NCASI METHOD NSV-99

NEUTRAL SEMIVOLATILE COMPOUNDS BY DICHLOROMETHANE EXTRACTION AND GC/MS

NCASI
West Coast Regional Center
Organic Analytical Program
November 1999

Acknowledgments

This method was prepared by Diana Cook, Research Chemist, and Nikki Frum, Research Associate, at the NCASI West Coast Regional Center. Other assistance was provided by Dean Hoy, Jon Jones, and Angela Parrish, Research Associates at the NCASI West Coast Regional Center.

For more information about this method, contact:

Diana Cook
Senior Research Scientist
NCASI West Coast Regional Center
P.O. Box 458
Corvallis, OR 97339
(541) 752-8801
dcook@ncasi.org

Robert Fisher, Ph.D.
Vice President, Health Effects
NCASI
P.O. Box 13318
Research Triangle Park, NC 27709-3318
(919) 558-1989
rfisher@ncasi.org

For information about NCASI publications, contact:

Publications Coordinator
NCASI
P.O. Box 13318
Research Triangle Park, NC 27709-3318
(919) 558-1999
publications@ncasi.org

National Council for Air and Stream Improvement, Inc. (NCASI). 2000. *Methods Manual - Neutral semivolatile compounds by dichloromethane extraction and gas chromatography/ mass spectrometry*. Research Triangle Park, NC: National Council for Air and Stream Improvement, Inc.

Disclaimer:

The mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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; therefore, those electing to apply the method are strongly encouraged to conduct rigorous QA/QC or validation so that the quality of the data generated can be evaluated. Proposed methods are not suitable for use as regulatory monitoring or compliance methods.

NCASI METHOD NSV-99

NEUTRAL SEMIVOLATILE COMPOUNDS BY DICHLOROMETHANE EXTRACTION AND GC/MS

1.0 Scope and Application

1.1 This method is designed to determine the concentration of neutral semivolatile compounds in wastewater treatment plant influents and biologically treated effluents. This method involves liquid/liquid extraction of the analytes at pH 11 using dichloromethane (DCM) and quantification by gas chromatography/mass spectrometry (GC/MS). Quantitation is accomplished using internal standard calibration with tridecane. Each sample is spiked with 2-acetyl-3-methylthiophene and p-chloroacetophenone to determine surrogate recovery.

1.2 These compounds can be confirmed or quantitated by GC/MS using NCASI NSV-99:

Compound	CAS Registry Number
2,3-Dimethylcyclopentenone	1121057
2,3,5-Trimethylcyclopentenone	28790865
Acetophenone	98862
3-Acetylthiophene	1468833
2-Acetylthiophene	88153
Dichlorodimethyl sulfone	37557963
2,3,4,5-Tetramethylcyclopentenone	54458616
Fenchyl alcohol	1632731
Camphor	464482
Terpinen-4-ol	562743
2-Propionylthiophene	13679759
p-Cymen-8-ol	1197019
alpha-Terpineol	10482561
Verbenone	1196016
Piperitone	89816

1.3 This method has been validated at the single laboratory level in wastewater treatment plant influents and in biologically treated effluents from kraft, thermomechanical (TMP), groundwood, deink, semi-chemical, and recycle pulp and paper mills. Demonstration of extraction efficiency and method performance for specific matrix types is recommended.

1.4 The estimated method detection limits (MDLs) were determined as specified in 40 CFR 136 Appendix B (1) using a biologically treated final effluent sample from a kraft mill producing bleached softwood pulp. The calculated method detection limits are listed in Section 17.0, Table 1. These values are provided as guidance. Due to improvements in instrumentation and changes in matrix effects, each laboratory should establish its own MDLs. The lower instrument calibration limit (LCL) for the target analytes is approximately 1 µg/L. The concentration range is from 1 to 100 µg/L.

1.5 The GC/MS portions of this method are for use only by analysts experienced with capillary GC/MS or under the close supervision of such qualified persons. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 Summary of Method

2.1 Biologically treated effluents

A 250-mL aliquot of pH 2 preserved effluent is fortified with p-chloroacetophenone and 2-acetyl-3-methylthiophene as surrogates and adjusted to pH 11 by the addition of a 10% sodium hydroxide (NaOH) solution. One gram of sodium chloride (NaCl) is added to the separatory funnel and the sample is shaken for one minute to dissolve the sodium chloride. The sample is then extracted three times with 40 milliliters of DCM. The combined extracts are concentrated and dried using sodium sulfate (Na_2SO_4). After the addition of 500 µL of cyclohexane, the extract is concentrated to a final volume of 500 µL. N-Tridecane is added as the internal standard and the extract is analyzed by GC/MS.

2.2 Wastewater treatment plant influents

A 125-mL aliquot of pH 2 preserved effluent is fortified with p-chloroacetophenone and 2-acetyl-3-methylthiophene as surrogates, diluted to 250 mL with deionized water, and adjusted to pH 11 by the addition of a 10% NaOH solution. One gram of NaCl is added and the sample is extracted three times with 40 milliliters of DCM. The combined extracts are concentrated and dried using Na_2SO_4. After the addition of 500 µL of cyclohexane, the extract is concentrated to a final volume of 500 µL. N-Tridecane is added as the internal standard and the extract is analyzed by GC/MS.

