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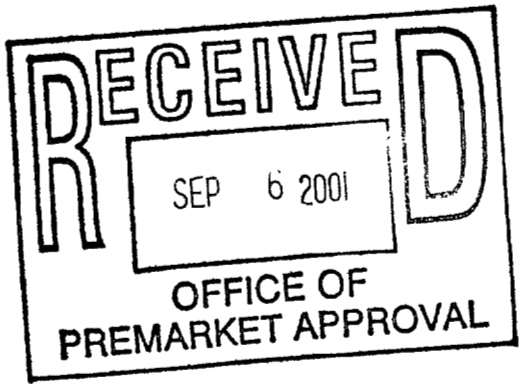

September 5, 2001

Dr. Linda S. Kahl
Office of Premarket Approval
Center for Food Safety and
Applied Nutrition (HFS-206)
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notice for Arabinogalactan from the Eastern Larch Tree

Dear Dr. Kahl:

We hereby submit, in triplicate, a GRAS notice each consisting of 2 volumes for FDA's acknowledgement of Larex's GRAS determination that arabinogalactan derived from the Eastern Larch tree is GRAS for use as a food ingredient. The two volumes are entitled: 1) *GRAS Notice Arabinogalactan From Eastern Larch Tree*, dated September 5, 2001, and 2) *Arabinogalactan From Eastern Larch*, dated February 12, 2001.

Please evaluate this GRAS notice at your most expeditious convenience. If you have any questions, please feel free to call me at 1-800-386-5300.

Sincerely,

Sanford W. Bigelow, Ph.D.
Vice President
Research & Development

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4815 White Bear Parkway
White Bear Lake, MN 55110-3325
(651) 636-2628
Fax. (651) 636-1583
www.larex.com

Toll Free: 800-386-5300

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PREMARKET APPROVAL

GRAS Notice

Arabinogalactan
from the
Eastern Larch Tree

Manufactured by:

Larex, Inc.
4815 White Bear Parkway
White Bear Lake, Minnesota USA
55110-3325

Ph: 1-800-386-5300
Fax: 651-426-8587

Prepared by:

Larex, Inc.
4815 White Bear Parkway
White Bear Lake, Minnesota USA
55110-3325

Phone: 1-800-386-5300
Fax: 651-426-8587

September 5, 2001

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Larex, Inc
GRAS Notice
Arabinogalactan
from
Eastern Larch

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Introduction

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Larex Inc.
GRAS Notice for Arabinogalactan

Introduction

Arabinogalactan is a polysaccharide found in nature in the cell walls of higher plants. As such, arabinogalactan has been consumed as part of the human diet for thousands of years. It is composed of galactose and arabinose units consisting of a highly substituted backbone of 1-3 linked β-D-galactopyranose units with side chains of galactose and arabinose. Larex, Inc., of White Bear Lake, Minnesota, has developed a unique process to extract arabinogalactan from Western larch (*Larix occidentalis*) using only water as the solvent. Larex has developed purification techniques that have improved the visual and organoleptic properties of arabinogalactan, enhancing its suitability for use as a food ingredient. Based on these improvements, Larex, Inc., submitted a 12-volume document to the FDA dated May 26, 2000 (GRN 000047), notifying the Agency of Larex's own GRAS determination that arabinogalactan is safe for use as an ingredient in the human food supply. On June 6, 2000, the FDA acknowledged in writing Larex's own determination that arabinogalactan from Western Larch *(Larix occidentalis)* is generally recognized as safe (GRAS) for use as an ingredient in the food supply.

In this subsequent notice, Larex, Inc., now requests FDA's acknowledgement of Larex's own determination that arabinogalactan from Eastern Larch (*Larix laricina*), also known as the Tamarack tree, is GRAS for use as an ingredient in the human food supply. Only the source of arabinogalactan differs, Eastern Larch vis-à-vis the Western Larch tree.

GRAS Notice No. 000047, dated May 26, 2000, incorporated herein by reference, and the information in this notice contains the information required in proposed §170.36 to allow the FDA to evaluate whether the submitted notice provides a sufficient basis for a determination that arabinogalactan derived from the Eastern Larch (*Larix laricina*) is GRAS as a food ingredient. For this determination, Larex, Inc., commissioned the same panel of experts, qualified by scientific training and experience to assess the safety of food ingredients, that was convened to determine the GRAS status of Western Larch arabinogalactan (GRN 000047). The expert panel evaluated the arabinogalactan from Eastern Larch (document titled: *Arabinogalactan from Eastern Larch*) prepared by Lee Dexter, a consultant to Larex, and GRN 000047, as well as other data and information relevant to the use and safety of this ingredient in a meeting held on February 12, 2001. The panel concluded that arabinogalactan derived from Eastern Larch is GRAS by scientific procedures for general use in food (Tab A). Based on the data and information contained in the Report and the opinion of the expert panel, Larex, Inc., explicitly accepts responsibility for the GRAS determination of arabinogalactan derived from Eastern Larch.

The information contained within shows that arabinogalactan derived from the Eastern Larch (*Larix laricina*) is substantially equivalent to the arabinogalactan derived from Western Larch (*Larix occidentalis*) on the following bases:

1) Eastern Larch arabinogalactan is produced using the same manufacturing processes as used for producing arabinogalactan from Western Larch (GRN 000047, Volume 2, Tabs 2 and 17; and *Arabinogalactan from Eastern Larch*, pp. 24-38),

2) Eastern Larch arabinogalactan is chemically equivalent to Western Larch arabinogalactan (GRN 000047, Volume 2, Tab 4; and *Arabinogalactan from Eastern Larch*, pp. 5-11 and 39; and Table 1 below). On a functional basis, the prebiotic activity of arabinogalactan from either Eastern or Western Larch is the same based on *in vitro* fermentation studies that shows that either arabinogalactan is equally capable of increasing populations of anaerobic bacteria in human fecal samples (*Arabinogalactan from Eastern Larch*, Section IV, pp. 39-44, Causey et al. 2000). In addition, quality of life (QOL) measures are similar in subjects who consumed Eastern or Western Larch arabinogalactan for six months (*Arabinogalactan from Eastern Larch*, Section IV, pp. 37-38, and Raw Data Section, pp. 50-51),
3) the same intake estimate used in GRN 000047 is provided in this notice (as Tables 2 and 3) because arabinogalactan derived from Eastern Larch will be used interchangeably as a food ingredient, on a gram-for-gram basis, as that used for arabinogalactan derived from Western Larch. Arabinogalactan derived from Eastern and Western Larch contains similar carbohydrate levels (Tab B, this volume), and forms the basis upon which arabinogalactan will be added as a food ingredient in food products (Table 5), and
4) in a study conducted at the University of Minnesota, both Eastern and Western Larch arabinogalactan were well tolerated with no adverse effects reported in 56 subjects who consumed up to 8.4 grams of either source of arabinogalactan daily for a period of 6 months (*Arabinogalactan from Eastern Larch*, Section IV, Safety, pp. 3-44; Robinson et al., 1999, Tab C, this volume). Acute toxicity studies in rats conducted at Celsis Laboratory Group (Edison NJ) demonstrated that both Eastern and Western Larch arabinogalactan are relatively nontoxic (*Arabinogalactan from Eastern Larch*, Section IV, Safety, pp. 45, and Raw Data Section, pp. 52-91).

In compliance with 21 CFR §170.30, Larex, Inc., determined that arabinogalactan is considered GRAS when used in accordance with current Good Manufacturing Practices. Larex, Inc., hereby wishes to voluntarily notify the FDA of that determination, and according to proposed §170.36 the company is submitting the following GRAS exemption claim.

GRAS
Exemption Claim

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GRAS Exemption Claim

Larex, Inc., hereby notifies the U.S. Food and Drug Administration that the use of arabinogalactan as a food ingredient is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Larex, Inc., has determined that such use is GRAS.

1. Notifier: Larex Inc.
   4815 White Bear Lake Parkway
   White Bear Lake, Minnesota USA 55110
   Telephone: (800) 386-5300
   Fax: (651) 426-8587

2. Common or Usual Name: Arabinogalactan

3. Applicable Conditions of Use:

   Applications for arabinogalactan include general use in foods as a multiple-use direct additive. The ingredient should be used under the conditions of current Good Manufacturing Practices. To classify the various effects that ingredients may have in food, FDA has published a list of 32 physical or technical functional effects for which direct food ingredients may be added to food. These technical effects are codified at 21 CFR §170.3(o)(1 through 32). Applications for arabinogalactan are covered under several of the following terms as listed under 21 CFR §170.3(o).

   (8) Emulsifiers and emulsifier salts: Substances which modify surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion.

   (14) Formulation aids: Substances used to promote or produce a desired physical state or texture in food, including carriers, binders, fillers, plasticizers, film-formers, and tableting aids, etc.

   (16) Humectants: Hygroscopic substances incorporated in food to promote retention of moisture, including moisture- retention agents and antidusting agents.

   (20) Nutrient supplements: Substances which are necessary for the body's nutritional and metabolic processes.

   (24) Processing aids: Substances used as manufacturing aids to enhance the appeal or utility of a food or food component, including clarifying agents, clouding agents, catalysts, flocculants, filter aids, and crystallization inhibitors, etc.

   (28) Stabilizers and thickeners: Substances used to produce viscous solutions or dispersions, to impart body, improve consistency, or stabilize emulsions, including suspending and bodying agents, setting agents, jellying agents, and bulking agents, etc.

(31) Synergist: Substances used to act or react with another food ingredient to produce a total effect different or greater than the sum of the effects produced by the individual ingredients.

(32) Texturizers: Substances which affect the appearance or feel of the food.

4. Basis of the GRAS Determination

The basis of the Larex, Inc., GRAS determination for arabinogalactan was the use of scientific procedures.

5. Availability of Data and Information and Key to References

The data and information that are the basis of the GRAS determination for Larex, Inc., will be available at the address of the notifier listed above, GRN 000047 (incorporated herein by reference), in Food Additive Master File No. 617, and the information contained within this GRAS notice. Larex, Inc., will be pleased to provide the agency copies of any of the references or other information pertinent to this GRAS notice upon written request.

6. Signature of an official for Larex, Inc.

Sept. 5, 2001

Sanford W. Bigelow, VP, Research & Development — Date

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Equivalency of Western and Eastern Larch Arabinogalactan

Table 1 below summarizes the equivalency of arabinogalactan derived from Eastern or Western Larch regarding its similarity in chemical structure and its functional actions.

Table 1
Comparisons between Eastern and Western Larch[a]

Characteristics	Western Larch	Eastern Larch
1. Distribution of Arabinogalactan	Decrease with tree height	Increase with tree height
2. Purity of Arabinogalactan (%)	91, 84, 88	79, 78, 81 (Fall harvest) 86, 83, 85 (Spring harvest)
3. Galactose: Arabinose[1]	5:2 - 6:2	3.8 - 5:7
4. Arabinogalactan Molecule[2] A: MW 100K B: MW 16K A: more spherical shape B: more linear shape	70 - 75% A 25 - 30% B	90 - 95% A 5 - 10% B
5. Lignin	18.1% - Bark of *L. Decidua* 17.4% - Bark of *L. Eurolepsis* 9.8% - Bark of *L. Kaempferi*	26% (dry wood weight)
6. Uronic acids	0.2%	2%
7. Likely structural differences	Backbone chain β-S-galactopyranosyl units linked: (1 → 3)	(1 → 3) and (1 → 6)
8. Ash	0.44	0.74

[1]Arabinogalactan from Eastern larch contains more L-arabinose residues than most AG from larch woods (Cote and Timmel 1967, Vol. 3 Tab 9, GRN 000047).
[2]From various sources in the literature (Cote and Timmel 1967, Vol. 3 Tab 9, GRN 000047).
[a]These data are also found in *Arabinogalactan from Eastern Larch*, Section I, Chemical Identity, page 39.

Intake Estimate for Arabinogalactan

Larex, Inc. was asked to provide the FDA with an intake estimate for arabinogalactan as part of GRN 000047 (Tables 2 and 3). Larex, Inc., used the information contained in the Agency's guidance document *Estimating Exposure to Direct Food Additives and Chemical Contaminants in the Diet* and USDA's *Continuing Survey of Food Intakes by Individuals* (CSFII, 1996) to estimate the maximum potential exposure to arabinogalactan produced by Larex, Inc. [Enns, et al., 1997, as mentioned in GRN 000047].

Larex, Inc., used the USDA's CSFII (1996) to estimate mean intakes of foods within various food categories and subcategories (Table 2). Realistic usage levels of arabinogalactan were assigned based on industry experience, applications trials, and economic considerations. The contribution of each food category to total

estimated arabinogalactan intake was calculated by multiplying the average usage level of arabinogalactan for recognized food subcategories by the average consumption for men and women as reported by the CSFII, 1996. This value was considered to be the mean level of estimated intake.

While the percentage of use assigned to each food subcategory was considered realistic, the exposure estimate remained conservative because 100% market penetration was assumed, and arabinogalactan was assumed to be included in all products in a given subcategory. Additionally, the values presented below assume that 100% of consumers are "eaters" of arabinogalactan containing products.

In reality, arabinogalactan use is expected in only certain products within a given subcategory. Further, arabinogalactan use within a subcategory will be limited by economics and organoleptic considerations. Market development in the U.S. is likely to be directed towards the functional properties of arabinogalactan, and certain functional properties may not be appropriate for a given consumer product. An example of this use limitation is that arabinogalactan tends to reduce viscosity in batter or fluid food systems (American Institute of Baking, November 1997; GRN 000047, Vol. 6, Tab 5). Thus, for these reasons, the use of arabinogalactan will be self-limiting in many applications.

Additionally, Larex, Inc. arabinogalactan is a relatively expensive food ingredient, costing several dollars per pound. Therefore, those applications in which it will be cost effective will need to be carefully chosen, and will most likely dictate the use of arabinogalactan at the minimum level necessary to produce a given technical or nutritional effect.

Table 2 below shows the realistic percentages of use assigned to each of the forty-three food categories listed at 21 CFR §170.3(n). Provided as a cross-reference, the correlation between the major food categories derived from the USDA food survey and those listed at §170.3(n) is shown in Table 4.

The mean intake for arabinogalactan in selected food subcategories is shown in Table 3 below. The sum mean daily intake of arabinogalactan is 10.474 grams per day. The Agency's guidance document indicates that an estimate of the 90th percentile level of intake can be calculated by doubling the total mean intake; this daily intake estimate is 20.95 grams per day. Larex, Inc., is also aware that certain individuals may consume arabinogalactan as a dietary supplement. The recommended dose of arabinogalactan as a dietary supplement is 4.5 grams per day. This dosage has been recommended based on the results of clinical trials with human volunteers [Robinson et al., 1999, and Kim et al., 1999].

Interestingly, the CSFII, 1996 shows that the mean fiber intake averaged for men and women is 16.1 grams per day [Enns, et al., 1997]. This level is still well below the 25-30 grams per day recommended by many health agencies. The addition of arabinogalactan to the diet, therefore, is not expected to create excessive fiber intake.

Larex, Inc. submits, therefore, that exposure to arabinogalactan will not result in a harmful level of fiber intake, and that arabinogalactan is safe for its intended use as a food ingredient.

Table 2: Maximum Use of Larex Arabinogalactan In the Food Categories Listed by the US FDA At 21 CFR §170.3 (n)

	Food Categories	Realistic Percentage of Use in the US Market*
01	Baked Goods, Baking Mixes	1.72%
02	Beverages alcoholic	0
03	Beverages and beverage bases, non-alcoholic	2.0%
04	Breakfast Cereals	1.7%
05	Cheeses	1.0%
06	Chewing Gum	0.278%
07	Coffee and tea	2.0%
08	Condiments and relishes	5.65%
09	Confections and frostings	3.16%
10	Dairy product analogs	0%
11	Egg Products	0.5%
12	Fats and oils	1.0%
13	Fish Products	0
14	Fresh eggs	0
15	Fresh fish	0
16	Fresh fruits and fruit juices	4.0%
17	Fresh meats	0.2%
18	Fresh poultry	0.2%
19	Fresh vegetables	0
20	Frozen dairy desserts and mixes	0.313%
21	Fruit and water ices	2.0%
22	Gelatins, puddings and fillings	1.5%
23	Grain products and pastas	0.166%
24	Gravies and Sauces	0.25%
25	Hard candy	2.72%
26	Herbs, seeds, spices, seasonings, blends	20.0%
27	Jams and jellies, home prepared	0
28	Jams and jellies commercial	5.0%
29	Meat products	0.2%
30	Milk, whole and skim	0
31	Milk products	1.5%
32	Nuts and Nut products	0
33	Plant protein products	0
34	Poultry products	0.2%
35	Processed fruits and fruit juices	4.0%
36	Processed vegetables and vegetable juices	0.214%
37	Snack foods	0.5%
38	Soft Candy	2.72%
39	Soups, home prepared	0
40	Soups and soup mixes	1.16%
41	Sugar, white granulated	0
42	Sugar substitutes	16.0%
43	Sweet Sauces, toppings, and syrups	1.25%

Table 3
Estimated Intake of Arabinogalactan in Selected Food Categories

Major Food Codes	Food Category	Use level (%)	Food Intake (g)	AG Intake (g)	CSFII
	Baked goods, baking mixes				
51	Yeast Breads and Rolls	0.5	50	0.25	1996
52-55	Quick breads, pancakes, etc.	0.5	0.1	0.694	1996
53	*Cakes, cookies, pastries, pies	3.475	38	1.42	1996
58	Mixtures mainly grain	0.5	107	0.535	1996
	Breakfast cereals				
571-574,578	Ready-to-eat cereals	1.7	17	0.289	1996
	Grain products and pastas				
562	Rice	0	19	0	1996
561	Pasta	0.5	21	0.105	1996
54	Snack foods (crackers, chips)	0.25	12	0.3	1996
	Total Vegetables		132		
	Fresh vegetables	0	0	0	1996
	Processed vegetables, juices	0	0	0	1996
	Total Fruits		162		
612	Citrus juices	1	59	0.59	1996
621	Dried fruits	0	1	0	1996
641,642	Non-citrus juices and nectars	1	26	0.26	1996
	Fruits and mixtures	0	0	0	1996
	Milk Products				
114	Yogurt	4	8	0.32	1996
131-134	Milk desserts	0.725	24	0.174	1996
140-147	Cheese	1	16	0.16	1996
116,118	Milk-Based Beverages	4	34	1.36	1996
	Meat Products				
252	Sausages, processed meats	0.2	21	0.42	1996
271-274	Mixtures mainly meat	0.2	99	0.198	1996
	Eggs				
321-324,33,34	Egg Products	0.5	9**	0.045	1996
	Legumes				
	Legumes	0	0	0	1996
	Nuts and Nut Products				
	Nuts and Nut Products	0	0	0	1996
	Fats and Oils				
832	Salad dressings	0.25	8	0.02	1996
	Total sugars and sweets				
917,918	Candy (soft and hard)	2.72	7	0.19	1996
911-916	Other Sugar Products***	3.16	15	0.474	1996
	Beverages Non-Alcoholic				
	Coffee	0	254	0	1996
923	Teas	2	128	2.56	1996
925	Low Cal. Fruit drinks and ades	2	18	0.36	1996
92400100	Low Cal. Carbonated drinks	2	74	1.48	1996
	Total Intake (grams)			**10.474**	
	Dietary Supplement			**4.5**	
	*Includes dietetic bars				
	**Includes 50% of egg intake for processed eggs				
	***Includes, Jams, Jellies, etc.				

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Table 4
USDA Major Food Categories And Corresponding Categories From 21 CFR 170.3 (n)

USDA Food Category[1]	Categories Listed In 170.3 (n)[2]
Total Grain Products	01, 04, 23,37
Dietary Supplement; Source of Soluble Fiber	
Vegetables and Vegetable Dishes	36
Fruits and Fruit Preparations	16, 35
Milk Desserts/Cheese Products	5, 20, 22, 31, 10
Meat, Poultry and Fish	29, 34
Total Fats and Oils	12
Legumes and Legume Dishes	33, 36
Sugars and Sweets	06, 09, 25, 25, 38, 42, 43
Nuts and Seeds	32
Total Non-Alcoholic Beverages (excluding coffee and fruit juices)	16, 35, 03

1. Major food categories are those used in USDA's Continuing Survey of Food Intake by Individuals [Enns, *et al*, 1997].
2. Column 2 contains the number assigned to the various food categories as listed in 21 CFR § 170.3 (n), which are contained within those reported by Enns, et al., 1997 [Enns, et al., 19971.]

Specifications for Arabinogalactan

Larex, Inc., provides herein the specifications for its arabinogalactan products, and are the same as those provided to the Agency in GRN 000047. Carbohydrate levels, as well as levels for other constituents, in arabinogalactan derived from Eastern Larch are provided below. Carbohydrate and lead levels for Eastern and Western Larch arabinogalactan are shown in tables 5 and 6, respectively. Tables 7 and 8 shows the specifications for the powdered and liquid forms of food grade arabinogalactan, respectively.

Carbohydrate levels. Carbohydrate levels for arabinogalactan derived from either Eastern or Western Larch are quite similar in which their carbohydrate ranges overlap (Table 5). Thus, the use level of arabinogalactan as a food ingredient in formulating a food product will be the same whether derived from the Eastern or Western Larch tree.

Table 5

Larex Arabinogalactan Carbohydrate Levels for Various Arabinogalactan Products		
Product	**Lot Number**	**Carbohydrates (%) d.w.b.**
	Western Larch Products	
FiberAid	2C9-991-08	96.78
FiberAid	2F9-00251-01	96.33
FiberAid	2C9-0016-01	95.67
	Eastern Larch Products	
FiberAid	2M500116-01	94.62
FiberAid	2C9-00120-01	94.42
FiberAid	2-IE-00120-01	93.26

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Lead levels. Recent analyses for lead allow a specification of < 0.1 ppm for all food-grade arabinogalactan products. Tables 6 below demonstrates that lead levels are either below the level of detection or well below 0.1 ppm (mg/kg) from arabinogalactan extracts from Western larch (*Larix occidentalis*) and Eastern Larch (*Larix laricina*). A more detailed description of the chemistry, molecular weight profiles, and specifications for arabinogalactan from the Eastern Larch is found in Section I, Chemical Identity, of *Arabinogalactan from Eastern Larch.*

Table 6

Larex Arabinogalactan[a] Lead Levels for Various Products		
Product	**Lot Number**	**Average (ppm)**
	Western Larch Products	
Decolorized	99022	0.051
Decolorized	99284	0.023
CT AG 99*	00054	N.D.***
CT 99*	99108	N.D.***
CT 99*	99358	N.D.***
Grade 100-Dried**	00054	0.052
	Eastern Larch Products	
Grade 100	2-A9-00120-01	N.D.***
ClearTrac	2-C9-00120-01	N.D.***
ImmunEnhancer	2-I9-00120-01	N.D.***
Detection Limit (D.L.)		0.020

*CT has been identified in GRN 000047 as Fiberaid.
**Grade I 00 has been identified as LSC-1. However, this sample of Grade I 00 has been dried.
***Non-detectable
[a]These data are also found in *Arabinogalactan from Eastern Larch*, page 22.

Final Product Specifications for Larex Decolorized Arabinogalactan

Tables 7 and 8 shows the specifications for food grade arabinogalactan for the powdered and liquid forms, respectively.

Table 7

Food Grade (Arabinogalactan)	
Chemical Specifications	
Carbohydrate,as arabinogalactan	90% (d.w.b.) min.
Total Ash	10% max.
Bulk Density	0.75 g/ml max.
Chloride	1,500 ppm max.
Lead	0.1ppm max.
Insoluble Matter	0.1% max.
Molecular Weight	16,000-25,000
pH	4.0 – 8.0
Phenol	5.0ppm max.
UV 280 nm	0.2 – 2.0
Viscosity	5.0 cps max.
Microbiological Specifications	
Aerobic Plate Count	1,000 CFU max.
Salmonella sp.	Negative
Staphylococci sp. (coagulase positive)	10 CFU max.
Eschericia coli	10 CFU max.
Yeast	100 CFU max.
Mold	100 CFU max.

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Table 8

Food Grade (LSC-1 Arabinogalactan)	
Chemical Specifications	
Carbohydrate, as arabinogalactan	50-55%
Total Ash	5% max.
Bulk Density	1.2 g/ml max.
Chloride	150 ppm max.
Lead	0.1ppm max.
Insoluble Matter	0.1% max.
Molecular Weight	16,000-25,000
pH	3.0 – 6.0
Phenol	1.0 – 3.0 ppm
UV 280 nm	0.55 – 1.20
Viscosity	500 cps max.
Microbiological Specifications	
Aerobic Plate Count	1,000 CFU max.
Salmonella sp.	Negative
Staphylococci sp. (coagulase positive)	10 CFU max.
Eschericia coli	10 CFU max.
Yeast	100 CFU max.
Mold	100 CFU max.

Summary

Larex Inc. has provided scientific data demonstrating the chemical and functional equivalence of Eastern Larch arabinogalactan with Western Larch arabinogalactan. This notice also contains intake estimates, demonstrating that the potential consumption of arabinogalactan derived from Eastern Larch will not reach levels of toxicological concern and are consistent with those intake levels expected for arabinogalactan derived from Western Larch. Further, the specifications for arabinogalactan derived from Eastern Larch mimic those for arabinogalactan derived Western Larch.

Larex, Inc. therefore submits that its arabinogalactan products can be considered generally recognized as safe, whether derived from Eastern or Western Larch, when used in accordance with current Good Manufacturing Practices.

Tab A

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Arabinogalactan Manufactured Using the Larex Process in Accordance with Current Good Manufacturing Practice for Use in Food

Larex, Incorporated (Larex) convened a panel of scientific experts (Expert Panel), qualified by training and experience in the areas of toxicology, nutrition, carbohydrate chemistry, biochemistry, microbiology, hydrocolloid applications, and regulatory compliance to render opinions on the GRAS status of arabinogalactan derived from Eastern larch produced by Larex, and meeting the specifications described herein, for use in foods to achieve certain technical effects. A comprehensive search of the scientific literature was conducted by Lee Dexter and was made available to the Expert Panel in the form of a thirteen (13) volume report. The Expert Panel individually and collectively critically evaluated this material and other materials deemed appropriate.

The Expert Panel conferred by telephone, and met with representatives of Larex in Minneapolis, Minnesota on 12 February, 2001. Following presentation by these representatives, and extensive discussion, the Expert Panel went into Executive Session and prepared the following report.

Based on the data and information critically evaluated, the Expert Panel provides the following statements about arabinogalactan from Eastern larch and its proposed general use in food: 1. Arabinogalactan produced by Larex from Western larch (*Larix occidentalis)* and Eastern larch, also known as Tamarack (*Larix laricina)* are chemically equivalent. 2. The arabinogalactan derived from trees of the genus *Larix* (larch) is a unique hemicellulosic product, in that it is easily extractable by water in a pure form from non-delignified plant tissues. Other hemicellulosic products have required chemical additives and/or mechanical treatments to extract them from their plant sources, and the literature has indicated that these extractants alter the polysaccharides to a certain degree [Whistler, *et al.*, 1970, Stephen, 1983, and Wilkie 1986]. Therefore, arabinogalactan produced by Larex from Eastern larch and Western larch can be considered equivalent to naturally occurring arabinogalactan [Whistler, *et al.*, 1970].

Arabinogalactan is a common constituent of plant cell walls, and as such has had a long history of consumption by humans [Pennell, *et al.*, 1989]. Arabinogalactans are often complexed with protein to form a glycoprotein which has various functions in the plant cell wall [Stephen, 1983, and Schindler, *et al.*, 1995]. Arabinogalactans have been commonly consumed by humans in such foods as maple syrup, carrots, tomatoes, soy beans, radishes, and wheat flour [Neukom, *et al.*, 1975, Morita, *et al.*, 1965, and Whistler, *et al.* 1970]. Arabinogalactan has a number of valuable properties for use in foods including moisture retention, emulsification, thickening, bulking, and the

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nutritional benefits of its soluble fiber content. The addition of extracted arabinogalactan to various foods, including baked goods such as cakes, cookies and yeast raised breads, has demonstrated a self-limiting level, beyond which the quality of the product begins to decline [American Institute of Baking, 1997].

The arabinogalactans manufactured by Larex are produced in a food grade plant in Cohasset, Minnesota, which complies with all state and federal regulations regarding food production, and includes a HACCP Plan which meets FDA guidelines.

Following it's dependent and critical evaluation of available information and data, the Expert Panel concluded that arabinogalactan derived from Eastern larch and manufactured using the Larex process in accordance with cGMP, and meeting the specifications described herein, is GRAS by scientific procedures for use in foods in the minimum quantity required to produce it's intended effects, as a binder, bodying agent, dough strengthener, emulsifier, firming agent, flavor enhancer, formulation aid, humectant, lubricant and release agent, processing aid, stabilizer and thickener, and surface finishing agent.

Signatures:

Joseph F. Borzelleca, Ph.D. Date: 12 Feb 2001

Darrell G. Medcalf, Ph.D. Date: Feb 12, 2001

Ralph Moorhouse, Ph.D. Date: Feb 12, 2001

Cleve Denny, B.A. Date: Feb. 12, 2001

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Tab B

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INGMAN LABORATORIES, INC.

Established in 1928

Grain · Feed · Water · Food Nutrition · Chemical · Microbiological Analyses

2945 34th Ave. So. (34th & E. Lake St.)
Minneapolis, MN 55406
• MAILING ADDRESS: P.O. Box 15305, Commerce Station •
Minneapolis, MN 55415
(612) 724-0121

Date 05/07/99

LAREX, INC.
2852 PATTON ROAD

ST.PAUL, MN 55113

This is to certify that there was a sample tested under the supervision of Ingman Laboratories, Inc. Ingman Laboratories, Inc. is not liable for negligence with respect to the selection of the sample, the test results of which are herein certified.

99108-01 C MATERIAL	Moisture 6.60 % Protein(Kjeldahl) 0.35 % Fat (Acid Hydrolysis) 0.30 % Ash 2.57 % Total Dietary Fiber 89.04 % Sugar Profile No sugars Det.

GRAIN EXCHANGE ☐
SUBMITTED SAMPLE, ☐
STATE SAMPLE, ☐

5361
LARE

INGMAN LABORATORIES, INC.

By _____

Form 110-286

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MVTL LABORATORIES, Inc.
P.O. BOX 249, 1126 N. FRONT STREET
NEW ULM, MN 56073-0249
PHONE (507) 354-8517 WATS (800) 782-3557 FAX (507) 359-2890

MEMBER ACIL

Report Date: 6 Dec 2000

Lab Number: 00-M38001
Work Order #: 13747
Account #: 019213
Purchase Order Number: 5860

BRYAN RODRIGUEZ
LAREX INC
4815 WHITE BEAR PARKWAY
WHITE BEAR LAKE MN 55110

Date Received: 28 Nov 2000

Sample Description: FIBER AID F99 POWDER 2C90025101 (2F9-00251-01 ON BAG) DRUM 315

ANALYTE	AS RECEIVED BASIS
Moisture, Vacuum Oven	5.86 %
	100 deg/5 hrs vac
Ash	3.24 %
Fiber, Total Dietary	82.59 %
Fat, Acid Hydrolysis	0.19 %
Protein N x 6.25	0.24 %
Ash at 825 C for 2 Hours	1.59 %
	RUN AT 800 DEG C

Comments: TDF NEEDS DRY BASIS

facsimile

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MVTL guarantees the accuracy of the analysis done on the sample submitted for testing. It is not possible for MVTL to guarantee that a test result obtained on a particular sample will be the same on any other sample unless all conditions affecting the sample are the same, including sampling by MVTL. As a mutual protection to clients, the public and ourselves, all reports are submitted as the confidential property of clients, and authorization for publication of statements, conclusions or extracts from or regarding our reports is reserved pending our written approval

000025

MINNESOTA VALLEY TESTING LABORATORIES, INC.
1126 N. Front St. - New Ulm, MN 56073 - 800-782-3557 - Fax 507-359-2890
1411 S. 12th St. - Bismarck, ND 58502 - 800-279-6885 - Fax 701-258-9724
710 S. 14th St. - Grand Forks, ND 58201 - 800-272-7645 - Fax 701-772-0028
35 W. Lincoln Way - Nevada, IA 50201 - 800-362-0855 - Fax 515-382-3885

MEMBER ACII

Report Date: 26 May 2000

Work Order #: 5114
Account #: 019213
Purchase Order Number: 50740

Date Received: 8 May 2000

BRYAN RODRIGUEZ
LAREX INC
4815 WHITE BEAR PARKWAY
WHITE BEAR LAKE MN 55110

LAB NUMBER	SAMPLE DESCRIPTION	RESULTS - AS RECEIVED	
00-M13416	2-C9-0016-01 POWDER		
	Ash	4.10	%
	Fat, Acid Hydrolysis	0.23	%
	Moisture, Vacuum Oven	6.14	%
		100 deg/5 hrs vac	
	Fiber, Total Dietary	83.92	%
	Protein N x 6.25	< 0.01	%
	Ash at 825 C for 2 Hours	2.35	%
	Calcium	1.271	%
	Potassium	0.099	%
	Oxalate	< 0.2	%
	Formate	1.786	%

Approved by: _____
Michael K. Grob, Laboratory Manager,
New Ulm, MN

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1411 S. 12th St. - Bismarck, ND 58502 - 800-279-6885 - Fax 701-258-9724
710 S. 14th St. - Grand Forks, ND 58201 - 800-272-7645 - Fax 701-772-0028
35 W. Lincoln Way - Nevada, IA 50201 - 800-362-0855 - Fax 515-382-3885

MEMBER ACII

Lot No. 2M500116-01

Report Date: 26 May 2000

Work Order #: 5114
Account #: 019213
Purchase Order Number: 50740

Date Received: 8 May 2000

BRYAN RODRIGUEZ
LAREX INC
4815 WHITE BEAR PARKWAY
WHITE BEAR LAKE MN 55110

LAB NUMBER	SAMPLE DESCRIPTION	RESULTS - AS RECEIVED	
00-M13417	CT TAMARACK POWDER		
	Ash	5.19	%
	Fat, Acid Hydrolysis	0.19	%
	Moisture, Vacuum Oven	3.56	%
		100 deg/5 hrs vac	
	Fiber, Total Dietary	79.91	%
	Protein N x 6.25	< 0.01	%
	Ash at 825 C for 2 Hours	3.72	%
	Calcium	1.086	%
	Magnesium	< 0.01	%
	Potassium	0.309	%
	Oxalate	< 0.2	%
	Formate	1.828	%

Approved by: _____
Michael K. Grob, Laboratory Manager,
New Ulm, MN

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P.O. BOX 249, 1126 N. FRONT STREET
NEW ULM, MN 56073-0249
PHONE (507) 354-8517 WATS (800) 782-3557 FAX (507) 359-2890

MEMBER ACII

Report Date: 20 Dec 2000

Work Order #: 13747
Account #: 019213
Purchase Order Number: 5860

Date Received: 28 Nov 2000

BRYAN RODRIGUEZ
LAREX INC
4815 WHITE BEAR PARKWAY
WHITE BEAR LAKE MN 55110

LAB NUMBER	SAMPLE DESCRIPTION	RESULTS - AS RECEIVED		
00-M38002	TAM 2C90012001 POWDER			
	Ash	4.79	%	
	Fat, Acid Hydrolysis	0.50	%	
	Moisture, Vacuum Oven	5.65	%	
		100 deg/5 hrs vac		
	Fiber, Total Dietary	78.58	%	
	Carbohydrate	88.82	%	
	Protein N x 6.25	0.29	%	
	Ash at 825 C for 2 Hours	3.16	%	800deg/2hrs
00-M38003	2LA0025301 DRUM 189 POWDER			
	Ash	10.87	%	
	Fat, Acid Hydrolysis	0.59	%	
	Moisture, Vacuum Oven	5.37	%	
		100 deg/5 hrs vac		
	Fiber, Total Dietary	51.06	%	
	Carbohydrate	83.20	%	
	Protein N x 6.25	< 0.01	%	
	Ash at 825 C for 2 Hours	5.92	%	800deg/2hrs

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rtech laboratories

alytical Laboratory
P. O. Box 64101, St. Paul, MN 55164-0101
Phone: 1.800.328.9687 or 651.481.2207
Fax: 651.486.0837

DNV Certification, Inc. USA ANSI-RAB QMS ACCREDITED DNV ISO 9002 REGISTERED FIRM DNV MSC The Netherlands Accredited by the RvA

ACCREDITED ISO 17025
Certificate No. 1765.01 and 1765.02

Work Order Number: 2001-08-0517
Page 2 of 2
COA Date: 8/22/01

Sample: 01 2-IE-001201 Tree Extract

Test Description	Test Date	Result	Units
Fat, Base Hydrol			
Fat, Base Hydrolysis	8/22/01	0.10	%
Protein			
Protein	8/20/01	0.11	%
Ash 550C			
Ash/550C	8/20/01	6.73	%
Ash/550C	8/20/01	6.53	%
Moisture,VAC/100C/5H			
Moisture/VAC/100C/5h	8/21/01	5.83	%

****

Our Analytical Laboratory is committed to providing technically valid results, as demonstrated by our adherence to the ISO quality standard. All products received have been sampled as per the Analytical Laboratory Sampling Plan, unless otherwise noted. Official references or validated methods have been used for sample Analyses. References and uncertainty data are available upon request.

Results reported relate only to the samples analyzed and do not represent approval of a product. Report may not be reproduced, except in full, without written authorization by the Analytical Laboratory.

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Tab C

Pages 000031 - 000032 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Tab D

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SOUTHWEST COLLEGE OF NATUROPATHIC MEDICINE & HEALTH SCIENCES
RESEARCH DEPARTMENT

CLINICAL TRIAL STUDY DESIGN

February 2, 1999

Investigator/Institution

Robert Waters, PhD — Chair, Research Department, Southwest College of Naturopathic Medicine
Linda Kim, NMD — Staff Physician, Southwest College of Naturopathic Medicine and Southwest Naturopathic Medical Center

Title (Tentative)

Effects of Low Dose Arabinogalactan from *Larix occidentalis* on Interferon and Other Immune Modulators.

Purpose

Preliminary human clinical trial to study the effects of arabinogalactan (Larix) produced from western larch, *Larix occidentalis* on blood immune modulators. Specifically three different products of Larix at two doses of 1.5 gram and 4.5 gram will be used, and response to the two doses assessed in the study. Eighteen subjects assessed with reduced or compromised immune system will be treated for 3 weeks with Larix. Six treatment groups with three subjects in each group for each of the Larix products at the two doses, and three subjects for the control group will be designed. The following parameter tests will be assessed before and after treatment in all twenty-one subjects: RBC, WBC, Monocytes, Gamma-Interferon, Tumor Necrosis Factor, Interleukin-6 and Total Anaerobes (including Lactobacilli and Enterobacteraceae). The study will also report any changes in health using *Health Appraisal - Comprehensive* (referenced questionnaire collected from research by Lyra Heller and Michael Katke, 1993) before and after treatment. The Larix products used in the study will be provided by Larix, Inc.

Background

Larix is produced from the western larch tree, *Larix occidentalis* through a counter current extraction process. The resultant liquor is refined into a light cream-colored powder having an indefinite shelf life. The unique properties of Larix, as compared with other natural polysaccharides, are: ease of solution, complete solubility; good body properties without viscosity buildup; excellent dispersing and surfactant properties; and stability over a wide range of concentrations, pH and temperature. The major component of Larix, is the long, branched polysaccharide, arabinogalactan. This polymer is composed of galactose and arabinose units in a 6:1 ration, with a trace of uronic acid. The molecular weights of the major fractions of arabinogalactan in Larix are 16,000 and 100,000. Current studies demonstrate favorable effects from using Larix on many conditions such as recurrent otitis media, asthma, chronic candidiasis, allergy symptoms, upper respiratory infections and influenza; thus Larix appears to have an effect on the immune system. The potential effects of Larix on the immune system are linked by current studies showing that Larix may stimulate and increase interferon and cytokines. Many samples are being made readily available for continued investigative studies and developmental work. Other studies and results of studies forthcoming and referenced on final study design protocol.

Results of Previous Human Trial (Trial In Progress at U of Minnesota)

A. Investigators
B. Initial Trial Study Parameters
C. Therapeutic Agents
D. Preliminary Results
E. Conclusion of Study

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Safety

Larix is FDA approved for use in food applications as per 121.1174 and 121.1219 (Code of Federal Regulations, Title 21, 1974). Larix may be safely used as an emulsifier, stabilizer, binder, or bodying agents in essential oils, non-nutritive sweeteners, flavor bases, non-standard dressings, and pudding mixes. The use of Larix has also been formally approved by the Canadian Governor-in-Council. Preliminary acute toxicity tests performed on albino rats by a western U.S. university have indicated that Larix is significantly less toxic than methyl cellulose.

Treatment Protocol

All subjects to follow general diet during the 3 week treatment.
Products provided by Larix, Inc. Three Larix products at 1.5 gram and 4.5 gram will be used in this study.
Specific instructions on how and when to take the products detailed later.
Length of treatment: 3 weeks (tentative schedule)
Protocol finalized upon review of current literature studies on arabinogalactan.

Procedures

Subjects selected according to selection/exclusion criteria. Initial entrance work up (diet and health assessment). Selected subjects with parameter tests and *Health Assessment - Comprehensive* results recorded prior to treatment and repeated at end of treatment. Follow-up at 14 days and after treatment. Treatment protocol implemented for 3 weeks. Parameter tests provided by Sonora Quest Lab (RBC, WBC, Monocytes, Tumor Necrosis Factor, Interleukin-6, Total Anaerobes) and Specialty Lab (Gamma-Interferon).
Procedures finalized upon review of current literature studies on arabinogalactan.

Nature of Study

Double Blind

Subject Population

Total Number: 21 Study: 18 Control: 3
Age Range: greater than 18 years, no upper limit
Sex: either male or female
Type of immune compromised: not restricted
Source: Patients at Southwest Naturopathic Medical Center
Naturopathic medical students
Patients at AIDS Project of Arizona (tentative)

Criteria for Selection

Clinical symptoms and/or signs of compromised immune system based on history and/or *Health Assessment - Comprehensive*.

Criteria for Exclusion

Use of drugs, medication, alcohol, including antibiotics and steroids during treatment.
Use of any other supplements during treatment.

Risks and Adverse Effects

None expected.

Clinical Trial Study Report

To include following: title, abstract, introduction/background, methods/procedures, data, discussion, conclusion, references.

LEE B. DEXTER & ASSOC.
TECHNOLOGY CONSULTANTS

15704 WEBBERVILLE ROAD
AUSTIN, TEXAS 78724 USA

TELEPHONE (512) 276-7408
FAX (512) 276-7489

CODE AND NUMBER OF BOTTLES NEEDED FOR SOUTHWEST COLLEGE OF NATUROPATHIC MEDICINE TRIAL

Number of Subjects	Treatment Type/ Daily Dose	Code	Total Capsules Needed*	Number of Bottles Needed**
3	Placebo 1.5g	A	252	3 with 84 capsules each[1]
3	Grade 100 1.5g	B	252	3 with 84 capsules each[1]
3	Grade 100 4.5g	C	756	9 with 84 capsules each[1]
3	Immun-Enhancer 1.5g	D	252	3 with 84 capsules each[1]
3	Immun-Enhancer 4.5g	E	756	9 with 84 capsules each[1]
3	ClearTrac 1.5g	F	252	3 with 84 capsules each[1]
3	ClearTrac 4.5g	G	756	9 with 84 capsules each[1]
3	AIDS Patients 15g (1E)	H	2520	30 with 84 capsules each[2]

*Assumes capsules are 500mg
** Assumes trial runs 28 days
1. Must be plain bottles
2. The number of capsules for each AIDS patient has not been finalized.

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Southwest College of Naturopathic Medicine & Health Sciences
Southwest College Research Institute
Southwest Naturopathic Medical Center

Fax
Cover Page

Date: 6/24/99

Number of Pages Including Cover: 3

To: Lee Dexter

From: Dr. Kim
602-549-0693
602-860-8210 fax
Southwest College Research Institute

RE: Arabinogalactan Study - Updated Test Results

Memo:
Lee
Attached please find the Total Anaerobes reported in colony counts. My apologies for not including them in previous memos. I am sending the test results of the Total Anaerobes, *Enterobacteraceae* and *Lactobacilli* in two report forms: colony counts and quantitative report based on a scale.

Total Anaerobes

Quantiative Report - Scale

Scale	Number of Colonies	Before		After
No	0			
Rare	1-9	II	2	(0)
Few	10-199	II	2	III (3)
Moderate	200-500	IIII	(4)	(0)
Many	>500	IIIIIIIIII	~~10~~ (9)	IIIIIIIIIIIIII (14)

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Southwest College of Naturopathic Medicine & Health Sciences
Southwest College Research Institute

Arabinogalactan Study

			Pre-Treatment								Post-Treatment							
Product	Dose	TAN	WBC K/MM3	Mono %	IL-6 pg/ml	G Inter pg/ml	TNF-a pg/ml	Total Anaerobes	Entero	Lacto	WBC K/MM3	Mono %	IL-6 pg/ml	G Inter pg/ml	TNF-a pg/ml	Total Anaerobes	Entero	Lacto
			normal 4.0-11.0	normal 3.0-15.0	normal <6.0	normal <10.0	normal <20.0	colony counts	colony counts	colony counts	normal 4.0-11.0	normal 3.0-15.0	normal <6.0	normal <10.0	normal <20.0	colony counts	colony counts	colony counts
Placebo	1.5 g	A-1	4	5	<6.0	0	12	†	†	†	4.2	5	<6.0	3	11	>500	>500	0
	1.5 g	A-2	6.8	5	18.1	0	13	10-199	10-199	10-199	5.3	8	15.1	0	13	>500	>500	0
	1.5 g	A-3	6	7	<6.0	0	12	>500	>500	200-500	6.1	6	<6.0	0	14	>500	>500	10-199
Grade 100	1.5 g	B-1	5.8	8	<6.0	0	8	200-500	200-500	0	5.2	9	<6.0	0	10	>500	>500	10-199
	1.5 g	B-2	5.7	8	26.8	0	11	>500	>500	10-199	4.4	10	19.6	0	9	>500	>500	0
	1.5 g	B-3	5.3	5	<6.0	0	17	1-9	1-9	0	4.8	5	<6.0	0	14	>500	>500	200-500
	4.5 g	C-1	4.8	7	<6.0	0	5	200-500	200-500	0	4.9	7	<6.0	0	5	>500	>500	0
	4.5 g	C-2	5.7	6	<6.0	0	12	>500	>500	0	5.6	5	<6.0	0	9	>500	>500	10-199
	4.5 g	C-3	4.4	8	<6.0	0	9	>500	>500	0	4.7	7	16.4	3	11	10-199	10-199	0
Grade 1000	1.5 g	D-1	6.3	6	<6.0	0	18	200-500	200-500	0	6.5	7	<6.0	0	20	>500	>500	0
	1.5 g	D-2	7.6	9	<6.0	0	10	10-199	10-199	10-199	6.5	3	<6.0	0	19	10-199	10-199	10-199
	1.5 g	D-3	6.8	7	<6.0	0	8	>500	>500	10-199	8.3	6	<6.0	0	8	>500	>500	200-500
	4.5 g	E-1	6	6	<6.0	0	40	1-9	1-9	0	5.8	6	<6.0	0	106	>500	>500	0
	4.5 g	E-2	3.1	7	<6.0	0	21	10-199	10-199	0	3	7	†	†	†	>500	>500	10-199
	4.5 g	E-3	7.3	6	<6.0	0	133	>500	>500	0	7.7	8	<6.0	0	9	†	†	†
Grade 3000	1.5 g	F-1	6.2	6	<6.0	0	10	>500	>500	0	5.4	6	<6.0	0	12	>500	200-500	>500
	1.5 g	F-2	6.1	7	<6.0	0	6	>500	>500	0	6.5	8	<6.0	0	8	>500	>500	200-500
	1.5 g	F-3	5.6	7	<6.0	0	8	200-500	1-9	200-500	6.3	7	<6.0	4	9	10-199	10-199	10-199
	4.5 g	G-1	4.2	6	<6.0	0	14	>500	>500	200-500	4.7	6	<6.0	0	10	>500	>500	>500
	4.5 g	G-2	5.5	8	25	0	11	>500	>500	200-500	7.5	8	17.7	0	8	>500	>500	0
	4.5 g	G-3	3.8	6	<6.0	0	6	>500	>500	0	4.5	6	<6.0	0	7	>500	>500	0
Grade 1000	15 g	H-1	5.3	6	<6.0	0	20	10-199	10-199	10-199	4.7	5	<6.0	0	15	10-199	10-199	0
	15 g	H-2	3.5	9	<6.0	0	28	10-199	10-199	10-199	3.3	9	<6.0	0	19	200-500	200-500	0
	15 g	H-3	6.7	8	<6.0	0	19	>500	>500	1-9	9.4	6	<6.0	2	16	>500	>500	0

† Waiting for results

Tab E

000039

Oral Administration of Arabinogalactan Affects Immune Status and Fecal Microbial Populations in the Dog

C. M. Grieshop, E. A . Flickinger, and G. C. Fahey, Jr.

Department of Animal Sciences, University of Illinois, Urbana, IL 61801

Abstract

Seven ileally cannulated dogs were randomly assigned to either a control or one of three arabinogalactan (AG) dietary treatments in a 7 x 7 Latin square design to evaluate the effects of oral administration of AG on immune status and fecal microbial populations Arabinogalactan treatments included a high (1 65 g/d) and low (0 55 g/d) dose of Grade 100, Grade 1000, or Grade 3000 AG provided via gelatin capsules Arabinogalactan grades differed in purity and synthesis procedures Each treatment period consisted of a 6-d adaptation followed by a 4-d collection period Blood and fresh fecal samples were collected on d 10 of each period All dogs were fed 500 g/d (as-fed basis) of a diet containing 33% protein and 22% fat. Total fecal anaerobic bacterial concentrations were not affected (P>0 05) by AG treatments, but dogs supplemented with the high dose of grade 1000 tended (P=0 13) to have a higher concentration of total aerobic fecal bacteria than control dogs Dogs supplemented with the low dose of grade 1000 and the high dose of grade 3000 tended to have a greater concentrations of fecal bifidobacteria (P=0 16) and lactobacilli (P<0 10) compared to control dogs In addition, dogs fed the low dose of grade 3000 had a lower concentration of *Clostridium perfringens* (P=0 06) than control dogs The effects of AG treatments on immune status were assessed by measurement of serum immunoglobulin concentrations, white blood cell distribution, and serum cytokine concentration Arabinogalactan treatments did not affect (P>0 05) serum immunoglobulin G, M, or A concentrations The effects of AG treatments on white blood cell concentration and distribution, and on cytokine concentrations, were dependent on both grade and level provided Specific grades of AG affected both fecal microbial populations and immune status of the dog.

Introduction

Arabinogalactans (AG) are a class of long, densely branched, polysaccharides with molecular weights ranging from 10,000 to 120,000 Many plants are rich sources of AG, including carrots, radishes, pears, and leek seeds The major commercial sources of AG are the Western Larch (*Larix occidentalis*) and Mongolian Larch (*Larix dahurica*) trees Larch AG is composed of galactose and arabinose units in a 6 2 ratio, with a trace of uronic acid. Unique properties of larch AG are its complete solubility and stability over a wide range of concentration, pHs and temperatures Larch AG has a stimulatory effect on the immune system of both humans and animals Cultures of human peripheral blood mononuclear cells and pre-separated peripheral non-adherent cells and monocytes showed enhancement of natural killer cytotoxicity against K562 tumor cells when pre-treated with AG from *Larix occidentalis* for 48-72 h (Hauer and Anderer, 1993). In addition to affecting the immune system, it was hypothesized that AG could also affect the digestive physiology of the animal

Objective

To determine the effects of AG on digestive events and immune status of healthy, adult dogs.

Materials and Methods

Animals and diets:

- 7 purpose bred adult female dogs with hound bloodlines (avg wt. 24 3 kg)
- Dogs were surgically fitted with a permanent "T" type cannula proximate to the ileocecal junction
- Dogs were housed in individual, solid floor pens in an environmentally controlled room.
- Water was offered ad libitum.
- Dogs were fed 250 g (as-fed basis) of a common diet (33% protein, 22% fat) at 0800 and 2000 h (total 500 g/d)
  - Protein source – chicken protein
  - Lipid source – mixture of poultry fat, corn oil, flax oil, tallow, and lard

Dietary treatments:

- Three grades of AG (AG100, AG1000, AG3000) were evaluated at two levels (0 55 and 1 65 g/d).
  - AG100 - hemicellulosic product extracted by water from non-delignified Larch plant tissues
  - AG1000 and AG3000 - hemicellulosic products that have undergone additional decoloration and spray agglomeration processes
- Daily allotment of AG provided in two equal doses via gelatin capsules

Sample collection and analyses:

- Each treatment period consisted of a 6-d adaptation phase followed by a 4-d sample collection phase.
- Blood samples and fresh fecal samples collected on d 10 of each treatment period
  - Fecal bacterial concentrations determined via serial dilution and inoculation onto petri dishes containing selective sterile agar
  - Serum immunoglobulins were determined by radial immunodiffusion
  - Blood lymphoid cell subset markers were determined by flow cytometry

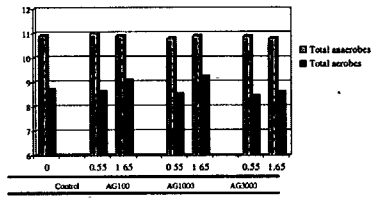
Statistical analysis:

- Data analyzed as 7 x 7 Latin square design using the GLM procedures of SAS (1996)
  - Sources of error in the model were treatment, period, and dog effects
  - Individual treatment means were compared to control means using non-orthogonal contrasts

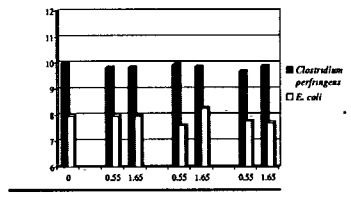
Results

Effect of AG supplementation on fecal microbial populations

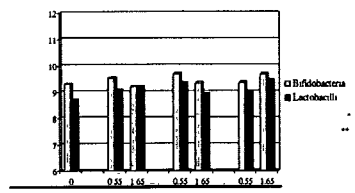
(log_{10} colony-forming units/g dry feces)



*Control vs AG1000 (1.65 g/d) P=0.13



*Control versus AG3000 (0.55 g/d) P=0.06.



*Control versus AG1000 (0.55 g/d) and AG3000 (1.65 g/d) P=0.16.
→ Control versus AG100 (1.65 g/d) P=0.17
→ Control versus AG1000 (0.55 g/d) and AG3000 (1.65 g/d) P< 0.10.

Effect of AG supplementation on serum immunological parameters

Item	Arabinogalactan Treatment							
Grade	Control	AG100		AG1000		AG3000		
Dose, g/d	0	0.55	1.65	0.55	1.65	0.55	1.65	SEM
Serum immunoglobulins, mg/dl								
IgA	13.1	13.4	12.3	12.3	12.3	13.1	12.9	0.9
IgM	95.0	95.1	98.4	99.6	99.1	97.6	106.1	5.8
IgG	2030.0	2050.1	2084.1	1976.7	1894.1	2149.4	2057.3	113.0
White blood cells[1]	9.35	9.71	9.85	10.74*	10.53	10.35**	10.64*	0.38
Neutrophils	6.00	6.26	6.21	6.82*	6.81*	6.63	7.05*	0.33
Lymphocytes	1.83	1.71	2.06	2.11	2.04	2.05	1.93	0.15
Monocytes	1.13	1.25	1.13	1.24	1.17	1.14	1.25	0.09
Eosinophils	0.38	0.47	0.42	0.50*	0.50*	0.50	0.41	0.04
Lymphoid cell subset markers, %								
CD3	74.29	68.00	72.43	70.14	71.00	65.00	72.00	2.71
CD4	10.43	8.43	9.71	9.14	10.86	9.29	10.71	1.25
CD8	26.71	21.57	27.00	28.00	28.14	24.86	26.71	1.57
CD19	15.43	16.29	14.71	14.71	17.43	13.86	13.71	1.90
CD56	2.57	3.00	3.43	4.14*	2.57	3.43	3.57	0.59

[1]Means are expressed as cells x 10^3/ul.
*Contrast of control versus specific form and dose P<0.05.
*Contrast of control versus specific form and dose P<0.10.

Summary:

Effects of AG supplementation on fecal bacteria concentrations:

- Total fecal anaerobic bacterial concentration was not affected by AG treatments,
  but dogs supplemented with the high dose of AG1000 tended to have a higher
  concentration of total aerobic fecal bacteria than control dogs
- Dogs supplemented with the low dose of AG1000 and the high dose of AG3000
  tended to have a greater concentrations of fecal bifidobacteria and lactobacilli than
  control dogs
- Dogs supplemented with the low dose of AG3000 had a lower fecal concentration
  of *Clostridium perfringens* than control dog

Effects of AG supplementation on immune status:

- Supplementation with either dose of AG1000 or the high dose of AG3000
  increased the number of circulating white blood cells and neutrophils compared to
  control dogs
- Supplementation with either dose of AG1000 or the low dose of AG3000
  increased circulating eosinophil number compared to control dogs

Conclusions

- Oral AG supplementation effected the fecal microbial populations and immune status, as determined by white blood cell and lymphocyte distributions, of healthy adult dogs
- Alteration in fecal microbial concentrations and immune status were dependent on specific dose and form of AG
- Further research in necessary to elaborate the specific mechanisms of actions of each of these compounds

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GRAS Report

Larex, Inc.
Arabinogalactan
From Eastern Larch

Manufactured by:

Larex, Inc.
4815 White Bear Lake Parkway
White Bear Lake, Minnesota
USA, 55110

Ph: 1-800-386-5300
Fax: 651-636-1583

Prepared by:

LEE B. DEXTER & ASSOC.
15704 Webberville Rd.
Austin, Texas 78724
Ph: 512 - 276-7408
Fax: 512 - 276-7489

February 12, 2001

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Executive Summary

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Executive Summary

Abbreviated Executive Summary

A. Introduction

Larex, Inc. has prepared a GRAS Report in support of the safety of arabinogalactan products derived from Eastern larch (*Larix laricina*). This report incorporates by reference, data and information contained in an earlier GRAS Report (December 27, 1997) which supported the safety of arabinogalactan from Western larch (*Larix. occidentalis*). The company has compiled data and information, which indicates that arabinogalactan from Eastern larch is chemically and functionally equivalent to that from Western larch. Clinical studies in humans and animals have further demonstrated that raw material sources are interchangeable, and that arabinogalactan derived from either Eastern or Western larch functions as a prebiotic soluble fiber.

B. Section I: Chemical Identity

Arabinogalactan occurs naturally in the wood of species of the genus *Larix*. Both *Larix occidentalis* and *Larix laricina* contain arabinogalactan in significant quantities (5-25%) on a wood weight basis. Distribution of arabinogalactan within the tree does differ by specie. In the Western larch arabinogalactan reaches its highest concentration in the lower portion of the trunk, whereas the greatest amount of arabinogalactan in Eastern larch is found in the lower parts of the branches [Cote`, *et al.*, 1967, Vol. 3 Tab 9].

Larex, Inc. has provided data, which demonstrates that the molecular weight of its arabinogalactan products from Eastern larch is virtually identical to that from Western larch (17,000 to 20,000 Daltons). Product derived from either source of arabinogalactan is capable of meeting the same set of physical and microbiological specifications.

C. Section II: Use and Functionality

Larex, Inc. has provided information on the nutritional components of its Eastern larch arabinogalactan products. The Western and Eastern arabinogalactan products were found to have a nutritional profile that was virtually identical. Of interest is the fact that arabinogalactan from Eastern larch contains an even lower amount of fatty acids than does the equivalent product from Western larch, although both products are very low in fat content.

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Executive Summary

A model system in which both Eastern and Western larch arabinogalactans were added to industrial inks, demonstrated that both products are capable of effecting the viscosity and dispersity of complex systems. These characteristics would also apply to food matrixes.

D. Section III: Analytical Methods

Larex, Inc. has provided a set of analytical methods for testing its products against published specifications. These methods are incorporated by reference from the Western larch GRAS Report of December 27, 1997.

E. Section IV: Safety

Results from a long-term (6 months) human feeding study showed that 8.4 grams per day of arabinogalactan from either Western or Eastern larch produced the same physiological effects in the test subjects. The subjects' response to a Health Related Quality of Life Assessment (HRQoIA) indicated that both products were well tolerated, and performed equally well as sources of fiber [Marett, *et al.*, 2000]. → see pp. 37-38, and Raw Data Section, pp. 50-51

An acute study in rats showed that arabinogalactan from both fiber sources had an LD_{50} of more than 5 g/kg bw. There were no mortalities or clinically significant signs observed.

F. Section V: Environmental Assessment

The Larex, Inc. processing plant in Cohasset Minnesota continues to operate under cGMP, and all discharges from the production of arabinogalactan are according to state and federal regulations.

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Section I
Chemical Identity

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Section I: Chemical Identity of Arabinogalactan

A. Common or Usual Name and Identity

Arabinogalactan is a polysaccharide composed of galactose and arabinose units with a highly substituted back bone of β 1-3 linked galactopyranose units and side chains of galactose and arabinose [Whistler, *et al.*, 1991, Vol. 5 Tab 9].

1. Arabinogalactan
2. Galactoarabinan
3. Arabogalactan
4. Larch Gum

B. Formal Names (IUPAC or Chemical Abstracts Names

1. Galactorabinan
2. L-arabino-D-galactan

C. Synonyms, Other Common Names, Tradenames

1. Stractan 2
2. Stractan 10
3. Stractan 15
4. LSC-1
5. L'Extra F500
6. L'Extra F100
7. FiberAid AG
8. L'Extra F2000
9. ClearTrac

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10. ImmunEnhancer AG

11. Laraceutical AG

12. Grade 100

13. AC-9

14. LaraCare

D. Chemical Formulae, Structures and Molecular Weights

1. The chemical structure of arabinogalactan can be described as $[(C_5H_8O_4)(C_6H_{10}O_5)_6]_x$.

2. Molecular Weight: 16,000 - 25,000

E. Chemical Abstracts Service Registry Number (CAS Registry No.)

1. CAS number 9036-66-2

F. Specifications for Food Grade Materials

Larex has developed a set of food grade specifications for the raw materials used in its manufacturing process, including larch logs, food grade hydrogen peroxide, and potassium hydroxide [Vol. 2 Tab 2]. It has also developed a set of final product specifications for its arabinogalactan products, which can be utilized to describe the products [Tables 1, and 2, below]. These specifications include a determination of the arabinogalactan percent by refractive index, specific gravity, color, phenol content, turbidity, chloride content, viscosity, molecular weight, ash content, lead, and insoluble matter [Vol. 1 Tab 3]. The products are of two basic types, wherein the first is primarily a liquid product, derived from the original pressate from the larch wood. The second type of product has been further refined and purified, and is described as "decolorized". This latter product occurs in various powdered forms.

Table 1
Final Product Specifications for Larex LSC-1 Arabinogalactan From Eastern Larch

Food Grade		
Chemical Specifications		
Arabinogalactan	50-55%	
Total Ash	5%	max.
Bulk Density	0.75 g/ml	max.
Chloride	150 ppm	max.
Lead	0.1 ppm	max.
Insoluble Matter	0.1%	max.
Molecular Weight	16,000 – 25,000	
pH	3.0 – 6.0	
Phenol	20.0 ppm	max.
UV 280 nm	0.2 – 1.20	
Viscosity	500 cps	max.
Microbiological Specifications		
Aerobic Plate Count	1,000 CFU	max.
Salmonella sp.	Negative	
Staphylococci sp. (coagulase positive)	10 CFU	max.
Eschericia coli	10 CFU	max.
Yeast	100 CFU	max.
Mold	100 CFU	max.

Table 2
Final Product Specifications for Larex Decolorized Arabinogalactan From Eastern Larch

Food Grade		
Chemical Specifications		
Arabinogalactan	90% (d.w.b.)	min.
Total Ash	10%	max.
Bulk Density	0.75 g/ml	max.
Chloride	1,500 ppm	max.
Lead	0.1 ppm	max.
Insoluble Matter	0.1%	max.
Molecular Weight	16,000 – 25,000	
pH	4.0 – 8.0	
Phenol	10.0 ppm	max.
UV 280 nm	0.2 – 2.0	
Viscosity	5.0 cps	max.
Microbiological Specifications		
Aerobic Plate Count	1,000 CFU	max.
Salmonella sp.	Negative	
Staphylococci sp. (coagulase positive)	10 CFU	max.
Eschericia coli	10 CFU	max.
Yeast	100 CFU	max.
Mold	100 CFU	max.

G. Quantitative Composition of Arabinogalactan from *Larix sp.*

Arabinogalactans from larch wood have been studied since the beginning of the century, and their chemistry has been the subject of many reviews [Whistler, *et al.*, 1991, Vol. 5 Tab 9, Wilkie, 1986, Vol. 5 Tab 12, and Timell, 1965, Vol. 4 Tab 11]. The larch wood arabinogalactans are a Type II arabinogalactan, the arabino-3, 6-galactans type which is the most prevalent class of arabinogalactan, and which is found in the exudate gums of gymnosperms such as trees of the genus *Larix, and* angiosperms such as the *Acacia* tree. The other types of arabinogalactans are Type I, the arabino-4-galactans, and the Type III, which are polysaccharides containing arabinogalactan side chains [Stephen, 1983, Vol. 4 Tab 8].

Distribution of Eastern Larch Arabinogalactan

Arabinogalactan concentrations in the Western larch, *Larix occidentalis* are highest at the lower end of the tree in the butt wood where concentrations have been reported to reach from 5-25% [CRC, 1972, Vol. 10 Tab 7, and BeMiller, 1989, Vol. 3 Tab 5]. High concentrations of arabinogalactan can also be extracted from the Tamarack or Eastern larch, (*Larix laricina)*, however, distribution of the arabinogalactan is significantly different in that species, reaching highest concentrations distally from the center of the tree [Cote`, *et al.*, 1967, Vol. 3 Tab 9].

Cote' , *et al.*, 1967, studied the distribution of arabinogalactan in tamarack (*Larix laricina*), and concluded that the polysaccharide was unevenly distributed in this species, with the content decreasing with distance from the pith, and from ground level, and in branches with distance from the stem. The author concluded that it was clear that Tamarack is a potential source of arabinogalactan, and could be utilized for industrial production. Microscopic examinations showed that the arabinogalactan in both Western larch and in Tamarack, was deposited in the lumen of the tracheids and ray cells [Cote', *et al.*, 1967, Vol. 3 Tab 9]. Table 3, below depicts the concentrations of arabinogalactan in various portions of the Eastern larch.

Table 3
Concentrations of Arabinogalactan in
Various Parts of Eastern Larch

Location and part of tree	Arabinogalactan (%)
Ground level	
Heartwood	5.00
Sapwood	0.27
Inner bark	3.70
Outer bark	0.39
36 Feet Above the Ground	
Sound Heartwood	16.8
Injured Heartwood	7.28
Injured Sapwood	0.44
Middle Portion	
Lower part of branches	20.8
Three Different Trees, Ground Level, Diameter	
3 inches	16.4
6 inches	12.9
12 inches	5.0

Composition of Eastern Larch Arabinogalactan

Arabinogalactans from Larch species have a backbone of (1-3)-linked β-D-galactopyranosyl units, each of which bears a substituent at the C-6 position. Most of these side chains are galactobiosyl units containing a (1-6)-β-D-linkage. Another common side chain consists of single L-arabinofuranosyl or of 3-O(β-L-arabinopyranosyl)-α-L-arabinofuranosyl units. Small amounts of D-glucuronopyranosyl units are present in arabinogalactans from European Larch and in Tamarack. In most species of arabinogalactan the ratio of L-arabinosyl units to D-galactosyl units is 1:6 [Whistler, *et al.*, 1970, Vol. 5 Tab 10 and Whistler *et al.*, 1991, Vol. 5 Tab 9].

Table 4, below lists the various extractives, and sugar residues from both the heartwood and the sapwood of Eastern larch [Cote', *et al.*, 1967, Vol. 3

Tab 9]. In this analysis arabinogalactan occurred primarily in the heartwood (10.5%), as opposed to the sapwood (0.59%).

Table 4
Composition and Extractives and Arabinogalactan Content of Various Areas of Eastern Larch

Component	Normal Core (%)	
	Sapwood	Heartwood
Summative Data		
Lignin	26.0	28.6
Ash	0.3	0.5
Acetyl	1.7	1.4
Uronic anhydride	5.0	4.9
Residues of:		
Galactose	1.7	10.9
Glucose	47.0	35.0
Mannose	11.6	9.0
Arabinose	1.3	3.7
Xylose	5.4	6.0
Ethanol-Benzene Extractives	1.66	1.4
Arabinogalactan	0.59	10.5

Molecular Weight Comparison of Eastern and Western Larch

As early as 1942, the molecular weight of arabinogalactan from larch wood was determined, and two fractions were identified: a high-molecular weight fraction of 100,000 Da, and a lower-molecular weight fraction of 16,000 Da [Borgin, 1949, Vol. 3 Tab 26]. Bouveng, and Lindberg, 1958, described studies on the fractionation of the arabinogalactan from *Larix occidentalis* [Bouveng, *et al.*, 1958, Vol. 3 Tab 7]. The homogeneity of the arabinogalactans from the *Larix* genus had long been under discussion according to the authors. In earlier ultracentrifuge studies, of a larch wood arabinogalactan (species not stated) Mosimann and Svedberg (1942) had found the two components with approximate molecular weights of 16,000 and 100,000. The authors reported that in the arabinogalactan from Western larch, Lystad-Borgin found only one component in the heartwood but two in the sapwood. The molecular weight of these components appeared to be of the same magnitude as those calculated earlier by

Mosimann and Svedberg, with the heartwood components having the higher molecular weight [Bouveng, *et al.*, 1958, Vol. 3 Tab 7]. In a different publication, Aspinall had concluded that the arabinogalactan from European larch was homogenous since no proof of heterogeneity could be found. According to Borgin, the variability of arabinogalactan content in different parts of the tree, or the effects of seasonal variations, as well as other factors, made the significance of this molecular weight disparity unclear.

Fractional precipitation of the polysaccharide either directly or after methylation or acetylation yielded results in a study by Bouveng, *et al.*, 1958, which were opposite to those reported by contemporary investigators. Therefore, the heterogeneity of the arabinogalactan from Western larch heartwood was re-investigated by Bouveng, *et al.*, 1958. Electrophoresis of the polysaccharide on glass fiber sheets using borate buffer showed the presence of two components, A and B which produced two distinct spots when the electrophoretograms were developed. When the authors utilized cetyl-trimethylammonium bromide, and similar quaternary ammonium salts for the fractionation of larch arabinogalactan, only one part of component A, the more mobile on electrophoresis, could be precipitated. The proportion of A to B was about 2:1, A, $[\alpha]_D^{20}$+7° (in water) contained 19% arabinose on a molar basis, and B, $[\alpha]_D^{20}$+10°, 21% arabinose. The authors concluded on the basis of these studies, and on the basis of sedimentation analysis, that the molecular weights of fractions A and B were of the same magnitude, 100,000 and 16,000 as those calculated for the two components by Mosimann and Svedberg. [Bouveng, *et al.*, 1958, Vol. 3 Tab 7].

Ettling *et al.*, 1968, studied the arabinogalactan from Western larch by gel filtration. A sample of arabinogalactan, which had been heated with magnesium oxide to remove phenolic impurities was passed through a Sephadex G-75 column. With the crude arabinogalactan, the dark-colored phenolic polymeric material eluted first, and accounted for 4% of the total. The main peak of arabinogalactan eluted second and accounted for 66% of the total. A shoulder from a low molecular weight arabinogalactan, which accounted for 21% of the material, followed the main peak, and the monomers, which accounted for 9% of the sample eluted last. The results showed that the colored phenolic impurities in crude arabinogalactan are a separate fraction from the predominant arabinogalactan. The arabinogalactans in this study consisted of two fractions which the author stated probably corresponded to the A and B fractions described by Bouveng, *et al.*, 1958 [Ettling, *et al.*, 1968, Vol. 3 Tab 11 and Bouveng, *et al.*, 1958, Vol. 3 Tab 7].

Section I, Chemical Identity

A study by Swenson, *et al.*, 1969, utilized gel permeation chromatography to separate arabinogalactan into fractions of the two polymers A , and B with molecular weights of 37,000, and 7,500 respectively. The fractions were characterized by viscometry, osmometry, and sedimentation equilibrium analyses. The analyses indicated that the B polymer of arabinogalactan has fewer or shorter branches than does the polymer A. The study also demonstrated the superiority of gel permeation chromatography over earlier fractionation methods, because of the monomolecularity of the fractions and by the ability of the method to separate cleanly the component polymers A and B. The author concluded also based on his studies that B polymer does not appear to be a breakdown product of polymer A. This was postulated because of the pronounced difference in configuration reflected by the viscosity studies [Swenson, *et al.*, 1969, Vol. 4 Tab 9].

Churms, *et al.*, 1978, studied the possible relationship between the arabinogalactan structure of *Larix occidentalis* and that of the branched framework of the plant gum exudate from *Acacia* species. The authors indicated that after a single Smith-degradation, the gums from the *Acacia* tree yielded a monodisperse polymer of low molecular weight. This polymer was essentially a galactan with a certain number of pendant arabinosyl units. Additionally, a mixture of polymers was obtained, but when this occurred, the molecular weights of those polymers were simply related to each other [Churms, *et al.*, 1978, Vol. 3 Tab 8].

The authors were interested to determine, if arabinogalactan might occur as a mixture of components of widely disparate molecular weights as did the acacia gum. Therefore, an arabinogalactan was extracted from the wood with warm water over a 48-hour period. The extract was centrifuged, and the clear solution was concentrated. The addition of ethanol and acetone precipitated the arabinogalactan. The colorless amorphous product had an optical rotation of $[\alpha]_D$+8°, was nitrogen free, and was shown by gel-permeation chromatography to contain 2 components of molecular weight 78,000 (20%) and 18,000 (80% by weight). Based on the results of acid hydrolysis, and a series of periodate degradation steps, the degraded polysaccharide was shown to consist of a single component of molecular weight 2200, The product, $[\alpha]_D$+16° contained galactosyl and arabinosyl residues in an 8:1 ratio. Partial hydrolysis with acid showed that the majority of the inter-galactose linkages were (1-3). The authors concluded that the arabinogalactan components of *Larix occidentalis* consisted mainly of (1-3)-linked blocks of β-D-galactopyranosyl residues separated at regular intervals by sugar units, which were vulnerable to periodate degradation. The authors stated

that this structural concept parallels exactly that postulated for the gums of *Acacia podalyriaefolia* and *A. filicifolia* [Chrums, *et al.*, 1978, Vol. 3 Tab 8].

Values of optical rotation that have been reported for arabinogalactan from gymnosperms are usually low and positive [Timell, 1965, Vol. 4 Tab 11]. The general conclusion from the many structural studies involving species of *Larix,* such as *occidentalis, desidua, laricina, lyallii, leptolplepis, and korean* is that there is considerable similarity among the constituent groups of sugar residues attached to the (1-3)-linked D-galactan chains [Stephen, 1983, Vol. 4 Tab 7, Odonmazig, 1994, Vol. 3 Tab 25, and Whistler, *et al.*, 1970, v 5 Tab 10].

Jones, *et al.*, 1968, studied the degradation of arabinogalactan in a 700-year old Western larch tree. The authors were interested in investigating the potential breakdown of arabinogalactan *in situ* in old trees because, other authors had shown that, the formation of acetic acid increases in wood as trees age. Using arabinogalactan extracted from growth rings, the author showed that at least in the heartwood of *Larix occidentalis,* a continuous removal of arabinose groups with a concomitant depolymerization resulted due to acid-catalyzed hydrolysis. Concurrently, the relative proportion of the low-molecular weight arabinogalactan B initially increases with increasing extent of hydrolysis prior to decreasing slightly. The authors indicated that data in the literature concerning the molecular weight of larch arabinogalactan has often been contradictory. They postulated that this situation could be largely explained by the fact that molecular weight characteristics may vary with the age of the heartwood used [Jones, *et al.*, 1968, Vol. 3 Tab 17].

Larex, Inc. recently studied the molecular weights of several of its products processed from either Western or Eastern larch. Samples were subjected to analysis via HPLC/GC, UV @ 280 nm, and refractive index [Analytical Methods, Vol 2 Tab 28]. The results are shown below in Table 5. Interestingly, the molecular weights were very similar between products and between species, despite differences in processing and purification. The most notable difference was the elution of an additional shoulder in Eastern larch samples. This shoulder eluted at approximately 10.1 minutes, and may account for the slightly higher average molecular weight in products derived from Eastern larch [Raw data, EL Vol 1 Tab X].
(pp. 26-29)

Table 5
Eastern and Western Larch
Molecular Weight Comparison by Product

Product	Eastern Larch		Western Larch	
	MW (Daltons)	Retention Time (min)	MW (Daltons)	Retention Time (min)
LSC-1	18,815	10.167 8.383	17,527	8.467
LaraCare	18,871	10.100 8.367	19,040	8.383
Immune Enhancer	18,153	10.133 8.383	17,978	8.417
ClearTrac	18,750	10.133 8.367	17,926	10.183 8.433

[Raw Data EL Tab Raw Data EL Vol 1] pp. 002 - 003.

