Resin and Fatty Acids in Water – PBM

Parameter Resin and Fatty Acids

Analytical Method Solvent extraction, methylation, florisil cleanup, GC/MS

Introduction This method is applicable to the quantitative determination of resin and fatty acids in water.

Resin and fatty acids are naturally carboxylic acids. Resin acids are a primary component of tree pitch (together with terpenes). Fatty acids are ubiquitous natural products with many common sources, particularly vegetable oils and animal fats, where they occur primarily within triglycerides. The majority of environmental testing for resin and fatty acids in BC is conducted for pulp mill effluents and wastewaters. There are no current CSR standards for resin or fatty acids, but BC Working Water Quality Guidelines exist for Total Resin Acids. Resin acids are toxic to freshwater aquatic life, with increasing toxicity at lower pH levels.

Method Summary Water samples are pH-adjusted and extracted with MTBE (methyl-t-butyl ether). Extracts are concentrated and derivatized with diazomethane (or equivalent) to produce the corresponding methyl ester derivatives, or other suitable derivatives. If required, extracts are cleaned-up by florisil adsorption column chromatography. The derivatives are analyzed by GC/MS or by an alternative mass spectrometric detection technique. LC/MS or preferably LC/MS/MS may alternatively be used (if performance criteria are met), which avoids the requirement for derivatization.

This method is performance-based. Laboratories may adopt alternative options to improve performance or efficiency provided that all stated performance requirements and prescribed (mandatory) elements are met.

MDL(s) and EMS Analyte Codes

Analyte	CAS #	Approx. MDL (mg/L)	EMS Analyte Code
Resin Acids:			
Abietic Acid	514-10-3	0.001	A030
Chlorodehydroabietic Acid, 12 + 14-	57055-38-6	0.002	C050
Chlorodehydroabietic Acid, 12-	65310-45-4	0.001	C048
Chlorodehydroabietic Acid, 14-	65281-76-7	0.001	C049
Dichlorodehydroabietic Acid, 12,14-	57055-39-7	0.001	D053
Dehydroabietic Acid	1740-19-8	0.001	D052
Isopimaric Acid	5835-26-7	0.001	I004
Levopimaric Acid	79-54-9	0.001	L003
Neoabietic Acid	471-77-2	0.001	N005
Pimaric Acid	127-27-5	0.001	P025
Sandaracopimaric Acid	471-74-9	0.001	S006
Total Resin Acids (BC WWQG)*	n/a	0.003	0128
Fatty Acids:			
Arachidic Acid	506-30-9	0.005	FA07
Behenic Acid	112-85-6	0.005	FA08
Lauric Acid	143-07-7	0.005	FA01
Lignoceric Acid	557-59-5	0.005	FA09
Linoleic Acid	60-33-3	0.005	FA05
Linolenic Acid	463-40-1	0.005	FA10
Myristic Acid	544-63-8	0.005	FA02
Oleic Acid	112-80-1	0.005	FA11
Palustric Acid	1945-53-5	0.02-0.05	FA12
Palmitic Acid	57-10-3	0.02-0.05	FA03
Stearic Acid	57-11-4	0.02-0.05	FA06

*Total Resin Acids (for purposes of the BC Working Water Quality Guidelines) is defined as the sum of abietic acid, neoabietic acid, pimaric acid, isopimaric acid, and sandaracopimaric acid.

EMS Method Code(s)

Resin and Fatty Acids by GC/MS: **P033**

***Refer to EMS Parameter Dictionary on the ministry website for all current EMS codes.

Matrix

Freshwater, Wastewater, Marine Water

Interferences and Precautions

a) Interferences may result from contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to artifacts and/or elevated baseline. All materials used should be routinely monitored and demonstrated to be free of interferences under the conditions of the analysis.

b) Matrix interferences may be caused by contaminants that could be co-extracted from the sample. The extent of the matrix interferences will vary from source to source. Mass spectrometric detection reduces or eliminates most common interferences.

c) Fatty Acids are ubiquitous, and trace contaminant levels of Fatty Acids are almost unavoidable, particularly for Palmitic, Stearic, and Palustric Acids. Common contaminant sources of fatty acids include soaps, detergents, and natural fats and oils. MDLs achievable for these substances are higher than for other Fatty Acids for this reason.

d) Recoveries for Neoabietic acid and Levopimaric acid can be highly variable due to isomerization reactions, especially at low pH.

e) Diazomethane is explosive and mutagenic. Generation and all handling of diazomethane must be conducted in a fume hood with appropriate safety precautions.

f) High purity resin acid reference standards may be difficult to source. Calibration standard reference values should be corrected for purity if < 96% (as per guidance from US EPA Method 8270E).

Sample Handling and Preservation

Container: Amber Glass containers with PTFE liners are required.

