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November 5, 2018

#826

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5001 Campus Drive
College Park, MD 20740-3835

Attention: Dr. Paulette Gaynor
Re: GRAS Notification—Dihydroquercetin

Dear Dr. Gaynor:

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,

(b) (6)

William J. Rowe
President
Agent for Blue California
GRAS Associates, LLC
27499 Riverview Center Blvd., Suite 212
Bonita Springs, FL 34134
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
000826	
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)*: N/A

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)*	
27499 Riverview Center Blvd., Suite 212	

City	State or Province	Zip Code/Postal Code	Country
Bonita Springs	Florida	34134	United States of America

Telephone Number	Fax Number	E-Mail Address
239-444-1724	239-444-1723	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)*

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)*

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 *(name of notifier)* 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, 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)
(b) (6)	Katrina Emmel on behalf of William J. Rowe, President	11/05/2018

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 7	

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

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

11/5/18

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FIGURES

TABLES

FOREWORD

Blue California based its Generally Recognized as Safe (GRAS) assessment of dihydroquercetin primarily on the composite safety information, i.e., scientific procedures with corroboration from history of use. The safety/toxicity of dihydroquercetin, history of use of dihydroquercetin, 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 September 10, 2018, 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 ("GA") convened an Expert Panel to complete an independent safety evaluation of Blue California's dihydroquercetin preparation. The purpose of the evaluation is to ascertain whether Blue California's conclusion that the intended food uses of dihydroquercetin as described in Part 3 are generally recognized as safe, i.e., GRAS, under the intended conditions of use. 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% dihydroquercetin preparation, BC-DHQ™, is GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic (FD&C) Act. 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

(b) (6)

William J. Rowe
President
GRAS Associates, LLC
27499 Riverview Center Blvd.
Suite 212
Bonita Springs, FL 34134

Date: 11/5/18

[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 4/7/18).

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% dihydroquercetin 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.

D. Conditions of Intended Use in Food

Blue California's BC-DHQ™ preparation (≥ 95% dihydroquercetin) is intended to be added as an ingredient into 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% dihydroquercetin) has been concluded to be GRAS on the basis of 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% dihydroquercetin preparation. The preparation of this safety evaluation also included a comprehensive literature search through September 10, 2018.

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.

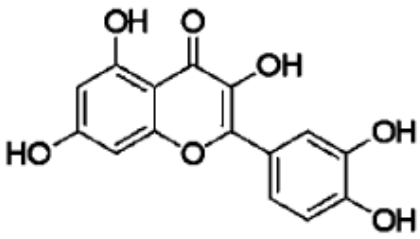
PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Chemical Identity of Ingredient

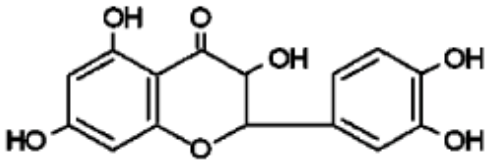
Flavonoids are a diverse chemical class of secondary metabolites universally found in the plant kingdom (Fowler and Koffas, 2009). Vegetables and fruits contain many flavonoids in the form of flavonols, flavones, and flavanones. It is estimated that the dietary intake of flavonoids ranges from 0.05 to 1 gram per person per day (Stevens et al., 1999). The total flavonol and flavone intake is 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 flavanonol that is structurally similar to quercetin. The chemical structures of quercetin and dihydroquercetin are provided in Figure 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; (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). Dihydroquercetin is currently marketed and sold as a pharmaceutical ingredient and as a "natural antioxidant additive" for food applications (Liu et al., 2014).

In certain foods, such as peanuts, white wine, and onions, dihydroquercetin is present as both an aglycone and a glycoside (Itaya and Igarashi, 1992; Oi et al., 2012; Singleton and Trousdale, 1983). Additionally, dihydroquercetin has also been identified as a component of bee pollen (Silva et al., 2009). A list of botanical species in which dihydroquercetin has been identified is provided in 0.

B. Manufacturing Processes

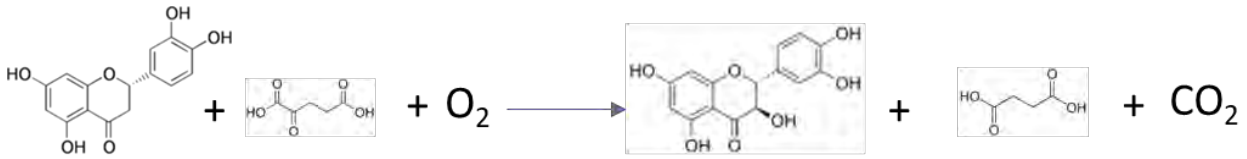
Blue California uses an enzymatic bioconversion reaction to produce BC-DHQ™ high purity dihydroquercetin from eriodictyol, a bitter-masking flavanone extracted from plant materials. Eriodictyol is converted to dihydroquercetin by flavanone 3β-hydroxylase (F3H), a ubiquitous enzyme found in higher order 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 dihydroquercetin, 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[2]. 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[3] 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

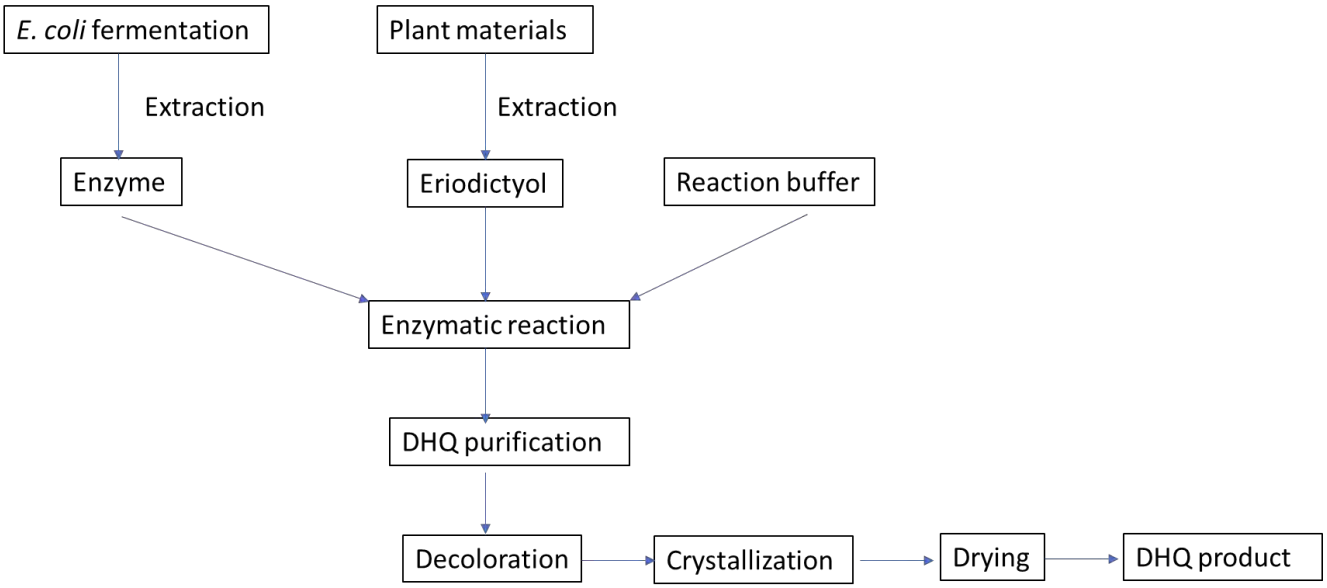
The enzymatic conversion mixture is centrifuged and the supernatant is transferred to an ion-exchange resin column. The column is washed with warm water and the dihydroquercetin is eluted with food grade ethanol. The eluent is condensed with a wipe-film evaporator, and the condensate is then transferred 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 dihydroquercetin preparation is dried in a baking oven and crushed into fine powder.

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

A manufacturing process flow chart for the production of the BC-DHQ™ high purity dihydroquercetin 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 dihydroquercetin; 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). Ametis JSC's specifications, compared with the specifications for Blue California's DHQ preparation, are shown in Table 1. These data demonstrate that Blue California's BC-DHQ™ is similar in composition to Ametis JSC's dihydroquercetin material.

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

Physical and Chemical Parameters	**Ametis JSC's** Dihydroquercetin Specifications[a]	**Blue California's BC-DHQ™**	
		Specification	Method
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

Blue California has adopted product specifications for its dihydroquercetin that are comparable to Ametis JSC's specifications, as reported to EFSA (Turck et al., 2017) for dihydroquercetin as a consumable human food substance. The compositions of five non-consecutive lots of Blue California's BC-DHQ™ preparation, as well as 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				
		(b) (6)				
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%

Physical and Chemical Parameters	Blue California BC-DHQ™ Specifications	Results of Batch Numbers				
		(b) (6)				
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

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

Blue California analyzes its high purity dihydroquercetin 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 dihydroquercetin 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 will be added to conventional foods and beverages as an ingredient as defined by 21 CFR 170.3(o)(20).

E. Stability

1. Published Stability on Dihydroquercetin

Ametis JSC submitted results of a stability study to EFSA on its dihydroquercetin material stored in dark glass containers over a time period of 3 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 1 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 the course of 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 6-month accelerated stability study on its BC-DHQ™ high purity dihydroquercetin 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 [redacted]				
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 [redacted]				
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 (b) (6)				
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 (b) (6)				
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 (b) (6)				
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 dihydroquercetin, 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 2-year shelf life for BC-DHQ™.

PART 3. DIETARY EXPOSURE

The subject dihydroquercetin 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% dihydroquercetin preparation (Turck et al., 2017). The intended food use categories and use levels for Blue California's BC-DHQ™ dihydroquercetin 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 9 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 Conventional Foods[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
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 dihydroquercetin 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 9 years)---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 dihydroquercetin 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	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 dihydroquercetin 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 combined dihydroquercetin intake from fortified foods (46 mg) and food supplements (100 mg) was estimated to be 146 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 estimates of daily dietary intakes of DHQ by the US population from the background diet. However, a non-exhaustive literature search indicates that DHQ is naturally-occurring in many foods common to the human diet, as shown in Table 8.

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	--	Turck (2017)
Olive oil	129.4 mg/kg	Carrasco Pancorbo et al. (2004)
Sorghum grain	--	Gujer et al. (1986)
White grapes	--	Masa et al. (2007)
Strawberries	--	Sun et al. (2014a)
Mulberries	21 μg/g (fresh weight)	Zhang et al. (2008)
Açaí	--	Gallori et al. (2004)
Peanuts	--	Pratt and Miller (1984)
Pine seeds	172 mg/100 g	Lantto et al. (2009)
Thyme	41.96-93.73 μg/g (essential oil)	Varga et al. (2015)
Citrus fruits	--	Kawaii et al. (1999)
White wine	--	Pozo-Bayón et al. (2003)
Beer	1 mg/L	Gerhäuser (2005)
Walnut	--	Zhao et al. (2017)
Mexican oregano	--	Lin et al. (2007)
Prickly pear	--	Dok-Go et al. (2003)
Fenugreek seeds	--	Yu et al. (2017)
Almond skin	9.0 μg/g	Fallico et al. (2011)

[a] Including dihydroquercetin derivatives such as taxifolin deoxyhexose found in açaí

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 daily consumption of various food types (FDA, 2006). FDA methodology is recognized as a method that overestimates consumption. Estimated Daily Intakes (EDIs) for these proposed conventional food categories, with respect to the intended use levels, are provided in Table 9.

Table 9. Conventional Foods Dietary Intake Estimations for Dihydroquercetin

Food Category	Maximum Use Level of DHQ (g/serving)	USDA Mean Grams of Food Consumed (All Individuals)[a]	RACC serving size (g)[b,c]	Mean mg DHQ Consumed (All Individuals)	Mean x 2 mg DHQ Consumed (All Individuals)	Reference Number, Page Number
Non-alcoholic beverages	0.0072	821	360	16.42	32.84	(2) table 9.7, page 32
Fermented milk and dairy products[d]	0.0034	8	170	0.16	0.32	(2) table 9.4, page 29
Candy containing chocolate	0.0021	4	30	0.28	0.56	(1) Appendix B, page 244
Total				16.86	33.72	

[a] Mean grams food consumed for all individuals taken from Reference 2 or calculated from Reference 1
[b] 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)
[c] For liquids, assume 1 mL = 1 g.
[d] Determined using yogurt USDA mean grams of food consumed and RACC serving size.

Reference List

[1] Foods Commonly Eaten in the United States Quantities Consumed Per Eating Occasion and in a Day, 1994-96 Helen Smiciklas-Wright, Diane C. Mitchell, Sharon J. Mickle, Annetta J. Cook, Joseph D. Goldman. Available at: http://www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/Portion.pdf (Accessed 8/25/18)
[2] DATA TABLES: Results from the USDA's 1994-96 Continuing Survey of Food Intakes by Individuals and 1994-96 Diet and Health Knowledge Survey Table Set 10 Food Surveys Research Group, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, 10300 Baltimore Ave., Bldg. 005, Rm 102, BARC-West, Beltsville, Maryland 20705-2350. Available at: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/Csfii3yr.pdf (Accessed 8/25/18)

The estimated daily mean intake of dihydroquercetin for the US population is slightly greater than the estimated daily mean intake for the European population (see Table 5); however, the highly conservative 'mean X 2' estimated daily intake of DHQ for the US population is less than the 97.5th percentile estimated daily intake for the European population (33.72 mg per day and 58.0 mg per day, respectively).

It should be noted that the EFSA Panel considered the total daily intake of DHQ from both conventional foods (at 58.0 mg per day for adults and 46.2 mg per day for adolescents) in addition to 100 mg DHQ per day from dietary supplements in its review, resulting in an estimated combined DHQ intake of 158 mg per day for adults and 146.2 mg per day for adolescents. No concerns about these dietary intake levels were raised, which supports the safety of the proposed use levels of BC-DHQ™ (Table 4) with a resulting estimated daily intake of 33.72 mg per day.

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 dihydroquercetin.

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 uses of dihydroquercetin prior to January 1, 1958.

Dihydroquercetin is naturally occurring in a number of foods that are part of the American diet, including apples, red onions, tomatoes, olive oil, sorghum, white grapes, strawberries, mulberries, açai, peanuts, pine nuts, 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 dosages 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 Notification (GRN) database 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 dihydroquercetin 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" resulted in no results relevant to food additive regulations.

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% dihydroquercetin) included non-alcoholic 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 9 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. Asian Regulatory History

A search of the websites of Japan's Ministry of Health, Labour and Welfare, FDA Philippines, the Agri-Food and Veterinary Authority of Singapore, and FDA Taiwan using the terms "taxifolin" and "dihydroquercetin" resulted in no results for use as a food additive.

6. Other Regulatory History

A search of the Food Standards Australia New Zealand (FSANZ) website using the terms "taxifolin" and "dihydroquercetin" resulted in no regulatory results for use as a food additive.

It has been reported that as of April 2009, over 250 dihydroquercetin-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 September 2018, using the terms "dihydroquercetin," "biological activity and dihydroquercetin," and "safety and dihydroquercetin," references were scanned for relevant biological effects and safety data on DHQ. Many of the studies found referred to DHQ by 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 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

Weidmann (2012) conducted a review to evaluate the therapeutic promise of DHQ in major disease states including cancer, cardiovascular disease, and liver disease based on the proposed mechanisms of action. Both in vitro and in vivo studies were reviewed and the authors concluded that DHQ works on many molecular targets that have beneficial effects in some disease conditions but the same effects may be detrimental in other disease conditions, specifically hypoxia mediated cardiovascular disease. This was the only comment made specifically regarding safety in this review.

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, 2005). Taxifolin was found to be slightly toxic to TIG-1 cells and to HUVE cells at levels up to 200 µM following incubation at 37°C for 24 hours.

To evaluate the phototoxic potential of taxifolin, a 3T3 Neutral Red Uptake Phototoxicity Test was conducted according to 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.

Blue California determined that the conclusions in the Weidmann (2012) review and the other reported studies do not raise any concerns about the safety of DHQ.

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 oral 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 7 consecutive days with 10,000 or 15,000 mg per kg bw of Lavitol® (90.94% DHQ) via oral 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 day 0 and 8. Animals were euthanized on day 8 and underwent a full necropsy with organ weights. There was no difference between test groups and control animals with respect to 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 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 was evaluated following oral dosing at 20 mg per kg bw per day for 6 weeks (Plotnikov, 2017a). No adverse effects related to the DHQ exposure were reported in the SHR rats. In another study, which evaluated the changes in angiotensin-converting enzyme activity in the aorta of male normotensive Wistar-Kyoto and SHR rats, animals were dosed by daily gavage with DHQ at 100 or 300 µg per kg for two weeks beginning at postnatal weeks 10 to 12 or 12 to 14 (Slashcheva, 2016). Blood pressure and the activity of angiotensin-converting enzyme in the aorta of SHR rats normally increases with age; however, dosing with DHQ had no effect on the increase of these parameters. DHQ administration to 14-week-old normotensive Wistar-Kyoto rats at 300 µg per kg was associated with reduced activity of the angiotensin-converting enzyme. No adverse effects were reported relating to DHQ exposure.

3. Subchronic Toxicity Studies

A 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 oral 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 DHQ 92.20%, aromadendrin 2.35%, eriodictyol 0.53%, quercetin 0.26%, naringenin 0.17%, and pinocembrin 0.11%. All of the animals in the study exhibited comparable weight gain throughout out the dosing period. The quantity of food and water consumed by the animals in all other groups was not significantly different compared with 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 group 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 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.

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 used in this study and exposed to 0, 50, 150, or 1,500 mg per kg bw per day by oral gavage for 90 days and included a recovery group, which were observed for 28 days following the treatment period (n = 5 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 groups 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. Gross pathological changes were noted in the stomach and adrenal glands in one or both sexes in multiple dose groups including the controls. These effects were not determined to be test article related, but were thought to be associated with the aggressive behavior seen during the study and were assumed to have led to stress and local irritation in the stomach. The EFSA panel concluded that the NOAEL was 1,500 mg per kg bw per day.

In a study to evaluate the process of peroxidation in male outbred albino rats following the administration of DHQ for 3 months, the diets of rats were supplemented daily with a DHQ dose of either 86, 860, or 3,000 mg per kg bw (Chernyak, 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. At 86 mg per kg bw, DHQ was as potent as rutin in modulating the process of peroxidation. Safety endpoints were not included in this study; 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 was evaluated (Plotnikov, 2017b). Animals were dosed with 50 mg per kg bw DHQ in 1% starch gel via oral gavage for 6 weeks. Concurrent controls, both SHR and normotensive Wistar Kyoto rats, received 1% starch gel only. An improvement in the microcirculation was noted and no adverse effects related to exposure to DHQ were reported.

4. Chronic Toxicity Studies

Booth and Deeds (1958) investigated the chronic oral toxicity of DHQ in albino rats. Ten 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. At the end of 226 days, 50 percent of the animals receiving 0.5% and 1.0% of the test material were terminated along with an equal number of animals that were fed the basal diet. The remaining animals in these dose groups were terminated 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 sacrificed along with an equal number of controls. The remaining animals in these dose groups were sacrificed after 650 days. Rats underwent a weekly clinical exam and were weighed weekly as well. During the course of 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, with the exception of 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 6-month chronic toxicity study in 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. In both studies, results did not show any evidence of DHQ toxicity.

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 a 13-day period 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. Females 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 oral gavage based on the most recent body weight taken on day 1, 8, 14, 17, 18, 19, 20, and 21 of gestation. The phytochemical composition of Lavitol® used in this study was DHQ 92.19%, aromadendrin 3.57%, eriodictyol 0.58%, quercetin 0.33%, naringenin 0.17%, and pinocembrin 0.17%. 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 prior to 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, 5 pregnant females were sacrificed on day 18 and another five on day 20 of gestation. The remaining pregnant females per 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 3 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® had no effect on 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 oral 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, 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.

Blue California concludes that the results of the significant number of acute, subacute, subchronic, chronic and reproductive/developmental toxicity studies reviewed do not raise any concerns about the safety of DHQ. 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 DHQ 97.51%, aromadendrin 1.55%, eriodictyol 0.1%, and quercetin 0.15%. 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; 5 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.