Analysis of Multiple Lots of Eastern Larch Arabinogalactan

Analysis of three lots of the LSC-1 product from Eastern larch (*Larix laricina*), revealed that the arabinogalactan met the specifications set out for a food grade product by Larex [Tables 1 and 2 above]. The product contained less than 3 ppm arsenic, less than 5% ash, and less than 10 ppm heavy metals as lead. The liquid product of the initial pressate contained 50-55% arabinogalactan in solution with a pH of 4.0 to 5.5, specific gravity was less than 1.5, and negligible insoluble matter (less than 0.1%). Phenol content ranged between 1.0 and 1.5 ppm. The turbidity of the solutions as measured in Nephelometric Turbidity Units (NTUs) was between 1.8 and 3.1. Molecular weights as determined by HPLC/GC were 19,000 to 21,000 and UV reflectance at 280 nm was between 0.55 and 0.70. Chloride content was less than 150 ppm, and viscosity was less than 500 cps.

Table 6
Summary of Analytical Results of Three Lots LSC-1
Arabinogalactan from Eastern Larch

Lot #	Refractive Index (%)	Ash (%)	Chloride (ppm)	Insoluble Matter (%)	MW	pH	Phenol (ppm)	Specific Gravity (g/ml)	Turbidity (NTU)	UV (280 nm)	Viscosity (cp)
0024-96242-01											
a	51.4	0.87	68	<0.01	19470	4.35	1.29	1.22	2.346	0.6860	226.4
b	51.4	0.87	68	<0.01	19284	4.35	1.25	1.22	2.106	0.6194	225.6
0024-96249-01											
a	51.3	0.70	98	<0.01	20835	5.21	0.96	1.22	1.805	0.6893	196.4
b	51.3	0.70	98	<0.01	20296	5.21	0.95	1.22	2.142	0.6761	197.6
0024-97160-01											
a	54	0.81	114	<0.01	19527	5.19	1.32	1.22	2.34	0.5685	177.2
b	54	0.81	114	<0.01	19468	5.19	1.23	1.22	3.096	0.5448	170.8

[Raw Data Vol 2 Tab 31] of GRN 000047

Section I, Chemical Identity

A second set of food-grade specifications was set forth by Larex to describe a decolorized arabinogalactan product. This product was derived from the LSC-1 products described above, however, a decolorizing process was added to improve color, taste, and odor. Specifications for this product differed somewhat from those describing the LSC-1 products [See Table 2, above]. In the decolorized product viscosity was much lower at <5.0 cps, and ash content was higher at <10% (most probably because of the use of either potassium hydroxide, or calcium hydroxide, and hydrogen peroxide in the decolorizing process [Vol. 2 Tab 20]). Color however, was reduced from a tan-cocoa colored liquid to a white powder.

In a subsequent set of experiments, Larex compared identical products processed from either Western Larch or Eastern Larch. The results are shown in Table 7 below. Color as measured by absorbance at 400nm was nearly identical between products. Phenol content as measured by absorbance at 280nm and by using CheMets® ampules was generally higher in samples from Eastern Larch. The molecular weight was similar across products. A comment from Dr. Glen Ponder, 1995 of the University of Montana, indicates that the molecular size of Tamarack (Eastern Larch) arabinogalactan may be as much as 30% higher than that of Western Larch [Ponder, 1995, Personal Communication, EL Tab EL Vol 1]. However the result of Larex studies using identical methods did not show this to be the case. Products from either raw material source maintained similar parameters despite differences in downstream processing. pp. 41-42 Raw Data

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Table 7
Comparison of Analytical Results of Decolorized Arabinogalactan Products from Eastern Larch and Western Larch

Product	Lot #	MW	Phenol (ppm)[1]	UV (280 nm)[2]	UV (400nm)[2]
Grade 100-EL	LSC1	18815	16.65	0.3535	0.0188
LSC1-WL	LSC1	17527	8.66	0.2456	0.0192
LaraCare-EL	00349706501	18871	2.13	0.5121	0.0070
LaraCare-WL	00349706501	19040	2.03	0.9432	0.0270
ImmunEnhancer-EL	2LB9910801	18153	5.46	1.8295	0.0178
ImmunEnhancer-WL	2LB9910801	17978	0.85	0.8025	0.0116
ClearTrac-EL	2C99935801	18750	0.29	0.2599	0.0062
ClearTrac-WL	2C99935801	17926	nd	0.3084	0.0074

WL denotes samples from Western larch. EL denotes samples from Eastern larch.
[Raw Data, 2000, EL Tab Raw Data EL Vol 1]

[1] All Measurements were made with 1% solution except for absorbance measurements for Grade 100-EL and LSC1-WL, which were made with 0.1% solutions.

[2] All Measurements were made with 1% solutions using CheMets® ampules.

[3] The processing steps for Tamarack products:

Grade 100-EL: Identical to LSC1 from Western Larch

LaraCare-EL: Grade 100-EL → cationic exchange → decolorize with KOH/H_2O_2 → anionic exchange → concentrate → dry.

ImmunEnhancer-EL: Grade 100-EL → cationic exchange → KOH/H_2O_2 → dry.

ClearTrac-EL: Grade 100-EL → cationic exchange → $Ca(OH)_2/H_2O_2$ → anionic exchange → carbon treatment → concentrate → dry.

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Microbial Content of Larex Eastern Larch Arabinogalactan

Microbial content of the Eastern larch product was remarkably low, easily meeting the specifications set forth by Larex of less than 1,000 CFUs for aerobic plate count, negative for *Salmonella*, mold counts of less than 100, yeast counts of less than 100, and *Staphylococci* less than 10. Actual values were negative for *Salmonella*, and 10 or <10 for all other microbial parameters [Table 8, below, and Vol. 2 Tab 10]. Raw data is contained in Volume 2 Tab 31 of the Western Larch GRAS Report (GRN 000047).

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Table 8
Microbiological Profile of Three Lots of LSC-1 Arabinogalactan from Eastern Larch

Lot #	Aerobic Plate Count (CFU/g)	Coliform Count (CFU/g)	*Eschericia coli* (CFU/g)	*Staphylococci*, Coag pos (CFU/g)	Mold Count (per g)	Yeast Count (CFU/g)	*Salmonella*	Weight for *Salmonella* (g)
00249716001	<10	<1	<1	<10	470	<10	Negative	100
00249624201	<10	<1	<1	<10	<10	<10	Negative	100
00249624901	50	<1	<1	<10	<10	<10	Negative	100

Heavy Metal and Contaminant Content of Larex Eastern Larch Arabinogalactan

Heavy metal content of the products showed that all USP heavy metals were below the level of detection, (5.0 ppm). Mercury was also below the level of detection (0.01 ppm), arsenic ranged from <0.01 ppm to 1.7 ppm, and chromium ranged from 0.06 ppm to <0.06 ppm [Table 12, 13, and 14, Vol. 2 Tabs 14, 15, and 16]. Benzene and organo-phosphorus pesticide results were below the level of detection for all products [Tables 9, 10, and 11 Vol. 2 Tabs 11, 12, and 13]. These data indicated that there was no species difference in the quantitation of the arabinogalactan product for heavy metals or contaminants, and that product from both species of larch met the food grade specifications set forth by Larex.

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Table 9

Heavy Metals and Mineral Analyses of Three Lots LSC-1 Arabinogalactan from Eastern Larch

Sample Lot Number	Iodine (%)	Potassium (mg/Kg)	Calcium (mg/Kg)	Magnesium (mg/Kg)	Sodium (mg/Kg)	Aluminum (mg/Kg)	Arsenic (mg/Kg)	Boron (mg/Kg)	Cadmium (mg/Kg)	Chromium (mg/Kg)
002496242-01	ND	2677	882.2	268.7	696.8	1.955	<0.1	<5.013	<0.551	0.952
002496249-01	ND	2422	614.1	236.6	544.2	1.697	0.123	<4.992	<0.549	<0.599
002497160-01	ND	2351	800.8	301.8	615.6	2.252	0..122	<5.005	<0.551	<.601

Sample Lot Number	Copper (mg/Kg)	Heavy Metals as Lead (ppm)	Iron (mg/Kg)	Manganese (mg/Kg)	Mercury (mg/Kg)	Nickel (mg/Kg)	Silicon (mg/Kg)	Sulfur (%)	Titanium (mg/Kg)	Zinc (mg/Kg)
002496242-01	1.905	<10	5.514	192	<0.105	2.11	<2.406	0	<0.602	46.12
002496249-01	0.649	<10	6.241	168	<0.091	<0.998	13.08	0	<0.599	3.345
002497160-01	1.151	<10	6.306	89.2	<0.095	<1	6.006	0	<0.601	4.654

ND= None Detected
[MVTL Laboratories, 1997, VOL. 2 TAB 31]

Section I, Chemical Identity

Specifications for Lead

In 1999 Larex developed a specification for lead separate from that developed for lead as heavy metals. Analysis of 5 lots of the LSC-1 arabinogalactan product derived from Eastern larch showed a mean level of 0.270 ppm. Analysis of 2 lots of decolorized arabinogalactan showed a mean level of 0.417 ppm lead. Based on these results, Larex, Inc. lead specifications have been reduced from a historical specification of <10-ppm lead as heavy metals to < 1.0-ppm lead. Table 10 (below) shows the raw data, which supported that change.

Table 10
Raw Data for Lead Analyses

Decolorized Arabinogalactan Lot No. From Eastern Larch	Results (mg/Kg)
0034-97065-01	0.525
0036-97149-01	0.309
Mean	**0.417**
LSC-1 Arabinogalactan Lot No. From Eastern Larch	
00189621301	<0.291
00189622601	<0.214
00249624201	<0.192
00249624901	<0.207
00189631801	<0.447
Mean	**0.270**

[MVTL Laboratories, 1999 EL Tab Raw Data EL Vol 1]. Tab 31

In accordance with discussions with FDA personnel, Larex again revised specifications for lead in its arabinogalactan products during 2000. Recent analyses for lead allow a specification of < 0.1-ppm for all food-grade products. This reduction is due in part to a change of raw materials in its manufacturing process.

Tables 11 and 12 below demonstrate that lead levels for a variety of arabinogalactan products are either below the level of detection, or well below 0.1 ppm. Table 11 depicts lead data from raw arabinogalactan extract from Western larch (*Larix occidentalis*). Table 12 compares lead data from arabinogalactan products, which have received further processing.

Table 11

Larex Western Larch Arabinogalactan	
LSC-1 Lead Levels	
Lots	**Results**
2-T3-99279-01	0.023 ppm Aver.
001B-97155-03	Non-detectable
Duplicate Samples	Detection Limit 0.008 ppm

[West Coast Analytical Service, 2000 EL Tab Raw Data EL Vol 1]. pp. 092-094

Table 12

Comparison of Lead Levels		
Larex Arabinogalactan from Western or Eastern Larch		
Western Larch Products	**Lot Number**	**Aver. (ppm)**
Decolorized	99022	0.051
Decolorized	99284	0.023
CT AG 99*	00054	N.D.***
CT 99*	99108	N.D. ***
CT 99*	99358	N.D. ***
Grade 100-Dried**	00054	0.052
Eastern Larch Products		
Grade 100	Tamarack	N.D.***
ClearTrac	Tamarack	N.D. ***
LaraCare	Tamarack	N.D.***
ImmunEnhancer	Tamarack	N.D.***
Detection Limit (D.L.)		0.020

[West Coast Analytical Service, 2000 EL Tab Raw Data EL Vol 1]. pp. 092-094.

* CT has been identified in GRN 000017 as Fiberaid.
** Grade 100 has been identified as LSC-1. However, this sample of Grade 100 has been dried.
*** Non-detectable

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Table 13

Benzene and Organo-Phosphorus Pesticide Analysis of Arabinogalactan LSC-1 from Western and Eastern Larch

Sample Lot Number	Benzene (ppb) Dilution Factor 50	Diazinon (ppm)	Ethion (ppm)	Malathion (ppm)	ethyl Parath (ppm)	Parathion (ppm)	Ronnell (ppm)
All lots of LSC-1 From Western Larch	< 55.0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
All lots of LSC-1 From Eastern Larch	< 55.0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[MVTL Laboratories, 1997, VOL. 2 TAB 29]

H. Manufacturing Process

The extraction of Larch arabinogalactan is unique among hemicellulosic extraction processes. Other hemicellulosic extraction procedures require harsh chemicals to release the polysaccharide from the plant matrix [Whistler, *et al.*, 1970, Vol.5 Tab 10, Wilkie, 1985, Vol. 5 Tab 12, and Wolf, *et al.*, 1953, Vol. 5 Tab 13]. The chemicals used for these other extraction processes are known to change the structure and the stereochemistry of the extracted polysaccharide [Whistler, *et al.*, 1970, Vol. 5 Tab 10, Wilkie, 1985, Vol. 5 Tab 12, and Stephen, 1983 Vol. 4 Tab 8]. In its native form, Larch arabinogalactan is present in sufficient quantities that it can be extracted with water. The resulting product is a pure and structurally unaltered form of hemicellulose in its natural state [Whistler, *et al.*, 1970, Vol 5 Tab 10, Whistler, *et al.*, 1991, Vol. 5 Tab 9, and Wilkie, 1986, Vol. 5 Tab 12].

Larex manufactures arabinogalactan at its plant in Cohasset, Minnesota. All processing is conducted under current Good Manufacturing Practices, and all raw materials comply with food grade specifications, or the specifications set forth by Larex for natural materials. HACCP and sanitation programs are in place to assure that the process produces a product consistent with the final product specifications [Vol. 2 ~~Page~~ Tab 18]. A written recall program has also been established to ensure that product can be tracked by lot number in the event of a general recall [Vol. 2 ~~Page~~ Tab 19].

In the processing scheme, the butt cut of large larch logs are brought to the plant via rail and truck. These log sections are first put through a debarker, and debarked logs are then split and chipped. The resulting wood chips are conveyed to a silo where they are stored until the chips are moved to a steam bin for heating. Steamed chips are fed via a plug screw feeder, extracted, and fed through a hot water impregnator. A second plug screw feeder further extracts the arabinogalactan, which is then screened to remove any cellulosic particles. Screened pressate is then sent to an extract storage tank where purified water is added. The extract is then further heated with steam before being sent through a pall separator. The permeate from this separation contains 7% arabinogalactan by weight. This dilute solution is then sent to an evaporator which utilizes steam for heat. Condensate from the evaporator is recycled after being solubilized with 50% sodium hydroxide.

The product of the evaporator contains 50-55% arabinogalactan, and is ready for drumming and shipping as either a food material or an industrial

processing aid. Typically, a small quantity of potassium sorbate is added to the product at this time as a preservative [Wilkie, 1985, Vol. 5 Tab 12]. Optionally, this liquid product can be sent through an ion exchange step to further purify the liquid before it is spray dried, or it can be decolorized, and further processed to reduce color, odor, and flavor. The decolorization is accomplished by treating the product with specified quantities of 45% potassium hydroxide and 35% hydrogen peroxide solutions alternatively; various concentrations of calcium hydroxide may be substituted for potassium hydroxide. After decolorization, the product is then cooled via cooling water. Following cooling, the concentration of the bulk arabinogalactan in solution is 35%. This product may enter one of several streams following decolorization and cooling: 1) it may be directly spray agglomerated; 2) it may be sent through a carbon filter followed by spray agglomeration; 3) it may be sent through a carbon filter and an ion exchange step prior to being spray agglomerated; or 4) it may go directly through an ion exchange step, a spray drying step and then be agglomerated.

HACCP and Recall Program

HACCP and sanitation programs are in place to assure that the Larex process produces a product consistent with the final product specifications [VOL. 2 TAB 18]. A written recall program has also been established to ensure that product can be tracked by lot number in the event of a general recall [VOL. 2 TAB 19]. The following forms and flow charts form the basis of the plant's HACCP program.

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Section I. Chemical Identity

LAREX, INC. RAW MATERIAL RECEIVING FORM

Date:_____ **Receiver:**_____

Shipper:_____
Supplier:_____

Product Receiving	Quantity	Lot#

Is vehicle in adequate condition to accept shipment? yes no
Is product in adequate condition to accept shipment? yes no
Accepting shipment: yes no

Comments:_____
Reason for Rejection:_____

Bring Form To The Front Desk.

For Office Use Only:	Initials:_____	
Purchase Order No./Invoice No:		
Items enclosed with shipment: _____MSDS _____Certificate of Analysis _____Shipping/packing ticket _____Other:_____	Requested from Supplier: _____ _____ _____ _____	Rec'd _____ _____ _____ _____

Comments:_____
Action taken on rejection of shipment:_____
_____
_____

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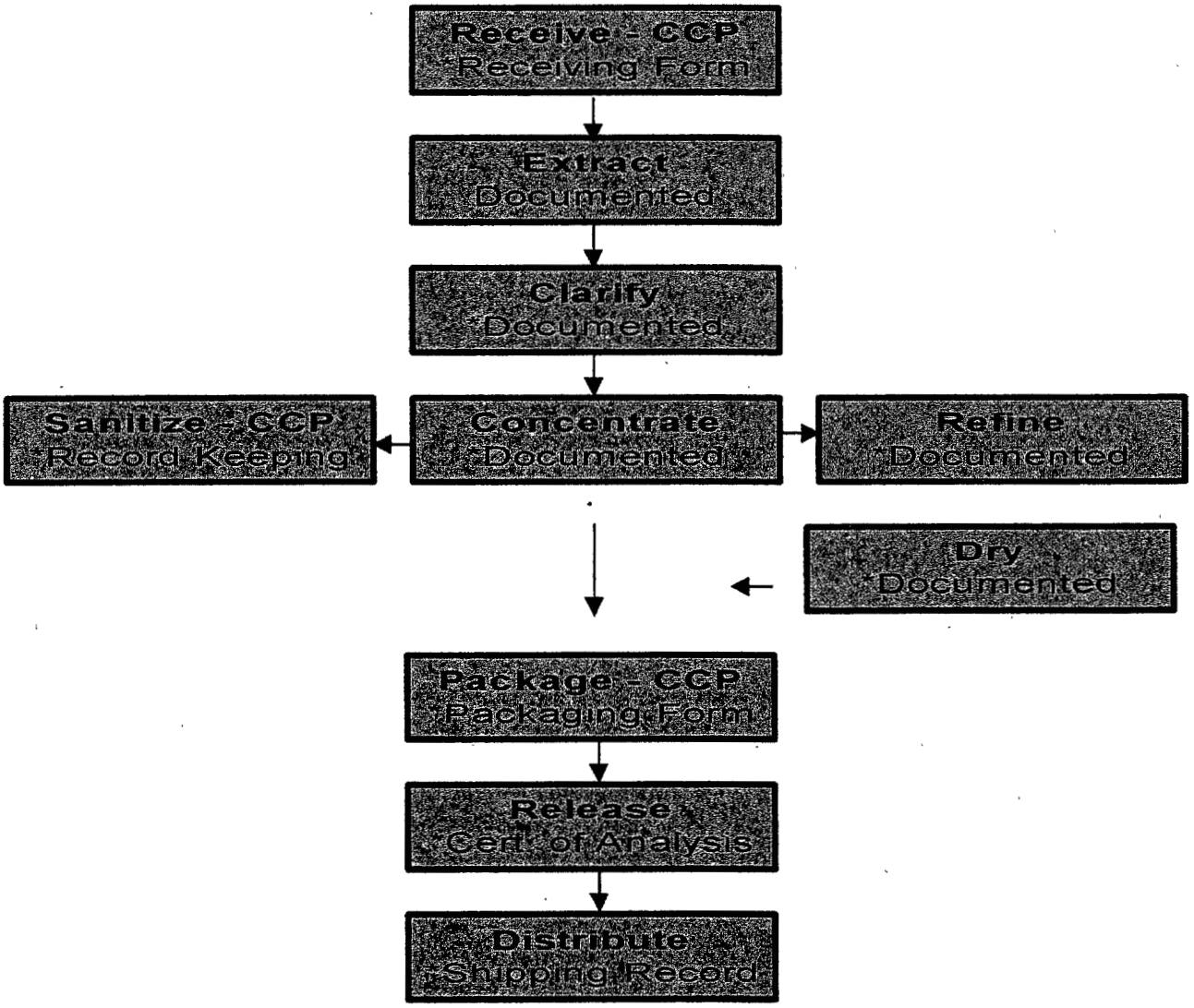
Section I, Chemical Identity

HACCP Plan Flow Chart



LAREX, Inc.
HACCP

QC04 **Page: 4 of 6**

OPERATIONAL STEP; RECEIVING OF RAW MATERIALS

Raw Materials: Hydrogen Peroxide, Potassium Hydroxide, and Logs.

Hazards:
1. Receipt of contaminated raw material (environmental).
2. Receipt of wrong raw materials (environmental, physical).
3. Species Substitution (other than Larch or Tamarack logs) (physical).

Critical Control Point:
1. Receive chemicals or logs.
2. Log lot number, or railcar number for logs.

Critical Limits:
1. Chemicals must meet the Food Chemical Codex specifications.
2. Damaged, badly soiled, or leaking containers shall be rejected.
3. No species substitution is to be admitted into the production facility, unless otherwise specified by the VP of Manufacturing, or Plant Supervisor.

Frequency:
Inspect each shipment upon arrival.

Monitoring Method:
1. Visual inspection for leaky containers.
2. Food Chemical Codex Code listed to each container, documenting the date received, initials of receiver, and lot number.
3. Attach an "Acceptance" label to each container, documenting the date received, initials of receiver, and lot number.
4. If log identification is in question, us the comparison chart located in the Lab Room.

Monitored By:
Receiver.

Corrective Actions:
1. Reject shipments that have open or leaky containers, damaged, or badly soiled.
2. The Office Manager will follow-up on all corrective actions.

Records:
Filed and maintained by the Office Manager.

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LAREX, Inc.
HACCP

QC04 **Page: 5 of 6**

OPERATIONAL STEP: PACKAGING

Hazards:
1. Microbial (Contamination of product).
2. Physical (Improper labeling of product).

Critical Control Points:
1. Prevent foreign material contamination.
2. Correct label and lot number on each container.
3. Retain sample.

Critical Limits:
1. All containers are to be visually inspected by a Production Technician before product is packaged. If a foreign object is found in the packaging unit, it shall be set aside and tagged for disposal.
2. Long hair must be pulled back. Hard hats are required in the plant at all times.
3. All products must be labeled properly.
4. Two samples are required for each Lot number packaged.

Frequency:
Each container shall be visually inspected before product is packaged.

Monitoring Method:
1. A Production Technician shall be responsible for visually checking containers before product is packaged.
2. The Production Technician assigned to packaging is responsible for the proper labeling of packaged material. The Office Manager is responsible for providing the labels to meet proper labeling requirements.
3. The Production Technician assigned to packaging is responsible for completing the "Packaging Lot Sheet".

Monitored By:
Production Technician assigned to packaging activity.

Corrective Action:
1. If a container is found to be unfit for packaging, the container will be tagged for disposal.
2. If a label is incorrect on any of the products, each package in the lot will be examined for correct labeling. Any mislabeled products will be put "On Hold" until corrective action has taken place.

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Records:
Filed and maintained by the Office Manager.

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LAREX, Inc.
HACCP

QC04 **Page: 6 of 6**

OPERATIONAL STEP: Cleaning of Equipment

Hazards:
Microbial, chemical, physical

Critical Control Points:
1. Prevent contamination of product.

Critical Limits:
1. Any unsanitary condition or filth shall be cleaned prior to operations.
2. Detailed cleaning procedures shall be followed as directed in MF03.

Frequency:
See MF03 (attached)

Monitoring Method:
1. Before a new production run starts, the "Master Cleaning Schedule" shall be reviewed to assure the tanks have been cleaned/sanitized.
2. A microbial test will be run on any product that will be labeled as a food product. The following tests will be run: Aerobic Plate Count, Coliform Count, E-Coli, Staphylococci, Mold and yeast count, Salmonella. A sample shall be sent to an independent agency for testing. Results shall be filed by the Technology team in St. Paul.

Monitored By:
Production Supervisor

Corrective Actions:
If the tanks were not sanitized/cleaned or not recorded sanitized/cleaned on the "Master Cleaning Schedule", a thorough sanitation/cleaning shall be competed before a new production run starts.

Records:
*Operators are responsible for documenting activities on the Master Cleaning Schedule.
*Master Cleaning Schedules are filed and maintained by Office Manger.

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I/J. Larex Product Identification

The Larex arabinogalactan products are of several types. The initial extractant from the process is purified through a pall separator, and is then evaporated to a 50% arabinogalactan concentration. This product is called LSC-1. At this point the product may either enter the printing industry as LaraPrint 100 or to the food industry as L'Extra F100, or it may be dried to Grade 100, or it may be further purified though ion exchange to improve color, taste, and odor. Additionally, the product may be decolorized utilizing hydrogen peroxide and potassium hydroxide. Finally, the products may be spray dried and agglomerated. A figure depicting the processing steps for each product type, and a listing of the various arabinogalactan products manufactured by Larex and their respective processing steps are given below.

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Larex Food Product Specific Processing Steps

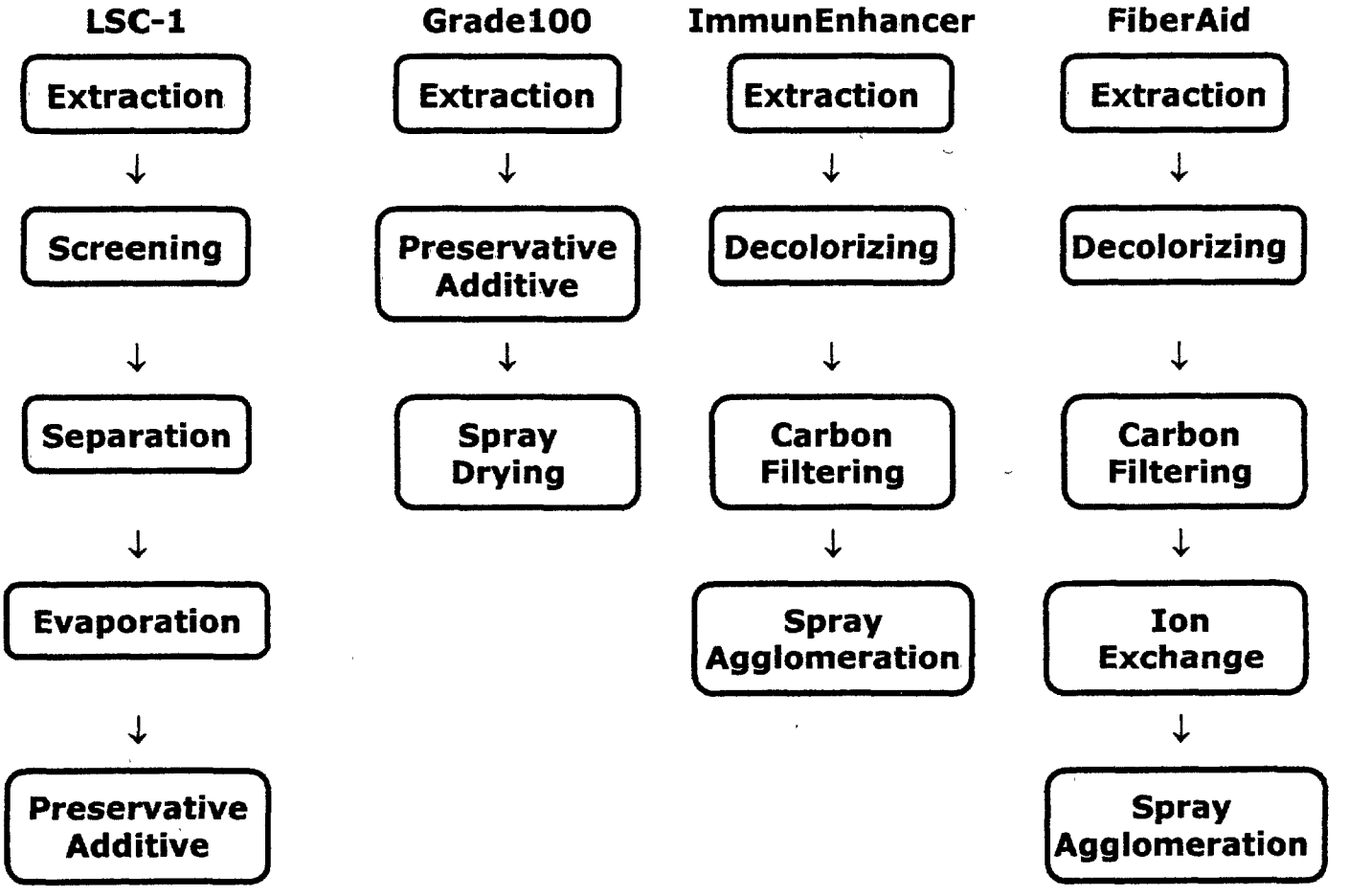

Table 13
Larex Arabinogalactan Products

Arabinogalactan Product Name	Market and Properties of Products	Market
LaraPrint ™ B50	Ink additive for decreasing viscosity while maintaining color density: caramel colored 50% solution preserved with potassium sorbate.	Printing and inks
LaraPrint ™ B100	Ink additive for decreasing viscosity while maintaining color density: tan colored powder	Printing and inks
L'Extra F100	A fiber source: off-white powder, very low taste and odor, no precipitation in liquids, excellent dissolution.	Food and Beverage
LaraCare™ A200	Emulsion stabilizer, film and gloss former, dispersant, moisture control, rheology control: off-white powder, mild odor, highly soluble, excellent dissolution, and biodegradable.	Personal Care
L'Extra™ F2000	Moisture control: mouthfeel, encapsulation, bulking agent: off-white powder, low viscosity, very low taste and odor, some precipitation in liquids, excellent dissolution, used for moisture control.	Food and Beverage
FiberAid™	A fiber source: off-white powder, very low taste and odor, no precipitation in liquids, excellent dissolution.	Food and Beverage
Laraceutical™ AG ImmunEnhancer™	Human and animal immune system enhancement: off-white powder, mild taste and odor, excellent dissolution	Nutraceuticals

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Table 14
Larex Product Specific Processing Steps

Arabinogalactan Product Name	Processing Steps
LaraPrint ™ B50	extraction, screening, separation, evaporation, preservative addition
LaraPrint ™ B100	extraction, screening, separation, evaporation, preservative addition, spray drying
L'Extra F100	extraction, screening, separation, evaporation, preservative addition, spray drying
LaraCare™ A200	extraction, screening, separation, evaporation, decolorizing, ion exchange, spray drying, agglomeration
L'Extra™ F2000	extraction, screening, separation, evaporation, decolorizing, carbon filtering, spray agglomeration
FiberAid™	extraction, separation, evaporation, decolorizing, carbon filtering, ion exchange, spray agglomeration
Laraceutical™ AG ImmunEnhancer™	extraction, screening, evaporation, decolorizing, spray agglomeration

K. Analytical Methods

Larex has provided written analytical methods and procedures for the determination of its specification parameters [Vol 2, Tab 28]. These methods include a description of the equipment to be used, the concentrations of any solutions to be used, and a set of sample calculations.

L. Raw Data

Raw data specific to Eastern larch arabinogalactan can be found in Eastern larch GRAS Report [EL Vol 1 EL Tab Raw Data].

M. Natural Sources of Arabinogalactan

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Arabinogalactans have been documented from many sources. A number of review articles indicate that arabinogalactan and arabinogalactan proteins are present in the plant cell walls of nearly all higher land plants. They have been documented in mosses, medicinal plants such as *Angelica acutiloba*, wheat flour, soy beans, *Pinus cortata*, carrots, grapes, and radishes [Geddes, *et al.*, 1971, Vol. 3 Tab 13, Laidlaw, *et al.*, 1962, Vol. 3 Tab 20, Morita, 1965, Vol. 3 Tab 22, Pennell, *et al.*, 1989, Vol. 4 Tab 1, Saulnier, 1992, Vol. 4 Tab 4, Neukom, *et al.*,

1975, Vol. 3 Tab 24, and Yamada, *et al.*, 1987, Vol. 5 Tab 14]. These references indicate that arabinogalactans are wide spread in the plant kingdom, and thus have historically been utilized by man as a food source.

Isolation Procedures

In general, arabinogalactans are present as a minor constituent in soft wood species; however, within the genus *Larix* arabinogalactan can constitute up to 35% of the wood. Because arabinogalactans have numerous branches, they are the only major wood polysaccharides that can be extracted in high yield from untreated wood with water. At the time of the authors publication, the defatted wood was extracted with hot water followed by alcohol precipitation to produce what was relatively pure, structurally unaltered, polysaccharide [Whistler, *et al.*, 1991, Vol. 5 Tab 9].

Hemicelluloses are usually extracted from plant tissue after removal of lipid and lignin. Removal of lignin exposes the hemicellulose for easy dissolution in alkali, and permits isolation of pure hemicellulose. Lipids are usually removed by extraction, with a hot, azeotropeic mixture of benzene and ethanol. One of the few major polysaccharides of wood that can be isolated in high yields by extracting the fully lignified wood with water is the arabinogalactan of Larch [Whistler, *et al.*, 1970, Vol. 5 Tab 10].

Delignification of plant tissue with acidified sodium chlorite causes slight degradation of the hemicelluloses. Reducing end groups are oxidized to aldonic acids, and slight depolymerization and oxidation of 2, 3-glycol groups may occur. Chlorine, which is also used for delignification, also appears to cause some degradation of the hemicelluloses [Whistler, *et al.*, 1970, Vol. 5 Tab 10]. Alkaline extraction of plant material or of holocellulose can result in many changes in the polysaccharide even under oxygen-free conditions. Alkaline degradation occurs at the reducing groups of the polysaccharide, and gives rise to saccharinic acid units by way of a β-elimination period. This leads to the exposure of new reducing groups, and the peeling reaction proceeds in stepwise manner unless interrupted by a "stopping" reaction. In a (1-4)-linked polysaccharide the action of alkali on a reducing-sugar end bearing a branch at C-3 has been though to cause elimination of the side chain as well as the reducing sugar [Whistler, *et al*, 1970, Vol. 5 Tab 10].

Most methods for fractionation and purification of hemicellulose on a macro scale make use of precipitation. Alkaline solutions of copper salts are often added to hemicellulose solutions to precipitate those containing significant amounts of D-mannose or D-xylose. The insoluble polysaccharide-copper complex is isolated, and the copper is removed by use of acidified alcohol solution or a chelating agent. Pure polysaccharides are frequently obtained from dilute solutions by the

gradual addition of ethanol. Acid hemicelluloses are easily separated from neutral hemicelluloses, by precipitation with quaternary ammonium salt [Whistler, *et al*, 1970, Vol. 5 Tab 10]. Other authors have indicated that even the superficially harmless procedure of air-drying certain plants, such as grasses, may lead to changes in the hemicellulose confirmation, denaturation, and absorptive or other associations between cell-wall components, including the hemicelluloses [Wilkie, 1979, Vol. 5 Tab 11 and Wilkie, 1985, Vol. 5, Tab 12].

Table 15
Arabinogalactan Physical Properties from Various Larch Species

Species	Yield (% of wood)	Gal/Ara	$[\alpha]_D$ (Degrees)
L. dahuricia (gmelini) Mongolian larch	16-24	5.5	
L. dahurica		4.1-5.5	
L. gmelini, var. japonica	15.7	4.3 (B)	
L. decidua European larch		5.8	+12.2
L. decidua			+16.5
L. decidua		5.6	+20
L. decidua		5.9	
L. decidua	3.4	5.7	
L. decidua	15.9	6.9	+17.6
L. laricina Tamarack	4	5.7	+13.0
L. laricina	3.9 (A)	5.4	
L. laricina	5.6	3.8	+11
L. leptolepis Japanese Larch	3	5.9	
L. leptolepis			
L. lyallii Alpine larch	8.0 (A)	7.8	
L. lyallii	5.6 (A)	4.9	
L. lyalli	2.1 (A)	2.6	
L. occidentalis Western larch		5.4	
L. occidentalis			+12.1
L. occidentalis		5.9	
L. occidentalis		5.2	
L. occidentalis	8.4 (heartwood)	5.3	
L. occidentalis	0.9 (sapwood)	6.2	
L. potaninni	8.6	4.3 (A) 3.8 (B)	+7 +10
L. sibirica Siberian larch	7.2 (A)	7.3	
L. sukaczevii		3.6	
	5.3	5.4	

[Timell, 1965, Vol. 4 Tab 11]

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N. Summary

The data and information presented above demonstrates that arabinogalactan produced from Eastern larch (*Larix laricina)* is equivalent to arabinogalactan produced from Western larch ((*Larix occidentalis)*. Both are derived from naturally occurring materials. Further, Larex, Inc. is capable of manufacturing arabinogalactan from either species of the larch tree to meet a given set of food grade specifications. The two products are virtually identical in every parameter tested, and Larex, Inc. submits that the two raw materials can be used interchangeably. Table 16 below summarizes the characteristics of each larch species.

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Table 16
Comparisons between Eastern and Western Larch

Characteristics	Western Larch	Eastern Larch
1. Distribution of Arabinogalactan	Decrease with tree height	Increase with tree height
2. Purity of Arabinogalactan (%)	91, 84, 88	79, 78, 81 (Fall harvest) 86, 83, 85 (Spring harvest)
3. Galactose: Arabinose[1]	5:2 – 6:2	3:8 – 5:7
4. Arabinogalactan Molecule[2] A: MW 100K B: MW 16K A: more spherical shape B: more linear shape	70 - 75% A 25 – 30% B	90 – 95% A 5 – 10% B
5. Lignin	18.1% - Bark of *L. Decidua* 17.4% - Bark of *L. Eurolepsis* 9.8% - Bark of *L. Kaempferi*	26% (dry wood weight)
6. Uronic acid	0.2%	2%
7. Likely structural differences	Backbone chain β-S-galactopyranosyl units linked: (1 → 3)	(1 → 3) and (1 → 6)
8. Ash	0.44	0.74

[1] Arabinogalactan from Eastern larch contains more L-arabinose residues than most AG from larch woods [Cote`, *et al.*, 1967, Vol. 3 Tab 9].
[2] From various sources in the literature [Cote`, *et al.*, 1967, Vol. 3 Tab 9].

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Section II
Use and Functionality

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Section II: Use and Functionality

A. Introduction

Arabinogalactan is a polysaccharide composed of galactose and arabinose units with a highly substituted backbone of β-1-3-linked galactopyranose units and side chains of galactose and arabinose. Most of the side chains contain 1-4 pyranose or furanose units with both galactose and arabinose present in the side chains [Meuser, *et al.*, 1986 Vol. 7 Tab 5]. Arabinogalactan occurs in most higher and lower plants, and is usually found attached to a protein component, making an arabinogalactan-protein complex [D'Adamo, 1990, Vol. 6 Tab 6].

Arabinogalactan and arabinogalactan proteins are common components of plant cell walls, and in their natural state enter the human food chain in such common food substances as wheat flour, radishes, and tomatoes, [Pogson, *et al.*, 1995, Vol. 7 Tab 10, and Kikuchi, *et al.*, 1993, Vol. 7 Tab 2]. Due to its highly branched structure, arabinogalactan is highly soluble in water, and relatively high concentrations of arabinogalactan are very low in viscosity [D'Adamo, 1990, Vol. 6 Tab 7]. This unique set of characteristics has led arabinogalactans to be considered as a fiber ingredient in a wide array of food materials. Much of the evidence for the applicability of arabinogalactan comes from the patent literature, where applications involving arabinogalactan have been wide ranging [U.S. Patent No. 3,264,114, Vol. 7 Tab 13, U.S. Patent No. 4,810,516, Vol. 8 Tab 5, and U.S. Patent No. 5,089,307, Vol. 8 Tab 9].

Recent human and animal clinical studies with arabinogalactan as a dietary supplement have shown that the addition of arabinogalactan to the diet increases the numbers of probiotic organisms in the colon, and improves regularity (Kim, *et al.*, 1999 Vol. 6 Tab 18, Grieshop, 1999 *In Press* Vol. 6 Tab 21, and Robinson, *et al.*, 1999 Vol. 6 Tab 19). Probiotic organisms have in themselves been associated with positive health benefits for both humans and animals. These studies have demonstrated that arabinogalactan has a variety of physiological attributes as a source of soluble fiber that would be desirable in the food supply. Larex, Inc. submits that its arabinogalactan product derived from *Larix laricina* could be utilized as a source of soluble prebiotic fiber. pp. 40-44

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B. Historical Consumption of Arabinogalactan

Arabinogalactan has always been a part of the diet of man, since it occurs in the cell walls of nearly all higher plants [Pennell, *et al.*, 1989 Vol. 4 Tab 1]. One common source of arabinogalactan in the human diet has been wheat flour. According to a review written by D'Appolonia, 1980, the primary non-starchy polysaccharides of wheat flour are the pentosans, which are classified as hemicelluloses. The reader should note, however, that certain researchers have used the term hemicellulose to refer to the water-insoluble non-starchy polysaccharides and the word "pentosan" to the water-soluble polysaccharides [D'Appolonia, 1980, Vol. 6 Tab 11].

Based on numerous investigations, researchers at North Dakota State University, Fargo, North Dakota, USA listed the yield of arabinoxylan and arabinogalactan protein complexes from wheat. This data is shown in Table 1 below [D'Appolonia, 1980, Vol. 6 Tab 11].

Table 1
Composition of the Fractionated Pentosan-Protein Complexes of Wheat

Fraction	Yield (g/100g flour d.m.)	Protein content (% d.m.)	Carbo-hydrate content (% d.m.)	Carbohydrate Composition (%)		
				Ara	Gal	Xyl
Arabinoxylan-protein complex	0.58	10.8	86.4	34.7	64.7	traces
Arabinogalactan-protein complex	0.14	5.2	91.2	41.0	traces	59.0

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Table 2

Occurrence of Arabinogalactan in Common Foods and Medicinal Plants

Source in Foods	Reference
Coffee Beans	[Fincher, *et al.*, 1974, Vol. 6 Tab 16]
Green Coffee Beans	[Wolfram, *et al.*, Vol. 8 Tab 17]
Roasted Coffee Beans	[DeMaria, *et al.*, 1994, Vol. 6 Tab 14
Soybean Seeds	[Fincher, *et al.*, 1974, Vol. 6 Tab 16]
Rape Seed	[Fincher, *et al.*, 1974, Vol. 6 Tab 16]
Apple Fruit	[Fincher, *et al.*, 1974, Vol. 6 Tab 16]
Broad Bean Leaves	[Fincher, *et al.*, 1974, Vol. 6 Tab 16]
Cultured Tomato Cells	[Fincher, *et al.*, 1974, Vol. 6 Tab 16] [Pogson, *et al.*, 1995, Vol. 7 Tab 10]
Wheat Flour	[Meuser, *et al.*, 1986, Vol. 7 Tab 5] [Medcalf, *et al.*, 1967, Vol. 7 Tab 4]
Rye Flour	[Meuser, *et al.*, 1986, Vol. 7 Tab 5]
Maple Syrup	[Adams, 1964, Vol. 6 Tab 1]
Radish	[Kikuchi, *et al.*, 1993, Vol. 7 Tab 2]
Black Gram	[Susheelamma, *et al.*,1994, Vol. 7 Tab 11]
Red Wine	[Waters, *et al.*, 1985, Vol. 8 Tab 14]

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C. Current Use

The product is currently sold as a food ingredient and as a dietary fiber supplement to improve intestinal and immune system health under the Dietary Supplement Health and Education Act in the United States [DSHEA Opinion, 1997. A Report Prepared for Larex by NFNC, St. Paul, Minnesota, Vol. 6 Tab 20]. Recent clinical studies in both humans and animals have shown that a dosage of between 1.5 and 4.5 grams per day (0.025 and 0.075 g/kg for a 60 kg human) has been effective in increasing the population of probiotic bacteria in the mammalian colon [Kim, *et al.,* 1999 Vol. 6 Tab 18 and Grieshop, 1999 *In Press* Vol. 6 Tab 21].

D. Exposure

Arabinogalactan is a naturally occurring compound which is found in the cell walls of most higher plants, and therefore, is a common constituent of the human diet [Kikuchi, *et al.*, 1993, Vol. 7 Tab 2, and D'Adamo, 1990, Vol. 6 Tab 7]. Products containing arabinogalactan have the connotation of safe use in the market place. There have been no known incidents of toxicity resulting from consumption of larch arabinogalactan as manufactured by Larex, Inc. or any earlier manufacturer. In light of a history of safe use, Larex asserts exposure levels for arabinogalactan from Eastern Western larch (*Larix laricina)* need be limited only by current Good Manufacturing Practices.

A Realistic Estimate of Exposure to Arabinogalactan

Larex, Inc. was asked to provide the FDA with a realistic estimate of exposure to arabinogalactan. The agency's guidance document "*Estimating Exposure to Direct Food Additives and Chemical Contaminants in the Diet"* and USDA's *Continuing Survey of Food Intakes by Individuals* (CSFII, 1996) were used as references to estimate the realistic exposure to the arabinogalactan produced by Larex, Inc. [Enns, *et al.,* 1997].

USDA's CSFII, 1996 was used to estimate mean intakes of the various food categories. Realistic usage levels of arabinogalactan were assigned based on industry experience, applications trials, and economic considerations. The contribution of each food category to arabinogalactan exposure was calculated by multiplying the average usage level of arabinogalactan for recognized food subcategories by the average consumption for men and women as reported by the CSFII, 1996. This value was considered to be the mean level of intake.

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In order to calculate the 90th percentile of consumption, the mean intake level was multiplied by 2 as indicated the agency's guidance document. Additional exposure to arabinogalactan may result from its use as a source of soluble prebiotic fiber in dietary supplement form [Enns, *et al.*, 1997 and Causey, *et al.*, 1999]. Recommended dosages for arabinogalactan as a prebiotic fiber have been determined by clinical trial [Causey, *et al.*, 1999].

While the percentage of use assigned to each food subcategory was considered realistic, the exposure estimate remained conservative because 100% market penetration was assumed, and arabinogalactan was assumed to be included in all products in a given subcategory. Additionally, the values presented below assume that 100% of consumers are "eaters" of arabinogalactan containing products.

In reality, arabinogalactan is expected to be used in only certain products within a given subcategory. Further, use within a subcategory will be limited by economics and organoleptic considerations. Market development in the US is likely to be directed towards the functional properties of arabinogalactan, and certain functional properties may not be appropriate for a given consumer product. An example of this is that arabinogalactan tends to reduce viscosity in batter or fluid food systems. This may not create a desirable product in all cases.

Additionally, Larex, Inc. arabinogalactan is a very expensive food ingredient, costing several dollars per pound. Therefore, those applications in which it will be cost effective will need to be carefully chosen, and will most likely dictate that arabinogalactan will be used at the minimum level necessary to produce a given technical or nutritional effect.

Table 3 below shows the realistic percentages of use assigned to each of the forty-three food categories listed at 21 CFR § 170.3 (n). The correlation between the major food categories derived from the USDA food survey, and those listed at § 170.3 (n) is shown in Table 4. 000094

The mean intake for selected major food subcategories is shown in Table 5 below. The sum of the grams of arabinogalactan calculated from the mean intakes for each major food category listed on Table 5 is 10.474 grams per day. The agency's guidance document indicates that an estimate of the 90th percentile level of intake can be calculated by doubling the total mean intake. This value is 20.95 grams per day. Larex, Inc. is also aware that certain individuals may consume arabinogalactan as a dietary supplement. The recommended dose of arabinogalactan as a dietary supplement is 4.5 grams per day. This dosage has been

recommended based on the results of clinical trials with human volunteers [Causey, *et al.,* 1999 and Kim, *et al.,* 1999].

Interestingly, the CSFII, 1996 shows that the mean fiber intake averaged for men and women is 16.1 grams per day [Enns, *et al.,* 1997]. This level is still well below the 25-30 grams per day recommended by many health agencies. The addition of arabinogalactan to the diet, therefore, is not expected to create excessive fiber intake.

Larex, Inc. submits, therefore, that exposure to arabinogalactan will not result in a harmful level of fiber intake, and that arabinogalactan is safe for its intended use as a food ingredient.

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Table 3: Maximum Use of Larex Arabinogalactan In the Food Categories Listed by the US FDA At 21 CFR §170.3 (n)

	Food Categories	Realistic Percentage of Use in the US Market*
01	Baked Goods, Baking Mixes	1.72%
02	Beverages alcoholic	0
03	Beverages and beverage bases, non-alcoholic	2.0%
04	Breakfast Cereals	1.7%
05	Cheeses	1.0%
06	Chewing Gum	0.278%
07	Coffee and tea**	2.0%
08	Condiments and relishes	5.65%
09	Confections and frostings	3.16%
10	Dairy product analogs	0
11	Egg Products	0.5%
12	Fats and oils	1.0%
13	Fish Products	0
14	Fresh eggs	0
15	Fresh fish	0
16	Fresh fruits and fruit juices	4.0%
17	Fresh Meats	0.2%
18	Fresh poultry	0.2
19	Fresh vegetables	0
20	Frozen dairy desserts and mixes	0.313%
21	Fruit and water ices	2.0%
22	Gelatins, puddings and fillings	1.5%
23	Grain products and pastas	0.166%
24	Gravies and Sauces	0.25%
25	Hard candy	2.72%
26	Herbs, seeds, spices, seasonings, blends	20.0%
27	Jams and jellies, home prepared	0
28	Jams and jellies commercial	5.0%
29	Meat products	0.2%
30	Milk, whole and skim	0
31	Milk products	1.5%
32	Nuts and Nut products	0
33	Plant protein products	0
34	Poultry products	0.2%
35	Processed fruits and fruit juices	4.0%
36	Processed vegetables and vegetable juices	0.214%
37	Snack foods	0.5%
38	Soft Candy	2.72%
39	Soups, home prepared	0
40	Soups and soup mixes	1.16%
41	Sugar, white granulated	0
42	Sugar substitutes	16.0%
43	Sweet Sauces, toppings, and syrups	1.25%

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Table 4
USDA Major Food Categories And Corresponding Categories From 21 CFR 170.3 (n)

USDA Food Category[1]	Categories Listed In 170.3 (n)[2]
Total Grain Products	01, 04, 23, 37
Dietary Supplement; Source of Soluble Fiber	
Vegetables and Vegetable Dishes	36
Fruits and Fruit Preparations	16, 35
Milk Desserts/Cheese Products	5, 20, 22, 31,10
Meat, Poultry and Fish	29, 34
Total Fats and Oils	12
Legumes and Legume Dishes	33, 36
Sugars and Sweets	06, 09, 25, 28, 38, 42, 43
Nuts and Seeds	32
Total Non-Alcoholic Beverages (excluding coffee and fruit juices)	16, 35, 03

1. Major food categories are those used in USDA's Continuing Survey of Food Intake by Individuals [Enns, *et al.*, 1997].
2. Column 2 contains the number assigned to the various food categories as listed in 21 CFR § 170.3 (n), which are contained within those reported by Enns, et al., 1997 [Enns, *et al.*, 1997].

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Table 5
Estimated Intake of Arabinogalactan in Selected Food Categories

Major Food Codes	Food Category	Use level (%)	Food Intake (g)	AG Intake (g)	CSFII
	Baked goods, baking mixes				
51	Yeast Breads and Rolls	0.5	50	0.25	1996
52, 55	Quick breads, pancakes, etc.	0.5	0.1	0.694	1996
53	*Cakes, cookies, pastries, pies	3.75	38	1.42	1996
58	Mixtures mainly grain	0.5	107	0.535	1996
	Breakfast cereals				
571 - 574, 578	Ready-to-eat cereals	1.7	17	0.289	1996
	Grain products and pastas				
562	Rice	0	19	0	1996
561	Pasta	0.5	21	0.105	1996
54	Snack foods(crackers, chips)	0.25	12	0.3	1996
	Total Vegetables		132		1996
	Fresh vegetables	0	0	0	1996
	Processed vegetables, juices	0	0	0	1996
	Total Fruits		162		1996
612	Citrus juices	1	59	0.59	1996
621	Dried fruits	0	1	0	1996
641,642	Non-citrus juices and nectars	1	26	0.26	1996
	Fruits and mixtures	0	0	0	1996
	Milk products				
114	Yogurt	4	8	0.32	1996
131,132, 133, 134	Milk desserts	0.725	24	0.174	1996
140-147	Cheese	1	16	0.16	1996
116, 118	Milk-Based Beverages	4	34	1.36	1966
	Meat Products				
252	Sausages, processed meats	0.2	21	0.42	1996
271,272,273,274	Mixtures mainly meat	0.2	99	0.198	1996
	Eggs				
321,323,324, 33,34	Egg Products	0.5	9**	0.045	1996
	Legumes				
	Legumes	0	0	0	1996
	Nuts and Nut Products				
	Nuts and Nut Products	0	0	0	1996
	Fats and Oils				
832	Salad dressings	0.25	8	0.02	1996
	Total sugars and sweets				
917,918	Candy (Soft and Hard)	2.72	7	0.19	1996
911 - 916	Other Sugar Products***	3.16	15	0.474	1996
	Beverages Non-Alcoholic				
	Coffee	0	254	0	1996
923	Teas	2	128	2.56	1996
925	Low Cal.Fruit drinks and ades	2	18	0.36	1996
92400100	Low Cal. Carbonated drinks	2	74	1.48	1996
	Total Intake (grams)			**10.474**	
	Dietary Supplement			**4.5**	

* Includes dietetic bars
** Includes 50% of egg intake for processed eggs
*** Includes Jams, Jellies, etc.

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Short-Term Clinical Studies

Clinical studies at the University of Minnesota with Larex, Inc. arabinogalactan have shown that free-living humans can consume up to 30 grams per day without ill effect or lack of tolerance [Robinson, *et al.*, 1999 Vol. 11 Tab Robinson, 1999]. Recently completed clinical trials in dogs and humans using low dosages, 0.025 and 0.075 g/kg (1.5 and 4.5 grams per day for free-living humans) demonstrated that arabinogalactan was well tolerated and positively effected populations of probiotic bacteria. Stool weight and frequency (dogs), and immune parameters associated with good health also increased [Kim, *et al.*, 1999 Vol. 6 Tab 18, Grieshop, 1999 *In Press* Vol. 6 Tab 21]. Tab D Tab E

see Section IV Safety. pp. 3-38). & Tab C, "GRAS Notice for Eastern Larch"

In order to demonstrate the safety of its product Larex, Inc. sponsored the first controlled human study on arabinogalactan at the University of Minnesota. The University of Minnesota study was designed to test arabinogalactan consumption in free-Living humans. The study design was a controlled crossover study, in which the subjects, (11 males and 11 females) consumed arabinogalactan in a drink mix at levels of either 15 or 30 grams per day. Each subject rotated at three-week intervals through both test levels and a control period, in which no arabinogalactan was taken.

The rationale for using these levels was that 30 grams is the level of dietary fiber recommended by the American Diabetic Association for persons with diabetes. This level has also been correlated with low incidences of heart disease and cancer through various epidemiological studies [ADA, 1995]. The 15 gram level was selected, because that is the level of fiber needed in the US diet to make up the difference between actual consumption levels for most Americans, and the 25 grams per day recommended by FDA.

Results of this study showed the consumption of arabinogalactan aided bowel habit and softened stools. Additionally, the population of anaerobic bacteria in the colons of the free-living human subjects increased after consumption of Larex arabinogalactan by 10X. An increase in total anaerobes in the human colon is considered beneficial because, additional fermentation lowers colonic pH, and increases the production of short chain fatty acids. Lower pH levels are known to protect against colon cancer, and other disorders of the bowel.

Populations of the probiotic bacterium *Lactobacillus* were shown to increase in the human subjects after consumption of Larex arabinogalactan by 3 fold. Toxic ammonia levels went down significantly in

the treatment groups, which follows from an increase in probiotic bacteria. In this study, free-living humans consuming an initial dose of 15g/day of Larex arabinogalactan lowered their population of the pathogenic bacterium *Clostridium perfringens* by 18.5% [Robinson, *et al.,* 1999]

Further, transit time in the gut was slower when arabinogalactan was consumed for both the 15 and 30 gram groups. This is typical for a soluble fiber, and shows that foods, especially sugars are being absorbed more slowly. Slower uptakes of sugars lead to lower spikes of insulin, and less fat deposition [Heller, *et al.,* 1997].

Both treatment groups tolerated the arabinogalactan well, and did not report excessive bloating or other GI distress, even though the dosage in this study was considered to be quite high. The investigator concluded that Larex arabinogalactan might have healthful benefits for humans.

Low Dose Human Studies

While high doses of Larex arabinogalactan had been shown to be safe and efficacious in humans, the question remained: "Could arabinogalactan at low dosages prove just as effective?" In order to answer this question, Larex, Inc. commissioned two pilot studies, one in dogs at the University of Illinois, and one in humans at Southwest College of Naturopathic Medicine in Phoenix (SWC). Both studies were designed to investigate two levels of three different arabinogalactan products. The human arm was designed as a double-blind placebo controlled study, with free-living subjects consuming either 1.5 or 4.5 grams per day of three different Larex arabinogalactan products for 28 days.

The University of Illinois dog study was conducted using a Latin Square design with an internal control and baseline. This meant that every dog received every treatment in a random sequence. Dosage levels of 20 or 60 mg/kg/day were equivalent to the Human SWC study described above. Each of the three products was fed for 10 days at each feeding level.

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E. Nutritional Analysis of Larex Decolorized Arabinogalactan

Five lots of Larex arabinogalactan manufactured over a period of time were analyzed for nutritional components and for caloric content. The results showed that moisture content of the arabinogalactan ranged from 3.10 to 6.20%, and protein content ranged from 0.35 to 0.55%. Fat as determined by acid hydrolysis ranged from 0.00 to 0.50%. Ash content varied from 0.19 to 7.66%. The carbohydrate percentage ranged from 85.34 to 96.01. Total dietary fiber content ranged from 77.80% to 80.24%. Calories in Kcal/100 g ranged from 346.31 to 387.59 [Vol. 6 Tabs 4-5].

It should be noted that these caloric values are considerably different from those reported earlier by the University of Wisconsin Research Foundation, who found that arabinogalactan extracted from Western larch contained about one third the calories of corn starch [Wisconsin Alumni Research Foundation Report, 1963, Vol.10 Tab 11]. This discrepancy is potentially due to the fact that the caloric content of soluble fiber under the US Nutritional Labeling and Education Act (NLEA) regulations of 1990 is, as of the issuance of the regulation, calculated as is any other carbohydrate (4 calories per gram), whereas previously, soluble fiber content was deducted from the total calories contributed by carbohydrates.

Arabinogalactan was found to be relatively low in mineral content, except for potassium levels which ranged from 0.033% to 3.93% [Ingman Laboratories Data, 1997, Vol. 6 Tab 9].

Calcium levels ranged from 0.01% to 0.056%, and sodium levels ranged from 0.05% to 0.37%. Iron values ranged from 0.00058 to 0.0085%. Zinc values ranged from 0.0002 to 0.0012, and magnesium levels ranged from 0.005 to 0.018%.

Table 6: Test Results for Sugar Components Of Larex Decolorized Arabinogalactan (5 Lots)

Five lots of Larex decolorized arabinogalactan products manufactured over a period of time were analyzed for four common sugars included in a sugar profile [Ingman Laboratories, Analytical Data, 1997, Vol. 6 Tab 9]. All samples showed that glucose, sucrose, maltose, and fructose were absent or non-detectable.

Table 6
Test Results on Sugar Components of Larex Decolorized Arabinogalactan
(5 Lots)
(Ingman Laboratories, Minneapolis, Minnesota)

Lot Number	Sugar Profile	Glucose	Sucrose	Maltose	Fructose
(Date of Manufacture)		(%)	(%)	(%)	(%)
36	N.D.	0.00	0.00	0.00	0.00
(7/15/97)					
38	N.D.	0.00	0.00	0.00	0.00
(7/15/97)					
0034-97065-01	N.D.	0.00	0.00	0.00	0.00
(3/18/97)					
2F097 201	N.D.	0.00	0.00	0.00	0.00
(8/29/97)					
29	N.D.	0.00	0.00	0.00	0.00
(7/15/97)					
(N.D. = None Detectable)					

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Table 7: Fatty Acid Analysis of Arabinogalactan

Three arabinogalactan products from either Eastern or Western larch were analyzed for fatty acid content by Medallion Laboratories in Minneapolis, Minnesota [Medallion Laboratories, Analytical Data, 2000, EL Tab Raw Data EL Vol 1]. The total fat of all samples measured by Medallion Laboratories, via acid hydrolysis, was less than 0.05%. Of the total fatty acids present, 0% were saturated fatty acids, 0.01% were monounsaturated fatty acids in a non-decolorized product, and 0% were polyunsaturated fatty acids (See Table 8 below). The major fatty acid constituents were oleic, 100.0%, for the Western larch pressate, trans Elaidic (40%) for the Eastern larch pressate, and oleic, 42.9%, for a product produced by a method developed by a former manufacturer.

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Table 7

Fat Analysis
Eastern and Western Larch

	Western Larch	Eastern Larch	Stractan 10
Sample Identification	***G100WL***	***G100EL***	***AC9***
Total Fat	0.00%	0.01%	0.02%
Saturated Fat	0.00%	0.00%	0.00%
Monounsaturated Fat	0.00%	0.00%	0.01%
Cis-Cis Polyunsaturated Fat	0.00%	0.00%	0.00%
Trans Fat	0.00%	0.00%	0.00%

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Table 8

Fatty Acid Profile
Percent of Fatty Acid Components Based on Total Fat

	G100WL Normalized by Weight	G100WL % (w/w) as Triglyceride in Product	G100EL Normalized by Weight	G100EL % (w/w) as Trigylceride in Product	AC9 Normalized by Weight	AC9 % (w/w) as Triglyceride in Product
Stearic					4.76%	0.001
Trans Elaidic			40.00%	0.002	4.76%	0.001
Oleic	100.00%	0.001	20.00%	0.001	42.86%	0.009
t-Octadecadienoic					4.76%	0.001
Linoleic				0.001	14.29%	0.003
Behenic			20.00%		4.76%	0.001
Palmitic				0.001	14.29%	0.003
Palmitoleic			20.00%		9.52%	0.002

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F. Functionality

The functional use of Larex, Inc. arabinogalactan is expected to be that of a prebiotic soluble fiber. As such it will be used to increase the daily intake of soluble fiber, and thereby increase the populations of probiotic bacteria in the colon. As stated above Larex, Inc. arabinogalactan has been shown to be effective in increasing desirable bacteria populations when consumed at levels between 1.5 and 4.5 grams per day.

The most frequent probiotic bacterial populations measured include those within the genera *Lactobacillus* and *Bifidus*. A number of independent researchers have investigated the effect of arabinogalactan on colonic populations of species within those groups. The following paragraphs provide a brief review of those studies.

Arabinogalactan Fermentation by Human Fecal Bacteria

As evidence of the long association of humans with arabinogalactans, Mazur, *et al.*, 1993, found that arabinogalactans were fermentable by human colonic flora [Mazur, *et al.*, 1993, Vol. 11 Tab 8]. According to the authors, arabinogalactans belong to a group of carbohydrates, which are classified as slowly fermentable soluble carbohydrates [Mazur, *et al.*, 1993, Vol. 11 Tab 8]. The author stated that all of the compounds for which the character of the anomeric or C-1 carbon was the only structural variable, such as L-galactose, maltitol, and polydextrose were metabolized by human colonic flora although at different relative rates. Their study found that highly branched oligo and polysaccharides exhibited consistently lower rates of metabolism than did linear, tetra, penta, or polysaccharides. The authors concluded that the character of the anomeric center or C-1 carbon had no significant effect on metabolism. The factor effecting the rate of metabolism of these compounds appeared to be the branching of oligo and polysaccharides. This suggests, according to the authors, that the branching may hinder access of glycosidases to glycosidic linkages [Mazur *et al.*, 1993 Vol. 11 Tab 8].

A study by Englyst, *et al.*, 1987, investigated the polysaccharide-degrading activity in different fractions of human feces, and found that non-starchy polysaccharides, including pectin, xylan, and arabinogalactan were fermented by mixed populations of human fecal bacteria in batched culture [Englyst, *et al.*, 1987, Vol. 10 Tab 12]. Detailed carbohydrate analysis demonstrated that starch and pectin were the most rapidly degraded substrates, and that arabinogalactan and xylan were broken down more slowly. Free sugars and oligosaccharides did not accumulate in the culture media with any of the polysaccharides tested. Time course

measurements of the polysaccharides remaining in the batch culture fermentation showed that the arabino side chains of pectin, xylan, and arabinogalactan were co-utilized with the backbone sugars. In these cultures polysaccharide-degrading activity was mainly cell associated, but extra cellular polysaccharidase activity increased as the various fermentations progressed. Molar ratios of the short-chain fatty acids including acetate, propionate, and butyrate produced by the fecal bacteria were dependent on the polysaccharide tested. The authors found that molar ratios of acetate, propionate, and butyrate in the starch, arabinogalactan, xylan, and pectin fermentations were 50:22:29, 50:42:8, 82:15:3, and 84:14:2 respectively. Importantly, the presence of starch did not inhibit the breakdown of arabinogalactan, xylan, or pectin by fecal bacteria.

The importance of the large gut fermentation in human physiology may be considerable according to the authors, since volatile fatty acids are sufficiently absorbed from the colon to provide a substantial proportion of the energy obtained by the host [Englyst, *et al.*, 1987, Vol. 10 Tab 12]. Volatile fatty acids also stimulate salt and water absorption, and are important components of intermediary metabolism in the colonic epithelium, since butyrate is the principal fuel for colonocytes. In the liver, propionate may influence gluconeogenesis and cholesterologenesis. Carbohydrate fermentation by the intestinal microflora can therefore be regarded as an important process controlling many aspects of large bowel function in man and may, through volatile fatty acids be a contributory factor in the control of hepatic and peripheral tissue metabolism.

In this study fresh feces from five individuals who had been eating normal western diets were suspended in anaerobic sodium phosphate buffer at pH 6.5 to produce 10% w/v fecal slurries [Englyst *et al.*, 1987, Vol. 10 Tab 12]. Each slurry was then centrifuged to yield a washed bacterial cell fraction, a particulate fraction, and a cell-free supernatant fraction.

To test polysaccharide fermentation by mixed bacterial populations, fecal samples were obtained from two individuals, and washed fecal bacteria cells were prepared as described above [Englyst, *et al.*, 1987 Vol. 10 Tab 12]. Batch cultures of these fecal bacteria were grown anaerobically in one liter fermenters on mineral salt medium with either starch, xylan, arabinogalactan, or pectin, all included at 10 mg/ml as the sole fermentable carbon source. Culture pH was 6.5 and was controlled using a Modular Fermenter pH Controller. The authors indicated that arabinogalactan was chosen as a non-starch polysaccharide because it is found in most plant tissues and is water-soluble. The rate of hydrolysis of the polysaccharide was found to be the rate-limiting step in the utilization

of arabinogalactan [Englyst, *et al.*, 1987 Vol. 10 Tab 12]. Results showed that colonic bacteria simultaneously degraded the arabino side chains and galactose backbone of arabinogalactan.

In a study by Crociani, *et al.*, 1994, the authors confirmed that arabinogalactan is degraded by 17 out of 20 strains of *Bifidobacterium longus* [Crociani, *et al.*, 1994,Vol. 10 Tab 8]. In the Englyst study about 43% of the polysaccharide fermented was recovered as volatile fatty acids. Molar ratios of acetate (50), propionate (42), and butyrate (8) differed from those that occurred in the starch fermentation in that more propionate and comparatively less acetate and butyrate were produced. Arabinogalactan-degrading activity was not detectable at zero time in the washed cells from either fecal sample, but cell-associated activity was found in samples taken at 3 hours. The rapid appearance of arabinogalactan-degrading activity would suggest that enzymes were being induced in bacteria already present in high numbers in the culture, rather than that selection of arabinogalactan-degrading populations had occurred. These data illustrated the nutritional versatility of colonic bacteria, which enables organisms to rapidly synthesize inducible catabolic enzyme systems, thereby enabling them to quickly adapt to the presence of new substrates. These studies also showed that mixed cultures of fecal bacteria fermented pectin, xylan, and arabinogalactan concomitantly with starch, and in fact, starch appeared, according to the authors, to stimulate arabinogalactan and xylan breakdown indicating that while catabolite control of metabolism may be important to individual bacterial species, it does not appear to significantly inhibit polysaccharide breakdown by mixed bacterial populations.

In a study published in 1981 by Salyers, *et al.*, continuous cultures were used in order to approximate some of the conditions encountered by colon bacteria in their normal habitats. Larchwood arabinogalactan was chosen as the carbohydrate for this study, because it is soluble in water, is commercially available, and has a complex branched structure comparable to that of many other plant cell wall polysaccharides. The author, citing a publication by Whistler and Richards, from 1972 [Salyers, *et al.*, 1981 Vol. 11 Tab 12], stated that Larchwood arabinogalactan was a mixture of two component polysaccharides, one having a molecular weight of around 160,000 and the other having a molecular weight of around 100,000. Both components were characterized by a β(1,3)-linked galactan backbone, with side chains containing galactose and arabinose.

Bacteroides thetaiotaomicron, one of the major species in the colonic micro flora, is able to ferment a variety of polysaccharides including arabinogalactan. In this study arabinogalactan was growth limiting, and

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the rate at which the medium was supplied determined the growth rate of the organisms in the vessel. To determine the effect of very slow growth rates, the authors equilibrated bacteria at steady-state growth rates of 3.5, 6.3, 11.6, and 27.7 hours per generation. At each growth rate the bacterial yield was determined and used as an indication of how efficiently the bacteria were using the arabinogalactan.

The ability of the bacteria to produce enzymes for degrading a new carbohydrate source was determined by measuring the rate at which a-glucosidase, an inducible enzyme, was produced when bacteria were exposed to maltose. Finally, the spent medium was analyzed to determine whether any undegraded portions of the arabinogalactan remained, and if so, what proportion of the original compound was comprised of this residual carbohydrate. Accordingly, the authors measured the amount of arabinogalactan which was utilized at growth rates ranging from 1.3 to 3.5 hr/generation and compared the results with those obtained at similar growth rates when galactose was the substrate. The transit time of most materials through the colon was known to be around 30-40 hours, thus, the stable bacterial mass which accounts for nearly 1/3 of the volume of colon contents can replace itself only once during this time [Salyers, *et al.*, 1981, Vol. 11 Tab 12]. However, the authors stated, that even these growth rates were likely to be much slower than the growth rates of 30 minutes to 2 hours per generation, which are customarily used in most *in vitro* systems by microbiologists. Growth rates of the organisms tested were found to be on the order of 10 hours per generation or longer.

To determine residual arabinogalactan in this experiment, medium, either spent or uninnoculated, was passed over a 1 x 3 cm column of Amberlite MB-3 mixed bed rosin and then neutralized with sodium hydroxide. Since the concentrations of residual carbohydrate in the spent medium were relatively low, spent medium was concentrated 10 fold by flash evaporation (30°C) after treatment with Amberlite. Portions (1ml) of this concentrated de-salted sample were chromatographed on a 1.5 x 85-cm column of Bio-Gel A 0.0 m with distilled water as the eluent. The flow rate was 20 cm per hour and fractions of 1.5 ml were collected. The concentration of carbohydrate in each fraction was determined by phenol-sulfuric assay [Salyers, *et al.*, 1981, Vol. 11 Tab 12].

When arabinogalactan in uninnoculated medium was taken through the de-salting and concentration procedures outlined above, its chromatographic profile was identical to that of arabinogalactan placed directly on the column without pre-treatment. Thus, the desalting and evaporation steps did not appear to alter the arabinogalactan. Results of

this investigation showed that the concentration of viable bacteria in the culture vessel (1.7 to 1.9 x 10^9 per ml) was the same at all dilution rates. Viability was approximately 100% since the total concentration of bacteria as determined using a counting chamber was 1.8 x 10^9 per ml. Although the concentration of bacteria did not change significantly with dilution rate, the dry weight of the bacteria or equivalently, the growth yield decreased as the dilution rate decreased (below 0.11 per hour) [Salyers, *et al.*, 1981, Vol. 11 Tab 12]. The growth yield in the study was computed by expressing the amount of arabinogalactan utilized as moles of equivalent galactose. This, the authors suggested, was reasonable since arabinose accounts for less than 10% of the total sugar in arabinogalactan. Total cellular carbohydrate declined, as growth rates became slower. The authors stated that this might reflect a decrease in storage polysaccharides [Salyers, *et al.*, 1981, Vol. 11 Tab 12].

The authors stated that to their knowledge, this study represented the first attempt to investigate polysaccharide breakdown by anaerobic bacteria growing in continuous culture, and that several conclusions could be drawn from the results. First, growth yields on arabinogalactan were quite high, in fact, they were comparable to yields obtainable when a monosaccharide such as glucose or galactose was the substrate. The affinity of *B. theataiotaomicron* for arabinogalactan was much lower than its affinity for a monosaccharide such as galactose, as indicated by the fact that the concentration of unutilized substrate increased much more rapidly with increasing growth rates when arabinogalactan was the substrate than when galactose was the substrate. When *Bacteroides* species degrade arabinoxylans as an example, a variety of fragments from disaccharides to much higher oligomers is left behind in the medium. Thus, when dietary fiber components are degraded by colonic bacteria, the original set of polysaccharides may be replaced by a different mixture of polysaccharides and oligomers, and the properties of this new mixture, for instance the ability to bind ions or toxic substances, may be very different from the properties of the original mixture. In conclusion, the ability of the organisms tested to produce the enzymes necessary to enable them to switch to a new carbohydrate source was significantly decreased when generation times were long [Salyers, *et al.*, 1981, Vol. 11 Tab 12].

The objectives of a study by Macfarlane *et al.*, 1995, were to investigate whether *Bifidobacterium adolescentis* was able to compete in carbon-limited continuous culture with *Bacteroides sp.* for the plant cell wall polysaccharide arabinogalactan, and to determine the effects of pH and growth rate on interactions between these organisms, since they are important environmental and physiological variables of the colon

[Macfarlane, *et al.*, 1995, Vol. 11 Tab 7]. Measurements of residual carbohydrate in pure and mixed culture chemostats showed that the *Bacteroides* species extensively digested the galactose back bone of the polymer, and to a lesser degree the arabinose side chains. Nevertheless, arabinose monomers and oligosaccharides accumulated in these cultures under all growth conditions. *Bifidobacterium* utilized considerably less arabinogalactan than did *Bacteroides*, as was reflected in the mixed culture studies. These experiments demonstrated that *Bacteroides thetaiotaomicron* was able to compete more successfully for this cell wall polysaccharide than *Bifidobacterium adolescentis* under nutritional, physiological, and environmental conditions broadly similar to those encountered in the human colon. The study indicated the existence of synergistic interactions between the two organisms that were growth rate dependent [Macfarlane, *et al.*, 1995, Vol. 11 Tab 7].

For bacteria in the human large intestine, the principal sources of carbon and energy are starches and plant cell wall polysaccharides that escape digestion in the small bowel. These substances are thought to be depolymerized by a relatively restrictive group of anaerobic bacteria, chief among them, the *Bacteroides* and the *Bifidobacteria*. According to the authors, these species were known to account for up to 30% and 25% respectively of the total cultivable gut flora in adults, with typical cell population densities in the region of 10^{11} per gram of dry weight of gut contents [Macfarlane, *et al.*, 1995, Vol. 11 Tab 7].

Ultimately, the host benefits in a variety of ways from the actions of these bacteria, including competitive exclusion of invading pathogens, and increased energy gain from short-chain fatty acid production. Results of this study showed that at low specific growth rates, cell population densities of *Bacteroides* were several orders of magnitude higher than those of *Bifidobacterium* irrespective of culture pH. However, bifidobacterial numbers increased contingent with dilution rate, and in chemostats operated at pH 6.5 these organisms predominated at dilution rates above 0.24 per hour. Both species begin to wash out of the culture vessels only when the highest dilution rate (D=0.38/hour) and a pH 5.0 medium was used. Neither *B. thetaiotaomicron* nor *B. adolescentis* were able to completely break down the polysaccharide, although digestion of the substrates was more comprehensive in the *Bacteroides* cultures. Free arabinose and arabinose-containing oligosaccharide with a degree of polymerization (d.p. < 10) accumulated in both cultures, but galactose was only present in the polymerized form [Macfarlane, *et al.*, 1995, Vol. 11 Tab 7].

