertin and Laviocard, which show that DHQ is well tolerated in humans, even those with various diseases, and agrees that they support the safety of the proposed use of DHQ.

8. Absorption, Distribution, Metabolism, and Excretion (ADME)

The absorption of a dietary flavonoid depends on its physiochemical properties such as molecular size, configuration, lipophilicity, solubility, and pH (Kumar, 2013). The flavonoids are then absorbed from either the small intestine or colon depending on the structure of the flavonoid. Following absorption, the flavonoids are conjugated in the liver by glucuronidation, sulfation or methylation, or are metabolized to smaller phenolic compounds.

In the late 1950s, Booth and Deeds reported on the metabolism of DHQ in humans. Two volunteers were given 2 grams of DHQ orally, and their urine samples were analyzed for metabolites; 3,4-dihydroxyphenylacetic acid, m-hydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid were observed. The authors noted that these are the same metabolites excreted following oral administration of quercetin or 3,4-dihydroxyphenylalanine (DOPA) in rats, rabbits, and humans (Booth and Deeds, 1958).

The urinary metabolites of French maritime pine bark extract, which is known to contain DHQ, were studied after oral administration of 5.28 grams and 1.06 grams in a human volunteer. Taxifolin conjugated as a glucuronide/sulfate was excreted in the urine within 18 hours post dosing, with peak excretion at two to three hours. The recovery of taxifolin in the urine ranged from 7-8% (Düweler and Rohdewald, 2000).

Single and multi-dose studies on Pycnogenol® (a maritime pine bark extract containing 14.35 µg taxifolin per mL) were conducted in human volunteers (Grimm et al., 2006). In a single-dose study, eleven volunteers (five female, six male) received 300 mg Pycnogenol® (calculated dose of 4.31 mg taxifolin) orally after 24-hours on a flavonoid-restricted diet. Taxifolin (both free and conjugated) was not detected in plasma prior to two hours post-dosing, and maximum concentrations were observed after eight hours, before dropping to a steady level until 14 hours post dosing (experiment end). The authors calculated a maximal plasma concentration (C_{max}) of approximately 33.34 ng per mL for taxifolin, with a time of maximal plasma concentration (T_{max}) of ~8.2 hours, and a terminal half-life ($T_{1/2}$) of ~8.89 hours based on the concentration of free taxifolin in the plasma samples. Based on this study, assuming linear absorption and a molecular weight of 304.25 g per mole, the C_{max} for taxifolin, when ingested at the 90th percentile estimated from use as described in this GRAS dossier (33.72 mg per day) is 260.8 ng per mL or 0.86 µM.

In a subsequent multiple-dose study, five volunteers (four female, one male) received 200 mg Pycnogenol® (equivalent to 2.87 mg taxifolin) via tablet for five days to reach steady state conditions, after 24-hours on a flavonoid-restricted diet (Grimm et al., 2006). Plasma samples were obtained 4 hours after the final dose; however, at this dose level, plasma taxifolin levels were below the limit of quantitation (10 ng per mL) in all samples. The authors indicated that the delayed observation of taxifolin after a single dose, as well as the lack of steady-state levels in plasma following multiple doses, may be due to metabolic degradation. The authors noted that following oral ingestion, *Clostridium orbiscindens* in the gastrointestinal tract can degrade taxifolin to 3,4-dihydroxyphenylacetic acid and phloroglucan; however, neither of these metabolites were observed in the plasma samples.

In an additional study with Pycnogenol®, 33 patients with severe osteoarthritis scheduled for a knee arthroplasty were randomized to two groups; one receiving 200 mg per day Pycnogenol® (equivalent to 2.87 mg taxifolin) for three weeks (n=16) and the other receiving no treatment (n=17) prior to surgery (Mulek et al., 2017). All participants were asked to consume a polyphenol-free diet before sample collection and were provided guidance on which foods to avoid. Blood samples were collected before treatment and during or shortly before surgery, approximately 12 hours after the last dose of Pycnogenol®. Concentrations of taxifolin in serum, blood cells and synovial fluid of treated patients were 0.20 ± 0.12 ng per mL, 0.56 ± 0.19 ng per mL, and 0.21 ± 0.03 ng per mL, respectively, slightly above the level of quantification (0.06 ng per mL, 0.12 ng per mL, and 0.08 mg per mL, respectively). Taxifolin was also found in blood cells of control patients (0.39 ± 0.16 ng per mL), but not serum or synovial fluid.

In 2003, Schoefer et al. investigated the anaerobic degradation of taxifolin by *C. orbiscindens*. When *C. orbiscindens* strain I2 cells were treated with 1 mM taxifolin, both 3,4-dihydroxyphenylacetic acid and alphitonin were observed. After treatment with 0.5 mM taxifolin, degradation to 3,4-dihydroxyphenylacetic acid was complete within five hours, while alphitonin was not detected. The authors concluded that *C. orbiscindens* might be as important as *Eubacterium ramulus* for flavonoid degradation in the human gastrointestinal tract (Schoefer et al., 2003).

In a study to evaluate the metabolism of taxifolin *in vivo*, twelve Sprague-Dawley rats were maintained in metabolic cages with *ad libitum* access to food and water and were divided into two groups following acclimation (Yang et al., 2016). Taxifolin was prepared in 0.5% analytical-grade sodium carboxymethyl cellulose (CMC-Na) solution and one group was dosed with the vehicle and the other with 200 mg per kg body weight of taxifolin, once daily for three days. During the dosing period, urine and feces were collected at 0 - 24 hours following the first and second dosing, in the treated and control groups, respectively. Following the last administration, blood samples were collected at 0.5, 1, and 1.5 hours from two rats per time point. There were 191 metabolites tentatively identified; of these, 154 were new metabolites, 69 were new compounds, and 32 were dimers. Seventeen metabolites were found to have various taxifolin-related bioactivities and the potential targets of taxifolin and 63 metabolites were predicted using PharmMapper, with results showing that more than 60 metabolites have the same five targets. These metabolites may exert the same pharmacological effects as taxifolin through an additive effect on the same drug targets. This observation indicated that taxifolin is bioactive not only in the parent form but also through its metabolites.

In a study to investigate the pharmacokinetics of plant phenolic compounds, rats were dosed i.v. with a single dose of DHQ at 1, 3, 10, and 30 mg per kg and with a single oral dose at 50 and 500 mg per kg (Voskoboinikova et al., 1993). Non-linear pharmacokinetic behavior was demonstrated following intravenous administration and after oral administration, DHQ was detected in only trace amounts in the plasma.

In vitro metabolism studies on taxifolin were conducted on human and rat hepatocytes in cell suspensions and primary cultures (Vacek et al., 2012). The major taxifolin metabolite was its sulfated conjugate and the methylated and dehydroxylated metabolites were also observed in human hepatocytes. Methylated and glucuronide conjugates were also observed in rat hepatocytes.

Blue California has reviewed these ADME studies conducted both in humans and animals and concludes that they do not raise any concerns about the safety of DHQ when used at the proposed levels.

9. Properties of Dihydroquercetin

As the biological action or mechanisms of action of an ingredient may reveal potential safety related concerns, a summary of the studies on the biological activity of DHQ that are pertinent for safety is presented here.

a. Estrogenic/Antiandrogenic Activity

The estrogenic potential of taxifolin was investigated with several other phytoestrogens using an *in vitro* assay that measured the transcriptional activation of the estrogen receptor (ER) in the BG1Luc4E2 cell line and an *in vivo* mouse uterotrophic bioassay in female CD-1 mice (Jefferson et al., 2002). For the *in vivo* assay, groups of 10 weanling mice were injected subcutaneously with various doses of taxifolin or other phytochemicals (0.1, 1, 10, 100, 1,000, 10,000, 100,000, or 500,000 μg per kg bw per day), corn oil (negative control), or varying doses of diethylstilbestrol or 17β-estradiol (positive controls) for three consecutive days. On the fourth day, animals were euthanized, and the uterus excised for examination. The positive controls gave the expected results in all assays. Taxifolin was the only phytochemical tested that did not induce transcriptional activation of the ER in the BG1Luc4E2 cell line at concentrations up to 10 mg per mL. In the mice, there was no effect of any dose of taxifolin tested on uterine wet weight or epithelial cell production of the estrogen responsive protein lactoferrin. The authors mentioned that taxifolin exhibited an increase in epithelial cell height at 1 μg per kg bw per day, but not at higher doses; however, data presented in Figure 3 of the study indicate that this result is for naringenin, rather than taxifolin. As shown in the study, taxifolin did not affect this endpoint at any concentration. Although taxifolin did not increase uterine wet weight, uterine gland numbers were slightly increased at a taxifolin dose of 500,000 μg per kg bw per day (500 mg per kg bw per day) compared to controls. It is unknown whether the result for uterine gland numbers was significantly different from control because the data for this endpoint were not analyzed statistically. The authors concluded that taxifolin appeared to be one of the least potent phytoestrogens tested in this study.

Liu et al. (2018) screened a panel of phytoestrogens for their role in estrogen receptor alpha (ERα) binding and transcriptional transcription and correlated the findings to anti-inflammatory activities in vascular endothelial cells stably expressing either a wild-type or mutant form of ERα deficient in its membrane association. Taxifolin was a “high binder” for ERα ligand binding, but was not a “high activator.” The investigators also found that in endothelial cells expressing wild-type ERα, the ERα “high activator,” but not the ERα “high binder,” promoted ERα nuclear translocation, estrogen response element reporter activity, and downstream gene expression. Further, only the ERα “high activator” inhibited nuclear translocation of nuclear factor ƘB, JNK, and p38, and the production of inflammatory cytokines IL-1β and TNFα. The results indicate that although taxifolin binds to the ERα receptor, it does not induce transcriptional activation of the ER, similar to the results of Jefferson (2002).

Taxifolin has been tested for antiandrogenic activity in a number of different assays in rat Leydig and human testes (Ge et al., 2018). Taxifolin (100 μM) significantly suppressed basal androgen production or androgen production stimulated by luteinizing hormone, 8-bromoadenosine 3′,5′-cyclic monophosphate, pregnenolone or progesterone basal in rat Leydig cells. Further study demonstrated that taxifolin inhibited rat 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase/17, 20-lyase (enzymes involved in androgen synthesis) with half maximal inhibitory concentration (IC_{50}) values of 14.55 ± 0.013 and 16.75 ± 0.011 μM (respectively) and no effect at approximately 1 μM. Taxifolin also inhibited these two enzyme activities in human testis with IC_{50} values of approximately 100 μM. As mentioned in the ADME section above, the maximum concentration of DHQ that could be present in plasma after consumption of DHQ at the 90th percentile as indicated in this GRAS dossier (approximately 260.84 ng per mL or 0.86 μM) is lower than the inhibitory concentrations for the assays examined in this study.

b. Antioxidant

Weidmann (2012) conducted a review on the properties of DHQ and noted that based on the presence of two of the three criteria for effective radical scavenging ability, the presence of the o-dihydroxy structure in the B ring which confers stability, and the 5- and 7-OH (hydroxyl) groups with 4-oxo function in the A and C rings which give the maximum radical scavenging potential, DHQ is classified as an antioxidant.

Several publications examined the ability of DHQ to act as an antioxidant *in vitro* and *in vivo*. Results of the *in vitro* studies are mentioned here because they support the hypothesis that DHQ may act as an antioxidant in the foods that DHQ will be added to per this GRAS dossier. DHQ is an effective antioxidant in vegetable oils, animal fat, milk powder, fat-containing pastry, and packaged veal (Dragoev et al., 2014).

DHQ was evaluated for the ability to act as a hydroxyl radical (•OH) scavenger in bone marrow-derived mesenchymal stem cells (Li, 2017). The study employed a variety of antioxidant assays, and the results showed that DHQ could act as an effective •OH scavenger via direct and indirect antioxidant effects.

The antioxidant and antiradical activities of taxifolin were investigated in another study by using different *in vitro* bioanalytical antioxidant methods including N,N-dimethyl-p-phenylene diamine radical cation (DMPD•+), 2,2′-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid radical cation (ABTS•+), superoxide anion radical (O_2•-), and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging effects, the total antioxidant influence, reducing capabilities, and ferrous iron (Fe^{2+}) chelating activities (Topal, 2015). Taxifolin demonstrated 81.02% inhibition of linoleic acid emulsion peroxidation at 30 μg per mL and demonstrated effective DMPD•+, ABTS•+, O_2•-, and DPPH• -scavenging effects, reducing capabilities, and Fe^{2+} chelating effects.

In another study, the *in vitro* antioxidant effects of taxifolin were studied in several assays, including a DPPH• scavenging assay, ABTS• scavenging assay, ferric reducing antioxidant

property (FRAP) assay, and •OH scavenging capacity (Manigandan et al., 2015). The activities of DPPH•, ABTS, FRAP, and •OH levels were significantly inhibited by taxifolin with IC_{50} values of 16.48, 66.34, 18.17, and 11.42 µg per mL, respectively.

There are indications that some flavonoids may have prooxidant effects (Metodiewa et al., 1999; Yang et al., 2012; Schmalhausen et al., 2007; Choi et al., 2003; Chobot et al., 2016). (Chobot et al., 2016) conducted an *in vitro* deoxyribose degradation assay to assess the pro- and antioxidant activity of three flavan type flavonoids, including taxifolin. The authors concluded that taxifolin demonstrated no prooxidant activity within the tested concentrations. The highest dose tested was 500 µM.

c. Effect on Drug Metabolizing Enzymes

Taxifolin is an inducer of some cytochrome P450 (CYP) isozymes *in vitro*. Jin et al. (2018) found that taxifolin induced CYP1A1 and UGT1A1 in Caco2 cells in a dose-dependent manner, with 50 and 100 µM (but not 10 µM) causing statistically significant increases in mRNA for these enzymes. Taxifolin did not have the same effect in K01 or K02 cells, which lack the aryl hydrocarbon receptor (AhR). As discussed in the ADME section above, the maximum concentration of DHQ that could be present in plasma after consumption of DHQ at the 90th percentile as indicated in this GRAS dossier (approximately 260.84 ng per mL or 0.86 µM) is considerably lower than the concentration that induced CYP1A1 and UGT1A1 in Caco2 cells.

10. Summary

Blue California's DHQ product is manufactured with suitable food-grade materials and analyzed using HPLC to prepare a method verification report. Analysis of Blue California's DHQ product showed that it is substantially equivalent to Ametis JSC's DHQ material Lavitol®, which has been approved by EFSA as a novel food.

Acute, subacute, subchronic, chronic, and reproductive and developmental animal studies were reviewed, and all showed that DHQ is well tolerated in laboratory animal models. There is a substantial amount of published literature that supports the safety of DHQ in human subjects. A number of ADME studies in both humans and animals were also reviewed. In December of 2016, and again in late 2017 for additional groups not evaluated in the first review, EFSA reviewed a novel food application for a taxifolin-rich extract for Ametis JCS and concluded that the extract would be safe under the proposed conditions of use.

B. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."[8]

Amplification is provided in that the conclusion of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

> "…General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances directly or indirectly added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use."
>
> "'Common knowledge' can be based on either "scientific procedures" or on experience based on common use of a substance in food prior to January 1, 1958." [9]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[10]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. General recognition of safety through scientific procedures shall be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are

[8] See 21 CFR 170.3 (e)(i) and 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 4/15/17).
[9] See 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 4/15/17).
[10] See Footnote 1.

published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods.

The apparent imprecision of the terms “appreciable,” “at the time,” and “reasonable certainty” demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

As noted below, this safety assessment to ascertain GRAS status for DHQ for the specified food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

C. Expert Panel Findings on Safety of BC-DHQ™

An evaluation of the safety and GRAS status of the intended use of Blue California’s BC-DHQ™ high purity dihydroquercetin preparation has been conducted by an Expert Panel convened by GRAS Associates; the Panel consisted of Kara Lewis, Ph.D. as Panel Chair; Margitta Dziwenka, DVM, DABT; and Stanley Omaye, Ph.D. The Expert Panel reviewed Blue California’s dossier as well as other publicly available information available to them. The individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 6.

D. Common Knowledge Elements for GRAS Conclusions

The first common knowledge element for a GRAS conclusion requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS conclusion requires that consensus exists within the broader scientific community.

1. Public Availability of Scientific Information

The majority of studies reviewed on DHQ have been published in the scientific literature; however, the Ametis JSC novel food dossier submitted to EFSA contained a number of unpublished studies or published studies with no English translation. EFSA published a critical evaluation on a taxifolin-rich extract from Dahurian Larch (~90% DHQ) in December 2016 and concluded that it is safe for use as a food supplement (EFSA et al., 2016). EFSA also released a second statement in late 2017 on the safety of the same extract, but this time considered all population groups, which was implemented as Commission Regulation (EU) 2018/461. Relevant toxicity studies, ADME studies, and a number of clinical studies found in the published literature support the conclusion that DHQ is well-tolerated in humans.

2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there be a basis for concluding that consensus exists among qualified scientists about the safety of the substance for its intended use. Blue California intends to use its DHQ preparation as an ingredient in a limited number of human food categories.

EFSA reviewed the body of data available on DHQ in December 2016, and again in late 2017, and concluded that the taxifolin-rich extract (~90% DHQ) was safe as a novel food under the intended conditions of use proposed by Ametis JSC. The proposed use was as an ingredient in non-alcoholic beverages, fermented milk and dairy products, and chocolate and as a dietary supplement at 100 mg DHQ per day.

Blue California's proposed levels of use for DHQ range from 0.02 g per L in non-alcoholic beverages and flavored fermented milk and dairy products to 0.07 g per kg in chocolate products. The intended uses proposed by Blue California result in estimated DHQ exposure of 33.72 mg per day (90th percentile) for adults, which are much lower than those reviewed by EFSA.

The relevant animal toxicity, ADME, reproductive and/or developmental toxicity, genotoxicity and mutagenicity studies, in addition to the human clinical studies, support the conclusion that the intended levels of use do not raise any safety concerns.

Blue California maintains that well-qualified scientists would conclude that DHQ is generally recognized as safe for use in food given the regulatory and safety data available.

E. Conclusion

The ingestion of Blue California's DHQ preparation from the intended uses of 0.02 g per L in non-alcoholic beverages, 0.02 g per kg in flavored fermented milk and dairy products, and 0.07 g per kg in chocolate products results in intakes that are safe within the limits of established historical use of 100 mg per day (EFSA, 2017; Turck et al., 2017) and published safety studies in animals with NOAELs in subchronic toxicity studies up to 1,500 mg per kg bw per day.

In consideration of the aggregate safety information available on DHQ, Blue California concludes that the DHQ preparation defined in this GRAS Assessment, and produced under Current Good Manufacturing Practices with food grade materials and processing aids, is safe for use as an ingredient in foods other than infant formulas and meat and poultry products, and is generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE.

A. Acronyms and References

1. List of Acronyms

^{1}H-NMR	Proton nuclear magnetic resonance
µM	Micromolar
ACE	Angiotensin-converting enzyme
ADME	Absorption, Distribution, Metabolism, and Excretion
AhR	Aryl hydrocarbon receptor
AOAC	Association of Official Analytical Chemists
bw	Body weight
CFR	Code of Federal Regulations
cfu	Colony forming unit
CGMP	Current Good Manufacturing Practice
C_{max}	Maximal plasma concentration
COSY 2D NMR	^{1}H-^{1}H Correlation spectroscopy 2-dimensional nuclear magnetic resonance
CVI	Chronic venous insufficiency
CYP	Cytochrome p450
DDT	Dichlorodiphenyltricholorethane
DHQ	Dihydroquercetin
DNA	Deoxyribonucleic acid
DOPA	3,4-Dihydroxyphenylalanine
EDIs	Estimated Daily Intakes
EFSA	European Food Safety Authority
ER	Estrogen receptor
F3H	flavanone 3β-hydroxylase
FD&C	Food, Drug, and Cosmetic Act
FOIA	Freedom of Information Act
FSANZ	Food Standards Australia New Zealand
GI	Gastrointestinal
GLP	Good laboratory practices
GRAS	Generally Recognized as Safe
GRN	GRAS Notice
HBA1c	Glycated hemoglobin
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
HUVE	Human umbilical vein endothelian cells
i.v.	Intravenous
IC_{50}	Half maximal inhibitory concentration
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IHD	Ischemic heart disease
L	Liter
LC	Lethal concentration
MDA	Malondialdehyde
mL	Milliliter
mRNA	Messenger ribonucleic acid
NA	Not applicable
NADPH	Nicotinamide adenine dinucleotide phopshate
ND	Not detected
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
ng	Nanogram
NGS	NADPH generating system
NIDDM	Non insulin dependent diabetes mellitus
NOAEL	No observed adverse effect level
NS	Not specified

OECD	Organisation for Economic Co-operation and Development
ppm	Parts per million
RACC	Reference Amounts Customarily Consumed
RBC	Red blood cell
SHR	Spontaneously hypertensive
$T_{1/2}$	Terminal half-life
tab	Tablet
TBARs	Thiobarbituric acid reactive substances
TIG-1	Cultured human lung embryonic fibroblasts
T_{max}	Time of maximal plasma concentration
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
v/v	Volume per volume
yrs	Years

2. References

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B. Appendices

Appendix 1 Determination of the Relative and Absolute Structural Configuration of Blue California's DHQ

Appendix 1.1 1H-NMR (Figure 1.1) and COSY (Figure 1.2) spectra confirmed the compound as trans-dihydroquercertin

Figure 1.1 1H-NMR spectrum of compound (500 MHz, DMSO-*d4*)

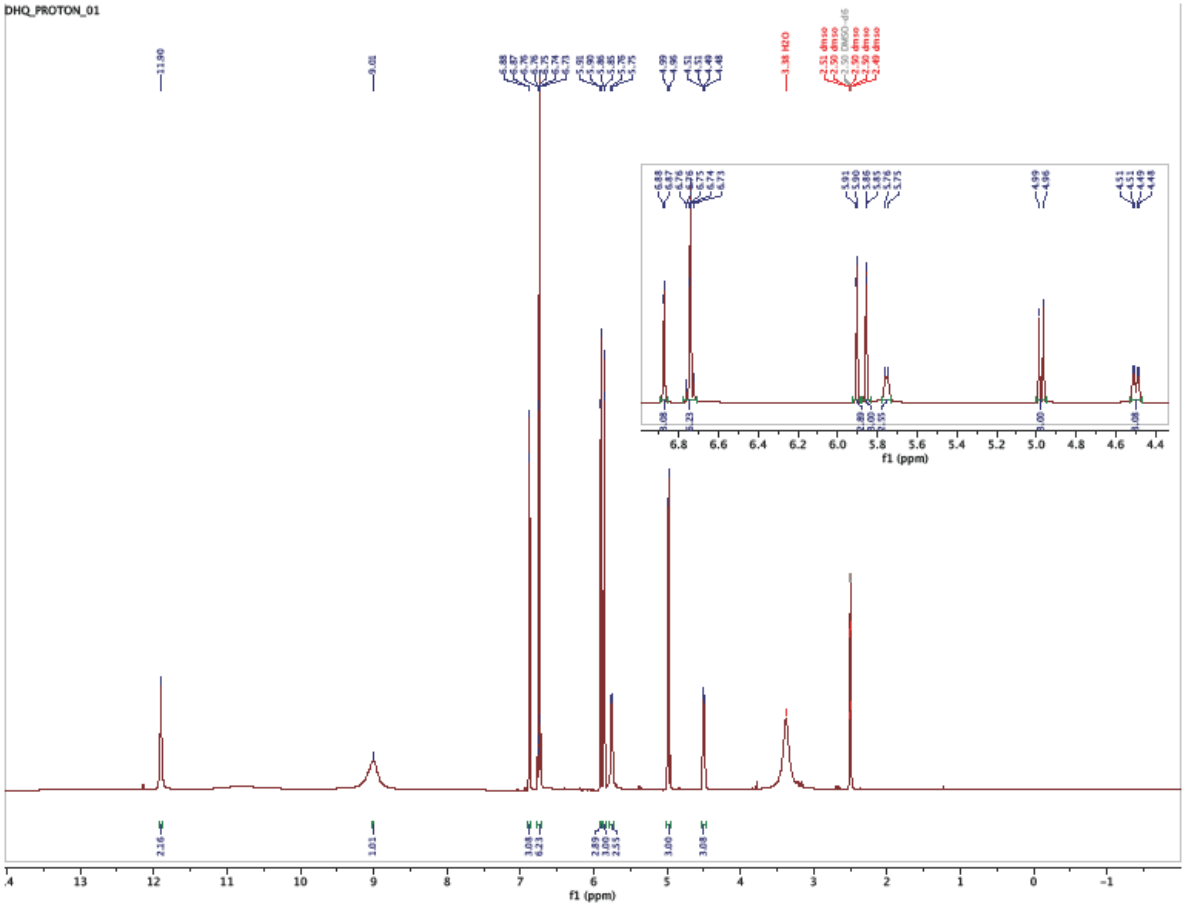
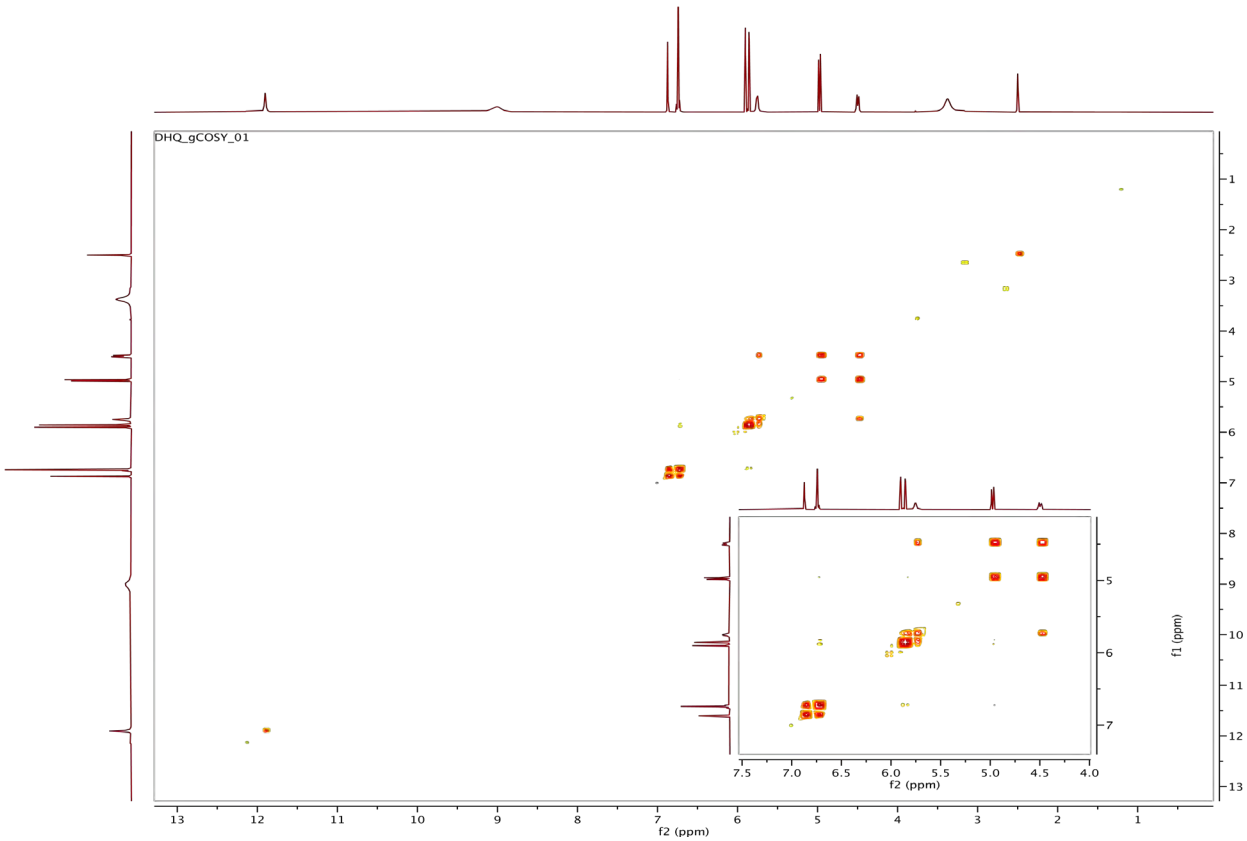
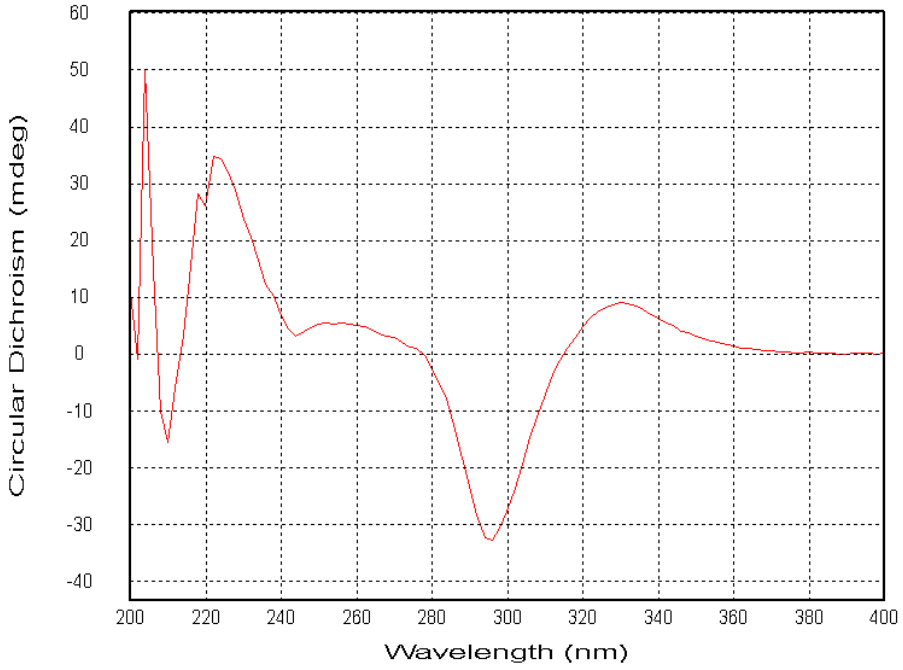


Figure 1.2 COSY spectrum of compound (500 MHz, DMSO-*d4*)



Appendix 1.2 Circular dichroism (CD) spectrum confirmed the compound as *2R,3R*-dihydroquercetin

Figure 1.3 Circular dichroism (CD) spectrum of compound



Appendix 2 Specifications and Certificates of Analyses for Production Processing Aids

Appendix 2.1 Glycerol

FSQD 0398156

KEMENTERIAN KESIHATAN MALAYSIA

MINISTRY OF HEALTH MALAYSIA

No. Rujukan: Bil (33) dlm. PKK 3712/11 Pt. 1(16)
Reference No.:

SIJIL KESIHATAN
HEALTH CERTIFICATE

(Makanan Am)
(General Food)

Penihal Konsainmen: *Description of Consignment:*	GLYCERIN, SUPEROL K - 99.7%
Kuantiti: *Quantity:*	80MT
Tanda Perdagangan: *Trade Mark:*	SUPEROL K GLYCERIN, USP, FCC
No. Konsainmen: *Consignment No.:*	XIAMEN FANGSHENGHUA IMPORT AND EXPORT TRADE CO LTD (CN-A16-193)
No. Kod dan Siri: *Code and Serial No.:*	70180388-GA-1 70220388-GB-1
Pembungkus: *Packer:*	FPG OLEOCHEMICALS SDN BHD, LOT 3831 KUANTAN PORT INDUSTRIAL AREA, TANJUNG GELANG, 26080 KUANTAN
Pengeksport: *Exporter:*	FPG OLEOCHEMICALS SDN BHD, LOT 3831 KUANTAN PORT INDUSTRIAL AREA, TANJUNG GELANG, 26080 KUANTAN
Pelabuhan Pengeksportan: *Port of Shipment:*	KUANTAN
Destinasi: *Destination:*	SHANGHAI, CHINA
Nama Kapal: *Name of Vessel:*	THALASSA PATRIS 0608-016E
Tempat Pensampelan: *Sampling Point:*	ANALYSIS TANK S1617A : FPG OLEOCHEMICALS ANALYSIS TANK S1617B: FPG OLEOCHEMICALS

Dengan ini adalah diesahkan bahawa konsainmen di atas telah diperiksa seperti berikut:
This is to certify that the above mentioned product has been duly inspected as specified:

THIS CERTIFICATE IS BASED ON HAZARDS ANALYSIS CRITICAL CONTROL POINT CERTIFICATE ISSUED BY THE MINISTRY OF HEALTH MALAYSIA VIDE REFERENCE NO KKM-HQ/VIS/07/1 AND SERIAL NO. 01413 DATED 13 NOVEMBER 2016

	70180388-GA-1	70220388-GB-1
LIMIT OF DEG &EG	< 0.10%	< 0.10%
FATTY ACID & ESTERS (USP)	0.1	0.1
SPECIFIC GRAVITY	1.2613	1.2614
COLOR, (APHA)	2	2
WATER	0.0	0.1
CHLORIDES	< 10ppm	< 10ppm
SULFATE	< 20ppm	< 20ppm
HEAVY METALS	< 1ppm	< 1ppm
CHLORINATED COMPOUNDS	< 30ppm	< 30ppm
SULPHATED ASH	0.00	0.00

Tandatangan: *Signature:*
Jawatan: *Designation:* DR. HJH FATIMAH BT A. MAJID
MEDICAL OFFICER OF HEALTH
DISTRICT HEALTH OFFICE, KUANTAN
PAHANG, MALAYSIA
Cop Rasmi: *Official Stamp:*

Tarikh Keluaran: *Date of Issue: JANUARY 31ST 2017*

Sijil ini adalah sah untuk dan tarikh keluarnya. Pengeluaran sijil ini bukannya bermaksud bagi pameran atau pengiklanan.
This certificate is valid for (3) THREE MONTHS from the date of issue. This certificate is not meant for display or use as an advertisement.