2.3 Quantitative analysis

The neutral semivolatile compounds are introduced into the GC with a narrow bore fused silica capillary column. The GC column is temperature programmed to separate the target analytes, which are then detected with a MS interfaced to the GC. Identification of target analytes by mass spectrometry is accomplished by comparing their mass spectra to that of an authentic standard. A compound is identified when its relative retention time and mass spectrum meet the criteria described in Section 12.0.

2.4 Quality assurance

Quality is assured through reproducible calibration and testing of the extraction and GC/MS system. A method blank is analyzed with each sample set (samples started through the extraction process on a given day, to a maximum of 20), along with a sample duplicate and a matrix spike to ensure quality data. In samples containing low concentrations of the target analytes, a matrix spike and matrix spike duplicate may provide a better assessment of method precision. Each sample is fortified with the surrogates and the surrogate recovery is calculated to assist in assessing data quality. A complete description of quality control procedures, calculations, and method performance criteria are listed in Section 9.0.

3.0 Definitions

3.1 These definitions 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 May—this action, activity, or procedural step is neither required nor prohibited

3.1.3 May not—this action, activity, or procedural step is prohibited

3.1.4 Must—this action, activity, or procedural step is required

3.1.5 Should—this action, activity, or procedural step is suggested, but not required

3.1.6 GC/MS—gas chromatograph with a mass spectrometer

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may contribute analytical interferences resulting in misinterpretation of chromatograms. Run method blanks initially and with each subsequent sample set to demonstrate that the solvents, reagents, glassware, and other sample processing hardware are free from interferences under the conditions of the method. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

4.2 Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled.

4.3 The surrogate compounds, p-chloroacetophenone and 2-acetyl-3-methylthiophene, may be detected in some effluent samples. Therefore, samples from new sources should be analyzed without the addition of the surrogates to determine if they are present. In the event that p-chloroacetophenone and 2-acetyl-3-methylthiophene are native to the sample, a sample-specific matrix spike experiment should be performed

instead of surrogate recovery using p-chloroacetophenone and 2-acetyl-3-methylthiophene to assess the accuracy of the method for that sample.

4.4 Contamination by carryover can occur when samples containing high concentrations of the target analytes are analyzed in sequence with low concentration samples. Whenever unusually concentrated samples are encountered, they should be followed by injections of a solvent blank to check for cross contamination prior to the analysis of additional samples.

5.0 Safety

5.1 The toxicity or carcinogenicity of the compounds or reagents used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to a level protective of human health. This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses.

5.2 Dichloromethane is a halogenated liquid that may be harmful if inhaled or absorbed through the skin, and is a suspected carcinogen. Use it in a laboratory fume hood or wear an approved respirator, and avoid contact by wearing chemical-resistant gloves, eye protection, and other protective clothing.

5.3 As with all samples, precautions should be taken to avoid exposure to potentially toxic, caustic, or nuisance odor compounds. Samples should be handled with gloves and opened in a fume hood.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers 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.*

6.1 Do not use glassware with any star fractures, cracks, or severe scratches. All fittings should be snug, and clamps and springs should be in good working order. All glassware should be washed with detergent, rinsed with tap water, then rinsed with reagent-grade water. If blank contamination is observed, the glassware may be solvent rinsed or baked prior to use.

6.2 Sampling equipment

6.2.1 It is recommended that glass containers, Teflon™ tubing, or stainless steel be utilized during sample collection. Use amber glass bottles equipped with Teflon-lined screw caps to store all samples.

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

6.3 Equipment for sample extraction (per sample)

6.3.1 One 500-mL (or larger) beaker

6.3.2 One 500-mL separatory funnel with ground glass stopper and Teflon stop-cock

6.3.3 Two 50-mL centrifuge tubes with Teflon-lined caps

6.3.4 One 250-mL graduated cylinder (for each sample set)

6.3.5 One 50-mL graduated cylinder (for each sample set)

6.3.6 One magnetic stir plate

6.3.7 One Teflon-coated stir bar

6.4 Equipment for sample concentration and Na_2SO_4 drying column (per sample)

6.4.1 Two 15-mL graduated concentrator tubes (part number 8080 Pyrex™ or equivalent); a ground-glass stopper may be used to prevent evaporation of extracts

6.4.2 500-mL evaporation flask

6.4.3 Half-inch springs

6.4.4 Three-ball macro-Snyder column

6.4.5 Micro-Snyder column

6.4.6 One 5¾-inch disposable Pasteur pipette

6.4.7 One 2-mL glass autosampler vial with Teflon-lined cap

6.4.8 Teflon boiling chips

6.4.9 Analytical filter pulp (No. 289 Schleicher and Schell or equivalent)

6.5 Other apparatus

6.5.1 Hot water bath in a fume hood, capable of ±5°C temperature control, preheated to a maximum temperature of 85°C

6.5.2 pH meter calibrated using a two-point calibration procedure at pH 7 and pH 11 using pH 7 and pH 11 buffer solutions