The authors stated that characteristically, polysaccharide utilization is

most extensive at low dilution rates. The proximal colon is a region of active fermentation and low pH (approximately 5.0 to 5.5), which receives substrates from the small gut [Macfarlane, *et al.*, 1995, Vol. 11 Tab 7]. In contrast, readily fermentable carbohydrates become depleted by the time digestive materials reach the distal bowel where gut pH rises and begins to approach neutrality as amino acid fermentation becomes increasingly significant. In this study ecological interactions between the two bacteria were investigated during growth on larch arabinogalactan. Results in their laboratory showed that approximately 88% of the arabinogalactan consisted of galactose with arabinose making up most of the remainder. Both the *Bifidobacterium* and *Bacteroides* species were able to utilize the arabinogalactan backbone and side chain sugars. However, arabinose uptake was found to be catabolite repressed by galactose in *B. adolescentis,* while *B. thetaiotaomicron* was able to assimilate both sugars concomitantly.

Results showed that the substrate was broken down most extensively by *B. thetaiotaomicron* particularly at low specific growth rates. However, although the chemostats were carbon-limited, substantial amounts of substrate remained at all dilution rates, showing that large parts of the molecule were not degraded by bacterial glycoside hydrolyses. The arabino side chains were particularly recalcitrant to hydrolysis by *Bifidobacterium* and *Bacteroides* glycosidases. Jeraci *et al.*, 1993, has indicated that arabinogalactan is partially fermentable, and its ability to be fermented varies with the subject being tested [Jeraci, *et al.*, 1993, Vol. 11 Tab 2]. Nevertheless, *B. thetaiotaomicron* adapted more effectively to growing at lower pH under more nutrient-limited conditions than *B. adolescentis* which competed most successively at higher pH values at high specific growth rates. The complex nature of the commercial arabinogalactan preparation used, and its bacterial hydrolysis products, effectively meant that in this study as in the large bowel, the bacteria were not growing on a single fermentable carbon source, but were growing under multi-substrate-limited conditions which enabled *B. adolescentis* to survive in this system, albeit at very low cell populations [Macfarlane, *et al.*, 1995, Vol. 11 Tab 7].

The authors concluded that bacterial interactions other than straightforward competition were observed in these experiments. Results indicted that *B. thetaiotaomicron* populations were stimulated in the presence of *B. adolescentis* at low dilution rates in pH 6.5 medium, but were partly repressed at high specific growth rates, or *Bacteroides* was not as competitive as *Bifidobacterium.* The authors posed the possibility that the *Bacteroides* were inhibited by low molecular-weight toxic substances produced by *B. adolescentis.* Both organisms produced cell-

associated arabinogalactan degrading enzymes (β-galactosidase, α-arabinofuranosidase), but regulation of enzyme synthesis differed markedly in these bacteria. These enzymes were maximal at high specific growth rates, but residual carbohydrate measurements showed that the enhanced competitiveness of *B. adolescentis* under high growth rate circumstances could not be attributed to increased synthesis of depolymerizing enzymes, since the majority of substrate was utilized at low dilution rates [Macfarlane, *et al.*, 1995, Vol. 11 Tab 7].

Vince *et al.*, 1990 reported that they had developed an *in vitro* method of stool incubation, which could be used to study the metabolic activity of intestinal bacteria. In their system the disaccharide lactulose was metabolized with the production of large amounts of short-chain fatty acids and a marked decrease in bacterial formation of ammonia. The author stated that these processes had both nutritional and therapeutic implications for the host, since short-chain fatty acids were absorbed rapidly through the colonic mucosa. A decrease in the production and absorption of ammonia was probably the major factor in the ability of lactulose to lower the blood ammonia concentration of patients with porto-systemic encephalopathy. They stated that similar effects might be expected from the carbohydrates of plant fiber which are known to be digested to varying degrees by the large bowel flora. The aim of their study was to compare the influence of four carbohydrates, lactulose, pectin, arabinogalactan, and cellulose on the production of short-chain fatty acids and ammonia. They proposed to determine whether previous exposure to increased amounts of these substrates significantly altered bacterial metabolism in the gut [Vince *et al.*, 1990, Vol. 11 Tab 15].

Fecal samples were obtained from subjects before and after they had ingested more than twice the normal dietary amounts of each substance. For this study five healthy subjects age 27-56 years and weighing 44-83 kg were studied, first while taking their usual diets, and later while eating their usual diets with the addition of the various carbohydrates listed above. Carbohydrates were taken in the order chosen by the subject with a minimum period of one month between the ingestion of each substance. Pectin and cellulose were taken in a dose of 0.3 g/kg per day, and lactulose and arabinogalactan were taken in a dose of 0.8 g/kg per day. These doses represented more than twice the amount of these carbohydrates likely to be present in the normal diet. Dietary additions were taken in three or four doses daily. Side effects were reported as minimal, consisting of wind, and particularly with cellulose, a sensation of fullness. For the fermentation studies, five 10 -100 ml portions of fecal homogenate were place in incubation vessels, and 4 vessels were supplemented with 10 g/l of either lactulose, cellulose, arabinogalactan, or

pectin [Vince *et al.*, 1990, Vol. 11 Tab 15].

Two subjects consumed their extra dietary carbohydrates for 14 days, with their feces being studied after days 2, 7, or 14. Because the observed effects of short-chain fatty acid production and ammonia metabolism were no greater after day 7or 14 than after day 2, the remaining subjects were studied only after the second day. During the first 48 hours of incubation of supplemented samples, lactulose, pectin and arabinogalactan formed amounts of organic anion which were more than twice those of their non-supplemented paired controls, but the quantity the organic anions generated did not differ significantly between these three carbohydrates. All three short-chain fatty acids increased markedly during incubation regardless of the experimental protocol used. However, acetate was the major short-chain fatty acid in all incubates at all times [Vince *et al.*, 1990, Vol. 11 Tab 15].

When additional carbohydrate had not been eaten, propionate and butyrate were present in approximately equal concentrations at zero hours and 48 hours as well as in the unsupplemented control incubates. After excess carbohydrate had been eaten, butyrate exceeded propionate in all incubates, both unsupplemented and supplemented at zero and 48 hours [Vince *et al.*, 1990, Vol. 11 Tab 15].

The concentration of ammonia increased steadily during incubation, in all samples except those supplemented with lactulose. In that sample ammonia concentrations fell before rising. Results from unsupplemented control incubates showed that ingestion of extra carbohydrates did not reduce net ammonia generation significantly at any of the individual sampling times, but, when 0, 1.5, and 3 hour results were pooled, significantly less ammonia was generated in incubates from subjects who had eaten lactulose or arabinogalactan. No such effect occurred in incubates from subjects who had eaten pectin or cellulose. In fact, pectin was the only substrate, which on ingestion significantly increased net ammonia generation. The author stated that these results were probably due to the increased excretion of fecal nitrogen by subjects consuming pectin. When the same values were expressed as percentages of total nitrogen converted to ammonia, the quantity of ammonia generated was less [Vince *et al.*, 1990, Vol. 11 Tab 15].

At the start of incubation the mean pH values of each group of fecal homogenates were in the range of 6.9 to 7.0 except when subjects had consumed either lactulose or arabinogalactan in which case mean pH values were 6.4 and 6.4 respectively. Of the four carbohydrates added to the fecal incubation system, only lactulose, pectin, and arabinogalactan

were all fermented vigorously with the production of very similar amounts of short-chain fatty acids within 48 hours. In their system the authors reported that the concentration of lactulose used was completely metabolized within 6 hours, and so the similar yield of short-chain fatty acids from pectin and arabinogalactan strongly suggested virtually complete fermentation of those three carbohydrates within 48 hours [Vince *et al.*, 1990, Vol. 11 Tab 15].

In their study the butyrate concentration usually exceeded that for propionate, especially after addition of fermentable carbohydrate. The authors proposed that if similar changes occurred in the intact large intestine after ingestion of fermentable carbohydrates, these might be of clinical benefit because butyrate is an important metabolic fuel for colon epithelial cells. Lactulose, pectin, and arabinogalactan each slowed the rate at which ammonia was generated in fecal incubates. The author stated that pectin and arabinogalactan were normal dietary constituents being present as structural polysaccharides in plant cell walls, and that small amounts of lactulose are found in food as a result of conversion from lactose during heating. The authors stated that bacteria in the intestine are therefor frequently exposed to these substrates, and have developed the ability to induce or inhibit the enzyme required for their metabolism fairly rapidly. The authors concluded that arabinogalactan might have some value in the treatment of porto-systemic encephalopathy since it will tend to lower ammonia absorption without the drastic purgation which might accompany the use of lactulose. As a dietary supplement pectin increases ammonia generation by fecal incubates probably because it increases the ileocaecal flow of total nitrogen [Vince *et al.*, 1990, Vol. 11 Tab 15].

Comparison of Arabinogalactan from Eastern and Western Larch in an Ink Model

A study was conducted by Flexotech to compare the effects of Western larch and Eastern larch when postadded to a red waterbased ink. A second objective of the study was to investigate pigment reduction effects with arabinogalactan formulated into cyan ink. These studies demonstrate the ability of arabinogalactan to effect viscosity and dispersion in a complex mixture, such as food.

Eight inks with postadded arabinogalactan were prepared: four with Western larch, and four with Eastern larch. The levels of arabinogalactan addition were 2, 6, 10, and 20%. Red ink was diluted with equal amounts of water served as a control.

In a high quality process cyan ink formula, the effect of reducing pigment

loading was evaluated in arabinogalactan-containing formulas for both Eastern larch and Western larch-based arabinogalactan.

Results showed that there were no major visual differences between arabinogalactan products from Western larch and Eastern larch when each was postadded red ink. The investigator concluded that, the products were equivalent, based on their ability to improve ink flowability and pigment dispersion. The investigator noted that, the Western larch arabinogalactan solution was more viscous than the Eastern larch solution, which made the Western larch-containing inks slightly higher in color density than the Eastern larch-containing inks. However, this difference in color did not affect the ink viscosities to any substantial degree.

Color strength was reported to be acceptably good at 2% arabinogalactan addition (4% reduction level in ink). Color intensity began to decrease at the 6% (12% reduction level) level for both arabinogalactans. Of interest, was the fact that at the 2% arabinogalactan level, the inks were much less viscous than ink to which no arabinogalactan had been added, yet the inks were similar in color strength to a standard ink. Higher levels of arabinogalactan addition (10 and 20%) produced inks that were lower in viscosity than either the starting ink or the arabinogalactan solution. The investigator stated that this would account in part, for the lower color density noted vs. the standard ink.

The indirect advantage of this result was that arabinogalactan solutions could be used as ink viscosity suppressants, allowing for the inclusion of higher polymer solids in the formula. The inks to which the water was added suffered from a severe viscosity reduction effect. Color loss was very extreme compared with the inks to which arabinogalactan was added.

In a second part of the study, Eastern larch and Western larch arabinogalactan were postadded at the same levels as above to a cyan ink (BCM 63A) prepared from a pigment chip. In this case, addition of arabinogalactan at all levels caused color loss. This formula was reported to be significantly different in its preparation than the red ink used above. The indication here was that benefits from arabinogalactan addition will be formulation-dependent. Once again, no significant differences between Western larch and Eastern larch were noted in this evaluation.

Lastly, both the Eastern larch and Western larch arabinogalactan were formulated (at the 4% addition level) into inks that had reduced pigment loadings. In an ink with 12% pigment reduction and equal polymer solids

to the standard, a higher color density ink was produced (LRX 22097-6), but it was also higher in viscosity than the standard. The other inks prepared were apparently too low in pigment loading and printed too weak, despite (in one case) equal viscosity to the standard (LRX 31397-1). The formula required additional optimization, but the author hypothesized that pigment could be reduced at least 12% but less than 16 %; with proper water and polymer balance, the formula would have acceptable color density versus standard inks at this level of arabinogalactan addition.

The conclusion from the studies was that arabinogalactans from Western and Eastern larch appeared to perform similarly in waterbased flexographic printing inks. Both the Western larch and Eastern larch products provided a synergistic viscosity reduction benefit when postadded to a red ink at higher levels. This effect has the indirect benefit of providing a formulator the latitude of incorporating higher polymer solids. The performance of either the Western larch or Eastern larch-derived arabinogalactan as a postadded ingredient was dependent on its interaction with other formula components. Therefore, it will provide color strength and viscosity benefits in some formulas and not in others. It appears that the greatest degree of benefit may occur in formulas that are prepared from high pigment to binder ratio pigment dispersions (i.e. inks that have an inherent instability). More stable formulas seemed to be subject to color and viscosity reduction effects induced by an arabinogalactan solution, however. Pigment reduction in inks in which arabinogalactan is formulated requires an adjustment in polymer loading.

G. Summary

Larex, Inc. has provided information from published peer-reviewed studies, which shows that arabinogalactan has been a natural component of the human diet, and that there is an arabinogalactan-degrading activity in the human colon, which derives from indigenous bacterial enzymes. Further, the data illustrated the nutritional versatility of colonic bacteria. These organisms are able to rapidly synthesize inducible catabolic enzyme systems, thereby enabling them to quickly adapt to the presence of new substrates. Published studies also showed that mixed cultures of fecal bacteria fermented pectin, xylan, and arabinogalactan concomitantly with starch, and in fact, starch appeared to stimulate arabinogalactan breakdown, indicating, that while catabolite control of metabolism may be important to individual bacterial species, it does not appear to significantly inhibit polysaccharide breakdown by mixed bacterial populations. This information would indicate that other common carbohydrate food sources would not inhibit the colonic degradation of arabinogalactan.

Larex, Inc. has also provided data from more recent studies in which larch arabinogalactan was administered to humans and dogs. These studies showed that low levels of larch arabinogalactan as a fiber supplement (from 1.5 to 4.5 grams per day) facilitated an increase in beneficial bacterial populations in the mammalian colon, and improved stool frequency.

Based on these results, Larex, Inc. submits that its arabinogalactan products would provide a valuable addition to the diet.

Section III
Analytical Method

000118

Section III: Analytical Method for Arabinogalactan

A. Summary

Arabinogalactan can be meaningfully characterized by molecular weight. The current method used by Larex, Inc. to determine the molecular weight of arabinogalactan is based on High Pressure Liquid Chromatography-Gel Permeation Chromatography (HPLC-GPC). A method is presented for the molecular weight determination at TAB 1. The method is based on the use of a Shodex KB-804 column made up of a cross-linked polyhydroxymethacrylate gel. The column dimensions are 8 mm I.D. x 300 mm. The column is supplied by Waters Corporation, and a Waters Millennium software system is used with the instrument. The eluent used is 0.05 M. $NaNO_3$, the flow rate is 1.0 ml/minute, and the sample concentration is 1% (w/v). The injection volume is 40 µl, and the detector used is a differential refractometer. The temperature of operation is ambient. The standards used for calibration are pullulans (molecular weights: 5800, 12200, 23700, 48000, 100000, and 186000 daltons). Retention time for arabinogalactan under the conditions listed above is 7.9 minutes.

The average molecular weight of arabinogalactan, or any other material can be described by the number average, the weight average, or the z average. These averages are numerically equal to each other only for an absolutely monodisperse material. For arabinogalactan, Larex typically finds all three averages in the range from 15000 - 20000 Daltons [VOL. 9 TAB 1]. The column described above must be calibrated using several well-characterized standards of a similar polymer with known molecular weights and very low polydispersities. Larex calculates polydispersitiy (Mw/Mn = dp) in the range of from 1.1 to 1.4 for refined grades of arabinogalactan. Pullulan standards are used for comparison in this system. After calibration the elution time from the column is correlated to molecular weights. The software (Millennium from Waters Corporation) calculates the molecular weight averages from the peak shape using this correlation. The standards used for calibration cover the molecular weight range of 5000 to 40000. Outside of this range, the apparent molecular weights are less reliable indications of the actual size of the molecules in the sample. In general, the correlation between molecular weight and elution time is inverse logarithmic (i.e. elution time decreases as the log of the molecular weight increases). With the Shodex column, a change in elution time of 45 seconds corresponds to 10-fold change in the molecular weight. At extremes of the chromatographic peak, it is possible to calculate the molecular weight, but for the reasons mentioned above, it is

unlikely that there are molecules in the sample that have the calculated extreme molecular weights. Additionally, the chromatographic dispersion produces peaks with a finite width, even from a monodisperse sample. The chromatographic dispersion from the Shodex columns is at least one minute (full width at half maximum) which corresponds to much of the apparent distribution of molecular weights.

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Section IV
Safety

000121

Section IV: Arabinogalactan from Eastern Larch: Its Safety when Consumed as an Ingredient in Processed Foods

A. Introduction and Description

Larex, Inc. of White Bear Lake, Minnesota manufactures, as an example, an arabinogalactan product, LSC-1, which is produced under cGMP in a state and federally inspected facility in Cohasset, Minnesota. The arabinogalactan products manufactured by Larex, Inc. and formulations thereof, are suitable for use in human foods, based on their production in a food grade inspected plant, and on the fact that numerous articles in the scientific literature and studies conducted by Larex, Inc. have indicated that arabinogalactan preparations are suitable for human consumption.

Arabinogalactan Description

Arabinogalactans belong to a major carbohydrate group called hemicelluloses which constitute 20-30% of woody plants [Whistler, *et al.*, Vol. 11 Tab 16]. Hemicelluloses are polysaccharides found in higher land plants. They are abundant in the primary and secondary cell walls of plants that have undergone lignification. Within the cell walls they form an aqueous gel in which bundles of cellulose microfibrils are embedded in regular or irregular orientations. Hemicelluloses characteristically dissolve in alkali, whereas cellulose fibers do not [Wilke, 1983, Vol. 12 Tab 17].

Hemicelluloses are generally classified according to the types of sugar residues present. The sugar units most often found in hemicelluloses are D-xylose, D-mannose, D-glucose, D-galactose, L-arabinose, 4-O-methyl-D-glucuronic acid, D-galacturonic acid and D-glucuronic acid [Wilkie, 1983, Vol. 12 Tab 17]. Isolated arabinogalactan has been reported to be composed of a major, highly branched b galactan fraction with a molecular weight of approximately 100,000 and a minor b galactan fraction with a molecular weight of approximately 16,000 in a 4:1 ratio.

Arabinogalactans as constituents of hemicellulose have been consumed by man in many plant foods including, fruits, wheat flour, and woody plant exudates [D'Appolonia, 1980, Vol. 10 Tab 10 and Chen, *et al.*, 1994, Vol. 10 Tab 5]. Familiar exudates include maple syrup, and gums such as arabic, karaya, tragacanth, and ghatti [BeMiller, 1989, Vol. 10 Tab 2, D'Appolonia, 1980, Vol. 10 Tab 10 and Wilke, 1983, Vol. 10 Tab 17].

Therefore, man has had a long history of arabinogalactan consumption "in situ".

Another form of arabinogalactan entering the human food chain are arabinogalactan-proteins which are functional complexes widely distributed in plants and believed to play a role in plant cell development [Chen, *et al.*, 1994, Vol. 10 Tab 5]. These proteoglycans contain a high proportion of carbohydrates (typically higher than 90%), which are linked to a protein backbone rich in hydroxyproline. The structure of the carbohydrate portion of the arabinogalactan-protein is known in detail, while the structure of the protein fraction is a subject of new investigations. These structures occur predominately in the intracellular spaces of plant tissues, but are also associated with membranes, some cytoplasmic organelles, and the cell wall. Scientists believe that the function of these arabinogalactan-proteins may be involved in development, cell-cell interactions and plant defense.

000123

B. Arabinogalactan Safety and Feeding Studies; Comparison of Two Species

Larex, Inc. has recently sponsored two feeding studies, which compared the effects of the Eastern and Western larch species, *Larix occidentalis* and *Larix laricina*. A long-term human feeding study was conducted by the University of Minnesota, St. Paul, MN in 2000, and an acute study in rats was completed in 1999. The human study was conducted to determine the long-term effects of ingestion of these fibers on blood lipids, glucose, and insulin.

The study was designed as a 6 month, randomized, double blind trial, in which subjects consumed their normal diet with the addition of arabinogalactan from either Western or Eastern larch. Healthy human subjects (n=56, 28 males and 28 females) ages 18-55 were chosen to consume up 8.4 g of arabinogalactan per day. Rice starch served as a placebo. Serum cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apo B, apo A-1, glucose and insulin were measured from a fasted blood sample on a once per month basis.

Compared to baseline measurements, subjects consuming arabinogalactan had significantly decreased HDL cholesterol after the second month. Results showed that there was no corresponding change in LDL cholesterol. However, subjects in the test group showed significantly decreased concentrations of apolipoprotein B at months 5 and 6.

Serum glucose decreased significantly in all groups. Serum insulin was significantly reduced after the 5th month in the Eastern larch group. Analysis of hyperlipidemic individuals within the subject population showed a significant decrease in total cholesterol for those supplemented with arabinogalactan after three months as compared to their baseline values. The investigators concluded that arabinogalactan supplementation along with a normal diet might have positive effects on blood lipids and glucose levels in healthy adults.

Background and Significance of the Study

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At least 400 species of bacteria inhabit the gastrointestinal tract at any given time. Some species such as the lactic acid bacteria *Bifidobacteria* and *Lactobacillus* spp., and the *eubacteria* are thought to exert beneficial influences on the health of the host by secreting B vitamins, vitamin K, absorbing procarcinogens, and fermenting dietary fiber. This fermentation results in the production of short-chain fatty acids (SCFA), principally acetic, butyric and propionic acids, which exert both local and systemic effects on host health.

000124

Effects of bacterial action include a reduction in the pH of the luminal contents, which inhibits the activity of certain enzymes (ß-glucuronidase, ß-glucosidase). These enzymes are capable of converting dietary substrates to precarcinogens. The reduction in pH provides an environment where beneficial microbes can proliferate at the expense of pathogenic organisms.

Systemic effects of SCFA include the lowering of serum cholesterol levels, by enterohepatic circulation of propionic acid to the liver and the inhibition of HMGCOA reductase, the rate-limiting step in de novo cholesterol synthesis. Beneficial microbes possess certain saccharolytic enzymes, which can degrade dietary carbohydrate substrates, including soluble fiber. Soluble fiber compounds have been distinguished as "prebiotics," by Gibson and Roberfroid. Those non-digestible food ingredients selectively promote the growth and activity of beneficial species of microorganisms, thereby improving the health of the host.

In vitro studies using fecal slurries have shown that the water soluble plant cell wall fraction obtained from the larch, *Larix occidentalis*, arabinogalactan (AG) supports the growth of bifidus and lactus species. Competitive inhibition is then responsible for the reduction of pathogenic species such as *E. coli*. An earlier pilot study in which 20 human subjects consumed arabinogalactan confirmed these findings *in vivo* [Marett, *et al.*, 2000].

The human immune system has the capacity to recognize foreign organisms such as bacteria fungi and yeast by virtue of lectin-like receptors on the surface of certain immune cells. These receptors bind carbohydrate cell walls and facilitate phagocytic degradation of pathogenic organisms. Further, the binding of these receptors results in an intracellular cascade that ends in chemokine synthesis and secretion by the nucleus [Marett, *et al.*, 2000].

A previous study by the same investigators at the University of Minnesota represented the first controlled human clinical evaluation of arabinogalactan [Robinson, 1999]. The group summarized the results as follows:

C. AG consumption at a dose of 15 or 30g/day resulted in a significant increase in *Lactobacillus* spp., one group of organisms considered to be beneficial to the gastrointestinal system of the host with trends toward decreased *E. coli*.

D. AG consumption at a dose of 15 and 30g/day resulted in a significant increase in total anaerobes.

000125

E. AG consumption as both 15 and 30g/day resulted in a significant increase in peripheral blood monocytes.

Goal of the Study

The goal of the study was to elucidate the mechanisms surrounding the prebiotic fiber effects of arabinogalactan, including any potential cholesterol lowering and immune stimulating properties in a large-scale human study during long term intake of AG.

Objectives Human Study

F. To investigate the prebiotic potential of AG on the growth of select beneficial gut microflora.
G. To examine the effects of AG on select fecal enzyme biomarkers of colon carcinogenesis and fecal sort-chain fatty acids
H. To investigate the cholesterol-lowering capacity of AG in hyperlipidemic individuals
I. To examine the immune stimulating capacity of AG in a long-term (6 months) human feeding study
J. To examine select biomarkers of immune stimulation after AG consumption

Study Design

The study consisted of several baseline measurements taken before the addition of AG to the diet. These baseline measurements were followed by a 6-month feeding schedule with periodic biological samplings. Each subject acted as his or her own control.

Inclusion Criteria

Selection criteria for the subjects included: no use of antibiotics or other medications within 6 months, consumption of a low fiber diet (<15g/day), moderate alcohol consumption (<2 week), no laxative or enema use, able to provide biological samples and comply with study protocols. One hundred subjects were selected from a pool of acceptable applicants. Both normal and hyperlipidemic men and women were be selected. Menstrual cycles were documented. Subjects remained free-living and met with study coordinators to pick up AG, drop off diet records and provide biological samples. AG was be provided as a powder or in capsule form at 10g/100 lb body weight.

Methods

A set of methods was designed by the study coordinators as follows:

K. Fecal flora taxonomic identification. Eleven-gram fecal composites will be mixed with PRAS peptone water and serial dilutions streaked on selective media. *Bifidus, Lactobacillus, clostridium, E. coli, bacteriodes,* total anaerobes and enterobactereaceae will be enumerated
L. 16s RNA will be used to further characterize the bifidus and lactobacillus species in fecal slurries
M. Fecal enzymes will be measured using commercially available kits purchased from Bio Merieux, Inc.
N. Fecal SCFA will be measured by gas chromatography
O. Luminal SCFA will be measured in microdialysis bags recovered from fecal samples. These values will be compared to fecal slurry values and may provide a better snapshot of gut SCFA profiles than that which remains in fecal samples
P. Serum lipid parameters (LDL, HDL and total cholesterol, triglycerides, apolipiproteins A1 and B) will be measured by the U of M Outreach Lab.
Q. Blood chemistry and cell types (monocytes, lymphocytes, granulocytes, NIK cells, platelets) will be measured by the Outreach Lab
R. Serum will be collected, flushed with nitrogen and frozen for later cytokine identification.

University guidelines required Institutional Review Board (IRB) approval of all studies dealing with humans.

Results

Results of the study and their statistical analysis are summarized in the Tables below (Tables 1-29). Table 30 identifies the mineral intake of the subjects as the result of fiber consumption. The well being of the subjects was measured subjectively, using a standard SF-36 Form. These data as well as the quantitative data from the biological measurements indicate that there was no significant difference in physiological effect between arabinogalactans derived from two species of the genus *Larix.*

Table 1

000127

Long-Term Human Trial: Serum Cholesterol Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	178.21	175.42	165.88	165.38	164.70	167.06	174.47
Std Dev.	37.77	30.16	33.53	30.41	29.13	24.07	30.68
p value		*0.40*	*0.15*	*0.13*	*0.11*	*0.14*	*0.37*
8.4 g Eastern Larch AG							
Mean	180.52	177.94	172.33	178.64	177.58	184.82	188
Std Dev	38.24	37.85	33.80	33.34	38.45	42.61	37.72
p value		*0.41*	*0.25*	*0.43*	*0.40*	*0.37*	*0.28*
4.4 g Larch AG							
Mean	165.10	168.00	169.60	167.24	164.59	169.12	162.92
Std Dev	25.91	21.62	16.18	19.89	21.74	22.59	22.89
p value		*0.36*	*0.27*	*0.39*	*0.47*	*0.31*	*0.40*
1.4 g Larch AG							
Mean	178.37	174.11	173.68	179.89	178.12	180.57	176.86
Std Dev	35.81	40.60	35.79	35.67	39.90	39.12	33.68
p value		*0.33*	*0.32*	*0.47*	*0.49*	*0.46*	*0.43*
Placebo							
Mean	179.67	175.44	167.20	175.00	174.66	170.88	166.87
Std Dev	39.00	39.76	38.56	41.44	41.71	40.04	35.56
p value		*0.37*	*0.18*	*0.37*	*0.36*	*0.26*	*0.17*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mg/dL; (range=0-200)

000128

Table 2
Long-Term Human Trial: Serum Triglyceride Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	98.15	97.21	110.44	102.83	101.65	106.69	101.76
Std Dev	41.52	46.42	65.79	65.19	59.93	94.32	62.68
p value		*0.47*	*0.25*	*0.40*	*0.42*	*0.37*	*0.42*
8.4 g Eastern Larch AG							
Mean	89.84	97.82	88.07	102.88	111.94	99.06	104.19
Std Dev	40.34	42.67	38.77	47.96	60.67	43.39	46.96
p value		*0.28*	*0.45*	*0.19*	*0.11*	*0.26*	*0.17*
4.4 g Larch AG							
Mean	107.65	112.31	109.07	104.53	106.41	115.88	89.38
Std Dev	61.80	49.27	62.19	47.77	41.61	70.09	25.08
p value		*0.40*	*0.47*	*0.43*	*0.47*	*0.35*	*0.12*
1.4 g Larch AG							
Mean	95.28	112.22	112.32	123.44	116.35	112.64	104.64
Std Dev	60.14	60.71	70.14	74.71	67.98	72.01	59.68
p value		*0.21*	*0.23*	*0.11*	*0.16*	*0.22*	*0.32*
Placebo							
Mean	106.21	103.31	84.20	87.56	97.73	90.35	89.60
Std Dev	75.43	55.43	35.69	40.37	67.33	41.99	48.29
p value		*0.45*	*0.14*	*0.18*	*0.37*	*0.22*	*0.22*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mg/dL

000129

Table 3
Long-Term Human Trial: Serum HDL-Cholesterol Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	50.05	47.84	43.94	45.78	47.00	46.25	48.06
Std Dev	11.49	12.18	9.21	11.43	11.45	10.60	9.51
p value		*0.28*	*0.04*	*0.13*	*0.22*	*0.16*	*0.29*
8.4 g Eastern Larch AG							
Mean	50.79	51.06	51.93	52.00	50.31	52.41	52.13
Std Dev	8.59	10.42	14.29	13.39	11.08	13.82	11.16
p value		*0.47*	*0.39*	*0.38*	*0.44*	*0.34*	*0.35*
4.4 g Larch AG							
Mean	48.63	47.88	51.20	49.71	48.41	49.71	49.92
Std Dev	11.01	12.64	10.98	11.76	11.16	12.16	12.67
p value		*0.43*	*0.25*	*0.39*	*0.48*	*0.39*	*0.38*
1.4 g Larch AG							
Mean	51.83	50.56	51.58	50.89	51.06	51.50	53.29
Std Dev	12.69	13.19	12.60	12.55	12.17	12.42	12.47
p value		*0.35*	*0.49*	*0.40*	*0.41*	*0.45*	*0.39*
Placebo							
Mean	51.84	50.94	50.20	51.44	50.67	50.88	50.20
Std Dev	9.85	13.63	11.54	8.45	11.28	10.90	8.37
p value		*0.41*	*0.33*	*0.44*	*0.38*	*0.39*	*0.30*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mg/dL

000130

Table 4
Long-Term Human Trial: Serum LDL-Cholesterol Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	109.63	108.05	99.89	99.00	97.29	99.50	105.76
Std Dev	37.68	27.15	32.74	26.50	22.03	25.02	24.94
p value		*0.44*	*0.20*	*0.16*	*0.12*	*0.17*	*0.36*
8.4 g Eastern Larch AG							
Mean	109.74	107.24	102.80	106.35	104.82	112.65	115.06
Std Dev	37.15	33.25	28.44	28.96	32.01	39.76	35.45
p value		*0.42*	*0.27*	*0.38*	*0.34*	*0.41*	*0.33*
4.4 g Larch AG							
Mean	92.05	97.63	96.60	96.59	94.94	96.24	94.77
Std Dev	26.05	24.74	17.15	25.95	26.09	21.60	24.94
p value		*0.26*	*0.27*	*0.30*	*0.37*	*0.30*	*0.38*
1.4 g Larch AG							
Mean	98.56	101.17	99.79	104.06	105.12	106.64	102.71
Std Dev	32.02	31.94	26.36	27.43	37.69	34.99	27.49
p value		*0.44*	*0.48*	*0.30*	*0.30*	*0.28*	*0.37*
Placebo							
Mean	105.92	103.75	100.07	106.13	101.00	101.82	98.67
Std Dev	30.59	32.98	33.36	36.11	33.42	35.63	29.42
p value		*0.43*	*0.31*	*0.48*	*0.35*	*0.37*	*0.25*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mg/dL as calculated by the Friedwald equation

000131

Table 5
Long-Term Human Trial: Serum Glucose Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	81.35	82.58	76.19	75.28	78.06	76.88	76.00
Std Dev	8.06	10.48	9.16	8.44	9.77	7.64	7.13
p value		*0.34*	*0.04*	*0.01*	*0.14*	*0.05*	*0.02*
8.4 g Eastern Larch AG							
Mean	82.05	80.13	78.07	74.35	75.18	75.71	76.20
Std Dev	5.24	5.04	7.45	6.96	7.90	9.24	6.20
p value		*0.1384*	*0.0505*	*0.0004*	*0.0026*	*0.0098*	*0.0034*
4.4 g Larch AG							
Mean	80.40	80.40	78.25	76.76	73.76	73.41	72.92
Std Dev	8.71	7.29	4.65	8.85	8.99	6.75	8.96
p value		*0.5000*	*0.1854*	*0.1091*	*0.0149*	*0.0047*	*0.0129*
1.4 g Larch AG							
Mean	82.75	80.25	75.61	73.78	78.88	77.27	75.14
Std Dev	6.96	13.84	6.97	6.57	8.09	8.04	6.13
p value		*0.2560*	*0.0039*	*0.0004*	*0.0820*	*0.0312*	*0.0018*
Placebo							
Mean	82.44	78.80	74.81	75.00	78.21	76.47	73.40
Std Dev	4.34	5.70	8.50	5.81	7.49	9.70	10.30
p value		*0.02*	*0.001*	*0.0001*	*0.03*	*0.01*	*0.003*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mg/dL

000132

Table 10
Long-Term Human Trial: White Blood Cell Counts

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	5.68E +09	5.26E +09	5.25E +09	5.56E +09	5.16E +09	5.04E +09	5.11E +09
Std Dev	1.24E +09	1.15E +09	1.15E +09	1.05E +09	9.99E +08	9.35E +08	1.16E +09
p value		*0.13*	*0.13*	*0.37*	*0.08*	*0.05*	*0.08*
8.4 g Eastern Larch AG							
Mean	6.11E +09	6.10E +09	5.73E +09	5.48E +09	5.44E +09	5.94E +09	5.60E +09
Std Dev	1.59E +09	1.77E +09	1.42E +09	1.30E +09	1.12E +09	1.66E +09	8.48E +08
p value		*0.49*	*0.23*	*0.10*	*0.07*	*0.37*	*0.12*
4.4 g Larch AG							
Mean	5.73E +09	5.90E +09	5.38E +09	5.93E +09	6.29E +09	6.42E +09	5.75E +09
Std Dev	1.74E +09	1.36E +09	1.39E +09	1.41E +09	1.68E +09	1.27E +09	1.35E +09
p value		*0.37*	*0.28*	*0.35*	*0.17*	*0.09*	*0.49*
1.4 g Larch AG							
Mean	5.96E +09	5.82E +09	6.41E +09	6.25E +09	6.28E +09	6.28E +09	3.48E +10
Std Dev	1.41E +09	2.07E +09	1.73E +09	2.08E +09	1.65E +09	1.40E +09	1.07E +11
p value		*0.41*	*0.20*	*0.31*	*0.28*	*0.27*	*0.17*
Placebo							
Mean	5.51E +09	5.26E +09	5.07E +09	5.36E +09	5.59E +09	5.72E +09	5.75E +09
Std Dev	1.74E +09	1.77E +09	1.45E +09	1.46E +09	1.38E +09	1.77E +09	1.30E +09
p value		*0.32*	*0.19*	*0.39*	*0.44*	*0.35*	*0.31*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (range= 9.0 E +09 – 11.0E +09)

000133

Table 6
Long-Term Human Trial: Serum Insulin Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	8.50	6.42	6.06	6.65	6.00	6.29	7.06
Std Dev	7.08	3.50	3.51	4.15	3.45	3.44	5.13
p value		*0.13*	*0.09*	*0.17*	*0.09*	*0.11*	*0.24*
8.4 g Eastern Larch AG							
Mean	5.79	6.63	5.53	6.31	5.71	4.59	4.47
Std Dev	2.23	3.46	2.50	5.21	4.45	1.91	2.17
p value		*0.21*	*0.37*	*0.36*	*0.47*	*0.05*	*0.05*
Placebo							
Mean	5.61	5.27	4.29	5.29	5.87	6.59	5.60
Std Dev	2.75	4.76	1.49	4.36	5.54	7.88	3.02
p value		*0.40*	*0.05*	*0.05*	*0.40*	*0.32*	*0.50*
4.4 g Larch AG							
not determined							
1.4 g Larch AG							
not determined							

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mU/L

000134

Table 7
Long-Term Human Trial: Monocytes

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	7.89	8.47	8.52	8.42	8.73	7.74	8.64
Std Dev	2.66	2.52	1.60	2.15	2.43	2.32	2.80
p value		*0.25*	*0.19*	*0.25*	*0.17*	*0.43*	*0.21*
8.4 g Eastern Larch AG							
Mean	8.01	8.91	8.98	8.43	7.72	8.31	8.79
Std Dev	2.23	2.53	2.11	1.80	1.49	2.47	1.37
p value		*0.14*	*0.10*	*0.26*	*0.32*	*0.35*	*0.10*
4.4 g Larch AG							
Mean	8.09	7.67	7.61	11.28	8.31	8.09	8.62
Std Dev	1.91	2.31	2.25	10.77	3.09	1.49	2.05
p value		*0.28*	*0.25*	*0.12*	*0.40*	*0.50*	*0.23*
1.4 g Larch AG							
Mean	8.89	8.49	8.47	8.36	8.99	8.29	8.66
Std Dev	1.77	1.91	2.05	2.11	2.90	1.97	1.72
p value		*0.25*	*0.18*	*0.16*	*0.49*	*0.18*	*0.31*
Placebo							
Mean	8.26	8.51	9.31	8.69	9.54	8.38	8.66
Std Dev	1.91	1.77	1.67	2.32	2.01	1.87	1.71
p value		*0.35*	*0.05*	*0.28*	*0.05*	*0.43*	*0.26*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as % in blood (range=0.0-12.0)

000135

Table 8
Long-Term Human Trial: Serum Apolipoprotein A1 Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	139.40	132.59	131.06	130.56	130.00	128.70	130.64
Std Dev	26.32	30.19	16.60	24.09	22.90	16.95	19.46
p value		*0.24*	*0.12*	*0.14*	*0.13*	*0.07*	*0.13*
8.4 g Eastern Larch AG							
Mean	140.21	136.25	142.22	138.82	137.19	138.00	139.18
Std Dev	23.04	17.59	30.91	22.43	20.92	18.62	20.13
p value		*0.28*	*0.41*	*0.42*	*0.34*	*0.38*	*0.44*
Placebo							
Mean	139.77	142.53	137.24	134.47	135.27	131.29	136.00
Std Dev	16.80	25.09	20.70	15.83	20.96	17.19	17.56
p value		*0.36*	*0.35*	*0.17*	*0.26*	*0.08*	*0.27*
4.4 g Larch AG							
not determined							
1.4 g Larch AG							
not determined							

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mg/dL; (range=none)

000136

Table 9
Long-Term Human Trial: Serum Apolipoprotein B Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	94.63	89.71	88.28	85.11	84.25	81.00	81.35
Std Dev	26.16	22.70	22.44	22.42	20.26	20.20	18.08
p value		*0.27*	*0.22*	*0.12*	*0.10*	*0.04*	*0.04*
8.4 g Eastern Larch AG							
Mean	91.73	90.88	87.39	86.64	87.88	81.75	86.47
Std Dev	29.52	31.82	28.59	28.92	32.14	32.82	28.24
p value		*0.47*	*0.33*	*0.30*	*0.36*	*0.17*	*0.29*
Placebo							
Mean	88.12	88.73	82.00	80.76	83.40	82.65	82.44
Std Dev	26.13	23.93	26.13	24.15	27.60	24.69	26.88
p value		*0.47*	*0.25*	*0.20*	*0.31*	*0.27*	*0.27*
4.4 g Larch AG							
not determined							
1.4 g Larch AG							
not determined							

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mg/dL; (range=none)

000137

Table 11
Long-Term Human Trial: Red Blood Cell Count

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	4.78E+12	4.75E+12	4.62E+12	4.64E+12	4.77E+12	4.76E+12	4.70E+12
Std Dev	5.82E+11	5.39E+11	5.26E+11	5.48E+11	4.42E+11	4.53E+11	4.04E+11
p value		*0.42*	*0.18*	*0.23*	*0.49*	*0.45*	*0.31*
8.4 g Eastern Larch AG							
Mean	4.54E+12	4.46E+12	4.44E+12	4.58E+12	4.26E+12	4.23E+12	4.48E+12
Std Dev	5.30E+11	5.62E+11	5.06E+11	5.23E+11	1.22E+12	1.23E+12	5.01E+11
p value		*0.33*	*0.28*	*0.42*	*0.20*	*0.17*	*0.37*
4.4 g Larch AG							
Mean	4.60E+12	4.59E+12	4.56E+12	4.60E+12	4.33E+12	4.56E+12	4.49E+12
Std Dev	3.45E+11	3.23E+11	4.20E+11	3.12E+11	1.16E+12	2.71E+11	3.41024E+11
p value		*0.45*	*0.39*	*0.49*	*0.18*	*0.34*	*0.18*
1.4 g Larch AG							
Mean	4.66E+12	4.62E+12	4.57E+12	4.55E+12	4.54E+12	4.56E+12	4.60E+12
Std Dev	5.87E+11	5.99E+11	6.22E+11	5.90E+11	4.97E+11	6.04E+11	5.91E+11
p value		*0.41*	*0.33*	*0.30*	*0.26*	*0.32*	*0.39*
Placebo							
Mean	4.75E+12	4.30E+12	4.65E+12	4.67E+12	4.55E+12	4.66E+12	4.64E+12
Std Dev	3.65E+11	1.27E+11	3.60E+11	3.65E+11	3.62E+12	3.96E+11	4.36E+11
p value		*0.09*	*0.20*	*0.25*	*0.07*	*0.25*	*0.22*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (range=none)

000138

Table 12
Long-Term Human Trial: Hemoglobin Levels

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	14.46	14.33	13.84	14.03	14.48	14.19	14.30
Std Dev	1.56	1.38	1.42	1.49	1.23	1.18	1.19
p value		*0.40*	*0.11*	*0.21*	*0.48*	*0.29*	*0.37*
8.4 g Eastern Larch AG							
Mean	14.04	13.73	13.65	13.94	13.94	13.87	13.92
Std Dev	1.45	1.45	1.43	1.46	1.41	1.57	1.38
p value		*0.26*	*0.22*	*0.42*	*0.42*	*0.37*	*0.40*
4.4 g Larch AG							
Mean	13.73	13.49	14.05	13.69	13.64	13.88	13.24
Std Dev	1.66	2.06	1.32	1.83	1.70	0.95	2.01
p value		*0.36*	*0.29*	*0.49*	*0.44*	*0.37*	*0.24*
1.4 g Larch AG							
Mean	14.21	14.06	13.73	13.71	13.81	13.82	14.03
Std Dev	1.53	1.53	1.84	1.71	1.21	1.43	1.51
p value		*0.39*	*0.21*	*0.19*	*0.21*	*0.24*	*0.37*
Placebo							
Mean	14.44	14.24	14.01	14.12	13.87	14.06	14.06
Std Dev	1.04	1.21	1.05	1.15	0.83	1.14	1.20
p value		*0.30*	*0.12*	*0.20*	*0.04*	*0.15*	*0.16*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as g/dL; (range=none)

000139

Table 13
Long-TermII Human Trial: Hematocrit

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	42.87	42.43	41.14	41.44	42.78	42.50	42.16
Std Dev	4.72	4.18	4.26	4.28	3.53	3.64	3.60
p value		*0.38*	*0.12*	*0.18*	*0.48*	*0.40*	*0.31*
8.4 g Eastern Larch AG							
Mean	41.31	40.44	40.46	41.44	41.49	41.28	40.83
Std Dev	4.23	4.21	4.43	4.33	4.09	4.68	3.91
p value		*0.28*	*0.29*	*0.46*	*0.45*	*0.49*	*0.37*
4.4 g Larch AG							
Mean	40.69	40.19	41.43	40.83	40.56	41.19	39.27
Std Dev	4.37	5.28	3.87	4.60	4.33	2.88	4.98
p value		*0.39*	*0.32*	*0.46*	*0.46*	*0.34*	*0.21*
1.4 g Larch AG							
Mean	42.09	41.64	40.73	40.74	40.91	41.19	41.29
Std Dev	4.68	4.47	5.16	4.87	3.61	4.44	4.20
p value		*0.39*	*0.21*	*0.21*	*0.22*	*0.30*	*0.31*
Placebo							
Mean	42.88	42.29	40.28	42.02	41.19	41.68	41.55
Std Dev	2.78	3.69	6.34	3.15	2.60	3.11	3.23
p value		*0.30*	*0.07*	*0.20*	*0.04*	*0.12*	*0.11*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as %; (range=none)

000140

Table 14
Long-Term Human Trial: Mean Corpuscular Volume

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	89.79	89.42	89.28	89.63	89.63	89.50	89.69
Std Dev	3.21	3.22	3.20	3.56	3.14	2.28	2.98
p value		*0.36*	*0.32*	*0.44*	*0.44*	*0.38*	*0.46*
8.4 g Eastern Larch AG							
Mean	91.28	91.13	91.33	90.78	91.24	91.29	91.39
Std Dev	4.11	3.50	3.96	4.02	3.80	3.29	4.30
p value		*0.45*	*0.48*	*0.36*	*0.49*	*0.49*	*0.47*
4.4 g Larch AG							
Mean	88.47	87.71	90.90	88.53	88.35	90.31	87.62
Std Dev	7.68	9.12	1.91	7.44	7.70	2.63	9.44
p value		*0.40*	*0.10*	*0.49*	*0.48*	*0.17*	*0.39*
1.4 g Larch AG							
Mean	90.31	90.56	89.22	89.67	90.31	90.57	90.14
Std Dev	2.98	3.40	3.59	3.91	2.98	3.55	3.28
p value		*0.41*	*0.17*	*0.29*	*0.50*	*0.42*	*0.44*
Placebo							
Mean	90.32	89.94	89.40	90.19	90.14	90.00	89.73
Std Dev	3.22	3.32	3.78	4.17	3.50	4.05	3.94
p value		*0.37*	*0.23*	*0.46*	*0.44*	*0.40*	*0.32*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as fL; (range=78-100)

000141

Table 15
Long-Term Human Trial: Mean Corpuscular Hemoglobin

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	30.31	30.22	30.60	30.33	30.23	29.84	30.44
Std Dev	1.27	1.26	3.22	1.26	1.27	0.98	1.04
p value		*0.42*	*0.36*	*0.48*	*0.43*	*0.12*	*0.36*
8.4 g Eastern Larch AG							
Mean	30.99	30.96	30.84	30.60	30.71	30.75	31.12
Std Dev	1.39	1.51	1.55	1.45	1.19	1.48	1.33
p value		*0.47*	*0.39*	*0.21*	*0.26*	*0.31*	*0.39*
4.4 g Larch AG							
Mean	29.84	29.37	30.80	29.68	29.72	30.42	29.52
Std Dev	3.00	3.64	0.71	2.99	3.14	0.93	3.96
p value		*0.35*	*0.10*	*0.44*	*0.45*	*0.22*	*0.40*
1.4 g Larch AG							
Mean	30.51	30.55	30.11	30.15	30.49	30.40	30.57
Std Dev	1.04	1.09	1.53	1.50	1.17	1.19	1.23
p value		*0.46*	*0.19*	*0.21*	*0.47*	*0.39*	*0.45*
Placebo							
Mean	30.42	30.42	30.11	30.28	30.36	30.38	30.37
Std Dev	1.25	1.23	1.64	1.67	1.31	1.42	1.69
p value		*0.50*	*0.28*	*0.40*	*0.45*	*0.47*	*0.47*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as pg; (range=26.5-35.0)

000142

Table 16
Long-Term Human Trial: Mean Corpuscular Hematocrit

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	33.75	33.77	33.65	33.84	33.85	33.41	33.89
Std Dev	0.56	0.52	0.46	0.55	0.42	0.44	0.41
p value		*0.44*	*0.28*	*0.32*	*0.27*	*0.03*	*0.19*
8.4 g Eastern Larch AG							
Mean	33.99	33.96	33.77	33.66	33.65	33.61	34.08
Std Dev	0.36	0.54	0.56	0.48	0.34	0.67	0.36
p value		*0.44*	*0.10*	*0.01*	*0.001*	*0.03*	*0.22*
4.4 g Larch AG							
Mean	33.65	33.44	33.93	33.44	33.58	33.70	33.55
Std Dev	0.86	1.12	0.37	1.01	1.00	0.58	1.31
p value		*0.29*	*0.12*	*0.27*	*0.42*	*0.42*	*0.41*
1.4 g Larch AG							
Mean	33.87	33.76	33.69	33.62	33.77	33.54	33.95
Std Dev	0.33	0.46	0.82	0.46	0.63	0.58	0.62
p value		*0.23*	*0.21*	*0.04*	*0.30*	*0.04*	*0.33*
Placebo							
Mean	33.65	33.82	33.62	33.57	33.64	33.75	33.86
Std Dev	0.41	0.65	0.54	0.53	0.52	0.42	0.68
p value		*0.19*	*0.42*	*0.30*	*0.46*	*0.25*	*0.15*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as g/dL; (range=32.0-36.0)

000143

Table 17
Long-Term Human Trial: RDW

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	12.29	12.19	12.30	12.23	12.31	12.28	12.31
Std Dev	0.53	0.47	0.60	0.51	0.64	0.52	0.38
p value		*0.26*	*0.49*	*0.36*	*0.46*	*0.46*	*0.45*
8.4 g Eastern Larch AG							
Mean	12.22	12.26	12.19	12.25	12.17	12.12	12.17
Std Dev	0.42	0.59	0.36	0.51	0.43	0.43	0.49
p value		*0.40*	*0.43*	*0.42*	*0.38*	*0.25*	*0.39*
4.4 g Larch AG							
Mean	12.50	14.39	12.20	12.74	12.41	12.10	12.73
Std Dev	1.62	6.16	0.49	2.52	1.77	0.54	2.33
p vlaue		*0.14*	*0.23*	*0.38*	*0.44*	*0.16*	*0.38*
1.4 g Larch AG							
Mean	12.21	12.02	12.35	12.31	12.16	12.19	12.30
Std Dev	0.39	0.46	0.99	1.04	0.50	0.57	0.48
p value		*0.11*	*0.29*	*0.35*	*0.38*	*0.45*	*0.28*
Placebo							
Mean	12.19	12.29	12.37	12.34	12.17	12.33	12.43
Std Dev	1..43	6.16	0.49	2.52	1.77	0.54	2.33
p vlaue		*0.29*	*0.20*	*0.27*	*0.46*	*0.30*	*0.19*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as %; (range=10.0-15.0)

000144

Table 18
Long-Term Human Trial: Platelets

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	2.29E+11	2.45E+11	2.23E+11	2.25E+11	1.30E+13	2.22E+11	2.19E+11
Std Dev	3.34E+10	4.63E+10	3.88E+10	3.55E+10	5.09E+13	2.70E+10	4.61E+10
p value		*0.12*	*0.29*	*0.35*	*0.17*	*0.25*	*0.23*
8.4 g Eastern Larch AG							
Mean	2.34E+11	2.44E+11	2.33E+11	2.28E+11	2.25E+11	2.29E+11	2.17E+11
Std Dev	3.82E+10	4.01E+10	3.27E+10	3.61E+10	3.53E+10	3.77E+10	3.15E+10
p value		*0.23*	*0.47*	*0.31*	*0.23*	*0.36*	*0.08*
4.4 g Larch AG							
Mean	2.41E+11	2.39E+11	2.03E+11	2.53E+11	2.46E+11	2.32E+11	2.35E+11
Std Dev	4.09E+10	1.13E+11	7.95E+10	6.37E+10	5.65E+10	3.51E+10	6.80E+10
p value		*0.48*	*0.10*	*0.26*	*0.37*	*0.25*	*0.40*
1.4 g Larch AG							
Mean	2.50E+11	2.47E+11	2.46E+11	2.40E+11	2.49E+11	2.47E+11	2.25E+11
Std Dev	5.19E+10	4.36E+10	4.64E+10	7.12E+10	5.00E+10	4.93E+10	7.81E+10
p value		*0.41*	*0.40*	*0.32*	*0.47*	*0.42*	*0.16*
Placebo							
Mean	2.54E+11	2.39E+11	2.43E+11	2.33E+11	2.30E+11	2.31E+11	2.35E+11
Std Dev	5.14E+10	5.46E+11	6.03E+10	6.47E+10	5.71E+10	4.96E+10	5.35E+10
p value		*0.20*	*0.29*	*0.15*	*0.11*	*0.09*	*0.14*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (1.5-4.5E+11)

000145

Table 19
Long-Term Human Trial: Mean Platelet Volume

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	8.97	8.65	8.77	8.76	8.81	8.94	8.59
Std Dev	0.71	0.86	0.88	0.70	0.89	0.90	0.90
p value		*0.11*	*0.23*	*0.19*	*0.29*	*0.46*	*0.09*
8.4 g Eastern Larch AG							
Mean	8.43	8.47	8.45	8.65	8.61	8.59	8.35
Std Dev	0.72	0.75	0.92	0.84	0.93	0.87	0.80
p value		*0.43*	*0.47*	*0.20*	*0.26*	*0.27*	*0.38*
4.4 g Larch AG							
Mean	8.54	8.56	8.57	8.39	8.47	8.61	8.58
Std Dev	0.83	0.70	0.56	0.69	0.87	0.75	0.68
p value		*0.47*	*0.46*	*0.29*	*0.41*	*0.40*	*0.43*
1.4 g Larch AG							
Mean	8.81	8.74	8.76	8.67	8.48	8.72	8.29
Std Dev	0.74	0.71	0.77	0.86	0.94	0.87	0.76
p value		*0.39*	*0.41*	*0.31*	*0.14*	*0.38*	*0.04*
Placebo							
Mean	838	8.45	8.53	8.68	8.50	8.45	8.34
Std Dev	0.90	1.03	1.09	1.02	1.03	1.04	0.99
p value		*0.42*	*0.34*	*0.19*	*0.37*	*0.42*	*0.45*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as fL; (range=6.5-10.0)

000146

Table 20
Long-Term Human Trial: Neutrophils

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	54.95	57.41	54.66	56.76	55.84	53.82	54.09
Std Dev	8.70	7.89	8.94	10.12	7.87	11.37	10.55
p value		*0.18*	*0.46*	*0.28*	*0.38*	*0.37*	*0.40*
8.4 g Eastern Larch AG							
Mean	58.69	53.62	59.04	55.52	56.51	58.08	54.53
Std Dev	10.07	15.87	10.74	6.43	8.78	12.46	7.72
p value		*0.14*	*0.46*	*0.13*	*0.25*	*0.44*	*0.08*
4.4 g Larch AG							
Mean	55.90	58.79	58.16	56.09	58.39	58.98	58.06
Std Dev	7.89	8.16	4.21	5.80	6.88	7.71	6.67
p value		*0.14*	*0.14*	*0.47*	*0.16*	*0.12*	*0.20*
1.4 g Larch AG							
Mean	57.76	59.10	57.96	59.26	61.78	58.24	57.30
Std Dev	7.40	8.68	7.69	9.35	10.61	7.77	6.79
p value		*0.31*	*0.47*	*0.30*	*0.11*	*0.43*	*0.43*
Placebo							
Mean	57.10	55.10	54.10	56.90	55.30	59.30	57.30
Std Dev	8.80	9.23	9.39	9.54	7.66	10.05	10.06
p value		*0.26*	*0.17*	*0.48*	*0.26*	*0.25*	*0.48*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as %; (range=40.0-75.0)

000147

Table 21
Long-Term Human Trial: Lymphocytes

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	32.73	31.05	33.46	31.99	32.28	34.89	34.17
Std Dev	7.23	6.62	8.52	9.22	7.03	11.01	9.40
p value		*0.23*	*0.39*	*0.40*	*0.43*	*0.25*	*0.31*
8.4 g Eastern Larch AG							
Mean	29.50	30.59	28.55	32.79	32.52	30.28	33.17
Std Dev	9.08	8.59	9.85	6.81	9.04		12.94
	8.20						
p value		*0.36*	*0.38*	*0.11*	*0.16*	*0.42*	*0.10*
4.4 g Larch AG							
Mean	32.51	29.99	31.05	37.21	29.80	29.35	29.67
Std Dev	7.39	7.05	3.77	21.95	5.02	7.81	6.73
p value		*0.15*	*0.22*	*0.20*	*0.10*	*0.11*	*0.13*
1.4 g Larch AG							
Mean	30.57	29.32	30.52	29.59	26.49	30.83	31.10
Std Dev	6.61	6.48	6.10	7.05	8.00	6.40	5.70
p value		*0.29*	*0.49*	*0.34*	*0.06*	*0.46*	*0.41*
Placebo							
Mean	31.80	35.20	33.20	31.50	32.10	29.40	31.00
Std Dev	9.73	11.36	9.19	9.74	6.32	8.99	8.69
p value		*0.17*	*0.33*	*0.47*	*0.46*	*0.23*	*0.40*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as %; (range=20.0-48.0)

000148

Table 22
Long-Term Human Trial: Eosinophils

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	2.68	2.34	2.52	2.27	2.68	2.82	2.41
Std Dev	1.54	1.35	1.25	1.37	1.85	1.69	1.55
p value		*0.24*	*0.36*	*0.19*	*0.50*	*0.40*	*0.30*
8.4 g Eastern Larch AG							
Mean	3.02	2.62	2.75	2.69	2.83	2.65	2.81
Std Dev	4.32	2.15	2.95	1.72	2.14	2.23	1.71
p value		*0.36*	*0.41*	*0.38*	*0.43*	*0.37*	*0.42*
4.4 g Larch AG							
Mean	2.46	2.89	2.42	6.28	2.89	2.96	2.82
Std Dev	1.18	1.78	1.32	14.16	1.61	1.51	1.48
p value		*0.20*	*0.47*	*0.14*	*0.18*	*0.14*	*0.23*
1.4 g Larch AG							
Mean	2.06	2.41	2.23	2.31	2.29	2.11	2.26
Std Dev	0.81	1.35	1.14	1.26	0.83	0.91	0.86
p value		*0.18*	*0.31*	*0.25*	*0.21*	*0.44*	*0.25*
Placebo							
Mean	2.22	2.29	2.57	2.24	2.47	2.25	2.47
Std Dev	1.60	1.77	1.49	1.38	1.61	1.27	1.94
p value		*0.45*	*0.26*	*0.48*	*0.34*	*0.47*	*0.35*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as %; (0.0-6.0)

000149

Table 23
Long-Term Human Trial: Basophils

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	0.89	0.77	0.89	0.58	0.74	0.84	0.83
Std Dev	0.44	0.32	0.32	0.26	0.36	0.76	0.47
p value		*0.18*	*0.48*	*0.01*	*0.15*	*0.41*	*0.35*
8.4 g Eastern Larch AG							
Mean	0.77	0.72	0.66	0.67	0.67	0.67	0.70
Std Dev	0.44	0.36	0.34	0.45	0.47	0.34	0.31
p value		*0.35*	*0.20*	*0.25*	*0.27*	*0.22*	*0.28*
4.4 g Larch AG							
Mean	0.79	0.75	0.74	0.68	0.61	0.88	0.82
Std Dev	0.44	0.30	0.36	0.35	0.31	0.98	0.51
p value		*0.37*	*0.37*	*0.19*	*0.08*	*0.37*	*0.42*
1.4 g Larch AG							
Mean	0.71	0.76	0.82	0.61	0.53	0.54	0.68
Std Dev	0.24	0.42	0.83	0.25	0.19	0.27	0.23
p value		*0.33*	*0.30*	*0.14*	*0.01*	*0.04*	*0.38*
Placebo							
Mean	0.72	0.86	0.85	0.61	0.56	0.56	0.61
Std Dev	0.29	0.42	0.38	0.26	0.29	0.29	0.34
p value		*0.23*	*0.26*	*0.04*	*0.03*	*0.02*	*0.09*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as %; (range=0.0-2.0)

000150

Table 24
Long-Term Human Trial: Abs Neutrophils

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	3.22E+09	3.07E+09	2.91E+09	3.14E+09	2.94E+09	2.78E+09	2.77E+09
Std Dev	1.22E+09	9.37E+08	9.13E+08	1.06E+09	9.66E+08	1.02E+09	1.05E+09
p value		*0.34*	*0.19*	*0.41*	*0.22*	*0.12*	*0.12*
8.4 g Eastern Larch AG							
Mean	3.70E+09	3.45E+09	3.45E+09	3.09E+09	3.15E+09	3.58E+09	3.09E+09
Std Dev	1.53E+09	1.29E+09	1.15E+09	9.42E+08	1.02E+09	1.63E+09	7.33E+08
p value		*0.30*	*0.29*	*0.08*	*0.11*	*0.41*	*0.07*
4.4 g Larch AG							
Mean	3.24E+09	3.28E+09	3.32E+09	3.33E+09	3.74E+09	3.59E+09	3.37E+09
Std Dev	1.27E+09	1.30E+09	9.14E+08	7.90E+08	1.32E+09	1.08E+09	9.13E+08
p value		*0.46*	*0.42*	*0.40*	*0.12*	*0.18*	*0.37*
1.4 g Larch AG							
Mean	3.49E+09	3.72E+09	3.74E+09	3.85E+09	3.91E+09	3.72E+09	3.59E+09
Std Dev	1.09E+09	1.42E+09	1.38E+09	1.92E+09	1.41E+09	1.17E+09	1.40E+09
p value		*0.29*	*0.28*	*0.25*	*0.17*	*0.29*	*0.42*
Placebo							
Mean	3.21E+09	2.82E+09	2.77E+09	3.13E+09	3.18E+09	3.54E+09	3.34E+09
Std Dev	1.11E+09	9.28E+08	1.20E+09	1.21E+09	1.20E+09	1.67E+09	1.05E+09
p value		*0.13*	*0.14*	*0.43*	*0.47*	*0.25*	*0.36*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (range=1.6-8.3E +09)

000151

Table 25
Long-Term Human Trial: Abs Lymphocytes

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	1.84E+09	1.61E+09	1.74E+09	1.68E+09	1.63E+09	1.72E+09	1.71E+09
Std Dev	3.18E+08	3.92E+08	5.31E+08	5.31E+08	2.96E+08	5.28E+08	5.09E+08
p value		*0.03*	*0.26*	*0.14*	*0.03*	*0.22*	*0.20*
8.4 g Eastern Larch AG							
Mean	1.72E+09	1.74E+09	1.61E+09	1.77E+09	1.72E+09	1.65E+09	1.84E+09
Std Dev	5.09E+08	3.39E+08	6.40E+08	4.70E+08	4.11E+08	5.12E+08	4.42E+08
p value		*0.42*	*0.29*	*0.36*	*0.50*	*0.36*	*0.22*
4.4 g Larch AG							
Mean	1.71E+09	1.58E+09	1.78E+09	1.86E+09	1.84E+09	1.88E+09	1.69E+09
Std Dev	6.68E+08	5.94E+08	5.50E+08	5.85E+08	4.40E+08	5.98E+08	5.17E+08
p value		*0.27*	*0.37*	*0.22*	*0.23*	*0.21*	*0.48*
1.4 g Larch AG							
Mean	1.79E+09	1.64E+09	1.88E+09	1.74E+09	1.65E+09	1.89E+09	1.81E+09
Std Dev	4.53E+08	4.55E+08	5.23E+08	4.71E+08	5.60E+08	4.25E+08	3.61E+08
p value		*0.17*	*0.31*	*0.38*	*0.21*	*0.27*	*0.45*
Placebo							
Mean	1.72E+09	1.95E+09	1.69E+09	1.44E+09	1.74E+09	1.58E+09	1.75E+09
Std Dev	5.18E+08	1.35E+09	6.97E+08	4.23E+08	4.07E+08	4.69E+08	5.95E+08
p value		*0.26*	*0.44*	*0.06*	*0.46*	*0.20*	*0.43*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (range=0.8-5.3E+09)

000152

Table 26
Long-Term Human Trial: Abs Monocytes

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	4.47E+08	4.37E+08	4.39E+08	4.50E+08	4.25E+08	3.63E+08	4.24E+08
Std Dev	1.50E+08	1.12E+08	1.04E+08	1.25E+08	1.13E+08	1.02E+08	1.25E+08
p value		*0.40*	*0.42*	*0.48*	*0.31*	*0.03*	*0.30*
8.4 g Eastern Larch AG							
Mean	4.74E+08	4.69E+08	5.06E+08	4.50E+08	4.18E+08	5.12E+08	4.78E+08
Std Dev	1.37E+08	1.74E+08	1.09E+08	1.20E+08	1.33E+08	2.03E+08	1.00E+08
p value		*0.46*	*0.22*	*0.29*	*0.11*	*0.26*	*0.46*
4.4 g Larch AG							
Mean	4.50E+08	4.29E+08	4.19E+08	5.06E+08	5.24E+08	5.12E+08	5.08E+08
Std Dev	1.67E+08	1.21E+08	1.56E+08	1.78E+08	1.99E+08	1.58E+08	1.85E+08
p value		*0.33*	*0.28*	*0.17*	*0.12*	*0.13*	*0.19*
1.4 g Larch AG							
Mean	5.18E+08	4.94E+08	5.22E+08	5.00E+08	5.47E+08	5.00E+08	5.21E+08
Std Dev	1.33E+08	1.21E+08	1.63E+08	1.46E+08	1.42E+08	1.04E+08	1.42E+08
p value		*0.30*	*0.46*	*0.36*	*0.27*	*0.34*	*0.47*
Placebo							
Mean	4.47E+08	4.44E+08	4.44E+08	4.50E+08	5.00E+08	4.71E+08	5.00E+08
Std Dev	1.65E+08	1.50E+08	1.90E+08	1.32E+08	1.22E+08	1.26E+08	1.60E+08
p value		*0.47*	*0.48*	*0.48*	*0.15*	*0.32*	*0.18*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (range=0.0-1.3E+09)

000153

Table 27
Long-Term Human Trial: Abs Eosinophils

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	1.47E+08	1.38E+08	1.39E+08	1.17E+08	1.38E+08	1.63E+08	1.26E+08
Std Dev	7.72E+07	8.78E+07	6.08E+07	6.08E+07	8.75E+07	8.06E+07	7.54E+07
p value		*0.36*	*0.36*	*0.10*	*0.37*	*0.29*	*0.21*
8.4 g Eastern Larch AG							
Mean	1.80E+08	1.69E+08	1.54E+08	1.51E+08	1.58E+08	1.60E+08	1.62E+08
Std Dev	2.19E+08	1.08E+08	1.77E+08	9.76E+07	1.29E+08	1.40E+08	1.08E+08
p value		*0.42*	*0.35*	*0.30*	*0.36*	*0.37*	*0.38*
4.4 g Larch AG							
Mean	1.56E+08	1.54E+08	1.51E+08	1.59E+08	1.65E+08	2.00E+08	1.46E+08
Std Dev	1.03E+08	9.23E+07	1.15E+08	9.29E+07	9.86E+07	8.66E+07	5.19E+07
p value		*0.48*	*0.44*	*0.46*	*0.39*	*0.08*	*0.36*
1.4 g Larch AG							
Mean	1.35E+08	1.22E+08	1.39E+08	1.48E+08	1.42E+08	1.36E+08	1.36E+08
Std Dev	6.06E+07	4.28E+07	7.67E+07	7.89E+07	6.04E+07	6.17E+07	7.31E+07
p value		*0.23*	*0.43*	*0.31*	*0.38*	*0.48*	*0.48*
Placebo							
Mean	1.14E+08	1.29E+08	1.61E+08	1.20E+08	1.38E+08	1.31E+08	1.41E+08
Std Dev	9.85E+07	1.01E+07	8.98E+07	6.31E+07	1.12E+07	7.51E+07	9.66E+07
p value		*0.34*	*0.08*	*0.42*	*0.27*	*0.29*	*0.22*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (range=0.0-0.7E +09)

000154

Table 28
Long-Term Human Trial: Abs Basophils

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	4.00E+07	4.00E+07	7.35E+07	4.50E+07	4.21E+07	2.80E+07	4.18E+07
Std Dev	4.37E+07	4.37E+07	4.23E+07	4.51E+07	4.48E+07	3.73E+07	4.43E+07
p value		*0.50*	*0.01*	*0.37*	*0.45*	*0.20*	*0.45*
8.4 g Eastern Larch AG							
Mean	3.84E+07	4.94E+07	5.50E+07	4.00E+07	3.40E+07	3.81E+07	3.50E+07
Std Dev	4.30E+07	4.61E+07	4.65E+07	4.37E+07	4.12E+07	4.31E+07	4.15E+07
p value		*0.24*	*0.14*	*0.46*	*0.38*	*0.49*	*0.40*
4.4 g Larch AG							
Mean	4.15E+07	5.80E+07	6.79E+07	4.71E+07	3.12E+07	3.25E+07	3.08E+07
Std Dev	4.40E+07	4.65E+07	4.48E+07	4.57E+07	3.94E+07	4.02E+07	3.95E+07
p value		*0.15*	*0.05*	*0.35*	*0.23*	*0.26*	*0.24*
1.4 g Larch AG							
Mean	3.65E+07	3.12E+07	4.06E+07	3.81E+07	2.59E+07	1.64E+07	3.57E+07
Std Dev	4.23E+07	3.94E+07	5.51E+07	4.31E+07	3.54E+07	2.41E+07	4.22E+07
p value		*0.35*	*0.40*	*0.46*	*0.22*	*0.05*	*0.48*
Placebo							
Mean	4.00E+07	4.44E+07	7.60E+07	3.25E+07	1.00E+07	2.59E+07	2.20E+07
Std Dev	4.37E+07	5.79E+07	4.12E+07	4.02E+07	0.0	3.54E+07	3.17E+07
p value		*0.40*	*0.01*	*0.30*	*0.000*	*0.15*	*0.09*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as cells/litre; (range=0.0-0.2E+09)

000155

Table 29
Long-Term Human Trial: Sedimentation Rate

Treatment	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
8.4 g Larch AG							
Mean	8.53	7.29	6.76	8.15	7.43	7.68	8.22
Std Dev	7.29	7.91	4.54	6.26	7.02	5.44	4.62
p value		*0.31*	*0.19*	*0.43*	*0.33*	*0.35*	*0.44*
8.4 g Eastern Larch AG							
Mean	10.68	13.37	10.93	8.88	8.74	12.23	9.83
Std Dev	5.59	6.19	6.08	6.21	6.15	5.46	5.35
p value		*0.10*	*0.45*	*0.18*	*0.17*	*0.20*	*0.32*
4.4 g Larch AG							
Mean	9.09	11.28	8.86	8.86	10.69	9.75	8.98
Std Dev	5.72	9.04	7.07	6.86	8.11	6.66	7.72
p value		*0.20*	*0.46*	*0.46*	*0.25*	*0.38*	*0.48*
1.4 g Larch AG							
Mean	7.86	6.98	10.53	8.75	9.75	8.62	8.70
Std Dev	6.60	5.23	9.29	8.40	8.84	6.62	6.94
p value		*0.34*	*0.17*	*0.37*	*0.25*	*0.38*	*0.37*
Placebo							
Mean	7.14	7.46	9.59	7.17	7.78	7.58	7.06
Std Dev	5.82	4.94	6.43	5.45	4.61	5.87	5.37
p value		*0.43*	*0.13*	*0.49*	*0.36*	*0.41*	*0.48*

p values denote One-Way ANOVA comparing each month to group baseline
expressed as mm/hr unc; (range=none)

000156

Table 30
Post-Dry Ash Mineral Content (ppm) of Arabinogalactan Preparations Fed to Subjects

	P	K	Ca	Mg	Mn	Al	Fe
4.4 g/day Larch AG KOH Extract	244.48	15571.60	96.52	84.18	4.46	<3.58	5.26
1.4 g/day Larch AG KOH Extract	401.58	5466.40	56.10	78.96	2.62	<3.58	2.18
8.4 g/day Larch AG Ca(OH)2 Extract	12.44	1046.70	13569.00	196.48	6.80	11.94	8.74
8.4 g/day Eastern Larch AG KOH Extract	23.27	108.93	46031.47	59.17	34.17	10.35	15.89

	Na	Zn	Cu	B	Pb	Ni	Cr
4.4 g/day Larch AG KOH Extract	837.22	1.64	<0.52	1.56	<1.68	<0.44	<0.28
1.4 g/day Larch AG KOH Extract	972.16	2.42	<0.52	0.80	<1.68	<0.44	<0.28
8.4 g/day Larch AG Ca(OH)2 Extract	1322.34	<0.14	<0.52	5.36	<1.68	0.66	0.70
8.4 g/day Eastern Larch AG KOH Extract	6947.62	0.92	2.29	0.75	108.01	2.10	0.54

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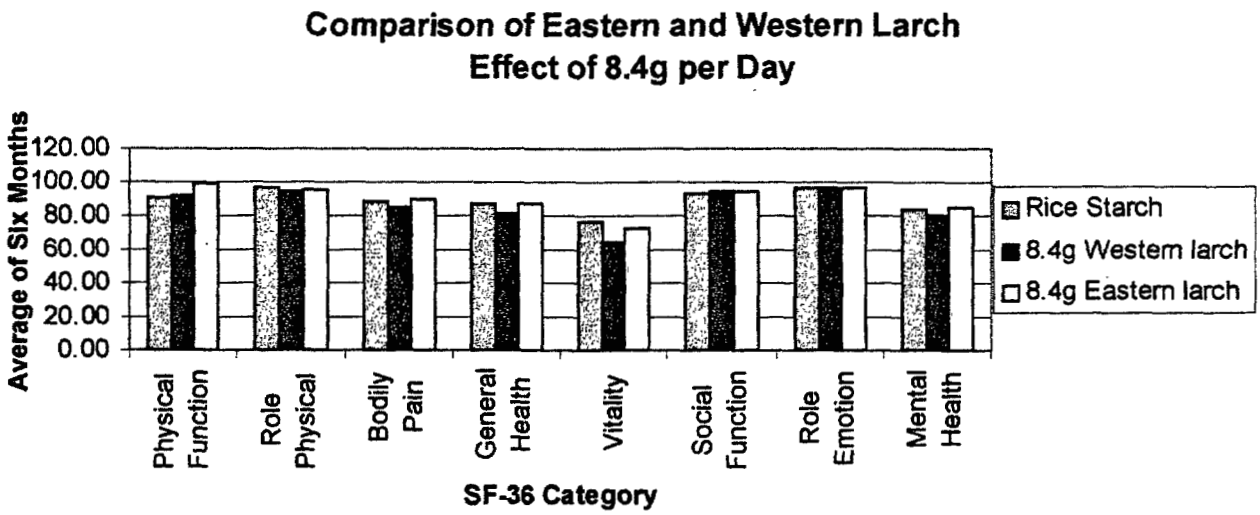
S. SF-36 Data

Over the years, health workers have built questionnaires that are designed to determine the state of a subject's health before and after a particular treatment regime. These questionnaires yield statistically significant results, which allow professionals to adjust the course of treatment for a particular disease state [Kim, 1999].



One of these questionnaires is called the SF-36 form, because it contains 36 questions, which clinical subjects can answer by themselves. There are over 1,000 references on the SF-36 in the scientific literature, and the form has now been translated into 11 languages. The number of people whose data has been published from the SF-36, now number in the tens of thousands in several countries [Kim, 1999].

The data shown graphically above and in Table 31 below, indicate that there was virtually no difference in effect between arabinogalactans from Eastern larch or Western larch.

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Table 31

Effect of 8.4 grams per day of Arabinogalactan

Derived from Two Species of the Genus *Larix*

SF-36 Parameter		"M" Rice Starch	"P" 8.4g Western larch	"H" 8.4g Eastern larch
Physical Function	Base	87.90	92.50	93.20
	Average	90.88	92.32	98.85
Role Physical	Base	89.30	92.50	100.00
	Average	96.67	94.33	95.70
Bodily Pain	Base	87.50	86.20	90.10
	Average	87.98	84.77	89.58
General Health	Base	83.30	79.60	88.50
	Average	86.62	81.27	87.32
Vitality	Base	68.10	65.00	72.40
	Average	76.98	64.80	72.73
Social Function	Base	93.50	96.30	96.10
	Average	92.72	93.98	93.72
Role Emotion	Base	95.20	98.30	89.50
	Average	96.58	96.23	96.15
Mental Health	Base	80.00	80.40	82.50
	Average	84.12	80.58	84.30

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D. Arabinogalactan Fermentation by Human Fecal Bacteria

Researchers at the University of Minnesota investigated the effect of various fibers including arabinogalactan derived from either Eastern or Western larch species on the proliferation of human fecal bacteria. Results are shown in Figures 1-5 below. Results showed that the population of anaerobic bacteria in human fecal samples increased with both types of arabinogalactan in comparison to time 0. Specific anaerobes, such as *Bacteroides sp.* were stimulated, whereas populations of *Clostridium sp.* were reduced. Neither sample of arabinogalactan had a stimulating effect on populations of Bifidobacteria [Causey, *et al.*, 2000].