Appendix 2.2 Yeast Peptone

Test Report

Check (Trade) Word No. 2016-SP13935

Product Name: Angel Yeast (yeast extract)

Specifications and Model: Powder

Unit Being Tested: Angel Yeast (Liuzhou) Co., Ltd.

Test Category: Commissioned inspection

Three Gorges Center for Food and Drug Control

Three Gorges Center for Food and Drug Control
Test Report

Check (Trade) Word No. 2016-SP13935 Page 1 out of 2

Product Name	Angel Yeast (yeast extract)	Specifications and Model	Powder
Sample Grade	N/A	Trademark	Angel
Unit Being Tested	Angel Yeast Co., Ltd.	Address of Unit Being Tested	N/A
Trust Unit Name	Angel Yeast Co., Ltd.	Test Category	Commissioned inspection
Manufacturer	Angel Yeast Co., Ltd.	Production Date / Lot Number	201611230288
Sampling Personnel	N/A	Commissioned By	Biying Luo
Sampling Site	N/A	Sampling Date	N/A
Sample Quantity	500g * 2	Sent Date	12/21/2016
Sample Batch	N/A	Test Date	12/22/2016 – 01/19/2017
Test Items	See attached pages	Sample Description	Normal, meet inspection requirement
Test Standard(s)	Q/YB 21875-2015		
Test Conclusion	The sample meets the requirements of Q/YB 21875-2015. (Stamp) Date of Issue: 01/20/2017		
Remarks	N/A		

Approver: Ailing Luo Examiner: Suyuan Li Major Tester: Dinghuan Zhao

Three Gorges Center for Food and Drug Control
Test Result

Check (Trade) Word No. 2016-SP13935

No.	Test Items		Unit	Specification	Test Results	Evaluation
1	Color		N/A	Light yellow to light brown	Yellow	Pass
2	Smell		N/A	Odor that yeast peptone should be	No strange smell	Pass
3	Exterior		N/A	Powder or paste	Powder	Pass
4	Impurities		N/A	No visible foreign impurities	No visible foreign impurities	Pass
5	Total Nitrogen (measured on dry basis)		%	>= 8.0	11.8	Pass
6	Amino Nitrogen (measured on dry basis)		%	>= 1.5	3.3	Pass
7	Moisture		%	<= 6.0	3.8	Pass
8	Ash		%	<= 15.0	9.0	Pass
9	NaCl		%	<= 2.0	0.5	Pass
10	pH		N/A	5.3 – 7.2	5.8	Pass
11	Pb		mg/kg	<= 2	< 0.1	Pass
12	Total As		mg/kg	<= 2	0.13	Pass
13	Total number of colonies		cfu/g	<= 50000	4200	Pass
14	Coliforms		MON/g	<= 0.3	< 0.3	Pass
15	Pathogens	Staphylococcus aureus	/25g	Cannot have any	Not detected any, /25g	Pass
		Salmonella	/25g	Cannot have any	Not detected any, /25g	Pass

Blank Below

Appendix 2.3 Ferrous Sulfate

Test Report

No: H2017WTS0156

Anti-counterfeiting code: 0XR64R

Product name	Ferrous sulfate
Unit being tested	-
Manufacturer	Jiangsu Kolod Food Ingredients Co., Ltd.
Entrusting Unit	Jiangsu Kolod Food Ingredients Co., Ltd.
Test Kind	Consigned Inspection

The Center of Lianyungang Product Quality Supervision and Inspection

The Center of Lianyungang Product Quality Supervision and Inspection

Test Report

No: H2017WTS0156 Page 1 of 2 pages

Product name	Ferrous sulfate	Trademark	Kolod
		Trademark (nominal)	-
Manufacturer	Jiangsu Kolod Food Ingredients Co., Ltd.		
Entrusting Unit/Address/Tel./ Postcode	Jiangsu Kolod Food Ingredients Co., Ltd./ South Side of Weier Road, Economic Development Zone, Guanyun County /0518-85110538/222000		
Unit being tested	-		
Test Kind	Consigned Inspection	Sample No.	H2017WTS0156
Quantity of Sample	100 g	Sample Grade	-
Date of Test	February 13, 2017 to February 27, 2017	Producing Date/Batch No.	—\—
Status of Samples	The sample has met the testing requirements	Date of Delivery	February 10, 2017
Status of Sealed Sample	-	Sealed Sample Examined by	Li Zhenzhen
Place of Test	The Center of Lianyungang Product Quality Supervision and Inspection		
Test Basis	GB 29211-2012 *National Food Safety Standard for Food Additive Ferrous Sulfate*		
Test Conclusion	Upon testing, the sample has met the standard requirements specified in GB 29211-2012 and the test conclusion is qualified.		
Notes	-		
Chiefly tested by: Lin Zexin Reviewed by: Gu Tiantian Approved by: Wang Lin*	(Seal of Inspection Unit) *(Special Seal of Inspection of the Center of Lianyungang Product Quality Supervision and Inspection (2))* Issued on: February 27, 2017		

Test Result

No.: H2017WTS0156 Page 2 of 2 pages

Serial No.	Test Items		Unit	Technical Requirements	Test Results	Individual Judge
1	Sensory Requirements	Color	-	Grey or blue green	Blue green	Qualified
		Texture	-	Granular crystals	Granular crystals	Qualified
2	Ferrous sulfate (measuring in $FeSO_4 \cdot 7H_2O$), w%		-	99.5-104.5	99.8	Qualified
3	Pb		mg/kg	⩽2	＜2	Qualified
4	Hg		mg/kg	⩽1	Undetected (detection limit: 0.002mg/kg)	Qualified
5	As		mg/kg	⩽3	＜3	Qualified
Notes	-					

Appendix 2.4 Disodium Phosphate

Test Report

No: H2017WTS0164

Anti-counterfeiting code: 8L00LN

Product name	Food additive disodium hydrogen phosphate (anhydrous)
Unitbeing tested	-
Manufacturer	Jiangsu Kolod Food Ingredients Co., Ltd.
Entrusting Unit	Jiangsu Kolod Food Ingredients Co., Ltd.
Test Kind	Consigned Inspection

The Center of Lianyungang Product Quality Supervision and Inspection

The Center of Lianyungang Product Quality Supervision and Inspection

Test Report

No: H2017WTS0164 Page 1 of 2 pages

Product name	Food additive disodium hydrogen phosphate (anhydrous)	Trademark	Kolod
		Trademark (nominal)	-
Manufacturer	Jiangsu Kolod Food Ingredients Co., Ltd.		
Entrusting Unit/Address/Tel./ Postcode	Jiangsu Kolod Food Ingredients Co., Ltd./South Side of Weier Road, Economic Development Zone, Guanyun County /0518-85110538/222000		
Unitbeing tested	-		
Test Kind	Consigned Inspection	Sample No.	H2017WTS0164
Quantity of Sample	100 g	Sample Grade	-
Date of Test	February 13, 2017 to February 15, 2017	Producing Date/Batch No.	—\—
Status of Samples	The sample has met the testing requirements	Date of Delivery	February 10, 2017
Status of Sealed Sample	-	Sealed Sample Examined by	Li Zhenzhen
Place of Test	The Center of Lianyungang Product Quality Supervision and Inspection		
Test Basis	GB 25568-2010 *National Food Safety Standard for Food Additive Disodium Hydrogen Phosphate*		
Test Conclusion	Upon testing, the sample has met the standard requirements specified in GB 25568-2010 and the test conclusion is qualified.		
Notes	-		
Chiefly tested by:Wang Yisheng Reviewed by: Gu Tiantian Approved by: Wang Lin*	(Seal of Inspection Unit) *(Special Seal of Inspection of the Center of Lianyungang Product Quality Supervision and Inspection (2))* Issued on: February 21, 2017		

Test Result

No: H2017WTS0164 Page 2 of 2 pages

Serial No.	Test Items		Unit	Technical Requirements	Test Results	Individual Judge
1	Sensory Requirements	Color	-	White	White	Qualified
		Texture	-	Powder	Powder	Qualified
2	Disodium hydrogen phosphate (Na_2HPO_4, measuring in a dry basis), w%		-	⩾98.0	98.5	Qualified
3	As		mg/kg	⩽3	<3	Qualified
4	Heavy metal (measuring in Pb)		mg/kg	⩽10	<10	Qualified
5	Pb		mg/kg	⩽4	<4	Qualified
6	Fluoride (measuring in F)		mg/kg	⩽50	5	Qualified
7	Insoluble substance, w/%		-	⩽0.2	Undetected	Qualified
8	Loss on drying (Na_2HPO_4), w%		-	⩽5.0	0.3	Qualified
Notes	-					

Appendix 2.5 Phosphoric Acid

2014100463Z

2014 S.Z.J.Y.Zi. No. 463

Inspection and Test Report

No.(2017) HGWJ0153

Sample Name	85% industrial phosphoric acid
Applicant:	Jiangsu ChengXing Phosph-Chemicals Co.,Ltd.
Inspection & Test Category:	Consigned Inspection

Jiangyin Product Quality Supervision and Testing Institute

Inspection and Testing Report

No.(2017) HGWJ0153 Page 1 of 2

Name of Applicant	Jiangsu ChengXing Phosph-Chemicals Co.,Ltd.
Address of Applicant	618 Meiyuan Avenue, Jiangyin City
Information of Manufacturer	Jiangsu ChengXing Phosph-Chemicals Co.,Ltd.\ 618 Meiyuan Avenue, Jiangyin City (The sample information is provided by the entrusting party and thus the entrusting party shall be responsible for the authenticity of such information.)

The following sample information is provided and confirmed by the entrusting party:

Sample Name	85% industrial phosphoric acid
Quantity of Sample	500ml
Sample description	Batch No.: 17020702 Sample Grade: First-rated product Date of Production: February 7, 2017
Method of Delivery\Date	Delivered by Entrusting Party February 24, 2017
Test Date	February 27, 2017 to March 16, 2017
Basis of Inspection and Test	GB/T 2091-2008 *Industrial Phosphoric Acid*
Conclusions of Inspection and Test	Upon testing, the sample has met the standard requirements specified in GB/T 2091-2008 *Industrial Phosphoric Acid.*
Notes	-

Approved by: Wang Wenjie	Reviewed by: Lu Yeqing	Prepared by: Li Juan	Issued on: March 16, 2017
Wang Wenjie/Deputy head of the Chemistry and Building Materials Department	Lu Yeqing	Li Juan	*(Special Seal of Inspection and Testing of Jiangyin Product Quality Supervision and Testing Institute)*

Inspection and Test Results

No. (2017) HGWJ0153 Page 2 of 2

Serial No.	Test Item	Unit	Technical Requirements	Test Results	Individual Judge
1	Appearance	-	Colorless and transparent or light colored viscous liquid	Qualified	Qualified
2	Chroma	Hei Zeng	≤20	<20	Qualified
3	Phosphoric acid (H_3PO_4)	%	≥85.0	85.5	Qualified
4	Chloride (measuring in Cl)	%	≤0.0005	<0.0005	Qualified
5	Sulfate (measuring in SO_4)	%	≤0.003	<0.003	Qualified
6	Fe	%	≤0.002	<0.002	Qualified
7	As	%	≤0.0001	<0.0001	Qualified
8	Heavy metal (measuring in Pb)	%	≤0.001	<0.001	Qualified
Notes	-				

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Appendix 2.6 Eriodictyol

Nantong Haitian Biotech Co., Ltd

Certificate Of Analysis

Product Name	ERIODICTYOL 98%
Lot No	20170915-19
Date Of Manufacturing	2017-08-02
Qty.	750kg
QC acceptance date QC	2017-08-07
Country Of Origin	China
Original Manufacturer	Nantong Haitian Biotech Co.,Ltd.
Sterilization Status	Treated by steam
Package Size	15kg/drum

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
Appearance	off-white Powder	CP2000	Pass
Odor	Characteristic	OLFACTORY	Pass
Taste	Tasteless	GUSTATORY	Pass
Loss On drying	≤5.0%	CP2000	0.1%
Heave Metals	≤10PPM	CP2000	Pass
Bulk density	0.15~0.3g/ml	CP2000	0.16g/ml
Tap density	≥0.2g/ml	CP2000	0.30g/ml
Particle Side	≥95%through Mesh#80 Sieve	CP2000	96.3%
Ash	≤10.0%	CP2000	0.16%
Assay	≥98%	HPLC	98.5%
Lead	≤3PPM	ICP	<3PPM
Arsenic	≤3PPM	ICP	<3PPM
Cadmium	≤3PPM	ICP	<3PPM
Hg	≤3PPM	ICP	<3PPM
Total Plate Count	≤1000cfu/gm	AOAC	50cfu/g
Total Coliform	≤100cfu/gm	AOAC	none
Yeast And Molds	≤100cfu/gm	AOAC	15cfu/g
E.Coli.	NEGATIVE	AOAC	none
Salmonella	NEGATIVE	AOAC	none

TESTED BY: GU- DANTONG DATE: 09-15-17

APPROVED BY: DATE: 09-15-17

Appendix 2.7 Sodium Chloride

Test Report

(2015) Commission Checked No. 4

Product Name: Non-iodized refined salt
Specifications and Model: N/A
Trademark: N/A
Trust Unit: Zhongyan Dongxing Yanhua Co., Ltd.
Manufacture: Zhongyan Dongxing Yanhua Co., Ltd.
Test Category: Commissioned inspection

QUALITY SUPERVISION INSPECTION CENTER OF NATIONAL LIGHT INDUSTRY WELL MINERAL SALT ADMINISTRATION

Description

1. Entrusted inspection is only responsible for the sample.
2. This Inspection Report is invalid if no official seal of the inspection unit.
3. The copy of this Inspection Report is invalid if no official seal of the re-stamped inspection unit.
4. Altered "Inspection Report" is invalid.
5. If there is any objection to the Inspection Report, please submit written opinions to the inspection unit within 15 days from the date of receipt of the Inspection Report, and shall be deemed to recognize the Inspection Report.
6. If no preparation, inspection, review, and approval of the signature, the Inspection Report is invalid.
7. If no objection to the Inspection Report within one month after receipt, the sample should be taken back, otherwise it will be dealt with in accordance with the relevant provisions.

Brief Introduction of Quality Supervision and Testing Center of National Light Industry Well Salt

The Center has passed the China National Accreditation Board for accreditation of Conformity Assessment Laboratory and Food Inspection Agency. The laboratory is in good condition and well equipped, mainly to carry out salt products, food, chemical products, food additives, and feed additives testing, but also bear the quality supervision and inspection, revision of national standards, industry standards and test methods of research, testing personnel technical training, and technical advice business.

Address: No. 11 Dongxing Temple, Zigong City, Sichuan Province
Zip code: 643000
Tel: (0813) 8104587
Fax: (0813) 8207279

QUALITY SUPERVISION INSPECTION CENTER OF NATIONAL LIGHT INDUSTRY WELL MINERAL SALT ADMINISTRATION

Test Report

Product Name	Non-iodized refined salt	Trademark	N/A
Trust Unit	Zhongyan Dongxing Yanhua Co., Ltd.	Specifications and Model	N/A
Address	Dindyuan Salt Mine, Dingyuan County, Chuzhou City, Anhui Province	Sampling Batch	80t
Zip Code	N/A	Sample Amount	500g
Product Unit	Zhongyan Dongxing Yanhua Co., Ltd.	Sample Grade	N/A
Sampling Date and Site	N/A	Sent Date	01/07/2015
Production Date / Lot Number	2015.01.05	Sent By	Sufang Chen
Test Date	01/13/2015	Test Category	Commissioned inspection
Test Standard(s)	GB5461-2000 GB/T5009.15-2003 GB/T5009.17-2003	Environment	11°C
Sample Reception Description	Mailed, plastic bag packaging, packaging intact, the sample is white granular solid.		
Test Conclusion	Based on GB 5461-2000 and GB2762-2012, the sample meets the requirement of non-iodized refined edible salt excellent grade. (Stamp) Date of Issue: 01/20/2015		
Remarks	All information related to the sample, except the inspection result, is provided by the client, who is responsible for the authenticity of the information provided.		

Approver: Wenjie Lei Examiner: Shuying Fu Major Tester: Qian Tan
Prepared by: Zhiyong Chen

QUALITY SUPERVISION INSPECTION CENTER OF NATIONAL LIGHT INDUSTRY WELL MINERAL SALT ADMINISTRATION

Test Report

Test Items	Specification	Test Results	Evaluation
Level of whiteness, degree	>= 80	88	Pass
Granularity (0.15 – 0.85) mm, %	>= 85	99	Pass
NaCl, %	>= 99.10	99.45	Pass
Moisture, %	<= 0.30	< 0.01	Pass
Water-insoluble, %	<= 0.05	< 0.01	Pass
As, mg/kg	<= 0.5	< 0.5	Pass
Pb, mg/kg	<= 2.0	< 2.0	Pass
Cd, mg/kg	<= 0.5	< 0.005	Pass
Total Hg, mg/kg	<= 0.1	< 0.025	Pass
Ba, mg/kg	<= 15.0	< 15.0	Pass
$[Fe(CN)_6]^{4-}$, mg/kg	<= 10.0	4.8	Pass
I, mg/kg	< 5	0.1	Pass
Sensation: white, taste salty, no strange smell, no obvious foreign substance that is not related to salt.	Meet the requirements	Meet the requirements	Pass

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Appendix 2.8 Methanol

Certification #(No.): 320816090024 – 1Q

CCIC JIANGSU CO., LTD Date: October 12, 2016

Quality Certification (Cabin)

Item Name: Methanol (in bulk)

Weight: --9,584.530- metric tons (4750.000 metric tons and 4,834.530 metric tons) (bill of lading)

Transportation tool: "VISINO ENERGY 1" Ship

Loading Berth: 1P/S, 2P/S, 3P/S, 4P/S, 5P/S, 6P/S & SLOP-P/S

Transit: from MIDDLE EAST to China Taicang

Inspection location: Taicang Power Shell Petrochemical Co. LTD

Inspection Date: October 10, 2016 to October 12, 2016

Contract #: HI/1608/6421

BL #: SEV1605-01& SEV1605-02

Inspection Results:

According to GB/T 6680-2003 Standards, our company inspector took samples from the items before unloading and did tests. Results are shown as below:

Inspected item	Inspection Method	Inspection Results
Specific gravity (20/20, °C)	ASTM D4052-15	0.7927
Color intensity (Pt-Co)	ASTM D1209-05(2011)	<5
Acidity (acetic acid)/(mg/kg)	ASTM D1613-06 (2012	12
Potassium permanganate test (15°C)/min	ASTM D1363-06 (2011	>60
Acetone (mg/kg)	IMPCA 001-14	<30
Sulfuric acid scrubbing color intensity (Pt-Co)	ASTM E346-08#1	20
Water miscibility test	GB/ T 6324.1-2004	Pass (1+3)
Water content (mass fraction)/%	ASTRM E1064-16	0.018
Distillation range (0°C, 760mmHg), °C	ASTM D1078-11	
Initial boiling point, °C		64.5
Dry point, °C		64.7
Purity(dry basis) (mass fraction)/%	IMPCA 001-14	99.98
Ethanol/ (mg/kg)	IMPCA 001-14	<5
Chlorinity/ (mg/kg)	SN/T 2994-2011	0.069
Sulfur content / (mg/kg)	ASTM D5453-16	<0.5
Iron content / (mg/kg)	ASTM E394-15	<0.01
Non-volatile matter / (mg/100mL)	ASTM D1353-13	0.1
Exterior condition	IMPCA 003-98	Transparent, no mechanical impurity
Aromatic hydrocarbon / (mg/kg)	GC.FID	<0.20
* *	END *	*

Based on our knowledge, we have tried our best to finish the above tests. Issuance of this certification does not imply the exemption of responsibility from the round turn and others beneficial partners.

Industrial and Commercial Registration #: 320191000002448

Appendix 2.9 Ethanol

ETHYL ALCOHOL

Certificate of analysis

Item	Quality		Result	
	Guaranteed reagent (GR)	Standard grade		
Color	Colorless and transparent		Colorless and transparent	Qualified
Odor	Characteristic	No foreign odor	No foreign odor	Qualified
Taste	Pure	Purer	Purer	Qualified
Colorimetric reading	≤10		8	Qualified
Ethanol (% Vol)	≥95.5	≥95.0	95.0	Qualified
Sulphuric acid color index	≤10	≤60	50	Qualified
Oxidation min	≥30	≥20	25	Qualified
Acetaldehyde (mg/L)	≤2	≤30	20	Qualified
Methanol (mg/L)	≤50	≤150	115	Qualified
1-propanol (mg/L)	≤15	≤100	70	Qualified
Isobutanol and isoamyl alcohol (mg/L)	≤2	≤30	25	Qualified
Acid (Acetic acid) (mg/L)	≤10	≤20	16	Qualified
Cyanide (HCN) (mg/L)	≤5		3	Qualified
Conclusion	The product is qualified according to GB10343-2008 standard Date： 2016.3.13 (YYYY.MM. DD)			

Inspector: Ling, Fen and Zhang, Shiyu Auditor: Li, Hongming

Appendix 2.10 Ion-Exchange Resin

Blue California
NATURE + SCIENCE + INNOVATION

A Perfect Blend of Science and Nature

July 20, 2018

FOOD GRADE STATEMENT

BLUE CALIFORNIA hereby certifies that all the processing aids and the following materials used in the manufacturing process of BC-DHQ ™ Dihydroquercetin 95% are food grade materials.

1. 0.22 µm sterile filter
2. Ion Exchange Resin

We certify this to be true to the best of our knowledge.

Sincerely,

Hadi Omrani

Hadi Omrani
Manager- Technical and Regulatory Affairs

Corporate Headquarters
30111 Tomas, Rancho Santa Margarita, CA 92688 Tel: 949-635-1990 Fax: 949-635-1984
Website: www.bluecal-ingredients.com

LANSHEN RESIN

Shaanxi Lanshen Special Resin Co., Ltd.
Creating more value for client

Quality Test Report

JL8.2.4-3

Product Name	LS-38	Serial Number	2017-015
Test Standard(s)	Enterprise Standards	Test Date	06/20/2017
Appearance of product	Light yellow or yellow opaque spherical particles		

No.	Test Items	Test Result	Remarks
1	Particle size range (0.315 – 1.25mm) %	95.98	Pass
2	Water content (%)	55.67	Pass
3	Weak base exchange capacity (mmol/g)	4.52	Pass
4	Strong base exchange capacity (mmol/g)	2.08	Pass
5	Bulk density in wet state (g/ml)	0.73	Pass
6	True density in wet state (g/ml)	1.10	Pass
Conclusion	Pass (Stamp)		
Tester	Songsong Zhang	Examiner	Jinhua Feng

LANSHEN RESIN—WWW.SXLANSHEN.COM TEL:86-29-86690026 FAX:86-29-892834

Appendix 2.11 Activated Charcoal

State Forestry Administration of the People's Republic of China

Quality Inspection and Supervision station of Forest Products

Laboratory Analysis Report

Analysis Method:

GB/T12496.1~12496.22-99

Testing Item and Results:

1. Material	Wood
2. Granularity	200 Mesh
3. Methylene (mg/g)	198
4. % Ferric Salt	0.02
5. % Moisture Content	9.3
6. % Heavy Metal	0.02
7. PH	5.63
8. % Chloride	0.1

Requesting Agent: Liyin City Jiangyin Active Carbon Facility Analyzed by:

Sample Description: 767 Type Active Carbon Approved by:

Sample Number:

February 16, 2017

Appendix 2.12 Ingredient Statement

Blue California
NATURE • SCIENCE • INNOVATION

A Perfect Blend of Science and Nature

October 3, 2018

INGREDIENT STATEMENT

Product: **BC-DHQ ™ Dihydroquercetin 95%**
Item Number: **BC0107730**

We hereby certify that all of the raw materials used in a manufacturing process of Dihydroquercetin (BC-DHQ™), are suitable food-grade materials, and are used in accordance with applicable US Federal Regulations and current Good Manufacturing Practices (cGMP).

We certify this to be true to the best of our knowledge.

Sincerely,

Hadi Omrani

Hadi Omrani
Manager, Technical and Regulatory Affairs

Corporate Headquarters
30111 Tomas, Rancho Santa Margarita, CA 92688 **Tel:** 949-635-1990 **Fax:** 949-635-1984 **Website:** www.bluecal-ingredients.com

Appendix 3 Analytical Method and Representative Chromatograms

Please refer to the Appendix 3 report, provided as a separate file:

Appendix 3 Method Verification of DHQ by HPLC Report.pdf

Appendix 4 Certificates of Analyses for Multiple Production Lots of BC-DHQ™

Appendix 4.1 Certificate of Analysis BC-DHQ™ Lot 7730-160823

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: BC-DHQ ™ Dihydroquercetin 95% (*Natural preservative*)
Item# BC0107730

Lot No:	**7730-160823**	**Original Manufacturer:**	**Blue California Co.**
Date of Manufacturing:	**August 19-2016**	**Expiration/Re-test date:**	**August 19-2018**
QC acceptance date:	**August 23-2016**	**Country of Origin:**	**China**

This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to white powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
DIHYDROQUERCETIN	**≥95%**	**HPLC**	**97.8% (dry base)**
LOSS ON DRYING	≤5%	USP 34	3.32%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.5 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.25 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.1 ppm
ETHANOL	< 1,000 ppm	USP 34	PASS
METHANOL	< 200 ppm	USP 34	PASS
BULK DENSITY	≥0.15 g/ml	USP 34	0.16 g/ml
TAP DENSITY	≥0.30 g/ml	USP 34	0.32 g/ml
PARTICLE SIZE:	> 95% through Mesh #60 Sieve	USP 34	100%
TOTAL PLATE COUNT	< 5,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	ND
SALMONELLA	NEGATIVE	AOAC	ND
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: J.H.Zhou (QC Manager) Revision date: 03-14-2018

Appendix 4.2 Certificate of Analysis BC-DHQ™ Lot 7730-161028

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: BC-DHQ ™ Dihydroquercetin 95% (*Natural preservative*)
Item# BC0107730

Lot No:	**7730-161028**	**Original Manufacturer:**	**Blue California**
Date of Manufacturing:	**October 28-2016**	**Expiration/Re-test date:**	**October 28-2018**
QC acceptance date:	**November 15-2016**	**Country of Origin:**	**China**

This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to white powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
DIHYDROQUERCETIN	**≥ 95%**	**HPLC**	**97.8% (dry base)**
LOSS ON DRYING	≤ 5%	USP 34	3.71%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.5 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.25 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.1 ppm
ETHANOL	< 1,000 ppm	USP 34	PASS
METHANOL	< 200 ppm	USP 34	PASS
BULK DENSITY	≥ 0.15 g/ml	USP 34	0.15 g/ml
TAP DENSITY	≥ 0.30 g/ml	USP 34	0.32 g/ml
PARTICLE SIZE:	> 95% through Mesh #60 Sieve	USP 34	100%
TOTAL PLATE COUNT	< 5,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 10 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 100 cfu/gm
E. COLI:	NEGATIVE	AOAC	ND
SALMONELLA	NEGATIVE	AOAC	ND
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: J.H.Zhou (QC Manager) Revision date: 04-06-18

Appendix 4.3 Certificate of Analysis BC-DHQ™ Lot 7730-170425

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: BC-DHQ ™ Dihydroquercetin 95% (*Natural preservative*)
Item# BC0107730

Lot No:	**7730-170425**	**Original Manufacturer:**	**Blue California**
Date of Manufacturing:	**April 25-2017**	**Expiration/Re-test date:**	**April 25-2019**
QC acceptance date:	**June 08-2017**	**Country of Origin of Raw Material:**	**China**

This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to cream powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
DIHYDROQUERCETIN	**≥ 95%**	**HPLC**	**97.3% (dry base)**
LOSS ON DRYING	≤ 5%	USP 34	3.25%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.5 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.25 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.10 ppm
ETHANOL	< 1,000 ppm	USP 34	PASS
METHANOL	< 200 ppm	USP 34	PASS
BULK DENSITY	≥ 0.15 g/ml	USP 34	0.16 g/ml
TAP DENSITY	≥ 0.30 g/ml	USP 34	0.34 g/ml
PARTICLE SIZE:	> 95% through Mesh #60 Sieve	USP 34	100%
TOTAL PLATE COUNT	< 5,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	ND
SALMONELLA	NEGATIVE	AOAC	ND
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: J.H.Zhou (QC Manager) Revision date: 04-06-2018

Appendix 4.4 Certificate of Analysis BC-DHQ™ Lot 7730-170525

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: BC-DHQ ™ Dihydroquercetin 95% (*Natural preservative*)
Item# BC0107730

Lot No:	**7730-170525**	**Original Manufacturer:**	**Blue California**
Date of Manufacturing:	**May 25-2017**	**Expiration/Re-test date:**	**May 25-2019**
QC acceptance date:	**June 08-2017**	**Country of Origin:**	**China**

This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to cream powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
DIHYDROQUERCETIN	**≥ 95%**	**HPLC**	**95.2% (dry base)**
LOSS ON DRYING	≤ 5%	USP 34	3.48%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.5 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.25 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.1 ppm
ETHANOL	< 1,000 ppm	USP 34	PASS
METHANOL	< 200 ppm	USP 34	PASS
BULK DENSITY	≥ 0.15 g/ml	USP 34	0.17 g/ml
TAP DENSITY	≥ 0.30 g/ml	USP 34	0.32 g/ml
PARTICLE SIZE:	> 95% through Mesh #60 Sieve	USP 34	100%
TOTAL PLATE COUNT	< 5,000 cfu/gm	AOAC	< 500 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	ND
SALMONELLA	NEGATIVE	AOAC	ND
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: X.Y. Mao (QC Manager) Revision date: 03-14-2018

Appendix 4.5 Certificate of Analysis BC-DHQ™ Lot 7730-170616

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: BC-DHQ ™ Dihydroquercetin 95% (*Natural preservative*)
Item# BC0107730

Lot No:	**7730-170616**	**Original Manufacturer:**	**Blue California**
Date of Manufacturing:	**June 12-2017**	**Expiration/Re-test date:**	**June 12-2019**
QC acceptance date:	**June 26-2017**	**Country of Origin:**	**China**