Preservation: Preserve samples with 0.5g/L Ascorbic Acid + 0.4g/L NaOH for 14 day hold time. A 7 day hold time applies to unpreserved samples.

Holding Time: Preserved: 14 days. Unpreserved: 7 days. Extracts: 40 days.

Storage: Chill to ≤ 10°C immediately after sampling and during transit to the laboratory. In the laboratory, samples must be refrigerated at ≤ 6°C. Avoid freezing to prevent sample breakage.

Procedure

Apparatus:

a) Separatory funnels, 1000 mL or as required.
b) 4-7 mL disposable glass extract vials.
c) Round bottom flasks, 500mL, 250 mL.
d) Diazomethane generator (Diazald kit, Sigma-Aldrich Z100250-1KT).
e) Rotary evaporator or equivalent.
f) Glass filter funnels (75 mm).
g) Glass chromatography column, 30 cm X 1.4 cm, with 150 mL reservoir.

Reagents:

a) Solvents, glass distilled, pesticide grade;
   1. MTBE (methyl-t-butyl ether)
   2. Petroleum ether or Hexanes
   3. Diethyl ether
   4. Hexanes (described as hexane)
b) Sulfuric acid, conc. (36 N) and 10% v/v, pre-extracted with hexane prior to use.
c) Sodium sulfate, anhydrous, granular, reagent grade, heat treated to 450-650°C.
d) Sodium hydroxide (aq): 10% w/v.
e) Florisil, pesticide residue grade, heat treated at 450-650°C, then deactivated with 1% water (w/w). For optional extract cleanup step.
f) Glass wool, solvent rinsed or heat treated at 450-650°C.
g) Diazald (N-methyl-N-nitroso-p-toluenesulfonamide) for diazomethane generation.
h) Diazomethane, ethereal alcoholic solution. Prepare as per instructions in Aldrich Technical Information Bulletin A180. Diazomethane is mutagenic and potentially explosive. Follow all listed safety precautions. Prepare in a fume hood with safety

shield. Never use ground glass joints to prepare diazomethane, due to spark hazards. Store ethereal alcoholic diazomethane solution over ether-washed NaOH in a glass bottle with Teflon lid (not ground glass).

Extraction Procedure:

a) Shake sample well before subsampling and accurately measure desired sample amount for extraction (typically 250 - 500 mL) into a separatory funnel.
b) Check sample pH. If required (e.g. for preserved samples), adjust pH to 6.5 – 7.5 with dilute (e.g. 10%) H_2SO_4 or NaOH.
c) Spike each sample with a known amount of one or more suitable surrogate compounds (e.g. d23-lauric acid, d35-stearic acid).
d) Extract each sample three times with 100 mL of MTBE. A higher ratio of solvent:sample helps to reduce emulsions in effluent samples.
e) Filter MTBE extracts through sodium sulfate, supported by glass wool in a glass funnel, collecting into a 500 mL round bottom flask.
f) Concentrate combined extracts to 1-2 mL using a rotary evaporator or equivalent.
g) Quantitatively transfer extract to a 4-7mL glass vial using ~ 1 mL portions of MTBE or methanol for rinsing.

Methylation Procedure:

a) Immediately prior to the methylation procedure, reduce volume just to dryness by nitrogen blowdown.
b) Dissolve extract in 1-4 mL of methanol.
c) Slowly add ethereal alcoholic diazomethane solution until a strong yellow colour persists in the extract.
d) Let stand in a fume hood for a minimum of 30 minutes.
e) If yellow colour does not persist for 30 minutes, re-concentrate to < 1 mL by nitrogen blowdown, and repeat derivatization procedure.
f) Remove residual diazomethane by concentrating just to dryness with a gentle stream of nitrogen.
g) Reconstitute extract with ~ 1 mL of hexane (or with an alternative desired instrumental solvent if cleanup is not required).

Florisil Cleanup (Optional):

a) If extract clean-up is required, fractionate on a chromatographic column containing 10g of 1% deactivated florisil topped with 1-2cm anhydrous sodium sulfate, as follows:
    1. Quantitatively transfer hexane extract to column.
    2. Elute with 100 mL of petroleum ether or hexanes (discard).
    3. Elute with 100 mL of 2% ethyl acetate in petroleum ether or hexanes. This fraction contains the resin and fatty acids.
b) Reduce the solvent to 1-2 mL using a rotary evaporator or equivalent and quantitatively transfer to a 4-7 mL glass vial.

Preparation of Extract for Analysis:

a) Add a quantitatively accurate amount of one or more suitable internal standards (e.g. deuterium labelled PAHs).
b) Blow down with nitrogen or dilute to an exact final volume (e.g. 1.00 – 2.00 mL).
c) Transfer to an autosampler vial.
d) Analyze by GC/MS.

