GRAS ASSOCIATES, LLC

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#734

September 25, 2017

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—Ergothioneine

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 *Ergothioneine.* 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 select conventional foods and beverages at a maximum use level of 5 mg ergothioneine per serving. 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)

Katrina V. Emmel, Ph.D.
Senior Scientist/Associate
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Enclosure: GRAS Notification for Blue California - *Ergothioneine*

GRAS ASSOCIATES, LLC

GRAS Notification

of

Ergothioneine

Food Usage Conditions for General Recognition of Safety

on behalf of

Blue California
30111 Tomas
Rancho Santa Margarita, CA 92688

9/25/17

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Foreword

At the request of Blue California, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of Blue California's ergothioneine (ErgoActive™). The purpose of the evaluation is to ascertain whether the intended food uses of ergothioneine 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 that GRAS Associates act as agent for the submission of this GRAS notification.

Blue California based its GRAS assessment on a large body of information that addressed the safety/toxicity of ergothioneine, history of use of ergothioneine and similar compounds, and compositional details, specifications, and method of preparation of the subject notification ingredient.

Safety/toxicity studies performed with animals and human clinical trials were noted to have value. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

In addition to the product specifications, chemical properties, manufacturing, and safety-related information, Blue California also provided consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through September 19, 2017. A GRAS assessment based primarily on the composite safety information, i.e., based on scientific procedures, was undertaken by Blue California, followed by an Expert Panel review coordinated by GRAS Associates. Those references that were deemed pertinent to the objective at hand are listed in Part 7.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

A. Basis of Exclusion From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

Blue California has concluded that its ergothioneine preparation, ErgoActive™, which meets the specifications described below, is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic 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 on scientific procedures as described in the following sections. The evaluation accurately reflects the intended conditions of food use for the designated ergothioneine preparation.

[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 4/15/17).

Signed:

(b) (6)

Agent for Blue California

Steven Overgaard
President
GRAS Associates, LLC
27499 Riverview Center Blvd.
Suite 212
Bonita Springs, FL 34134

Date: 9/25/17

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 ergothioneine preparation, which is also referred to ErgoActive™, as described in the subject safety evaluation; consequently, ergothioneine preparation, which meet 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 ergothioneine.

D. Conditions of Intended Use in Food

Blue California's ErgoActive™ preparation (5% ergothioneine with maltodextrin) is intended to be added for a variety of technical effects 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 to accomplish its intended technical effect.

E. Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30(a) and (b), Blue California's ErgoActive™ preparation (5% ergothioneine with maltodextrin) has been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

ErgoActive™ 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 and GRAS Associates certify, to the best of our knowledge, that this GRAS notice is a complete, representative, and balanced assessment that includes all relevant information, both favorable and unfavorable, available and pertinent to the evaluation of safety and GRAS status of ergothioneine.

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 and GRAS Associates, LLC certify 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

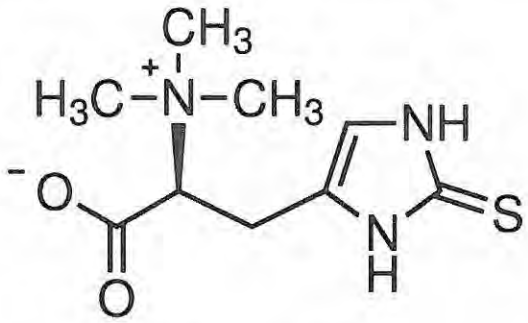
A. Chemical Identity of Ingredient

Ergothioneine is a thiolhistidine derivative, first isolated from rye ergot (*Claviceps purpurea*) in 1909. It occurs naturally in common foods, including mushrooms, offal, cereals, and some varieties of black and red beans (*Phaseolus vulgaris*) (EFSA et al., 2016).

Common or Usual Name: Ergothioneine
Chemical Name: L-Ergothioneine
Synonyms: (2S)-3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)-2-(trimethylammonio)-propanoate; (S)-α-Carbosy-N,N,N-trimethyl-2-mercapto-1H-imidazole-4-ethanaminium inner salt; Thioneine; L(+)-Ergothioneine
CAS Number: 497-30-3
Molecular Formula: $C_9H_{15}N_3O_2S$
Molecular Mass: 229.30 Da

The chemical structure of ergothioneine is shown in Figure 1.

Figure 1. Chemical Structure of Ergothioneine[a]



[a] From Sigma (2017).

B. Manufacturing Processes

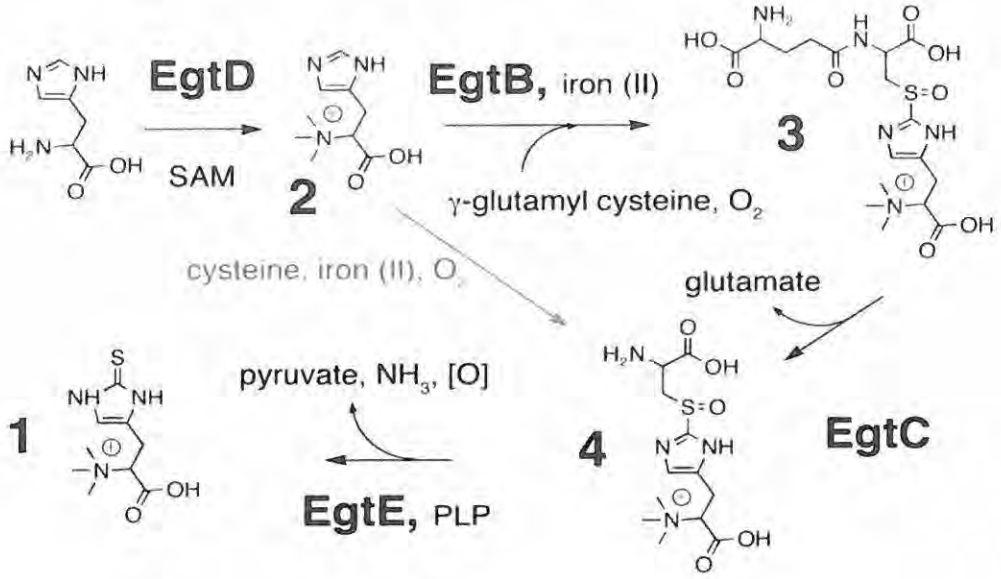
Blue California uses a novel multi-step biosynthesis pathway process to manufacture ergothioneine using *Escherichia coli* (*E. coli*) that contain four biosynthesis proteins that facilitate the enzymatic conversion of histidine to ergothioneine.

1. Production of the Microorganism

The parental strain is *E. coli* K12 MG1655. This bacterium normally lives in the intestines of people and animals. Most *E. coli* are harmless and are an important part of a healthy human intestinal tract. The microbe is gram-negative, non-spore forming, facultative anaerobe, nonpathogenic and nontoxigenic, and has a long history of safe industrial use. The *E. coli* K12 strain is the most commonly used industrial strain, and complies with 21 CFR 170.36 specifications.

Four genes encoding for a formylglycine-generating enzyme-like protein (EgtB), a glutamine amidotransferase (EgtC), a histidine methyltransferase (EgtD), and a pyridoxal 5-phosphate binding protein (EgtE), respectively, were cloned and expressed in *E. coli* K12 MG1655. The plasmids carrying related genes were constructed following standard molecular cloning protocols. EgtD and EgtB were cloned into pConB7A with ampicillin (Amp) resistance, whereas EgtC and EgtE were inserted into pConA5K with kanamycin (Kan) resistance. These two constructs have been reported in the patent application PCT/US2015/027977. Together, the EgtB, EgtC, EgtD, and EgtE proteins convert histidine to ergothioneine, as shown in Figure 2.

Figure 2. Bioconversion of Histidine to Ergothioneine



The two plasmids were confirmed by sequencing and integrated into the genome to avoid using any antibiotic selection. They were then transformed into the *E. coli* parent strain to obtain one working strain.

2. Fermentation Process

The glycerol stocks of the transformed *E. coli* are stored at -80°C. To prepare the inoculating culture, the stock is removed from the freezer, thawed in a water bath, and streaked on a plate prepared with appropriate antibiotics to separate a single colony. The plate medium is prepared with yeast peptone, yeast extract, sodium chloride, and agar. The media is sterilized at 121°C for 30 minutes, and after streaking, the plates are cultured at 37°C for 10-16 hours, under static conditions.

A single colony is chosen from the plate and inoculated into seed culture medium comprised of yeast peptone, yeast extract, and sodium chloride, and which has been previously sterilized at 121°C for 30 minutes. The inoculated seed culture medium is maintained at 37°C for 8-10 hours while shaken at 220 rpm, to an OD_{600} ~2.0.[2]

A basal medium is prepared from monopotassium phosphate, dipotassium phosphate, citric acid, yeast extract, antifoaming agent, ammonium hydroxide, and distilled water, adjusted to pH 6.7 with aqueous ammonia, and sterilized in the fermenter. Added to that is a pre-sterilized media composed of magnesium sulfate heptahydrate, thiamine, and glucose dissolved in distilled water and sulfuric acid, which is filter-sterilized with a 0.22 μm sterile filter. Feed medium, composed of magnesium sulfate heptahydrate, glucose, and ferric citrate, prepared in distilled water with sulfuric acid, and which is filter-sterilized with a 0.22 μm sterile filter is also added to the fermenter.

[2] Blue California uses older, larger cells to perform the measurement.

The seed culture is inoculated into the fermenter at a concentration of 7% of the initial fermentation volume, along with ammonium iron (II) sulfate. The culture temperature is controlled at 37°C, at pH 7.0 (with aqueous ammonia), and a dissolved oxygen level of 30%, with agitation and aeration cascading. After 2 hours, aliquots are sampled to detect the sugar concentration and OD_{600}. The glucose concentration is maintained between 0.1-0.2 g per L, for a specific growth rate of 0.1-0.3 h^{-1}. For protein expression, the genes are induced in the mid-exponential phase (OD_{600} 35-40). The fermenter is held at 25°C until a final concentration of 500 ng per mL is achieved. Fermentation runs are terminated after 16-20 hours of induction. The supernatant is then collected by centrifugation.

3. Extraction and Purification

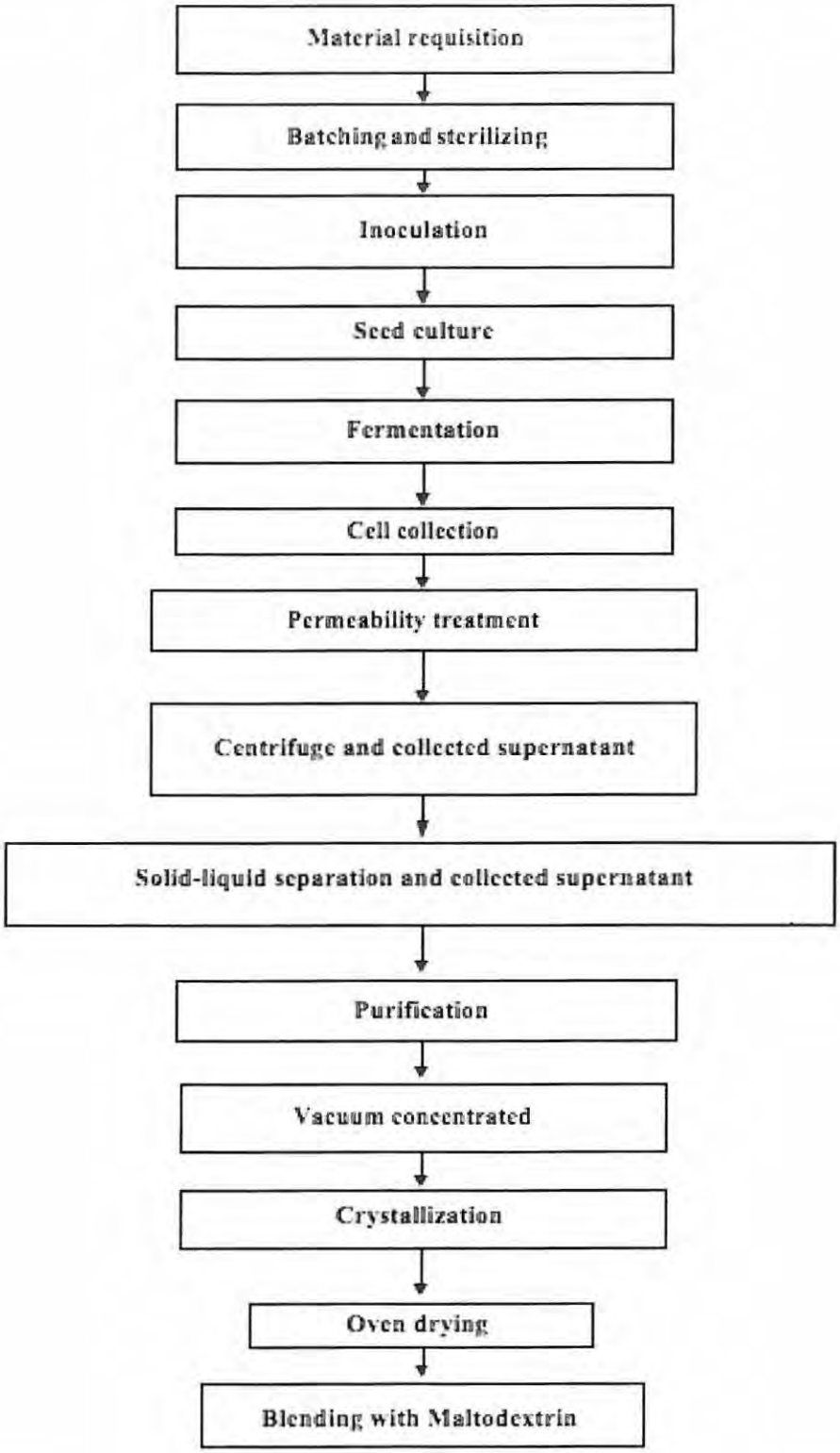
The supernatant is transferred to an ion-exchange resin column, which is washed with warm water and eluted with sodium chloride solution. The eluent is condensed with a wipe-film evaporator. The condensate is transferred to a crystallization tank and chilled. The resulting crystals are re-dissolved in water, decolorized with activated charcoal, and dried in a baking oven before being crushed into fine powder.

A manufacturing process flow chart for the production of the ergothioneine raw material is provided in Figure 3.

The ergothioneine (≥ 98%) raw material is then blended with a maltodextrin carrier derived from *Zea mays* seed, resulting in Blue California's ErgoActive™ finished product, which contains 5% ergothioneine.

Raw materials used in the manufacturing process are suitable food-grade materials, and are used in accordance with applicable US Federal Regulations. Maltodextrin from *Zea mays* (corn) is GRAS for food use, with no limitation other than good manufacturing practice, under 21 CFR 184.1444. All filters and resins comply with 21 CFR 173 specifications. Supporting documentation for the raw materials and processing aids is provided Appendix 1.

Figure 3. General Reaction Scheme of Ergothioneine Raw Material Production Process



C. Product Specifications

1. Specifications for Ergothioneine

There are no known established standardized specifications for ergothioneine; however, specifications for Tetrahedron's ergothioneine preparation were reviewed and reported by EFSA et al. (2016). Tetrahedron's specifications, compared with the specifications and analytical results for Blue California's ergothioneine raw material, are shown in Table 1. These data demonstrate that Blue California's ergothioneine raw material is substantially chemically equivalent to Tetrahedron's ergothioneine material.

Table 1. Specifications and Analysis for Blue California's Ergothioneine Raw Material

Physical and Chemical Parameters	Tetrahedron Specifications[a]	Blue California's Ergothioneine Raw Material				
		Specification	Method	Lot No 3330-160524	Lot No 3330-160605	Lot No 3330-160610
Appearance Form & Color	White Powder	White Powder	Visual	White Powder	White Powder	White Powder
Optical Rotation	$[\alpha]_D \geq (+)$ 122° (c=1, H_2O)	NS	NA	NS	NS	NS
Assay- HPLC	≥ 99.5%	≥ 98%	HPLC	99.1%	99.2%	99.2%
Total Residual Solvents[b]	< 1,000 ppm	NS	NA	NS	NS	NS
Loss on Drying	< 0.5%	≤ 5%	USP 34	1.80%	< 2%	1.70%
Impurities	< 0.8%	NS	NA	NS	NS	NS
Heavy Metals	NS	< 10 ppm	USP 34	Pass	Pass	Pass
Lead	< 3 ppm	< 1 ppm	ICP-MS	<0.20 ppm	<0.20 ppm	<0.20 ppm
Arsenic	NS	< 1 ppm	ICP-MS	< 0.50 ppm	< 0.50 ppm	< 0.50 ppm
Cadmium	< 1 ppm	< 1 ppm	ICP-MS	< 0.25 ppm	< 0.25 ppm	< 0.25 ppm
Mercury	< 0.1 ppm	< 1 ppm	ICP-MS	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm
Total Viable Count	≤ 1,000 cfu/g	< 5,000 cfu/g	AOAC	< 1,000 cfu/g	< 1,000 cfu/g	< 1,000 cfu/g
Total Coliform	NS	< 100 cfu/g	AOAC	< 3 cfu/g	< 3 cfu/g	< 3 cfu/g

Physical and Chemical Parameters	Tetrahedron Specifications[a]	Blue California's Ergothioneine Raw Material				
		Specification	Method	Lot No 3330-160524	Lot No 3330-160605	Lot No 3330-160610
Total Yeast & Mold	≤ 100 cfu/g	< 100 cfu/g	AOAC	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
E. coli	Absent in 1 g	Negative	AOAC	ND	ND	ND
Salmonella	NS	Negative	AOAC	Pass	Pass	Pass

[a] From EFSA et al. (2016)
[b] Total residual solvents as sum of methanol, ethyl acetate, isopropanol, and ethanol.
NS Not specified; NA Not applicable; ND Not detected

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

Blue California has adopted product specifications for its ergothioneine raw material that meet or exceed Tetrahedron's specifications, as reported by EFSA (2016), for ergothioneine as a consumable human food substance. This raw material is then blended with maltodextrin to produce a 5% ergothioneine finished product. The compositions of five lots of Blue California's ErgoActive™ 5% ergothioneine preparation, as well as product specifications, are provided in Table 2.

Table 2. Specifications for Blue California's Ergothioneine Preparation

Physical and Chemical Parameters	Blue California ErgoActive™ Specifications	Results of Batch Numbers				
		3332-1606-01	3332-1610-03	3332-1611-04	3332-1611-05	3332-1608-02
Appearance Form & Color	White Powder	Pass	Pass	Pass	Pass	Pass
Bulk Density	0.3-0.6 g/mL	0.48 g/mL	0.50 g/mL	0.50 g/mL	0.48 g/mL	0.50 g/mL
Tap Density	≥ 0.6 g/mL	0.70 g/mL	0.72 g/mL	0.72 g/mL	0.71 g/mL	0.72 g/mL
Particle Size	> 90% through mesh #80 sieve	97%	97%	97%	96%	97%
Assay- HPLC	≥ 5%	5.28%	5.38%	5.35%	5.38%	5.35%
Loss on Drying	≤ 6%	5.30%	5.20%	5.20%	5.30%	5.20%
Heavy Metals	< 10 ppm	Pass	Pass	Pass	Pass	Pass
Lead	< 1 ppm	<0.20 ppm	<0.20 ppm	<0.20 ppm	<0.20 ppm	<0.20 ppm
Arsenic	< 1 ppm	<0.50 ppm	<0.50 ppm	<0.50 ppm	<0.50 ppm	<0.50 ppm
Cadmium	< 1 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm

Physical and Chemical Parameters	Blue California ErgoActive™ Specifications	Results of Batch Numbers				
		3332-1606-01	3332-1610-03	3332-1611-04	3332-1611-05	3332-1608-02
Mercury	< 1 ppm	<0.10 ppm	<0.10 ppm	<0.10 ppm	<0.10 ppm	<0.10 ppm
Total Plate Count	< 5,000 cfu/g	< 500 cfu/g	< 500 cfu/g	< 500 cfu/g	< 500 cfu/g	< 500 cfu/g
Total Coliform	< 100 cfu/g	< 3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
Total Yeast & Mold	< 100 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
E. coli	Negative	ND	ND	ND	ND	ND
Salmonella	Negative	Pass	Pass	Pass	Pass	Pass

ND = not detected

Blue California analyzes its ergothioneine preparation by HPLC. A method verification report, which includes representative chromatograms, is provided in Appendix 2. 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 ergothioneine are provided in Appendix 3.

Blue California has also analyzed representative lots of material for pesticides (Appendix 4), and no concerns were noted upon review.

The collection of these reports demonstrates that the substance is well characterized and meets the established purity criteria.

D. Physical or Technical Effect

Ergothioneine will be added to conventional foods and beverages as a nutrient supplement as defined by 21 CFR 170.3(o)(20).

E. Stability

1. Published Stability Data on Ergothioneine

Tetrahedron reported that results of a stability study on its ergothioneine material was expected to remain stable and within specification under normal storage conditions (2-8°C) for one year, based upon a six-month accelerated stability study (40 ± 2°C, 75 ± 5% relative humidity) and a 12-month intermediate stability study (30 ± 2°C, 65 ± 5% relative humidity). The EFSA panel "considers that the data provided sufficient information with respect to the stability" of Tetrahedron's ergothioneine (EFSA et al., 2016).

2. Stability Data for Blue California's Ergothioneine Raw Material

Blue California is currently conducting a 36-month shelf-stability study on its ergothioneine raw material under ambient storage conditions. A summary of the shelf-stability results is presented in Table 3, and a product stability report is provided in Appendix 6.

Table 3. Blue California's Ergothioneine (≥ 98%) Raw Material Stability Data

Ergothioneine Lot# FB160307		
Duration	**Appearance**	**Ergothioneine**
t=0	White Crystalline Powder	99.5%
6 months	White Crystalline Powder	99.3%
12 months	White Crystalline Powder	99.4%
Ergothioneine Lot# FB160310		
Duration	**Appearance**	**Ergothioneine**
t=0	White Crystalline Powder	99.3%
6 months	White Crystalline Powder	99.1%
12 months	White Crystalline Powder	99.2%
Ergothioneine Lot# FB160313		
Duration	**Appearance**	**Ergothioneine**
t=0	White Crystalline Powder	99.6%
6 months	White Crystalline Powder	99.5%
12 months	White Crystalline Powder	99.5%

The stability data in the scientific literature for ergothioneine, along with Blue California's stability testing results for their ergothioneine (≥ 98%) raw material, support the position that Blue California's ErgoActive™ 5% ergothioneine preparation is well-suited for the intended food uses, as no change in stability is expected to be observed after blending with maltodextrin.

In addition, Blue California claims a 2-year shelf life for ErgoActive™ 5% ergothioneine.

PART 3. DIETARY EXPOSURE

The subject ergothioneine preparation is intended to be used in a limited number of human food categories as a nutrient supplement. The intended food use categories and use levels of ErgoActive™ are presented in Table 4.

Table 4. Blue California's Intended ErgoActive™ Food Uses

FOOD CATEGORY	LEVEL OF USE
Cakes, cookies, pastries (including granola bars)	5 mg/serving
Coffee	5 mg/serving
Tea	5 mg/serving
Fruit drinks and ades	5 mg/serving
Carbonated soft drinks	5 mg/serving
Candy containing chocolate	5 mg/serving

A. Estimate of Dietary Exposure to ErgoActive™

In 2016, EFSA reviewed a petition for the use of synthetic L-ergothioneine as a novel food ingredient at levels of up to 5 mg per serving in specific conventional foods: alcohol-free beverages, cereal bars, milk, fresh dairy products, and chocolates, as well as in dietary supplements with a recommended daily dose of 30 mg ergothioneine per day for adults and 20 mg ergothioneine per day for children (EFSA et al., 2016).

As part of their evaluation of estimated dietary intakes of ergothioneine in the European population, EFSA reviewed a recently published intake assessment by Ramirez-Martinez et al. (2016). EFSA noted that the mean chronic dietary intake of ergothioneine in a number of European countries ranged from 0.051 to 0.255 mg per kg bw per day for adult consumers and 0.306 to 0.409 mg per kg bw per day for adolescent consumers.

The estimated combined intake from all sources, including the background diet, was estimated to be less than 1.7 mg per kg body weight (bw) per day for adults (excluding pregnant and breastfeeding women) and less than 3.7 mg per kg bw per day for children over 3 years of age. The EFSA Panel concluded that based on a no observed effect level (NOAEL) of 800 mg per kg by per day for ergothioneine, the estimated combined intake values were within sufficient margins of safety (470 for adults and 261 for children) (EFSA et al., 2016).

Ramirez-Martinez et al. (2016) also estimated the dietary intakes of ergothioneine in the US population, where the consumption of mushrooms, beans, meat organs, and oat meal ---the main dietary sources of ergothioneine--- was considered. In the US, the mean chronic dietary intake of ergothioneine was estimated to be 0.153 mg per kg bw per day for adult consumers and 0.299 mg per kg bw per day for adolescent consumers. As compared with the European populations, US consumers are estimated to have a lower overall consumption of ergothioneine in the diet.

Blue California intends to use ErgoActive™ in conventional foods similar to those identified in the EFSA review, at a use level to provide 5 mg of ergothioneine per serving[3], as detailed in Table 4. FDA's methodology was used 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 5.

Table 5. Conventional Foods Dietary Intake Estimations for Ergothioneine

Food Category	Maximum Use Level of Ergothioneine (mg/serving)	USDA Mean Grams of Food Consumed (All Individuals)[a]	RACC serving size (g)[b,c]	Mean mg Ergothioneine Consumed (All Individuals)	Mean x 2 mg Ergothioneine Consumed (All Individuals)	Reference Number, Page Number
Cakes, Cookies, pastries (including granola bars)	5	38	40	4.8	9.5	(2) table 9.1, page 26
Coffee	5	259	360	3.6	7.2	(2) table 9.7, page 32
Tea	5	133	360	1.8	3.7	(2) table 9.7, page 32
Fruit drinks and ades	5	95	360	1.3	2.6	(2) table 9.7, page 32
Carbonated soft drinks	5	332	360	4.6	9.2	(2) table 9.7, page 32
Candy containing chocolate	5	4	30	0.7	1.3	(1) Appendix B, page 244
Total				16.79	33.58	

[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.

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 7/6/17).
[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 7/6/17).

In addition, the estimated daily intake of ergothioneine from ErgoActive™ was determined for specific subpopulations, as shown in Table 6.

[3] This is equivalent to a use level of 100 mg ErgoActive™, as the finished product contains 5% ergothioneine.

Table 6. Estimated Daily Intake of Ergothioneine from Conventional Foods by Subpopulation

Subpopulation	Default Body Weight[a] (kg)	Estimated Daily Intake (mg/kg bw/day)	
		Mean mg ErgoActive™ Consumed (All Individuals)	Mean x 2 mg ErgoActive™ Consumed (All Individuals)
Adults	70	0.24	0.48
Children, 3-10 years old	21.6	0.78	1.55
Children, 10-14 years old	42	0.4	0.8
Children, 14-18 years old	60	0.28	0.56

[a] Default body weight values obtained from EFSA (2011).

In adults, the combined intake of ergothioneine from fortified conventional foods (0.48 mg per kg bw per day) plus high consumption from the background diet (0.153 mg per kg bw per day) would result in a maximum ergothioneine intake of 0.633 mg per kg bw per day. For adolescents, the combined intake from fortified conventional foods from the high-consumption subpopulation of children 3 to 10 years old (1.55 mg per kg bw per day) plus high consumption from the background diet (0.299 mg per kg bw per day) would result in a maximum ergothioneine consumption of 1.849 mg per kg bw per day.

In all subpopulations, the estimated combined daily intake of ergothioneine is lower than the levels evaluated by EFSA: 1.7 mg per kg bw per day for adults and 3.7 mg per kg bw per day for children. It is important to note that for the subpopulation of children aged 3 to 10, the combined daily intake in Table 6 is highly overestimated by including coffee and tea drinks in their total consumption, as this subpopulation is not expected to be high consumers of these conventional foods.

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 ErgoActive™.

C. Dietary Exposure to Contaminants or Byproducts

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

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 ergothioneine prior to January 1, 1958.

Ergothioneine is naturally occurring in a number of foods that are part of the typical American diet, including mushrooms, red and black beans, cereal, and offal (EFSA et al., 2016).

2. U.S. Regulatory History

A search of FDA's GRAS Notification database using the term "ergothioneine" resulted in no results.

On April 20, 2012, NutraIngredients reported that Oxis International completed the "self-affirmed GRAS process" for their L-ergothioneine product, EGT™, in the fall of 2011 (Watson, 2012).

Tetrahedron, a company based in Paris, France, announced an independent GRAS assessment of their ergothioneine product, ERGONEINE™, in February, 2015 (Tetrahedron, 2015).

3. Canadian Regulatory History

A search of the Health Canada website, using the term "ergothioneine" resulted in no results relevant to food additive regulations.

4. European Regulatory History

In October 2016, the European Food Safety Authority (EFSA) responded to a novel food application for synthetic L-ergothioneine (Ergoneine®) submitted by Tetrahedron. The EFSA Panel noted that the specifications, representative batch data, and stability data presented for Tetrahedron's Ergoneine® was sufficient and did not present any safety concerns. For non-alcoholic beverages, cereal bars, milk, fresh dairy products, and chocolate, up to 5 mg ergothioneine per serving use level was indicated. As a food supplement, a daily dose of 30 mg per day for adults and 20 mg per day for children was also proposed. Using a NOAEL of 800 mg per kg bw per day derived from two subchronic toxicity studies in rats and estimated intake levels, the EFSA Panel determined a margin of safety of 470 for adults and 216 for children three and older. It should be noted that pregnant and breastfeeding women are not part of the target population. The Panel concluded that Ergoneine® is safe as a novel food under the intended conditions of use proposed by Tetrahedron (EFSA et al., 2016).

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 (AVA), and FDA Taiwan using the term "ergothioneine" resulted in no regulatory results for use as a food additive.

6. Other Regulatory History

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

PART 6. NARRATIVE

A. Discussion on Safety Data on Ergothioneine

1. Acute Toxicity Studies

The acute oral toxicity of L-ergothioneine (Tetrahedron, Vincennes, France, >98% purity) was evaluated in six, nulliparous female, 8-week-old Sprague-Dawley Rj Han:SD rats (Forster et al., 2015). Ergothioneine (EGT, 100 mg per mL, dissolved in water) was administered as a single dose by gavage to three rats according to OECD Technical Guidance 423. The first group of rats was fasted overnight and treated with 2,000 mg EGT per kg bw at a dosage volume of 20 mL per kg bw. The second group of three females was treated 7 days later. All animals were observed for 14 days post-treatment. Daily observations included mortality, morbidity, and clinical signs. Body weight was recorded on days 1, 8, and 15. Post mortem macroscopic examination was conducted on all animals and included digestive tract, heart, kidneys, liver, lungs, pancreas, and spleen. There was no mortality and no treatment-related findings of weight gain, morbidity or pathology. The authors concluded the Acute Toxicity Estimate was >2,000 mg per kg bw, the highest dose tested. EGT is not classified for toxicity according to European Regulation 1272/2008. The median lethal dose (LD_{50}) of EGT is greater than 2,000 mg per kg bw.

2. Subacute Toxicity Studies

In a 2-week, subacute toxicity, dose-ranging study, EGT (Tetrahedron, Vincennes, France, >98% purity) was evaluated in Sprague-Dawley Crl CD (SD) rats (n=2 per sex per dose) (Forster et al., 2015). EGT was mixed with SAFE A04 C P2.5 diet (SAFE, Augy, France) at 0, 0.3, 0.5, and 0.9% by weight. Animals were observed twice daily for mortality and morbidity and once daily for clinical signs. Body weight was recorded before treatment, on day 1 and then twice per week. Food consumption was recorded twice per week. Macroscopic post-mortem examination was performed on all animals at sacrifice.

There was no mortality and no differences among the groups in clinical signs of toxicity, body weight or food consumption, or in treatment-related findings in the post-mortem macroscopic examination. The achieved dose levels of EGT in the 0.3%, 0.5%, and 0.9% groups were 222 ±

28.4, 348 ± 19.6, and 662 ± 82.5 mg per kg bw per day for males and 236 ± 13.8, 398 ± 27.4, and 721 ± 57.6 mg per kg bw per day for females, respectively.

In a 28-day, repeated dose toxicity study, EGT (Mironova Labs Inc, Fairfield, New Jersey, >98% purity) was administered daily by gavage to Sprague Dawley Crl CD (SD) rats (n=5 per sex per dose) (Marone et al., 2016). Animals were treated with 0, 50, 200, or 500 mg per kg bw per day. The study was conducted under OECD Guidelines for the Testing of Chemicals, Section 4, No. 407. Animals were observed for changes in clinical signs, body weight, body weight gain, food consumption, food efficiency, or macroscopic findings at necropsy. Gross necropsy included examination of the external surface, all orifices, musculoskeletal system, cranial, thoracic, abdominal, and pelvic cavities, and associated organs and tissues.