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 terminated 3 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 terminated 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 DHQ 97.5%, aromadendrin 1.55%, eriodictyol 0.10%, and quercetin 0.15%. 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 2 single doses (15 and 2,000 mg per kg bw) compared to a single dose-treated cyclophosphamide control and negative control groups (Schauss et al., 2015*).* The 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 in another experiment, mice were dosed orally with 15 mg per kg bw for 5 consecutive days. Prior to 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 5 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 5 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

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

a. Clinical Studies on Dihydroquercetin

Rohdewald (2018) completed a review on the effects of Pycnogenol® in mild stage 1 and 2 osteoarthritis (OA). Pycnogenol® contains multiple polyphenols and other components, and it mimics a sustained release formulation by its natural combination of short and long-acting anti-inflammatory substances. Based on other published reports, Pycnogenol® contains 14.35 μg taxifolin per mg. The maximum plasma concentration of taxifolin following Pycnogenol® intake is at 8 hours, and it is still present at 14 hours, while other components have peak concentrations at 1 to 4 hours. Taxifolin could also be found in the synovial fluid taken from the OA patients suggesting a local anti-inflammatory action. This review identified three identically designed clinical trials that investigated the role of Pycnogenol® in OA treatment. All three studies were randomized, double-blind, and placebo-controlled and study participants were middle aged (48-54 years old) patients suffering from mild stage 1 or 2 OA. The patients were all treated three times daily with total doses of either 100 or 150 mg per day Pycnogenol® added to their existing nonsteroidal anti-inflammatory drugs (NSAID) therapy. The success of the add-on supplementation was objectivated by using the Western Ontario McMasters University (WOMAC) questionnaire for OA for a period of 3 months. In all 3 Pycnogenol® groups, the use of NSAIDs was significantly reduced. In one study, the evaluation of the NSAID use was more precisely evaluated. This study reported that the consumption of Pycnogenol® was associated with a reduction of the intake of NSAIDs by 58%. No unwanted side effects linked to Pycnogenol® intake were reported in any of the three studies. The authors concluded that Pycnogenol® acts as an anti-inflammatory and chondroprotective add-on supplement providing long-lasting positive effects in OA patients.

A two-week study investigated the effect of different treatments, including diquertin (not less than 90% dihydroquercetin), on patients suffering from acute pneumonia and hospitalized within the first 3 days of the disease (Kolhir et al., 1998). The 112 patients were divided into 3 groups: group 1 (n=50) received standard therapy, group 2 (n=32) received standard therapy plus α-tocopherol acetate and sodium thiosulphate; and group 3 (n=30) received the standard therapy plus 40-60 mg of diquertin per day. Patients in group 3 showed a more rapid disappearance of

physical signs of disease as compared to the control group, more rapid lung tissue restoration, decreased pulmonary fibrosis, and a tendency towards improved indicators of ventilatory function of the lung as well as increased levels of endogenous antioxidants. No safety endpoints were discussed, and no side effects were observed.

Twenty-nine patients, aged 56-78 years, with discirculatory encephalopathy were treated with 80 mg taxifolin per day over the course of 18-21 days. Patients displayed significant improvement in psychoemotional conditions. No safety endpoints were discussed, and no side effects were observed (Zavolokov and Ilyuhina, 2001; Ametis, 2010).

In single and multi-dose studies on Pycnogenol®, a maritime pine bark extract containing 14.35 µg taxifolin per mg, in human volunteers discussed earlier, no safety endpoints were discussed and no adverse effects were reported (Grimm et al., 2006).

In a randomized, double-blind, placebo-controlled study, the effects of taxifolin supplementation were studied on 68 hypertensive patients, aged 50-70 years, who suffered from atherosclerosis. The effects on hemodynamic and biochemical parameters, endothelial function and neurological status were evaluated. Subjects were treated with 80 mg taxifolin per day, along with standard therapy over the course of 12 weeks. Positive effects on lipid metabolism and cerebral microcirculation were observed. No safety endpoints were discussed, and no side effects were observed (Britov and Aparina, 2006; Ametis, 2010).

Twenty-eight patients, aged 50-76 years, with chronic microcirculatory disturbances were treated with 75 mg taxifolin per day over the course of 3 months. Treatment resulted in improved blood microcirculation and rheological indices and strengthened capillary walls. No safety endpoints were discussed, and no side effects were observed (Kozlov et al., 2006; Ametis, 2010).

Koshkin and Nastavsheva (2008) studied the effect of taxifolin on forty patients, aged 39-75 years, suffering from atherosclerosis of the lower extremities. Patients received 60 mg taxifolin per day (Capilar), over the course of 2 months, in the form of oral, or oral and topical, doses. Improved microcirculation, decrease in ischemic pain, and the ability of patients to walk longer distances without pain were observed. No safety endpoints were discussed, and there were no side effects (Koshkin and Nastavsheva, 2008; Ametis, 2010).

The effects of taxifolin supplementation (Capilar) were studied on 20 patients, aged 32-68 years, suffering from post-operative ischemic heart disease after aorta-coronary shunting. Subjects were treated with 60 mg taxifolin per day for 12-17 days. A control group (n=10) was included and received basic rehabilitation only. Treatment resulted in significant improvement in microcirculation, blood oxygenation, and psychoemotional conditions. No safety endpoints were discussed and no side effects were observed (Shakula et al., 2007; Ametis, 2010).

A 12-week study on taxifolin treatment in 40 patients with diabetes mellitus (aged 56.2 ± 8.5 years) was conducted by Nedosugova (2006). Patients received 120 mg taxifolin per day in

addition to basic therapy and the control group (n=20) received a placebo plus basic therapy. A significant decrease in HbA1x levels and an improved sensitivity to insulin were observed. No safety endpoints were discussed, and no side effects were observed (Nedosugova, 2006; Ametis, 2010).

A 12-week study was conducted on taxifolin treatment in 20 patients (aged 30-68 years) with diabetes-related onychomycosis of feet and hands. Patients received 120 mg taxifolin per day in addition to basic therapy. The level and value of oligopeptides indicated significantly decreased malondialdehyde (MDA) levels and coefficient of intoxication. No safety endpoints were discussed, and there were no side effects reported (Davudova and Zoloeva, 2009; Ametis, 2010).

The effect of taxifolin supplementation on male patients with chronic pulmonary obstructive disease (n=20, aged 30-65 years) was investigated in an 18-21 day study. Patients were treated with 80 mg taxifolin (Capilar) per day. Increased blood oxygenation, improved rheological blood parameters, and normalized indices of tissue and organ microcirculation were observed, as well as a reported increase in tolerance to physical exercise and improved functioning of the respiratory and cardiovascular systems. No safety endpoints were discussed, and no side effects were observed (Shakula et al., 2008; Ametis, 2010).

b. Clinical Studies on Ascovertin and Laviocard

Ascovertin is a complex of DHQ and ascorbic acid, 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.[4] Laviocard is a very similar preparation containing both taxifolin (30 mg) and ascorbic acid (70 mg).

Twenty patients with ischemic heart disease were treated with Ascovertin (1 tablet, 3 times per day; 20 mg taxifolin and 50 mg ascorbic acid per tablet) over the course of 3 months. A control group of 20 patients receiving basic therapy and a placebo was included in the study. Ametis reported significantly decreased anginal episodes per week, and positive effects on the hemorheological status were observed. Safety endpoints and side effects were not discussed (Tyukavkina et al., 2001; Ametis, 2010).

Two clinical studies were conducted by The Russian Academy of Medical Science. In the first study, 31 patients with arteriosclerosis and stage I-II discirculatory encephalopathy and 20 healthy volunteers were given 2 tablets of Ascovertin (40 mg dihydroquercetin and 100 mg ascorbic acid per 2 tablets) three times per day in week 1 and then 1 tablet (20 mg dihydroquercetin and 50 mg ascorbic acid per tablet) three times daily during weeks 2 and 3 (Plotnikov et al., 2004a). The authors concluded that treatment with Ascovertin improved attention, memory, mental performance, normalized sleep, relieved headaches and decreased

[4] 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.

fatigability in patients with discirculatory encephalopathy. In the second study by Plotnikov et al. (2004b), 21 patients with stage I or II vascular encephalopathy with atherosclerosis, aged 45-65 years and a control group of 10 age-matched patients, were given Ascovertin for 21 days (abstract only). Patients experienced a decrease in blood viscosity and blood-clotting tendency, which improved attention, relieved vertigo and headaches, and normalized sleep compared to age-matched controls. The authors did not observe any adverse effects.

Subsequently, Plotnikov et al. conducted additional 3-week studies in patients suffering from cerebral atherosclerosis (n=21, median age 60.4 ± 4.8 years), arterial hypertension (n=38, median age 60 ± 5 years), and type 2 diabetes mellitus (n=19, median age 56 ± 4 years). The dosing schedule was the same as reported in the studies above. In all studies, a significant decrease in blood viscosity was observed, along with an increase in time of erythrocyte aggregation and significant decrease in quantity of primary and secondary products of lipid peroxidation. Patients with cerebral atherosclerosis displayed improved short-term memory and ability to concentrate. Patients with arterial hypertension and type 2 diabetes had decreased blood pressure and an increase in the systolic index. No safety endpoints were discussed, and no side effects were observed in any of the studies (Plotnikov et al., 2005; Ametis, 2010).

In a three-month clinical study, Plotnikov et al. also studied the effect of Ascovertin treatment on 31 patients (aged <65 years) with ischemic heart disease. Dosing was in the form of tablets that contained 20 mg DHQ and 50 mg ascorbic acid. During the first week of the study, patients received 1 tablet four times per day, for total doses of 80 mg DHQ and 200 mg ascorbic acid. For the second and third months, patients received 1 tablet three times per day, for a total of 60 mg DHQ and 150 mg ascorbic acid. Two control groups were included in the study; one with 20 patients (10 with ischemic heart disease; 10 with a history of myocardial infarction) who received basic therapy only and another group of healthy volunteers (n=10). Patients displayed an increase in the deformability index of the erythrocytes, a decreased level of fibrinogen, episodes of stenocardia, and level of primary and secondary products of lipid peroxidation. In addition, there was a decrease in nitroglycerine administration and tolerability to physical exercise. No safety endpoints were discussed, and no side effects were observed (Plotnikov et al., 2005; Ametis, 2010).

In a two-week study, Plotnikov et al. (2005) investigated the effect of taxifolin and ascorbic acid treatment on 25 female patients, aged 20-34, who were scheduled to undergo ovarian surgery. Patients received 120 mg taxifolin and 300 mg ascorbic acid per day for four days prior to, and 10 days following, surgery. A control group of 23 patients underwent basic therapy. Patients displayed decreased MDA levels, while levels of catalase and superoxide dismutase (SOD) were significantly increased. No safety endpoints were discussed, and no side effects were reported (Plotnikov et al., 2005; Ametis, 2010).

In a similar study, Tikhonov (2008) studied the effect of Laviocard on patients with chronic venous insufficiency (n=15) or atherosclerosis (n=15). A control group of 30 patients receiving only the basic therapy were included in the study. Patients received 1 tablet per day, providing 30

mg taxifolin and 70 mg ascorbic acid, for 30 days as well as basic therapy. Positive changes in hemodynamic indices, rheological blood parameters and normalization of cholesterol metabolism was seen in the group receiving taxifolin. No safety endpoints were discussed and no side effects were observed (Tikhonov, 2008; Ametis, 2010).

Blue California has reviewed these clinical studies, which show that DHQ is well tolerated in humans, even those with various diseases, and agree 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 dihydroquercetin in humans. Two volunteers were given 2 grams of dihydroquercetin 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 dihydroquercetin, 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 2-3 hours. The recovery of taxifolin in the urine ranged from 7-8% (Düweler and Rohdewald, 2000).

In a 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® for three weeks and the other receiving no treatment (Mulek, 2017). The authors concluded that the results of this study provided the first evidence that polyphenols are distributed in the synovial fluid of patients with osteoarthritis.

Single and multi-dose studies on Pycnogenol®, a maritime pine bark extract containing 14.35 µg taxifolin per mg, 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 2 hours post-dosing, and maximum concentrations were observed after 8 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.

In a subsequent multiple-dose study, five volunteers (4 female, 1 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 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 has the ability to degrade taxifolin to 3,4-dihydroxyphenylacetic acid and phloroglucan; however, neither of these metabolites were observed in the plasma samples.

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 5 hours, while alphitonin was not detected. The authors concluded that *C. orbiscindens* may 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, 2016). Taxifolin was prepared in 0.5% analytical-grade sodium carboxymethyl cellulose (CMC-Na) solution and one group was dosed with vehicle and the other with 200 mg per kg body weight of taxifolin, once daily for 3 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 2 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 intravenously 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 (Vosoboinikova, 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. Biological Activity of Dihydroquercetin

Dihydroquercetin has been used in used as an ingredient in dietary supplements in Russia, Switzerland, Canada, and the US (Turck et al., 2017) as a home remedy for conditions related to oxidative stress as it is a known antioxidant. However, scientific studies have shown that dihydroquercetin may provide some benefit in antioxidative, chemoprotective, hepatoprotective, and anti-inflammatory activities. As the biological action or mechanisms of action of an ingredient may reveal potential safety related concerns, a summary of the studies on biological activity of dihydroquercetin is presented in Appendix 6. It should be noted that no adverse effects related to dihydroquercetin were reported in any of the studies.

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, which is used as a dietary ingredient in Russia.

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."[5]

[5] 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).

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." [6]

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:[7]

- 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 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).

[6] 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).
[7] See Footnote 1.

As noted below, this safety assessment to ascertain GRAS status for dihydroquercetin 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 7.

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 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 to conclude 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 levels proposed were 100 mg per day for use as an ingredient in non-alcoholic beverages, fermented milk and dairy products, and chocolate.

Blue California's proposed levels of use 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 levels of use proposed by Blue California result in estimated exposures of 46.2 mg (97.5th percentile) for adolescents from 10 to 17 years of age and 58.0 mg (97.5th percentile) for adults, which are much lower than those reviewed by EFSA.

The relevant animal toxicity studies, ADME studies, 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, as well as the report from the designated Expert Panel provided in Appendix 7, Blue California concludes that the DHQ preparation defined in this comprehensive 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. List of Acronyms

4-HNE	4-hydoxynonenal
ADME	Absorption, Distribution, Metabolism and Excretion
AOAC	Association of Official Agricultural Chemists
Apo-A1	apolipoprotein A-1
ApoB	apolipoprotein B
ARPE-19	human RPE cells
bw	body weight
CCl_4	tetrachloromethane
CFR	Code of Federal Regulations
CFU or cfu	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
C_{max}	maximum serum concentration
CMC-Na	sodium carboxymethyl cellulose
DDT	dichlorodiphenyltrichloroethane
DHQ	Dihydroquercetin
DN	Diabetic neuropathy
DOPA	3,4-dihydroxyphenylalanine
DPPH	2,2-diphenyl-1-picrylhydrazyl
DU145	human prostate cancer cells
EC_{50}	half maximal effective concentration
EDI	Estimated daily intake
EFSA	European Food Safety Authority
EGFR	EGF receptor
ER	Estrogen receptor

F3H	flavanone 3β-hydroxylase
FDA	Food and Drug Administration
FD&C	Federal Food, Drug, and Cosmetic Act
FOIA	Freedom of Information Act
FRAP	Ferric reducing antioxidant property
FSANZ	Food Safety Authority Australia/New Zealand
g	gram
GA	GRAS Associates
GLP	Good Laboratory Practice
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
GSH-Px	glutathione peroxidase
GSSG-R	glutathione reductase
HaCat	immortalized human keratinocytes
HeLa	Human cervical cancer cells
HepG2	human liver cells
HFD	high fat diet
HPLC	High Performance Liquid Chromatography
HUVE	Human umbilical vein endothelial cells
IC_{50}	half maximal inhibitory concentration
ICP-MS	Inductively coupled plasma mass spectrometry
IgM	immunoglobulin M
JEFCA	The Joint FAO/WHO Expert Committee on Food Additives
kg	kilogram
L	Liter
LLC	Limited Liability Corporation

MCAO	Middle cerebral arterial occlusion
MDA	malondialdehyde
mg	milligram
mL	milliliter
mM	millimolar
mMol	millimole
n	number
ng	nanogram
NA	Not applicable
NADPH	nicotinamide adenine dinucleotide phosphate
ND	Not detected
NDA	The EFSA Panel of Dietetic Products, Nutrition and Allergies
NGS	NADPH generating system
NOAEL	No Observed Adverse Effect Level
NS	Not specified
NSAID	Nonsteroidal anti-inflammatory drug
OA	Osteoarthritis
OECD	Organisation for Economic Co-operation and Development
OH	Hydroxyl
OVCAR-3	human ovarian cancer cells
Ph.D.	Doctor of Philosophy
PI3K	phosphoinositide 3-kinase
ppm	parts per million
RACC	Reference Amounts Customarily Consumed
RBC	red blood cell
SHR	spontaneously hypertensive

SOD	Superoxide dismutase
$T_{1/2}$	Half-life
TBARS	Thiobarbituric acid reactive substance
TIG-1	Human lung embryonic fibroblasts
T_{max}	Time to maximum plasma concentration
μg	microgram
US or U.S.	United States
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
VEGF	vascular endothelial growth factor
v/v	volume per volume
WOMAC	Western Ontario McMasters University

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C. Appendices

Appendix 1 Botanical Sources of Dihydroquercetin

Table 1-A. Botanical Sources of Dihydroquercetin

Plant Species	Common name	Source	Dihydroquercetin Form	Reference
Agrimonia pilosa Ledeb	Hairy agrimony	Herb	Taxifolin-3-glucoside	Wei et al. (2009b)
Allium cepa L.	Onion	Bulb	Taxifolin Taxifolin-3-glucoside Taxifolin-7-glucoside Taxifolin-4'-gluocoside	Slimestad et al. (2007)
Amygdalus lycioides Spach	NA	Branchelets	(*2R,3R*)-Taxifolin	Gaggeri et al. (2012)
Arachia hypogoea	Spanish peanut	Peanut	Dihydroquercetin	Pratt and Miller (1984)
Berchemia floribunda	NA	Bark	*Cis*-Dihydroquercetin *Trans*-Dihydroquercetin	Wang et al. (2006a)
Blumea balsamifera DC	Capa, Sambong	Leaves	Dihydroquercetin-4'-methyl ether Dihydroquercetin-7,4'-dimethyl ether	Nessa et al. (2005)
Casearia gosspiosperma Briq.	NA	Leaves	(+)-Taxifolin	Vieira Júnior et al. (2017)
Cedrus deodara	Himalayan cedar	NR	Dihydroquercetin	Awad et al. (2015)
Chromolaena odorata	Siam weed	Leaf, stem, and root	Taxifolin 4'-methyl ether Taxifolin 7-methyl ether	Ling et al. (2007)
Coreopsis tinctoria	Plains coreopsis; garden tickseed; golden tickseed	Capitula	Taxifolin-7-O-β-D-glucopyranoside	Han et al. (2016)[b]
Equisetum arvense	Common horsetail	NR	Dihydroquercetin	Syrchina et al. (1975)
Fragaria vesca	Strawberry	Fruit	Taxifolin-3-*O*-arabinoside	Sun et al. (2014a)
Glycomis pentaphylla	Orangeberry; ginberry	NR	*Cis*-Dihydroquercetin *Trans*-Dihydroqueretin	Chen et al. (2016)
Hovenia dulcis Thunberg	Oriental Raisin	Fruit	Taxifolin	Park et al. (2016)
Hydnocarpus alpina	NA	NR	(*2R,3R*)-Taxifolin 3-O-rhamnoside	Balamurugan et al. (2015)[b]
Hymenaea courbaril L.	West Indian Locust	Xylem sap	Taxifolin	da Costa et al. (2014)
Juglans mandshurica	Manchurian walnut	Stem-bark	Taxifolin	Min et al. (2003)
Juglans regia L.	English walnut, Persian walnut, common walnut	NR	(*2S,3S*)-Taxifolin-3-O-α-D-arabinofuranoside (*2S,3S*)-Taxifolin-3-O-α-L-arabinofuranoside	(Zhao et al., 2017)[b]
Larix gmelinii	Dahurian larch	Wood Wood, roots, branches, needles	Dihydroquercetin Taxifolin	Hemingway and Hillis (1969); Kolhir et al. (1996); Ma et al. (2014); Ma et al. (2012) Liu et al. (2014)