This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to cream powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
DIHYDROQUERCETIN	**≥ 95%**	**HPLC**	**97.7% (dry base)**
LOSS ON DRYING	≤ 5%	USP 34	3.82%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.5 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.25 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.1 ppm
ETHANOL	< 1,000 ppm	USP 34	PASS
METHANOL	< 200 ppm	USP 34	PASS
BULK DENSITY	≥ 0.15 g/ml	USP 34	0.16 g/ml
TAP DENSITY	≥ 0.30 g/ml	USP 34	0.32 g/ml
PARTICLE SIZE:	> 95% through Mesh #60 Sieve	USP 34	100%
TOTAL PLATE COUNT	< 5,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	ND
SALMONELLA	NEGATIVE	AOAC	ND
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: J.H.Zhou (QC Manager) Revised date: 03-21-2018

Appendix 5 Pesticide Analyses for Multiple Production Batches of BC-DHQ™

Appendix 5.1 Pesticide Analysis BC-DHQ™ Lot 7730-160823

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 94954
Tel.+1 707 792 7300
Fax:+1 707 792 7309

July 17, 2017

Cecilia Cecilia McCollum
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

CERTIFICATE OF ANALYSIS
AR-17-KK-008895-01
Batch #: EUCAPE-00091741

Sample Identification:
Sample #: 740-2017-07030094
Description: BC-DHQ, Powder, Lot #7730-160823
Condition: Beige powder in a double ziplock bag received at room temperature.
Date Received: July 03, 2017

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/17/2017

	Result	Theoretical Level
Acephate	<0.10 mg/kg	
[Method performed by an outsource lab.]		
Alachlor	<0.02 mg/kg	
Aldrin and Dieldrin (sum of)	<0.02 mg/kg	
Azinphos-ethyl	<0.02 mg/kg	
Azinphos-methyl	<0.05 mg/kg	
Bromophos-ethyl	<0.02 mg/kg	
Bromophos-methyl	<0.02 mg/kg	
Bromopropylate	<0.05 mg/kg	
Chlordane (sum of cis-, trans- and Oxychlordane)	<0.05 mg/kg	
Chlorfenvinphos	<0.02 mg/kg	
Chlorpyrifos-ethyl	<0.02 mg/kg	
Chlorpyrifos-methyl	<0.02 mg/kg	
Chlorthal-dimethyl	<0.01 mg/kg	
Cyfluthrin (sum of)	<0.10 mg/kg	
Cyhalothrin, lambda-	<0.02 mg/kg	
Cypermethrin and isomers (sum of)	<0.1 mg/kg	
DDT (total)	<0.02 mg/kg	
Deltamethrin	<0.10 mg/kg	
Diazinon	<0.02 mg/kg	
Dichlofluanid	<0.02 mg/kg	
Dichlorvos	<0.02 mg/kg	
Dicofol	<0.02 mg/kg	
Dimethoate/Omethoate (sum)	<0.10 mg/kg	
Endosulfan (sum of isomers and endo. sulfate)	<0.02 mg/kg	
Endrin	<0.02 mg/kg	
Ethion	<0.02 mg/kg	
Etrimfos	<0.05 mg/kg	
Fenchlorphos (sum)	<0.10 mg/kg	
Fenitrothion	<0.02 mg/kg	
Fenpropathrin	<0.03 mg/kg	

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Sample #: 740-2017-07030094

Blue California Co.
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Rancho Santa Margarita, CA
92688

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/17/2017

	Result	Theoretical Level
Fensulfothion (sum of parent, -oxons and sulfones)	<0.05 mg/kg	
Fenthion (sum of fenthion, -oxons, -sulfones)	<0.05 mg/kg	
Fenvalerate	<0.20 mg/kg	
Flucythrinate	<0.05 mg/kg	
Fluvalinate, tau-	<0.05 mg/kg	
Fonofos	<0.02 mg/kg	
Heptachlor (heptachlor+ cis-, trans- h. epoxide	<0.03 mg/kg	
Hexachlorobenzene	<0.01 mg/kg	
Hexachlorocyclohexane isomers (other than gamma)	<0.02 mg/kg	
Lindane (gamma-HCH)	<0.01 mg/kg	
Malathion and malaoxon (sum of)	<0.02 mg/kg	
Mecarbam	<0.05 mg/kg	
Methacriphos	<0.05 mg/kg	
Methamidophos	<0.05 mg/kg	
Methidathion	<0.02 mg/kg	
Methoxychlor	<0.05 mg/kg	
Mirex	<0.01 mg/kg	
Monocrotophos	<0.10 mg/kg	
Parathion-ethyl and Paraoxon-ethyl (sum of)	<0.20 mg/kg	
Parathion-methyl and Paraoxon-methyl (sum of)	<0.20 mg/kg	
Pendimethalin	<0.10 mg/kg	
Pentachloranisole	<0.01 mg/kg	
Permethrin and isomers (sum of)	<0.2 mg/kg	
Phosalone	<0.04 mg/kg	
Phosmet	<0.05 mg/kg	
Piperonyl butoxide (PBO)	<1.0 mg/kg	
Pirimiphos-ethyl	<0.05 mg/kg	
Pirimiphos-methyl (incl. N-desethyl-)	<0.10 mg/kg	
Procymidone	<0.10 mg/kg	
Profenofos	<0.10 mg/kg	
Prothiofos	<0.05 mg/kg	
Pyrethrum (sum of cinerins, jasmolins, pyrethrins)	<3.0 mg/kg	
Quinalphos	<0.05 mg/kg	
Quintozene (sum quintozene,pentachloraniline,MPPS)	<0.1 mg/kg	
S 421	<0.02 mg/kg	
Tecnazene	<0.05 mg/kg	
Tetradifon	<0.05 mg/kg	
Vinclozolin	<0.05 mg/kg	

QA23Q: Bromide, inorganic (GC)
Method Reference: EURL-SRM, Bromine Containing Fumigants
Completed: 07/17/2017

	Result	Theoretical Level
Bromide	<10 mg/kg	

[Method performed by an outsource lab.]

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Sample #: 740-2017-07030094

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QA602: EBDCs (Dithiocarbamates) (CS2 method, GC-MS)
Method Reference: J. Agric. Food Chem. Vol. 49 pp 2152, 2001
Completed: 07/17/2017

	Result	Theoretical Level
Total Dithiocarbamates, as CS2	<0.01 mg/kg	
[Method performed by an outsource lab.]		

Results pertain only to the items tested.
All results are reported on an as-is basis unless otherwise stated.
Estimation of uncertainty of measurement is available upon request.
Results shall not be reproduced except in full without written permission from Eurofins Scientific, Inc.

Kent Rader
BU Manager

Appendix 5.2 Pesticide Analysis BC-DHQ™ Lot 7730-161028

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 94954
Tel.+1 707 792 7300
Fax:+1 707 792 7309

July 17, 2017

Cecilia Cecilia McCollum
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

CERTIFICATE OF ANALYSIS
AR-17-KK-008897-01
Batch #: EUCAPE-00091741

Sample Identification:
Sample #: 740-2017-07030097
Description: BC-DHQ, Powder, Lot #7730-161028
Condition: Beige powder in a double ziplock bag received at room temperature.
Date Received: July 03, 2017

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/17/2017

	Result	Theoretical Level
Acephate	<0.10 mg/kg	
[Method performed by an outsource lab.]		
Alachlor	<0.02 mg/kg	
Aldrin and Dieldrin (sum of)	<0.02 mg/kg	
Azinphos-ethyl	<0.02 mg/kg	
Azinphos-methyl	<0.05 mg/kg	
Bromophos-ethyl	<0.02 mg/kg	
Bromophos-methyl	<0.02 mg/kg	
Bromopropylate	<0.05 mg/kg	
Chlordane (sum of cis-, trans- and Oxychlordane)	<0.05 mg/kg	
Chlorfenvinphos	<0.02 mg/kg	
Chlorpyrifos-ethyl	<0.02 mg/kg	
Chlorpyrifos-methyl	<0.02 mg/kg	
Chlorthal-dimethyl	<0.01 mg/kg	
Cyfluthrin (sum of)	<0.10 mg/kg	
Cyhalothrin, lambda-	<0.02 mg/kg	
Cypermethrin and isomers (sum of)	<0.1 mg/kg	
DDT (total)	<0.02 mg/kg	
Deltamethrin	<0.10 mg/kg	
Diazinon	<0.02 mg/kg	
Dichlofluanid	<0.02 mg/kg	
Dichlorvos	<0.02 mg/kg	
Dicofol	<0.02 mg/kg	
Dimethoate/Omethoate (sum)	<0.10 mg/kg	
Endosulfan (sum of isomers and endo. sulfate)	<0.02 mg/kg	
Endrin	<0.02 mg/kg	
Ethion	<0.02 mg/kg	
Etrimfos	<0.05 mg/kg	
Fenchlorphos (sum)	<0.10 mg/kg	
Fenitrothion	<0.02 mg/kg	
Fenpropathrin	<0.03 mg/kg	

eurofins Sample #: 740-2017-07030097

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Rancho Santa Margarita, CA
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QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/17/2017

	Result	Theoretical Level
Fensulfothion (sum of parent, -oxons and sulfones)	<0.05 mg/kg	
Fenthion (sum of fenthion, -oxons, -sulfones)	<0.05 mg/kg	
Fenvalerate	<0.20 mg/kg	
Flucythrinate	<0.05 mg/kg	
Fluvalinate, tau-	<0.05 mg/kg	
Fonofos	<0.02 mg/kg	
Heptachlor (heptachlor+ cis-, trans- h. epoxide	<0.03 mg/kg	
Hexachlorobenzene	<0.01 mg/kg	
Hexachlorocyclohexane isomers (other than gamma)	<0.02 mg/kg	
Lindane (gamma-HCH)	<0.01 mg/kg	
Malathion and malaoxon (sum of)	<0.02 mg/kg	
Mecarbam	<0.05 mg/kg	
Methacriphos	<0.05 mg/kg	
Methamidophos	<0.05 mg/kg	
Methidathion	<0.02 mg/kg	
Methoxychlor	<0.05 mg/kg	
Mirex	<0.01 mg/kg	
Monocrotophos	<0.10 mg/kg	
Parathion-ethyl and Paraoxon-ethyl (sum of)	<0.20 mg/kg	
Parathion-methyl and Paraoxon-methyl (sum of)	<0.20 mg/kg	
Pendimethalin	<0.10 mg/kg	
Pentachloranisole	<0.01 mg/kg	
Permethrin and isomers (sum of)	<0.2 mg/kg	
Phosalone	<0.04 mg/kg	
Phosmet	<0.05 mg/kg	
Piperonyl butoxide (PBO)	<1.0 mg/kg	
Pirimiphos-ethyl	<0.05 mg/kg	
Pirimiphos-methyl (incl. N-desethyl-)	<0.10 mg/kg	
Procymidone	<0.10 mg/kg	
Profenofos	<0.10 mg/kg	
Prothiofos	<0.05 mg/kg	
Pyrethrum (sum of cinerins, jasmolins, pyrethrins)	<3.0 mg/kg	
Quinalphos	<0.05 mg/kg	
Quintozene (sum quintozene,pentachloraniline,MPPS)	<0.1 mg/kg	
S 421	<0.02 mg/kg	
Tecnazene	<0.05 mg/kg	
Tetradifon	<0.05 mg/kg	
Vinclozolin	<0.05 mg/kg	

QA23Q: Bromide, inorganic (GC)
Method Reference: EURL-SRM, Bromine Containing Fumigants
Completed: 07/17/2017

	Result	Theoretical Level
Bromide	<10 mg/kg	

[Method performed by an outsource lab.]

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Sample #: 740-2017-07030097

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92688

QA602: EBDCs (Dithiocarbamates) (CS2 method, GC-MS)
Method Reference: J. Agric. Food Chem. Vol. 49 pp 2152, 2001
Completed: 07/17/2017

	Result	Theoretical Level
Total Dithiocarbamates, as CS2	<0.01 mg/kg	
[Method performed by an outsource lab.]		

Results pertain only to the items tested.
All results are reported on an as-is basis unless otherwise stated.
Estimation of uncertainty of measurement is available upon request.
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Kent Rader
BU Manager

Appendix 5.3 Pesticide Analysis BC-DHQ™ Lot 7730-170425

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 94954
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Fax:+1 707 792 7309

July 11, 2017

Cecilia Cecilia McCollum
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

CERTIFICATE OF ANALYSIS
AR-17-KK-008606-01
Batch #: EUCAPE-00091741

Sample Identification:
Sample #: 740-2017-07030100
Description: BC-DHQ, Powder, Lot #7730-170425
Condition: Beige powder in a double ziplock bag received at room temperature.
Date Received: July 03, 2017

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/11/2017

	Result	Theoretical Level
Acephate *[Method performed by an outsource lab.]*	<0.10 mg/kg	
Alachlor	<0.02 mg/kg	
Aldrin and Dieldrin (sum of)	<0.02 mg/kg	
Azinphos-ethyl	<0.02 mg/kg	
Azinphos-methyl	<0.05 mg/kg	
Bromophos-ethyl	<0.02 mg/kg	
Bromophos-methyl	<0.02 mg/kg	
Bromopropylate	<0.05 mg/kg	
Chlordane (sum of cis-, trans- and Oxychlordane)	<0.05 mg/kg	
Chlorfenvinphos	<0.02 mg/kg	
Chlorpyrifos-ethyl	<0.02 mg/kg	
Chlorpyrifos-methyl	<0.02 mg/kg	
Chlorthal-dimethyl	<0.01 mg/kg	
Cyfluthrin (sum of)	<0.10 mg/kg	
Cyhalothrin, lambda-	<0.02 mg/kg	
Cypermethrin and isomers (sum of)	<0.1 mg/kg	
DDT (total)	<0.02 mg/kg	
Deltamethrin	<0.10 mg/kg	
Diazinon	<0.02 mg/kg	
Dichlofluanid	<0.02 mg/kg	
Dichlorvos	<0.02 mg/kg	
Dicofol	<0.02 mg/kg	
Dimethoate/Omethoate (sum)	<0.10 mg/kg	
Endosulfan (sum of isomers and endo. sulfate)	<0.02 mg/kg	
Endrin	<0.02 mg/kg	
Ethion	<0.02 mg/kg	
Etrimfos	<0.05 mg/kg	
Fenchlorphos (sum)	<0.10 mg/kg	
Fenitrothion	<0.02 mg/kg	
Fenpropathrin	<0.03 mg/kg	

eurofins Sample #: 740-2017-07030100

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Rancho Santa Margarita, CA
92688

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/11/2017

	Result	Theoretical Level
Fensulfothion (sum of parent, -oxons and sulfones)	<0.05 mg/kg	
Fenthion (sum of fenthion, -oxons, -sulfones)	<0.05 mg/kg	
Fenvalerate	<0.20 mg/kg	
Flucythrinate	<0.05 mg/kg	
Fluvalinate, tau-	<0.05 mg/kg	
Fonofos	<0.02 mg/kg	
Heptachlor (heptachlor+ cis-, trans- h. epoxide	<0.03 mg/kg	
Hexachlorobenzene	<0.01 mg/kg	
Hexachlorocyclohexane isomers (other than gamma)	<0.02 mg/kg	
Lindane (gamma-HCH)	<0.01 mg/kg	
Malathion and malaoxon (sum of)	<0.02 mg/kg	
Mecarbam	<0.05 mg/kg	
Methacriphos	<0.05 mg/kg	
Methamidophos	<0.05 mg/kg	
Methidathion	<0.02 mg/kg	
Methoxychlor	<0.05 mg/kg	
Mirex	<0.01 mg/kg	
Monocrotophos	<0.10 mg/kg	
Parathion-ethyl and Paraoxon-ethyl (sum of)	<0.20 mg/kg	
Parathion-methyl and Paraoxon-methyl (sum of)	<0.20 mg/kg	
Pendimethalin	<0.10 mg/kg	
Pentachloranisole	<0.01 mg/kg	
Permethrin and isomers (sum of)	<0.2 mg/kg	
Phosalone	<0.04 mg/kg	
Phosmet	<0.05 mg/kg	
Piperonyl butoxide (PBO)	<1.0 mg/kg	
Pirimiphos-ethyl	<0.05 mg/kg	
Pirimiphos-methyl (incl. N-desethyl-)	<0.10 mg/kg	
Procymidone	<0.10 mg/kg	
Profenofos	<0.10 mg/kg	
Prothiofos	<0.05 mg/kg	
Pyrethrum (sum of cinerins, jasmolins, pyrethrins)	<3.0 mg/kg	
Quinalphos	<0.05 mg/kg	
Quintozene (sum quintozene,pentachloraniline,MPPS)	<0.1 mg/kg	
S 421	<0.02 mg/kg	
Tecnazene	<0.05 mg/kg	
Tetradifon	<0.05 mg/kg	
Vinclozolin	<0.05 mg/kg	

QA23Q: Bromide, inorganic (GC)
Method Reference: EURL-SRM, Bromine Containing Fumigants
Completed: 07/11/2017

	Result	Theoretical Level
Bromide *[Method performed by an outsource lab.]*	<10 mg/kg	

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Sample #: 740-2017-07030100

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

QA602: EBDCs (Dithiocarbamates) (CS2 method, GC-MS)
Method Reference: J. Agric. Food Chem. Vol. 49 pp 2152, 2001
Completed: 07/11/2017

	Result	Theoretical Level
Total Dithiocarbamates, as CS2	<0.01 mg/kg	
[Method performed by an outsource lab.]		

Results pertain only to the items tested.
All results are reported on an as-is basis unless otherwise stated.
Estimation of uncertainty of measurement is available upon request.
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Kent Rader
BU Manager

Appendix 5.4 Pesticide Analysis BC-DHQ™ Lot 7730-170525

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 94954
Tel.+1 707 792 7300
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July 11, 2017

Cecilia Cecilia McCollum
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

CERTIFICATE OF ANALYSIS
AR-17-KK-008605-01
Batch #: EUCAPE-00091741

Sample Identification:
Sample #: 740-2017-07030103
Description: BC-DHQ, Powder, Lot #7730-170525
Condition: Beige powder in a double ziplock bag received at room temperature.
Date Received: July 03, 2017

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/11/2017

	Result	Theoretical Level
Acephate *[Method performed by an outsource lab.]*	<0.10 mg/kg	
Alachlor	<0.02 mg/kg	
Aldrin and Dieldrin (sum of)	<0.02 mg/kg	
Azinphos-ethyl	<0.02 mg/kg	
Azinphos-methyl	<0.05 mg/kg	
Bromophos-ethyl	<0.02 mg/kg	
Bromophos-methyl	<0.02 mg/kg	
Bromopropylate	<0.05 mg/kg	
Chlordane (sum of cis-, trans- and Oxychlordane)	<0.05 mg/kg	
Chlorfenvinphos	<0.02 mg/kg	
Chlorpyrifos-ethyl	<0.02 mg/kg	
Chlorpyrifos-methyl	<0.02 mg/kg	
Chlorthal-dimethyl	<0.01 mg/kg	
Cyfluthrin (sum of)	<0.10 mg/kg	
Cyhalothrin, lambda-	<0.02 mg/kg	
Cypermethrin and isomers (sum of)	<0.1 mg/kg	
DDT (total)	<0.02 mg/kg	
Deltamethrin	<0.10 mg/kg	
Diazinon	<0.02 mg/kg	
Dichlofluanid	<0.02 mg/kg	
Dichlorvos	<0.02 mg/kg	
Dicofol	<0.02 mg/kg	
Dimethoate/Omethoate (sum)	<0.10 mg/kg	
Endosulfan (sum of isomers and endo. sulfate)	<0.02 mg/kg	
Endrin	<0.02 mg/kg	
Ethion	<0.02 mg/kg	
Etrimfos	<0.05 mg/kg	
Fenchlorphos (sum)	<0.10 mg/kg	
Fenitrothion	<0.02 mg/kg	
Fenpropathrin	<0.03 mg/kg	

eurofins Sample #: 740-2017-07030103

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/11/2017

	Result	Theoretical Level
Fensulfothion (sum of parent, -oxons and sulfones)	<0.05 mg/kg	
Fenthion (sum of fenthion, -oxons, -sulfones)	<0.05 mg/kg	
Fenvalerate	<0.20 mg/kg	
Flucythrinate	<0.05 mg/kg	
Fluvalinate, tau-	<0.05 mg/kg	
Fonofos	<0.02 mg/kg	
Heptachlor (heptachlor+ cis-, trans- h. epoxide	<0.03 mg/kg	
Hexachlorobenzene	<0.01 mg/kg	
Hexachlorocyclohexane isomers (other than gamma)	<0.02 mg/kg	
Lindane (gamma-HCH)	<0.01 mg/kg	
Malathion and malaoxon (sum of)	<0.02 mg/kg	
Mecarbam	<0.05 mg/kg	
Methacriphos	<0.05 mg/kg	
Methamidophos	<0.05 mg/kg	
Methidathion	<0.02 mg/kg	
Methoxychlor	<0.05 mg/kg	
Mirex	<0.01 mg/kg	
Monocrotophos	<0.10 mg/kg	
Parathion-ethyl and Paraoxon-ethyl (sum of)	<0.20 mg/kg	
Parathion-methyl and Paraoxon-methyl (sum of)	<0.20 mg/kg	
Pendimethalin	<0.10 mg/kg	
Pentachloranisole	<0.01 mg/kg	
Permethrin and isomers (sum of)	<0.2 mg/kg	
Phosalone	<0.04 mg/kg	
Phosmet	<0.05 mg/kg	
Piperonyl butoxide (PBO)	<1.0 mg/kg	
Pirimiphos-ethyl	<0.05 mg/kg	
Pirimiphos-methyl (incl. N-desethyl-)	<0.10 mg/kg	
Procymidone	<0.10 mg/kg	
Profenofos	<0.10 mg/kg	
Prothiofos	<0.05 mg/kg	
Pyrethrum (sum of cinerins, jasmolins, pyrethrins)	<3.0 mg/kg	
Quinalphos	<0.05 mg/kg	
Quintozene (sum quintozene,pentachloraniline,MPPS)	<0.1 mg/kg	
S 421	<0.02 mg/kg	
Tecnazene	<0.05 mg/kg	
Tetradifon	<0.05 mg/kg	
Vinclozolin	<0.05 mg/kg	

QA23Q: Bromide, inorganic (GC)
Method Reference: EURL-SRM, Bromine Containing Fumigants
Completed: 07/11/2017

	Result	Theoretical Level
Bromide	<10 mg/kg	

[Method performed by an outsource lab.]

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Sample #: 740-2017-07030103

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

QA602: EBDCs (Dithiocarbamates) (CS2 method, GC-MS)
Method Reference: J. Agric. Food Chem. Vol. 49 pp 2152, 2001
Completed: 07/11/2017

	Result	**Theoretical Level**
Total Dithiocarbamates, as CS2	<0.01 mg/kg	
[Method performed by an outsource lab.]		

Results pertain only to the items tested.
All results are reported on an as-is basis unless otherwise stated.
Estimation of uncertainty of measurement is available upon request.
Results shall not be reproduced except in full without written permission from Eurofins Scientific, Inc.

Kent Rader
BU Manager

Appendix 5.5 Pesticide Analysis BC-DHQ™ Lot 7730-170616

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 94954
Tel.+1 707 792 7300
Fax:+1 707 792 7309

July 17, 2017

Cecilia Cecilia McCollum
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

CERTIFICATE OF ANALYSIS
AR-17-KK-008896-01
Batch #: EUCAPE-00091741

Sample Identification:
Sample #: 740-2017-07030091
Description: BC-DHQ, Powder, Lot #7730-170616
Condition: Beige powder in a double ziplock bag received at room temperature.
Date Received: July 03, 2017

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/17/2017

	Result	Theoretical Level
Acephate	<0.10 mg/kg	
[Method performed by an outsource lab.]		
Alachlor	<0.02 mg/kg	
Aldrin and Dieldrin (sum of)	<0.02 mg/kg	
Azinphos-ethyl	<0.02 mg/kg	
Azinphos-methyl	<0.05 mg/kg	
Bromophos-ethyl	<0.02 mg/kg	
Bromophos-methyl	<0.02 mg/kg	
Bromopropylate	<0.05 mg/kg	
Chlordane (sum of cis-, trans- and Oxychlordane)	<0.05 mg/kg	
Chlorfenvinphos	<0.02 mg/kg	
Chlorpyrifos-ethyl	<0.02 mg/kg	
Chlorpyrifos-methyl	<0.02 mg/kg	
Chlorthal-dimethyl	<0.01 mg/kg	
Cyfluthrin (sum of)	<0.10 mg/kg	
Cyhalothrin, lambda-	<0.02 mg/kg	
Cypermethrin and isomers (sum of)	<0.1 mg/kg	
DDT (total)	<0.02 mg/kg	
Deltamethrin	<0.10 mg/kg	
Diazinon	<0.02 mg/kg	
Dichlofluanid	<0.02 mg/kg	
Dichlorvos	<0.02 mg/kg	
Dicofol	<0.02 mg/kg	
Dimethoate/Omethoate (sum)	<0.10 mg/kg	
Endosulfan (sum of isomers and endo. sulfate)	<0.02 mg/kg	
Endrin	<0.02 mg/kg	
Ethion	<0.02 mg/kg	
Etrimfos	<0.05 mg/kg	
Fenchlorphos (sum)	<0.10 mg/kg	
Fenitrothion	<0.02 mg/kg	
Fenpropathrin	<0.03 mg/kg	

eurofins

Sample #: 740-2017-07030091

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 07/17/2017

	Result	Theoretical Level
Fensulfothion (sum of parent, -oxons and sulfones)	<0.05 mg/kg	
Fenthion (sum of fenthion, -oxons, -sulfones)	<0.05 mg/kg	
Fenvalerate	<0.20 mg/kg	
Flucythrinate	<0.05 mg/kg	
Fluvalinate, tau-	<0.05 mg/kg	
Fonofos	<0.02 mg/kg	
Heptachlor (heptachlor+ cis-, trans- h. epoxide	<0.03 mg/kg	
Hexachlorobenzene	<0.01 mg/kg	
Hexachlorocyclohexane isomers (other than gamma)	<0.02 mg/kg	
Lindane (gamma-HCH)	<0.01 mg/kg	
Malathion and malaoxon (sum of)	<0.02 mg/kg	
Mecarbam	<0.05 mg/kg	
Methacriphos	<0.05 mg/kg	
Methamidophos	<0.05 mg/kg	
Methidathion	<0.02 mg/kg	
Methoxychlor	<0.05 mg/kg	
Mirex	<0.01 mg/kg	
Monocrotophos	<0.10 mg/kg	
Parathion-ethyl and Paraoxon-ethyl (sum of)	<0.20 mg/kg	
Parathion-methyl and Paraoxon-methyl (sum of)	<0.20 mg/kg	
Pendimethalin	<0.10 mg/kg	
Pentachloranisole	<0.01 mg/kg	
Permethrin and isomers (sum of)	<0.2 mg/kg	
Phosalone	<0.04 mg/kg	
Phosmet	<0.05 mg/kg	
Piperonyl butoxide (PBO)	<1.0 mg/kg	
Pirimiphos-ethyl	<0.05 mg/kg	
Pirimiphos-methyl (incl. N-desethyl-)	<0.10 mg/kg	
Procymidone	<0.10 mg/kg	
Profenofos	<0.10 mg/kg	
Prothiofos	<0.05 mg/kg	
Pyrethrum (sum of cinerins, jasmolins, pyrethrins)	<3.0 mg/kg	
Quinalphos	<0.05 mg/kg	
Quintozene (sum quintozene,pentachloraniline,MPPS)	<0.1 mg/kg	
S 421	<0.02 mg/kg	
Tecnazene	<0.05 mg/kg	
Tetradifon	<0.05 mg/kg	
Vinclozolin	<0.05 mg/kg	

QA23Q: Bromide, inorganic (GC)
Method Reference: EURL-SRM, Bromine Containing Fumigants
Completed: 07/17/2017

	Result	Theoretical Level
Bromide	<10 mg/kg	

[Method performed by an outsource lab.]

eurofins

Sample #: 740-2017-07030091

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

QA602: EBDCs (Dithiocarbamates) (CS2 method, GC-MS)
Method Reference: J. Agric. Food Chem. Vol. 49 pp 2152, 2001
Completed: 07/17/2017

	Result	Theoretical Level
Total Dithiocarbamates, as CS2	<0.01 mg/kg	
[Method performed by an outsource lab.]		

Results pertain only to the items tested.
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Estimation of uncertainty of measurement is available upon request.
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Kent Rader
BU Manager

Appendix 6 GRAS Associates Expert Panel Report

The Generally Recognized as Safe (GRAS) Status of the Proposed Uses of BC-DHQ™

February 5, 2020

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC, on behalf of their client, Blue California, to evaluate the safety and Generally Recognized as Safe (GRAS) status of BC-DHQ™ high purity dihydroquercetin. The members of this Expert Panel† are qualified to serve in this capacity by their scientific training and experience in the safety of food and food ingredients.

The Expert Panel, having reviewed the information summarized in Blue California's dossier, the available published studies, and the EFSA expert committee evaluation on taxifolin rich extract concludes that Blue California's DHQ preparation is generally recognized as safe in foods at the usage levels described herein.

Blue California's DHQ™ is substantially chemically equivalent to Ametis JSC's dihydroquercetin preparation already in commercial use. The Expert Panel considered the following evidence as evidence for the safety of Blue California's DHQ™:

- **BC-DHQ™** is produced from eriodictyol using an enzymatic bioconversion reaction. This reaction utilizes a nonpathogenic and nontoxigenic stain of wild type *Escherichia coli*, K12 W3110. The manufacturing process also uses suitable food-grade materials that are used in accordance with applicable US Federal Regulations. The substance was confirmed to be in the (2R,3R)-*trans* form. BC-DHQ™ is shown to be stable in a six-month accelerated stability study.

- **ADME studies** in animals and humans indicate that, following absorption, DHQ is conjugated in the liver by glucuronidation, sulfation, or methylation or is metabolized to smaller phenolic compounds.

† Dr. Dziwenka holds a Doctor of Veterinary Medicine degree from the University of Saskatchewan and is a Diplomat with the American Board of Toxicology. She has over 22 years' experience as a practicing veterinarian and 19 years of experience in research, preclinical regulatory toxicology, and safety evaluation in food and animal feed additives and GRAS dossier preparation. Dr. Lewis is a biologist with more than 10 years of experience preparing GRAS dossiers. Dr. Omaye is a nutritionist, toxicologist, and professor in the department of Agriculture, Nutrition, and Veterinary Sciences at the University of Nevada, Reno. He is a Fellow of the Academy of Toxicological Sciences, a Certified Nutrition Specialist, and a Certified Food Scientist. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in the deliberations of GRAS Expert Panels. Dr. Lewis served as Chair of the Panel.

- **Acute and subacute animal toxicity studies** show that DHQ is well tolerated in rats at a single dose of 1,500 mg per kg (at 91-98% DHQ) as well as following seven days of exposure at up to 15,000 mg per kg bw (90.94% DHQ) in a GLP-compliant study.

- **Subchronic animal studies** revealed no changes in mortality or body weights, no clinical signs of toxicity or changes in feed consumption, and no treatment-related histopathological findings and other toxicity endpoints following 90 days of exposure by gavage in rats at doses up to 1,500 mg per kg bw (92.20% DHQ).

- **Chronic animal studies** showed that dietary levels up to 1% DHQ for up to 650 days are well tolerated in male and female rats. The Expert Panel recognized that this is a study from 1958 and was non-GLP but considered it useful to support the safety conclusion.

- **Reproductive and/or developmental toxicity** was not observed in a GLP-compliant study when given daily to pregnant female rats via gavage at doses of up to 1,500 mg per kg bw per day from gestational day 6 to 16 or up to 75 mg per kg bw in pregnant female rats from gestational day 1 to 19.

- **Genotoxicity and mutagenicity studies** have shown no *in vitro* or *in vivo* genotoxicity or *in vivo* mutagenesis. Studies conducted included a GLP-compliant single-cell gel electrophoresis Comet assay and a DNA-comet assay, a GLP-compliant micronucleus assay in human lymphocytes, and a mutagenicity study in *S. typhimurium* and *E. coli* strains.

- **Clinical studies** show that DHQ is well-tolerated in humans with various disease conditions. No adverse effects were observed in these studies, which included some well-designed clinical studies in patients with various disease conditions. The Expert Panel noted that some of the studies cited in the Ametis (2010) novel food application were obscure and difficult to find. EFSA has previously reviewed these studies in the Ametis application.