Instrumental Analysis:

Detailed instrumental procedures are not provided in this method. The procedures described in the following reference are suitable for general guidance for GC/MS:

- USEPA Method 8270E, “Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)”, Revision 6, June 2018.

GC/MS or LC/MS techniques must be used. Selective ion monitoring (SIM) mode is commonly employed to achieve lower detection limits.

Gas chromatography is recommended (but not prescribed) to be performed using a 30 m x 0.25 or 0.32 mm ID capillary column with 0.25, 0.5, or 1 μm film thickness of 100% dimethyl polysiloxane phase (e.g. DB1 or equivalent). The recommended column achieves separation of all the target compounds, as methyl esters.

A minimum five-point initial calibration over the desired working range is required.

The use of one or more internal standards is required. Internal standards greatly improve method precision. Deuterium labeled PAHs are recommended (e.g. anthracene-d10, benzo(a)pyrene-d12, d10-Pyrene, d12-Chrysene etc.). Internal standards must not introduce significant interferences on test analytes or surrogates.

Analysis and Calculation of Methylated Resins and Fatty Acids

For GCMS data acquisition and calculations, use quantitation and qualifier ions as indicated in the table below.

Recommended Quantitation and Qualifier Ions

Compound	*Category*	*Quant Ion (m/z)*	*Qualifier1 (m/z)*	*Qualifier2 (m/z)*
Myristic acid	Fatty Acid (methyl ester)	242	199	143
Palmitic acid	Fatty Acid (methyl ester)	270	227	143
Oleic acid	Fatty Acid (methyl ester)	296	264	222
Linolenic acid	Fatty Acid (methyl ester)	292	236	261
Linoleic acid	Fatty Acid (methyl ester)	294	263	262
Stearic acid	Fatty Acid (methyl ester)	298	255	199
Arachidic acid	Fatty Acid (methyl ester)	326	283	143
9,10-Dichlorostearic acid	Fatty Acid (methyl ester)	262	294	263
Behenic acid	Fatty Acid (methyl ester)	354	311	199
Lauric Acid	Fatty Acid (methyl ester)	183	171	143
Lignoceric Acid	Fatty Acid (methyl ester)	339	382	283
Pimaric acid	Resin Acid (methyl ester)	316	257	180
Sandaracopimaric acid	Resin Acid (methyl ester)	316	257	180
Isopimaric acid	Resin Acid (methyl ester)	241	257	301
Palustric acid	Resin Acid (methyl ester)	301	241	185
Levopimaric acid	Resin Acid (methyl ester)	146	181	134
Dehydroabietic acid	Resin Acid (methyl ester)	239	299	314
Abietic acid	Resin Acid (methyl ester)	316	241	256
Neoabietic acid	Resin Acid (methyl ester)	316	135	148
14-Chlorodehydroabietic acid	Resin Acid (methyl ester)	273	275	348
12-Chlorodehydroabietic acid	Resin Acid (methyl ester)	273	275	348
12,14-Dichlorodehydroabietic acid	Resin Acid (methyl ester)	307	309	382

Recommended Options for Surrogates & Internal Standards:

o-Methylpodocarpic Acid	Surrogate (methyl ester)	302	227	
d23-Lauric Acid	Surrogate (methyl ester)	237	187	
d35-Stearic Acid	Surrogate (methyl ester)	333	283	
Tricosanoic Acid	Surrogate (methyl ester)	368	325	143
Butyl Stearate	Internal Std	285	56	
Methyl Heneicosanoate	Internal Std	340	297	143
d10-Pyrene	Internal Std	212	211	
d12-Chrysene	Internal Std	240	241	

Performance Requirements

Any analytical method options selected for this analysis must meet or exceed the performance requirements specified below.

Accuracy and Precision requirements are distinct from daily QC requirements and apply to measures of long-term method performance (averages and standard deviations). Achievement of these requirements is to be demonstrated during initial and ongoing method re-validation studies. For Initial Validations, averages of at least 8 Lab Control Samples or RMs must be assessed. Ongoing Re-validations (performance reviews) should assess QC data encompassing longer timeframes (e.g. 6 months to 1 year). A

minimum frequency of 2 years is recommended for Ongoing Re-validations.

Accuracy Requirement: Laboratories must demonstrate method accuracy (measured as average recovery) of 70-130% (60-140% for levopimaric and neoabietic acid) for Lab Control Samples or Certified Reference Materials at concentrations above ten times the MDL.

Precision Requirement: Laboratories must demonstrate method precision equal to or better than 20% relative standard deviation (25% for levopimaric acid and neoabietic acid) for clean matrix spikes at concentrations above ten times the MDL.

Sensitivity Requirement: Where possible, the method should support Reporting Limits (and MDLs) that are less than 1/5 of applicable numerical standards. The method is not fit-for-purpose if an MDL exceeds a guideline, standard, or regulatory criteria against which it will be used for evaluation of compliance.