Plant Species	Common name	Source	Dihydroquercetin Form	Reference
Ledum procumbens	Labrador tea	Shoots	Dihydroquercetin	Ganina and Popova (2015)
Lippia graveolens	Mexican oregano	Herb	Taxifolin	Lin et al. (2007)
Machilus japonica	NA	Stem	(+)-Taxifolin	Joo et al. (2014)
Malus x domestica	Apple	Skin and flesh	(*2S,3R*)-(+)Taxifolin (*2S,3S*)-(-)Taxifolin (*2R,3R*)-(+)Taxifolin (*2R,3S*)-(-)Taxifolin (*2S,3R*)-(+)Taxifolin glycoside (*2S,3S*)-(-)Taxifolin glycoside (*2R,3R*)-(+)Taxifolin glycoside (*2R,3S*)-(-)Taxifolin glycoside	Vega-Villa et al. (2009)
Mimusops manilkara	NA	NR	Dihydroquercetin	Baky et al. (2016)
Morus alba L.	Mulberry	Seeds	(+)-dihydroquercetin	Lee et al. (2011)[a]
Mutingia calabura	Calabur tree	Leaves	Dihydroquercetin	Zakaria et al. (2014)
Opuntia ficus-indica var. *saboten*	Prickly pear	Fruit and stem	(+)-Dihydroquercetin	Dok-Go et al. (2003); Kim et al. (2017)
Picea abies Karst.	Norway spruce	Phloem	Taxifolin glycoside Taxifolin 3-O-β-D-glucoside	Brignolas et al. (1995); Lieutier et al. (2003)
Piceaa smitiana (wall) Boiss	West Himalayan Spruce	Aerial parts	Dihydroquercetin	Bashir et al. (2018)
Pinus brutia	Turkish pine	Bark	Taxifolin Taxifolin-O-hexoside	Cretu et al. (2013)
Pinus pinaster	French Maritime Pine	Bark	Taxifolin	Nishioka et al. (2007)
Polygonum amphibium	Water knotweed	Herb	Taxifolin	Smolarz (2002)
Polygonum aviculare	Common knotgrass	Herb	Taxifolin	Smolarz (2002)
Polygonum bistorta	Bistort	Herb	Taxifolin	Smolarz (2002)
Polygonum convolvulus	Wild buckwheat	Herb	Taxifolin	Smolarz (2002)
Polygonum hydropiper	Water pepper	Sprout Herb	(*2R,3R*)-(+)-Taxifolin Taxifolin	Miyazawa and Tamura (2007) Smolarz (2002)
Polygonum lapathifolium spp. *Nodosum*	NA	Herb	Taxifolin	Smolarz (2002)
Polygonum lapathifolium spp. *Tomentosum*	NA	Herb	Taxifolin	Smolarz (2002)
Polygonum mite	Tasteless water pepper	Herb	Taxifolin	Smolarz (2002)
Polygonum orientale	NA	Leaves, wood, and seeds	Taxifolin	Wei et al. (2009a)
Polygonum persicaria	Lady's thumb	Herb	Taxifolin	Smolarz (2002)
Pouteria campechiana	NA	Leaves	Taxifolin-3-O-α-L-rhamnopyranoside	Baky et al. (2016)

Plant Species	Common name	Source	Dihydroquercetin Form	Reference
			trans-Taxifolin-3-O-α-L-arabinopyranoside Taxifolin-3-O-α-L-arabinofuranoside	
Pseudotsuga menziesii Franco	Douglas fir	Wood and bark Needles	Dihydroquercetin Dihydroquercetin-3'-glucoside	Hemingway and Hillis (1969) Stafford and Lester (1981)
Rhizoma smilacis glabrae	Tu Fu Ling	'herb extract powder'	(*2S,3R*)-(+)Taxifolin (*2S,3S*)-(-)Taxifolin (*2R,3R*)-(+)Taxifolin (*2R,3S*)-(-)Taxifolin	Vega-Villa et al. (2009)
Rosa canina L.	Dog-rose	Rose hip	(+)-Taxifolin (+)-Taxifolin 3-O-β-D-xylopyranoside (+)-Taxifolin 3-O-α-L-arabinofuranoside	Fujii and Saito (2009)
Rosa davurica	Amur rose	Roots	(+)-Taxifolin 3-O-β-D-apio-D-furanoside	Yoshida et al. (1989)
Silybum marianum	Milk Thistle	Seeds	Taxifolin (+)-Taxifolin	Tedesco et al. (2004); Polyak et al. (2010); Sato et al. (2013); Zholobenko and Modriansky (2014)
Trigonella foenum-graecum	Fenugreek	Seeds	Dihydroquercetin	Yu et al. (2017)
Vitus vinifera c.v. Cabernet Gernischet	Cabernet grapes	Grape skin	Dihydroquercetin-3-*O*-rhamnoside	Meng et al. (2012)
Vitus vinifera 'Malbec'	Malbec grapes	Red wine	Dihydroquercetin-3-*O*-glucoside	Fanzone et al. (2015)

[a] Article in Korean; Information based on abstract (in English) only
[b] Abstract only
NA – Not Applicable; NR – Not reported

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: BH (23) 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.:*	70190388-GA-1 70220388-GB-1
Pembungkus: *Packer:*	FPG OLEOCHEMICALS SDN BHD, LOT 5831 KUANTAN PORT INDUSTRIAL AREA, TANJUNG GELANG, 26080 KUANTAN
Pengeksport: *Exporter:*	FPG OLEOCHEMICALS SDN BHD, LOT 5831 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 0509-016E
Tempat Pensampelan: *Sampling Point:*	ANALYSIS TANK S1617A - FPG OLEOCHEMICALS ANALYSIS TANK S1617B- FPG OLEOCHEMICALS

Dengan ini adalah disahkan 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/HE/SF7 AND SERIAL NO. 2314/2 DATED 13 NOVEMBER 2016

	70190388-GA-1	70220388-GB-1
LIMIT OF DEGREE	> 0.15%	> 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	< 50ppm	< 50ppm
SULPHATED ASH	0.00	0.00

(b) (6)

Tandatangan: *Signature:*
Jawatan: *Designation:*
Cap Rasmi: *Official Stamp:*

BT A. MAJID
F HEALTH
FFICE, KUANTAN

Tarikh Keluaran: *Date of Issue: 13 JANUARY 2017*

Sijil ini adalah sah untuk dan tarikh keluarnya. Pengeluaran sijil ini bukannya bermaksud bagi penerian 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. (b) (6)

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	(b) (6)
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)	(b) (6)		
Test Conclusion	The sample meets the requirements of (b) (6) (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. (b) (6)

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

(b) (6)

Test Report

No: (b) (6)

(b) (6)

Anti-counterfeiting code

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: (b) (6) 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.	(b) (6)
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.: (b) (6) 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

(b) (6)

Test Report

No: (b) (6)

(b) (6)

Anti-counterfeiting cod

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.	(b) (6)
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: (b) (6) 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

(b) (6)

(b) (6)

Inspection and Test Report

(b) (6)

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

(b) (6) 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.: (b) (6) 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	(b) (6)
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: (b) (6)
Fax:

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	(b) (6)	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

Blank Below

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 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™ (b) (6)

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:	(b) (6)	**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

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- THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

Appendix 4.2 Certificate of Analysis BC-DHQ™ (b) (6)

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:	(b) (6)	**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

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* THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

Appendix 4.3 Certificate of Analysis BC-DHQ™ (b) (6)

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: (b) (6)
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

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- THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

Appendix 4.4 Certificate of Analysis BC-DHQ™ (b) (6)

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:	(b) (6)	**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

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* THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

Appendix 4.5 Certificate of Analysis BC-DHQ™ (b) (6)

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:	(b) (6)	**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

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- THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

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

Appendix 5.1 Pesticide Analysis BC-DHQ™ (b) (6)

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 #: (b) (6)

Sample Identification:
Sample #: (b) (6)
Description: BC-DHQ, Powder, (b) (6)
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 #: (b) (6)

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.]

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Sample #: (b) (6)

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.
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.

(b) (6)

Kent Rader
BU Manager

Appendix 5.2 Pesticide Analysis BC-DHQ™ (b) (6)

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 #: (b) (6)

Sample Identification:
Sample #: (b) (6)
Description: BC-DHQ, Powder, (b) (6)
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 #: (b) (6)

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 #: (b) (6)

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.
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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(b) (6)

Kent Rader
BU Manager

Appendix 5.3 Pesticide Analysis BC-DHQ™ (b) (6)

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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-008606-01
Batch #: (b) (6)

Sample Identification:
Sample #: (b) (6)
Description: BC-DHQ, Powder, (b) (6)
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	

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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 #: (b) (6)

Blue California Co.
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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.]		

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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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(b) (6)

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BU Manager

Appendix 5.4 Pesticide Analysis BC-DHQ™ (b) (6)

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 11, 2017

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

CERTIFICATE OF ANALYSIS
AR-17-KK-008605-01
Batch #: (b) (6)

Sample Identification:
Sample #: (b) (6)
Description: BC-DHQ, Powder, (b) (6)
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	<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 #: (b) (6)

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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 #: (b) (6)

Blue California Co.
30111 Tomas
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92688

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

	Result	Theoretical Level
Total Dithiocarbamates, as CS2	<0.01 mg/kg	

[Method performed by an outsource lab.]

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BU Manager

Appendix 5.5 Pesticide Analysis BC-DHQ™ (b) (6)

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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 #: (b) (6)

Sample Identification:
Sample #: (b) (6)
Description: BC-DHQ, Powder, (b) (6)
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 #: (b) (6)

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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 #: (b) (6)

Blue California Co.
30111 Tomas
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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.]		

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(b) (6)

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BU Manager

Appendix 6 Summary of Studies on the Biological Activity of Dihydroquercetin

The bioavailability, metabolism and biological activity of flavonoids is dependent upon the configuration, total number of hydroxyl groups, and substitution of functional groups around the nuclear structure (Kumar, 2013). Many flavonoids have been shown to have multiple biological activities including antioxidative activity, chemoprotective, hepatoprotective, and anti-inflammatory activity in many published studies. A summary of studies related to the biological activity of dihydroquercetin is provided below.

A. Estrogenic 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 (Jefferson, 2002). Taxifolin was the only phytochemical tested that did not induce transcriptional activation of the ER. In addition, taxifolin was one of the compounds tested *in vivo* that did not increase uterine wet weight. However, all compounds tested induced sensitive and morphological biological parameters such as uterine epithelial cell height increase and uterine gland number increase, demonstrating that all tested phytoestrogens gave some measure of estrogenicity in this assay. Taxifolin demonstrated a non-dose dependent increase in epithelial cell height, with the increase occurring at the lowest dose tested instead of the highest. Although taxifolin did not increase uterine wet weight, uterine gland numbers were significantly increased over control at a taxifolin dose of 500,000 µg per kg per day. The authors concluded that taxifolin appeared to be one of the least potent phytoestrogens tested in this study.

B. Anti-Cancer Activity

The chemopreventive effects of flavonoids has been well characterized and there are many published reports on the chemoprotective and preventative effects of DHQ. DHQ can affect the process of carcinogenesis through multiple mechanism including induction of phase II detoxifying enzymes (which results in the detoxification of carcinogenic intermediates), suppression of cytochrome P450-dependent monooxygenases, fatty acid synthase and antioxidant response element with the effect of DHQ on lipid peroxidation resulting in the most promising chemopreventive and chemotherapeutic action across multiple disease states (Weidmann, 2012).

A study was conducted to investigate the anti-cancer effects of taxifolin on human osterosarcoma cancer cells (Chen, 2018). The study included a control group (8 mice with 14 tumors) and a treatment group (8 mice with 13 tumors). Mice in the treatment group received 25 mg per kg taxifolin once every 2 days over the course of 24 days, while the control group received an equal volume of the saline vehicle. Taxifolin was shown to inhibit the proliferation and diminished

colony formation of U2OS and Saos-2 osteosarcoma cell lines in soft agar in a dose dependent manner. When nude mice bearing U2OS xenograft tumors were treated with taxifolin by intraperitoneal injection, there was a significant inhibition of tumor growth.

In another study, DHQ showed good antitumor activity against HepG2 cell lines, having an inhibition rate of 44.1% at a concentration of 400 μmol, and that complexing DHQ with either lecithin or β-cyclodextrin significantly increased the inhibition rate (Zhang, 2017). Furthermore, it was demonstrated that taxifolin suppresses UV-induced skin cancer *in vitro* and in a mouse model by inhibiting EGF-induced cell transformation by targeting the EGF receptor (EGFR) and phosphoinositide 3-kinase (PI3K) (Oi et al., 2012).

Several other *in vitro* studies have demonstrated the chemoprotective effect of DHQ. Zhai et al. (2011) found that taxifolin is an inhibitor of human cervical cancer cells (HeLa) and the mechanism of cell death was related to cell apoptosis. The authors reported that the mechanism of cell death by taxifolin is related to cell apoptosis due to the upregulation of p53 mRNA and P21 mRNA and independent of Bcl-2 mRNA/Bax mRNA apoptosis protein transcription. In a study where the effects of flavonoids on cell proliferation and vascular endothelial growth factor (VEGF) expression in human ovarian cancer cells (OVCAR-3) were evaluated, taxifolin inhibited ovarian cancer cell growth in a dose dependent matter via inhibition of VEGF expression (Luo et al., 2008). Taxifolin was the least potent of the 9 flavonoids tested.

A study by Zhang et al. (2013) demonstrated that taxifolin enhances the apoptotic effect of andrographolide in human prostate cancer (DU145) cells. Taxifolin alone did not significantly affect the DU145 cell proliferation; however, the anti-cancer effect of andrographolide was significantly enhanced by taxifolin. Taxifolin was also a potent inhibitor of the growth of two human lymphoid tissue cell lines, IM-9 and Molt-4, at concentrations ranging from 10 – 50 μM (Devi and Das, 1993). The authors concluded that the natural plant polyphenols could be considered for use in the treatment of lymphocyte malignancy. Analysis of anti-lipogenic effects on intact cancer cells, the human LNCaP prostate cancer cell line and the MDA-MB-231 breast cancer cell line, showed that taxifolin was an inhibitor of lipogenesis and had marked effects on cancer cell growth and survival (Brusselmans, 2005).

Lee et al. (2007) studied the chemopreventive effects of taxifolin by measuring quinone reductase activity in HCC 116 cells. To identify the target genes regulated by taxifolin, a DNA microarray was performed using a 3K human cancer chip containing 3,096 human genes associated with carcinogenesis. In the presence of 60 μM taxifolin, sixty-five genes, including a few detoxification enzymes (NQO1, GSTM1) and an antioxidant enzyme (TXNRD1), were up-regulated. This study indicates that taxifolin acts a chemoprotective agent by upregulating genes associated with antioxidant activity and P450 metabolism.

C. Antioxidant

Many of the biological effects of DHQ, such as protection of neuronal injury, cardio protection, diabetes, and antiaging, are attributable to antioxidant activity. A review was completed to examine the evidence that DHQ is a potent flavonoid antioxidant and the therapeutic promise it may have for conditions such as cancer, cardiovascular disease, and liver disease (Weidmann, 2012). Weidmann (2012) 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 groups with 4-oxo function in the A and C rings which give the maximum radical scavenging potential, DHQ is classified as an antioxidant which had a similar anti-oxidant activity profile to α-tocopherol.

When the antioxidant activity of taxifolin was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, the half maximal effective concentration (EC_{50}) was determined to be 48 µg per mL, which was comparable to the activity of α-tocopherol (51 µg per mL) (Boniface, 2017). In another study, DHQ was evaluated for its protective effects on Fenton reagent-treated bone marrow-derived mesenchymal stem cells (Li, 2017). The study employed a variety of antioxidant assays, and the results showed that DHQ can act as an effective •OH scavenger via direct and indirect antioxidant effects.

The antioxidant and antiradical activities of taxifolin was investigated in another study by using different *in vitro* bioanalytical antioxidant methods including DMPD•+, ABTS•+, O_2•-, and DPPH• scavenging effects, the total antioxidant influence, reducing capabilities, and 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 radical scavenging assay, ABTS radical scavenging assay, ferric reducing antioxidant property (FRAP) assay, and hydroxyl (OH) radical scavenging capacity at concentrations of 20, 40, 60, 80, and 100 µg per mL (Manigandan et al., 2015). The activities of DPPH, ABTS, FRAP, and OH radical levels were significantly inhibited by taxifolin with half maximal inhibitory concentration (IC_{50}) values of 16.48, 66.34, 18.17, and 11.42 µg per mL, respectively. This study also investigated the impact of taxifolin on DNA integrity on pUC19 plasmid DNA, and found that there was a strong protective effect at 1.0 µg per mL.

Manigandan et al. (2015) also investigated the protective mechanism of taxifolin against cadmium intoxicated zebrafish embryos and found that treatment with taxifolin at 0.1, 1.0, and 10 µM significantly enhanced the antioxidant enzyme levels by reducing lipid peroxidation in the embryos.

The potential protective effects of taxifolin on human RPE (ARPE-19) cells cultured under oxidative stress conditions was investigated and the underlying mechanism evaluated (Xie,

2017). The cells were treated with different doses of taxifolin (10, 20, 50, or 100 µg per mL) and 0.4 mM of H_2O_2 for 24 hours, and taxifolin was found to protect the cells against oxidative stress-induced apoptosis.

In a study comparing the antioxidant effects of quercetin and DHQ in human blood cells, DHQ was more potent than quercetin in inhibiting superoxide (Chen and Deuster, 2009). In another study, there was a concentration-dependent inhibition of the oxidative neuronal injuries in primary cultured rat cortical cells with DHQ, which was less potent than quercetin (Dok-Go et al., 2003).

Taxifolin was successful in reducing adult rat brain injury induced by middle cerebral arterial occlusion (MCAO) by reducing oxidative damage (Wang et al., 2006b). Taxifolin administration [0.1 and 1.0 µg per kg body weight (bw) via i.v.] sixty minutes after MCAO protected the rat brain from injury by diminishing cerebral lipid peroxidation and protein nitrosylation.

The role of the antioxidant effect of DHQ has also been demonstrated in *in vivo* and *in vitro* diabetes models. A study by Sun et al. (2014b) demonstrated the potential therapeutic role of taxifolin against diabetic cardiomyopathy using streptozotocin-induced diabetic mice and H9c2 cardiac myoblasts. Taxifolin was dosed at 25, 50, and 100 mg per kg bw. In diabetic mice, taxifolin improved diastolic function, reduced myocardium structure abnormality at the 50 and 100 mg per kg bw dose levels, inhibited myocyte apoptosis and enhanced antioxidant enzyme activity at all dose levels tested. In addition, taxifolin maintained the clarity of the rat lens when incubated with a high concentration of glucose. The antioxidant capacity of DHQ was also responsible for protection against hepatic injury caused by tetrachloromethane (CCl_4) in male Wistar rats (Teselkin et al., 2000). Animals received 100 mg DHQ per kg bw for 4 days prior to the first administration of CCl_4, resulting in a higher antioxidant activity in the blood plasma. In a study to determine the effect of taxifolin on cardiac hypertrophy and fibrosis in mouse myocytes, taxifolin arrested the oxidative stress and decreased the expression of 4-hydoxynonenal (4-HNE) induced by pressure overload Guo et al. (2015).

The dietary administration of DHQ to aged mice resulted in the restoration of mitochondrial enzyme activity (Bronnikov et al., 2009). Kv:SHK mice, 23 months old, were divided into two groups: one received 0.05 mg of DHQ by daily oral gavage (n=5); and the other group (n=5) received water only as a control. Mice were dosed for 6 weeks and the activity of citrate synthase, NADH-coenzymeQ1-oxidoreductase (complex 1), and cytochrom-c-oxidase (complex 4) were assessed. The biochemical alterations seen in the DHQ treated group increased the animals' mobility and improved fur and skin condition. In addition, a study found that 86 mg per kg bw DHQ was as potent as rutin in modulating the process of peroxidation in male rats (Chernyak and Shchukina, 2009). In an *in vivo* study, taxifolin was found to have a potential protective effect against oxidative damage and cataracts in the rat lens by inhibiting the hyperosmotic effect that is often seen in diabetes (Haraguchi et al., 1997).

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, 2016). Chobot (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.

D. Hepatoprotective Effects

DHQ has demonstrated hepatic effects and potential protection against liver injury. The potential hepato-protective mechanisms include lipid peroxidation, apoptosis and some anti-viral effects (Weidmann, 2012). Taxifolin was found to slightly but significantly lower the atherogenic index and serum cholesterol level in rats fed a cholesterol-enriched diet (Itaya and Igarashi, 1992), as well as lower the liver phospholipid concentration, and the serum and liver thiobarbituric acid reactive substances (TBARS) concentration, in rats.

Five-week-old male weanling Wistar rats were divided into three groups of 5-6 rats each and then exposed to either a control, astilbin (0.074%) added, or taxifolin (0.05%) added diet for 10 days (Igarashi et al., 1996). At the end of the dosing period, animals were anesthetized, blood collected from the heart, and the liver was immediately removed and frozen. The serum TBARS concentration, total cholesterol, high density lipoprotein (HDL)-cholesterol, triacylglycerol and phospholipid were determined as well as liver total cholesterol, liver TBARS, catalase, glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) activity. Erythrocyte superoxide dismutase, catalase, and glutathione peroxidase were measured as well. The activity of GSH-Px and GSSG-R were not impacted by feeding astilbin or taxifolin. The study authors concluded that taxifolin may exert cholesterol-lowering activity by its influence on the endogenous cholesterol metabolism. The effect of taxifolin on lipid, apolipoprotein B, and apolipoprotein A-1 synthesis and secretion was determined in human liver (HepG2) cells (Theriault et al., 2000). Taxifolin was found to decrease hepatic lipid synthesis coupled with a decrease in apoB and an increase in apoA-1 secretion, and the authors concluded that this study supports the theory that taxifolin has the potential to help control atherogenesis. Dihydroquercetin was shown to ameliorate concanavalin A-induced mouse experimental fulimant hepatitis (Zhao et al., 2015). A follow-up *in vitro* study indicated that the mechanism of action DHQ was to scavenge oxidative stress and inhibit the release of inflammatory mediators in mouse RAW264 cell lines.

E. Immunological/Anti-inflammatory Effects

Low concentrations of DHQ as a food supplement have been shown to increase the immune status of the gilthead seabream (Awad et al., 2015). Fish that received 0.1% DHQ showed the most significant difference in phagocytosis, respiratory burst, immunoglobulin M (IgM) level, total protein, complement, antiprotease and bactericidal activities compared to control.