Note: Certain fiber samples have been coded in the following figures to protect confidential information.

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Figure 1

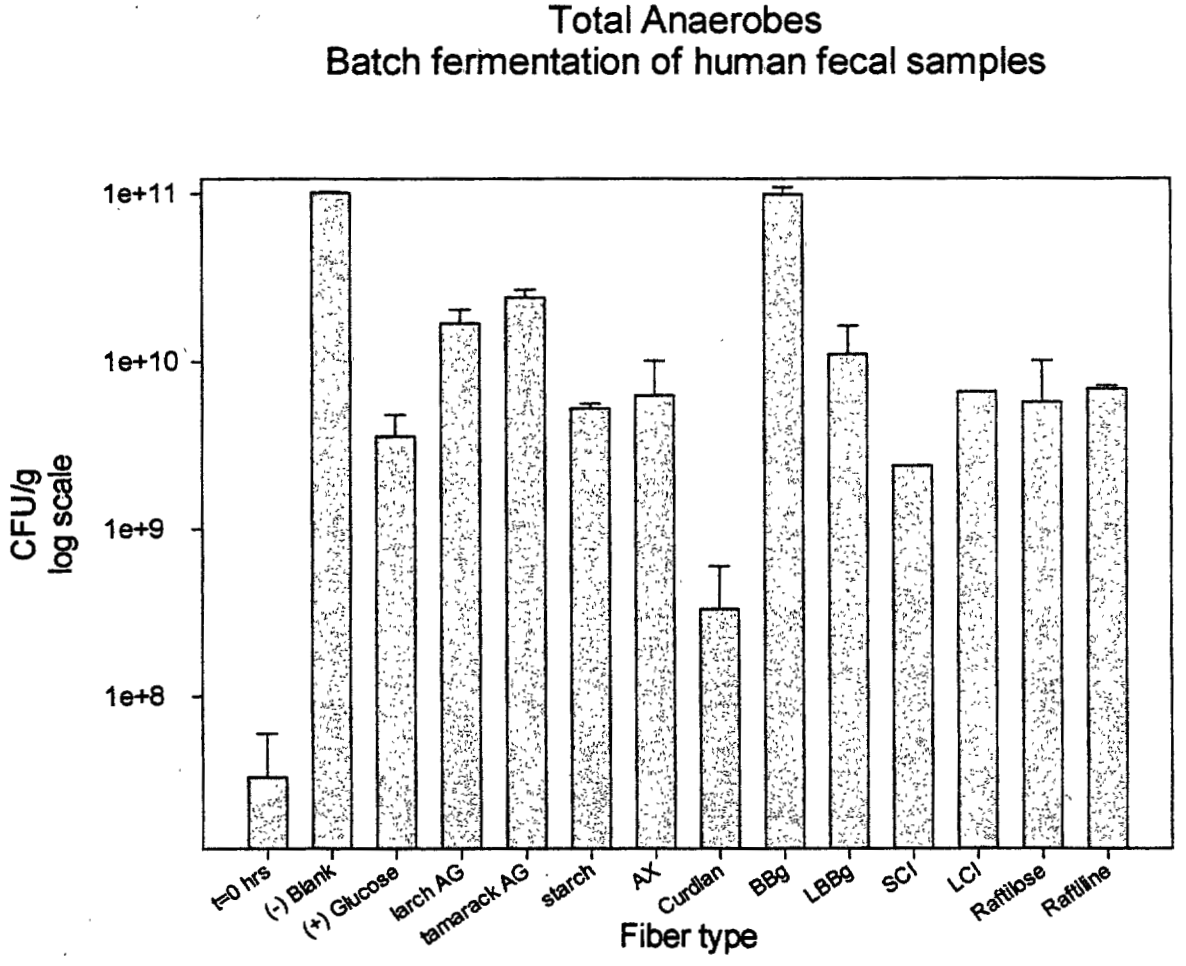
Total Anaerobes
Batch fermentation of human fecal samples
1e+11
1e+10
1e+9
1e+8
CFU/g
log scale
t=0 hrs
(-) Blank
(+) Glucose
larch AG
tamarack AG
starch
AX
Curdlan
BBg
LBBg
SCI
LCI
Raftilose
Raftiline
Fiber type

Figure 2

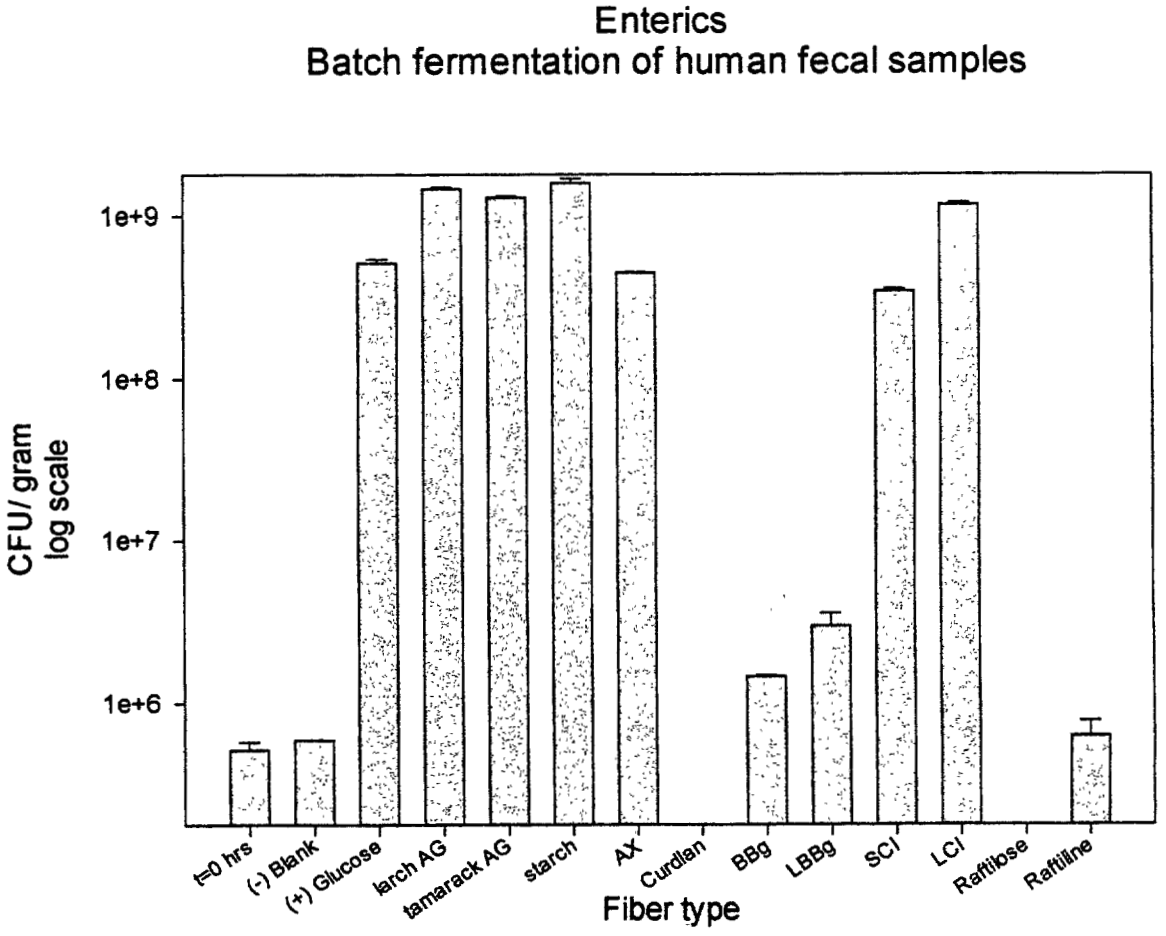

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Figure 3

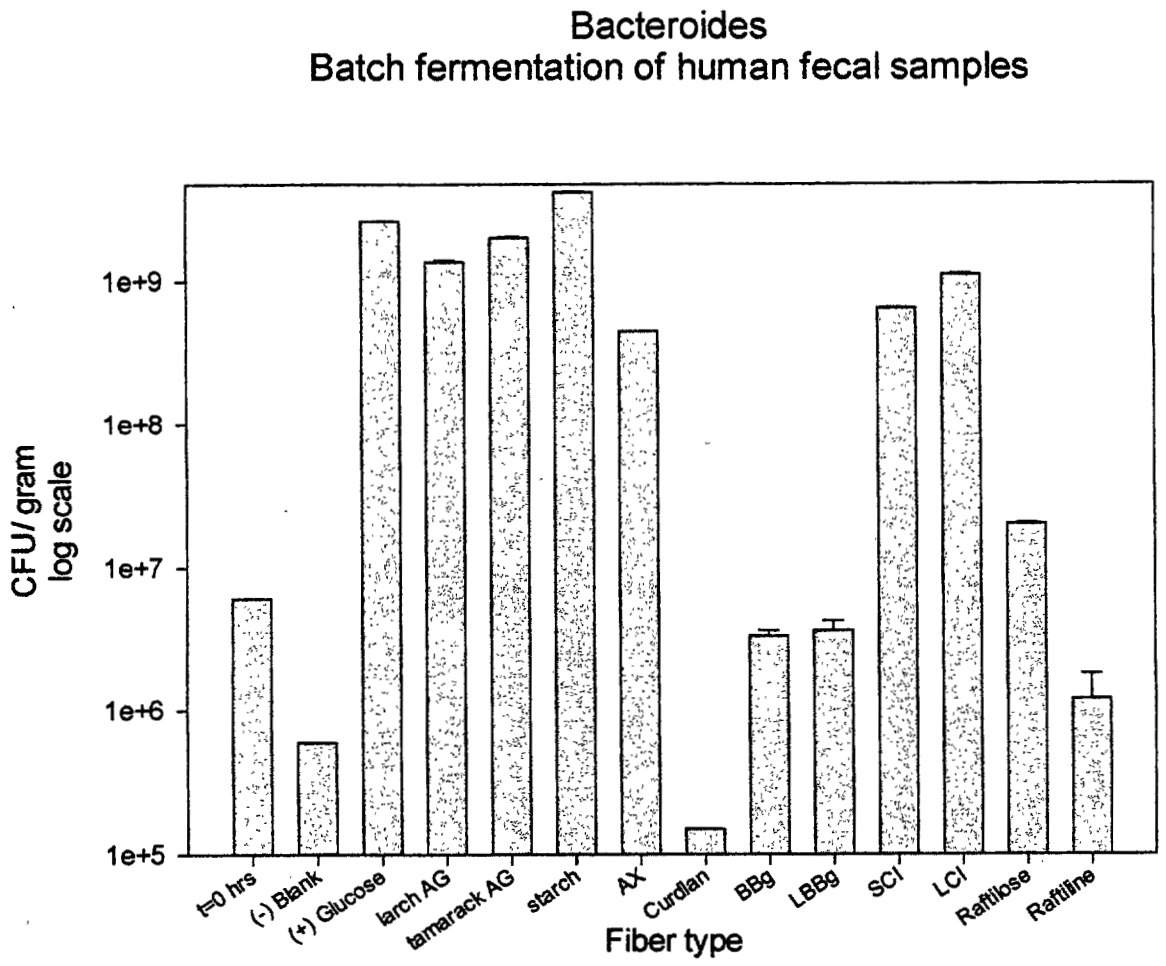

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Figure 4

Clostridia
Batch fermentation of human fecal samples

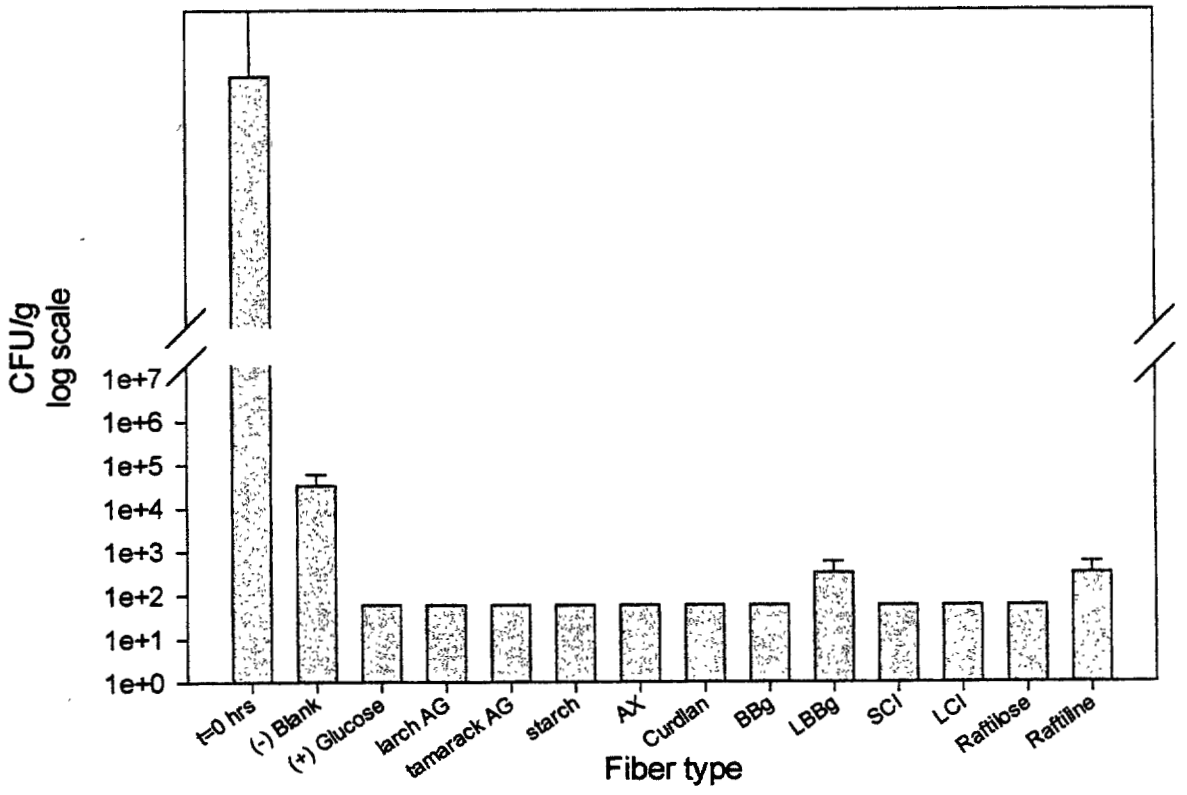

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Figure 5

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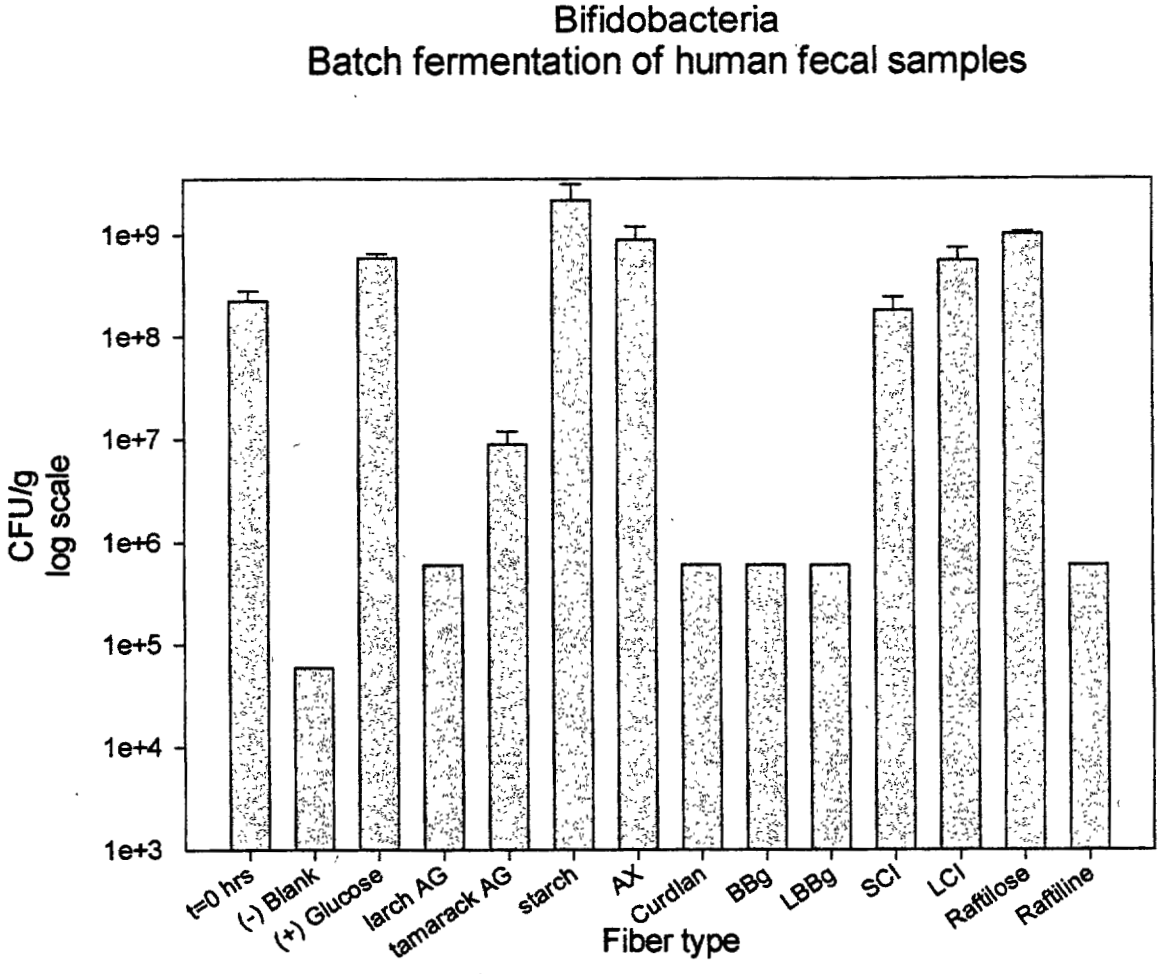

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Acute Study in Rats

Larex, Inc. engaged Celsis Laboratory Group, Edison, New Jersey to conduct an acute study in rats. Five male and 5 female Sprague Dawley rats were assigned to each treatment group. Treatment groups received 5g/kg bw of a Larex arabinogalactan product as a single dose. Animals were dosed by syringe and intubation tube. The test materials were dosed as a 1:2 weight to volume concentration in D.I. water. All animals were observed for signs of toxicity and mortality for 4 hours after dosing, and then twice daily for a total of 14 days [Gilman, 1999].

Results showed that rats receiving arabinogalactan from Eastern larch suffered no mortalities or other clinically significant effects. An LD_{50} was described as greater than 5g/kg.

Two other arabinogalactan samples derived from Western larch were also tested in the study (Grade 3000 and Grade 4000). Four of the five animals receiving Grade 3000 experienced a slight loss of weight between days 7 and 14. However, all had gained weight by day 14 in comparison to Day 0. The LD_{50} was described as greater than 5g/kg for both samples of Western larch arabinogalactan [Gilman, 1999].

F. Current Safe Use of Arabinogalactan

Arabinogalactan was approved as a food additive at 21 CFR §172.230 and §172.610 [Federal Register, 1965 Vol. 10 Tab 14]. It was listed as GRAS in 1972 by the Flavor Extract Manufacturer's Association (FEMA) by an expert panel which affirmed that arabinogalactan was a safe ingredient for use with flavoring ingredients in food [Oser, *et al.*, 1972 Vol. 11 Tab 10]. In Canada arabinogalactan is given the designation CEPA-NDSL No. 9036-66-2 section B16 100 Table 4. In Japan it is listed on the MITI list as a natural food additive. The substance has also been given a harmonization code at Schedule B1301.90.0000. [Larex, 1997, Vol. 11 Tab 5].

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Arabinogalactan has also been used as a dietary supplement since 1990 by the NANCI Corporation, and others in the dietary supplement industry. As such, arabinogalactan has been considered "grandfathered" as a safe dietary supplement since its use began prior to October 15, 1994 [Federal Register, 1994, Vol. 10 Tab 14]. Arabinogalactan from Western larch was self-affirmed as GRAS on January 16, 1998. Larex, Inc. received a letter

from the US Food and Drug Administration on June 6, 2000, indicating that the agency had no objection to the company's determination that arabinogalactan from Western larch was GRAS based on scientific procedures.

G. Summary

Larex, Inc. has presented data from acute animal studies, *in vitro* human bacteriological studies, and long-term human feeding studies, that indicate that arabinogalactan from Eastern larch can be considered generally recognized as safe, when produced and used under current Good Manufacturing Practices. Results of the studies demonstrated that arabinogalactan from Eastern larch has identical physiological effects on the human body as does arabinogalactan from Western larch. Therefore, Larex submits that arabinogalactan derived from Eastern larch should be considered generally recognized as safe when used for its intended purpose as a food ingredient.

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Section V
Environmental Assessment

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Section V: Environmental Assessment

A. Summary

The Larex, Inc. manufacturing plant for arabinogalactan products is located in Cohasset, Minnesota. It is the position of Larex, Inc. that the manufacture of its products poses no threat to the environment. All manufacturing is performed under cGMP, and any waste materials from the plant are disposed of through proper channels. All liquid waste from the plant is disposed of through the Cohasset, Minnesota City sewer system, and solid waste from the plant is sold to secondary end users or sent to an appropriate land-fill facility.

The Larex plant manufactures arabinogalactan from a renewable resource with a positive growth rate. The raw material, the butt cuts of Larch logs, is delivered to the plant via rail and truck. These logs are chipped at the plant, and extracted with hot water to produce a liquid arabinogalactan product. The spent chips are sold to local fiberboard manufacturers, and hence pose no threat to the local environment.

To further establish that the production of arabinogalactan poses no threat to the environment, Larex, Inc. subjected its arabinogalactan product to a OECD screening test for biodegradability [BioScreen Testing Services, Inc. Report, 7/10/96, Vol. Tab 1]. Results showed that the arabinogalactan material obtained greater than 70% dissolved organic carbon (DOC) removal within 28 days (the required level for a valid test). The loss of dissolved organic carbon from the arabinogalactan sample was above the 70% level at day 17, and remained above that level for the duration of the 28 day test. The conclusion from this experiment was that arabinogalactan can be regarded as readily biodegradable.

% DOC Removal from Arabinogalactan

Accession #	Flask #	Calculation	%DOC removal after x days				
			7 day	14 day	17 day	27 day	28 day
960000843	1	D_1	24.3	86.5	91.9	91.9	89.2
	2	D_2	15.8	51.3	89.5	86.8	84.2
	mean	D	20	69	91	89	87

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Section I: Chemical Identity of Arabinogalactan
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	Tab	Vol.
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Raw Data

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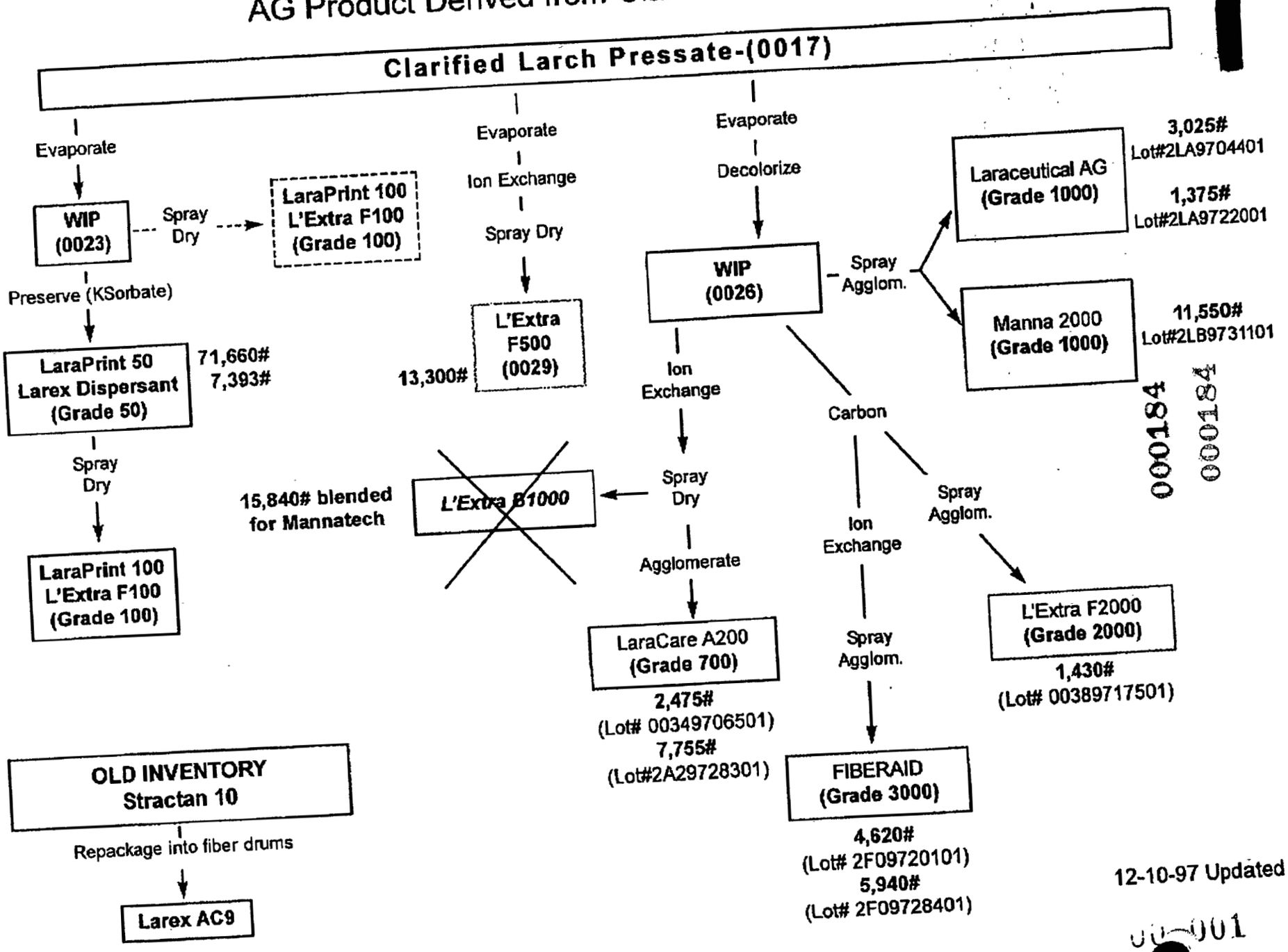
AG Product Derived from Clarified Larch Pressate



12-10-97 Updated

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Tamarack vs Western Larch Products

Cohasset Plant produced four spray-dried products from Tamarack : Grade 100, LaraCare, ImmunEnhancer and ClearTrac.

The processing steps for Tamarack products:
Grade 100-EL: same as LSC1 from Western Larch.

LaraCare-EL: Grade 100-EL → cationic exchanged → decolorized with KOH/H_2O_2 → anionic exchanged → concentrated → dried.

ImmunEnhancer-EL: Grade 100-EL → cationic exchanged → KOH/H_2O_2 → dried.

ClearTrac-EL: Grade 100-EL → cationic exchanged → $Ca(OH)_2/H_2O_2$ → anionic exchanged → carbon treated → concentrated → dried.

The above tamarack products were compared with the corresponding western larch products, which were processed the same way with the exception that the cationic exchanged step is not used in processing western larch products. The western larch products used were LSC1, LaraCare 00349706501, ImmunEnhancer 2LB9910801, and ClearTrac 2C99935801.

(Tamarack is also known as Eastern Larch. I'll use EL for Tamarack and WL for Western Larch.)

Results:

	Absorbance at*			
Sample	400nm	280nm	Phenol,ppm**	Mw
Grade 100-EL	0.0188	0.3535	16.65	18,815
LSC1-WL	0.0192	0.2456	8.66	17,527
LaraCare-EL	0.0070	0.5121	2.13	18,871
LaraCare-WL	0.0270	0.9432	2.03	19,040
ImmunEnhancer-EL	0.0178	1.8295	5.46	18,153
ImmunEnhancer-WL	0.0116	0.8025	0.85	17,978
ClearTrac-EL	0.0062	0.2599	0.29	18,750
ClearTrac-WL	0.0074	0.3084	nd	17,926

*All measurements were made with 1% solution except for Absorbance measurements for Grade 100-EL and LSC1-WL, which were measured with 0.1% solutions.
**All measurements were made with 1% solutions using CheMets ampules.

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Color - as indicated by abs @ 400nm
Tamarack products were very comparable to the existing Western Larch products in terms of color.

Phenols – abs @ 280nm and CheMets ampules
All EL samples have higher phenol level than their WL counterpart using CheMets ampules.

Turbidity
ImmunEnhancer-EL is the only product that is more turbid, otherwise, all tamarack products were comparable to their counterparts.

HPLC
From the RI, AG from EL was found to have similar Mw to that of WL in all samples. In his letter dated 1994, Dr. Glen Ponder found that the Mw from EL is 30% larger than that from WL , based upon the calibration with Pullulan standards. However, in his letter in 1995, he quoted that Timell and coworkers reported a larger Mw in WL(58,500) than EL(36,000).

From UV @ 280nm there is a peak eluted before the AG peak in all EL but not WL products. This peak only showed up in the UV but not in RI. The impurities in the EL samples seemed to be present in larger quantities than WL as indicated by the larger area under the curve for the EL samples.

CHM
4-17-2000

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[redacted]

Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 280.0

Function	Reading
Scan	0.35379

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

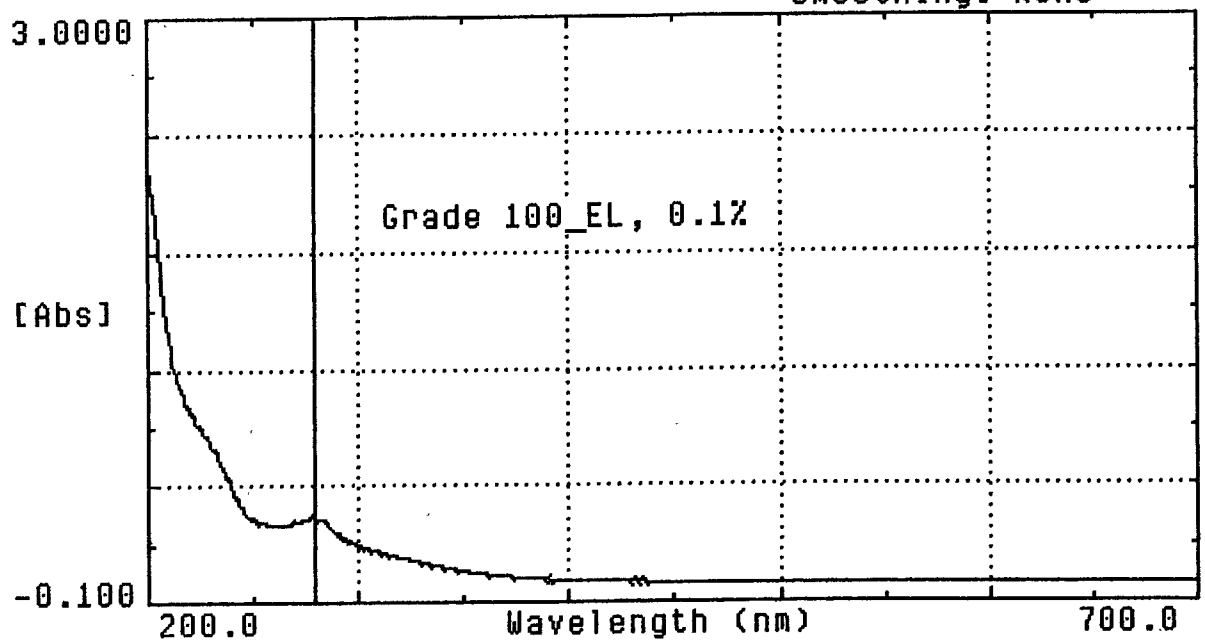
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.3538	pt
400.0	0.0188	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



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MATCH OFF	[UV ON]	RediRead	PrtScrn	-0.0025 Abs	02:42	07/04/00	N/A	N/A

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Wavelength Scan HELP

ReadSamples Tabula

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

A:\WORK_001 (1200)

Wavelength: 278.0

Function	Reading
Scan	0.52056

NetA Method SaveClear Print Quit

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

Print ↓ ↑ ← → Exit

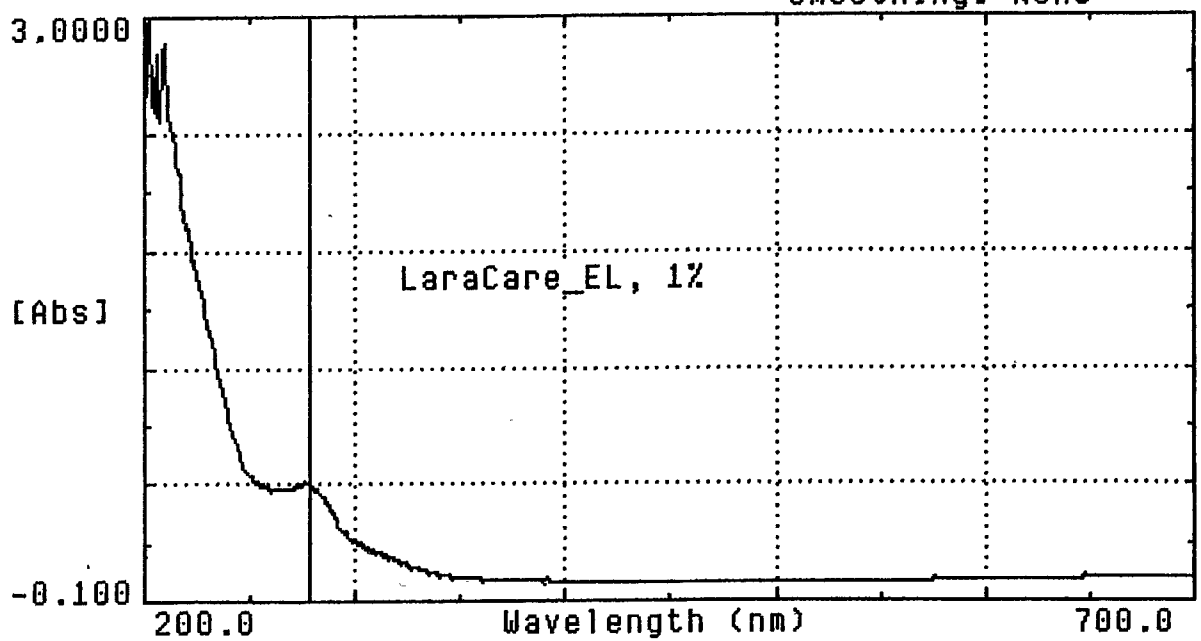
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.5121	pt
400.0	0.0070	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



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MATCH OFF	[UV ON]	RediRead	PrtScrn	0.0312 Abs	02:26	07/04/00	N/A	N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 277.0

Function	Reading
Scan	1.85813

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

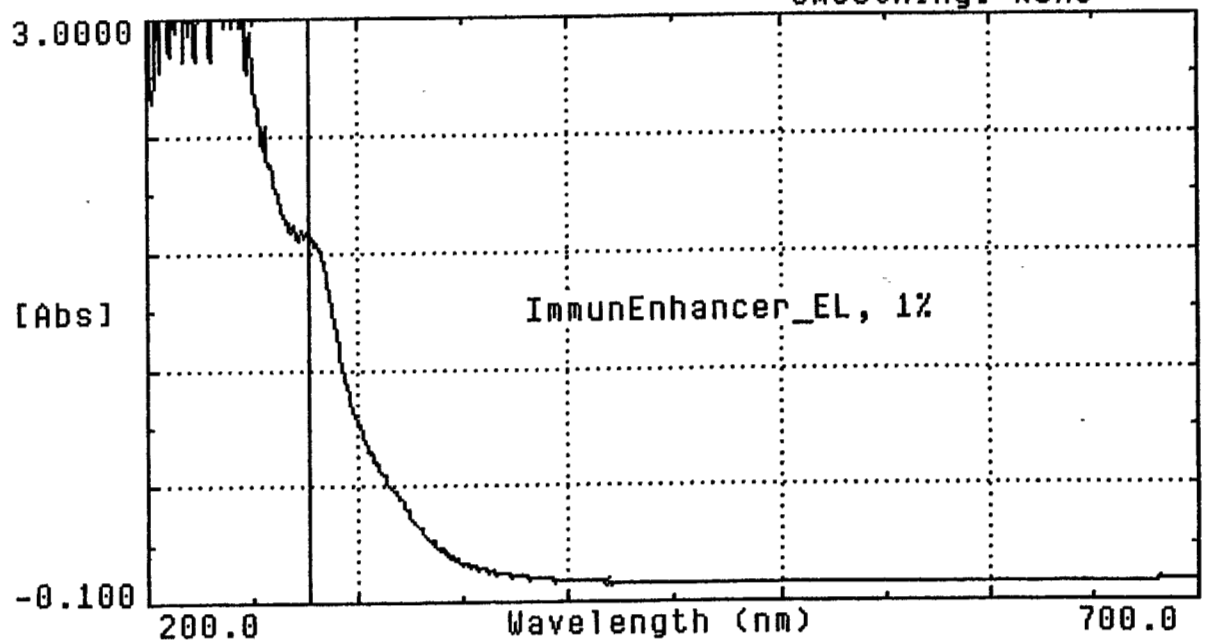
Print ↓ ↑ ← → Exit | Zoom ZoomOut Trace Function Autoscale Annotate Print

A:\WORK_001 Scan

wl	Abs	Pick
280.0	1.8295	pt
400.0	0.0178	pt

Functions: Scan

Smoothing: None



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MATCH OFF	[UV ON]	RediRead	PrtScrn	0.0110 Abs	02:32	07/04/00	N/A	N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 274.0

Function	Reading
Scan	0.26415

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

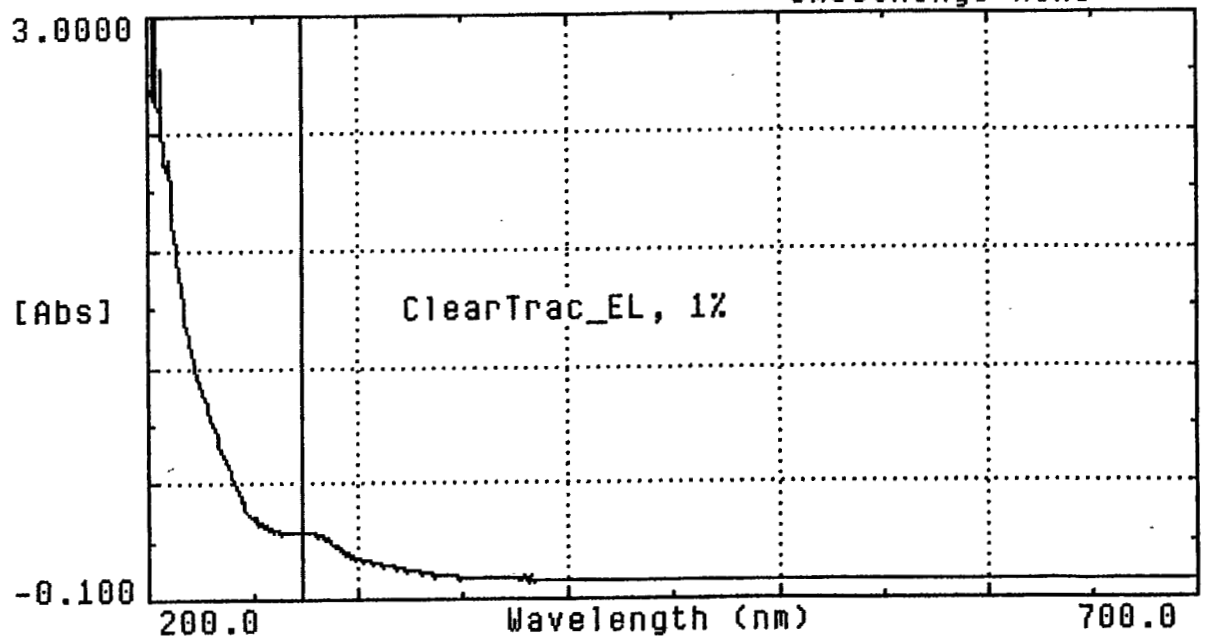
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.2599	pt
400.0	0.0062	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



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MATCH OFF [UV ON] RediRead PrtScrn -0.0000 Abs 02:19 07/04/00 N/A N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 280.0

Function	Reading
Scan	1.11660

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

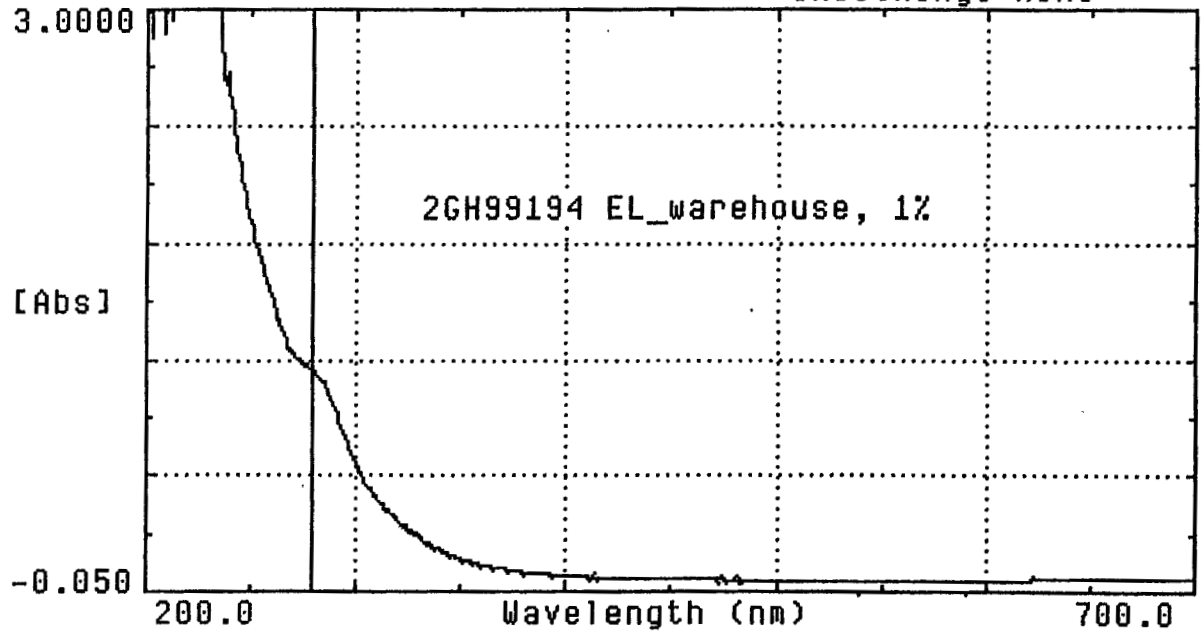
A:\WORK_001 Scan

wl	Abs	Pick
280.0	1.1166	pt
400.0	0.0374	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



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MATCH OFF	[UV ON]	RediRead	PrtScrn	0.0018 Abs	10:08	24/07/00	N/A	N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 255.0

Function	Reading
Scan	0.61669

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

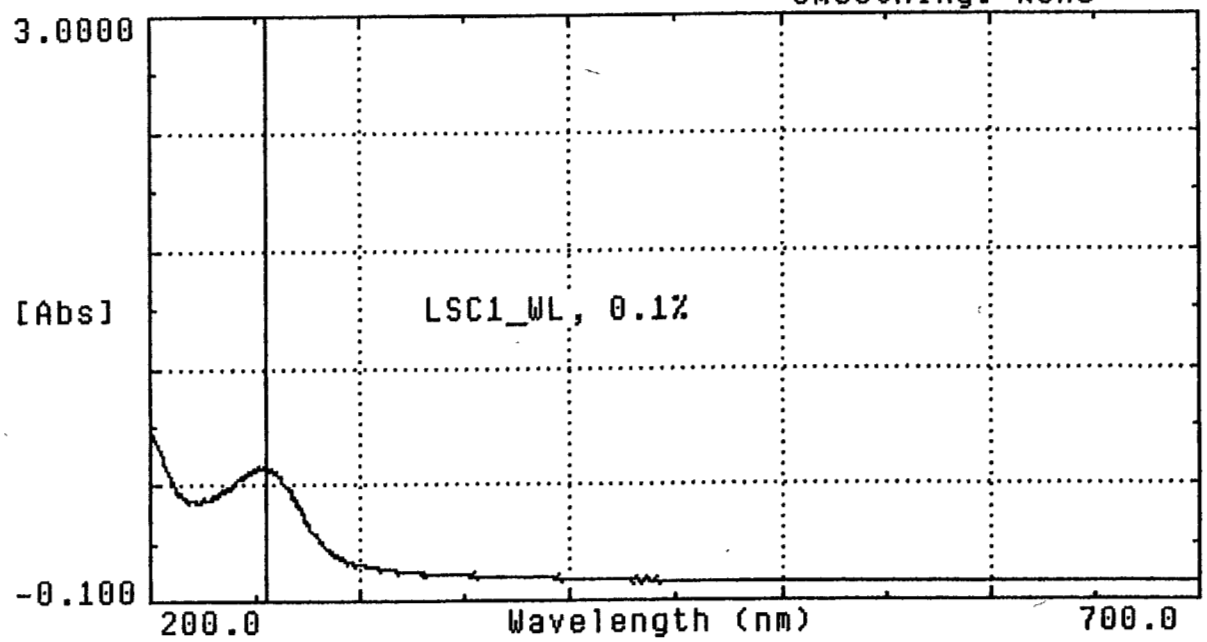
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.2456	pt
400.0	0.0192	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 278.0

Function	Reading
Scan	0.96417

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

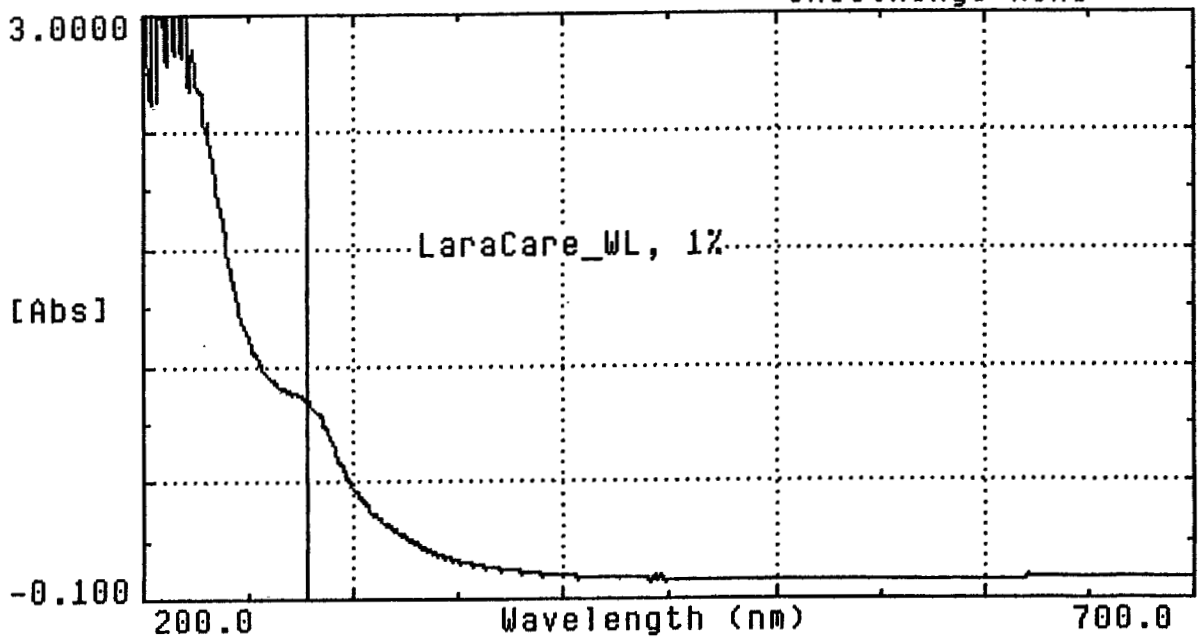
Print ↓ ↑ ← → Exit | Zoom ZoomOut Trace Function Autoscale Annotate Print

A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.9432	pt
400.0	0.0270	pt

Functions: Scan

Smoothing: None



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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 276.0

Function	Reading
Scan	0.82902

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

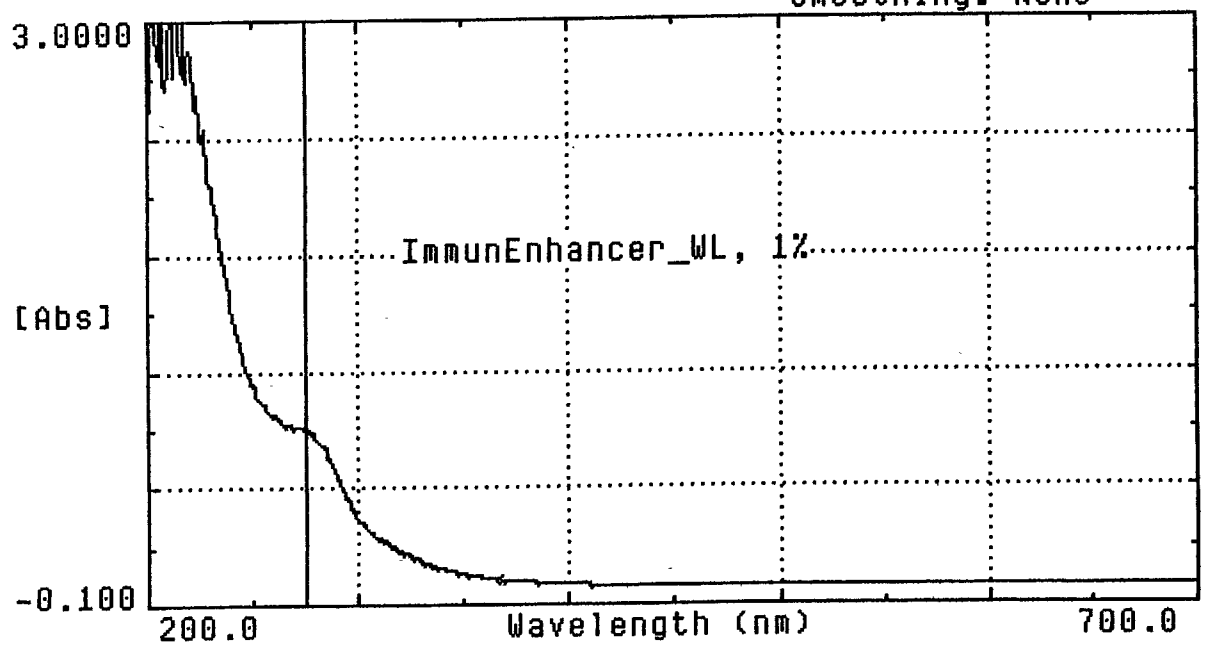
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.8025	pt
400.0	0.0116	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



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Wavelength Scan HELP

ReadSamples Tabula NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 269.0

Function	Reading
Scan	0.33173

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_002 (1200)

Print ↓ ↑ ← → Exit

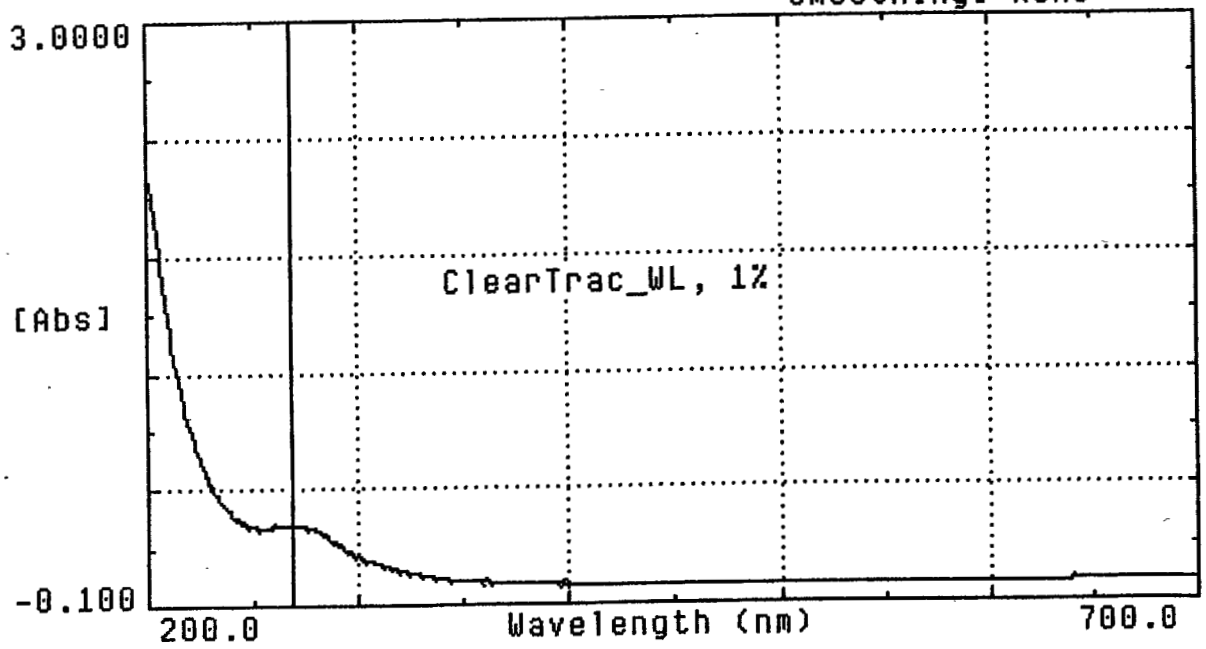
A:\WORK_002 Scan

wl	Abs	Pick
280.0	0.3084	pt
400.0	0.0074	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



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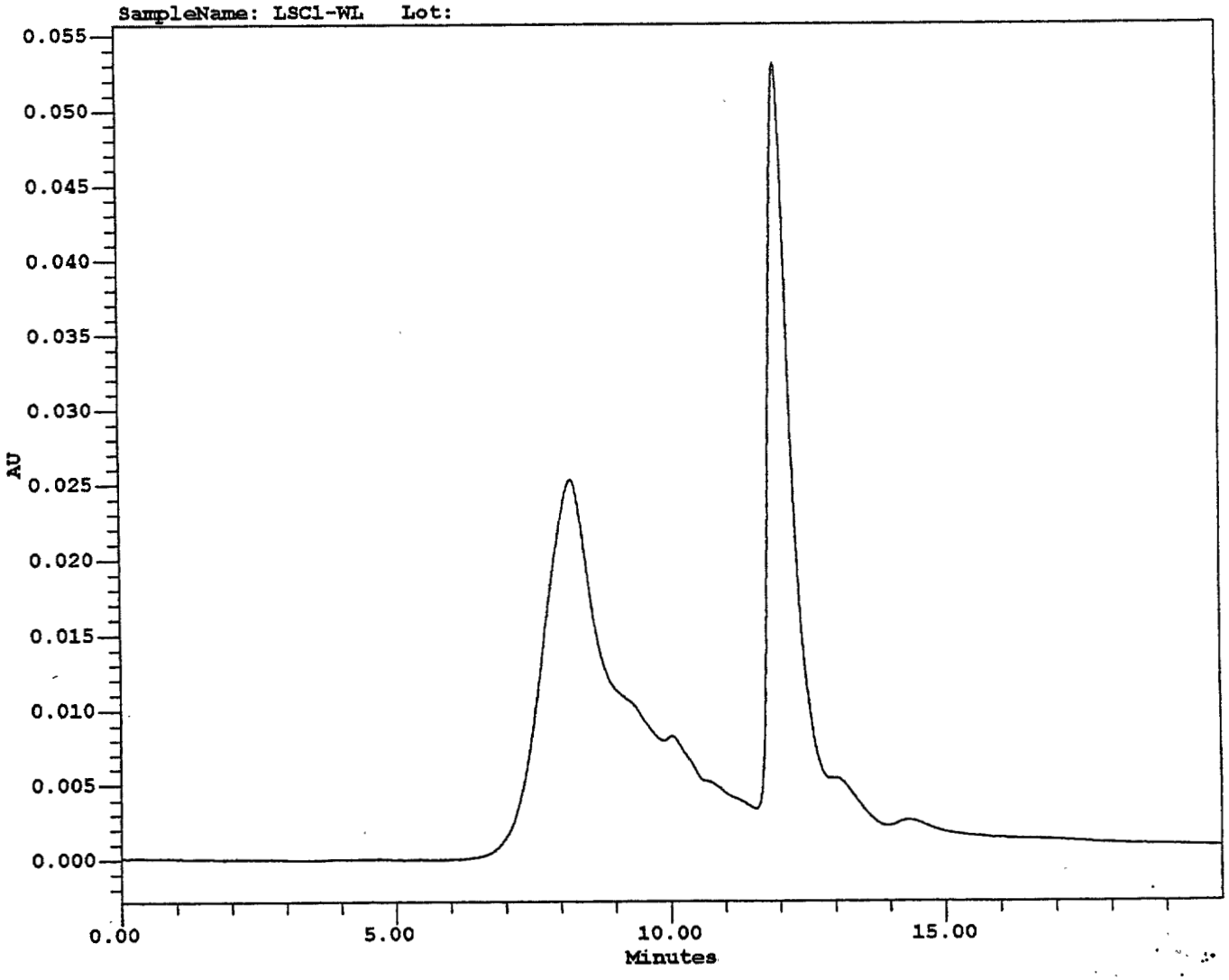
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LSC1-WL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 02:22:51 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:25 PM	Vial:	12
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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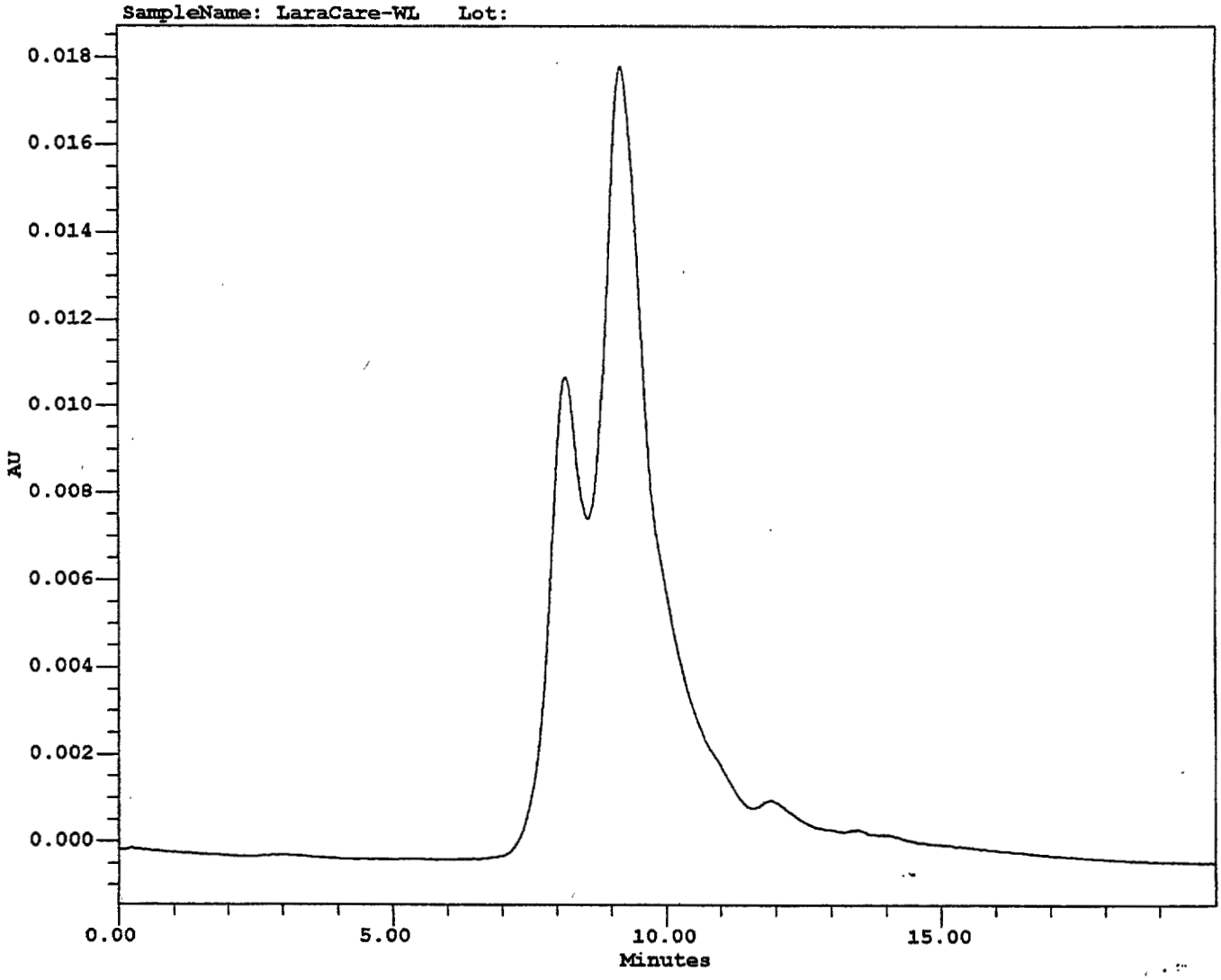
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LaraCare-WL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 01:39:34 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:44 PM	Vial:	10
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-WL
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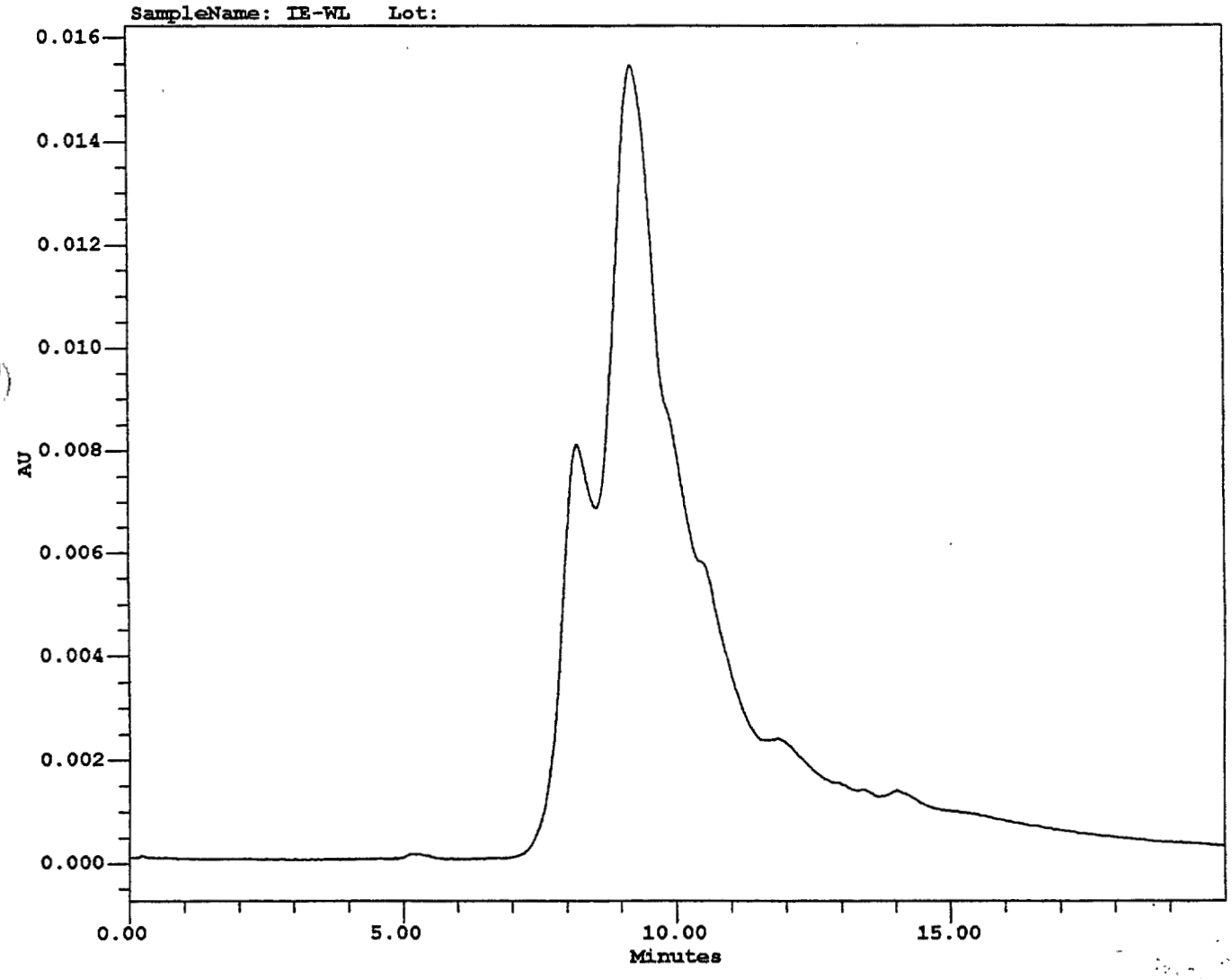
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 12:56:09 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:58 PM	Vial:	8
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-WL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 12:12:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:43:13 PM	Vial:	6
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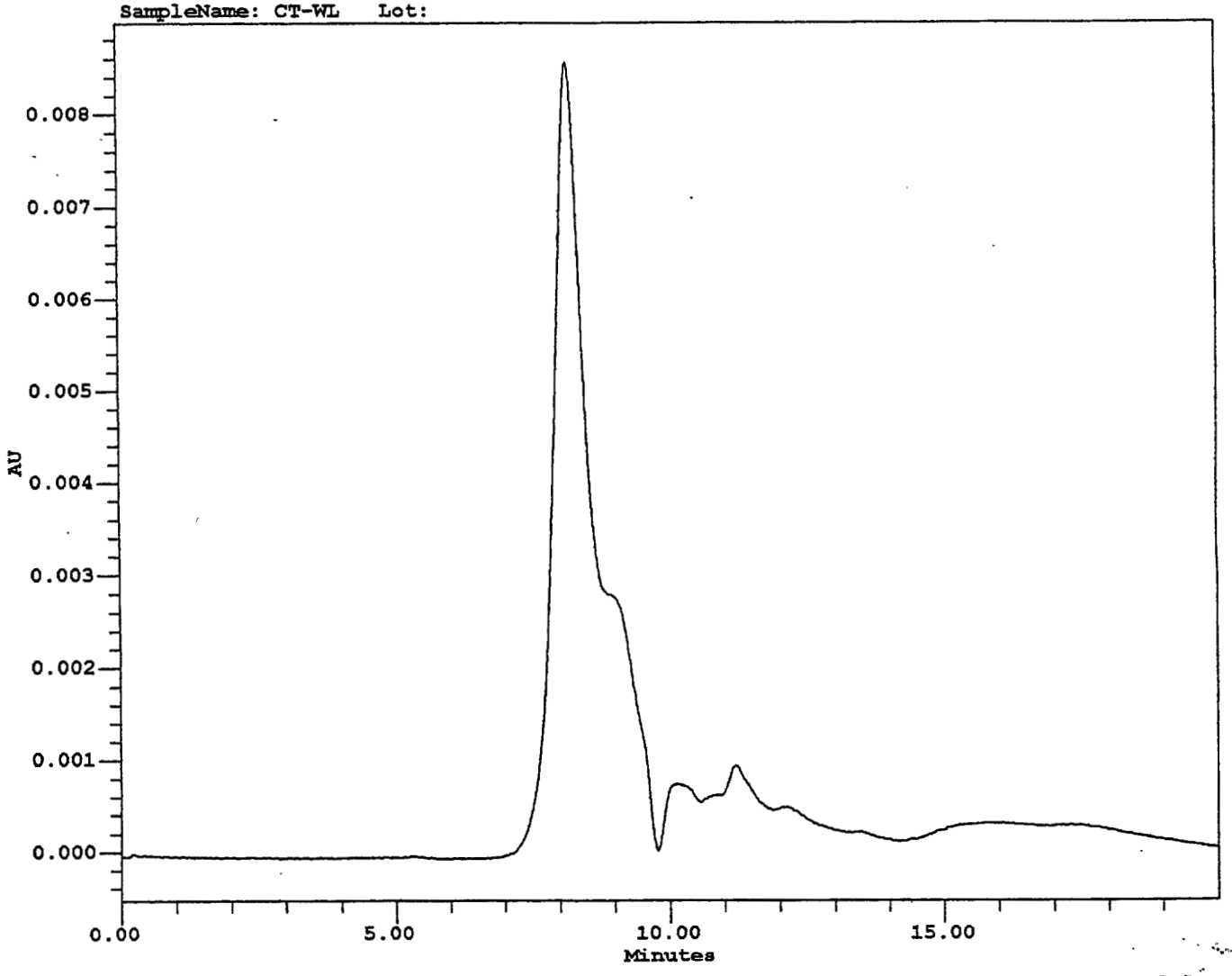
Channel Descr.	UV 280nm		



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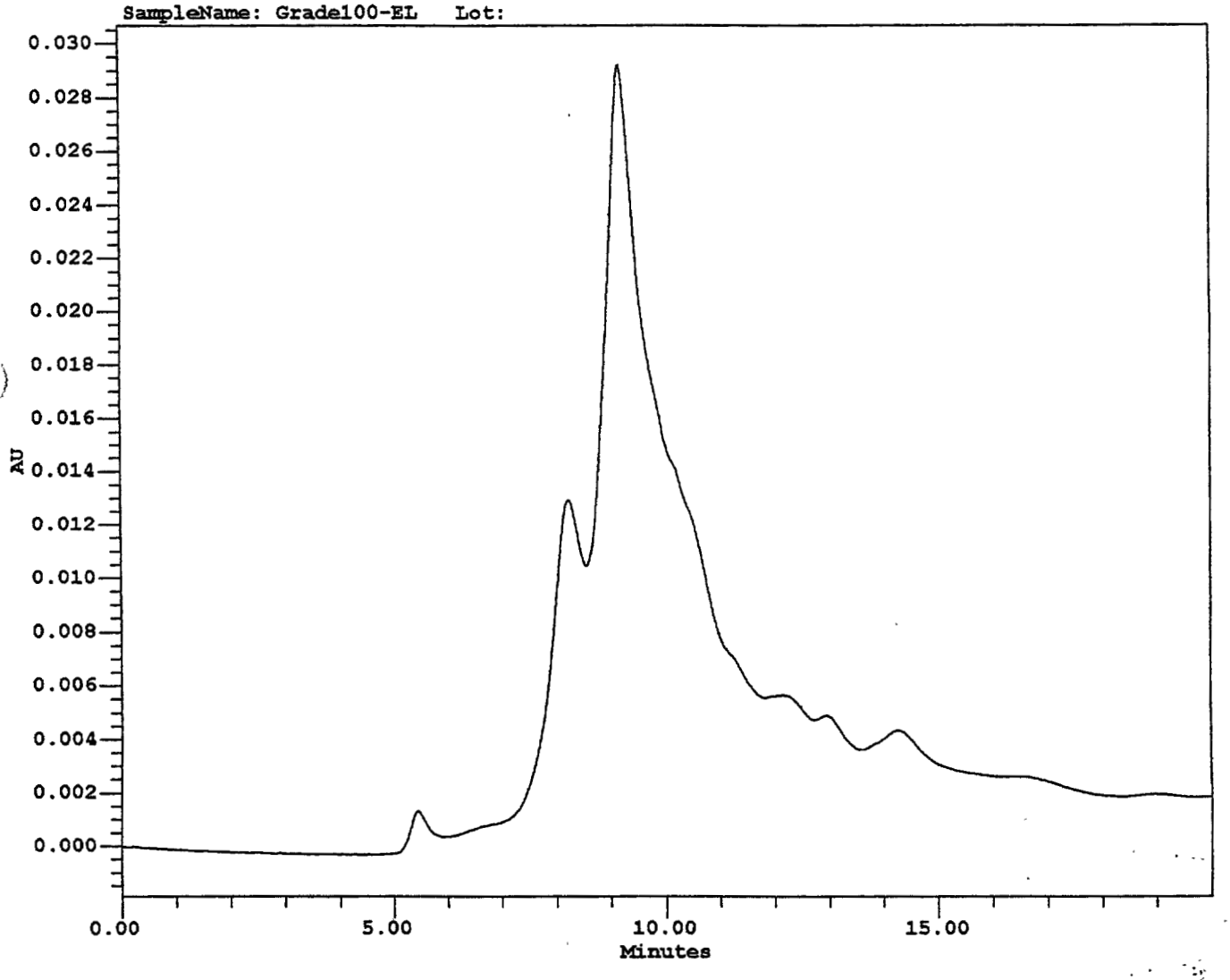
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Grade100-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 02:44:30 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:16 PM	Vial:	13
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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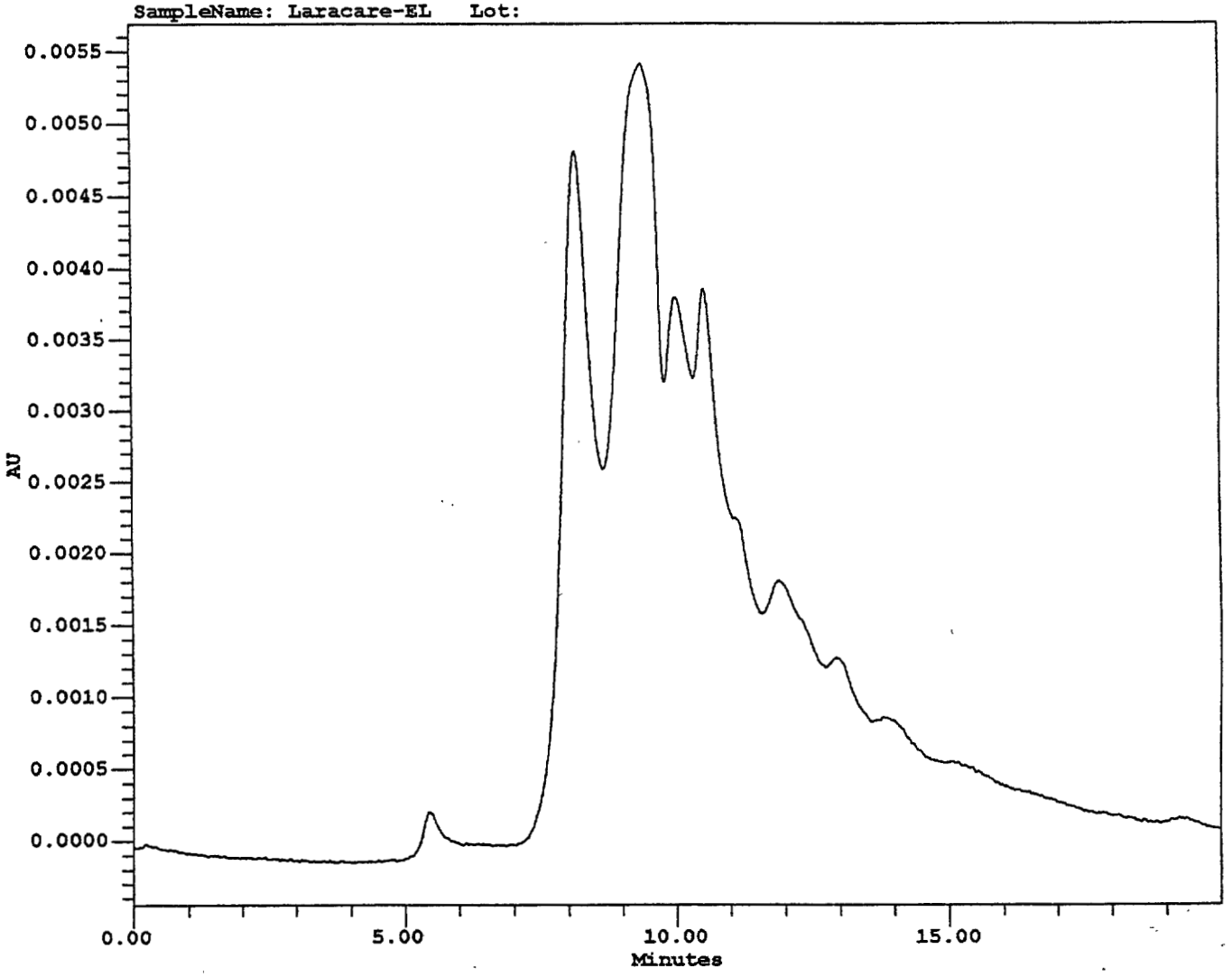
7.24.02

Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Laracare-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 02:01:13 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:33 PM	Vial:	11
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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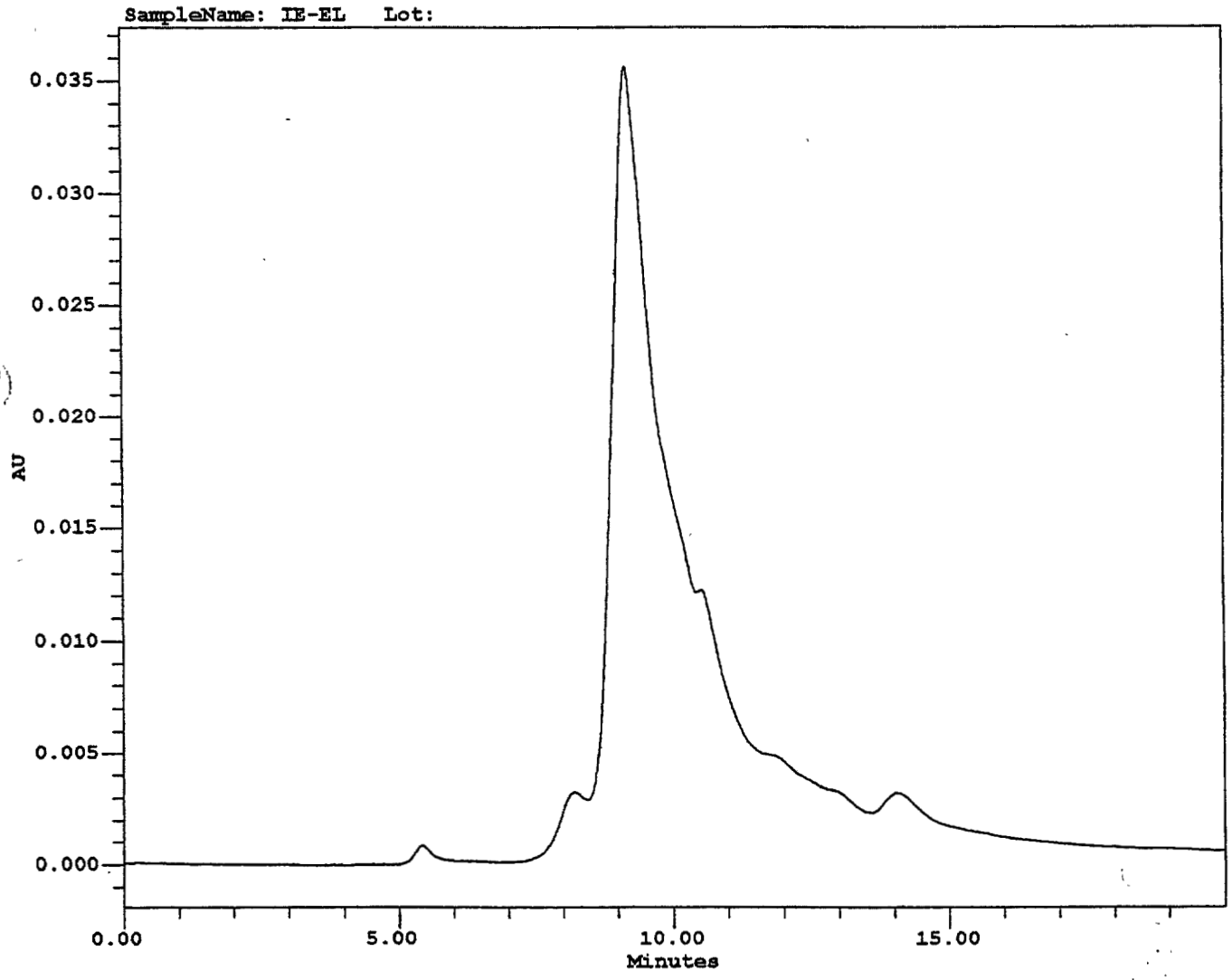
7.24.

Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 01:17:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:50 PM	Vial:	9
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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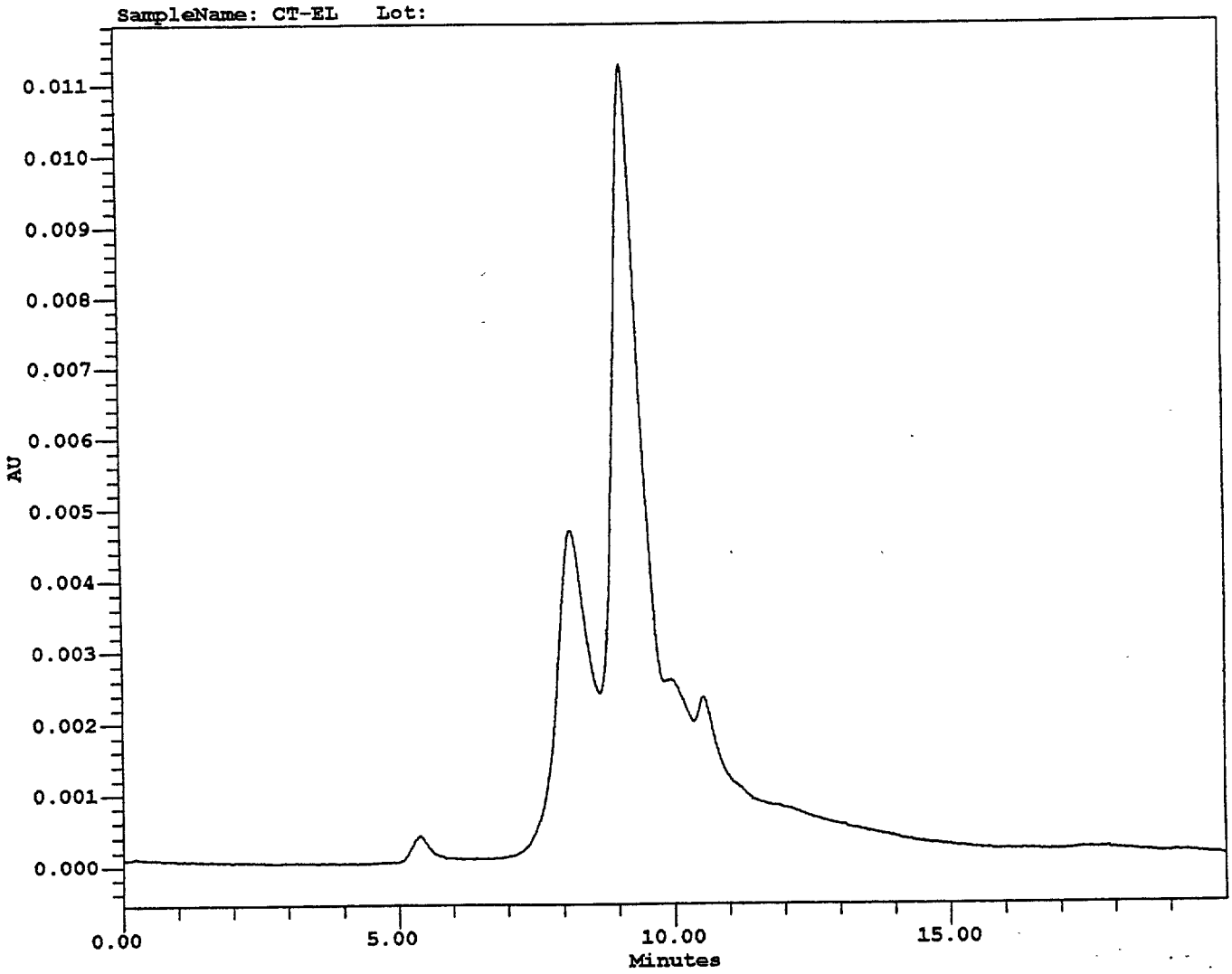
7.24.

Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 12:34:29 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:43:06 PM	Vial:	7
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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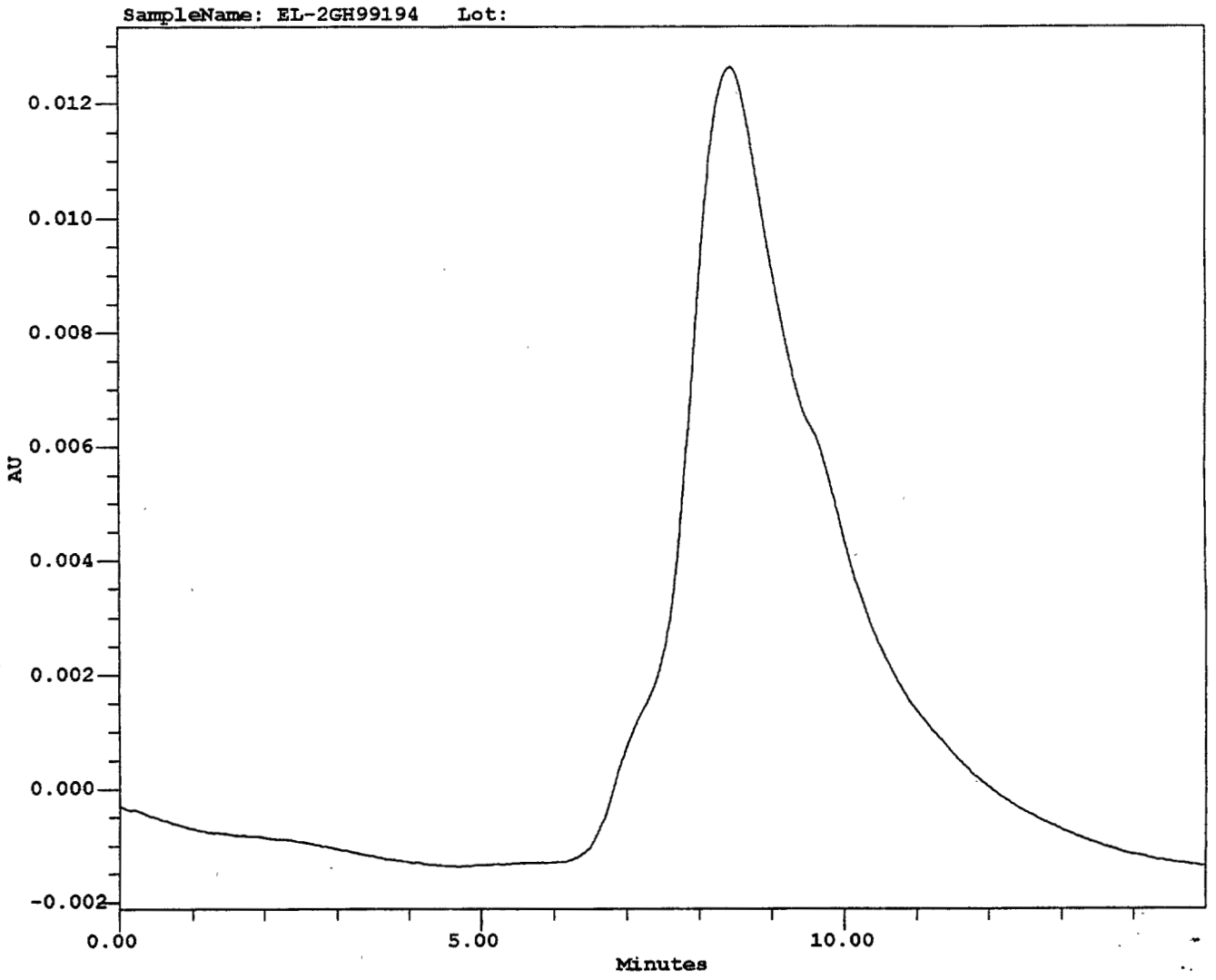
Larex International

July 24, 2000

Project Name:	AG_July_Y2K	Sample Name:	EL-2GH99194
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	07/21/00 03:26:05 PM	Inj Volume, ul:	40.0
Date Processed:	07/24/00 10:30:49 AM	Vial:	29
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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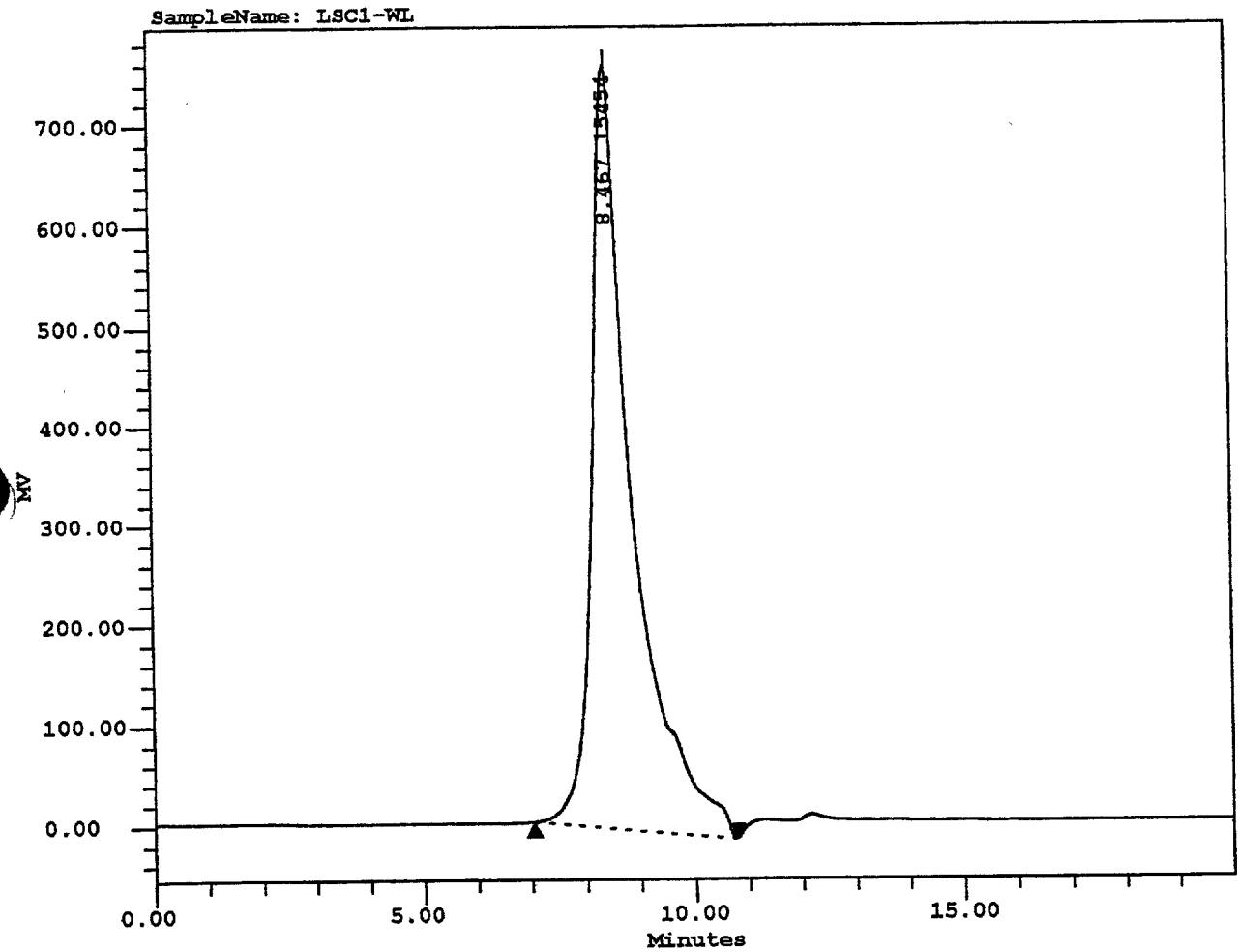
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LSC1-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	12
Date Acquired:	04/10/00 02:22:51 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:42 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.467	15454	17527	14086	1.244	8393678	162742	51.577

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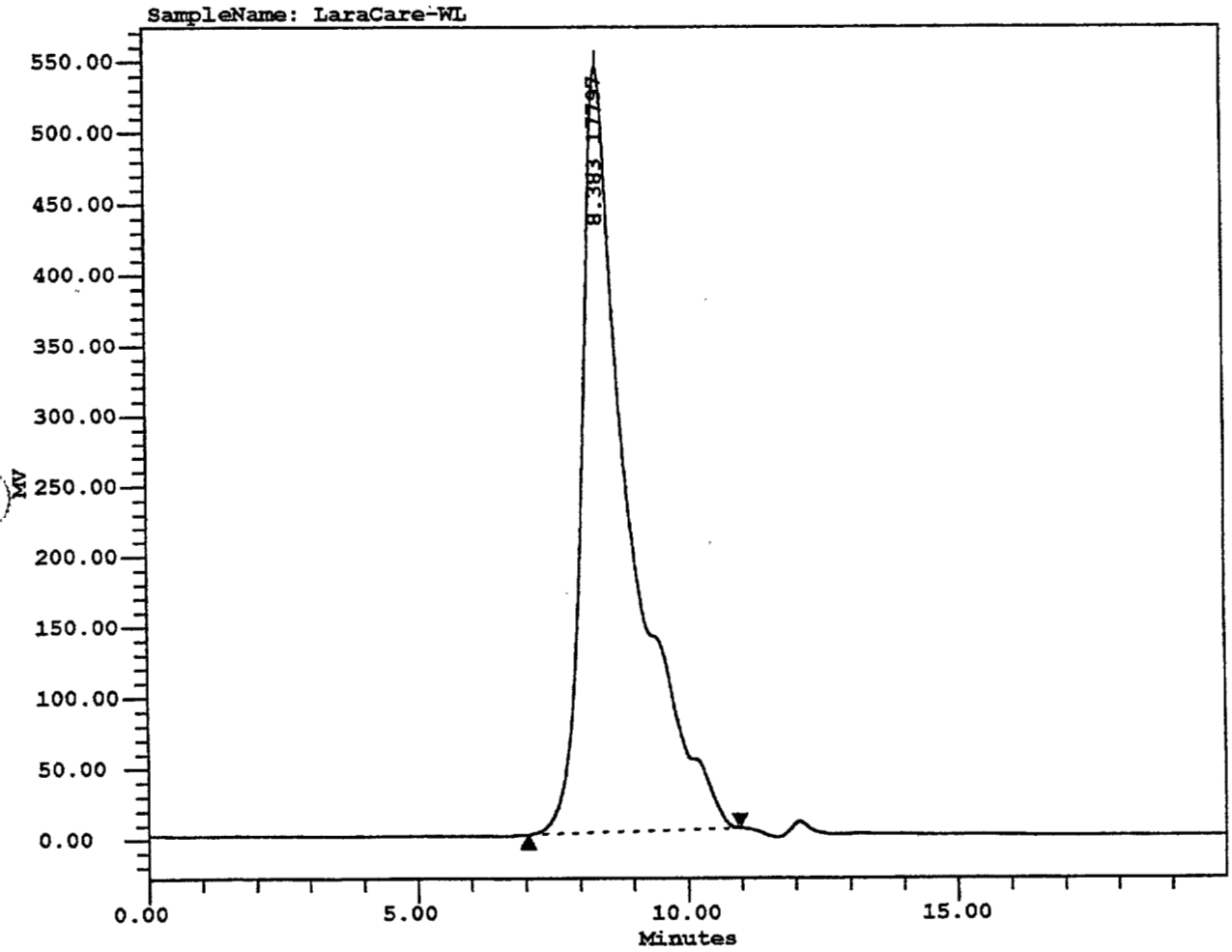
7.24.00

Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LaraCare-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	10
Date Acquired:	04/10/00 01:39:34 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:08 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.383	17797	19040	14953	1.273	7025777	115781	60.682

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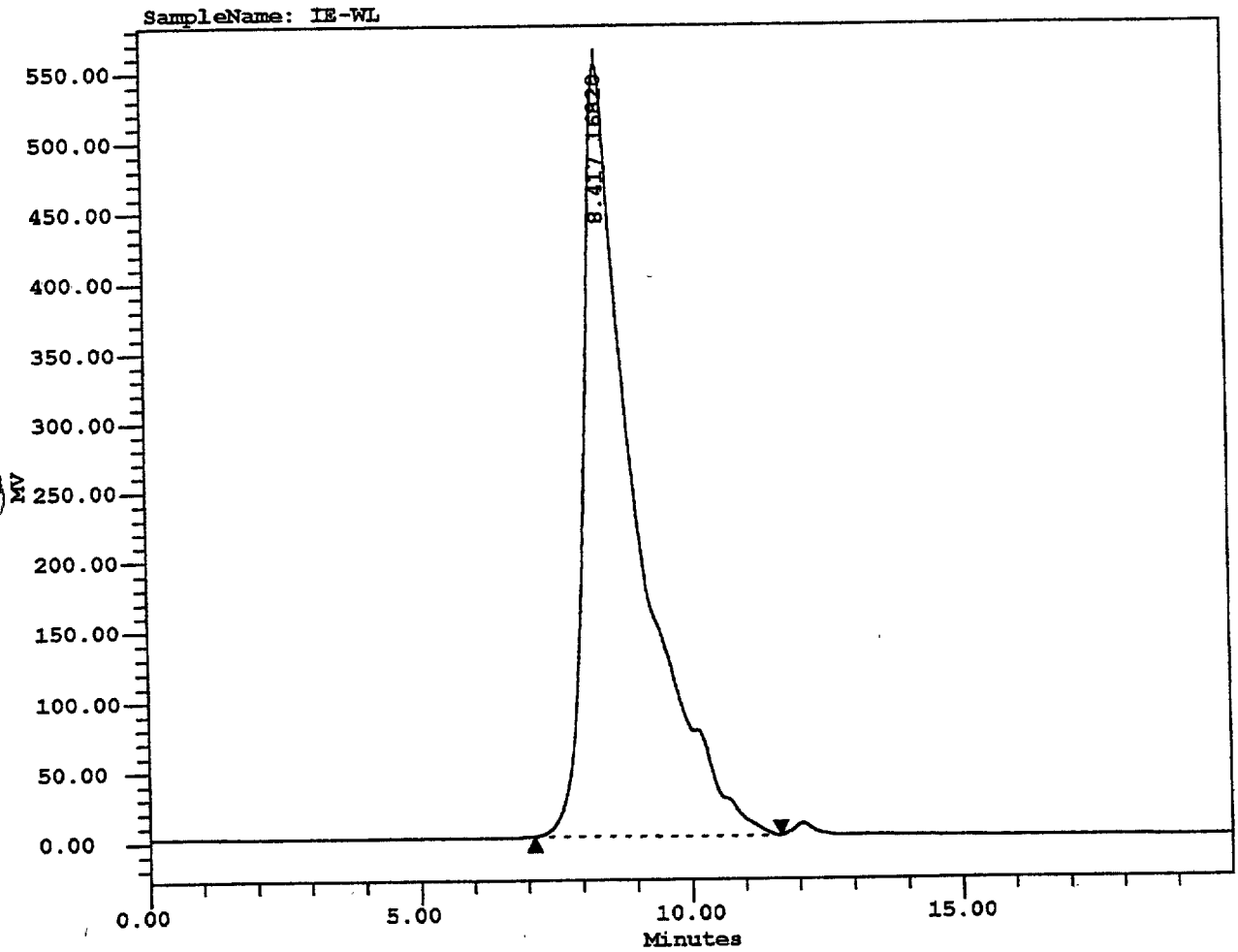
7.24.00

Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	8
Date Acquired:	04/10/00 12:56:09 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:02:11 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.417	16820	17978	14272	1.260	7751456	117936	65.726

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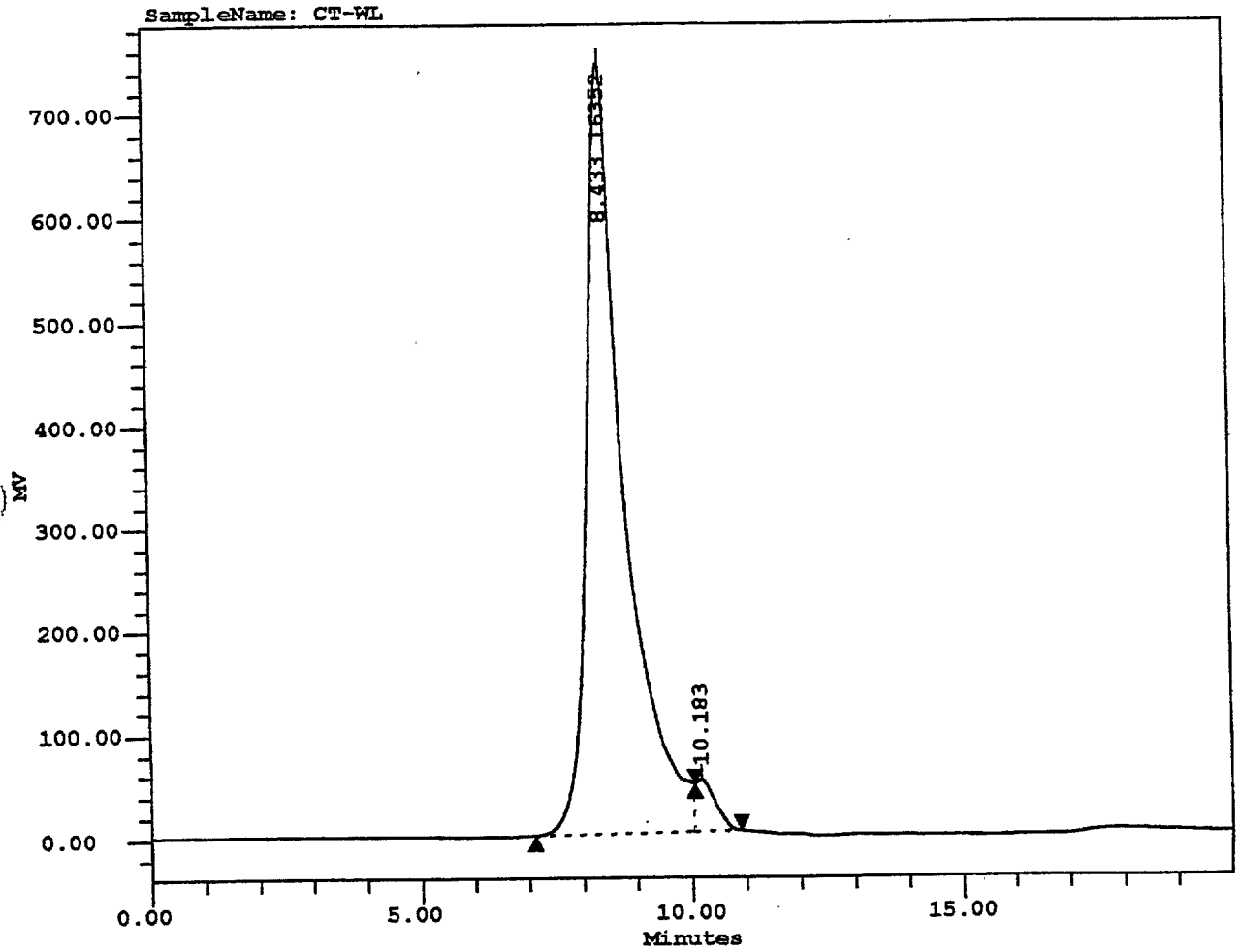
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	6
Date Acquired:	04/10/00 12:12:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:01:50 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.433	16352	17926	14636	1.225	7596724	158803	47.837
2	10.183					260984	10432	25.018

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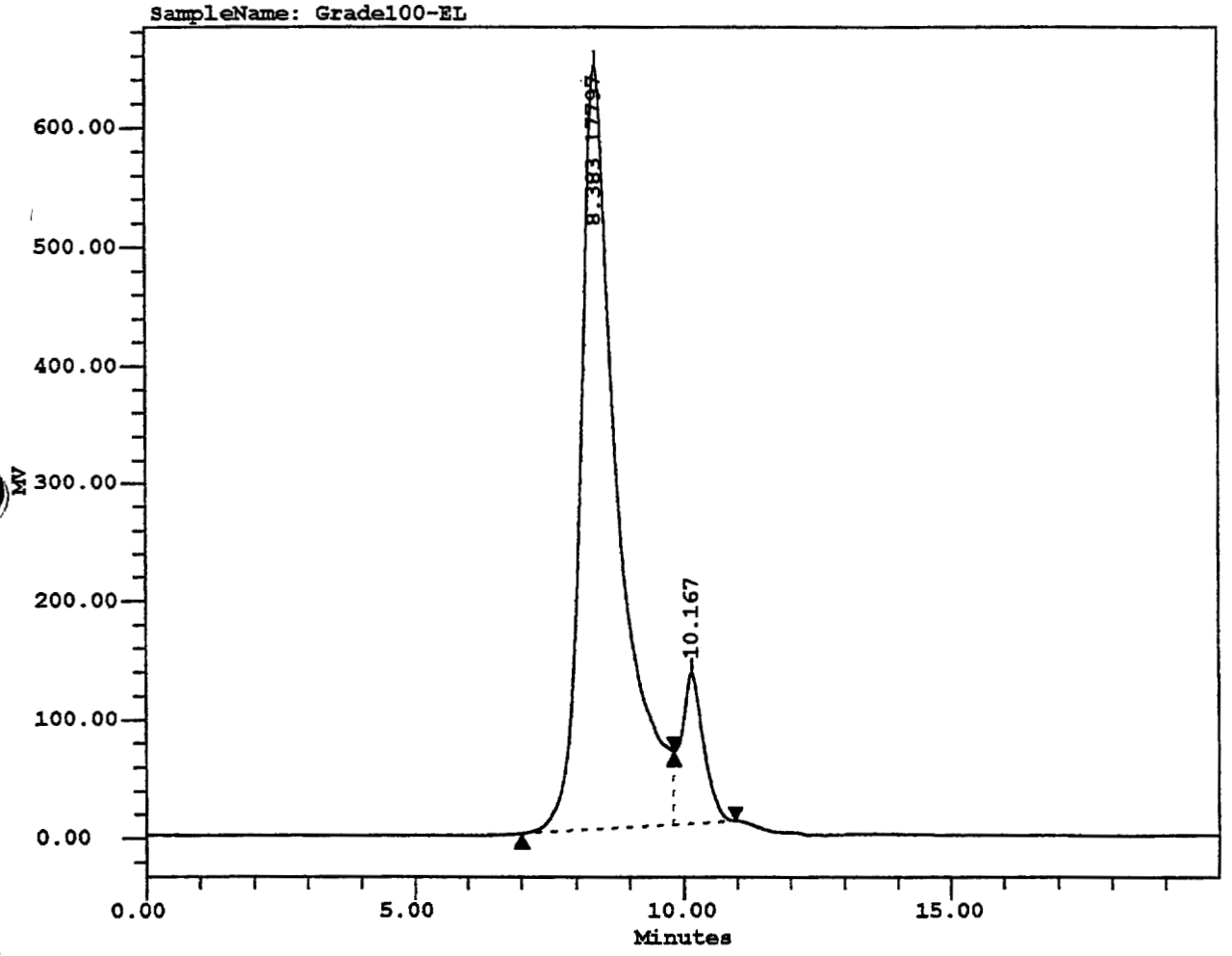
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Grade100-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	13
Date Acquired:	04/10/00 02:44:30 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:58 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.383	17797	18815	15174	1.240	6580753	137672	47.800
2	10.167					810068	27087	29.906

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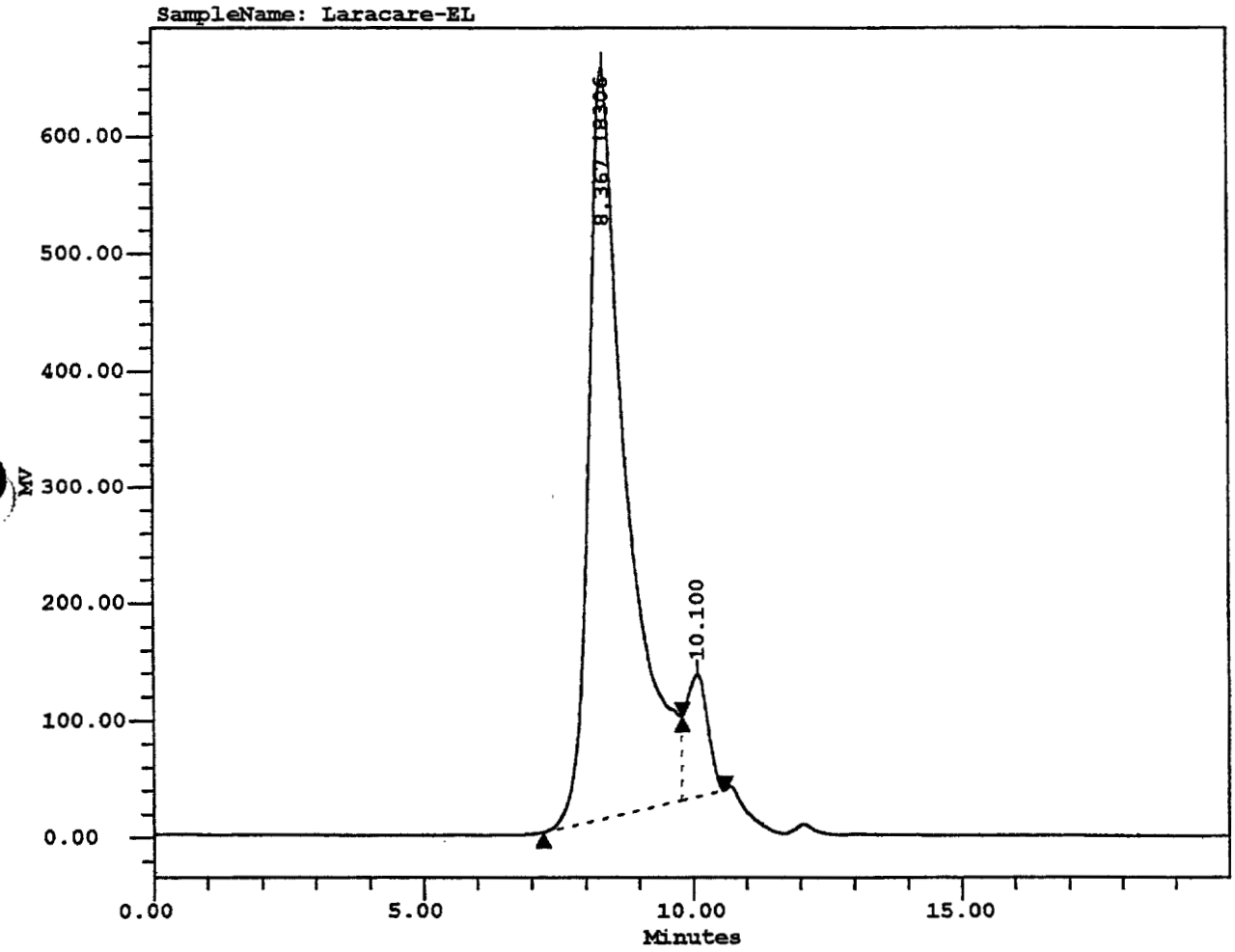
7.24.00

Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Laracare-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	11
Date Acquired:	04/10/00 02:01:13 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:25 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.367	18306	18871	15318	1.232	6674420	137232	48.636
2	10.100					642775	22172	28.991

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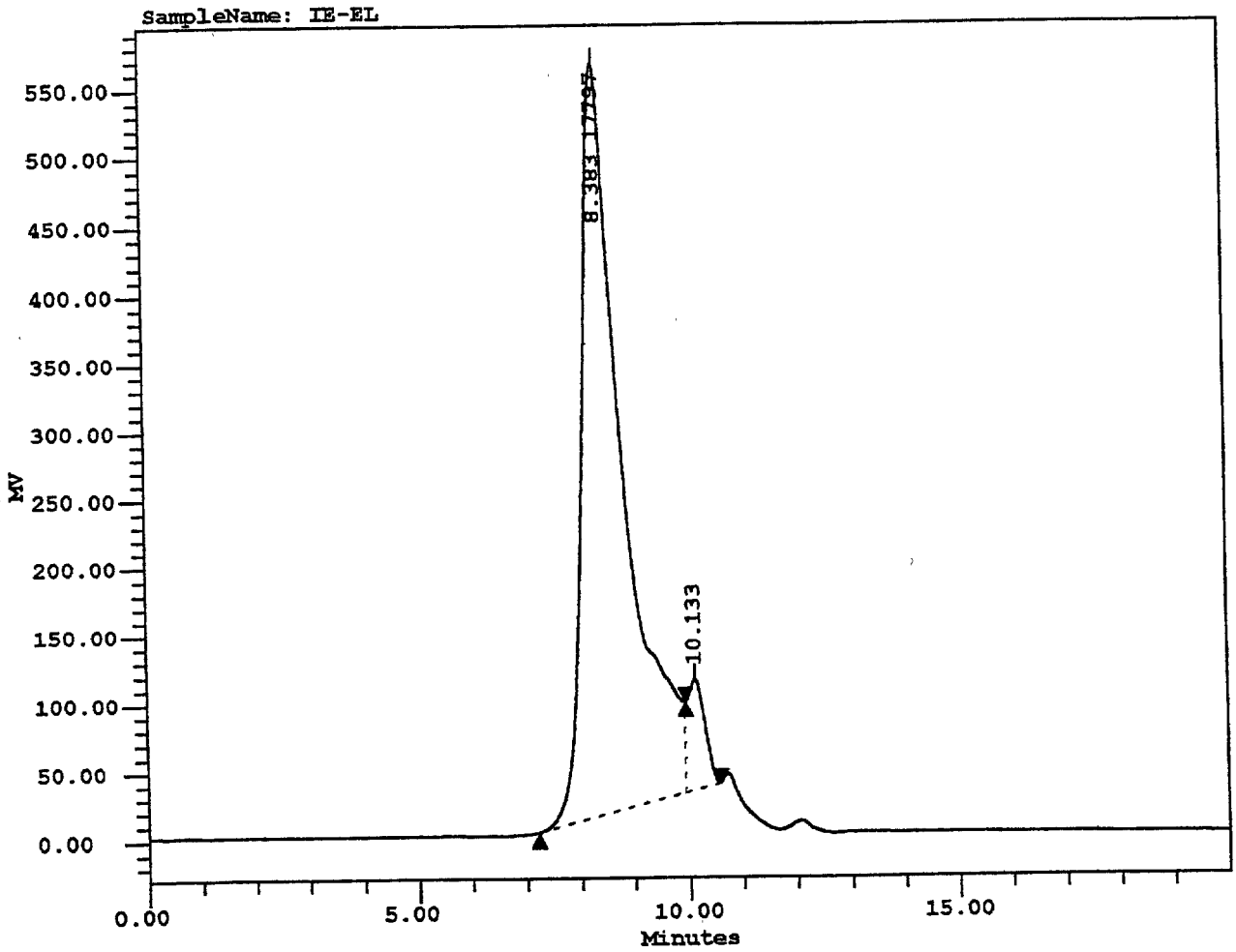
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	9
Date Acquired:	04/10/00 01:17:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:02:50 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.383	17797	18153	14771	1.229	6258076	117685	53.177
2	10.133					386162	17261	22.372

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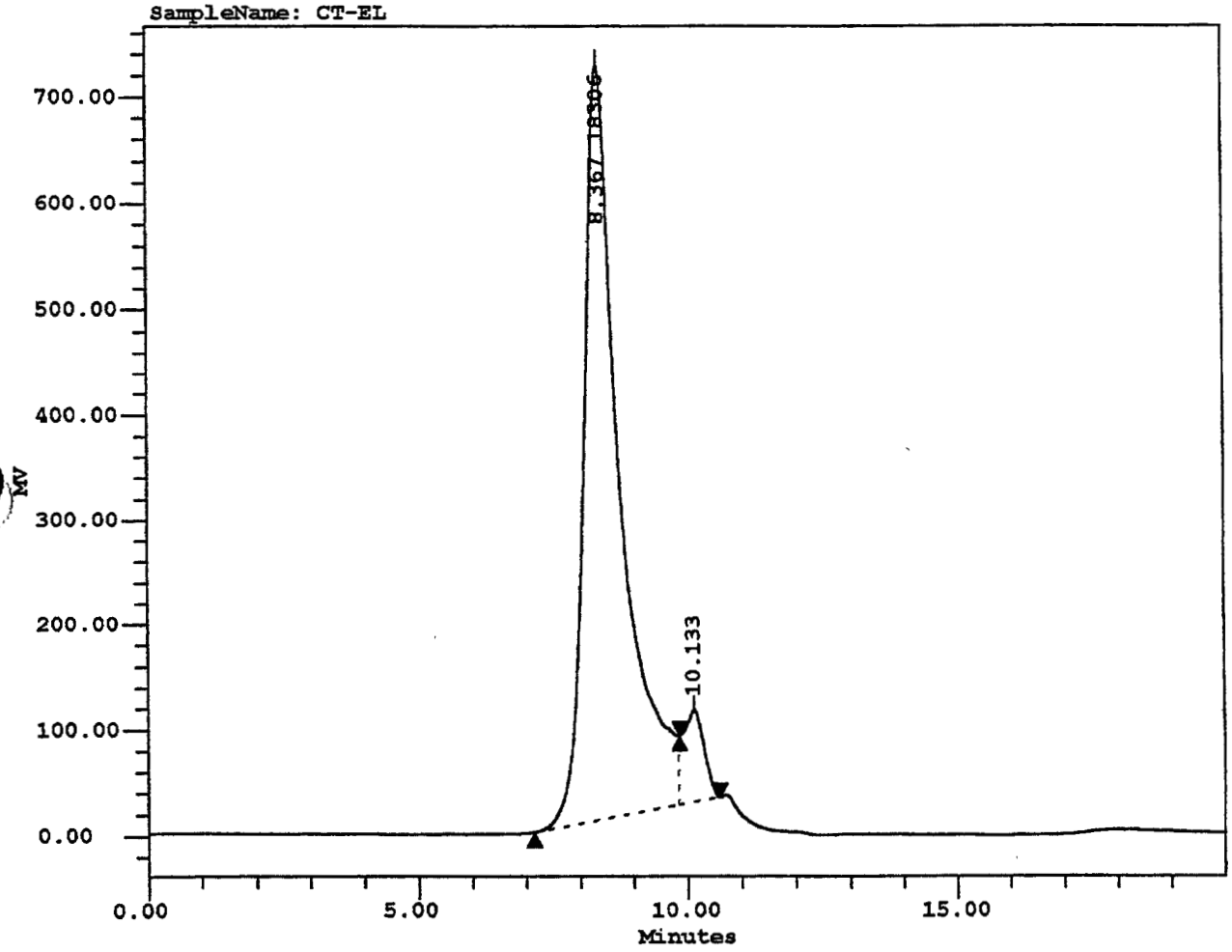
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	7
Date Acquired:	04/10/00 12:34:29 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:02:32 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.367	18306	18750	15335	1.223	7162551	152672	46.915
2	10.133					508077	18429	27.569

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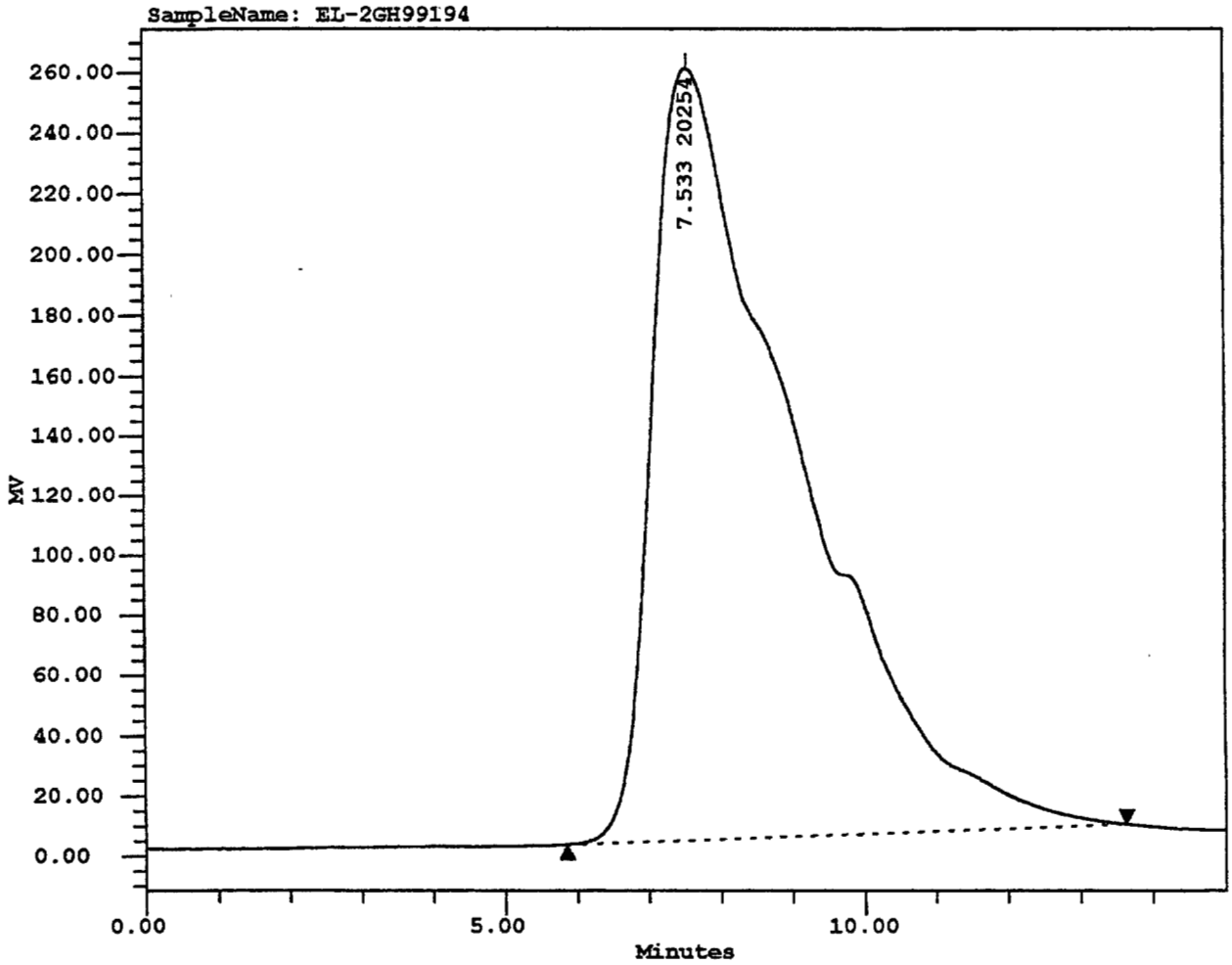
7.24.

Larex Incorporated

July 24, 2000

Project Name:	AG_July_Y2K	Sample Name:	EL-2GH99194
Processing Method:	pullulan07192k	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	29
Date Acquired:	07/21/00 03:26:05 PM	Inj Volume, ul:	40.0
Date Processed:	07/24/00 09:23:19 AM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	7.533	20254	25007	15167	1.649	7637910	54694	139.649

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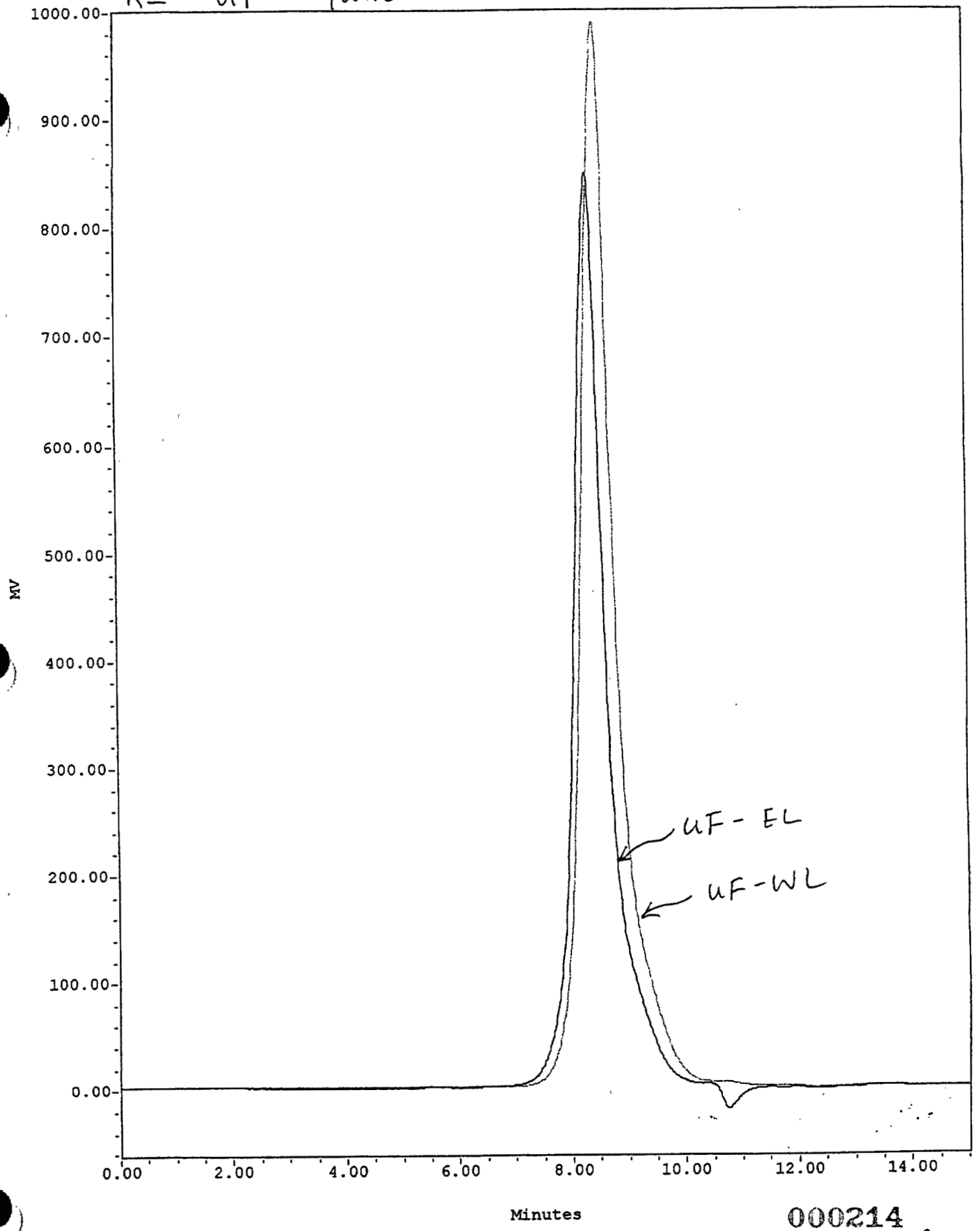
RI: UF-- Tamarack vs W. Larch



000214

Abs. scale

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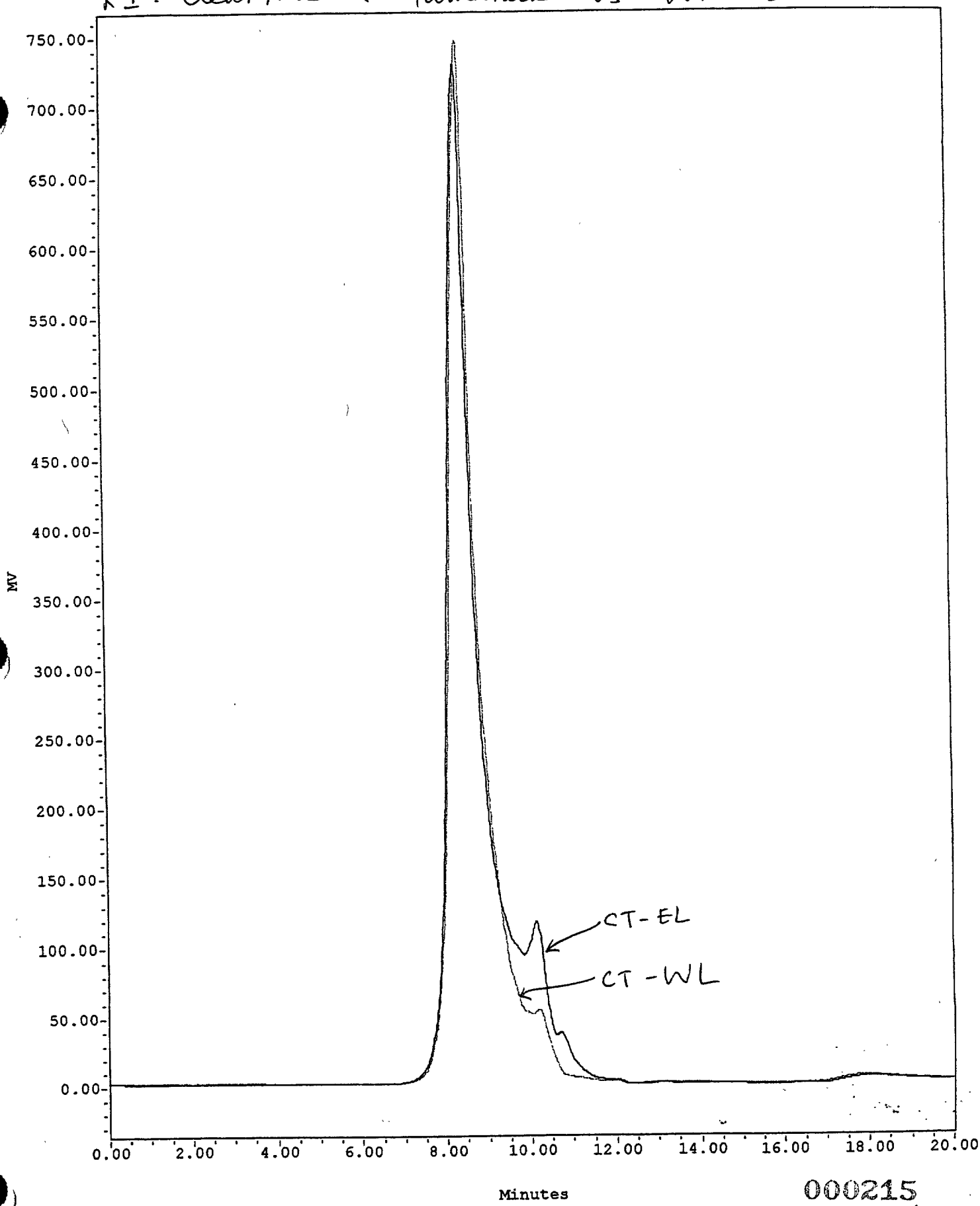
000214

RI: ClearTrac : Tamarack vs W. Larch



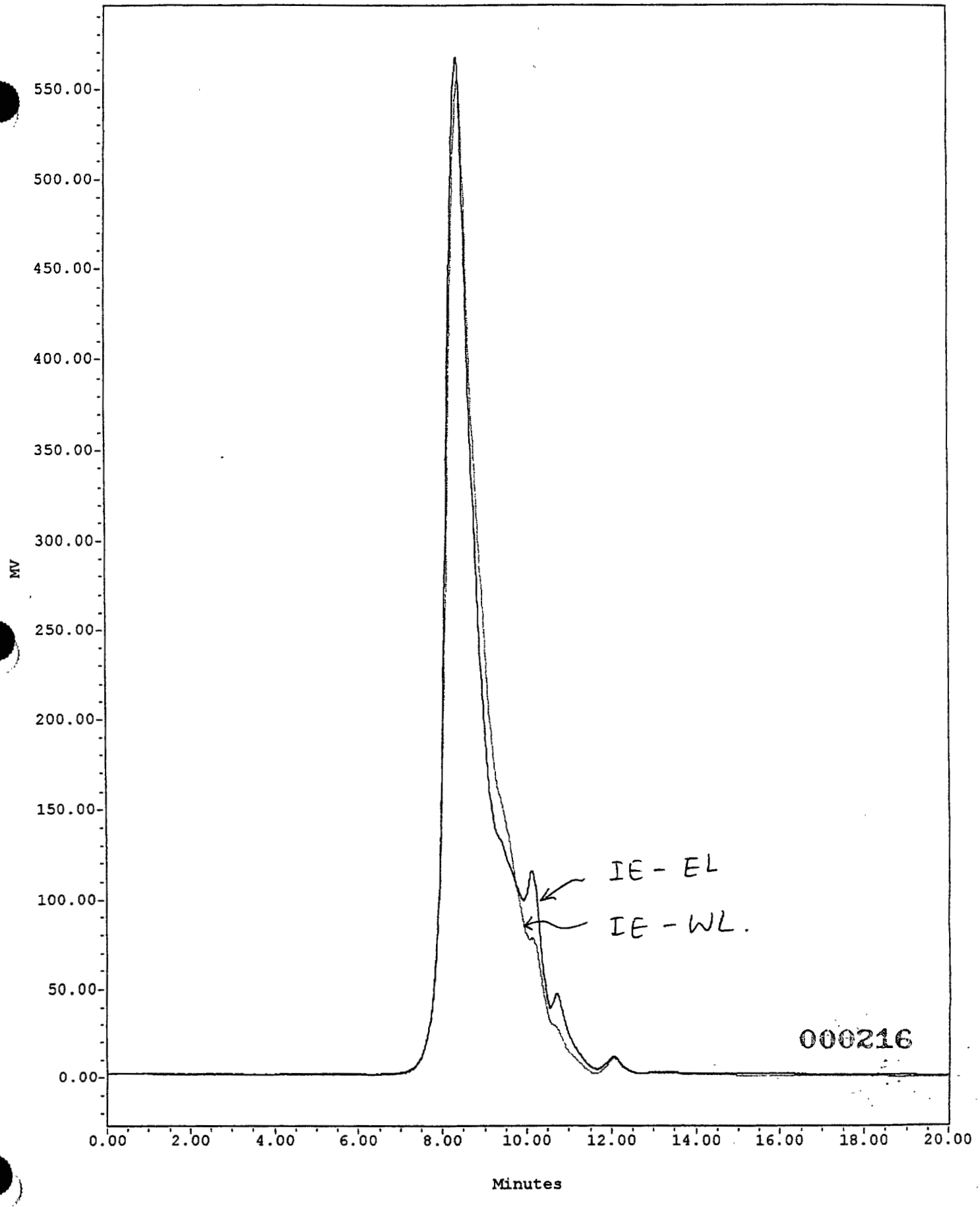
000215

Abs. scale

00 032

000215

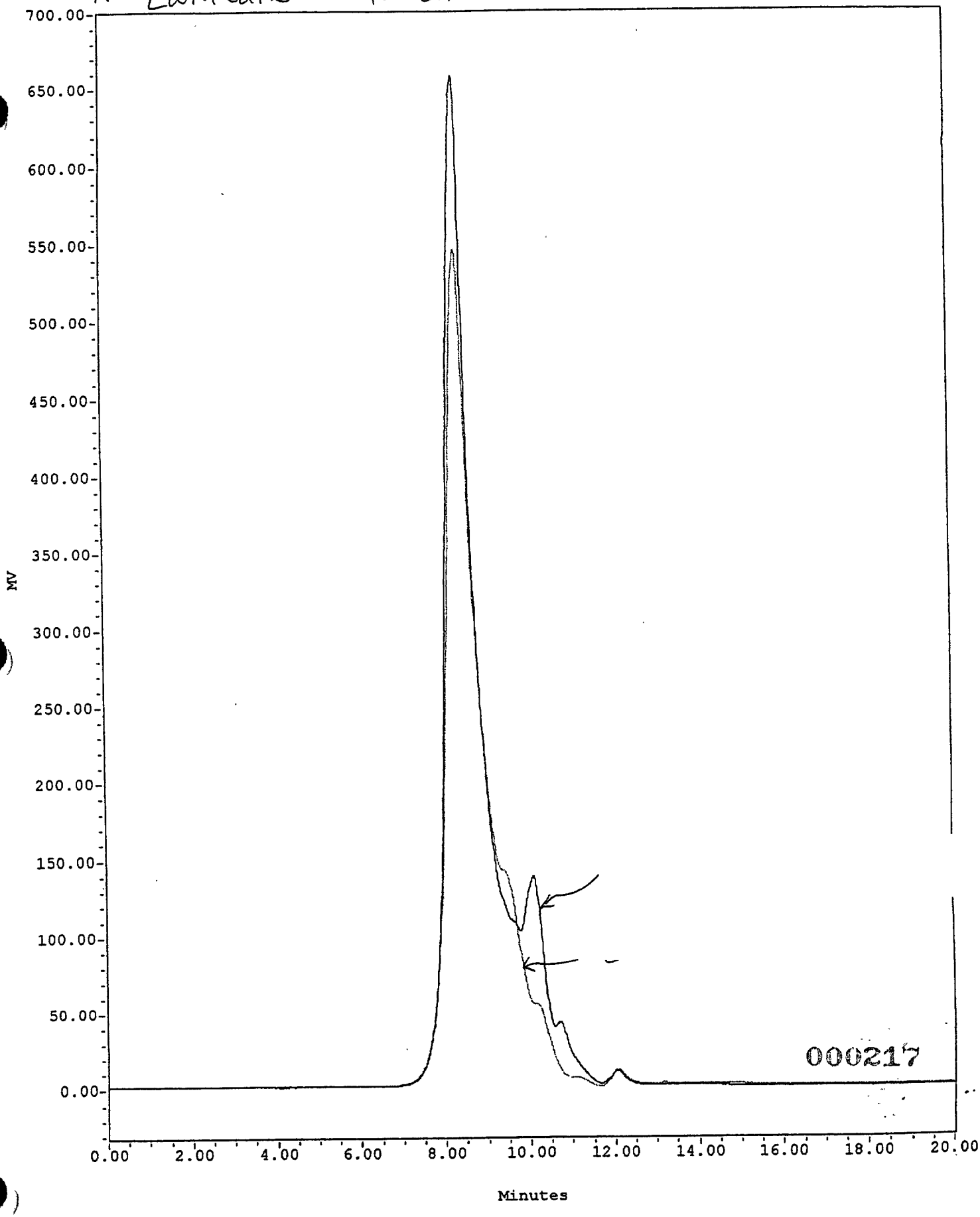
K1 : Immuntenhancer - Tamarack vs W. Larch



00 033 Abs. scale

000216

RL: LaraCare - Tamarack vs W. Larch

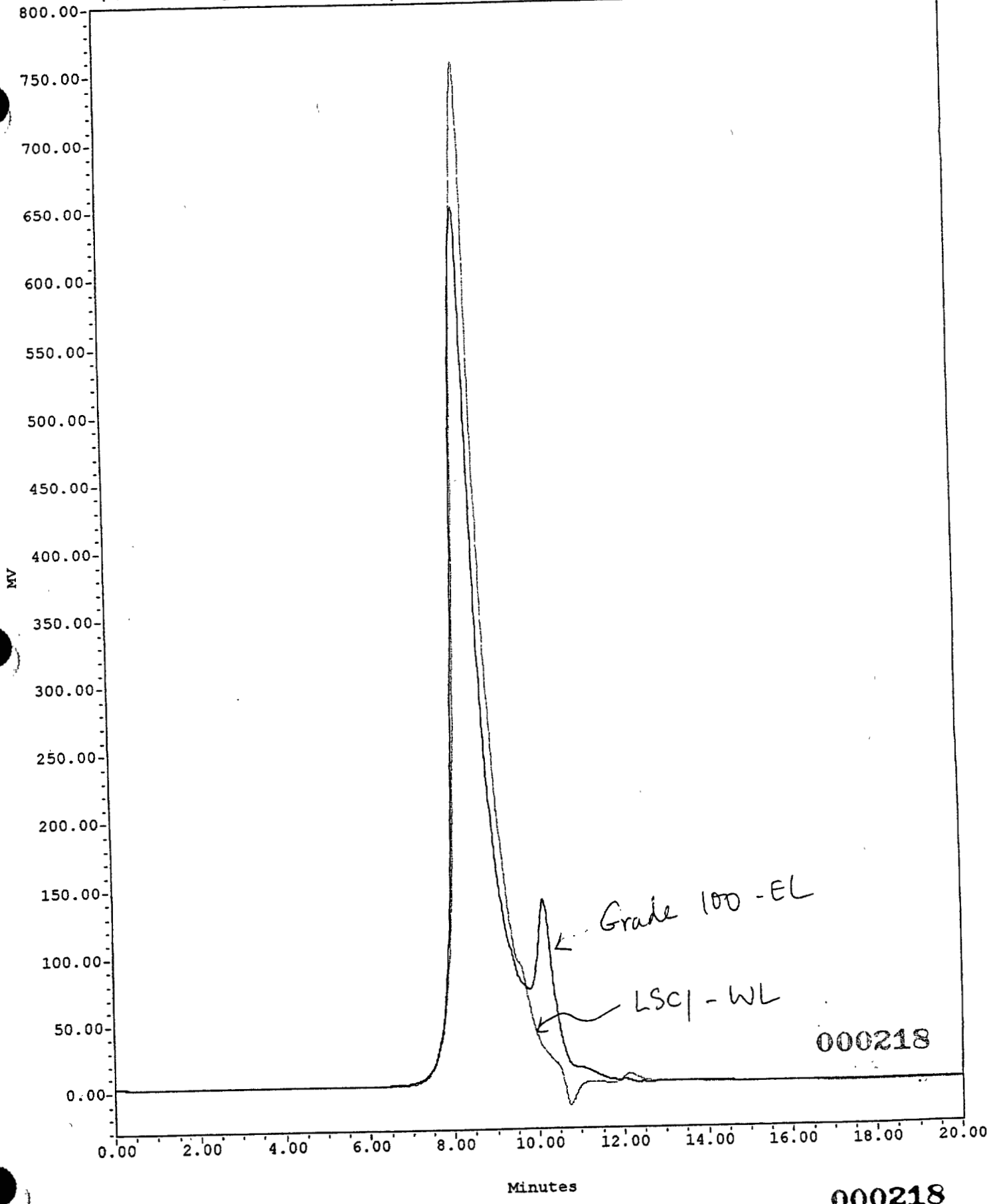


00 034

Abs. scale

000217

K1:- Grade 100 - Tamarack vs LSC1 - W. Larch



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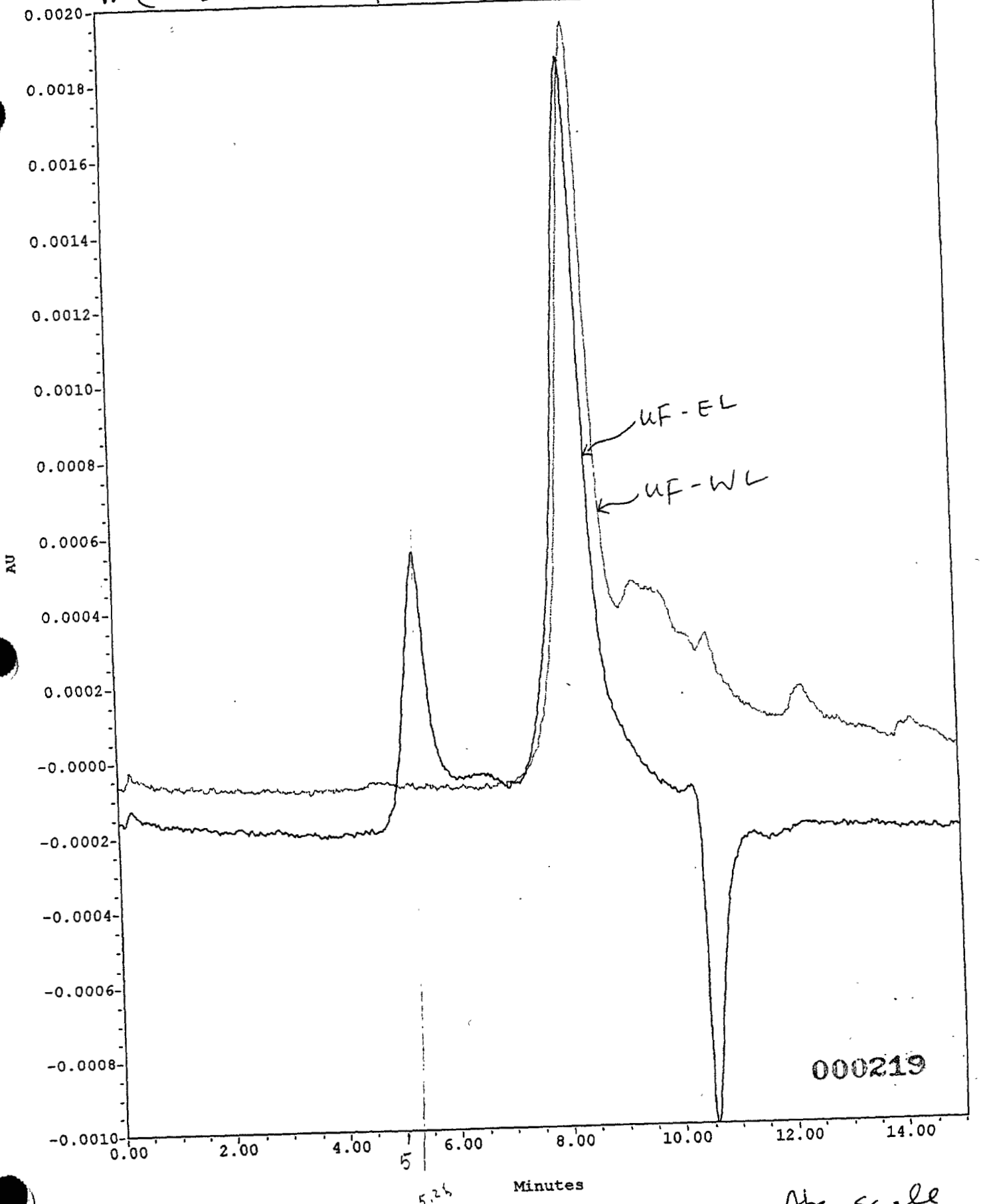
000218

00 035

absolute scale

UV @ 280 nm: UF - Tamarack vs W. Larch



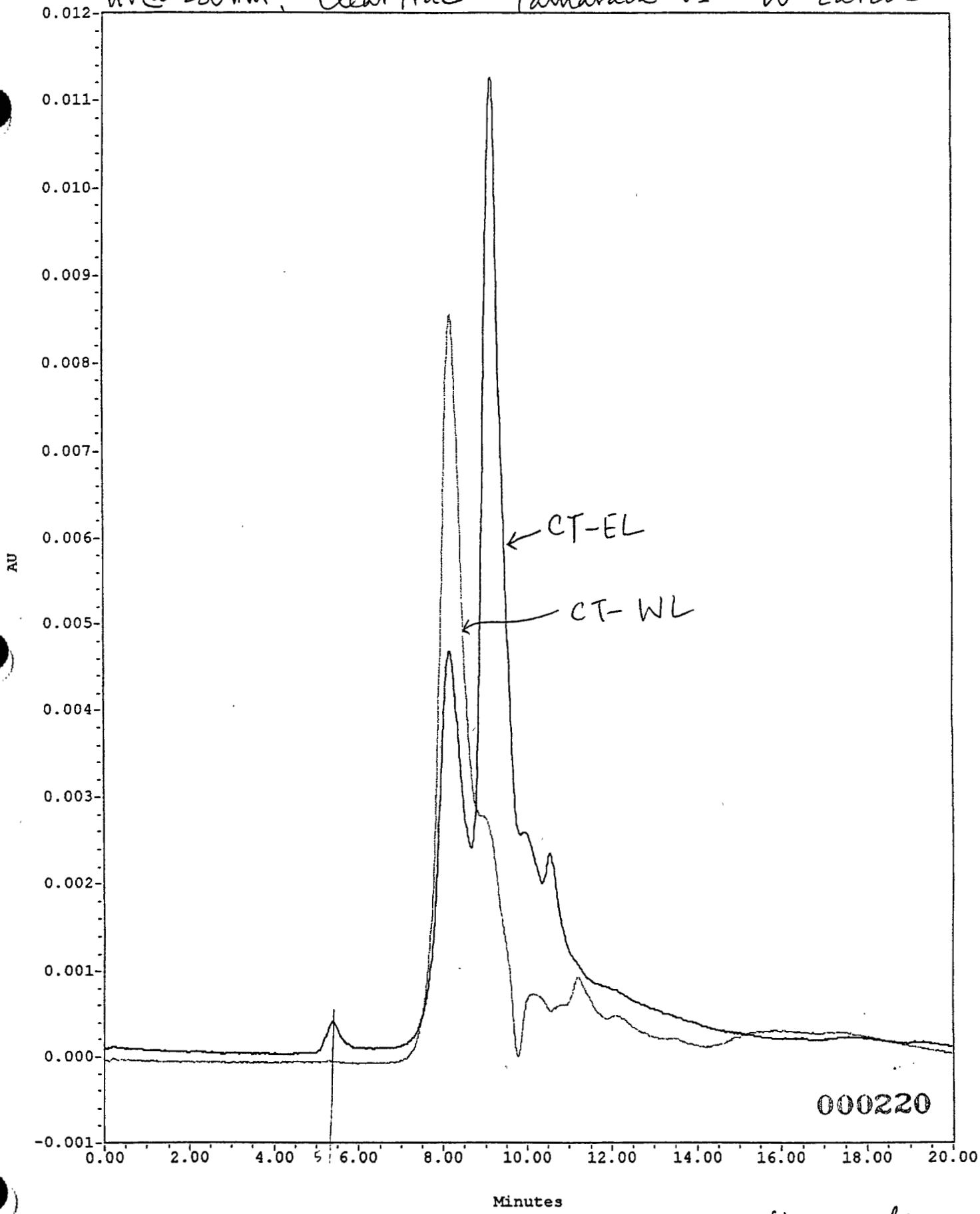
5

5.26

Abs. scale

00 036

UV @ 280 nm: ClearTrac Tamarack vs W Larch



Abs. scale

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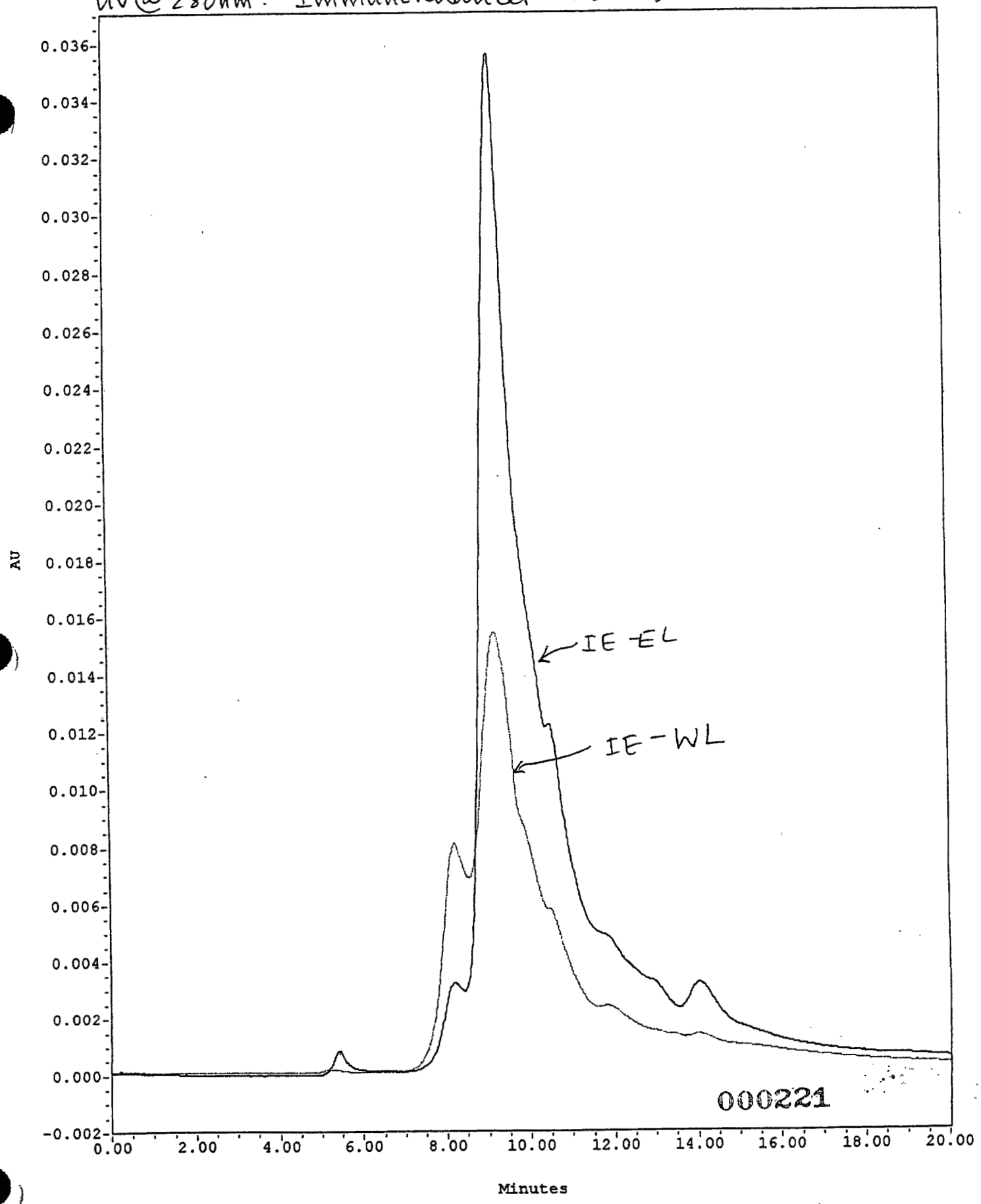
000220

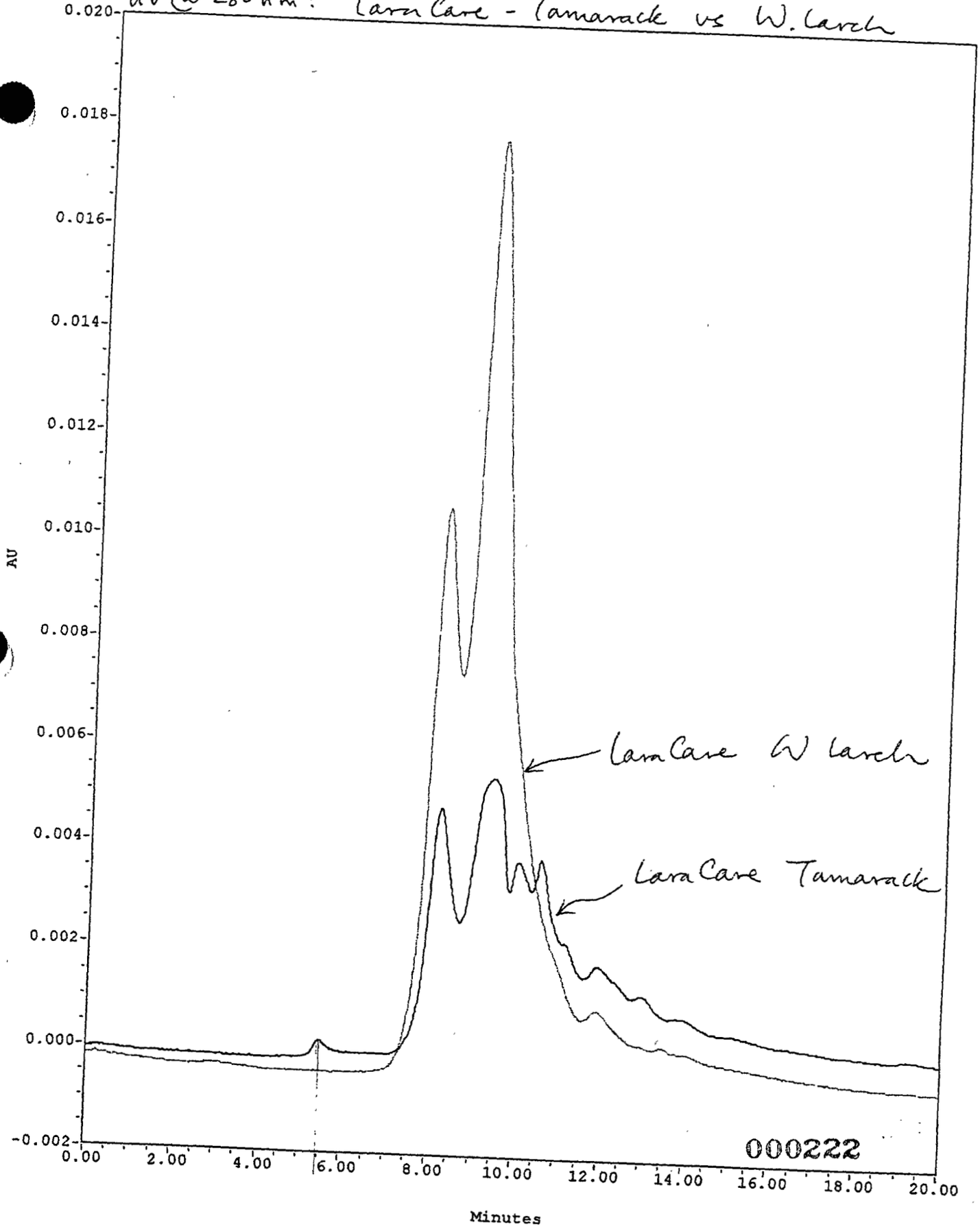
UV@280nm: ImmunEnhancer EL vs WL



Abs. scale

00 038

UV @ 280 nm: LaraCare - Tamarack vs W. Larch



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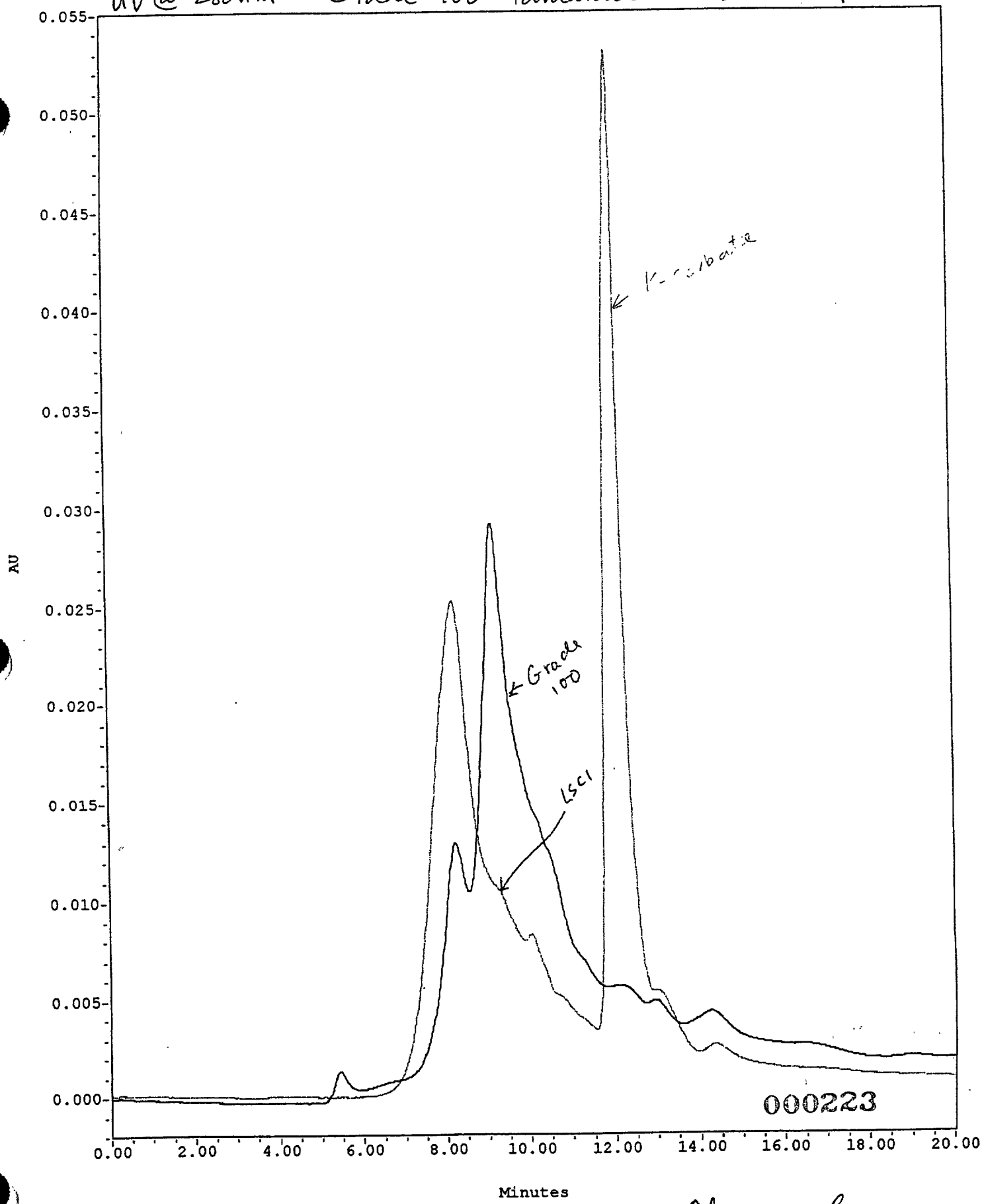
Abs. scale

000222

UV @ 280nm Grade 100 - Tamarack vs LSCI - W. Larch



Abs. scale

00 040

000223

The University of
Montana

Shafizadeh Center for Wood and Carbohydrate Chemistry
The University of Montana
Missoula, Montana 59812
(406) 243-4435
(406) 243-4227 FAX

FAX TRANSMISSION

f

DATE: January 19, 1995

TO: Dr. Christopher H. Price
Larex International, Inc.
Fax (612) 636-1583

FROM: Dr. G.R. Ponder

SIX PAGES FOLLOW

Dear Dr. Price,

The matters in this letter flow from your telephone conversation with Dr. Richards yesterday.

