Appendix A:

Certificates of Analysis for Tall Oil Phytosterol Product Lots

PO Box 678
Paris, IL 61944
217-465-6577

ABITEC GROUP **ABITEC Corporation**

October 24, 2005

CERTIFICATE OF ANALYSIS

Peter Cremer NA
3117 Southside Avenue
Cincinnati, OH 45204

Product:	PCNA Wood Sterols
Lot Number:	349M4
Product Number:	N/A
Mfg. Date:	12/14/04

Wood Sterols

TEST	RESULT
Color (Gardner)	+2 -3
Sterol Profile (by GC) %	
Brassicasterol	0.0 %
Campesterol	6.17 %
Campestanol	0.79 %
Stigmasterol	0.84 %
Beta-Sitosterol	83.76 %
Sitostanol	10.48 %
Total Identified Sterols & Stanols	100 %

Analyst: Mary Ann Guiney & Justin Sweitzer
Title: Quality Control Laboratory

Reviewed and Authorized By: Suella Dye
Suella Dye
Quality Control Manager

PO Box 878
Paris, IL 61944
217-466-8577

ABITEC GROUP **ABITEC Corporation**

October 24, 2005

CERTIFICATE OF ANALYSIS

Peter Cremer NA
3117 Southside Avenue
Cincinnati, OH 45204

Product:	PCNA Wood Sterols
Lot Number:	192M5
Product Number:	N/A
Mfg. Date:	07/10/05

Wood Sterols

TEST	RESULT
Color (Gardner)	N/A
Sterol Profile (by GC) %	
Brassicasterol	0.0 %
Campsterol	6.05 %
Campestanol	0.83 %
Stigmasterol	0.48 %
Beta-Sitosterol	83.09 %
Sitostanol	10.53 %
Total Identified Sterols & Stanols	100 %

Analyst: Mary Ann Guiney & Justin Sweitzer
Title: Quality Control Laboratory

Reviewed and Authorized By: Suella Dyo
Suella Dyo
Quality Control Manager

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Paris, IL 61944
217-466-8577

ABITEC GROUP **ABITEC Corporation**

October 24, 2005

CERTIFICATE OF ANALYSIS

Peter Cremer NA
3117 Southside Avenue
Cincinnati, OH 45204

Product:	PCNA Wood Sterols
Lot Number:	194M5
Product Number:	N/A
Mfg. Date:	07/12/05

Wood Sterols

TEST	RESULT
Color (Gardner)	+4 -5
Sterol Profile (by GC) %	
Brassicasterol	0.0 %
Campsterol	6.15 %
Campestanol	0.71 %
Stigmasterol	0.82 %
Beta-Sitosterol	86.27 %
Sitostanol	10.02 %
Total Identified Sterols & Stanols	100 %

Analyst: Mary Ann Guiney & Justin Sweitzer
Title: Quality Control Laboratory

Reviewed and Authorized By: Suella Dye
Suella Dye
Quality Control Manager

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ABITEC GROUP **ABITEC Corporation**

October 24, 2005

CERTIFICATE OF ANALYSIS

Peter Cremer NA
3117 Southside Avenue
Cincinnati, OH 45204

Product:	PCNA Wood Sterols
Lot Number:	222M5
Product Number:	N/A
Mfg. Date:	08/11/05

Wood Sterols

TEST	RESULT
Color (Gardner)	+5 -6
Sterol Profile (by GC) %	
Brassicasterol	0.0 %
Campesterol	5.92 %
Campestanol	0.84 %
Stigmasterol	0.80 %
Beta-Sitosterol	83.11 %
Sitostanol	11.10 %
Total Identified Sterols & Stanols	100 %

Analyst: Mary Ann Guiney & Justin Sweitzer
Title: Quality Control Laboratory

Reviewed and Authorized By: _____
Suella Dye
Quality Control Manager

ABITEC GROUP ABITEC Corporation

PO Box 878
Paris, IL 61944
217-465-6677

October 24, 2005

CERTIFICATE OF ANALYSIS

Peter Cremer NA
3117 Southside Avenue
Cincinnati, OH 45204

Product:	PCNA Wood Sterols
Lot Number:	267M5
Product Number:	N/A
Mfg. Date:	09/23/05

Wood Sterols

TEST	RESULT
Color (Gardner)	+5 -6
Sterol Profile (by GC) %	
Brassicasterol	0.0 %
Campsterol	5.62 %
Campestanol	0.74 %
Stigmasterol	0.78%
Beta-Sitosterol	81.95 %
Sitoetanol	10.31 %
Total Identified Sterols & Stanols	99.41 %