- **The European Food Safety Authority (EFSA)** released a scientific opinion on a novel food, taxifolin-rich extract from Dahurian Larch in December of 2016 containing a minimum of 90% taxifolin (Turck et al., 2017). The novel food was intended to be added to non-alcoholic beverages at concentrations up to 0.02 g per L, to yogurt up to 0.02 g per kg, and chocolate confectionery up to 0.07 g per kg with the target population from nine years and older. It was also intended to be added to food supplements at 100 mg per day for the general population ages 14 years and above. The Panel concluded that the taxifolin-rich extract was safe under these proposed conditions of use. In late 2017, EFSA put out a statement on the safety of the same extract but was asked to take into account all population groups for this review (Turck et al., 2017). The Panel concluded that the highest intake estimate per kg bw per day from fortified foods would be in toddlers and

children at approximately 1.5 mg per kg bw per day and that the extract would be safe under the proposed conditions of use.

- **The estimated daily mean intake of DHQ** for the US population using the 90th percentile estimated daily intake of DHQ for the US population, 33.72 mg per day, is less than the 97.5th percentile estimated daily intake for the European population, 58.0 mg per day, which was considered safe by EFSA.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of Blue California's DHQ™ in support of a GRAS conclusion under the conditions of its intended use.

Conclusion

The Expert Panel critically reviewed the data provided by Blue California for their DHQ, as well as publicly available published information obtained from peer reviewed journals and other safety assessments prepared by well-respected international regulatory bodies.

The ingestion of Blue California's DHQ from the intended uses results in intakes that are safe within the limits of established historical use, those evaluated by EFSA, and published safety studies. The levels at which Blue California intends to use its DHQ are the same as those authorized by Commission Regulation (EU) 2018/431.

The Expert Panel unanimously concluded that the proposed uses of Blue California's DHQ, as described in their dossier, and when manufactured using suitable food-grade materials which are used in accordance with applicable US Federal Regulations, is generally recognized as safe (GRAS) when added to the specified human food categories at the proposed levels.

This declaration is made in accordance with FDA's food ingredient safety standard, i.e., reasonable certainty of no harm under the intended conditions of use.



Kara Lewis, Ph.D. Margitta Dziwenka, DVM, DABT Stanley Omaye, Ph.D.

Panel Chair

END

Eurofins Scientific, Inc.
1365 Redwood Way
Petaluma, Ca 94951

Summary Report

Method Verification of the Determination of Dihydroquercetin (BC-DHQ™) by High Performance Liquid Chromatography (HPLC) and Purity Analysis of Five Production Samples

Prepared by: _____
Hong You, Ph.D., Principal Scientist
Eurofins Scientific, Inc.

_____
Darlene Enriquez, QA Manager
Eurofins Scientific, Inc.

_____
Kent Rader, Business Unit Manager
Eurofins Scientific, Inc.

Approved by: _____
Cecilia McCollum, Executive Vice President
Blue California.

Date Issued: July 26th, 2017

I. Study Identification

1. Study Title:

Method Verification of the Determination of Dihydroquercetin (BC-DHQ™) by High Performance Liquid Chromatography (HPLC) and Purity Analysis of Five Production Samples

2. Study Objective:

The objective of this study was to verify the assay for dihydroquercetin by High Performance Liquid Chromatography (HPLC) and purity analysis of five production samples using a method modified based on a fully validated ISO-accredited Eurofins in-house method.

3. Study Coordinator/Performing Laboratory:

Hong You, Ph.D., Principal Scientist
Eurofins Scientific, Inc.

Timothy Sit, Analyst
Eurofins Scientific, Inc.

Darlene Enriquez, QA Manager
Eurofins Scientific, Inc.

Kent Rader, Business Unit Manager
Eurofins Scientific, Inc

4. Study Monitors:

Cecilia McCollum, Executive Vice President
Blue California

5. Method References:

K0195 Determination of Dihydroquercetin
LC-K0023 HPLC Determination of Bioflavonoids (Eurofins ISO-accredited method)

II. Study Description

1. Scope:

This method is applicable to the determination and quantification of dihydroquercetin, in raw materials and BC-DHQ™ products. Dihydroquercetin quantitation was determined using the Sigma standard. HPLC-DAD (HPLC with Diode Array Detector) was used as the analytical instrument.

2. Test Materials:

Dihydroquercetin dietary supplement finished product

(1) Eurofins sample 740-2017-07030039 BC-DHQ, Powder, Lot #7730-170616

(2) Eurofins sample 740-2017-07030040 BC-DHQ, Powder, Lot #7730-160823

(3) Eurofins sample 740-2017-07030041 BC-DHQ, Powder, Lot #7730-161028

(4) Eurofins sample 740-2017-07030042 BC-DHQ, Powder, Lot #7730-170425

(5) Eurofins sample 740-2017-07030043 BC-DHQ, Powder, Lot #7730-170525

3. Test Reagents:

(1) Acetonitrile (HPLC Grade), Fisher Catalog #: A998-4, C.A.S #: 75-05-8

(2) Methanol (HPLC Grade), Fisher Catalog #: A452-4, C.A.S #: 67-56-1

(3) O-Phosphoric acid (HPLC Grade), Fisher Catalog #: A260-500, C.A.S #: 7664-38-2

(4) Taxifolin (dihydroquercetin), Sigma Catalog #: 78666, C.A.S #: 480-18-2

(5) Milli-Q water, fresh daily

4. Mobile Phase Preparation:

Mobile phase A: 0.2% phosphoric acid in Milli-Q water

Mobile phase B: 100% acetonitrile

Mobile phase C: 100% methanol

5. Reference Standards:

A. Stock standards.

1. Adjust standard concentration for purity and moisture levels (Sigma). Corrections were made based on supplier's Certificate of Analysis.

2. On a microbalance, accurately weighed about 12 mg of dihydroquercetin Sigma standard; quantitatively added 40 mL methanol. This is stock solution.

B. Calibration working standards were prepared by diluting standard stock solution with methanol. The range of quantitation was approximately between 10 ug/mL and 280 ug/mL in solution. A 5 point curve was utilized for determination of linearity for this study. A minimum of 3 point curve will be used for routine quantitation for the current and future samples. The sample test concentration was approximately 75 ug/mL dihydroquercetin, based on the expected test sample

concentration. The adjusted dihydroquercetin standard curve covered the targeting dihydroquercetin sample concentration.

C. Accuracy test was performed by testing routine samples that were spiked with three different levels of the standard stock solution.

D. Sigma dihydroquercetin standard was utilized for system suitability test and as calibration standards. See results section for concentrations.

6. Single Lab Verification Study Results:

A. Primary method: See provided method.

B. System Suitability:

1. Minimum of 5 injections of an approximately 145 ug/ml standard solution were injected during the analysis sequence for dihydroquercetin.

2. Acceptance criteria: The system is considered suitable if
   USP tailing factor of the standard peak must be $T \leq 2.0$
   Critical resolution must be > 1.5
   Standard peak area %RSD ≤ 2.0
   Standard retention time %RSD ≤ 2.0

   Standard peak area and retention time results are as follows:

	Dihydroquercetin	PASS/FAIL
Retention time (RT) Range (minutes)	7.61 – 7.71	-
RT % RSD	**0.568**	**PASS**
Peak area range	1642	-
Peak area RSD	**1.87**	**PASS**
Number of Data Points	5	-

Dihydroquercetin standard retention time %RSD passed the criteria of less than 2%.
Dihydroquercetin standard peak area %RSD passed the criteria of less than 2%.

3. A Peak Performance Evaluation report was generated using Agilent Chem Station software to include the resolution and USP tailing for dihydroquercetin. Results are as follows:

Resolution to Next Peak Dihydroquercetin = 3.66 **PASS**
USP Tailing Dihydroquercetin = 1.00 **PASS**

4. The retention time and identity for dihydroquercetin in samples were confirmed using the Sigma dihydroquercetin commercial standards.

C. Linearity:

1. A 5 point calibration curve for dihydroquercetin was developed. The stock standard was diluted into working solutions and then injected. The 5 point calibration curve for this project with relative concentrations for dihydroquercetin was as follows (adjusted for standard purity):

Stock used (mL)	Final working solution (mL)	Relative Concentration (mg/mL)
5	5	0.278
3.75	5	0.208
2.5	5	0.139
1.25	5	0.0695
0.167	5	0.00928

Linearity Results Dihydroquercetin:

Correlation Coefficient	Criteria	PASS/FAIL
0.99944	**> 0.999**	**PASS**

2. The relative standard deviation (RSD) for the response factor ((amount/area) mg/mL/mAU) was determined between calibration levels. The RSD expressed as a percent is to achieve a specification of **<5%**. The %RSDs achieved between calibration levels was acceptable at **2.58%** for dihydroquercetin.

D. Specificity: For purposes of this study, selectivity is specificity

1. Perform selectivity procedures:
   a. Analyze at least one prep solvent blank.
2. Results:
   a. Three preparation solvent blanks were tested. The chromatograms were free of interfering peaks. Dihydroquercetin was also shown to not interfere (baseline resolution) with other flavonoids that have similar chemical structures including eriocitrin, rutin, narirutin, naringin, hesperidin, neohesperidin, quercetin, naringenin, and hesperitin.

E. Accuracy (Recovery):

Accuracy was determined by spiking a sample of known value (740-2017-07030039) with different levels of standard stock solution at the beginning of the study. The analyzed final results were used to compare to their theoretical results for the percentage recovery result. This test was used to determine if the method can accurately determine the analyte results without significant matrix interference.

Spiked stock(mL)	Recovery%	Acceptance criteria	PASS/FAIL
3 (low level)	**99.0**	**95-102%**	**PASS**
5 (mid level)	**97.5**	**95-102%**	**PASS**
10 (high level)	**96.3**	**95-102%**	**PASS**

F. Precision (Repeatability):

Five lots of BC-DHQ™ testing samples were analyzed for purity concentration. Dihydroquercetin stock standard was prepared at about 0.278 mg/mL (5 mL, 3.75 mL, 2.5 mL, 1.25 mL, and 0.167 mL stock solution were used to prepare 5 levels of working calibration standard solution). The range of dihydroquercetin quantitation was approximately between 10 ug/mL and 280 ug/mL. The testing purity samples were prepared at approximately 75 ug/mL with 95% as their expected concentration level. Based on Eurofins' in-house criteria, % RSD for precision measurements shall be less than 5.

Only one dihydroquercetin signal was found in corresponding chromatograms.

Sample results are as follows:

740-2017-07030039	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	% Relative Standard Deviation
Dihydroquercetin	93.7	93.7	94.5	94.0	0.492
740-2017-07030040	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	% Relative Standard Deviation
Dihydroquercetin	94.5	94.9	94.1	94.5	0.423
740-2017-07030041	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	% Relative Standard Deviation
Dihydroquercetin	93.5	94.8	94.1	94.1	0.691
740-2017-07030042	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	% Relative Standard Deviation
Dihydroquercetin	94	94	94.4	94.1	0.245
740-2017-07030043	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	% Relative Standard Deviation
Dihydroquercetin	92.2	91.5	92	91.9	0.392

G. Moisture Correction:

Moisture determination tests were conducted. Sample results after moisture correction are listed below:

740-2017-07030039		Run 1	Run 2	Run 3		
Compound	Moisture %	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Average	% Relative Standard Deviation
Dihydroquercetin	3.82	97.4	97.4	98.3	97.7	0.492
740-2017-07030040		Run 1	Run 2	Run 3		
Compound	Moisture %	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Average	% Relative Standard Deviation
Dihydroquercetin	3.32	97.7	98.2	97.3	97.8	0.423
740-2017-07030041		Run 1	Run 2	Run 3		
Compound	Moisture %	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Average	% Relative Standard Deviation
Dihydroquercetin	3.71	97.1	98.5	97.7	97.8	0.691
740-2017-07030042		Run 1	Run 2	Run 3		
Compound	Moisture %	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Average	% Relative Standard Deviation
Dihydroquercetin	3.25	97.2	97.2	97.6	97.3	0.245
740-2017-07030043		Run 1	Run 2	Run 3		
Compound	Moisture %	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Result (%w/w) on dry-basis	Average	% Relative Standard Deviation
Dihydroquercetin	3.48	95.5	94.8	95.3	95.2	0.392

7. Conclusions:

The results generated met and exceed the acceptance criteria as established in the method verification proposal. All analyses were performed on an Agilent 1100 series HPLC-DAD (HPLC with diode array detector) and processed using Agilent ChemStation software. The primary objective of the study was to accurately determine the concentration of dihydroquercetin in BC-DHQ™ products without significant matrix interference.

Quantitation of dihydroquercetin was accomplished against Sigma's dihydroquercetin reference material (standard) as described in Eurofins K0195 Determination of Dihydroquercetin.

Limit of detection and limit of quantitation were beyond the scope of this project and considered not necessary because of the high purity of target samples (dihydroquercetin raw material).

Five lots of BC-DHQ™ samples were tested by this method. All testing results have met client's expected level after moisture corrections. The results showed that the method is linear, specific, suitable, precise and accurate for dihydroquercetin determination in BC-DHQ™ product matrix.

LINEARITY & PRECISION (REPEATABILITY)

PREP SHEETS

eurofins

Supplement Analysis Center

FRM-476.04
HPLC Sample Preparation Sheet

Replace: FRM-476.03
Effective Date: 05/25/2017
QA Approval: 05/24/2017

Date Entered into e-LIMS:	7/17/17	**Analyst:**	TS	**Earliest Sample Due Date:**		
Date Started	7/11/17			**Log #:**	i7-1360	
Prepped By	[redacted]			**Method:**	KD145	**Sequence:** LCK0023-17-1360
Method Name	DHQ			**Column Type:**	SB-C18	**Instrument:** HPLC-7
Balance	XP26#2 BP211D#2			**Column ID:**	4086	CI#/Lot # / Exp.
Vol. Device	Dispenser #2			Eluent A:	2Phos	1456 / 7/25/17
Prep Solvent	MeOH / ACN / Milli-Q	Lot #	16154	Eluent B:	ACN	17823 / 1/11/18
Prep Solvent	—	Lot #	—	Eluent C:	MeOH	18131 / 12/7/17
Prep Solvent	—	Lot #	—	Other Chemicals:	—	

Prep Solvent	—	Lot #	—
Prep Solvent	—	Lot #	—

*Note: Mark "X" or "√" if sample was Ground. Mark "-" if sample was NOT Ground.
**Final Dilution to be entered into ChemStation.

Sample Preparation

Val/Rep Use Only Δ	Ground *	Sample ID	Amount (mg)	Volume: Dilution Vol. (mL)	Volume: 2º Dilution Vol. (mL)	Volume: Injection Vol. (µL)	Volume: Final Dilution (mL)**	Notes
—	—	Control Total	62.75	40	—	5	40	
—		Control RR	45.020	40	—			
—		Control Hesp	3.488	40	—			
Δ		07030034A	3.031	40	—			
		07030034B	3.076	40	—			
		07030034C	3.294	40	—			
		07030040A	3.139	40	—			
		07030040B	3.416	40	—			
		07030040C	3.201	40	—			
		07030041A	3.072	40	—			
		" " B	3.494	40	—			
		" " C	3.696	40	—			
		07030042A	3.271	40	—			
		" " B	3.433	40	—			
Δ	—	" " C	3.804	40	—			

Δ Note: R (Reported), OOS (Out of Specification), INC (Incomplete)

Δ Ready to report MT 7/17/17

Reviewed By: [redacted] Date: 7/17/17

Validated By: [redacted] Date: 7/17/17

Date Entered into e-LIMS:			**Analyst:**		
Date Started					
Prepped By					
Method Name					
Balance					
Vol. Device				TS 7/17/17	
Prep Solvent	MeOH ACN Milli-Q	Lot #		Exp.	
Prep Solvent		Lot #		Exp.	
Prep Solvent		Lot #		Exp.	

Earliest Sample Due Date:		
Log #:		
Method:	**Sequence:**	
Column Type:	**Instrument:**	
Column ID:	CI#/Lot #	Exp.
Eluent A:		
Eluent B:		
Eluent C:		
Other Chemicals: TS 7/17/17		

*Note: Mark "X" or "√" if sample was Ground. Mark "-" if sample was NOT Ground.
**Final Dilution to be entered into ChemStation.

Sample Preparation

Val/Rep Use Only Δ	Ground *	Sample ID	Amount (mg)	Volume				Notes:
				Dilution Vol. (mL)	2º Dilution Vol. (mL)	Injection Vol. (µL)	Final Dilution (mL)**	
D	–	07030043.A	2.947	40	–	5	40	
D	–	" " B	3.087	40	–	5	40	
D	–	" " C	2.886	40	–	5	40	
					TS 7/17/17			

Δ Note: R (Reported), OOS (Out of Specification), INC (Incomplete)

Reviewed By: _____ Date: 7/17/17

Validated By: _____ Date: 7/17/17

eurofins
Supplement Analysis Center

FRM-474.02
HPLC Multiple Reference Material Preparation Sheet

Replace: FRM-474.01
Effective date: 03/06/2017
QA Approval: AKO3 03/02/2017

Date Prepared	7/11/17		
Prepared by	[redacted]		
Method Name	DHQ		
Method #	KC0145		
Balance	XP26#3		
Vol. Device	Class A		
Prep Solvent	~~MeOH~~ ACN Milli-Q	Lot# 181574	Exp. 9/27/17
Prep Solvent	—	Lot# —	Exp. —

Log #: 17-1360	
Lot Number*: —	Expires*: —
Other Chemicals or Notes: —	

When reusing previously made material, annotate the Lot number, attach a copy of the prep sheet to the data packet, and record the expiration date.

Reference Material Preparation*

Analyte	CI#	Exp.	Purity	Amt (mg)	Vol (mL)	Concentration (mg/mL) Dilutions/Injection Volumes		
Taxifolin	13354	12/18	85.6	1.045	10	—	—	—
#2 Taxifolin	13354	12/18	85.6	3.137	10	stk 0.2685272 ✓	3.75→5 0.2013954 ✓	2.5→5 0.1342636 ✓
—	—	—	—	—	—	1.25 0.0671318 ✓	1.67→5 0.0089648055 ✓	—
standard used for BlueCal method verification project. HY 7/27/17 Taxifolin	18294	7/20	95.4	2.913	10	stk 0.2774002 ✓	3.75mL→5mL 0.20842515 ✓	2.5mL→5mL 0.1389501 ✓
						1.25mL→5mL 0.0694 7505 ✓	1.67mL→5mL 0.0092818667 ✓	—
							TS 7/17/17	

Reviewed By [redacted] Date 7/17/17

ACCURACY

PREP SHEETS

eurofins
Supplement Analysis Center

FRM-476.04
HPLC Sample Preparation Sheet

Replace: FRM-476.03
Effective Date: 05/25/2017
QA Approval: 05/24/2017

Date Entered into e-LIMS: 7/25/17		**Analyst:** TS	
Date Started	7/21/17		
Prepped By	[redacted]		
Method Name	DHQ		
Balance	XP26#2		
Vol. Device	Class A		
Prep Solvent	MeOH ACN Milli-Q	Lot # 18164	Exp. 9/27/17
Prep Solvent	—	Lot # —	Exp. —
Prep Solvent	—	Lot # —	Exp. —

Earliest Sample Due Date: N/A		
Log #: 17-1425		
Method: K00203	**Sequence:**	
Column Type: SB-C18	**Instrument:** HPLC-7	
Column ID: 4086	CI#/Lot #	Exp.
Eluent A: 0.2% Phosphoric Acid in MilliQ	2Phos-1962	8/4/17
Eluent B: Acetonitrile	17823	1/11/18
Eluent C: Methanol	18131	12/7/17
Other Chemicals: —		

*Note: Mark "X" or "√" if sample was Ground. Mark "-" if sample was NOT Ground.
**Final Dilution to be entered into ChemStation.

Sample Preparation								Notes:
Val/Rep Use Only Δ	Ground *	Sample ID	Amount (mg)	Volume: Dilution Vol. (mL)	Volume: 2º Dilution Vol. (mL)	Volume: Injection Vol. (µL)	Volume: Final Dilution (mL)**	
	—	Control Hesp	4.305	40	—	5	40	
	—	Control Total	50.757	40	—	↓	↓	
	—	control RQ	46.040	40	—			
	—	07030039	3.034	40	—			
	—	07030039 d	3.272	40	—			
	—	07030039 S1	3.030	40	—			
	—	07030039 S2	3.031	40	—			
	—	07030039 S3	3.022	40	—			
				TS	7/25/17			

Δ Note: R (Reported), OOS (Out of Specification), INC (Incomplete)

Reviewed By: [redacted] Date: 7/28/17

Validated By: [redacted] Date: 8/9/17 (Validated previously, NK absent) M- 8/9/17

eurofins Supplement Analysis Center

FRM-474.02
HPLC Multiple Reference Material Preparation Sheet

Replace: FRM-474.01
Effective date: 03/06/2017
QA Approval: AKO3 03/02/2017

Date Prepared	7/21/17		
Prepared by	[redacted]		
Method Name	DHQ		
Method #	IC0025		
Balance	XP26#2		
Vol. Device	class A		
Prep Solvent	MeOH ACN Milli-Q	Lot# 18154	Exp. 9/27/17
Prep Solvent	—	Lot# —	Exp. —

Log #: 17-1425	
Lot Number*: —	Expires*: —
Other Chemicals or Notes: —	

When reusing previously made material, annotate the Lot number, attach a copy of the prep sheet to the data packet, and record the expiration date.

Reference Material Preparation*

Analyte	CI#	Exp.	Purity	Amt (mg)	Vol (mL)	Concentration (mg/mL) Dilutions/Injection Volumes		
						Do not use below point. Four point curve used for calibration. HY 7/28/17		
Taxifolin	18294	7/20	95.4	12.034	40	STK 0.2870109	3.75→75 mL 0.215258175	2.5→75 mL 0.143505545
						1.25→5 mL 0.071752725	1.67→1 →5mL 0.009586164	
					TS 7/25/17			

Reviewed By [redacted] Date 7/28/17

REFERENCE MATERIAL CERTIFICATION OF ANALYSIS

TAXIFOLIN (dihydroquercetin)

18294

SIGMA-ALDRICH

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	TAXIFOLIN analytical standard
Product Number:	78666
Batch Number:	BCBQ3955V
Brand:	Sigma-Aldrich
CAS Number:	480-18-2
Formula:	$C_{15}H_{12}O_7$
Formula Weight:	304.25
Quality Release Date:	07 JUL 2015

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	WHITE TO LIGHT BROWN	FAINT BROWN
APPEARANCE (FORM)	POWDER	POWDER
PURITY (HPLC AREA %)	≥ 85.0 %	95.4 %
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

[redacted]

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

[redacted]

PRECISION (REPEATABILITY)

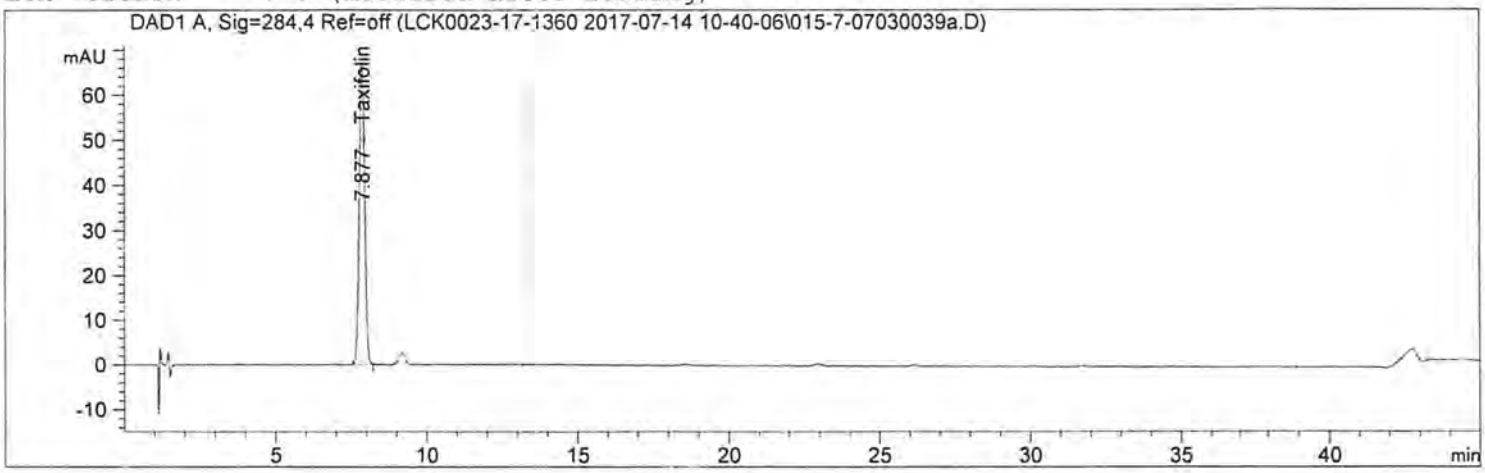
SAMPLE CHROMATOGRAMS

PERCENT WEIGHT RESULTS

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\015-7-07030039a.D
Sample Name: 07030039a

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  15
Acq. Instrument : HPLC-07                       Location :   7
Injection Date  : 7/14/2017 11:24:37 PM            Inj :   1
                                                Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by Timothy Sit
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.03100  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.877	BB	845.34656	8.40135e-5	93.725505		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\015-7-07030039a.D
Sample Name: 07030039a

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				93.725505		

1 Warnings or Errors :

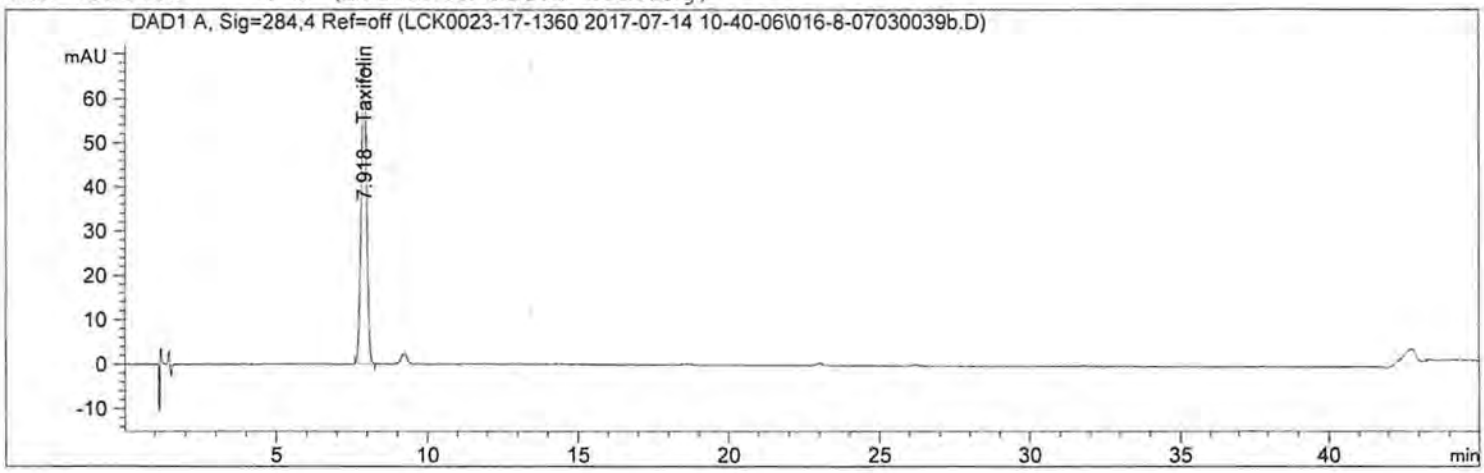
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\016-8-07030039b.D
Sample Name: 07030039b

```
==========================================================================
Acq. Operator   :                                    Seq. Line :  16
Acq. Instrument : HPLC-07                             Location :   8
Injection Date  : 7/15/2017 12:19:08 AM                    Inj :   1
                                                    Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by Timothy Sit
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    :
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.07600  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.918	BB	857.26685	8.40763e-5	93.726650		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\016-8-07030039b.D
Sample Name: 07030039b

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				93.726650		

1 Warnings or Errors :

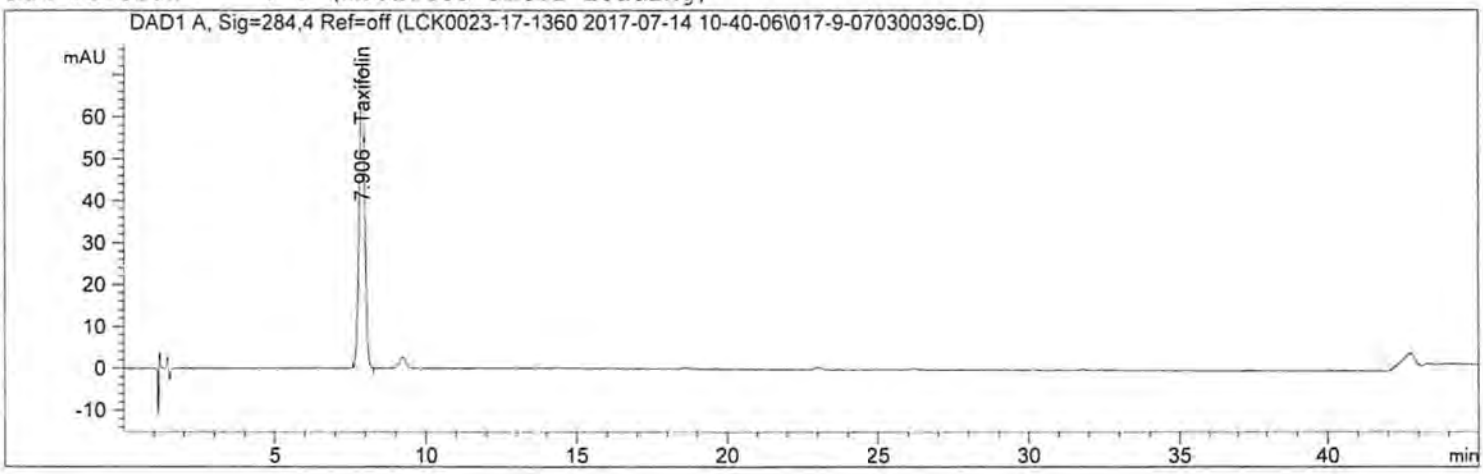
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\017-9-07030039c.D
Sample Name: 07030039c

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  17
Acq. Instrument : HPLC-07                       Location :   9
Injection Date  : 7/15/2017 1:13:40 AM             Inj :   1
                                                Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by Timothy Sit
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.29400  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.906	BB	921.91217	8.43885e-5	94.473329		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\017-9-07030039c.D
Sample Name: 07030039c

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				94.473329		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\019-10-07030040a.D
Sample Name: 07030040a

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :  19
Acq. Instrument : HPLC-07                           Location :   10
Injection Date  : 7/15/2017 3:02:38 AM                  Inj :    1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
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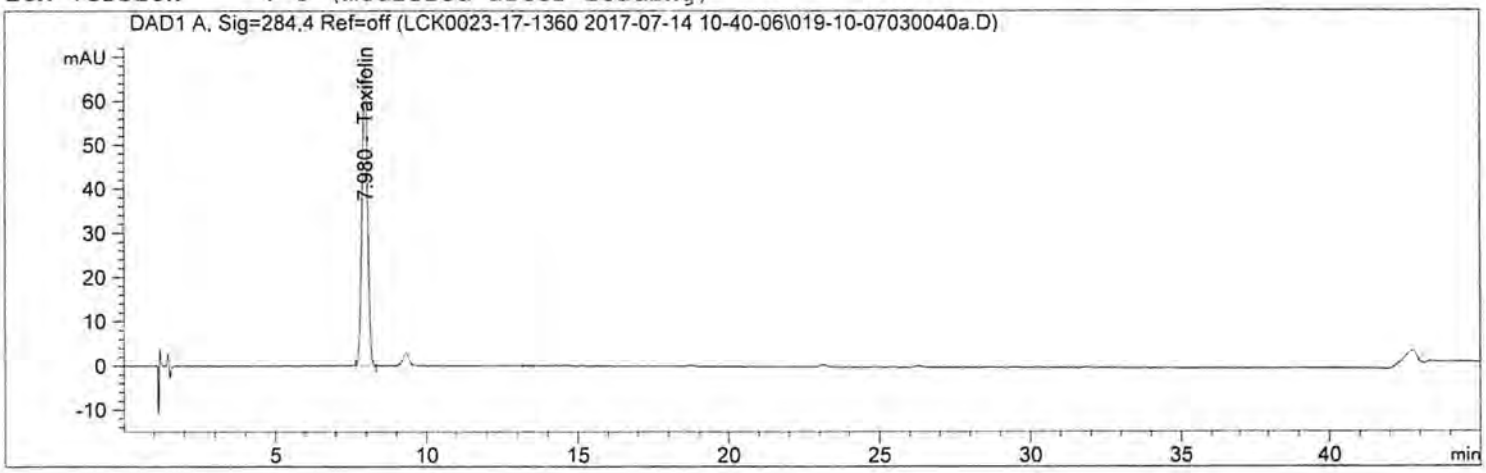


