METHOD RA/FA-85.02

**RESIN AND FATTY ACIDS BY EXTRACTION/ETHYLATION GC/FID
AND GC/MS ANALYSIS**

**NCASI
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Acknowledgments

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This method is included in the NCASI Methods manual as a PROPOSED method. The purpose of including proposed methods in the NCASI Methods Manual is to make it known that a method is under development and to solicit comment regarding the technical merit and applicability of the method. This method should not be construed as having been rigorously validated on any matrix and therefore, those electing to apply the method are strongly encouraged to conduct rigorous QA/QC or validation such that the quality of the data generated can be evaluated. Please note that PROPOSED methods are not suitable for use as regulatory monitoring or compliance methods.

NCASI METHOD RA/FA-85.02

RESIN AND FATTY ACIDS BY EXTRACTION/ETHYLATION GC/FID AND GC/MS ANALYSIS

1.0 Scope and Application

1.1 This method is used to determine the concentration of resin and fatty acid compounds in extracts prepared from water samples and analyzed by gas chromatography/flame ionization detection (GC/FID) or mass spectrometry (GC/MS). This method is an updated version of NCASI Method RA/FA 85.01 (NCASI 1986). Modifications to the method include; (a) the option to use methyl-O-methyl podocarpate as the internal standard instead of propyl dehydroabietate, which must be synthesized and is not commercially available, (b) substitution of methyl-t-butyl ether (MTBE) for the diethyl ether due to the hazards associated with the formation of peroxides when using diethyl ether, (c) the addition of ascorbate solution prior to extraction due to matrix effects exhibited in some sample matrices. The following compounds can be confirmed or quantitated by this method.

1.2

Compound	CAS Registry Number
Oleic Acid	112-80-1
Linoleic Acid	60-33-3
9,10-Dichlorostearic Acid	5829-48-1
Sandracopimaric Acid	471-74-9
Isopimaric Acid	5835-26-7
Dehydroabietic Acid	1740-19-8
Pimaric Acid	127-27-5
Neoabietic Acid	471-77-2
Abietic Acid	514-10-3
Palustric Acid	1945-53-5
14 & 12-Chlorodehydroabietic Acid	57055-38-6
Dichlorodehydroabietic Acid	57055-39-7

1.3 The method has been found to be satisfactory for biologically treated and untreated pulp mill effluents, receiving waters, and some process waters. The method has been shown to be problematic in some pulp and paper matrices, such as effluents from facilities employing de-inking processes. Dilution of the matrix may be necessary for analysis. Demonstration of extraction efficiency and method performance for specific matrix types is recommended.

1.4 The method has been single laboratory validated and is a proposed method.

1.5 The estimated method detection limits (MDL) established for Method RA/FA 85.01 are given in Section 17, Table 1. These values are provided as guidance, due to

improvements in instrumentation and changes in matrix effects, each laboratory should establish their own MDL's. The lower calibration level (LCL) of the instrument is approximately 1 µg/L. The concentration range is from 1 µg/L to 80 µg/L. The lower quantitation level is dependent on matrix specific dilutions required to avoid saturation of the detector and to obtain concentrations within the calibration range of the instrument.

1.6 Although the analysis of resin acids can be routinely performed using GC/FID, it is recommended that confirmation by mass spectral identification be performed using GC/MS.

1.7 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatographs, and skilled in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0   Summary of the Method

2.1 A 250 mL sample is acidified and extracted using methyl-t-butyl ether (MTBE) as the solvent in a separatory funnel. Extractions are performed at pH 5 and pH 2. After concentration, the combined extracts are ethylated by formation of the diisopropyl-ethylamine salts and formation of the ethyl esters using triethyloxonium tetrafluoroborate (TEOTFB). An activated silica gel cleanup is then employed to remove potential interferences. The cleanup step has also been found to reduce the frequency of instrument maintenance such as changing injection port liners and preserving column life.

2.2 The resin and fatty acid compounds are introduced into the gas chromatograph (GC) with a narrow bore fused silica capillary column. The GC column is temperature programmed to separate the analytes which are then detected with a flame ionization detector or mass spectrometer interfaced to the gas chromatrograph.

2.3 Identification of target analytes by mass spectrometry is accomplished by comparing their mass spectra with the mass spectra of known standards. The response of a major quantitation ion relative to an internal standard with a six-point calibration curve determines the quantitation of the analyte. Identification of target analytes by flame ionization detection is determined by comparison of their retention times with the retention times of known standards. Confirmation of analytes detected by FID is performed by GC/MS.

2.4 The sensitivity of the method usually depends on the level of interferences rather than on instrumental limitations.

2.5 Quality is assured through testing of the extraction and analytical systems. This is accomplished through the use of tune checks, calibration checks, and surrogate spike recovery standards. A method blank, duplicate and matrix spike are also analyzed with each analytical batch (set of no more than 20 samples of similar matrix type) to ensure data quality.

3.0   Definitions

3.1   The definitions and purposes below are specific to this method, but conform to common usage as much as possible.

3.1.1   µg/L–micrograms per liter

3.1.2   Ethylation–derivatization to the ethyl esters of the carboxylic acid group of resin and fatty acids

3.1.3   May–this action, activity or procedural step is neither required nor prohibited

3.1.4   Must not–this action, activity or procedural step is prohibited

3.1.5   Must–this action, activity or procedural step is required

3.1.6   Should–this action, activity or procedural step is suggested, but not required.

4.0   Interferences

4.1   Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing hardware. All these materials must be routinely demonstrated to be free from interferences under the conditions of the analyses by running laboratory blanks as described in Section 9.4.

4.2   Glassware must be scrupulously cleaned. Clean all glassware by detergent washing with hot water and rinsing with tap water. The glassware should then be drained dry and either baked at 400°C for at least six hours, or solvent rinsed with the extraction solvent.