There was no mortality in any of the test groups and no treatment-related abnormal clinical findings. While there were no treatment-related clinical observations noted in any of the dose groups, intermittent alopecia in males and females was observed in all groups. Ocular and nasal discharge was observed in 1 male in the high dose group; this was attributed to malocclusion of the upper incisors. There were no test substance-related changes in body weight, body weight gain, food consumption, food efficiency, or macroscopic observations. A consistent increase in overall mean body weight, body weight gain, and food consumption was observed in the low-dose male group. Because this was not dose-dependent it was not considered test substance-related. Other than fluid-filled uterus in 1 female each in the control, low- and mid-dose groups, there were no macroscopic observations at necropsy. The observed changes in the uterus were attributed to normal variation in the estrus cycle and were not considered test substance-related.

3. Subchronic Toxicity Studies

In a combined subchronic toxicity and reproductive/developmental toxicity study, Sprague-Dawley Crl CD (SD) (n=10 per dose per sex) were fed EGT (Tetrahedron, Vincennes, France, >98% purity) mixed with the commercial rat chow (SAFE, France) diet at 0, 0.1, 0.3 and 0.9% by weight (Forster et al., 2015). Males received the diet for 13 weeks (10 weeks before pairing and during the 3-week pairing period) and females received the diet for 13 weeks before pairing, during the pairing and gestation period and through day 5 of lactation. The study was based on OECD No. 422 and OECD No. 408 guidance. F_0 rats were checked twice daily for mortality and morbidity and once daily for clinical signs. A weekly, detailed clinical examination included evaluation of skin, fur, eyes, mucous membranes, clonic/tonic movements, stereotypes and behavior, gait, posture, and response to handling. Body weight and food consumption were recorded weekly until mating and periodically throughout gestation and lactation. The mean achieved doses in the 0.1, 0.5, and 0.9% groups were 67, 203, and 615 mg per kg bw per day for males and 73, 238, and 721 mg per kg bw per day for females, respectively for the pre-mating period.

Five male and five female animals per dose group were examined at the conclusion of the pre-mating period. There was no mortality, no clinical signs of toxicity, and no differences in feed consumption for any of the groups. Compared to the control group, the group mean body weights

for the male rats trended lower but the difference was not statistically significant and was not dose-dependent, which the authors suggested was a palatability issue. There were no differences in body weight among the female rat groups during the pre-mating period. Compared to the control group, the highest female dose group trended towards a lower body weight during gestation and lactation but the difference was not statistically significant. There were no between group differences in organ weight changes; any observed organ weight changes were of low magnitude and not dose-related. Necropsy findings were described as commonly reported for rats of this strain and age, and the normal incidence and severity were similar across the different groups. There were no treatment-related histopathological findings. The NOAEL was estimated based on the highest equivalent dose tested, 615 mg per kg bw per day and 725 mg per kg bw per day for males and females, respectively.

In a 90-day, subchronic toxicity study, EGT (Mironova Labs Inc, Fairfield, New Jersey, >98% purity) was administered daily by gavage to Sprague Dawley Crl CD (SD) rats (n=10 per sex per dose) (Marone et al., 2016). Animals were treated with 0, 400, 800, or 1,600 mg per kg bw per day. The study was conducted under OECD Guidelines for the Testing of Chemicals, No. 408. Animals were observed for changes in clinical signs, body weight, body weight gain, food consumption, food efficiency, or macroscopic findings at necropsy. Gross necropsy included examination of the external surface, all orifices, musculoskeletal system, and cranial, thoracic, abdominal, and pelvic cavities and associated organs and tissues. Ophthalmologic evaluations were conducted prior to commencement of treatment and at day 89. Blood samples were collected during week 12 and evaluated for hematology and clinical chemistry. Urine was collected during week 12 and evaluated for quality, pH, ketone, color, glucose, bilirubin, clarity, specific gravity, blood, volume, protein, urobilinogen, and sediment. Histopathological examinations were conducted on preserved organs and tissues from control and high-dose groups and any gross lesions of potential toxicological significance of the low- and mid-dose groups were recorded.

One male in the mid-dose group died on day 66, which the authors attributed to problems with gavage administration, and one female in the mid-dose group was found dead on day 85 with pathology consistent with moderate epidermal necrosis but with no obvious cause of death. The absence of dose-response effects or significant macroscopic or microscopic findings led the authors to conclude the death was not related to treatment. Thus, there were no treatment-related mortalities. The only clinical findings attributed to EGT treatment were soft feces and diarrhea in the high-dose group. Other clinical observations were not dose-dependent, mild, and not considered treatment-related. There were no treatment-related changes in body weight, body weight gain, food consumption and food efficiency. Hematology, coagulation, and clinical chemistry results revealed decreases in mean corpuscular volume, absolute eosinophil counts, and absolute reticulocyte counts and an increase in red cell distribution width in the high dose females. There were increased alkaline phosphatase levels in the high-dose males and females and decreases in creatinine, total protein and albumin in the high-dose females. All but the eosinophil counts fell within historical control levels. There were no absolute or relative organ weight changes in the males but a decrease in absolute and relative thymus weights and increase in relative kidney and

liver weights in the high-dose females. These changes were not associated with hematological or histological changes and were therefore considered non-adverse and incidental. The high incidence of microscopic findings in the control animals complicated interpretation of these findings, therefore, the increased incidence of microscopic findings in the high-dose group males and females were not considered in the evaluation of adverse effects. Based on hematological, clinical chemistry, and organ weight changes in hematopoietic system, liver and kidney in the high dose group, the NOAEL is considered to be 800 mg per kg bw per day.

4. Reproductive/Developmental Toxicity Studies

The reproductive/developmental toxicity of EGT (Tetrahedron, Vincennes, France, >98% purity) was evaluated in the F_0 parents (n=10 per sex per group) and F_1 offspring following the subchronic dietary administration described above (Forster et al., 2015). The study was based on OECD No. 422 and OECD No. 408 guidance. F_0 females were mated with F_0 males treated at the same dietary concentration. Confirmation of mating was confirmed by presence of a vaginal plug or sperm in the vaginal lavage. Females were allowed to litter normally and rear the F_1 progeny until day 5 postpartum.

There was no effect on mating index, days to mating, fertility index, number of females with live born pups, gestation index, number of females that delivered, duration of gestation, corpora lutea, implantations, pre-implantation loss, number of pups, or post-implantation loss. There was no treatment-related effect on litter size, mean pup body weight, incidence or nature of clinical signs or the repartition of found dead/cannibalized pups. A slight decrease in mean body weight compared to control was observed in the high-dose group in both male and female pups but this did not attain statistical significance and fell within historical control range for day 5 postpartum rat pups. The NOAEL is based on the maximum dose tested, 615 mg per kg bw per day and 725 mg per kg bw per day for males and females, respectively.

The effect of EGT on embryopathy was evaluated in diabetic Wistar rats (Guijarro et al., 2002). Diabetes was induced in females by administration of streptozotocin and confirmed 5 days later by analysis of glucose in the urine. The rats were administered a daily subcutaneous dose of insulin throughout the remainder of the study. After mating with untreated males, the pregnant females were divided into 4 experimental groups: diabetic control (n=5), diabetic plus EGT (n=6), control (n=9), and control plus EGT (n=8). EGT (Oxis International, >99% purity) was administered by gavage at a dose of 1.147 mg per kg bw per day. All animals were sacrificed on day 11.5 of gestation. The number of somites and the crown-rump length was lower for both groups of diabetic rats compared to their corresponding control groups. The incidence of neural tube defects for the diabetic rats was higher than in the control groups but the diabetic plus EGT group had fewer malformations than the diabetic group and was not different from the control plus EGT group. Thus, the authors concluded that EGT "modulated the hyperglycemia-dependent malformations."

5. Genotoxicity Studies

EGT (Oxis International, >99% purity) was tested for its potential mutagenic activity using a bacterial reverse mutation assay (Schauss et al., 2010). The study used four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and one strain of *Escherichia coli* (WP2 pKM101). The test was in compliance with OECD Guidelines for Testing of Chemicals, No. 471, ICH Guidance on specific aspects of regulatory genotoxicity tests, and principles of good laboratory practice. The test was conducted both in the presence and absence of metabolic activation with post-mitochondrial supernatant S9. EGT was tested over a range of concentration from 15.8 to 5,000 µg per plate and mutagenic activity was compared to the appropriate positive and negative controls. No biologically relevant increases compared to solvent controls were observed in the number of revertant colony numbers of any of the five test strains with EGT at any concentration. The authors concluded EGT did not induce gene mutations and has no mutagenic activity under the test conditions used.

Marone et al. (2016) evaluated the potential mutagenic activity of EGT (Mironova Labs, >98% purity) using both the plate incorporation and preincubation assay. The test used four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and one strain of *Escherichia coli* (WP2uvrA) both in the presence and absence of metabolic activation with post-mitochondrial supernatant S9. The test was conducted in conformance with OECD Good Laboratory Practices and under OECD Guidelines for Testing of Chemicals, No. 471. EGT concentrations ranged from 1.58 – 5,000 µg per plate and the results were compared to the appropriate positive and negative controls. Assessed both by revertant colony counts and mutation ratio, EGT did not cause gene mutation activity in any of the tester strains. There was no precipitation, no toxic effects, or biologically relevant increases in revertant colony numbers at any dose of EGT.

In an unpublished study [Vivotecnia (2013) as cited in EFSA et al. (2016)], EGT (Tetrahedron, > 99% purity) was tested in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and one strain of *Escherichia coli* (WP2 pKM101) in compliance with the OECD test guideline, No. 471. The EGT concentration ranged from 60 – 5,000 µg per plate both in the presence and absence metabolic activation with post-mitochondrial supernatant S9. No increase in mutant colonies was observed for any strain at any concentration of EGT.

Schauss et al. (2011) evaluated the clastogenic and mutagenic potential of EGT (Oxis International, >99% purity) in an *in vitro* chromosome aberration assay with V79 CHO cells and an *in vivo* mammalian erythrocyte micronucleus test. The chromosome aberration study was conducted in compliance with OECD No. 473 and Good Laboratory Practices. EGT at concentrations ranging from 1,250 to 5,000 µg per mL both in the presence and absence of S9 mix was incubated with the CHO cells for various treatment and sampling times. Toxicity was determined by cytotoxicity and polyploidy. EGT did not induce structural chromosome aberrations. For the erythrocyte micronucleus test, healthy (SPF) CRL:NMRI BR mice (n=5 per sex per dose) were treated with 375, 750, and 1,500 mg per kg bw EGT under assay conditions compliant with OECD No. 474, Guidance S2A, and EC 220/2008 guidelines. Bone marrow was obtained 24 and

48 h after administration of the EGT; 2,000 polychromatic erythrocytes (PCE) were scored for each animal. Frequencies of micronucleated PCEs were compared to the values in the concurrent negative control groups. The very low frequency of micronucleated PCEs observed were within acceptable range. There was no biologically or statistically significant increase in micronucleated PCEs in either the 24 or 48 hr samples compared to the control group. The authors concluded that EGT had no clastogenic or mutagenic activity under these experimental conditions.

6. Human Studies

In a randomized, placebo-controlled, double-blinded study healthy male volunteers (n=15 per group) were divided into three groups: placebo, 5 mg EGT (Tetrahedron), or 25 mg EGT (Cheah et al., 2017). Subjects were administered treatment daily for 7 days, blood and urine samples were collected over 35 days. EGT was quickly absorbed and retained with significant elevations in plasma and whole blood during the entire observation period, while only < 4% of administered EGT was excreted in the urine. Allantoin, 8-hydroxy-2'-deoxyguanosine (8OHdG), plasma protein carbonyl, F_2-isoprostanes, and C-reactive protein were measured as markers of oxidative damage and inflammation. There were no differences in blood allantoin levels. Urinary 8OHdG levels were decreased only in the low-dose group on day 35. There were no changes in plasma protein carbonyl or C-reactive protein. Although the trend towards decreasing levels of F_2-isoprostanes was temporally correlated with blood EGT levels, the trend did not attain statistical significance. There were no differences observed in liver function tests (albumin, bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase) or lipid profiles (fasting glucose, low/high-density lipoprotein cholesterol, triglycerides, and gamma-glutamyl transpeptidase) during the study. No adverse effects were observed and subjects reported no side-effects.

The effect of ergothioneine-supplementation on kidney health and quality of life in participants with impaired kidney function (Chronic Kidney Disease stages 3 and 4) is being assessed in an unpublished, ongoing one year clinical study, as reported by Walley (2017). Over the course of the study, 60 patients will receive 2 supplement capsules per day (Ergo4Health™/Kidney, a proprietary formulation of *Pleurotus eryngii* mushrooms containing 0.75 mg L-ergothioneine and 1,250 IU Vitamin D2 per 500 mg capsule). Various clinical measurements are to be assessed at 0, 3, 6, 9, and 12 months, along with a Kidney Quality of Life (KDQOL) survey at 3 months. Preliminary results obtained after 3 months demonstrate improvements in estimated glomerular filtration rate (eGFR) and mean blood urea nitrogen (BUN) levels from baseline values, and indicate an overall improvement in kidney function.

Benson et al. (2012) evaluated the anti-inflammatory effects of a L-ergothioneine-containing supplement on 12 subjects who consumed the treatment over a period of 6 weeks. No safety parameters were reported.

An EFSA review on the safety of EGT addressed concerns about the effect of EGT on glucose homeostasis (EFSA et al., 2016). Several studies of observational design were considered as part

of this review. Salt (1931) determined the EGT content in healthy, diabetic, nephritic, and patients with miscellaneous diseases. Salt found no correlation between EGT and blood glucose levels and no association related to any stage of the disease or treatment. Fraser and Jegard (1950) reported elevated EGT levels in diabetic patients compared to healthy subjects but no correlation with duration of diabetes, age, sex, blood cholesterol, uric acid or non-protein nitrogen. Epand et al. (1988) observed higher EGT concentrations in males than females, subjects with type 2 diabetics than type 1 diabetics, and in diabetics receiving a higher insulin dose. EGT was not correlated with HbA1c. The variability of the findings among these studies and the observational study designs do not support a causal relationship between blood EGT and diabetes. These studies are not considered relevant to the evaluation of safety of EGT.

Calvo et al. (2016) evaluated 37 adult subjects with metabolic syndrome in a retrospective analysis of a clinical trial in which the subjects consumed white button mushrooms for 16 weeks with a 4-week post treatment follow-up. Because white button mushrooms are a good source of EGT, serum EGT levels increased from 317 ng per mL to 677 ng per mL and remained elevated at the conclusion of the follow-up period (496 ng per mL). The retrospective analysis focused on serum risk factors for diabetes, including inflammatory and oxidative stress markers, as well as measures of insulin resistance and glucose metabolism. Although as part of the original trial design subjects received various levels of vitamins D2 and D3, vitamin D status had no influence on these parameters and the results of all the experimental groups were combined for this retrospective analysis (Mehrotra et al., 2014). Associated with the increased EGT levels were increased serum oxygen radical absorption capacity (ORAC) and the anti-inflammatory hormone, adiponectin and decreased measures of serum oxidative stress inducing factors, carboxymethyllysine and methyl glyoxal. There were no changes in 8-isoprostane, leptin, insulin resistance or glucose metabolism. The authors concluded that white button mushrooms rich in EGT may provide anti-inflammatory and antioxidant benefits in adults predisposed to type 2 diabetes and do not appear to effect glucose metabolism or the risk of diabetes.

The EFSA review on the safety of EGT addressed concerns about the effect of EGT on autoimmune diseases such as Crohn's Disease (CD) and rheumatoid arthritis (RA) (EFSA et al., 2016). Studies have associated mutations in the EGT transporter OCTN1/ETT with susceptibility to CD in Caucasian populations (Peltekova et al., 2004, Taubert et al., 2005, Taubert et al., 2009, Leung et al., 2006, Huff et al., 2012) but not in Japanese patients with CD (Kato et al., 2010). A particular OCTN1/ETT variant, 503F, was singled out for association with higher EGT concentrations in cultured fibroblast cells and CaCo-2 epithelial cells (Taubert et al., 2005) and peripheral blood mononuclear cells (Taubert et al., 2009). EGT levels were elevated in mucosal biopsies from inflamed segments of CD patients correlating with an increase in OCTN1/ETT expression (Taubert et al., 2009). Huff et al. (2012) attributed the apparent increase in frequency of the 503F variant in CD patients to positive selection and not to an association with the inflammatory bowel disease 5 gene, rather a result of genetic hitchhiking. The EFSA panel concluded that "no relationship can be inferred from the above described studies with regard to dietary or supplemental EGT and the susceptibility for CD."

One observational study reported lower EGT concentrations in red blood cells in patients with rheumatoid arthritis (RA) compared to healthy volunteers (Reglinski et al., 1991), while another observational study reported increased EGT levels in RA patients compared to patients with coronary heart disease or osteoarthritis (Taubert et al., 2006). Susceptibility to RA has been negatively associated in the Japanese population with SLC22A4, a 22 member, 4 gene family encoding OCTN1 (Tokuhiro et al., 2003). The variability of the findings and the observational study designs do not support a causal relationship between blood EGT and RA and thus, these studies are not considered relevant to the evaluation of safety of EGT.

Various observational studies investigated the association of EGT and various disease states. Red blood cell EGT levels were decreased in patients with chronic granulocytic leukemia but not in other leukemia patients compared to normal subjects (McMenamy et al., 1960), were increased in pre-eclamptic pregnant women compared to normotensive women (Turner et al., 2009), and were decreased in cataractous lenses (Shukla et al., 1981). These studies do not support a causal relationship between EGT and the respective disease states and are not considered relevant to the evaluation of safety of EGT.

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

The absorption, distribution, metabolism, and excretion (ADME) of ergothioneine has been recently reviewed (EFSA et al., 2016). EGT is absorbed from the diet in the gastrointestinal tract, selectively taken up by the tissues, and resorbed by the kidneys via a specific transporter, OCTN1/ETT. Tissue concentrations are tightly regulated and metabolic turnover in organs is low. There is no proportional increase with dose or duration and excretion of EGT metabolites occurs primarily through the kidney. Information on the overall percentage of the dose absorbed and excreted could not be found in the literature.

8. Summary

Three subchronic oral toxicity studies, two subacute oral toxicity studies, one acute toxicity study, and a reproductive/developmental toxicity study in rats administering EGT from different commercial sources all support the determination of a NOAEL of 800 mg per kg bw per day. All of these studies have been published in peer review journals. In addition, three bacterial reverse mutation studies, a chromosomal aberration study, and a mouse micronucleus study all support the conclusion that EGT is not genotoxic. A seven-day human safety study in which EGT was administered at 5 and 25 mg per person per day followed by a 28-day observation period supports the conclusion of safety. With the exception of one of the bacterial reverse mutation studies, all of these studies have been published in the peer review literature. Various studies of observational design and *in vitro* studies were evaluated to address potential relationship between EGT and diabetes, autoimmune disease, leukemia, and other disease states. None of this evidence supports a causal relationship between EGT and any disease state.

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 conditions of its intended use."[4]

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 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 in food prior to January 1, 1958." [5]

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:[6]

- 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

[4] 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).
[5] 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).
[6] See Footnote 1.

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

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

As noted below, this safety assessment to ascertain GRAS status for ergothioneine 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[7] Findings on Safety of Blue California's Ergothioneine Preparation

The Expert Panel, having reviewed the available published studies, and the EFSA expert committee evaluation on ergothioneine, concludes that Blue California's ErgoActive™ preparation is generally recognized as safe in foods at the usage levels described herein.

Blue California's ergothioneine is substantially chemically equivalent to other synthetic ergothioneine preparations already in commercial use. The Expert Panel considered the following evidence developed using the other commercial preparations as evidence for the safety of Blue California's ergothioneine:

- **ADME studies** in animals and humans indicate that tissue concentrations of ergothioneine are highly regulated, and there was no evidence of a dose- or duration-dependent increase in tissue concentrations in multiple studies.
- **Animal toxicity studies** show ergothioneine displays a low order of toxicity in animal testing, yielding a LD_{50} value > 2,000 mg per kg bw in mice. Subchronic rat studies determined the NOAEL to range between 615 and 800 mg per kg bw per day.
- **Subchronic animal studies** revealed no changes in mortality, no clinical signs of toxicity, no differences in feed consumption, and no treatment-related histopathological findings. Minor gastro-intestinal effects were observed in certain high-dose groups, which included soft feces and diarrhea.
- **Reproductive and/or developmental toxicity** was not observed at dietary doses of up to 615 mg per kg bw per day and 725 mg per kg bw per day for male and female rats, respectively. Ergothioneine was found to "modulate the hyperglycemia-dependent malformations" in diabetic rats at doses of 1.147 mg per kg bw per day by gavage.
- **Genotoxicity and mutagenicity studies** have shown no *in vitro* mutagenesis or genotoxicity.

[7] Drs. Falk, Lewis, and Emmel have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in the deliberations of GRAS Expert Panels. Dr. Emmel served as chair of the Panel.

- **Clinical studies** also show ergothioneine is well-tolerated in humans. No adverse effects were observed and no side-effects were reported by subjects administered 25 mg ergothioneine for 7 days.

- Synthetic ergothioneine (Ergoneine®, > 99% ergothioneine) was reviewed by EFSA et al. (2016) and the Panel concluded that it was safe for use in alcohol-free beverages, cereal bars, milk, fresh dairy products and chocolates at levels up to 5 mg per serving, and as a dietary supplement with a daily dose of up to 30 mg per day for adults and 20 mg per day for children. Blue California's ErgoActive™ is intended to be used in similar conventional foods and at the same levels as those presented in EFSA's review.

- Blue California's ErgoActive™ is produced under cGMP.

- Based on results from an on-going shelf stability study on the raw material, Blue California's ErgoActive™ is expected to be stable for at least twelve months when stored under ambient conditions.

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

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 ergothioneine have been published in the scientific literature.

EFSA has published a critical evaluation of ergothioneine, and has concluded that ergothioneine is safe for use as a food ingredient (EFSA et al., 2016)

Furthermore, clinical studies have shown ergothioneine is well-tolerated in humans (Cheah et al., 2016, Cheah et al., 2017, Kumosani, 2001), and it has a low order of toxicity in single dose studies as well as repeated dose studies (Forster et al., 2015, Marone et al., 2016).

2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there must be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

EFSA reviewed the body of data available on ergothioneine and concluded that a synthetic ergothioneine preparation with >99% purity was safe under the intended conditions of use in conventional foods and dietary supplements.

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

E. Conclusion

In consideration of the aggregate safety information available on ergothioneine, Blue California and the Expert Panel conclude that ergothioneine, when consumed in foods as described within this GRAS notification, is generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

Blue California's ergothioneine preparation, when produced in accordance with FDA Good Manufacturing Practices requirements and when meeting at a minimum the FCC purity specifications for ergothioneine and those specifications presented by Blue California in Table 2, is Generally Recognized As Safe when consumed in certain conventional foods and non-alcoholic beverages at a use level of up to 5 mg per serving. It is important to observe good manufacturing practices principles in that the quantity of a substance added to food should not exceed the amount reasonably required to accomplish its intended technical effect.

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.

(b) (6)

Katrina V. Emmel, Ph.D.
Chair

(b) (6)

Michael Falk, Ph.D.

(b) (6)

Kara Lewis, Ph.D.

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

A. List of Acronyms

8OHdG	8-hydroxy-2'-deoxyguanosine
ADI	Acceptable Daily Intake
ADME	Absorption, distribution, metabolism, and elimination
AMP	Ampicillin
AVA	Agri-Food & Veterinary Authority of Singapore
BMI	Body Mass Index
bw	body weight
CAS	Chemical Abstract Service
CD	Crohn's Disease
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
cGMP	current good manufacturing practices
Co	Company
Da	dalton
DABT	Diplomat of the American Board of Toxicology
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EGT	L-Ergothioneine
EgtB	Formylglycine-generating enzyme-like protein
EgtC	Glutamine amidotransferase
EgtD	Histidine methyltransferase
EgtE	Pyridoxal 5-phosphate binding protein
EU	European Union
FAO	Food and Agriculture Organization of the United Nations

FCC	Food Chemical Codex
FDA	Food and Drug Administration
FD&C	Federal Food, Drug, and Cosmetic Act
FOIA	Freedom of Information Act
FSANZ	Food Standards Australia New Zealand
FSSAI	Food Safety and Standards Authority of India
g	gram
GA	GRAS Associates
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
HbA1c	glycated hemoglobin
HPLC	High-Performance Liquid Chromatography
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
JECFA	Joint FAO/WHO Expert Committee on Food Additives
Kan	Kanamycin
kg	kilogram
L	Liter
LD_{50}	Median lethal dose
LLC	Limited Liability Corporation
Ltd.	Limited
mg	milligram
min.	minimum
mL	milliliter
n	number
NA	Not applicable

ND	Not detected
NHANES	National Health and Nutrition Examination Survey
No.	Number
NOAEL	No Observed Adverse Effect Level
NS	Not Specified
OECD	Organization for Economic Cooperation and Development
ORAC	Oxygen radical absorption capacity
PCE(s)	Polychromatic erythrocytes
Ph.D.	Doctor of Philosophy
ppm	parts per million
RA	Rheumatoid arthritis
RACC	Reference amounts customarily consumed
rpm	revolutions per minute
t	Time
µg	microgram
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
WHO	World Health Organization

B. References

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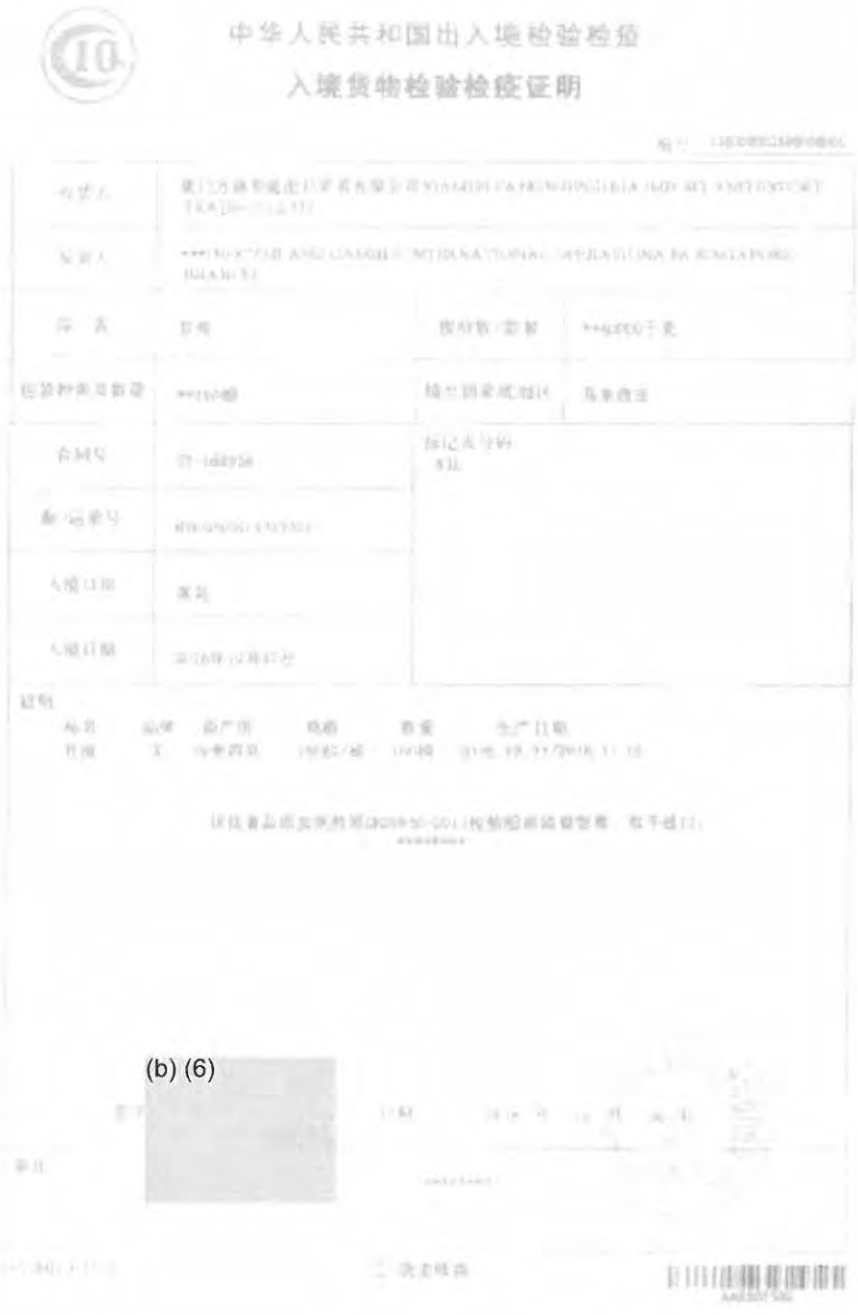
C. Appendices

Appendix 1 Specifications and Certificates of Analysis for Production Processing Aids

Appendix 1.1	**Glycerin**
Appendix 1.2	**Yeast Peptone**
Appendix 1.3	**Yeast Extract**
Appendix 1.4	**Sodium Chloride**
Appendix 1.5	**Ampicillin**
Appendix 1.6	**Agar**
Appendix 1.7	**Monopotassium Phosphate**
Appendix 1.8	**Dipotassium Phosphate**
Appendix 1.9	**Citric Acid**
Appendix 1.10	**Antifoam**
Appendix 1.11	**Ammonium Hydroxide**
Appendix 1.12	**Ammonia**
Appendix 1.13	**Magnesium Sulfate Heptahydrate**
Appendix 1.14	**Thiamine**
Appendix 1.15	**Glucose**
Appendix 1.16	**Sulfuric Acid**
Appendix 1.17	**Iron (III) Citrate**
Appendix 1.18	**Ammonium Iron (II) Sulfate**
Appendix 1.19	**Ion-Exchange Resin**
Appendix 1.20	**Activated Charcoal**

Appendix 1.1 Glycerin


中华人民共和国出入境检验检疫

入境货物检验检疫证明

(b) (6)

Entry-Exit Inspection and Quarantine of the People's Republic of China

Inspection and Quarantine Certificate of Import and Export goods

No.116000880219605400J

Consignee: Xiamen Fangshenghua Import and Export Trade Co., LTD

Consignor: Procter and Gamble International Operations SA Singapore Branch

Item Name: Glycerin Net Weight: 40,000kg

Number and Kind of Packages: 160 Drums Country/Place of Export: Malaysia

Contract No: SY-160928 Marks and Number: NIL

Bill of Lading No: NYKSPKG515763700

Port of Entry: Huangdao

Entry Date: December 07, 2016

Certification

Item Description	Brand	Place of Origin	Specification	Quantity	Date of Manufacturing
Glycerin	None	Malaysia	250KG/Drum	160 Drums	2016.10.31/ 2016.11.22

This batch of food additive is approved for import in accordance to GB29950-2013 inspection and quarantine supervision.