```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.13900  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.980	BB	880.84595	8.41955e-5	94.505550		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\019-10-07030040a.D
Sample Name: 07030040a

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				94.505550		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\020-11-07030040b.D
Sample Name: 07030040b

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :  20
Acq. Instrument : HPLC-07                           Location :   11
Injection Date  : 7/15/2017 3:57:06 AM                  Inj :   1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
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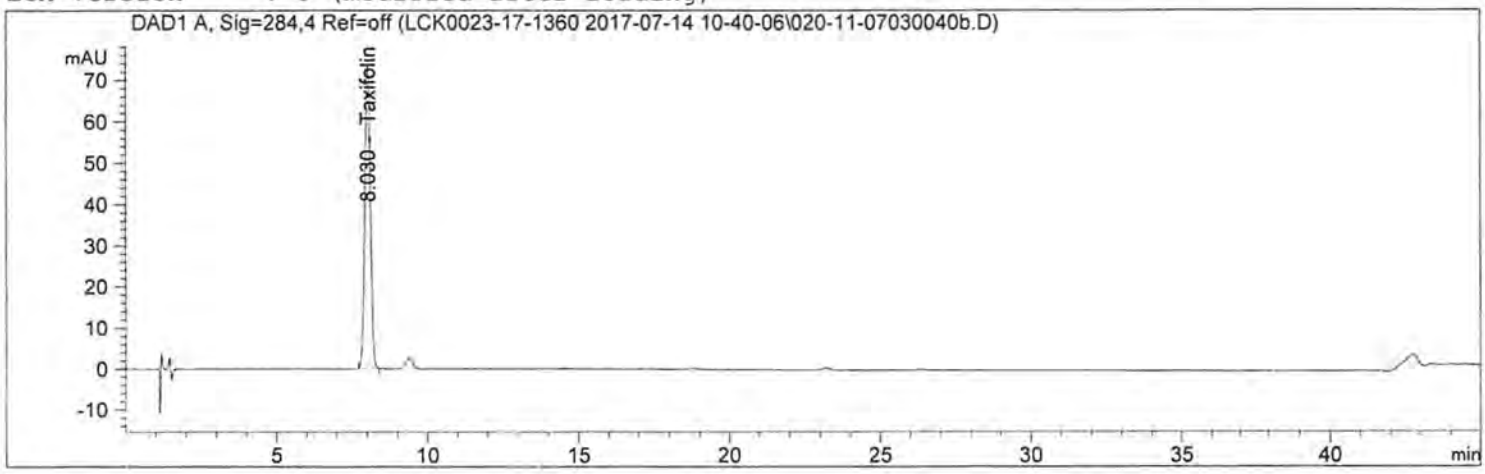


```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.41600  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
8.030	BB	958.33020	8.45459e-5	94.874522		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\020-11-07030040b.D
Sample Name: 07030040b

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				94.874522		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\021-12-07030040c.D
Sample Name: 07030040c

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  21
Acq. Instrument : HPLC-07                       Location :   12
Injection Date  : 7/15/2017 4:51:34 AM             Inj :    1
                                                Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
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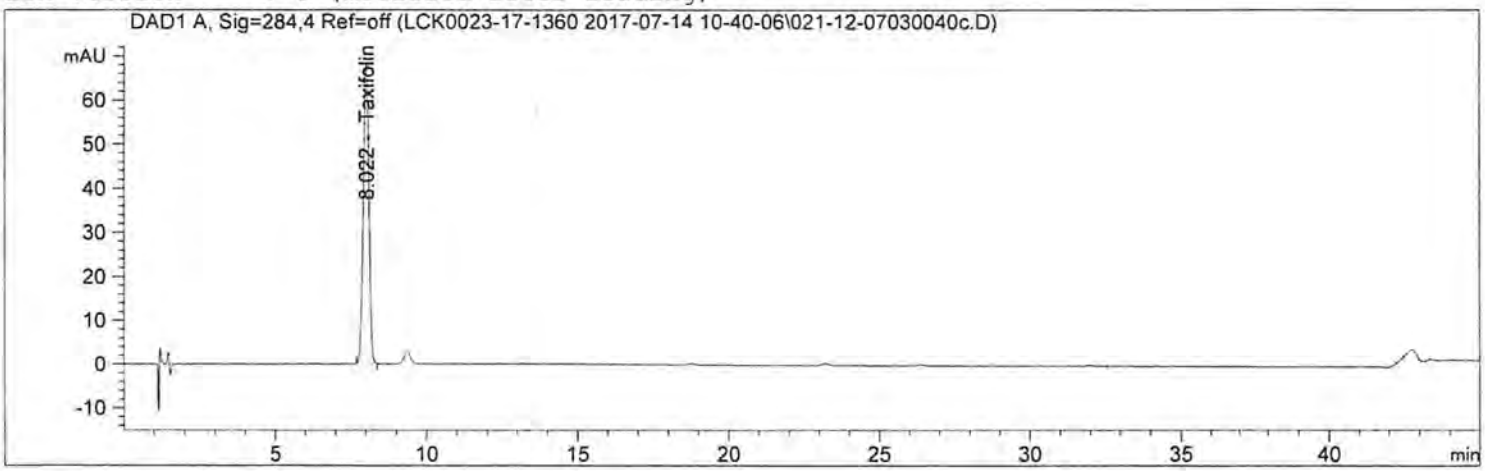


```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.20100  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
8.022	BB	894.12799	8.42598e-5	94.144427		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\021-12-07030040c.D
Sample Name: 07030040c

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				94.144427		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\022-13-07030041a.D
Sample Name: 07030041a

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  22
Acq. Instrument : HPLC-07                       Location :   13
Injection Date  : 7/15/2017 5:46:02 AM             Inj :    1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
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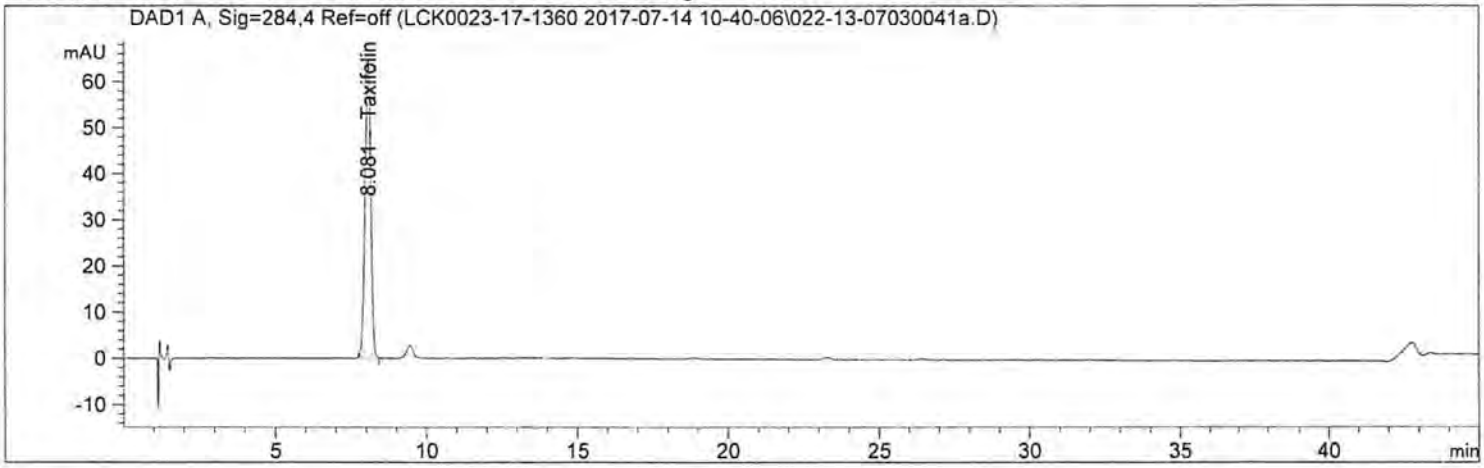



```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.07200  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
8.081	BB	854.61871	8.40625e-5	93.543433		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\022-13-07030041a.D
Sample Name: 07030041a

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				93.543433		

1 Warnings or Errors :

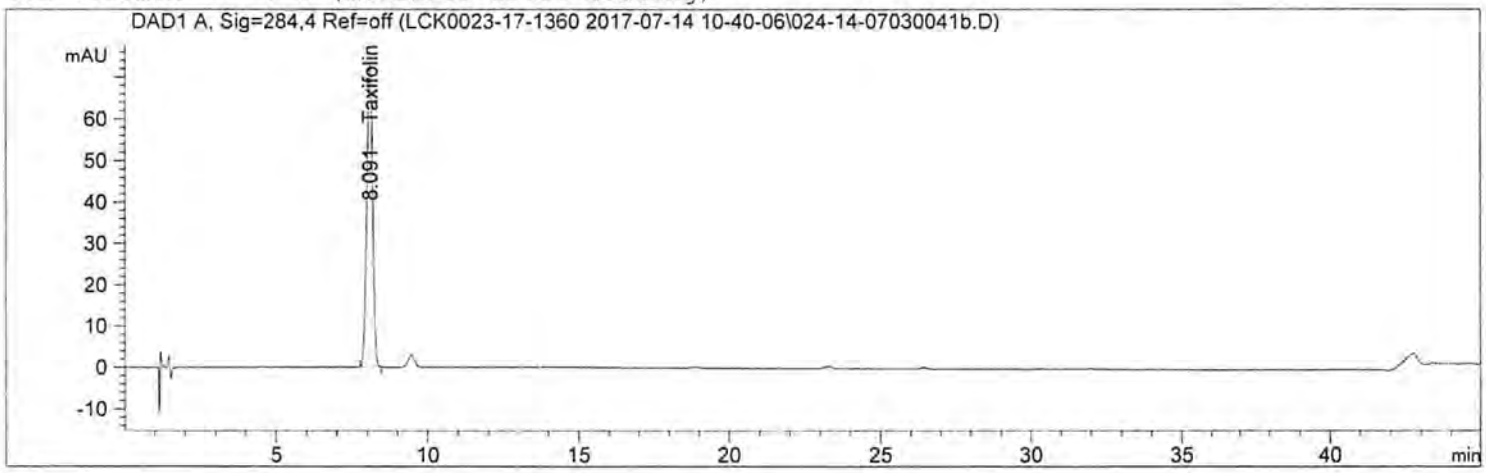
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\024-14-07030041b.D
Sample Name: 07030041b

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  24
Acq. Instrument : HPLC-07                       Location :   14
Injection Date  : 7/15/2017 7:34:55 AM             Inj :    1
                                                Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.49400  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
8.091	BB	978.23102	8.46269e-5	94.773490		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\024-14-07030041b.D
Sample Name: 07030041b

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				94.773490		

1 Warnings or Errors :

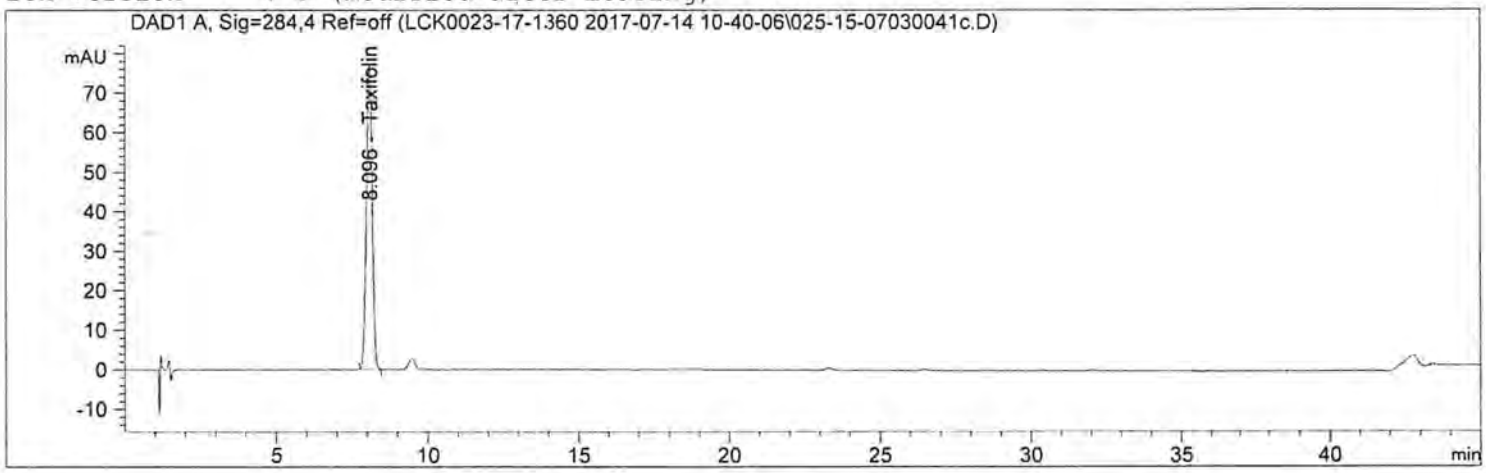
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\025-15-07030041c.D
Sample Name: 07030041c

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :  25
Acq. Instrument : HPLC-07                           Location :   15
Injection Date  : 7/15/2017 8:29:28 AM                  Inj :   1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.69600  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
8.096	BB	1024.93567	8.48047e-5	94.068576		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\025-15-07030041c.D
Sample Name: 07030041c

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				94.068576		

1 Warnings or Errors :

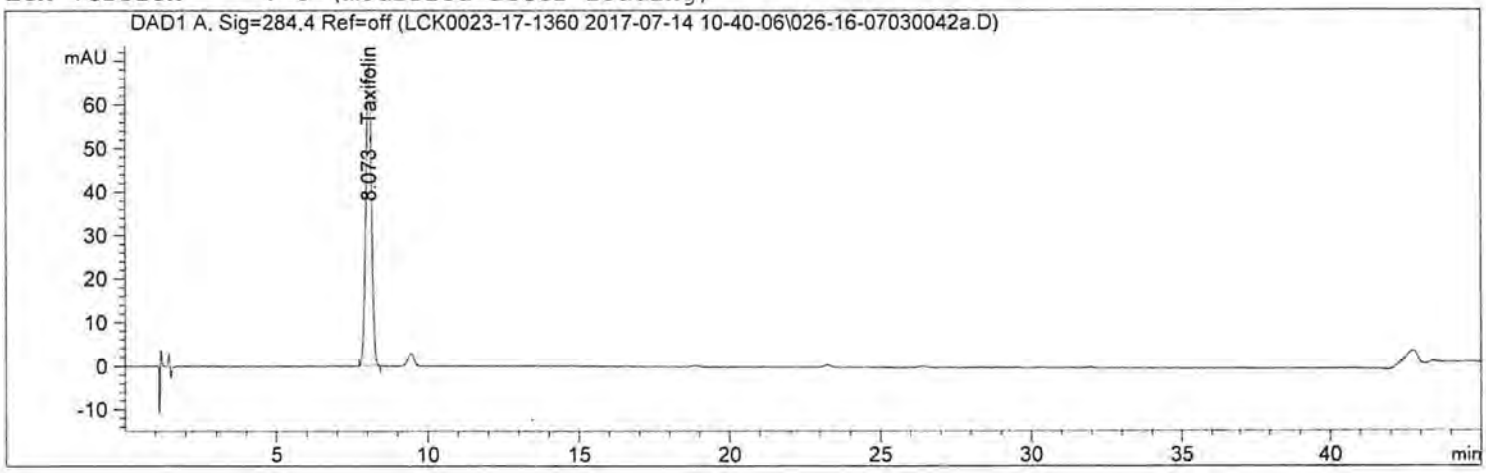
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\026-16-07030042a.D
Sample Name: 07030042a

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  26
Acq. Instrument : HPLC-07                       Location :   16
Injection Date  : 7/15/2017 9:24:04 AM              Inj :    1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Timothy Sit
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.27100  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
8.073	BB	911.09961	8.43394e-5	93.967056		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\026-16-07030042a.D
Sample Name: 07030042a

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				93.967056		

1 Warnings or Errors :

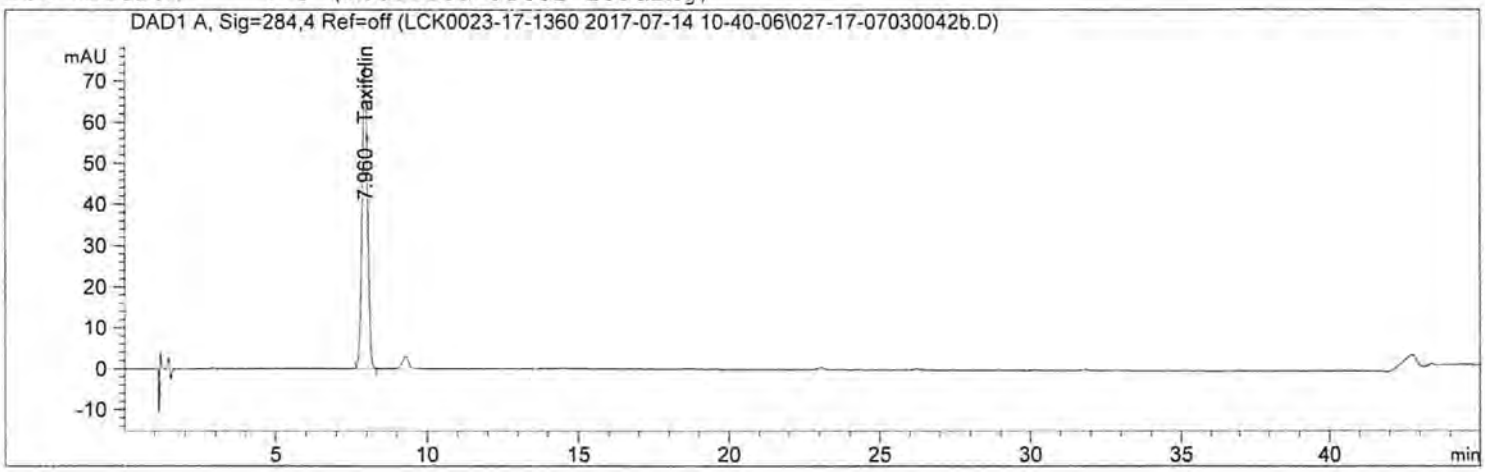
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\027-17-07030042b.D
Sample Name: 07030042b

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  27
Acq. Instrument : HPLC-07                       Location :   17
Injection Date  : 7/15/2017 10:18:31 AM             Inj :    1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Timothy Sit
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.43300  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.960	BB	954.15601	8.45284e-5	93.974138		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\027-17-07030042b.D
Sample Name: 07030042b

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				93.974138		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\028-18-07030042c.D
Sample Name: 07030042c

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  28
Acq. Instrument : HPLC-07                       Location :   18
Injection Date  : 7/15/2017 11:13:01 AM             Inj :    1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
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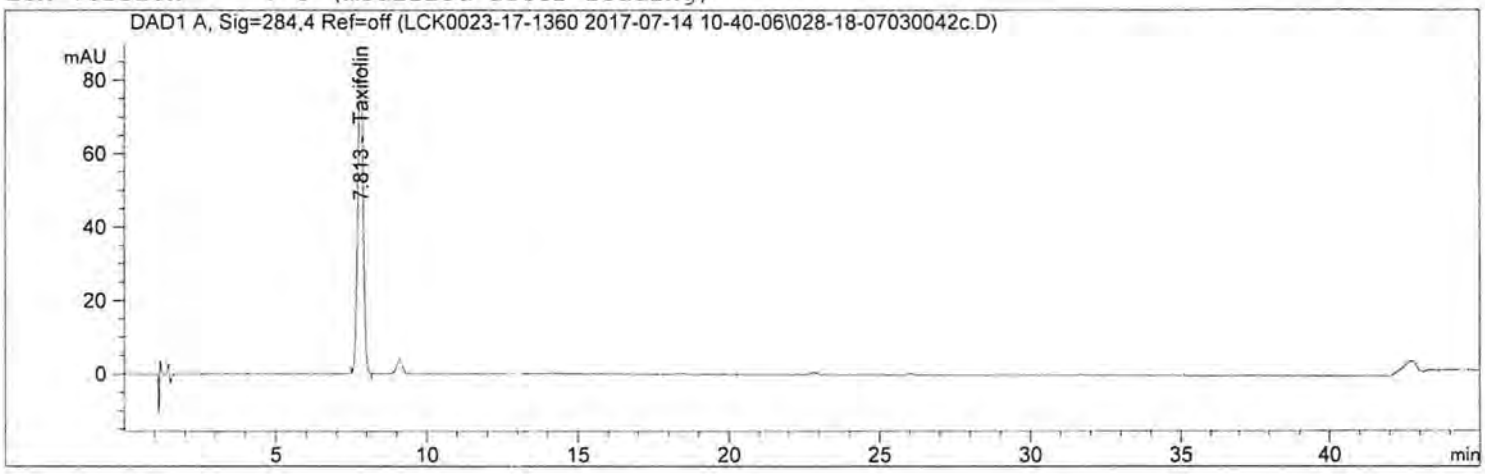



```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.80400  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.813	BB	1056.75745	8.49169e-5	94.360166		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\028-18-07030042c.D
Sample Name: 07030042c

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				94.360166		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

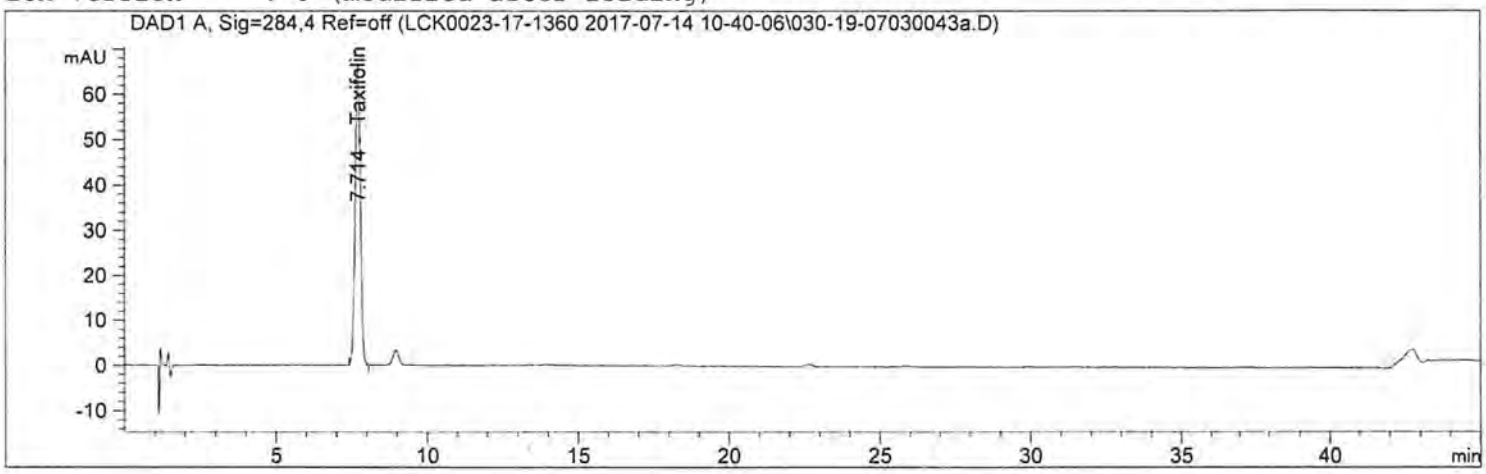
==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\030-19-07030043a.D
Sample Name: 07030043a

```
==========================================================================
Acq. Operator   :                                   Seq. Line :  30
Acq. Instrument : HPLC-07                            Location :  19
Injection Date  : 7/15/2017 1:02:01 PM                    Inj :  1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by Timothy Sit
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Timothy Sit
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      2.94700  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.714	BB	810.14398	8.38173e-5	92.167016		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\030-19-07030043a.D
Sample Name: 07030043a

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				92.167016		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

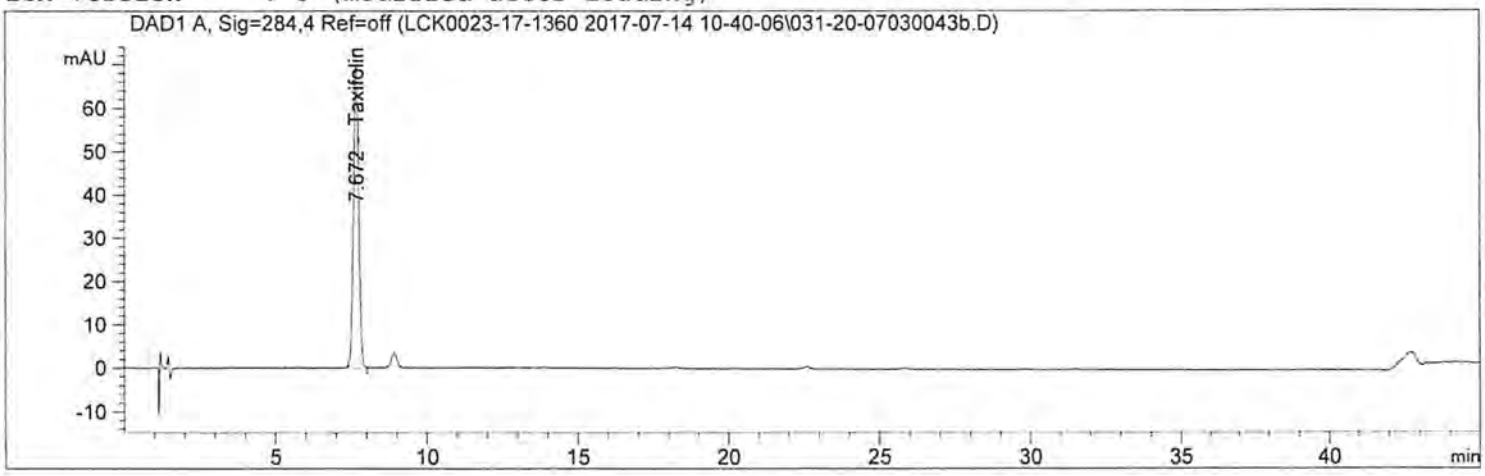
==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\031-20-07030043b.D
Sample Name: 07030043b

```
==========================================================================
Acq. Operator   : Timothy Sit                      Seq. Line :  31
Acq. Instrument : HPLC-07                          Location :  20
Injection Date  : 7/15/2017 1:56:35 PM                 Inj :  1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by '
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    :
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.08700  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.672	BB	840.58362	8.39879e-5	91.478902		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\031-20-07030043b.D
Sample Name: 07030043b

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				91.478902		

1 Warnings or Errors :

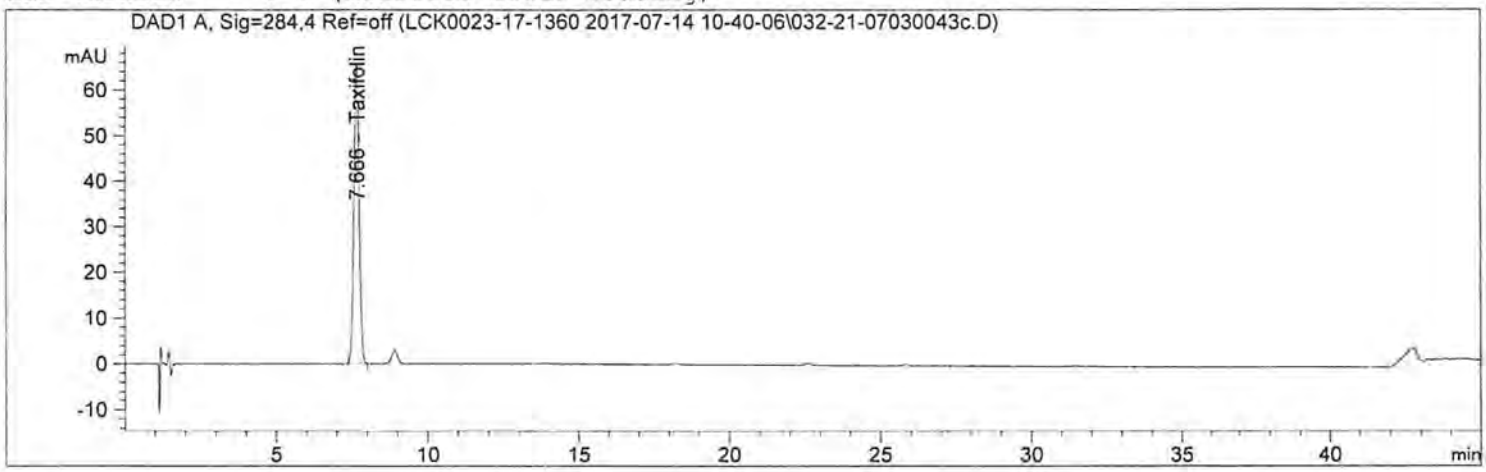
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\032-21-07030043c.D
Sample Name: 07030043c

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  32
Acq. Instrument : HPLC-07                       Location :   21
Injection Date  : 7/15/2017 2:51:06 PM             Inj :    1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 10:15:22 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      2.88600  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.414		-	-	-		Eriocitrin
7.666	BB	792.86896	8.37146e-5	91.995435		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\032-21-07030043c.D
Sample Name: 07030043c

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				91.995435		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

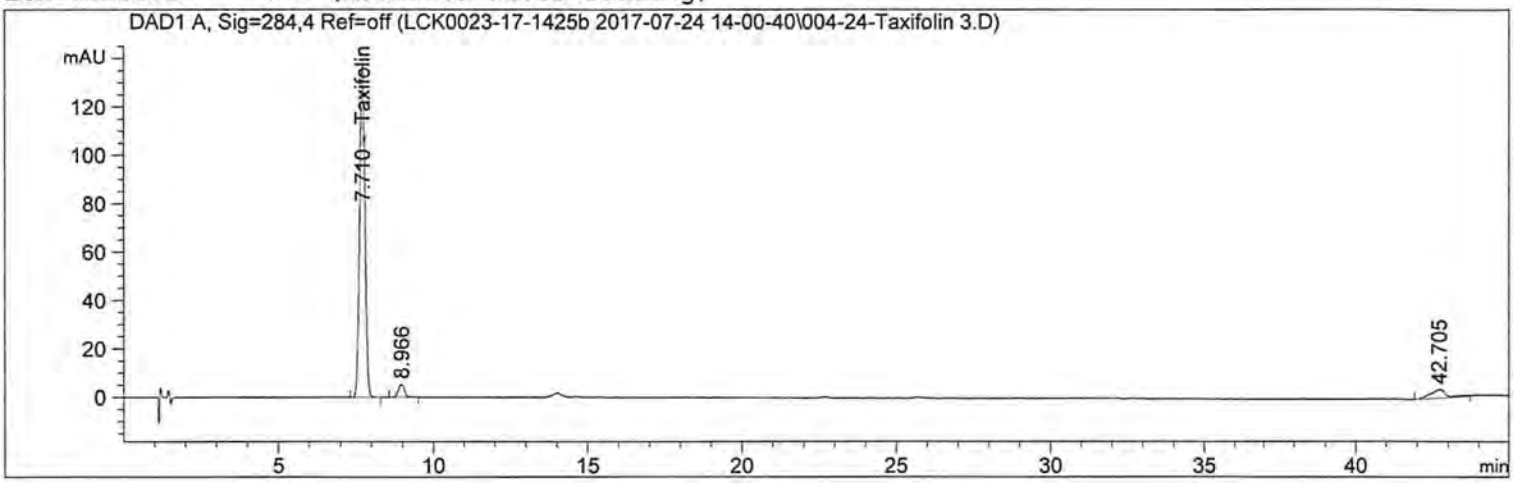
SYSTEM SUITABILITY

CHROMATOGRAMS

Data File D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\004-24-Taxifolin 3.D
Sample Name: Taxifolin 3