4.3   Matrix interferences may be caused by other compounds that are co-extracted from the sample. The extent of these interferences, which have usually proved to be fatty acids, (C20 and higher) will vary depending upon the matrix and diversity of the wastewater being sampled. In cases where the quantitation of the interfered compounds is critical, the GC/MS analytical procedure as outlined in Sections 10.2 and 12.2 are employed.

5.0   Safety

5.1   The toxicity or carcinogenicity of each analyte or reagent has not been precisely determined; however, each chemical should be treated as a potential health hazard. It is recommended that prudent practices for handling chemicals in the laboratory be employed.

5.2   This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness of OSHA

regulations regarding safe handling of chemicals used in this method. Material safety data sheets (MSDS) should be available to all personnel involved in these analyses.

5.3   TEOTFB is a slightly hydroscopic crystalline solid. Although TEOTFB's stability and crystalline form make it safer and more convenient to handle than the commonly used methylation reagent diazomethane, it is still a potent alkylating agent and should be handled with care. Conduct weighing in a laboratory hood and wear appropriate gloves and other protective clothing.

5.4   MTBE is a flammable liquid which may be harmful if inhaled or absorbed through the skin. Use in a laboratory fume hood, or wear an approved respirator and avoid contact by wearing chemical resistant gloves, eye protection and other protective clothing.

6.0   Equipment and Supplies

6.1   Brand names and suppliers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.2   **Sampling equipment**

   6.2.1   Automatic sampling equipment which comes in contact with a sample should be constructed of glass, Teflon™, or stainless steel.

   6.2.2   Samples are to be collected in brown glass bottles with Teflon™-lined screw caps.

6.3   **Laboratory glassware and supplies**

   6.3.1   Separatory funnel, 500 mL with Teflon™ stopcock

   6.3.2   Beaker, 400 mL

   6.3.3   Erlenmeyer flask, 250 mL

   6.3.4   Graduated cylinders, 250 mL and 100 mL

   6.3.5   Centrifuge tubes, 15 mL and 50 mL with Teflon™ lined screw cap

   6.3.6   Centrifuge tube, 15 mL graduated conical with ground glass stopper

   6.3.7   Kuderna-Danish (KD) apparatus

   6.3.8   Concentrator tube, 15 mL

6.3.9 Evaporation flask, 250 mL attached to a concentrator tube using springs, clamps etc.

6.3.10 Snyder column, three-ball macro

6.3.11 Chromatogaphic clean-up column: constructed by joining a 10 cm x 8.0 mm OD, 6.0 mm ID glass tube to the bottom of a 10 cm test tube. The end of the tubing is tapered to give a small orifice.

6.3.12 Automatic injection vials with Teflon™ lined lids

6.3.13 Pipets, pasteur disposable with one mL latex bulb

6.3.14 Pipets, 1 mL to 10 mL

6.3.15 Syringes, 10 μL to 250 μL

6.3.16 Boiling Chips−Teflon™

6.3.17 Analytical Filter Pulp−used to plug the chromatographic clean-up column

6.4 Laboratory equipment

6.4.1 Magnetic stirrer

6.4.2 Centrifuge−bench top model

6.4.3 Water bath

6.4.4 pH meter−calibrated using two point procedure

6.4.5 Drying oven−set at $130 \pm 5°C$ for activating the silica gel

6.4.6 Balance, analytical, capable of weighing to the nearest 0.0001 g

6.5 Analytical equipment

6.5.1 Gas chromatograph/detector system. Gas chromatography analytical system complete with a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories including syringes, analytical columns and gases.

6.5.2 Column. 30 m $\pm$ 5 m x 0.25 mm $\pm$ 0.02 mm x 0.25 micron bonded phase DB-5 fused silica capillary column. (J&W scientific or equivalent) or Restek RTX-5 with five meter guard column. The guard column potentially may prolong column life for analyses without clean-ups.

6.5.3 GC Detector. Flame ionization with appropriate data system

6.5.4 Mass Spectrometer. Capable of scanning from 35 to 450 amu every one sec or less, (utilizing 70 volts nominal) electron energy in the electron impact ionization mode. A computer system must be interfaced to the mass spectrometer. The system must allow the continuous acquisition and storage of machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer must have software that can search any GC/MS data file for ions of a specific mass, and that can plot such ion abundances versus time or scan number. This type of plot is defined as an extracted ion current profile (EICP). Software must also be available that allows integrating the abundance in any EICP between specified time or scan number limits.

7.0 Reagents and Standards

7.1 Non-spectrograde, hexane, cyclohexane, methylene chloride, methanol, distilled in glass (Burdick and Jackson).

7.2 Methyl-t-butyl ether (Burdick and Jackson).

7.3 Reagent water. Organic free such as produced by a Barnstead Model D2798 NANO-pure-A water purification system.

7.4 Sodium Hydroxide (5%). Dissolve 25 g NaOH in reagent water and dilute to 500 mL.

7.5 Sulfuric acid (1:3): Slowly add one part concentrated H_2SO_4 to three parts of reagent water.

7.6 Ascorbate Solution. Dissolve 11.2 grams of ascorbic acid, sodium salt into 100 mL of reagent water. Prepare fresh daily.

7.7 Diisopropylethylamine (1M). Dissolve 3.88 g of diisopropylethylamine (Aldrich) in 30 mL of dichloromethane (Burdick and Jackson). Store in a Teflon™-lined screw capped bottle in a freezer.

7.8 Triethyloxonium tetrafluoroborate (TEOTFB) (1M). Dissolve 6.09 g of TEOTFB (Fluka) in 32 mL of dichloromethane and store in a freezer. Caution: weigh the material in a hood and wear chemical resistant gloves. The crystalline TEOTFB should be stored in a freezer. If the reagent becomes saturated with water, it can be washed with anhydrous ether since it is virtually insoluble.