Taxifolin demonstrated potent anti-inflammatory activity in albino rats and had a therapeutic index almost equal to hydrocortisone in carrageenan-induced oedema (Gupta et al., 1971). The

intraperitoneal administration of taxifolin prevented the increase in serum aminotransferase activity during inflammation, which was similar to the hydrocortisone controls. In another study, taxifolin demonstrated potent and dose-dependent analgesic activity when administered orally and intraperitoneally during a writhing test and formalin test in mice, and against paw edema caused by carrageenan, dextran, and bradykinin in rat (Cechinel-Filho et al., 2000).

F. Other Effects

Ding et al. (2018) investigated the kidney protective effects of DHQ in rats with diabetic neuropathy (DN) induced by a high fat diet and streptozotocin *in vivo*. Male Sprague-Dawley rats were used in this study and divided into 2 groups: control (n=10) and high fat diet (HFD) group (n=63). The rats in the HFD group were fed the HFD (40 kJ per kg, 20% fat) for 4 weeks and then given a single intraperitoneal dose of streptozotocin. Animals in the control group received a single intraperitoneal injection of the sodium citrate buffer. When animals in the HFD groups were confirmed to be diabetic, they were randomized to 5 groups (n=10) and treated for 12 weeks: DN control; three DN + DHQ groups – 25, 50, and 100 mg per kg bw per day; and a group of DN + Losartan at 20 mg per kg bw per day. Body weights and fasting serum glucose were determined every 2 weeks and urine was collected at the end of the experiment. The authors concluded that DHQ possessed kidney protection effects.

The effect of DHQ on mean blood pressure and macro- and micro-rheological blood parameters in hypertensive SHR rats was evaluated following oral dosing at 20 mg per kg per day for 6 weeks (Plotnikov, 2017a). The mean blood pressure in the experimental rats decreased by 11%, the left ventricular mass index by 2%, and the whole blood viscosity by 7-10% as compared to the control SHR rats, which received no DHQ.

In a study utilizing cultured AML12 hepatocytes and a hyperuricemic mouse model to test the anti-hyperuricemic effect of DHQ, the results suggested that DHQ possesses a potent hypouricemic effect (Adachi, 2017). The inhibitory effect of DHQ on uric acid production was significantly stronger when hepatocytes were treated for 4 hours than those treated for 2 hours when treated with doses of 25, 50, and 100 μM, respectively, indicating a time dependent effect. For the mouse model, animals were dosed with guanosine-5’-monophosphate and inosine-5’-monophosphate via intraperitoneal injection at a dose of 300 mg each per kg to induce hyperuricemia one hour after DHQ administration.

Appendix 7 GRAS Associates Expert Panel Report

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

November 5, 2018

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 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 other dihydroquercetin preparations 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. BC-DHQ™ is shown to be stable in a 6-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.

- **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 7 days of exposure at up to 15,000 mg per kg bw (90.94% DHQ) in a GLP-compliant study.

† 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 21 years' experience as a practicing veterinarian and 18 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.

- **Subchronic animal studies** revealed no changes in mortality or body weights, no clinical signs of toxicity or feed consumption, and no treatment-related histopathological findings and other toxicity endpoints following 90 days of exposure by oral 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 was 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 oral 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 females 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 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, 2017b). 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 to chocolate confectionery up to 0.07 g per kg with the target population from 9 years and older. It was also intended to be added to food supplements at 100 mg per day for the general population in ages of 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, 2017a). 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 ‘mean x 2’ 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.

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

Panel Chair

END

Eurofins Scientific, Inc.
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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: (b) (6)
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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 (b) (6) BC-DHQ, Powder, (b) (6)

(2) Eurofins sample BC-DHQ, Powder,

(3) Eurofins sample BC-DHQ, Powder,

(4) Eurofins sample BC-DHQ, Powder,

(5) Eurofins sample BC-DHQ, Powder,

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:

(b) (6)	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
(b) (6)	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
(b) (6)	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
(b) (6)	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
(b) (6)	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:

(b) (6)		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
(b) (6)		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
(b) (6)		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
(b) (6)		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
(b) (6)		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

Date Entered into e-LIMS:	7/17/17		**Analyst:**	TS	**Earliest Sample Due Date:**		
Date Started	7/11/17				**Log #:**	(b) (6)	
Prepped By	Tvm				**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	Dispense tip				Eluent A:	2Phos	1956 / 7/25/17
Prep Solvent	MeOH / ACN / Milli-Q	Lot #	(b) (6)	Exp. 9/27/17	Eluent B:	ACN	178223 / 1/11/18
Prep Solvent	—	Lot #		Exp. —	Eluent C:	MeOH	181311 / 12/7/17
Prep Solvent	—	Lot #		Exp. —	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

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	—	|	|	
↓	—	" " C	3.804	40	—	↓	↓	

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

Δ Ready to report MT 7/17/17

Reviewed By: MT Date: 7/17/17

Validated By: MT 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: Dilution Vol. (mL)	Volume: 2º Dilution Vol. (mL)	Volume: Injection Vol. (µL)	Volume: Final Dilution (mL)**	Notes:
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: M Date: 7/17/17

Validated By: M 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	Tim		
Method Name	DH2		
Method #	KOR45		
Balance	XP26#3		
Vol. Device	Class A		
Prep Solvent	MeOH ACN Milli-Q	Lot# [redacted]	Exp. 9/27/17
Prep Solvent	—	Lot# [redacted]	Exp. —

Log #: [redacted] (6) (a)	
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	167ul 0.0089648065	—
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 0.0694505	167ul→5mL 0.0092818667	—
							TS 7/17/17	

Reviewed By MT 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	Tim		
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 #: [redacted]		
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

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 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: (b) (6) Date: 7/28/17

Validated By: _____ Date: 8/9/17 (Validated previously, NK absent) MT 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	Tim		
Method Name	DHQ		
Method #	K 0023		
Balance	XP26#2		
Vol. Device	class A		
Prep Solvent	MeOH ACN Milli-Q	Lot# (b) (6)	Exp. 9/27/17
Prep Solvent	—	Lot# (b) (6)	Exp. —

Log #: (b) (6)	
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→5 mL 0.215258175	2.5→5 mL 0.143505545
						1.25→5 mL 0.071752725	1.67 mL →5 mL 0.009586164	
					TS 7/25/17			

Reviewed B (b) (6) 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:	(b) (6)
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

(b) (6)

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

(b) (6)

PRECISION (REPEATABILITY)

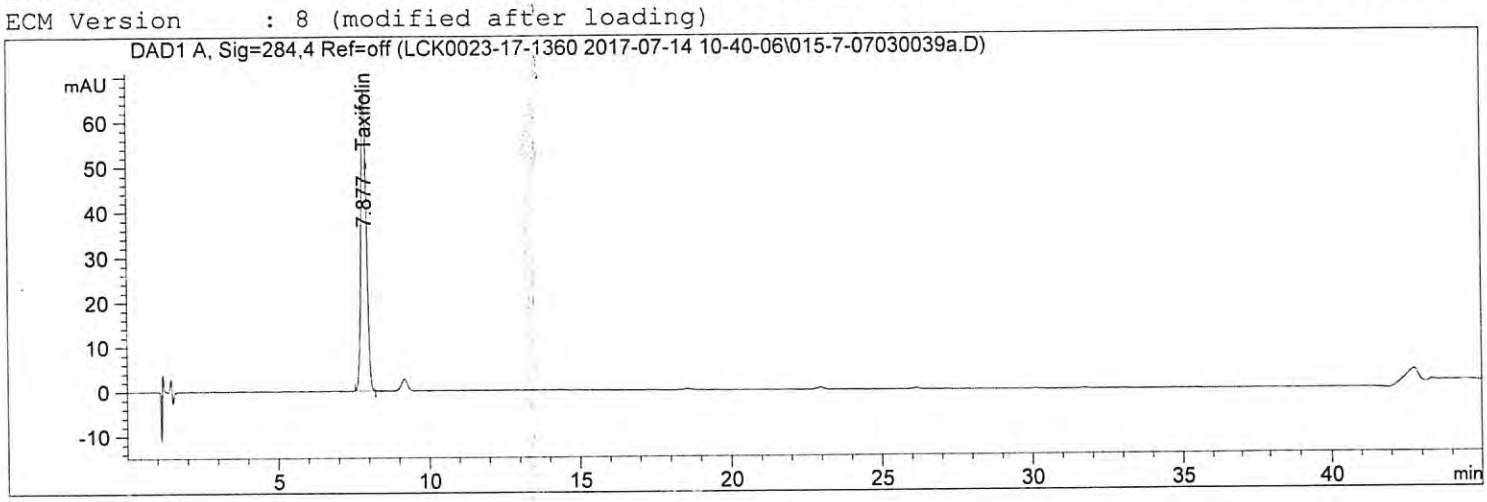
SAMPLE CHROMATOGRAMS

PERCENT WEIGHT RESULTS

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\015-7-07030039a.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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     : 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:                :      3.03100  [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: (b) (6)

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

1 Warnings or Errors :

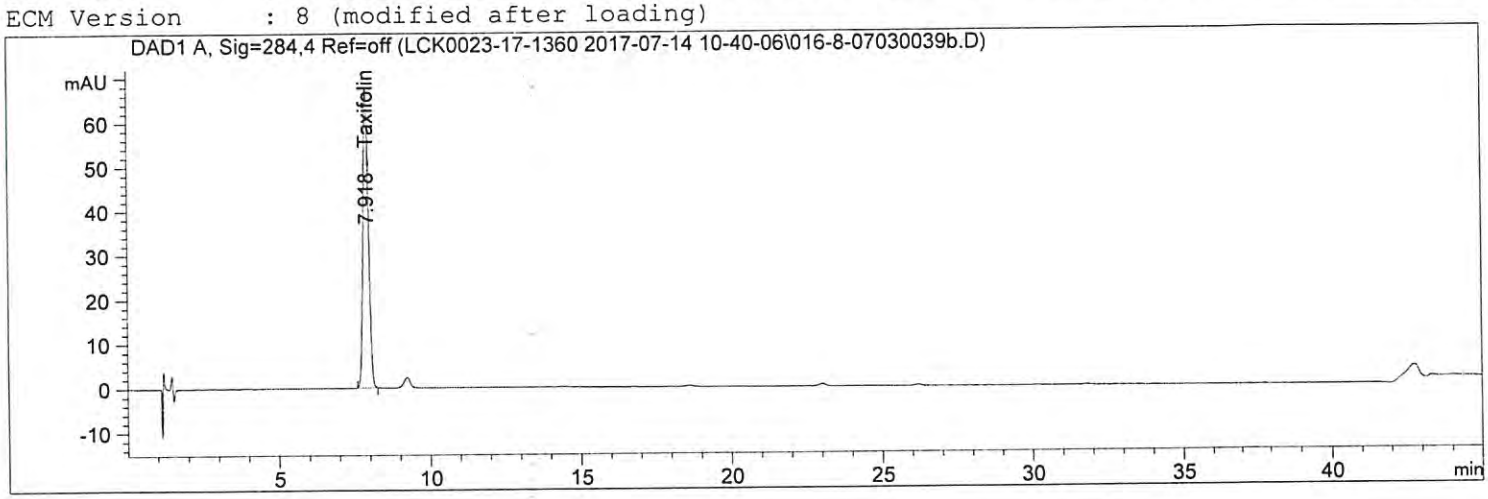
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\016-8-07030039b.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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     : 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:                :      3.07600  [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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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     : 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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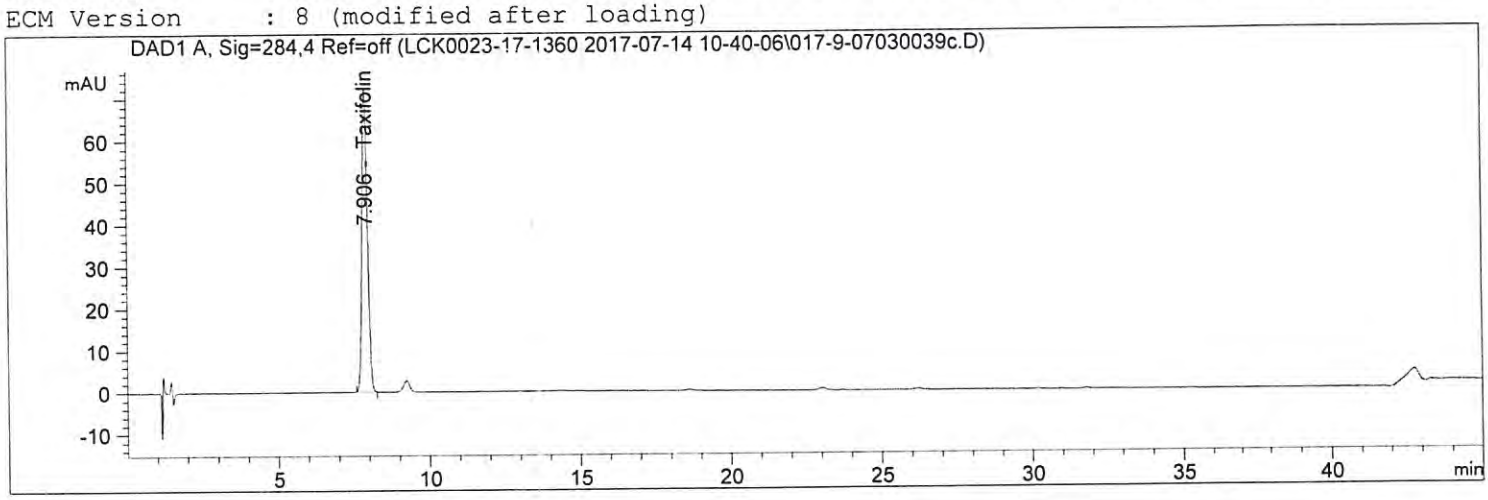


```
==========================================================================
                          ESTD Percent Report
==========================================================================

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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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 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)
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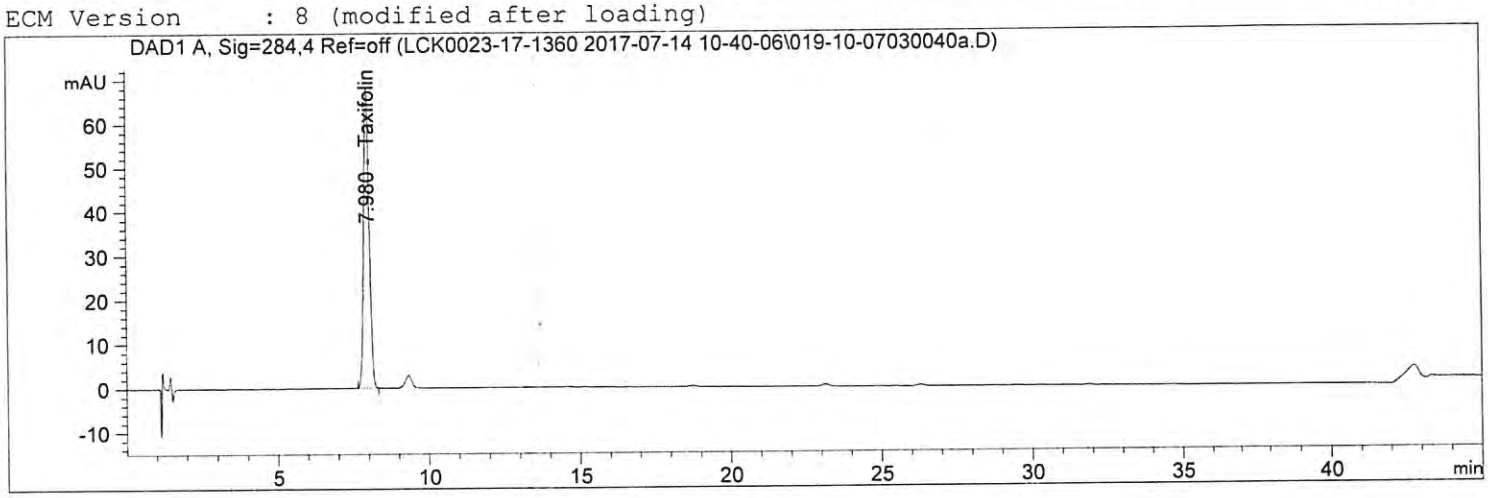


```
==========================================================================
                          ESTD Percent Report
==========================================================================

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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                    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 Methcd)
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)
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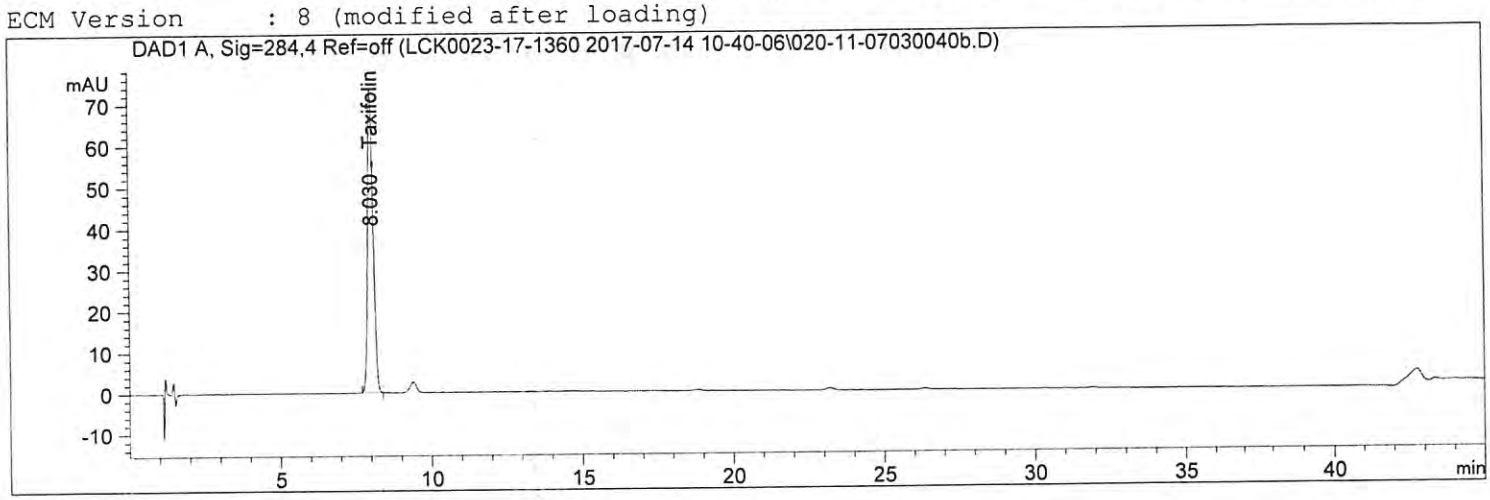


```
==========================================================================
                          ESTD Percent Report
==========================================================================

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  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          [mAU*s]                   %
-------|------|----------|----------|----------|--|-----------------
  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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator   : Timothy Sit                      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 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)
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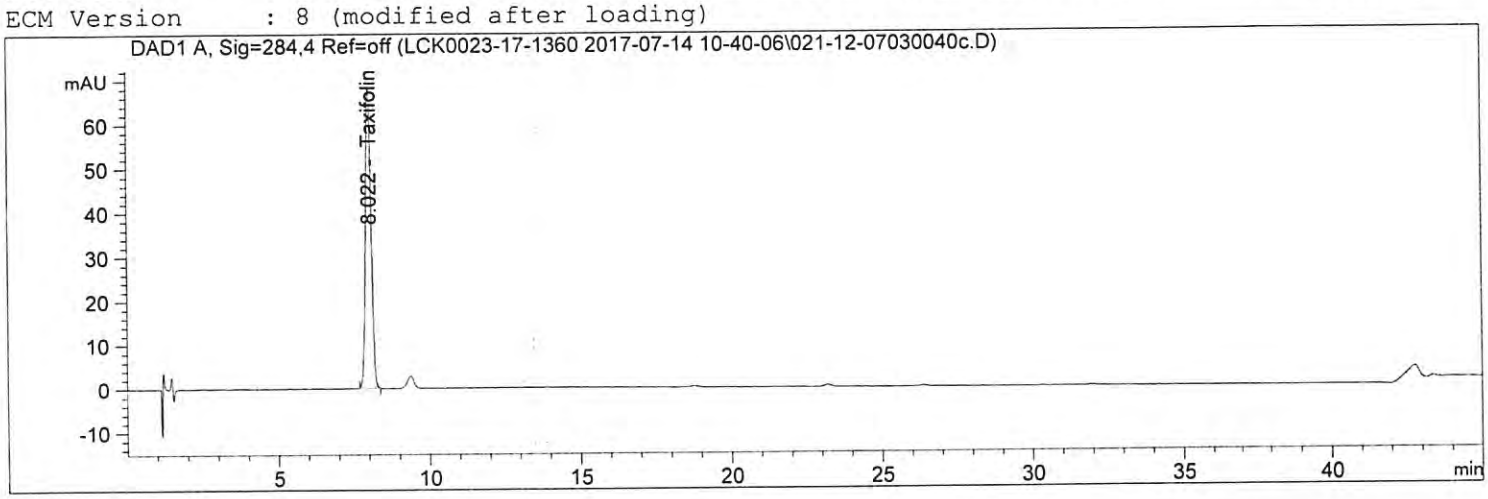