6.5.3 Magnetic stirrer

6.5.4 Balances—an analytical balance capable of weighing to the nearest 0.1 mg with an accuracy of ±0.1 mg, and a top-loading balance capable of weighing to the nearest 10 mg with an accuracy of ±10 mg

6.5.5 Gas chromatograph—must be equipped with a mass spectrometer and a splitless injection port for capillary column, and have the capacity of running the temperature program and performance specifications outlined in Sections 9.2 and 10.1

6.5.6 Gas chromatographic column—30 ±5 m x 0.25 ±0.02 mm ID x 0.25 μm, 5% phenyl, 95% methyl (DB-5 or equivalent)

6.5.7 Mass spectrometer—70 eV electron impact ionization; must repetitively scan from 42 to 360 AMU in 0.7 to 1.3 second, and must produce a unit resolution (valley between m/z 441-442 less than 10% of the height of the 441 peak), background corrected mass spectrum from 50 ηg decafluorotriphenylphosphine (DFTPP) introduced through the GC inlet; spectrum must meet the mass intensity criteria listed in Section 9.2 and Section 17, Table 2; mass spectrometer must be interfaced to the GC via a directly coupled column with a heated transfer line per the manufacturer's specifications; all portions of the column which connect the GC to the ion source must remain at or above the oven temperature during analysis to preclude condensation of less volatile compounds

6.5.8 The gas chromatograph data system should collect and record the GC data, process and store GC/MS data, generate reports, and compute and record response factors.

7.0 Reagents and Standards

7.1 Solvents

7.1.1 Cyclohexane, dichloromethane, and acetone supplied by Burdick & Jackson, or equivalent high purity solvent suitable for gas chromatography and pesticide residue analysis; the acetone is distilled prior to use for the preparation of standards

7.1.2 Organic-free reagent water in which the compounds of interest and interfering compounds are not detected by this method; all organic-free water should be stored in glass to prevent the leaching of contaminants from plastic containers; containers must have tightly-fitting Teflon-lined caps

7.2 Standards

7.2.1 2,3-Dimethylcyclopentenone (2,3-DMCP), 3-acetylthiophene, 2-acetylthiophene, 2,3,4,5-tetramethycyclopentenone (2,3,4,5-TMCP), fenchyl alcohol, camphor, terpinen-4-ol, p-cymen-8-ol, verbenone, 2-acetyl-3-methylthiophene, p-chloroacetophenone, and n-tridecane can be purchased from Aldrich or an equivalent supplier. Use standards of the highest purity available. If the standards have a chemical purity of <98%, correct all calculations, calibrations, and matrix spikes for the difference in purity.

7.2.2 Dichlorodimethyl sulfone can be purchased from Helix Biotech, piperitone from Pfaltz & Bauer, and acetophenone from TCI. Both 2-propionylthiophene and alpha-terpineol can be purchased from Alpha Products. An equivalent supplier may replace any of the listed suppliers. If standards have a chemical purity of <96%, correct all calculations, calibrations, and matrix spikes for the difference in purity.

7.2.3 2,3,5-trimethylcyclopentenone (2,3,5-TMCP) is not commercially available; therefore synthesis is required.

7.2.4 Prepare primary standards of the target analytes (Section 1.2) in distilled acetone at a concentration of 1 mg/mL ±0.1 mg. Place the solutions into glass vials with Teflon-lined caps.

7.2.5 Prepare a working stock by transferring 2 mL of the primary standards (Section 7.2.4) of 2,3-DMCP, 2,3,5-TMCP, acetophenone, 3-acetylthiophene, 2-acetylthiophene, dichlorodimethyl sulfone, 2,3,4,5-TMCP, and fenchyl alcohol into a 25-mL volumetric flask with distilled acetone, yielding a final concentration of approximately 80 µg/mL for each component. Label this stock as working stock solution A.

7.2.6 Prepare a working stock by transferring 2 mL of the primary standards (Section 7.2.4) of camphor, terpinen-4-ol, 2-propionylthiophene, p-cymen-8-ol, alpha-terpineol, verbenone, and piperitone into a 25-mL volumetric flask with distilled acetone, yielding a final concentration of approximately 80 µg/mL for each component. Label this stock as working stock solution B.

7.2.7 Prepare each surrogate primary standard, p-chloroacetophenone and 2-acetyl-3-methylthiophene, in distilled acetone at a concentration of 1 mg/mL. Place each solution into a glass vial with a Teflon-sealed cap.

7.2.8 Prepare the surrogate working stock by diluting 2 mL of each of the primary surrogate stocks (Section 7.2.7) into a 25-mL volumetric flask with distilled acetone, yielding a final concentration of approximately 80 µg/mL per component.

7.2.9 N-Tridecane, 99+% pure, is available from Aldrich or an equivalent supplier. Prepare an internal standard working stock solution of 1 mg/mL in distilled acetone.