Signature: Date: December 20, 2016

Notes:

(1) For Consignee

KEMENTERIAN KESIHATAN
MALAYSIA

MINISTRY OF HEALTH
MALAYSIA

SIJIL KESIHATAN
HEALTH CERTIFICATE

(b) (6)

FATIMAH BT A MAJID
OFFICER OF HEALTH
HEALTH OFFICE KUANTAN
MALAYSIA

Appendix 1.2 Yeast Peptone

检 验 报 告

Test Report

№：检(业)字2016-SP13935号

样品名称 安琪酵母多肽（酵母浸出物）

规格型号 粉状

受检单位 安琪酵母股份有限公司

检验类型 委托检验

三峡食品药品检验检测中心

Three Gorges Center for Food and Drug Control

三峡食品药品检验检测中心
检 验 报 告

No. 检(食)字[2016-SP]3825号 共 1 页 第 1 页

样品名称	灵芝酵母菌粉（等级出售）	样品编号	3825
样品信息	———	样品状态	完好
受检单位名称	宜昌[illegible]有限公司	受检单位地址	[illegible]
委托单位名称	宜昌[illegible]有限公司	检验类别	委托检验
生产单位（标称）	宜昌[illegible]有限公司	生产日期或批号	20161230ZB5
抽样人员	———	委托人员	李金荣
抽样地点	———	抽样日期	———
样品数量	93kg×2	收样日期	2016-12-31
样品基数	———	检验日期	2016-12-22 至 2017-01-13
检测项目	详见报告	样品描述	样品完好，符合检验要求
检验依据或判定依据	Q/YB 2185-2015		
检验结论	该样品所检项目符合Q/YB 2185-2015标准要求。		
备 注	———		

批准：罗爱玲 审核：李素媛 主检：赵宏玮

一球食品药品检验检测中心
检 验 结 果

序号	检验项目		单位	标准（技术）要求	实测结果	单项结论
1	色泽		——	本品为无色结晶	符合	合格
2	气味		——	具有麦角硫因固有的气味	具有	合格
3	外观		——	粉末或晶体	粉末	合格
4	杂质		——	无肉眼可见的外来杂质	无肉眼可见异物	合格
5	总氮（以干基计）		%	≥9.0	13.9	合格
6	氨基酸态氮（以干基计）		%	≥1.5	2.3	合格
7	水分		%	≤6.0	4.4	合格
8	灰分		%	≤15.0	9.0	合格
9	氯化钠		%	≤2.0	0.7	合格
10	pH		——	5.1～5.2	5.2	合格
11	铅（以Pb计）		mg/kg	≤2	0.1	合格
12	总砷（以As计）		mg/kg	≤2	0.13	合格
13	菌落总数		cfu/g	≤10^4	430	合格
14	大肠菌群		MPN/g	≤0.3	0.3	合格
15	霉菌酵母	金黄色葡萄球菌	25g	不得检出	未检出（25g）	合格
		沙门氏菌	25g	不得检出	未检出（25g）	合格

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Test Report

No.2016-SO13935

Product Name:	Angel Yeast Peptone (Yeast Extract)
Specification:	Powder
Requestor:	Angel Yeast Co., Ltd
Test type:	Analysis Request

Three Gorges Center for Food and Drug Control

Test Report

No: 2016-SP13935 Total 2 Pages / Page 1

Sample Name	Angel Yeast Peptone (Yeast Extract)	Specification Type	Powder
Sample Grade	-	Trademark	Angel
Test Unit Name	Angel Yeast Co., Ltd	Test Unit Address	-
Requestor Name	Angel Yeast Co., Ltd	Test Type	Analysis Request
Manufacturer (trademark)	Angel Yeast Co., Ltd	Manufacturing Date or Batch Number	20161230288
Sampling Personnel	-	Requestor	Ruo, Biying
Sampling Location		Sampling Date	
Sampling Quantity	500g x 2	Received Date	2016-12-21
Sampling Base		Testing Date	2016-12-22 - 2017-01-19
Test Item	See attached	Sample Description	Normal, requirements met
Test Compliance	Q/YB2187S-2015		
Test Conclusion	Sample meets the standard of Q/YB2187S-2015. Inspection Stamp Approval Date:		
Notes			

Approved by: Reviewed by: Analyzed by:

Three Gorges Center for Food and Drug Control

Test Results

No: 2016-SP13935 Total 2 Pages / Page 2

No.	Test Items		Units	Standard/Technical Requirements	Test Results	Evaulation
1	Color			Light yellow to light brown	Yellow	PASS
2	Odor			Standard odor of yeast peptone	No odor	PASS
3	Appearance			Powder or paste	Powder	PASS
4	Impurities			No visible impurities	No visible impurities	PASS
5	Total Nitrogen (dry basis)		%	≥8.0	11.8	PASS
6	Amino Nitrogen (dry basis)		%	≥1.5	3.3	PASS
7	Moisture Content		%	≤6.0	3.8	PASS
8	Ash		%	≤15.0	9.0	PASS
9	Sodium Chloride		%	≤2.0	0.5	PASS
10	pH			5.3~7.2	5.8	PASS
11	Lead (based on Pb)		mg/kg	≤2	<0.1	PASS
12	Arsenic (based on As)		mg/kg	≤2	0.13	PASS
13	Total Number of Colonies		cfu/g	<50000	4200	PASS
14	Coliforms		MPN/g	≤0.3	<0.3	PASS
15	Pathogens	Staphylococcus aureus	mg/L	Negative	Negative, /25g	PASS
		Salmonella		Negative	Negative, /25g	PASS
Blank Below						

Appendix 1.3 Yeast Extract

CMA

P201617198S

检 验 报 告

Test Report

№：检(业)字2016-SP13951号

样品名称 安琪酵母浸粉

规格型号 FM802

受检单位 安琪酵母股份有限公司

检验类型 委托检验

三峡食品药品检验检测中心

Three Gorges Center for Food and Drug Control

三峡食品药品检验检测中心
检 验 报 告

No: [illegible]号　　　　共 3 页 第 1 页

样品名称	[illegible]	规格型号	[illegible]
样品等级	——	商标	[illegible]
受检单位名称	安琪酵母股份有限公司	受检单位地址	——
委托单位名称	安琪酵母股份有限公司	检验类型	委托检验
生产单位（标称）	安琪酵母股份有限公司	生产日期或批号	2016110385[illegible]9
抽样人员	——	委托人员	[illegible]
抽样地点	——	抽样日期	——
样品数量	500g×2	到样日期	2016-12-21
样品基数	——	检验日期	2016-12-22～2017-01-13
检验项目	详见附页	样品描述	样品正常，符合检测要求
检验依据判定依据	Q/YB 21475-2016		
检验结论	该样品所检指标符合Q/YB 21475-2016 标准要求。 签发日期：[illegible]		
备注	——		

批准：刘健　　审核：李素媛　　主检：[illegible]

三峡食品药品检验检测中心
检 验 结 果

序号	检验项目		单位	标准（技术）要求	实测结果	单项结论
1	感官要求	色泽	——	黄色至灰黄色	黄色	合格
		性状	——	粉状	粉状	合格
		气、滋味	——	具有原辅料固有的气味，无异臭异味	具有原辅料固有的气味，无异臭异味	合格
		杂质	——	无肉眼可见外来杂质	无肉眼可见杂质	合格
2	总菌（以干基计）		%	≥95.0	96.0	合格
3	氨基氮（以干基计）		%	≥3.0	5.3	合格
4	水分		%	≤6.0	3.9	合格
5	灰分		%	≤15.0	9.8	合格
6	pH值（2%水溶液）		——	5.3~7.2	5.6	合格
7	铅		mg/kg	≤1.0	0.11	合格
8	总砷（以As计）		mg/kg	≤0.5	0.12	合格
9	菌落总数		CFU/g	≤1000	1000	合格
10	大肠菌群		MPN/g	≤0.3	<0.3	合格
11	致病菌	沙门氏菌	/25g	不得检出	未检出（/25g）	合格
		金黄色葡萄球菌	/25g	不得检出	未检出（/25g）	合格

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Test Report

No.2016-SO13931

Product Name:	Angel Yeast Extract Powder
Specification:	FM802
Requestor:	Angel Yeast Co., Ltd
Test type:	Analysis Request

Three Gorges Center for Food and Drug Control

Test Report

No: 2016-SP13931 Total 2 Pages / Page 1

Sample Name	Angel Yeast Extract Powder	Specification Type	FM802
Sample Grade	-	Trademark	Angel
Test Unit Name	Angel Yeast Co., Ltd	Test Unit Address	-
Requestor Name	Angel Yeast Co., Ltd	Test Type	Analysis Request
Manufacturer (trademark)	Angel Yeast Co., Ltd	Manufacturing Date or Batch Number	20161030389
Sampling Personnel	-	Requestor	Ruo, Biying
Sampling Location		Sampling Date	
Sampling Quantity	500g x 2	Received Date	2016-12-21
Sampling Base		Testing Date	2016-12-22 2017-01-13
Test Item	See attached	Sample Description	Normal, requirements met
Test Compliance	Q/YB2147S-2016		
Test Conclusion	Sample meets the standard of Q/YB2147S-2016. Inspection Stamp Approval Date:		
Notes			

Approved by: Reviewed by: Analyzed by:

Three Gorges Center for Food and Drug Control

Test Results

No. 2016-SP13931 Total 2 Pages / Page 2

No.	Test Items		Units	Standard/Technical Requirements	Test Results	Evaluation
1	Appearance	Color		Yellow to light yellow	Yellow	PASS
		Traits		Powder	Powder	PASS
		Odor, taste		Standard oder of yeast extract, no corrupt smell	Standard odor and taste of item tested	PASS
		Impurities		No visible impurities	No visible impurities	PASS
2	Total Nitrogen (dry basis)		%	≥9.0	12	PASS
3	Amino Nitrogen (dry basis)		%	≥3.0	5.2	PASS
4	Moisture Content		%	≤6.0	3.8	PASS
5	Ash		%	≤15.0	9.8	PASS
6	pH (2% solution)			5.3~7.2	5.6	PASS
7	Lead (based on Pb)		mg/kg	≤2	<0.1	PASS
8	Arsenic (based on As)		mg/kg	<2	0.13	PASS
9	Total Number of Colonies		cfu/g	≤50000	1600	PASS
10	Coliforms		MPN/g	≤0.3	<0.3	PASS
11	Pathogens	Staphylococcus aureus	mg/L	Negative	Negative, /25g	PASS
		Salmonella		Negative	Negative, /25g	PASS
Blank Below						

Appendix 1.4 Sodium Chloride

Test Report

(2015) Commission Checked No. 4

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

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

Description

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

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

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

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

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

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

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

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

Test Report

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

Blank Below

Appendix 1.5 Ampicillin

Tianq°

苏州天可贸易有限公司

Suzhou Tianqe Trading Co.,LTD
Tel: 13776026955 fax:0512-66366729
E-mail:tkdaling@163.com 网址: www.tianq.cn

CERTIFICATION OF ANALYSIS

Product Name	Ampicillin sodium salt (氨苄青霉素钠)
Quantity	20kg
Batch number	0325K4017
Date of production	2017.03.05
Date of Analysis	2017.03.25
Structural formula	$C_{16}H_{18}N_3O_4SNa$
MW	371.39
Standard of analysis	BR

Items	Requirements	Results
Assay	≥99.0%	99.02%
Description	white crystal powder	Pass
Potency	845~988 mcg/mg	Pass
Specific Rotation	+258° ~ +287°	+270°
pH (1%, H2O)@25℃	8.5-10.0	9.0
Water	≤2.0%	1.64%
Heavy metals	≤20ppm	2ppm
Endotoxin	<0.1EU	Not detected
Water solubility	easy to soluble in wat	

QC Dept. Manager: Zhanghua Checker: Shijian

苏州天可贸易有限公司 质检专用章

Appendix 1.6 Agar

SCECSHZQZG-05R-25A
FQC No.: CCP2016009063

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co.,Ltd
中国医药集团

上海市宁波路52号
No.52 Ningbo Road
Shanghai China
P.C.:200002
TEL:86-21-63219651
Fax:86-21-63214037
www.reagent.com.cn

检 验 报 告 单

Certification of Analysis

商品编号 Commodity No.10000561
品名：琼脂粉，纯化 Name: Agar-agar powder
性状：浅黄色细粉末。
Property condition: Pale yellow fine powder.
分子式 Formula: (C12H18O9)n 分子量 Molecular Weight: NA
规格 Specification: 生化试剂 BR 包装 Packing: 250g
批号 Lot No: 20161118
检验依据 Standard of Analysis: Q/CYDZ 488-2014

技术条件
Specification

检验项目 Item of Analysis	标准值 Standard	实测值 Result
水不溶物,% Water-insoluble matter	≤1.0	<1.0
凝胶强度(15g/L，20℃)/(g/cm2) Gel strength	≥400.0	400.0
淀粉试验 Starch test	合格	合格
微生物灵敏度试验 Sensitivity to microbial	合格	合格
吸水率/mL Water absorption	≤75.0	75.0
灼烧残渣，% Residue on ignition	≤5.0	3.2
干燥失量，% Loss on drying	≤15.0	7.3

结论 Result: 合格(To pass test)

检验人 Analyer: 方诚 审核人 Reviewed by: 马骏
检验日期 Date of Analysis: 2016-12-08 批准人 Approved by: 郑琦

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co.,Ltd
质量管理部
QA/QC Center
检测地：上海市金山工业区月工路1600号

Appendix 1.7 Monopotassium Phosphate

检 验 报 告

Test Report

№:H2017WTS0166

防伪码:BL6JD

产品名称： Product Name	磷酸二氢钾
经销单位： Unitbeing tested	——
生产单位： Manufacturer	江苏科伦多食品配料有限公司
委托单位： Entrusting Unit	江苏科伦多食品配料有限公司
检验类别： Test Kind	委送检验

连云港市产品质量监督检验中心

The Center of Lianyungang Product Quality Supervision and Inspection

连云港市产品质量监督检验中心
检 验 报 告

№ H2017WTS0166 共 2 页 第 1 页

产品名称	磷酸二氢钾	商标	科多
		规格型号	——
生产单位	江苏科伦多食品配料有限公司		
委托单位\地址\联系电话\邮编	江苏科伦多食品配料有限公司\灌云县经济开发区纬二路南侧\0518-85110538\222000		
经销单位	——		
检验类别	委托检验	样品编号	H2017WTS0166
样品数量	100g	样品等级	——
检验日期	2017-02-07~2017-02-10	生产日期\批号	—\—
样品状态	符合检验要求	送样日期	2017-02-10
封样状态	——	检查封样人员	李真真
检测地点	连云港市产品质量监督检验中心		
检验依据	GB 25560-2010《食品安全国家标准 食品添加剂 磷酸二氢钾》		
检验结论	样品经检验，符合GB 25560-2010标准规定的要求，检验结论为合格。		
备 注			

主 检：王海波

审 核：顾丽丽

批 准：王村红

（检验检测专用章）
（2）

签发日期：2017年 月 日

检验结果

第 3 页 第 2 页

序号	检验项目		单位	技术要求	检验结果	单项评价
1	感官要求	色泽	——	白色	白色	合格
		组织状态	——	晶状粉末或颗粒	晶状粉末	合格
2	磷酸二氢钾（KH_2PO_4）（以干基计），w/%		——	≥98.0	98.6	合格
3	水不溶物，w/%		——	≤0.2	未检出	合格
4	砷（As）		mg/kg	≤3	<3	合格
5	重金属（以Pb计）		mg/kg	≤10	<10	合格
6	铅（Pb）		mg/kg	≤2	<2	合格
7	氟化物（以F计）		mg/kg	≤10	5	合格
8	pH值（10g/L溶液）		——	4.2～4.7	4.5	合格
9	干燥减量，w/%		——	≤1.0	0.6	合格
备注	——					

Test Report

No.H2017WTS0166

Security Code: BL6JT0

Product Name: Potassium Phosphate Monobasic

Distributor:

Manufacturer: Jiangsu Kolod Food Ingredients Co., Ltd.

Requestor: Jiangsu Kolod Food Ingredients Co., Ltd.

Test Kind: Analysis Request

Lianyungang Product Quality Supervision & Inspection Institute

Analysis Report

No: H2017WTS0166 Total 2 Pages / Page 1

Product Name	Potassium Phosphate Monobasic	Trademark	Kolod
		Specification	
Manufacturer	Jiangsu Kolod Food Ingredients Co., Ltd.		
Requestor/Address/ Phone/ Postal Code	Jiangsu Kolod Food Ingredients Co., Ltd./Guanyun Economic Development Zone Wei'er Rd South/0518-85110538/222000		
Distributor			
Test Kind	Analysis Request	Sample Number	H2017WTS0166
Sampling Quantity	100g	Sample Grade	
Testing Date	2017-02-07~2017-02-10	Manufacturing Date/ Batch Number	
Sample Condition	Analysis requirements met	Date Received	2017-02-10
Sealing State		Sealing State Inspector	Li. ZhenZhen
Test Location	Lianyungang Product Quality Supervision & Inspection Institute		
Test Compliance	GB25560-2010 (Potassium Phosphate Monobasic National Standard of Food Safety of Food Additives)		
Test Result	Sample in compliance with GB25560-2010 standards, result as PASS		
Notes			

Analyzed by:

Reviewed by: (Analyzing unit stamp)

Approved by: Approval Date: February 13, 2017

Analysis Result

No: H2017WTS0166 Total 2 Pages / Page 2

No.	Testing Item		Unit	Standard	Result	Evaluation
1	Appearance	Color		White	White	PASS
		State		Crystallized powder or granules	Crystallized powder	PASS
2	Potassium Phosphate Monobasic			≥98.0	98.6	PASS
3	Water insoluble matter			≤0.2	Negative	PASS
4	Arsenic (As)		mg/kg	≤3	<3	PASS
5	Heavy Metal (amount of Pb)		mg/kg	≤10	<10	PASS
6	Lead (Pb)		mg/kg	≤2	<2	PASS
7	Fluoride (amount of F)		mg/kg	≤10	5	PASS
8	PH (10g/L solution)			4.2~4.7	4.3	PASS
9	Loss on Drying			≤1.0	0.6	PASS
Notes						

Appendix 1.8 Dipotassium Phosphate

Jiangsu Kolod Food Ingredients Co., Ltd

CERTIFICATE OF ANALYSIS

Production name		Dibasic potassium phosphate(Anhydrous)		Product standard		GB 25561-2010
Lot No.	16122801	Production date	December 28, 2016	Delivery date		January 2, 2016

Items	Requirements	Analysis method	Test value	Analysis results
Color	White	Transfer certain amount of product into 50ml beaker and check its color and form in natural light	White	Qualified
Form	Powder		Powder	Qualified

Quality Indicators

Items	Indicators	Test values	Analysis basis	Analysis results
Dibasic potassium phosphate(based on dry mass), %W	≥98.0	99.67	GB 25561-2010 Appendix A. A4	Qualified
Insoluble, %W	≤0.2	0.03	GB 25561-2010 Appendix A. A5	Qualified
Arsenic(As), (mg/kg)	≤3.0	<3	GB 25561-2010 Appendix A. A6	Qualified
Heavy metals(based on Pb), (mg/kg)	≤10.0	<10	GB 25561-2010 Appendix A. A7	Qualified
Lead(Pb), (mg/kg)	≤2.0	<2	GB 25561-2010 Appendix A. A8	Qualified
Fluoride(based on F), (mg/kg)	≤10.0	7	GB 25561-2010 Appendix A. A9	Qualified
pH value(10g/L solution)	9.0±0.4	9.11	GB 25561-2010 Appendix A. A10	Qualified
Loss on drying, %W	≤2.0	0.21	GB 25561-2010 Appendix A. A11	Qualified
Analysis results	Passed the requirements of GB 25560-2010 (Food additive monobasic potassium phosphate).			

Analyzed by: Chen, Hui Auditor: Xu, Guangsheng

Appendix 1.9 Citric Acid

SCRCSHQCZG-05R-05A
BQC No.: FQC0014120

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co.,Ltd

上海市宁波路52号
No. 52 Ningbo Road
Shanghai China
P. C.: 200002
Tel: 86-21-63219631
Fax: 86-21-63214037
www.reagent.com.cn

检 验 报 告 单

Certification of Analysis

商品编号 Commodity No. 10007118
品名: 柠檬酸, 水
Name: Citric acid monohydrate
性状Property condition:: 无色结晶或白色颗粒。
分子式 Formula: C6H8O7·H2O
分子量 Molecular Weight: 210.14
规格 Specification: 分析纯 AR
包装 Packing: 500g
批号 Lot No. 20151208
检验依据 Standard of Analysis: GB/T 9855-2008

技 术 条 件

Specification

检验项目 Item of Analysis	标准值 Standard	实测值 Result
草酸盐(C2O4), % Oxalate(C2O4)	≤0.05	0.05
澄清度试验/号 Transparency of solution	≤4	4
钙(Ca),% Calcium(Ca)	≤0.005	0.001
含量, % Assay	≥99.5	99.9
磷酸盐(PO4), % Phosphate(PO4)	≤0.001	0.001
硫酸盐(SO4), % Sulphate(SO4)	≤0.005	0.005
氯化物(Cl), % Chloride(Cl)	≤0.0005	0.0005
铅(Pb),% Lead(Pb)	≤0.0005	0.0004
水不溶物,% Water-insoluble matter	≤0.005	0.005
铁(Fe),% Iron(Fe)	≤0.0005	0.0003
铜(Cu),% Copper(Cu)	≤0.0005	0.00006
易炭化物质 Really carbonized substances	合格	合格

结论 Result: 合格 (To pass test)

检验人 Analyer: 王英
复核人 Verified By: 马骏
检验日期 Date of Analysis: 2016-01-04
审批人 Approved By: 郑琦

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co., Ltd
质量管理检测中心
QC/QA Center

第1页 共2页

BQC No.: FQCZ014120

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co., Ltd
No. 52 Ninghe Road
Shanghai China
P.C. 200060
Tel: 86-21-63210668
Fax: 86-21-63214027
www.reagent.com.cn

检 验 报 告 单

Certification of Analysis

商品编号 Commodity No. I0007118
品名: 柠檬酸 一水
Name: Citric acid monohydrate
性状Property condition: 无色结晶或白色颗粒。
分子式 Formula: C6H8O7·H2O 分子量 Molecular Weight: 210.14
规格 Specification: 分析纯 AR 包装 Packing: 500g
批号 Lot No. 20151208
检验依据 Standard of Analysis: GB/T 9835-2008

技 术 条 件

Specification

检验项目 Item of Analysis	标准值 Standard	实测值 Result
灼烧残渣(以硫酸盐计), % Residue on ignition(as SO4)	≤0.02	0.01

结论 Result: 合格 (To pass test)

检验人 Analyst: 王燕 复核人 Verified By: 马骏
检验日期 Date of Analysis: 2016-01-04 审核人 Approved By: 郑琦

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co., Ltd
质量管理检测中心
QC/QA Centre

Appendix 1.10 Defoamant

Date 2016-11-24 (YYYY-MM-DD) Time 02:40:19 (Greenwich Mean Time) Page 1 of 1

DOW

DOW CHEMICAL PACIFIC LIMITED

200031
中国

Ship From: Nankang
NANTOU
Taiwan

分析证明/
Certificate of Analysis

产品编号/Product Number 00000101924

产品名称/Product Name

DOWFAX™ DF 103. 消泡剂

送货单号/Delivery No. 809219769 / 000010

订单号码/Order Number 105906354

装货日期/Date Shipped 2016-11-23 (YYYY-MM-DD)

装运单号/Shipment No. 28705027

客户资讯/Customer Information

客户名称/Customer Name

客户订单号/Customer PO number KANDO201625

集装箱编号/Container ID WHLU2877849

车辆号/Vehicle# 320-KQ

规格号/Specification Number 000000056144

批号/Batch Number C499GBL205

生产日期/Manufacturing Date 2016-11-19 (YYYY-MM-DD)

测试/Test	单位/Unit	下限制/Lower Limit	上限制/Upper Limit	值/Value	方法/Method
羟值 Hydroxyl Number 如氢氧化钾	mg/g	52.8	58.5	56.9	ASTM D4274 D
水分 Water	WT%	-	0.080	0.026	ASTM D4672
颜色, APHA Color, APHA		-	40	7	ASTM D4890
粘度 Viscosity 在25摄氏度	cSt	425	495	461	ASTM D4878

Quality Coordinator

如有问题, 请咨询客户服务人员或当地销售人员
For inquiries please contact Customer Service or local sales

Blue California

A Perfect Blend of Science and Nature

April 13, 2017

FOOD GRADE STATEMENT

BLUE CALIFORNIA hereby certifies that DOWFAX DF 103 is a food-grade defoaming agent. We further certify that this product is compliant with 21 CFR 173.340.

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

Sincerely,

Hadi Omrani

Hadi Omrani
Technical Manager-Regulatory Affairs

Corporate Headquarters
30111 Tomas, Rancho Santa Margarita, CA 92688 Tel: 949-635-1900 Fax: 949-635-1984
Website: www.blue-california.com

Appendix 1.11 Ammonium Hydroxide

TEST REPORT

2017201F3000004

Product Name: Ammonium hydroxide (Food Grade)

Trust Unit: Yimin Taixing Chemical Co., Ltd.

Manufacture: Yimin Taixing Chemical Co., Ltd.

Test Category: Commissioned sample inspection

TEST REPORT

2017201F3000004 Total 2 pages Page 1

Product Name		Ammonium hydroxide (Food Grade)	Specification Type	27%
			Trademark	-
Trust Unit	Name	Yimin Taixing Chemical Co., Ltd.	Unit being tested	-
	Address	6 Beierbuan west road, Binjian, Taixing city, Jiangsu Province	Manufacturer	Yimin Taixing Chemical Co., Ltd.
Sampling site		-	Sampling Date	March 15, 2017
Sample Quantity		500ml	Sample Grade	-
Sampling Batch		-	Samples Conditions	Qualified
Serial Number or Manufacture Date		-	Test Category	Commissioned sample inspection
Test Date		March 15, 2017 to March 22, 2017	Test place	Quality Supervising and Inspecting Center of Taizhou City, Medicine city
Test Standard(s)		GB329201-2012 «National Food Safety Standard. Food additive. Ammonium hydroxide».		
Test Conclusion		The product is qualified based on the standard of GB329201-2012. Date: March 23, 2017		
Remarks		The test results of this report are based on the basis of the inspection only on the inspection items, does not mean that the unexamined items or functions meet the requirements.		

Approver: Gao, Junwei Examiner: Zhu, Lei Major tester: Yang, Min

TEST RESULTS

2017201F3000004 Page 2

Serial	Test Items	Unit	Specification	Test Results	Evaluation
1	Color	-	Colorless	Colorless	Qualified
2	Appearance	-	Transparent liquid	Transparent liquid	Qualified
3	NH3 content. w%	-	27.0-30.0	28.0	Qualified
4	Pb	Mg/kg	≤0.5	0.03	Qualified
5	Residue after evaporation. w%	-	≤0.02	0.001	Qualified
6	Readily oxidation substance	-	Pass	Pass	Qualified

Appendix 1.12 Ammonia

Test Report

2017201F3000004

Product Name: Food-grade ammonia

Trust Unit: Taixing Yimin Chemical Co., Ltd.

Manufacture: Taixing Yimin Chemical Co., Ltd.

Test Category: Commissioned inspection

PRODUCT QUALITY SUPERVISING AND INSPECTING CENTER OF TAIZHOU CITY

Test Report

201701F3000004 Page 1 out of 2

Product Name		Food-grade ammonia	Specification Type	27%
			Trademark	N/A
Trust Unit	Name	Taixing Yimin Chemical Co., Ltd.	Unit being tested	N/A
	Address	No. 6 West Road, 2nd North Ring, Binjiang Town, Taixing City	Manufacturer	Taixing Yimin Chemical Co., Ltd.
Sampling Site		N/A	Sampling Date	03/15/2017
Sampling Quantity		500ml	Sample Grade	N/A
Sampling Batch		N/A	Samples Conditions	Meet the requirement of the inspection
Serial Number or Manufacture Date		N/A	Test Category	Commissioned inspection
Test Date		03/15/2017 – 03/22/2017	Test Place	Taizhou Product Quality Supervising & Inspecting Center, Medical City
Test Standard(s)		GB 29201-2012 "National standards for food safety: Food additives, Ammonia"		
Test Conclusion		The sample meets the standards of GB 29201-2012. (stamp) Signature Date: 03/23/2017		
Remarks		The results of this report are based on the test basis of items inspected only. This does not mean that the untested project or function to meet the requirements.		

Approver: Junwei Gao Examiner: Lei Zhu Major tester: Min Yang

Test Results

201701F3000004

Serial	Test items	Unit	Specification	Test results	Evaluation
1	Color	N/A	Colorless	Colorless	Pass
2	Status	N/A	Transparent liquid	Transparent liquid	Pass
3	NH_3, ω/%	N/A	27.0 – 30.0	28.0	Pass
4	Pb	mg/kg	<= 0.5	0.03	Pass
5	Evaporation residue	N/A	<= 0.02	0.001	Pass
6	Readily oxidizable substances	N/A	Pass the test	Passed the test	Pass

Points for attention

1. This report is invalid if in any form by any means altered or without the official seal of the Institute.
2. Any objection to the result of this report in test, please present to the Institute for the consigned test within 15 days upon the receiving date.
3. The Institute is just responsible for the test results of the sample sent by the client. Thus, the test result is only meant to help the client know the quality of the sample.
4. This report may not be copied in any form without the prior permission of the Institute. The permitted report copy is valid under the terms that they are copied in full and confirmed by the official seal of the Institute.
5. The client should get back the remained sample in three months upon the receiving date of the report. For sample effective in a given period time, does according to its limitation period. Otherwise sample is at the Institute's disposal.

The Institute Add: 9 Tianhong Road, Taizhou, Jiangsu, China
G21, No.1 Avenue China Medical City. Taizhou City
No.8, Tongjiang Road, Taixing Economic Development Zone
The Institute Complain Tel: 0523-86999978
The Institute Business Tel: 0523-86999900 86882122 (Headquarters)
86999981 (Yaocheng) 80736218 (Taixing)
The Institute Fax: 0523-86999986, 86882122, 86999985, 80736216
The Institute Post: 225300
The Institute Email: taizhouzjs@jsqts.gov.cn
Web: http://www.tzj.gov.cn www.cntgs.com

Appendix 1.13 Magnesium Sulfate Heptahydrate

Test Report

No. H2017WT50167

Product Name: Magnesium sulfate heptahydrate
Unitbeing Tested: N/A
Manufacturer: Jiangsu Kolod Food Ingredients Co., Ltd.
Trust Unit: Jiangsu Kolod Food Ingredients Co., Ltd.
Test Category: Commissioned inspection

The Center of Lianyungang Product Quality Supervision and Inspection

The Center of Lianyungang Product Quality Supervision and Inspection Test Report

No. H2017WTS0167 Page 1 out of 2

Product Name	Magnesium sulfate heptahydrate	Trademark	Keduo
		Specifications and Model	N/A
Manufacturer	Jiangsu Kolod Food Ingredients Co., Ltd.		
Trust Unit\ Address\ Tel\ Zip Code	Jiangsu Kolod Food Ingredients Co., Ltd.\ Economic Development Zone Of Guanyun County 2nd Wei Road, South side\ 0510-85110530\ 222000		
Distribution Unit	N/A		
Test Category	Commissioned inspection	Sample No.	H2017WTS0167
Sample Quantity	100g	Sample Grade	N/A
Test Date	02/21/2017 – 02/27/2017	Production date / lot number	N/A\ N/A
Sample Condition	Meet the inspection requirements	Send date	02/10/2017
Sealed State	N/A	Staff that checked the sample	Zhenzhen Li
Test Site	The Center of Lianyungang Product Quality Supervision and Inspection		
Test Standard(s)	GB 29207-2012 "Food Safety National Standard, Food Additive, Magnesium Sulfate"		
Test Conclusion	After tested, the sample is in line with the requirements of the GB 29207-2012 standard. Therefore, the product is qualified.		
Remarks	N/A		

Major Tester: Shanshan Xu Inspection Unit

Examiner: Tiantian Gu (Stamp)

Approver: Linguo Wang Date of Issue: 02/27/2017

Test Result

No. H2017WTS0167

No.	Test Items		Unit	Specification	Test Results	Evaluation
1	Sensory Requirements	Color	N/A	Colorless or white	White	Pass
		Status	N/A	Crystal or powder	Crystal	Pass
2	Content of $MgSO_4$ after burning, w%		N/A	>= 99.0	99.5	Pass
3	Heavy Metals (measured based on Pb)		mg/kg	<= 10	< 10	Pass
4	Pb		mg/kg	<= 2	< 2	Pass
5	Se		mg/kg	<= 30	< 30	Pass
6	pH (50g/L solution)		N/A	5.5 – 7.5	6.2	Pass
7	Chloride (measured based on Cl), w%		N/A	<= 0.03	< 0.03	Pass
8	As		mg/kg	<= 3	< 3	Pass
9	Fe		mg/kg	<= 20	2	Pass
10	Weight loss of Magnesium sulfate heptahydrate after burning, w%		N/A	40.0 – 52.0	45.2	Pass
Remarks		N/A				

Appendix 1.14 Thiamine

SCRC 国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co.,Ltd

上海市宁波路52号
No. 52 Ningbo Road Shanghai China
P.C.:200002
Tel: 86-21-63219651
Fax: 86-21-63214037
www.reagent.com.cn

检 验 报 告
Certification of Analysis

商品编号 Commodity No. 67002134
名称 盐酸硫胺(维生素B1)
Name Thiamine hydrochloride
性状 Property condition 白色结晶或结晶性粉末。
分子式 Formula C12H17ClN4OS·HCl 分子量 Molecular Weight 337.27
规格 Specification 生化试剂 BR 包装 Packing 25g
批号 Lot No. 20150810
检验依据 Standard of Analysis Q/CYDZ 72-2008

技 术 条 件
Specification

检验项目 Item of Analysis	标准值 Standard	实测值 Result
含量(C12H17ClN4OS HCl) Assay	≥98.0%	98.5%
干燥失量 Loss on drying	≤5.0%	1.2%
灼烧残渣(以硫酸盐计) Residue on ignition	≤0.2%	0.02%
硫酸盐(SO4) Sulfate	≤0.05%	0.05%
pH(12.5g/L, 25℃)	≥2.5	3.1

检验日期 Date of Analysis 2015-8-17 检验人 Analyst 王海平
结论 Conclusion 合格 To pass test 复核人 Reviewed by 赵丽娟
批准人 Approved by 郑瑞

Sinopharm Chemical Reagent Co.,Ltd
国药集团化学试剂有限公司
QC/QA Center
质量管理检测中心

Appendix 1.15 Glucose

Test Report

No. W-S-20170229

Product Name: Edible glucose

Model Specifications: 500g/bag

Trust Unit: Xiwang Pharmaceutical Co., Ltd, Zouping Branch

Test Category: Commissioned Test

Binzhou City Food and Drug Inspection and Testing Center

Precautions

1. This report is invalid if no "inspection report special seal" or inspection unit official seal.
2. This report is invalid if no chief inspector, auditor, and signature of approver.
3. The copy of this report is invalid if no re-stamped "inspection report special seal" or inspection unit official seal.
4. The altered report is invalid.
5. The test results are only responsible for this batch of samples. Without the consent of the inspection agency, the client shall not use the test results for publicity.