```
==========================================================================
                          ESTD Percent Report
==========================================================================

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: (b) (6)

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

1 Warnings or Errors :

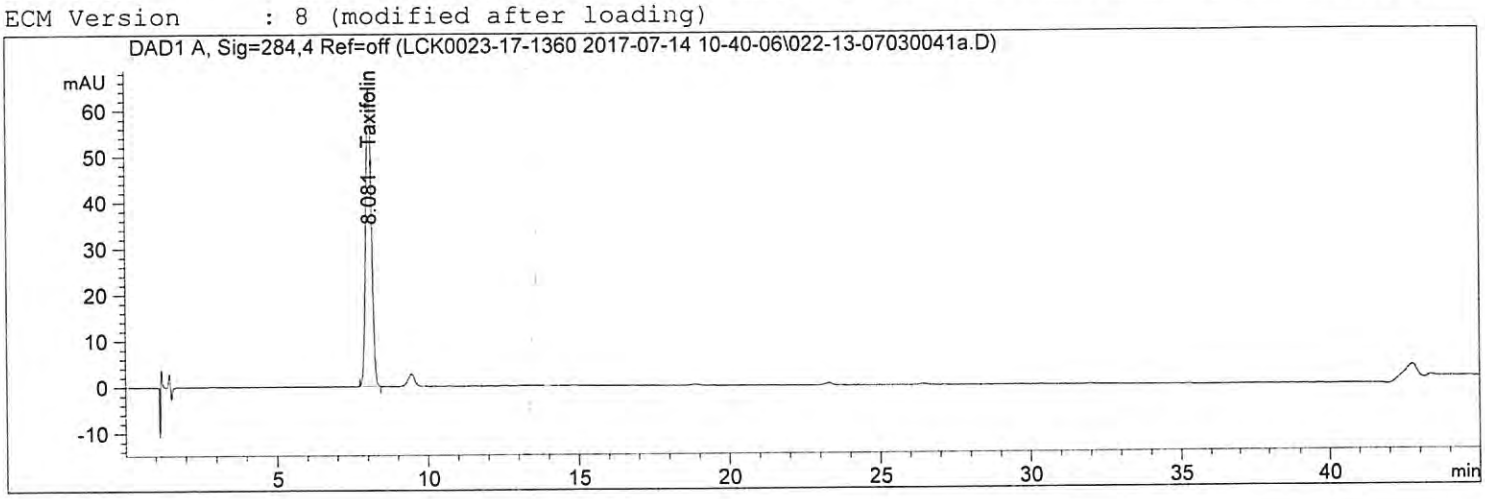
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\022-13-07030041a.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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 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:                :      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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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 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)
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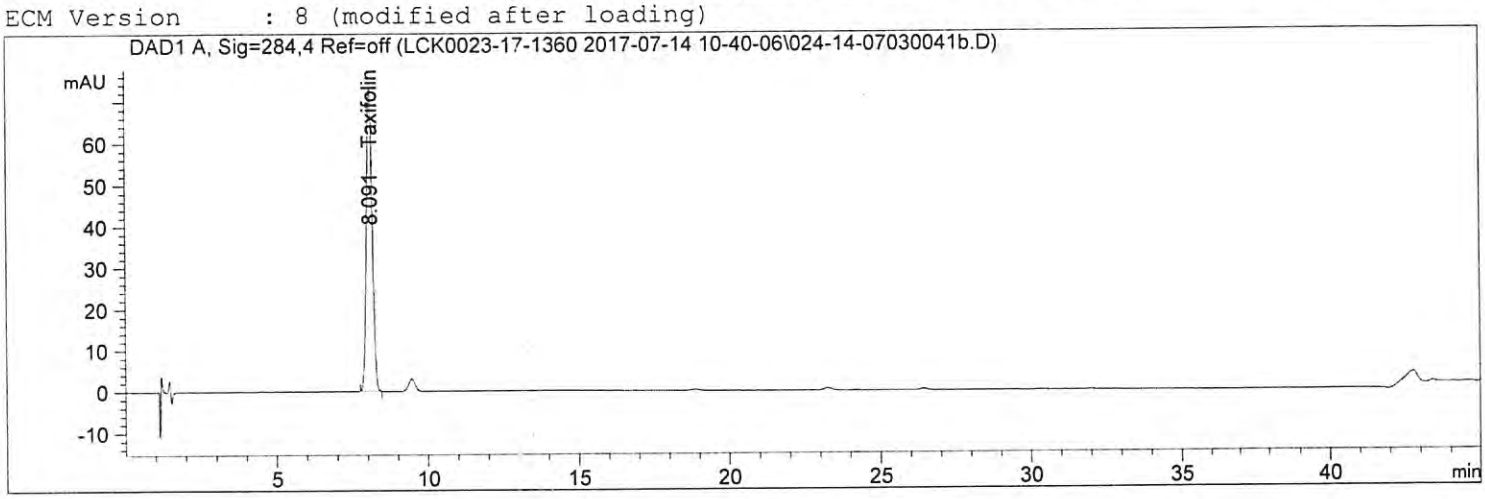



```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.49400  [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: (b) (6)

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

1 Warnings or Errors :

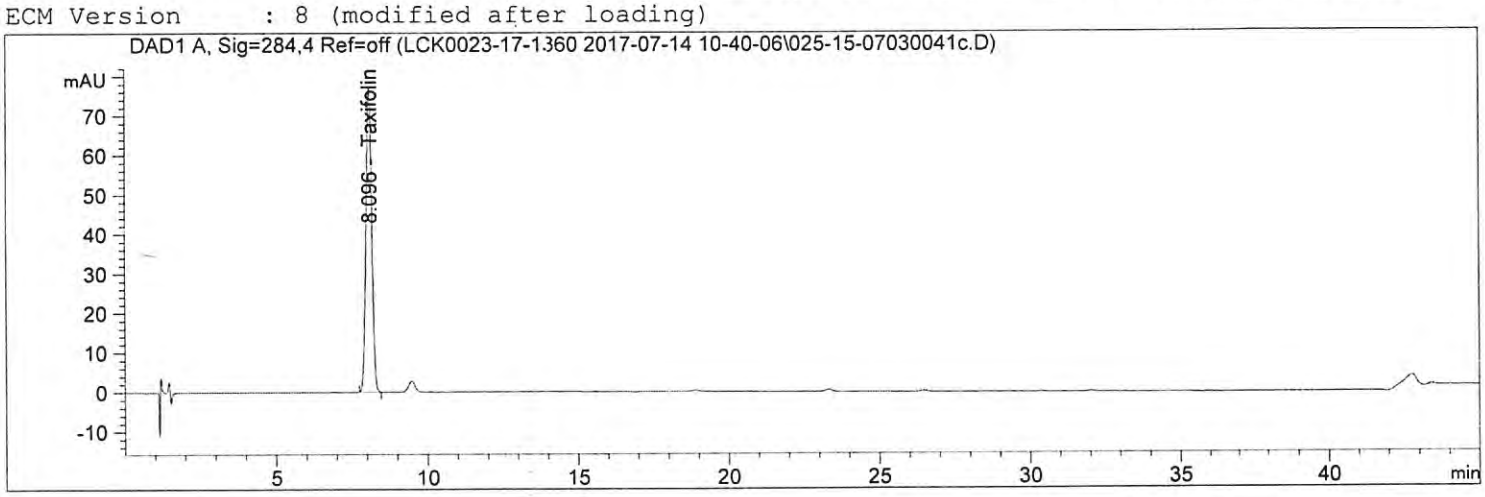
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\025-15-07030041c.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator   : Timothy Sit                      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 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:                :      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: (b) (6)

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

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

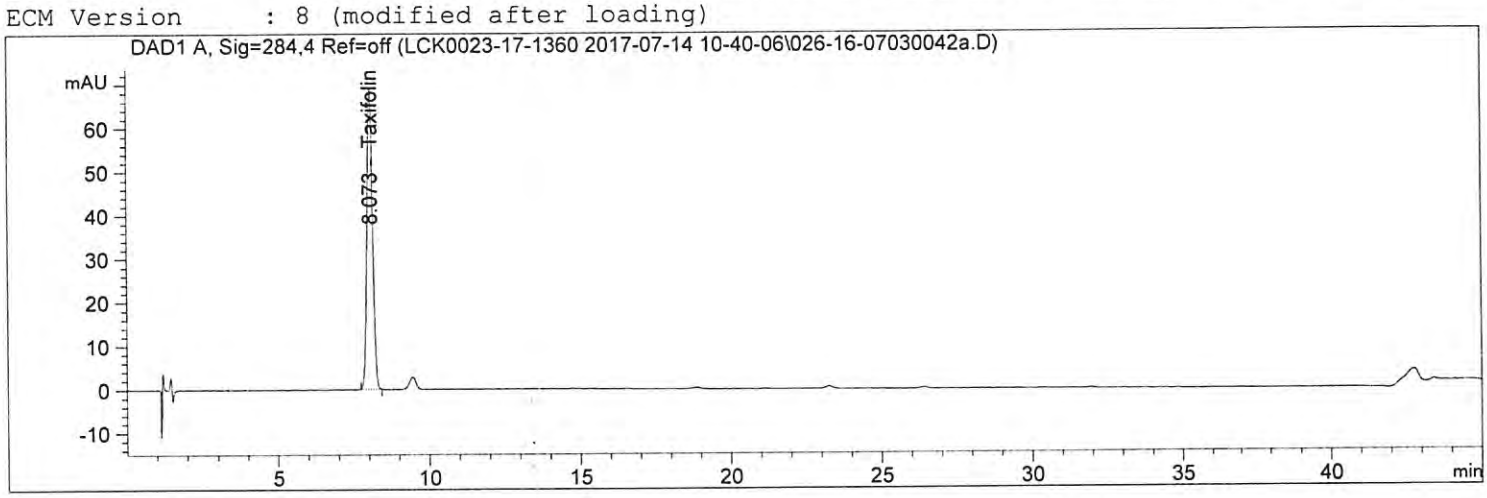
==========

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\026-16-07030042a.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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 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:                :      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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator   : Timothy Sit                      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 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)
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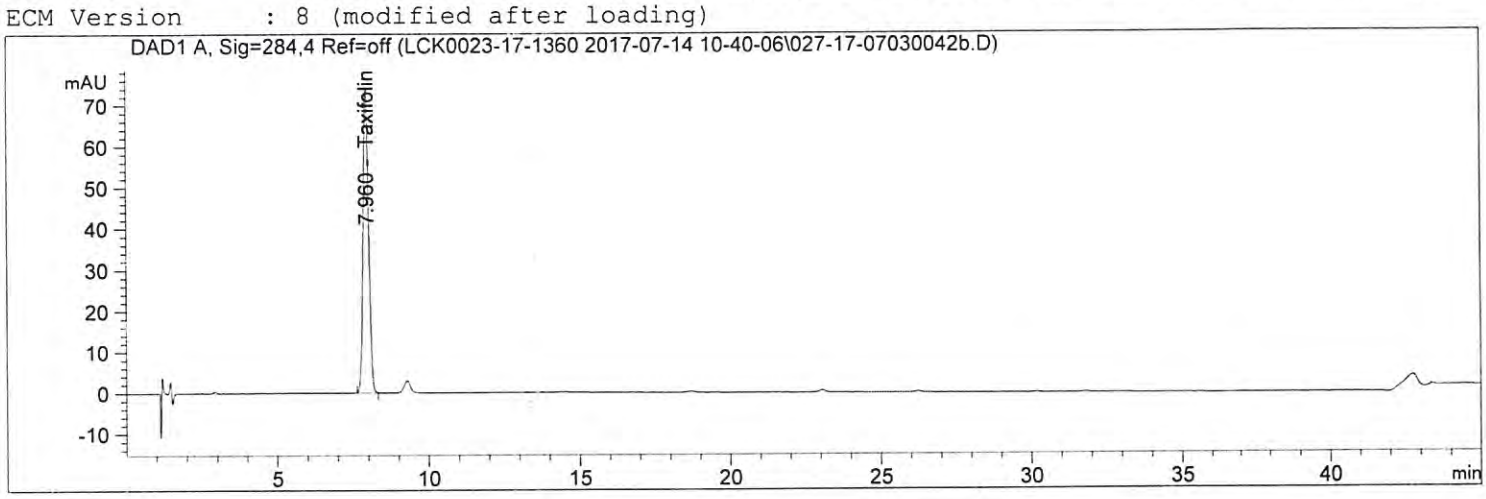



```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      3.43300  [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: (b) (6)

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

1 Warnings or Errors :

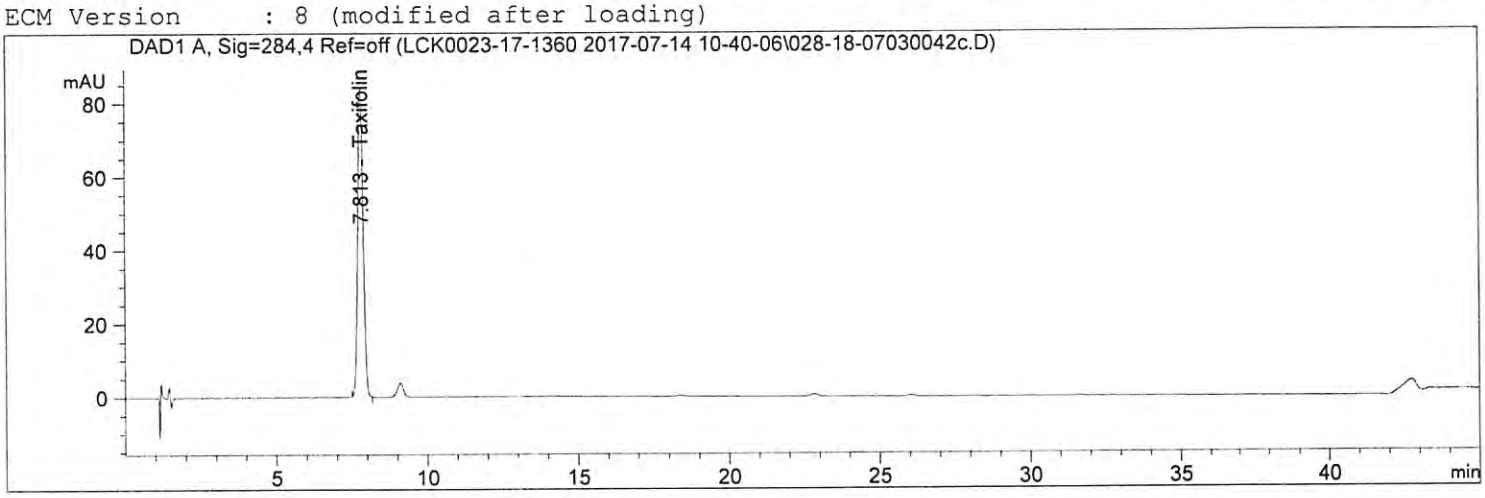
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\028-18-07030042c.D
Sample Name: (b) (6)

```
Acq. Operator    : Timothy Sit                    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 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:                :      3.80400  [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]                   %
-------|------|----------|----------|----------|--|-----------------
  6.414           -           -          -          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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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)
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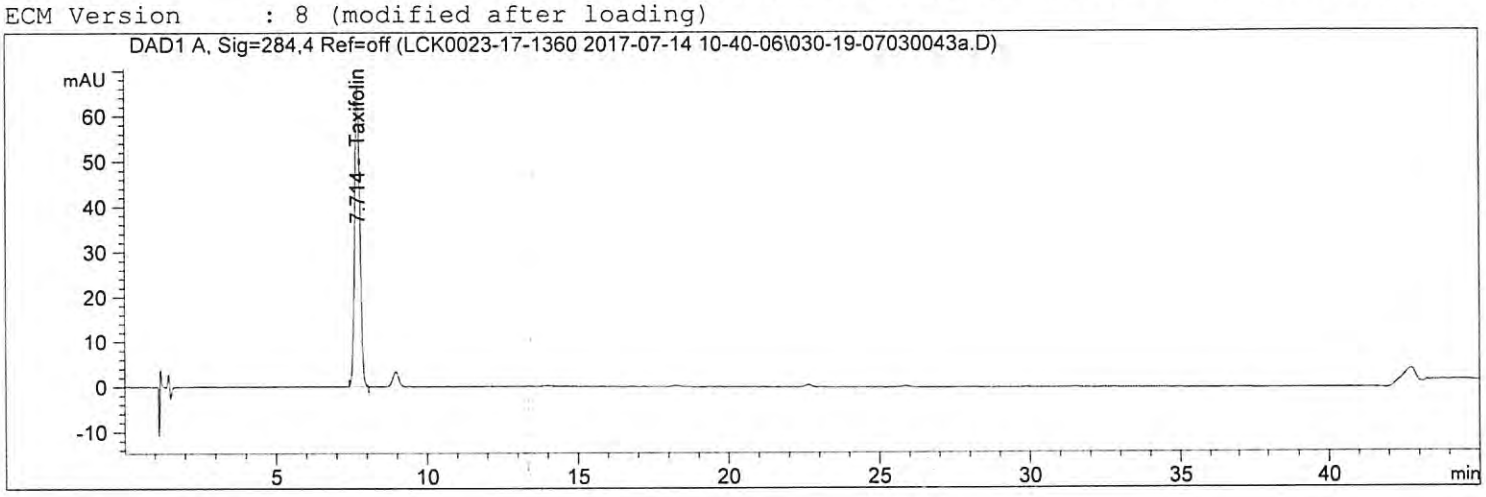



```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      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: (b) (6)

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

1 Warnings or Errors :

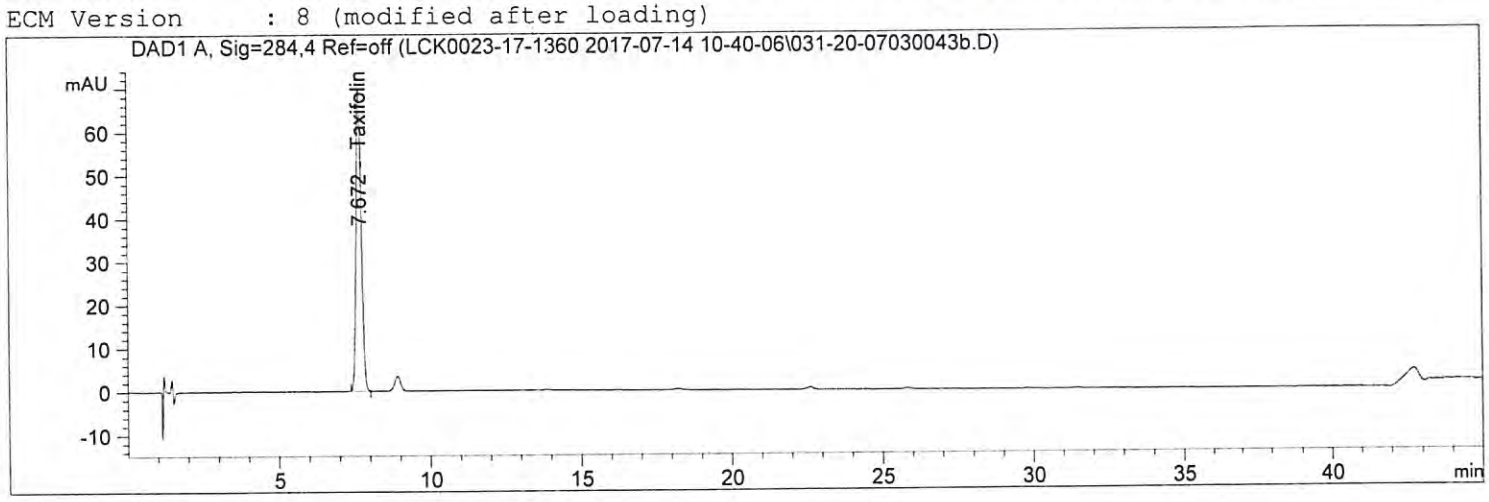
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File D:\Chem32\3\Data\LCK0023-17-1360 2017-07-14 10-40-06\031-20-07030043b.D
Sample Name: (b) (6)

```
==========================================================================
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 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:                :      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: (b) (6)

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: (b) (6)