7.9 Potassium Chloride (KCL) (saturated). Excess KCL is added to reagent water and is allowed to stand overnight. Solid KCL should remain present to insure the solution is saturated. If blank problems are traced to this reagent, the KCL can be baked at 400°C for several hours prior to preparing the solution.

7.10 Sodium Sulfate. Anhydrous powder, store at 130°C.

7.11 Silica gel. grade 62, 60 to 200 mesh, 150A, activated at 130°C.

7.12 Indicator solution. Dissolve 10 mg of Sudan I and 150 mg of azulene in 20 mL hexane, store in Teflon™-lined screw cap scintillation vial.

7.13 Preservative solution. Dissolve 15 mg of Ethanox 702™ [4,4′-methylene bis(di-t-butylphenol)] in 20 mL of hexane, store in Teflon™-lined screw cap scintillation vial.

7.14 Surrogate spike primary stock solution. Prepare a 1 mg/mL standard stock solution by weighing 25 ±0.5 mg each of O-methylpodocarpic acid (Helix Biotech Corp.) and heptadecanoic acid (J.T. Baker, Aldrich) and dissolving in methanol, in a 25 mL volumetric flask.

7.15 Surrogate spiking solution. Prepare a spiking solution by diluting the primary stock solution 1:10 in methanol. Final concentration 100 ug/mL.

7.16 Internal standard spike primary stock solution. Internal standard solutions of n-propyl dehydroabietate *or* methyl-o-methyl podocarpate (Aldrich) may be used for the internal standard. The n-propyl dehydroabietate is not readily available and must be synthesized prior to use following the procedure described in Technical Bulletin 501 (NCASI 1986). Potential interferences should be evaluated for complex matrices when using the methyl-o-methyl podocarpate. Prepare the primary stock solution by weighing 60 to 65 mg of the standard and dissolving in 25 mL of hexane in a volumetric flask. Approximately 2.5 mg/mL stock solution.

7.17 Internal standard spiking solution. Prepare internal standard spiking solutions by diluting the primary solution 1:5 with hexane. Approximately 500 µg/mL spiking solution.

7.18 Calibration and matrix spike primary stock solutions. Prepare stock solutions of individual compounds (Section 1.2, many available from Helix Biotech Corporation) by weighing, to the nearest 0.1 mg, 60 to 65 mg of standard and dissolving in 25 mL of methanol. (2.5 mg/mL primary stocks). Individual constituents must be purity-checked prior to analysis. Abietic acid is unstable and may require preparation from freshly isomerized and crystallized levopimaric acid. The concentration of standards that are not at least 95% pure should be corrected for purity.

7.19 Calibration and matrix spike solutions. Prepare standard solutions by transferring 1 mL of each of the stock solutions to a 25 mL volumetric flask with methanol. Approximately 100 ug/mL.

7.20 Calibration standards are prepared by spiking 2.5, 5, 25, 50, 100 and 200 µL of the calibration spike solution and the surrogate spike solution into 15 mL screw-capped centrifuge tubes containing approximately 0.25 mL of cyclohexane. Exchange the methanol/cyclohexane mixture with additional aliquots of cyclohexane in a hot water bath

until all of the methanol is removed. Proceed with the derivatization outlined in Section 11.2. Adding 200 µL of ethanox preservative and 25 µL of the internal standard. Transfer the final derivatized standards into autosampler vials for analysis.

7.21   Decafluorotriphenylphosphine (DFTPP) can be purchased from Supelco or an equivalent supplier as a 25,000 µg/mL solution in dichloromethane. A working stock solution at a concentration of 50 µg/mL is prepared in hexane. Store in the dark in autosampler vials with Teflon™ seal crimp caps prior to use.

8.0   Sample Collection, Preservation, and Storage

8.1   Collection. Grab samples must be collected in glass containers having a Teflon™-lined screw cap. Composite samples may be collected using automatic sampling equipment. The parts of the automatic sampling equipment that come in contact with the sample should be constructed of glass, Teflon™, or stainless steel. Composite samples should be refrigerated during the sampling period

8.2   Preservation. All samples must be preserved by adjusting to pH 10, with NaOH and refrigerating. This should be done as soon as possible after sample collection. Samples must be shipped in iced containers as quickly as possible.

8.3   Storage. Samples may be stored in the refrigerator (4°C) for up to 30 days. Extracts are maintained at 4°C prior to analysis. The extracts must be analyzed within 30 days of extraction.

9.0   Quality Control

9.1   Each field sampling program or laboratory that uses this method is required to operate a formal quality assurance program. Laboratory or field performance is compared to established criteria to determine if the results of analyses meet the performance characteristics of the method.

9.2   **GC/MS performance**

9.2.1   DFTPP Tune Check. Prior to analyzing any samples or standards, the GC/MS performance must be verified by a DFTPP tune. This tune check must be analyzed just prior (± 6 hours) to the calibration standard analyses, and must meet the specifications listed in Section 17, Table 2.

9.2.2   Calibration Verification. Before each set of samples is analyzed, a calibration check will be done to determine that the GC/MS system is operating within acceptable parameters. The calibration check involves the analysis of a calibration standard in the mid range of the calibration curve. The concentration for each of the analytes must be within ± 20% of the initial calibration. If the calibration fails to meet the expected criteria, the GC/MS system may require

maintenance. If routine maintenance does not correct the problem the instrument will need to be recalibrated.

9.3   Frequency

One sample per analytical batch of no more than 20 samples of similar matrix type, should be allocated for quality control. In addition to this, representative samples from each new or untested source or sample matrix should be treated as a quality control sample. Laboratory replicates and fortification (matrix spikes) should be conducted on each quality control sample to document method performance as indicated by precision and recovery.