7.2.10 Prepare a five-point calibration curve encompassing the sample concentration range of approximately 1.0 to 100 µg/L for a 250-mL sample in the following manner. Place 500 µL of cyclohexane into a 15-mL conical tube. To prepare the 1 µg/L calibration level spike 3 µL of each working stock solution A and B (Section 7.2.5 and 7.2.6) and 3 µL of the surrogate working stock solution (Section 7.2.8) into the cyclohexane. Add an appropriate amount of acetone so that the total volume of each point is approximately 925 µL. Include a sample blank with the curve consisting of 500 µL of cyclohexane and 400 µL of acetone. Concentrate each point to 0.5 mL, add 25 µL of 1.0 mg/mL n-tridecane (Section 7.2.9) and proceed with GC/MS sample analysis as described for the samples in Section 11.5 and 12.0. Repeat this procedure using 10, 25, 125, and 300 µL of the analyte working stock solutions A and B as well as the surrogate working stock solution, to result in a five-point calibration curve. No addition of acetone will be needed for the highest calibration level.

7.2.11 Decafluorotriphenylphosphine (DFTPP) for GC/MS confirmation analyses can be purchased from Supelco or an equivalent supplier as a 25,000 µg/mL solution in DCM. Prepare a working stock solution in hexane at a concentration of 50 µg/mL. Prior to use, store in the dark in autosampler vials with Teflon-seal crimp caps.

7.2.12 Stock solutions of all standards must be stored under refrigeration (4°C). Stock solutions of all standards should be checked for signs of concentration or formation of precipitates prior to the preparation of calibration or performance test standards. Replace the stock solutions if a change in concentration is indicated by the inability to meet the criteria specified in Sections 9.2 and 10.3.

7.3 Reagents for sample preservation and pH adjustment

7.3.2 Sodium hydroxide, ACS reagent grade, is used to adjust sample pH during extraction. Prepare a 10% solution in reagent grade water by dissolving 50 grams of sodium hydroxide pellets in 500 mL of reagent grade water.

7.4 Reagent for extract drying

Sodium sulfate, 10 to 60 mesh, granular, can be purchased from Aldrich or a comparable supplier. Dry overnight at 130 to 135°C prior to use.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample collection

Collect grab samples in glass containers with Teflon-lined screw caps. 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 Sample preservation

Preserve all samples in the field by acidification, pH 2 to pH 3, using 1:4 sulfuric acid, then refrigerate. This should be done as soon as possible after sample collection. Ship samples in iced containers as quickly as possible.

8.3 Sample and extract storage

Results of storage stability studies indicate that samples and sample extracts may be stored for up to 30 days in the refrigerator (4°C) prior to analysis.

9.0 Quality Control

9.1 Each laboratory that uses this method should operate a formal Quality Assurance Program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and ongoing analyses of standards and blanks as a test of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.2 GC/MS performance and calibration verification

9.2.1 Verify the GC/MS instrument performance by conducting a DFTPP tune prior to analyzing any samples, blanks, or standards. Analyze the tune check just prior to the calibration standard analyses, and confirm that it meets the specifications listed in Section 17, Table 2.

9.2.2 Determine that the GC/MS system is operating within acceptable parameters by conducting a calibration check before each set of samples (samples started through the extraction process on a given day, to a maximum of 20) is analyzed. The calibration check involves reanalyzing one of the extracts used in the calibration curve (Section 7.2.10 and 10.2). The response factors determined for the calibration check should not deviate by more than ±15% from the average response factors determined for the calibration curve. The analytes may be sensitive to GC/MS instrument conditions such as contamination of the injection port, detector, and/or column. If the calibration check fails to meet the ±15% acceptance criterion, appropriate GC/MS maintenance is necessary. Reanalyze the calibration verification upon completion of all necessary instrument maintenance. If all recommended instrument maintenance fails to correct the calibration verification difficulties, the calibration curve should be reprepared and reanalyzed.

9.3 Frequency

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

9.4 Blanks

9.4.1 Demonstrate that the analytical system is free of contamination by preparing and analyzing a blank with each sample set. Prepare a method blank using the same procedure outlined in Section 11.0 utilizing reagent grade water for the sample.

9.4.2 If any of the compounds of interest (Section 17, Table 1) or any potentially interfering compounds are found in the blank at greater than 10% of the method detection limit or the lowest calibration limit (assuming a response factor of one relative to the internal standard tridecane for compounds not listed in Section 17, Table 1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

9.5 Surrogate recovery spikes

Spike all samples with the surrogate compounds to monitor surrogate recovery. Compute the recovery of the surrogate compound as the ratio of concentration found to concentration spiked, using Equation 1.

Equation 1

$$Percent\ recovery = \frac{Concentration\ \ found\ x\,100}{Concentration\ spiked}$$

Performance criteria for acceptable surrogate recovery as determined during a single laboratory validation of this method are presented in Section 17, Table 3. The criteria were determined by calculating the average recovery ±2 times the standard deviation of the recoveries for biologically treated effluent samples. If the recovery is greater or less than the acceptable criteria range, action should be taken to resolve the problem and the samples should be re-extracted and re-analyzed. Analyze samples from new sources without the addition of the surrogates to determine if p-chloroacetophenone or 2-acetyl-3-methylthiophene is present. In the event that p-chloroacetophenone or 2-acetyl-3-methylthiophene is native to the sample, a sample-specific matrix spike experiment should be performed instead of surrogate recovery to assess the accuracy of the method for that sample.