Food and Drug Inspecting and Testing Center of Binzhou City
Test Report

No. W-5-20170229 page 1 out of 2

Product Name	Edible glucose	Model Specification	500g/bag
Trust Unit	Xiwang Pharmaceutical Co., Ltd, Zouping Branch	Trademark	N/A
Manufacturer	Xiwang Pharmaceutical Co., Ltd, Zouping Branch	Test Category	Commissioned test
Sampling Site	N/A	Sample Grade	Excellent
Sample Quantity	2 bags	Person who sent the sample	Mingzhao He
Sample Batch	N/A	Sent Date	03/21/2017
Sample Description	White powder in bags, good condition	Production Date or Lot Number	03/19/2017 2017031933
Specifications	Appearance, smell, taste, color, specific rotary power, glucose content, pH, etc.		
Test Standard(s)	GB/T 20880-2007		
Test Conclusion	The sample passed the test items. (stamp) Signature Date: 04/06/2017		
Remarks	1. The report is invalid if it does not have the inspection special seal, or does not have the approver's signature. 2. The report is invalid if it is modified without additional inspection special seal.		

Approver: Wenjun Han 04/06/2017, Examiner: Cui Gao 04/06/2017, Major Tester: Yanming Li 04/06/2017

Food and Drug Inspection and Testing Center of Binzhou City
Test Report

No. W-S-20170229 page 2 out of 2

No.	Test Item	Specification	Test Result	Evaluation
1	Appearance	Crystalline powder, no visible impurities	Crystalline powder, no visible impurities	Pass
2	Smell	No strange smell	No strange smell	Pass
3	Taste	Mild sweet, pure taste, no strange taste	Mild sweet, pure taste, no strange taste	Pass
4	Color	White or colorless	White	Pass
5	Specific rotary power, °	52.0 – 53.5	53.0	Pass
6	Glucose content (based on dry substance), %	>= 99.5	99.7	Pass
7	pH	4.0-6.5	5.4	Pass
8	Chloride, %	<= 0.01	< 0.01	Pass
9	Moisture, %	<= 10.0	8.8	Pass
10	Sulfuted ash, %	<= 0.25	0.00	Pass
11	Pb, mg/kg	<= 0.5	Not detected (< 0.005)	Pass
12	Total As, mg/kg	<= 0.5	Not detected (< 0.04)	Pass
13	Cu, mg/kg	N/A	Not detected (< 0.1)	N/A
14	SO_2 residue, g/kg	<= 0.04	0.003	Pass
15	Total number of colonies, cfu/g	N/A	< 10	N/A
16	Coliforms, MPN/100g	N/A	< 30	N/A
17	Pathogens (Salmonella, Shigella, Staphylococcus aureus)	N/A	Not detected	N/A
18	Aflatoxin B_1, μg/kg	N/A	Not detected (< 1)	N/A

Blank below

To provide users with accurate testing conclusions and quality service, Food and Drug Inspecting and Testing Center of Binzhou City commits to the community

1. Comply with the relevant laws, regulations and related regulations, according to the law.
2. In accordance with standards, procedures for product testing, so that the test data is accurate, the conclusion is correct.
3. Resist any interference, impartial service, based on the standard to ensure the authenticity of the data.
4. Fulfill their duties, abide by the rules and regulations, not abuse of power, not part-time work in the inspected units.
5. Not provide the test data to any party other than the client or the inspected unit.
6. Strictly keep the technology, patents, and business secrets of the inspected unit.
7. The technical information provided by the inspected unit is for inspection only.

Appendix 1.16 Sulfuric Acid

SCRCSHQCZG-05R-03A
FQC No.: IQC0004824

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co.,Ltd

检 验 报 告 单
Certification of Analysis

上海市宁波路82号
No.82 Ningbo Road
Shanghai China
P.C.:200002
Tel: 86-21-63210651
Fax: 86-21-63211037
www.reagent.com.cn

商品编号 Commodity No. 10021618
名称: 硫酸
Name: Sulfuric acid
性状 Character: 无色透明液体
分子式 Formula: H2SO4
分子量 Molecular Weight: 98.08
规格 Specification: 分析纯 AR
包装 Packing: 500ml
批号 Lot No. 20160803
检验依据 Standard of Analysis: GB/T 625-2007

技 术 条 件
Specification

检验项目 Item of Analysis	标准值 Standard	实测值 Result
铵盐(NH4), % Ammonium	≤0.0002	0.0002
含量, % Assay	95.0~98.0	96.8
还原高锰酸钾物质(以SO2计), % Permanganate reducing substances(as SO2)	≤0.0005	0.0005
氯化物(Cl), % Chloride(Cl)	≤0.00003	0.00003
铅(Pb), % Lead(Pb)	≤0.00001	0.0000003
色度/黑曾单位 Color, Hazen units	≤10	10
砷(As), % Arsenic(As)	≤0.000003	0.000003
铁(Fe), % Iron(Fe)	≤0.00005	0.00005
铜(Cu), % Copper(Cu)	≤0.00001	0.0000006
硝酸盐(NO3), % Nitrate(NO3)	≤0.00005	0.00005
灼烧残渣(以硫酸盐计), % Residue on ignition(as SO4)	≤0.001	0.001

结论 Result: 合格 (To pass test)

检验人 Analyer: 沈静
审核人 Verified By: 马骏
检验日期 Date of Analysis: 2016-08-19
批准人 Approved By: 郑琦

国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co., Ltd.
品量管理部
QA/QC Center
检测单: 江苏省太仓市沙溪镇工业开发区陆鸿路18号

第1页 共1页

Appendix 1.17 Iron (III) Citrate

SCRC 国药集团化学试剂有限公司
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上海中宁波路52号
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检 验 报 告
Certification of Analysis

商品编号 Commodity No. 30107418
名称 柠檬酸铁,水合
Name Iron(III) citrate tribasic hydrate
性状 Property condition 暗红或棕色鳞片或粒状。(避光)
分子式 Formula C6H5O7Fe·nH2O 分子量 Molecular Weight 244.95(以无水
规格 Specification 分析纯 AR 包装 Packing 500g
批号 Lot No. 20160510
检验依据 Standard of Analysis Q/CYDZ 52-2005

技 术 条 件
Specification

检验项目 Item of Analysis	标准值 Standard	实测值 Result
稀盐酸溶解试验 Solubility in diluted HCl	合格 to pass test	合格 to pass test
铵盐 Ammonium	合格 to pass test	合格 to pass test
含量(以Fe计) Assay	16.5~18.5%	16.6%
氯化物(Cl) Chloride	≤0.005%	0.001%
硫酸盐(SO4) Sulfate	≤0.05%	0.05%
碱金属(Na2CO3) Alkali metal	≤0.15%	0.15%
重金属(以Cu计) Heavy metals	≤0.005%	0.005%

检验日期 Date of Analysis 2016-5-10 检验人 Analyst 干海平
结论 Conclusion 合格 To pass test 复核人 Reviewed by 胡晓
批准人 Approved by 郑玲

Sinopharm Chemical Reagent Co.,Ltd
国药集团化学试剂有限公司
QC/QA Center
质量管理检测中心

Appendix 1.18 Ammonium Iron (II) Sulfate

SCRC 国药集团化学试剂有限公司
Sinopharm Chemical Reagent Co.,Ltd

上海市宁波路51号
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Fax: 86-21-63214037
www.reagent.com.cn

检 验 报 告
Certification of Analysis

商品编号 Commodity No. 10001918
名称 六水合硫酸铁(II)铵(硫酸亚铁铵)
Name Ammonium iron(II) sulfate hexahydrate
性状 Property condition 浅蓝绿色结晶
分子式 Formula (NH4)2Fe(SO4)2·6H2O **分子量 Molecular Weight** 392.14
规格 Specification 分析纯 AR **包装 Packing** 500g
批号 Lot No. F20110307
检验依据 Standard of Analysis GB T 661-1992

技 术 条 件
Specification

检验项目 Item of Analysis	标准值 Standard	实测值 Result
含量[(NH4)2Fe(SO4)2·6H2O] Assay	≥99.5%	100.1%
pH(50g L,25℃)	3~5	3.8
水不溶物 Insoluble in water	≤0.005%	<0.005%
氯化物(Cl) Chloride	≤0.001%	0.001%
磷酸盐(PO4) Phosphate	≤0.0005%	0.0005%
氨水不沉淀物(以硫酸盐计) Substances not precipitated by NH4OH	≤0.1%	0.1%
锰(Mn) Manganese	≤0.05%	0.05%
高铁(Fe) Iron (III)	≤0.01%	0.005%
锌(Zn) Zinc	≤0.005%	0.0005%
铜(Cu) Copper	≤0.002%	0.0004%
铅(Pb) Lead	≤0.002%	0.0002%

检验日期 Date of Analysis 2011-3-16 **检验人 Analyst** 陈阳 倪佐东
结论 Conclusion **合格 To pass test** **复核人 Reviewed by** 薛丽
批准人 Approved by 郑琦

Sinopharm Chemical Reagent Co.,Ltd
国药集团化学试剂有限公司
QC/QA Center
质量管理检测中心

Appendix 1.19 Ion-Exchange Resin

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) %	95.90	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 1.20 Activated Charcoal

中 国 林化产品质量监督检验站
国 家 林 业 局

分 析 化 验 报 告 单

分析检验方法：

GB/T12496.1~12496.22-99

检验项目及结果：

1、材质	木质型
2、粒度	200 目
3、亚甲基兰 mg/g	198
4、铁盐%	0.02
5、水份%	9.3
6、重金属%	0.02
7、PH	3.63
8、氯化物%	0.1

委托单位：[illegible]
样品名称：767 型活性炭
样品编号：

分析检验人：[illegible]
审核人：[illegible]

2017 年 2 月 16 日

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 Analytical Method and Representative Chromatograms

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

Summary Report

Method Verification of the Determination of L-Ergothioneine (ErgoActive™) by High Performance Liquid Chromatography (HPLC) and Purity Analysis of Five Production Samples

Prepared by (b) (6)

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

(b) (6)

Hilary Rogers, Operations Manager
Eurofins Scientific, Inc.

Approved by: _____

Cecilia McCollum, Executive Vice President
Blue California.

Date Issued: February 16th, 2017

I. Study Identification

1. Study Title:

Method Verification of the Determination of L-Ergothioneine (ErgoActive™) 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 L-Ergothioneine by High Performance Liquid Chromatography (HPLC) and Purity Analysis of Five Production Samples (modified Sotgia et al. PLOS ONE method).

3. Study Coordinator/Performing Laboratory:

Jeff Lin, Senior Scientist
Eurofins Scientific, Inc.

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

Hilary Rogers, Operations Manager
Eurofins Scientific, Inc.

4. Study Monitors:

Cecilia McCollum, Executive Vice President
Blue California

5. Method References:

Sotgia S, Pisanu E, Pintus G, Erre GL, Pinna GA, Deiana L, Carru C, Zinellu A. Plasma L-ergothioneine measurement by high-performance liquid chromatography and capillary electrophoresis after a pre-column derivatization with 5-iodoacetamidofluorescein (5-IAF) and fluorescence detection. PLoS one. 2013 Jul 29;8(7):e70374.

II. Study Description

1. Scope:

This method is applicable to the determination and quantification of L-ergothioneine, in raw materials and ErgoActive™ products. L-Ergothioneine quantitation was determined using the Sigma standard. 5-Iodoacetamidofluorescein (5-IAF) was used as a pre-column derivatization reagent, and HPLC-FLD (HPLC with fluorescence detector) was used as the analytical instrument.

2. Test Materials:

L-Ergothioneine dietary supplement finished product

(1) Eurofins sample 740-2016-00017587 ErgoActive 5%, Lot #3332-1606-01

(2) Eurofins sample 740-2016-00017591 ErgoActive 5%, Lot #3332-1606-02

(3) Eurofins sample 740-2016-00017595 ErgoActive 5%, Lot #3332-1606-03

(4) Eurofins sample 740-2016-00017599 ErgoActive 5%, Lot #3332-1606-04

(5) Eurofins sample 740-2016-00017603 ErgoActive 5%, Lot #3332-1606-05

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) Potassium phosphate dibasic (Analytical Grade), Sigma Catalog #: P3786, C.A.S #: 7758-11-4

(4) L-(+)-Ergothioneine, Sigma Catalog #: E7521, C.A.S #: 497-30-3

(5) Dimethyl sulfoxide, VWR Catalog #: 081-1, C.A.S #: 67-68-5

(6) Sodium phosphate tribasic dodecahydrate, Sigma Catalog #: 222003-500G, C.A.S #: 10101-89-0

4. Mobile Phase Preparation:

Mobile phase A: 40% 100mM potassium phosphate dibasic: 7% acetonitrile : 3% methanol: 50% water
Mobile phase B: 40% 100mM potassium phosphate dibasic: 20% acetonitrile : 3% methanol: 37% water

5. Reference Standards:

A. Stock standards.

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

2. On a microbalance, accurately weighed 1.0 ± 0.1 mg of L-ergothioneine Sigma standard; quantitatively added 1 mL water. This is stock solution.

B. Calibration working standards were prepared by diluting standard stock solution with water. The range of quantitation was approximately between 5 ug/mL and 35 ug/mL in solution. A 5 point curve was utilized for determination of linearity for this study. A 3 point curve will be used for routine quantitation for the current and future samples. The sample test concentration was approximately 16 ug/mL L-ergothioneine, based on the expected test sample concentration. The adjusted L-ergothioneine standard curve covered the targeting L-ergothioneine 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 L-ergothioneine 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 20 ug/ml standard solution were injected during the analysis sequence for L-ergothioneine.

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:

	L-Ergothioneine	PASS/FAIL
Retention time (RT) Range (minutes)	4.90 - 4.92	-
RT % RSD	**0.106**	**PASS**
Peak area range	272 - 275	-
Peak area RSD	**0.331**	**PASS**
Number of Data Points	8	-

L-Ergothioneine standard retention time %RSD passed the criteria of less than 2%.
L-Ergothioneine standard peak area %RSD passed the criteria of less than 2%.

3. An Extended Performance report was generated using Agilent Chem Station software to include the resolution and USP tailing for L-ergothioneine. Results are as follows;

Resolution Tangent method L-Ergothioneine = 2.257 PASS
USP Tailing L-Ergothioneine = 1.136 PASS

4. The retention time and identity for L-Ergothioneine were confirmed using the derivatized Sigma L-Ergothioneine commercial standards.

C. Linearity:

1. A 5 point calibration curve for L-Ergothioneine was developed. The stock standard was diluted into working standard and then injected after derivatization. The 5 point calibration curve for validation with relative concentrations for L-Ergothioneine was as follows (adjusted for standard purity):

Stock used (uL)	Diluent (mL)	Relative Concentration (mg/mL)
70	2	0.0345
55	2	0.0273
40	2	0.0200
25	2	0.0126
10	2	0.00508

Linearity Results L-Ergothioneine:

Correlation Coefficient	Criteria	PASS/FAIL
0.99955	**> 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 **2.71%** for L-ergothioneine.

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. Five preparation solvent blanks were tested throughout the duration of the study. The chromatograms were free of interfering peaks.

E. Accuracy (Recovery):

Accuracy was determined by spiking a sample of known value (740-2016-00017587) 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(uL)	Recovery%	Acceptance criteria	PASS/FAIL
60 (low level)	98.5	80-115%	PASS
120 (mid level)	102	80-115%	PASS
180 (high level)	102	80-115%	PASS

F. Repeatability (Precision):

Five ErgoActive testing samples were analyzed for purity. L-Ergothioneine stock standard was prepared at 1 mg/mL (70 uL, 55 uL, 40 uL, 35 uL, and 10 uL stock solution were used to prepare 5 levels of working calibration standard solution). The range of L-ergothioneine quantitation was approximately between 5 ug/mL and 35 ug/mL. The testing purity samples were prepared at approximately 0.32 mg/mL with 5% as their expected purity level. Because the samples were low purity finished product, % RSD for precision measurements shall be less than 5 according to Eurofins' in-house criteria.

Only one L-ergothioneine signal was found in corresponding chromatograms.

Sample results are as follows:

740-2016-17587	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	Relative Standard Deviation %
L-Ergothioneine	6.54	6.61	6.14	**6.43**	**3.96 (PASS)**
740-2016-17591	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	Relative Standard Deviation %
L-Ergothioneine	5.57	5.84	5.75	**5.72**	**2.38 (PASS)**
740-2016-17595	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	Relative Standard Deviation %
L-Ergothioneine	6.08	6.02	5.98	**6.03**	**0.82 (PASS)**
740-2016-17599	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	Relative Standard Deviation %
L-Ergothioneine	6.42	6.46	6.93	**6.60**	**4.33 (PASS)**
740-2016-17603	Run 1	Run 2	Run 3		
Compound	Result (%w/w)	Result (%w/w)	Result (%w/w)	Average (%w/w)	Relative Standard Deviation %
L-Ergothioneine	6.06	6.50	5.81	**6.12**	**5.66 (FAIL!!)**

G. Moisture Correction:

A moisture correction was not conducted because samples are not known to be hygroscopic. However, this test can be done upon request.

7. Conclusions:

The results generated meet and exceed the acceptance criteria as established in the method verification proposal **with sample 740-2016-17603 as the exception (%RSD > 5%)**. All analyses were performed on an Agilent 1260 series HPLC-FLD (HPLC with fluorescence detector) and Agilent ChemStation software. The primary objective of the study has been to show that the method as designed can accurately determine the concentration of L-ergothioneine in "ErgoActive 5%" products. The results showed that the method is precise and accurate.

Quantification of L-ergothioneine was accomplished using Sigma L-ergothioneine reference material (standard) as described in Sotgia et al. PLOS ONE method with modifications.

The optimal operational parameters of the fluorescence detector were investigated for 5-IAF derivatized L-ergothioneine and a GAIN of 5 was found to be able to provide an optimal signal-to-noise ratio. The derivatization reagent (5-IAF) was found to be unstable and therefore needs to be freshly prepared.

Limit of detection and limit of quantitation were beyond the scope of this project. However determination of the impurities can be performed at the low levels that are found in these samples. The ICH signal/noise method (ICH Validation of Analytical Procedures: Methodology, section 6.2) for determining limit of detection and limit of quantitation was utilized. Limit of detection and limit of quantitation for these compounds are roughly estimated at 0.28% and 0.95% respectively. In the future additional work can be performed to statistically determine these limits upon request.

Five lots of "ErgoActive 5%" were tested by this method. The results have shown accurate and precise determination of L-ergothioneine without significant matrix interference. **Sample 740-2016-17603 failed Eurofins repeatability criteria (%RSD > 5%)**

ACCURACY & REPEATABILITY #1

NOTEBOOK PAGES

TITLE

Work continued from Page ——→

PROJECT NO. L-ergothioneine 27

BOOK NO. JL-01

Mobile phase A: 100 mmol/l potassium phosphate dibasic/ACN/MeOH/water (40:7:5:50), log #: 02072017-0003
made: 2/7/17, exp: 5/7/17

To make 2 liters:

800 ml of 100 mmol/l potassium phosphate dibasic

140 ml acetonitrile CI# 17667

60 ml methanol CI# 17677

1000 ml water

JL 2/7/17
~~To make~~

Mobile phase B: 100 mmol/l potassium phosphate dibasic/ACN/MeOH/water (40:20:3:37)
log #: 02072017-0004
made: 2/7/17, exp: 5/7/17

To make 1 liter:

400 ml potassium phosphate dibasic

200 ml acetonitrile CI# 17667

30 ml methanol CI# 17677

370 ml water

To make 100 mmol/l potassium phosphate dibasic solution
17.418 g/L, CI# 13375, exp. 12/31/18

To make 2 liters:

target weight	weight (g)	
JL 2/7/17 +17.418 = 34.836 g	19.9095 + 14.92713 = 34.83663	→ 2 liters of water log # L02020917B made 2/7/17, exp. 2/21/17

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Work continued to Page 28

SIGNATURE (b) (6)			DATE 2/7/17
DISCLOSED TO AND UNDERSTOOD BY (b) (6)	DATE	WITNESS	DATE 2/14/17

28 TITLE Micro Balance = XP26H1 PROJECT NO. L-ergothioneine
Work continued from Page 27 Pipettes used: E5000 For whole experiment K20 S200 BOOK NO. JL-01

standard prep: L-ergothioneine CI# 17589, exp. 12/31/21

target weight (mg)	weight (mg)	prep method
0.999748	1.005	@ dilute with 1 mL millIQ water

Dilution scheme:

amount of stock standard	dilution (mL) (millIQ water)	
10 µL	2	→ For information only
30 µL	2	Used in calibration curve to quantify samples 17587 and 17599.
50 µL	2	
70 ~~90~~ µL JL 2/16/17	2	Used for accuracy test.
90 µL	2	→ For information only

① Took 150 µL of each standard dilution and transfered to amber autosampler vial
② Added 50 µL of 5-IAF working solution
③ Let solution sit for 30 minutes at room temperature in a darkened area.
④ Transfer solution to an insert.

vortex for 1 minute

JL 2/16/17

www.scientificbindery88yrs.com Work continued to Page 29

SIGNATURE (b) (6)			DATE 2/8/17
DISCLOSED TO AND UNDERSTOOD BY (b) (6)	DATE	WITNESS	DATE 2/14/17

TITLE Balance - XP26# 1 **PROJECT NO.** L-ergothioneine **29**
Work continued from Page 28 **BOOK NO.** JL-01

5-IAF prep: 5-(Iodoacetamido)-fluorescein CI# 17685
exp: 11/17/20

target weight (mg)	weight (mg)	prep method (stock)
10.305	10.354	① dilute with 1 mL of dimethyl sulfoxide ② Vortex 10 seconds, sonicate for 15 minutes ③ To make working solution, take 963 µL of stock, disperse into 25 mL amber volumetric flask, fill to volume with sodium phosphate tribasic diluent

5-IAF working solution diluent = sodium phosphate tribasic dodecahydrate CI# 17582, exp. 12/31/21

target weight (mg)	weight (mg)	prep method
2850	2850.24	→ 50 mL milliQ water

JL 2/14/17

SIGNATURE (b) (6)			DATE 2/8/17
DISCLOSED TO & UNDERSTOOD BY (b) (6)	DATE	WITNESS	DATE 2/14/17

30 TITLE

Balance: XP26 #1 PROJECT NO. L-ergothioneine

Work continued from Page 29

BOOK NO. JL-01

Sequence: ERT_020807

Instrument: 30

~~MPA: 02072017-0005~~

MPB: 02072017-0004

Sample prep:

sample #	weight (mg)	prep method
17587-A	8.121	
17587-B	8.097	
17587-C	8.033	
17587-D	8.168	→ spike with 60 µL of stock solution standard before dilution
17587-E	8.159	→ spike with 120 µL of stock solution standard before dilution
17587-F	8.089	→ spike with 180 µL of stock solution standard before dilution
17599-A	8.073	
17599-B	8.106	
17599-C	8.107	

Note: all samples have been ground

① add 12.5 mL milliQ water and 12.5 mL acetonitrile

② vortex for 30 seconds

③ sonicate for 30 minutes

④ centrifuge 1mL of each sample at 14,000g for 10 minutes

⑤ Take 150 µL of the supernatant and dispense into into an amber autosampler vial

⑥ Add 50 µL of 5-IAF working solution into the amber autosampler vial with the sample solution

⑦ vortex vial for a minute

⑧ Let sit for 30 minutes at room temperature in a darkened area

⑨ Transfer sample into an insert.

SIGNATURE	(b) (6)		DATE 2/8/17
DISCLOSED TO AND UNDERSTOOD BY (b) (6)	DATE	WITNESS	DATE 2/14/17

LINEARITY & REPEATABILITY #2

NOTEBOOK PAGES

TITLE Balance: XP26#1 Pipettes: E5000 K20 S200 **PROJECT NO.** L-ergothioneine **31**
Work continued from Page _____ **BOOK NO.** JL-01
Sequence: ERT_021007
Instrument: 30
~~MPA: 02072017-0003~~
MPB: 02072017-0004

Sample prep:

sample #	weight (mg)
17591-A	8.063
17591-B	8.141
17591-C	8.108
17595-A	8.027
17595-B	8.068
17595-C	8.085
17603-A	8.104
~~17603~~ 17603-B	8.160
17603-C	8.103

Note: All samples were ground prior to weighing

① add 12.5 mL MilliQ water and 12.5 mL acetonitrile
② Vortex for 30 seconds
③ Sonicate for 30 minutes
④ Centrifuge 1 mL of each sample at 14,000g for 10 minutes
⑤ Take 150 µL of the supernatant and dispense into an amber autosampler vial.
⑥ Add 50 µL of S-IAF working solution to the sample
⑦ Vortex for a minute
⑧ Let sit for 30 minutes at room temperature in a darkened area.
⑨ Transfer sample into an insert.

JL 2/16/17

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Work continued to Page 32

SIGNATURE (b) (6)			DATE 2/10/17
~~DISCLOSED TO AND UNDERSTOOD BY~~ (b) (6)	DATE	WITNESS	DATE 2/14/17

32 TITLE Balance: XP26#1

PROJECT NO. L-ergothioneine

Work continued from Page 31 BOOK NO. JL-01

standard prep: L-ergothioneine CI# 17761, exp: 12/31/21
Lot: BCBK8742V, E7521

target weight (mg)	weight (mg)	
0.999748	1.034	→ 1 mL milliQ water

Purity = 98.8 %

Dilution scheme:

amount of stock standard from JL 2/10/17	dilution with milliQ (mL)	
10	2	used for calibration curve to quantitate samples 17591, 17595, 17603. used for linearity test.
25	2	
40	2	
55	2	
70	2	

① Took 150 µl of each standard dilution and transferred to amber autosampler vial.
② Add 50 µl of 5-IAF working solution.
③ Let solution sit for 30 minutes at room temperature in darkened area.
④ Transfer to an insert
∴ vortex for a minute.

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Work continued to Page 33

SIGNATURE (b) (6)			DATE 2/10/17
DISCLOSED TO AND UNDERSTOOD BY (b) (6)	DATE	WITNESS	DATE 2/14/17

Balance: XP26 #1
BP211D #2

TITLE

Work continued from Page 32

PROJECT NO. L-ergothioneine **33**

BOOK NO. JL-01

5-IAF prep: 5-(Iodoacetamido)fluorescein, CI# 17685
exp. 11/17/20

target weight (mg)	weight (mg)	
10.305	10.356	① Dilute with 1 mL dimethyl sulfoxide CI# 16514 exp. 5/18/17
		② Vortex for 10 seconds, sonicate for 15 minutes
		③ working solution: Take 963 µl of stock, dispense into 25 mL amber volumetric flask, fill to volume with sodium phosphate tribasic diluent

5-IAF working solution diluent:

sodium phosphate tribasic dodecahydrate, CI# 17582, exp: 12/31/21

target weight (mg)	weight (mg)	
2850	2850.48	→ 50 mL milliQ water in volumetric flask

JL 2/14/17

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Work continued to Page —

SIGNATURE (b) (6)			DATE 2/10/17
DISCLOSED TO AND UNDERSTOOD BY (b) (6)	DATE	WITNESS	DATE 2/14/17

REFERENCE MATERIAL CERTIFICATE of ANALYSIS

L-(+)-Ergothioneine

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:	L-(+)-ERGOTHIONEINE
Product Number:	E7521
Batch Number:	BCBK8742V
Brand:	Sigma
CAS Number:	497-30-3
Formula:	$C_9H_{15}N_3O_2S$
Formula Weight:	229.30
Storage Temperature:	-20 C
Quality Release Date:	26 OCT 2016

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	COLORLESS OR WHITE	WHITE
APPEARANCE (FORM)	POWDER	POWDER
PURITY (TLC AREA %)	≥ 98.0 %	98.8 %
SOLUBILITY (COLOR)	COLORLESS	COLORLESS
SOLUBILITY (TURBIDITY)	CLEAR	CLEAR
SOLUBILITY (METHOD)	50MG IN 1ML WATER	50MG IN 1ML WATER
WATER	REPORT RESULT	0.79 %
CARBON CONTENT	45.0 % - 48.3 %	46.39 %
NITROGEN CONTENT	17.3 % - 18.8 %	17.55 %

(b) (6)

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

REPEATABILITY

SAMPLE CHROMATOGRAMS

%WEIGHT RESULTS

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\017-P1-A7-17591-A.D
Sample Name: 17591-A

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Acq. Operator   : Brandi Glover                    Seq. Line :  17
Acq. Instrument : HPLC-30                         Location :   P1-A7
Injection Date  : 2/10/2017 5:28:13 PM                 Inj :   1
                                                  Inj Volume : 2.000 µl
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Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7 (modified after loading)
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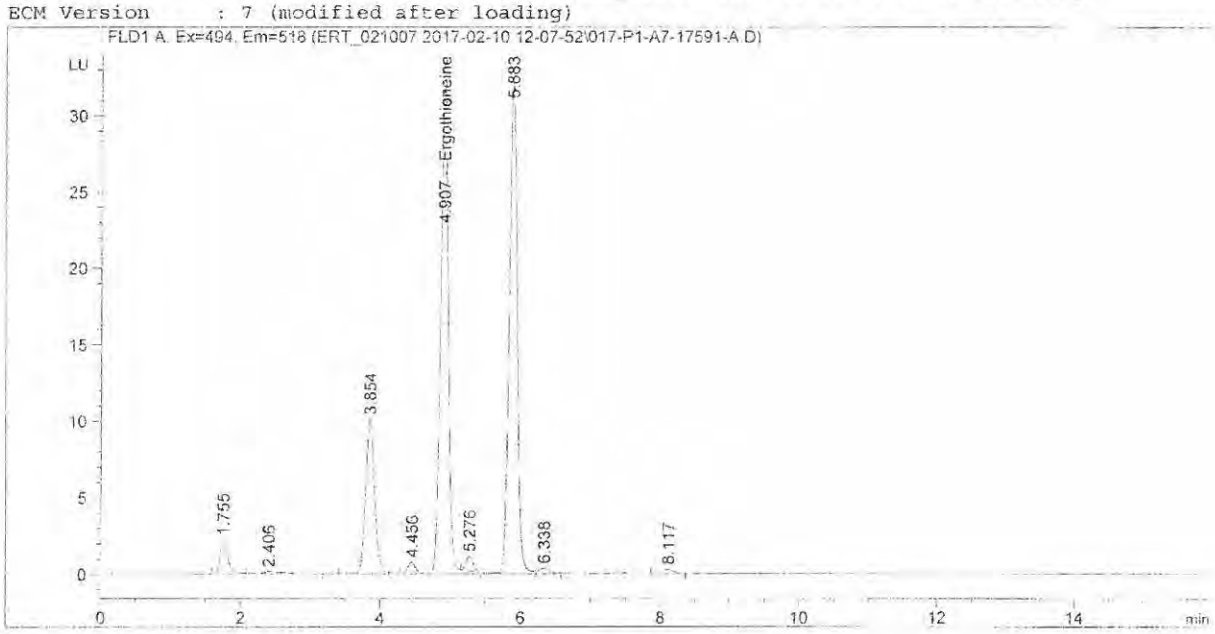


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==========================================================================
                        ESTD Percent Report
==========================================================================

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Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.06300  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]           [LU*s]                   %
-------|------|----------|----------|----------|--|------------------
  4.907 VV      243.121353 7.38716e-5  5.568573    Ergothioneine

Totals :                               5.568573
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\017-P1-A7-17591-A.D
Sample Name: 17591-A