9.4   Blanks

In addition to the quality control sample, one method blank that utilizes all glassware and reagents required for sample analyses should be prepared per analytical batch to demonstrate that all materials are interference free.

9.4.1   Reagent blanks. Whenever new reagents are prepared, or when blank problems develop, the analyst should demonstrate that the reagents are free from contamination by performing the steps required for the reagent's use. Derivatization reagent blanks would not require the extraction step but would require performing the derivatization step.

9.4.2   Method blanks. Blanks prepared using reagent water should be carried through all stages of the sample preparation and measurement to demonstrate that all glassware, reagents, and instrumentation are free from contamination.

9.5   Replicates

Replicates consist of running two or more separate aliquots of the sample through the entire analytical procedure. If detectable levels of analytes are not found in the sample matrix, a duplicate matrix spike may be performed to measure method precision. The concentration determined for each analyte, the range of concentrations determined by the replicates, the relative percent difference and the mean should be tabulated in a method precision log.

9.6   Matrix spike recovery

Using the mean concentration determined by the replicate analyses, or the background level determined from a single measurement, determine the spiking level which will give three to five times the background. If the background sample does not have detectable levels of analytes, spike the sample at five times the lowest calibration level of the instrument. Spike the sample with the determined amount of the calibration standard/matrix spike solution (Section 7.19) and proceed to analyze the sample in the normal manner. Single laboratory matrix spike recovery data is reported in Section 17, Table 3 for FID analyses and Table 4 for GC/MS analyses. If the levels determined are

outside the control limits (average plus or minus three times the standard deviation), the extraction and analyses must be repeated. If the results are outside the warning limits (average plus or minus two times the standard deviation), the analyst should review the analytical data and procedure for possible degradation of standards or other analytical problems. Calculate the percent recovery as follows in Equation 1.

Equation 1

$$Percent\ Recovery\ =\ \frac{Level\ Measured\ -\ Background}{Level\ Spiked}\ x\ 100$$

where the background is the mean of the replicate determinations described above.

9.7   Surrogate spike recovery

The analyst must spike all samples with the surrogate standard (Section 7.15) to monitor surrogate spike recoveries. The spiking level should be in the mid-range of the calibration (approximately 40 to 50 µg/L). The acceptable range for recoveries of the surrogate spike is 65 to 120%. If the recovery for any surrogate does not fall within these limits, steps must be taken to isolate the problem before proceeding any further. The calculations for recovery are in Equation 2.

Equation 2

$$Percent\ Recovery\ =\ \frac{Level\ of\ Surrogate\ Measured}{Level\ of\ Surrogate\ Spiked}\ x\ 100$$

10.0   Calibration and Standardization

10.1   FID operating conditions. Assemble the GC/FID and establish the operating conditions outlined below. Once the GC/FID system is optimized for analyte separation and sensitivity, the same operating conditions must be used to analyze all samples, blanks, calibration standards, and quality assurance samples.

GC/FID Operating Conditions for Resin and Fatty Acid Analysis

Injector Temperature:	270°C
FID Detector Temperature	320°C
Splitless Valve Time:	0.5 min
Carrier Gas	Hydrogen @ 42 cm/sec @200°C
Column:	DB-5, 30 m x 0.25 mm id x 0.25 micron or Restek RTx-5, 30 m with 5 m guard column

Temperature Program °C:

Initial:	130°C for one min
Ramp 1:	6°C/min for 6 minutes
Ramp 2:	2°C/min to 280°C
Final Hold time:	10 minutes

10.2 GC/MS operating conditions. Assemble the GC/MS and establish the operating conditions outlined below. Once the GC/MS system is optimized, the same operating conditions must be used to analyze all samples, blanks, calibration standards and quality assurance samples.

GC/MS Operating Conditions for Resin and Fatty Acid Analysis

Injector Temperature:	270°C
Interface Temperature	280°C
Splitless Valve Time:	0.8 min
Carrier Gas	Helium @ 30-35 cm/sec @200°C
Column:	DB-5, 30 m x 0.25 mm id x0.25 micron or Restek RTx-5, 30 m with 5 m guard column

Temperature Program °C:

Initial:	60°C for 5 min
Ramp 1:	8°C/min to 200°C for 17.5 minutes
Ramp 2:	4°C/min to 280°C for 5 minutes
Ramp 3:	20°C/min to 300°C for two minutes

MS Conditions:

Scan Start Time:	10 min
Scan Range:	42 to 500 AMU
Cycle Time:	0.95 to 1.0 seconds

10.3   Preparation of the calibration curve

10.3.1   Prepare a six-point calibration curve for each of the target compounds and the surrogate spikes.

10.3.2   Prepare the calibration standards by spiking 2.5, 5.0, 25, 50, 100 and 200 uL of the calibration spiking solution (Section 7.19) and the surrogate spiking solution (Section 7.15) into 0.25 mL of cyclohexane in a 15 mL screw cap centrifuge tube. Since the standards are prepared in methanol, each standard must be exchanged into cyclohexane by concentration and addition of cyclohexane until all of the methanol is removed.

10.3.3   Following exchange into cyclohexane, each standard is ethylated using the same procedure used for sample preparation, outlined in Section 11.2.

10.3.4   It is not necessary to perform clean-up steps on the standard solutions. However, the addition of 200 µL of the anti-oxidant ethanox prior to final concentration is required. Add 25 µL of internal standard (Section 7.17) and transfer to autosampler vials for analysis.

10.4   GC/FID analysis of calibration standards

10.4.1   Inject 1 µL of each of the calibration standards and determine the retention time of the target analytes relative to the internal standard. Each analyst should optimize the temperature program or instrument conditions, as necessary, to establish distinct separate peaks.