9.6 Matrix spikes

9.6.1 Assess the accuracy of the method by analyzing a matrix spike with each set of samples. Wastewater treatment plant influents and biologically treated effluents contain variable levels of the target analytes; for samples with a high ratio of non-detects, a duplicate matrix spike may be more appropriate. Demonstrate performance throughout the working concentration range of the method by varying the spike level of the target analyte working stock solutions A and B (Section 7.2.5 and 7.2.6) added to the sample prior to pH adjustment and extraction (Section 11.3). Adjust the amount of working stock added to the sample to give a final concentration in the sample that is a minimum of twice the native level present, and preferably three to five times the native level. Prepare the matrix spike sample in exactly the same manner as a routine sample, using the pH adjustment, extraction, and concentration procedures outlined in Section 11.0.

9.6.2 Compare the recovery of the spiked compounds with the single laboratory matrix spike recovery data reported in Section 17, Table 4. If the levels determined are outside the action limits (the average recovery ±3 times the standard deviation), repeat the extraction and analysis of the sample. If the results are outside the warning limits (the average recovery ±2 times the standard deviation), the analyst should review the analytical data and procedure for possible degradation of standards or other analytical problems.

9.7 Sample and duplicate precision

Analyze a sample and duplicate with each set of samples to assess the precision of the analyses. For effluent and influent samples that may contain low levels of analytes or a high frequency of non-detects, a duplicate matrix spike may be used to assess precision. Calculate the relative percent difference in concentration for each sample and duplicate pair (matrix spike and spike duplicate pair) using Equation 2.

Equation 2

$$Relative\ Percent\ Difference = \frac{(Highest\ concentration - Lowest\ concentration)\ x\ 100}{Average\ concentration\ of\ the\ sample\ and\ duplicate}$$

A summary of the precision determined in a single laboratory is provided in Section 17, Table 5 for treatment system influent and biologically treated effluent samples.

9.8 Field replicates and field spikes

Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10.0 Calibration and Standardization

10.1 Assemble the GC/MS and establish the operating conditions outlined below. Optimize the GC conditions as specified by the criteria outlined in Section 9.2. Once the operating conditions are optimized, use the same operating conditions to analyze all samples, blanks, calibration curves, calibration verification samples, and matrix spikes.

GC/MS Operating Conditions for NCASI Method NSV-99

Injector Temperature:	200°C
Splitless Valve Time:	0.8 min
Carrier Gas:	Helium @ 30-35 cm/sec & 130°C
Injection Volume:	1 µL
Temperature Program °C	
Initial:	35 for 2 min
Ramp:	35 to 140 @ 5°C/min
Ramp 2:	140 to 300 @ 50°C/min
Post Run:	300 for 6 min
Oven Equilibration:	0.50 min
Run Time:	32.2 min
Interface Temperature:	260°C
MS Conditions	
Scan Start Time:	5.00 min
Scan Range:	42 to 360 AMU
Scans/Sec:	0.7 to 1.3

10.2 Internal standard quantitation

10.2.1 Analyze the calibration standards (Section 7.2.10) using the procedure described in Section 11.5. Compute the relative response factors using Equation 3.

Equation 3

$$RRF = [(A_S / A_{IS}) \times (C_{IS} / C_S)]$$

where:
A_S = *area of the target compound in the calibration standard*
A_{IS} = *area of the internal standard in the calibration standard*
C_{IS} = *concentration of the internal standard in the calibration standard*
C_S = *concentration of the target compound in the calibration standard*

10.2.2 If the average of the relative response factors (RRF) calculated across the calibration range is constant (i.e., less than 20%) the calibration is acceptable and the average RRF can be used in all target analyte quantifications. Otherwise, evaluate the problem, undertake the appropriate remedial action, and reanalyze the calibration curve extracts. If remedial actions and reanalysis fail to produce a constant RRF, prepare new calibration curve extracts and analyze. The statistics for response factors determined during a single laboratory validation of this method are included in Section 17, Table 6.

10.3 Verify calibration prior to the analysis of each set of samples (Sections 9.2). Analyze one of the calibration standards (Section 7.2.10) prior to the analysis of each set of samples. It is recommended that the selected calibration standard vary over time in order to verify the calibration over the calibration range of the method. Recalibrate if the relative response factor for the target compounds in the analyzed calibration verification point differ by ±15% of the relative response factor determined for that calibration point in the current calibration curve. Calculate the percent difference between the calibration curve and the calibration verification relative response factors using Equation 4.

Equation 4

$$Percent\ Difference = [(RRF_{AVG} - RRF_V)/RRF_{AVG}] * 100$$

where:
RRF_{AVG} = *the average relative response factor from the initial calibration curve*
RRF_V = *the relative response factor from the calibration verification*

10.4 Process a blank with the curve to confirm that the glassware, reagents, and other components are free from contamination. Prepare the blank using the procedure used to prepare the calibration standards, omitting the addition of the target analytes and surrogates (Section 7.2.10).