```
==========================================================================
Acq. Operator   : Timothy Sit                      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 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)
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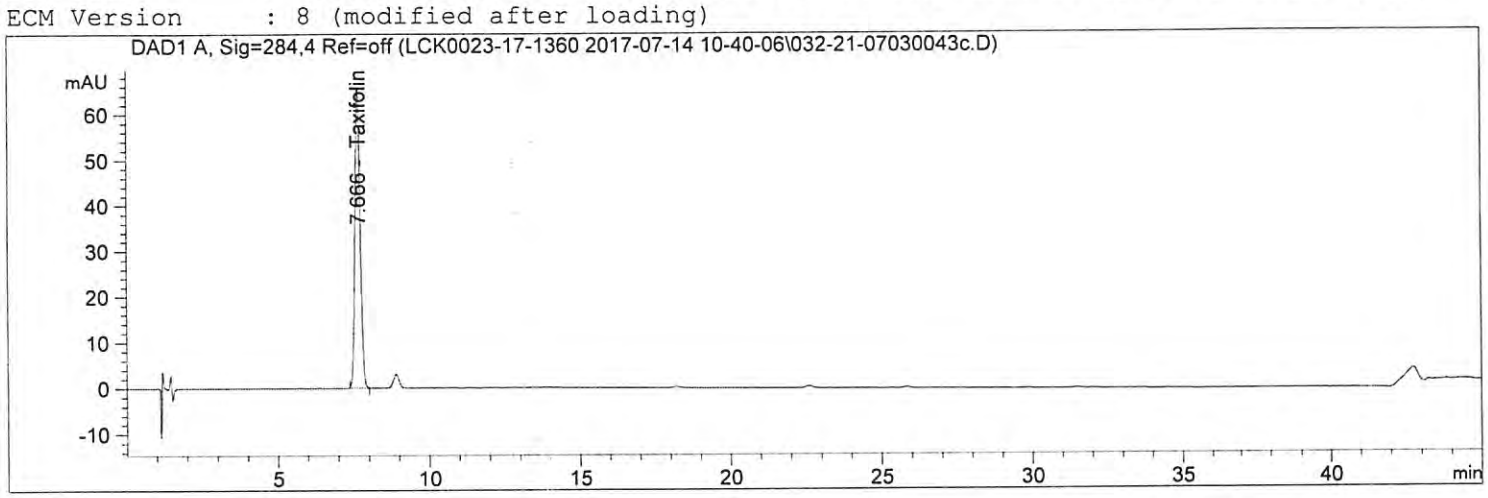



```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Monday, July 17, 2017 9:49:42 AM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :      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: [redacted]

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 ***

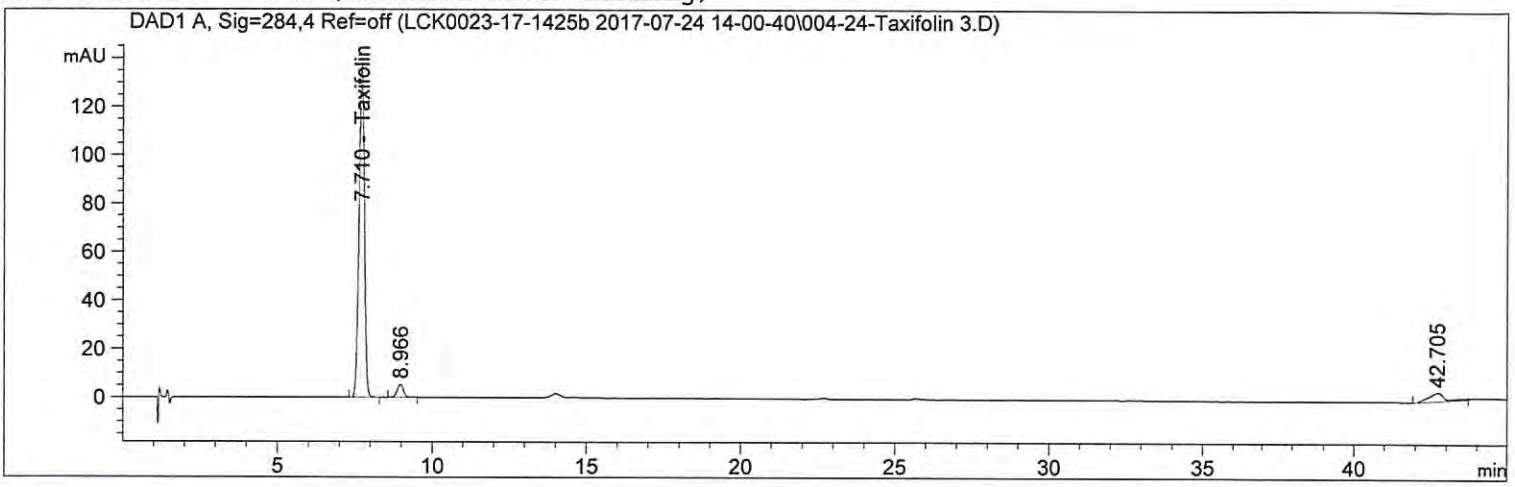
SYSTEM SUITABILITY

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   : Timothy Sit                    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 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    : 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
7.710	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

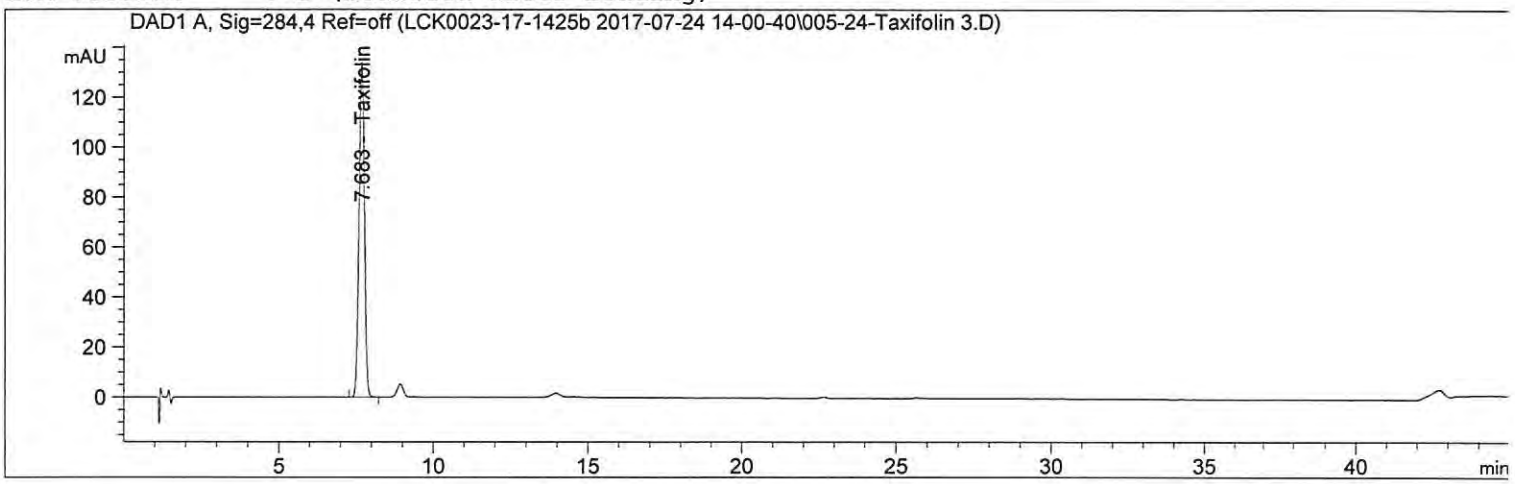
==========

*** 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   : Timothy Sit                    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 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    : 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

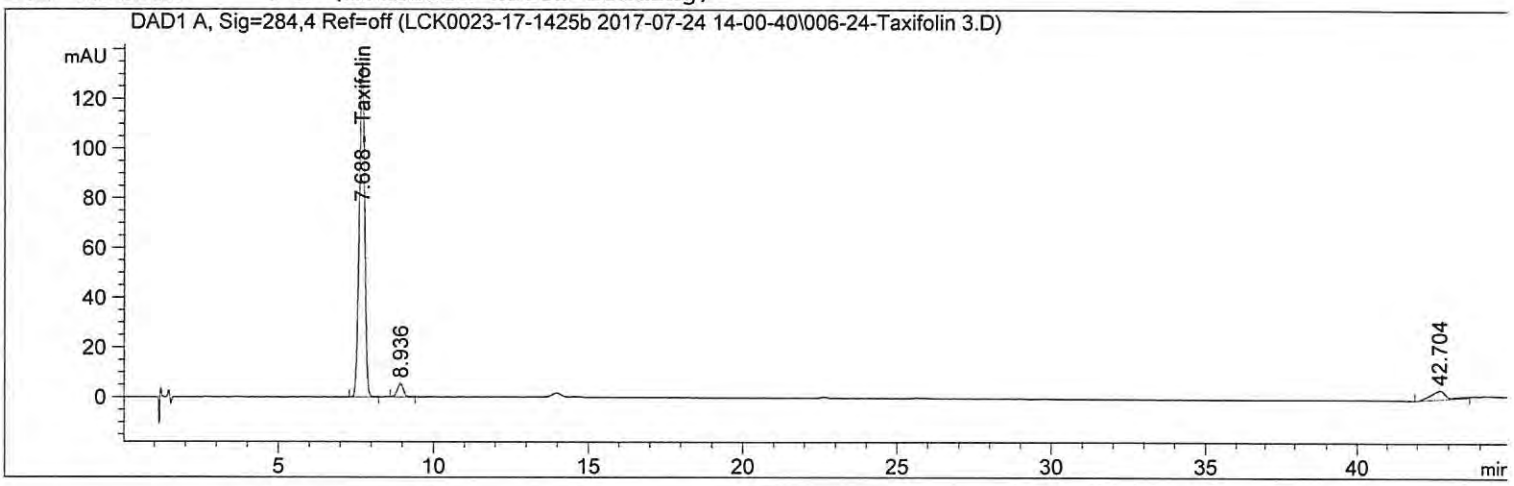
==========

*** 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   : Timothy Sit                      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 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    : 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

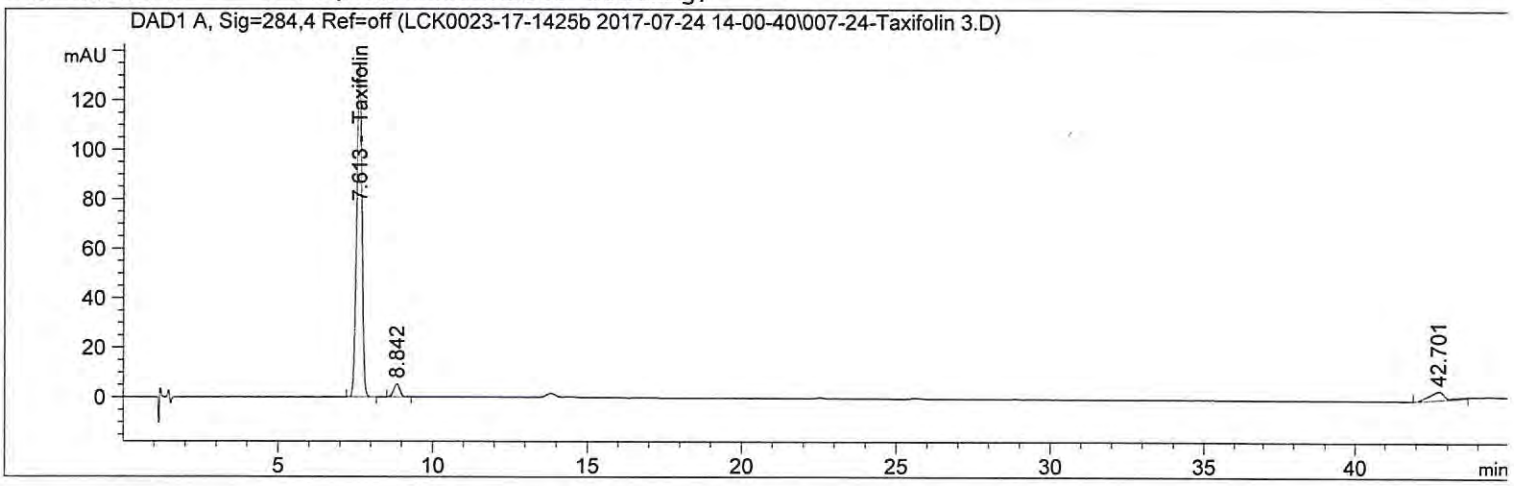
==========

*** 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    : Timothy Sit                    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     : 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
7.613	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

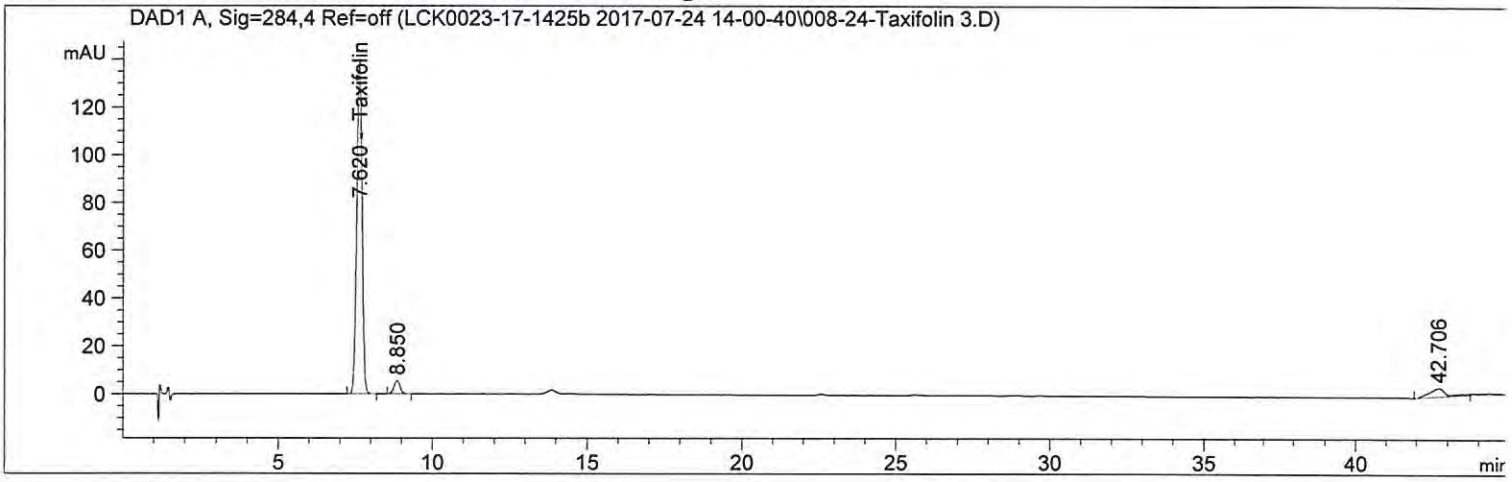
==========

*** 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    : Timothy Sit                    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 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     : 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 ***

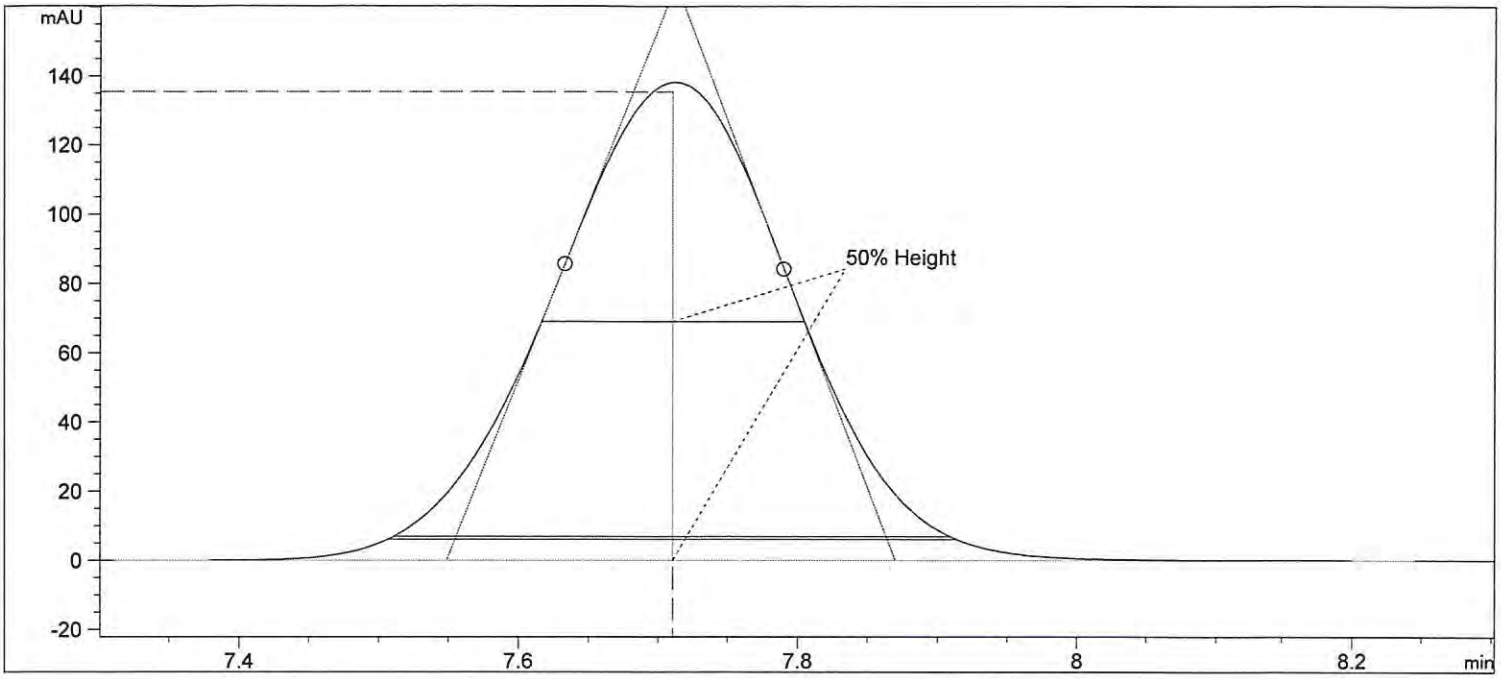
SYSTEM SUITABILITY

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)

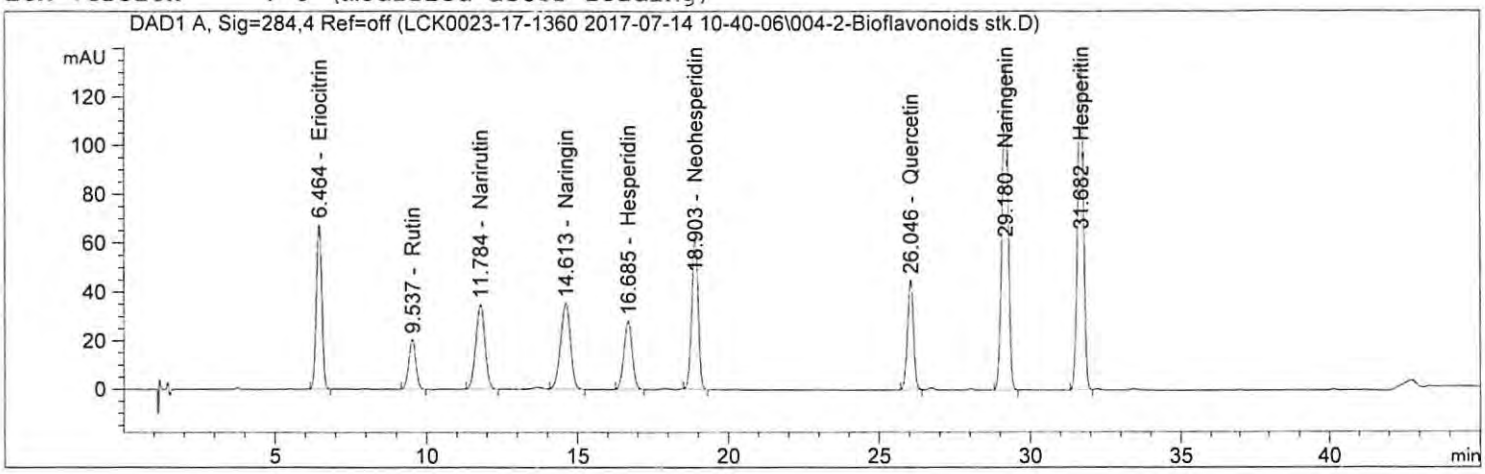
SPECIFICITY

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    : Timothy Sit                    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     : 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)
```




```
==========================================================================
          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 from other common flavonoids in this method.
HY 7/28/17

Totals : 1.05613

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

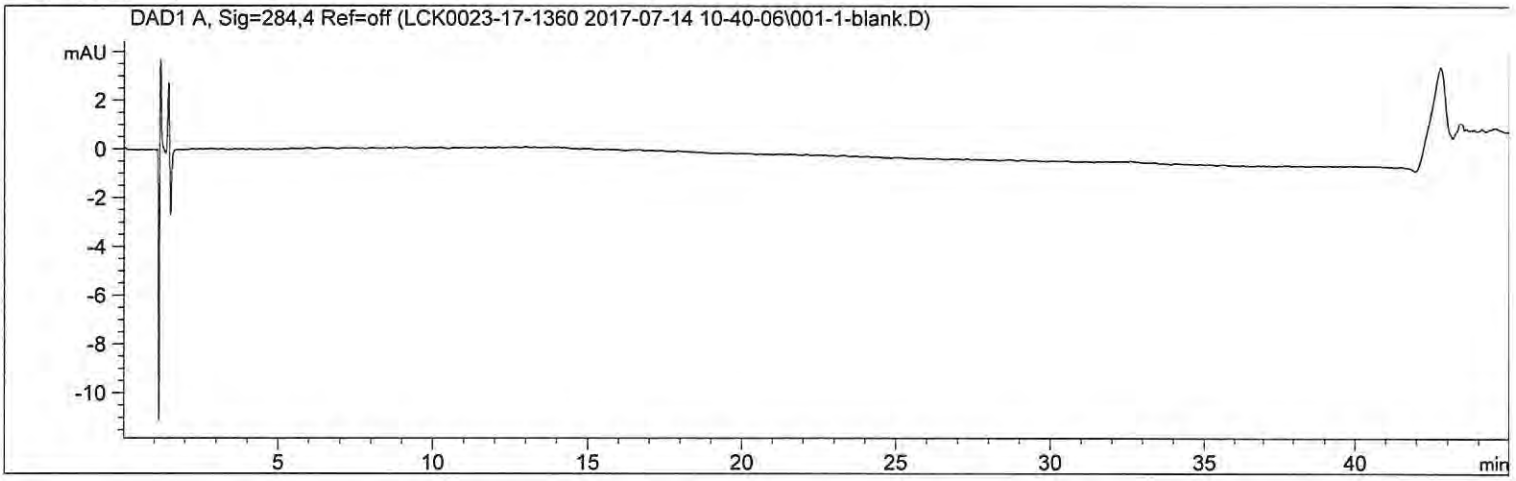
==========

*** 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    : Timothy Sit                    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 Timothy Sit
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 Timothy Sit
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\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 :

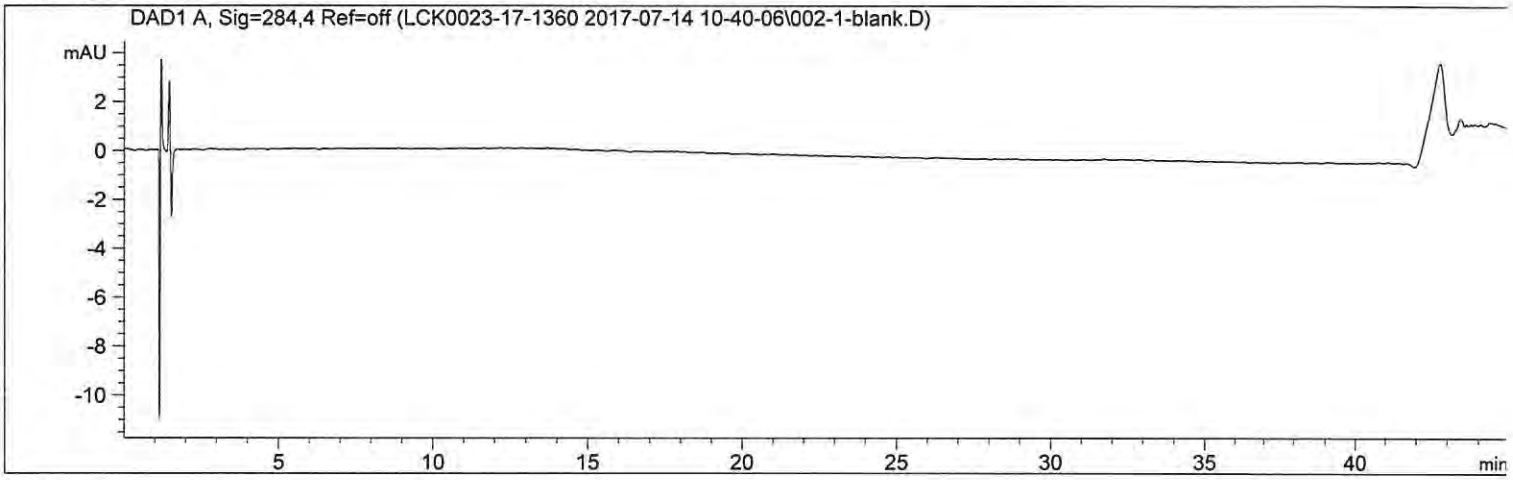
Warning : Calibrated compound(s) not found

==========
*** 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   : Timothy Sit                    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 Timothy Sit
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 Timothy Sit
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 ***

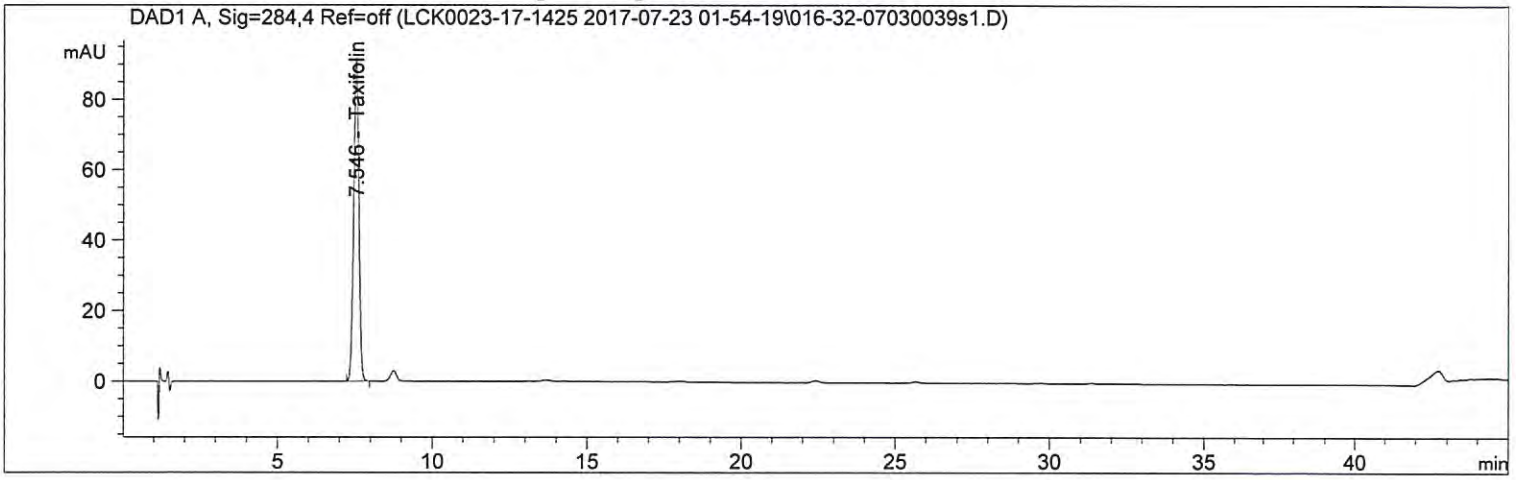
ACCURACY

CHROMATOGRAMS

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\016-32-07030039s1.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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 Timothy Sit
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     : 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 [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
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: (b) (6)

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

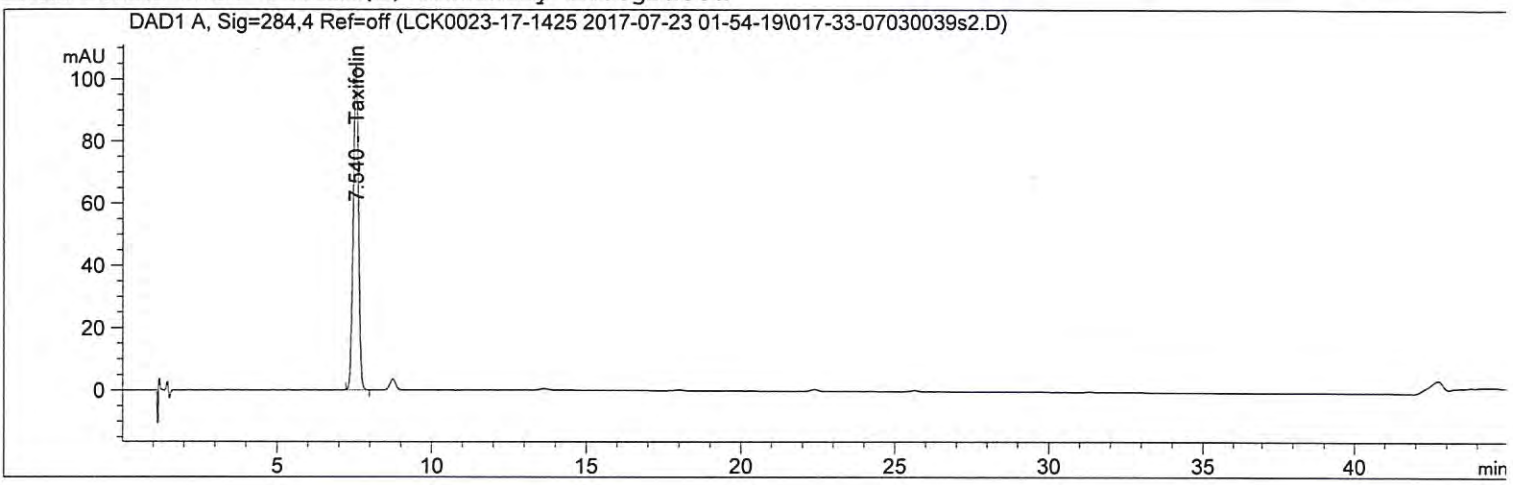
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\017-33-07030039s2.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator   : Brandi Glover                    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 Timothy Sit
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    : 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:                :      3.03100  [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: (b) (6)

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

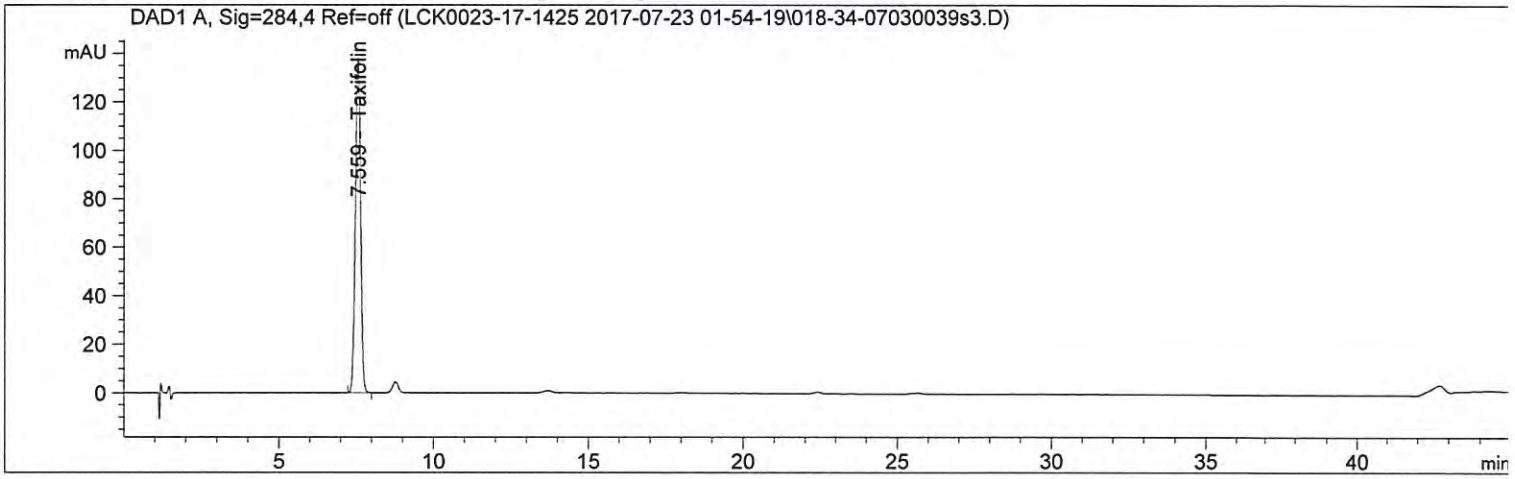
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\018-34-07030039s3.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator   : Brandi Glover                    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 Timothy Sit
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    : 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: (b) (6)

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

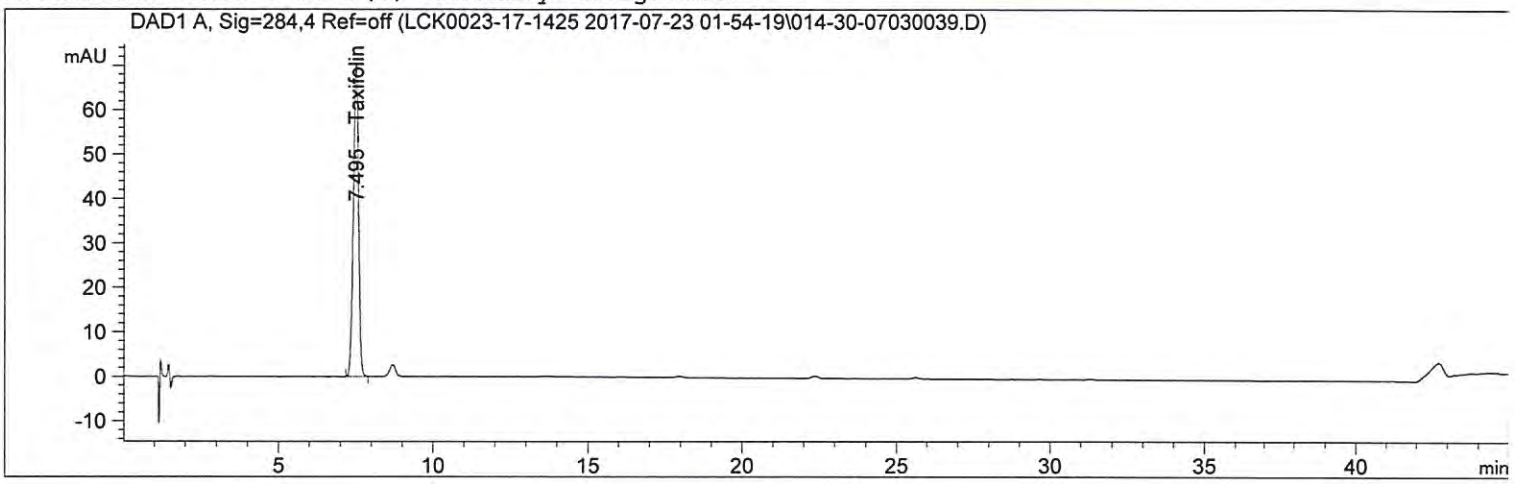
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\014-30-07030039.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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 Timothy Sit
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     : 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  Type     Area      Amt/Area    Amount    Grp   Name
 [min]           [mAU*s]                  %
-------|------|----------|----------|----------|--|------------------
  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: (b) (6)

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

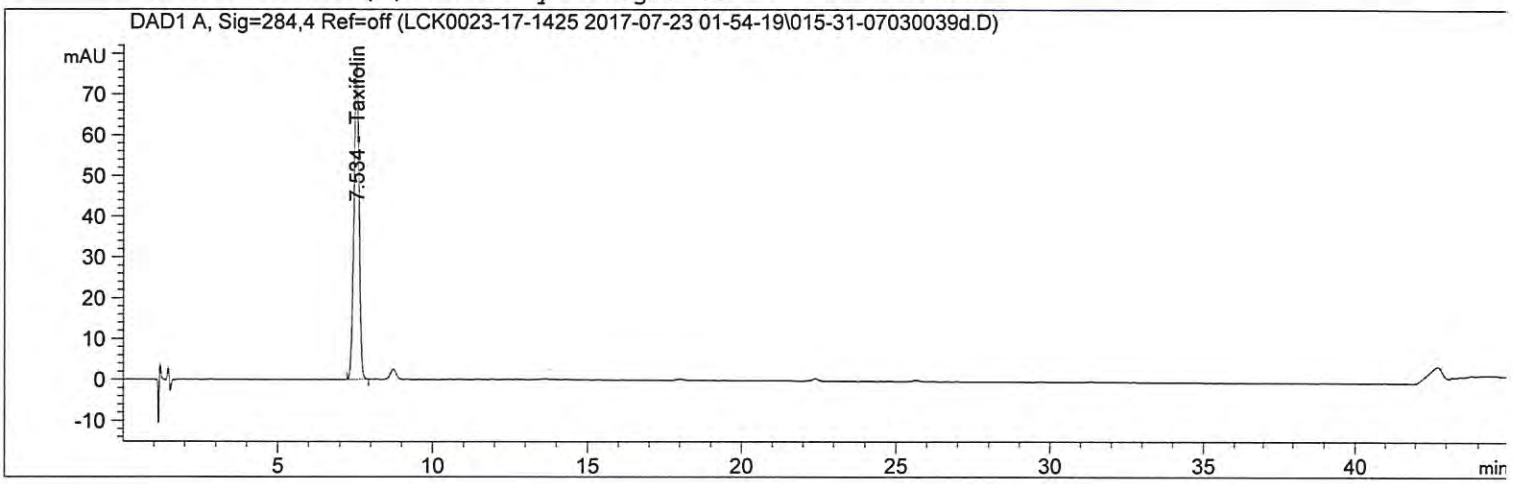
==========

*** End of Report ***

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\015-31-07030039d.D
Sample Name: (b) (6)