Regarding the different HPSEC elution times for AG's from Western larch and Tamarack, the numbers reported in the fax of Jan.12 correspond to molecular weights of 16,600 and 21,400, respectively, based on Pullulan calibration. It is noteworthy that Timell and coworkers also found indications of size differences between these polymers, but in an opposite sense. Thus, they reported number average molecular weights, determined by osmotic pressure measurements, as follows.

Western larch AG: 58,500 Tamarack AG: 36,000.

[Simson, Côte & Timell, "Studies on larch arabinogalactan. IV. Molecular properties" *Svensk Papper.* 19 (1968) 699-710.] In the same paper, they reported additional molecular weight determinations based on ultracentrifugation and gel permeation chromatography, and although the numbers vary widely they generally agree in attributing a larger size to the Western larch AG.

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The fact that the difference which we observe is consistent for more than one tree sample (and in the case of Tamarack, for more than one time of year) suggests that it may be more

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yield from the current 320 sample. It would seem that wood selection only partly solves the problem and that scum will be an intrinsic problem for Western larch. (The preceding comments are based on the assumption that the defectiveness of the first P.S.F. had no effect on the proportion of scum in the exudate.)

As for Tamarack, a tentative conclusion from the data in the table is that spring may be a better time of year to harvest trees for AG than fall. The table also presents an unexpected result: Tamarack AG (whether from fall or spring wood) consistently elutes from our HPSEC column about 0.15 minutes faster than Western larch AG. Based on Pullulan calibration, this suggests that the molecular size of Tamarack AG is 30% larger than that of Western larch AG. However, there could also be structural differences involved.

I have included the UV and RI chromatograms for the impregnator solutions of each run. These are representative of the four types of wood, since the chromatograms for the corresponding pressates are very similar, the only differences being minor alterations in proportions of the UV peaks. On the other hand, some major differences can be seen when comparing the four types of wood. For example, the proportions of various phenolics in Tamarck appear to be dependent on the time of year. This is not surprising, and it probably applies to Western larch as well. Note also the comparison of UV chromatograms for Western larch heartwood and whole wood. The virtual absence of the strong absorbance at 7.75 min in the 230 chromatogram suggests that this absorbance is due to a sapwood component, whereas the excluded light-absorbing material at 5.48 min is predominantly derived from heartwood.

Sincerely,

(b)(6)

Glenn R. Ponder

Jan 12, 1994
letter to C. Price

Medallion Laboratories

Analytical Report

900 Plymouth Avenue • Minneapolis, MN 55427

1-800-245-5615
(612) 540-4453
Fax: (612) 540-4010

Chew-Hung Mak
Larex Inc.
4815 White Bear Pkwy
White Bear Lake MN 55110

Date Submitted: 30May2000
Date Reported : 6Jun2000
P.O. Number : 5748
Library Number: 2000-04503
Company Code : LAREX01

F I N A L R E P O R T

* * * * * * * * * *

Customer ID: G100WL Grade 100 Western Larch Medallion Labs ID: 2000033251
Sample Descr.: Extract from Trees

Test Description	Results	Units	Estimate
Fatty Acid Analysis w/Profile			
Total Fat	0.00	%	
Saturated Fat	0.00	%	
Monounsaturated Fat	0.00	%	
cis-cis Polyunsaturated Fat	0.00	%	
trans Fat	0.00	%	

* * * * * * * * * *

Customer ID: G100EL Grade 100 Tamarack Medallion Labs ID: 2000033252
Sample Descr.: Extract from Trees

Test Description	Results	Units	Estimate
Fatty Acid Analysis w/Profile			
Total Fat	0.0100	%	
Saturated Fat	0.00	%	
Monounsaturated Fat	0.00	%	
cis-cis Polyunsaturated Fat	0.00	%	
trans Fat	0.00	%	

* * * * * * * * * *

Customer ID: AC9 Stractan 10 Medallion Labs ID: 2000033253
Sample Descr.: Extract from Trees

Test Description	Results	Units	Estimate
Fatty Acid Analysis w/Profile			
Total Fat	0.0200	%	
Saturated Fat	0.00	%	
Monounsaturated Fat	0.0100	%	
cis-cis Polyunsaturated Fat	0.00	%	
trans Fat	0.00	%	

000226

00 043

Signed [redacted] (b)(6)
For Medallion Laboratories

General Mills

Fatty Acid Profile/Fat Analysis (with cis-cis polyunsaturates)

TO: Medallion Labs

.IMS SAMPLE ID: 2000033251

Library No.: 2000-04503

:[redacted]MER ID: G100WL

Date Analyzed: 6Jun00

Reference #: C060500A0004

Analyst: Kerri Gustafson

Description: Extract from Trees

	Normalized by Weight	% (w/w) as Triglyceride in Product	% (w/w) Fatty Acids in Product: Saturated Fatty Acids	Monounsaturated Fatty Acids	cis-cis Polyunsaturated Fatty Acids	trans Fatty Acids
4:0 Butyric						
6:0 Caproic						
8:0 Caprylic						
10:0 Capric						
12:0 Lauric						
13:0 Tridecanoic						
14:0 Myristic						
14:1 t-Tetradecenoic						
14:1 Myristoleic						
15:0 Pentadecanoic						
15:1 Pentadecenoic						
16:0 Palmitic						
16:1 t-Hexadecenoic						
16:1 Palmitoleic						
17:0 Margaric						
17:1 Margaroleic						
18:0 Stearic						
18:1 trans Elaidic						
18:1 Oleic	100.000%	0.001		0.001		
18:2 t-Octadecadienoic						
18:2 Linoleic						
20:0 Arachidic						
18:3 g-Linolenic						
20:1 Gadoleic						
18:3 Linolenic						
21:0 Heneicosanoic						
18:2 conj-Linoleic						
18:4 Octadecatetraenoic						
20:2 Eicosadienoic						
22:0 Behenic						
20:3 g-Eicosatrienoic						
22:1 Erucic						
20:3 Eicosatrienoic						
20:4 Arachidonic						
23:0 Tricosanoic						
22:2 Docosadienoic						
24:0 Lignoceric						
20:5 Eicosapentaenoic						
24:1 Nervonic						
22:3 Docosatrienoic						
22:4 Docosatetraenoic						
22:5 Docosapentaenoic						
22:6 Docosahexaenoic						
Totals	100.0%	0.00		0.00		
Percent of Fatty Acid Components based on Total Fat				100.00%		

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000227

Fatty Acid Profile/Fat Analysis (with cis-cis polyunsaturates)

TO: Medallion Labs

IMS SAMPLE ID: 2000033252

Library No.: 2000-04503

:U[redacted]MER ID: G100EL

Date Analyzed: 6Jun00

Reference #: C060500A0005

Analyst: Kerri Gustafson

Description: Extract from Trees

	Normalized by Weight	% (w/w) as Triglyceride in Product	% (w/w) Fatty Acids in Product: Saturated Fatty Acids	Monounsaturated Fatty Acids	cis-cis Polyunsaturated Fatty Acids	trans Fatty Acids
:0 Butyric						
:0 Caproic						
:0 Caprylic						
0:0 Capric						
2:0 Lauric						
3:0 Tridecanoic						
4:0 Myristic						
4:1 t-Tetradecenoic						
4:1 Myristoleic						
5:0 Pentadecanoic						
5:1 Pentadecenoic						
16:0 Palmitic	20.000%	0.001	0.001			
16:1 t-Hexadecenoic						
16:1 Palmitoleic						
17:0 Margaric						
17:1 Margaroleic						
1[redacted]earic						
18:[redacted]ns Elaidic	40.000%	0.002				0.002
18:1 Oleic	20.000%	0.001		0.001		
18:2 t-Octadecadienoic						
18:2 Linoleic	20.000%	0.001			0.001	
20:0 Arachidic						
18:3 g-Linolenic						
20:1 Gadoleic						
18:3 Linolenic						
21:0 Heneicosanoic						
18:2 conj-Linoleic						
18:4 Octadecatetraenoic						
20:2 Eicosadienoic						
22:0 Behenic						
20:3 g-Eicosatrienoic						
22:1 Erucic						
20:3 Eicosatrienoic						
20:4 Arachidonic						
23:0 Tricosanoic						
22:2 Docosadienoic						
24:0 Lignoceric						
20:5 Eicosapentaenoic						
24:1 Nervonic						
22:3 Docosatrienoic						
[redacted]Docosatetraenoic						
[redacted]ocosapentaenoic						
22:6 Docosahexaenoic						
Totals	100.00%	0.01	0.00	0.00	0.00	0.00
Percent of Fatty Acid Components based on Total Fat			19.93%	20.02%	20.01%	40.04%

00 045

000228

Fatty Acid Profile/Fat Analysis (with cis-cis polyunsaturates)

TO: Medallion Labs

Library No.: 2000-04503

Date Analyzed: 6Jun00

Analyst: Kerri Gustafson

MS SAMPLE ID: 2000033253

JS[redacted]MER ID: AC9

eference #: C060500A0006

escription: Extract from Trees

	Normalized by Weight	% (w/w) as Triglyceride in Product	% (w/w) Fatty Acids in Product: Saturated Fatty Acids	Monounsaturated Fatty Acids	cis-cis Polyunsaturated Fatty Acids	trans Fatty Acids
0 Butyric						
0 Caproic						
0 Caprylic						
:0 Capric						
:0 Lauric						
:0 Tridecanoic						
:0 Myristic						
:1 t-Tetradecenoic						
:1 Myristoleic						
:0 Pentadecanoic						
:1 Pentadecenoic						
:0 Palmitic	14.286%	0.003	0.003			
:1 t-Hexadecenoic						
:1 Palmitoleic	9.524%	0.002		0.002		
7:0 Margaric						
7:1 Margaroleic						
[redacted]aric	4.762%	0.001	0.001			0.001
[redacted]ns Elaidic	4.762%	0.001				
8:1 Oleic	42.857%	0.009		0.009		
8:2 t-Octadecadienoic	4.762%	0.001				0.001
8:2 Linoleic	14.286%	0.003			0.003	
0:0 Arachidic						
8:3 g-Linolenic						
0:1 Gadoleic						
8:3 Linolenic						
1:0 Heneicosanoic						
8:2 conj-Linoleic						
8:4 Octadecatetraenoic						
20:2 Eicosadienoic						
22:0 Behenic	4.762%	0.001	0.001			
20:3 g-Eicosatrienoic						
22:1 Erucic						
20:3 Eicosatrienoic						
20:4 Arachidonic						
23:0 Tricosanoic						
22:2 Docosadienoic						
24:0 Lignoceric						
20:5 Eicosapentaenoic						
24:1 Nervonic						
22:3 Docosatrienoic						
[redacted]ocosatetraenoic						
[redacted]ocosapentaenoic						
22:6 Docosahexaenoic						
Totals	100.0%	0.02	0.00	0.01	0.00	0.00
Percent of Fatty Acid Components based on Total Fat			23.80%	52.38%	14.29%	9.53%

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FORMULAS:

Table X
Postadded Eastern Larch and Larch AG to Red Ink 42-7315

	LRX 21297-1	-2	-3	-4
42-7315	144.0	132.0	120.0	90.0
Eastern Larch AG solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

	LRX 21297-5	-6	-7	-8
42-7315	144.0	132.0	120.0	90.0
Sample 00184 AG Solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

For formulas 22097-1 through -4, water was added to ink 42-7315 at the same levels as the AG additions above.

	LRX 22097-1	-2	-3	-4
42-7315	144.0	132.0	120.0	90.0
Distilled water	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% water	6% water	10% water	20% water

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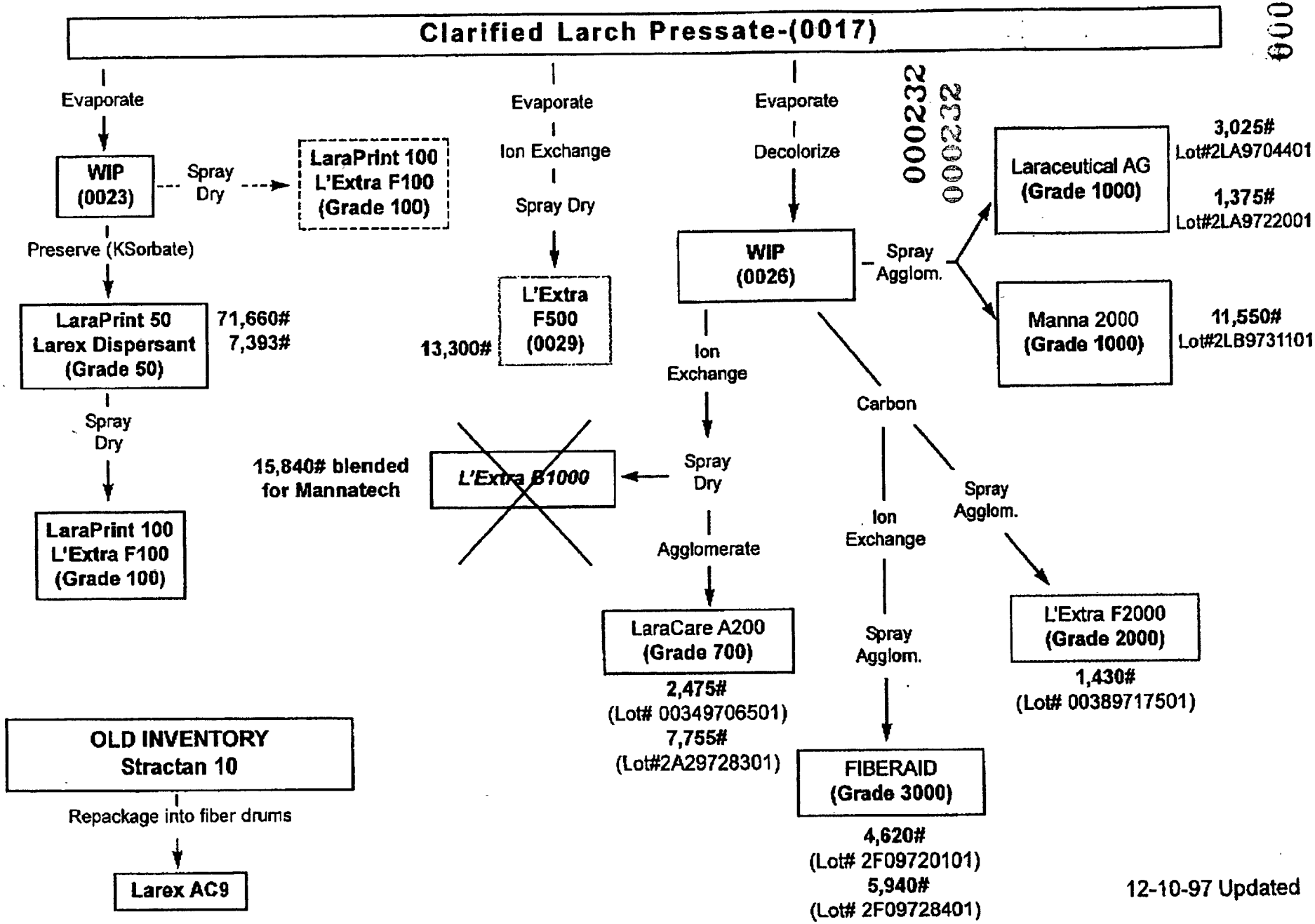
Table X
Postadded Eastern Larch and Larch AG to Cyan ink BCM 63A

	LRX 21297-9	-10	-11	-12
BCM 63A	144.0	132.0	120.0	90.0
Eastern Larch AG solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

	LRX 21297-13	-14	-15	-16
BCM 63A	144.0	132.0	120.0	90.0
Sample 00184 AG solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

AG Product Derived from Clarified Larch Pressate



12-10-97 Updated

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Tamarack vs Western Larch Products

Cohasset Plant produced four spray-dried products from Tamarack : Grade 100, LaraCare, ImmunEnhancer and ClearTrac.

The processing steps for Tamarack products:
Grade 100-EL: same as LSC1 from Western Larch.

LaraCare-EL: Grade 100-EL → cationic exchanged → decolorized with KOH/H_2O_2 → anionic exchanged → concentrated → dried.

ImmunEnhancer-EL: Grade 100-EL → cationic exchanged → KOH/H_2O_2 → dried.

ClearTrac-EL: Grade 100-EL → cationic exchanged → $Ca(OH)_2/H_2O_2$ → anionic exchanged → carbon treated → concentrated → dried.

The above tamarack products were compared with the corresponding western larch products, which were processed the same way with the exception that the cationic exchanged step is not used in processing western larch products. The western larch products used were LSC1, LaraCare 00349706501, ImmunEnhancer 2LB9910801, and ClearTrac 2C99935801.

(Tamarack is also known as Eastern Larch. I'll use EL for Tamarack and WL for Western Larch.)

Results:

	Absorbance at*			
Sample	400nm	280nm	Phenol,ppm**	Mw
Grade 100-EL	0.0188	0.3535	16.65	18,815
LSC1-WL	0.0192	0.2456	8.66	17,527
LaraCare-EL	0.0070	0.5121	2.13	18,871
LaraCare-WL	0.0270	0.9432	2.03	19,040
ImmunEnhancer-EL	0.0178	1.8295	5.46	18,153
ImmunEnhancer-WL	0.0116	0.8025	0.85	17,978
ClearTrac-EL	0.0062	0.2599	0.29	18,750
ClearTrac-WL	0.0074	0.3084	nd	17,926

*All measurements were made with 1% solution except for Absorbance measurements for Grade 100-EL and LSC1-WL, which were measured with 0.1% solutions.
**All measurements were made with 1% solutions using CheMets ampules.

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Color - as indicated by abs @ 400nm
Tamarack products were very comparable to the existing Western Larch products in terms of color.

Phenols – abs @ 280nm and CheMets ampules
All EL samples have higher phenol level than their WL counterpart using CheMets ampules.

Turbidity
ImmunEnhancer-EL is the only product that is more turbid, otherwise, all tamarack products were comparable to their counterparts.

HPLC
From the RI, AG from EL was found to have similar Mw to that of WL in all samples. In his letter dated 1994, Dr. Glen Ponder found that the Mw from EL is 30% larger than that from WL , based upon the calibration with Pullulan standards. However, in his letter in 1995, he quoted that Timell and coworkers reported a larger Mw in WL(58,500) than EL(36,000).

From UV @ 280nm there is a peak eluted before the AG peak in all EL but not WL products. This peak only showed up in the UV but not in RI. The impurities in the EL samples seemed to be present in larger quantities than WL as indicated by the larger area under the curve for the EL samples.

CHM
4-17-2000

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Wavelength Scan HELP

ReadSamples Tabula NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 280.0

Function	Reading
Scan	0.35379

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

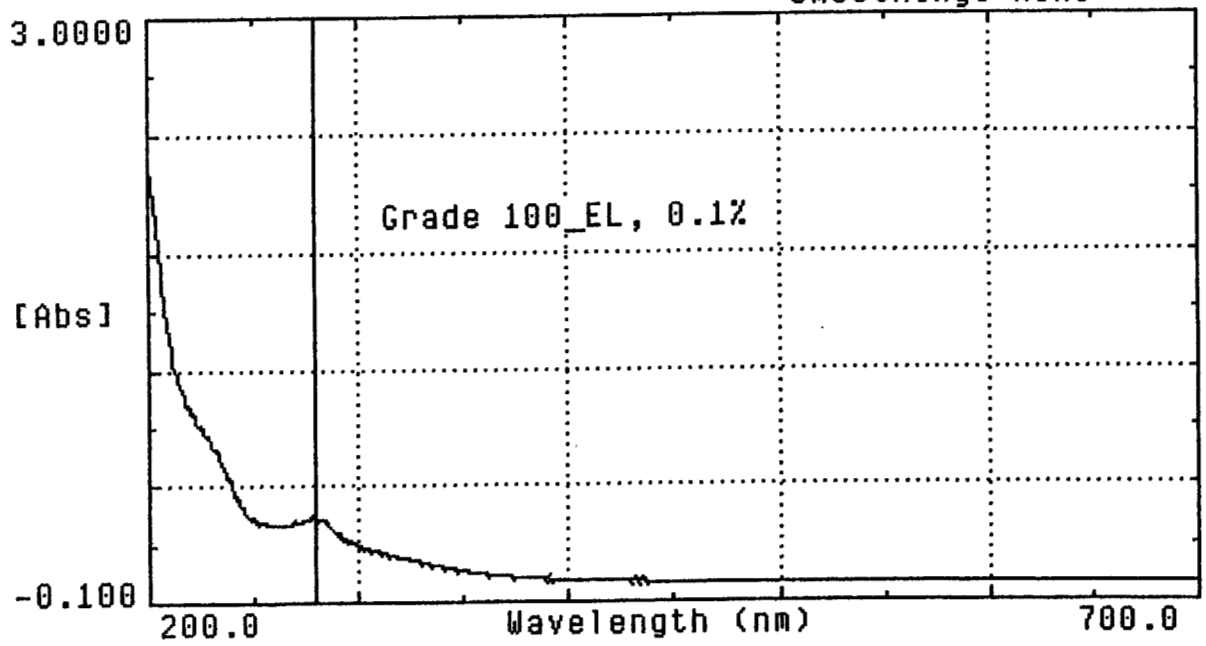
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.3538	pt
400.0	0.0188	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK [VIS ON] RediScan DEVICES 700.0 nm TIME DATE TEMP CELL
MATCH OFF [UV ON] RediRead PrtScrn -0.0025 Abs 02:42 07/04/00 N/A N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 278.0

Function	Reading
Scan	0.52056

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

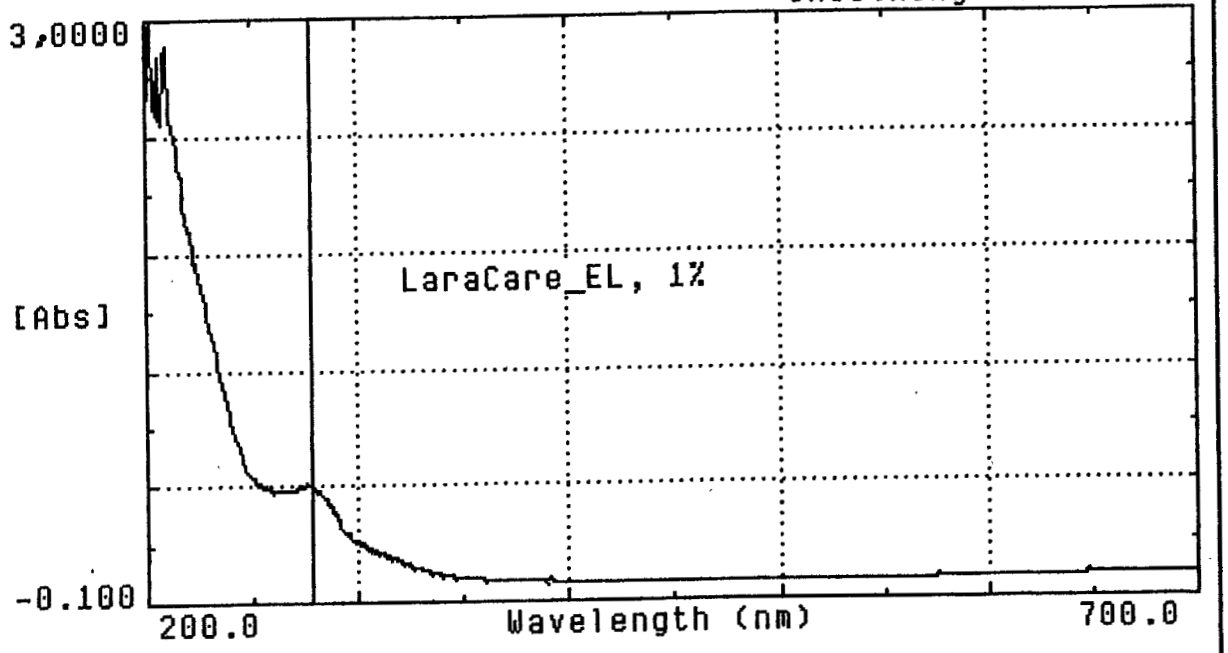
Print ↓ ↑ ← → Exit | Zoom ZoomOut Trace Function Autoscale Annotate Print

A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.5121	pt
400.0	0.0070	pt

Functions: Scan

Smoothing: None



BLANK [VIS ON] RediScan DEVICES 700.0 nm TIME DATE TEMP CELL
MATCH OFF [UV ON] RediRead PrtScrn 0.0312 Abs 02:26 07/04/00 N/A N/A

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7.24.

Wavelength Scan HELP

ReadSamples Tabula

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

A:\WORK_001 (1200)

Wavelength: 277.0

Function	Reading
Scan	1.85813

NetA Method SaveClear Print Quit

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

Print ↓ ↑ ← → Exit

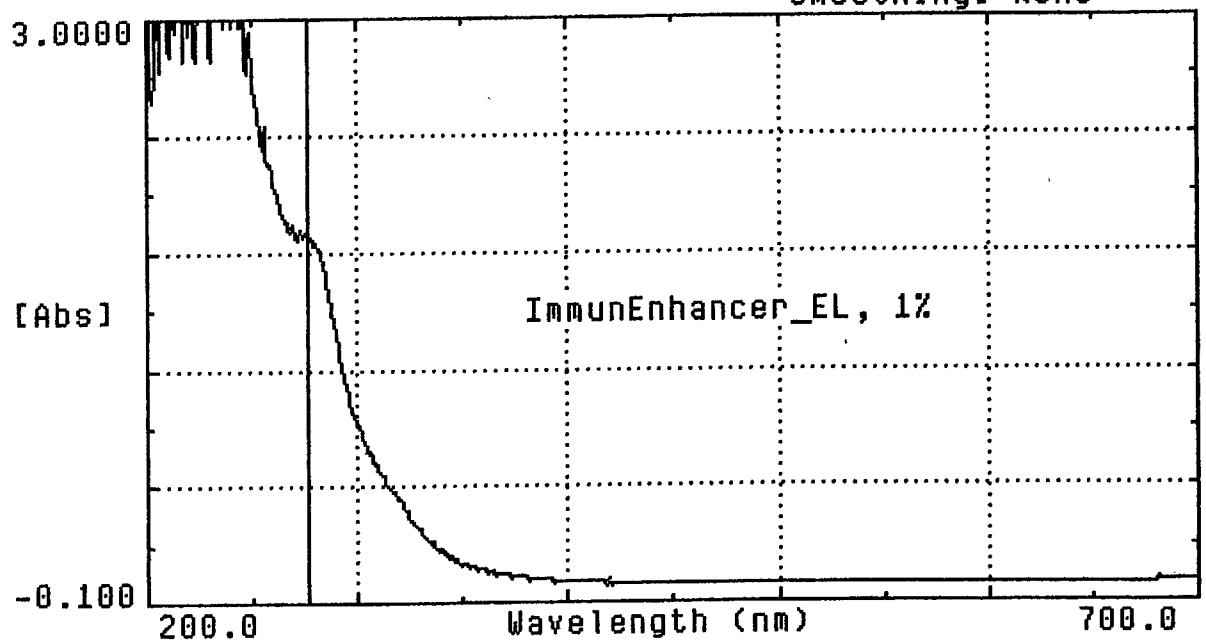
A:\WORK_001 Scan

wl	Abs	Pick
280.0	1.8295	pt
400.0	0.0178	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK	[VIS ON]	RediScan	DEVICES	700.0 nm	TIME	DATE	TEMP	CELL
MATCH OFF	[UV ON]	RediRead	PrtScrn	0.0110 Abs	02:32	07/04/00	N/A	N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 274.0

Function	Reading
Scan	0.26415

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

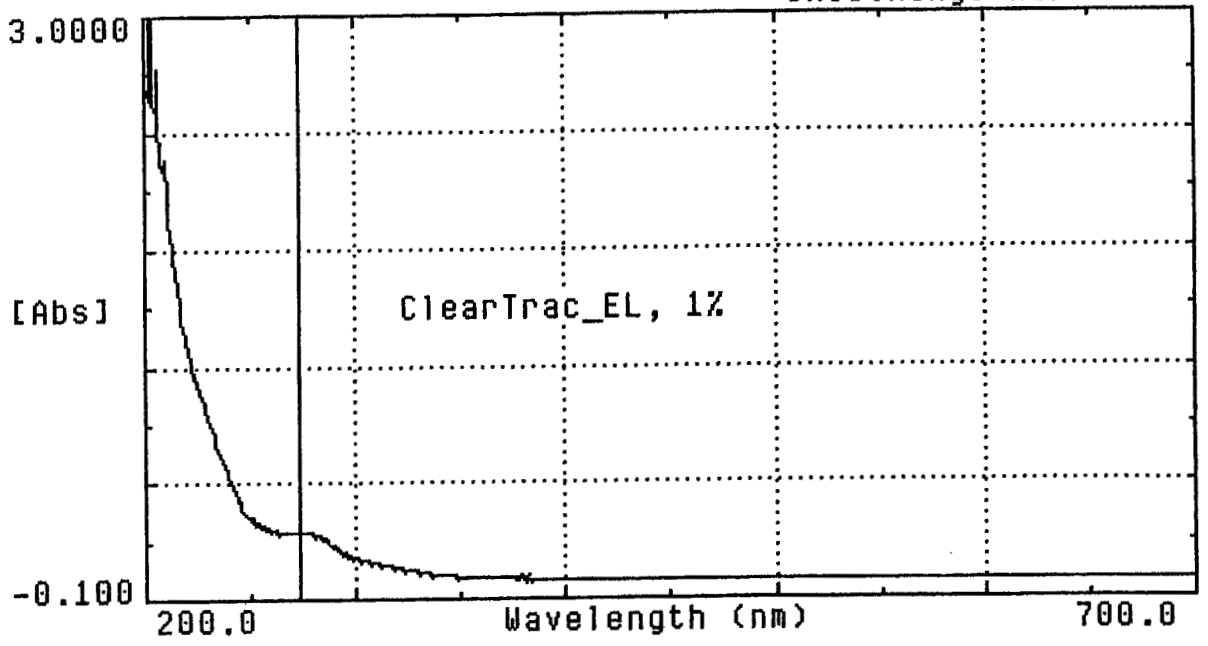
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.2599	pt
400.0	0.0062	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK [VIS ON] RediScan DEVICES 700.0 nm TIME DATE TEMP CELL
MATCH OFF [UV ON] RediRead PrtScrn -0.0000 Abs 02:19 07/04/00 N/A N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 280.0

Function	Reading
Scan	1.11660

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

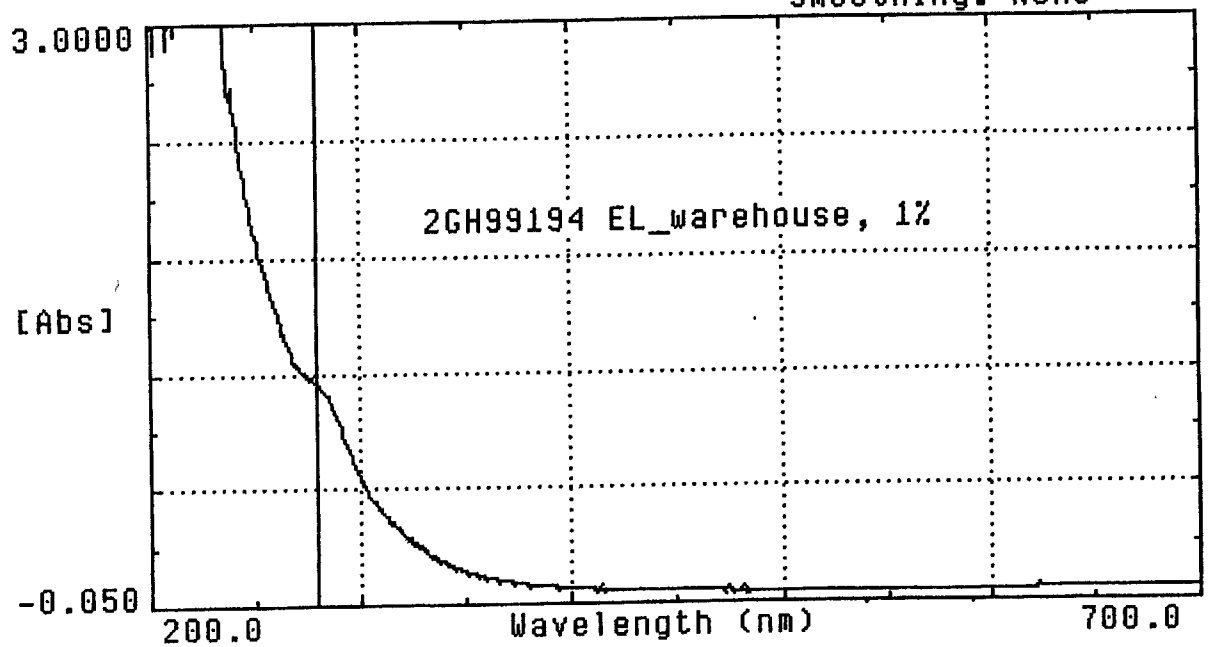
A:\WORK_001 Scan

wl	Abs	Pick
280.0	1.1166	pt
400.0	0.0374	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK	[VIS ON]	RediScan	DEVICES	700.0 nm	TIME	DATE	TEMP	CELL
MATCH OFF	[UV ON]	RediRead	PrtScrn	0.0018 Abs	10:08	24/07/00	N/A	N/A

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7.24.00

Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 255.0

Function	Reading
Scan	0.61669

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

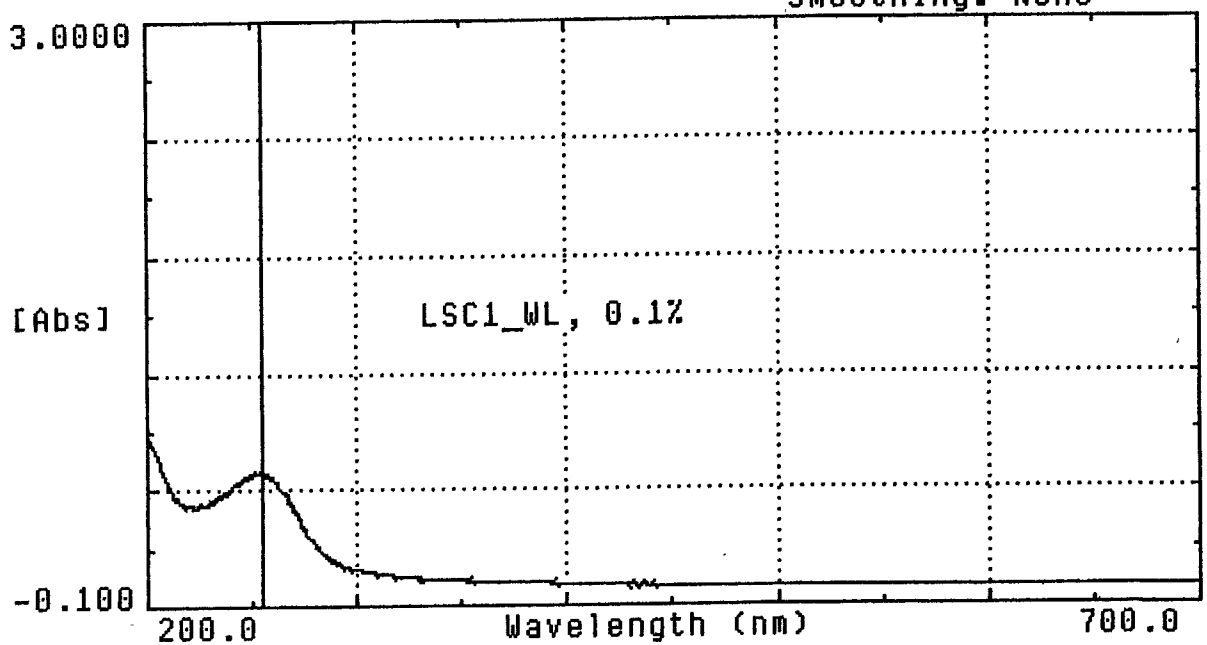
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.2456	pt
400.0	0.0192	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK	[VIS ON]	RediScan	DEVICES	700.0 nm	TIME	DATE	TEMP	CELL
MATCH OFF	[UV ON]	RediRead	PrtScrn	-0.0040 Abs	02:46	07/04/00	N/A	N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 278.0

Function	Reading
Scan	0.96417

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

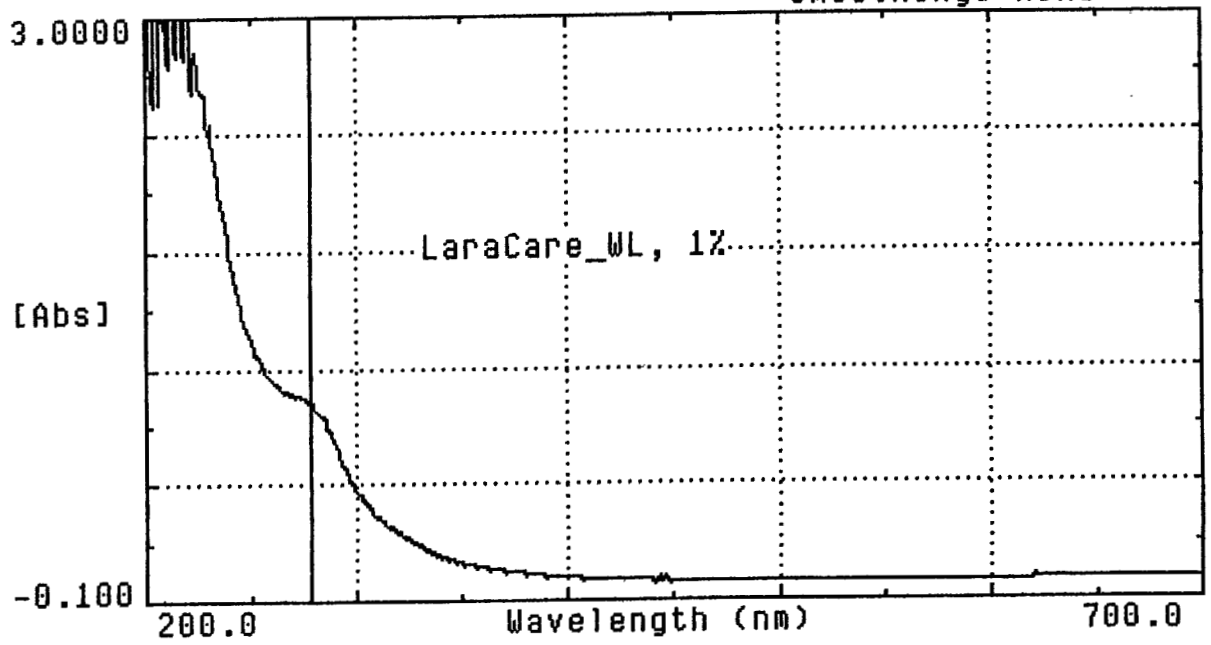
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.9432	pt
400.0	0.0270	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK	[VIS ON]	RediScan	DEVICES	700.0 nm	TIME	DATE	TEMP	CELL
MATCH OFF	[UV ON]	RediRead	PrtScrn	-0.0008 Abs	02:23	07/04/00	N/A	N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 276.0

Function	Reading
Scan	0.82902

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_001 (1200)

Print ↓ ↑ ← → Exit

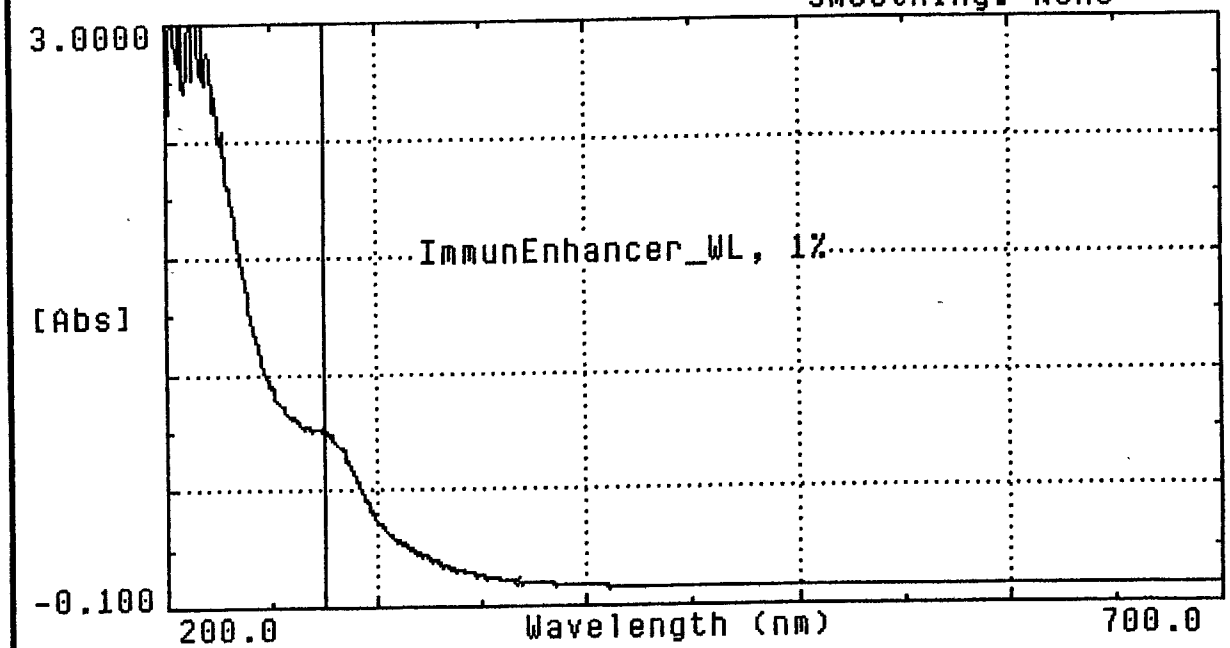
A:\WORK_001 Scan

wl	Abs	Pick
280.0	0.8025	pt
400.0	0.0116	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK [VIS ON] RediScan DEVICES 700.0 nm TIME DATE TEMP CELL
MATCH OFF [UV ON] RediRead PrtScrn -0.0005 Abs 02:29 07/04/00 N/A N/A

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Wavelength Scan HELP

ReadSamples Tabula | NetA Method SaveClear Print Quit

Scan directory: VI
Start wl: 200 nm
End wl: 700 nm
Overlay scans: [No

Wavelength: 269.0

Function	Reading
Scan	0.33173

: 1
[sec]

Method name: A:\DEFAULT
Autosave name: [A:\]SCANS
Sampling device: Sipper
Scan speed: 1200 nm/min

A:\WORK_002 (1200)

Print ↓ ↑ ← → Exit

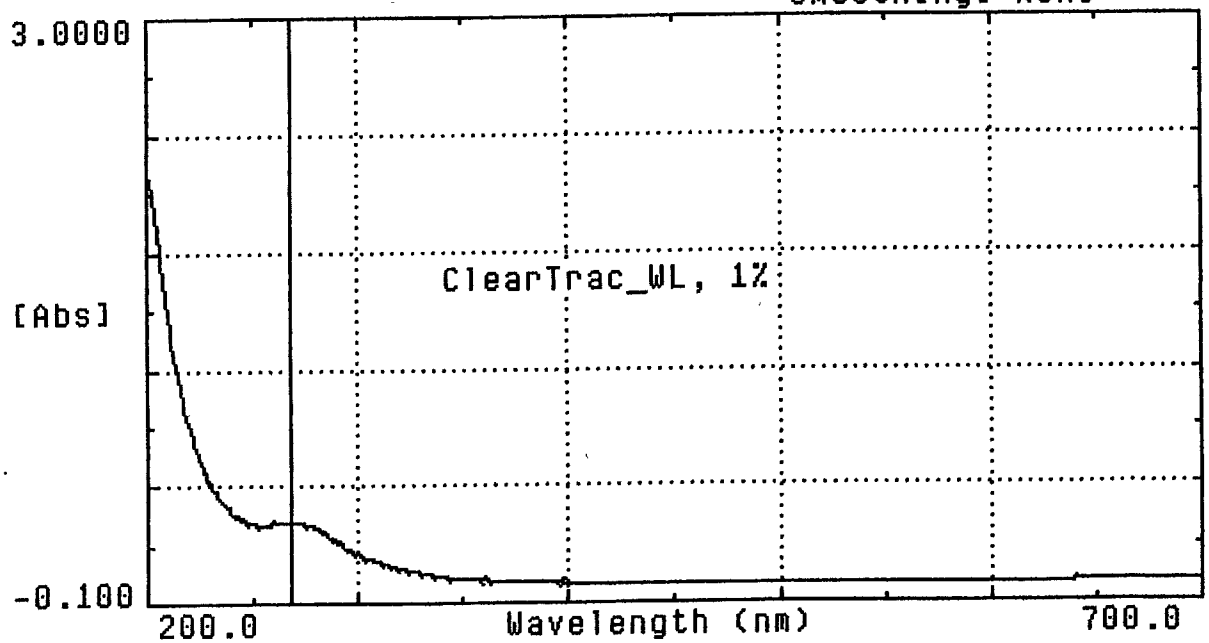
A:\WORK_002 Scan

wl	Abs	Pick
280.0	0.3084	pt
400.0	0.0074	pt

Zoom ZoomOut Trace Function Autoscale Annotate Print

Functions: Scan

Smoothing: None



BLANK	[VIS ON]	RediScan	DEVICES	700.0 nm	TIME	DATE	TEMP	CELL
MATCH OFF	[UV ON]	RediRead	PrtScrn	-0.0174 Abs	02:16	07/04/00	N/A	N/A

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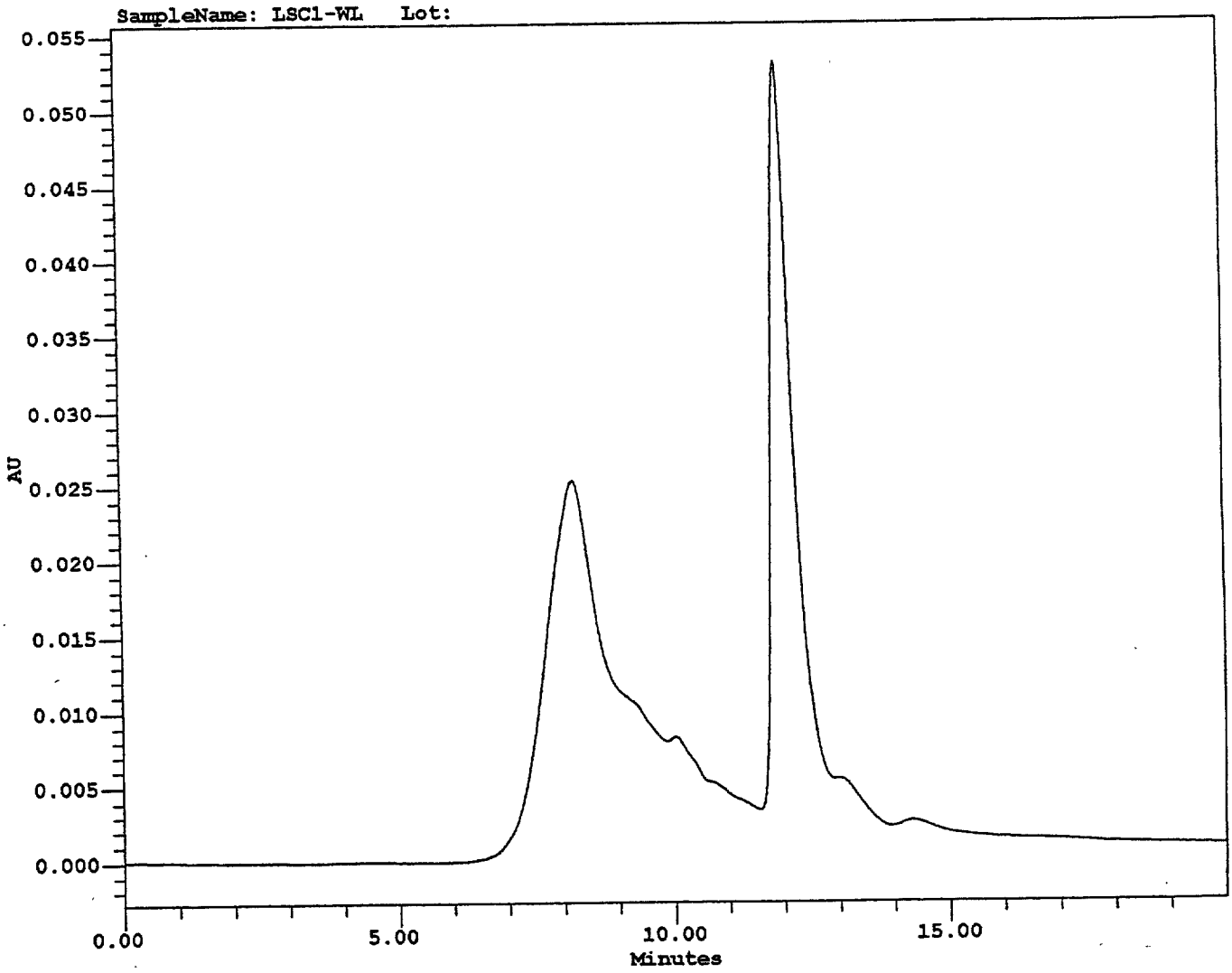
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LSC1-WL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 02:22:51 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:25 PM	Vial:	12
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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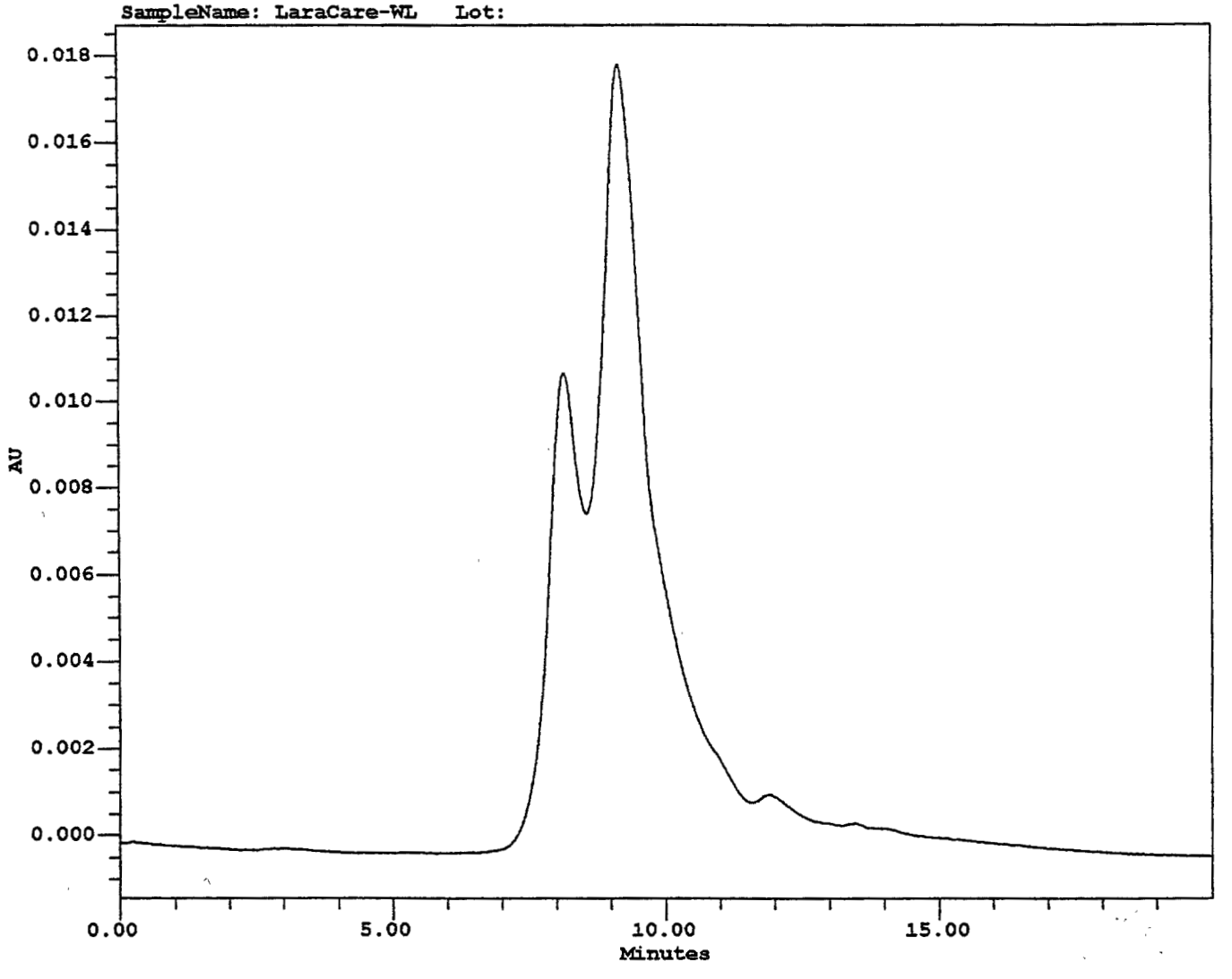
7.24.00

Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LaraCare-WL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 01:39:34 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:44 PM	Vial:	10
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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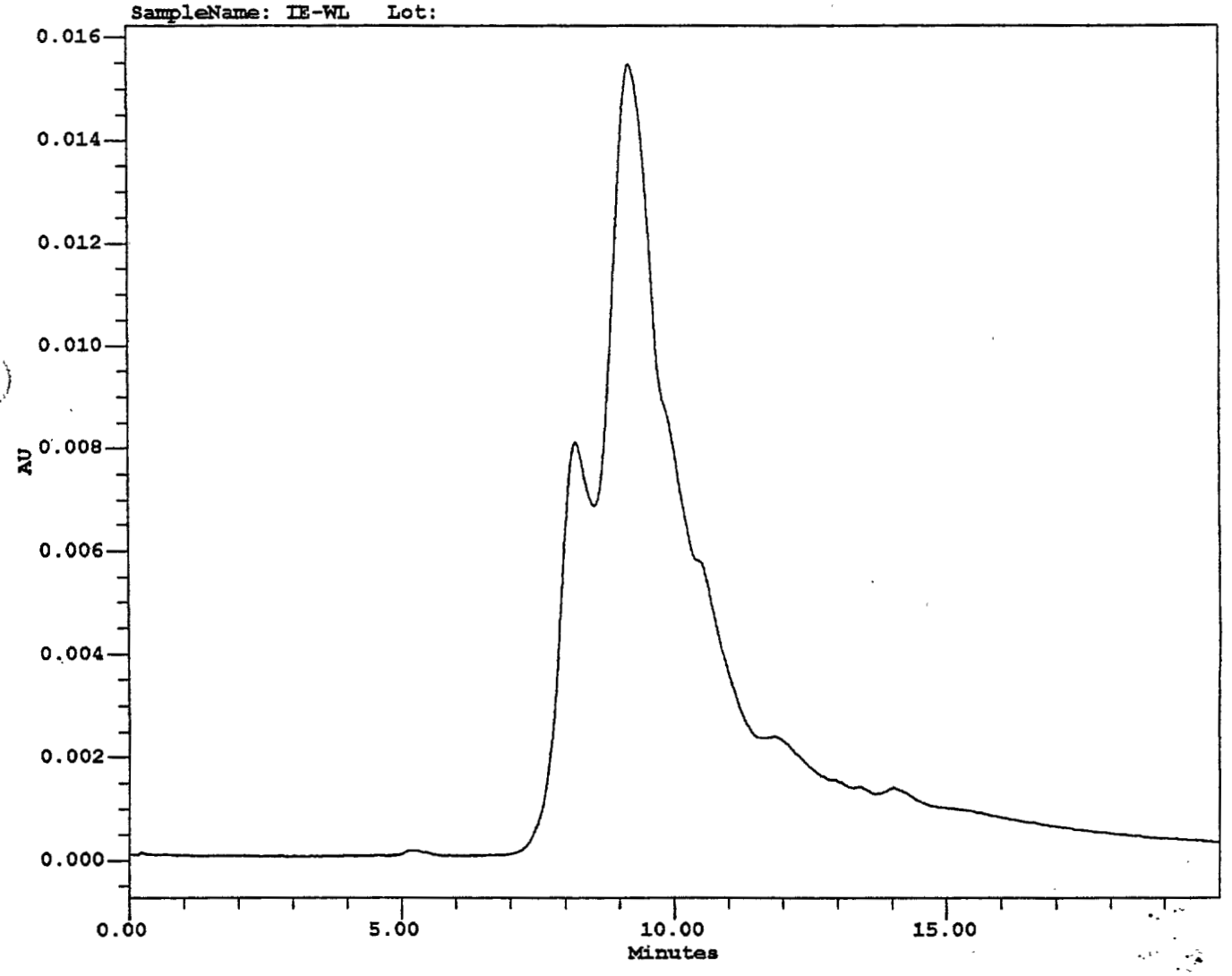
(b)(6)

7.24.0

Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-WL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 12:56:09 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:58 PM	Vial:	8
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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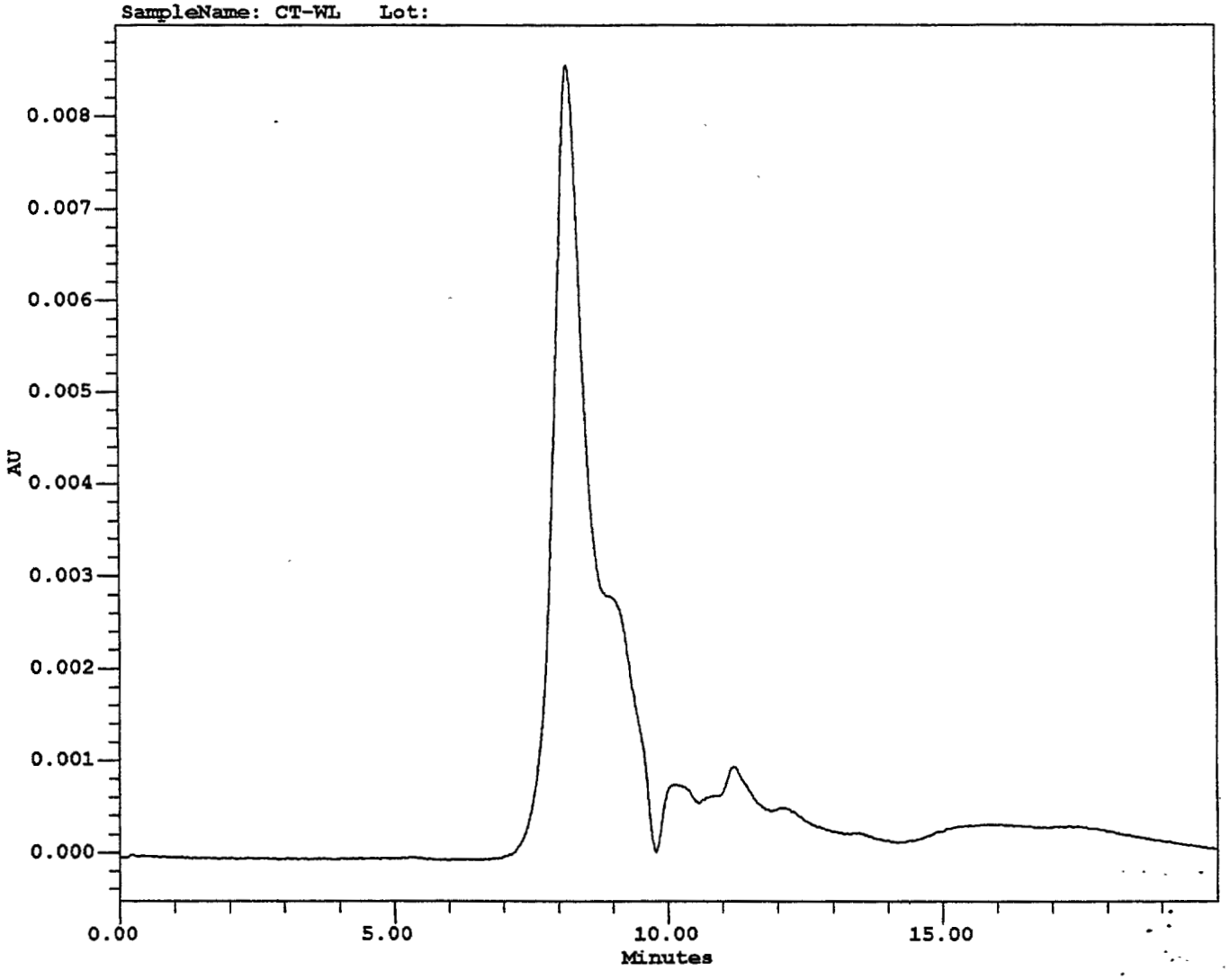
7.24.00

Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-WL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 12:12:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:43:13 PM	Vial:	6
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		

SampleName: CT-WL Lot:



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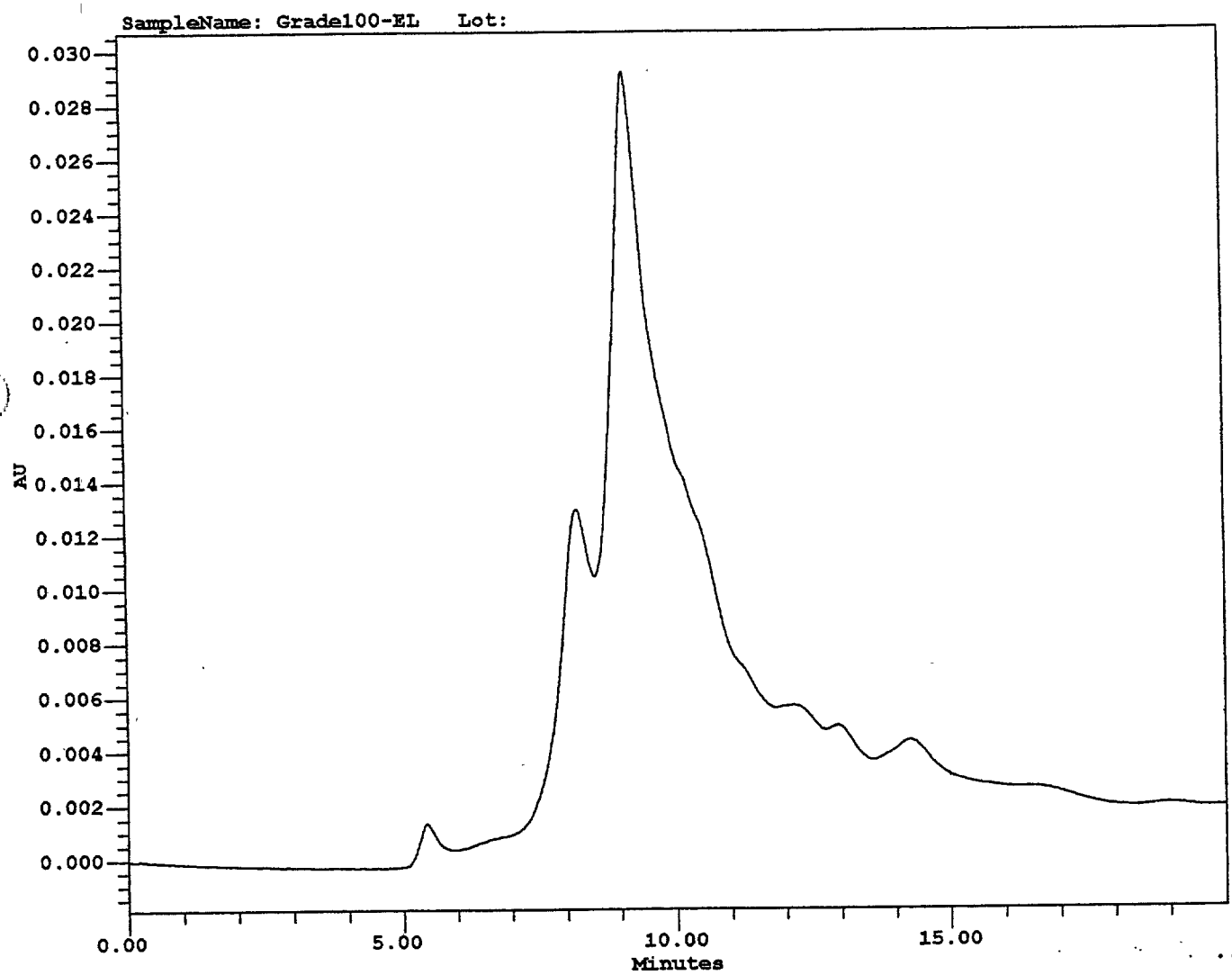
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Grade100-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 02:44:30 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:16 PM	Vial:	13
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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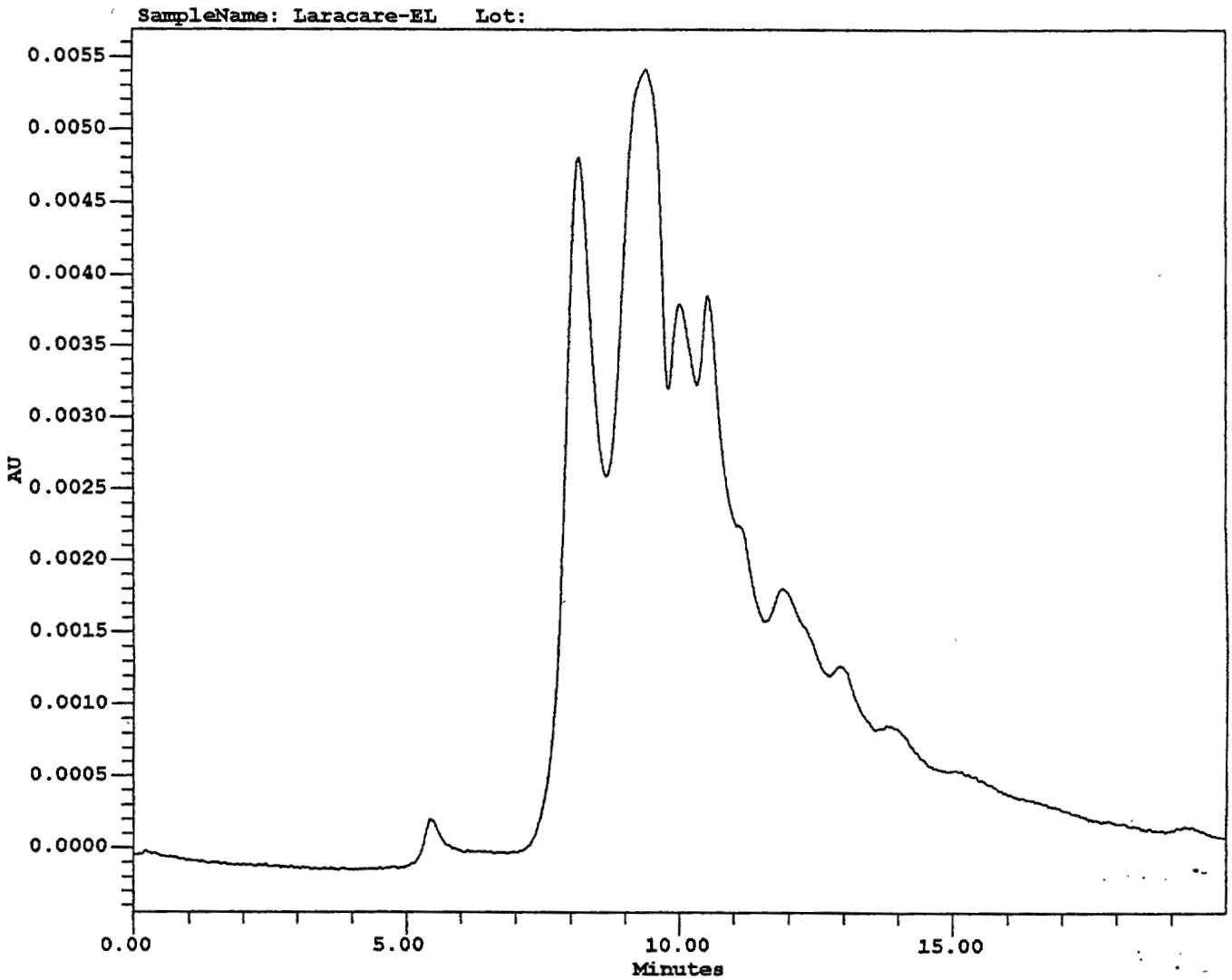
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Laracare-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 02:01:13 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:33 PM	Vial:	11
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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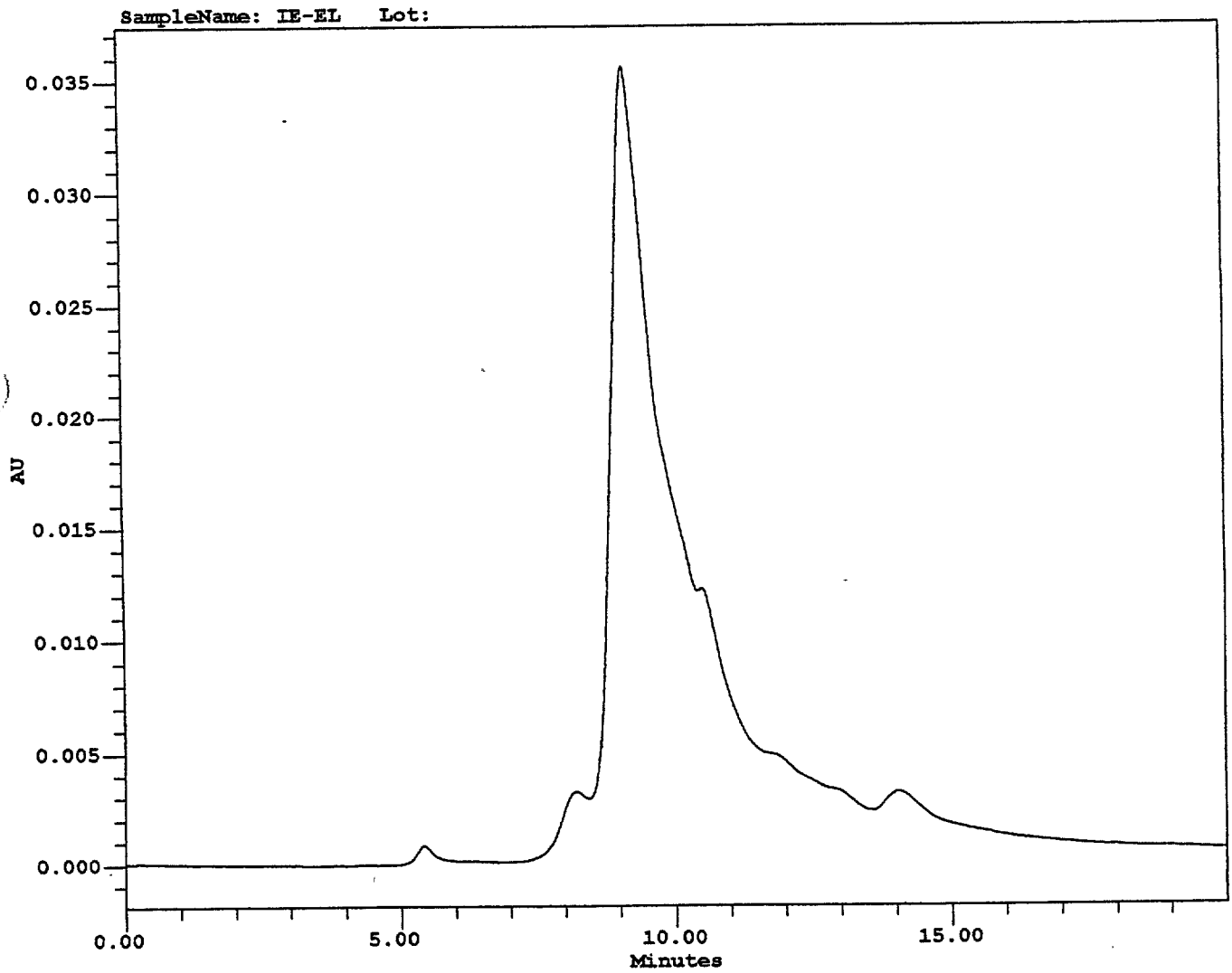
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 01:17:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:42:50 PM	Vial:	9
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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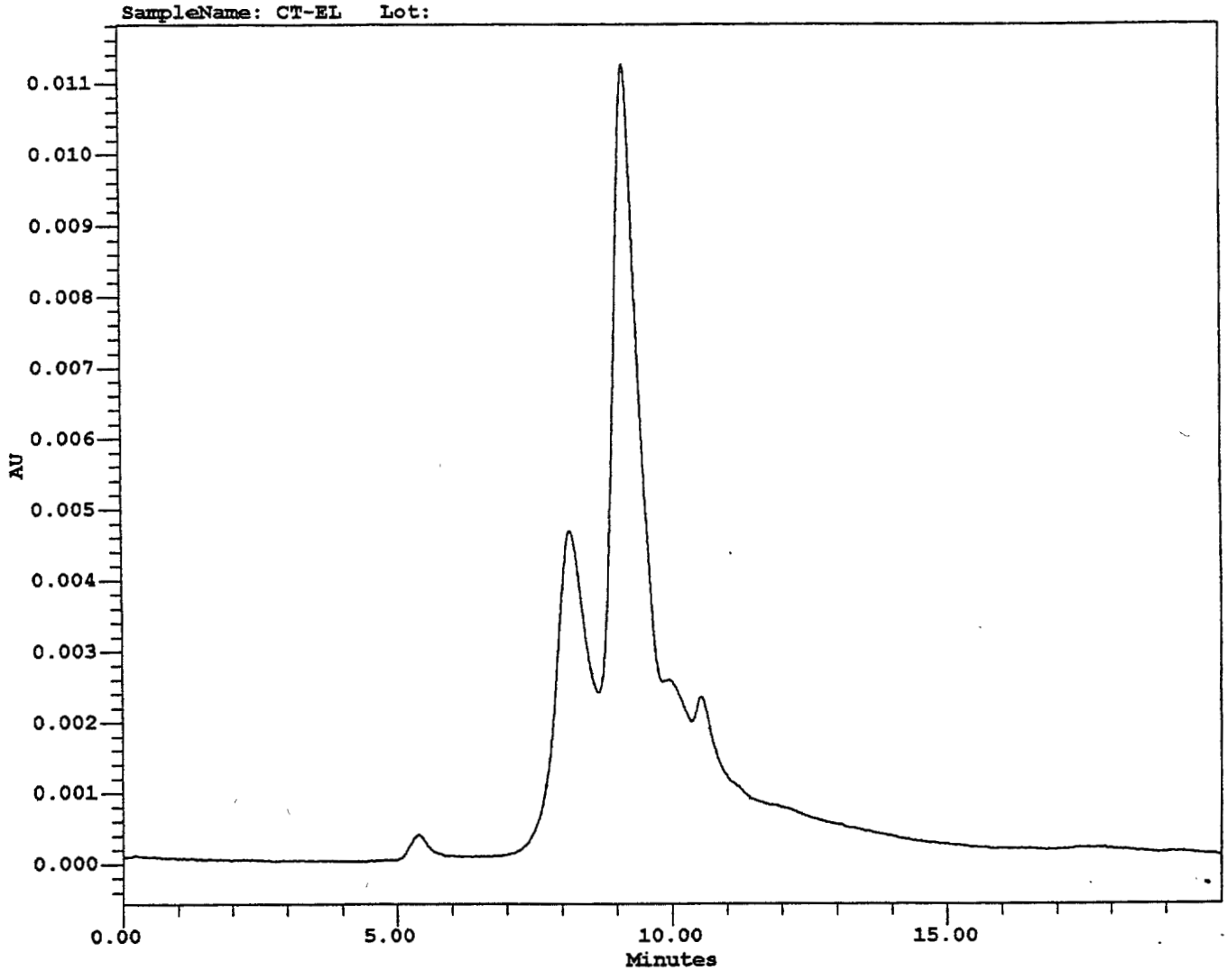
Larex International