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\018-P1-A8-17591-B.D
Sample Name: 17591-B

```
==========================================================================
Acq. Operator   : Brandi Glover                    Seq. Line :  18
Acq. Instrument : HPLC-30                         Location :   P1-A8
Injection Date  : 2/10/2017 5:45:53 PM                Inj :    1
                                                  Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7 (modified after loading)
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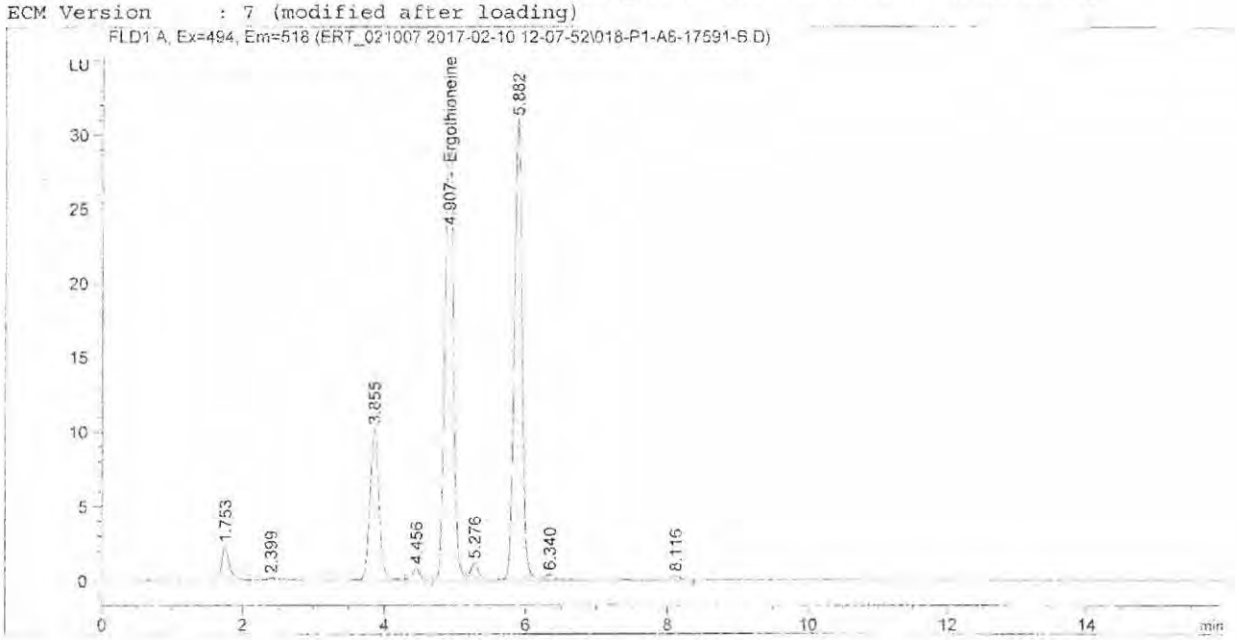


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==========================================================================
                        ESTD Percent Report
==========================================================================

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Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.14100  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]           [LU*s]                   %
-------|------|----------|----------|----------|--|-------------------
  4.907 VV      256.671967 7.40344e-5  5.835450     Ergothioneine

Totals :                               5.835450
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\018-P1-A8-17591-B.D
Sample Name: 17591-B

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\019-P1-A9-17591-C.D
Sample Name: 17591-C

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :  19
Acq. Instrument : HPLC-30                        Location :  P1-A9
Injection Date  : 2/10/2017 6:03:32 PM               Inj :  1
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7 (modified after loading)
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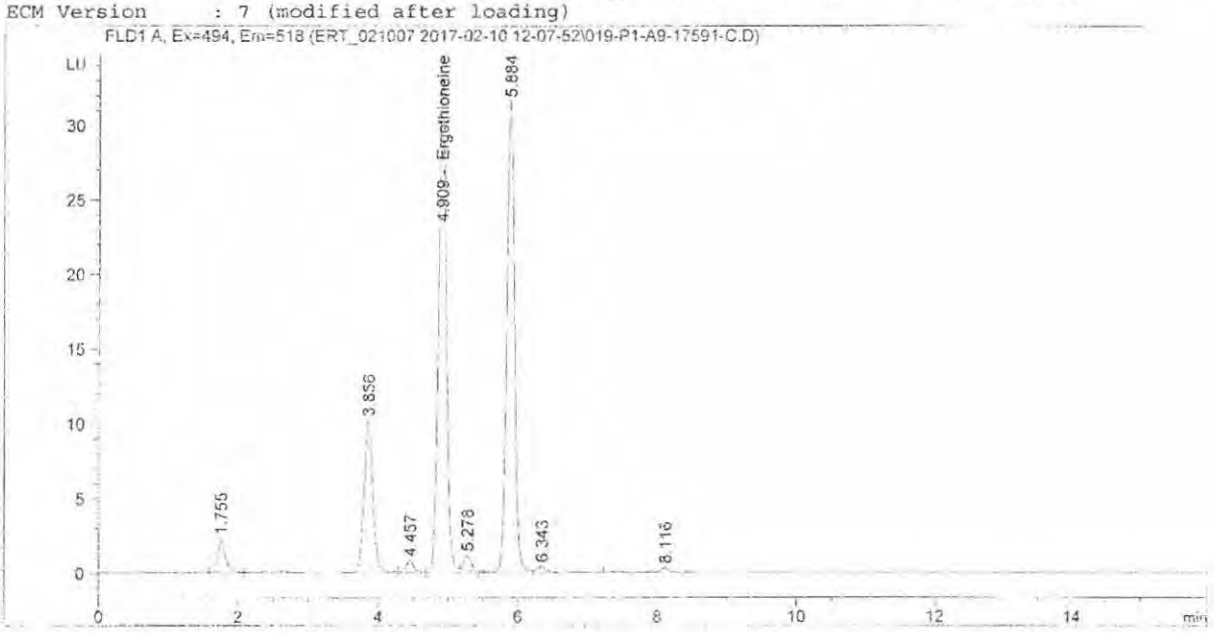


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==================================================================
                        ESTD Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.10800  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                   %
-------|------|----------|----------|----------|--|------------------
  4.909 VV      252.198914 7.39826e-5  5.753062    Ergothioneine

Totals :                                5.753062
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\019-P1-A9-17591-C.D
Sample Name: 17591-C

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\020-P1-B1-17595-A.D
Sample Name: 17595-A

```
==================================================================
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Acq. Instrument : HPLC-30                        Location :   P1-B1
Injection Date  : 2/10/2017 6:21:11 PM               Inj :   1
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
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ECM Version     : 7 (modified after loading)
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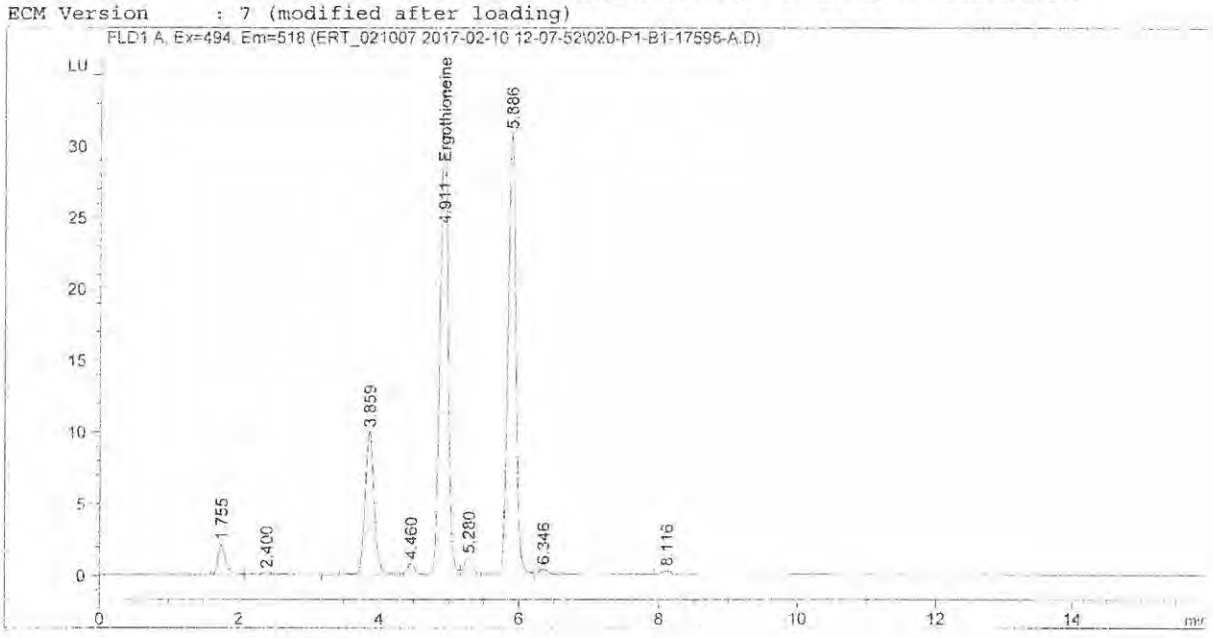


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==================================================================
                        ESTD Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.02700  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]           [LU*s]                  %
-------|------|----------|----------|----------|--|------------------
  4.911 VV      263.305298 7.41080e-5  6.077311     Ergothioneine

Totals :                               6.077311
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\020-P1-B1-17595-A.D
Sample Name: 17595-A

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\021-P1-B2-17595-B.D
Sample Name: 17595-B

```
==================================================================
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Acq. Instrument : HPLC-30                        Location :  P1-B2
Injection Date  : 2/10/2017 6:38:50 PM               Inj :  1
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
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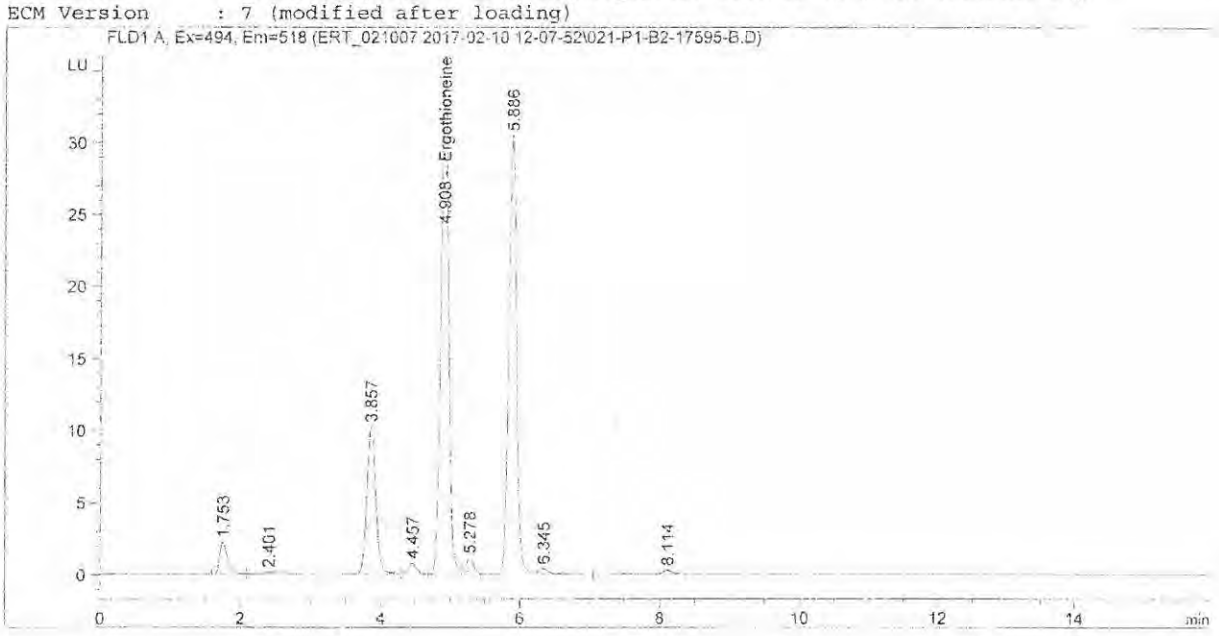


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==================================================================
                        ESTD Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.06800  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]           [LU*s]                  %
-------|------|----------|----------|----------|--|------------------
  4.908 VV      262.342468 7.40976e-5  6.023468     Ergothioneine

Totals :                               6.023468
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\021-P1-B2-17595-B.D
Sample Name: 17595-B

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\023-P1-B3-17595-C.D
Sample Name: 17595-C

```
==========================================================================
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Acq. Instrument : HPLC-30                         Location :   P1-B3
Injection Date  : 2/10/2017 7:14:15 PM                 Inj :   1
                                                  Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7 (modified after loading)
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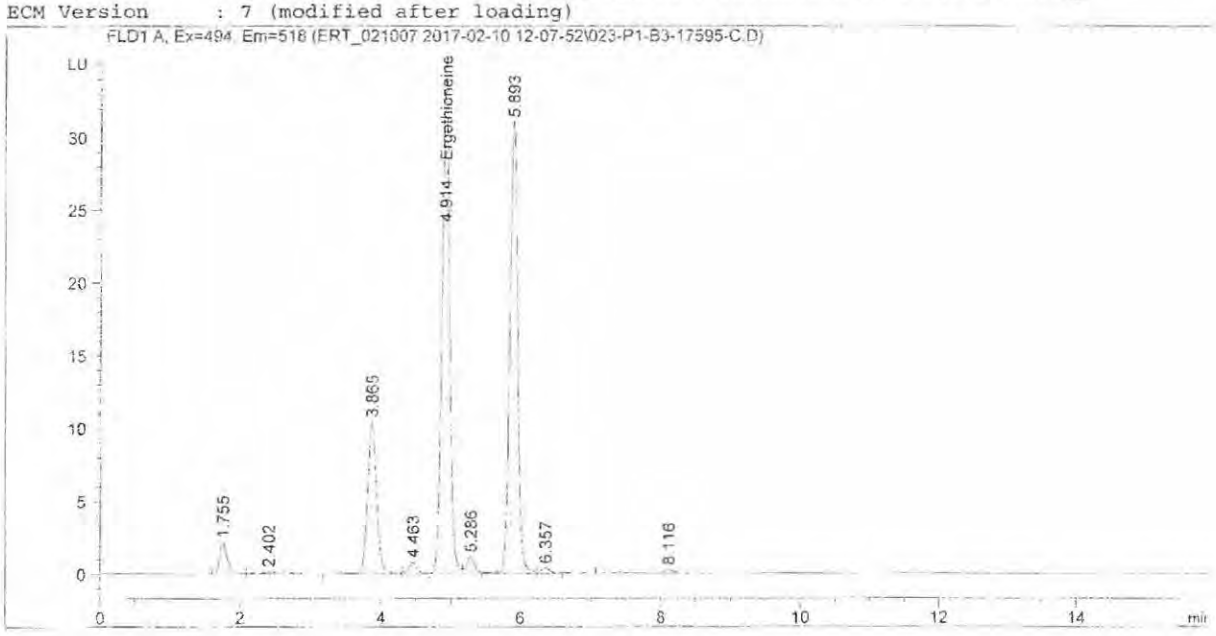


```
==========================================================================
                        ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.08500  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                   %
-------|------|----------|----------|----------|--|------------------
  4.914 VV      260.962769 7.40824e-5  5.977972     Ergothioneine

Totals :                               5.977972
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\023-P1-B3-17595-C.D
Sample Name: 17595-C

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\024-P1-B4-17603-A.D
Sample Name: 17603-A

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :  24
Acq. Instrument : HPLC-30                        Location :  P1-B4
Injection Date  : 2/10/2017 7:31:55 PM               Inj :  1
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7 (modified after loading)
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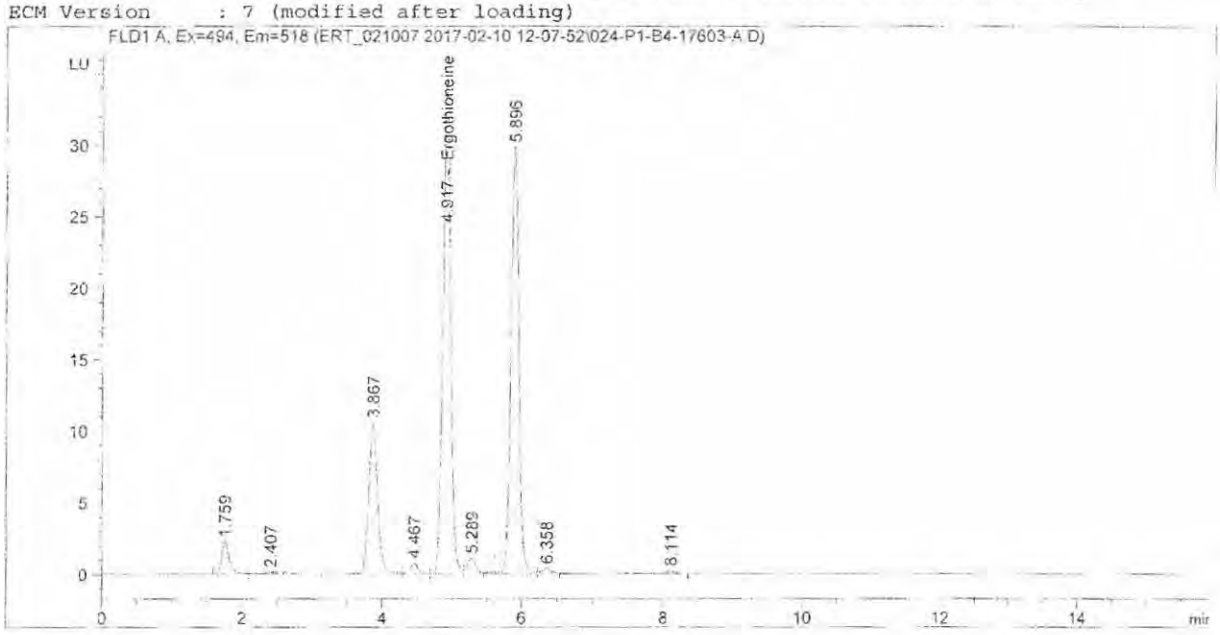


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==================================================================
                        ESTD Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.10400  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                   %
-------|------|----------|----------|----------|--|------------------
  4.917 VV      264.828705 7.41244e-5  6.055734     Ergothioneine

Totals :                               6.055734
```

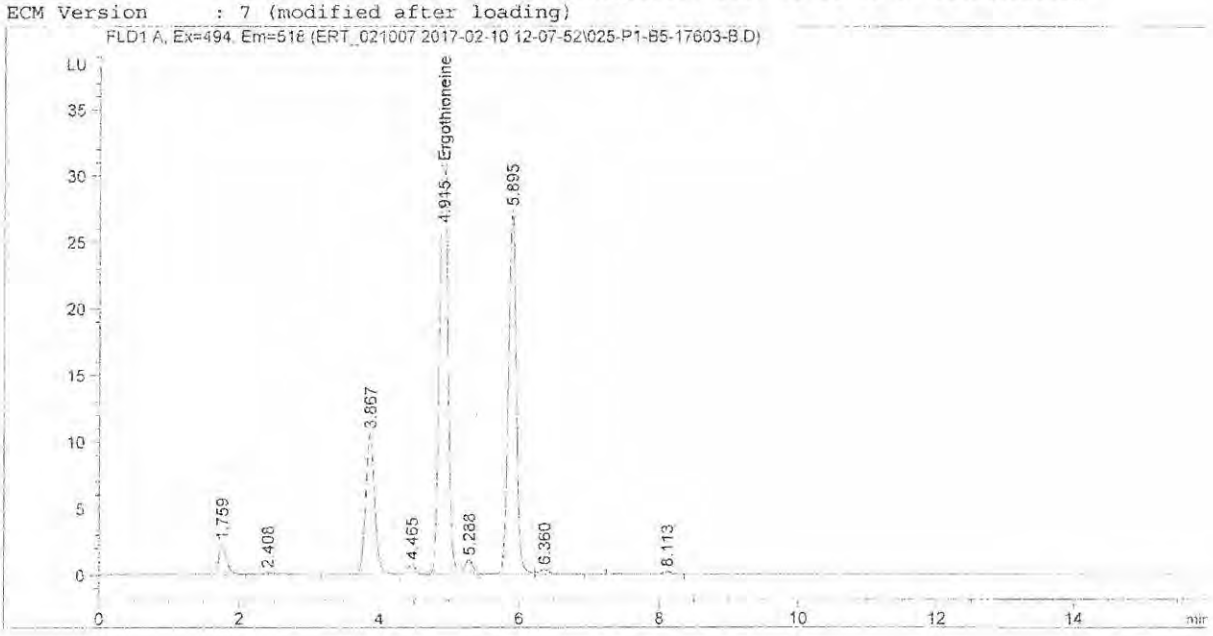
Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\024-P1-B4-17603-A.D
Sample Name: 17603-A

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\025-P1-B5-17603-B.D
Sample Name: 17603-B

Acq. Operator	: Brandi Glover	Seq. Line	: 25
Acq. Instrument	: HPLC-30	Location	: P1-B5
Injection Date	: 2/10/2017 7:49:35 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M		
Last changed	: 2/10/2017 12:07:54 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 2:11:45 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip		
ECM Version	: 7 (modified after loading)		



ESTD Percent Report

Sorted By	:	Signal	
Calib. Data Modified	:	2/14/2017 1:47:14 PM	
Multiplier	:	1.0000	
Dilution	:	25.0000	
Sample Amount:	:	8.16000	[mg/ml]

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.915	VV	285.322083	7.43278e-5	6.497350		Ergothioneine
Totals :				6.497350		

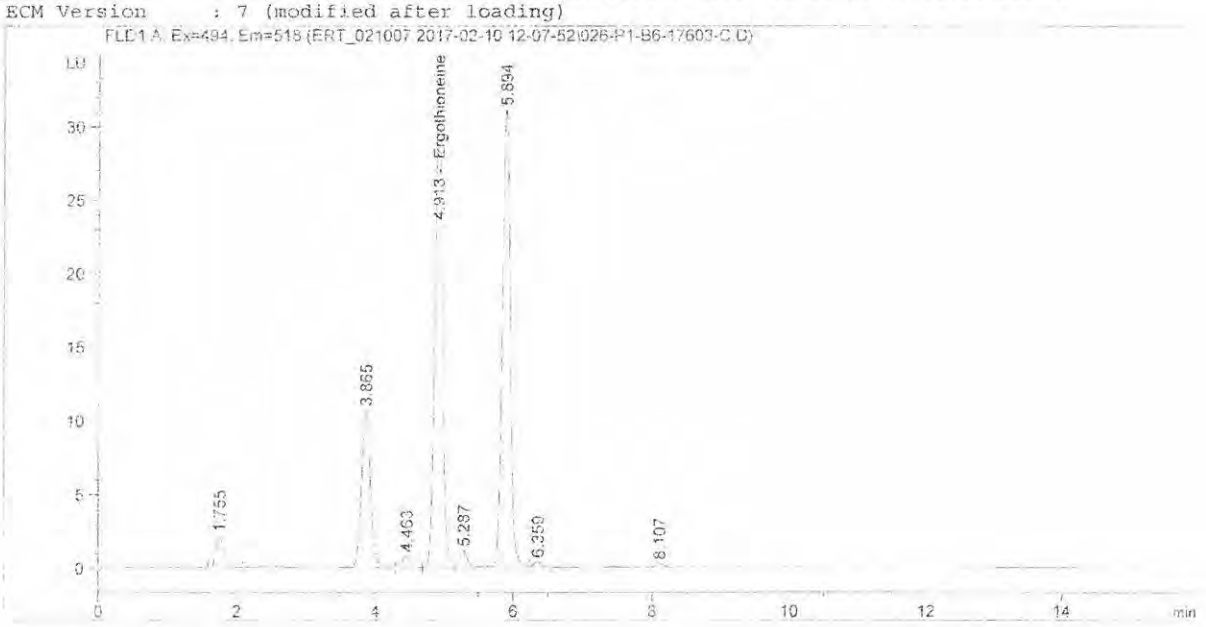
Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\025-P1-B5-17603-B.D
Sample Name: 17603-B

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\026-P1-B6-17603-C.D
Sample Name: 17603-C

Acq. Operator	: Brandi Glover	Seq. Line	: 26
Acq. Instrument	: HPLC-30	Location	: P1-B6
Injection Date	: 2/10/2017 8:07:15 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M		
Last changed	: 2/10/2017 12:07:54 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 2:11:45 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip		
ECM Version	: 7 (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 2/14/2017 1:47:14 PM
Multiplier : 1.0000
Dilution : 25.0000
Sample Amount : : 8.10300 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.913	VV	254.594559	7.40106e-5	5.813492		Ergothioneine
Totals :				5.813492		

DA-15B 2/14/2017 2:12:37 PM Hong You Page 1 of 2

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\026-P1-B6-17603-C.D
Sample Name: 17603-C

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\017-P1-A7-17587-A.D
Sample Name: 17587-A

```
==========================================================================
Acq. Operator   : Brandi Glover                    Seq. Line :  17
Acq. Instrument : HPLC-30                          Location :  P1-A7
Injection Date  : 2/8/2017 10:12:49 PM                 Inj :  1
                                                  Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M
Last changed    : 2/8/2017 4:52:36 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:10:20 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip
ECM Version     : 9
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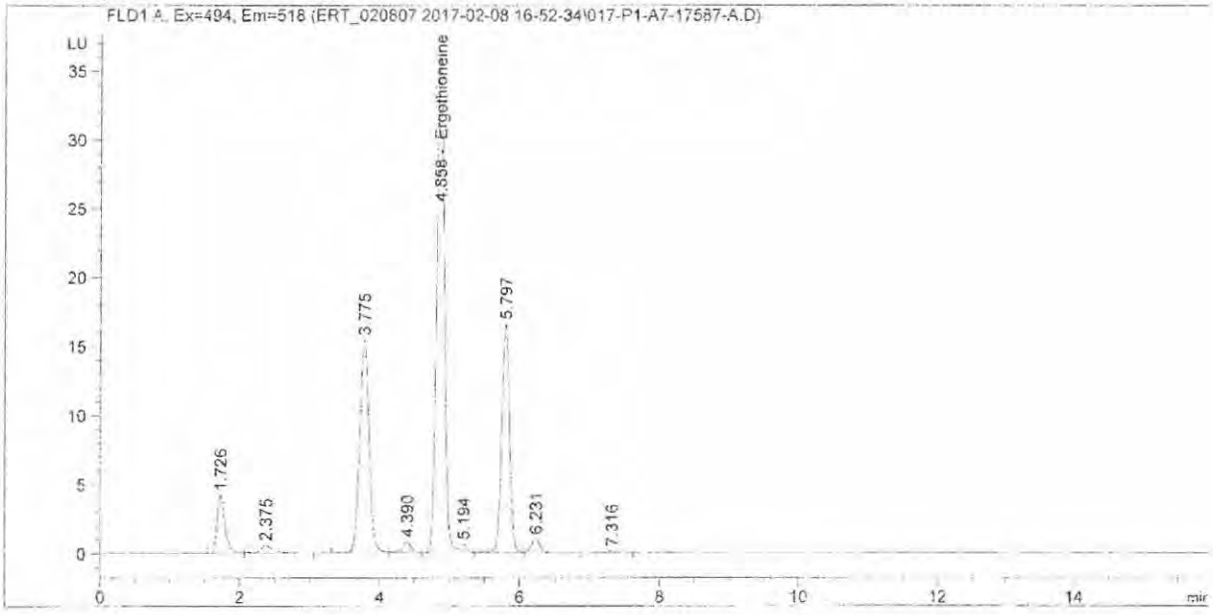



```
==========================================================================
                        ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:10:18 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.12100  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]           [LU*s]                  %
-------|------|----------|----------|----------|--|------------------
  4.858 VV      272.854095 7.78833e-5  6.541925     Ergothioneine

Totals :                               6.541925
```

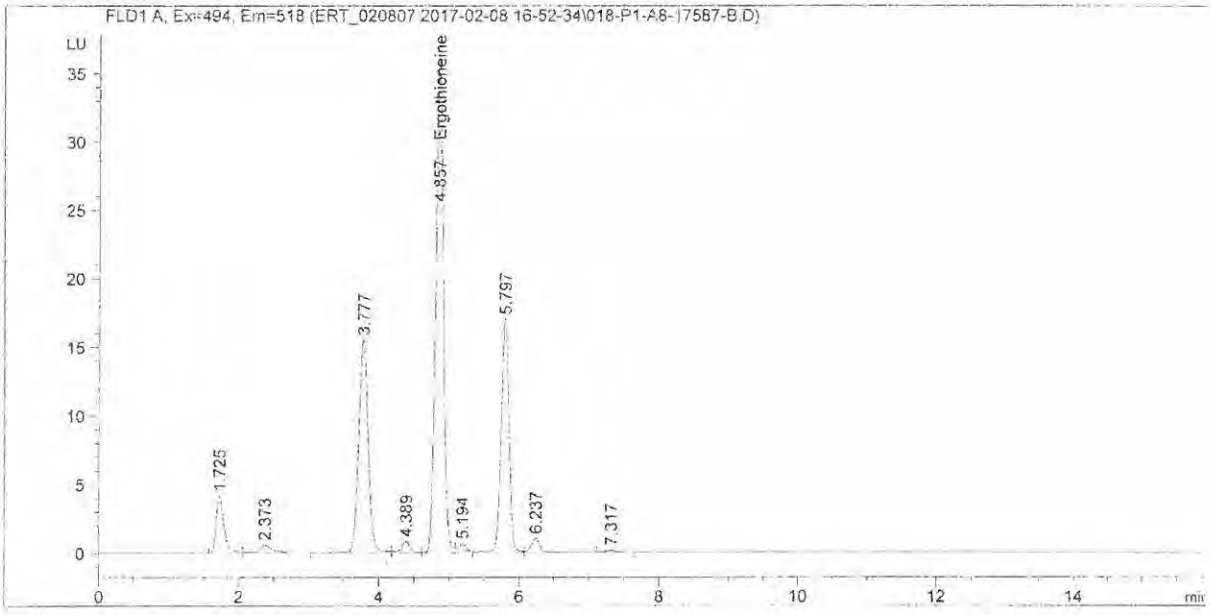
Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\017-P1-A7-17587-A.D
Sample Name: 17587-A

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\018-P1-A8-17587-B.D
Sample Name: 17587-B

Acq. Operator	: Brandi Glover	Seq. Line	: 18
Acq. Instrument	: HPLC-30	Location	: P1-A8
Injection Date	: 2/8/2017 10:30:29 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M		
Last changed	: 2/8/2017 4:52:36 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:10:20 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip		
ECM Version	: 9		



ESTD Percent Report

Sorted By	: Signal		
Calib. Data Modified	: 2/14/2017 1:10:18 PM		
Multiplier	: 1.0000		
Dilution	: 25.0000		
Sample Amount:	: 8.09700 [mg/ml]		

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.857	VV	274.713379	7.79643e-5	6.612896		Ergothioneine

Totals : 6.612896

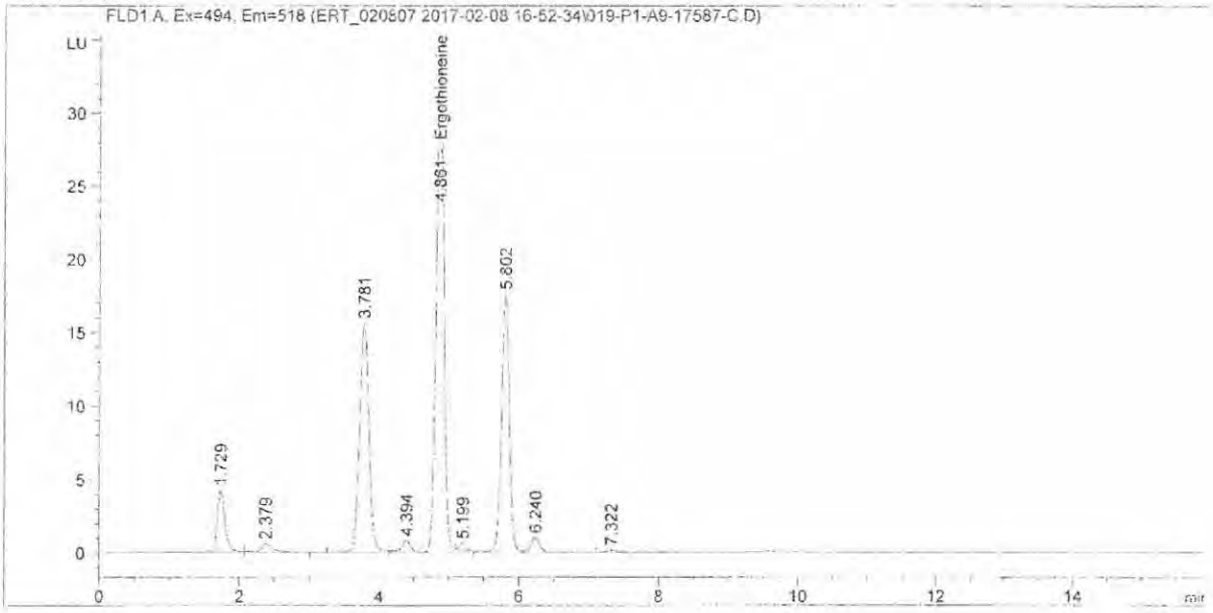
Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\018-P1-A8-17587-B.D
Sample Name: 17587-B

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\019-P1-A9-17587-C.D
Sample Name: 17587-C

Acq. Operator	: Brandi Glover	Seq. Line	: 19
Acq. Instrument	: HPLC-30	Location	: P1-A9
Injection Date	: 2/8/2017 10:48:07 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M		
Last changed	: 2/8/2017 4:52:36 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:10:20 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip		
ECM Version	: 9		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 2/14/2017 1:10:18 PM
Multiplier : 1.0000
Dilution : 25.0000
Sample Amount: : 8.03300 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.861	VV	255.954819	7.70932e-5	6.141040		Ergothioneine

Totals : 6.141040

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\019-P1-A9-17587-C.D
Sample Name: 17587-C

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\024-P1-B4-17599-A.D
Sample Name: 17599-A