Analyst: Mary Ann Guiney & Justin Sweitzer
Title: Quality Control Laboratory

Reviewed and Authorized By: Stella Dye
Stella Dye
Quality Control Manager

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ABITEC GROUP **ABITEC Corporation**

October 24, 2005

CERTIFICATE OF ANALYSIS

Peter Cremer NA
3117 Southside Avenue
Cincinnati, OH 45204

Product:	PCNA Wood Sterols
Lot Number:	271M5
Product Number:	N/A
Mfg. Date:	09/27/05

Wood Sterols

TEST	RESULT
Color (Gardner)	+6 -7
Sterol Profile (by GC) %	
Brassicasterol	0.0 %
Campesterol	5.54 %
Campestanol	0.78 %
Stigmasterol	0.91 %
Beta-Sitosterol	81.75 %
Sitostanol	10.77 %
Total Identified Sterols & Stanols	99.75 %

Analyst: Mary Ann Guiney & Justin Sweitzer
Title: Quality Control Laboratory

Reviewed and Authorized By: _____
Suella Dye
Quality Control Manager

Appendix B

Methods of Analysis for Phytosterols and Phytosterol Ester Product Lots

ANALYTICAL METHOD
Method Number: PCNA046

DETERMINING PHYTOSTEROL BY GC-FID

(Derivitized with BSTFA/TMCS)

1. SCOPE: This procedure is intended for the analysis of phytosterols products and intermediates that have been saponified. Samples are silylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA in 1% Trimethylchlorosilane (TMCS)) and separated by gas chromatography. Pyridine is used as a catalyst. Detection is by FID. Dihydrocholesterol is used as an internal standard, and stigmasterol is used to generate response factors for the sterols.
2. REFERENCE: NA
3. EQUIPMENT: *Equivalent equipment may be substituted.*
   HP6890 Gas Chromatograph
   HP-5 Column 30m x 0.32mm x 0.25µm film thickness
   Balance, analytical, capable of measurements to 0.1mg
   Oven capable of maintaining temperature at 80ºC ±2ºC
4. PERSONAL PROTECTIVE EQUIPMENT: Review MSDS for chemicals used. Gloves and safety glasses.
5. CRITICAL OPERATING CONDITIONS: NA
6. REAGENTS AND SUPPLIES: *Equivalent reagents may be substituted.*
   Stigmasterol reference standard (Aldrich, S440-9)
   Toluene, 99.5% min. purity
   Dihydrocholesterol, reference standard (D-6128, Sigma)
   Pyridine, anhydrous (270013, Regis Technologies)
   BSTFA + 1% TMCS (230127, Regis Technologies)
7. PROCEDURE:
   7.1. Note the identification number of the balance used when weighing samples, and weigh all samples for a batch run on the same balance whenever possible.
   7.2. Dihydrocholesterol Internal Standard Solution
      7.2.1. Accurately weigh 5g of Dihydrocholesterol into a 1 L volumetric flask. Record the weight to 4 decimal places.
      7.2.2. Fill to volume with toluene. Cap and invert, mixing until all the Dihydrocholesterol has dissolved.
      7.2.3. Shelf-life of 1 year, or until response ratios or reference standard checks begin to drift.
   7.3. Calibration Standard Preparation
      7.3.1. Weigh out 80 mg of Stigmasterol into a 1/2 oz bottles. Record weights to the nearest 0.1 mg.
      7.3.2. Add 0.5 ml of pyridine, then 1 ml of BSTFA (+1% TMCS) to the bottles and vortex.

7.3.3. Add 5.00 ml (4.365 gm) of the Dihydrocholesterol solution to the bottle and record the weight added to the nearest 0.1 mg. Vortex to mix.

7.3.4. Heat in an oven for 20 minutes at 80°C.

7.3.5. Remove from oven and cool to room temperature.

7.3.6. Transfer 300 µl of solution to a test vial and dilute with 1 ml of toluene. Cap and mix well.

7.4. Sample Preparation

7.4.1. Weigh 100 mg of sample into a 1/2 oz bottle. Record weight to the nearest 0.1 mg.

7.4.2. Add 0.5 ml of pyridine, then 1 ml of BSTFA (+1% TMCS) to the bottle and vortex.

7.4.3. Add 5.00 ml (4.365 gm) of the Dihydrocholesterol solution to the bottle and record the weight added to the nearest 0.1 mg. Vortex to mix.