10.5 Demonstrate that the target analytes are detectable at the minimum level using the lowest level calibration curve standard.

11.0 Procedure

11.1 This section includes the procedures used to extract and concentrate treatment plant influent and biologically treated effluent samples. The extraction, concentration, and extract drying procedures are used for all types of samples and method blanks.

11.2 Remove the sample, surrogate working stock (Section 7.2.8), internal standard working stock (Section 7.2.9), and appropriate working stock solutions A and B (Section 7.2.5 and 7.2.6) from the refrigerator and bring to room temperature.

11.3 Extraction of samples

11.3.1 Shake the sample to ensure homogeneity and immediately measure a 250-mL portion of the biologically treated effluent sample (125 mL of the treatment system influent diluted to 250 mL with deionized water) into a 500-mL beaker using a graduated cylinder. For method blanks, measure 250 mL of reagent grade water.

11.3.2 Spike with 125 µL of approximately 80 µg/mL surrogate working stock (Section 7.2.8). For matrix spikes, add the appropriate amount of the analyte working stock solutions A and B (Section 7.2.5 and 7.2.6).

11.3.3 Adjust to pH 11 by the addition of 10% sodium hydroxide.

11.3.4 Transfer the beaker contents into a 500-mL separatory funnel containing 1 gram of sodium chloride (NaCl) and mix thoroughly to dissolve the NaCl.

11.3.5 Pour a 40-mL portion of DCM into each empty beaker to help transfer any residual sample. Transfer the DCM to the separatory funnel, stopper the separatory funnel, and shake vigorously with frequent venting for a minimum of one minute. Allow the phases to separate for a minimum of ten minutes, then drain the DCM layer and any emulsions into a centrifuge tube. Two tubes may be necessary to contain the DCM layer and emulsions.

11.3.6 Centrifuge the samples for approximately two minutes and transfer the DCM layer to a Kuderna-Danish (KD) apparatus. Pour any remaining sample back into the separatory funnel to be re-extracted with the aqueous phase.

11.3.7 Repeat Section 11.3.5 to 11.3.6 two additional times, for a total of three extractions, with 40-mL portions of DCM. Combine the three DCM extracts in a KD apparatus.

11.4 Concentration and drying of the extract

11.4.1 Confirm that the water bath temperature is at a maximum of 70°C. Add a clean boiling chip to the KD apparatus, and attach a three-ball Snyder column. Pre-wet the Snyder column by adding 1 mL of DCM to the top of the column. Place the KD apparatus in the water bath and concentrate the extract until the apparent volume of liquid reaches 1 to 2 mL. Do not allow the extract to go to dryness.

11.4.2 Using approximately 2 mL of DCM, rinse down the Snyder column and 500-mL KD flask and remove them.

11.4.3 Construct a drying column by plugging a 5 ¾-inch Pasteur pipette tip with a small amount of filter pulp. Tap a 5 cm column of dried Na_2SO_4 into the Pasteur pipette. Dry the extract by loading it onto the top of the Na_2SO_4 column followed by three rinses with DCM, using 1 mL for each rinse. Place each rinse of DCM on the column just as the surface layer of the Na_2SO_4 has dried. Fill the column reservoir with a 2- to 3-mL aliquot of DCM to ensure that the extract is carried through the column. Collect all transfers and rinses (total volume 5 to 7 mL) in a 15-mL receiving tube. Add 500 µL of cyclohexane and a boiling chip to each receiving tube. Attach a micro-Snyder to the top of the tube, and concentrate the extract to 0.5 mL in a 85°C hot water bath.

11.4.4 Add 25 µL of the tridecane stock (Section 7.2.9) to each sample, mix thoroughly, and transfer qualitatively to a 2-mL autosampler vial. Cap the vial with a Teflon-lined cap. If the extracts are not analyzed immediately, store at 4°C. Always allow the extract to come to room temperature prior to GC/MS analysis.

11.5 GC/MS analysis

11.5.1 The GC/MS conditions should be set according to the criteria described in Section 10.1.

11.5.2 Bring the DFTPP tune solution to room temperature. Perform the DFTPP tune as outlined in Section 9.2.1.

11.5.3 Bring the daily calibration solution to room temperature. Perform the daily calibration verification as outlined in Section 9.2.2.

11.5.4 Bring the sample extract or standard to room temperature and verify that any precipitate has redissolved. Inject a 1-µL volume of the standard solution or extract, using a splitless injection.

12.0 Data Analysis and Calculations

12.1 GC/MS data analysis

An analyte is identified by comparison of the sample mass spectrum with the mass spectrum of a standard of the suspected compound which has been previously stored in a mass spectral library. Refer to Section 17, Table 7 for a list of the characteristic ions. Identification of a compound is confirmed when the following criteria are met.

12.1.1 Verify that the selected ions specified in Section 17, Table 7 are present and maximize within the same two consecutive scans.

12.1.2 The relative percent abundance of the ions designated in Section 17, Table 7 must agree within ±20% of those observed for the mid-point calibration curve standard during the most current calibration curve analysis.