10.4.2   If the average of the relative response factors for each analyte (RRF_a) is constant, i.e., exhibits a coefficient of variation less than 20%, the calibration is acceptable and the average RRF_a can be used in all subsequent calculations; otherwise, the calibration curve extracts must be reanalyzed and reevaluated. It may be necessary to perform instrument maintenance prior to reanalysis. If reanalysis also fails to produce a linear curve, new calibration standards must be prepared and analyzed.

10.4.3   Analyze and calculate the relative response factor of a mid-range calibration standard, daily, prior to each sample set, using the formula below to verify the calibration. The relative response factors must be within the acceptance range given in Section 17, Table 5. If they are not, either prepare a new standard or perform instrument maintenance. If necessary, re-calibrate the instrument.

Equation 3

$$RRF_a = [A_a/A_{is}) \times (C_{is}/C_a)]$$

Where: A_a = *area of the analyte peak*
A_{IS} = *area of the internal standard peak*
C_a = *concentration of the analyte injected*
C_{IS} = *concentration of the internal standard injected*

10.5   GC/MS analysis of calibration standards

10.5.1   The relative response factors are calculated using the integrated areas of EICP for the characteristic ions shown in Section 17, Table 4.

10.5.2   Inject 1 μL of each of the calibration standards. If the average of the RRF_a for each analyte is constant, i.e., exhibits a coefficient of variation less than 20%, the calibration is acceptable and the average RRF_a can be used in all subsequent calculations; otherwise, the calibration curve extracts must be reanalyzed and reevaluated. If reanalysis also fails to produce a linear curve, new calibration standards must be prepared and analyzed.

10.5.3   Analyze and calculate the relative response factor of a mid-range standard daily, prior to sample analysis using the formula below. The relative response factors must be within ±20% of the average response factor for the calibration curve. If they are not, either prepare a new standard or perform instrument maintenance. If necessary, re-calibrate the instrument.

Equation 4

$$RRF_a = [A_a/A_{is}) \times (C_{is}/C_a)]$$

Where:

A_a	=	*area of the peak for characteristic mass of the analyte*
A_{IS}	=	*area of the peak for the characteristic mass of the internal standard*
C_a	=	*concentration of the analyte*
C_{IS}	=	*concentration of the internal standard*

10.6   Analytical range and minimum calibration level

10.6.1   Demonstrate that the calibration curve is linear (relative response factors exhibit a coefficient of variation less than 20%) throughout the range of the calibration curve.

10.6.2   Demonstrate that the target analytes are detectable at the minimum levels using the lowest level calibration curve extract.

11.0   Procedure

11.1   Extraction

11.1.1   Remove working surrogate spiking solution, working matrix spike solution, if necessary, and sample(s) from the refrigerator and bring to room temperature.

11.1.2   Obtain pre-baked glassware or rinse glassware with MTBE, assemble and label the necessary glassware.

11.1.3   Calibrate the pH meter, using a two point procedure with pH 7 and pH 2 buffers.

11.1.4   Shake sample to ensure homogeneity, measure out 250 mL of sample using a 250 mL graduated cylinder and place in a 400 mL beaker.

11.1.5   Spike all samples with 100 µL (40 µg/L spike) of the surrogate spiking solution (Section 7.15) using a 100 or 250 µL gas tight syringe. Spike the QA/QC sample with matrix spike solution (Section 7.19) at three to five times the background level of the matrix or at five times the lower calibration level, if no analytes are detected.

11.1.6   While stirring the sample with a magnetic stir bar, add 10 mL ascorbate solution (Section 7.6), then adjust the sample to pH 5.0 ±0.2 by dropwise addition of 1:3 H_2SO_4. Transfer the sample to a 500 mL separatory funnel.

11.1.7   Add 60 mL of MTBE to the beaker, swirl, then add to the sample in the separatory funnel.

11.1.8   Stopper the separatory funnel and shake *vigorously* with frequent venting for a minimum of two minutes.

11.1.9   Allow the organic and aqueous phases to separate a *minimum of ten minutes*. Drain the aqueous layer back into the beaker. Drain the organic layer into Teflon™-lined, screw-capped, 50 mL centrifuge tubes. Cap and centrifuge on high speed for two to three minutes to break emulsions.

11.1.10 Transfer the solvent layer from the centrifuge tube(s) using a disposable pipet, leaving behind the residual emulsion, into an assembled 250 mL KD or a 250 mL Erlenmeyer flask. It is often necessary to break the emulsion by stirring to free up the solvent, and additional centrifugation may also be required. Recovery of the solvent should be no less than 30 mL. It is necessary to avoid transfer of water to the receiving vessel, therefore, it may be easier to first transfer the extract to an Erlenmeyer flask and decant to the KD just prior to concentration. Storage in an Erlenmeyer flask is more practical if the extract will not be concentrated on the same day.

11.1.11 Transfer the residual emulsion and aqueous phase back into the separatory funnel. It may help to rinse the centrifuge tube with some of the aqueous sample to transfer all of the emulsion to the separatory funnel.

11.1.12 Repeat the extraction two more times using 40 mL volumes of MTBE. Rinse both the beaker and centrifuge tube with portions of the MTBE, and transfer the rinse and any remaining MTBE into the separatory funnel. Repeat steps 11.1.8 to 11.1.12, combining the organic phases.

11.1.13 Verify the calibration of the pH meter at pH 2 using a one point procedure and pH 2 buffer. Adjust the sample to pH 2.0 ±0.2 using 1:3 H_2SO_4 solution.

11.1.14 Extract as described in steps 11.1.8 to 11.1.12 two more times using 40 mL portions of MTBE, combining the organic phases. If the extract is not to be concentrated the same day, cover the flask with aluminum foil, or stopper with a ground glass stopper and store overnight in an explosion-proof refrigerator.