Quality Control

Summary of QC Requirements

QC Component	Minimum Frequency	Minimum Data Quality Objectives
Calibration Verification Standard (CVS) – 2nd source	1 per Initial Calibration	80 - 120%
Continuing Calibration Verification (CCV)	At least every 12 hours (max 20 samples), and at end of each batch.	80 - 120% for mid-level standards
Method Blank (MB)	One per batch (max 20 samples)	Less than reported DL
Lab Control Sample (LCS)	One per batch (max 20 samples)	60-140% Levopimaric acid 30-140% Neoabietic acid 30-140% Palustric acid 30-140%
Lab Duplicate (DUP)	One per batch (max 20 samples)	50% RPD [or within 2x reported DL for low level results]
Matrix Spike (MS)	One per batch (max 20 samples)	50-140% Levopimaric acid 20-140% Neoabietic acid 20-140% Palustric acid 20-140%
Surrogate Compounds (e.g. d23-lauric acid, d35-stearic acid)	All samples	60-140% Not applicable where valid surrogate recoveries cannot be obtained due to interferences.

If DQOs are not met, repeat testing or report qualified test results. DQOs do not apply to MS results where sample background exceeds spike amount.

Prescribed Elements

The following components of this method are mandatory:

1. Analysis must utilize mass spectrometric detection. Permitted techniques include GC/MS (single quadrupole, triple quadrupole, or high resolution magnetic sector) or LC/MS (single or triple quadrupole). Procedures described in this method are applicable to GC/MS analysis. Refer to ECCC Method for Resin Acids by LC/MS/MS for further guidance on analysis by LC/MS techniques. At least one qualifier ion per analyte must be monitored (two recommended where possible).
2. Initial calibrations must include at least five points.
3. Derivatization (typically methylation or ethylation) is required for Gas Chromatographic methods. Numerous alternative derivatization procedures are described in literature.
4. The use of one or more internal standards is required.
5. All Sample Handling and Preservation Requirements, Performance Requirements, and Quality Control requirements must be met.
6. Where Total Resin Acids or Total Fatty Acids are reported, laboratory reports must

indicate which substances are included in the sum.

Apart from these limitations, and provided performance requirements are met, laboratories may introduce modifications to this method in order to improve quality or efficiency.

References

1. Alberta Environment AE129.0, Resin and Fatty Acids in Pulp Mill Effluents and Receiving Waters, Alberta Environment, August 1990 (reference for preservatives and recommended extraction pH).
2. Environment Canada, Pacific and Yukon Region Laboratories, Resin Acids, Gas Chromatographic / Mass Spectrometric Method, v 2.3, August 1990.
3. RA/FA-85.02 Resin and Fatty Acids by Extraction/Ethylation GC/FID and GC/MS Analysis, NCASI West Coast Regional Center Organic Analytical Program, March 1997.
4. Environment and Climate Change Canada (ECCC), Pacific and Yukon Laboratory for Environmental Testing, Standard Operating Procedure for Resin Acids by Liquid Chromatography Pseudo Triple Quadrupole Mass Spectrometry, version 1.1, May 27, 2019.
5. US EPA 8270E, Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), Revision 6, June 2018.
6. US EPA 3510C, Separatory Liquid-Liquid Extraction, Revision 3, Dec 1996.
7. US EPA 3520C, Florisil Cleanup, Revision 4, July 2014.
8. Aldrich Technical Information Bulletin A180, Diazald® and Diazomethane Generators, Sigma-Aldrich, 2007.

Revision History

Jun 23, 2021	Method revised to PBM format, with new requirement for mass spectrometric detection (GC/MS or LC/MS) to replace legacy GC-FID method. Florisil cleanup is normally not required with MS detection (optional). Changed recommended extraction pH from <2 to 6.5-7.5 and deleted acid treatment of sodium sulfate and glass wool to minimize isomerization of neoabietic acid and levopimaric acid as per AE 129.0. Changed recommended extraction solvent from DCM to MTBE as per AE 129.0 and NCASI Method RA/FA-85.02. QC Requirements updated with DQOs. Preservation and hold time requirements updated to reflect newer BC ENV Sample Preservation and Holding Time Requirements (ascorbic acid used as anti-oxidant, NaOH prevents isomerization). CAS numbers added. Definition of Total Resin Acids (for BC WWQG) added. MDLs for fatty acids revised to reflect more realistic values with consideration of background. New method references added. Added guidance to correct standards for purity if <96% pure. Upon director approval, this method will supersede and replace method “Resin and Fatty Acids in Water” from the 2020 BC Lab Manual.
Dec 31, 2000	SEAM codes replaced by EMS codes. Requirement for NaOH preservative removed as suggested by PESC and confirmed by the BCQAAC Technical Subcommittee.
Feb 14, 1994	Publication in 1994 Laboratory Manual.