```
==========================================================================
Acq. Operator    : Timothy Sit                      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 Timothy Sit
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     : 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:                :      3.27200  [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 7/28/17

Data File D:\Chem32\4\Data\LCK0023-17-1425 2017-07-23 01-54-19\015-31-07030039d.D
Sample Name: (b) (6)

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

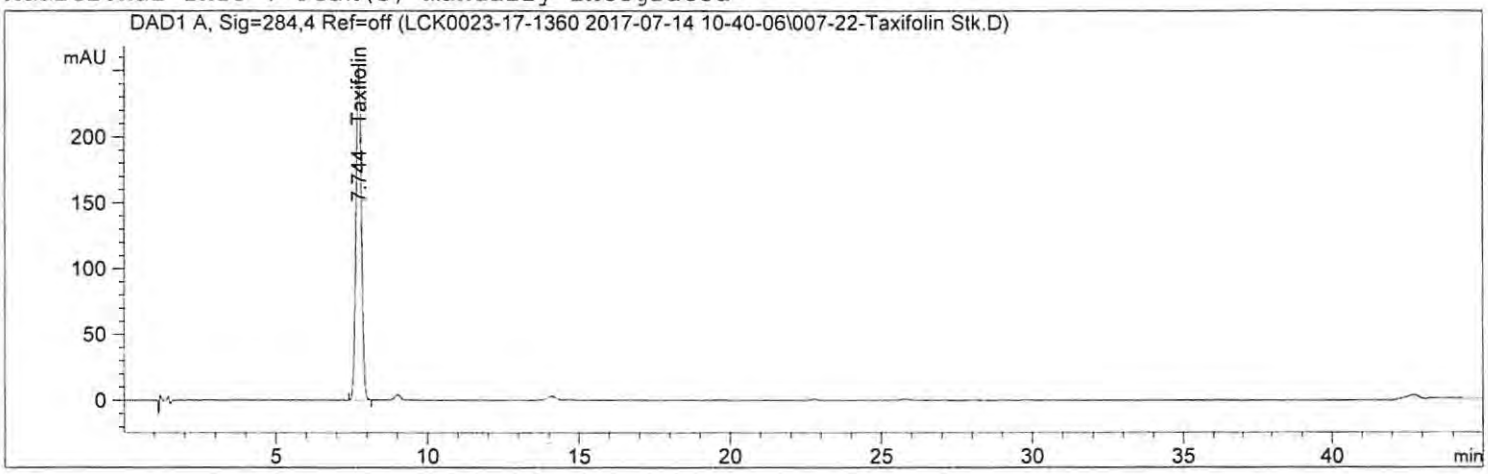
CHROMATOGRAMS

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   : Timothy Sit                      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 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 9:53:52 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)
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