11.1.15 Transfer the extract, if necessary, into a 250 mL KD assembled with a 15 mL receiving tube. Be careful to avoid transferring any water to the KD apparatus. Rinse the Erlenmeyer flask twice with five to 10 mL portions of MTBE and combine in KD assembly.

11.1.16 Place one to two Teflon™ boiling chips into the assembled KD containing the extract.

11.1.17 Pre-wet the Snyder column with MTBE (~l mL).

11.1.18 Place the KD into a heated 80°C water bath and concentrate the extract until 2 to 3 mL remain in the receiving tube. *Do not concentrate to dryness.*

11.1.19 Remove the KD from the water bath. When the KD is cool, start with the Snyder column and rinse the components with MTBE, disassembling the KD until only the receiving tube remains.

11.1.20 Transfer the extract to a 15 mL Teflon™-lined screw cap culture tube using two 0.5 to 1.0 mL MTBE rinses of the collection tube. Do not concentrate

further until derivatization is to be performed. Cap and store the extracts in the explosion-proof refrigerator.

11.2   Ethylation of samples and standards

11.2.1   Allow the prepared calibration standards and/or the concentrated sample extract(s) in culture tubes to reach room temperature.

11.2.2   Allow the diisopropylethylamine and TEOTFB to warm to room temperature.

11.2.3   Add a boiling chip to the culture tube and concentrate the extract to approximately 0.5 mL using a hot water bath at 80°C. *Do not permit the extract to concentrate to dryness*. Cool to room temperature.

11.2.4   Add 1 mL of the diisopropylethylamine to the centrifuge tube using an autopipet.

11.2.5   Add 1 mL of the TEOTFB to the centrifuge tube using an autopipet. Cap immediately and shake for 30 seconds. Proceed immediately to the next step.

11.2.6   Autopipet 1 mL of saturated KCl solution to the centrifuge tube, cap and shake for 30 seconds.

11.2.7   Add 2 mL of hexane using an autopipet to the extract, cap and shake for one minute.

11.2.8   Centrifuge, if necessary, to break up emulsions, and transfer the extract to a graduated conical tube using a pasteur pipet.

11.2.9   Repeat steps 11.2.7 and 11.2.8 two more times. Calibration standards do not require clean-up. Continue with steps 11.2.10 to 11.2.13 when preparing calibration standards. Most sample extracts require clean-up. Proceed to Section 11.3. Do not concentrate the sample extracts until clean-up procedures are going to be performed. Extracts can be stored for 14 days prior to clean-up.

11.2.10   Add 200 µL of the Ethanox solution to the calibration standard extracts.

11.2.11   Add a boiling chip and concentrate the standard extract to 0.5 mL using a hot water bath. The DCM in the extract from derivatization will evaporate off more rapidly than the hexane. Therefore, it will be necessary to slowly lower the tube, a little at a time, into the 80°C bath as the standard is concentrated. *Do not concentrate to dryness*.

11.2.12   Spike the concentrated calibration standards with 25 uL of the current working internal standard solution, either methyl-o-methyl podocarpate or propyl dehydroabietate (Section 7.17). Gently mix by tapping the conical tube or by vortex mixing.

11.2.13   Transfer the calibration standard into a 2 mL auto injection vial.

11.3   **Preparation of clean-up columns**

11.3.1   Gently push a small plug of filter pulp to the bottom of the column (Section 6.3.11). Mark the column using a marking pen at 5 and 6 cm above the filter plug.

11.3.2   Dry pack the column with 5 cm of the activated silica gel (Section 7.11), gently tapping the column sides while packing.

11.3.3   Add 1.0 cm of powdered anhydrous sodium sulfate to the top of the silica gel.

11.3.4   The columns can be used immediately or stored in the drying oven at 130°C until needed. Prepare only the columns which will be used for an analytical batch of samples to ensure consistency from batch-to-batch of the activated silica.

11.4   **Clean-up procedure**

11.4.1   Add a Teflon™ boiling chip to the sample extract and concentrate to 0.5 mL using a hot water bath. The DCM in the extract from derivatization will evaporate more rapidly than the hexane. Therefore, it will be necessary to slowly lower the tube, a little at a time, into the 80°C bath as the extract is concentrated to prevent bumping. *Do not concentrate to dryness.*

11.4.2   Remove the pre-packed column from the oven and allow it to cool to the touch, use immediately. Do not allow the column to stand at room temperature for extended periods prior to use.

11.4.3   Add 20 ul of the color indicator solution (Section 7.12) to the concentrated hexane extract of the derivatized sample.

11.4.4   Place a 15 mL screw-cap culture tube as a receiver under the column. Pre-elute the column by placing approximately 1 to 2 mL of hexane on the column.

11.4.5   Just prior to exposure of the sodium sulfate layer to air, transfer the sample to the column. Use two washes of 0.2 to 0.3 mL of hexane for quantitative transfer.

11.4.6   Rinse the sample tube with 0.2 to 0.3 mL 95:5 hexane to MTBE, adding it to the column just prior to the exposure of the sodium sulfate layer. Fill the column reservoir with the 95:5 hexane/MTBE mix after this rinse.

11.4.7   Just before the azulene (purple-blue band) reaches the bottom of the column, replace the culture tube with a graduated conical centrifuge tube. Continue to collect the eluant until the Sudan I (orange band) begins to elute from the

column. Replace the graduated tube with the original screw-cap culture tube to collect the remaining solvent. Expect to collect between 6 and 10 mL of sample.

11.4.8   Add 200 uL of Ethanox solution to the extract and a Teflon™ boiling chip, concentrate in a 80°C hot water bath to 0.5 mL. *Do not concentrate to dryness*. Add 25 uL of the current internal standard spiking solution of methyl-o-methylpodocarpate acid *or* propyl dehydroabietate (Section 7.17). Gently mix by tapping the tube or by vortex mixing. Transfer the sample extract into an auto injection vial.