7.4.4. Heat in an oven for 20 minutes at 80°C.

7.4.5. Remove from oven and cool to room temperature.

7.4.6. Transfer 300 µl of solution to a test vial and dilute with 1 ml of toluene. Cap and mix well.

7.5. Analysis Conditions

Equipment	HP-6890 gas chromatograph
Column	HP-5 Length: 30m ID: 0.32mm Film: 0.25µm
Carrier Gas	Hydrogen with constant flow Initial: 0.8 ml/min Velocity: 33 cm/sec
Injector	Temperature: 320 °C Split Ratio: 1:175 (split flow 140 ml/min) Gas Saver ON
Temperature Profile	Initial Temperature: 200°C Final Temperature: 300°C Rate: 3°C / min
Detector	Temperature: 320 °C H_2: 30 ml/min. Air: 300 ml/min Makeup Flow: 35 ml/min.

Injection technique

Manual Injections (use a syringe rinse technique)

Pull up 0.5µl of toluene, then 0.5µl airspace, and 1.0µl of sample

Auto injector Injections

1.0µl injection

Post-injection rinse: 10 times with Toluene

Integration - Manually integrate each phytosterol peak

7.6. When entering sample information into the sequence table include the balance identification number for each sample weighed.

7.7. Calibration Run Order:

7.7.1. Toluene blank

7.7.2. Calibration Standard (run in triplicate)

7.7.2.1. The calibration standard only needs to be run when a new lot of Dihydrocholesterol Internal Standard solution is prepared.

7.8. Sample Run Order

7.8.1. Toluene blank
7.8.2. Calibration standard
7.8.2.1. Do not run daily.
7.8.2.2. Run when a control sample is out of acceptable range.
7.8.2.3. Run when a new internal standard solution is prepared.
7.8.3. Samples (run in triplicate)

7.9. Peak Identification

Analyte	*Approx. Retention Time (minutes)*
Dihydrocholesterol	27.7
Campesterol	29.7
Stigmasterol	30.4
Sitosterol	31.6
Campestanol	29.9
Stiostanol	31.8
Δ5- Avenasterol	31.9

8. CALCULATIONS

8.1. Determine the purity of Stigmasterol by Loss on Drying and GC.

$$\%Purity = (GCAreaPercent) \times (\%Solids)$$

Where:

%Solids is written as a decimal

8.2. Determine the amount of Stigmasterol in the calibration standard

$$g = \%P \times gStigmasterol$$

Where:

%P = %Purity (8.1)

gStigmasterol = weight of Stigmasterol from calibration standard solution (7.3.1)

8.3. Calculate the response ratio

$$Responce\ Ratio = \frac{\left(\frac{DA}{IS}\right)}{\left(\frac{SA}{Sg}\right)}$$

Where:

DA = Dihydrocholesterol Area from chromatogram
IS = Internal Standard weight (7.3.3)
SG = Stigmasterol Area from chromatogram
Sg = Stigmasterol weight (7.3.1)

8.4. Calculate the amount of each analyte in the sample

$$\%Analyte\ in\ Sample = \frac{(ARR)(AA)(ISs)}{(ISsA)(S)} \times 100$$

Where:

ARR = Analyte Response Ratio
AA = Analyte Area
ISs = Internal Standard weight (7.4.3) from the sample preparation
ISsA = Internal Standard Area from the sample chromatogram
S = Sample weight (7.4.1)

9. RECORDS:
   9.1. Record the weight percent of each analyte for the sample on the appropriate documents.
   9.2. Attach a copy of the sample and response ratio chromatograms, with the peaks labeled, to the documents.
      9.2.1. If a spreadsheet is used to calculate percent sterols include the balance identification number in the appropriate fields for both standard preparation and sample weigh-up. Include this spreadsheet with the chromatograms.
   9.3. Have the analysis approved by a peer or QC supervisor.
   9.4. Immediately report the detection of any atypical peak or out-of-specification condition to the quality control manager.