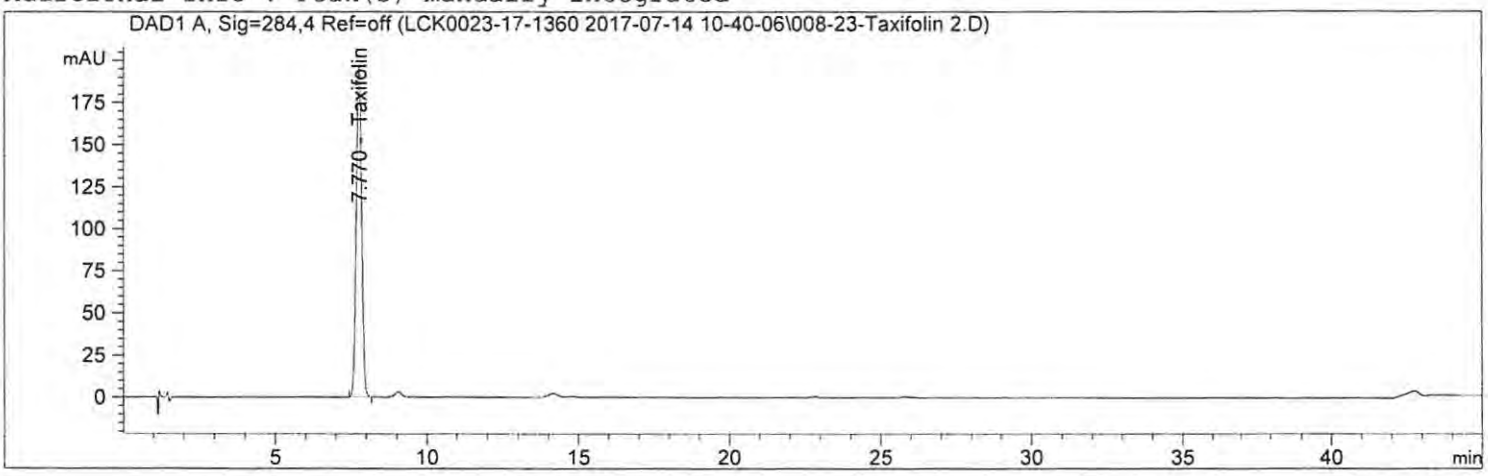
==========

*** 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    : Timothy Sit                    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 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 9:53:52 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)
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   : Timothy Sit                    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 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 9:53:52 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)
Additional Info : Peak(s) manually integrated
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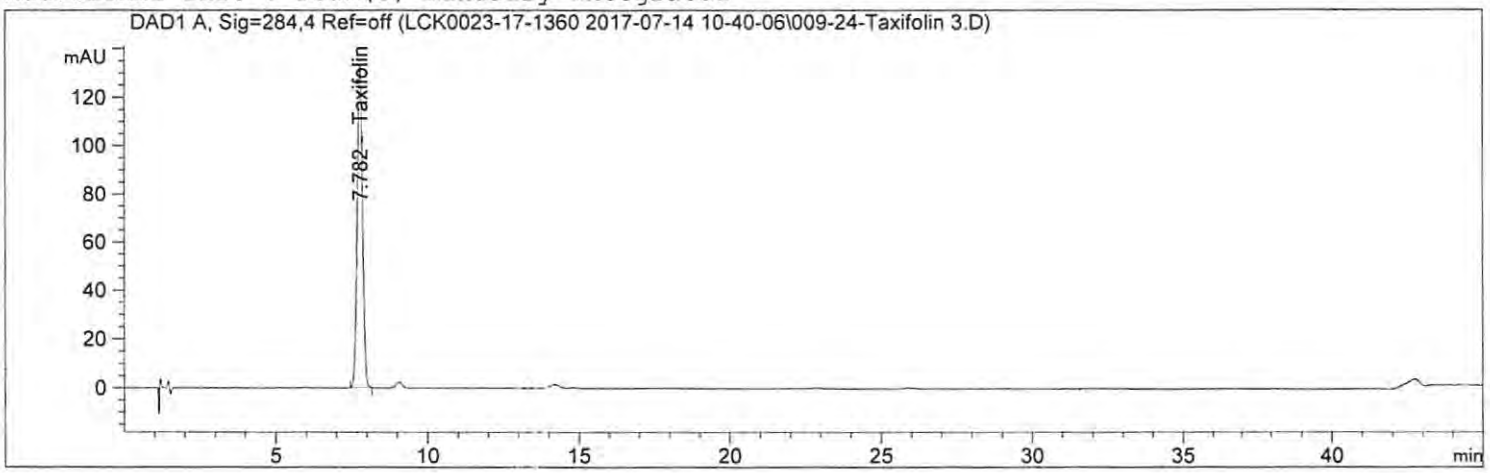


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==========================================================================
           External Standard Report (Sample Amount is 0!)
==========================================================================

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
7.782	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    : Timothy Sit                      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 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 9:53:52 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)
Additional Info  : Peak(s) manually integrated
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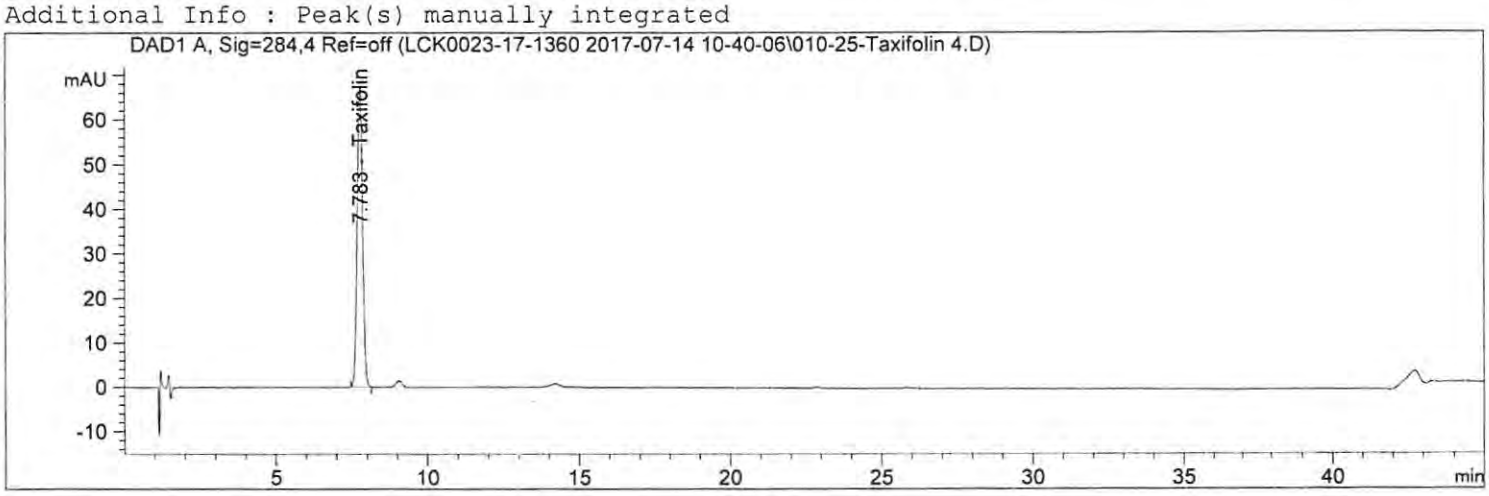


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==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

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
7.783	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   : Timothy Sit                      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 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 9:53:52 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)
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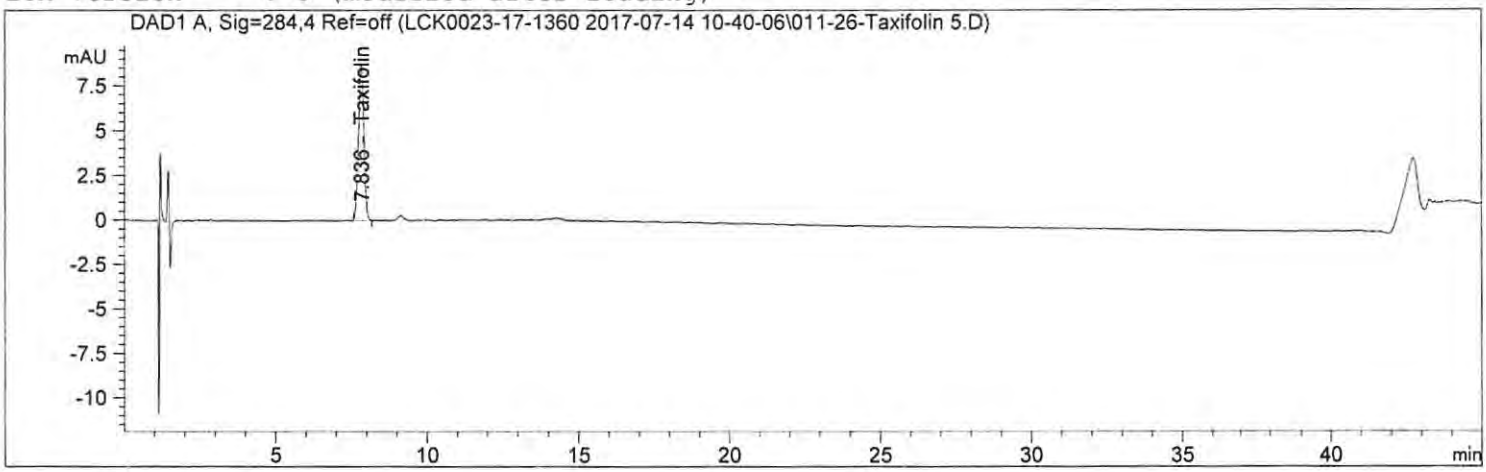


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==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

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

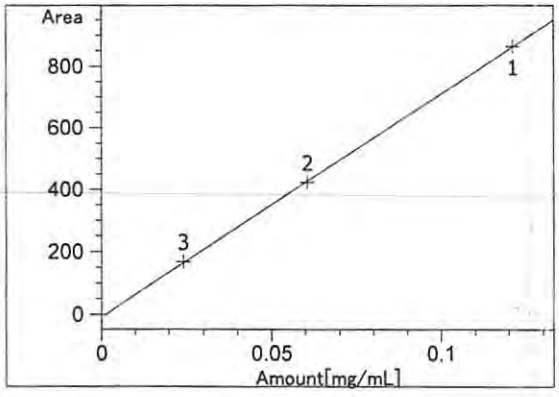
RT	Sig	Lvl	Amount [mg/mL]	Area	Rsp.Factor	Ref	ISTD	#	Compound
6.414	1	3	2.42183e-2	167.28256	1.44775e-4	No	No		Eriocitrin
		2	6.05457e-2	421.37521	1.43686e-4				
		1	1.21091e-1	863.27692	1.40270e-4				
7.836	1	5	9.28187e-3	109.35014	8.48821e-5	No	No		Taxifolin

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				

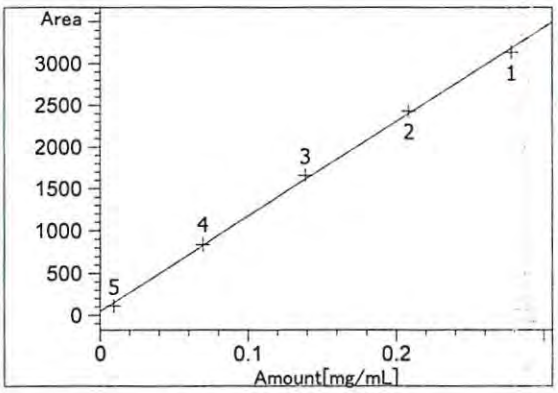
Peak Sum Table

No Entries in table

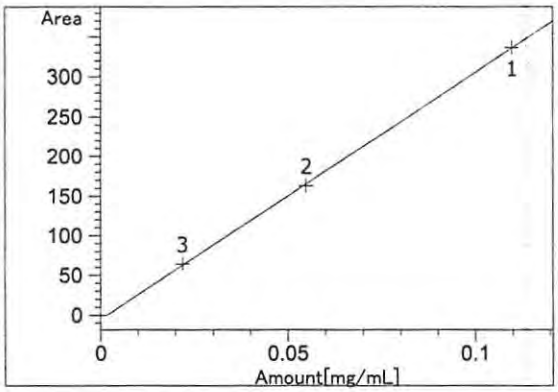
Calibration Curves



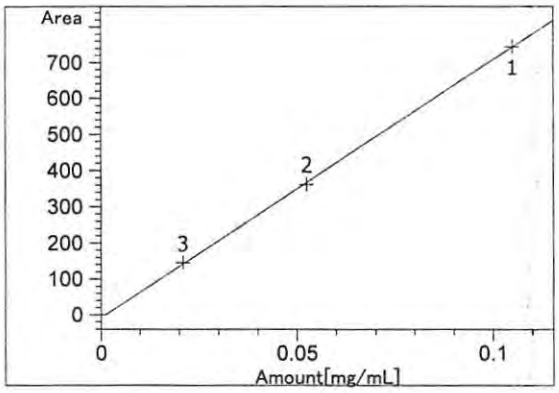
Eriocitrin at exp. RT: 6.414
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99994
Residual Std. Dev.: 5.58027
Formula: y = mx + b
m: 7196.22908
b: -9.81633
x: Amount[mg/mL]
y: Area



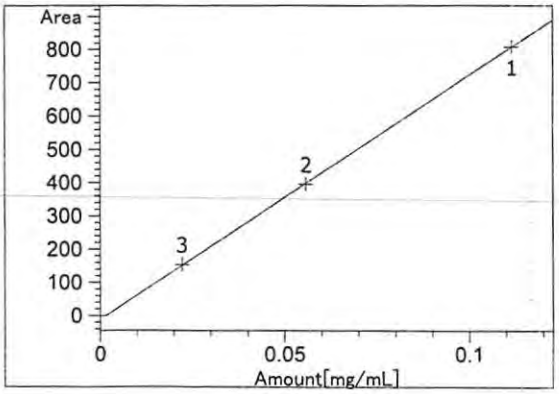
Taxifolin at exp. RT: 7.836
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99944
Residual Std. Dev.: 46.58433
Formula: y = mx + b
m: 11295.72044
b: 43.11883
x: Amount[mg/mL]
y: Area



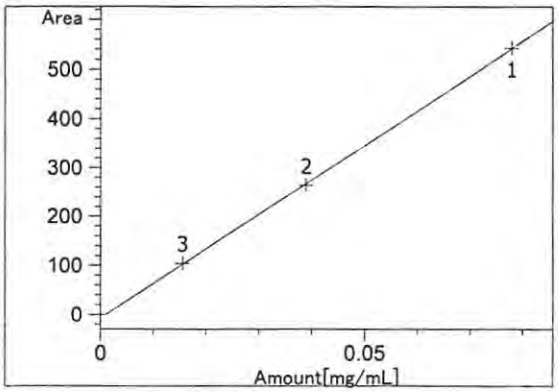
Rutin at exp. RT: 9.420
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99989
Residual Std. Dev.: 2.93082
Formula: y = mx + b
m: 3106.46104
b: -5.42185
x: Amount[mg/mL]
y: Area



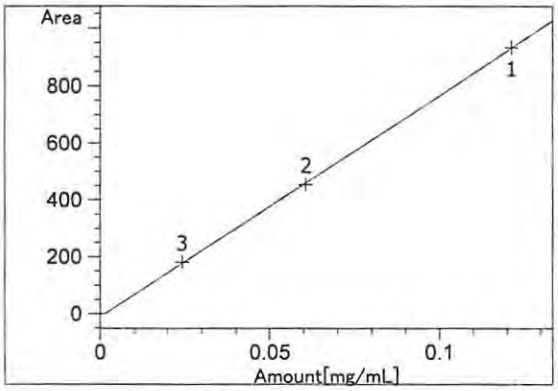
Narirutin at exp. RT: 11.667
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99990
Residual Std. Dev.: 5.93728
Formula: y = mx + b
m: 7164.72135
b: -9.15763
x: Amount[mg/mL]
y: Area



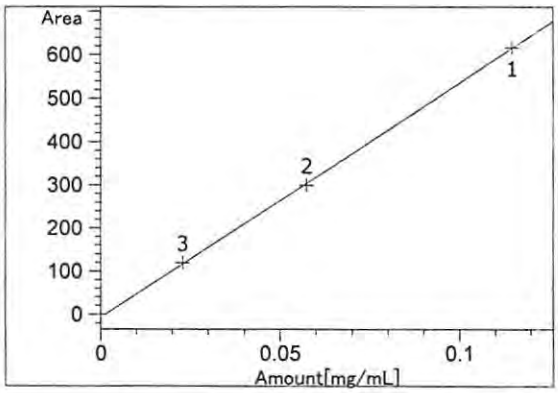
Naringin at exp. RT: 14.472
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99996
Residual Std. Dev.: 4.01579
Formula: y = mx + b
m: 7347.55905
b: -12.53698
x: Amount[mg/mL]
y: Area



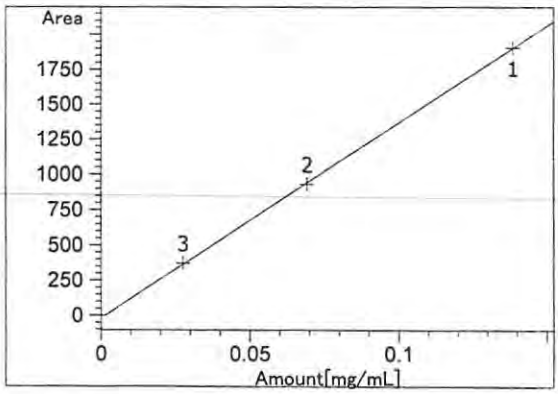
Hesperidin at exp. RT: 16.574
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99993
Residual Std. Dev.: 3.74633
Formula: y = mx + b
m: 7046.58356
b: -7.43117
x: Amount[mg/mL]
y: Area



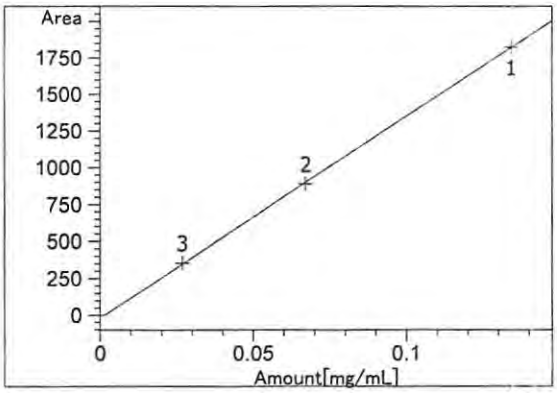
Neohesperidin at exp. RT: 18.801
DAD1 A, Sig=284,4 Ref=off
Correlation: 0.99992
Residual Std. Dev.: 6.94893
Formula: y = mx + b
m: 7769.18633
b: -11.90750
x: Amount[mg/mL]
y: Area



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	—
17-0903-0040	87.0216	88.7567	88.6991	3.3197	—
17-0703-0041	93.5920	95.3157	95.2917	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

End

Work continued to Page ____

SIGNATURE		DATE
Robert C. Zoellner		7/15/2017
DISCLOSED TO AND UNDERSTOOD BY	**DATE**	**WITNESS** / **DATE**
	7/15/17	

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	

From: Katrina Emmel
To: DiFranco, Stephen
Subject: Re: GRN 000826 for dihydroquercetin Cease to Evaluate
Date: Wednesday, September 25, 2019 4:02:05 PM
Attachments: image001.png
image002.png

Good Afternoon Dr. DiFranco,

I am confirming receipt of the cease to evaluate letter on behalf of Blue California.

Thank you,

Katrina

Katrina Emmel, Ph.D.
Senior Scientist/Project Manager/Associate

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> On Sep 25, 2019, at 12:46 PM, DiFranco, Stephen <Stephen.DiFranco@fda.hhs.gov> wrote:
>
> Dear Dr. Emmel,
>
> Please see the attached letter regarding GRAS notice GRN 000826 on dihydroquercetin submitted on behalf of Blue California. If you have any additional questions or concerns, please don't hesitate to contact me atstephen.difranco@fda.hhs.gov or by phone at 240.402.2710.
>
> Best,
>
> Steve

Stephen DiFranco, PhD
Chemist/Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Food Ingredients
U.S. Food and Drug Administration
Tel: 240-402-2710
stephen.difranco@fda.hhs.gov

<image013.png>

<image014.jpg> <image015.jpg> <image016.jpg> <image017.jpg> <image018.jpg>

<GRN 826 Cease to Evaluate Letter_final trans.pdf>

Bonnette, Richard

From: Katrina Emmel <emmel@gras-associates.com>
Sent: Tuesday, December 04, 2018 2:33 PM
To: Bonnette, Richard
Cc: William J. Rowe; Amy Mozingo GRAS Associates
Subject: Submission to the FDA GRAS notification for Dihydroquercetin and Reb E on behalf of Blue California

Hello Mr. Bonnette,

We can confirm that no confidential information is included in either the Dihydroquercetin or Rebaudioside E notifications, and the pages are releasable under FOIA.

Thank you,

Katrina

Katrina Emmel, Ph.D.
Senior Scientist/Project Manager/Associate
GRAS Associates, LLC.

emmel@gras-associates.com

From: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Sent: December-04-18 1:23 PM
To: William J. Rowe <wrowe@nutrasource.ca>
Subject: submission to the FDA GRAS notification program for Reb E

Mr. Rowe,
Similar to my email from earlier, we noted some pages labelled as confidential in the rebaudioside E submission dated November 15, 2018 (on behalf of Blue California). The pages confidential notes are 48-49, and 62-66. Can you confirm that these pages are releasable under FOIA?
Thanks,
Richard

From: Bonnette, Richard
Sent: Tuesday, December 04, 2018 9:50 AM
To: wrowe@nutrasource.ca
Subject: submission to the FDA GRAS notification program for dihydroquercetin

Dear Mr. Rowe,
We've completed a prefiling evaluation of the submission dated November 5, 2018 regarding GRAS uses of dihydroquercetin in food and had a quick detail to clarify before we move forward with filing. We see that in Part 1 and on Form 3667 you have noted that the submission does not contain trade secret or confidential information. We note that on pages 79-83 of the submission and pages 100-103 of the appendix contain stamps or language suggesting these documents are confidential. Just wanted to confirm with you that these pages are indeed releasable. If this is the case,

I'll append your response to this email to the submission and we'll move forward with filing. Let me know if this isn't the case and we can talk about options.
Thanks,
Richard

Richard E. Bonnette, M.S.
Center for Food Safety and Applied Nutrition
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