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :   4
Acq. Instrument : HPLC-07                          Location :   24
Injection Date  : 7/24/2017 5:00:46 PM                 Inj :   1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M
Last changed    : 7/24/2017 2:00:42 PM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/26/2017 4:48:06 PM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425b 2017-07-24 14-00-40.SC.SSIzip
ECM Version     : 2 (modified after loading)
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/26/2017 4:47:54 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.500		-	-	-		Eriocitrin
7.710	BB	1675.05542	8.61369e-5	1.44284e-1		Taxifolin
9.800		-	-	-		Rutin
12.100		-	-	-		Narirutin
15.000		-	-	-		Naringin
16.700		-	-	-		Hesperidin
18.492		-	-	-		Neohesperidin
25.588		-	-	-		Quercetin
28.707		-	-	-		Naringenin
31.267		-	-	-		Hesperitin

```
Totals :                              1.44284e-1
```

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

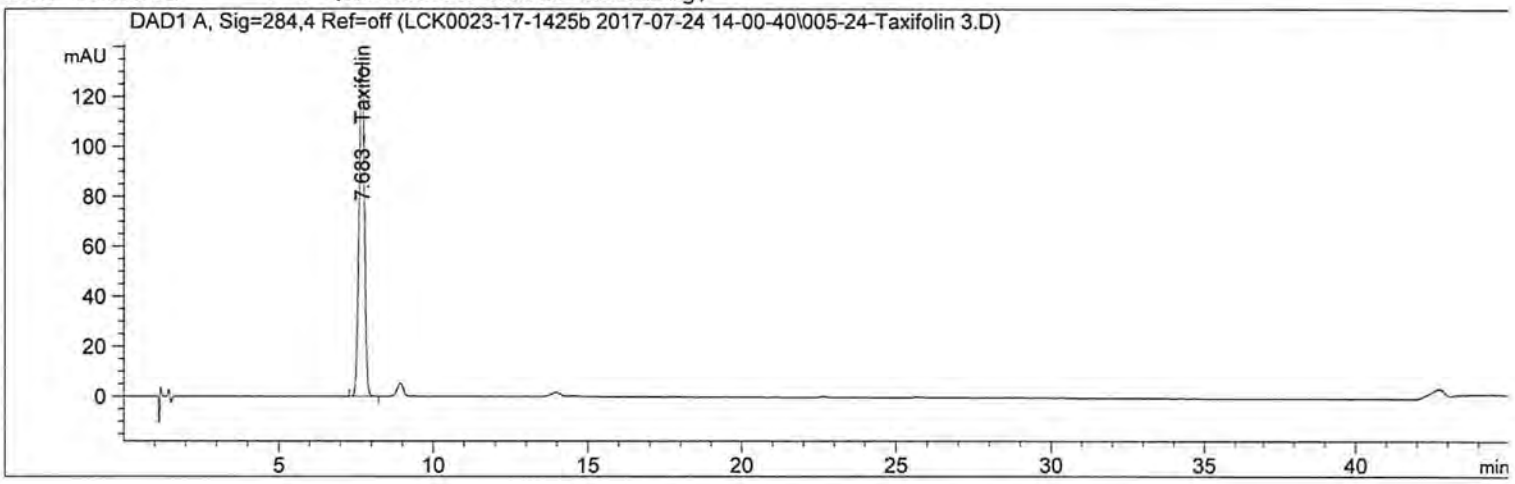
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\005-24-Taxifolin 3.D
Sample Name: Taxifolin 3

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :   5
Acq. Instrument : HPLC-07                       Location :   24
Injection Date  : 7/24/2017 5:55:18 PM             Inj :   1
                                              Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M
Last changed    : 7/24/2017 5:45:50 PM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/26/2017 4:48:06 PM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425b 2017-07-24 14-00-40.SC.SSIzip
ECM Version     : 2 (modified after loading)
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/26/2017 4:47:54 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.500		-	-	-		Eriocitrin
7.683	BB	1621.12231	8.61369e-5	1.39638e-1		Taxifolin
9.800		-	-	-		Rutin
12.100		-	-	-		Narirutin
15.000		-	-	-		Naringin
16.700		-	-	-		Hesperidin
18.492		-	-	-		Neohesperidin
25.588		-	-	-		Quercetin
28.707		-	-	-		Naringenin
31.267		-	-	-		Hesperitin

Totals : 1.39638e-1

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

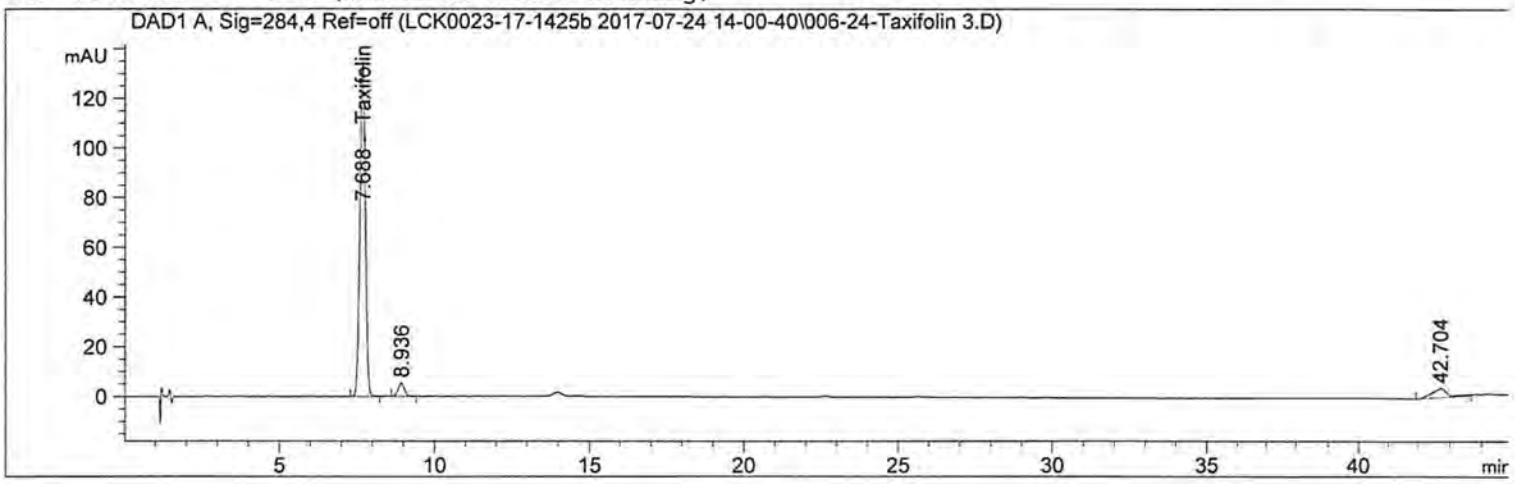
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\006-24-Taxifolin 3.D
Sample Name: Taxifolin 3

```
==========================================================================
Acq. Operator   :                                   Seq. Line :   6
Acq. Instrument : HPLC-07                           Location :   24
Injection Date  : 7/24/2017 6:49:50 PM                 Inj :   2
                                                   Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M
Last changed    : 7/24/2017 6:40:26 PM by
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/26/2017 4:48:06 PM by
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425b 2017-07-24 14-00-40.SC.SSIzip
ECM Version     : 2 (modified after loading)
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/26/2017 4:47:54 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.500		-	-	-		Eriocitrin
7.688	BB	1626.20020	8.61369e-5	1.40076e-1		Taxifolin
9.800		-	-	-		Rutin
12.100		-	-	-		Narirutin
15.000		-	-	-		Naringin
16.700		-	-	-		Hesperidin
18.492		-	-	-		Neohesperidin
25.588		-	-	-		Quercetin
28.707		-	-	-		Naringenin
31.267		-	-	-		Hesperitin

Totals : 1.40076e-1

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

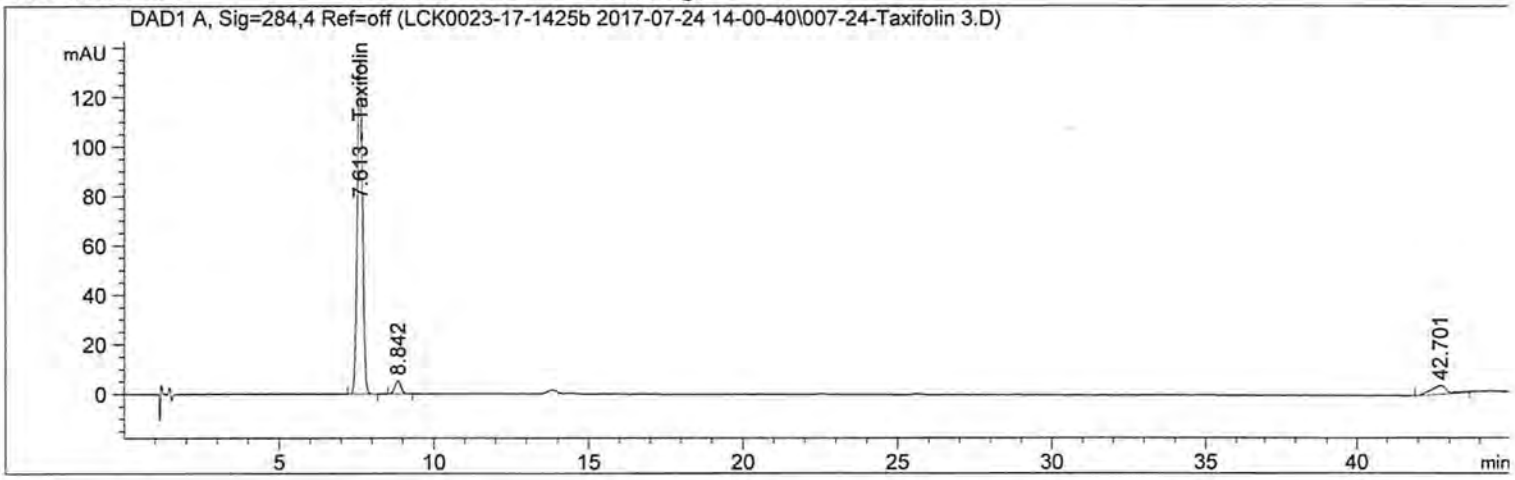
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\007-24-Taxifolin 3.D
Sample Name: Taxifolin 3

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :   7
Acq. Instrument : HPLC-07                           Location :   24
Injection Date  : 7/24/2017 7:44:25 PM                 Inj :   3
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M
Last changed    : 7/24/2017 7:34:54 PM by Timothy Sit
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/26/2017 4:48:06 PM by Hong You
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425b 2017-07-24 14-00-40.SC.SSIzip
ECM Version     : 2 (modified after loading)
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/26/2017 4:47:54 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.500		-	-	-		Eriocitrin
7.613	BB	1612.90723	8.61369e-5	1.38931e-1		Taxifolin
9.800		-	-	-		Rutin
12.100		-	-	-		Narirutin
15.000		-	-	-		Naringin
16.700		-	-	-		Hesperidin
18.492		-	-	-		Neohesperidin
25.588		-	-	-		Quercetin
28.707		-	-	-		Naringenin
31.267		-	-	-		Hesperitin

Totals : 1.38931e-1

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

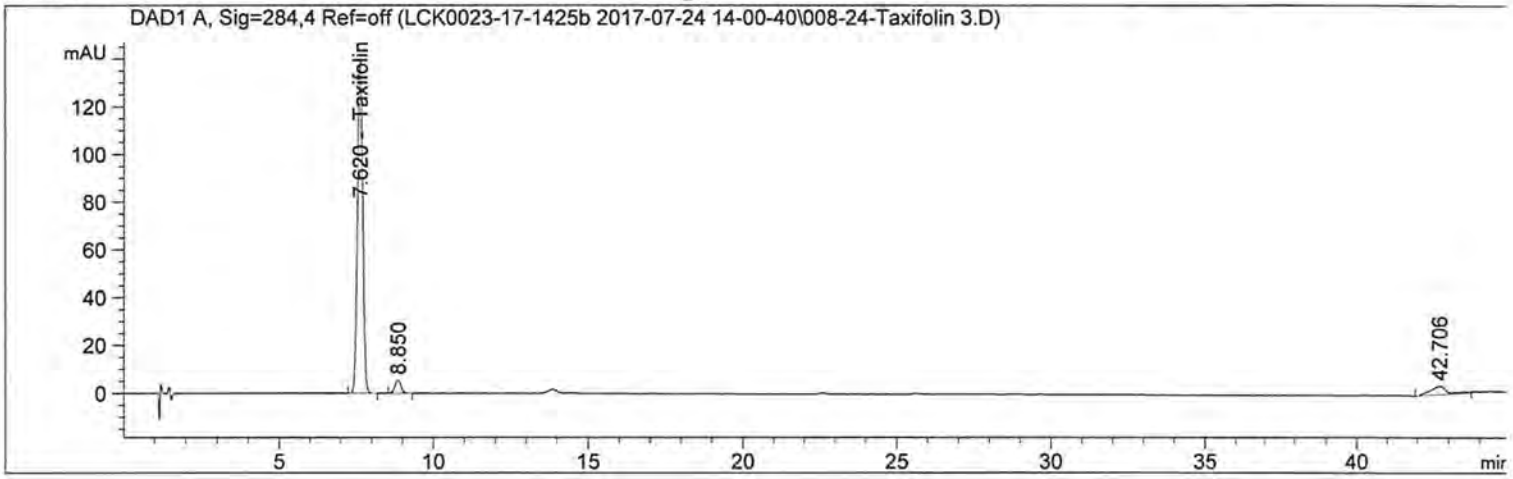
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\008-24-Taxifolin 3.D
Sample Name: Taxifolin 3

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :   8
Acq. Instrument : HPLC-07                          Location :   24
Injection Date  : 7/24/2017 8:39:01 PM                 Inj :   4
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M
Last changed    : 7/24/2017 8:29:31 PM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425b 2017-07-24 14-00-40\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/26/2017 4:48:06 PM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425b 2017-07-24 14-00-40.SC.SSIzip
ECM Version     : 2 (modified after loading)
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/26/2017 4:47:54 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.500		-	-	-		Eriocitrin
7.620	BB	1676.10657	8.61369e-5	1.44375e-1		Taxifolin
9.800		-	-	-		Rutin
12.100		-	-	-		Narirutin
15.000		-	-	-		Naringin
16.700		-	-	-		Hesperidin
18.492		-	-	-		Neohesperidin
25.588		-	-	-		Quercetin
28.707		-	-	-		Naringenin
31.267		-	-	-		Hesperitin

Totals : 1.44375e-1

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

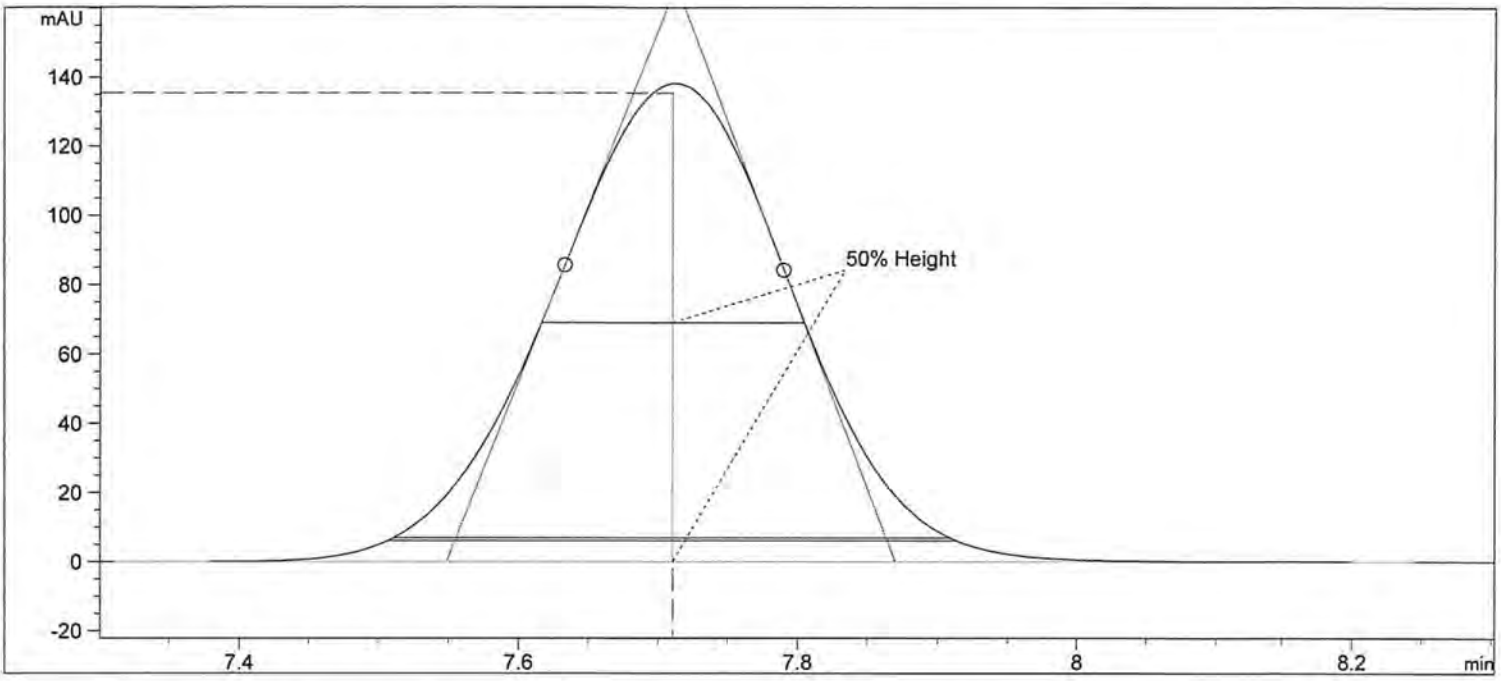
SYSTEM SUITABILITY

PEAK PERFORMANCE REPORT

Sample Name Taxifolin 3

Peak Performance Evaluation

DAD1 A, Sig=284,4 Ref=off RT 7.71042 min



Ret.Time [min] t (integrator)	7.71042
Ret.Time [min] t (peak model)	7.70917
Void time [min] (Column) t0	-
k'	-
Height [mAU] (integrator)	135.43806
Height [mAU] (peak model)	138.17695
Area [mAU*s]	1675.0554
Peakwidth method	Half height (EP)
Peakwidth [min]	0.18838
Peak Start [min]	7.31042
Peak End [min]	8.29250
Skew	0.05173
Excess	0.18116
Symmetry (integrator)	0.97427
Symmetry (Foley Dorsey at 10% height)	0.99939
Symmetry (USP at 10% height)	1.00030
USP Tailing (at 5% height)	1.00400
Noise of classic noise range [mAU]	-
Signal to noise ratio(classic range)	1298.903244
Integration Type	BB
Time Increment [ms]	400.00000
Data Points	180
Moment0	1673.5133
Moment1	7.712033
Moment2	0.006679
Moment3	0.000028
Moment4	0.000142
Efficiency [Plates/Column]	9278
Efficiency [Plates/Meter]	-
Foley Dorsey [Plates/Column]	9106
Foley Dorsey [Plates/Meter]	-
Selectivity to prev peak	-
Selectivity to next peak	1.16286
Resolution to prev peak	-
Resolution to next peak	3.66611

Sample Name Taxifolin 3

Configuration settings

Void time and Column Configured : From Data File
Void Time(min) : -
Column Length(cm) : -
Peak Width method selected : Half height (EP)

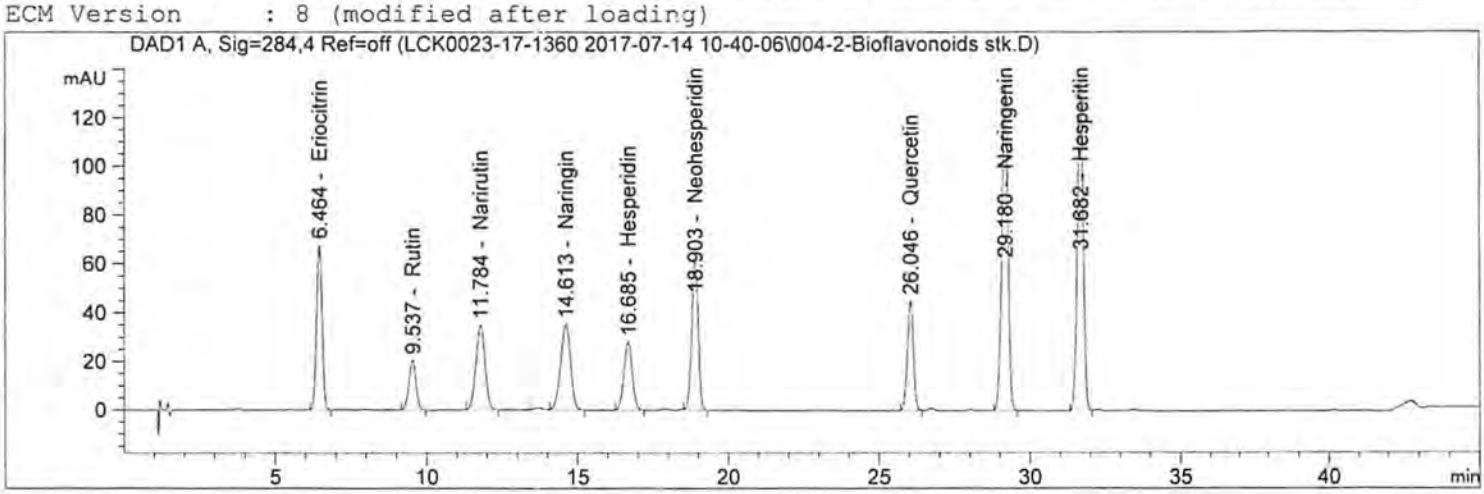
SPECIFICITY

CHROMATOGRAMS

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\004-2-Bioflavonoids stk.D
Sample Name: Bioflavonoids stk

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :   4
Acq. Instrument : HPLC-07                       Location :   2
Injection Date  : 7/14/2017 1:25:01 PM              Inj :   1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 10:40:08 AM by Timothy Sit
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 9:35:12 AM by Timothy Sit
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:35:01 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.464	BB	863.27692	1.42884e-4	1.23349e-1		Eriocitrin
7.836		-	-	-		Taxifolin
9.537	BB	336.36642	3.34952e-4	1.12667e-1		Rutin
11.784	BB	744.35327	1.43785e-4	1.07027e-1		Narirutin
14.613	BB	808.25098	1.41595e-4	1.14444e-1		Naringin
16.685	BB	542.63300	1.46808e-4	7.96627e-2		Hesperidin
18.903	BB	932.78711	1.32791e-4	1.23866e-1		Neohesperidin
26.046	BB	616.32721	1.90232e-4	1.17245e-1		Quercetin
29.180	BB	1904.35022	7.41449e-5	1.41198e-1		Naringenin
31.682	BB	1823.41907	7.49558e-5	1.36676e-1		Hesperitin

* Taxifolin is well-separated fro. other common flavonoids in this method.
HY 7/28/17

Totals : 1.05613

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

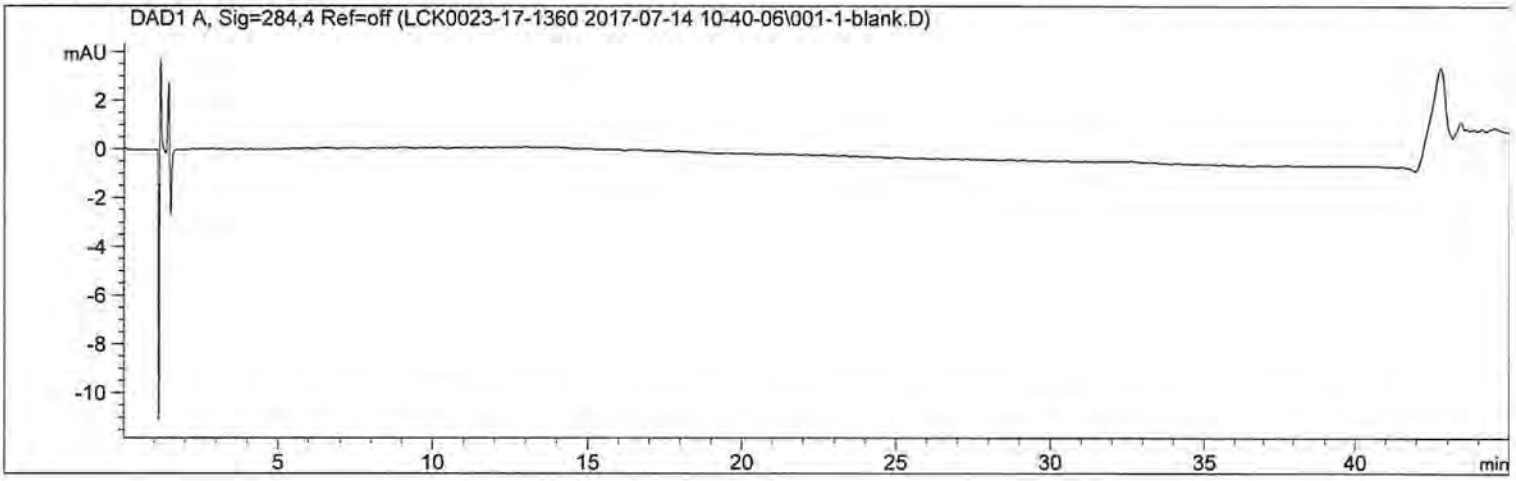
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1360 2017-07-14 10-40-06\001-1-blank.D
Sample Name: blank

```
==========================================================================
Acq. Operator    : [redacted]                        Seq. Line :   1
Acq. Instrument : HPLC-07                            Location :   1
Injection Date   : 7/14/2017 10:41:42 AM                 Inj :   1
                                                   Inj Volume : 5.000 µl
Acq. Method      : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed     : 7/14/2017 10:40:08 AM by [redacted]
Analysis Method  : D:\Chem32\4\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (Sequence
                   Method)
Last changed     : 7/17/2017 4:40:26 PM by [redacted]
Method Info      : Bioflavonoids

ECM Server       : http://us05apvp001/ecmwg
ECM Operator     : [redacted]
ECM Path         : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version      : 9
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.414		-	-	-		Eriocitrin
7.836		-	-	-		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Totals : 0.00000

Data File D:\Chem32\4\Data\LCK0023-17-1360 2017-07-14 10-40-06\001-1-blank.D
Sample Name: blank

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========
==========
Area Percent Report
==========

Sorted By : Signal
Calib. Data Modified : Monday, July 17, 2017 9:49:42 AM
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	6.414		0.0000	0.00000	0.0000	Eriocitrin
2	7.836		0.0000	0.00000	0.0000	Taxifolin
3	9.420		0.0000	0.00000	0.0000	Rutin
4	11.667		0.0000	0.00000	0.0000	Narirutin
5	14.472		0.0000	0.00000	0.0000	Naringin
6	16.574		0.0000	0.00000	0.0000	Hesperidin
7	18.801		0.0000	0.00000	0.0000	Neohesperidin
8	25.952		0.0000	0.00000	0.0000	Quercetin
9	29.084		0.0000	0.00000	0.0000	Naringenin
10	31.605		0.0000	0.00000	0.0000	Hesperitin

Totals : 0.00000

1 Warnings or Errors :

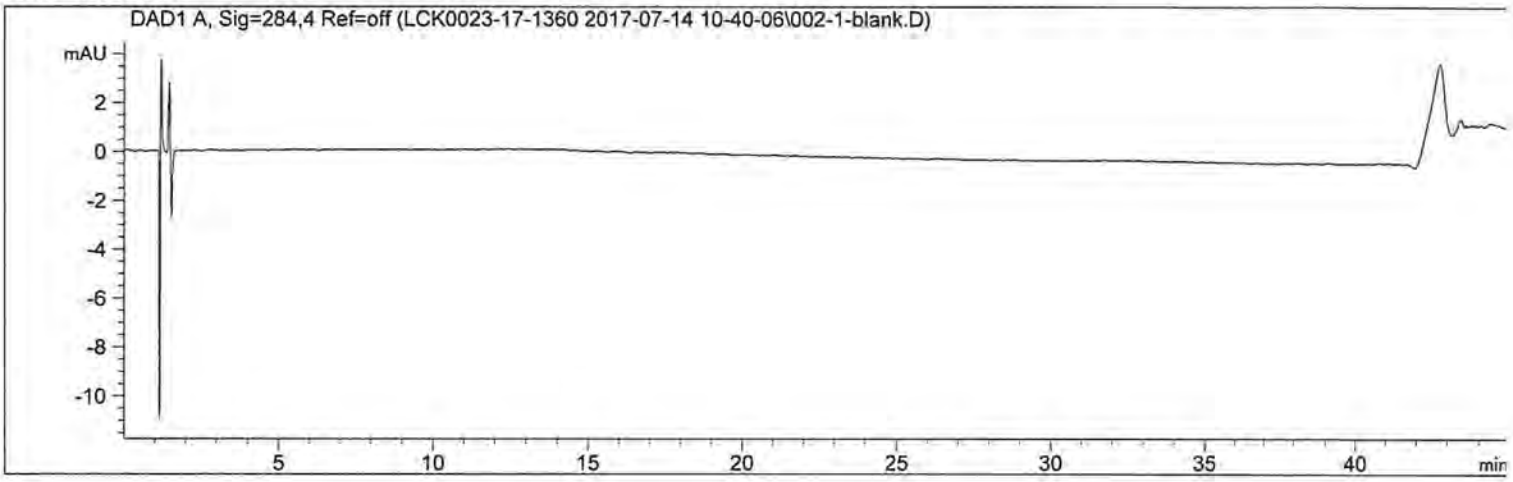
Warning : Calibrated compound(s) not found

==========
*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1360 2017-07-14 10-40-06\002-1-blank.D
Sample Name: blank

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :   2
Acq. Instrument : HPLC-07                           Location :   1
Injection Date  : 7/14/2017 11:36:07 AM                 Inj :   2
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 10:40:08 AM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (Sequence
                  Method)
Last changed    : 7/17/2017 4:40:26 PM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 9
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.414		-	-	-		Eriocitrin
7.836		-	-	-		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Totals : 0.00000

Data File D:\Chem32\4\Data\LCK0023-17-1360 2017-07-14 10-40-06\002-1-blank.D
Sample Name: blank

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========
==========
Area Percent Report
==========

Sorted By : Signal
Calib. Data Modified : Monday, July 17, 2017 9:49:42 AM
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	6.414		0.0000	0.00000	0.0000	Eriocitrin
2	7.836		0.0000	0.00000	0.0000	Taxifolin
3	9.420		0.0000	0.00000	0.0000	Rutin
4	11.667		0.0000	0.00000	0.0000	Narirutin
5	14.472		0.0000	0.00000	0.0000	Naringin
6	16.574		0.0000	0.00000	0.0000	Hesperidin
7	18.801		0.0000	0.00000	0.0000	Neohesperidin
8	25.952		0.0000	0.00000	0.0000	Quercetin
9	29.084		0.0000	0.00000	0.0000	Naringenin
10	31.605		0.0000	0.00000	0.0000	Hesperitin

Totals : 0.00000

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========
*** End of Report ***