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-EL
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	04/10/00 12:34:29 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:43:06 PM	Vial:	7
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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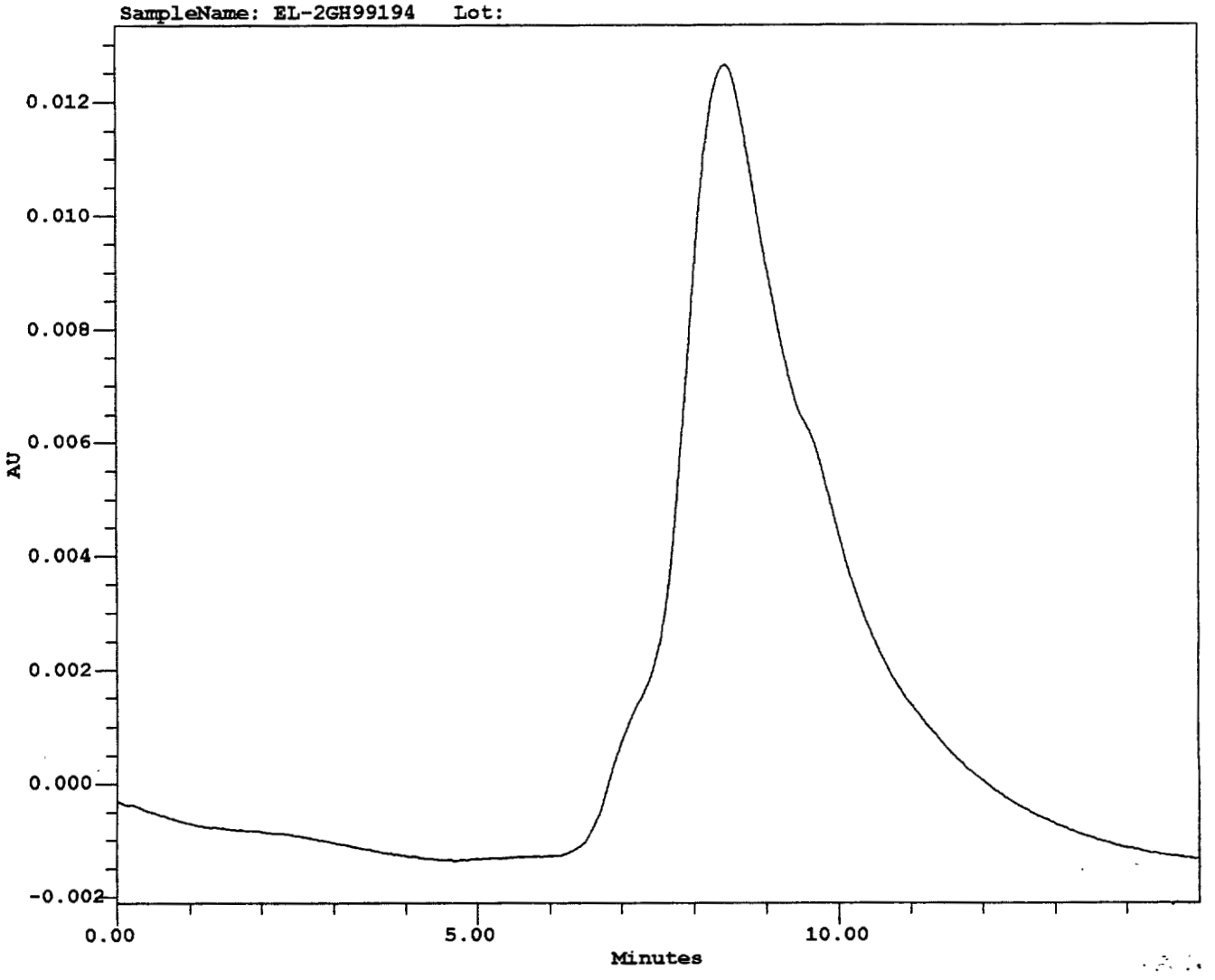
Larex International

July 24, 2000

Project Name:	AG_July_Y2K	Sample Name:	EL-2GH99194
Processing Method:	LC_analysis	Lot:	
Acq Meth Set:	GPC_LC_meth_set	Sample Type:	Broad Unknown
Date Acquired:	07/21/00 03:26:05 PM	Inj Volume, ul:	40.0
Date Processed:	07/24/00 10:30:49 AM	Vial:	29
Run Time:	20.0 min	Injection:	1
Channel:	486	Level:	
Channel Descr.	UV 280nm		



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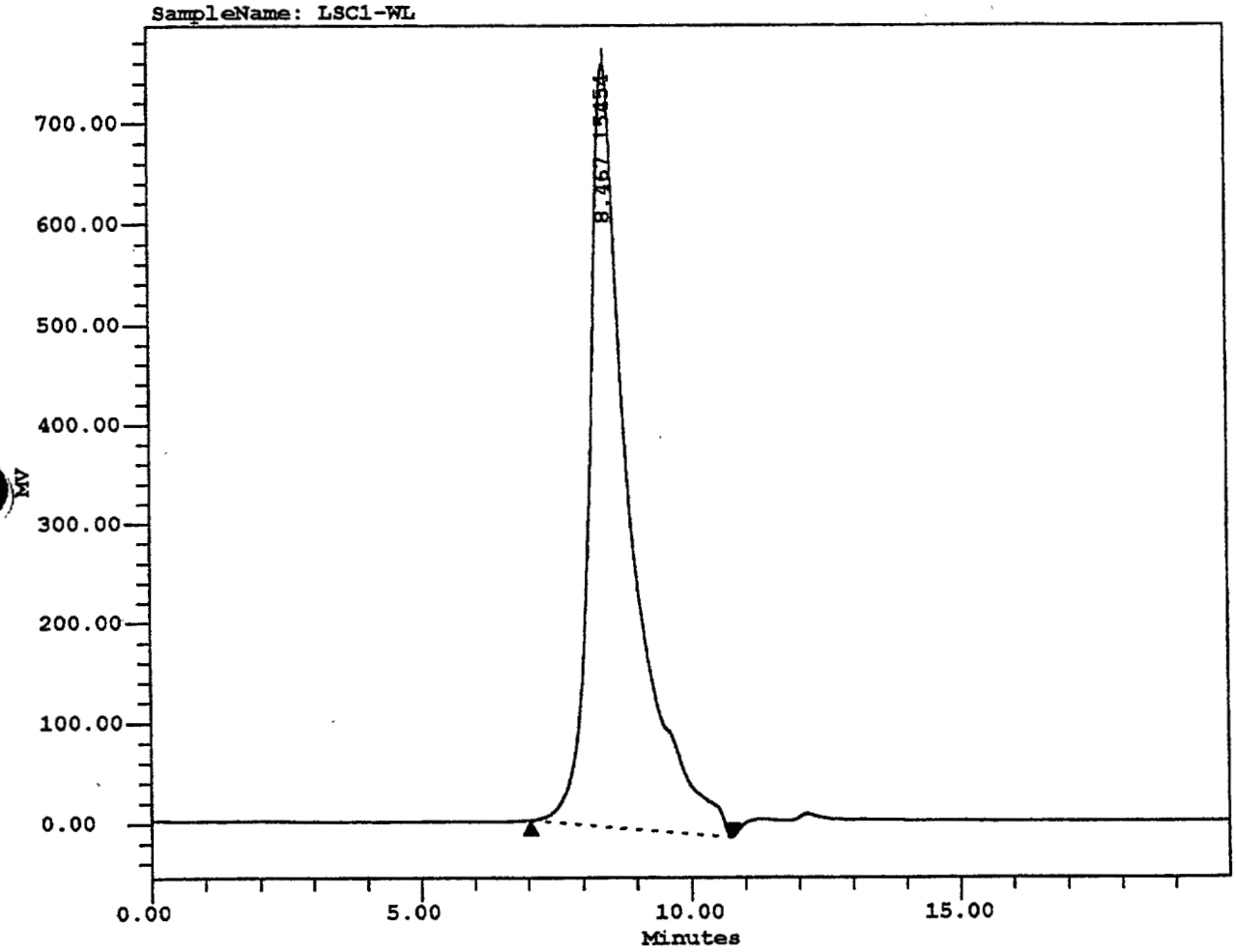
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LSC1-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	12
Date Acquired:	04/10/00 02:22:51 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:42 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.467	15454	17527	14086	1.244	8393678	162742	51.577

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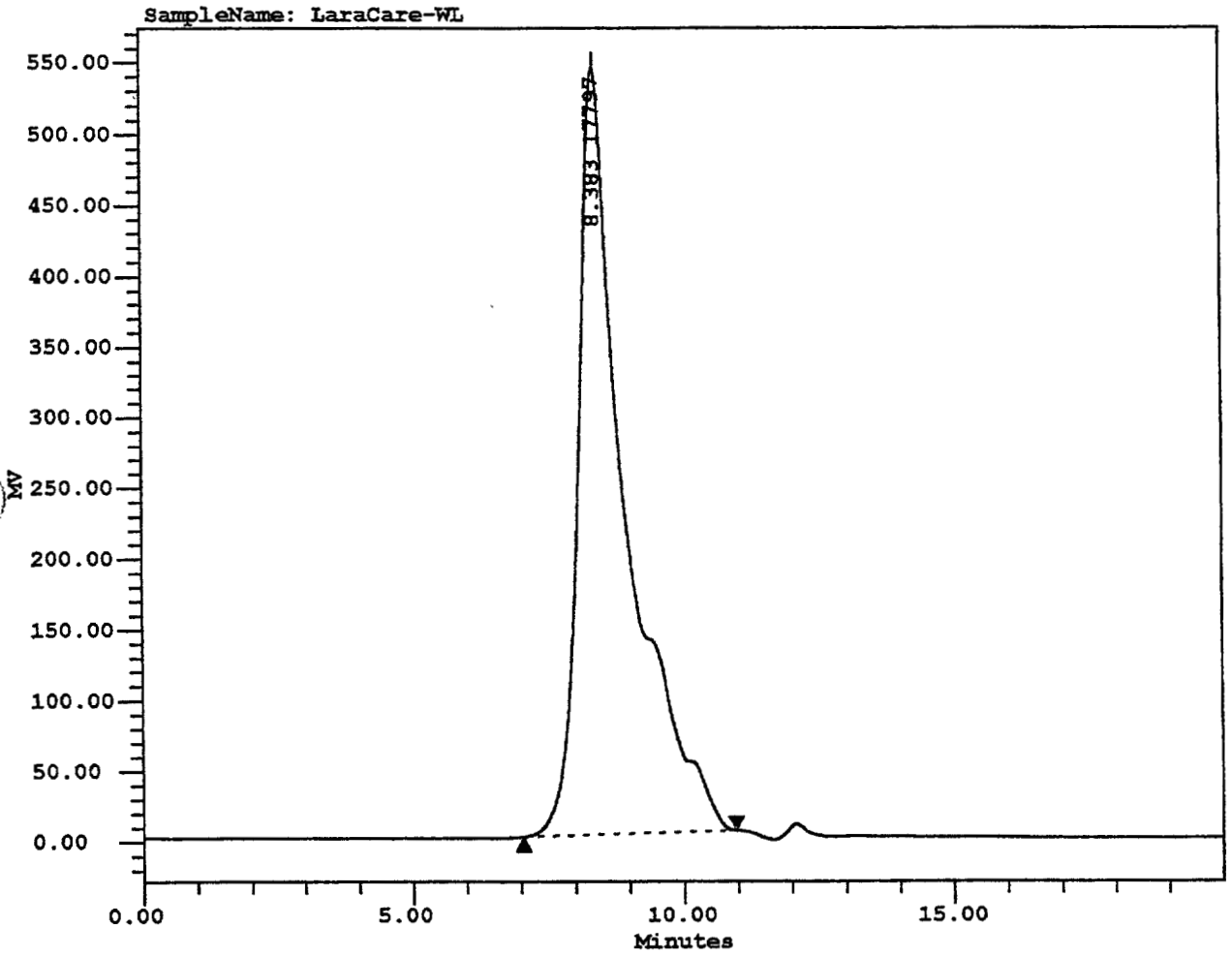
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	LaraCare-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	10
Date Acquired:	04/10/00 01:39:34 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:08 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.383	17797	19040	14953	1.273	7025777	115781	60.682

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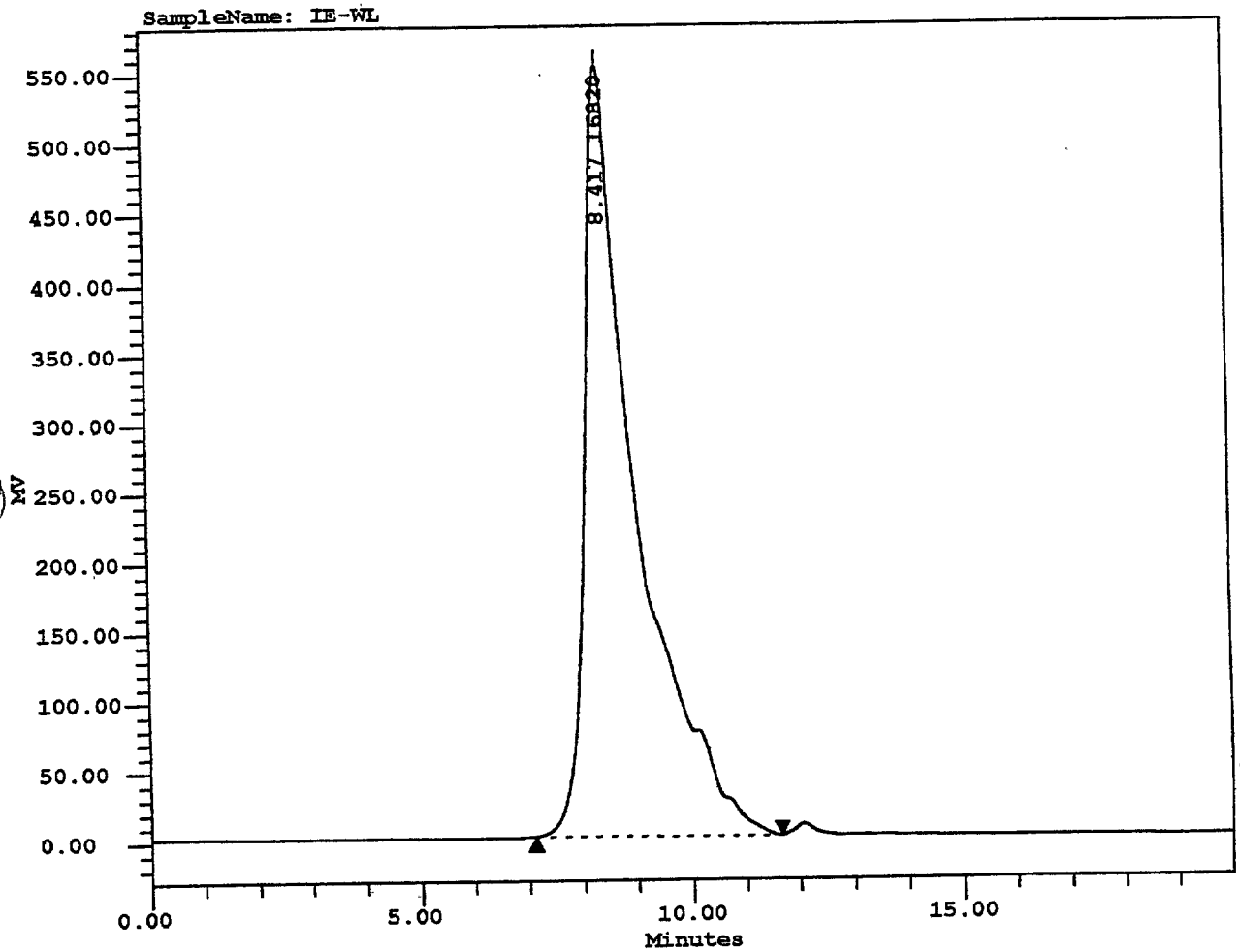
7.24.00

Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	8
Date Acquired:	04/10/00 12:56:09 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:02:11 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.417	16820	17978	14272	1.260	7751456	117936	65.726

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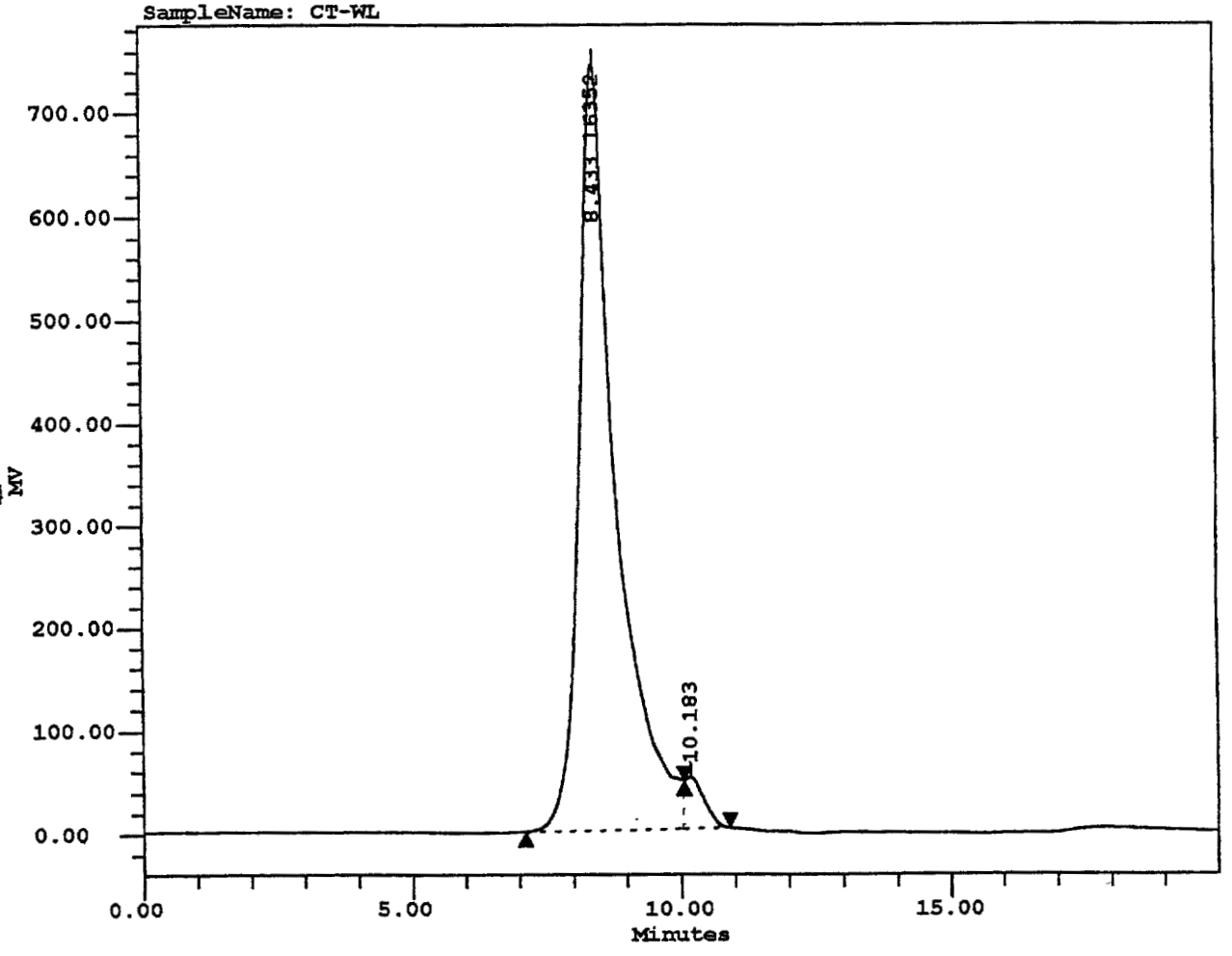
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-WL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	6
Date Acquired:	04/10/00 12:12:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:01:50 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.433	16352	17926	14636	1.225	7596724	158803	47.837
2	10.183					260984	10432	25.018

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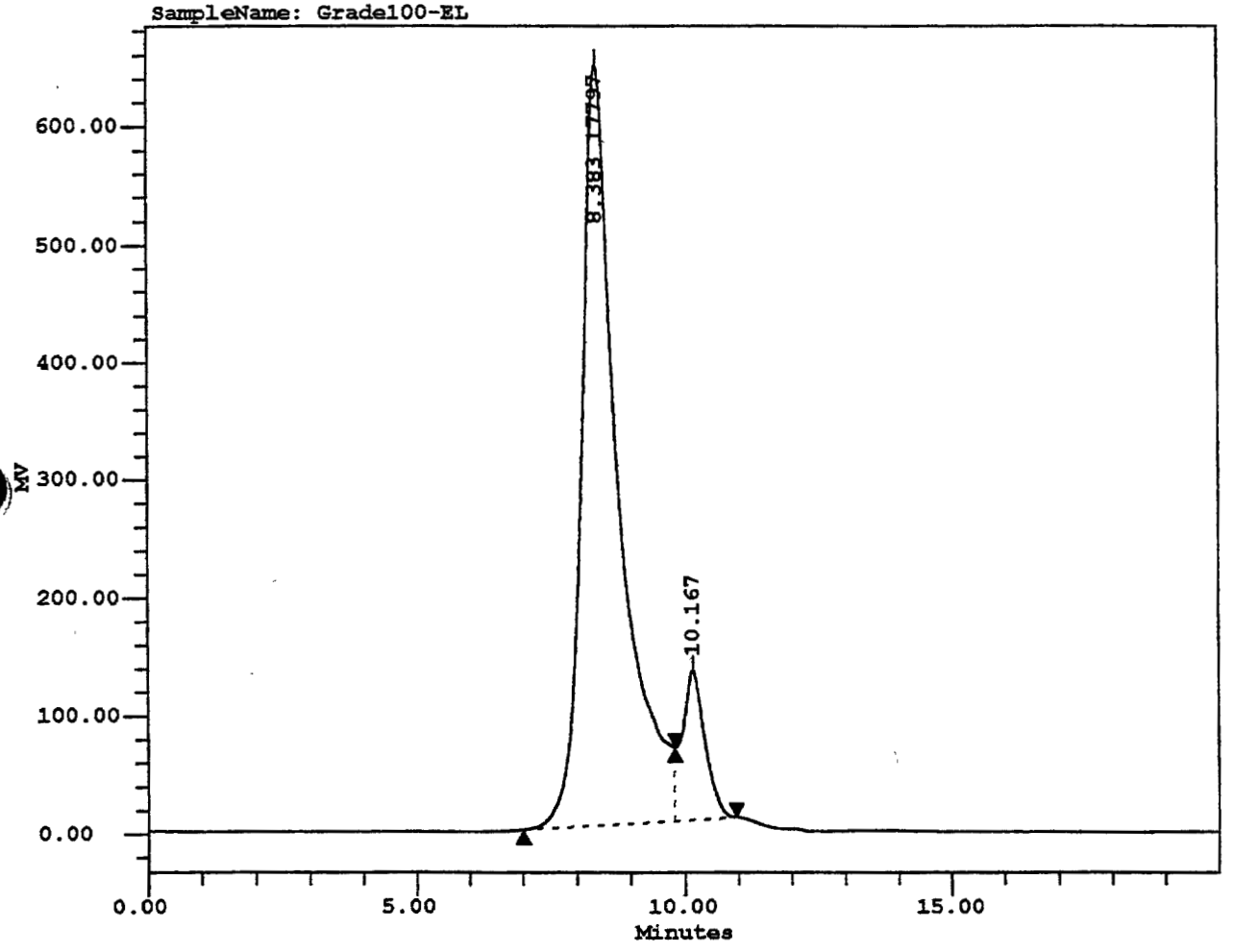
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Grade100-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	13
Date Acquired:	04/10/00 02:44:30 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:58 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.383	17797	18815	15174	1.240	6580753	137672	47.800
2	10.167					810068	27087	29.906

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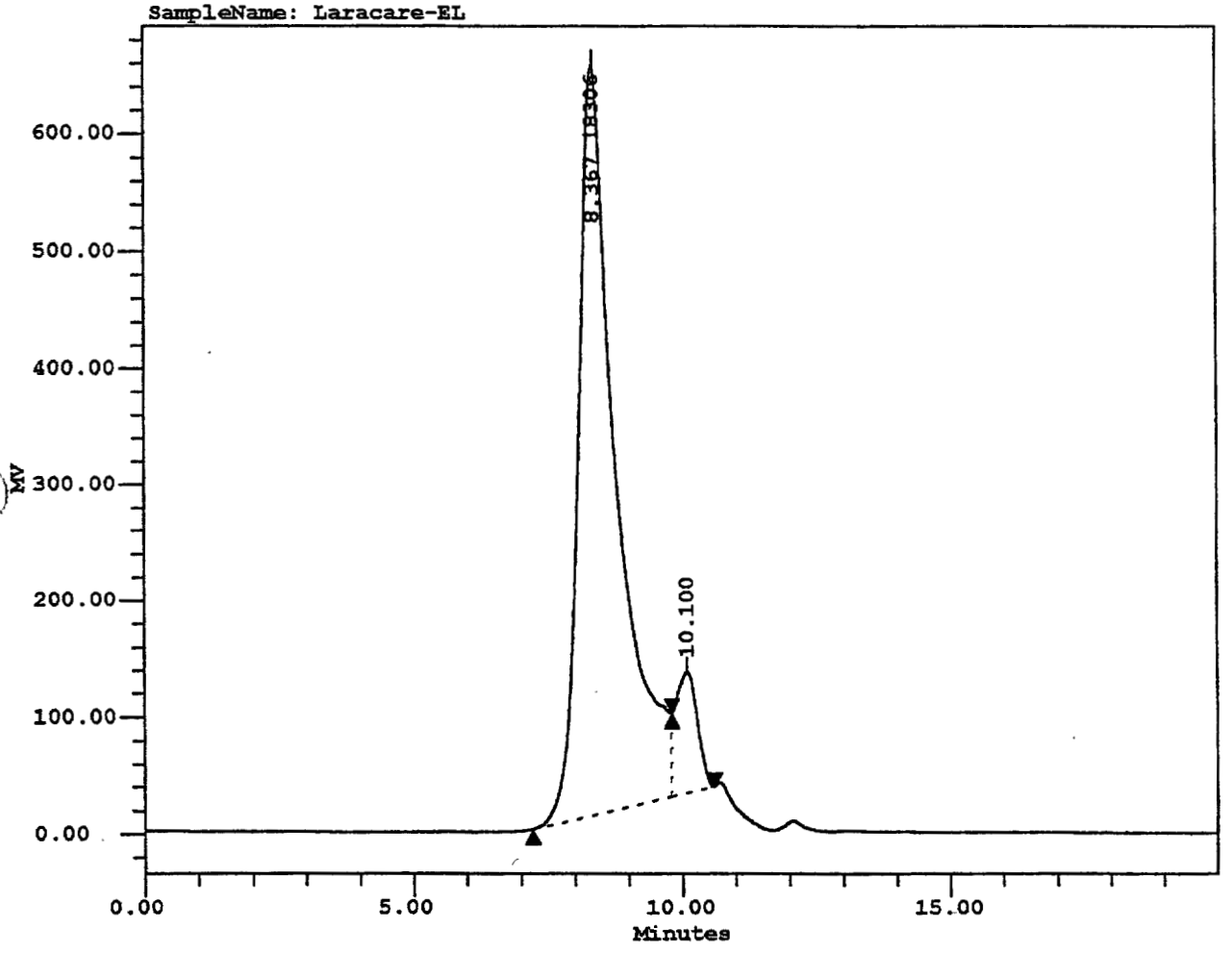
7.24.00

Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	Laracare-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	11
Date Acquired:	04/10/00 02:01:13 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:03:25 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.367	18306	18871	15318	1.232	6674420	137232	48.636
2	10.100					642775	22172	28.991

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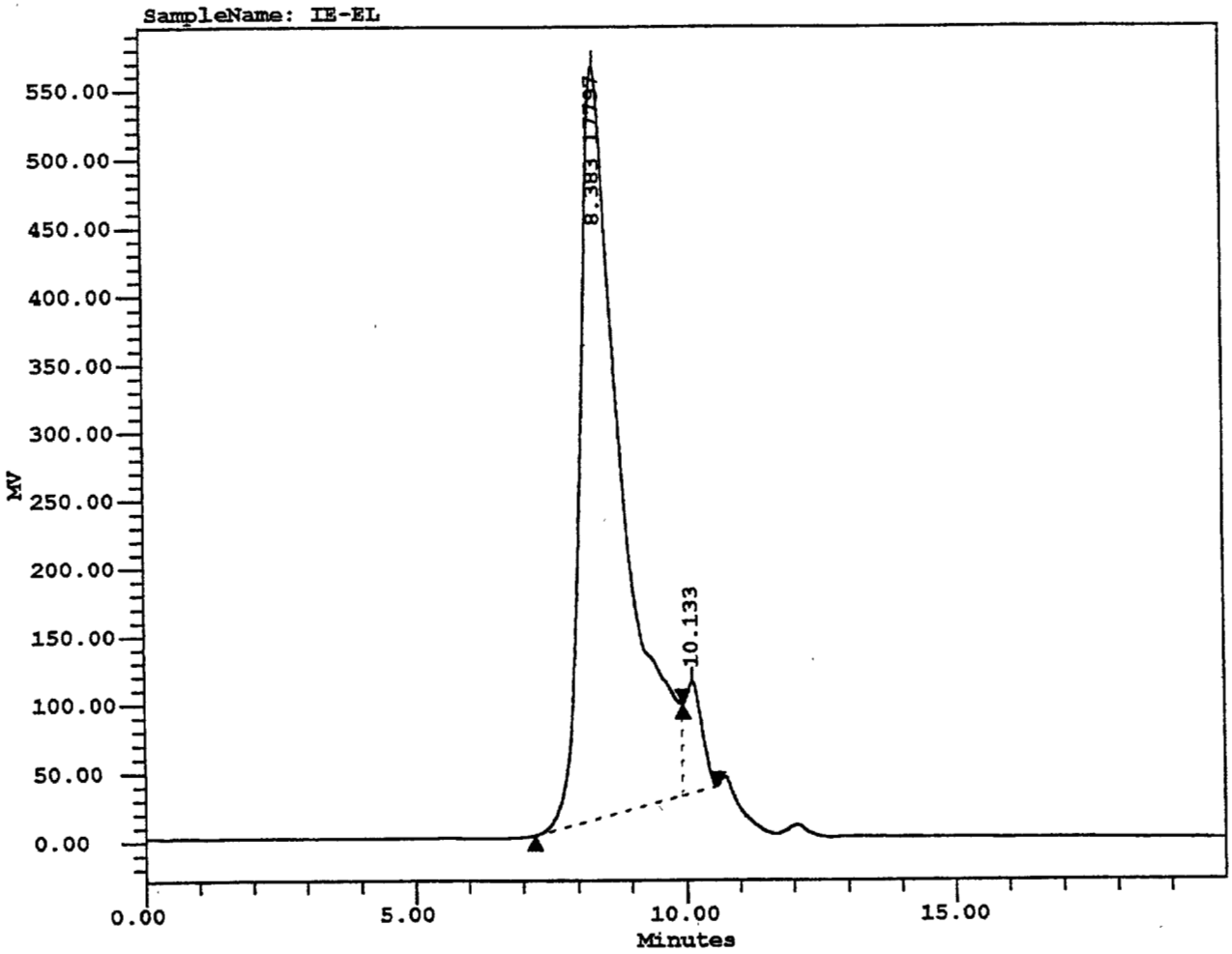
Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	IE-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	9
Date Acquired:	04/10/00 01:17:49 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:02:50 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.383	17797	18153	14771	1.229	6258076	117685	53.177
2	10.133					386162	17261	22.372

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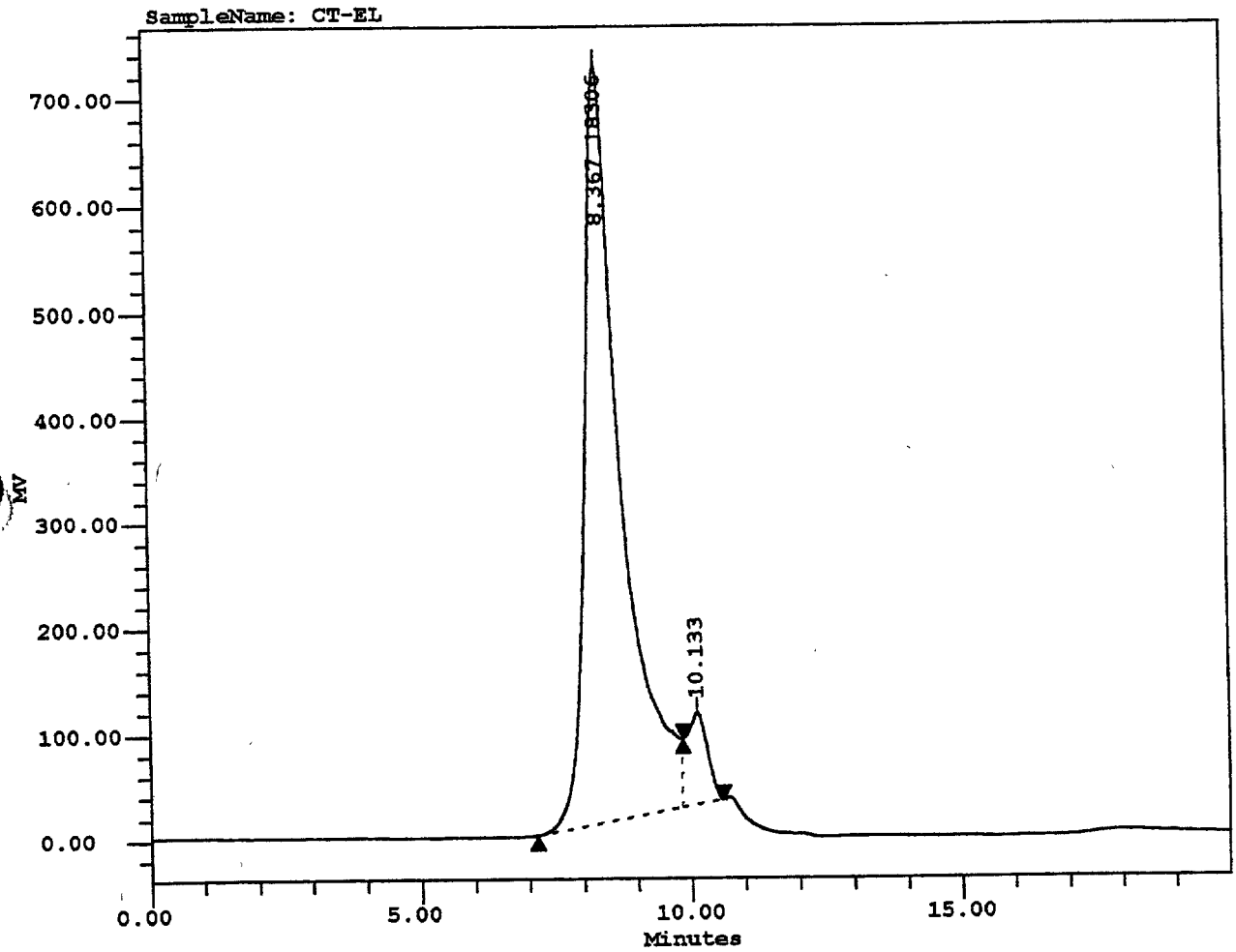
7.24.

Larex Incorporated

April 10, 2000

Project Name:	AG_April2000	Sample Name:	CT-EL
Processing Method:	Pullulam04102000	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	7
Date Acquired:	04/10/00 12:34:29 PM	Inj Volume, ul:	40.0
Date Processed:	04/10/00 04:02:32 PM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	8.367	18306	18750	15335	1.223	7162551	152672	46.915
2	10.133					508077	18429	27.569

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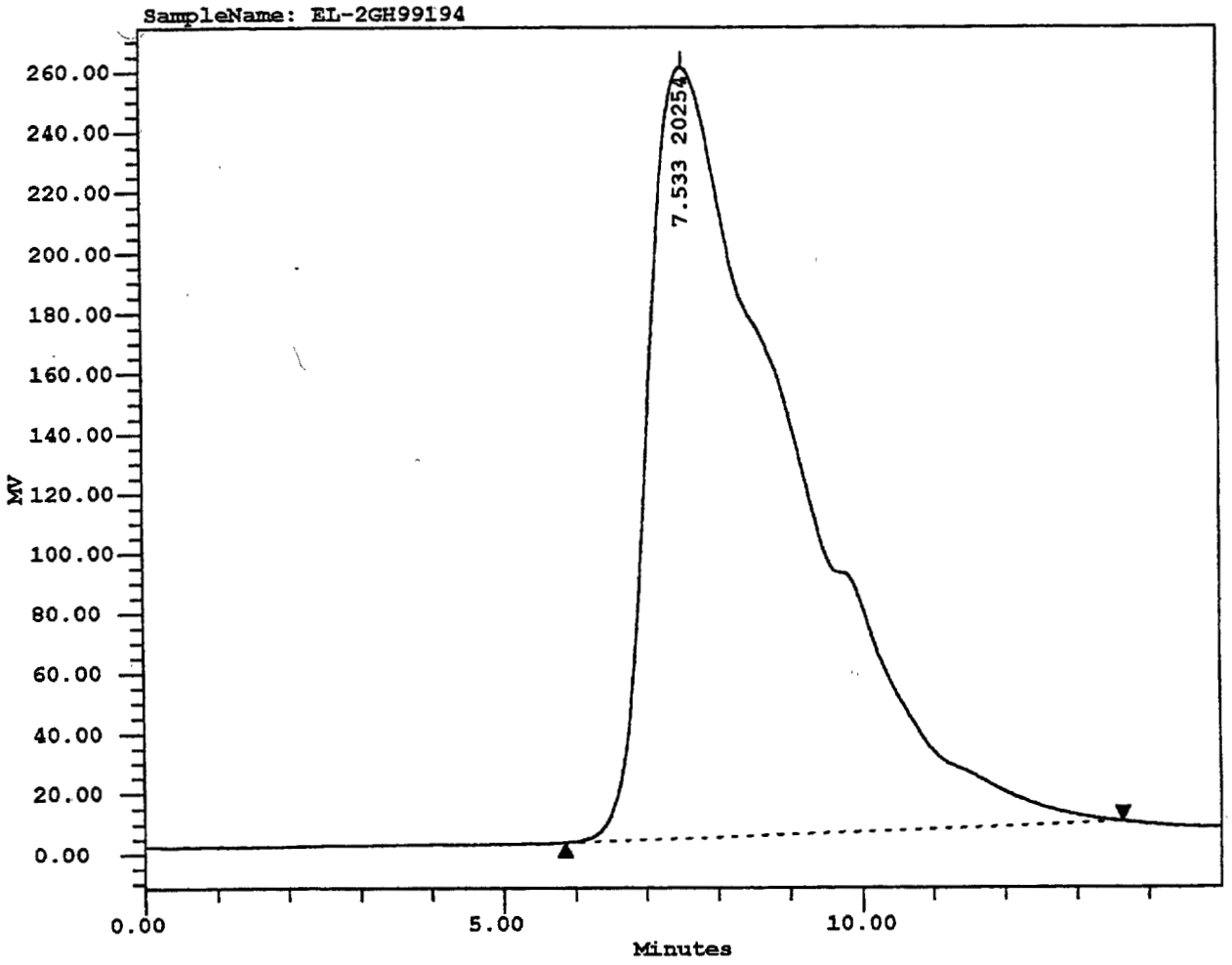
Larex Incorporated

July 24, 2000

Project Name:	AG_July_Y2K	Sample Name:	EL-2GH99194
Processing Method:	pullulan07192k	Sample Type:	Broad Unknown
Acq Meth Set:	GPC_LC_meth_set	Vial:	29
Date Acquired:	07/21/00 03:26:05 PM	Inj Volume, ul:	40.0
Date Processed:	07/24/00 09:23:19 AM	Injection:	1
Channel:	410	Run Time:	20.0 min
Channel Descr.:	RI		



RI Results

#	Ret Time (min)	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	PD	Area (uV*sec)	Height (uV)	Area Height (sec)
1	7.533	20254	25007	15167	1.649	7637910	54694	139.649

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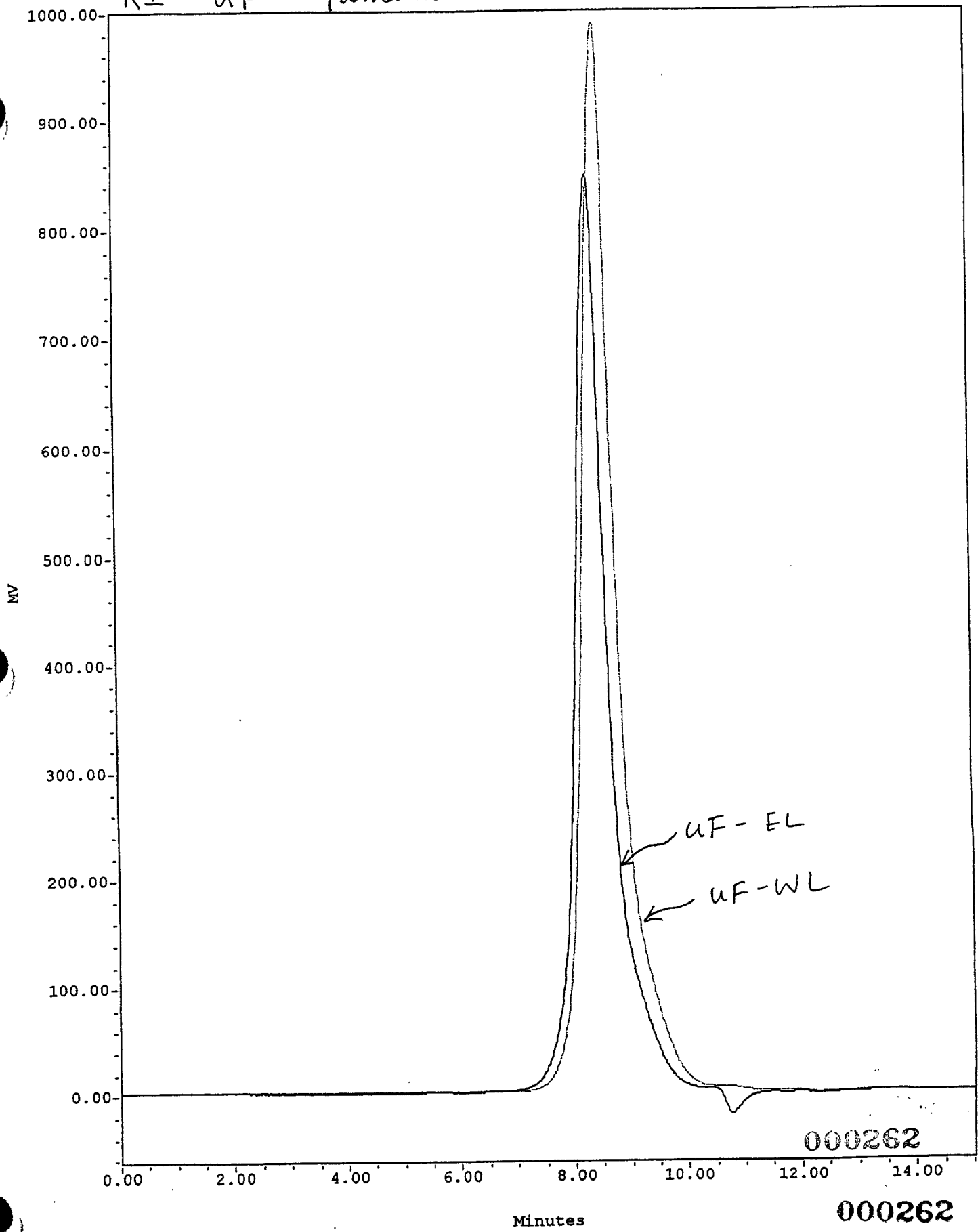
RI: UF - Tamarack vs W. Larch



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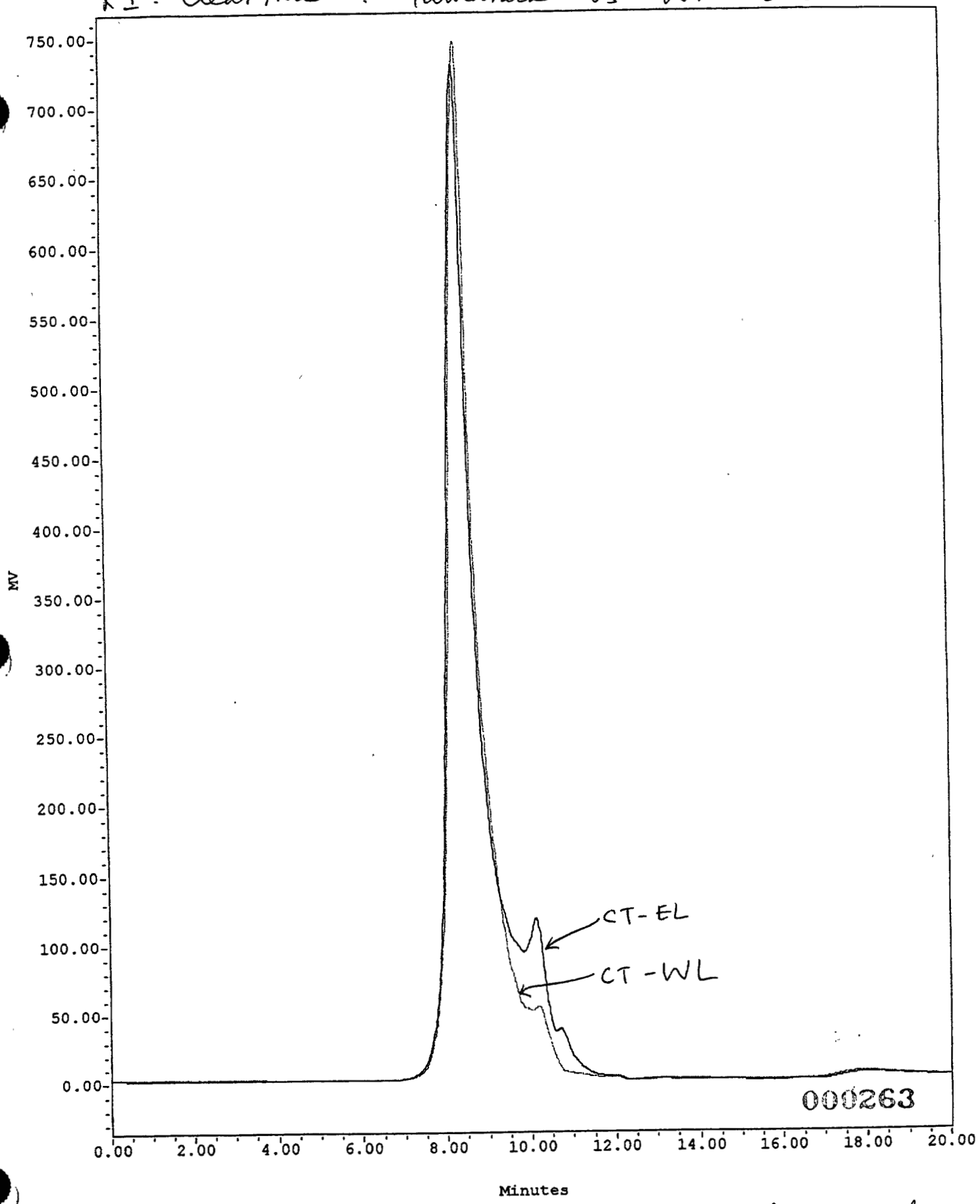
Abs. scale

RI: ClearTrac : Tamarack vs W. Larch



Abs. scale

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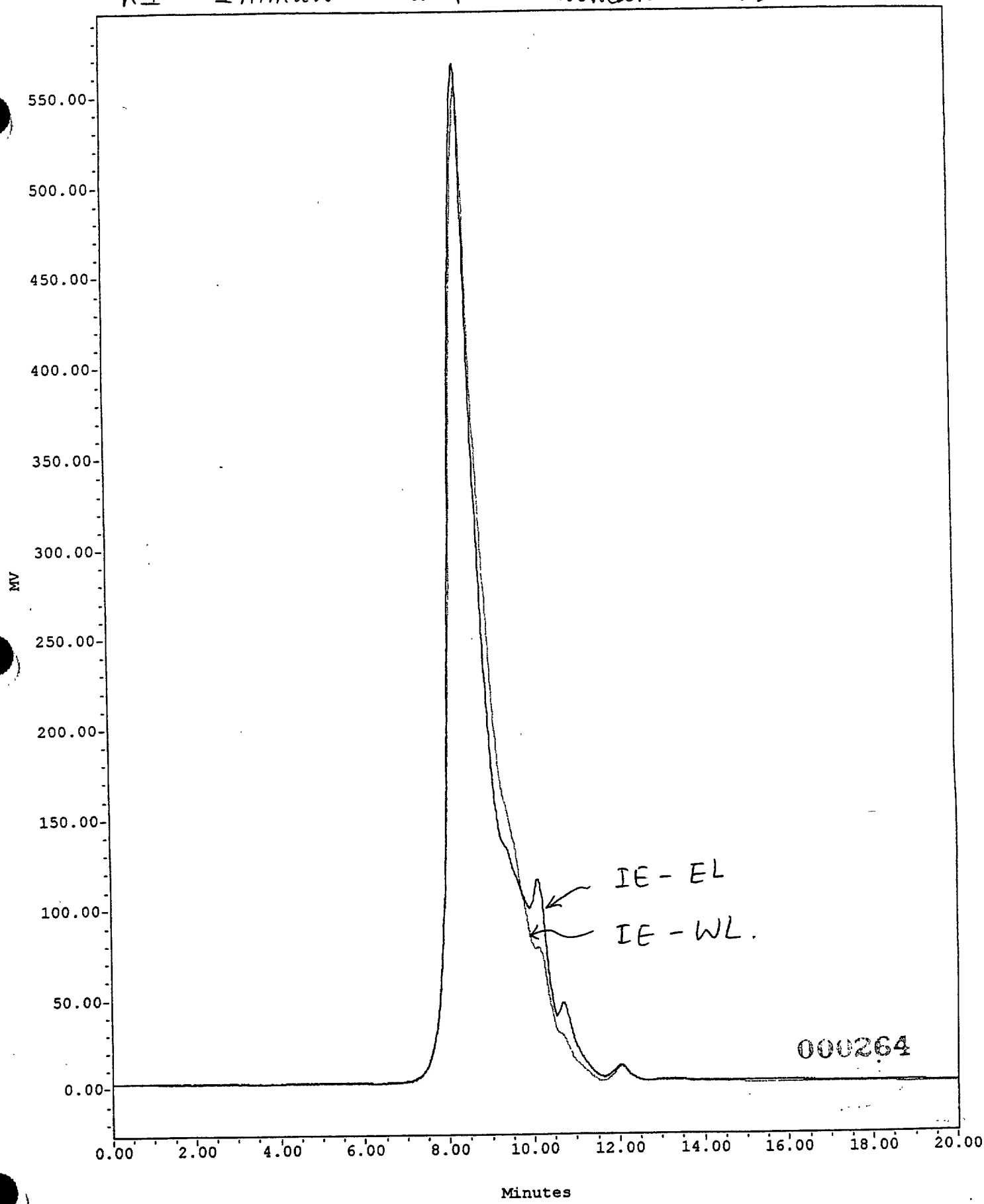
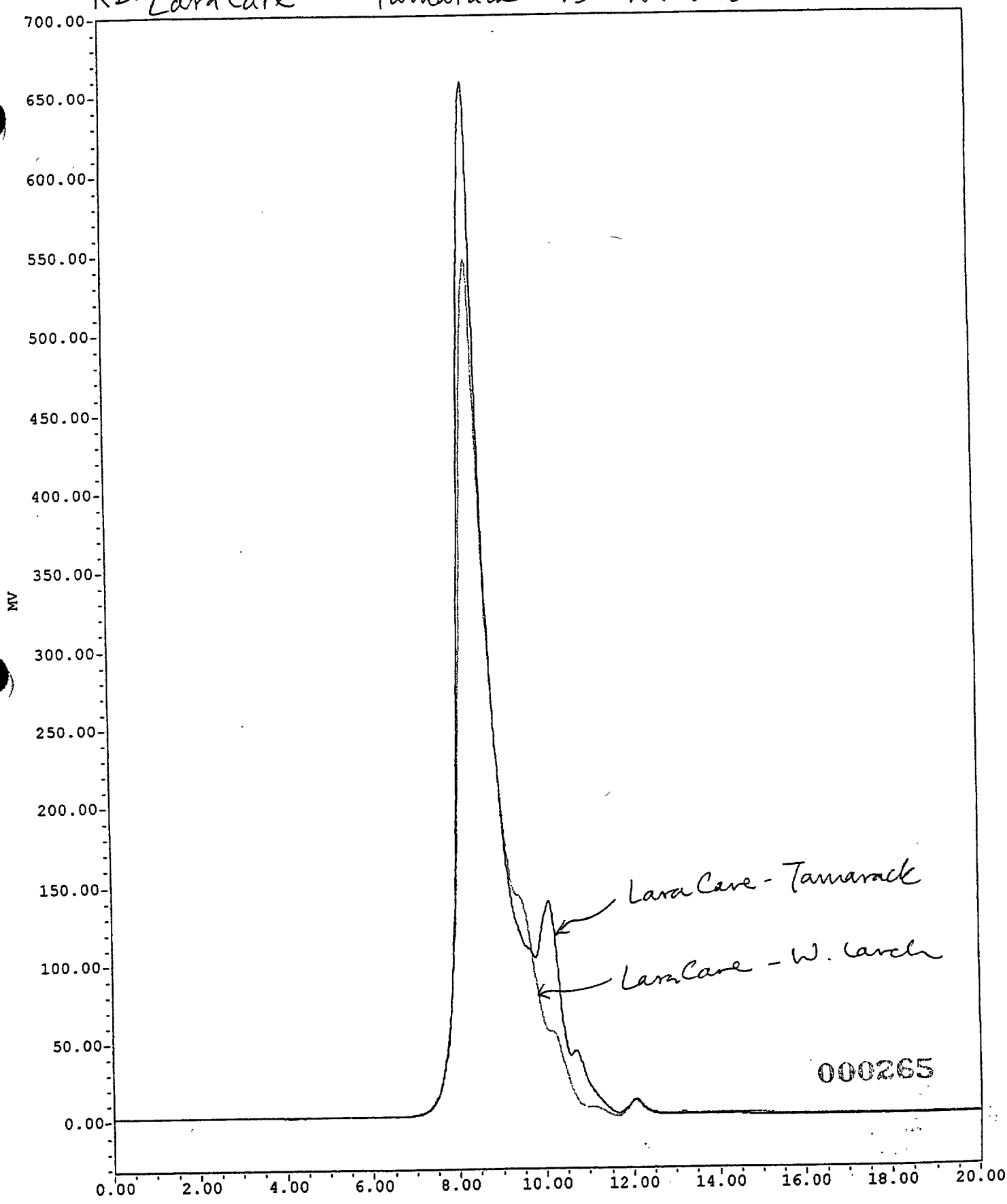
K1 : Immuntenhancer - Tamarack vs W. Larch



00 033 Abs. scale

RI: Lara Care - Tamarack vs W. Larch



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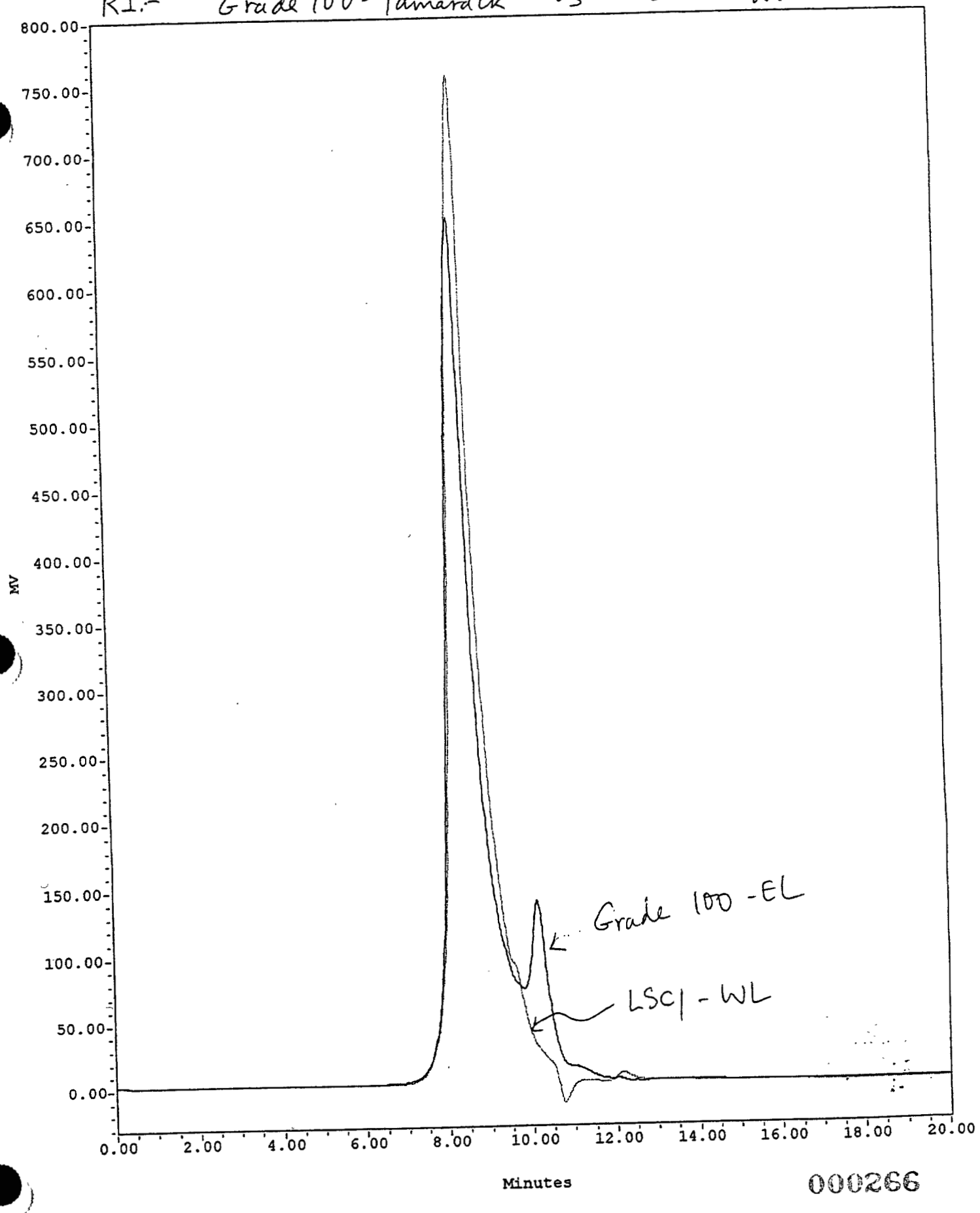
Abs. scale

K1:- Grade 100 - Tamarack vs LSC1 - W. Larch



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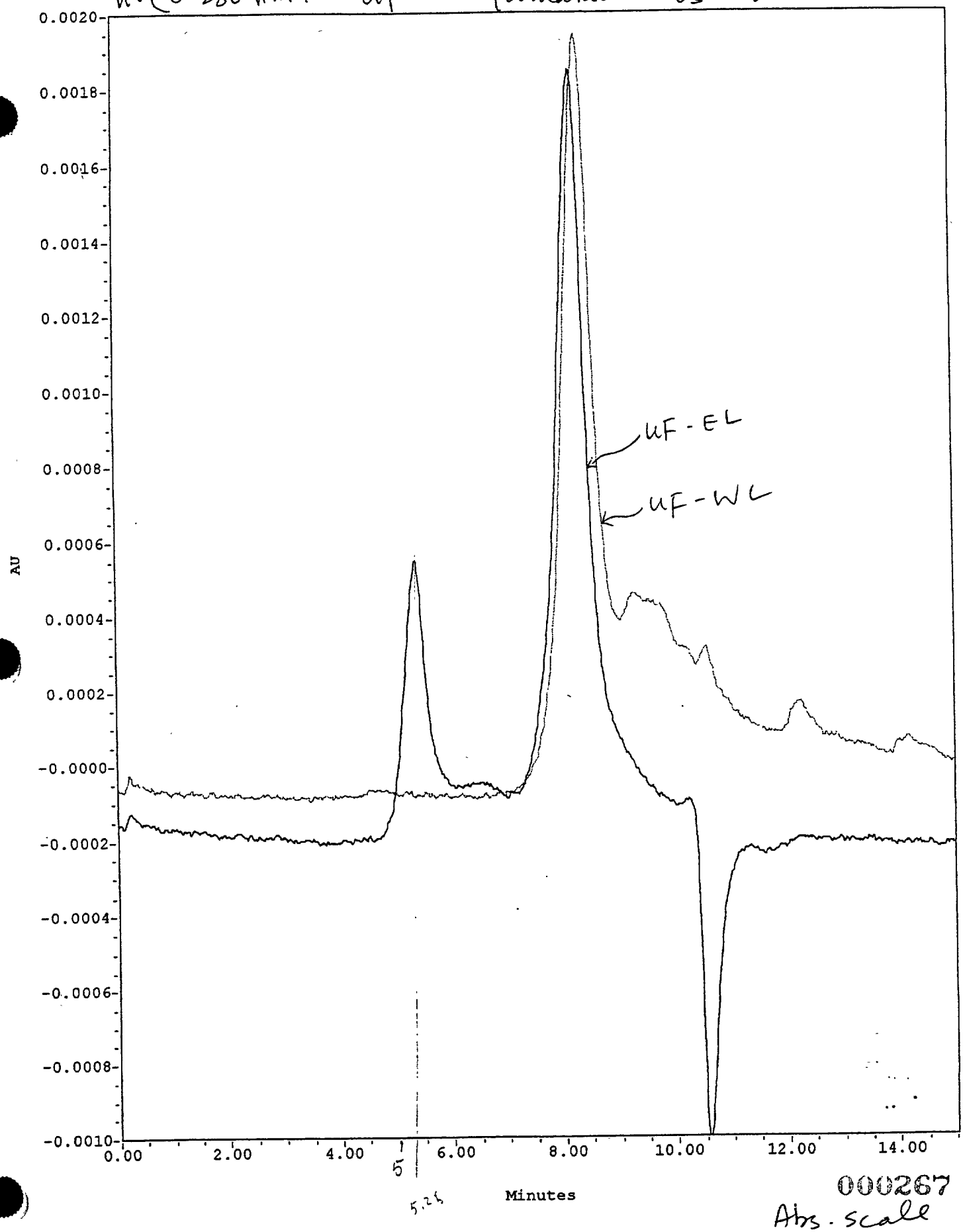
absolute scale

UV (@ 280 nm: UF - Tamarack vs W. Larch



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Abs. scale

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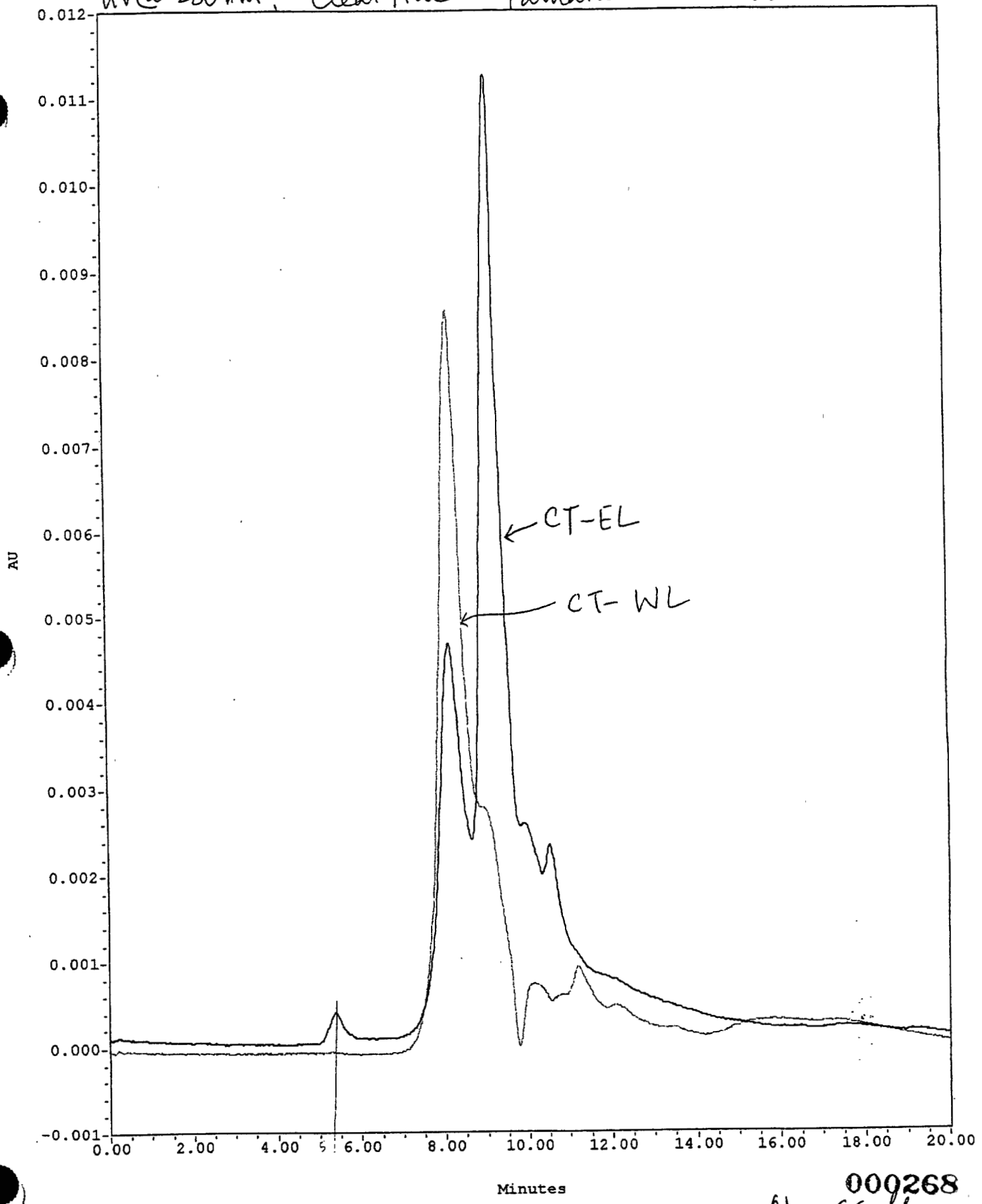
UV @ 280 nm: ClearTrac Tamarack vs W Larch



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Abs. scale

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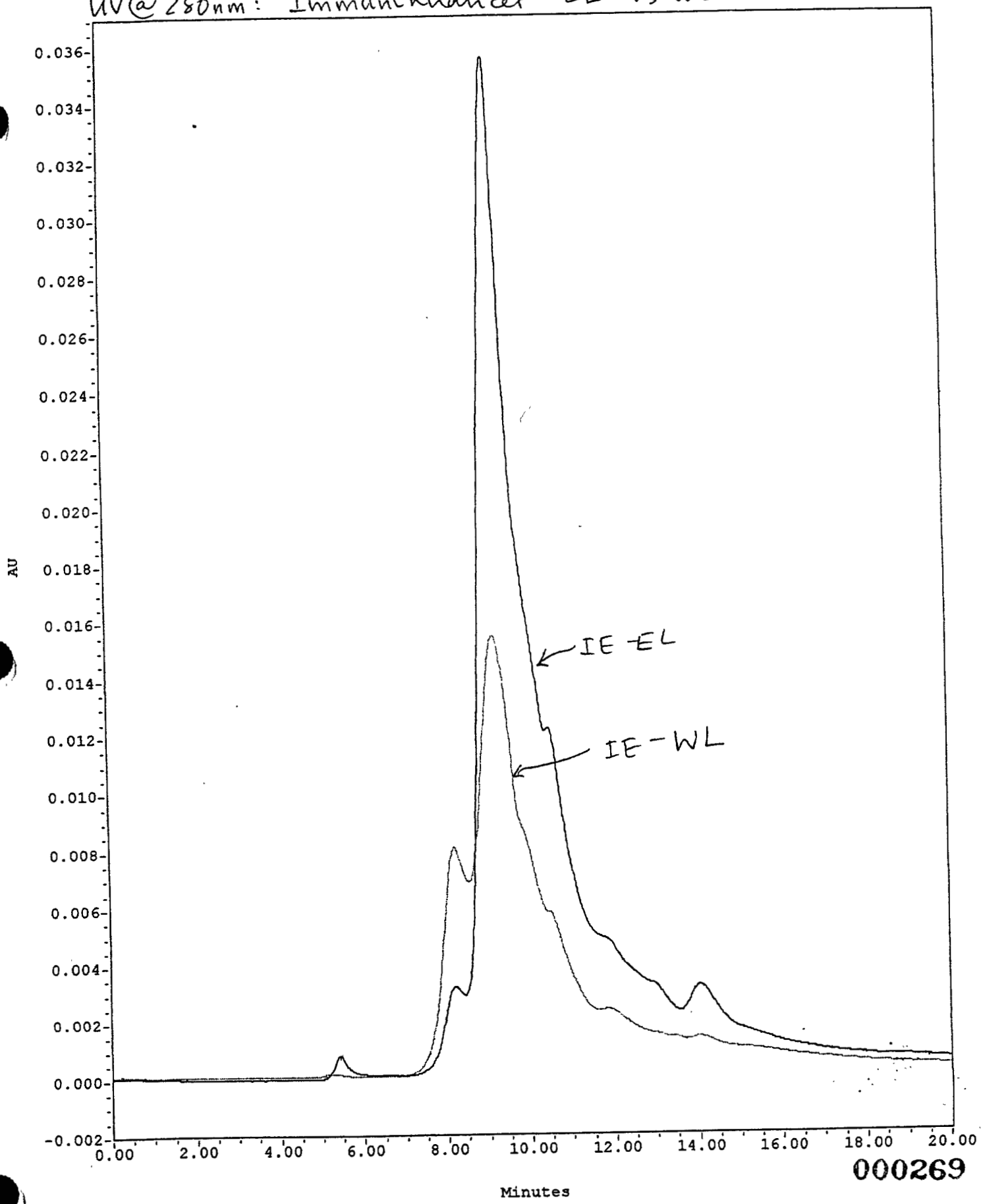
UV@280nm: ImmunEnhancer EL vs WL



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Abs. scale

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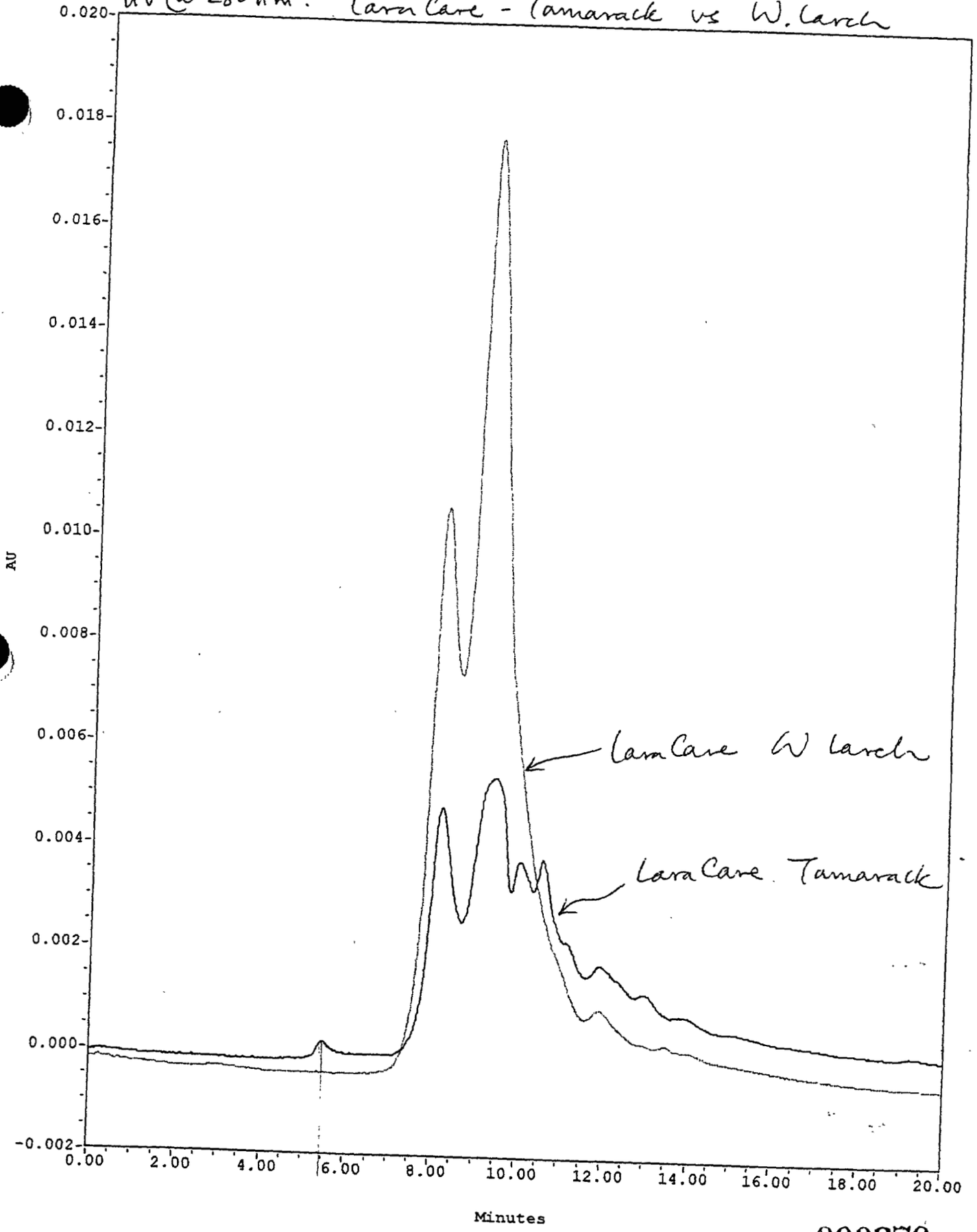
UV @ 280 nm: LaraCare - Tamarack vs W. Larch



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Abs. scale

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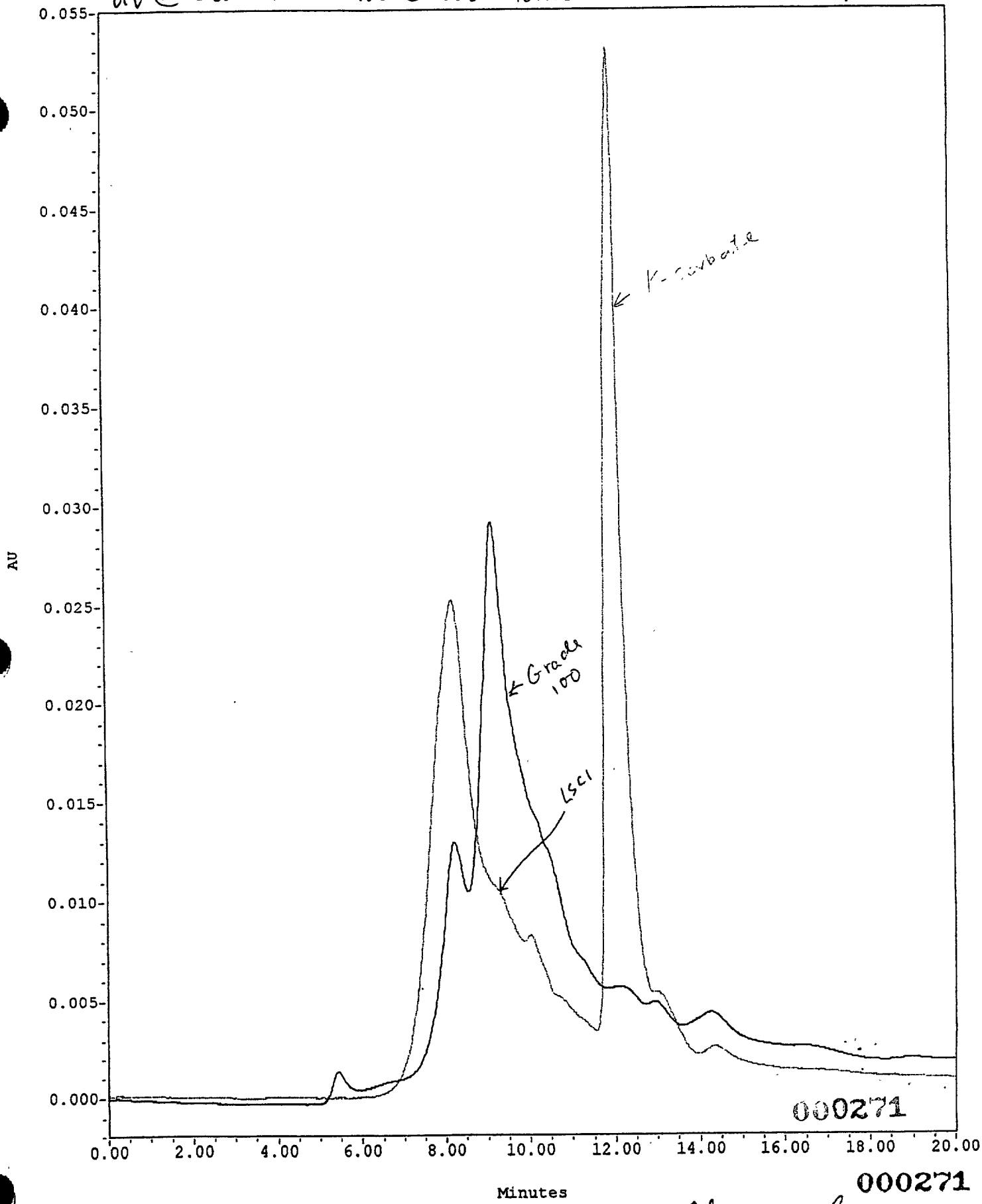
UV @ 280nm Grade 100 - Tamarack vs LSCI - W. Larch



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Abs. scale

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The University of
Montana

Shafizadeh Center for Wood and Carbohydrate Chemistry
The University of Montana
Missoula, Montana 59812
(406) 243-4435
(406) 243-4227 FAX

FAX TRANSMISSION

f

DATE: January 19, 1995

TO: Dr. Christopher H. Price
Larex International, Inc.
Fax (612) 636-1583

FROM: Dr. G.R. Ponder

SIX PAGES FOLLOW

Dear Dr. Price,

The matters in this letter flow from your telephone conversation with Dr. Richards yesterday.