```
==================================================================
Acq. Operator   : Brandi Glover                 Seq. Line :  24
Acq. Instrument : HPLC-30                       Location :  P1-B4
Injection Date  : 2/9/2017 12:16:27 AM              Inj :  1
                                                Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M
Last changed    : 2/8/2017 4:52:36 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:39:01 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip
ECM Version     : 10
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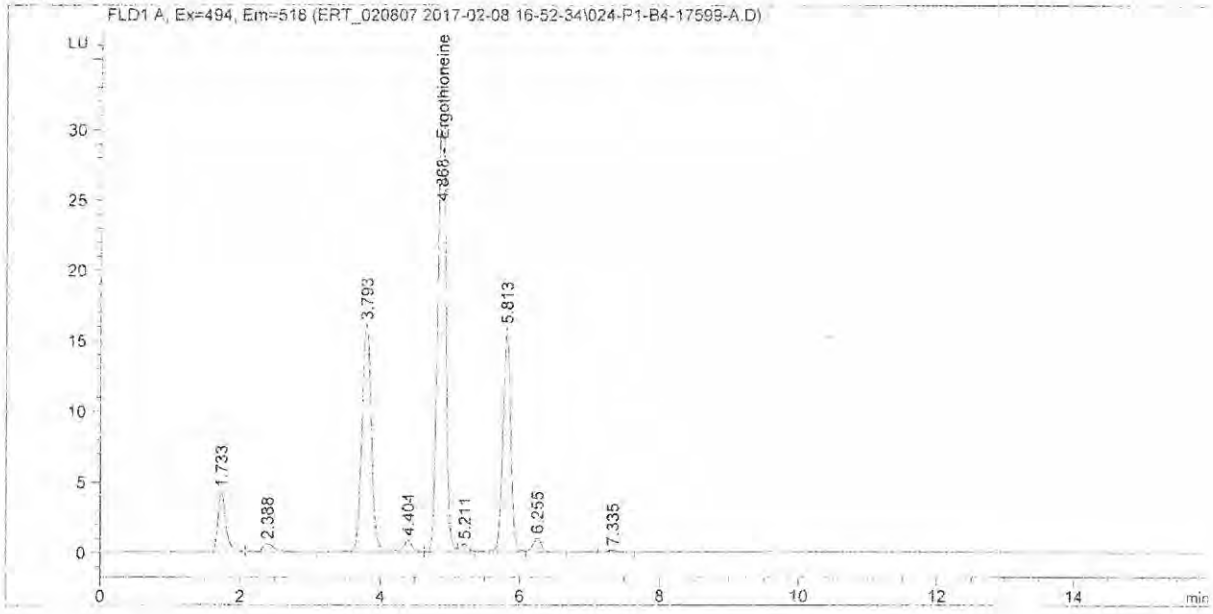


```
==================================================================
                        ESTD Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:39:00 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.07300  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                   %
-------|------|----------|----------|----------|--|------------------
  4.868 VV      266.897430 7.76163e-5  6.415082    Ergothioneine

Totals :                               6.415082
```

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\024-P1-B4-17599-A.D
Sample Name: 17599-A

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\025-P1-B5-17599-B.D
Sample Name: 17599-B

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :  25
Acq. Instrument : HPLC-30                         Location :  P1-B5
Injection Date  : 2/9/2017 12:34:06 AM                Inj :  1
                                                Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M
Last changed    : 2/8/2017 4:52:36 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:39:01 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip
ECM Version     : 10
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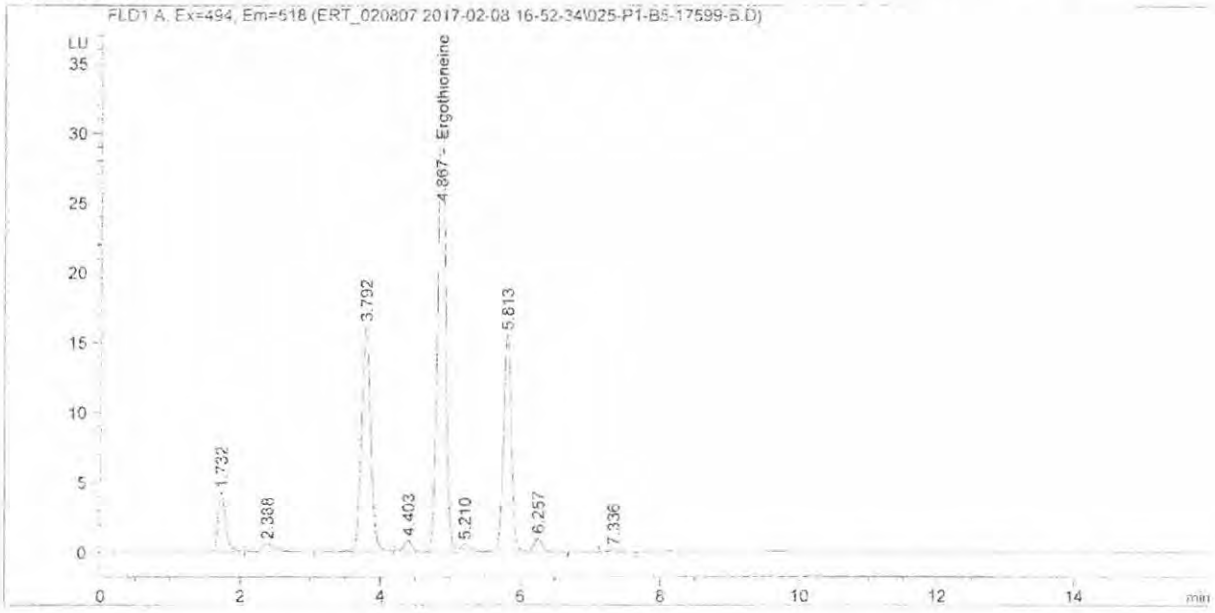


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==================================================================
                        ESTD Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:39:00 PM
Multiplier            :      1.0000
Dilution              :     25.0000
Sample Amount:                :      8.10600  [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]           [LU*s]                  %
-------|------|----------|----------|----------|---|------------------
  4.867 VV      269.573700 7.77377e-5  6.463138     Ergothioneine

Totals :                               6.463128
```

DA-15B 2/14/2017 1:45:39 PM Hong You Page 1 of 2

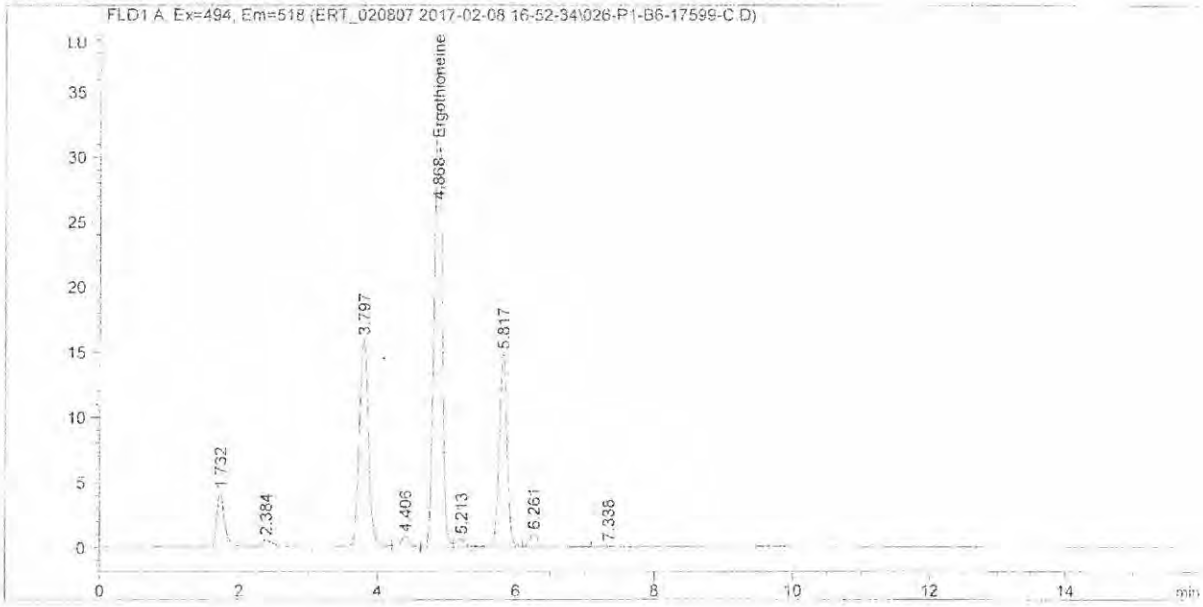
Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\025-P1-B5-17599-B.D
Sample Name: 17599-B

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\026-P1-B6-17599-C.D
Sample Name: 17599-C

Acq. Operator	: Brandi Glover	Seq. Line	: 26
Acq. Instrument	: HPLC-30	Location	: P1-B6
Injection Date	: 2/9/2017 12:51:46 AM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M		
Last changed	: 2/8/2017 4:52:36 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:39:01 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip		
ECM Version	: 10		



ESTD Percent Report

Sorted By	:	Signal	
Calib. Data Modified	:	2/14/2017 1:39:00 PM	
Multiplier	:	1.0000	
Dilution	:	25.0000	
Sample Amount:	:	8.10700	[mg/ml]

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.868	VV	286.550903	7.84553e-5	6.932728		Ergothioneine
Totals :				6.932728		

DA-15B 2/14/2017 1:45:44 PM Hong You Page 1 of 2

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\026-P1-B6-17599-C.D
Sample Name: 17599-C

==========

*** End of Report ***

SYSTEM SUITABILITY

CHROMATOGRAMS

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\015-P1-A6-ERT Standard_70ul.D
Sample Name: ERT Standard_70ul

E x t e n d e d P e r f o r m a n c e R e p o r t

Instrument: HPLC-30

Module	Type	Firmware rev.	Serial number
Binary Pump	G1312B	A.06.55 [004]	DEACB09243
HiP Sampler 2	G1367E	A.06.54 [006]	DEACO05514
Column Comp. 3	G1316A	A.06.50 [005]	DEACN34335
DAD 4	G4212B	B.06.77 [0004]	DEAA309619
FLD 5	G1321B	A.07.01 [001]	DEABW06878

Software Revision: Rev. C.01.07 SR1 [110] Copyright © Agilent Technologies

Analysis method:
Path: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\
Name: ERT.M

Sample information for Location: P1-A6

Sample Name: ERT Standard_70u Multiplier: 1.00
Injection#: 1 Dilution: 1.00
Injection volume: 2.000 µl

Acquisition information:

Operator: Brandi Glover
Date/Time: 2/10/2017 4:52:54 PM
Data file:
Path: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\
Name: 015-P1-A6-ERT Standard_70ul.D
Method file:
Path: d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\
Name: ERT.M

ECM Information:
ECM Server: http://us05apvp001/ecmwg
ECM Operator: Hong You
ECM Path: \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version: 7 (modified after loading)

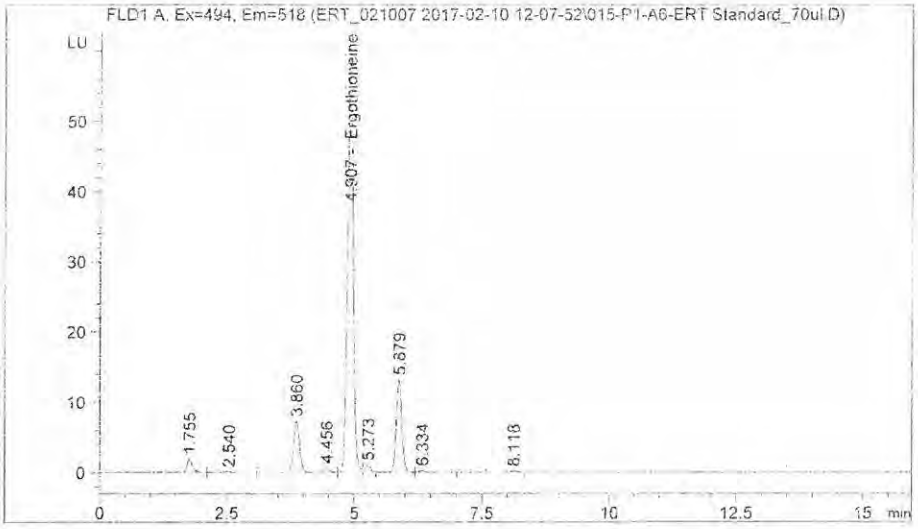
Flow: 1.200 ml/min
Pressure at start: 170 bar Pressure at end: 182 bar
Left Temp. at start: 25.0°C Left Temp. at end: 25.0 °C
Right Temp. at start: 25.0°C Right Temp. at end: 25.0 °C

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\015-P1-A6-ERT Standard_70ul.D
Sample Name: ERT Standard_70ul

Solvents:	PMP1, Solvent A40:7:3:50 Potassium phosphate/ACN/MeOH/water
	PMP1, Solvent A
	PMP1, Solvent B40:20:3:37 Potassium phosphate/ACN/MeOH/water
	PMP1, Solvent B

Cell Tag (DAD1)	
Product Number:	G4212-60008
Serial Number:	DE43815609
Production Date:	02/05/2015 14:00:16
Path Length:	10 mm
Volume:	1 nl
Max. Pressure:	60 bar
Last Cell Test:	02/05/2015 15:30:20
UV Lamp Tag (DAD1)	
Product Number:	5190-0917
Serial Number:	G36045
Production Date:	03/03/2016 12:00:20
Accum. UV On Time:	2162 h
Number of Ignitions:	400
Last Intensity Test:	07/01/2016 09:34:40

Signal description: FLD1 A, Ex=494, Em=518

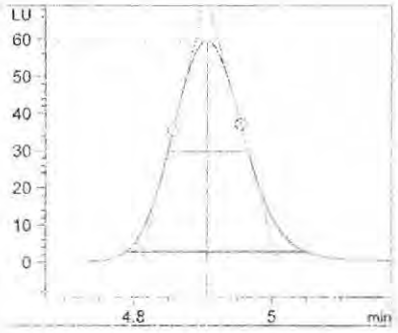


Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\015-P1-A6-ERT Standard_70ul.D
Sample Name: ERT Standard_70ul

Compound# 5: Ergothioneine
Amount [mg/ml]: 0.03423

Peak description [min]:
Signal: FLD1 A, Ex=494, Em=518

RetTime:	4.90650	k':	-
Height:	59.45682	Area:	454.56802
Start:	4.67745	End:	5.16957
Skew:	0.32480	Excess:	0.45849
Width at ..		half height:	0.11711
		5 sigma:	0.26451
		tangent:	0.19870
		tailing:	0.25728
Symmetry (from integrator):			0.88820
USP Tailing:			1.13558
Integration type:			VV
Time increment [msec]:			432.00276
Data points:			80



Statistical moments (BB peak detection):

M0:	448.042742
M1:	4.908887
M2:	0.002719
M3:	0.000046
M4:	0.000026

Efficiency: Plates per ..

	column	meter
Tangent method:	9743	-
Halfheight method:	9712	-
5 sigma method:	8591	-
Statistical:	8862	-

Relationship to preceeding peak:

Selectivity:	1.10100		
Resolution Tangent method:	2.25683	5 sigma method:	1.87095
Halfheight method(Classic):	2.24667	Statistical method:	2.22423
Halfheight method (EP/JP):	2.25623		

Relationship to other peaks:

RT Ratio:	1.00000
Peak to Valley Ratio (Front):	3449.2552
Peak to Valley Ratio (Tail):	-

Additional calculations:

USP Asymmetry at 10% height:	1.10834
Foley-Dorsey Asymmetry at 10% height:	0.82191

	per column	per meter
Foley-Dorsey Plates:	10009.924	-

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\015-P1-A6-ERT Standard_70ul.D
Sample Name: ERT Standard_70ul

#	Ret.Time [min]	Amount [mg/ml]	Name	Page #
1	4.907	0.0342	Ergothioneine	3
	Total:	0.0342		

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\009-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

```
==========================================================================
Acq. Operator    : Brandi Glover                    Seq. Line :   9
Acq. Instrument  : HPLC-30                           Location :   P1-A4
Injection Date   : 2/10/2017 3:06:54 PM                  Inj :   1
                                                  Inj Volume : 2.000 µl
Acq. Method      : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed     : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method  : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed     : 2/14/2017 2:11:45 PM by Hong You
Method Info      : L-ergothioneine

ECM Server       : http://us05apvp001/ecmwg
ECM Operator     : Hong You
ECM Path         : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version      : 7 (modified after loading)
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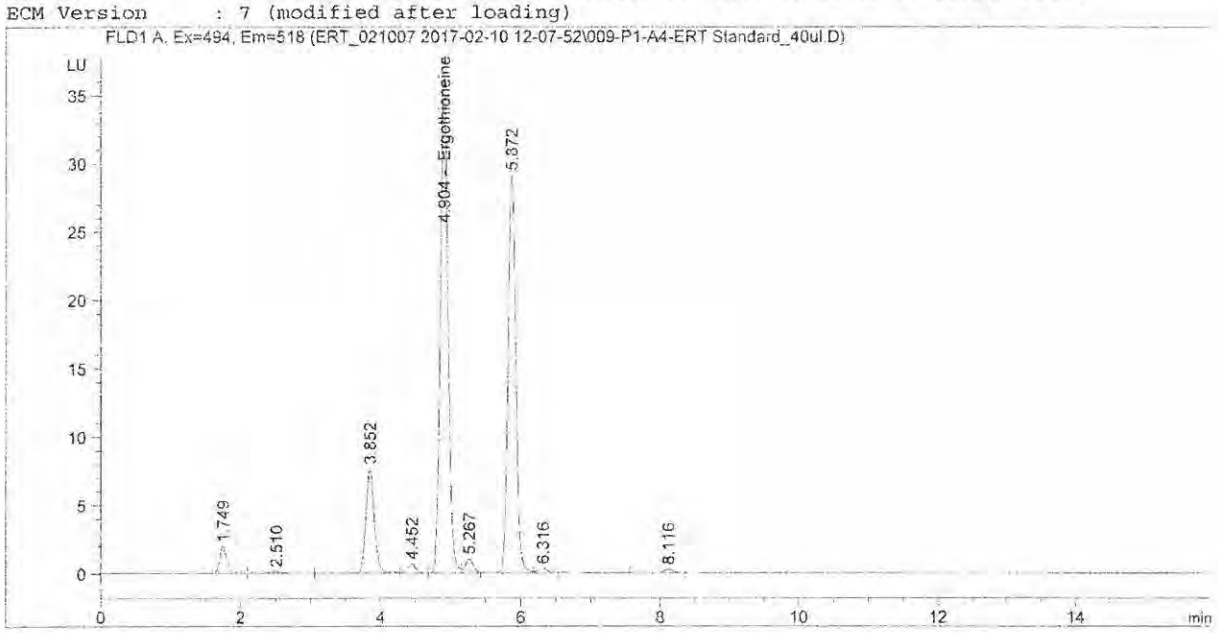


```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]           [LU*s]                [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.904 VV       274.49606 7.42241e-52.03742e-2    Ergothioneine

Totals :                              2.03742e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\009-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\010-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :  10
Acq. Instrument : HPLC-30                        Location :  P1-A4
Injection Date  : 2/10/2017 3:24:34 PM               Inj :  1
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:47:16 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7
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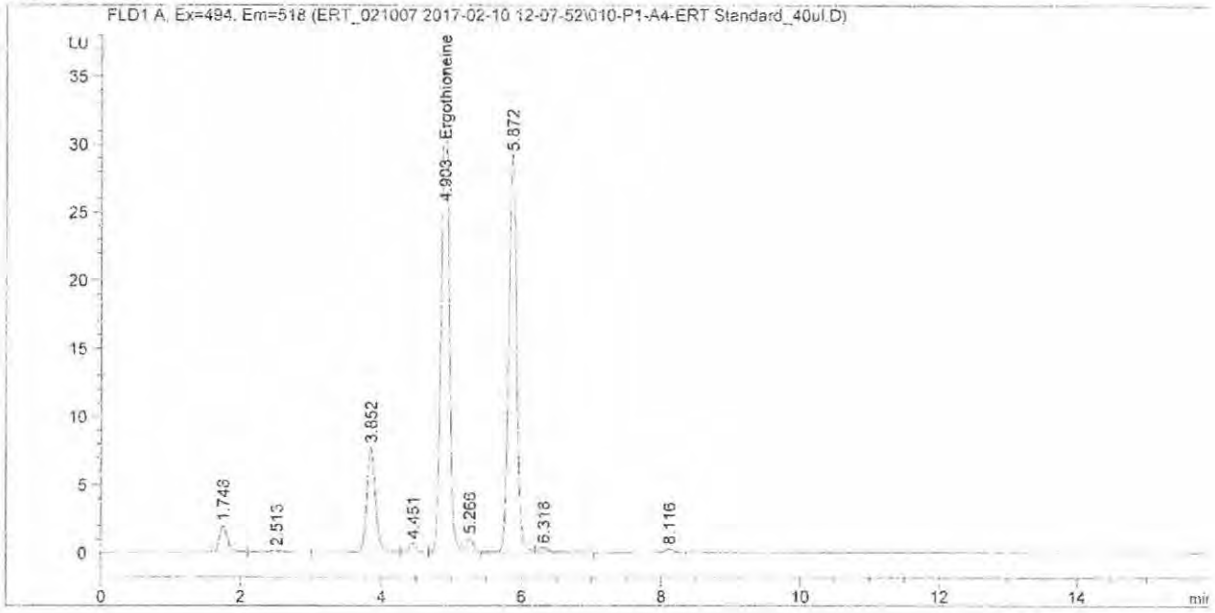


```
==================================================================
          External Standard Report (Sample Amount is 0!)
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                 [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.903 VV       275.20953 7.42312e-52.04291e-2    Ergothioneine

Totals :                              2.04291e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\010-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

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*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\011-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :  11
Acq. Instrument : HPLC-30                        Location :  P1-A4
Injection Date  : 2/10/2017 3:42:13 PM               Inj :  2
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:47:16 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7
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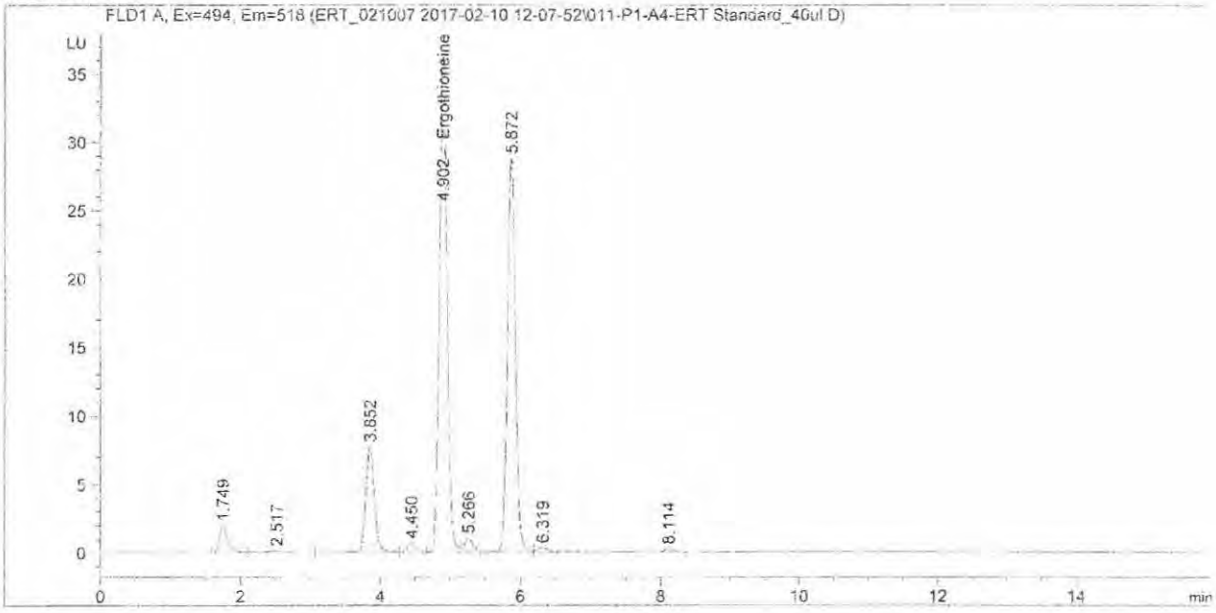


```
==================================================================
          External Standard Report (Sample Amount is 0!)
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]           [LU*s]                [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.902 VV       274.04996 7.42197e-52.03399e-2    Ergothioneine

Totals :                              2.03399e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\011-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

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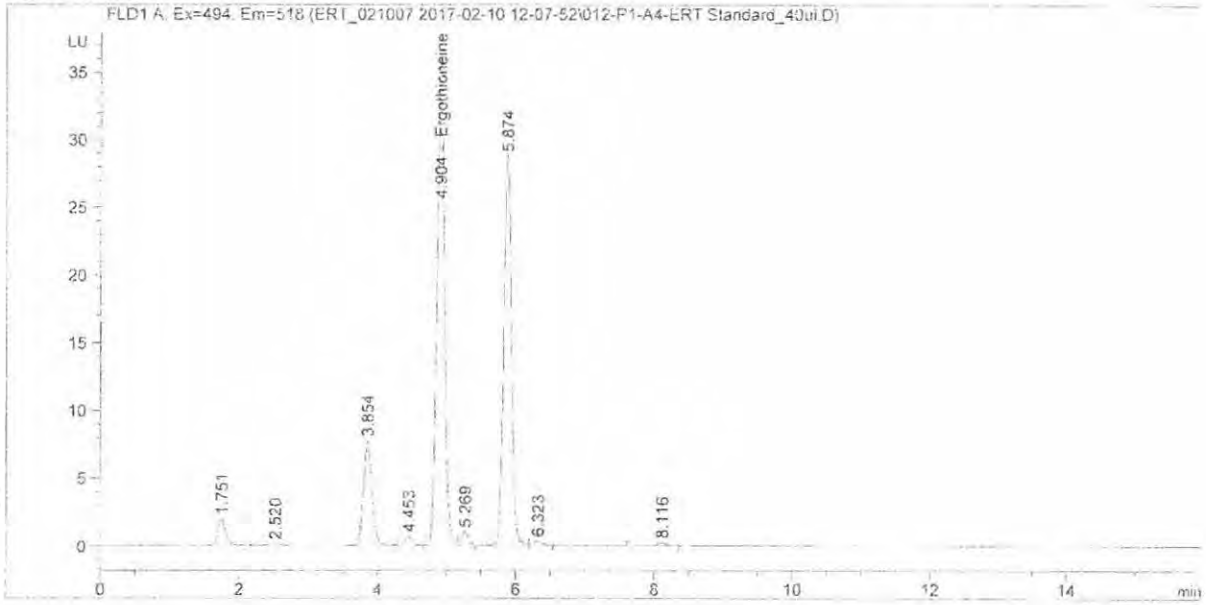
*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\012-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

Acq. Operator	: Brandi Glover	Seq. Line	: 12
Acq. Instrument	: HPLC-30	Location	: P1-A4
Injection Date	: 2/10/2017 3:59:53 PM	Inj	: 3
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M		
Last changed	: 2/10/2017 12:07:54 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:47:16 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip		
ECM Version	: 7		



External Standard Report (Sample Amount is 0!)

Sorted By	:	Signal	
Calib. Data Modified	:	2/14/2017 1:47:14 PM	
Multiplier	:	1.0000	
Dilution	:	1.0000	

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [mg/ml]	Grp	Name
4.904	VV	273.70810	7.42162e-5	2.03136e-2		Ergothioneine
Totals :				2.03136e-2		

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\012-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

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*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\013-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :  13
Acq. Instrument : HPLC-30                        Location :  P1-A4
Injection Date  : 2/10/2017 4:17:33 PM               Inj :  4
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:47:16 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7
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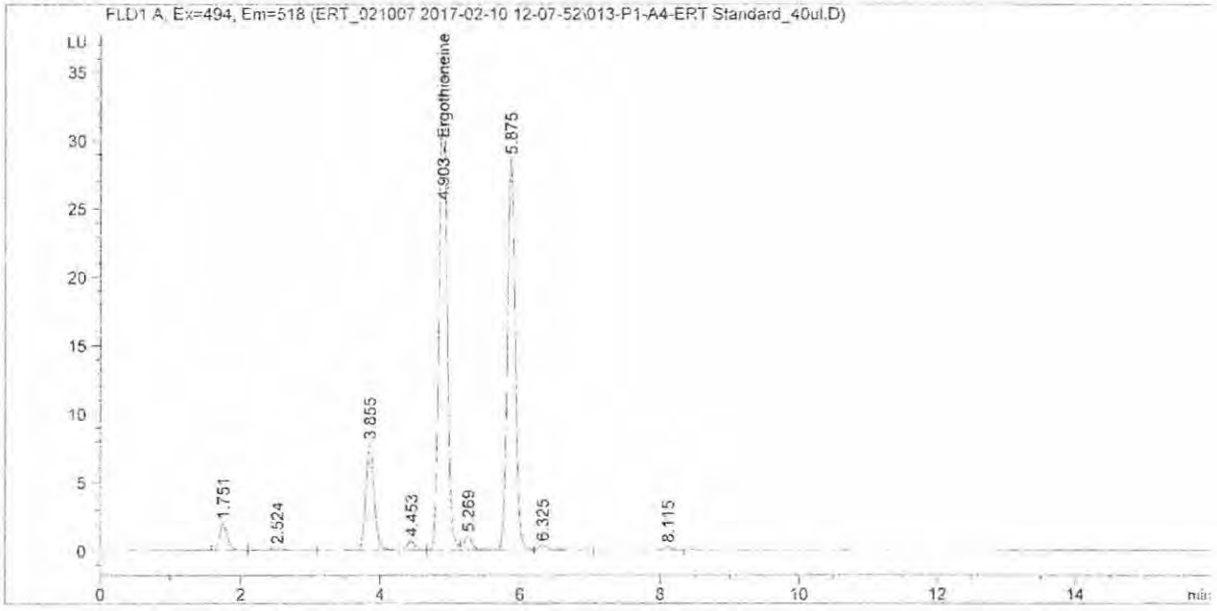


```
==================================================================
          External Standard Report (Sample Amount is 0!)
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                 [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.903 VV       273.39633 7.42131e-5 2.02896e-2    Ergothioneine

Totals :                              2.02896e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\0i3-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\022-P1-A4-ERT Standard_40ul_cc.D
Sample Name: ERT Standard_40ul_cc

```
==========================================================================
Acq. Operator    : Brandi Glover                    Seq. Line :  22
Acq. Instrument  : HPLC-30                           Location :  P1-A4
Injection Date   : 2/10/2017 6:56:35 PM                  Inj :  1
                                                   Inj Volume : 2.000 µl
Acq. Method      : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed     : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method  : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed     : 2/14/2017 2:11:45 PM by Hong You
Method Info      : L-ergothioneine

ECM Server       : http://us05apvp001/ecmwg
ECM Operator     : Hong You
ECM Path         : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version      : 7 (modified after loading)
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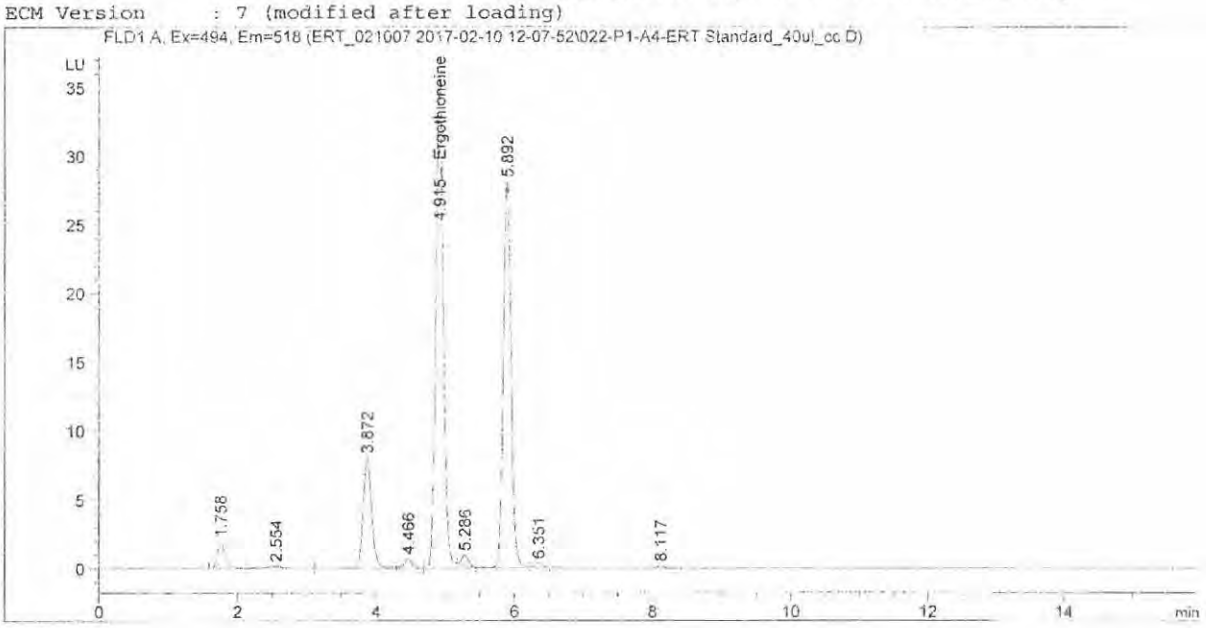