12.1.3 The m/zs 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, an experienced spectrometrist must determine the presence or absence of the compound.

12.2 Internal standard quantitation

12.2.1 The tridecane internal standard is used to quantitate the corresponding neutral semivolatile compounds. Calculate the concentration of the target compound in the sample according to Equation 5.

Equation 5

$$Concentration\ of\ target\ (\mu g/L) = [(A_S \times C_{IS})/(A_{IS} \times RRF_{AVE})]$$

where:
A_S = *area of the compound being measured*
C_{IS} = *concentration (µg/L) of the tridecane internal standard in the sample*
A_{IS} = *area of the internal standard*
RRF_{AVE} = *averaged relative response from the initial calibration curve*

12.2.2 The concentration of target compounds found in the influent samples is corrected by using a multiplier of 2 to account for the extraction of 125 mL of sample versus 250 mL of sample.

12.3 Data review requirements

12.3.1 Review the data for accuracy of the identification, GC problems, interferences, and bias. Correct any problems prior to reporting the analytical results.

12.3.2 Manually review the chromatograms to confirm internal standard and analyte identification and area integrations. As part of this review, assess the need for sample/extract dilutions. The procedure for conducting extract dilution and reanalysis is described in Section 12.4.

12.3.3 Visually inspect the total ion chromatogram for obvious problems which might result in poor internal standard recoveries or false negatives/false positives. The presence of non-target species can become apparent from this review.

12.3.4 Resolve any inconsistencies between duplicate analyses (i.e., if a compound shows up in one replicate but not the other) and attempt to determine the reason.

12.3.5 Generate a GC/MS report that includes the retention time of the compound, area of the compound, width of the peak, and calculated concentration of the target compound detected. If review of the data shows any problems which could affect subsequent analyses, analyses are discontinued until the problems are resolved.

12.4 Results outside the calibration range

If the calculated concentration of any of the target analytes exceeds the concentration of the highest calibration point, dilute an aliquot of the extract with cyclohexane to bring the concentration within the calibration range of the method, and reanalyze. A maximum dilution of 1 to 10 is allowed in order to maintain sufficient internal standard concentrations in the extracts.

13.0 Method Performance

13.1 Single laboratory performance for this method is detailed in Section 17, Tables 3, 4, 5, 6, and 7. Acceptance criteria were established from single laboratory use of the draft method.

14.0 Pollution Prevention

Pollution prevention approaches have not been evaluated for this method. It is recommended that the laboratory check with state and local requirements to determine if pollution prevention equipment, such as solvent recovery devices, are required or recommended in their area. Use of these devices to reclaim solvents can be part of a pollution prevention program to reduce air emissions.

15.0 Waste Management

15.1 It is the laboratory's responsibility 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 and controlling releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 Instructions for sample and waste handling and disposal

15.2.1 Dispose of all waste solvents as required by federal, state and local regulations.

15.2.2 Neutralize the sodium hydroxide solution and pour it down the drain with copious amounts of water.

15.2.3 Neutralize the aqueous portion of the extracted sample to pH 7 and pour the aqueous portion of the extracted sample aliquot down the drain with copious amounts of water.

15.3 For further information on waste management, the Environmental Protection Agency suggests you consult *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

1. *Federal Register*, "Appendix B to Part 136-Definition and procedure for the determination of the method detection limit-revision 1.11," Vol. 49, No. 209, October 26, 1984.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Method Detection Limits for Biologically Treated Effluents[a]

Compound	MDL[b] (µg/L)
2,3-Dimethylcyclopentenone	1.1
2,3,5-Trimethylcyclopentenone	0.5
Acetophenone	0.5
3-Acetylthiophene	0.5
2-Acetylthiophene	0.5
Dichlorodimethyl Sulfone	0.6
2,3,4,5-Tetramethylcyclopentenone	0.5
Fenchyl Alcohol	0.4
Camphor	0.6
Terpinen-4-ol	0.5
2-Propionylthiophene	0.4
p-Cymen-8-ol	NA
Alpha-Terpineol	0.6
Verbenone	0.6
Pipertone	0.5

[a] an MDL has not been determined for treatment system influents
[b] MDLs determined using 40 CFR 136, Appendix B, *Federal Register* 1984
NA = MDL not available for this compound

Table 2. DFTPP Criteria for NCASI NSV-99

m/z	Ion Abundance Criteria
51	10-80% of mass 198
68	< 2% of mass 69
69	10-100% 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	1-100% of mass 198
441	10-100% of mass 443
442	50-100% of mass 198
443	15-24% of mass 442

Table 3. Surrogate Recovery in Biologically Treated Effluents During NCASI NSV-99 Method Validation Studies[a]

Compound	Spike Concentration (µg/L)	Average Recovery (%)	Acceptable Recovery Range (%)	n
2-Acetyl-3-methylthiophene	40	94	67-120	52
p-Chloroacetophenone	40	84	67-101	22

[a] data not available for influents due to change in surrogate selection during the initial method development experiments