ANALYTICAL METHOD
Method Number: PCNA044

PHYTOSTEROL ESTER BY HPLC

1. SCOPE: This procedure shall be used for HPLC analysis of phytosterol esters.
2. REFERENCE: NA
3. PERSONAL PROTECTION EQUIPMENT (PPE): safety glasses or goggles, gloves, lab coat or rubberized apron
4. CRITICAL OPERATING CONDITIONS: NA
5. EQUIPMENT: *Equivalent equipment may be substituted.*
   Hewlett-Packard Series 1100 series Liquid Chromatograph
   1 x Varian Microsorb-MV 100-5 C18, 250 x 4.6m, Part Number R0086200C5
   1 x Phenominex Luna C18 250 x 4.6 100Å serial number 00G-4252-E0
   Balance, analytical, capable of measurements to 0.1mg
6. REAGENTS: *Equivalent reagents may be substituted.*
   HPLC-Grade Acetone
   Standard, PS lot 19038NF, 92%
   Standard, FAME Agrimul 2232, lot 19026NF, 99%
   Standard, PSE, lot 19288NF, 94.6%
7. PROCEDURE:
   7.1. Standard Preparation
      7.1.1. Stock Solutions (These solutions are used for area calibration.)
         7.1.1.1.For each of the three standards:
            7.1.1.1.1. Dilute 100mg of standard with 80g of acetone.
            7.1.1.1.2. Record all weights to the nearest 0.1 mg.
         7.1.1.2.FAME and PS require further dilution.
         7.1.1.3.Take ~8.0g aliquot of the stock solution and dilute with ~50.0g of acetone.
   7.2. Area Calibration
      7.2.1. Place ~1ml of each stock solution in a 1.5ml septa cap vial.
      7.2.2. Inject 25, 50, and 75µl of each stock solution in duplicate volume using an auto injector.
   7.3. Integration
      7.3.1. Manually integrate each peak for the calibration and samples.
   7.4. Sample Preparation
      7.4.1. Dilute 100mg of sample with 80g of acetone.
      7.4.2. Record all weights to the nearest 0.1 mg.
   7.5. System Suitability
      7.5.1. Chromatographic performance shall be evaluated on the basis of consistent area count per mg of contained PS (slope) for the standard solution. Determination of the slope for multiple injections has shown the relative standard deviation (RSD) of slope to be = 2.0%.
      7.5.2. Prior to running any sample analysis, run a system suitability check.
         7.5.2.1.Inject an acetone blank to ensure the column is clean.
         7.5.2.2.Inject a reference standard.
         7.5.2.3.Calculate the dividend of area vs. contained FAME, PS, and PSE.

7.5.2.3.1. If the slope is = 2.0% from the average slope for the previous 10 standard analyses, the system is not suitable for sample analysis, and the cause must be found and repaired.

7.5.2.4. Repeat the standard analysis after every 5-10 sample analysis to ensure that the calibration is still valid.

7.6. Inject 50 µl of each sample preparation into the HPLC under the following conditions:

Chromatographic Operating Conditions

Instrument	Hewlett-Packard Series 1100 series Liquid Chromatograph	
Columns	1 x Varian Microsorb-MV 100-5 C18, 250 x 4.6m 1 x Phenominex Luna C18 250 x 4.6 100Å	
Temperature	35 ºC (ambient)	
Mobile Phase	Isocratic	100% acetone
Run Time	30.0 minutes	
Flow Rate	1.0 ml/minute	
Injection Size	50 µl	
Detection	RI	

7.7. Approximate Retention Times (Minutes)

Methyl ester	6.4-7.3
Phytosterol	9.3
Phytosterol esters	13.6-17.2

8. CALCULATIONS:

Construct the response curve for each analyte by the following:

8.1. Calculate the on-column amount for each analyte for each analysis:

$$On-column(mcg)=\frac{(Sw)(P)(Sv)(Iv)}{(Sw+Dw)(Sv+Fv)}$$

Where:

Sw = Sample wt (mg) into solution
P = Purity
Sv = Sample solution wt (g) into vial
Dw = Diluent wt (g) into solution
Fv = Acetone solution wt into vial
Iv = Injection volume (µl)

8.2. Calculate the slope, intercept and correlation coefficient for each set of data from the standard runs.

Reference equations:

$$m=\frac{y_1-y_2}{x_1-x}$$

$$y=mx+b$$

$$r=\frac{n\sum xy-\left(\sum x\right)\left(\sum y\right)}{\sqrt{n\left(\sum x^2\right)-\left(\sum x\right)^2}\sqrt{n\left(\sum y^2\right)-\left(\sum y\right)^2}}$$

Where:

y = area from the chromatogram
x = on-column amount

8.3. Calculate the on-column weight in the analytical sample:

$$On-columnwt.=\frac{(Sw)(Sv)(Iv)}{(Sw+Dw)(Sv+Fv)}$$

Where:

Sw = Sample wt (mg) into solution
Sv = Sample solution wt (g) into vial
Dw = Diluent wt (g) into solution
Fv = 80/20 solution wt into vial
Iv = Injection volume (µl)

8.4. Calculate Percent PSE:

$$\%PSE=\frac{(Aa-Ba)}{(Ma)(Sc)}$$

Where:

Aa = Area of analyte
Ba = intercept for analyte
Ma = slope for analyte
Sc = Sample on-column amount

9. RECORDS: Record the PSE concentration on the appropriate documents. Immediately report the detection of any atypical peak or atypical distribution to the quality control manager.