```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                 [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.915 VV       272.43707 7.42035e-52.02158e-2    Ergothioneine

Totals :                              2.02158e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\022-P1-A4-ERT Standard_40ul_cc.D
Sample Name: ERT Standard_40ul_cc

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\027-P1-A4-ERT Standard_40ul_cc.D
Sample Name: ERT Standard_40ul_cc

```
==========================================================================
Acq. Operator   : Brandi Glover                    Seq. Line :  27
Acq. Instrument : HPLC-30                          Location :  P1-A4
Injection Date  : 2/10/2017 8:24:55 PM                 Inj :  1
                                                  Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 2:11:45 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7 (modified after loading)
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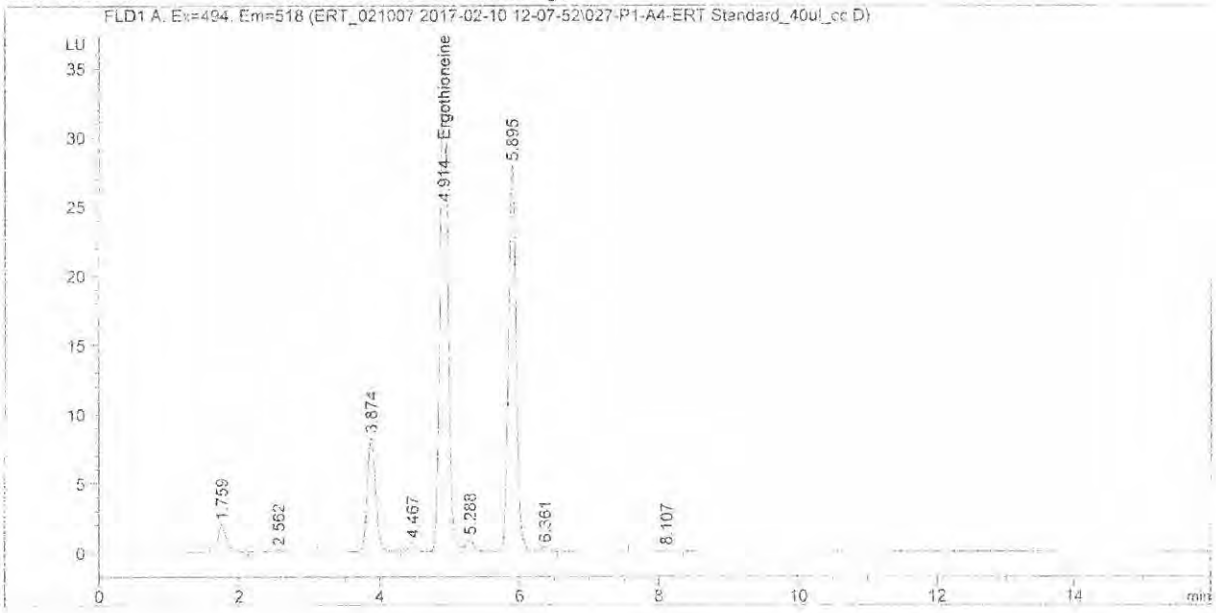



```
==========================================================================
           External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                 [mg/ml]
--------|------|----------|----------|---------|--|-----------------
  4.914 VV       272.68890 7.42060e-52.02352e-2    Ergothioneine

Totals :                               2.02352e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\027-P1-A4-ERT Standard_40ul_cc.D
Sample Name: ERT Standard_40ul_cc

==========

*** End of Report ***

SPECIFICITY

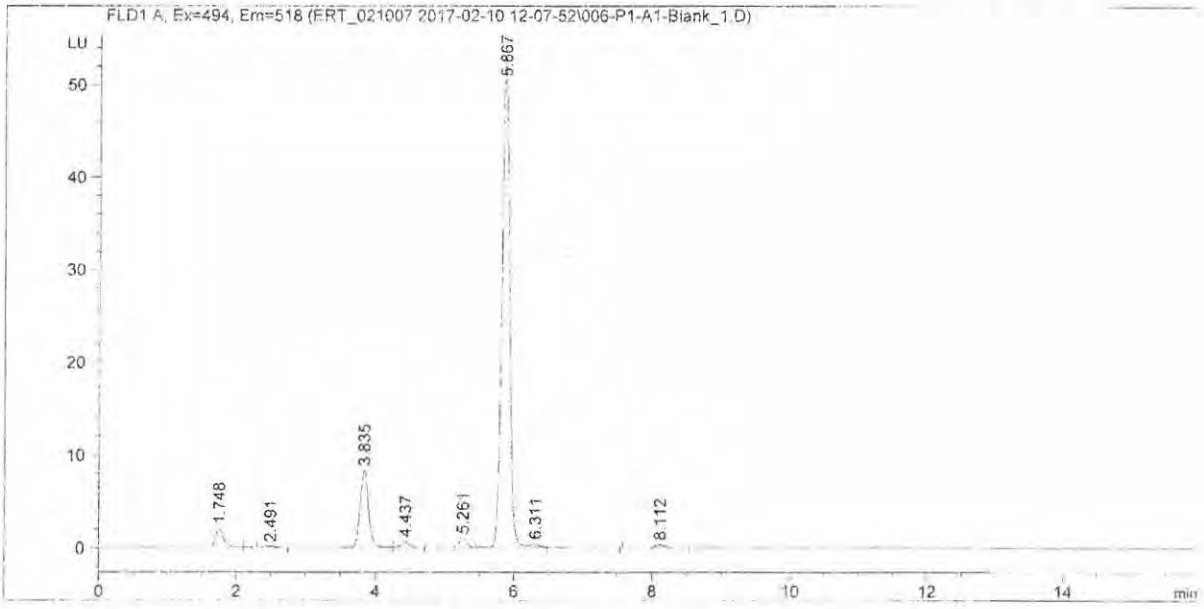
CHROMATOGRAMS

BLANKS

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\006-P1-A1-Blank_1.D
Sample Name: Blank_1

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :   6
Acq. Instrument : HPLC-30                        Location :   P1-A1
Injection Date  : 2/10/2017 2:13:56 PM               Inj :   4
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:47:16 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7
```




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

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]           [LU*s]                [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.907             -          -          -          Ergothioneine

Totals :                                0.00000
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\006-P1-A1-Blank_1.D
Sample Name: Blank_1

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2/14/2017 1:47:14 PM
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

Peak #	RetTime [min]	Type	Width [min]	Area [LU*s]	Area %	Name
1	1.748	BV	0.1224	15.91637	3.0527	?
2	2.491	VV T	0.1955	1.27725	0.2450	?
3	3.835	VV R	0.1309	71.76747	13.7647	?
4	4.437	VB	0.1261	5.32445	1.0212	?
5	4.907		0.0000	0.00000	0.0000	Ergothioneine
6	5.261	VV R	0.1242	7.88681	1.5127	?
7	5.867	VV R	0.1219	413.87885	79.3804	?
8	6.311	VV T	0.1165	2.16792	0.4158	?
9	8.112	BB	0.1576	3.16754	0.6075	?

Totals : 521.38665

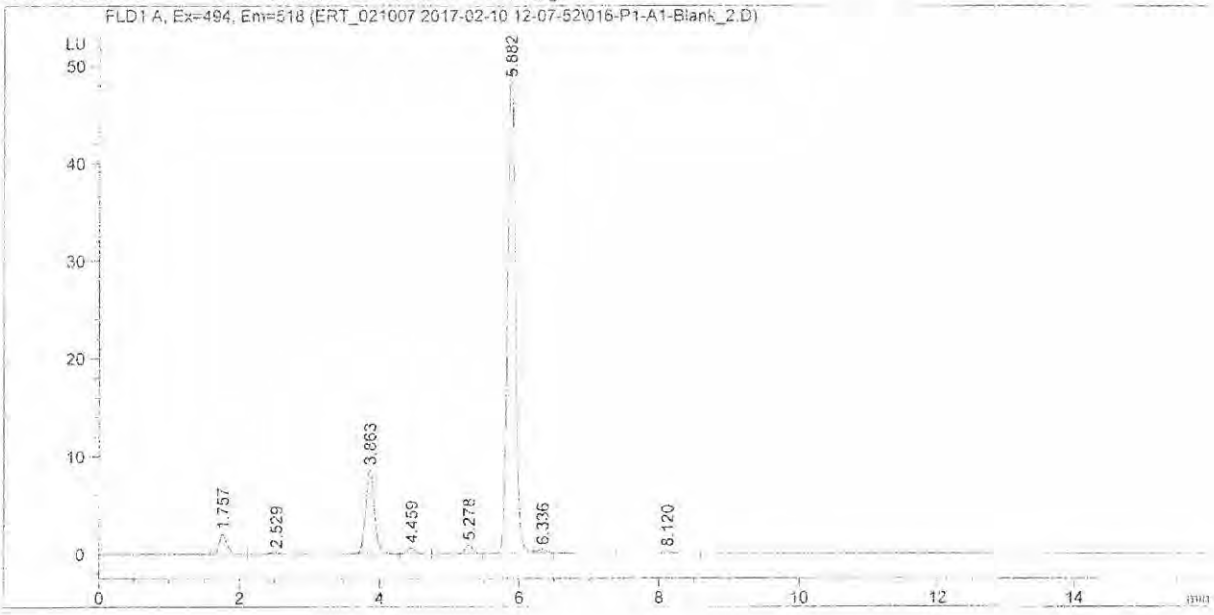
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\016-P1-A1-Blank_2.D
Sample Name: Blank_2

Acq. Operator	: Brandi Glover	Seq. Line	: 16
Acq. Instrument	: HPLC-30	Location	: P1-A1
Injection Date	: 2/10/2017 5:10:33 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M		
Last changed	: 2/10/2017 12:07:54 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 2:11:45 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip		
ECM Version	: 7 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By	:	Signal
Calib. Data Modified	:	2/14/2017 1:47:14 PM
Multiplier	:	1.0000
Dilution	:	1.0000

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [mg/ml]	Grp	Name
4.907		-	-	-		Ergothioneine
Totals :				0.00000		

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\016-P1-A1-Blank_2.D
Sample Name: Blank_2

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2/14/2017 1:47:14 PM
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

Peak #	RetTime [min]	Type	Width [min]	Area [LU*s]	Area %	Name
1	1.757	BV	0.1186	16.18838	3.2047	?
2	2.529	VV R	0.2181	2.09660	0.4150	?
3	3.863	VV R	0.1307	74.08631	14.6663	?
4	4.459	VB	0.1205	5.26897	1.0431	?
5	4.907		0.0000	0.00000	0.0000	Ergothioneine
6	5.278	VV R	0.1243	7.20478	1.4263	?
7	5.882	VV R	0.1216	395.81470	78.3563	?
8	6.336	VV T	0.1165	2.35009	0.4652	?
9	8.120	VB	0.1582	2.13735	0.4231	?

Totals : 505.14717

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

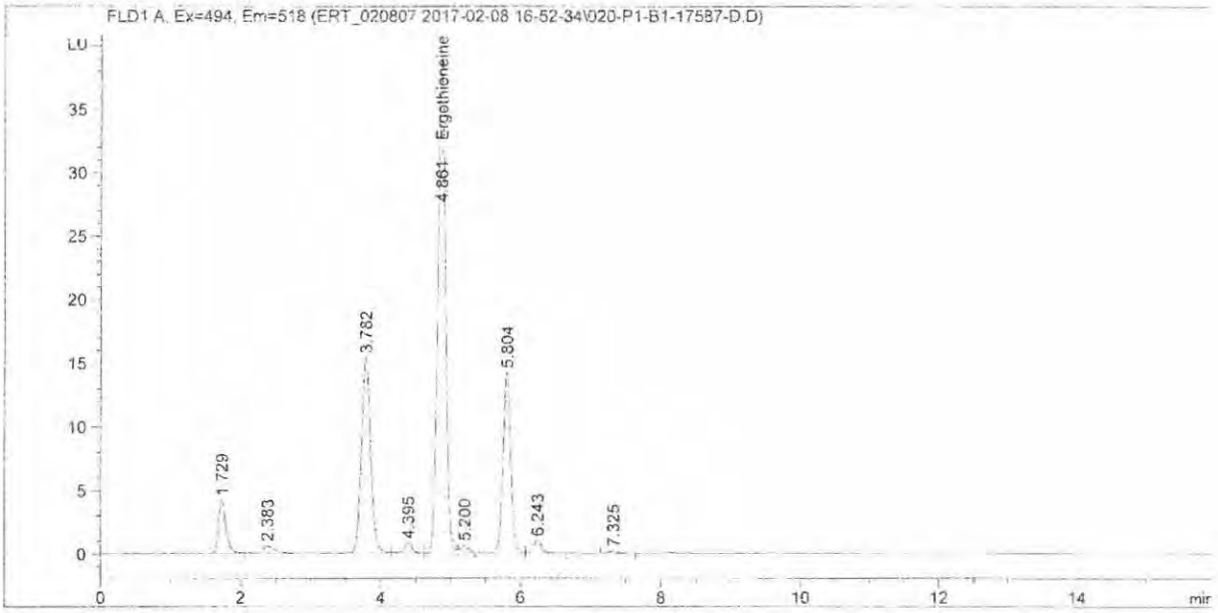
*** End of Report ***

ACCURACY

CHROMATOGRAMS

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\020-P1-B1-17587-D.D
Sample Name: 17587-D (low level spike)
lead stock

Acq. Operator	: Brandi Glover	Seq. Line	: 20
Acq. Instrument	: HPLC-30	Location	: P1-B1
Injection Date	: 2/8/2017 11:05:47 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M		
Last changed	: 2/8/2017 4:52:36 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:10:20 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip		
ECM Version	: 9		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 2/14/2017 1:10:18 PM
Multiplier : 1.0000
Dilution : 25.0000
Sample Amount: : 8.16800 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.861	VV	296.590271	7.88411e-5	7.157042		Ergothioneine
Totals :				7.157042		

*Theoretical result = 7.27%
Recovery = 98.5%

DA-15B 2/14/2017 1:37:58 PM Hong You Page 1 of 2

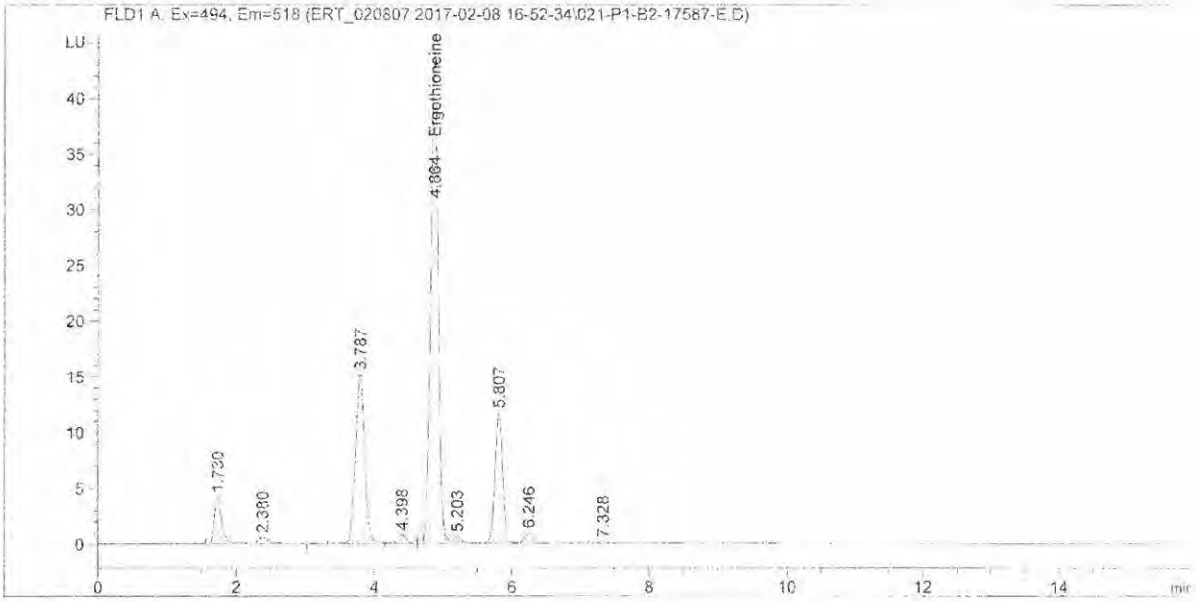
Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\020-P1-B1-17587-D.D
Sample Name: 17587-D

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\021-P1-B2-17587-E.D
Sample Name: 17587-E (mid level spike - 1ml std stock)

Acq. Operator	: Brandi Glover	Seq. Line	: 21
Acq. Instrument	: HPLC-30	Location	: P1-B2
Injection Date	: 2/8/2017 11:23:26 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M		
Last changed	: 2/8/2017 4:52:36 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:10:20 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip		
ECM Version	: 9		



ESTD Percent Report

Sorted By	:	Signal
Calib. Data Modified	:	2/14/2017 1:10:18 PM
Multiplier	:	1.0000
Dilution	:	25.0000
Sample Amount:	:	8.15900 [mg/ml]

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.864	VV	333.768391	8.00679e-5	8.189038*		Ergothioneine
Totals :				8.189038		

*theoretical result = 8.00%
Recovery = 102%

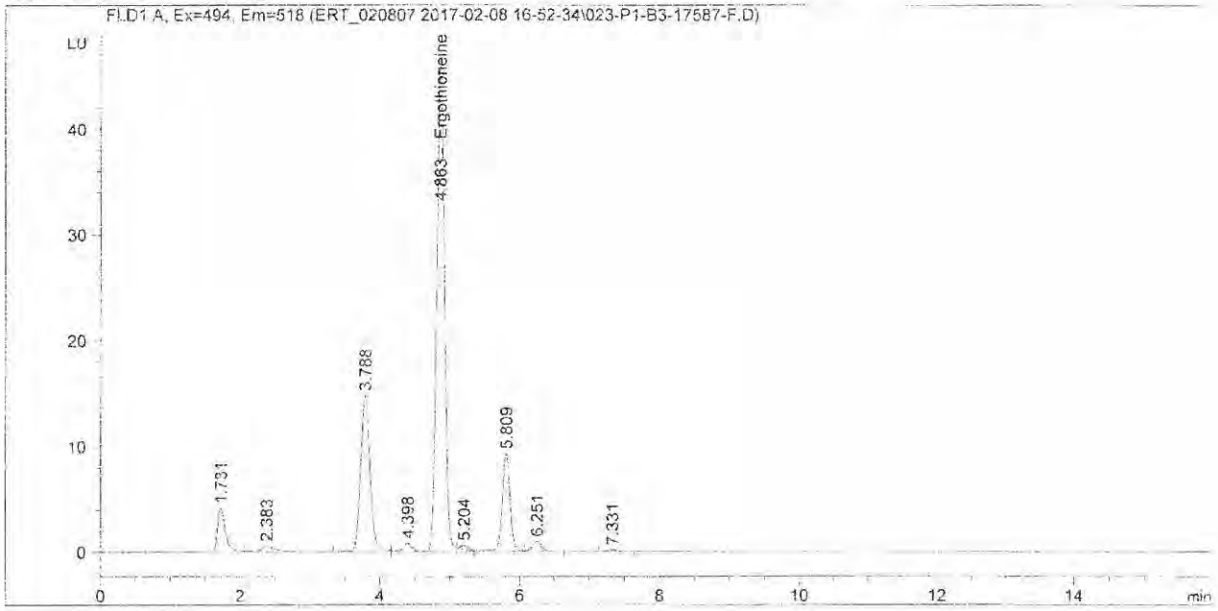
Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\021-P1-B2-17587-E.D
Sample Name: 17587-E

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\023-P1-B3-17587-F.D
Sample Name: 17587-F (high level spike - 180 ml stock)

Acq. Operator	: Brandi Glover	Seq. Line	: 23
Acq. Instrument	: HPLC-30	Location	: P1-B3
Injection Date	: 2/8/2017 11:58:45 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_020807 2017-02-08 16-52-34\ERT.M		
Last changed	: 2/8/2017 4:52:36 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:39:01 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_020807 2017-02-08 16-52-34.SC.SSIzip		
ECM Version	: 10		



ESTD Percent Report

Sorted By	:	Signal	
Calib. Data Modified	:	2/14/2017 1:39:00 PM	
Multiplier	:	1.0000	
Dilution	:	25.0000	
Sample Amount:	:	8.08900	[mg/ml]

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount %	Grp	Name
4.863	VV	356.756134	8.06977e-5	8.897700*		Ergothioneine
Totals :				8.897700		

* Theoretical result = 8.75%
Recovery = 102%

Data File d:\Chem32\6\Data\ERT_020807 2017-02-08 16-52-34\023-P1-B3-17587-F.D
Sample Name: 17587-F

==========

*** End of Report ***

LINEARITY

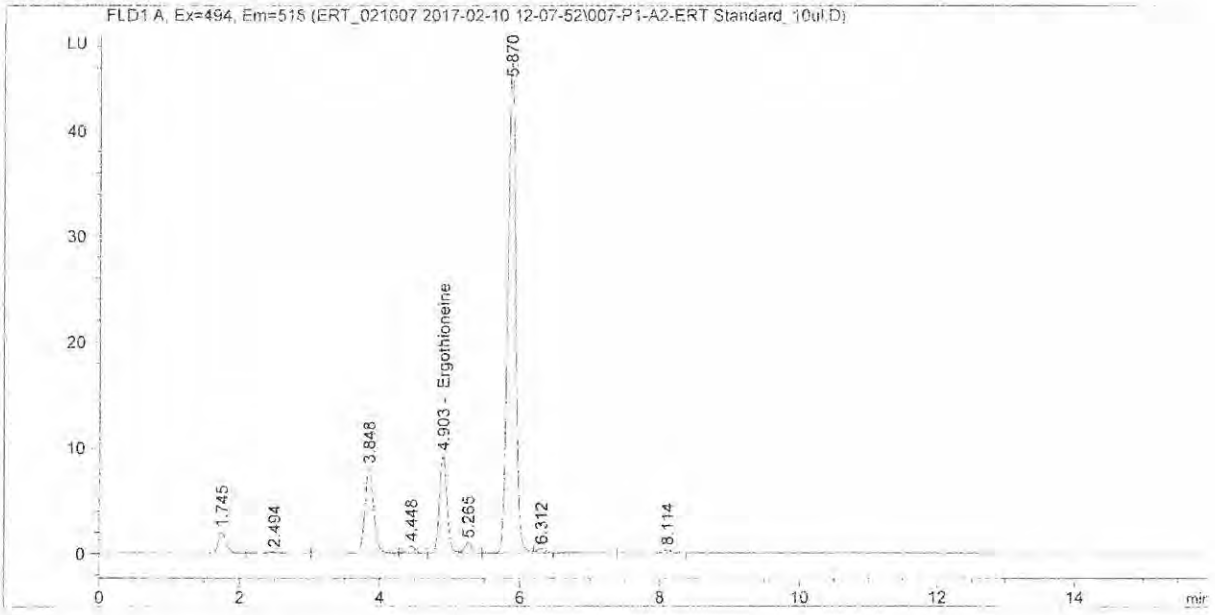
CHROMATOGRAMS

5 POINT CALIBRATION

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\007-P1-A2-ERT Standard_10ul.D
Sample Name: ERT Standard_10ul

```
==========================================================================
Acq. Operator   : Brandi Glover                    Seq. Line :   7
Acq. Instrument : HPLC-30                          Location :   P1-A2
Injection Date  : 2/10/2017 2:31:36 PM                 Inj :   1
                                                  Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:47:16 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7
```




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

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]           [LU*s]                [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.903 VV        70.33330 6.62947e-5 4.66273e-3     Ergothioneine

Totals :                              4.66273e-3
```

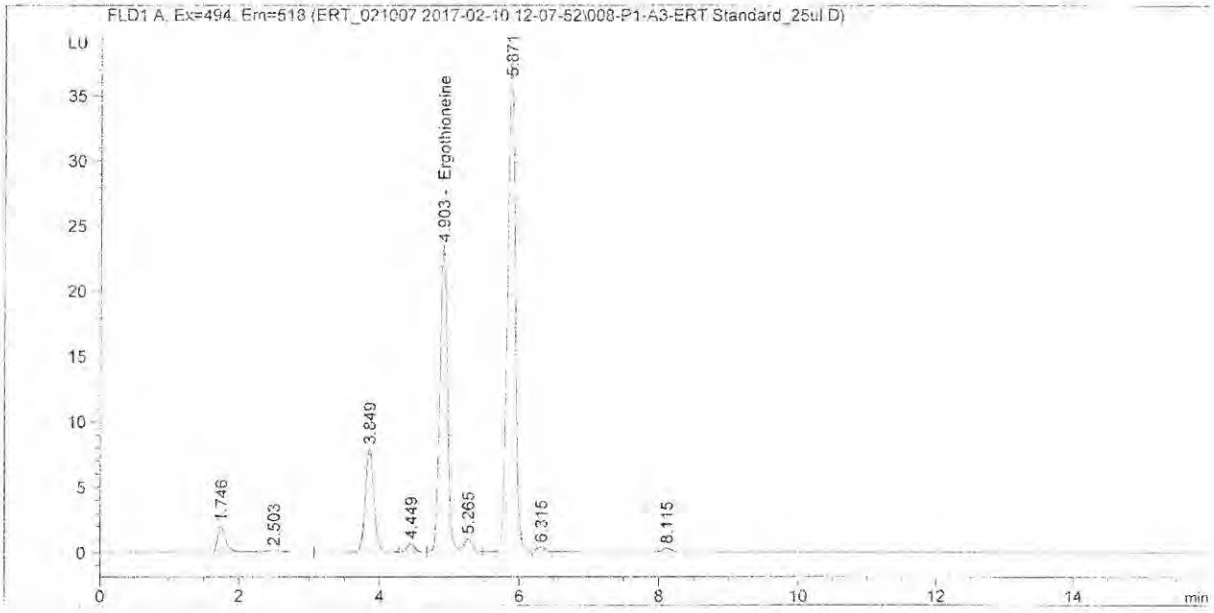
Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\007-P1-A2-ERT Standard_10ul.D
Sample Name: ERT Standard_10ul

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\008-P1-A3-ERT Standard_25ul.D
Sample Name: ERT Standard_25ul

Acq. Operator	: Brandi Glover	Seq. Line	: 8
Acq. Instrument	: HPLC-30	Location	: P1-A3
Injection Date	: 2/10/2017 2:49:16 PM	Inj	: 1
		Inj Volume	: 2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M		
Last changed	: 2/10/2017 12:07:54 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:47:16 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip		
ECM Version	: 7		



External Standard Report (Sample Amount is 0!)

Sorted By	:	Signal
Calib. Data Modified	:	2/14/2017 1:47:14 PM
Multiplier	:	1.0000
Dilution	:	1.0000

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [mg/ml]	Grp	Name
4.903	VV	177.82634	7.27391e-5	1.29349e-2		Ergothioneine
Totals :				1.29349e-2		

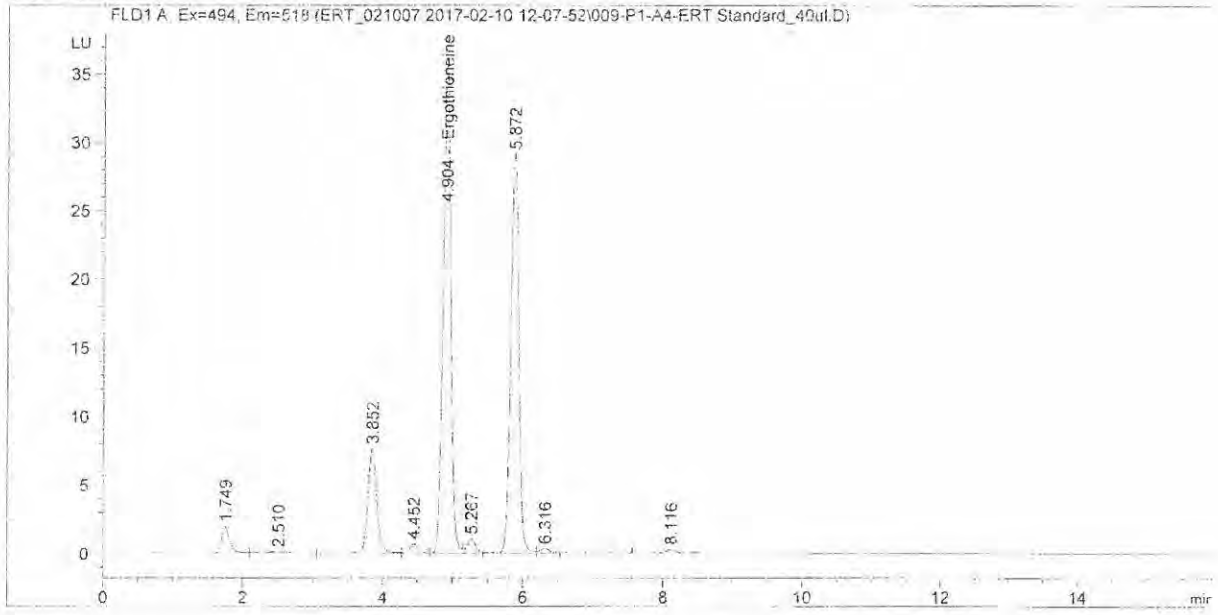
Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\008-P1-A3-ERT Standard_25ul.D
Sample Name: ERT Standard_25ul

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\009-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

Acq. Operator	: Brandi Glover	Seq. Line :	9
Acq. Instrument	: HPLC-30	Location :	P1-A4
Injection Date	: 2/10/2017 3:06:54 PM	Inj :	1
		Inj Volume :	2.000 µl
Acq. Method	: d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M		
Last changed	: 2/10/2017 12:07:54 PM by Brandi Glover		
Analysis Method	: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)		
Last changed	: 2/14/2017 1:47:16 PM by Hong You		
Method Info	: L-ergothioneine		
ECM Server	: http://us05apvp001/ecmwg		
ECM Operator	: Hong You		
ECM Path	: \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip		
ECM Version	: 7		



External Standard Report (Sample Amount is 0!)