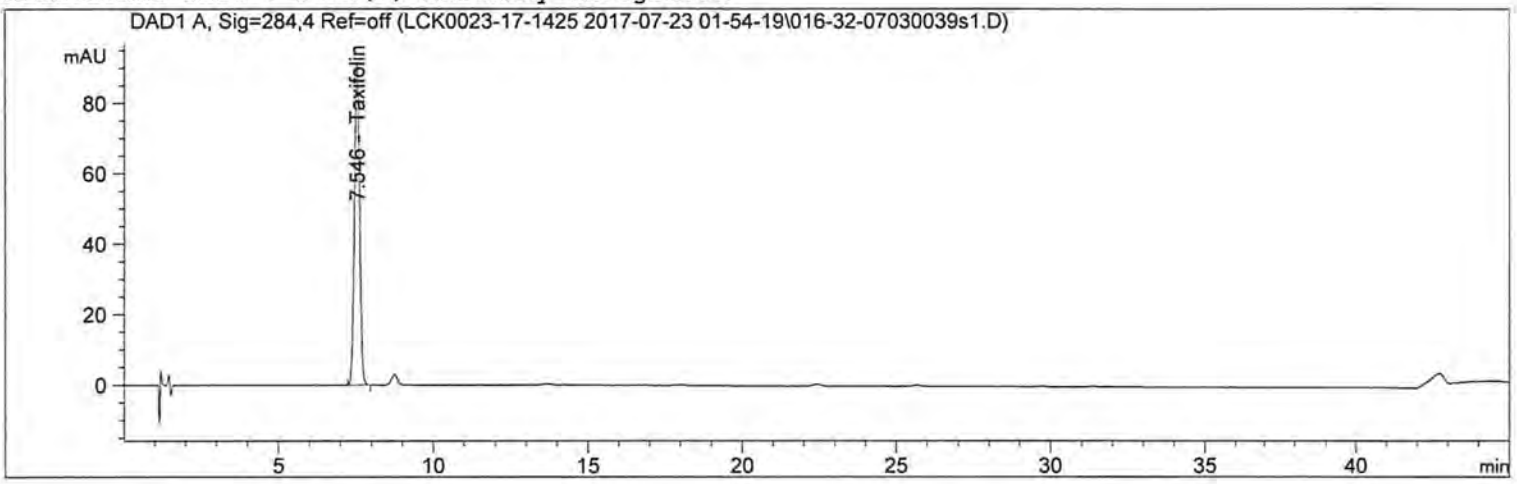
ACCURACY

CHROMATOGRAMS

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\016-32-07030039s1.D
Sample Name: 07030039s1

```
==========================================================================
Acq. Operator   :                                   Seq. Line :  16
Acq. Instrument : HPLC-07                            Location :   32
Injection Date  : 7/23/2017 3:33:24 PM                   Inj :   1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M
Last changed    : 7/23/2017 9:02:18 AM by
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M (Sequence
                  Method)
Last changed    : 7/28/2017 1:49:49 PM by
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425 2017-07-23 01-54-19.SC.SSIzip
ECM Version     : 5 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/28/2017 1:48:52 PM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.03000  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]           [mAU*s]                   %
-------|------|----------|----------|----------|--|------------------
  6.570           -           -          -          Eriocitrin
  7.546 BB     1079.22522 8.40351e-5 119.726468      Taxifolin
  9.730           -           -          -          Rutin
 12.001           -           -          -          Narirutin
 14.866           -           -          -          Naringin
 16.864           -           -          -          Hesperidin
 19.037           -           -          -          Neohesperidin
 26.218           -           -          -          Quercetin
 29.353           -           -          -          Naringenin
 31.840           -           -          -          Hesperitin
```

* low level spike HY 7/28/17

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\016-32-07030039s1.D
Sample Name: 07030039s1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				119.726468		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

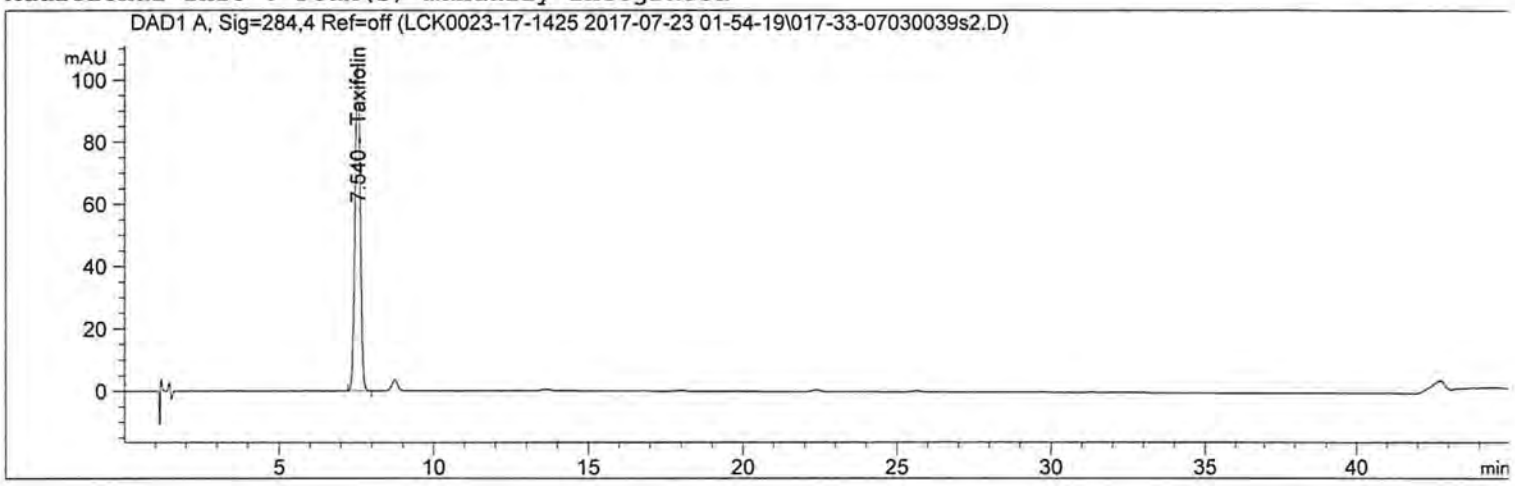
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\017-33-07030039s2.D
Sample Name: 07030039s2

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  17
Acq. Instrument : HPLC-07                       Location :   33
Injection Date  : 7/23/2017 4:28:13 PM              Inj :    1
                                              Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M
Last changed    : 7/23/2017 9:02:18 AM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M (Sequence
                  Method)
Last changed    : 7/28/2017 1:49:49 PM by Hong You
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425 2017-07-23 01-54-19.SC.SSIzip
ECM Version     : 5 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/28/2017 1:48:52 PM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.03100   [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.570		-	-	-		Eriocitrin
7.540	BB	1230.38611	8.39427e-5	136.300774		Taxifolin
9.730		-	-	-		Rutin
12.001		-	-	-		Narirutin
14.866		-	-	-		Naringin
16.864		-	-	-		Hesperidin
19.037		-	-	-		Neohesperidin
26.218		-	-	-		Quercetin
29.353		-	-	-		Naringenin
31.840		-	-	-		Hesperitin

* mid level spike
HY 7/28/17

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\017-33-07030039s2.D
Sample Name: 07030039s2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				136.300774		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

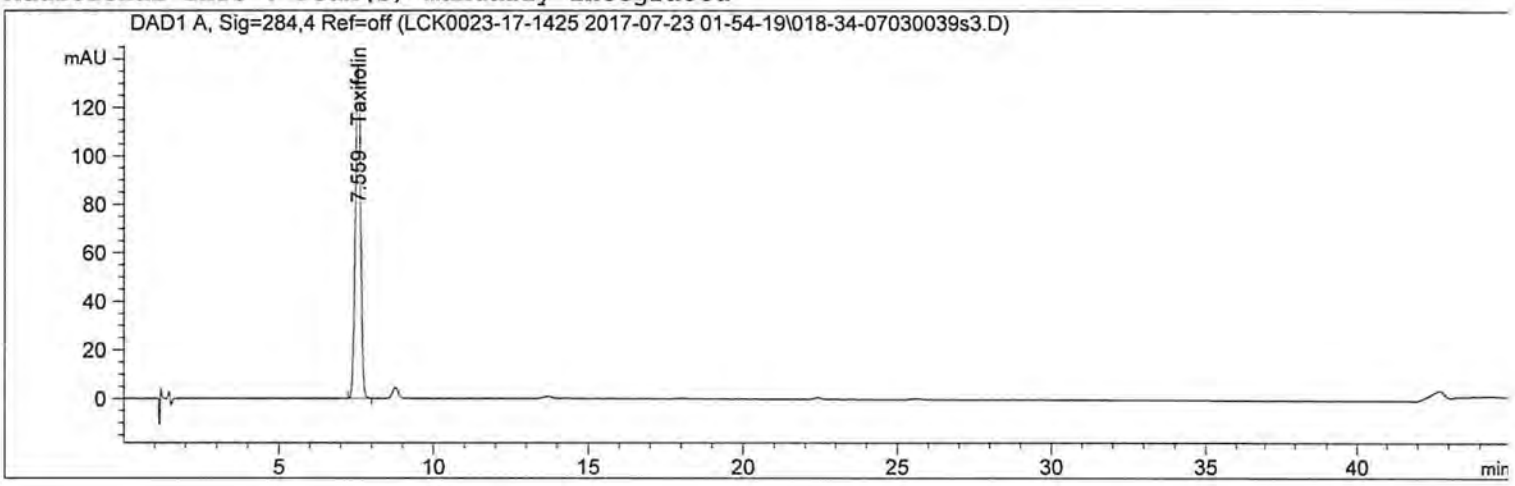
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\018-34-07030039s3.D
Sample Name: 07030039s3

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  18
Acq. Instrument : HPLC-07                       Location :   34
Injection Date  : 7/23/2017 5:22:43 PM             Inj :   1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M
Last changed    : 7/23/2017 9:02:18 AM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M (Sequence
                  Method)
Last changed    : 7/28/2017 1:49:49 PM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425 2017-07-23 01-54-19.SC.SSIzip
ECM Version     : 5 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/28/2017 1:48:52 PM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.02200   [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.570		-	-	-		Eriocitrin
7.559	BB	1628.23657	8.37815e-5	180.563915		Taxifolin
9.730		-	-	-		Rutin
12.001		-	-	-		Narirutin
14.866		-	-	-		Naringin
16.864		-	-	-		Hesperidin
19.037		-	-	-		Neohesperidin
26.218		-	-	-		Quercetin
29.353		-	-	-		Naringenin
31.840		-	-	-		Hesperitin

* high level spike
HY 7/28/17

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\018-34-07030039s3.D
Sample Name: 07030039s3

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				180.563915		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

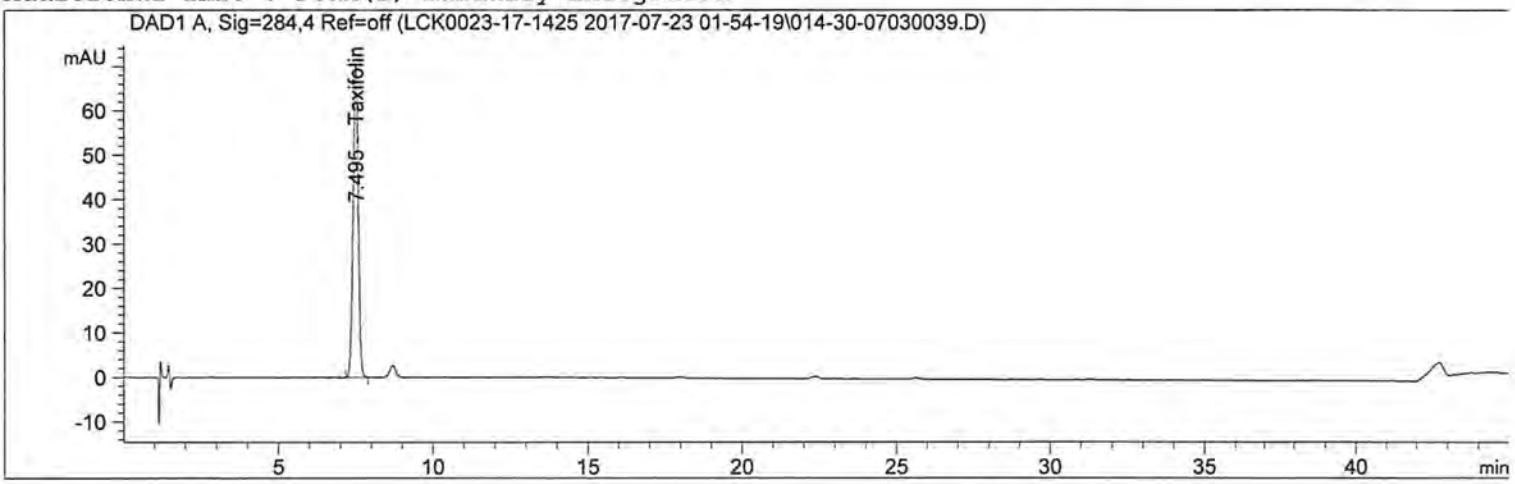
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\014-30-07030039.D
Sample Name: 07030039

```
==========================================================================
Acq. Operator   : [redacted]                        Seq. Line :  14
Acq. Instrument : HPLC-07                           Location :   30
Injection Date  : 7/23/2017 1:44:23 PM                  Inj :   1
                                                  Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M
Last changed    : 7/23/2017 9:02:18 AM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M (Sequence
                  Method)
Last changed    : 7/28/2017 1:49:49 PM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425 2017-07-23 01-54-19.SC.SSIzip
ECM Version     : 5 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/28/2017 1:48:52 PM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.03400  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.570		-	-	-		Eriocitrin
7.495	BB	824.30042	8.42677e-5	91.578025		Taxifolin
9.730		-	-	-		Rutin
12.001		-	-	-		Narirutin
14.866		-	-	-		Naringin
16.864		-	-	-		Hesperidin
19.037		-	-	-		Neohesperidin
26.218		-	-	-		Quercetin
29.353		-	-	-		Naringenin
31.840		-	-	-		Hesperitin

* 0 level spike HY 7/28/17

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\014-30-07030039.D
Sample Name: 07030039

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				91.578025		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

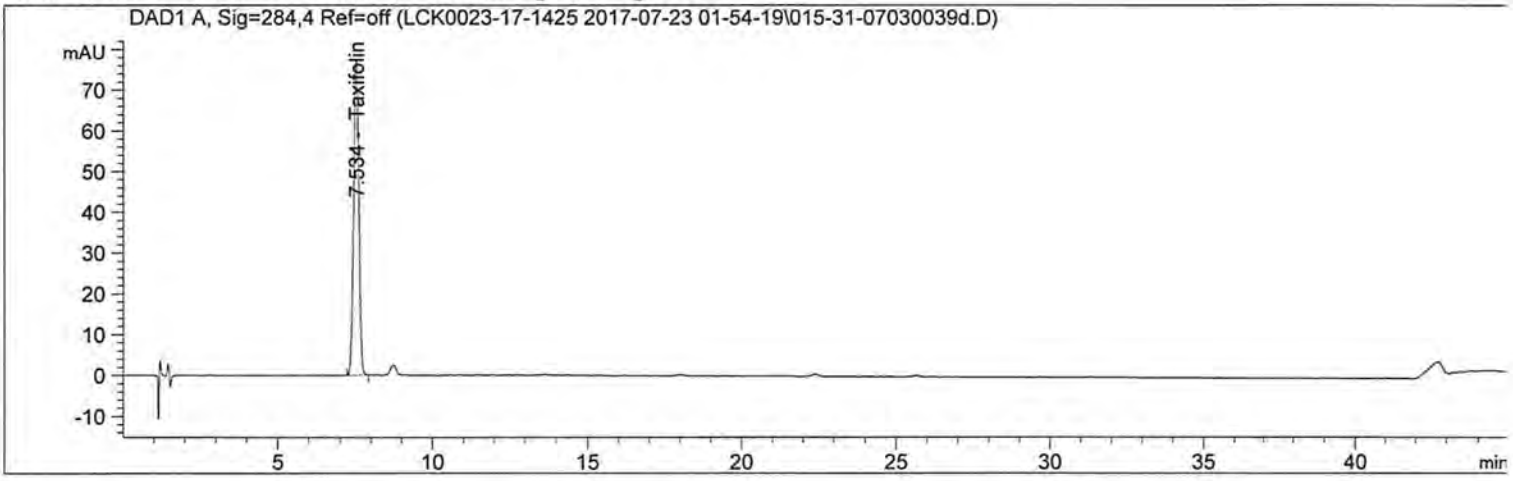
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\015-31-07030039d.D
Sample Name: 07030039d

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  15
Acq. Instrument : HPLC-07                       Location :   31
Injection Date  : 7/23/2017 2:38:54 PM             Inj :   1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M
Last changed    : 7/23/2017 9:02:18 AM by [redacted]
Analysis Method : D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\LCK0023-7.M (Sequence
                  Method)
Last changed    : 7/28/2017 1:49:49 PM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1425 2017-07-23 01-54-19.SC.SSIzip
ECM Version     : 5 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      7/28/2017 1:48:52 PM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.27200  [mg/mL]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
6.570		-	-	-		Eriocitrin
7.534	BB	908.13861	8.41768e-5	93.452588		Taxifolin
9.730		-	-	-		Rutin
12.001		-	-	-		Narirutin
14.866		-	-	-		Naringin
16.864		-	-	-		Hesperidin
19.037		-	-	-		Neohesperidin
26.218		-	-	-		Quercetin
29.353		-	-	-		Naringenin
31.840		-	-	-		Hesperitin

* a level Spike HY 7/18/17

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\015-31-07030039d.D
Sample Name: 07030039d

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
Totals :				93.452588		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

LINEARITY

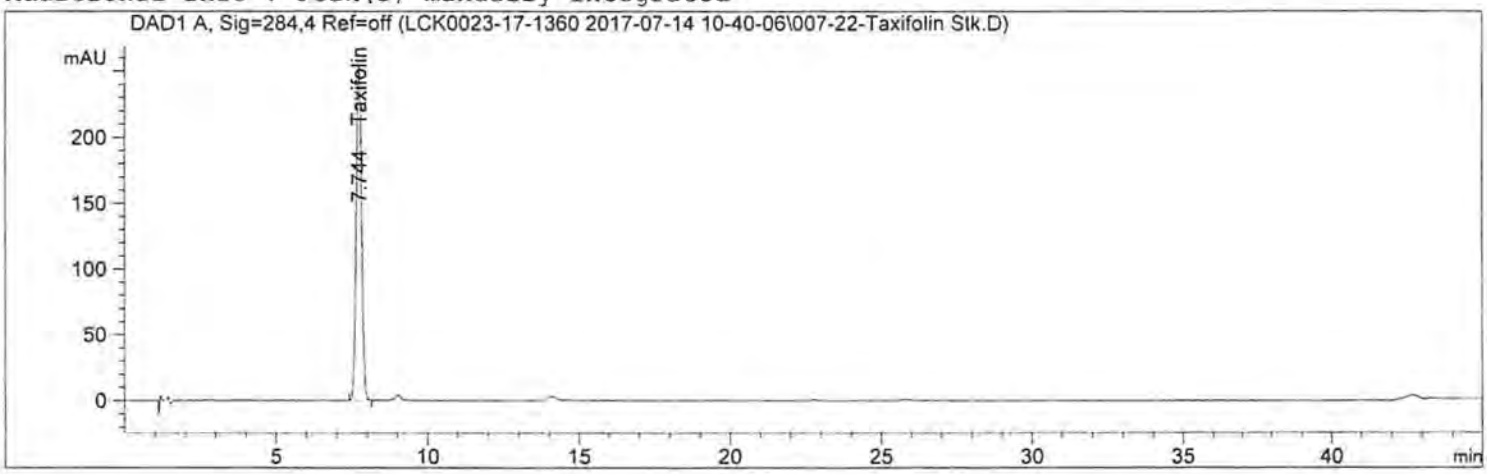
CHROMATOGRAMS

DIHYDROQUERCETIN 5 POINT CALIBRATION FOR PURITY DETERMINATION

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\007-22-Taxifolin Stk.D
Sample Name: Taxifolin Stk

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :   7
Acq. Instrument : HPLC-07                       Location :   22
Injection Date  : 7/14/2017 4:08:25 PM             Inj :   1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 3:59:03 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 9:53:52 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.414		-	-	-		Eriocitrin
7.744	BB	3136.09863	8.73119e-5	2.73819e-1		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\007-22-Taxifolin Stk.D
Sample Name: Taxifolin Stk

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
Totals :				2.73819e-1		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

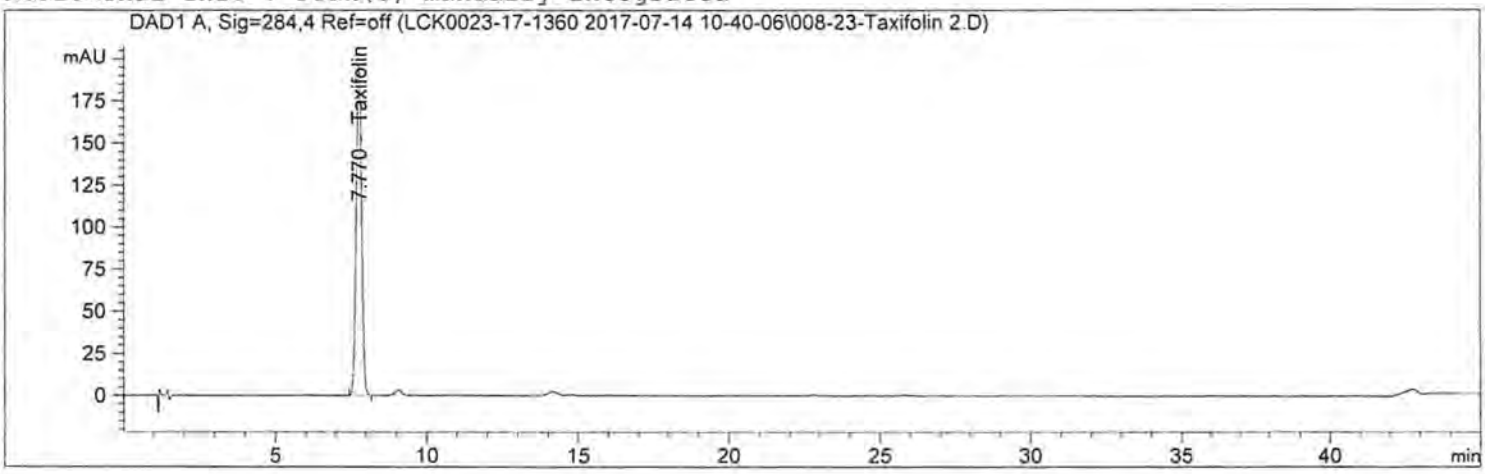
==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\008-23-Taxifolin 2.D
Sample Name: Taxifolin 2

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :   8
Acq. Instrument : HPLC-07                       Location :   23
Injection Date  : 7/14/2017 5:02:57 PM             Inj :   1
                                              Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 4:53:29 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 9:53:52 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Timothy Sit
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.414		-	-	-		Eriocitrin
7.770	BB	2428.68335	8.69574e-5	2.11192e-1		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\008-23-Taxifolin 2.D
Sample Name: Taxifolin 2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
Totals :				2.11192e-1		

1 Warnings or Errors :

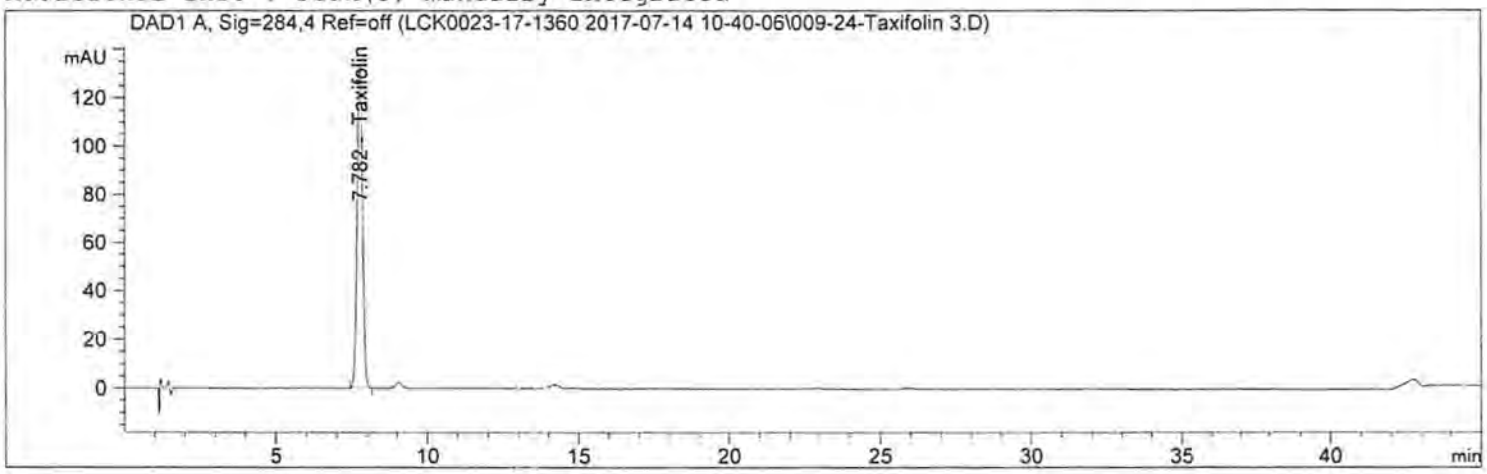
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\009-24-Taxifolin 3.D
Sample Name: Taxifolin 3

Acq. Operator	: [redacted]	Seq. Line	: 9
Acq. Instrument	: HPLC-07	Location	: 24
Injection Date	: 7/14/2017 5:57:28 PM	Inj	: 1
		Inj Volume	: 5.000 µl
Acq. Method	: D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M		
Last changed	: 7/14/2017 5:48:00 PM by [redacted]		
Analysis Method	: D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (Sequence Method)		
Last changed	: 7/17/2017 9:53:52 AM by [redacted]		
Method Info	: Bioflavonoids		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: [redacted]		
ECM Path	: \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip		
ECM Version	: 8 (modified after loading)		
Additional Info	: Peak(s) manually integrated		



External Standard Report (Sample Amount is 0!)

Sorted By	:	Signal	
Calib. Data Modified	:	Monday, July 17, 2017 9:49:42 AM	
Multiplier	:	1.0000	
Dilution	:	1.0000	

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.414		-	-	-		Eriocitrin
7.782	BB	1655.01001	8.62226e-5	1.42699e-1		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\009-24-Taxifolin 3.D
Sample Name: Taxifolin 3

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
Totals :				1.42699e-1		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

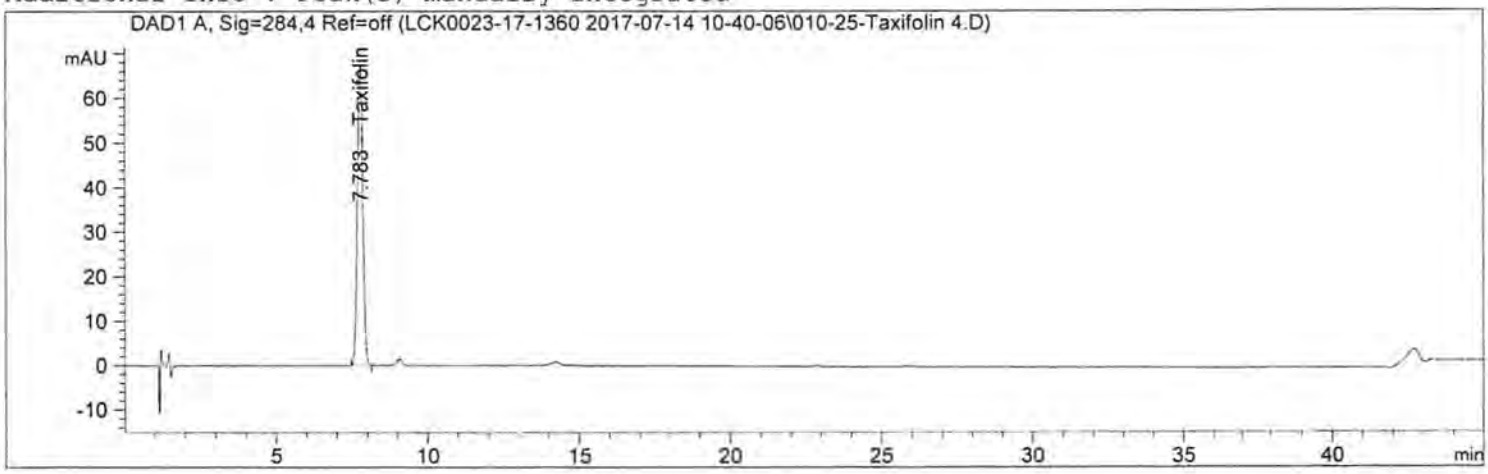
==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\010-25-Taxifolin 4.D
Sample Name: Taxifolin 4

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  10
Acq. Instrument : HPLC-07                        Location :  25
Injection Date  : 7/14/2017 6:52:00 PM               Inj :  1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 9:53:52 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : [redacted]
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.414		-	-	-		Eriocitrin
7.783	BB	839.00482	8.39793e-5	7.04591e-2		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\010-25-Taxifolin 4.D
Sample Name: Taxifolin 4

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
Totals :				7.04591e-2		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\011-26-Taxifolin 5.D
Sample Name: Taxifolin 5

```
==========================================================================
Acq. Operator   : [redacted]                    Seq. Line :  11
Acq. Instrument : HPLC-07                       Location :   26
Injection Date  : 7/14/2017 7:46:34 PM             Inj :   1
                                               Inj Volume : 5.000 µl
Acq. Method     : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M
Last changed    : 7/14/2017 6:42:32 PM by [redacted]
Analysis Method : D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\LCK0023-7.M (
                  Sequence Method)
Last changed    : 7/17/2017 9:53:52 AM by [redacted]
Method Info     : Bioflavonoids