12.0   Data Analysis and Calculations

12.1   GC/FID data analysis

12.1.1   The clean-up procedure is necessary when analyzing samples using the FID to remove any potential interferences from the matrix. Confirmation of the presence of an analyte by GC/MS should be performed whenever a new matrix is tested, and at a laboratory established frequency to verify correct identification of the analyte for routine analyses.

12.1.2   The analytes are identified by comparison of the relative retention times established in the calibration to the retention times in the sample. The sample component relative retention time (RRT) must fall within ±0.06 RRT units of the RRT of the standard component.

12.1.3   Calculate the sample concentration, using the internal standard response factors established in Section 10.4, according to the Equation 5.

Equation 5

Concentration (ug/L) = (A_s x C_{IS})/(A_{IS} x RRF_a)

Where:
A_s = *Area of the peak of the target analyte*
C_{IS} = *Concentration of the internal standard (ug/L)*
A_{IS} = *Area of the internal standard peak*
RRF_a = *Relative response factor of the target analyte (Section 10.4)*

12.2   GC/MS data analysis

12.2.1   The clean-up procedure is necessary when analyzing samples using the FID to remove any potential interferences from the matrix. Clean-up steps, although not required for GC/MS analyses, are recommended to reduce the frequency of instrument maintenance, such as replacing injection port liners, and preserving column life. The GC/MS procedure may be used either for routine analyses or as confirmation of FID analyses.

12.2.2   The analytes are identified by comparison of the relative retention times established in the calibration to the retention times in the sample and when all characteristic m/z signals stored for the reference spectra are present and maximize within the same two consecutive scans.

12.2.3   The sample component relative retention time (RRT) must fall within ±0.06 RRT units of the RRT of the standard component. The RRT should be assigned by using the EICP's for ions unique to the component of interest. Relative retention time guidelines, based on propyl dehydroabietate are given in Table 6.

12.2.4   The m/z's present in the mass spectrum from components in the samples that are not present in the reference spectrum should be accounted for by contamination or background ions. If the experimental mass spectrum is contaminated, or if identification is ambiguous, the analysis and/or sample extraction, ethylation, and clean-up should be repeated.

12.2.5   Calculate the sample concentration, using the internal standard response factors established in Section 10.5, according to Equation 6.

Equation 6

Concentration ($\mu g/L$) = (A_s x C_{IS})/(A_{IS} x RRF_a)

Where:

A_s = *Area of the characteristic ion of the target analyte*
C_{IS} = *Concentration of the internal standard ($\mu g/L$)*
A_{IS} = *Area of the characteristic ion of the internal standard peak*
RRF_a = *Relative response factor of the target analyte (Section 10.5)*

12.3   Data review requirements

12.3.1   The data is reviewed for accuracy of the identification, GC problems, interferences, and bias. Any problems should be corrected prior to reporting of analytical results.

12.3.2   All the chromatograms are manually reviewed to confirm internal standard and analyte identification and area integrations. As part of this review, the analyst assesses whether or not the concentration is within the calibration range of the instrument. The analyst should determine if the level of interferences and baseline noise can be corrected with dilution of the extract. Low recoveries of surrogates and high baseline noise are often indicators that the matrix affected

the clean-up column profile. For samples containing high levels of resin and fatty acids, this can often be corrected by diluting the sample, re-extracting and repeating the clean-up procedures.

12.3.3   Any inconsistencies between replicate analyses are resolved (i.e., if a compound is detected in one replicate and not the other), and attempts are made to determine the reason.

12.3.4   A report is generated that includes the retention time of the compound, the area of the compound (FID) or the area of the characteristic ion (GC/MS), and the calculated concentration of the target compound detected in µg/L.

12.4   Data reporting requirements

12.4.1   Report results in µg/L to two significant figures.

12.4.2   Report all corresponding blanks, replicates, and matrix spike recoveries for each analytical batch of samples.

13.0   Method Performance

13.1   Single laboratory method validation studies were performed during the development of the method and are reported in NCASI Technical Bulletin 501. The method performance data for FID analyses presented in Section 17, Table 3 is from Technical Bulletin 501. Following changes to the method, additional monitoring of QA/QC parameters were performed for GC/MS analyses. The results of the matrix spike recovery data are presented in Section 17, Table 4.

14.0   Pollution Prevention

14.1   The laboratory should check with state and local requirements to determine if pollution prevention equipment, such as solvent recovery devises, are required or recommended in their area. Use of these devises to reclaim solvents can be part of a pollution prevention program to reduce air emissions.

15.0   Waste Management

15.1   It is the responsibility of the laboratory to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing releases into the environment. Compliance with all sewage discharge permits and regulations is also required.

15.2   Recommendations for sample and waste handling

15.2.1   The pH 2 adjusted aqueous phase from extractions must be neutralized prior to disposal down the drain with large amounts of water.

15.2.2   Waste solvents must be handled under federal, state and local guidelines for proper disposal of flammable and chlorinated solvents.

15.2.3   The solid waste from the silica gel clean-up is considered a flammable waste and should also be handled in accordance with federal, state, and local guidelines for proper disposal.

15.2.4   For further information on waste management, the EPA recommends consulting "The Waste Management Manual for Laboratory Personnel," and "Less is Better: Laboratory Chemical Management for Waste Reduction." Both are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC, 20036.

16.0   References

16.1   National Council for the Paper Industry for Air and Stream Improvement, Inc. (NCASI). 1986. *Resin and fatty acids by extraction/ethylation GC/FID and GC/MS determination, Method RA/FA 85.01*. Technical Bulletin No. 501. Research Triangle Park, NC: National Council of the Paper Industry for Air and Stream Improvement, Inc.