Sorted By	:	Signal	
Calib. Data Modified	:	2/14/2017 1:47:14 PM	
Multiplier	:	1.0000	
Dilution	:	1.0000	

Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [mg/ml]	Grp	Name
4.904	VV	274.49606	7.42241e-5	2.03742e-2		Ergothioneine
Totals :				2.03742e-2		

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\009-P1-A4-ERT Standard_40ul.D
Sample Name: ERT Standard_40ul

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\014-P1-A5-ERT Standard_55ul.D
Sample Name: ERT Standard_55ul

```
==========================================================================
Acq. Operator    : Brandi Glover                    Seq. Line :  14
Acq. Instrument  : HPLC-30                           Location :  P1-A5
Injection Date   : 2/10/2017 4:35:13 PM                 Inj :  1
                                                   Inj Volume : 2.000 µl
Acq. Method      : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed     : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method  : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed     : 2/14/2017 1:47:16 PM by Hong You
Method Info      : L-ergothioneine

ECM Server       : http://us05apvp001/ecmwg
ECM Operator     : Hong You
ECM Path         : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version      : 7
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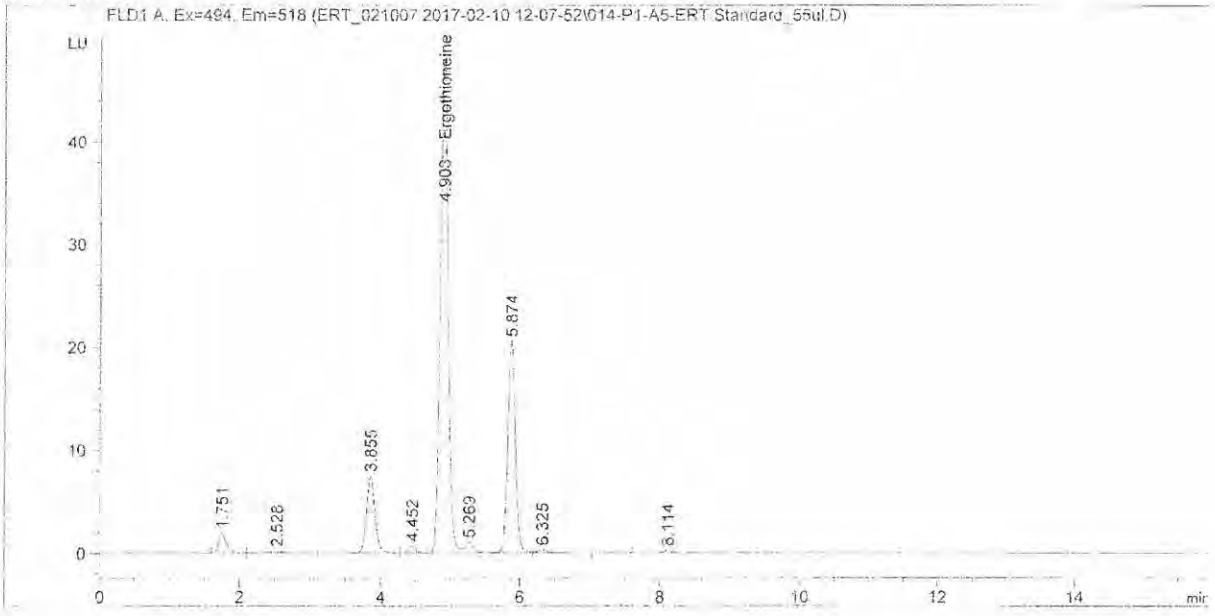


```
==========================================================================
          External Standard Report (Sample Amount is 0!)
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [LU*s]                 [mg/ml]
-------|------|----------|----------|----------|--|------------------
  4.903 VV       366.26349 7.49085e-52.74363e-2    Ergothioneine

Totals :                               2.74363e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\014-P1-A5-ERT Standard_55ul.D
Sample Name: ERT Standard_55ul

==========

*** End of Report ***

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\015-P1-A6-ERT Standard_70ul.D
Sample Name: ERT Standard_70ul

```
==================================================================
Acq. Operator   : Brandi Glover                  Seq. Line :  15
Acq. Instrument : HPLC-30                        Location :  P1-A6
Injection Date  : 2/10/2017 4:52:54 PM               Inj :  1
                                                 Inj Volume : 2.000 µl
Acq. Method     : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed    : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed    : 2/14/2017 1:47:16 PM by Hong You
Method Info     : L-ergothioneine

ECM Server      : http://us05apvp001/ecmwg
ECM Operator    : Hong You
ECM Path        : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version     : 7
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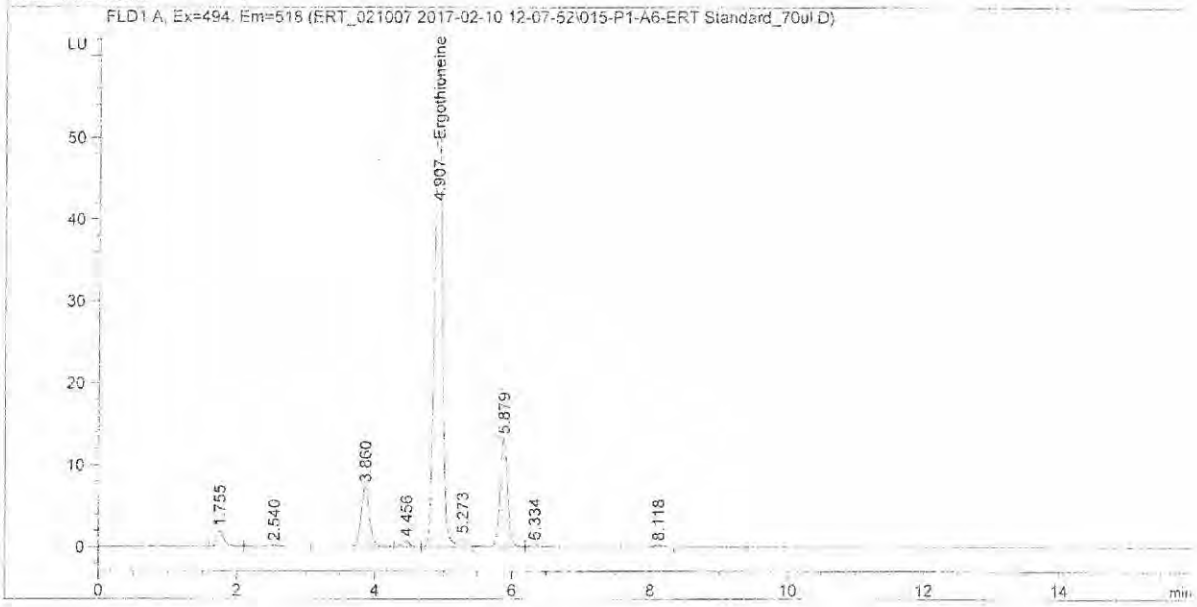



```
==================================================================
          External Standard Report (Sample Amount is 0!)
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]           [LU*s]                 [mg/ml]
-------|------|----------|----------|----------|--|--------------------
  4.907 VV       454.56802 7.53062e-5 3.42318e-2     Ergothioneine

Totals :                               3.42318e-2
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\015-P1-A6-ERT Standard_70ul.D
Sample Name: ERT Standard_70ul

==========

*** End of Report ***

LINEARITY CALIBRATION TABLES

CALIBRATION CHROMATOGRAMS

Method d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M

Calibration Table

General Calibration Setting

Calib. Data Modified : 2/14/2017 1:47:14 PM
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 : 0.000 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: FLD1 A, Ex=494, Em=518

Overview Table

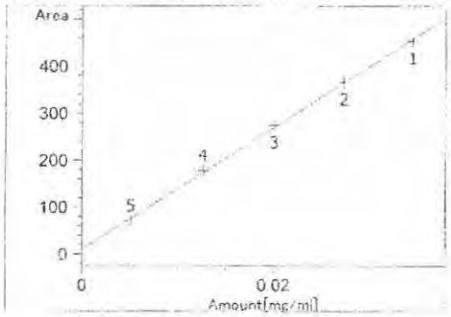
RT	Sig	Lvl	Amount [mg/ml]	Area	Rsp.Factor	Ref	ISTD	#	Compound
4.907	1	5	5.08300e-3	70.33330	7.22702e-5	No	No		Ergothioneine
		4	1.26120e-2	177.82634	7.09231e-5				
		3	2.00310e-2	274.17200	7.30600e-5				
		2	2.73420e-2	366.26349	7.46512e-5				
		1	3.45470e-2	454.56802	7.59996e-5				

Method d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M

Peak Sum Table

No Entries in table

Calibration Curves



Ergothioneine at exp. RT: 4.907
FLD1 A, Ex=494, Em=518
Correlation: 0.99955
Residual Std. Dev.: 5.25412
Formula: y = mx + b
m: 12994.48145
b: 9.74358
x: Amount [mg/ml]
y: Area

LIMIT OF DETECTION AND QUANTIFICATION

eurofins

Effective Date: 02/05/2016
Version 1

Spreadsheet For Calculating LOQ and LOD

Please Read Instruction Sheet Before Use (see Page 1)

I. Calculation using manual input information

Sample Amnt	8.00
Sample DF	25.00
Serving Size	

[Sample]	0.32

Formulas used:
LOQ area: =(Area of Std)/(Signal to Noise/10)
LOD area: =(Area of Std)/(Signal to Noise/3)
LOQ value: =(LOQ Area * [Standard] * Serv. Size)/([Sample] * Area of Std)
LOD value: =(LOD Area * [Standard] * Serv. Size)/([Sample] * Area of Std)

		A1	A2	A3	A4	A5	A6	A7	A8
Signal/Noise		1659							
Low Stk Area		70.3							
Low Stk Conc.		0.00508							
Area Limits	LOD:	0.12712							
	LOQ:	0.42375							
Values	LOQ:	0							
	LOD:	0							

II. Calculation using information from Sequence Table

					LOQ Values (Lowest Quantifiable Amount)							
Sample ID	Amnt	DF	Multiplier	Conc.	A1	A2	A3	A4	A5	A6	A7	A8
17591-A	8.063	25	1	0.32252	0.0095							
17591-B	8.141	25	1	0.32564	0.0094							
17591-C	8.108	25	1	0.32432	0.009447							
17595-A	8.027	25	1	0.32108	0.009542							
17595-B	8.068	25	1	0.32272	0.009494							
17595-C	8.085	25	1	0.3234	0.009474							
17603-A	8.104	25	1	0.32416	0.009452							
17603-B	8.16	25	1	0.3264	0.009387							
17603-C	8.103	25	1	0.32412	0.009453							

Lab Analyst Date: _____

GRAS Notice –Ergothioneine
Blue California

GRAS ASSOCIATES, LLC

eurofins

Effective Date: 02/05/2016
Version 1

Spreadsheet For Calculating LOQ and LOD

Please Read Instruction Sheet Before Use (see Page 1)

I. Calculation using manual input information

Sample Amnt	8.00
Sample DF	25.00
Serving Size	0.00

[Sample]	0.32

Formulas used:
LOQ area: =(Area of Std)/(Signal to Noise/10)
LOD area: =(Area of Std)/(Signal to Noise/3)
LOQ value: =(LOQ Area * [Standard] * Serv. Size)/([Sample] * Area of Std)
LOD value: =(LOD Area * [Standard] * Serv. Size)/([Sample] * Area of Std)

		A1	A2	A3	A4	A5	A6	A7	A8
Signal/Noise		1659							
Low Stk Area		70.3							
Low Stk Conc.		0.005083							
Area Limits	LOD:	0.127125							
	LOQ:	0.423749							
Values	LOQ:								
	LOD:								

II. Calculation using information from Sequence Table

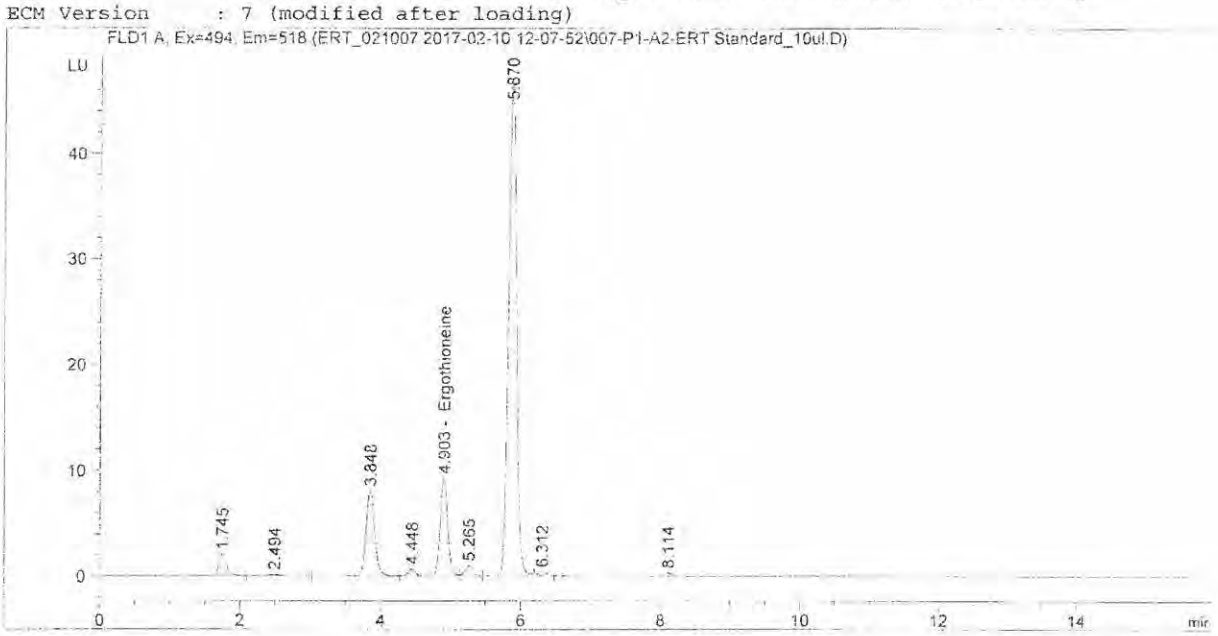
					LOD Values (Lowest Detectable Amount)							
Sample ID	Amnt	DF	Multiplier	Conc.	A1	A2	A3	A4	A5	A6	A7	A8
17591-A	8.063	25	1	0.32252	0.00285							
17591-B	8.141	25	1	0.32564	0.002823							
17591-C	8.108	25	1	0.32432	0.002834							
17595-A	8.027	25	1	0.32108	0.002863							
17595-B	8.068	25	1	0.32272	0.002848							
17595-C	8.085	25	1	0.3234	0.002842							
17603-A	8.104	25	1	0.32416	0.002836							
17603-B	8.16	25	1	0.3264	0.002816							
17603-C	8.103	25	1	0.32412	0.002836							

Lab Analyst Date: _____

```
Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\007-P1-A2-ERT Standard_10ul.D
Sample Name: ERT Standard_10ul

==========================================================================
Acq. Operator    : Brandi Glover                    Seq. Line :   7
Acq. Instrument  : HPLC-30                           Location :   P1-A2
Injection Date   : 2/10/2017 2:31:36 PM                  Inj :   1
                                                   Inj Volume : 2.000 µl
Acq. Method      : d:\Chem32\2\Data\ERT_021007 2017-02-10 12-07-52\ERT.M
Last changed     : 2/10/2017 12:07:54 PM by Brandi Glover
Analysis Method  : d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT.M (Sequence Method)
Last changed     : 2/15/2017 12:29:06 PM by Hong You
Method Info      : L-ergothioneine

ECM Server       : http://us05apvp001/ecmwg
ECM Operator     : Hong You
ECM Path         : \Petaluma\LC\HPLC-30\Data\ERT_021007 2017-02-10 12-07-52.SC.SSIzip
ECM Version      : 7 (modified after loading)
```




```
==========================================================================
External Standard Report (Sample Amount is 0!) with Performance and Noise
==========================================================================

Calib. Data Modified  :      2/14/2017 1:47:14 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=494, Em=518
```

Data File d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\007-P1-A2-ERT Standard_10ul.D
Sample Name: ERT Standard_10ul

Noise determination:

Time range from [min]	to [min]	Noise (6*SD) [LU]	Noise (PtoP) [LU]	Noise (ASTM) [LU]	Wander [LU]	Drift [LU/h]
9.000	10.000	5.582e-3	3.123e-3	-	-	-2.910e-2

RetTime [min]	k'	Sig	Amount [mg/ml]	Symm.	Width [min]	Plates	Signal /Noise	Name
4.903	-	1	4.66273e-3	0.89	0.1167	9777	1659.2	Ergothioneine

*** End of Report ***

Sequence: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT_021007.S

Sequence Table:

Method and Injection Info Part:

Line	Location	SampleName DataFile	Method AutoBalance	Inj LimsID	SampleType	InjVolume
1	P1-A1	Blk_1 001-P1-A1-Blk_1	ERT_Startup	1	Blank	0
2	P1-A1	Blk_2 002-P1-A1-Blk_2	ERT_Equil	1	Blank	0
3	P1-A1	Blank_1 003-P1-A1-Blank_1	ERT	1	Blank	
4	P1-A1	Blank_1 004-P1-A1-Blank_1	ERT	1	Blank	
5	P1-A1	Blank_1 005-P1-A1-Blank_1	ERT	1	Blank	
6	P1-A1	Blank_1 006-P1-A1-Blank_1	ERT	1	Blank	
7	P1-A2	ERT Standard_10ul 007-P1-A2-ERT Standa	ERT	1	Calibration	
8	P1-A3	ERT Standard_25ul 008-P1-A3-ERT Standa	ERT	1	Calibration	
9	P1-A4	ERT Standard_40ul 009-P1-A4-ERT Standa	ERT	1	Calibration	
10	P1-A4	ERT Standard_40ul 010-P1-A4-ERT Standa	ERT	1	Calibration	
11	P1-A4	ERT Standard_40ul 011-P1-A4-ERT Standa	ERT	1	Calibration	
12	P1-A4	ERT Standard_40ul 012-P1-A4-ERT Standa	ERT	1	Calibration	
13	P1-A4	ERT Standard_40ul 013-P1-A4-ERT Standa	ERT	1	Calibration	
14	P1-A5	ERT Standard_55ul 014-P1-A5-ERT Standa	ERT	1	Calibration	
15	P1-A6	ERT Standard_70ul 015-P1-A6-ERT Standa	ERT	1	Calibration	
16	P1-A1	Blank_2 016-P1-A1-Blank_2	ERT	1	Blank	
17	P1-A7	17591-A 017-P1-A7-17591-A	ERT	1	Sample	
18	P1-A8	17591-B 018-P1-A8-17591-B	ERT	1	Sample	
19	P1-A9	17591-C 019-P1-A9-17591-C	ERT	1	Sample	
20	P1-B1	17595-A 020-P1-B1-17595-A	ERT	1	Sample	
21	P1-B2	17595-B 021-P1-B2-17595-B	ERT	1	Sample	
22	P1-A4	ERT Standard_40ul_cc 022-P1-A4-ERT Standa	ERT	1	Calibration	
23	P1-B3	17595-C 023-P1-B3-17595-C	ERT	1	Sample	
24	P1-B4	17603-A 024-P1-B4-17603-A	ERT	1	Sample	
25	P1-B5	17603-B 025-P1-B5-17603-B	ERT	1	Sample	

DA-15B 2/14/2017 2:13:07 PM Hong You Page 1 of 3

Sequence: d:\Chem32\5\Data\ERT_021007 2017-02-10 12-07-52\ERT_021007.S

Line	Location	SampleName DataFile	Method AutoBalance	Inj LimsID	SampleType	InjVolume
26	P1-B6	17603-C 026-P1-B6-17603-C	ERT	1	Sample	
27	P1-A4	ERT Standard_40ul_cc 027-P1-A4-ERT Standa	ERT	1	Calibration	
28	P1-A1	Blank_3 028-P1-A1-Blank_3	Column Flush 1-6	1	Blank	0

Calibration Part:

Line	Location	SampleName	Method	CalLev	Update RF	Update RT	Interval
7	P1-A2	ERT Standard_10- ul	ERT	5	Replace	Replace	
8	P1-A3	ERT Standard_25- ul	ERT	4	Replace	Replace	
9	P1-A4	ERT Standard_40- ul	ERT	3	Replace	Replace	
10	P1-A4	ERT Standard_40- ul	ERT	3	Average	Average	
11	P1-A4	ERT Standard_40- ul	ERT	3	Average	Average	
12	P1-A4	ERT Standard_40- ul	ERT	3	Average	Average	
13	P1-A4	ERT Standard_40- ul	ERT	3	Average	Average	
14	P1-A5	ERT Standard_55- ul	ERT	2	Replace	Replace	
15	P1-A6	ERT Standard_70- ul	ERT	1	Replace	Replace	
22	P1-A4	ERT Standard_40- ul_cc	ERT	3	No Update	No Update	
27	P1-A4	ERT Standard_40- ul_cc	ERT	3	No Update	No Update	

Quantification Part:

Line	Location	SampleName	SampleAmount	ISTDAmt	Multiplier	Dilution
1	P1-A1	Blk_1				
2	P1-A1	Blk_2				
3	P1-A1	Blank_1				
4	P1-A1	Blank_1				
5	P1-A1	Blank_1				
6	P1-A1	Blank_1				
7	P1-A2	ERT Standard_10- ul				
8	P1-A3	ERT Standard_25- ul				
9	P1-A4	ERT Standard_40- ul				
10	P1-A4	ERT Standard_40- ul				

Sequence: d:\Chem32\6\Data\ERT_021007 2017-02-10 12-07-52\ERT_021007.S

Line	Location	SampleName	SampleAmount	ISTDAmt	Multiplier	Dilution
11	P1-A4	ERT Standard_40-ul				
12	P1-A4	ERT Standard_40-ul				
13	P1-A4	ERT Standard_40-ul				
14	P1-A5	ERT Standard_55-ul				
15	P1-A6	ERT Standard_70-ul				
16	P1-A1	Blank_2				
17	P1-A7	17591-A	8.063			25
18	P1-A8	17591-B	8.141			25
19	P1-A9	17591-C	8.108			25
20	P1-B1	17595-A	8.027			25
21	P1-B2	17595-B	8.068			25
22	P1-A4	ERT Standard_40-ul_cc				
23	P1-B3	17595-C	8.085			25
24	P1-B4	17603-A	8.104			25
25	P1-B5	17603-B	8.160			25
26	P1-B6	17603-C	8.103			25
27	P1-A4	ERT Standard_40-ul_cc				
28	P1-A1	Blank_3				

Pages 194-200 have been removed in accordance with copyright laws. The removed reference is:

Sotgia, Salvatore Published: July 29, 2013 Plasma L-Ergothioneine Measurement by High-Performance Liquid Chromatography and Capillary Electrophoresis after a Pre-Column Derivatization with 5-Iodoacetamidofluorescein (5-IAF) and Fluorescence Detection, https://doi.org/10.1371/journal.pone.0070374

Appendix 3 Certificates of Analyses for Multiple Production Batches of ErgoActive™

Appendix 3.1 Certificate of Analysis ErgoActive™ Lot 3332-1606-01

Appendix 3.2 Certificate of Analysis ErgoActive™ Lot 3332-1610-03

Appendix 3.3 Certificate of Analysis ErgoActive™ Lot 3332-1611-04

Appendix 3.4 Certificate of Analysis ErgoActive™ Lot 3332-1611-05

Appendix 3.5 Certificate of Analysis ErgoActive™ Lot 3332-1608-02

Appendix 3.1 Certificate of Analysis ErgoActive™ Lot 3332-1606-01

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

CERTIFICATE OF ANALYSIS

Product: ErgoActive ™ 5%With Maltodextrin
Item# ER0203332

Lot No:	**3332-1606-01**	**Original Manufacturer:**	**Anhui Lonking Biotechnology***
Date of Manufacturing:	**06-05-16**	**Exclusive Distributor**	**Blue California**
Expiration/re-test date:	**06-05-18**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**07-22-16**	**Carrier: Maltodextrin**	
Country of Origin :	**China**	**This Product has NOT been treated**	

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	WHITE POWDER	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
L-Ergothioneine	**≥5%**	**HPLC**	**5.28%**
LOSS ON DRYING	≤ 6%	USP 34	5.30%
HEAVY METALS	< 10 ppm	USP 34	PASS
LEAD	< 1 ppm	ICP-MS	< 0.20 ppm
ARSENIC	< 1 ppm	ICP-MS	< 0.50 ppm
CADMIUM	< 1 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 1 ppm	ICP-MS	< 0.10 ppm
BULK DENSITY	0.3-0.6 g/ml	USP 34	0.48 g/ml
TAP DENSITY	≥ 0.6 g/ml	USP 34	0.70 g/ml
PARTICLE SIZE:	> 90% through Mesh #80 Sieve	USP 34	97%
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	N/D
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	2 YEARS	AOAC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 02-23-2017

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Appendix 3.2 Certificate of Analysis ErgoActive™ Lot 3332-1610-03

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

CERTIFICATE OF ANALYSIS

Product: ErgoActive ™ 5%With Maltodextrin
Item# ER0203332

Lot No:	**3332-1610-03**	**Original Manufacturer:**	**Anhui Lonking Biotechnology***
Date of Manufacturing:	**06-28-16**	**Exclusive Distributor:**	**Blue California**
Expiration/re-test date:	**06-28-18**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**07-22-16**	**Carrier: Maltodextrin**	
Country of Origin :	**China**	**This Product has NOT been treated**	

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	WHITE POWDER	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
L-Ergothioneine	**≥ 5%**	**HPLC**	**5.38%**
LOSS ON DRYING	≤ 6%	USP 34	5.20%
HEAVY METALS	< 10 ppm	USP 34	PASS
LEAD	< 1 ppm	ICP-MS	< 0.20 ppm
ARSENIC	< 1 ppm	ICP-MS	< 0.50 ppm
CADMIUM	< 1 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 1 ppm	ICP-MS	< 0.10 ppm
BULK DENSITY	0.3-0.6 g/ml	USP 34	0.50 g/ml
TAP DENSITY	≥ 0.6 g/ml	USP 34	0.72 g/ml
PARTICLE SIZE:	> 90% through Mesh #80 Sieve	USP 34	97%
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	N/D
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	2 YEARS	AOAC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 02-23-2017

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Appendix 3.3 Certificate of Analysis ErgoActive™ Lot 3332-1611-04

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

CERTIFICATE OF ANALYSIS

Product: ErgoActive ™ 5%With Maltodextrin
Item# ER0203332

Lot No:	**3332-1611-04**	**Original Manufacturer:**	**Anhui Lonking Biotechnology***
Date of Manufacturing:	**06-28-16**	**Exclusive Distributor:**	**Blue California**
Expiration/re-test date:	**06-28-18**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**07-22-16**	**Carrier:**	**Maltodextrin**
Country of Origin :	**China**	**This Product has NOT**	**been treated**

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	WHITE POWDER	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
L-Ergothioneine	**≥5%**	**HPLC**	**5.35%**
LOSS ON DRYING	≤ 6%	USP 34	5.20%
HEAVY METALS	< 10 ppm	USP 34	PASS
LEAD	< 1 ppm	ICP-MS	< 0.20 ppm
ARSENIC	< 1 ppm	ICP-MS	< 0.50 ppm
CADMIUM	< 1 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 1 ppm	ICP-MS	< 0.10 ppm
BULK DENSITY	0.3-0.6 g/ml	USP 34	0.50 g/ml
TAP DENSITY	≥ 0.6 g/ml	USP 34	0.72 g/ml
PARTICLE SIZE:	> 90% through Mesh #80 Sieve	USP 34	97%
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	N/D
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	2 YEARS	AOAC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 02-23-2017

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Appendix 3.4 Certificate of Analysis ErgoActive™ Lot 3332-1611-05

Blue California
30111 Tomay
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1989

CERTIFICATE OF ANALYSIS

Product: ErgoActive ™ 5%With Maltodextrin
Item# ER0203332

Lot No:	**3332-1611-05**	**Original Manufacturer:**	**Anhui Lonking Biotechnology***
Date of Manufacturing:	**11-03-16**	**Exclusive Distributor:**	**Blue California**
Expiration/re-test date:	**11-03-18**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**11-18-16**	**Carrier: Maltodextrin**	
Country of Origin :	**China**	**This Product has NOT been treated**	

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	WHITE POWDER	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
L-Ergothioneine	**≥5%**	**HPLC**	**5.38%**
LOSS ON DRYING	≤6%	USP 34	5.30%
HEAVY METALS	< 10 ppm	USP 34	PASS
LEAD	< 1 ppm	ICP-MS	< 0.20 ppm
ARSENIC	< 1 ppm	ICP-MS	< 0.50 ppm
CADMIUM	< 1 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 1 ppm	ICP-MS	< 0.10 ppm
BULK DENSITY	0.3-0.6 g/ml	USP 34	0.48 g/ml
TAP DENSITY	≥ 0.6 g/ml	USP 34	0.71 g/ml
PARTICLE SIZE	> 90% through Mesh #80 Sieve	USP 34	96%
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	N/D
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	2 YEARS	AOAC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 02-23-2017

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Appendix 3.5 Certificate of Analysis ErgoActive™ Lot 3332-1608-02

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

CERTIFICATE OF ANALYSIS

Product: ErgoActive ™ 5%With Maltodextrin
Item# ER0203332

Lot No:	**3332-1608-02**	**Original Manufacturer:**	**Anhui Lonking Biotechnology***
Date of Manufacturing:	**06-28-16**	**Exclusive Distributor:**	**Blue California**
Expiration/re-test date:	**06-28-18**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**07-22-16**	**Carrier: Maltodextrin**	
Country of Origin :	**China**	**This Product has NOT been treated**	

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	WHITE POWDER	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
L-Ergothioneine	**≥ 5%**	**HPLC**	**5.35%**
LOSS ON DRYING	≤ 6%	USP 34	5.20%
HEAVY METALS	< 10 ppm	USP 34	PASS
LEAD	< 1 ppm	ICP-MS	< 0.20 ppm
ARSENIC	< 1 ppm	ICP-MS	< 0.50 ppm
CADMIUM	< 1 ppm	ICP-MS	< 0.25 ppm
MERCURY	< 1 ppm	ICP-MS	< 0.10 ppm
BULK DENSITY	0.3-0.6 g/ml	USP 34	0.50 g/ml
TAP DENSITY	≥ 0.6 g/ml	USP 34	0.72 g/ml
PARTICLE SIZE	> 90% through Mesh #80 Sieve	USP 34	97%
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	N/D
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	2 YEARS	AOAC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 02-23-2017

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Appendix 4 Pesticide Analyses for Multiple Production Batches of ErgoActive™

Appendix 4.1 Pesticide Analysis for ErgoActive™ Lot 3332-1606-01
Appendix 4.2 Pesticide Analysis for ErgoActive™ Lot 3332-1608-02

Appendix 4.1 Pesticide Analysis for ErgoActive™ Lot 3332-1606-01

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

February 23, 2017

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

CERTIFICATE OF ANALYSIS

AR-16-KK-017973-02

Batch #: EUCAPE-00086225

This analytical report supersedes AR-16-KK-017973-01.

Sample Identification:

Sample #: 740-2016-00017588
Description: ErgoActive 5%, Lot #3332-1606-01
Condition: White powder in a double ziplock bag received at room temperature.
Date Received: December 07, 2016

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 12/28/2016

	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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QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 12/28/2016

	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: 12/28/2016

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

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Sample #: 740-2016-00017588

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QA602: EBDCs (Dithiocarbamates) (CS2 method, GC-MS)
Method Reference: J. Agric. Food Chem. Vol. 49 pp 2152, 2001
Completed: 12/28/2016

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

Results pertain only to the items tested.
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Hilary Rogers
Operations Manager

Appendix 4.2 Pesticide Analysis for ErgoActive™ Lot 3332-1608-02

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

February 23, 2017

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

CERTIFICATE OF ANALYSIS

AR-16-KK-017972-02

Batch #: EUCAPE-00086225

This analytical report supersedes AR-16-KK-017972-01.

Sample Identification:

Sample #: 740-2016-00017592
Description: ErgoActive 5%, Lot #3332-1608-02
Condition: White powder in a double ziplock bag received at room temperature.
Date Received: December 07, 2016

QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 12/28/2016

	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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QA12C: Pesticides - USP 561 Screen (USP 39)
Method Reference: USP 561
Completed: 12/28/2016

	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: 12/28/2016

	Result	Theoretical Level
Bromide	<10 mg/kg	

[Method performed by an outsource lab.]

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Sample #: 740-2016-00017592

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QA602: EBDCs (Dithiocarbamates) (CS2 method, GC-MS)
Method Reference: J. Agric. Food Chem. Vol. 49 pp 2152, 2001
Completed: 12/28/2016

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

[Method performed by an outsource lab.]

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Hilary Rogers
Operations Manager

Appendix 5 Product Stability Report

Product Stability Report

1. Purpose
   This report helps us to identify the quality and stability parameter of the product as well as its storage condition.

2. Background
   In order to access the valid safety consumable period, as well as the stability performance of the product during its storage process, the Company has developed a new sampling method. This method evaluates 3 consecutive samples for 36 months to detect any quality change indicators. During this period, the color, state, and content of the samples are evaluated based on sampling method.

3. Experimental Method
   HPLC

4. Sample Source
   Provided by R&D department

5. Experimental Conditions
   Room temperature

6. Analytical Methods and Criteria
   6.1 Color and Traits: Sample placed on a clean white paper and analyzed by visual method
   6.2 Content: Analyzed based on Company's SOP for product content detection

7. Sampling Method
   Sample every 6 months for 36 months.

8. Appendix
   Appendix A: Result of the stability test of the product under room temperature storage.

9. Precaution
   Products should be thoroughly mixed to consider qualifying product batch for testing.

10. Conclusion
    The content, trait, and color stability from three batches of the sample products were evaluated for 12 months as of current. The data show the product being stable under current conditions and will continue to be evaluated for product stability and quality.

Appendix A:

Results of stability test of the product during the storage at room temperature

Description	Product				
Batch number	FB160307	Date of production	2016.03.14	Experimental conditions	Room temperature
Type of product	98%	Size of Package	20 g/bag	Starting time of testing	2016.03.27
stability items tested	Product criteria	Measuring methods	Month 0	Month 6	Month 12
Color	white color	samples placed on a clean/white	white color	white color	white color
State	crystalline powder	paper and visualized by naked eyes	crystalline powder	crystalline powder	crystalline powder
Content (%)	≥98%	SOP-QC-41	99.5%	99.3%	99.4%

Description	Product				
Batch number	FB160310	Date of production	2016.03.17	Experimental conditions	Room temperature
Type of product	98%	Size of Package	20 g/bag	Starting time of testing	2016.03.27
stability items tested	Product criteria	Measuring methods	Month 0	Month 6	Month 12
Color	white color	samples placed on a clean/white	white color	white color	white color
State	crystalline powder	paper and visualized by naked eyes	crystalline powder	crystalline powder	crystalline powder
Content (%)	≥98%	SOP-QC-41	99.3%	99.1%	99.2%

Description	Product				
Batch number	FB160313	Date of production	2016.03.21	Experimental conditions	Room temperature
Type of product	98%	Size of Package	20 g/bag	Starting time of testing	2016.03.27
stability items tested	Product criteria	Measuring methods	Month 0	Month 6	Month 12
Color	white color	samples placed on a clean/white	white color	white color	white color
State	crystalline powder	paper and visualized by naked eyes	crystalline powder	crystalline powder	crystalline powder
Content (%)	≥98%	SOP-QC-41	99.6%	99.5%	99.5%

Reporter/Date: Reviewer/Date: Approver/Date:

END