Regarding the different HPSEC elution times for AG's from Western larch and Tamarack, the numbers reported in the fax of Jan.12 correspond to molecular weights of 16,600 and 21,400, respectively, based on Pullulan calibration. It is noteworthy that Timell and coworkers also found indications of size differences between these polymers, but in an opposite sense. Thus, they reported number average molecular weights, determined by osmotic pressure measurements, as follows.

Western larch AG: 58,500 Tamarack AG: 36,000.

[Simson, Côte & Timell, "Studies on larch arabinogalactan. IV. Molecular properties" *Svensk Papper.* 19 (1968) 699-710.] In the same paper, they reported additional molecular weight determinations based on ultracentrifugation and gel permeation chromatography, and although the numbers vary widely they generally agree in attributing a larger size to the Western larch AG.

The fact that the difference which we observe is consistent for more than one tree sample (and in the case of Tamarack, for more than one time of year) suggests that it may be more

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An Equal Opportunity University

yield from the current 320 sample. It would seem that wood selection only partly solves the problem and that scum will be an intrinsic problem for Western larch. (The preceding comments are based on the assumption that the defectiveness of the first P.S.F. had no effect on the proportion of scum in the exudate.)

As for Tamarack, a tentative conclusion from the data in the table is that spring may be a better time of year to harvest trees for AG than fall. The table also presents an unexpected result: Tamarack AG (whether from fall or spring wood) consistently elutes from our HPSEC column about 0.15 minutes faster than Western larch AG. Based on Pullulan calibration, this suggests that the molecular size of Tamarack AG is 30% larger than that of Western larch AG. However, there could also be structural differences involved.

I have included the UV and RI chromatograms for the impregnator solutions of each run. These are representative of the four types of wood, since the chromatograms for the corresponding pressates are very similar, the only differences being minor alterations in proportions of the UV peaks. On the other hand, some major differences can be seen when comparing the four types of wood. For example, the proportions of various phenolics in Tamarck appear to be dependent on the time of year. This is not surprising, and it probably applies to Western larch as well. Note also the comparison of UV chromatograms for Western larch heartwood and whole wood. The virtual absence of the strong absorbance at 7.75 min in the 230 chromatogram suggests that this absorbance is due to a sapwood component, whereas the excluded light-absorbing material at 5.48 min is predominantly derived from heartwood.

Sincerely,

(b)(6)

Glenn R. Ponder

Jan 12, 1994
letter to C. Price

Medallion Laboratories

Analytical Report

900 Plymouth Avenue • Minneapolis, MN 55427

1-800-245-5615
(612) 540-4453
Fax: (612) 540-4010

Chew-Hung Mak
Larex Inc.
4815 White Bear Pkwy
White Bear Lake MN 55110

Date Submitted: 30May2000
Date Reported : 6Jun2000
P.O. Number : 5748
Library Number: 2000-04503
Company Code : LAREX01

F I N A L R E P O R T

* * * * * * * * * *

Customer ID: G100WL Grade 100 Western Larch Medallion Labs ID: 2000033251
Sample Descr.: Extract from Trees

Test Description	Results	Units	Estimate
Fatty Acid Analysis w/Profile			
Total Fat	0.00	%	
Saturated Fat	0.00	%	
Monounsaturated Fat	0.00	%	
cis-cis Polyunsaturated Fat	0.00	%	
trans Fat	0.00	%	

* * * * * * * * * *

Customer ID: G100EL Grade 100 Tamarack Medallion Labs ID: 2000033252
Sample Descr.: Extract from Trees

Test Description	Results	Units	Estimate
Fatty Acid Analysis w/Profile			
Total Fat	0.0100	%	
Saturated Fat	0.00	%	
Monounsaturated Fat	0.00	%	
cis-cis Polyunsaturated Fat	0.00	%	
trans Fat	0.00	%	

* * * * * * * * * *

Customer ID: AC9 Stractan 10 Medallion Labs ID: 2000033253
Sample Descr.: Extract from Trees

Test Description	Results	Units	Estimate
Fatty Acid Analysis w/Profile			
Total Fat	0.0200	%	
Saturated Fat	0.00	%	
Monounsaturated Fat	0.0100	%	
cis-cis Polyunsaturated Fat	0.00	%	
trans Fat	0.00	%	

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Signed [redacted] (b)(6)
For Medallion Laboratories

General Mills

Fatty Acid Profile/Fat Analysis (with cis-cis polyunsaturates)

TO: Medallion Labs

LIMS SAMPLE ID: 2000033251 | Library No.: 2000-04503

CUSTOMER ID: G100WL | Date Analyzed: 6Jun00

Reference #: C060500A0004 | Analyst: Kerri Gustafson

Description: Extract from Trees

	Normalized by Weight	% (w/w) as Triglyceride in Product	% (w/w) Fatty Acids in Product: Saturated Fatty Acids	Monounsaturated Fatty Acids	cis-cis Polyunsaturated Fatty Acids	trans Fatty Acids
4:0 Butyric						
6:0 Caproic						
8:0 Caprylic						
10:0 Capric						
12:0 Lauric						
13:0 Tridecanoic						
14:0 Myristic						
14:1 t-Tetradecenoic						
14:1 Myristoleic						
15:0 Pentadecanoic						
15:1 Pentadecenoic						
16:0 Palmitic						
16:1 t-Hexadecenoic						
16:1 Palmitoleic						
17:0 Margaric						
17:1 Margaroleic						
18:0 Stearic						
18:1 trans Elaidic						
18:1 Oleic	100.000%	0.001		0.001		
18:2 t-Octadecadienoic						
18:2 Linoleic						
20:0 Arachidic						
18:3 g-Linolenic						
20:1 Gadoleic						
18:3 Linolenic						
21:0 Heneicosanoic						
18:2 conj-Linoleic						
18:4 Octadecatetraenoic						
20:2 Eicosadienoic						
22:0 Behenic						
20:3 g-Eicosatrienoic						
22:1 Erucic						
20:3 Eicosatrienoic						
20:4 Arachidonic						
23:0 Tricosanoic						
22:2 Docosadienoic						
24:0 Lignoceric						
20:5 Eicosapentaenoic						
24:1 Nervonic						
22:3 Docosatrienoic						
22:4 Docosatetraenoic						
22:5 Docosapentaenoic						
22:6 Docosahexaenoic						
Totals	100.0%	0.00		0.00		
Percent of Fatty Acid Components based on Total Fat				100.00%		

00 044

Fatty Acid Profile/Fat Analysis (with cis-cis polyunsaturates)

TO: Medallion Labs

IMS SAMPLE ID: 2000033252

Library No.: 2000-04503

CUSTOMER ID: G100EL

Date Analyzed: 6Jun00

Reference #: C060500A0005

Analyst: Kerri Gustafson

Description: Extract from Trees

	Normalized by Weight	% (w/w) as Triglyceride in Product	% (w/w) Fatty Acids in Product: Saturated Fatty Acids	Monounsaturated Fatty Acids	cis-cis Polyunsaturated Fatty Acids	trans Fatty Acids
:0 Butyric						
:0 Caproic						
:0 Caprylic						
0:0 Capric						
2:0 Lauric						
3:0 Tridecanoic						
4:0 Myristic						
4:1 t-Tetradecenoic						
4:1 Myristoleic						
5:0 Pentadecanoic						
5:1 Pentadecenoic						
16:0 Palmitic	20.000%	0.001	0.001			
16:1 t-Hexadecenoic						
16:1 Palmitoleic						
17:0 Margaric						
17:1 Margaroleic						
1[illegible] Stearic						
18:[illegible]ns Elaidic	40.000%	0.002				0.002
18:1 Oleic	20.000%	0.001		0.001		
18:2 t-Octadecadienoic						
18:2 Linoleic	20.000%	0.001			0.001	
20:0 Arachidic						
18:3 g-Linolenic						
20:1 Gadoleic						
18:3 Linolenic						
21:0 Heneicosanoic						
18:2 conj-Linoleic						
18:4 Octadecatetraenoic						
20:2 Eicosadienoic						
22:0 Behenic						
20:3 g-Eicosatrienoic						
22:1 Erucic						
20:3 Eicosatrienoic						
20:4 Arachidonic						
23:0 Tricosanoic						
22:2 Docosadienoic						
24:0 Lignoceric						
20:5 Eicosapentaenoic						
24:1 Nervonic						
22:3 Docosatrienoic						
[illegible] Docosatetraenoic						
[illegible] Docosapentaenoic						
22:6 Docosahexaenoic						
Totals	100.054	0.01	0.00	0.00	0.00	0.00
Percent of Fatty Acid Components based on Total Fat			19.93%	20.02%	20.01%	40.04%

00 045

000276

Fatty Acid Profile/Fat Analysis (with cis-cis polyunsaturates)

TO: Medallion Labs

MS SAMPLE ID: 2000033253
Library No.: 2000-04503
JS[illegible]ER ID: AC9
Date Analyzed: 6Jun00
eference #: C060500A0006
Analyst: Kerri Gustafson
escription: Extract from Trees

	Normalized by Weight	% (w/w) as Triglyceride in Product	% (w/w) Fatty Acids in Product: Saturated Fatty Acids	Monounsaturated Fatty Acids	cis-cis Polyunsaturated Fatty Acids	trans Fatty Acids
0 Butyric						
0 Caproic						
0 Caprylic						
1:0 Capric						
2:0 Lauric						
3:0 Tridecanoic						
4:0 Myristic						
4:1 t-Tetradecenoic						
4:1 Myristoleic						
5:0 Pentadecanoic						
5:1 Pentadecenoic						
6:0 Palmitic	14.286%	0.003	0.003			
6:1 t-Hexadecenoic						
6:1 Palmitoleic	9.524%	0.002		0.002		
7:0 Margaric						
7:1 Margaroleic						
6[illegible]aric	4.762%	0.001	0.001			
8:[illegible]ns Elaidic	4.762%	0.001				0.001
8:1 Oleic	42.857%	0.009		0.009		
8:2 t-Octadecadienoic	4.762%	0.001				0.001
8:2 Linoleic	14.286%	0.003			0.003	
0:0 Arachidic						
8:3 g-Linolenic						
0:1 Gadoleic						
8:3 Linolenic						
1:0 Heneicosanoic						
8:2 conj-Linoleic						
6:4 Octadecatetraenoic						
0:2 Eicosadienoic						
2:0 Behenic	4.762%	0.001	0.001			
0:3 g-Eicosatrienoic						
2:1 Erucic						
0:3 Eicosatrienoic						
0:4 Arachidonic						
3:0 Tricosanoic						
2:2 Docosadienoic						
4:0 Lignoceric						
0:5 Eicosapentaenoic						
4:1 Nervonic						
2:3 Docosatrienoic						
[illegible]Docosatetraenoic						
2[illegible]ocosapentaenoic						
2:6 Docosahexaenoic						
Totals	100.0%	0.02	0.00	0.01	0.00	0.00
Percent of Fatty Acid Components based on Total Fat			23.80%	52.38%	14.29%	9.53%

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FORMULAS:

Table X
Postadded Eastern Larch and Larch AG to Red Ink 42-7315

	LRX 21297-1	-2	-3	-4
42-7315	144.0	132.0	120.0	90.0
Eastern Larch AG solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

	LRX 21297-5	-6	-7	-8
42-7315	144.0	132.0	120.0	90.0
Sample 00184 AG Solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

For formulas 22097-1 through -4, water was added to ink 42-7315 at the same levels as the AG additions above.

	LRX 22097-1	-2	-3	-4
42-7315	144.0	132.0	120.0	90.0
Distilled water	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% water	6% water	10% water	20% water

Table X
Postadded Eastern Larch and Larch AG to Cyan ink BCM 63A

	LRX 21297-9	-10	-11	-12
BCM 63A	144.0	132.0	120.0	90.0
Eastern Larch AG solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

	LRX 21297-13	-14	-15	-16
BCM 63A	144.0	132.0	120.0	90.0
Sample 00184 AG solution	6.0	18.0	30.0	60.0
TOTAL	150.0	150.0	150.0	150.0
	2% AG	6% AG	10% AG	20% AG

000279

Table X
AG Solutions Formulated into Process Cyan Ink

	LRX 22097-5	-6	-7	-9
PCN Blue Paste	78.9	70.0	64.0	64.0
Water	24.5	17.4	23.4	23.4
Lucidene 614	87.6	87.6	87.6	87.6
AG 00184	0.0	16.0	16.0	----
FG 249624201	0.0	----	----	16.0
Liquitron 251	9.0	9.0	9.0	9.0
TOTAL	200.0	200.0	200.0	200.0
% Pigment	12.0	10.5	9.73	9.73

	LRX 31397-1	-2
PCN Blue Paste	66.0	69.0
Water	21.4	21.4
Lucidene 614	87.6	84.6
AG 00184	0.0	0.0
FG 249624201	16.0	16.0
Liquitron 251	9.0	9.0
TOTAL	200.0	200.0
% Pigment	10.04	10.49

	Rice Starch	SD	p	n	8.4 g Larch	SD	p	n	8.4 g Tamarack	SD	p	n	4.5 g Larch	SD	p	n	1.5 g Larch	SD	p
Vitality																			
Base	68.1	12.3		20	65	15.13		20	72.4	15.93		19	65.5	9.72		20	69.3	15	
Month 1	75.8	5.75	0.02	18	65.8	12.94	0.86	19	70.6	13.6	0.70	18	63.3	15.15	0.60	18	68.7	13.3	0.9
Month 2	76.2	7.19	0.02	17	65	14.95	1.00	18	76.3	8.14	0.32	19	63.6	17.97	0.69	18	62.4	21	0.25
Month 3	76.3	12.58	0.06	16	62.4	20.32	0.65	17	74.2	12.39	0.68	19	65.6	14.67	0.98	17	68.8	12.9	0.93
Month 4	77.2	8.94	0.02	16	63.8	18.59	0.83	17	71.4	12.93	0.84	18	63.8	18.03	0.71	16	69.1	18.1	0.98
Month 5	77.9	14.22	0.05	12	66.8	15.3	0.73	17	69.7	24.84	0.70	16	70	13.63	0.26	15	68.2	16	0.85
Month 6	78.5	11.56	0.03	10	65	19.61	1.00	14	74.2	19.05	0.78	12	73.3	8.62	0.03	12	65.5	19.2	0.56
Social Function																			
Base	93.5	10.91		20	96.3	7.14		20	96.1	8..39		19	95	11.03		20	93.8	11.1	
Month 1	97.2	6.85	0.21	18	93.4	12.75	0.40	19	89.6	16.74	0.12	18	92.4	10.62	0.46	18	92.1	16.3	0.71
Month 2	90.4	16.85	0.51	17	96.5	7.18	0.91	18	89.5	18.29	0.14	19	97.2	6.85	0.47	18	92.6	15.4	0.8
Month 3	89.8	20.01	0.49	16	91.9	16.5	0.29	17	91.4	18.66	0.31	19	97.1	8.3	0.53	17	95.6	7.58	0.57
Month 4	92.2	19.3	0.80	16	97.1	7.03	0.73	17	99.3	3.03	0.15	18	88.3	25.19	0.29	16	98.5	4.15	0.1
Month 5	97.9	7.22	0.22	12	91.2	15.13	0.19	17	97.7	6.8	0.54	16	93.3	10.42	0.65	15	95.5	13.5	0.68
Month 6	88.8	19.94	0.40	10	93.8	13.65	0.49	14	94.8	9.91	0.79	12	90.6	18.56	0.41	12	91.3	18.7	0.65
Role Emotion																			
Base	95.2	21.82		20	98.3	7.45		20	89.5	27.34		19	85	33.29		20	96.7	10.3	
Month 1	98.1	7.86	0.60	18	93	23.78	0.34	19	96.3	10.78	0.30	18	90.7	25.06	0.56	18	93	21	0.49
Month 2	100	0	0.38	17	98.1	7.86	0.94	18	98.2	7.65	0.16	19	98.1	7.86	0.11	18	94.1	17.6	0.59
Month 3	95.8	11.39	0.92	16	96.1	11.07	0.47	17	94.4	23.57	0.54	19	93.8	25	0.39	17	96.1	11.1	0.87
Month 4	91.7	25.82	0.65	16	94.1	17.62	0.34	17	96.3	15.71	0.36	18	97.9	8.33	0.14	16	98	8.08	0.66
Month 5	97.2	9.62	0.77	12	96.1	16.17	0.58	17	100	0	0.13	16	100	0	0.09	15	97.6	8.91	0.78
Month 6	96.7	10.54	0.85	10	100	0	0.41	14	91.7	28.87	0.83	12	94.4	19.25	0.38	12	93.3	14.1	0.47
Mental Health																			
Base	80	9.63		20	80.4	8.09		20	82.5	10.17		19	77.6	13		20	81.6	12.7	
Month 1	86	7.67	0.04	18	80.8	9.85	0.88	19	82.3	7.2	0.95	18	75.1	16.67	0.61	18	81.5	12.4	0.98
Month 2	84.2	7.68	0.15	17	80.2	10.31	0.95	18	81.7	13.35	0.82	19	77.1	11.3	0.90	18	81.9	11.8	0.95
Month 3	81.8	9.12	0.58	16	80.5	11.39	0.98	17	82.7	10.67	0.95	19	79.1	9.85	0.71	17	87.3	6.04	0.1
Month 4	84.5	6	0.11	16	78.1	12.18	0.50	17	85.3	7.39	0.35	18	75.5	13.3	0.64	16	84.5	8.82	0.43
Month 5	85	9.96	0.17	12	81.6	7.88	0.64	17	87.5	5.03	0.09	16	76.3	16.73	0.79	15	84.9	11.1	0.45
Month 6	83.2	11.59	0.43	10	82.3	10.37	0.56	14	86.3	8.44	0.29	12	83.7	10.44	0.18	12	82	10.2	0.93

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SF-36 Parameter	"M"				"P"				"H"				"K"				"L"		
	Rice Starch	SD	p	n	8.4 g Larch	SD	p	n	8.4 g Tamarack	SD	p	n	4.5 g Larch	SD	p	n	1.5 g Larch	SD	p
Physical Function																			
Base	87.9	26.91		20	92.5	19.7		20	93.2	18.2		19	94.5	14.5		20	98	3.77	
Month 1	87.8	31.31	0.99	18	92.6	21.43	0.98	19	98.1	3.49	0.24	18	96.7	6.64	0.57	18	89.7	25.1	0.15
Month 2	93.8	19.65	0.45	17	91.4	22.87	0.87	18	97.6	7.52	0.30	19	94.2	14.68	0.94	18	86.5	29	0.09
Month 3	92.5	24.97	0.60	16	96.8	9.67	0.42	17	98.7	3.67	0.18	19	96.8	8.47	0.58	17	90.9	24.6	0.21
Month 4	92.5	25.17	0.60	16	91.2	24.21	0.86	17	99.4	1.62	0.15	18	93.4	13.51	0.82	16	92.4	20.9	0.24
Month 5	91.7	28.87	0.71	12	91.5	23.1	0.88	17	99.7	1.25	0.16	16	97	7.97	0.55	15	87.1	28.6	0.1
Month 6	87	31.99	0.94	10	90.4	22.83	0.77	14	99.6	1.44	0.24	12	96.7	7.49	0.64	12	99.5	1.58	0.24
Role Physical																			
Base	89.3	28.03		20	92.5	23.08		20	100	0		19	91.3	24.7		20	87.5	31.9	
Month 1	95.8	17.68	0.40	18	92.1	20.5	0.96	19	98.6	5.89	0.29	18	94.4	23.57	0.69	18	88.2	31.6	0.95
Month 2	94.1	16.61	0.54	17	97.2	8.08	0.42	18	93.4	23.34	0.20	19	100	0	0.14	18	86.8	29.5	0.94
Month 3	98.4	6.25	0.21	16	89.7	29.39	0.75	17	93.1	20.66	0.13	19	94.1	24.25	0.73	17	97.1	8.3	0.24
Month 4	93.8	25	0.62	16	100	0	0.19	17	100	0		18	81.3	37.08	0.34	16	92.6	21.2	0.57
Month 5	97.9	7.22	0.31	12	94.1	24.25	0.84	17	95.3	18.75	0.28	16	96.7	12.91	0.45	15	91.1	27.1	0.74
Month 6	100	0	0.24	10	92.9	26.73	0.97	14	93.8	21.65	0.21	12	95.8	14.43	0.56	12	100	0	0.23
Bodily Pain																			
Base	87.5	17.93		20	86.2	13.22		20	90.1	13.12		19	87.1	18		20	80	19.3	
Month 1	89.4	13.08	0.71	18	86.8	15.89	0.89	19	81.9	18.28	0.11	18	88.2	10.37	0.82	18	87.1	20.3	0.27
Month 2	86.8	14.44	0.89	17	87.7	15.09	0.74	18	89.1	10.92	0.79	19	89.6	9.01	0.61	18	84.6	18.5	0.47
Month 3	87.8	18.5	0.96	16	84.7	19.91	0.79	17	90.9	9.85	0.82	19	89.4	11.74	0.66	17	84.4	14.8	0.45
Month 4	87.1	19.54	0.95	16	83.8	15.54	0.61	17	93.8	8.03	0.31	18	81.1	19.38	0.34	16	88.7	11.2	0.11
Month 5	90.2	10.97	0.65	12	88.2	17.53	0.69	17	91.6	10.39	0.70	16	84.6	17.62	0.68	15	78.1	21	0.78
Month 6	86.6	11.47	0.88	10	77.4	24.68	0.19	14	90.2	10.97	0.98	12	92	8.36	0.38	12	87	12.1	0.31
General Health																			
Base	83.3	13.07		20	79.6	14.26		20	88.5	10.68		19	88.8	9.66		20	86.1	11.7	
Month 1	84.8	10.87	0.70	18	79.8	11.32	0.94	19	84.3	10.34	0.21	18	82.2	9.94	0.04	18	86.5	11.4	0.92
Month 2	85.1	15.82	0.71	17	80	14.18	0.92	18	86.7	12.74	0.61	19	87.3	9.84	0.65	18	82.4	14.3	0.39
Month 3	88.8	10.91	0.18	16	81.5	14.6	0.69	17	84.6	14.43	0.32	19	87.1	9.2	0.58	17	84.2	14.7	0.67
Month 4	85.3	11.77	0.65	16	79.9	15.03	0.95	17	88	9.99	0.88	18	78.6	15.48	0.02	16	86.5	15	0.92
Month 5	89	13.23	0.24	12	83.2	15.49	0.46	17	88.8	9.68	0.95	16	85.4	12.75	0.38	15	84.7	14.6	0.76
Month 6	86.7	10.95	0.49	10	83.2	14.52	0.47	14	91.5	8.53	0.43	12	88	13.54	0.85	12	80.4	17.3	0.29

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0002282

Celsis Laboratory Group

REPORT OF ANALYSIS

ATTN: Mr. Bryan Rodriguez
Larex, Inc.
2852 Patton Road

Roseville, MN 55113

ID#: 830585

Report Date: 08/31/1999
Received : 07/21/1999
Page : 1 of 1

SAMPLE: Grade 3000

LOT# : None
P.O.# : 5572

TEST : Acute Oral Toxicity
METHOD: FDA-GLP
CLAIM : N/A
LIMIT : Report Results
RESULT: Date Assayed: 08/18/1999

See Attached Report.

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Celsis Laboratory Group

by: [redacted] (b)(6) [redacted]

Unless alternate arrangements have been made, samples will be retained for 30 days and raw data for 7 years after report date.

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Celsis Laboratory Group

GR

New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

Telephone
732 • 346 • 5100

Fax
732 • 346 • 5115

website
www.celsislabs.com

email
clg@celsis.com

STUDY TITLE

Acute Oral Toxicity Study

Larex, Inc.
Grade 3000

DATA REQUIREMENTS

21 CFR 58

IN VIVO STUDY COMPLETED ON

August 18, 1999

PERFORMING LABORATORY

Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

LABORATORY PROJECT ID

Assay # 830585

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Celsis Laboratory Group

830585

GOOD LABORATORY PRACTICE STATEMENT

This study was performed in conformance with the Good Laboratory Practice Standards as outlined in the requirements of 21 CFR Part 58.

Sample: Larex, Inc.
Grade 3000

Sponsor: Larex, Inc.
2852 Patton Road
Roseville, MN 55113

Testing Laboratory: Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

(b)(6)

DATE 8/6/15

Martin R. Gilman, Ph.D.
Study Director

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Celsis Laboratory Group

830585

QUALITY ASSURANCE UNIT STATEMENT:

Inspections of study events are listed below. The study operations, records and the final report were reviewed by the Quality Assurance Unit.

Inspections	Dates of Inspections	Date Reported Management	Date Reported Study Director
Protocol Review	8-2-99	8-13-99	8-13-99
Dosing	8-4-99	8-13-99	8-13-99
Raw Data Review	8-24-99	8-24-99	8-27-99
Draft Report Review	8-25-99	8-27-99	8-27-99
Final Report Review	8-27-99	827-99	8-27-99

No deviations from Good Laboratory Practices were found. The final report accurately describes the methods and procedures and accurately reflects the raw data.

(b)(6) DATE 8-27-99

Quality Assurance Auditor

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I. SUMMARY:

Five male and five female Spraque Dawley derived rats were administered Larex, Inc., sample Grade 3000, at a dose level of 5 g/kg body weight. There were no mortalities during the 14 day observation period. The LD_{50} is greater than 5 g/kg. (See section V).

II. INTRODUCTION:

1.0 TITLE: ACUTE ORAL TOXICITY STUDY, 21 CFR 58

2.0 PURPOSE: To determine the potential toxicity and/or toxic effects of a test substance by a single oral dosing in rats. Data from this study may be used to support applications for research or marketing permits for products regulated by the Food and Drug Administration, and to establish dosage requirements for subsequent toxicity testing.

3.0 SPONSOR: Larex, Inc.
2852 Patton Road
Roseville, MN 55113

4.0 TESTING FACILITY: Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

5.0 STUDY DIRECTOR: Martin R. Gilman, Ph. D.

6.0 SCHEDULE: Start: August 4, 1999
End: August 18, 1999

III. MATERIAL AND METHODS:

1.0 TEST ANIMALS:

1.1 Species: *Rattus norvegicus*

1.2 Strain: Sprague Dawley derived

1.3 Sex: Male and female (females nulliparous and non-pregnant).

1.4 Weight Range (at initiation): Male: 200 - 300 grams
Female: 200- 300 grams

1.5 Number/Sex/Dose Level: 5 male, 5 female

1.6 Supplier: Ace Animals, Inc.
Boyertown, Pa
USDA #: 23-B-009

1.7 Identification: Animals placed on test were identified with ear tags and cage labels which identified the animal and project number.

1.8 Husbandry: During the acclimation and testing periods, each animal was housed and maintained according to "The Guide For The Care and Use of Laboratory Animals (NIH 86-23)".

1.8.1 Diet: Agway Prolab 3000 rodent feed; food and city water were available ad libitum.

1.8.2 Housing: Animals were either single or double housed in suspended stainless steel wire-mesh cages in a room controlled for temperature.

1.8.3 Acclimation: Animals were randomly assigned to caging on arrival and were acclimated to the testing facility at least 7 days prior to the start of testing. Animals were observed for general health and suitability for testing during this period.

2.0 TEST MATERIAL:

2.1 Identification: Larex, Inc., sample Grade 3000, white powder.

2.2 Storage: The test material was stored at room temperature in the container in which it was received.

2.3 Reserve Sample: Remaining test material will be returned to the sponsor upon acceptance of the final report

2.4 Test Material Formulation: Supplied as a white powder.

3.0 EXPERIMENTAL DESIGN:

3.1 Animal Requirements:

3.1.1 Number of Animals/Sex/Dose Level: 5 male, 5 female

3.1.3 Number of Animals/Group: 10

3.2 Assignment to Dose Groups: Animals placed on test were randomly assigned to dose groups. Only rats with body weight within +/- 20% of the mean body weight of rats of the same age, strain, and sex were used.

3.3 Route of Administration: The test material was administered by syringe and suitable intubation tube.

3.4 Dose Level: 5 g/kg body weight.

3.5 Frequency: Once

3.6 Test Duration: 14 days.

4.0 DOSING PROCEDURE:

4.1 Animal Preparation: The rats were fasted overnight between 18 and 24 hours prior to dosing.

4.2 Sample Preparation: The test material was dosed as a 1:2 w:v concentration in deionized water.

4.3 Treatment: The test material was measured by syringe and dosed via syringe and intubation tube. The dose volume was calculated using the following equation: Dose Volume (ml) = [Animal Weight (kg) x Limit Dose (5 g/kg)] divided by the Test Material Density (g/ml).

5.0 IN LIFE OBSERVATION:

5.1 Body Weight: Body weight was recorded in grams for each animal just prior to administration of test substance and weekly thereafter. Body weights were also recorded prior to schedule sacrifices.

5.2 Signs of Toxicity and Mortality: All test animals were observed for signs of toxicity and mortality 4 hours after dosing and then twice daily seven days a week after test material administration. Test animals were observed for a total of 14 days after dosing.

Observations included the following: circulatory, respiratory, autonomic and central nervous systems, somatomotor activity, behavior patterns, onset of tremors, convulsions, salivation, lethargy, sleep and coma, skin and fur, and eyes and mucous membranes.

5.3 Post-Mortem Observations: A gross necropsy was performed on all surviving test animals 14 days after dosing. The animals were sacrificed with CO_2.

5.4 Tissue Preservation: Tissue samples for possible future histological examination were taken from each of the test animals. Tissues taken and preserved in 10% formalin were the lungs, both kidneys and representative sections of the liver.

6.0 QUALITY ASSURANCE UNIT AUDITS: A Quality Assurance Unit statement is attached with this report.

7.0 RECORD RETENTION: All study records, raw-data, specimens, study protocols, final reports, protocol and report revisions and any correspondence concerning the conduct of the study are maintained at the testing facility for five years.

8.0 PERSONNEL:

8.1 Toxicology Department:

M. Gilman	Study Director, Director Toxicology
J. Barbera	Associate Director, Toxicology

8.2 Quality Assurance Unit:

A. Perewiznyk	Director, Quality Assurance
R.H. Gensler	Auditor
R. Taylor	Auditor

IV. RESULTS:

1.0 MORTALITY:

Test Group: Ten of ten test animals survived the 14 day observation period.

2.0 OBSERVATIONS: All animals appeared normal for the duration of the study period.

3.0 BODY WEIGHT: All animals had a weight gain on days seven and fourteen of the study. (See Table 1).

4.0 GROSS PATHOLOGY: No abnormalities were observed at necropsy.

5.0 STATISTICAL EVALUATION: Statistical evaluation of the study data was not considered necessary.

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TABLE II

GROSS NECROPSY

No.	Ear tag #	Sex	Observation
1	10433	F	NGA
2	10434	F	NGA
3	10435	F	NGA
4	10436	F	NGA
5	10437	F	NGA
6	10438	M	NGA
7	10439	M	NGA
8	10440	M	NGA
9	10441	M	NGA
10	10442	M	NGA

NGA = No Gross Abnormalities

830585

TABLE I

BODY WEIGHT DATA

TEST GROUP

No.	Eartag #	Sex	Day 0 Initial Weight (g)	Day 7 Intermediate Weight (g)	Day 14 Final Weight (g)	Change in Weight (g)
1	10433	F	241	264	267	+26
2	10434	F	263	284	282	+19
3	10435	F	218	249	242	+24
4	10436	F	236	277	273	+37
5	10437	F	260	295	293	+33
6	10438	M	257	328	357	+ 100
7	10439	M	248	311	331	+83
8	10440	M	282	366	417	+135
9	10441	M	254	329	367	+113
10	10442	M	257	327	361	+104

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VI. TABLE/FIGURES:

Table I. Body Weight Data

Table II. Gross Necrospy

V. DISCUSSION: Five male and five female Spraque Dawley derived rats were administered Larex, Inc., sample Grade 3000, at a dose level of 5 g/kg body weight.

Four of the five animals had a slight weight loss between day 7 and 14; all animals gained weight on day 14 as compared with the initial weight . There were no mortalities during the 14 day observation period. The LD_{50} is greater than 5 g/kg according to the definitions in 16 CFR 1500.3 (c) (2) (i).

V. SIGNATURE(S):

Work Performed Under the Supervision of:

Celsis Laboratory Group

(b)(6)

Martin R. Gilman, Ph. D.
Study Director

8/31/99
Date

Celsis Laboratory Group

REPORT OF ANALYSIS

ATTN: Mr. Bryan Rodriguez
Larex, Inc.
2852 Patton Road
Roseville, MN 55113

ID#: 830586

Report Date: 08/27/1999
Received : 07/21/1999
Page : 1 of 1

SAMPLE: Grade 4000

LOT# : None
P.O.# : 5572

TEST : Acute Oral Toxicity
METHOD: FDA-GLP
CLAIM : N/A
LIMIT : Report Results
RESULT: Date Assayed: 08/04/1999

See Attached Report

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Celsis Laboratory Group
by: (b)(6)

Unless alternate arrangements have been made, samples will be retained for 30 days and raw data for 7 years after report date.

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Celsis Laboratory Group

New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

Telephone
732 • 346 • 5100

Fax
732 • 346 • 5115

website
www.celsislabs.com

email
clg@celsis.com

STUDY TITLE

Acute Oral Toxicity Study

Larex, Inc.
Grade 4000
DATA REQUIREMENTS

21 CFR 58

IN VIVO STUDY COMPLETED ON

August 18, 1999

PERFORMING LABORATORY

Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

LABORATORY PROJECT ID

Assay # 830586

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Celsis Laboratory Group

830586

GOOD LABORATORY PRACTICE STATEMENT

This study was performed in conformance with the Good Laboratory Practice Standards as outlined in the requirements of 21 CFR Part 58.

Sample: Larex, Inc.
Grade 4000

Sponsor: Larex, Inc.
2852 Patton Road
Roseville, MN 55113

Testing Laboratory: Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

(b)(6) DATE 8/27/97

Martin R. Gilman, Ph.D.
Study Director

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Celsis Laboratory Group

830586

QUALITY ASSURANCE UNIT STATEMENT:

Inspections of study events are listed below. The study operations, records and the final report were reviewed by the Quality Assurance Unit.

Inspections	Dates of Inspections	Date Reported Management	Date Reported Study Director
Protocol Review	8-2-99	8-13-99	8-13-99
Dosing	8-4-99	8-13-99	8-13-99
Raw Data Review	8-25-99	8-27-99	8-27-99
Draft Report Review	8-25-99	8-27-99	8-27-99
Final Report Review	8-26-99	8-27-99	8-27-99

No deviations from Good Laboratory Practices were found. The final report accurately describes the methods and procedures and accurately reflects the raw data.

(b)(6) DATE 8-27-99

Quality Assurance Auditor

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I. SUMMARY:

Five male and five female Spraque Dawley derived rats were administered Larex, Inc., sample Grade 4000, at a dose level of 5 g/kg body weight. There were no mortalities during the 14 day observation period. The LD_{50} is greater than 5 g/kg. (See section V).

II. INTRODUCTION:

1.0 TITLE: ACUTE ORAL TOXICITY STUDY, 21 CFR 58

2.0 PURPOSE: To determine the potential toxicity and/or toxic effects of a test substance by a single oral dosing in rats. Data from this study may be used to support applications for research or marketing permits for products regulated by the Food and Drug Administration, and to establish dosage requirements for subsequent toxicity testing.

3.0 SPONSOR: Larex, Inc.
2852 Patton Road
Roseville, MN 55113

4.0 TESTING FACILITY: Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

5.0 STUDY DIRECTOR: Martin R. Gilman, Ph. D.

6.0 SCHEDULE: Start: August 4, 1999
End: August 18, 1999

III. MATERIAL AND METHODS:

1.0 TEST ANIMALS:

1.1 Species: *Rattus norvegicus*

1.2 Strain: Sprague Dawley derived

1.3 Sex: Male and female (females nulliparous and non-pregnant).

1.4 Weight Range (at initiation): Male; 200 - 300 grams
Female: 200- 300 grams

1.5 Number/Sex/Dose Level: 5 male, 5 female

1.6 Supplier: Ace Animals, Inc.
Boyertown, Pa
USDA #: 23-B-009

1.7 Identification: Animal placed on test were identified with ear tags and cage labels which identified the animal and project number.

1.8 Husbandry: During the acclimation and testing periods, each animal was housed and maintained according to "The Guide For The Care and Use of Laboratory Animals (NIH 86-23)".

1.8.1 Diet: Agway Prolab 3000 rodent feed; food and city water were available ad libitum.

1.8.2 Housing: Animals were either single or double housed in suspended stainless steel wire-mesh cages in a room controlled for temperature.

1.8.3 Acclimation: Animals were randomly assigned to caging on arrival and were acclimated to the testing facility at least 7 days prior to the start of testing. Animals were observed for general health and suitability for testing during this period.

2.0 TEST MATERIAL:

2.1 Identification: Larex, Inc., sample Grade 4000, white powder.

2.2 Storage: The test material was stored at room temperature in the container in which it was received.

2.3 Reserve Sample: Remaining test material was retained by Celsis Laboratory Group.

2.4 Test Material Formulation: Supplied as an off- white powder.

3.0 EXPERIMENTAL DESIGN:

3.1 Animal Requirements:

3.1.1 Number of Animals/Sex/Dose Level: 5 male, 5 female

3.1.2 Number of Groups: 1

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3.1.3 Number of Animals/Group: 10

3.2 Assignment to Dose Groups: Animals placed on test were randomly assigned to dose groups. Only rats with body weight within +/- 20% of the mean body weight of rats of the same age, strain, and sex were used.

3.3 Route of Administration: The test material was administered by syringe and suitable intubation tube.

3.4 Dose Level: 5 g/kg body weight.

3.5 Frequency: Once

3.6 Test Duration: 14 days.

4.0 DOSING PROCEDURE:

4.1 Animal Preparation: The rats were fasted overnight between 18 and 24 hours prior to dosing.

4.2 Sample Preparation: The test material was dosed as a 1:2 w:v concentration in deionized water.

4.3 Treatment: The test material was measured by syringe and dosed via syringe and intubation tube. The dose volume was calculated using the following equation: Dose Volume (ml) = [Animal Weight (kg) x Limit Dose (5 g/kg)] divided by the Test Material Density (g/ml).

5.0 IN LIFE OBSERVATION:

5.1 Body Weight: Body weight was recorded in grams for each animal just prior to administration of test substance and weekly thereafter. Body weights were also recorded prior to schedule sacrifices.

5.2 Signs of Toxicity and Mortality: All test animals were observed for signs of toxicity and mortality 4 hours after dosing and then twice daily seven days a week after test material administration. Test animals were observed for a total of 14 days after dosing.

Observations included the following: circulatory, respiratory, autonomic and central nervous systems, somatomotor activity, behavior patterns, onset of tremors, convulsions, salivation, lethargy, sleep and coma, skin and fur, and eyes and mucous membranes.

5.3 Post-Mortem Observations: A gross necropsy was performed on all surviving test animals 14 days after dosing. The animals were sacrificed with CO_2.

5.4 Tissue Preservation: Tissue samples for possible future histological examination were taken from each of the test animals. Tissues taken and preserved in 10% formalin were the lungs, both kidneys and representative sections of the liver.

6.0 QUALITY ASSURANCE UNIT AUDITS: A Quality Assurance Unit statement is attached with this report.

7.0 RECORD RETENTION: All study records, raw-data, specimens, study protocols, final reports, protocol and report revisions and any correspondence concerning the conduct of the study are maintained at the testing facility for five years.

8.0 PERSONNEL:

8.1 Toxicology Department:

M. Gilman	Study Director, Director Toxicology
J. Barbera	Associate Director, Toxicology

8.2 Quality Assurance Unit:

A. Perewiznyk	Director, Quality Assurance
R.H. Gensler	Auditor
R. Taylor	Auditor

IV. RESULTS:

1.0 MORTALITY:

Test Group: Ten of ten test animals survived the 14 day observation period.

2.0 OBSERVATIONS: All animals appeared normal for the duration of the study period.

3.0 BODY WEIGHT: All animals had a weight gain on days seven and fourteen of the study. (See Table 1).

4.0 GROSS PATHOLOGY: No abnormalities were observed at necropsy.

5.0 STATISTICAL EVALUATION: Statistical evaluation of the study data was not considered necessary.

V. DISCUSSION: Five male and five female Spraque Dawley derived rats were administered Larex, Inc., sample Grade 4000, at a dose level of 5 g/kg body weight.

There were no mortalities during the 14 day observation period. The LD_{50} is greater than 5 g/kg according to the definitions in 16 CFR 1500.3 (c) (2) (i).

V. SIGNATURE(S):

Work Performed Under the Supervision of:

Celsis Laboratory Group

(b)(6)

Martin R. Gilman, Ph. D.
Study Director

8/27/99
Date

830586

VI. TABLE/FIGURES:

Table I. Body Weight Data

Table II. Gross Necrospy

Celsis Laboratory Group

830586

TABLE I

BODY WEIGHT DATA

TEST GROUP

No.	Eartag #	Sex	Day 0 Initial Weight (g)	Day 7 Intermediate Weight (g)	Day 14 Final Weight (g)	Change in Weight (g)
1	10443	F	214	229	248	+34
2	10444	F	254	274	284	+30
3	10445	F	230	259	272	+42
4	10446	F	241	271	279	+38
5	10447	F	240	255	264	+24
6	10448	M	266	350	400	+134
7	10449	M	261	329	354	+93
8	10450	M	252	313	345	+93
9	10451	M	255	323	353	+98
10	10452	M	260	335	368	+108

TABLE II

GROSS NECROPSY

No.	Ear tag #	Sex	Observation
1	10443	F	NGA
2	10444	F	NGA
3	10445	F	NGA
4	10446	F	NGA
5	10447	F	NGA
6	10448	M	NGA
7	10449	M	NGA
8	10450	M	NGA
9	10451	M	NGA
10	10452	M	NGA

NGA = No Gross Abnormalities

Celsis Laboratory Group

REPORT OF ANALYSIS

ATTN: Mr. Bryan Rodriguez
Larex, Inc.
2852 Patton Road

Roseville, MN 55113

ID#: 830583

Report Date: 08/27/1999
Received : 07/21/1999
Page : 1 of 1

SAMPLE: Grade TAM

LOT# : None
P.O.# : 5572

TEST : Acute Oral Toxicity
METHOD: FDA-GLP
CLAIM : N/A
LIMIT : Report Results
RESULT: Date Assayed: 08/05/1999

See Attached Report

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Celsis Laboratory Group

by: [redacted] (b)(6) [redacted]

Unless alternate arrangements have been made, samples will be retained for 30 days and raw data for 7 years after report date.

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Celsis Laboratory Group

New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

Telephone
732 • 346 • 5100

Fax
732 • 346 • 5115

website
www.celsislabs.com

email
clg@celsis.com

STUDY TITLE

Acute Oral Toxicity Study

Larex, Inc.
Grade TAM
DATA REQUIREMENTS

21 CFR 58

IN VIVO STUDY COMPLETED ON

August 19, 1999

PERFORMING LABORATORY

Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

LABORATORY PROJECT ID

Assay # 830583

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Celsis Laboratory Group

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VI. Signature (s) 9

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Celsis Laboratory Group

830583

GOOD LABORATORY PRACTICE STATEMENT

This study was performed in conformance with the Good Laboratory Practice Standards as outlined in the requirements of 21 CFR Part 58.

Sample: Larex, Inc.
Grade TAM

Sponsor: Larex, Inc.
2852 Patton Road
Roseville, MN 55113

Testing Laboratory: Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

(b)(6) DATE 8/27/97

Martin R. Gilman, Ph.D.
Study Director

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000312

00 081

Celsis Laboratory Group

830583

QUALITY ASSURANCE UNIT STATEMENT:

Inspections of study events are listed below. The study operations, records and the final report were reviewed by the Quality Assurance Unit.

Inspections	Dates of Inspections	Date Reported Management	Date Reported Study Director
Protocol Review	8-2-99	8-13-99	8-13-99
7 Day Body Weight	8-12-99	8-13-99	8-13-99
Raw Data Review	8-25-99	8-27-99	8-27-99
Draft Report Review	8-25-99	8-27-99	8-27-99
Final Report Review	8-26-99	8-27-99	8-27-99

No deviations from Good Laboratory Practices were found. The final report accurately describes the methods and procedures and accurately reflects the raw data.

(b)(6) _____ DATE 8-27-99

Quality Assurance Auditor

000313

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Celsis Laboratory Group

830583

I. SUMMARY:

Five male and five female Spraque Dawley derived rats were administered Larex, Inc., sample Grade TAM, at a dose level of 5 g/kg body weight. There were no mortalities during the 14 day observation period. The LD_{50} is greater than 5 g/kg. (See section V).

II. INTRODUCTION:

1.0 TITLE: ACUTE ORAL TOXICITY STUDY, 21 CFR 58

2.0 PURPOSE: To determine the potential toxicity and/or toxic effects of a test substance by a single oral dosing in rats. Data from this study may be used to support applications for research or marketing permits for products regulated by the Food and Drug Administration, and to establish dosage requirements for subsequent toxicity testing.

3.0 SPONSOR: Larex, Inc,
2852 Patton Road
Roseville, MN 55113

4.0 TESTING FACILITY: Celsis Laboratory Group
New Jersey Division
165 Fieldcrest Avenue
Edison, NJ 08837

5.0 STUDY DIRECTOR: Martin R. Gilman, Ph. D.

6.0 SCHEDULE: Start: August 5, 1999
End: August 19, 1999

III. MATERIAL AND METHODS:

1.0 TEST ANIMALS:

1.1 Species: *Rattus norvegicus*

1.2 Strain: Sprague Dawley derived

1.3 Sex: Male and female (females nulliparous and non-pregnant).

1.4 Weight Range (at initiation): Male: 200 - 300 grams
Female: 200- 300 grams

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Celsis Laboratory Group

830583

1.5 Number/Sex/Dose Level: 5 male, 5 female

1.6 Supplier: Ace Animals, Inc.
Boyertown, Pa
USDA #: 23-B-009

1.7 Identification: Animal placed on test were identified with ear tags and cage labels which identified the animal and project number.

1.8 Husbandry: During the acclimation and testing periods, each animal was housed and maintained according to "The Guide For The Care and Use of Laboratory Animals (NIH 86-23)".

1.8.1 Diet: Agway Prolab 3000 rodent feed; food and city water were available ad libitum.

1.8.2 Housing: Animals were either single or double housed in suspended stainless steel wire-mesh cages in a room controlled for temperature.

1.8.3 Acclimation: Animals were randomly assigned to caging on arrival and were acclimated to the testing facility at least 7 days prior to the start of testing. Animals were observed for general health and suitability for testing during this period.

2.0 TEST MATERIAL:

2.1 Identification: Larex, Inc., sample Grade TAM, white powder.

2.2 Storage: The test material was stored at room temperature in the container in which it was received.

2.3 Reserve Sample: Remaining test material was retained by Celsis Laboratory Group.

2.4 Test Material Formulation: Supplied as a white powder.

3.0 EXPERIMENTAL DESIGN:

3.1 Animal Requirements:

000315

3.1.1 Number of Animals/Sex/Dose Level: 5 male, 5 female

000315

3.1.2 Number of Groups: 1

00 084

3.1.3 Number of Animals/Group: 10

3.2 Assignment to Dose Groups: Animals placed on test were randomly assigned to dose groups. Only rats with body weight within +/- 20% of the mean body weight of rats of the same age, strain, and sex were used.

3.3 Route of Administration: The test material was administered by syringe and suitable intubation tube.

3.4 Dose Level: 5 g/kg body weight.

3.5 Frequency: Once.

3.6 Test Duration: 14 days.

4.0 DOSING PROCEDURE:

4.1 Animal Preparation: The rats were fasted overnight between 18 and 24 hours prior to dosing.

4.2 Sample Preparation: The test material was dosed as a 1:2 w:v concentration in deionized water.

4.3 Treatment: The test material was measured by syringe and dosed via syringe and intubation tube. The dose volume was calculated using the following equation: Dose Volume (ml) = [Animal Weight (kg) x Limit Dose (5 g/kg)] divided by the Test Material Density (g/ml).

5.0 IN LIFE OBSERVATION:

5.1 Body Weight: Body weight was recorded in grams for each animal just prior to administration of test substance and weekly thereafter. Body weights were also recorded prior to schedule sacrifices.

5.2 Signs of Toxicity and Mortality: All test animals were observed for signs of toxicity and mortality 4 hours after dosing and then twice daily seven days a week after test material administration. Test animals were observed for a total of 14 days after dosing.

Observations included the following: circulatory, respiratory, autonomic and central nervous systems, somatomotor activity, behavior patterns, onset of tremors, convulsions, salivation, lethargy, sleep and coma, skin and fur, and eyes and mucous membranes.

5.3 Post-Mortem Observations: A gross necropsy was performed on all surviving test animals 14 days after dosing. The animals were sacrificed with CO_2.

5.4 Tissue Preservation: Tissue samples for possible future histological examination were taken from each of the test animals. Tissues taken and preserved in 10% formalin were the lungs, both kidneys and representative sections of the liver.

6.0 QUALITY ASSURANCE UNIT AUDITS: A Quality Assurance Unit statement is attached with this report.

7.0 RECORD RETENTION: All study records, raw-data, specimens, study protocols, final reports, protocol and report revisions and any correspondence concerning the conduct of the study are maintained at the testing facility for five years.

8.0 PERSONNEL:

8.1 Toxicology Department:

M. Gilman	Study Director, Director Toxicology
J. Barbera	Associate Director, Toxicology

8.2 Quality Assurance Unit:

A. Perewiznyk	Director, Quality Assurance
R.H. Gensler	Auditor
R. Taylor	Auditor

IV. RESULTS:

1.0 MORTALITY:

Test Group: Ten of ten test animals survived the 14 day observation period.

2.0 OBSERVATIONS: All animals appeared normal for the duration of the study period.

3.0 BODY WEIGHT: All animals had a weight gain on days seven and fourteen of the study. (See Table 1).

000317

4.0 GROSS PATHOLOGY: No abnormalities were observed at necropsy.

5.0 STATISTICAL EVALUATION: Statistical evaluation of the study data was not considered necessary.

Celsis Laboratory Group

830583

V. DISCUSSION: Five male and five female Spraque Dawley derived rats were administered Larex, Inc., sample Grade TAM, at a dose level of 5 g/kg body weight.

There were no mortalities during the 14 day observation period. The LD_{50} is greater than 5 g/kg according to the definitions in 16 CFR 1500.3 (c) (2) (i).

V. SIGNATURE(S):

Work Performed Under the Supervision of:

(b)(6)

Martin R. Gilman, Ph. D.
Study Director

Celsis Laboratory Group

8/27/98
Date

000318

000318

00 087

Celsis Laboratory Group

830583

VI. TABLE/FIGURES:

Table I. Body Weight Data

Table II. Gross Necrospy

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TABLE I

BODY WEIGHT DATA

TEST GROUP

No.	Eartag #	Sex	Day 0 Initial Weight (g)	Day 7 Intermediate Weight (g)	Day 14 Final Weight (g)	Change in Weight (g)
1	10453	F	223	268	260	+37
2	10454	F	224	260	275	+51
3	10455	F	230	258	262	+32
4	10456	F	232	264	278	+46
5	10457	F	248	289	287	+39
6	10458	M	280	353	397	+117
7	10459	M	272	340	374	+102
8	10460	M	268	341	373	+105
9	10461	M	268	351	396	+128
10	10462	M	279	359	399	+120

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00 089

Celsis Laboratory Group

830583

TABLE II

GROSS NECROPSY

No.	Ear tag #	Sex	Observation
1	10453	F	NGA
2	10454	F	NGA
3	10455	F	NGA
4	10456	F	NGA
5	10457	F	NGA
6	10458	M	NGA
7	10459	M	NGA
8	10460	M	NGA
9	10461	M	NGA
10	10462	M	NGA

NGA = No Gross Abnormalities

000321

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00 091

WEST COAST ANALYTICAL SERVICE, INC.
Analytical Chemists
9840 Alburtis Avenue Santa Fe Springs, CA 90670
562-948-2225 Fax 562-948-5850
http://www.wcaslab.com

FAX REPORT

Date: Friday, April 21, 2000

Fax No: 651.426.8587

Company: Larex

Location: St Paul

Attn:

REFERENCE: Data - WCAS job number 46564

Here is the data that you requested. Please give me a call if you have any questions.

From: Elena Orozco Time: 11:50am Faxed pages: ~~2~~ 3

West Coast Analytical Service, Inc. is not responsible for fax transmission errors. Unless otherwise noted, this data is preliminary and has not had final quality control review.

000322

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00 092

Client: LAREX
Job Number: 46564

Lead per CA AG Protocol for Proposition 65
Quantitative Analysis Report
Inductively Coupled Plasma-Mass Sp

Parts Per Million (µg/g)

Sample ID	Rep 1	Rep 2	Avg.	
2-LA-99022-01	0.055	0.047	0.051	Decolorized Immun Enhancer
2-LA-99284-01	0.020	0.025	0.023	ditto above
2-A2-99152-01	0.086	0.082	0.084	LaraCare A200
2XP9910801	ND	ND	ND	Ca(OH)2 CT 99
2C99935801	ND	ND	ND	ditto above
2C19800501	* 0.051	0.052	0.052	Grade 100 Spray dried
2C90005401	* ND	ND	ND	Ca(OH)2 CTAG099
Grade 100 Tamarack	ND	ND	ND	Tamarack
ClearTrac Tamarack	ND	ND	ND	
LaraCare Tamarack	ND	ND	ND	
ImmunEnh. Tamarack	ND	ND	ND	

Detection Limit: 0.020

Date Analyzed: 4-17-00, * 4-19-00

Quality Control Summary

Sample: SRM 1486 Bone Meal

	Mass	Certified Value µg/g	Found µg/g	% Acceptable Error	% Error
Lead	206-8	1.335	1.320	20	-1.1%

Date Analyzed: 4-19-00

000323

000323

00 093

Client: LAREX
Job Number: 46564

Lead per CA AG Protocol for Proposition 65
Quantitative Analysis Report
Inductively Coupled Plasma-Mass Spectrometry

Sample ID	Parts Per Million (µg/g) Rep 1	Rep 2	Avg.						
2-T3-99279-01	0.023	0.024	0.023	LSC-1	WL				
0018-97155-03	ND	ND	ND	LSC-1	WL				

Detection Limit: 0.008

Date Analyzed: 4-17-00

Quality Control Summary

Sample: 2C99935801

	Lead Parts Per Million (µg/g)
Sample:	ND
Duplicate:	ND
Average:	ND
Sample RPD:	NR
Spike Conc:	0.98
MS Result:	0.960
% Recovery	97.9
Control Limits:	
RPD	<25%
% Recovery	80-120%

Date Analyzed: 4-17-00

000324

SUBMISSION END

Reference List for Industry Submission, GRN 000084

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000031 - 000032	Robinson, R.; Rapp, J.; Fiertag, J.; Slavin, J.	Effect of Arabinogalactin Consumption on Fecal Microflora, Frequency, Weight, Transit Time and pH in Healthy Humans	March 15, 1999	The FASEB Journal	Volume 13, Number 5, Abstract 668.4

NA- Not applicable

AM

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DATE: 11/13/2001 TIME: 4:40 PM

TO:	Dr. Jason Dietz
COMPANY:	U.S. Food and Drug Administration
FAX:	202-418-3131
FROM:	Sandy Bigelow
SUBJECT:	Larex Responses for GRN 000084

Total Number of pages (including cover) - 4

Jason:

Attached are responses from Larex, Inc. Thanks for your help,

Sandy (b)(6)

000329

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Fax (651) 636-1583 • www.larex.com

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November 13, 2001

Dr. Jason Dietz
Office of Premarket Approval
Center for Food Safety and
Applied Nutrition (HFS-206)
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notice (GRN 000084) for Arabinogalactan from *Larix laricina*

Dear Dr. Dietz:

We hereby respond to your questions and clarifications given to us regarding FDA's acknowledgement of Larex's GRAS determination that arabinogalactan derived from the Eastern Larch tree is generally recognized as safe (GRAS) for use as a food ingredient. On September 21, 2001, the FDA had informed Larex, Inc., that the Agency received two volumes, entitled: 1) *GRAS Notice Arabinogalactan From Eastern Larch Tree*, dated September 5, 2001, and 2) *Arabinogalactan From Eastern Larch*, dated February 12, 2001, hereinafter referred to as GRN 000084. Arabinogalactan has also been recognized as larch gum or larch fiber. Each of FDA's questions (which are presented below in bold) is followed by a response provided by Larex, Inc.

What were the experimental conditions used to measure 1) UV absorbance at 280 nm and 2) viscosity, as listed for Chemical Specifications in Table 7 (page 14) of the GRAS Notice?

The conditions used to measure UV absorbance and viscosity was as follows. Solutions containing 1.0% decolorized or 0.1% Grade 50 (LSC-1) material were measured with the use of a Beckman DU640 Spectrophotometer set at 280 nm and conducted at room temperature. The viscosity measurements were conducted at room temperature with solutions containing 10% decolorized or 50% grade 50 (LSC-1) material with the use of a Brookfield LVT viscometer, fitted with a #18 spindle, and set at 60 rpm or 6 rpm for the decolorized and LSC-1 materials, respectively. These conditions were the experimental conditions mentioned in the Chemical Identity section (page 15) in *Arabinogalactan From Eastern Larch* (described under footnote 1 of Table 7, wherein footnotes 1 and 2 should have been switched in the table).

4815 White Bear Parkway
White Bear Lake, MN 55110-3325
(651) 636-2628
Fax. (651) 636-1583
www.larex.com

Toll Free: 800-386-5300

Please provide a copy of Gilman, 1999 for our records.

The document, Gilman 1999, is already contained within the document, *Arabinogalactan From Eastern Larch*, in the Raw Data section (pages 00 052 to 00 091). Gilman, 1999 is cited on page 45 in the Safety section of this document, and his name and affiliation are listed on page 00 057 within the Raw Data section. Dr. Martin Gilman is the study director of the Celsis Laboratory Group who conducted the acute feeding study in rats.

Please identify the source of the samples listed in Tab B of the GRAS Notice.

Tab B contains analytical reports for manufactured lots of arabinogalactan derived from Eastern or Western Larch trees, all of which correspond to the numbered lots listed in Table 5 (page 12) in the *GRAS Notice*. Each analytical report contains a lot number. Arabinogalactan derived from Western Larch is designated as 99108-01 C (or as 2C9-991-08 in Table 5), 2F9-00251-01 (or also as 2C90025101 in the analytical report), and 2-C9-0016-01. Arabinogalactan derived from Eastern Larch is represented by 2M500116-01, 2C9-00120-01, and 2-IE-0012001.

How does formic acid end up in the final product?

Formate salts (e.g., calcium) are natural constituents found in the raw extract of the Western and Eastern Larch tree. Some formate salts may also be formed during the decolorizing step in the manufacturing process. The panel of experts considered the presence of formate salts when they made their determination that arabinogalactan, derived from the Eastern Larch, was GRAS for use in food (see third paragraph of the panel's GRAS determination in Tab A of the *GRAS Notice*).

Were commercial manufactured lots of arabinogalactan derived from the Eastern Larch used to conduct the acute feeding studies in rats and the human clinical trials?

Yes. The lots used in the acute feeding studies and the human clinical studies were commercial lots manufactured by the same process used to manufacture arabinogalactan derived from the Western Larch tree.

Why was the reference, Kim et al. (1999), included in the GRAS Notice?

Kim et al. (1999) is a pilot study conducted with arabinogalactan, and represents a portion of the totality of the information that the panel of experts had reviewed and considered in making their GRAS determination. Specifically, Kim et al. (1999) is cited in the Use and Functionality section (page 1) in *Arabinogalactan From Eastern Larch*.

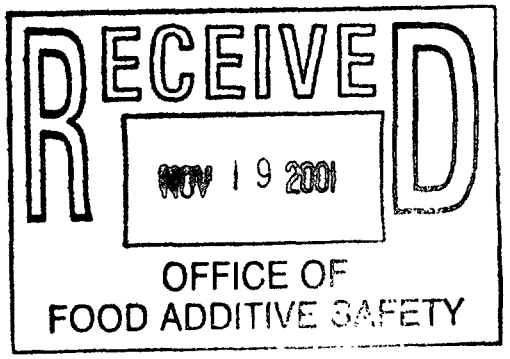
Please evaluate our response regarding this GRAS notice at your most expeditious convenience. If you have any questions, please feel free to call me at 1-800-386-5300.

Sincerely,

(b)(6)

Sanford W. Bigelow, Ph.D.
Vice President
Research & Development

LAREX®



November 13, 2001

Dr. Jason Dietz
Office of Premarket Approval
Center for Food Safety and
Applied Nutrition (HFS-206)
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notice (GRN 000084) for Arabinogalactan from *Larix laricina*

Dear Dr. Dietz:

We hereby respond to your questions and clarifications given to us regarding FDA's acknowledgement of Larex's GRAS determination that arabinogalactan derived from the Eastern Larch tree is generally recognized as safe (GRAS) for use as a food ingredient. On September 21, 2001, the FDA had informed Larex, Inc., that the Agency received two volumes, entitled: 1) *GRAS Notice Arabinogalactan From Eastern Larch Tree*, dated September 5, 2001, and 2) *Arabinogalactan From Eastern Larch*, dated February 12, 2001, hereinafter referred to as GRN 000084. Arabinogalactan has also been recognized as larch gum or larch fiber. Each of FDA's questions (which are presented below in bold) is followed by a response provided by Larex, Inc.

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Toll Free. 800-386-5300

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Please evaluate our response regarding this GRAS notice at your most expeditious convenience. If you have any questions, please feel free to call me at 1-800-386-5300.

Sincerely,

(b)(6)

Sanford W. Bigelow, Ph.D.
Vice President
Research & Development

AM

LAREX

DATE: December 5th, 2001 TIME: 9:30 C

TO: Dr. Jason Dietz
COMPANY: FDA
FAX: 202-418-3131
FROM: Richard Lamb
SUBJECT: Larex Contract

Total pages including cover -

Dear Dr. Dietz:

Thanks for your call this morning. I've sent an original letter via US Mail this morning. Looking forward to hearing from you.

With best regards,

(b)(6)

000338

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(651) 636-2628 • Fax (651) 636-1583 • www.larex.com

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December 5, 2001

Dr. Jason Dietz
Office of Premarket Approval
Center for Food Safety and Applied Nutrition (HFS-206)
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notice GRN 000084 for Arabinogalactan from *Larix laricina*

Dear Dr. Dietz:

This letter serves to officially inform you and the Office of Premarket Approval that Dr. Sandy Bigelow, who submitted the referenced Notice on behalf of Larex and with whom you've had previous correspondence regarding that matter, is no longer associated with Larex. I, Mr. Richard Lamb, Technical Director at Larex, will be the primary contact for the above referenced subject as of this date.

Larex is, of course, anxious to support your office in whatever way it can with regards to the GRAS Notice. Please feel free to call, email or write me at any time regarding this important regulatory matter.

With best regards,

(b)(6)

Richard Lamb
Technical Director

000339

4815 White Bear Parkway
White Bear Lake, MN 55110-3325
(651) 636-2628
Fax: (651) 636-1583
www.larex.com

Toll Free: 800-386-5300

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JAN 25 2002
OFFICE OF
FOOD ADDITIVE SAFETY

December 5, 2001

Dr. Jason Dietz
Office of Premarket Approval
Center for Food Safety and Applied Nutrition (HFS-206)
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notice GRN 000084 for Arabinogalactan from *Larix laricina*

Dear Dr. Dietz:

This letter serves to officially inform you and the Office of Premarket Approval that Dr. Sandy Bigelow, who submitted the referenced Notice on behalf of Larex and with whom you've had previous correspondence regarding that matter, is no longer associated with Larex. I, Mr. Richard Lamb, Technical Director at Larex, will be the primary contact for the above referenced subject as of this date.

Larex is, of course, anxious to support your office in whatever way it can with regards to the GRAS Notice. Please feel free to call, email or write me at any time regarding this important regulatory matter.

With best regards,

(b)(6)

Richard Lamb
Technical Director

000340

4815 White Bear Parkway
White Bear Lake, MN 55110-3325
(651) 636-2628
Fax: (651) 636-1583
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Toll Free: 800-386-5300

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JAN 17 2002
OFFICE OF
FOOD ADDITIVE SAFETY

December 13, 2001

Dr. Jason Dietz
Office of Premarket Approval
Center for Food Safety and Applied Nutrition (HFS-206)
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Notice GRN 000084 for Arabinogalactan from *Larix laricina*

Dear Dr. Dietz:

I trust that by now you have received the official letter informing the FDA and your office that I will be the primary contact for the above referenced subject. Our US mail system has slowed because of the current international problems, but please feel free to call me on that issue if needed.

We would like to inquire about whether an official review start date for the Larex GRAS Notice has been established, understanding that once it is set we can be guided by the ninety day target review timeline proposed earlier by the agency. We at Larex are attempting to anticipate a future date in time when we will be able to make strategic business decisions as a result of a completed review.

Larex is, as indicated before, anxious to support your office in whatever way it can with regards to the GRAS Notice. Please feel free to call, email or write me at any time regarding this important regulatory matter.

With best regards,

(b)(6)

Richard Lamb
Technical Director

000341

cc: Mr. Bo Nickoloff, President and CEO, Larex, Inc.

4815 White Bear Parkway
White Bear Lake, MN 55110-3325
(651) 636-2628
Fax. (651) 636-1583
www.larex.com

Toll Free: 800-386-5300

AM

LAREX®

DATE: 1/8/2002 TIME: 9:22 AM

TO:	Mr. Jason Dietz
COMPANY:	FDA
FAX:	(202) 418-3131
FROM:	Richard D. Lamb
SUBJECT:	Attached document

Total Number of pages (including cover) -

Dear Jason –

Original will be sent overnight. Thanks again.

With best regards,

(b)(6)

000342

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Fax (651) 636-1583 • www.larex.com

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January 08, 2002

Mr. Jason Dietz
Center for Food Safety and Applied Nutrition (HFS-206)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: FDA Questions Regarding GRAS Notice GRN 000084 for Arabinogalactan from *Larix laricina*

Dear Mr. Dietz:

Thank you, Jason, for your call this morning regarding the new mailing address and the two questions related to the Larex Notice (No. 000084). Please let me know if you find the following answers sufficient or whether more information is needed:

1. **Question: Were the materials used in the UIUC Grieshop study "Oral Administration of Arabinogalactan Affects Immune Status and Fecal Microbial Populations in the Dog" of Western or Eastern (Tamarack) larch origin?**

   Answer: All UIUC Grieshop clinical trial materials were of Western larch origin.

2. **Question: With respect to the 6-month human feeding study conducted at the University of Minnesota and described in the Safety Section, Page 3 of that section reports intake consumptions of up to 8.4 grams/day while Page 5 reports that the Study Inclusion Criteria had consumption at 10g/100 lb body weight; which is correct?**

   Answer: The original study inclusion criteria were amended to three levels of consumption, either 1.4, 4.4 or 8.4 grams/day. As a result, arabinogalactan from Eastern larch was consumed at 8.4 grams per day for a period of six months (one study arm). Arabinogalactan from Western larch was consumed at all three levels (three study arms).

With best regards,
(b)(6)
Richard Lamb
Technical Director

000343

4815 White Bear Parkway
White Bear Lake, MN 55110-3325
(651) 636-2628
Fax: (651) 636-1583
www.larex.com

Toll Free: 800-386-5300

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JAN 11 2002
OFFICE OF
FOOD ADDITIVE SAFETY

January 08, 2002

Mr. Jason Dietz
Center for Food Safety and Applied Nutrition (HFS-206)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: FDA Questions Regarding GRAS Notice GRN 000084 for Arabinogalactan from *Larix laricina*

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1. **Question: Were the materials used in the UIUC Grieshop study "Oral Administration of Arabinogalactan Affects Immune Status and Fecal Microbial Populations in the Dog" of Western or Eastern (Tamarack) larch origin?**

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With best regards,

(b)(6)

Richard Lamb
Technical Director

000344

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Toll Free 800-386-5300