Table 4. Matrix Spike Recovery for Compounds During Method Validation Studies Using NCASI NSV-99 by GC/MS

Compound	Average Recovery (%)	Standard Deviation	Warning Limits[a] (%)	Action Limits[b] (%)	n
2,3-DMCP	108	15	78-137	63-151	76
2,3,5-TMCP	105	13	78-132	65-145	69
Acetophenone	100	12	77-124	65-136	77
3-Acetylthiophene	104	12	80-128	67-141	77
2-Acetylthiophene	104	14	77-131	63-145	77
Dichlorodimethyl Sulfone	102	13	75-128	62-142	72
2,3,4,5-TMCP	103	14	76-131	63-144	77
Fenchyl Alcohol	100	16	69-132	53-148	73
Camphor	99	11	76-122	65-133	76
Terpinen-4-ol	104	12	79-128	67-140	77
2-Propionylthiophene	112	12	89-135	77-147	76
p-Cymen-8-ol	104	12	80-128	68-140	74
Alpha-Terpineol	105	12	82-128	71-140	76
Verbenone	109	12	85-133	73-144	77
Piperitone	106	13	81-131	69-144	77

[a] warning limits determined by taking the average plus or minus two times the standard deviation on n recovery experiments

[b] action limits determined by taking the average plus or minus three times the standard deviation of n recovery experiments

Table 5. Single Laboratory Precision Results Using NCASI NSV-99[a]

Compound	Range of RPDs Observed[b] (%)	Average RPD (%)	n[c]
2,3-DMCP	4.6-8.1	6.3	3
2,3,5-TMCP	1.0-9.7	5.6	8
Acetophenone	2.6-8.7	5.5	11
3-Acetylthiophene	0.29-8.0	3.0	13
2-Acetylthiophene	0.21-6.3	2.7	14
Dichlorodimethyl Sulfone	0.07-10.4	3.7	22
2,3,4,5-TMCP	0.39-16.2	4.6	19
Fenchyl Alcohol	5.6-7.4	6.5	2
Camphor	7.0-13.3	9.5	3
Terpinen-4-ol	1.6-2.8	2.2	2
2-Propionylthiophene	0.36-10.3	3.4	9
p-Cymen-8-ol	0.8-10.5	4.5	3
Alpha-Terpineol	1.7-19.4	8.4	6
Verbenone	5.2-8.6	6.4	3
Piperitone	0.31-10.6	5.4	2

[a] precision of the target analytes native to treatment system influents and biologically treated effluents

[b] range of relative percent differences observed between a sample and a duplicate

[c] number of duplicate data points with both values above the MDL

Table 6. Response Factor Statistics For NCASI NSV-99

Compound	Average Relative Response Factor[a]	Standard Deviation[b]	Relative Response Factor Range[c]
2,3-DMCP	0.211	0.049	0.146-0.305
2,3,5-TMCP	0.438	0.064	0.332-0.547
Acetophenone	1.140	0.093	1.015-1.359
3-Acetylthiophene	1.211	0.100	1.091-1.400
2-Acetylthiophene	1.272	0.113	1.110-1.424
Dichlorodimethyl Sulfone	0.977	0.149	0.717-1.246
2,3,4,5-TMCP	0.693	0.049	0.638-0.774
Fenchyl Alcohol	0.567	0.088	0.457-0.701
Camphor	0.627	0.067	0.509-0.744
Terpinen-4-ol	0.557	0.206	0.338-0.977
2-Propionylthiophene	1.818	0.111	1.626-1.945
p-Cymen-8-ol	0.649	0.089	0.499-0.769
Alpha-Terpineol	0.289	0.026	0.262-0.342
Verbenone	0.382	0.069	0.272-0.476
Piperitone	0.719	0.165	0.496-1.011
2-Acetyl-3-methylthiophene (S)	1.197	0.116	1.002-1.390

[a] average relative response factors determined for ten five-point calibration curves

[b] average relative standard deviation expressed as a percent for the ten five-point calibration curves

[c] relative response factor range observed in a single lab over ten five-point calibration curves

(S) surrogate

Table 7. Characteristic Ions for NCASI NSV-99 Compounds Using GC/MS

Compound	Primary Ion	Secondary Ions
2,3-Dimethylcyclopentenone	110	95, 67
2,3,5-Trimethylcyclopentenone	109	124, 81
Acetophenone	105	77, 120
3-Acetylthiophene	111	126, 83
2-Acetylthiophene	111	126, 83
Dichlorodimethyl Sulfone	83	85
2,3,4,5-Tetramethylcyclopentenone	123	138, 95
Fenchyl Alcohol	81	111, 121
Camphor	95	108, 152
Terpinen-4-ol	111	136, 154
2-Propionylthiophene	111	140, 83
p-Cymen-8-ol	135	150, 91
Alpha-Terpineol	121	93, 136
Verbenone	107	91, 135, 150
Piperitone	110	137, 152
2-Acetyl-3-Methylthiophene (S)	125	140, 97
p-Chloroacetophenone(S)	139	141, 111, 154
Tridecane(IS)	71	85, 184

(S) Surrogate
(IS) Internal Standard