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Timothy Sit
ECM Path        : \Petaluma\LC\HPLC-07\Data\LCK0023-17-1360 2017-07-14 10-40-06.SC.SSIzip
ECM Version     : 8 (modified after loading)
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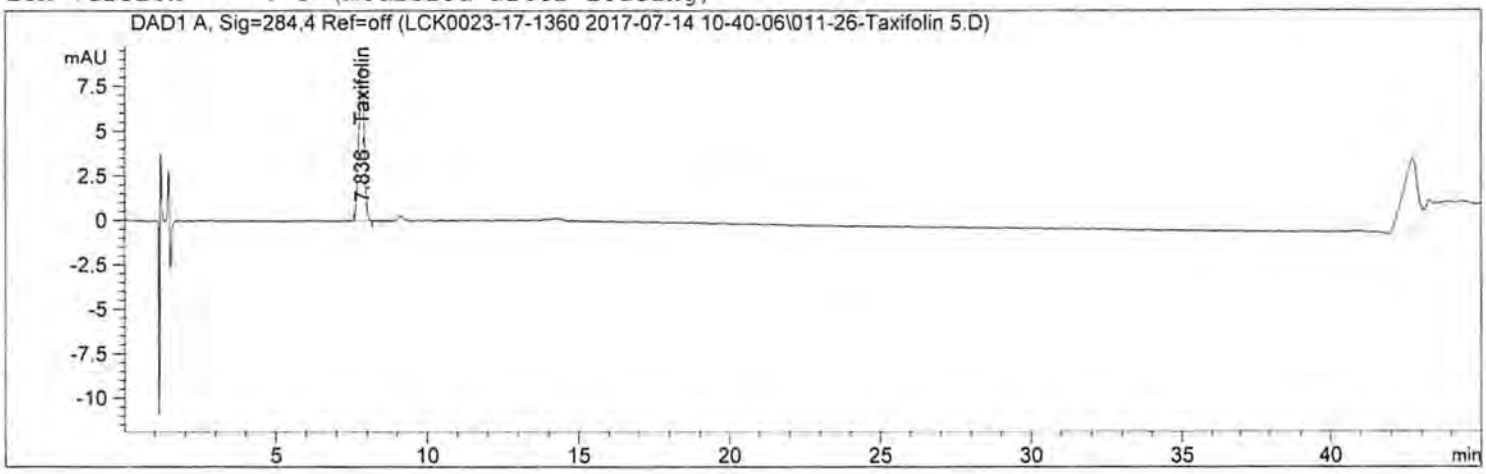



```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=284,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
6.414		-	-	-		Eriocitrin
7.836	BB	109.35014	5.36204e-5	5.86340e-3		Taxifolin
9.420		-	-	-		Rutin
11.667		-	-	-		Narirutin
14.472		-	-	-		Naringin
16.574		-	-	-		Hesperidin
18.801		-	-	-		Neohesperidin
25.952		-	-	-		Quercetin
29.084		-	-	-		Naringenin
31.605		-	-	-		Hesperitin

Totals : 5.86340e-3

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

LINEARITY

CALIBRATION TABLE

Calibration Table

General Calibration Setting

Calib. Data Modified : Monday, July 17, 2017 9:49:42 AM
Signals calculated separately : No

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 1.000 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Ignored
Weight : Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal Details

Signal 1: DAD1 A, Sig=284,4 Ref=off

Overview Table

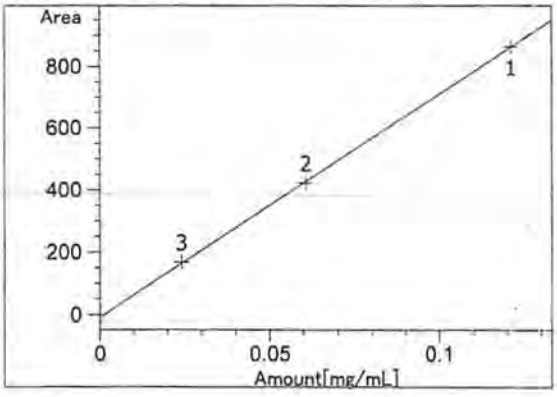
RT	Sig	Lvl	Amount [mg/mL]	Area	Rsp.Factor	Ref	ISTD	#	Compound
6.414	1	3	2.42183e-2	167.28256	1.44775e-4	No	No		Eriocitrin
		2	6.05457e-2	421.37521	1.43686e-4				
		1	1.21091e-1	863.27692	1.40270e-4				
7.836	1	5	9.28187e-3	109.35014	8.48821e-5	No	No		Taxifolin

RT	Sig	Lvl	Amount [mg/mL]	Area	Rsp.Factor	Ref	ISTD	#	Compound
		4	6.94750e-2	839.00482	8.28065e-5				
		3	1.38950e-1	1655.01001	8.39573e-5				
		2	2.08425e-1	2428.68335	8.58182e-5				
		1	2.77900e-1	3136.09863	8.86133e-5				
9.420	1	3	2.19478e-2	64.23846	3.41662e-4	No	No		Rutin
		2	5.48695e-2	162.65974	3.37327e-4				
		1	1.09739e-1	336.36642	3.26249e-4				
11.667	1	3	2.09837e-2	144.18347	1.45535e-4	No	No		Narirutin
		2	5.24592e-2	361.90015	1.44955e-4				
		1	1.04918e-1	744.35327	1.40953e-4				
14.472	1	3	2.23087e-2	153.40550	1.45423e-4	No	No		Naringin
		2	5.57716e-2	394.00327	1.41551e-4				
		1	1.11543e-1	808.25098	1.38006e-4				
16.574	1	3	1.55800e-2	104.24678	1.49453e-4	No	No		Hesperidin
		2	3.89500e-2	264.00577	1.47535e-4				
		1	7.79000e-2	542.63300	1.43559e-4				
18.801	1	3	2.42648e-2	180.12000	1.34715e-4	No	No		Neohesperidin
		2	6.06620e-2	453.77130	1.33684e-4				
		1	1.21324e-1	932.78711	1.30066e-4				
25.952	1	3	2.29516e-2	118.93430	1.92977e-4	No	No		Quercetin
		2	5.73790e-2	299.37958	1.91660e-4				
		1	1.14758e-1	616.32721	1.86197e-4				
29.084	1	3	2.77049e-2	369.43033	7.49935e-5	No	No		Naringenin
		2	6.92622e-2	926.69012	7.47415e-5				
		1	1.38524e-1	1904.35022	7.27410e-5				
31.605	1	3	2.68380e-2	353.78403	7.58598e-5	No	No		Hesperitin
		2	6.70950e-2	889.21442	7.54542e-5				
		1	1.34190e-1	1823.41907	7.35925e-5				

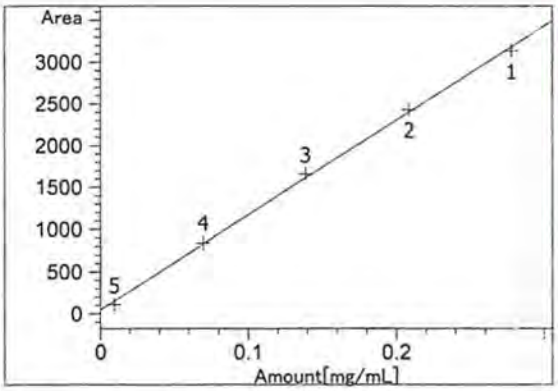
Peak Sum Table

No Entries in table

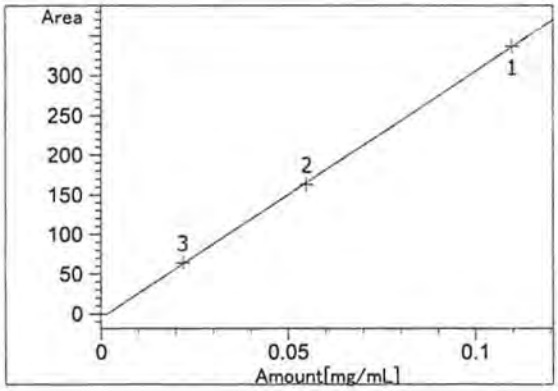
Calibration Curves



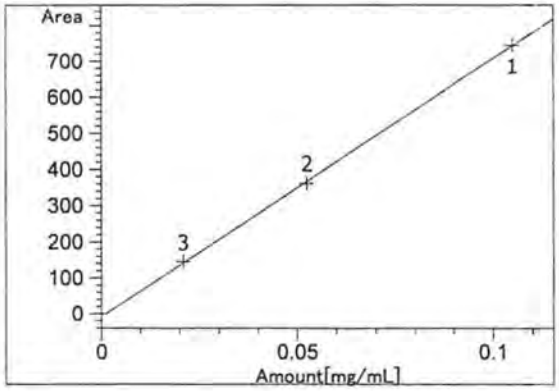
Eriocitrin at exp. RT: 6.414
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99994
Residual Std. Dev.: 5.58027
Formula: y = mx + b
m: 7196.22908
b: -9.81633
x: Amount[mg/mL]
y: Area



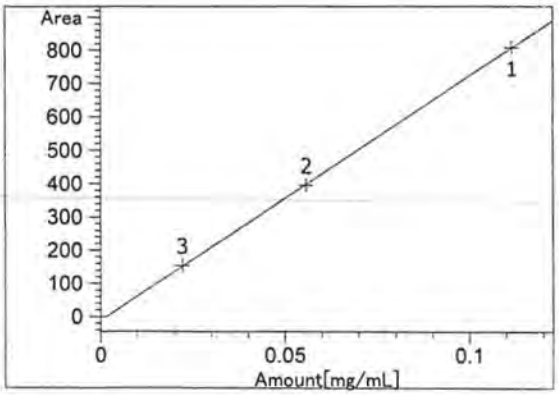
Taxifolin at exp. RT: 7.836
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99944
Residual Std. Dev.: 46.58433
Formula: y = mx + b
m: 11295.72044
b: 43.11883
x: Amount[mg/mL]
y: Area



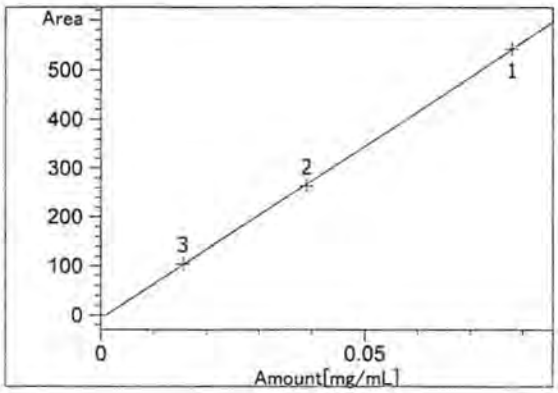
Rutin at exp. RT: 9.420
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99989
Residual Std. Dev.: 2.93082
Formula: y = mx + b
m: 3106.46104
b: -5.42185
x: Amount[mg/mL]
y: Area



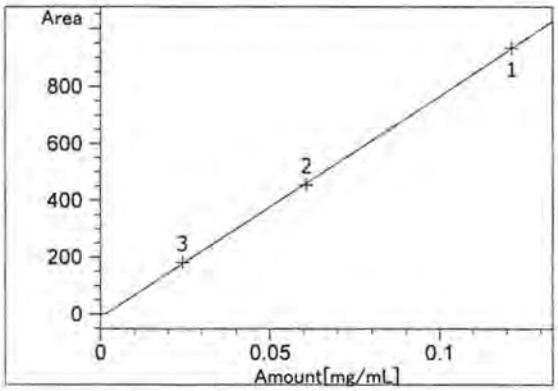
Narirutin at exp. RT: 11.667
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99990
Residual Std. Dev.: 5.93728
Formula: y = mx + b
m: 7164.72135
b: -9.15763
x: Amount[mg/mL]
y: Area



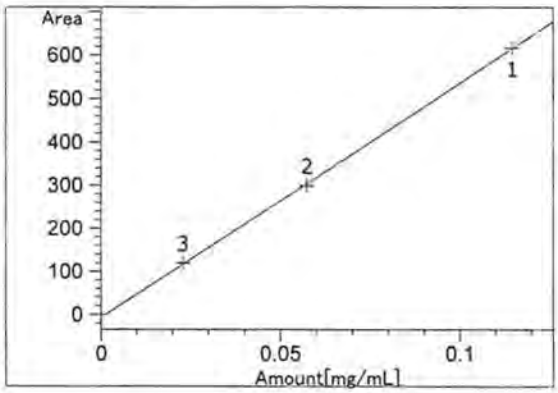
Naringin at exp. RT: 14.472
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99996
Residual Std. Dev.: 4.01579
Formula: y = mx + b
m: 7347.55905
b: -12.53698
x: Amount[mg/mL]
y: Area



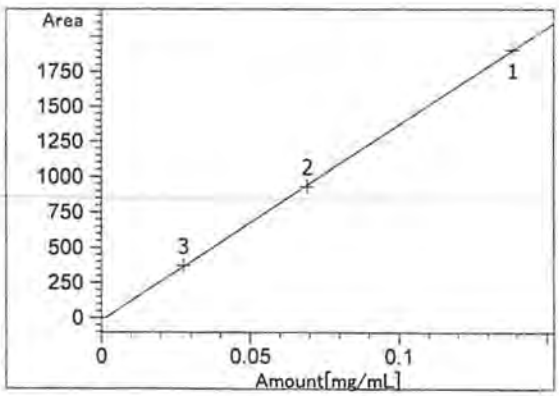
Hesperidin at exp. RT: 16.574
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99993
Residual Std. Dev.: 3.74633
Formula: y = mx + b
m: 7046.58356
b: -7.43117
x: Amount[mg/mL]
y: Area



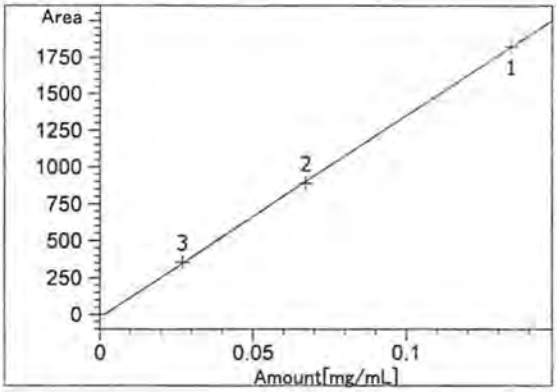
Neohesperidin at exp. RT: 18.801
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99992
Residual Std. Dev.: 6.94893
Formula: y = mx + b
m: 7769.18633
b: -11.90750
x: Amount[mg/mL]
y: Area



Quercetin at exp. RT: 25.952
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99990
Residual Std. Dev.: 4.91101
Formula: y = mx + b
m: 5428.65298
b: -8.14241
x: Amount[mg/mL]
y: Area



Naringenin at exp. RT: 29.084
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99991
Residual Std. Dev.: 14.81705
Formula: y = mx + b
m: 13877.63985
b: -22.53176
x: Amount[mg/mL]
y: Area



Hesperitin at exp. RT: 31.605
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99993
Residual Std. Dev.: 12.67358
Formula: y = mx + b
m: 13713.72205
b: -20.66597
x: Amount[mg/mL]
y: Area

==========

MOISTURE DETERMINATION

NOTEBOOK PAGE

TITLE Loss on Drying Start

Work continued from Page ____

PROJECT NO. eLIMS entry: 7/15/2017

BOOK NO. RT-005

Method: K0148

condition: 105°C | 2 hours

Start Time: 5:02pm

End Time: 7:05pm

oven: T007

Balance: BP211D#1

start Temp.: 105°C

End temp.: 105°C

RCT 7/15/2017

Sample ID	Empty Dish(g)	wet sample + Dish(g)	dry Sample + Dish(g)	% Moisture	Notes
17-0703-0039	94.7967	96.7556	96.6808	3.8185	—
17-0703-0040	87.0216	88.7567	88.6991	3.3197	—
17-0703-0041	93.5920	95.3157	95.2517	3.7129	—
17-0703-0042	91.8534	93.8424	93.7778	3.2479	—
17-0703-0043	88.2170	89.9927	89.9309	3.4803	—
17-0710-0044	90.1886	91.9518	91.9507	0.0624	—
17-0710-0046	84.1988	86.0259	86.0248	0.0602	—

RCT 7/15/2017

Work continued to Page End

SIGNATURE [redacted] **DATE** 7/15/2017

DISCLOSED TO AND UNDERSTOOD BY [redacted] **DATE** 7/15/17 **WITNESS** **DATE**

1) Procedure

This method is for the determination of dihydroquercetin (taxifolin) by high performance liquid chromatography (HPLC). This method has been verified for use on raw material (purities).

2) Definitions

N/A

3) Responsibility

Senior operations will implement this method. Only properly trained personnel may perform this method. The revision of, or any deviation from, this method requires written approval of supervisory personnel prior to initiation of work.

4) Safety

Follow all applicable safety, health, and environmental programs.

5) Environmental Conditions

N/A

6) Equipment

HPLC, Agilent 1100 HPLC or equivalent
Column, Agilent Zorbax SB-C18 Column, 4.6 X 150 mm, 3.5 micron or equivalent
Analytical balance, 0.00001 g resolution
Microbalance, 0.000001 g resolution
Sonicator
Serological pipets, various sizes
Class A pipettes, various sizes
Disposable glass pipets, various sizes
0.45 µm PTFE filter
Graduated cylinder, 1000-mL
Glass eluent bottles, 1000-mL
VOA vials, 20-mL and 40-mL sizes

Amber autosampler vials
Disposable syringes, 5-mL

7) Reference Materials/Reagents

Taxifolin (dihydroquercetin), Sigma #78666, CAS# 480-18-2
Methanol, HPLC grade
Phosphoric Acid (H_3PO_4), HPLC grade
Acetonitrile, HPLC grade
Milli-Q water, fresh daily

8) Quality Control Plan

1. A preparation solvent blank must be free of interfering peaks, and is analyzed every ten samples.
2. Linearity must be demonstrated by a 3-point calibration reference material or other means. Correlation coefficients of reference material curves must be greater than 0.999.
3. Response factors of reference material calibration levels must agree within 10% of the average of the response factors for the complete calibration curve.
4. Bracket each run with reference material injections, and include an additional reference material injection after every five sample injections.
5. Every tenth sample in a set must be prepared and analyzed in duplicate. If the set is fewer than ten samples, one sample in the set must be run in duplicate. The percent difference between duplicate results must be less than ten for finished products and less than two for purity samples.
6. If estimated levels or specifications have been provided, the sample area count must not fall more than 10% above the area counts of the reference material curve.
7. Beer's Law must be met.

9) Procedure

Mobile Phase Preparation:

0.2% phosphoric acid in Milli-Q water:

1. Using a graduated cylinder, measure 1000 mL of Milli-Q water and transfer to a fresh 1000-mL eluent bottle.
2. Transfer 2.0 mL of phosphoric acid, via a 1.0-mL serological pipet to the eluent bottle.
3. Swirl to mix and label appropriately.

Note: This solution may be stored at room temperature for up to two weeks.

Reference Material Preparation:

1. Using commercially available reference materials, on a microbalance, accurately weigh 1.0 ± 0.1 mg of taxifolin and transfer to a 20-mL VOA vial.
2. Dilute with 10.0 mL of methanol via a 10.0-mL class A volumetric pipet.
3. Sonicate for 15 ± 2 minutes to dissolve.
4. If warming during sonication has occurred, allow the solution to cool to room temperature.
5. Prepare the following two dilutions of this stock solution for use as calibration standards along with the stock solution to create a 3-point calibration curve:
    a. Using a class A pipet, transfer 2.5 mL into a 5-mL volumetric flask, fill to volume with

methanol, and invert to mix several times.

b. Using a class A pipet, transfer 1.0 mL into a 5-mL volumetric flask, fill to volume with methanol, and invert to mix several times.

6. Transfer the reference material solutions to separate amber autosampler vials and cap.

Note: Correct the reference material concentration using the following calculation:

$$[\text{reference material}_{mg/mL}]_{corrected} = \frac{[\text{reference material}_{mg/mL}] \times \% \text{ purity}}{100}$$

Sample Preparation:

1. Sample size should be based on client specifications or estimates and prepared according to the calibration reference material levels. Weigh an accurate amount into a 40-mL VOA vial.
2. Dilute with 40.0 mL of methanol via a 40.0-mL class A volumetric pipet.
3. Sonicate for 20 ± 2 minutes.
4. If warming during sonication has occurred, allow the solution to cool to room temperature.
5. Filter through a 0.45-µm PTFE filter into an amber autosampler vial, cap, and analyze.

Instrument Conditions:

Column Temperature: 35°C
Detection: UV 284 nm
Flow Rate: 1.25 mL/minute
Injection Volume: 5.0 µL
Gradient Program:

Time (min)	% H_3PO_4 (0.2% in Milli-Q Water)	% Acetonitrile	% Methanol
1.0	74.0	11.0	15.0
12.0	74.0	11.0	15.0
31.0	60.0	25.0	15.0
40.0	45.0	40.0	15.0
41.0	3.0	82.0	15.0
44.0	2.0	96.0	2.0
45.0	74.0	11.0	15.0

Run Time: 45.0 minutes
Post Time: 8.0 minutes
Retention Times: Taxifolin (Dihydroquercetin) ~7.7 minutes

10) Calculations

$$\%\ \text{dihydroquercetin (taxifolin)} = \frac{(\text{Area (sample)} - \text{Calibration intercept}) \times 100}{\text{Calibration slope} \times [\text{sample}]}$$

Where,
[ ] sample concentration is in mg/mL

Calibration curve settings:
Type: Linear
Origin: Ignore
Weight: Equal

11) References

1. *HPLC Determination of Bioflavonoids, LC-K0023.01, Effective 03/23/2012.*

End of document

Version history

Version	Approval	Revision information
1	09.AUG.2017	

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July 15, 2020

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
Division of Petition Review
5001 Campus Drive
College Park, MD 20740-3835

Attention: Dr. Stephen DiFranco
Re: GRN 916 –Dihydroquercetin –Response to Questions Posed in an Email Dated 6/30/2020

Dear Dr. DiFranco:

Per your request, GRAS Associates, LLC, acting as the agent for Blue California, is providing a response to complete FDA's request for additional clarification as denoted in your email dated June 30, 2020, as follows:

Administrative:

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Blue California and GRAS Associates, LLC certify that no data or information contained in GRN 916 are exempt from disclosure under FOIA. Furthermore, Blue California does not view the information contained on pages 100-103 of Appendix 3 as confidential.

Intended Uses:

1. *The intended uses for dihydroquercetin described in your notice includes use in "flavored fermented milk and dairy products" and for the purpose of determining probable dietary exposure, data on consumption of yogurt is used to represent this intended category. Please clarify if the intended use in "flavored fermented milk and dairy products" is limited to yogurt products or includes other types of foods.*

• • •

Blue California confirms that “flavored fermented milk and dairy products” is limited to yogurt products.

Specifications:

1. *We note that some [of] the methodologies listed in Table 1 of your notice are from USP and AOAC or refer to an instrument type (e.g., ICP-MS). Please provide a confirmatory statement that all methods used are validated and appropriate for the analytes tested.*

All methods used to analyze Blue California’s dihydroquercetin are validated and appropriate for the analytes tested.

Enzyme identity question[s]:

1. *Please provide the Enzyme Commission Number for the flavanone 3β-hydroxylase (F3H) used in the manufacture of dihydroquercetin.*

The Enzyme Commission Number for the F3H used in the manufacture of dihydroquercetin is EC 1.14.11.9.

2. *On page 7 of your notice, you state that the GRAS status of the* E. coli *K12 microorganism that produces the enzyme used in the manufacture of dihydroquercetin is established as GRAS under 21 CFR 170.36. We note that this is not an existing regulation, please clarify this statement with the correct citation.*

Blue California concurs that this is not an existing regulation with regard to *E. coli* K12. Furthermore, Blue California is not aware of an existing regulation under which *E. coli* K12 has been established as GRAS.

Enzyme Production questions:

1. *It is unclear how the wild type* E. coli *W3110 produced the F3H enzyme that is used in the manufacture of dihydroxyquercetin. You state in your notice that the f3h gene is present in higher order plants. On page 8 of the notice, you also state that the f3h gene is from an apple species. Please provide a narrative of the construction of the* E. coli *W3110 production strain that carries the f3h gene to produce the F3H enzyme.*

Blue California did not use wild type *E. coli* W3110, as it does not have the F3H gene. Blue California used our engineered W3110 strain to produce the F3H enzyme. The strain harbors

an apple F3H gene. The F3H gene ubiquitously exists in higher plants (not higher order plants) including apple.

Manufacturing:

1. *Please indicate whether the F3H enzyme is secreted extra- or intracellularly during the fermentation process.*

The engineered W3110 cells produce the F3H enzyme in the cells during the fermentation process. The enzyme is released from the cell by cell homogenization in the extraction step.

2. *Please provide a narrative to support the absence of potential allergens that maybe [sic] used in the fermentation medium during the production of the F3H enzyme, in the final dihydroxyquercetin product.*

LB culture seed media used for the production of the F3H enzyme contains tryptone (derived from casein), which is consumed by the microorganism. Subsequent steps of harvesting the cells by centrifugation, followed by homogenization, and additional centrifugation and purification of dihydroxyquercetin would likely result in the removal of any residual tryptone peptides. While tryptone is derived from a milk product, there is no evidence in the published literature of a food allergy associated with tryptone.

3. *Please indicate in Figure 3 of your notice where 2-oxoglutarate is utilized. We note in Appendix 2 of the notice, specifications and certificates of analysis are provided for materials used, however, this information is not provided for 2-oxoglutarate.*

2-Oxoglutarate is used together with eriodictyol, F3H enzyme, and reaction buffer for the bioconversion reacted. An updated manufacturing flow chart and representative certificate of analysis for 2-oxoglutarate (syn. 2-oxo-pentanedioic acid) is provided in Attachments A and B of this letter, respectively.

Toxicology:

1. *Is the test article. Used in the Schauss et al (2015) studies comparable to the subject of this notice? Please provide a clear explanation discussing the similarities and any differences present.*

As the test article studied in the Schauss et al. (2015) was prepared from Larch and Blue California's dihydroquercetin is a fermentation product, we expect that there will be some small differences in composition. The table below compares the composition of Lavitol as

described by Schauss et al. with Blue California's dihydroquercetin preparation. As shown, both materials are high purity preparations of dihydroquercetin.

Component	Lavitol[a]	Blue California's DHQ
DHQ	90.94-97.51%	95.2-97.8%
Aromadendrin (dihydrokaempferol)	1.6-3.6%	0.07%
Quercetin	0-0.7%	Not detected
Naringenin	0-0.2%	0.06%
Eriodictyol	0.1-1.1%	2.0%
Pinocembrin	0-0.17%	Not detected

[a] Based on results of batches tested in Schauss et al. (2015).

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Assuming linear extrapolation from the Grimm et al. (2016) paper, the maximum plasma level from the 90th percentile cumulative dihydroquercetin intake (i.e., 217.72 mg per d) is 5.5 μM. This concentration is lower than concentrations that appeared to cause toxicity (by visual interpretations of graphical results) to either cell type tested in the Matsuo et al. (2005) study (≥ 50 μM), concentrations that appeared to cause inhibition of human testosterone biosynthetic enzyme activities (by visual interpretations of graphical results) in the Ge et al. (2018) study (≥ 10 μM), and effects on drug metabolizing enzymes in the Jin et al. (2018) study (≥ 50 μM). It is important to note that all data in the Matsuo et al. (2006) study and data for concentrations other than 100 μM in the Ge et al. (2018) study were not analyzed statistically.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via email.

We look forward to your feedback.

Sincerely,

Katrina V. Emmel, Ph.D.
Senior Scientist/Project Manager/Associate
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Suite 500
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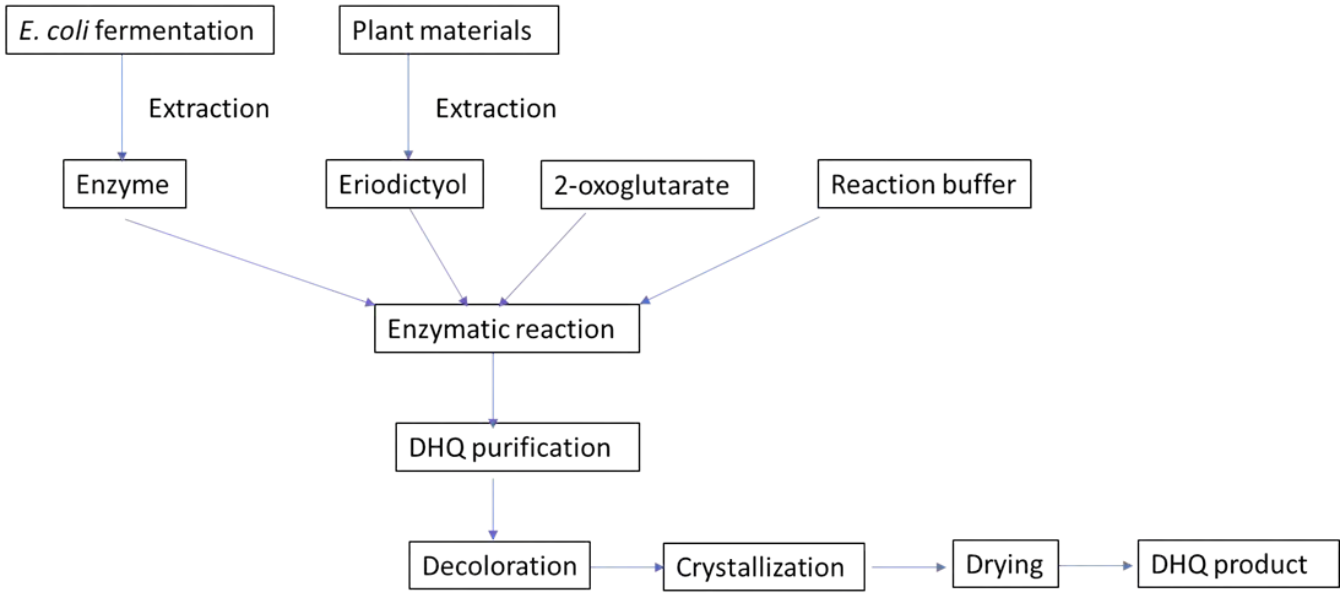
emmel@gras-associates.com

Attachment A **Manufacturing Flow Chart for Blue California's Dihydroquercetin**



Attachment B Certificate of Analysis for 2-Oxoglutarate

苏州亚科科技股份有限公司
地址：苏州工业园区金海路 17 号
邮编：215021
电话：0512-87182055 传真：0512-87182056
网址:http://www.yacoo.com.cn
电子邮件：sales@yacoo.com.cn

质量检验报告

CERTIFICATE OF ANALYSIS

货号（Product number）：T0007
中文名称(Chinese Name)：α-酮戊二酸
英文名称(English Name)：2-oxo-Pentanedioic acid
分子式(Molecular Formula)：$C_5H_6O_5$
分子量(Molecular Wt)：146.10
CAS：328-50-7
有效期：两年
Shelf life：two years

分析项目 SPECIFICATION PROPERTIES	技术指标 STANDARD	实测结果 RESULTS
外观 Appearance	白色至微黄色结晶性粉末 White to micro-yellow crystalline powder	符合 Conforms
含量(T) Assay,%	99.0–101.0	99.25
熔点 Melting point,℃	113.0-115.0	113.8-115.0
干燥失重 Loss on drying,%	≤0.5	0.36
重金属 Heavy Metals ,ppm	≤10	符合 Conforms

储存条件：2-8℃
Storage：2-8℃

生产日期(Product Date)：2020-05-29 检验日期(Test Date)：2020-07-02

生产批次(Batch No.)：YK2020052901 检验员(Checker)：009

END

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July 15, 2020

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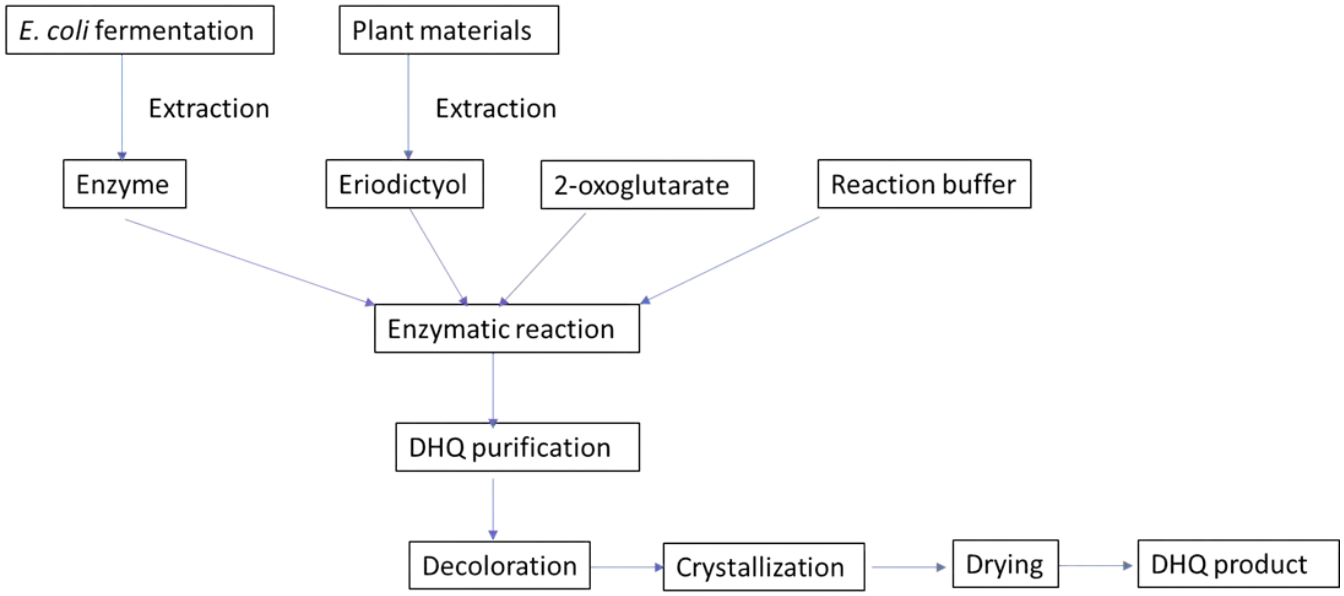

Attachment B Certificate of Analysis for 2-Oxoglutarate

苏州亚科科技股份有限公司
地址：苏州工业园区金海路 17 号
邮编：215021
电话：0512-87182055 传真：0512-87182056
网址:http://www.yacoo.com.cn
电子邮件：sales@yacoo.com.cn

质量检验报告

CERTIFICATE OF ANALYSIS

货号（Product number）：T0007
中文名称(Chinese Name)：α-酮戊二酸
英文名称(English Name)：2-oxo-Pentanedioic acid
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生产批次(Batch No.)：YK2020052901 检验员(Checker)：009

END