17.0   Tables and Validation Data

Table 1 Estimated FID and GC/MS Method Detection Limits[a]

Compound	GC/FID MDL(µg/L)	GC/MS MDL(µg/L)
Oleic Acid	2.2	4.2
Linoleic Acid	0.8	0.8
Pimaric Acid	0.8	3.3
Sandracopimaric Acid	1.3	0.9
Isopimaric Acid	0.8	3.0
Palustric Acid	1.1	2.6
Dehydroabietic Acid	1.7	8.5
Abietic Acid	4.7	9.6
Neoabietic Acid	2.3	0.7
14-Chlorodehydroabietic Acid	2.0	10
12-Chlorodehydroabietic Acid	Na[b]	1
Dichlordehydroabietic acid	5.4	20

[a] Method detection limit estimates from RA/FA 85.01
[b] NA indicates data not available

Table 2 DFTPP Criteria for RA/FA 85.02

m/z	Ion Abundance Criteria
51	10-80% of mass 198
68	< 2% of mass 69
69	11-91% of mass 198
70	< 2% of mass 69
127	10-80% of mass 198
197	< 2% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-60% of mass 198
365	> 1% of mass 198
441	less than mass 443
442	greater than 50% of mass 198
443	15-24% of mass 442

Table 3 FID QA Matrix Spike Recovery Data[a]

Compound	Mean Percent	Standard Deviation	Relative Standard Deviation
Oleic Acid	81	12	15
Linoleic Acid	86	9.2	11
Pimaric Acid	91	15	16
Sandracopimaric Acid	88	5.1	5.8
Isopimaric Acid	96	11	11
Palustric Acid	81	8.6	11
Dehydroabietic Acid	91	6	6.6
Abietic Acid	89	5.4	6.1
Neoabietic Acid	79	7.2	9.2
14-Chlorodehydroabietic Acid	87	6.3	7.2
Dichlordehydroabietic acid	88	8.3	9.5

[a] Based on single laboratory data of 26 effluent sample determinations using RA/FA 85.01.

Table 4 GC/MS Matrix Spike Recovery Data

Compound	Mean Percent	Standard Deviation	Relative Standard Deviation
Oleic Acid	86	17	20
Linoleic Acid	94	17	18
Pimaric Acid	98	11	12
Sandracopimaric Acid	98	10	10
Isopimaric Acid	97	9.2	9.4
Palustric Acid	78	13	17
Dehydroabietic Acid	94	11	12
Abietic Acid	98	12	12
Neoabietic Acid	74	13	17
14-Chlorodehydroabietic Acid	94	6.2	6.6
12-Chlorodehydroabietic Acid	92	6.9	7.5
Dichlorodehydroabietic acid	90	6.4	7.1
Dichlorostearic Acid	98	15	15

[a] Based on single laboratory data of 42 effluent sample determinations using RA/FA 85.02.

Table 5 Relative Response Factor Criteria for Calibration Checks by GC/FID

Compound	Propyl Dehydroabeitate Mean	Range	Methyl-o-Methyl Podocarpate Mean	Range
Oleic Acid	1.32	1.21 - 1.42	1.27	1.16 - 1.36
Linoleic Acid	1.16	1.05 - 1.30	1.11	1.01 - 1.25
Pimaric Acid	1.20	1.12 - 1.30	1.15	1.08 - 1.25
Sandracopimaric Acid	1.19	1.12 - 1.26	1.14	1.08 - 1.21
Isopimaric Acid	1.18	1.11 - 1.26	1.13	1.06 - 1.21
Palustric Acid	1.14	1.05 - 1.23	1.09	1.01 - 1.18
Dehydroabietic Acid	1.12	1.06 - 1.19	1.07	1.02 - 1.14
Abietic Acid	1.14	1.05 - 1.25	1.09	1.01 - 1.20
Neoabietic Acid	1.06	0.98 - 1.16	1.02	0.94 - 1.11
14-Chlorodehydroabietic Acid	0.88	0.81 - 0.95	0.84	0.78 - 0.91
12-Chlorodehydroabietic Acid	0.90	0.83 - 0.99	0.86	0.80 - 0.95
Dichlorodehydroabietic acid	0.82	0.74 - 0.93	0.79	0.79 - 0.89
n-Heptadecanoic Acid (SS[a])	1.15	1.03 - 1.16	1.10	0.99 - 1.11
O-Methylpodocarpic Acid (SS)	1.06	1.00 - 1.14	1.02	0.96 - 1.09

[a] SS = Surrogate

Table 6 GC/MS Characteristic Quantitation Ions

Compound	Relative Retention Time	Quantitation Ions Primary	Secondary
Ethyl Linoleate	0.791	67	81, 308
Ethyl Oleate	0.829	55	264, 310
Ethyl Pimarate	0.903	121	257, 330
Ethyl Sandracopimarate	0.912	121	257, 330
Ethyl Isopimarate	0.928	241	256, 330
Ethyl Palustrate	0.935	241	315, 330
Ethyl Dehydroabietate	0.951	239	328
Ethyl Abietate	0.971	256	241, 330
Ethyl Neoabietate	1.009	135	330
Ethyl Dichlorostearate	1.037	88	101, 262
Ethyl 14-Chlorodehydroabietate	1.049	273	275
Ethyl 12-Chlorodehydroabietate	1.065	273	275
Ethyl Dichlorodehydroabietate	1.148	307	309
Ethyl Heptadecanoate (SS[a])	0.791	88	101, 298
Ethyl-O-Methylpodocarpate (SS)	0.940	227	316
Propyl Dehydroabietate (IS[b])	1.00	239	240
Methyl-O-Methyl Podocarpate (IS)	0.908	227	302, 171

[a] SS = Surrogate
[b] IS = Internal Standard